Diss. ETH N^{o} 21499

ENGINEERING OF SYNTHETIC GENE CIRCUITS FOR THE TREATMENT OF METABOLIC DISEASES

A dissertation submitted to

ETH ZURICH

for the degree of

Doctor of Sciences

presented by

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2013

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SUMMARY

Biomedical science is aiming towards the development of customized therapies against multifactorial chronic diseases, such as diabetes, hypercholesterolemia and obesity. Synthetic biology is a novel discipline in this scientific community focusing on reprogramming cells that are able to sense and process external signals with almost computer-like precisions. This work collectively illustrates the potential of clinical applications in synthetic biology, where the detection of specific disease markers can be directly linked to the timely production of a therapeutic protein.

In the first project we have developed a gene regulation network able to sense various fatty acids derived from plants or animals. The regulatory core of this device is the hybrid protein LSR (lipid-sensing receptor) consisting of the peroxisome proliferator-activated receptor PPARα (a key mammalian regulator of lipid metabolism) and the *Pseudomonas putida* DOT-TE1 derived transcription factor TtgR. Taking advantage of LSR's sequence-specific binding ability to chimeric TtgR promoters, this hybrid protein was able to translate the lipid levels in the cellular environment to tightly regulated gene transcription. The overall gene regulation network showed excellent performance in several mammalian cell types as well as in animal experiments. To test the clinical utility of this network, we expressed the clinically licensed anorectic drug pramlintide in diet-induced obese mice that showed increased blood fat levels, which caused a change in food intake, decreasing blood fat levels and weight reduction.

Similarly, the gene regulation circuit developed in the second project could be implemented as a biosensor for physiological bile acid concentrations. Abnormal bile acid levels in the blood can damage the liver, thereby representing a potential source of different metabolic disorders. By transferring the bile acid multidrug efflux pump CmeR-O_{CmeABC} of the bacteria *Campylobacter jejuni* into mammalian cells, we were able to design the precise, robust and adjustable bile acid sensors $BEAR_{OFF}$ and $BEAR_{ON}$, which interact with several bile acids to regulate gene expression *in vitro*. Furthermore, $BEAR_{ON}$ was able to sense bile acids in mice indicating its potential for future gene- and cell-based therapies.

In nature activation of stimuli-specific cell-surface receptors is an adapted response of cells upon changing environmental conditions. When activated by a specific trigger molecule, the receptor protein initiates a biochemical signal cascade that is eventually transduced into the nucleus where the expression of response-specific genes is initiated. In the third project we focused on the development of a synthetic brain-dopamine interface by establishing a cell-surface receptor-based gene regulation system activated by dopamine as a trigger molecule. Dopamine is a neurotransmitter in the brain that achieves several functions in the central and peripheral nervous

system (movement, emotional responses etc.) and also functions outside the nervous system as a chemical messenger. It is released into the blood by sympathetic nerves that enmeshing blood vessels throughout the body and correlates with brain dopamine produced in reward situations. By rewiring the human dopamine receptor D1 with a response-specific promoter (P_{CRE}), we have designed a gene regulation network that was functional in several mammalian cell lines with high expression levels and tight regulatory control *in vitro*. In mice experiments this engineered system could be activated via direct dopamine administration or in an auto-regulated fashion by dopamine released in response to pleasure-associated situations. Furthermore, we were able to use the circuit for the expression of a vasodilator to reduce blood pressure in hypertensive mice. The designed synthetic circuit is versatile and highly flexible and further provides new gene- and cell-based strategies that require seamless and self-sufficient drug dosing.

ZUSAMMENFASSUNG

Die biomedizinische Wissenschaft zielt auf die Entwicklung maßgeschneiderter Therapien gegen multifaktorielle chronischen Krankheiten, wie z.B. Diabetes, Hypercholesterinämie und Fettleibigkeit, ab. Synthetische Biologie ist ein neuer Bereich dieser Wissenschaft, welche sich auf die Neuprogrammierung von Zellen fokussiert, die in der Lage sind externe Signale mit beinahe Computer-ähnlicher Genauigkeit zu erfassen und zu verarbeiten. Die vorliegende Arbeit gibt einen zusammenfassenden Überblick über das Potential klinischer Anwendungen in der synthetischen Biologie, in der die Detektion von spezifischen Krankheitsmarkern direkt mit der zeitnahen Produktion eines therapeutischen Proteins verknüpft werden kann.

In dem ersten Projekt haben wir ein Genregulationnetzwerk entwickelt, das verschiedene Fettsäuren aus Pflanzen und Tieren erkennen kann. Der regulatorische Kern dieser Baugruppe ist das Hybrid-Protein LSR (Lipid-erkennender Rezeptor) bestehend aus dem Peroxisomproliferator-aktivierten Rezeptor PPARα (ein wichtiger Säugerzellen-Regulator des Fettstoffwechsel) und dem aus *Pseudomonas putida* DOT -TE1 stammenden Transkriptionsfaktors TtgR. Dieses Hybrid-Protein war fähig, unter Verwendung der LSR' Sequenz-spezifischen Bindefähigkeit zu chimärischen TtgR Promotoren, Blutfettwerte der zellulären Umgebung in eine genau regulierte Gentranskription zu übersetzen. Das gesamte Genregulationsnetzwerk zeigte eine hervorragende Funktionalität in verschiedenen Säuger- Zelltypen sowie im Tierversuch. Um die klinische Durchführbarkeit dieses Netzwerk zu testen haben wir einen klinisch lizenzierten Appetitzügler produziert, der in Diät-induzierte fettleibigen Mäusen mit erhöhten Blutfettwerten zur Appetitssättigung, sinkenden Blutfettwerten und Gewichtsreduzierung führte.

In ähnlicher Weise könnte die im zweiten Projekt entwickelte Genregulation als Biosensor für physiologische Gallensäure-Konzentrationen verwendet werden. Abnormale Gallensäurekonzentrationen im Blut können die Leber schädigen was die potentielle Quelle verschiedener Stoffwechselstörungen sein kann. Durch die Anwendung der Gallensäure „Multidrug-Efflux" Pumpe CmeR-O_{CmeABC} von *Campylobacter jejuni* in Säugerzellen konnten wir die präzisen, robusten und einstellbaren Gallensäure-basierenden Biosensoren $BEAR_{OFF}$ und $BEAR_{ON}$ entwickeln, die mit verschiedenen Gallensäuren interagieren und die Expression von Genen *in vitro* realisieren können. Darüber hinaus war der $BEAR_{ON}$ Sensor in der Lage, Gallensäuren in Mäusen wahrzunehmen, was dessen biomedizinischen Einsatzbereich darlegt.

In der Natur ist die Aktivierung von Stimuli-spezifischen Zelloberflächen-Rezeptoren eine angepasste Reaktion von Zellen gegenüber wechselnden Umgebungsbedingungen. Nach der Aktivierung durch ein bestimmtes Auslösermolekül stösst der Rezeptor eine biochemische

Signalkaskade an, die dem Zellkern übermittelt wird, wo die Expression der Ansprech-spezifischen Gene gestartet werden kann. Dieser Mechanismus stellt ein nützliches Werkzeug für die neuen Strategien zur Behandlung von Krankheiten in der synthetischen Biologie dar. Im dritten Projekt haben wir uns auf die Entwicklung einer Gehirn-Dopamin-Schnittstelle durch die Herstellung eines Zelloberflächen-Rezeptor-basierten Genregulationssystem konzentriert, das durch Dopamin als Auslösermolekül aktiviert wird. Dopamin ist ein Neurotransmitter im Gehirn, der mehrere Funktionen im zentralen und peripheren Nervensystem (Bewegung, emotionale Reaktionen) und Funktionen in mehreren Teilen außerhalb des Nervensystems als Botenstoff erfüllt. Es wird ins Blut über sympathische Nerven, welche die Blutgefässe im gesamten Körper umgeben, freigesetzt und korreliert mit Dopamin im Gehirn, das durch Belohnungssituationen produziert wurde. Durch die Kombination des humanen Dopamin-D1-Rezeptors mit einem Ansprech-Promotor (P_{CRE}), haben wir ein Genregulationsnetzwerk entwickelt, das in mehreren Säugetier-Zelllinien mit hoher Expression und enger regulatorischer Kontrolle *in vitro* funktionell war. In Mausexperimenten konnte das erstellte System direkt durch Administration von Dopamin oder auto-reguliert durch Dopamin, welches als Reaktion auf Genuss-assoziierte Situationen ausgeschüttet wurde, aktiviert werden. Darüber hinaus konnten wir mittels des entwickelten Systems erfolgreich einen Vasodilator zur Blutdrucksenkung in hypertonischen Mäusen exprimieren. Das entworfene synthetische System ist vielseitig und sehr flexibel und stellt für die Zukunft neue Gen- und Zellbasierenden Strategien zur Verfügung, welche eine nahtlose und unabhängige Medikamentdosierung erfordern.

INTRODUCTION

General Introduction

Worldwide, scientists work to improve the quality of human life and to benefit society. Large amounts of biological data concerning cell life, cell metabolism and cell function are generated and different research areas such as bioinformatics, biophysics, biochemistry, systems biology and medicine implement this new knowledge. Additionally, synthetic biology as a new research field has been established, in which naturally or modified cell components are recombined to generate gene regulation networks controlling gene expression and thereby regulating cellular behavior.

Although, the volume of biological data is consistently increasing, many medical issues remain unsolved. Unsuccessful treatment therapies for metabolic diseases, such as diabetes and obesity make research and further development of alternative therapies crucial. Several lifestyle-associated medical conditions in modern society like type 2 diabetes, cardiovascular disorders or osteoarthritis harbor major challenges such as drug delivery, potential side effects, drug-specific targeting and sustainable therapeutic efficacy for the development of biomedical applications. Synthetic biology could provide an elegant solution to manage the high demand on modern molecular medicine. Several synthetic engineered devices, which will be illustrated in the next chapter, have recently been described overcoming such concerns and provide solutions for novel cell-based therapeutic strategies to improve the treatment of human diseases.

In this work we have developed (i) a pioneering fatty acid-triggered synthetic circuit for the treatment of diet-induced obesity, which constantly monitors blood fatty acid level and processes the expression of an anti-obesity peptide hormone to reduce food intake, blood fat level and body weight in response to specific diets; (ii) bile acid-triggered gene regulation circuits ($BEAR_{OFF}$ and $BEAR_{ON}$), which precisely expresses the human secreted alkaline phosphatase in response to different bile acids *in vitro* and *in vivo* (only $BEAR_{ON}$); and (iii) a reward-controlled gene regulation network based on dopamine enabling gene expression of the antihypertensive peptide hormone ANP to reduce blood pressure in hypertensive mice.

Synthetic Biology

Synthetic biology is a rapidly developing interdisciplinary scientific field involving engineers, biologists, mathematicians and chemists. It focuses on the design of new biological parts, systems and even cells to build up living devices, which do not naturally exist, as well as to target the re-design of existing, natural biological systems (IDEKER *et al.* 2001; BRENT 2004; ADRIANANTOANDRO *et al.* 2006). Furthermore, those devices can be used as molecular-scale factories, to clean up pollutants, to diagnose human metabolic diseases, to deliver vaccines or to design new hybrid materials (SCHMIDT 2008; KEASLING 2008; KARLSSON AND WEBER 2012).

Several decisive accomplishments in synthetic biology have been achieved over the past years, including different gene regulation systems which were combined to create numerous gene networks like toggle switches (GARDNER *et al.* 2000; KRAMER *et al.* 2004), hysteretic circuits (KRAMER AND FUSSENEGGER 2005), oscillators (ATKINSON *et al.* 2003; ELOWITZ AND LEIBLER 2000; TIGGES *et al.* 2009; TIGGES *et al.* 2010), band-pass filters (BASU *et al.* 2005; WEBER *et al.* 2007), inter-cellular communication networks (BACCHUS *et al.* 2012) and logic metabolic computers (FRIEDLAND *et al.* 2009; RINAUDO *et al.* 2007; XIE *et al.* 2011; AUSLÄNDER *et al.* 2012). Furthermore, regulatory biosensors that respond to metabolic input signals or external sources, such as light or clinically licensed drugs, have been developed to reprogram cellular behavior in order to employ a feedback-controlled regulation system for therapeutic use (KEMMER *et al.* 2010; KEMMER *et al.* 2011; YE *et al.* 2011; BACCHUS AND FUSSENEGGER 2012; YE *et al.* 2013; AUSLÄNDER AND FUSSENEGGER 2013). Despite its immense improvements over the last years, synthetic biology is still in its infancy. Environmental safety, versatility and long-term applicability as well as complexity and physiology of the mechanism of action within host organism continuously raise the need for further development.

The following sections will introduce the general principles of the regulatory building blocks essential for synthetic biology.

Transcription-based Gene Regulation Systems

The regulation of gene expression is an essential tool used by cells to increase or decrease specific protein production. It enhances the versatility and adaption of an organism to changing environmental conditions and therefore represents an interesting and useful instrument for synthetic biology. In the last years several tunable and oscillating gene expression applications have been successfully implemented in the field of functional genomics (BAUMGARTEL *et al.* 2008), biopharmaceutical manufacturing of complex proteins (WURM 2004; ULMER *et al.* 2006; WEBER AND FUSSENEGGER 2007; GITZINGER *et al.* 2009a), tissue engineering & functional materials (GREBER AND FUSSENEGGER 2007; SANCHEZ-BUSTAMANTE *et al.* 2006; WEBER and FUSSENEGGER 2006; EHRBAR *et al.* 2008), drug discovery (SHARPLESS and DEPINHO 2006; WEBER *et al.* 2008), the design of complex synthetic networks (DEANS *et al.* 2007; KRAMER AND FUSSENEGGER 2005; TIGGES *et al.* 2010; TIGGES *et al.* 2009; XIE *et al.* 2011; AUSLÄNDER *et al.* 2012) and gene therapy (GERSBACH *et al.* 2006; WEBER AND FUSSENEGGER 2006; KEMMER *et al.* 2010). This illustrates the importance of precise control of target gene expression for different research areas, including basic biology and applied sciences like synthetic biology. Although early gene regulation systems were implemented in prokaryotes, biomedical applications and human therapy now focus more and more on mammalian cell-based systems.

In this work we will focus on small-molecule-based design for controlling gene regulation in eukaryotic cells, which are based on and therefore comparable to prokaryotic systems. Gene regulation systems contain a regulator element, which can interact with an operator. The DNA-binding protein (DBP) is fused to a mammalian transactivator (TA), such as the transactivation domain of Herpes simplex virus (VP16), the p65 transactivation domain or the human E2F4 transactivation domain (URLINGER *et al.* 2000; AKAGI *et al.* 2001), thereby forming the transactivator that activates gene expression by binding to its cognitive operator sequence placed downstream of an eukaryotic minimal promoter (P_{min}). The DBP can also be fused to a transrepressor (TR) domain, like the human Kruppel-associated box-protein (KRAB), to generate a transrepressor, which represses transgene expression while binding to its hybrid promoter engineered from a specific operator upstream of a constitutive promoter. (DEUSCHLE *et al.* 1995; FUSSENEGGER *et al.* 2000; WEBER *et al.* 2002a; WEBER *et al.* 2002b).

Combining these regulators with its specific operators enables the creation of two systems: the OFF- and the ON-type system (Figure 1). In the OFF-type system gene expression is activated in the non-induced state, since the transactivator is continuously binding to its operator. Upon addition of the inducer the transactivator releases from the DNA, which stops further transgene

production. This mechanism is reversed in the ON-type systems. In the inducer's absence, the transrepressor binds to the DNA and gene expression is shut down. Whereas, its presence causes the release of the regulator from the DNA segment, similar to the OFF-type system, and induces target gene production (GOSSEN AND BUJARD 1992; FUSSENEGGER *et al.* 2000; WEBER AND FUSSENEGGER 2004).

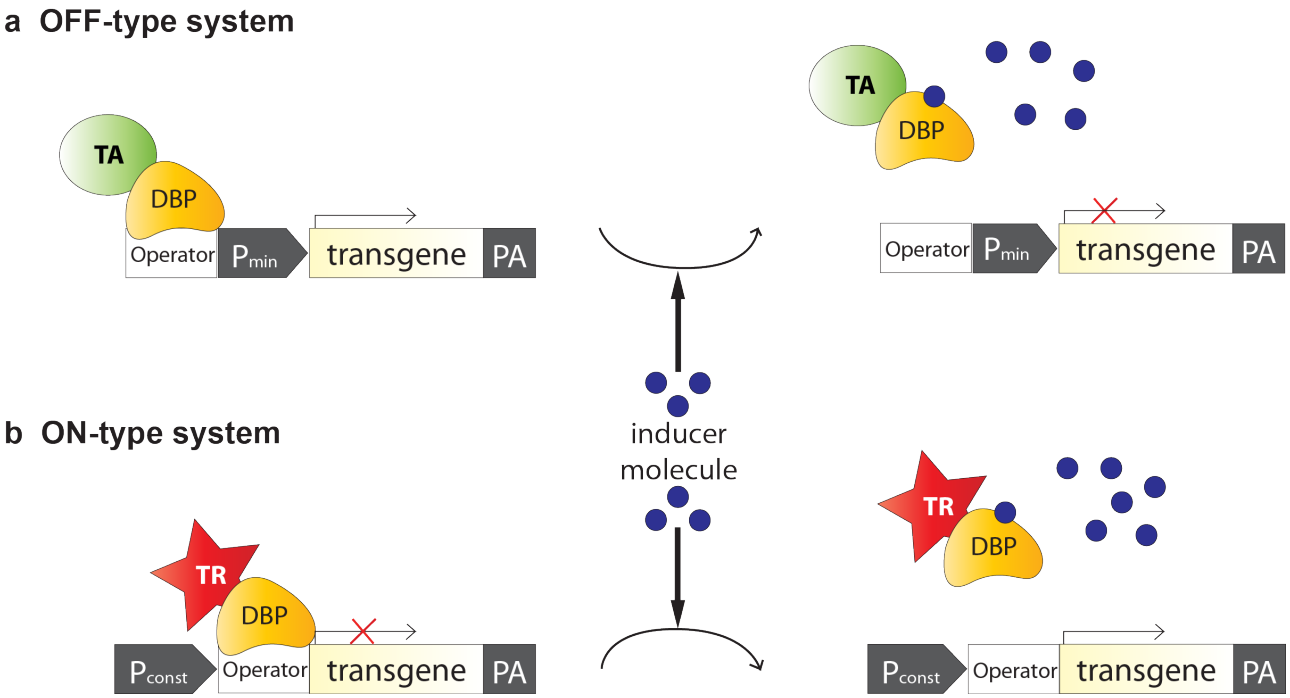

Figure 1: Controlled gene expression by two different variants. (a) OFF-type system: Gene expression is initiated in absence of the ligand by precise binding of the transactivator (TA) to its cognitive operator sequence, which is located directly in front of a minimal promoter (P_{min}). Upon addition of the inducer TA is released from its operator and hence gene expression is switched off. (b) ON-type system: the regulator functions as a transrepressor (TR) in order to repress gene expression in ligand-free medium while TR is continuously binding to its operator, which is located immediately after a constitutive promoter (P_{const}). Supplementation of the inducer changes TR's conformation, TR releases from the operator and gene expression is initiated.

Optimization of the robustness and reducing the leakiness of the inducible system can be generated by several strategies, including the variety of the operator, the number of operator repeats or by varying the length of the linker sequence between the operator and the minimal promoter for optimal binding and regulation (GITZINGER *et al.* 2009b).

Early gene regulations systems were induced by several small molecules, such as antibiotics (e.g. macrolide, streptogramin, tetracycline), hormones, immunosupressors, quorum-sensing molecules or gaseous compounds (WEBER *et al.* 2002a, FUSSENEGGER *et al.* 2000; GOSSEN *et al.*

1995; NORDSTROM 2002; PALLI *et al.* 2005; NEDDERMANN *et al.* 2003; WEBER *et al.* 2006; POLLOCK AND CLACKSON 2002; POLLOCK *et al.* 2002; ROLLINS *et al.* 2000). These established systems showed (i) precise target gene titration, (ii) high target gene adjustability (iii) tight regulation (low leakiness), and (iv) high transgene expression levels. However, the use of such inducer molecules in gene and cell-based treatment strategies still remains limited due to several drawbacks, e.g.: (i) undesired side effects, (ii) undefined pharmacodynamics and -kinetics and (iii) biosafety (production of new antibiotic-resistant strains) (LAUTERMANN *et al.* 2004; SANCHEZ *et al.* 2004; ALANIS 2005). Most recent biological gene regulation networks are designed to understand the complexity of diseases, treat diseases, or to improve commercial drugs, e.g. by using endogenous metabolites, such as amino acids (BACCHUS *et al.* 2012; BACCHUS *et al.* 2013; HARTENBACH *et al.* 2007), vitamins (WEBER *et al.* 2007a; WEBER *et al.* 2009), several licensed food additives (GITZINGER *et al.* 2012, WEBER *et al.* 2008) or pathological molecules, like urate (KEMMER *et al.* 2010) and even light (YE *et al.* 2011) as trigger molecules.

Some of these successfully established gene regulation systems have as well demonstrated proof-of-concept strategies for the treatment of metabolic diseases such as type 2 diabetes, acute hyperuricemic disorders or the metabolic syndrome (KEMMER *et al.* 2010, YE *et al.* 2011, YE *et al.* 2013), illustrating the direction synthetic biology is likely to take, towards clinical practice.

In Chapter I (Lipid-sensing receptor to control obesity) and Chapter II (bile-acid responsive gene regulation network) we will provide first insights on how synthetic gene regulation systems such as the ones described in these chapters can be used and have the potential in treating metabolic diseases, e.g. obesity.

GPCR-based Gene Regulation systems in Mammalian cells

Living cells are sophisticated systems that mediate important physiological processes, such as the immune system, taste, smell or the fight-or-flight response in humans. G protein-coupled receptors (GPCRs) are essential tools for cells to respond to variation in the environment in a fast, specific and sensitive way (DORSAM and GUTKIND 2007). These receptors are signal transducers on the cell surface in the form of seven transmembrane helices (PIERCE *et al.* 2002), which are connected by loops, three on the intracellular and three on the extracellular side (Fig. 2). Their primary function is to transmit signals from the outside to the inside of the cell to make the cell respond as well as adapt to environmental changes. Within the cell, GPCRs are bound to small subunits (α, β, γ) of the guanine nucleotide binding protein (G protein), a key signaling molecule that acts as a sensor and messenger to transfer signals further within the cell (GILMAN 1987; NEVES *et al.* 2002). A large range of molecules, like biogenic amine (dopamine, noradrenaline etc.), lipids, peptides, proteins, clinically licensed drugs or even light and odorants can stimulate a GPCR (YE *et al.* 2011; YE *et al.* 2013). After binding of a ligand, the GPCR undergoes a conformational change that results in the dissociation of one of the G protein subunits. This subunit is then able to interact with several downstream effectors to activate a signaling cascade. By implementing synthetic biology, scientists have provided specific response elements that interact with such endogenous intercellular signaling molecules, thereby creating custom-designed signaling circuits with novel functions (KEMMER *et al.* 2011; YE *et al.* 2011; YE *et al.* 2013).

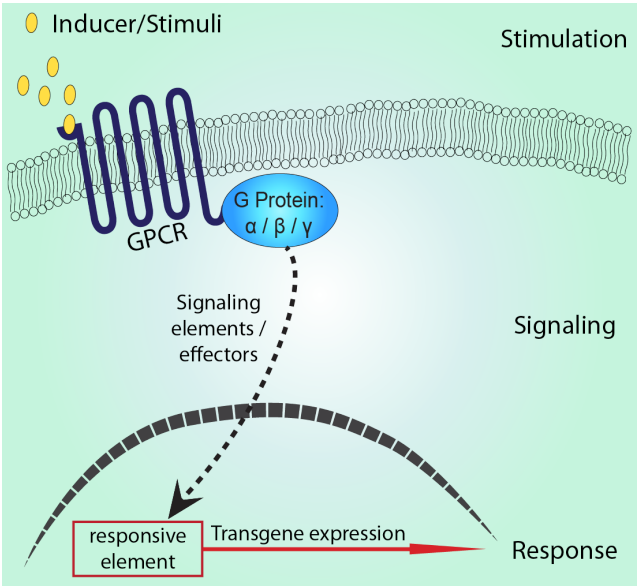

Figure 2: Elementary demonstration of G protein-coupled receptor signaling for the generation of gene regulation networks. Binding and stimulation of exogenous inducer to the G protein-coupled receptor (GPCR) triggers the G protein to stimulate effector proteins, whereby a signaling cascade including several effectors becomes activated. Those effectors can be recognized by specific responsive elements, which can be developed by synthetic biology and which are further able to initiate transgene expression, e.g. of specific target proteins.

In chapter III, we have focused on the dopamine receptor D1 (DRD1), a GPCR activated by dopamine, to generate a synthetic gene regulation circuit. Dopamine is the predominant neurotransmitter in the brain that controls several central nervous system events including movement and emotion (MISSALE *et al.* 1998). Many important diseases, such as Parkinson's disease or schizophrenia are associated with dysfunction of the dopamine system (LEKNES and TRACEY 2008). Additionally, dopamine functions in several parts outside the nervous system as chemical messenger to control respiration and glucose homeostasis (RUBÍ and MAECHLER 2010). All this combined represents dopamine as a highly interesting trigger molecule for synthetic biology. After dopamine binding and thereby DRD1 activation, the G protein $G\alpha_S$ stimulates the adenylyl cyclase which catalyzes the conversion of ATP to cyclic AMP (cAMP). cAMP functions as a second messenger and activates the protein kinase A (PKA), which further activates the cAMP-responsive element binding protein 1 (CREB1) by phosphorylation (ZHOU *et al.* 1990; DEARRY *et al.* 1990). Rewiring of the activated CREB1 with the synthetic responsive promoter P_{CRE} allowed the construction of a dopamine-triggered synthetic gene regulation network with a novel treatment strategy to cure hypertension (chapter III).

Lifestyle-associated Diseases related to this work

Lifestyle-associated diseases, including heart diseases, type 2 diabetes, obesity, atherosclerosis, metabolic syndrome or asthma, are diseases that appear more often in industrialized countries than in other areas of the world (FRASER and SHAVLIK 2001). They have to be seen separately from other diseases, since they can be prevented or diminished with a change in diet, lifestyle or environment (WADDEN *et al.* 2007). However, dieting or lifestyle changes often only show limited success and mostly work in a low number of patients (MANN *et al.* 2007). Over the past years many drugs have been invented to treat these diseases, but due to several side effects they are inconvenient and some have even been withdrawn from the markets again. Therefore, the use of synthetic biology could overcome these problems and may offer new treatment strategies by developing specific gene regulation networks that sense disease-related biomarkers in order to generate therapeutic proteins to cure those lifestyle-associated diseases.

Obesity

Obesity is an abnormal fat accumulation within the body, which shows a prevalence of more than 30% in the USA (> 15% of children and adolescents) (HASLAM AND JAMES 2005; WANG *et al.* 2011; ZHUO *et al.* 2012) as well as in other industrialized countries. Obesity represents a major public health problem, as its risk to promote chronic diseases such as diabetes, cardiovascular diseases, high blood pressure and cancer is evident.

The change in lifestyle through dieting or improving physical exercises is the fundamental treatment in reducing obesity and thereby other related diseases like hypertension. However, low efficacy and sustainability limit the success in reducing body weight (MANN *et al.* 2007).

Although, many companies focus on obesity and try to find new anti-obesity drugs, there are currently just a few drugs available on the market for the long-term treatment of obesity, which only show moderate efficacy (GRAY *et al.* 2012; KHAN *et al.* 2012; BAYS 2011). Most invented drugs have been withdrawn from the market again, due to side effects (NISSEN AND WOLSKI 2007; BOUCHI 2012). Surgery also represents an option to reduce body weight, but due to significant risk to the patient it is not recommended (LALMOHAMED *et al.* 2012).

Beside the production and development of new cost-effective drugs, the treatment of obesity requires new strategies. Synthetic biology might therefore provide a solution. Based on fatty acids, we have established a synthetic gene regulation circuit by combining human fat responsive host elements with regulatory elements to express an anorectic target protein. This approach allowed for

the reduction of body weight in diet-induced obese (DIO) mice by expressing the anti-obesity peptide hormone pramlintide in response to fatty acids (chapter I).

Hypertension

Another chronic disease associated with lifestyle dependent diseases such as obesity, is high blood pressure or hypertension. Elevated blood pressure in the arteries requires the heart to work harder than normal in order to circulate blood through the blood vessels. In mammals blood pressure is regulated by the atrial natriuretic peptide (ANP), a powerful vasodilator released by the heart muscle cells, which controls the body water, sodium, potassium and fat homeostasis in the circulatory system (SCHILLINGER *et al.* 2005; THERRIEN *et al.* 2010; LIN *et al.* 1995). Normal blood pressure values are in the range of 90 – 140mmHg systolic blood pressure (SBP), whereas the SBP of hypertensive people rises above 140mmHg (CHOBANIAN *et al.* 2003, TULMAN *et al.* 2012). Approximately 30% of the adult population is diseased by hypertension worldwide. Since 2003, high blood pressure became the most common chronic medical problem in the US (between 2003 and 2006 74.500.000 US adults ≥ 20 years of age suffered from hypertension), resulting in immense healthcare costs, as the majority of hypertensive people are usually treated with more than 1 drug to control their high blood pressure (LLOYD-JONES *et al.* 2010). Even if the combination of two drugs seems to be efficient, side effects still remain since these medications affect the heart and blood vessels and can therefore change patient's heartbeat and –rate (ROTH *et al.* 1986; BADESCH *et al.* 2004; GRADMAN *et al.* 2010).

Also in this case, the use of synthetic biology could provide new approaches and solutions in terms of hypertension treatment. Taking advantage of a gene regulation system to express ANP as a target, which controls blood pressure, we were able to decrease high blood pressure in hypertensive mice (chapter III).

Contribution of this work

Chapter I: A Designer Circuit Controlling Diet-Induced Obesity.

Obesity is the world's number one killer and recently it has been shown that its health burden even tops hunger as the biggest problem worldwide. The success of several treatment procedures, such as physiological persistence, medical care or surgery remains low and often harbors drastic side effects and risks. In this chapter, we developed a novel precise synthetic gene regulation circuit that allows the detection of a variety of plant- and animal derived fatty acids and which could even score diet-dependent blood fat levels in mice. On top of that, the system was able to correct diet-induced obesity in mice by coupling the lipid sensor to a building block expressing a clinically licensed anorectic drug, which caused satiety, reduction in body weight and decreased blood fat levels. Substitution of the clinically licensed apple metabolite phloretin resulted in an additional safety switch, which could prevent undesired side effects. Taking all these features together, this novel transgene expression system offers broad application possibilities and opens new ways in synthetic biology to treat diseases based on feedback-loop mechanism.

Chapter II: Bile acid-controlled transgene expression in mammalian cells and mice.

Several diseases have metabolic abnormalities as a source of harm, which represents a starting point for future gene regulations systems to focus on the detection and correction of metabolic pathological molecules. Cholesterol and its derivative bile acids play major roles in the human metabolism and are the origin of different regulatory processes. Variations of bile acids in the human circulatory system have a tremendous influence on other pathways, which makes it important to focus on the regulation of this metabolite.

Capitalizing on the interaction between *Campylobacter jejunis* CmeR repressor and its inverted repeat operator O_{Cme}, we have designed the synthetic mammalian bile acid-triggered control devises $BEAR_{OFF}$ and $BEAR_{ON}$. Both systems offer precise gene expression in response to a critically important metabolic trigger compound in an adjustable manner in cells grown in culture. Additionally, $BEAR_{ON}$ performed very well when implanted into mice. Through interaction with several bile acids this device represents a useful tool for future applications and is qualified for its

use in diagnostic or prosthetic networks monitoring and correcting pathophysiology associated with bile acid.

Chapter III: Reward-based hypertension control by a synthetic brain-dopamine interface.

A large number of signaling pathways is used by cells to regulate their activity. The majority responds to external stimuli, such as dopamine, which represents a useful trigger molecule, since it plays an important physiological role in the body of animals and is considered to be a key component of the reward system. By rewiring the G protein coupled dopamine receptor D1 with a response-specific transgene expression block we could take advantage of the second messenger molecule cAMP and protein kinases originated within the cell. We developed a dopamine specific gene regulation network that shows a broad applicability in different cell lines and excellent functionality *in vivo* when the dopamine flux was initiated by the reward system. Furthermore, we expressed a peptide hormone that serves as a vasodilator to reduce blood pressure in hypertensive mice based on sexual arousal. In general, this synthetic network might be useful for a lot of different applications besides hypertension and represents a valid alternative to existing gene regulation systems in synthetic biology.

References

AKAGI, K., KANAI, M., SAYA, H., KOZU, T. & BERNS, A. 2001. A novel tetracycline-dependent transactivator with E2F4 transcriptional activation domain. *Nucleic Acids Res. 29*, E23.

ALANIS, A. J. 2005. Resistance to antibiotics: are we in the post-antibiotic era? *Arch. Med. Res. 36*, 697-705.

ANDRIANANTOANDRO, E., BASU, S., KARIG, D. K. & WEISS, R. 2006. Synthetic biology: new engineering rules for an emerging discipline. *Mol. Syst. Biol. 2*, 2006.0028.

ATKINSON, M. R., SAVAGEAU, M. A., MYERS, J. T. & NINFA, A. J. 2003. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in Escherichia coli. *Cell 113,* 597-607.

AUSLÄNDER, S., AUSLÄNDER, D., MÜLLER, M., WIELAND, M., & FUSSENEGGER, M. 2012. Programmable single-cell mammalian biocomputers. *Nature* 487,123-127.

AUSLÄNDER, S. & FUSSENEGGER, M. 2013. From gene switches to mammalian designer cells: present and future prospects. Trends Biotechnol. 31, 155-68.

BACCHUS, W., LANG, M., EL-BABA, M. D., WEBER, W., STELLING, J. & FUSSENEGGER, M. 2012. Synthetic two-way communication between mammalian cells. *Nat. Biotechnol*. 30, 991-996.

BACCHUS, W. & FUSSENEGGER, M. 2012. Engineering of synthetic intracellular communication systems. *Metab. Eng. 16*, 33-41.

BACCHUS, W., WEBER, W. & FUSSENEGGER, M. 2013. Increasing the dynamic control space of mammalian transcription devices by combinatorial assembly of homologous regulatory elements from different bacterial species. *Metab. Eng. 15*, 144-150.

BADESCH, D. B., ABMAN, S. H., AHEARN, G. S., BARST, R. J., MCCRORY, D. C., SIMONNEAU, G. & MCLAUGHLIN, V. V. 2004. Medical Therapy For Pulmonary Arterial HypertensionACCP Evidence-Based Clinical Practice Guidelines. *CHEST 126*, 35S-62S.

BASU, S., GERCHMAN, Y., COLLINS, C. H., ARNOLD, F. H. & WEISS, R. 2005. A synthetic multicellular system for programmed pattern formation. *Nature 434*, 1130-1134.

BAUMGARTEL, K., GENOUX, D., WELZL, H., TWEEDIE-CULLEN, R. Y., KOSHIBU, K., LIVINGSTONE-ZATCHEJ, M., MAMIE, C. & MANSUY, I. M. 2008. Control of the establishment of aversive memory by calcineurin and Zif268. *Nat. Neurosci. 11*, 572-578.

BAYS, H. E. 2011. Lorcaserin: drug profile and illustrative model of the regulatory challenges of weight-loss drug development. *Expert Rev. Cardiovasc. Ther. 9*, 265-277.

BOUCHIE, A. 2012. Regulatory fog lifts on obesity drugs. *Nat. Biotechnol. 30*, 810-811.

BRENT, R. 2004. A partnership between biology and engineering. *Nat. Biotechnol. 22*, 1211-1214.

CHOBANIAN, A. V., BAKRIS, G. L., BLACK, H. R., CUSHMAN, W. C., GREEN, L. A., IZZO, J. L., JONES,

D. W., MATERSON, B. J., OPARIL, S., WRIGHT, J. T. & ROCCELLA, E. J. 2003. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 42, 1206-52.

DEANS, T. L., CANTOR, C. R. & COLLINS, J. J. 2007. A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells. *Cell 130*, 363-372.

DEARRY, A., GINGRICH, J. A., FALARDEAU, P., FREMEAU, R. T., BATES, M. D. & CARON, M. G. 1990. Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature 347*, 72-76.

DEUSCHLE, U., MEYER, W. K. & THIESEN, H. J. 1995. Tetracycline-reversible silencing of eukaryotic promoters. *Mol. Cell Biol. 15*, 1907-1914.

DORSAM, R. T., & GUTKIND, J. S. 2007. G-protein-coupled receptors and cancer. *Nat. Rev. Cancer 7*, 79-94.

EHRBAR, M., SCHOENMAKERS, R., CHRISTEN, E. H., FUSSENEGGER, M. & WEBER, W. 2008. Drug-sensing hydrogels for the inducible release of biopharmaceuticals. *Nat. Mater. 7*, 800-804.

ELOWITZ, M. B. & LEIBLER, S. 2000. A synthetic oscillatory network of transcriptional regulators. *Nature 403*, 335-338.

FRASER, G. E. & SHAVLIK, D. J. 2001. Ten years of life: Is it a matter of choice. *Arch Intern Med 161*, 1645-1652.

FRIEDLAND, A. E., LU, T. K., WANG, X., SHI, D., CHURCH, G. & COLLINS, J. J. 2009. Synthetic gene networks that count. *Science 324*, 1199-1202.

FUSSENEGGER, M., MORRIS, R. P., FUX, C., RIMANN, M., VON STOCKAR, B., THOMPSON, C. J. & BAILEY, J. E. 2000. Streptogramin-based gene regulation systems for mammalian cells. *Nat. Biotechnol. 18*, 1203-1208.

GARDNER, T. S., CANTOR, C. R. & COLLINS, J. J. 2000. Construction of a genetic toggle switch in Escherichia coli. *Nature 403*, 339-342.

GERSBACH, C. A., LE DOUX, J. M., GULDBERG, R. E. & GARCIA, A. J. 2006. Inducible regulation of Runx2-stimulated osteogenesis. *Gene Ther. 13*, 873-882.

GILMAN, A. G. 1987. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem. 56*, 615-649.

GITZINGER, M., PARSONS, J., RESKI, R. & FUSSENEGGER, M. 2009A. Functional cross-kingdom conservation of mammalian and moss (Physcomitrella patens) transcription, translation and secretion machineries. Plant Biotechnol. J. 7, 73–86.

GITZINGER, M., KEMMER, C., EL-BABA, M. D., WEBER, W. & FUSSENEGGER, M. 2009b. Controlling transgene expression in subcutaneous implants using a skin lotion containing the apple metabolite phloretin. *Proc. Natl. Acad. Sci. USA 106*, 10638-10643.

GITZINGER, M., KEMMER, C., FLURI, D. A., EL-BABA, M. D., WEBER, W. & FUSSENEGGER, M. 2012. The food additive vanillic acid controls transgene expression in mammalian cells and mice. *Nucleic Acids Res. 40*, e37.

GOSSEN, M. & BUJARD, H. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA 89*, 5547-5551.

GRADMAN, A. H., BASILE, J. N., CARTER, B. L. & BAKRIS, G. L. 2010. Combination therapy in hypertension. *J. Am. Soc. Hypertens. 4*, 90-98.

GRAY, L. J., COOPER, N., DUNKLEY, A., WARREN, F. C., ARA, R., ABRAMS, K., DAVIES, M. J., KHUNTI, K. & SUTTON, A. 2012. A systematic review and mixed treatment comparison of pharmacological interventions for the treatment of obesity. *Obes. Rev. 13*, 483-498.

GREBER, D. & FUSSENEGGER, M. 2007. Multi-gene engineering: simultaneous expression and knockdown of six genes off a single platform. *Biotechnol. Bioeng. 96*, 821-834.

HARTENBACH, S., DAOUD-EL BABA, M., WEBER, W. & FUSSENEGGER, M. 2007. An engineered L-arginine sensor of Chlamydia pneumoniae enables arginine-adjustable transcription control in mammalian cells and mice. *Nucleic Acids Res. 35*, e136.

HASLAM, D. W. & JAMES, W. P. T. 2005. Obesity. *Lancet 366*, 1197-1209.

IDEKER, T., GALITSKI, T. & HOOD, L. 2001. A new approach to decoding life: systems biology. *Ann. Rev. Genomics Hum. Genet. 2*, 343-372.

KARLSSON, M. & WEBER, W. 2012. Therapeutic synthetic gene networks. *Curr. Opin. Biotechnol. 23*, 703-711.

KEASLING, J. D. 2008. Synthetic biology for synthetic chemistry. *ACS Chem. Biol. 3*, 64-76.

KEMMER, C., FLURI, D. A., WITSCHI, U., PASSERAUB, A., GUTZWILLER, A. & FUSSENEGGER, M. 2011. A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. *J. Control Release 150*, 23-29.

KEMMER, C., GITZINGER, M., DAOUD-EL BABA, M., DJONOV, V., STELLING, J. & FUSSENEGGER, M. 2010. Self-sufficient control of urate homeostasis in mice by a synthetic circuit. *Nat. Biotechnol. 28*, 355-360.

KHAN, A., RAZA, S., KHAN, Y., AKSOY, T., KHAN, M., WEINBERGER, Y. & GOLDMAN, J. 2012. Current updates in the medical management of obesity. *Recent Pat. Endocr. Metab. Immune Drug Discov. 6*, 117-128.

KRAMER, B. P. & FUSSENEGGER, M. 2005. Hysteresis in a synthetic mammalian gene network. *Proc. Natl. Acad. Sci. USA 102*, 9517-9522.

KRAMER, B. P., VIRETTA, A. U., DAOUD-EL BABA, M., AUBEL, D., WEBER, W. & FUSSENEGGER, M. 2004. An engineered epigenetic transgene switch in mammalian cells. *Nat. Biotechnol. 22*, 867-870.

LALMOHAMED, A., DE VRIES, F., BAZELIER, M. T., COOPER, A., VAN STAA, T. P., COOPER, C. & HARVEY, N. C. 2012. Risk of fracture after bariatric surgery in the United Kingdom: population based, retrospective cohort study. *BMJ 345*, e5085.

LAUTERMANN, J., DEHNE, N., SCHACHT, J. & JAHNKE, K. 2004. [Aminoglycoside- and cisplatin-ototoxicity: from basic science to clinics]. *Laryngorhinootologie 83*, 317-323.

LEKNES, S. & TRACEY, I. 2008. A common neurobiology for pain and pleasure. *Nat Rev Neurosci 9*, 314-320.

LIN, K. F., CHAO, J. & CHAO, L. 1995. Human atrial natriuretic peptide gene delivery reduces blood pressure in hypertensive rats. *Hypertension 26*, 847-853.

LLOYD-JONES, D., ADAMS, R. J., BROWN, T. M., CARNETHON, M., DAI, S., DE SIMONE, G., FERGUSON, T. B., FORD, E., FURIE, K., GILLESPIE, C., GO, A., GREENLUND, K., HAASE, N., HAILPERN, S., HO, P. M., HOWARD, V., KISSELA, B., KITTNER, S., LACKLAND, D., LISABETH, L., MARELLI, A., MCDERMOTT, M. M., MEIGS, J., MOZAFFARIAN, D., MUSSOLINO, M., NICHOL, G., ROGER, V. L., ROSAMOND, W., SACCO, R., SORLIE, P., STAFFORD, R., THOM, T., WASSERTHIEL-SMOLLER, S., WONG, N. D. & WYLIE-ROSETT, J. 2010. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation 121*, e46-e215.

MANN, T., TOMIYAMA, A. J., WESTLING, E., LEW, A. M., SAMUELS, B. & CHATMAN, J. 2007. Medicare's search for effective obesity treatments: diets are not the answer. *Am. Psychol. 62*, 220-233.

MARINISSEN, M. J. & GUTKIND, J. S. 2001. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol. Sci. 22*, 368-376.

MILLS, G. B. & MOOLENAAR, W. H. 2003. The emerging role of lysophosphatidic acid in cancer. *Nat. Rev. Cancer 3*, 582-591.

MISSALE, C., NASH, S. R., ROBINSON, S. W., JABER, M. & CARON, M. G. 1998. Dopamine receptors: from structure to function. *Physiol. Rev. 78*, 189-225.

NEDDERMANN, P., GARGIOLI, C., MURAGLIA, E., SAMBUCINI, S., BONELLI, F., DE FRANCESCO, R. & CORTESE, R. 2003. A novel, inducible, eukaryotic gene expression system based on the quorum-sensing transcription factor TraR. *EMBO Rep. 4*, 159-165.

NEVES, S. R., RAM, P. T., & IYENGAR, R. 2002. G protein pathways. *Science 296*, 1636-1639.

NISSEN, S. E. & WOLSKI, K. 2007. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N. Engl. J. Med. 356*, 2457-2471.

NORDSTROM, J. L. 2002. Antiprogestin-controllable transgene regulation in vivo. *Curr. Opin. Biotechnol. 13*, 453-458.

PALLI, S. R., KAPITSKAYA, M. Z. & POTTER, D. W. 2005. The influence of heterodimer partner ultraspiracle/retinoid X receptor on the function of ecdysone receptor. *FEBS J. 272*, 5979-5990.

PIERCE, K. L., PREMONT, R. T. & LEFKOWITZ, R. J. 2002. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol. 3*, 639-650.

POLLOCK, R. & CLACKSON, T. 2002. Dimerizer-regulated gene expression. *Curr. Opin. Biotechnol. 13*, 459-467.

POLLOCK, R., GIEL, M., LINHER, K. & CLACKSON, T. 2002. Regulation of endogenous gene expression with a small-molecule dimerizer. *Nat. Biotechnol. 20*, 729-733.

RINAUDO, K., BLERIS, L., MADDAMSETTI, R., SUBRAMANIAN, S., WEISS, R. & BENENSON, Y. 2007. A universal RNAi-based logic evaluator that operates in mammalian cells. *Nat. Biotechnol. 25*, 795-801.

ROLLINS, C. T., RIVERA, V. M., WOOLFSON, D. N., KEENAN, T., HATADA, M., ADAMS, S. E., ANDRADE, L. J., YAEGER, D., VAN SCHRAVENDIJK, M. R., HOLT, D.A., GILMAN, M. & CLACKSON, T. 2000. A ligand-reversible dimerization system for controlling protein-protein interactions. *Proc. Natl. Acad. Sci. USA 97*, 7096-7101.

ROTH, A., HARRISON, E., MITANI, G., COHEN, J., RAHIMTOOLA, S. H. & ELKAYAM, U. 1986. Efficacy and safety of medium- and high-dose diltiazem alone and in combination with digoxin for control of heart rate at rest and during exercise in patients with chronic atrial fibrillation. *Circulation 73*, 316-324.

RUBÍ, B. & MAECHLER, P. 2010. Minireview: new roles for peripheral dopamine on metabolic control and tumor growth: let's seek the balance. *Endocrinology 151*, 5570-5581.

SANCHEZ, A. R., ROGERS, R. S., 3RD & SHERIDAN, P. J. 2004. Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. *Int. J. Dermatol. 43*, 709-715.

SANCHEZ-BUSTAMANTE, C. D., KELM, J. M., MITTA, B. & FUSSENEGGER, M. 2006. Heterologous protein production capacity of mammalian cells cultivated as monolayers and microtissues. *Biotechnol. Bioeng. 93*, 169-180.

SCHILLINGER, K. J., TSAI, S. Y., TAFFET, G. E., REDDY, A. K., MARIAN, A. J., ENTMAN, M. L., OKA, K., CHAN, L. & O'MALLEY, B. W. 2005. Regulatable atrial natriuretic peptide gene therapy for hypertension. *Proc Natl Acad Sci USA 102*, 13789-13794.

SCHMIDT, M. 2008. Diffusion of synthetic biology: a challenge to biosafety. *Syst. Synth. Biol. 2*, 1-6.

SHARPLESS, N. E. & DEPINHO, R. A. 2006. The mighty mouse: genetically engineered mouse models in cancer drug development. *Nat. Rev. Drug Discov. 5*, 741-754.

TIGGES, M., DENERVAUD, N., GREBER, D., STELLING, J. & FUSSENEGGER, M. 2010. A synthetic low-frequency mammalian oscillator. *Nucleic Acids Res. 38*, 2702-2711.

TIGGES, M., MARQUEZ-LAGO, T. T., STELLING, J. & FUSSENEGGER, M. 2009. A tunable synthetic mammalian oscillator. *Nature 457*, 309-312.

THERRIEN, J.-P., KIM, S. M., TERUNUMA, A., QIN, Y., TOCK, C. L., PFÜTZNER, W., OHYAMA, M., SCHNERMANN, J. & VOGEL, J. C. 2010. A gene therapy approach for long-term

normalization of blood pressure in hypertensive mice by ANP-secreting human skin grafts. *Proc Natl Acad Sci USA 107*, 1178-1183.

TULMAN, D. B., STAWICKI, S. P. A., PAPADIMOS, T. J., MURPHY, C. V. & BERGESE, S. D. 2012. Advances in management of acute hypertension: a concise review. *Discov. Med. 13*, 375-383.

ULMER, J. B., VALLEY, U. & RAPPUOLI, R. 2006. Vaccine manufacturing: challenges and solutions. *Nat. Biotechnol. 24*, 1377-1383.

URLINGER, S., HELBL, V., GUTHMANN, J., POOK, E. & GRIMM, S. 2000. The p65 domain from NF-κB is an efficient human activator in the tetracycline-regulatable gene expression system. *Gene 18*, 103-110.

WADDEN, T. A., BUTRYN, M.L. & WILSON, C. 2007. Lifestyle modification for the management of obesity. *Gastroenterology 132*, 2226-2238.

WANG, Y. C., MCPHERSON, K., MARSH, T., GORTMAKER, S. L. & BROWN, M. 2011. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet 378*, 815-825.

WEBER, W., BACCHUS, W., DAOUD-EL BABA, M. & FUSSENEGGER, M. 2007. Vitamin H-regulated transgene expression in mammalian cells. *Nucleic Acids Res. 35*, e116.

WEBER, W. & FUSSENEGGER, M. 2004. Approaches for trigger-inducible viral transgene regulation in gene-based tissue engineering. *Curr. Opin. Biotechnol. 15*, 383-391.

WEBER, W. & FUSSENEGGER, M. 2006. Pharmacologic transgene control systems for gene therapy. *J. Gene Med. 8*, 535-556.

WEBER, W. & FUSSENEGGER, M. 2007. Inducible product gene expression technology tailored to bioprocess engineering. *Curr Opin Biotechnol 18*, 399-410.

WEBER, W., FUX, C., DAOUD-EL BABA, M., KELLER, B., WEBER, C. C., KRAMER, B. P., HEINZEN, C., AUBEL, D., BAILEY J. E. & FUSSENEGGER, M. 2002. Macrolide-based transgene control in mammalian cells and mice. *Nat. Biotechnol. 20*, 901-907.

WEBER, W., KRAMER, B. P., FUX, C., KELLER, B. & FUSSENEGGER, M. 2002. Novel promoter/transactivator configurations for macrolide- and streptogramin-responsive transgene expression in mammalian cells. *J. Gene Med. 4,* 676-686.

WEBER, W., LIENHART, C., DAOUD-EL BABA, M. & FUSSENEGGER, M. 2009. A biotin-triggered genetic switch in mammalian cells and mice. *Metab. Eng, 11*, 117-124.

WEBER, W., LINK, N. & FUSSENEGGER, M. 2006. A genetic redox sensor for mammalian cells. *Metab. Eng. 8*, 273-280.

WEBER, W., SCHOENMAKERS, R., KELLER, B., GITZINGER, M., GRAU, T., DAOUD-EL BABA, M., SANDER, P. & FUSSENEGGER, M. 2008. A synthetic mammalian gene circuit reveals antituberculosis compounds. *Proc. Natl. Acad. Sci. USA 105*, 9994.

WEBER, W., STELLING, J., RIMANN, M., KELLER, B., DAOUD-EL BABA, M., WEBER, C., AUBEL, D. & FUSSENEGGER, M. 2007. A synthetic time-delay circuit in mammalian cells and mice. *Proc. Natl. Acad. Sci. USA 104*, 2643.

WURM, F. M. 2004. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat. Biotechnol. 22*, 1393-1398.

XIE, Z., WROBLEWSKA, L., PROCHAZKA, L., WEISS, R. & BENENSON, Y. 2011. Multi-input RNAi-based logic circuit for identification of specific cancer cells. *Science 333*, 1307-1311.

YE, H., CHARPIN-EL HAMRI, G., ZWICKY, K., CHRISTEN, M., FOLCHER, M. & FUSSENEGGER, M. 2013. Pharmaceutically controlled designer circuit for the treatment of the metabolic syndrome. *Proc. Natl. Acad. Sci. USA 110*, 141-146.

YE, H., DAOUD-EL BABA, M., PENG, R.-W. & FUSSENEGGER, M. 2011. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science 332*, 1565-1568.

ZHOU, Q. Y., GRANDY, D. K., THAMBI, L., KUSHNER, J. A., VAN TOL, H. H., CONE, R., PRIBNOW, D., SALON, J., BUNZOW, J. R. & CIVELLI, O. 1990. Cloning and expression of human and rat D1 dopamine receptors. *Nature 347*, 76-80.

ZHUO, Q., YANG, W., CHEN, J. & WANG, Y. 2012. Metabolic syndrome meets osteoarthritis. *Nat. Rev. Rheumatol*.

CHAPTER I

A closed-loop synthetic gene circuit for the treatment of diet-induced obesity in mice

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Nature Communications 2013, 4:2825.

Abstract

Diet-induced obesity is a lifestyle-associated medical condition that increases the risk of developing cardiovascular disease, type 2 diabetes and certain types of cancer. Here we report the design of a closed-loop genetic circuit that constantly monitors blood fatty acid levels in the setting of diet-associated hyperlipidemia and coordinates reversible and adjustable expression of the clinically licensed appetite-suppressing peptide hormone pramlintide. Grafting of the peroxisome proliferator-activated receptor-a onto the phloretin-responsive repressor TtgR produces a synthetic intracellular lipid-sensing receptor (LSR) that reversibly induces chimeric TtgR-specific promoters in a fatty acid-adjustable manner. Mice with dietinduced obesity in which microencapsulated cells engineered for LSR-driven expression of pramlintide are implanted show significant reduction in food consumption, blood lipid levels and body weight when put on a high-fat diet. Therapeutic designer circuits that monitor levels of pathologic metabolites and link these with the tailored expression of protein pharmaceuticals may provide new opportunities for the treatment of metabolic disorders.

Diet-induced obesity typically results from a combination of excessive food energy intake, favored by an evolutionary remain associating energy-dense food with greater taste, lack of physical activity and genetic susceptibility[1]. Obesity is at the origin of a wide range of diseases such as cardiovascular disorders, type 2 diabetes, obstructive sleep apnea, osteoarthritis, asthma and certain types of cancer and has become a leading preventable cause of death with dramatically increasing prevalence, affecting up to 1.5 billion people in industrialized and developing countries[2-4]. A sustainable change in lifestyle including dieting and physical exercise is the pillar for the treatment of obesity that can be complemented by gastric balloon and bariatric surgery to assist weight loss[4] and by taking anti-obesity drugs to reduce appetite or fat absorption[5-7]. However, dieting often shows limited success[8], surgery is associated with significant risks[9] and only some anti-obesity drugs with modest efficacy[10] remain on the market after many have been withdrawn because of side effects[11-14] and few have entered clinical trials since[15]. The amylin analogue pramlintide has recently been licensed as injection-based adjunctive type 2 diabetes therapy[16] as it reduces adsorption of glucose and other nutrients such as fat by slowing gastric emptying, promotes satiety via GLP-1-independent hypothalamic receptors and inhibits inappropriate secretion of glucagon[17]. However, precise injection-based dosing at mealtime remains challenging[18].

Synthetic biology-inspired designer networks that coordinate caloric intake to satiety responses could control dietary energy homeostasis and may provide a new strategy for the treatment of obesity. In humans, peroxisome proliferator-activated receptor alpha (PPARα) is a nuclear lipid receptor heterodimerizing with the retinoic X receptor (RXR) to form a transcription factor that constitutively binds to specific target promoters[19,20]. Through interaction with regulatory co-activators such as the steroid receptor coactivator 1 (ref.21), the peroxisome proliferator-activated receptor-γ coactivator 1 (ref. 22), the CREB-binding protein (CBP)[22] and the E1A binding protein p300 (ref. 22) or with specific co-repressors like the nuclear receptor corepressor (NCoR)[23-25], the G-protein pathway suppressor 2 (GPS2)[26] and the histone deacetylase (HDAC3)[26] PPARα manages uptake, utilization and catabolism of fatty acids in a variety of tissues such as liver, kidney, heart, muscle and adipose. PPARα's activity is modulated by binding of fatty acids[27,28] (e.g., linoleic acid, a plant-derived fatty acid and palmitic acid, an animal-derived fatty acid) and synthetic fibrate drugs licensed for the treatment of hyperlipidemia[29].

In this study we take advantage of PPARα`s capacity to detect a variety of fatty acids to score diet-dependent blood fat levels and to correct diet-induced obesity in mice by expressing a clinically licensed appetite-suppressing peptide hormone. Closed-loop synthetic gene networks operating inside vascularized designer cell implants that constantly monitor circulating disease-

relevant metabolites, process off-level concentrations and coordinate production and release of a specific protein therapeutic in a selfsufficient manner may become a treatment strategy of the future.

Results

Design of a synthetic mammalian lipid-sensing receptor. We have fused the ligand-binding domain of PPARα to the bacterial DNA-binding repressor TtgR[30] to create a binary synthetic transcription factor, the lipid-sensing receptor (LSR), that retains dual-input sensitivity to fatty acids (via PPARα) as well as to the transdermal cosmetic additive phloretin[31] (via TtgR) and specifically binds (via TtgR) and modulates (via PPARα) transcription of chimeric promoters (P_{TtgR1}) in an adjustable manner (Fig. 1a). Whereas LSR's PPARα domain recruits co-repressors (NCoR, GPS2, HDAC3) in the absence of fatty acids, which silence P_{TtgR1}-driven transgene expression (Fig. 1a,b), they become replaced by co-activators (PPAR-γ coactivator 1, CBP, steroid receptor coactivator 1 and p300) upon binding of lipids, which activates P_{TtgR1}-mediated transcription in a dose-dependent manner. Lipid-dependent modulation of P_{TtgR1} exclusively occurs when LSR is bound to the promoter via its TtgR domain in the absence of phloretin, while in the presence of this trigger compound, LSR is released from P_{TtgR1} and transcription shut down irrespective of the lipid status of the cells. Thus, LSR's TtgR domain ensures promoter specificity and provides a potential trigger-inducible safety switch to override lipid-dependent promoter activation (Fig. 1a). Cotransfection of several rodent, primate and human cell lines with the constitutive LSR expression vector pKR135 (P_{hCMV}-LSR-pA; LSR, TtgR-PPARα) and the LSR-dependent secreted alkaline phosphatase (SEAP) reporter construct pMG10 (ref. 30; P_{TtgR1}-SEAP-pA) showed that SEAP was exclusively and dose-dependently induced whenever the cells were exposed to exogenous linoleic acid (Fig. 1c). As the isogenic transcription factor $TtgA_1$ (pMG11 (ref. 30), TtgR-VP16) was insensitive to fatty acids in an identical experimental set-up, LSR's lipid-sensing capacity indeed resides in its PPARα domain while the TtgR moiety mediates sequence-specific binding (Supplementary Fig. S1a). Differences in the cellular portfolio of promiscuous PPARα-coregulatory components and lipid metabolism may in part explain the differences in basal expression and induction profiles among different cell lines (Fig. 1c). Control experiments showed that neither ectopic expression of LSR (SEAP level: + LSR (pKR135/pSEAP2-Control), 87.9 ± 2.7 U l^{-1}; - LSR (pcDNA3.1/pSEAP2-Control), 87.4 ± 6.9 U l^{-1}) nor exposure of the cells to excessive fatty acid concentrations had a negative impact on their viability (Supplementary Fig. S1b). In addition, specificity and leakiness tests confirmed that LSR's target promoter P_{TtgR1} was neither induced by endogenous components nor activated by unrelated synthetic transcription factors

(Supplementary Fig. S2a). Further experiments using different constitutive promoters to drive LSR and different LSR-specific target promoters confirmed that the combination of pKR135, pMG10 and HT-1080 cells showed the best fatty acid-triggered transgene induction and was therefore used in all follow-up experiments (Fig. 1d,e).

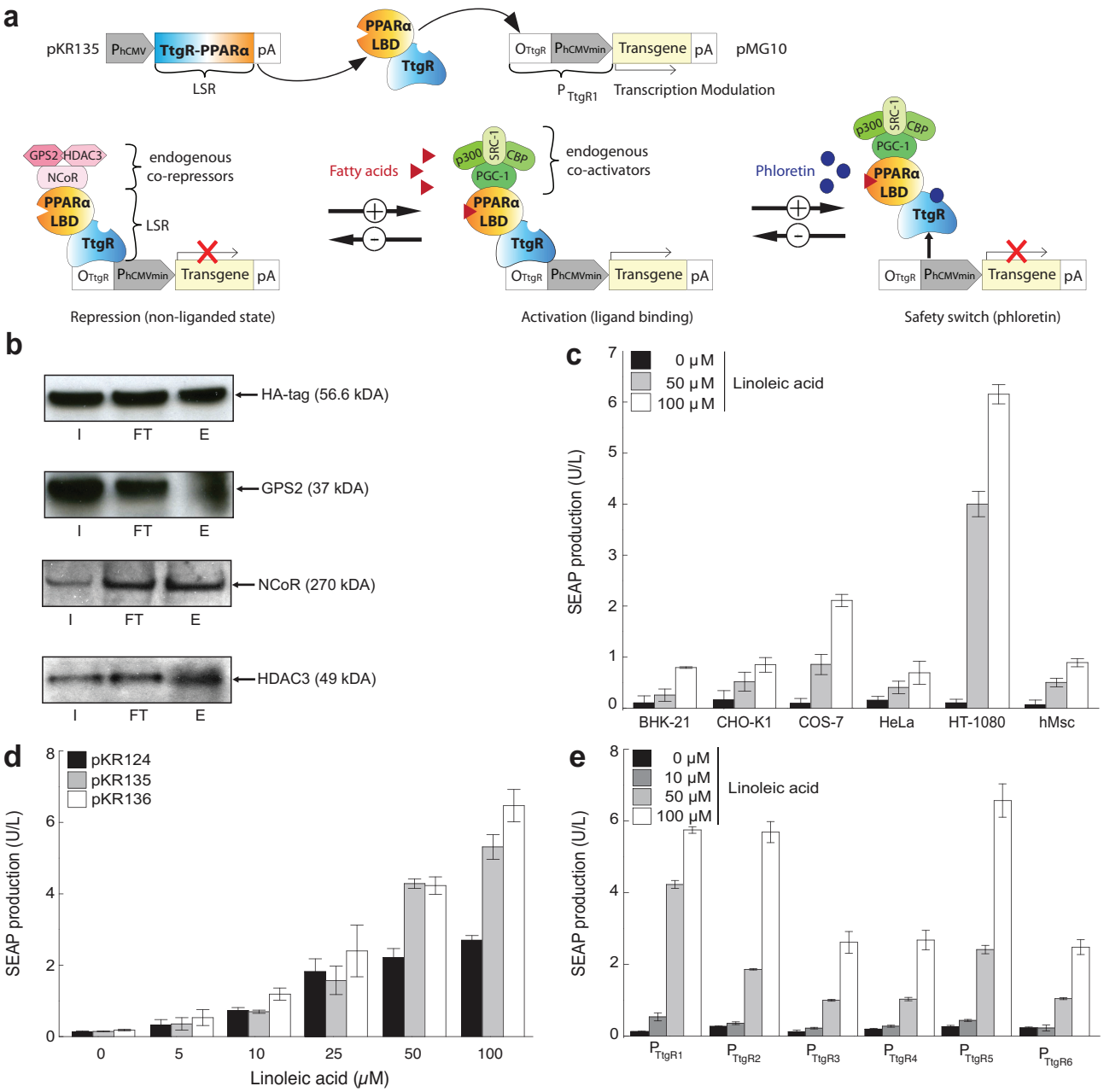


Figure 1 | A synthetic fatty acid-responsive mammalian gene switch. (**a**) The synthetic lipid-controlled mammalian transcription device consists of an intracellular LSR, a fusion protein combining the phloretin-responsive repressor (TtgR) and the human PPARα, which binds a TtgR-specific operator (O_{TtgR}) linked to a minimal promoter ($P_{hCMVmin}$) (P_{TtgR1}) to control transgene expression. In the absence of fatty acids, LSR associates with an inhibitory complex (GPS2, G-protein pathway suppressor 2; HDAC, histone deacetylase; NCoR, nuclear receptor corepressor) to repress transgene expression, which switches to full induction in the presence of fatty acids when LSR associates with the activation complex (SRC1, steroid receptor coactivator 1; p300, E1A

binding protein; CBP, CREB-binding protein; PGC-1, peroxisome proliferator-activated receptor-g coactivator). Transgene expression can also be shut down by disrupting LSR-promoter binding by addition of the clinically licensed skin-penetrating apple metabolite phloretin. (**b**) Coimmunoprecipitation. HT-1080 were cotransfected with pKR151 (encoding HA-tagged LSR) and pMG10, and the co-repressors (NCoR, GPS2 and HDAC3) interacting with LSR were identified by protein complex CoIP after 48 h. E, eluate; FT, flow through; I, input. (**c**) Linoleic acid-induced SEAP expression in different cell lines. Cells were co-transfected with the LSR-encoding expression vector (pKR135; P_{hCMV}-LSR-pA) and the PTtgR1-driven SEAP expression plasmid (pMG10; $P_{hCMV*-1}$-SEAP-pA), grown in the presence of different linoleic acid concentrations, and SEAP levels were profiled in the culture supernatant after 48 h. (**d,e**) Optimization of the LSR's fatty acid sensitivity. (**d**) HT-1080 were (co-)transfected with pMG10 (P_{TtgR1}-SEAP-pA) and an expression vector encoding LSR under control of different constitutive promoters (pKR124, P_{SV40}-LSR-pA; pKR135, P_{hCMV}-LSR-pA; pKR136, $P_{hEF1\alpha}$-LSR-pA) and cultivated in the presence of increasing concentrations of linoleic acid for 48 h before SEAP was profiled in the culture supernatant. (**e**) HT-1080 were (co-)transfected with the LSR expression vector pKR135 (P_{hCMV}-LSR-pA) and reporter constructs encoding SEAP under control of different LSR-specific promoter variants containing 0 (P_{TtgR1}; pMG10 (ref. 30)), 2 (P_{TtgR2}; pMG20 (ref. 30)), 4 (P_{TtgR3}; pMG21 (ref. 30)), 6 (P_{TtgR4}; pMG22 (ref. 30)), 8 (P_{TtgR5}; pMG23 (ref. 30)) and 10 (P_{TtgR6}; pMG24 (ref. 30)) base pair linkers between the TtgR operator (O_{TtgR}) and the minimal promoter ($P_{hCMVmin}$). Transfected HT-1080 were cultivated in the presence of different linoleic acid concentrations and SEAP expression was profiled after 48 h. Error bars indicate s.d. (n=4).

LSR characterization and control by fatty acids and fibrates. Detailed characterization of pKR135/pMG10-transgenic HT-1080 cells revealed that the synthetic fatty acid sensor circuit was fully reversible (Fig. 2a) and precisely adjusted transgene expression levels in response to a wide range of fatty acid concentrations (Fig. 2b). At the same time LSR retained its dose-dependent sensitivity to the licensed cosmetic additive phloretin[32] that could release LSR from P_{TtgR1}, override fatty acid induced transcription control and shut down transgene expression (Fig. 2c).

Interestingly, LSR was sensitive to a broad spectrum of fatty acids including unsaturated (linoleic acid, oleic acid) and saturated (palmitic acid, myristic acid, stearic acid, lauric acid) ones of plant (linoleic acid) and animal (palmitic acid, stearic acid, myristic acid) origin as well as to complex fatty acids mixtures such as dietary oils (Fig. 2d). For example, although linoleic and palmitic acids mediated up to 55-fold induction of SEAP expression, rapeseed oil reached induction factors of up to 140 (Fig. 2d). LSR is also sensitive to fibrates such as WY-14643, gemfibrozil (LOPID) and bezafibrate (BEZALIP) (Fig. 2e), a class of synthetic amphipathic carboxylic acids that are clinically licensed for the treatment of hyperlipidemia[29]. This suggests that the LSR controller device could also be used for drug-controlled expression of therapeutic transgenes and combined with fibrate-based therapies.

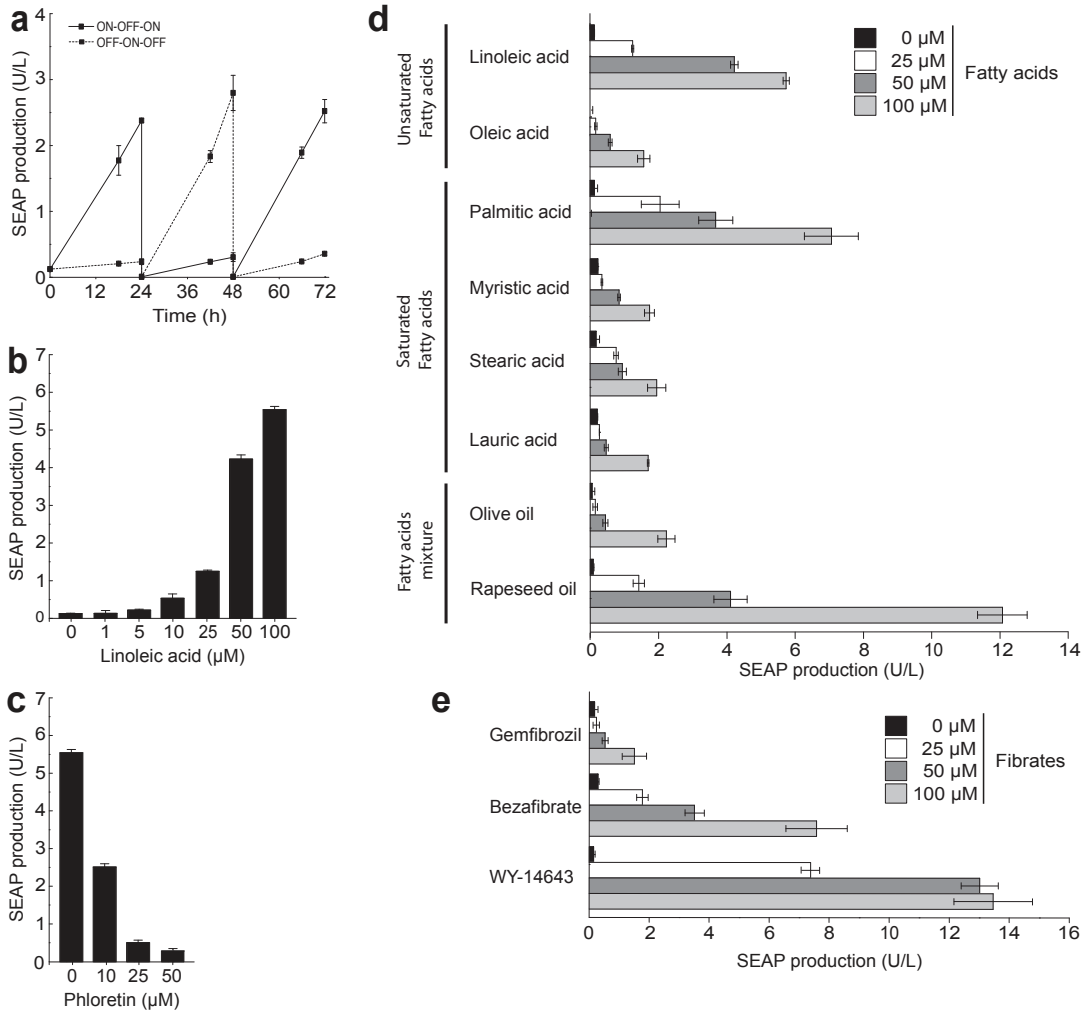


Figure 2 Characterization of fatty acid-controlled transgene expression. (**a**) Reversibility of fatty acid-responsive SEAP expression was assessed by cultivating transgenic HT-1080 (pKR135/pMG10) for 72 h while alternating the linoleic acid status of the culture (100 µM ON, 0 µM OFF) at 24 and 48 h. (**b**) Adjustability of fatty-acid-inducible SEAP expression. pKR135-/pMG10-transgenic HT-1080 were cultivated in medium containing increasing linoleic acid concentrations and corresponding SEAP production was quantified in the culture supernatant after 48 h. (**c**) Phloretin-triggered safety switch. pKR135-/pMG10-engineered HT-1080 were programmed for maximum expression by 100 µM linoleic acid followed by addition of different concentrations of phloretin before SEAP expression was scored after 48 h. (**d**) Sensitivity of the LSR control switch to different fatty acids. HT-1080 containing the LSR device were exposed for 48 h to different concentrations of specific fatty acids before SEAP expression was assessed. (**e**) Tunability of the LSR control device in response to clinically licensed fibrate drugs. LSR-transgenic HT-1080 (pKR135/pMG10) were cultivated in the presence of different concentrations of fibrate drugs and SEAP production was profiled after 48 h. Error bars indicate s.d. (n=3).

Fatty acid-mediated transgene expression in mice. Covering a wide range of dietary and synthetic fatty acids at high sensitivity, the LSR-based sensor device has all its takes to precisely score physiologic fatty acid levels. To validate fatty acid-triggered product gene expression *in vivo*, pKR135/pMG10-transgenic HT-1080 variants engineered for fatty acid-controlled SEAP expression were microencapsulated in coherent alginate-poly-(L-lysine)-alginate beads[33], which is

the preferred cell implant material known for its optimal pore-size tunability[34], metabolic and immuno-isolation[34] and vascularization[35,36] as well as for long-term support of cell functionality[34-38] and low inflammatory reaction. The microencapsulated circuit-transgenic cells were implanted into the peritoneal cavity of wild-type mice kept on a standard diet. Previous studies have shown that intraperitoneally implanted alginate-encapsulated cells become vascularized and connected to the bloodstream[36], and that fatty acids reach a rapid diffusion-based equilibrium between the peritoneal cavity and the peripheral circulation[39]. The animals were treated with diverse concentrations of linoleic and palmitic acid or received different oral doses of rapeseed oil while control mice obtained an identical fatty acid-free care. Mice with LSR implants that were treated with lipid supplements showed a significant dose-dependent increase of SEAP in their serum (Fig. 3a,b). This suggests that the LSR sensor device was able to monitor, process and report hyperlipidemia *in vivo* while the circuit remains shut down at physiologic blood fat levels reached during a standard diet. To confirm the *in vivo* precision of the LSR sensor device, we also implanted microencapsulated pKR135/pMG10-transgenic HT-1080 cells into diet-induced obese mice put on medium-fat (MF; 10kcal% fat) as well as high-fat (HF; 60kcal% fat) diets and compared their blood fatty acid and SEAP levels to non-obese control animals with a standard caloric low-fat intake (LF; 5kcal% fat) (Fig. 3c,d). LSR-transgenic cell implants were able to indirectly score the food quality by sensing specific blood fat levels represented by serum cholesterol and phospholipids[40-43] (Fig. 3c) and produced the corresponding SEAP expression response that resulted in serum SEAP levels specific for each diet group (Fig. 3d). Control animals receiving isogenic pMG10 (P_{TtgR1}-SEAP-pA)-containing implants lacking LSR had no detectable SEAP circulating in their bloodstream (Supplementary Fig. S2b).

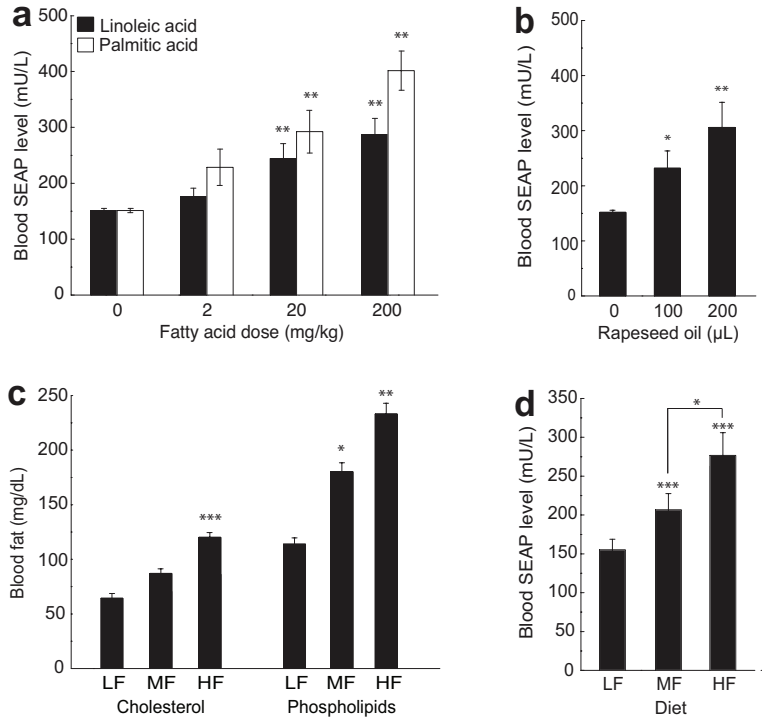


Figure 3 | Performance of fatty acid-inducible expression control in wild-type mice. (**a–d**) Standard low fat-fed wild-type mice were implanted with per microencapsulated pKR135-/pMG10-transgenic HT-1080 (2x106 cells per mouse) and received different doses of linoleic/palmitic acids (**a**), rapeseed oil (**b**) or were put on standard LF, (5 kcal% fat), MF (10 kcal% fat) and HF (60 kcal% fat) diets (**c,d**). Resulting blood SEAP levels (**a,b,d**) and blood fat levels represented by serum cholesterol and phospholipid concentrations (**c**) were quantified after 48 h (a,b,d) or 72h (**c**). Data are mean ± s.e.m., statistics by two-tailed Student's t-test; n=8 mice per group. *P<0.05, **P<0.005, ***P<0.0001 versus zero lipid doses (**a,b**) or LF mice (**c,d**).

Application of LSR in diet-induced obese mice. In order to automatically control diet-induced obesity in a self-sufficient manner we functionally coupled the LSR sensor device to expression of the clinically licensed anorectic peptide hormone pramlintide[7], which suppresses appetite, promotes satiety[5] and slows gastric emptying[44], thereby limiting high-caloric food intake, attenuating hyperlipidemic blood levels, reducing body weight and restoring the energy homeostasis of the organism. Therefore, we replaced SEAP of pMG10 by pramlintide and validated the resulting vector pK146 (P_{TtgR1}-Pram-pA) for LSR-controlled pramlintide production using enzyme-linked immunosorbent assay (ELISA; Supplementary Fig. S3a) as well as a cell-based assay, confirming pramlintide's capacity to activate its target CALC/RAMP receptor and trigger the corresponding signaling cascades (Supplementary Fig. S3b). To test the designer circuit's capacity to control diet-induced obesity we implanted pKR135/pKR146-engineered HT-1080 into diet-induced obese mice receiving HF (60kcal% fat) or MF (10kcal% fat) diets as well as wild-type mice receiving standard food (LF; 5kcal% fat) and profiled pramlintide levels, blood fat concentrations, food intake and body weight for several days (Fig. 4). Although treated diet-induced obese mice showed a

significant diet-dependent increase in circulating pramlintide levels (Fig. 4a) leading to reduction of blood fat levels (Fig. 4b), food consumption (Fig. 4c) and body weight (Fig. 4d), all of these values remained unchanged in control animals receiving standard food (Fig. 4a-d). In addition, pramlintide levels in the plasma of animals receiving isogenic LSR-free but pKR146 (P_{TtgR1}-SEAP-pA)-containing control implants were almost undetectable (Supplementary Fig. S2c). It is of particular importance that standard-fed wild-type mice implanted with the LSR-driven pramlintide device kept their body weight, suggesting that the designer network is only active in an obesity risk situation involving excess fat and stops pramlintide production to prevent underfeeding (Fig. 4a,d). Visual analysis of the capsules in theperitoneal cavity (Fig. 4e) and profiling of the inflammatory indicators interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) in treated animals of different diet groups (LF, MF and HF diets) showed no detectable inflammation (Supplementary Fig. S4).

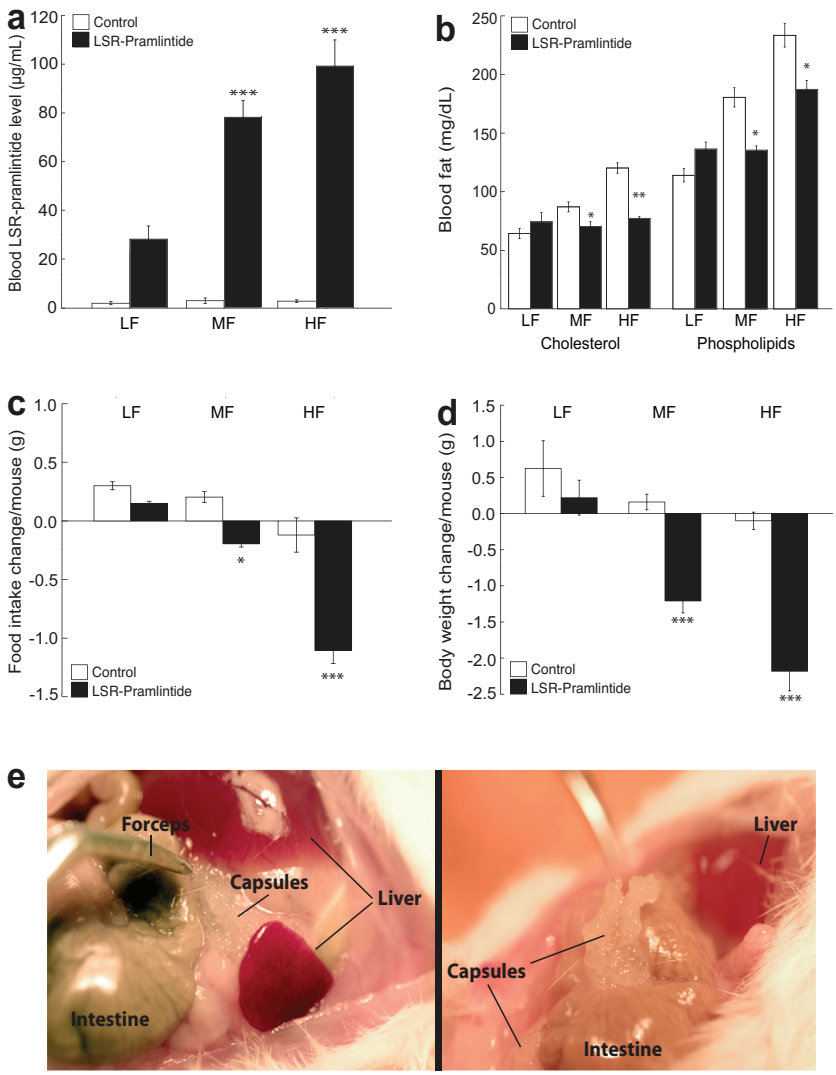


Figure 4 | Self-sufficient LSR-based control of pramlintide expression in diet-induced obese mice. (a–d) Mice fed for 14 weeks with standard LF (5 kcal% fat), MF (10 kcal% fat) and HF (60

kcal% fat) diets were implanted with pKR135/pKR146-transgenic HT-1080 ($2x10^6$ cells per mouse) (LSR-Pramlintide) or the isogenic pKR135/pMG10-transgenic HT-1080 ($2x10^6$ cells per mouse) (Control). Seventy-two hours after implantation, blood pramlintide levels (**a**) and blood fat levels represented by serum cholesterol and phospholipid concentrations (**b**), as well as change in food intake (**c**) and weight loss (**d**) were profiled. (**e**) Illustrations showing implanted microencapsulated circuit-transgenic HT-1080 (pKR135/pKR146) of two different dissected mice. Data are mean ± s.e.m., statistics by two-tailed Student's t-test; n=8 mice per group. *P<0.05, **P<0.005, ***P<0.0001 versus LF mice.

Discussion

Synthetic biology-inspired control devices and gene networks that precisely reprogram the dynamic behavior, metabolism and physiology of mammalian cells have reached a level of sophistication[45] which enables the community to rapidly move forward and start to shape new clinical applications and treatment concepts[46-48]. Therapeutic networks combining sensor and effector devices that, upon integration into cells and functional connection to their metabolism, monitor disease-relevant metabolites, process on/off level control and coordinate adjusted therapeutic responses that restore metabolite homeostasis in a seamless, automatic and self-sufficient manner are particularly attractive for future gene and cell-based therapies. The closed-loop LSR-pramlintide circuit represents a prototype for such a therapeutic designer circuit. The LSR sensor captures a wide range of lipids within their physiologic concentration range, becomes dose-dependently activated by peak fatty acid levels and shuts down at native concentrations. In combination with the pramlintide-encoding effector component and implanted into diet-induced obese mice the designer circuit (i) constantly monitored blood fatty acid levels, (ii) processed peak fat values, (iii) produced a coordinated pramlintide production response that (iv) reduced food intake, (v) decreased blood fat levels, (vi) dropped the body weight, and (vii) automatically switched off at normal blood fat levels. Also, since the LSR used TtgR as DNA-binding component the designer device can be switched off at any time using a skin lotion containing the clinically licensed apple metabolite phloretin[32], which may represent an additional safety latch in future clinical applications. With obesity being on its way to develop into a global epidemic, available pharmacotherapies showing limited success and an almost dried-out drug pipeline[14-18] synthetic biology-inspired cell-based therapies may foster new opportunities in the treatment of obesity and related metabolic diseases. It is now conceivable that autologous designer cells that are implanted into humans inside vascularizing semi-permeable microcontainers monitor disease-relevant metabolites in the peripheral circulation and coordinate expression of relevant therapeutic proteins in an emerging pathologic situation[36,38,49,50]. As designer cell implants are scalable to the patients' needs, could be replaced by ambulant interventions and removed in acute situations or after completion of the therapy, they may become a pillar in future personalized medicine programs[51,52].

Methods

Lipid sensor components. Comprehensive design and construction details for all expression vectors are provided in supplementary **Table S1**. Key plasmids include the following: **pKR135** encoding constitutive expression of the LSR (P_{hCMV}-LSR-pA); **pMG10** (ref. 30) harboring a SEAP expression unit driven by the LSR-specific phloretin-responsive promoter (P_{TtgR1}; P_{TtgR1}-SEAP-pA); and **pKR146** containing a P_{TtgR1}-driven pramlintide expression unit (P_{TtgR1}-Pram-pA).

Cell culture, transfection. Human embryonic kidney cells (HEK-293T, ATCC: CRL-11268), Baby hamster kidney cells (BHK-21, ATCC: CCL-10), African green monkey kidney cells (COS-7, ATCC: CRL-1651), human cervical adenocarcinoma cells (HeLa, ATCC: CCL-2), human fibrosarcoma cells (HT-1080, ATCC: CCL-121) and immortalized human mesenchymal stem cells (hMSC[53]) were cultivated in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Basel, Switzerland) supplemented with 1% (v/v) penicillin/streptomycin solution (Sigma-Aldrich, Munich, Germany). Wild-type Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultured in ChoMaster® HTS (Cell Culture Technologies GmbH, Gravesano, Switzerland) containing 1% penicillin/streptomycin. All cell types were cultivated at 37°C in a humidified atmosphere containing 5% CO_2. For (co-)transfection of BHK-21, CHO-K1, COS-7, HeLa, HT-1080 and hMSCs, 40,000 cells seeded per well of a 24-well plate 12h prior to transfection were incubated for 6h with a 4:1 PEI:DNA mixture (Polyethyleneimine; MW 40,000, Polysciences, Inc., Warrington, USA). After transfection, all cells were cultivated in their specific media containing different inducer concentrations and reporter protein levels were profiled after 48h unless stated otherwise. For cotransfection of circuits components (pKR135, pKR153) into HT-1080 a typical transfection efficiency of 61% was reached (Supplementary Fig. S5).

SEAP production. Production levels of the human placental SEAP were quantified in cell culture supernatant using a *p*-nitrophenylphosphate-based light absorbance time course[54]. One hundred microlitres of cell-culture supernatant were heat inactivated for 30 min at 65 °C and centrifuged for 2min at 14,000g to remove cell debris. Eighty microlitres of the sample were transferred into a well of a 96-well plate and adjusted to 37 °C. One hundred microlitres of 2x SEAP buffer (20mM homoarginine, 1mM MgCl2, 21% (v/v) diethanolamine, pH 9.8) were mixed with 20 ml substrate solution (120mM para-nitrophenyl phosphate), adjusted to 37 °C and added to the well containing the heat-inactivated sample. The absorbance time course was recorded at 405nm using an Envision 2104 multilabel plate reader (Perkin Elmer, Waltham, USA). SEAP concentrations in blood samples were quantified using a chemiluminescence-based assay (Roche Diagnostics GmbH,

Mannheim, Germany). In brief, 50 ml of heat-inactivated serum (30 min, 65 °C), centrifuged for 30s at 14,000g, were transferred to a well of a 96-well plate containing 50 ml inactivation buffer and incubated for 10 min at 22 °C. Next, 50 ml of freshly prepared substrate reagent (50 ml CSPD [3-(4-methoxyspiro[1,2-dioxetane-3,20(50-chloro)-tricyclo(3.3.1.1^{3,7}decane]-4-yl)phenylphosphate] mixed with 950 ml substrate buffer) was added to each well and incubated for 10 min at 22 °C before light emission was recored at 477nm using the Envision 2104 multilabel plate reader (Perkin Elmer).

Flow cytometry. pDSRed-N1- or pKR135/pKR153-(co)-transfected HT-1080 cell populations were diluted in 1% BSA (Sigma-Aldrich)-containing PBS (137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.4mM KH_2PO_4, pH 7.4; 1x106 cells per ml) and analyzed using a LSRII Fortessa flow cytometer (Becton Dickinson, Allschwil, Switzerland) equipped for DsRed (561 nm laser excitation and 586/15 emission filter) detection and set to exclude dead cells and cell doublets. At least 10,000 cells were recorded per data set and analyzed using FACSDiva (BD Biosciences, Franklin Lakes, NJ, USA, version no. 6.1.3.).

Blood fat levels. Blood cholesterol levels were profiled using a total cholesterol assay kit (Fluoro Cholesterol; Cell Technology, Mountain View, USA). In brief, 50 µl of appropriately ddH2O-diluted serum was added per well of a clear-bottom black 96-well plate containing 50 ml of freshly prepared reaction cocktail (2µ ml resuspended enzyme mix, 20 µl resuspended cholesterol probe and 960 µl reaction buffer). The plate was incubated in the dark for 1 h at 22 °C and fluorescence was quantified at excitation 530nm and emission 585 nm using the Envision 2104 multilabel plate reader (Perkin Elmer). Blood phospholipid levels were quantified using a phospholipid assay kit (Abnova GmbH, Heidelberg, Germany). In brief, 20 µl of appropriately Triton X-100 (Sigma-Aldrich)-diluted serum was added per well of a clear-bottom black 96-well plate containing 80 µl of freshly prepared working reagent (85 µl assay buffer, 1 µl PLD enzyme, 1 µl Enzyme Mix and 1 µl Dye Reagent). The plate was incubated in the dark for 30 min at 22 °C and fluorescence was quantified at excitation 530 nm and emission 585 using the Envision 2104 multilabel reader (Perkin Elmer).

Pramlintide quantification and activity tests. *In vitro* activity of pramlintide was measured by (co-)transfecting HT-1080 with pKR135/pKR146 or pJWS17 (6µg DNA, 800,000 cells, 10cm Petri dish). 48h after transfection, the culture medium was transferred to 60,000 HEK-293 cells that had been cotransfected with pCALCR, pRAMP3, pCK53 and pcDNA3.1 at a ratio of 1:1:0.5:7.5 (0.6µg

of total DNA) and SEAP levels were profiled after 48h. Alternatively, pramlintide was quantified using the human amylin EIA ELISA kit (Phoenix Pharmaceuticals, Burlingame, USA) and pure pramlintide as standard (Feldan, Quebec, Canada). For quantification of pramlintide in mouse serum samples, 96-well plates were coated with 5µg ml^{-1} (100µL/well) rabbit polyclonal anti-human amylin (Amylin H-50, Santa Cruz Biotechnology, Santa Cruz, USA; cat. no. sc-20936, lot. no. I0303) in dilution buffer (PBS, 0.5% BSA, 0.01% Tween80, pH7.4) at 4°C overnight. Plates were blocked with 200µL per well PBS/1% BSA pH7.4 for 1h at 22°C. Pramlintide standard (1µg/mL-500µg/mL) and samples (100µL/well) were diluted (PBS, 0.5% BSA, 0.01% Tween80, pH7.4) and incubated for 1.5h at 22°C. Plates were washed three times with 250µL/well PBS containing 0.15% Tween-20 and then incubated with 100µL/well of a mouse monoclonal anti-Amylin (Abcam, Cambridge, UK; cat. no. ab115766, lot no. GR81110-2) for 1h at 22°C. After three washing steps (250µL PBS/0.15% Tween-20), the plates were incubated with an anti-mouse horseradish peroxidase-conjugated IgG (GE Healthcare, Buckinghamshire, UK, cat. no. NA931V, lot no. 399402) for 1h at 22°C, washed again (250µL PBS/0.15% Tween-20) and bound pramlintide was visualized by incubation of the samples with 100µL/well of TMB substrate (Interchim, Montluçon, France) at 22°C for 6min. Reactions were stopped by addition of 2M sulfuric acid (100 µL/well) and pramlintide was quantified at 450nm using an Envision plate reader.

Co-Immunoprecipitation: Six microgram of Protein A Dynabeads (Invitrogen Dynal AS, Oslo, Norway) were washed twice with 300µL CoIP buffer (50mM Tris, pH8, 150mM NaCl, 1mM EDTA, 1% Triton X-100) containing 6µL protease inhibitor cocktail (Roche Diagnostics GmbH, Basel, Switzerland) and incubated with 6µg of the rabbit polyclonal anti-HA antibody (Y-11; Santa Cruz Biotechnology Inc; cat. no. sc-805, lot no. G1210) for 4h while mixing at room temperature on an end-over rotator. $1.2x10^8$ pKR151/pMG10-cotransfected HT-1080 cells were washed twice with 30mL cold PBS and the cell nuclei were extracted with 45mL nuclear isolation buffer (300mM sucrose, 10mM Tris, pH8, 5mM Mg chloride and 0.5% Triton X-100, 0.1% protease mix) for 10min at 4°C. Isolated cell nuclei were resuspended in 2mL CoIP buffer containing 200µL protease inhibitor cocktail (Roche Diagnostics GmbH) and 1µL/mL Benzonase® (Invitrogen) and sonicated for 15min à 30sec at 4°C (320W, Biorupter Plus, Diagenode S.A, Liège, Belgium). 2mL of the resulting nuclear extract was incubated overnight at 4°C with 30µL of anti-HA antibody-linked Protein A Dynabeads while mixing on an end-over rotator. The Immunocomplexes were washed three times with 500µL CoIP buffer for 10min and the immunoprecipitated protein complexes were then visualized by Western blot analysis.

Western blot: Twenty microliter of the immunoprecipitated protein complexes was mixed with 5µl of NuPAGE LSD sample buffer (4x; Invitrogen) and 1µL of 1M DTT, resolved on a 4-12% Bis-Tris gel (NuPAGE; Invitrogen) or a 3-8% Tris-Acetate Gel (NuPAGE Novex; Invitrogen) and electroblotted (Trans-Blot® SD, Bio-Rad, Reinach, Switzerland) onto a Amersham Hybond-ECL membrane (GE Healthcare, Glattbrugg, Switzerland). The membrane was blocked with 5% (w/v) nonfat dried milk (AppliChem GmbH, Darmstadt, Germany) diluted in PBST (PBS, 0.1% Tween-20) for 30min at 22°C and incubated over night at 4°C with recommended dilutions of the following primary antibodies: mouse monoclonal anti-GPS2 antibody (Abcam; cat. no. ab53406, lot no. GR61606-3), goat polyclonal anti-NCoR antibody (C-20; Santa Cruz Biotechnology Inc.; cat. no. sc-1609, lot no. A2813); goat polyclonal anti-HDAC3 antibody (N-19; Santa Cruz Biotechnology Inc.; cat. no. sc-8138, lot no. C1913) and goat polyclonal anti-HA antibody (F-7; Santa Cruz Biotechnology Inc.; cat. no. sc-7392, lot no. C081). The membrane was then washed three times in PBST and incubated for 1h with a horseradish peroxidase-coupled sheep anti-mouse IgG (GE Healthcare; cat. no. NA931V, lot no. 399402) or rabbit anti-goat IgG (Sigma-Aldrich; cat. no. A5420, lot no. 043M4763). The super signal West Femto Western blot detection reagent (Thermo Scientific, Rockford, USA) and the X-ray apparatus Konika Minolta SRX-101A (Konika Minolta GmbH, Langenhagen, Germany) were used for detection.

Inflammation: TNF-α and IL-6 levels were profiled in the blood of treated animals using murine TNF-α- and IL-6-specific ELISA assays (PeproTech, Rocky Hill, NJ, USA, catalogue numbers 900-M54 (TNF-α) and 900-M50 (IL-6)) according to the manufacturer's instructions. Intraperitoneal injections of 1 ml 1% (w/v) thioglycollate in ddH2O (Sigma-Aldrich) were used as an inflammation inducing positive control set-up.

Animal experiments. Intraperitoneal implants were produced by encapsulating pKR135/pMG10- or pKR135/146-transgenic HT-1080 into coherent alginate-poly-(L-lysine)-alginate beads (400µm; 200 cells/capsule) using an Inotech Encapsulator Research Unit IE-50R (Buchi Labortechnik AG, Flawil, Switzerland) set to the following parameters: 200µm nozzle with a vibration frequency of 1023Hz and 900 V for bead dispersion, 20mL syringe operated at a flow rate of 403 units. 400µL of serum-free DMEM containing $2x10^6$ microencapsulated transgenic cells (pKR135/pMG10- or pKR135/pKR146-transgenic HT-1080; 200 cells/capsule) were injected intraperitoneally into 14-week-old female CD1 (normal 5kcal% fat diet; Janvier S.A.S., Le Genest-Saint-Isle, France) or 14-week-old diet-induced obese mice (DIO, C57BL/6J, The Jackson Laboratory, Maine, USA) that

were on a 10kcal% (D12450Bi, Research Diets, Inc., NJ) or a 60kcal% fat diet (D12492i, Research Diets, New Brunswick, USA). CD1 mice implanted with pKR135/pMG10-transgenic HT-1080 received twice daily injections of linoleic or palmitic acid (200µl, 0-200mg/kg; linoleic acid, Thermo Fisher Scientific, Geel, Belgium; palmitic acid: Sigma-Aldrich) or oral doses of rapeseed oil (100µL and 200µL; Naturaplan Rapsöl, Coop, Basel, Switzerland). Control mice were treated with capsules containing non-engineered parental HT-1080. Every day, the food intake and body weight of the animals was profiled. After a starvation period of 4h blood samples were collected and the serum was isolated using microtainer SST tubes according to the manufacturer's protocol (Beckton Dickinson, Plymouth, UK) before serum SEAP, blood fat and pramlintide levels were scored as described above. All experiments involving animals were performed according to the directives of the European Community Council (2010/63/EU), approved by the French Republic (no. 69266309), and carried out by Ghislaine Charpin-El Hamri at the Institut Universitaire de Technology, IUTA, F-69622 Villeurbanne Cedex, France.

Acknowledgments. We thank Julian Sikorski for providing pJWS17, Tommy Schlumpf for assistance with the Co-IP, Marc Folcher for visualizing microencapsulated cells in the peritoneal cavity and Pratik Saxena, Barbara Geering, Simon Ausländer as well as Henryk Zulewski for critical comments on the manuscript. This work was supported by an European Research Council (ERC) advanced grant (No. 321381) and in part by the Cantons of Basel and the Swiss Confederation within the INTERREG IV A.20 tri-national research program.

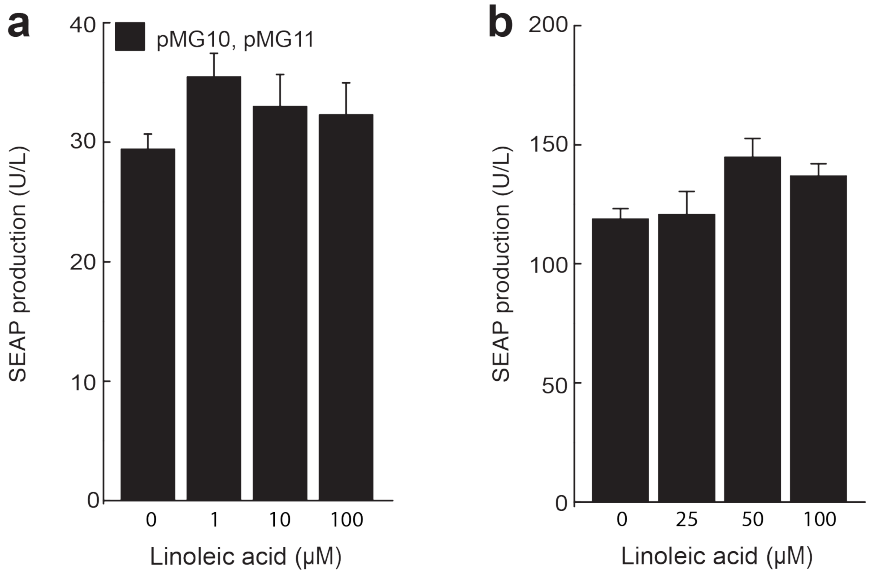
References

1. Rejeski, W.J. *et al.* Lifestyle change and mobility in obese adults with type 2 diabetes. *N. Engl. J. Med.* **366**, 1209-1217 (2012).
2. Haslam, D.W. & James, W.P.T. Obesity. *Lancet* **366**, 1197-1209 (2005).
3. Wang, Y.C., McPherson, K., Marsh, T., Gortmaker, S.L. & Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* **378**, 815-825 (2011).
4. Zhuo, Q., Yang, W., Chen, J. & Wang, Y. Metabolic syndrome meets osteoarthritis. *Nat. Rev. Rheumatol.* **8**, 729-737(2012)
5. Halford, J.C.G., Boyland, E.J., Blundell, J.E., Kirkham, T.C. & Harrold, J.A. Pharmacological management of appetite expression in obesity. *Nat. Rev. Endocrinol.* **6**, 255-269 (2010).
6. Gray, L.J. *et al.* A systematic review and mixed treatment comparison of pharmacological interventions for the treatment of obesity. *Obes. Rev.* **13**, 483-498 (2012).
7. Khan, A. *et al.* Current updates in the medical management of obesity. *Recent Pat. Endocr. Metab. Immune Drug Discov.* **6**, 117-128 (2012).
8. Mann, T. *et al.* Medicare's search for effective obesity treatments: diets are not the answer. *Am. Psychol.* **62**, 220-233 (2007).
9. Lalmohamed, A. *et al.* Risk of fracture after bariatric surgery in the United Kingdom: population based, retrospective cohort study. *BMJ* **345**, e5085 (2012).
10. Bays, H.E. Lorcaserin: drug profile and illustrative model of the regulatory challenges of weight-loss drug development. *Expert Rev. Cardiovasc. Ther.* **9**, 265-277 (2011).
11. Bray, G.A. Drug treatment of obesity. *Rev. Endocr. Metab. Disord.* **2**, 403-418 (2001).
12. Nissen, S.E. & Wolski, K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N. Engl. J. Med.* **356**, 2457-2471 (2007).
13. Ham, J. *et al.* Discovery, design and synthesis of Y-shaped peroxisome proliferator-activated receptor δ agonists as potent anti-obesity agents in vivo. *Eur. J. Med. Chem.* **53C**, 190-202 (2012).
14. Bouchie, A. Regulatory fog lifts on obesity drugs. *Nat Biotechnol* **30,** 810-811 (2012).
15. Hofbauer, K.G. & Nicholson, J. R. Pharmacotherapy of obesity. *Exp. Clin. Endocrinol. Diabetes* **114**, 475-484 (2006).
16. Younk, L.M., Mikeladze, M. & Davis, S.N. Pramlintide and the treatment of diabetes: a review of the data since its introduction. *Expert Opin. Pharmacother.* **12**, 1439-1451 (2011).
17. Singh-Franco, D., Robles, G. & Gazze, D. Pramlintide acetate injection for the treatment of type 1 and type 2 diabetes mellitus. *Clin. Ther.* **29**, 535-562 (2007).
18. Young, A. Tissue expression and secretion of amylin. *Adv. Pharmacol.* **52**, 19-45 (2005).
19. Kumar, R. & Thompson, E.B. The structure of the nuclear hormone receptors. *Steroids* **64**, 310-319 (1999).
20. Chinetti, G. *et al.* PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat. Med.* **7**, 53-58 (2001).
21. Tien, E. S., Hannon, D.B., Thompson, J.T. & Vanden Heuvel, J.P. Examination of ligand-dependent coactivator recruitment by peroxisome proliferator-activated receptor-alpha (PPARalpha). *PPAR Res.* **2006**, 69612 (2006).
22. Vega, R.B., Huss, J.M. & Kelly, D.P. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol. Cell. Biol.* **20**, 1868-1876 (2000).
23. Daynes, R.A. *et* Jones, D.C. Emerging roles of ppars in inflammation and immunity. *Nat. Rev. Immunol.* **2**, 748-759 (2002).
24. Stanley, T.B. *et al.* Subtype specific effects of peroxisome proliferator-activated receptor ligands on corepressor affinity. *Biochemistry* **42**, 9278-9287 (2003).
25. Dowell, P. *et al.* Identification of nuclear receptor corepressor as a peroxisome proliferator-

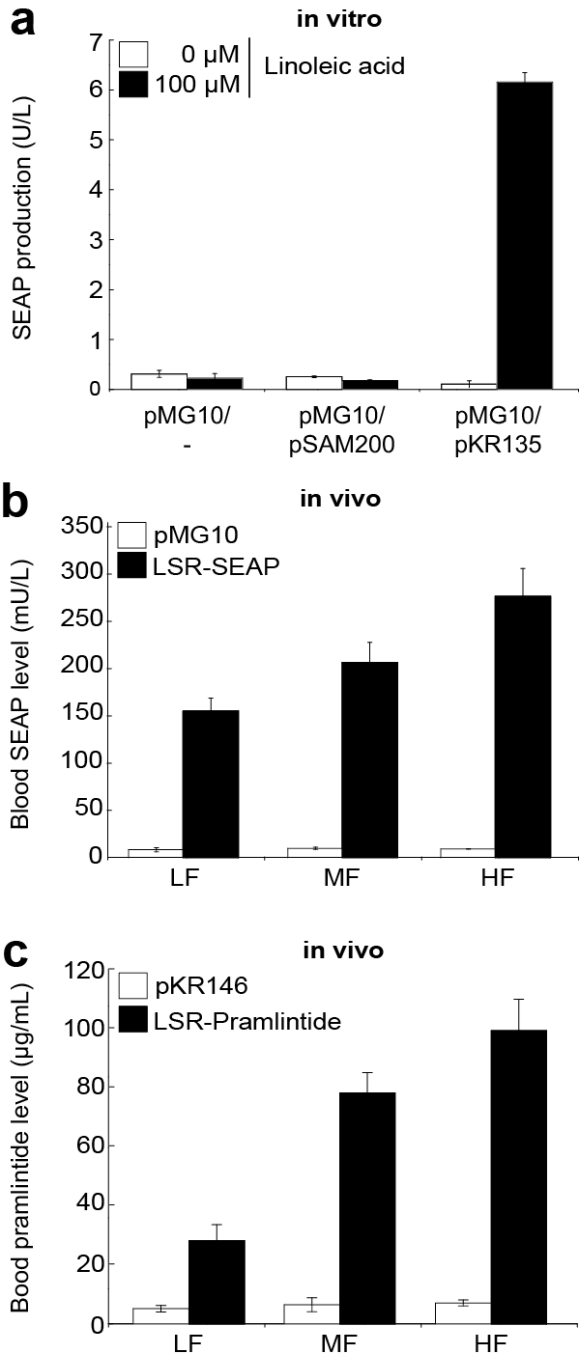
activated receptor alpha interacting protein. *J. Biol. Chem.* **274**, 15901-15907 (1999).
26. Oberoi, J. *et al.* Structural basis for the assembly of the SMRT/NCoR core transcriptional repression machinery. *Nat. Struct. Mol. Biol.* **18**, 177-84 (2011).
27. Hanley, K. *et al.* Activators of the nuclear hormone receptors PPARalpha and FXR accelerate the development of the fetal epidermal permeability barrier. *J. Clin. Invest.* **100**, 705-712 (1997).
28. Krey, G. *et al.* Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **11**, 779-791 (1997).
29. Kersten, S., Desvergne, B. & Wahli, W. Roles of PPARs in health and disease. *Nature* **405**, 421-424 (2000).
30. Gitzinger, M., Kemmer, C., El-Baba, M.D., Weber, W. & Fussenegger, M. Controlling transgene expression in subcutaneous implants using a skin lotion containing the apple metabolite phloretin. *Proc. Natl. Acad. Sci. USA* **106**, 10638-10643 (2009).
31. Terán, W., Krell, T., Ramos, J.L. & Gallegos, M.-T. Effector-repressor interactions, binding of a single effector molecule to the operator-bound TtgR homodimer mediates derepression. *J. Biol. Chem.* **281**, 7102-7109 (2006).
32. Oresajo C, *et al*. Protective effects of a topical antioxidant mixture containing vitamin C, ferulic acid, and phloretin against ultraviolet-induced photodamage in human skin. *J. Cosmet. Dermatol.* **7**, 290–297 (2008).
33. Weber, W. *et al.* Macrolide-based transgene control in mammalian cells and mice. *Nat. Biotechnol.* **20**, 901-907 (2002).
34. Soon-Shiong, P. Treatment of type I diabetes using encapsulated islets. *Adv. Drug Deliv. Rev.* **35**, 259-270 (1999).
35. Orive, G. *et al.* Long-term expression of erythropoietin from myoblasts immobilized in biocompatible and neovascularized microcapsules. *Mol. Ther.* **12**, 283-289 (2005).
36. Jacobs-Tulleneers-Thevissen, D. *et al.* Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient. *Diabetologia* **56**, 1605-1614 (2013).
37. Duvivier-Kali, V. F., Omer, A., Parent, R. J., O'Neil, J. J. & Weir, G. C. Complete protection of islets against allorejection and autoimmunity by a simple barium-alginate membrane. *Diabetes* **50**, 1698-1705 (2001).
38. Chang, T. M. S. Therapeutic applications of polymeric artificial cells. *Nat. Rev. Drug Discov.* **4**, 221-235 (2005).
39. Mermier, P. & Baker, N. Flux of free fatty acids among host tissues, ascites fluid, and Ehrlich ascites carcinoma cells. J. Lipid Res. **15**, 339–351 (1974).
40. Arab, L. Biomarkers of fat and fatty acid intake. *J. Nutr.* **133 Suppl 3**, 925S-932S (2003).
41. Hegsted, D.M., McGandy, R.B., Myers, M.L. & Stare, F.J. Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **17**, 281-295 (1965).
42. Keys, A., Anderson, J.T. & Grande. F. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism* **14**, 376-387 (1965).
43. Kris-Etherton, P.M. & Yu, S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *Am. J. Clin. Nutr.* **65**, 1628S-1644S (1997).
44. Lebovitz, H.E. Adjunct therapy for type 1 diabetes mellitus. *Nat. Rev. Endocrinol.* **6**, 326-334 (2010).
45. Alper, H. & Weber, W. Building synthetic cell systems from the ground up. *Curr. Opin. Biotechnol.* (2012).
46. Wieland, M. & Fussenegger, M. Reprogrammed cell delivery for personalized medicine. *Adv. drug deliv. Rev.* **64**, 1477-1487 (2012).
47. Ruder, W.C., Lu, T. & Collins, J.J. Synthetic biology moving into the clinic. *Science* **333**,

1248-1252 (2011).
48. Khalil, A.S. & Collins, J.J. Synthetic biology: applications come of age. *Nat. Rev. Genet.* **11**, 367-379 (2010).
49. Orive, G. *et al.* History, challenges and perspectives of cell microencapsulation. *Trends Biotechnol.* **22**, 87-92 (2004).
50. Catena, R. *et al.* Improvement of the monitoring and biosafety of encapsulated cells using the SFGNESTGL triple reporter system. *J. Control. Release* **146**, 93-98 (2010).
51. Ausländer, S., Wieland, M. & Fussenegger, M. Smart medication through combination of synthetic biology and cell microencapsulation. *Metab. Eng* **14**, 252-260 (2012).
52. Fischbach, M. A., Bluestone, J. A. & Lim, W. A. Cell-based therapeutics: the next pillar of medicine. *Sci. Transl. Med.* **5**, 179ps177 (2013).
53. Simonsen, J.L. *et al*. Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. *Nat. Biotechnol.* **20**, 592-596 (2002).
54. Schlatter, S., Rimann, M., Kelm, J. & Fussenegger, M. SAMY, a novel mammalian reporter gene derived from Bacillus stearothermophilus alpha-amylase. *Gene* **282**, 19-31 (2002).

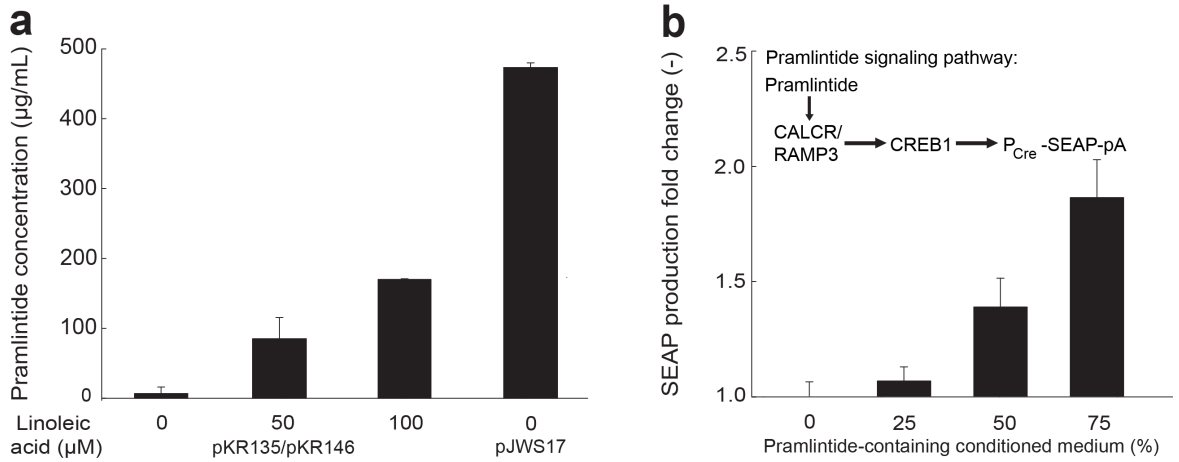
Supplementary information



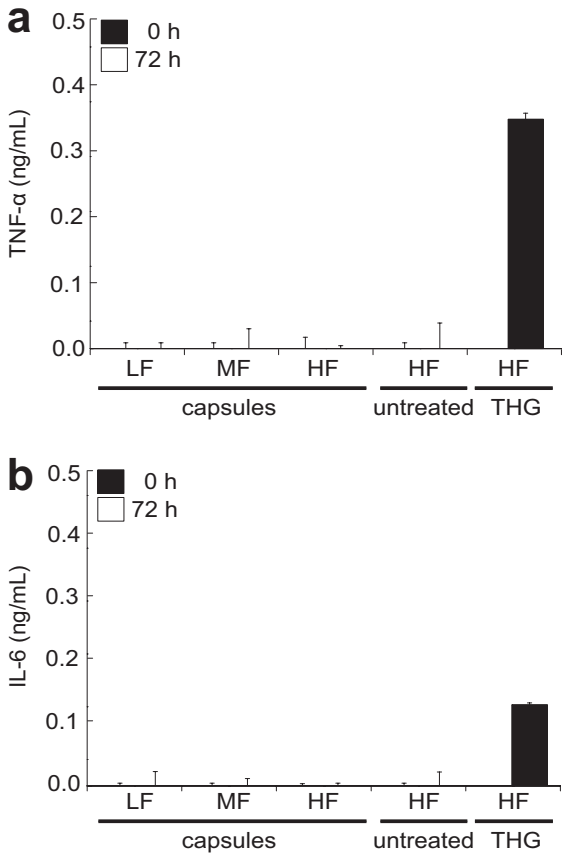
Supplementary Figure S1. Control specificity and viability impact of linoleic acid. (**a**) Insensitivity of the phloretin-responsive control element to linoleic acid. HT-1080 were cotransfected with the components encoding the phloretin-responsive gene switch (pMG10, (P_{TtgR1}-SEAP-pA) and pMG11, (P_{SV40}-$TtgA_1$-pA))[1] and grown for 48h in the presence of increasing linoleic acid concentrations before SEAP was profiled in the cell culture supernatant. (**b**) Viability of HT-1080 exposed to increasing concentrations of Linoleic acid. HT-1080 were transfected with pSEAP2-control (P_{SV40}-SEAP-pA) and exposed to increasing concentrations of linoleic acid. Any negative impact of excessive fatty acid on the metabolism or viability of the cells would impair their overall SEAP production capacity. The error bars indicate the s.d. (n = 3).



Supplementary Figure S2. Specificity and leakiness of LSR components. (**a**) HT-1080 cells were (co-)transfected with pMG10 (P_{TtgR1}-SEAP-pA), pSAM200 (P_{hCMV}-tTA-pA) and pMG10 or pKR135 (P_{hCMV}-LSR-pA) and pMG10 and cultivated for 48h in the presence and absence of linoleic acid before SEAP production was profiled in the culture supernatant. (**b, c**) Wild-type mice kept on a standard low-fat diet were implanted with microencapsulated pMG10-, pKR135-/pMG10$^{\#}$- (**b**), pKR146 (P_{TtgR1}-Pram-pA)- or pKR135/pKR146$^{\#\#}$- (**c**) transgenic HT-1080 ($2x10^6$ cells/mouse) and put on standard low fat (LF, 5kcal% fat), medium fat (MF, 10kcal% fat) and high fat (HF, 60kcal% fat) diets. $^{\#,\#\#}$ For comparison and clarity, data from Figs. 3d ($^{\#}$) and 4a ($^{\#\#}$) of the main text were replicated, respectively. The error bars indicate the s.d. (n = 4).

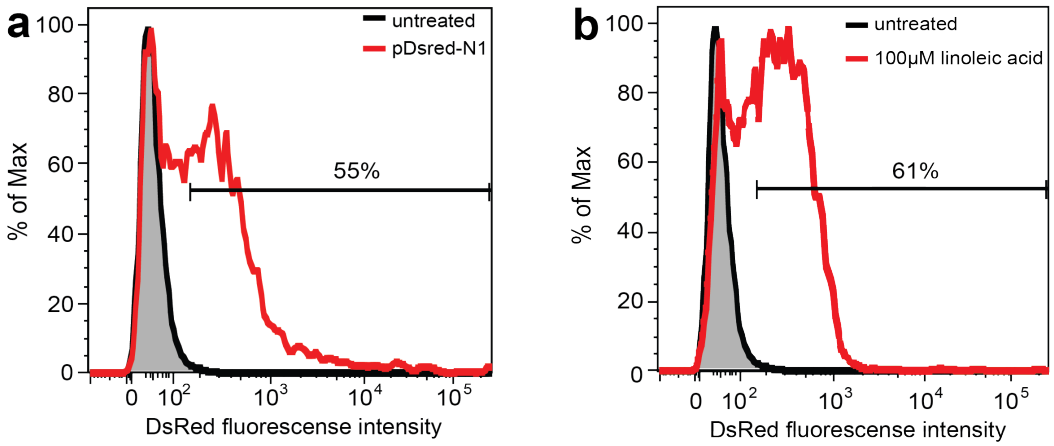


Supplememtary Figure S3. Characterization of pramlintide expression. (**a**) Comparative analysis of linoleic acid (LA)-induced LSR-driven (pKR135/pKR146) and constitutive (pJWS17, P_{hCMV}-Pram-pA) pramlintide expression by HT-1080 (co-)transfected with indicated vectors and cultivated for 48h. (**b**) Validation of functional pramlintide production. The culture supernatant of pKR135/pKR146-transgenic pramlintide-producing HT-1080 cultivated for 48h in the presence of 100µM linoleic acids was mixed at different percentages with DMEM and used to cultivate HEK-293 (co-)transfected with expression vectors encoding the pramlintide-specific receptors pCALCR, pRAMP3 and the corresponding reporter unit (pCK53; P_{CRE}-SEAP-pA[2]). Pramlintide-triggered SEAP expression was profiled after 48h. The error bars indicate the s.d. (n = 3).



Supplememtary Figure S4. Profiling of the inflammatory response after implantation of microencapsulated circuit-transgenic HT-1080. Wild-type mice kept on a standard diet were implanted with circuit-transgenic (pKR135/pKR146) HT-1080 ($2x10^6$ cells/mouse), non-treated (negative control) or injected with thioglycollate (THG; positive control) and put on different

caloric diets (low fat, LF = 5kcal% fat; medium fat, MF = 10kcal% fat and high fat, HF = 60kcal% fat) before circulating TNF-α (**a**) as well as IL-6 (**b**) levels were profiled after 72h. The error bars indicate the s.d. (n = 3).



Supplememtary Figure S5. FACS-based analysis of HT-1080's circuit transfection efficiency. (**a**) Constitutive expression of DsRed (red) in HT-1080 48h after transfection compared to untreated viable cells (black). (**b**) DsRed-based expression of the LSR circuit in pKR135/pKR153 cotransfected HT-1080 48h after transfection and cultivation with 100µM linoleic acid (green) compared to untreated viable cells (black).

Supplementary Table S1. Plasmids used and designed in this study

Plasmid	Description and Cloning Strategy	Reference or Source
pcDNA3.1	Mammalian expression vector (P_{hCMV}-MCS-pA).	Life Technologies, Carlsbad, CA, USA
pSEAP2-control	Constitutive mammalian SEAP expression vector (P_{SV40}-SEAP-pA).	Clontech, Mountain View, CA,USA
pDsRed-N1	Constitutive mammalian DsRed expression vector (P_{hCMV}-DsRed-pA).	Clontech, Mountain View, CA, USA
pCALCR	Constitutive pcDNA3.1-derived CALCR expression vector (P_{hCMV}-CALCR-pA).	www.cdna.org
pRAMP3	Constitutive pcDNA3.1-derived RAMP3 expression vector (P_{hCMV}-RAMP3-pA).	www.cdna.org
pUC57-Pram	Custom-designed pUC57-derived vector containing pramlintide.	GenScript Inc. Piscataway, NJ, USA
pCK53	Vector encoding a P_{CRE}-driven SEAP expression unit (P_{CRE}-SEAP-pA).	55
pMG10	Vector encoding a P_{TtgR1}-driven SEAP expression unit (P_{TtgR1}-SEAP-pA).	30
pMG11	Constitutive mammalian $TtgA_1$ expression vector (P_{SV40}-$TtgA_1$-pA; $TtgA_1$, TtgR-VP16).	30
pSAM200	Constitutive tTA expression vector (P_{SV40}-tTA-pA, tTA, TetR-VP16).	56
pWW29	Constitutive mammalian MphR(A) expression vector ($P_{hEF1\alpha}$-MphR(A)-pA).	33
pJWS17	Constitutive pcDNA3.1-derived pramlintide expression vector (P_{hCMV}-Pram-pA). Pram was excised from pUC57-Pram with *Xba*I/*Apa*I and cloned into the corresponding sites (*Xba*I/*Apa*I) of pcDNA3.1.	This work
pKR124	Constitutive mammalian LSR expression vector (P_{SV40}-LSR-pA; LSR, TtgR-PPARα). PPARα was PCR-ampified from human genomic cDNA using oligonucleotides OKR189 (5'-gtacagccgcgcgcATCTCAA ATCTCTGGCCAAGAG-3') and OKR190 (5'-ctatcccggatccTTAGTAC ATGTCCCTGTAGATC-3'), digested with *BSS*HII/*Bam*HI and cloned into the corresponding sites (*BSS*HII/*Bam*HI) of pMG11.	This work
pKR135	Constitutive mammalian LSR expression vector (P_{hCMV}-LSR-pA). P_{hCMV} was exised from pcDNA3.1 with *Mlu*I/*Eco*RI and cloned into the corresponding sites (*Mlu*I/*Eco*RI) of pKR124.	This work
pKR136	Constitutive mammalian LSR expression vector ($P_{hEF1\alpha}$-LSR-pA). $P_{hEF1\alpha}$ was excised from pWW29 with *Ssp*I/*Kpn*I and cloned into the corresponding sites (*Ssp*I/*Kpn*I) of pKR135.	This work
pKR146	P_{TtgR1}-driven pramlintide expression vector (P_{TtgR1}-Pram-pA). Pram was excised from pUC57-Pram with *Eco*RI/*Hin*dIII and cloned into the corresponding sites (*Eco*RI/*Hin*dIII) of pMG10.	This work
pKR151	Constitutive mammalian HA-tagged LSR expression vector (P_{hCMV}-LSR-HA-pA). TtgR-PPARα-HA was PCR-amplified from pKR135 using oligonucleotides OKR237 (5'- gggaattcaagcttccaccATGGTCCGT CGAACCAAAGAAG-3') and OKR239 (5'-catacctctagaTTAGGTG GATCCGAGCTCGGTACCGGCATAGTCAGGAACATCGTATGGG TACATGTACATGTCCCTGTAGATCTC-3'), digested with *Hin*dIII/*Xba*I and cloned into the corresponding sites (*Hin*dIII/*Xba*I) of pKR135.	This work

Supplementary Table S1. Continued

Plasmid	Description and Cloning Strategy	Reference or Source
pKR153	Vector encoding a P_{TtgR1}-driven DsRed expression unit (P_{TtgR1}-DsRed-pA). DsRed was PCR-amplified from pDsRed-N1 using oligonucleotides OMM50 (5'-cgggatccgtcgacgaattcaccATGGCCTCC TCCGAGAACGTC-3') and OMM51 (5'- ccatcgataagctttctagaCTACA GGAACAGGTGGTG-3'), digested with *Eco*RI/*Hin*dIII and cloned into the corresponding sites (*Eco*RI/*Hin*dIII) of pMG10.	This work

Restriction endonuclease-specific sites are underlined in oligonucleotide sequences. Annealing base pairs contained in oligonucelotide sequences are shown in capital letters.

Abbreviations: **CALCR**, human calcitonin receptor; **DsRed,** *Discosoma sp.* red fluorescent protein; **EGFP**, enhanced green fluorescent protein; **LSR**, lipid-sensing receptor (TtgR-PPARα); **MCS**, multiple cloning site; **MphR(A)**, repressor of the *Escherichia coli* 2'-phosphotransferase I; **pA**, SV40-derived polyadenylation site; $\mathbf{P_{hCMV}}$, human cytomegalovirus immediate early promoter; $\mathbf{P_{hEF1\alpha}}$, human elongation factor 1α promoter; $\mathbf{P_{SV40}}$, simian virus 40 promoter; $\mathbf{P_{TtgR1}}$, phloretin-responsive promoter; $\mathbf{P_{CRE}}$, synthetic mammalian promoter containing a cAMP-response element; **PPARα**, human peroxisome proliferator-activated receptor alpha; **Pram**, pramlintide, a stabilized variant of human peptide hormone amylin; **RAMP3**, human calcitonin receptor-like receptor activity modifying protein 3; **SEAP**, human placental secreted alkaline phosphatase; **TetR**, Tetracycline repressor from *Escherichia coli*; **tTA**, Tetracycline-dependent transactivator; $\mathbf{TtgA_1}$, phloretin-dependent transactivator; **TtgR**, repressor of the *Pseudomonas putida* DOT-T1E ABC multi-drug efflux pump; **VP16**, *Herpes simplex*-derived transactivation domain.

Supplementary References

55. Kemmer, C. *et al.* A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. *J. Control. Release* 150, 23-29 (2011).
56. Fussenegger, M., Moser, S., Mazur, X., Bailey, J.E. 1997. Autoregulated multi- cistronic expression vectors provide one-step cloning of regulated product gene expression in mammalian cells. Biotechnol. Prog. **13**, 733–740 (1997).

CHAPTER II

Bile acid-controlled transgene expression in mammalian cell and mice.

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Metabolic Engineering 2014, 21:81-90.

Abstract

In recent years, using trigger-inducible mammalian gene switches to design sophisticated transcription-control networks has become standard practice in synthetic biology. These switches provide unprecedented precision, complexity and reliability when programming novel mammalian cell functions. Metabolite-responsive repressors of human-pathogenic bacteria are particularly attractive for use in these orthogonal synthetic mammalian gene switches because the trigger compound sensitivity often matches the human physiological range. We have designed both a bile acid-repressible ($BEAR_{OFF}$) as well as a bile-acid-inducible ($BEAR_{ON}$) gene switch by capitalizing on components that have evolved to manage bile acid resistance in *Campylobacter jejunis,* the leading causative agent of human food-borne enteritis. We have shown that both of these switches enable bile acid-adjustable transgene expression in different mammalian cell lines as well as in mice. For the $BEAR_{OFF}$ device, the *C. jejunis* repressor CmeR was fused to the VP16 transactivation domain to create a synthetic transactivator that activates minimal promoters containing tandem operator modules (O_{cme}) in a bile acid-repressible manner. Fusion of CmeR to a transsilencing domain resulted in an artificial transsilencer that binds and represses a constitutive O_{cme}-containing promoter until it is released by addition of bile acid ($BEAR_{ON}$). A tailored multi-step tuning program for the inducible gene switch, which included the optimization of individual component performance, control of their relative abundances, the choice of the cell line and trigger compound, resulted in a $BEAR_{ON}$ device with significantly improved bile acid-responsive control characteristics. Synthetic metabolite-triggered gene switches that are able to interface with host metabolism may foster advances in future gene and cell-based therapies.

Innovations in synthetic biology have made the design of trigger-controlled devices that can precisely fine-tune the expression of transgenes possible. These recent developments are significant because they can be used to reprogram mammalian cells to execute desired activities or interface with complex metabolic pathways (Kemmer et al., 2010; Ye et al., 2011; Ye et al., 2013). Synthetic transcription control devices have been instrumental for gene function analysis and functional genomic research (Malleret et al., 2001, Baumgartel et al., 2008), constructing genetic toggle switches and genetic counters (Gardner et al., 2000; Kramer et al., 2004, Friedland et al., 2009), discovering new drugs and manufacturing biopharmaceuticals (Sharpless and Depinho, 2006; Weber et al., 2008, Wurm, 2004; Ulmer et al., 2006;Weber and Fussenegger, 2007; Gitzinger et al., 2009b) as well as designing specialized biomaterials (Greber and Fussenegger, 2007; Weber and Fussenegger, 2006; Ehrbar et al., 2008). Artificial transcription controllers have become essential components of molecular design, allowing construction of highly complex gene networks such as oscillators (Elowitz and Leibler, 2000; Deans et al., 2007; Tigges et al., 2009; Tigges et al. 2010), biocomputers (Xie et al., 2011; Ausländer et al., 2012), inter-cellular communication devices (Bacchus et al., 2012), and closed-loop control circuits known as prosthetic networks that correct metabolic disorders (Kemmer et al., 2010). Trigger-adjustable repressors found in prokaryotes have been an especially fruitful source of molecular parts for use in the assembly of orthogonal mammalian transcription-control devices (Ausländer and Fussenegger, 2013; Urlinger et al., 2000; Weber et al., 2002a; Gitzinger et al., 2009b; Gitzinger et al., 2012). Repressors derived from human-pathogenic bacteria are particularly attractive components because their trigger sensitivity often matches the human physiological range. This is a result of years of co-evolution during which the pathogen was selected to optimally interface and synchronize with its host's metabolism (Ausländer and Fussenegger, 2013; Bacchus et al., 2013; Hartenbach et al., 2007; Weber et al., 2007; Kemmer et al., 2010).

Campylobacter jejuni is the leading bacterial cause of human food-borne enteritis in industrialized countries and has become increasingly resistant to antimicrobials (Klančnik et al., 2012). Over time, *C. jejuni* has evolved multiple mechanisms to adapt to antibiotic treatments as well as the human gastrointestinal environment. As part of this adaptation, *C. jejuni* uses a three-gene operon called *cmeABC* that encodes a multidrug efflux system able to confer resistance to structurally diverse antimicrobial compounds (Lin et al., 2002; Lin et al., 2005b). Expression of *cmeABC* is controlled by the transcriptional repressor CmeR which directly binds to the inverted repeat operator O_{Cme} within the P_{cmeABC} promoter and represses the transcription of *cmeABC* (Lin et al., 2005a; Routh et al., 2009). CmeR is a member of the TetR family of transcriptional regulators and contains typical motifs of this family, namely a N-terminal DNA-binding helix-turn-helix

domain as well as a C-terminal region predicted to be involved in recognizing the inducer molecule (Routh et al., 2009; Gu et al., 2007). Bile acids, a key compound family that defines the gastrointestinal microenvironment, have recently been shown to bind and dose-dependently inactivate the CmeR repressor resulting in induction of the CmeABC multidrug efflux pump (Lei et al., 2011; Kurinčič et al., 2012). Timely induction of the multidrug efflux pump, which contributes to *C. jejuni*'s resistance to a broad spectrum of antimicrobials as well as to bile acids, is essential for the pathogen's colonization of the intestinal tract (Lin et al., 2005b; Young et al., 2007).

Bile acids have long been known to act as emulsifiers that improve digestion and absorption of lipids and fat-soluble vitamins in the small intestine (Insull, 2006; Lefebvre et al., 2009; Hofmann, 2009). Recently, they have been shown to act as hormones involved in the regulation of metabolic processes including the maintenance of energy homeostasis (Staels and Fonseca, 2009), and therefore to have a much greater impact on human physiology than previously understood. Bile acids are synthesized from cholesterol in the liver (Hofmann, 1999a; Hofmann, 2009). The immediate products of the bile acid synthetic pathways are the primary bile acids cholic acid and chenodeoxycholic acid, which are conjugated with taurine or glycine for improved hydrophilicity before storage in the gallbladder as mixed micelles. Primary bile acids are also converted to secondary bile acids such as deoxycholic or lithocholic acids by the action of the intestinal bacterial flora (Thomas et al., 2008; Enhsen et al., 1998; Ridlon, 2005). Upon ingesting food, gallbladder contraction releases micellar bile acids into the intestinal lumen to facilitate digestion. These bile acids are subsequently reabsorbed and recycled via enterohepatic circulation (Hofmann, 1999b).

Capitalizing on *C. jejuni*'s bile acid-sensing components, we have engineered a bile acid-responsive transcription-controlling device that is able to reversibly fine-tune transgene expression in different mammalian cell lines as well as in mice. By constructing this device, we have made a gene switch responsive to a critically important metabolic trigger compound available. It will expand the metabolic intervention portfolio and may foster novel advances in gene- and cell-based therapies.

Materials and Methods

Bile acid sensor components. Comprehensive design and construction details for all expression vectors are provided in **Table 1**.

Cell culture and transfection. Human embryonic kidney cells (HEK-293T, ATCC: CRL-11268), Baby hamster kidney cells (BHK-21, ATCC: CCL-10), African green monkey kidney cells (COS-7, ATCC: CRL-1651), human cervical adenocarcinoma cells (HeLa, ATCC: CCL-2) and human fibrosarcoma cells (HT-1080, ATCC: CCL-121) were cultivated in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Basel, Switzerland) supplemented with 10% (v/v) fetal calf serum (FCS, PAN Biotech GmbH, Aidenbach, Germany, lot no. P251110) or 10% (v/v) charcoal-stripped fetal bovine serum (cFBS, Sigma-Aldrich, Munich, Germany, cat. no. F6765, lot. no. 11C151) and 1% (v/v) penicillin/streptomycin solution (Sigma-Aldrich, Munich, Germany). Wild-type Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultured in ChoMaster® HTS (Cell Culture Technologies GmbH, Gravesano, Switzerland) containing 1% penicillin/streptomycin. All cell types were cultivated at 37°C in a humidified atmosphere containing 5% CO_2. For co-transfection of BHK-21, CHO-K1, COS-7, HEK-293, HeLa and HT-1080 cells, 40,000 cells were seeded per well of a 24-well plate 12h prior to transfection and were incubated for 6h with a 4:1 PEI:DNA mixture (Polyethyleneimine; MW 40,000, Polysciences, Inc., Warrington, USA). After transfection, all cells were maintained in their specific media containing different inducer concentrations. Reporter protein levels were measured after 48 h unless stated otherwise.

Colorimetric SEAP assay. Production of the human placental secreted alkaline phosphatase (SEAP) was quantified in culture supernatants according to a p-nitrophenylphosphate-based light absorbance time course (Schlatter et al., 2002). Serum levels of SEAP were profiled using a chemiluminescence-based assay (Roche Diagnostics GmbH, Mannheim, Germany).

Inducer compounds, cholic acid and its derivatives. Cholic acid (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland; cat. no. C9282), chenodeoxycholic acid (Sigma-Aldrich Chemie GmbH; cat. no. C8261), lithocholic acid (Sigma-Aldrich Chemie GmbH; cat. no. L6250), glycocholic acid (Sigma-Aldrich Chemie GmbH; cat. no. G7132), taurocholic acid (Calbiochem, La Jolla, USA; cat. no. 580217), deoxycholic acid (Acros Organics, Geel, Belgium; cat. no. 21859), and water soluble cholesterol (Sigma-Aldrich Chemie GmbH; cat. no. C4951) were prepared as 10mM stock solutions in water and adjusted to pH 7.

Animal experiments. Intraperitoneal implants were produced by encapsulating pKR69/pKR114-transgenic HEK-293 cells in coherent alginate-poly-(L-lysine)-alginate beads (400µm; 200cells/capsule) using an Inotech Encapsulator Research Unit IE-50R (Buchi

Labortechnik AG, Flawil, Switzerland) set to the following parameters: 200µm nozzle with a vibration frequency of 1023Hz and 900V for bead dispersion, 20mL syringe operated at a flow rate of 403 units. 400µL of serum-free DMEM containing $2x10^6$ microencapsulated pKR69/pKR114-transgenic HEK-293 cells (200 cells/capsule) were injected intraperitoneally into 14-week-old female CD1 mice (Janvier S.A.S., Le Genest-Saint-Isle, France), which received twice-daily injections of cholic acid (200µl, 0-100mg/kg), chenodeoxycholic acid or deoxycholic acid (20 µl, 0-30mg/kg). Blood samples were collected and the serum was isolated using BD Microtainer® SST tubes according to the manufacturer's protocol (Beckton Dickinson, Plymouth, UK) before serum SEAP levels were scored as described above. All experiments involving animals were performed according to the directives of the European Community Council (2010/63/EU), approved by the French Republic (no. 69266309), and carried out by Ghislaine Charpin-El Hamri at the Institut Universitaire de Technology (IUTA, F-69622 Villeurbanne Cedex, France).

Statistical analysis. Data represent mean ± SD of at least three independent experiments. Comparisons among the groups were made using the Student's two-tailed *t* test expressed as mean ± SEM. Differences were considered statistically significant at *P < 0.05, **P < 0.005, ***P < 0.0001.

Table 1. Plasmids used and designed in this study

Plasmid	Description and Cloning Strategy	Reference or Source
pcDNA3.1	Mammalian expression vector (P_{hCMV}-MCS-pA).	Life Technologies, Carlsbad, CA, USA
pSEAP2-control	Constitutive mammalian SEAP expression vector (P_{SV40}-SEAP-pA).	Clontech, Mountain View, CA, USA
pSAM200	Constitutive tTA expression vector (P_{SV40}-tTA-pA).	Fussenegger et al. (1997)
pWW29	Constitutive mammalian MphR(A) expression vector ($P_{hEF1\alpha}$-MphR(A)-pA).	Weber et al. (2002b)
pWW43	Constitutive ET4 expression vector (P_{SV40}-ET4-pA; ET4, MphR(A)-KRAB).	Weber et al. (2002b)
pWW1088	Biotin-inducible SEAP expression vector (P_{BIT1}-SEAP-pA).	Weber et al. (2009)
pKR1	Constitutive $CmeA_1$ expression vector (P_{SV40}-$CmeA_1$-pA; $CmeA_1$, CmeR-VP16). CmeR was PCR-amplified from *Campylobacter jejuni* genomic cDNA using oligonucleotides OKR115 (5'-ccgatttccg*gaattc*caccATG AACTCAAATAGAACACCATC-3') and OKR113 (5'-cgc*ggatcc*c*ggcgcgc*GGCTGTACGCGGAAGCTTTGG AGCTATTGATTCC-3'; restriction sites lower case italics, annealing sequence upper case), digested with *Eco*RI/*Bss*HII and cloned into the corresponding sites (*Eco*RI/*Bss*HII) of pSAM200.	This work
pKR64	Vector encoding a $P_{CmeOFF4}$-driven SEAP expression unit ($P_{CmeOFF4}$-SEAP-pA; $P_{CmeOFF4}$, O_{Cme4}-$P_{hCMVmin}$). Oligonucleotides OKR124 (5'-ccttaacgc*gtcgacTGTAAT AAATATTACATGTAATAAATATTACAagatctTGTAATA AATATTACgatatcTGTAATAAATATTACAagatctatgcatc ctgcagg*tattccgc-3') and OKR125 (5'-gcggaata*cctgcagg atgcatagatctTGTAATATTTATTACAgatatcGTAATATTT ATTACAagatct*T*GTAATATTTATTACATGTAATATTTA TTACAgtcgac*gcgttaagg-3'; restriction sites lower case, O_{Cme4} upper case italics) were annealed and ligated directly into pWW1088 using *Sal*I/*Sbf*I.	This work
pKR66	Constitutive $CmeA_2$ expression vector (P_{SV40}-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB). KRAB was excised from pWW43 with *Bss*HII/*Bam*HI and cloned into the corresponding sites (*Bss*HII/*Bam*HI) of pKR1.	This work
pKR69	Constitutive $CmeA_2$ expression vector ($P_{hEF1\alpha}$-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB). $P_{hEF1\alpha}$ was PCR-amplified from pWW29 using oligonucleotides OKR83 (5'-gaaaaacctag*ctagc*GGGAAAGTGATGTCGTGTAC-3') and OKR84 (5'-accaaatagtttag*cggccgc*GAATTCGTAC AAGGGCAATTC-3'; restriction sites lower case italics, annealing sequence upper case), digested with *Nhe*I/*Not*I and cloned into the corresponding sites (*Nhe*I/*Not*I) of pKR66.	This work

Table 1. Continued

Plasmid	Description and Cloning Strategy	Reference or Source
pKR75	Vector encoding a P_{CmeON2}-driven SEAP expression unit (P_{CmeON2}-SEAP-pA; P_{CmeON2}, P_{SV40}-O_{Cme2}). SEAP was PCR-amplified from pSEAP2-control using oligonucleotides OKR119 (5'-cttaaccccg*gaattcTGTAATAAAT ATTACATGTAATAAATATTACA*ccaccATGCTGCTGC TGCTGCTGC-3') and OKR81 (5'-ggaattc*catatg*GGAA GCGGTCCATG-3'; restriction sites lower case italics, annealing sequence upper case, O_{Cme2} upper case italics), digested with *Eco*RI/*Nde*I and cloned into the corresponding sites (*Eco*RI/*Nde*I) of pSEAP2-control.	This work
pKR76	Vector encoding a P_{CmeON4}-driven SEAP expression unit (P_{CmeON4}-SEAP-pA; P_{CmeON4}, P_{SV40}-O_{Cme4}). SEAP was PCR-amplified from pSEAP2-control using oligonucleotides OKR121 (5'-cttaaccccg*gaattcTGTAATAAAT ATTACATGTAATAAATATTACATGTAATAAATATTAC ATGTAATAAATATTACA*ccaccATGCTGCTGCTGCTG CTGC-3) and OKR81 (5'-ggaattc*catatg*GGAAGCGGT CCATG-3'; restriction sites lower case italics, annealing sequence upper case, O_{Cme4} upper case italics), digested with *Eco*RI/*Nde*I and cloned into the corresponding sites (*Eco*RI/*Nde*I) of pSEAP2-control.	This work
pKR98	Vector encoding a P_{CmeON8}-driven SEAP expression unit (P_{CmeON8}-SEAP-pA; P_{CmeON8}, P_{SV40}-O_{Cme8}). Oligonucleotides OKR165 (5'-cgctac*aagcttGGACTGTAATAA ATATTACAT GTAATAAATATTACAagatctTGTAATAAA TATTACgatatcTGTAATAAATATTACAagatctATGCATC CTGgaattc*tagaatg-3') and OKR166 (5'-cattcta*gaattcCA GGATGCATagatctTGTAATATTTATTACAgatatcGTAA TATTTATTACAagatctTGTAATATTTATTACATGTAATA TTTATTACAGTCCaagctt*gtagcg-3'; restriction site lower case italics, O_{Cme4} upper case italics) were annealed and ligated directly into pKR76 using *Hin*dIII/*Eco*RI.	This work
pKR104	Constitutive $CmeA_2$ expression vector (P_{hCMV}-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB). P_{hCMV} was excised from pcDNA3.1 using *Ssp*I/*Eco*RI and cloned into the corresponding sites (*Ssp*I/*Eco*RI) of pKR69.	This work
pKR113	Vector encoding a $P_{CmeON12}$-driven SEAP expression unit ($P_{CmeON12}$-SEAP-pA; $P_{CmeON12}$, P_{SV40}-O_{Cme12}). Oligonucleotides OKR171 (5'-gcttgatt*aagcttTGTAATA AATATTACATGTAATAAATATTACATGTAATAAATAT TACATGTAATAAATATTACAaagctt*aatcaagc-3') and OKR172 (5'-gcttgatt*aagcttTGTAATATTTATTACATGTA ATATTTATTACATGTAATATTTATTACATGTAATATTT ATTACAaagctt*aatcaagc-3'; restriction sites lower case, O_{Cme4} upper case italics) were annealed and ligated directly into pKR98 using *Hin*dIII.	This work

Table 1. Continued

Plasmid	Description and Cloning Strategy	Reference or Source
pKR114	Vector encoding a $P_{CmeON14}$-driven SEAP expression unit ($P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-O_{Cme14}). Oligonucleotides OKR173 (5'-ctgatt*aagcttTGTAATAAAT ATTACATGTAATAAATATTACATGTAATAAATATTACA TGTAATAAATATTACATGTAATAAATATTACATGTAAT AAATATTACAaagctt*aatcag-3') and OKR174: (5'-ctgatt *aagcttTGTAATATTTATTACATGTAATATTTATTACATG TAATATTTATTACATGTAATATTTATTACATGTAATATT TATTACATGTAATATTTATTACAaagctt*aatcag-3'; restriction sites lower case, O_{Cme6} upper case italics) were annealed and ligated directly into pKR98 using *Hin*dIII.	This work
pKR132	Vector encoding a $P_{CmeOFF8}$-driven SEAP expression unit ($P_{CmeOFF8}$-SEAP-pA; $P_{CmeOFF8}$, O_{Cme8}- $P_{hCMVmin}$). Oligonucleotides OKR197 (5'- gattacg*cctgcaggTGGACTGTAA TAAATATTACATGTAATAAATATTACAAGATCTTGTAA TAAATATTACGATATCTGTAATAAATATTACAAGATCT cctgcagg*tagaatg-3') and OKR198: (5'-cattcta*cctgcagAG ATCTTGTAATATTTATTACAGATATCGTAATATTTATTA CAAGATCTTGTAATATTTATTACATGTAATATTTATTA CAGTCCAcctgcagg*cgtaatc-3'; restriction sites lower case, O_{Cme4} upper case italics) were annealed and ligated directly into pKR64 using *Sbf*I.	This work

Abbreviations: **$CmeA_1$**, bile acid-dependent transactivator (CmeR-VP16); **$CmeA_2$**, bile acid-dependent transsilencer (CmeR-KRAB); **CmeR**, *Campylobacter jejuni* bile acid-dependent repressor; **ET4**, macrolide-dependent transsilencer (E-KRAB); **KRAB**, human Krueppel-associated box domain; **MCS**, multiple cloning site; **MphR(A)**, repressor of the *Escherichia coli* 2'-phosphotransferase I; **$O_{Cme(n)}$**, n tandem CmeR-specific operator site; **pA**, SV40-derived polyadenylation site; **P_{BIT1}**, biotin-inducible promoter; **$P_{CmeOFF4,8}$**, bile acid-responsive promoter harboring 4 and 8 operator repeats (O_{Cme}) 5' of $P_{hCMVmin}$ ($O_{Cme4,8}$-$P_{hCMVmin}$)**;** **$P_{CmeON2,4,8,12,14}$,** bile acid-responsive promoter containing different operator repeats after P_{SV40}; **P_{hCMV}**, human cytomegalovirus immediate early promoter; **$P_{hCMVmin}$**, minimal version of P_{hCMV}; **$P_{hEF1\alpha}$**, human elongation factor 1α promoter; **P_{SV40}**, simian virus 40 promoter; **SEAP**, human placental secreted alkaline phosphatase; **tTA**, tetracycline-dependent transactivator; **VP16**, *Herpes simplex*-derived transactivation domain.

Results

Design and validation of the bile acid-responsive mammalian transcription control system (BEAR). By fusing the bile acid-dependent transcriptional repressor CmeR of *C. jejuni* to different transactivation and transsilencing domains we created synthetic bile acid-sensitive transcription factors which either repress ($BEAR_{OFF}$) or induce ($BEAR_{ON}$) chimeric target promoters containing CmeR-specific operator modules (O_{Cme}) in the presence of cholic acid. The design of the $BEAR_{OFF}$ system consists of the bile acid-dependent transactivator $CmeA_1$ (CmeR-VP16), assembled by fusing CmeR to the VP16 transactivation domain of *Herpes simplex* virus (VP16; Triezenberg et al., 1988), and a cognate promoter $P_{CmeOFF8}$ (O_{Cme8}-$P_{hCMVmin}$) that contains an octameric $CmeA_1$-specific operator module immediately 5' of a minimal version of the human cytomegalovirus immediate-early promoter ($P_{hCMVmin}$) (Fig. 1A). HEK-293 cells co-transfected with the $CmeA_1$ expression vector (pKR1, P_{SV40}-$CmeA_1$-pA; $CmeA_1$, CmeR-VP16) and the $P_{CmeOFF8}$-driven SEAP reporter plasmid (pKR132, $P_{CmeOFF8}$-SEAP-pA; $P_{CmeOFF8}$, O_{Cme8}- $P_{hCMVmin}$), and treated with increasing concentrations of cholic acid, confirmed dose-dependent shut down of SEAP expression (Fig. 1B). Cholic acid concentrations able to regulate the circuit had no impact on cell viability as the cellular SEAP production capacity remained constant even at the highest cholic acid concentrations (Fig. 1B).

The design of the $BEAR_{ON}$ system consists of the bile acid-dependent transsilencer $CmeA_2$, assembled by fusing CmeR to the human Krueppel-associated box (KRAB; (Moosmann et al., 1997), transsilencing domain, and a $CmeA_2$-specific promoter $P_{CmeON14}$ (P_{SV40}-O_{Cme14}) that contains 14 tandem O_{Cme} (O_{Cme14}) immediately 3' of the constitutive simian virus 40 promoter (P_{SV40}) (Fig. 1C). Co-transfection of the $CmeA_2$ expression vector (pKR66, P_{SV40}-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB) and the $P_{CmeON14}$-driven SEAP reporter plasmid (pKR114, $P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-O_{Cme14}) into HEK-293 cells was used to validate dose-dependent induction of SEAP expression in the presence of increasing concentrations of cholic acid (Fig. 1D). Bile salts present in the fetal calf serum used as a cultivation media supplement may account for some basal expression of the $BEAR_{ON}$ system (Neimark et al., 2004). We have therefore also cultivated pKR66/pKR114-cotransfected HEK-293 cells in bile-acid-free charcoal-stripped fetal bovine serum (cFBS)-containing culture medium supplemented with different cholic acid concentrations. This experimental set-up resulted in lower basal expression of the $BEAR_{ON}$ system, suggesting that the basal leakiness indeed originates in part from bile salts contained in serum supplements (Fig. 1D).

The $BEAR_{OFF}$ as well as the $BEAR_{ON}$ systems were responsive within the clinically relevant concentration range (pathological serum levels are between 10 and 300 µM; Bacq et al., 1995; Glantz et al., 2004; Palmeira and Rolo, 2004), indicating that the gene switches could double as

biosensors detecting pathological bile acid levels in future clinical applications. Because ON-type gene switches are the preferred configuration for biosensor circuits as well as transgene expression fine-tuning in vitro, we used the $BEAR_{ON}$ system in all follow-up experiments.

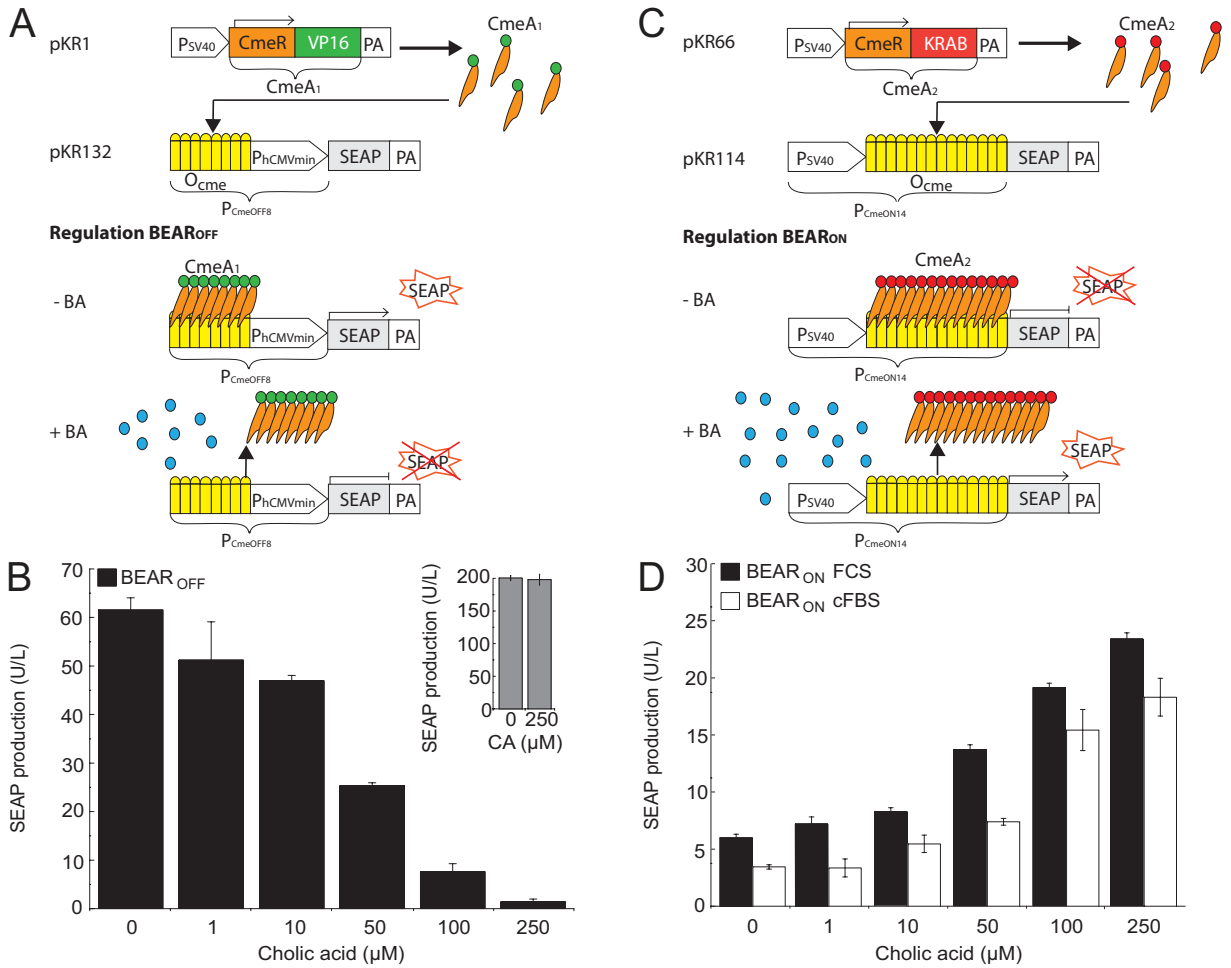

Fig. 1. Synthetic bile-acid-responsive mammalian gene switch. (A and B) Design and functionality of $BEAR_{OFF}$. (A) CmeR was fused to the *Herpes simplex*-derived transactivation domain (VP16) to create a bile acid-dependent transactivator $CmeA_1$ (CmeR-VP16), which is driven by the simian virus 40 promoter (pKR1, P_{SV40}-$CmeA_1$-pA). In the absence of bile acids (-BA), $CmeA_1$ binds to a chimeric target promoter $P_{CmeOFF8}$ (pKR132; $P_{CmeOFF8}$-SEAP-pA; $P_{CmeOFF8}$, O_{Cme8}-$P_{hCMVmin}$) containing a CmeR-specific octameric operator module and activates SEAP expression. In the presence of bile acids (+BA), $CmeA_1$ is released from $P_{CmeOFF8}$ and SEAP expression is shut down. (B) HEK-293 cells were co-transfected with pKR1 (P_{SV40}-$CmeA_1$-pA; $CmeA_1$, CmeR-VP16) and pKR132 ($P_{CmeOFF8}$-SEAP-pA; $P_{CmeOFF8}$, O_{Cme8}-$P_{hCMVmin}$) and SEAP expression was assessed 48h after cultivation of the cells in medium containing different concentrations of cholic acid (0-250µM). Metabolic integrity and viability of the cells was confirmed via SEAP production of HEK-293 cells transfected with pSEAP2-control (P_{SV40}-SEAP-pA) and cultivated for 48h in the presence and absence of cholic acid (0, 250 µM). (C and D) Design and functionality of $BEAR_{ON}$. (C) CmeR was fused to the human Krueppel-associated box (KRAB) domain to produce a bile acid-dependent transsilencer $CmeA_2$ (CmeR-KRAB) which is driven by the constitutive simian virus 40 promoter (pKR66, P_{SV40}-$CmeA_2$-pA). In the absence of bile acids (-BA), $CmeA_2$ binds to the tandem operator sites (O_{cme}) within $P_{CmeON14}$ (P_{SV40}-O_{cme14}; pKR114, $P_{CmeON14}$-SEAP-pA) and represses P_{SV40}-driven SEAP expression. In the presence of bile acids (+BA), $CmeA_2$ is released from $P_{CmeON14}$ which

results in dose-dependent induction of P_{SV40}-driven SEAP expression. (D) Cholic acid-inducible SEAP expression in HEK-293 cells. HEK-293 cells were co-transfected with pKR66 (P_{SV40}-$CmeA_2$-pA) and pKR114 ($P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-$O_{CmeON14}$) and grown for 48h in the presence of different cholic acid concentrations (0-250µM) in the culture media containing fetal calf serum (FCS) or charcoal-stripped fetal bovine serum (cFBS) before SEAP levels were profiled in the culture supernatant.

Functionality of the $BEAR_{ON}$ device in different mammalian cell lines. In order to assess the versatility and broad applicability of $BEAR_{ON}$, we co-transfected several rodent, monkey and human cell lines with pKR66 (P_{SV40}-$CmeA_2$-pA) and pKR114 ($P_{CmeON14}$-SEAP-pA) and cultivated them for 48h in the presence of different cholic acid levels (0, 50, 100 µM) or in the absence of cholic acid, before measuring SEAP production in the culture supernatant (Table 2). The $BEAR_{ON}$ device was functional in all tested cell lines, suggesting that this gene switch has broad applicability. Since HEK-293 cells showed the best induction level they were used for all further experiments.

Table 2. $BEAR_{ON}$-controlled gene expression in various mammalian cell lines.

	Cholic acid (µM)				Fold
Cell line	**0**	**50**	**100**	**250**	**induction**
BHK-21	38.8 ± 3.3	48.0 ± 2.4	62.0 ± 5.8	73.2 ± 5.3	1.9x
CHO-K1	10.2 ± 0.4	15.5 ± 0.9	17.5 ± 1.7	21.9 ± 2.7	2.1x
COS-7	69.7 ± 0.3	81.7 ± 6.4	101.0 ± 12.6	150.0 ± 21.1	2.2x
HEK-293	6.0 ± 0.3	13.6 ± 0.4	19.0 ± 0.4	23.7 ± 0.4	4.0x
HeLa	8.7 ± 0.4	5.3 ± 1.1	23.6 ± 1.7	30.4± 2.0	3.5x
HT-1080	49.4 ± 6.6	63.1 ± 1.1	91.5 ± 2.6	112.2 ± 3.7	2.3x

SEAP production (U/L) was quantified 48h after transient co-transfection of pKR66 (P_{SV40}-$CmeA_2$-pA) and pKR114 ($P_{CmeON14}$-SEAP-pA).

Optimization of the $BEAR_{ON}$ gene switch I – promoter variants with different tandem operators. The sensitivity and leakiness of gene switches can be optimized by varying the number of tandem operators contained in the chimeric promoters because these operators recruit the synthetic transcription factors (Weber et al., 2009; Malphettes et al., 2005). To improve the transcription control performance of the $BEAR_{ON}$ system, we designed $CmeA_2$-specific promoter variants containing two (pKR75, P_{CmeON2}-SEAP-pA; P_{CmeON2}, P_{SV40}-*Hin*dIII-*Nru*I-*Eco*RI-O_{Cme}-O_{Cme}-ccaccATG), four (pKR76, P_{CmeON4}-SEAP-pA; P_{CmeON4}, P_{SV40}-*Hin*dIII-*Nru*I-*Eco*RI-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-ccaccATG), eight (pKR98, P_{CmeON8}-SEAP-pA; P_{CmeON8}, P_{SV40}-*Hin*dIII-O_{Cme}-O_{Cme}-*Bgl*II-O_{Cme}-*Eco*RV-O_{Cme}-*Bgl*II-*Nsi*I-*Eco*RI-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-ccaccATG), twelve (pKR113,

$P_{CmeON12}$-SEAP-pA; $P_{CmeON12}$, P_{SV40}-*Hin*dIII-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-*Hin*dIII-O_{Cme}-O_{Cme}-*Bgl*II-O_{Cme}-*Eco*RV-O_{Cme}-*Bgl*II-*Nsi*I-*Eco*RI-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-ccaccATG) or fourteen (pKR114, $P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-*Hin*dIII-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-*Hin*dIII-O_{Cme}-O_{Cme}-*Bgl*II-O_{Cme}-*Eco*RV-O_{Cme}-*Bgl*II-*Nsi*I-*Eco*RI-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-ccaccATG) operator repeats immediately 3' of the SV40 promoter. After co-transfection of HEK-293 cells with pKR66 (P_{SV40}-$CmeA_2$-pA) and SEAP-driving promoter variants, the cells were cultivated for 48h in the presence or absence of 100µM cholic acid before SEAP levels were profiled in the culture supernatant (Fig. 2A). All promoter configurations were induced in the presence of cholic acid, although the extent of induction as well as the basal and maximum expression levels varied. Beyond 8 operators, the overall induction factor increased with the number of tandem operators contained in the synthetic promoter. $P_{CmeON14}$ performed the best; it had the lowest leaky SEAP expression (6.0 ± 0.3 U/L) and highest induction factor (4-fold induction). This promoter was therefore used for further experiments (Fig. 2A).

Optimization of the $BEAR_{ON}$ gene switch II – different $CmeA_2$ expression levels. Besides the promoter configuration, the expression level of the transsilencer can also impact the overall performance of a gene switch (Qin et al., 2010; Gitzinger et al., 2009a). We therefore tested $CmeA_2$ expression using constitutive promoters of different strengths including the simian virus 40 promoter (P_{SV40}; pKR66, P_{SV40}-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB), the human cytomegalovirus immediate-early promoter (P_{hCMV}; pKR104, P_{CMV}-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB), and the human elongation factor 1α promoter ($P_{hEF1\alpha}$; pKR69, $P_{hEF1\alpha}$-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB). The respective $CmeA_2$ expression vectors were co-transfected together with pKR114 ($P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-*Hin*dIII-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-*Hin*dIII-O_{Cme}-O_{Cme}-*Bgl*II-O_{Cme}-*Eco*RV-O_{Cme}-*Bgl*II-*Nsi*I-*Eco*RI-O_{Cme}-O_{Cme}-O_{Cme}-O_{Cme}-ccaccATG) into HEK-293 cells and cultivated for 48h in the presence of different cholic acid concentrations (0 - 250µM) or the absence of cholic acid before SEAP production was profiled in the culture supernatant (Fig. 2B). Since $P_{hEF1\alpha}$ had the lowest basal SEAP expression and largest range of induction (7.3-fold), it was chosen to drive $CmeA_2$ in all follow-up experiments.

Optimization of the $BEAR_{ON}$ gene switch III – transfection ratio optimization. Since the transsilencer directly interacts with its target promoter, the relative abundance of both components impacts the dynamic range of the gene switch (Lin et al., 2005a; Lin et al., 2005b; Cagliero et al., 2007). To further optimize the $BEAR_{ON}$ gene switch, we analyzed which ratio between the transsilencer (pKR69; $P_{hEF1\alpha}$-$CmeA_2$-pA) and its target expression unit (pKR114;

$P_{CmeON14}$-SEAP-pA) would achieve optimal cholic acid-inducible reporter gene expression. Therefore, HEK-293 cells were co-transfected with different component-encoding expression vectors (pKR114 : pKR69 = 1:5, 1:3, 1:1, 3:1 5:1) and cultivated for 48h in the presence or absence of 100µM cholic acid before SEAP production was scored in the culture supernatant (Fig. 2C). Precise reporter gene expression profiling revealed that relative increases in the reporter vector were correlated with higher leaky expression and lower induction ratios **(**Fig. 2C). In contrast, when increasing the relative abundance of the transsilencer expression vector, the induction ratio increased significantly while basal expression was reduced considerably (Fig. 2C). At a 5:1 transsilencer to reporter ratio in the cell, the least leakiness and an up to 12-fold induction could be achieved (Fig. 2C).

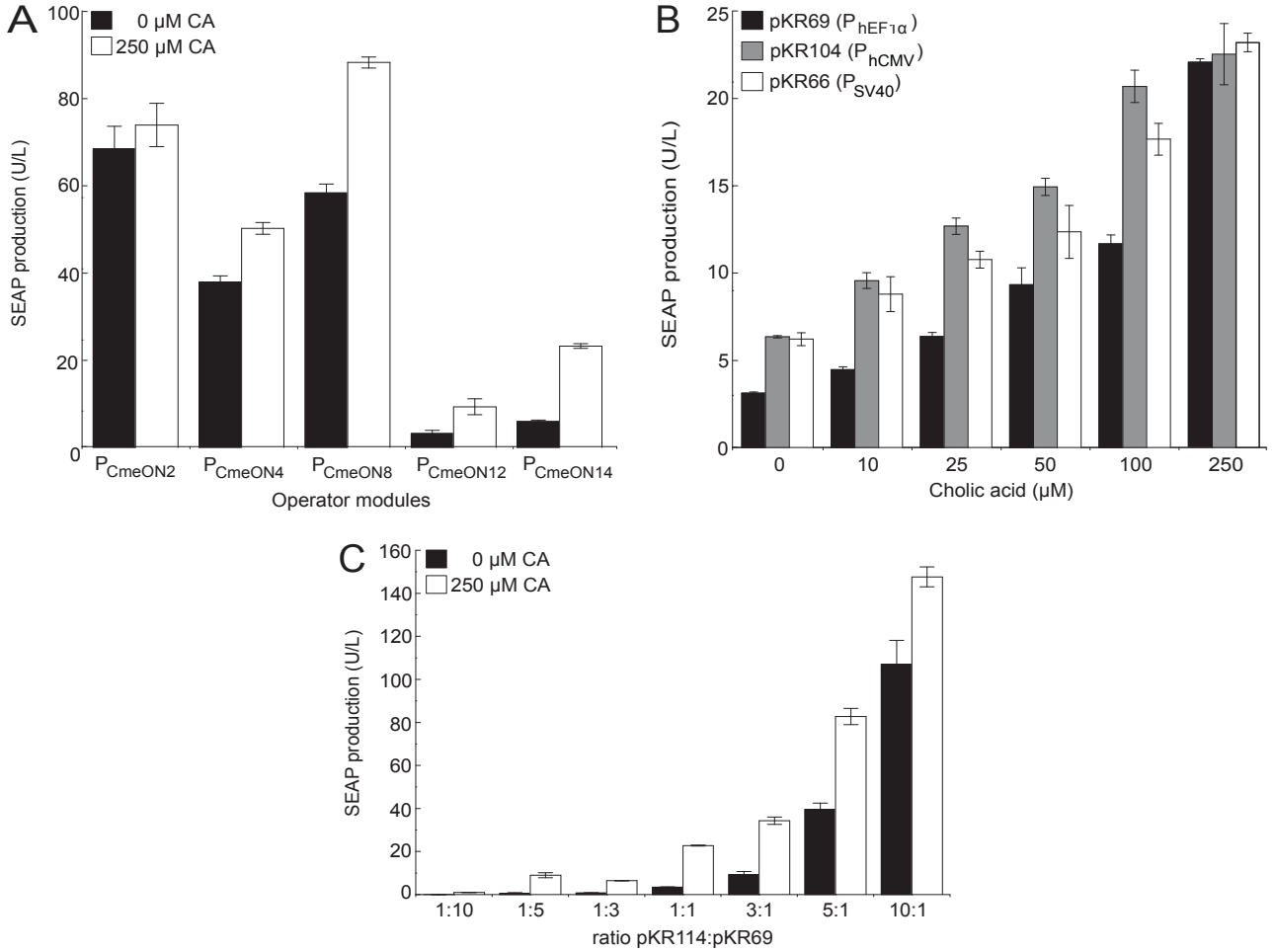

Fig. 2. Refinement of the $BEAR_{ON}$ gene switch. (A) Optimization of the bile acid-responsive promoter. The number of tandem operator repeats within P_{cmeON} were increased from two (P_{cmeON2}, pKR75) to four (P_{cmeON4}, pKR76), eight (P_{cmeON8}, pKR98), twelve ($P_{cmeON12}$, pKR113), and fourteen ($P_{cmeON14}$, pKR114) and the corresponding SEAP expression vectors were co-transfected with the transsilencer-encoding plasmid pKR66 (P_{SV40}-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB) into HEK-293 cells. Transfected populations were grown for 48h in the presence or absence of cholic acid before SEAP levels were scored in the culture supernatant. (B) Tuning the constitutive $CmeA_2$ expression level. $CmeA_2$ was placed under control of different constitutive promoters with increasing

expression strength including P_{SV40} (pKR66; P_{SV40}-$CmeA_2$-pA), P_{hCMV} (pKR104, P_{hCMV}-$CmeA_2$-pA) and $P_{hEF1\alpha}$ (pKR69; $P_{hEF1\alpha}$-$CmeA_2$-pA). The resulting transsilencer-encoding vectors were co-transfected with pKR114 ($P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-$O_{CmeON14}$) into HEK-293 cells, cultivated for 48h in the presence or absence of cholic acid before SEAP levels were quantified in the culture supernatant. (C) Impact of the relative abundance of transsilencer and bile acid-responsive reporter expression vectors on $BEAR_{ON}$ performance. pKR69 ($P_{hEF1\alpha}$-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB) and pKR114 ($P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-$O_{CmeON14}$) were co-transfected at different ratios into HEK-293 cells and cultivated for 48h in the presence and absence of cholic acid before SEAP was profiled in the culture supernatant.

Characterization of the triple-optimized $BEAR_{ON}$ gene switch. In order to quantify adjustability, induction kinetics, and reversibility of the $BEAR_{ON}$ gene switch, HEK-293 cells were co-transfected with the product-encoding expression plasmid pKR114 ($P_{CmeON14}$-SEAP-pA) and the transsilencer-containing vector pKR69 ($P_{hEF1\alpha}$-$CmeA_2$-pA) at a 1:5 ratio. The $BEAR_{ON}$-containing HEK-293 cells showed precise cholic-acid-adjustable SEAP expression over a wide and clinically relevant range (Fig. 3A). These cells also displayed exponential induction kinetics exclusively in the presence of cholic acid (Fig. 3B), and exhibited full expression reversibility, which was tested by repeatedly switching the system ON and OFF by alternating between presence and absence of cholic acid (Fig. 3C).

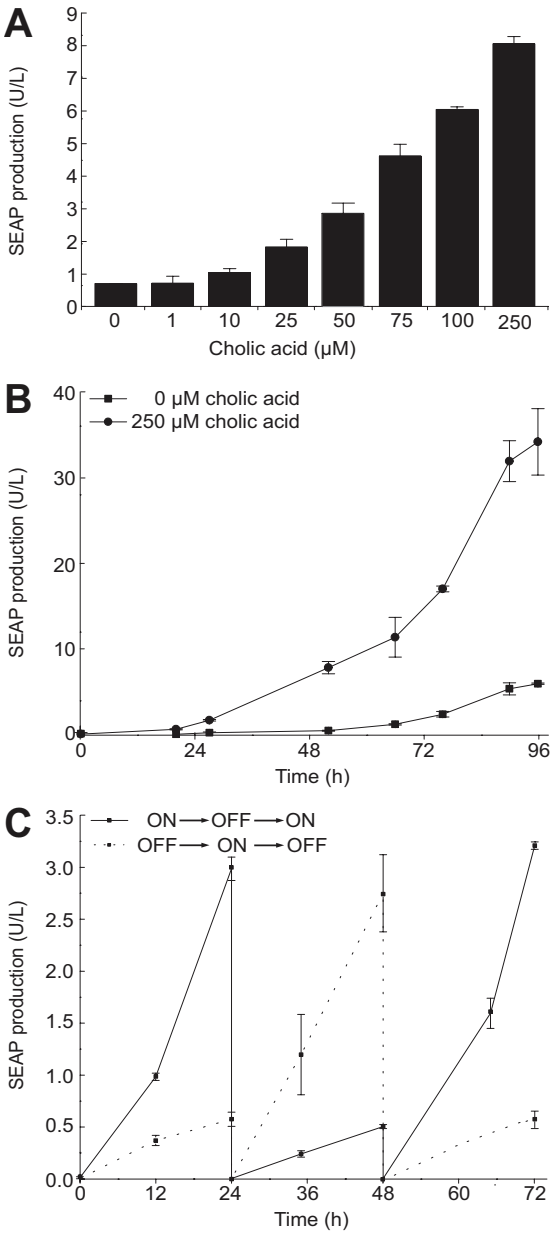

Fig. 3. Characterization of bile acid-controlled transgene expression. (A) Adjustability of bile acid-inducible SEAP expression in HEK-293 cells co-transfected with pKR69 ($P_{hEF1\alpha}$-$CmeA_2$-pA; $CmeA_2$, CmeR-KRAB) and pKR114 ($P_{CmeON14}$-SEAP-pA; $P_{CmeON14}$, P_{SV40}-$O_{CmeON14}$). Transfected cell populations were cultivated for 48h in culture medium containing increasing concentrations of cholic acid before SEAP was profiled in the culture supernatant. (B) SEAP expression kinetics of pKR69/pKR114-co-transfected HEK-293 cells cultured for 96h in the presence or absence of cholic acid before SEAP levels were assessed. (C) Reversibility of cholic acid-inducible SEAP expression. pKR69/pKR114-co-tranfected HEK-293 cells were cultivated for 72h while alternating the presence and absence of cholic acid (100µM ON; 0µM, OFF) and scoring SEAP levels every 24h.

Trigger-compound specificity of $BEAR_{ON}$ gene switch. In order to be effective, gene switches and biosensor devices will have to be specific for a particular trigger molecule or compound class and largely unable to be triggered by unrelated chemicals. Mammals produce several bile acids from cholesterol, including primary bile acids (cholic acid (CA) and

chenodeoxycholic acid), secondary bile acids (deoxycholic acid and lithocholic acid), and glycine- or taurine-conjugated derivatives of bile acids (Hofmann, 1999a). To assess the bile acid specificity of $BEAR_{ON}$ we co-transfected HEK-293 cells with pKR114 ($P_{CmeON14}$-SEAP-pA) and pKR69 ($P_{hEF1\alpha}$-$CmeA_2$-pA) (1:5 ratio). To assess viability, metabolic integrity, and the control of cytotoxicity, we transfected with pSEAP2-control alone. We cultivated the populations for 48h in the presence of different concentrations (0, 50, 100, 250 μM) of cholesterol or bile acids (cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), glycocholic acid (GCA) and taurocholic acid (TCA)), and scored the resulting SEAP levels in the culture supernatant (Fig. 4A-B). DCA and CDCA showed higher SEAP expression levels (24-fold and 23-fold induction, respectively) at non-toxic levels compared to LCA (9-fold induction) and CA and its derivatives (CA 12-fold, GCA 7-fold, TCA, 11-fold induction). These varying expressin levels might result from different binding affinities or different locations of the compounds in respect to the CmeR part of the transsilencer (Lei et al., 2011). Cholesterol, the precursor of all bile acids, did not activate the $BEAR_{ON}$ control device, which indicates that this gene switch is indeed exclusively controlled by bile acids (Fig. 4A).

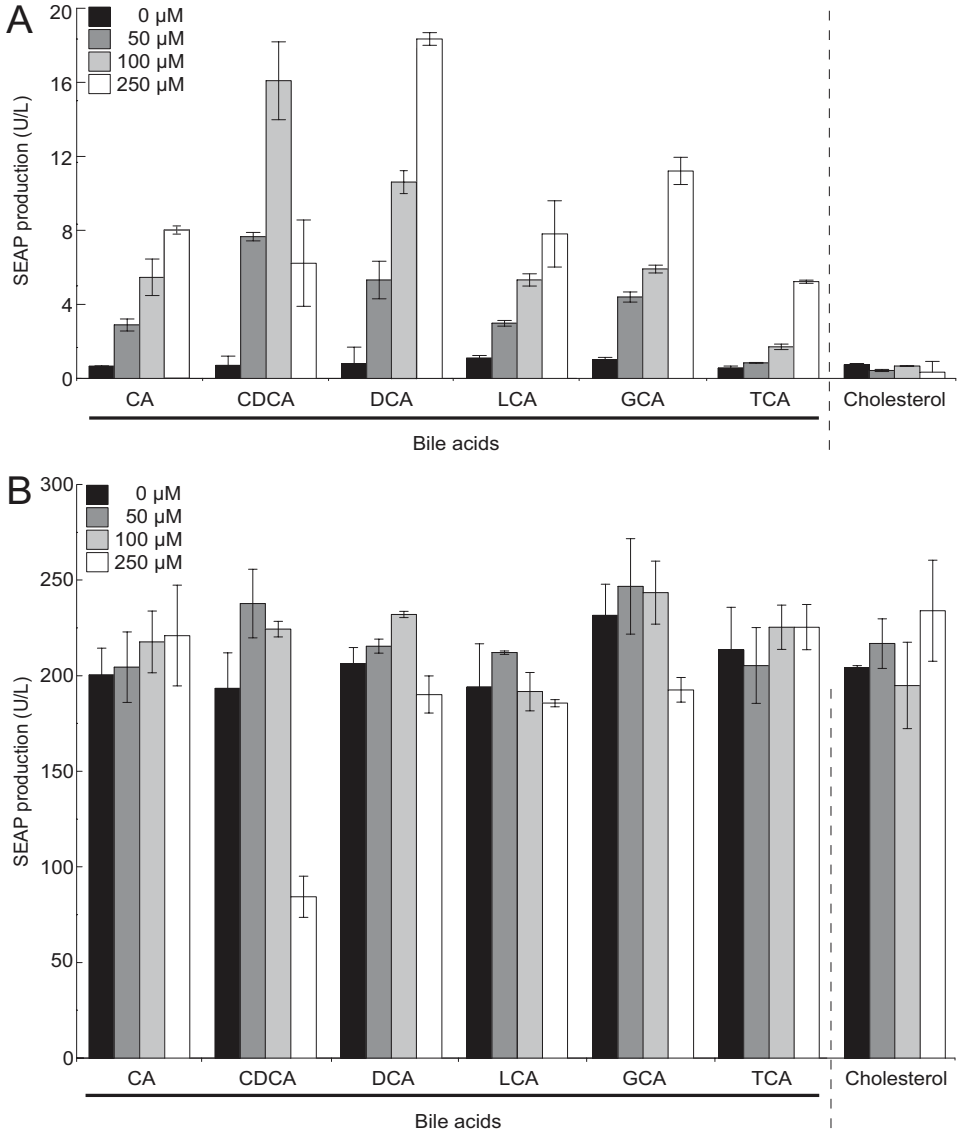

Fig. 4. Responsiveness of $BEAR_{ON}$ to cholesterol and different bile acid variants. HEK-293 cells co-transfected with (A) pKR69/pKR114 or (B) pSEAP2-control were cultivated for 48h in culture medium containing increasing concentrations of different bile acids as well as cholesterol before SEAP expression was profiled in the culture supernatant.

Validation of the $BEAR_{ON}$ gene switch in mice. To validate the $BEAR_{ON}$ device in vivo, we microencapsulated pKR69/pKR114-transgenic HEK-293 cells into coherent alginate-poly-L-lysine-alginate microcontainers (200 cells/capsule) and implanted them intraperitoneally into mice. The mice received daily injections of different doses of CA, CDCA, or DCA. SEAP levels in the bloodstream of treated animals were quantified 48h after implantation, and the measured levels confirmed bile acid-adjustable transgene expression *in vivo* (Fig. 5A-C). The low basal SEAP levels detected in the absence of bile acid treatment likely result from endogenous bile acids present in the animals' peripheral circulation. As observed in cell cultures, the $BEAR_{ON}$ device showed higher SEAP production levels in mice treated with CDCA and DCA compared to animals treated with CA

(Fig. 5A-C). Overall, performance validation in mice suggests that the $BEAR_{ON}$ device could be used as a precise and robust gene switch to control desired transgene expression by administering an endogenous trigger metabolite. This device may serve as a biosensor able to monitor the entire range of bile acids in a diagnostic setting, or be used in future cell-based therapy.

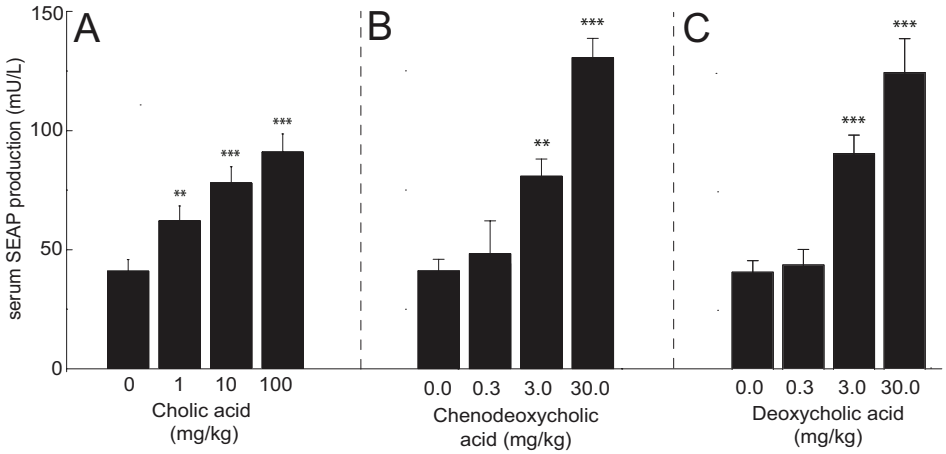

Fig. 5. Performance of bile acid-inducible transgene expression in mice. (A-C) Wild-type mice were implanted with microencapsulated pKR69-/pKR114-transgenic HEK-293 cells ($2x10^6$ cells/mouse) and received different daily doses of (A) cholic acid (0-100mg/kg), (B) chenodeoxycholic acid or (C) deoxycholic acid (0-30 mg/kg). Resulting SEAP levels were quantified in the bloodstream of treated animals after 48h. Data are mean ±SEM, statistics by two-tailed *t* test; *n*=8mice. $*P < 0.05$, $**P < 0.005$, $***P < 0.0001$ vs. zero bile acid dose.

Discussion

The co-evolution of pathogenic bacteria with their hosts has resulted in bacteria able to optimally coordinate nutrient supplies and synchronize their metabolism via a complex adaptive host-pathogen crosstalk (Lim, 2010; Benghezal et al., 2006). This host-pathogen crosstalk often involves compound-sensing repressors that continually adjust the pathogen's transcriptome to the changing microenvironment found inside the host (Lin et al., 2005b). As a consequence, most metabolite-inducible bacterial repressors are responsive to metabolite concentrations that are within the host's native or pathophysiological range (Thomas et al., 2008). Therefore, metabolite-inducible bacterial repressors have become a unique resource for the design of orthogonal transcription-control devices that express desired transgenes in response to metabolites (Hartenbach et al., 2007; Weber et al., 2009; Gitzinger et al., 2009a; Gitzinger et al., 2012) or act as biosensors that monitor pathological metabolites and program therapeutic responses (Kemmer et al., 2010). The repressor CmeR, which controls expression of the CmeABC multidrug efflux pump in *C. jejuni,* is a perfect candidate for use in a mammalian transcription control device. When fused to transactivating ($BEAR_{OFF}$) and transsilencing domains ($BEAR_{ON}$), the chimeric CmeR-based transcription factors specifically respond to bile acids and can be used to fine-tune the response of engineered target

promoters within the (patho-)physiological bile acid concentration range. Although the initial design already showed bile acid responsiveness in a variety of mammalian cell lines, there are several strategies that can be employed to improve the dynamic range of synthetic transcription controllers. We have used a simple sequential multi-step strategy to improve the overall performance in a specific cell line without resorting to protein engineering. The number of tandem operators in the chimeric promoter, the expression level of the transsilencer and the relative abundance of the transsilencer and its target expression unit all had a critical impact on the performance of the $BEAR_{ON}$ device in mammalian cells. Together with the choice of the cell line and a specific bile acid, these design parameters are ways in which devices can be modified to provide an optimal fit for a specific application. The optimized $BEAR_{ON}$ device showed reduced leakiness, high expression levels, robust induction kinetics, precise adjustability, and full reversibility. With these modifications, the $BEAR_{ON}$ gene switch also performed very well in mice, thus qualifying it for use in diagnostic or prosthetic networks employed to monitor and correct pathophysiologies associated with bile acid.

Continuous expansion of the portfolio of synthetic devices that control transgene expression in a metabolite-responsive manner is essential for the design of prosthetic networks that interface with the host metabolism to sense and correct metabolic disorders. The biosensor-based gene switches $BEAR_{OFF}$ and $BEAR_{ON}$ combine bile acid-specific sensor capacity with dose-dependent expression of a specific transgene. Capitalizing on a dramatically increasing toolbox of improved mammalian controller devices $BEAR_{OFF}$ and $BEAR_{ON}$ devices could also be combined with other gene switches to design higher-order gene networks such as oscillators (Elowitz and Leibler, 2000; Tigges et al., 2009; Tigges et al., 2010), genetic toggle switches (Gardner et al., 2000; Kramer et al., 2004), genetic counters (Friedland et al., 2009), synthetic hormone systems (Kemmer et al., 2011), biocomputers (Ausländer et al., 2012), T-cell expansion controllers (Culler et al., 2010) and cancer kill switches (Nissim and Bar-Ziv, 2010; Xie et al., 2011) that will likely become of high relevance for new treatment strategies of gene- and cell-based therapies.

Acknowledgments. We thank Barbara Geering as well as John and Claire Bendix for critical comments on the manuscript. This work was supported by an European Research Council (ERC) advanced grant (No. 321381) and in part by the Cantons of Basel and the Swiss Confederation within the INTERREG IV A.20 tri-national research program.

References

Ausländer, S., Ausländer, D., Müller, M., Wieland, M., Fussenegger, M., 2012. Programmable single-cell mammalian biocomputers. Nature. 487, 123-127.

Ausländer, S., Fussenegger, M., 2013. From gene switches to mammalian designer cells: present and future prospects. Trends Biotechnol. 31, 155-68.

Bacchus, W., Weber, W., Stelling, J., Fussenegger, M., 2013. Increasing the dynamic control space of mammalian transcription devices by combinatorial assembly of homologous regulatory elements from different bacterial species. Metab. Eng. 15, 144-150.

Bacchus, W., Lang, M., El-Baba, M. D., Weber, W., Stelling, J., Fussenegger, M., 2012. Synthetic two-way communication between mammalian cells. Nat. Biotechnol. 30, 991-996.

Bacq, Y., Myara, A., Brechot, M. C., Hamon, C., Studer, E., Trivin, F., Metman, E. H., 1995. Serum conjugated bile acid profile during intrahepatic cholestasis of pregnancy. J. Hepatol. 22, 66-70.

Baumgartel, K., Genoux, D., Welzl, H., Tweedie-Cullen, R. Y., Koshibu, K., Livingstone-Zatchej, M., Mamie, C., Mansuy, I. M., 2008. Control of the establishment of aversive memory by calcineurin and Zif268. Nat. Neurosci. 11, 572-578.

Benghezal, M., Fauvarque, M.-O., Tournebize, R., Froquet, R., Marchetti, A., Bergeret, E., Lardy, B., Klein, G., Sansonetti, P., Charette, S. J., Cosson, P., 2006. Specific host genes required for the killing of Klebsiella bacteria by phagocytes. Cell. Microbiol. 8, 139-148.

Cagliero, C., Maurel, M.-C., Cloeckaert, A., Payot, S., 2007. Regulation of the expression of the CmeABC efflux pump in Campylobacter jejuni: identification of a point mutation abolishing the binding of the CmeR repressor in an in vitro-selected multidrug-resistant mutant. FEMS Microbiol. Lett. 267, 89-94.

Culler, S. J., Hoff, K. G., Smolke, C. D., 2010. Reprogramming cellular behavior with RNA controllers responsive to endogenous proteins. Science 330, 1251-1255.

Deans, T. L., Cantor, C. R., Collins, J. J., 2007. A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells. Cell 130, 363-372.

Ehrbar, M., Schoenmakers, R., Christen, E. H., Fussenegger, M., Weber, W., 2008. Drug-sensing hydrogels for the inducible release of biopharmaceuticals. Nat. Mater. 7, 800-804.

Elowitz, M. B., Leibler, S., 2000. A synthetic oscillatory network of transcriptional regulators. Nature 403, 335-338.

Enhsen, A., Kramer, W., Wess, G., 1998. Bile acids in drug discovery. Drug Discovery Today 3, 409-418.

Friedland, A. E., Lu, T. K., Wang, X., Shi, D., Church, G., Collins, J. J., 2009. Synthetic gene networks that count. Science 324, 1199-1202.

Gardner, T. S., Cantor, C. R., Collins, J. J., 2000. Construction of a genetic toggle switch in Escherichia coli. Nature 403, 339-342.

Gitzinger, M., Kemmer, C., Fluri, D. A., El-Baba, M. D., Weber, W., Fussenegger, M., 2012. The food additive vanillic acid controls transgene expression in mammalian cells and mice. Nucleic Acids Res. 40, e37.

Gitzinger, M., Kemmer, C., El-Baba, M. D., Weber, W., Fussenegger, M., 2009a. Controlling transgene expression in subcutaneous implants using a skin lotion containing the apple metabolite phloretin. Proc. Nat. Acad. Sci. U.S.A. 106, 10638–10643.

Gitzinger, M., Parsons, J., Reski, R., Fussenegger, M., 2009b. Functional cross-kingdom conservation of mammalian and moss (Physcomitrella patens) transcription, translation and secretion machineries. Plant Biotechnol. J. 7, 73-86.

Glantz, A., Marschall, H., Mattsson, L., 2004. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. Hepatology 40, 467-474.

Greber, D., Fussenegger, M., 2007. Multi-gene engineering: simultaneous expression and knockdown of six genes off a single platform. Biotech. Bioeng. 96, 821-834.

Gu, R., Su, C., Shi, F., Li, M., McDermott, G., Zhang, Q., Yu, E., 2007. Crystal structure of the transcriptional regulator CmeR from Campylobacter jejuni. J. Mol. Biol. 372, 583-593.

Hartenbach, S., Daoud-El Baba, M., Weber, W., Fussenegger, M., 2007. An engineered L-arginine sensor of Chlamydia pneumoniae enables arginine-adjustable transcription control in mammalian cells and mice. Nucleic Acids Res. 35, e136.

Hofmann, A., 1999a. Bile acids: the good, the bad, and the ugly. Physiology 14, 24-29.

Hofmann, A. F., 1999b. The continuing importance of bile acids in liver and intestinal disease. Arch. Intern. Med. 159, 2647-2658.

Hofmann, A. F., 2009. Bile acids: Trying to understand their chemistry and biology with the hope of helping patients. Hepatology 49, 1403-1418.

Insull, W., Jr., 2006. Clinical utility of bile acid sequestrants in the treatment of dyslipidemia: a scientific review. South Med J. 99, 257-273.

Kemmer, C., Gitzinger, M., Daoud-El Baba, M., Djonov, V., Stelling, J., Fussenegger, M., 2010. Self-sufficient control of urate homeostasis in mice by a synthetic circuit. Nat. Biotechnol. 28, 355-360.

Kemmer, C., Gitzinger, M., Daoud-El Baba, M., Djonov, V., Stelling, J., Fussenegger, M., 2011. A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. J. Control. Realease 150, 23-29.

Klančnik, A., Možina, S. S., Zhang, Q., 2012. Anti-Campylobacter activities and resistance mechanisms of natural phenolic compounds in Campylobacter. PLoS ONE. 7, e51800.

Kramer, B. P., Viretta, A. U., Daoud-El Baba, M., Aubel, D., Weber, W., Fussenegger, M., 2004. An engineered epigenetic transgene switch in mammalian cells. Nat. Biotechnol. 22, 867-870.

Kurinčič, M., Klančnik, A., Smole Možina, S., 2012. Effects of efflux pump inhibitors on erythromycin, ciprofloxacin, and tetracycline resistance in Campylobacter spp. isolates. Microb Drug Resist. 18, 492-501.

Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., Staels, B., 2009. Role of bile acids and bile acid receptors in metabolic regulation. Physiol. Rev. 89, 147-191.

Lei, H.-T., Shen, Z., Surana, P., Routh, M. D., Su, C.-C., Zhang, Q., Yu, E. W., 2011. Crystal structures of CmeR-bile acid complexes from Campylobacter jejuni. Protein Sci. 20, 712-723.

Lim, W. A., 2010. Designing customized cell signalling circuits. Nat. Rev. Mol. Cell Biol. 11, 393-403.

Lin, J., Akiba, M., Sahin, O., Zhang, Q., 2005a. CmeR functions as a transcriptional repressor for the multidrug efflux pump CmeABC in Campylobacter jejuni. Antimicrob. Agents Chemother. 49, 1067-1075.

Lin, J., Cagliero, C., Guo, B., Barton, Y., Maurel, M., Payot, S., Zhang, Q., 2005b. Bile salts modulate expression of the CmeABC multidrug efflux pump in Campylobacter jejuni. J. Bacteriol. 187, 7417-7424.

Lin, J., Michel, L., Zhang, Q., 2002. CmeABC functions as a multidrug efflux system in Campylobacter jejuni. Antimicrob Agents Chemother. 46, 2124-2131.

Malleret, G., Haditsch, U., Genoux, D., Jones, M. W., Bliss, T. V., Vanhoose, A. M., Weitlauf, C., Kandel, E. R., Winder, D. G., Mansuy, I. M., 2001. Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell 104, 675-686.

Malphettes, L., Weber, C. C., El-Baba, M. D., Schoenmakers, R. G., Aubel, D., Weber, W., Fussenegger, M., 2005. A novel mammalian expression system derived from components coordinating nicotine degradation in arthrobacter nicotinovorans pAO1. Nucleic Acids Res. 33, e107.

Moosmann, P., Georgiev, O., Thiesen, H. J., Hagmann, M., Schaffner, W., 1997. Silencing of RNA polymerases II and III-dependent transcription by the KRAB protein domain of KOX1, a Kruppel-type zinc finger factor. Biol. Chem. 378, 669-677.

Neimark, E., Chen, F., Li, X., Shneider, B. L., 2004. Bile Acid-Induced Negative Feedback Regulation of the Human Ileal Bile acid Transporter. Hepatology 40, 49-156.

Nissim, L., Bar-Ziv, R. H., 2010. A tunable dual-promoter integrator for targeting of cancer cells. Mol. Syst. Biol. 6, 444.

Palmeira, C. M., Rolo, A. P., 2004. Mitochondrially-mediated toxicity of bile acids. Toxicology 203, 1-15.

Qin, J. Y., Zhang, L., Clift, K. L., Hulur, I., Xiang, A. P., Ren, B.-Z., Lahn, B. T., 2010. Systematic comparison of constitutive promoters and the doxycycline-inducible promoter. PLoS ONE 5, e10611.

Ridlon, J. M., 2005. Bile salt biotransformations by human intestinal bacteria. J Lipid Res. 47, 241-259.

Routh, M. D., Su, C.-C., Zhang, Q., Yu, E. W., 2009. Structures of AcrR and CmeR: Insight into the mechanisms of transcriptional repression and multi-drug recognition in the TetR family of regulators. Biochim. Biophys. Acta 1794, 844-851.

Schlatter, S., Rimann, M., Kelm, J., Fussenegger, M., 2002. SAMY, a novel mammalian reporter gene derived from Bacillus stearothermophilus alpha-amylase. Gene 282, 19-31.

Sharpless, N. E., Depinho, R. A., 2006. The mighty mouse: genetically engineered mouse models in cancer drug development. Nat. Rev. Drug Discov. 5, 741-754.

Staels, B., Fonseca, V. A., 2009. Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. Diabetes care. 32 Suppl 2, S237-245.

Thomas, C., Pellicciari, R., Pruzanski, M., Auwerx, J., Schoonjans, K., 2008. Targeting bile-acid signalling for metabolic diseases. Nat. Rev. Drug Discov. 7, 678-693.

Tigges, M., Denervaud, N., Greber, D., Stelling, J., Fussenegger, M., 2010. A synthetic low-frequency mammalian oscillator. Nucleic Acids Res. 38, 2702-2711.

Tigges, M., Marquez-Lago, T. T., Stelling, J., Fussenegger, M., 2009. A tunable synthetic mammalian oscillator. Nature 457, 309-312.

Triezenberg, S. J., Kingsbury, R. C., McKnight, S. L., 1988. Functional dissection of VP16, the trans-activator of herpes simplex virus immediate early gene expression. Genes Dev. 2, 718-729.

Ulmer, J. B., Valley, U., Rappuoli, R., 2006. Vaccine manufacturing: challenges and solutions. Nat. Biotechnol. 24, 1377-1383.

Urlinger, S., Helbl, V., Guthmann, J., Pook, E., Grimm, S., 2000. The p65 domain from NF-κB is an efficient human activator in the tetracycline-regulatable gene expression system. Gene 247, 103-110.

Weber, W., Bacchus, W., Daoud-El Baba, M., Fussenegger, M., 2007. Vitamin H-regulated transgene expression in mammalian cells. Nucleic Acids Res. 35, e116.

Weber, W., Fussenegger, M., 2006. Pharmacologic transgene control systems for gene therapy. J. Gene. Med. 8, 535-556.

Weber, W., Fussenegger, M., 2007. Inducible product gene expression technology tailored to bioprocess engineering. Curr. Opin. Biotechnol. 18, 399-410.

Weber, W., Fux, C., Daoud-El Baba, M., Keller, B., Weber, C. C., Kramer, B. P., Heinzen, C., Aubel, D., Bailey, J. E., Fussenegger, M., 2002a. Macrolide-based transgene control in mammalian cells and mice. Nat. Biotechnol. 20, 901-907.

Weber, W., Lienhart, C., Baba, M. D.-E., Fussenegger, M., 2009. A biotin-triggered genetic switch in mammalian cells and mice. Metab. Eng. 11, 117-124.

Weber, W., Schoenmakers, R., Keller, B., Gitzinger, M., Grau, T., Daoud-El Baba, M., Sander, P., Fussenegger, M., 2008. A synthetic mammalian gene circuit reveals antituberculosis compounds. Proc. Nat. Acad. Sci. U.S.A. 105, 9994-9998.

Wurm, F. M., 2004. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat. Biotechnol. 22, 1393-1398.

Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R., Benenson, Y., 2011. Multi-input RNAi-based logic circuit for identification of specific cancer cells. Science 333, 1307-1311.

Ye, H., Daoud-El Baba, M., Peng, R.-W., Fussenegger, M., 2011. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. Science 332, 1565-1568.

Ye, H., Hamri, G. C.-E., Zwicky, K., Christen, M., Folcher, M., Fussenegger, M., 2013. Pharmaceutically controlled designer circuit for the treatment of the metabolic syndrome. Proc. Nat. Acad. Sci. U.S.A. 110, 141-146.

Young, K., Davis, L., DiRita, V., 2007. Campylobacter jejuni: molecular biology and pathogenesis. Nat. Rev. Microbiol. 5, 665-679.

CHAPTER III

Reward-based hypertension control by a synthetic brain-dopamine interface

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Proceedings of the National Academy of Science USA. 2013, 110:18150-18155.

Abstract

Synthetic biology has significantly advanced the design of synthetic trigger-controlled devices that can reprogram mammalian cells to interface with complex metabolic activities. In the brain, the neurotransmitter dopamine coordinates communication with target neurons via a set of dopamine receptors that control behavior associated with reward-driven learning. This dopamine transmission has recently been suggested to increase central sympathetic outflow resulting in plasma dopamine levels that correlate with corresponding brain activities. By functionally rewiring the human dopamine receptor 1 (DRD1) via the second messenger cyclic adenosine monophosphate (cAMP) to synthetic promoters containing cAMP-response element-binding protein 1 (CREB1)-specific cAMP-responsive operator modules, we have designed a synthetic dopamine-sensitive transcription controller that reversibly fine-tunes specific target gene expression at physiologically relevant brain-derived plasma dopamine levels. Following implantation of circuit-transgenic human cell lines insulated by semi-permeable immunoprotective microcontainers into mice, the designer device interfaced with dopamine-specific brain activities and produced a systemic expression response when the animals' reward system was stimulated by either food, sexual arousal or addictive drugs. Reward-triggered brain activities were able to remotely program peripheral therapeutic implants to produce sufficient amounts of the atrial natriuretic peptide, which reduced the blood pressure of hypertensive mice to the normal physiologic range. Seamless control of therapeutic transgenes by subconscious behavior may provide novel opportunities for the treatment strategies of the future.

Significance

Essential activities such as feeding and reproduction as well as social, emotional and mental behavior is reinforced by the brain's reward system. Pleasure status directly correlates with the dopamine levels released in the brain. Because dopamine leaks into the bloodstream via the sympathetic nervous system, brain and blood dopamine levels are interrelated. We designed a synthetic dopamine sensor-effector device that enables engineered human cells, insulated by immunoprotective microcontainers and implanted into the abdomen of mice, to monitor blood dopamine levels and drive dopamine-dependent secretion of product proteins in pleasure situations associated with palatable food, drugs or sexual arousal. Hypertensive animals treated with this device, which produces a clinically licensed antihypertensive peptide, had their high blood pressure corrected when exposed to sexual arousal.

The constant iteration between memory, providing consolidated information on past experience, and reward, confirming success of current activities, represents the central learning cycle of higher organisms that programs essential processes such as drinking, eating and reproduction and reinforces complex somatic, social, emotional, mental and cognitive behavior (1-4). The neurotransmitter dopamine is a key endogenous molecule that coordinates memory consolidation with learning, controls emotions and motivates individuals to perform reward- and pleasure-seeking behavior, excel and improve fitness (4-6). The dopaminergic reward system is finely tuned and major imbalances have severe pathologic consequences. Excessive reward-seeking behavior may lead to obesity (eating), addiction (alcohol, nicotine), drug abuse (cocaine, methylamphetamine), schizophrenia or depression, whereas decreased dopamine availability has been associated with Parkinson's disease (6-11). Although the precise dynamics of memory/learning processing remains a little-understood process it involves dopaminergic neurons of the mesolimbic pathway that release the neurotransmitter into the presynaptic space in response to a motivation-related action potential (12). Dopamine then binds and activates dopamine receptors, such as the dopamine receptor D1 (DRD1), a G protein-coupled receptor (GPCR) that modulates the cyclic adenosine monophosphate (cAMP) second messenger to produce a cellular response ultimately triggering an action potential by opening plasmalemmal ion channels (13,14). Besides the brain, dopamine receptors are also expressed in peripheral tissues such as kidney, adrenal glands or blood vessels and serum dopamine has been found to regulate respiration, and glucose homeostasis, suggesting that central and peripheral actions of dopamine converge to mediate homeostasis in central metabolic pathways (15-17). For example, palatable food stimuli engage dopamine in the reward circuits and increase dopamine levels in circulation where it regulates pancreatic endocrine function including insulin release and modulation of insulin impact on adipocytes (17, 18). Because the liver removes and metabolizes virtually all catecholamines including dopamine delivered from the gut via the portal vein and dopamine does not cross the blood-brain barrier, peripheral dopamine in the blood is predominantly derived from networks of sympathetic nerves enmeshing blood vessels throughout the body (17, 19-21). Because sympathetic neurons synthesize norepinephrine from dopamine in rapid-release vesicles containing the dopamine-β-hydroxylase unconverted neurotransmitter may reach the bloodstream at concentrations that correlate with the reward system in the brain (20, 22).

To demonstrate a possible correlation of dopamine levels between the central and peripheral systems we have created designer cells containing a synthetic dopamine-responsive sensor-effector device that taps into the peripheral dopaminergic network and can be controlled by motivational

stimuli such as food, addictive drugs and sexual arousal, which are known to trigger the central reward system.

Results

Design and Functionality of a Synthetic Mammalian Reward-based Biosensor. The dopamine sensor device was designed by ectopically expressing the human dopamine receptor D1 in mammalian cells and rewiring its native signaling cascade involving $G_{s\alpha}$ proteins via the second messenger cAMP to cAMP-responsive protein kinase A (PKA)-mediated phospho-activation of cAMP response element-binding protein (CREB1) that triggers transcription from synthetic promoters containing CREB1-specific cAMP response elements (CRE) (Fig. 1*A*). Co-transfection of several rodent, primate and human cell lines with the constitutive human dopamine receptor D1 expression vector pDRD1 (P_{hCMV}-DRD1-pA) (Table S1) and the DRD1-dependent P_{CRE}-driven human placental secreted alkaline phosphatase (SEAP) reporter construct pCK53 (P_{CRE}-SEAP-pA) (23) showed that SEAP was exclusively induced whenever the cells were exposed to the neurotransmitter dopamine which confirms the broad applicability as a mammalian gene switch (Fig. 1*B*). Possible differences in availability and compatibility of endogenous signal-transduction components with dopamine-triggered DRD1-mediated input may in part explain the differences in basal expression and induction profiles among different cell lines (Fig. 1*B*). Despite capitalizing on the endogenous cAMP signaling pathway to achieve a most minimalistic all-human design, basal transcription remains very low in the absence of dopamine, which indicates that pleiotropic input into this endogenous signaling cascade is insignificant under standard culture conditions (Fig. 1*B*). The human dopamine-sensor device combining human DRD1 and human HEK-293 equipped with a synthetic human signaling cascade and transgene showed the best dopamine-triggered transgene expression profile and was therefore used in all follow-up studies. A basic set of control experiments confirmed (i) that HEK-293 express endogenous DRD1 (24) but that these levels were insufficient to drive significant dopamine-responsive P_{CRE} activation (Fig. 1*B* and Fig. S1*A*), (ii) that neither ectopic expression of DRD1 nor exposure of cells to excessive dopamine concentrations had a negative impact on HEK-293 viability (Fig. S1*B*) and protein production capacity (Fig. S1*C*), (iii) that DRD1 was localized in the cell membrane and activated by dopamine (Fig. S1*D* and *E*) and (iv) that the dopamine-triggered DRD1-mediated signaling was inactivated by the DRD1 antagonist and antipsychotic drug Fluphenazine (Fig. S1*F*) and the PKA inhibitor H-89 (Fig. S1*G*).

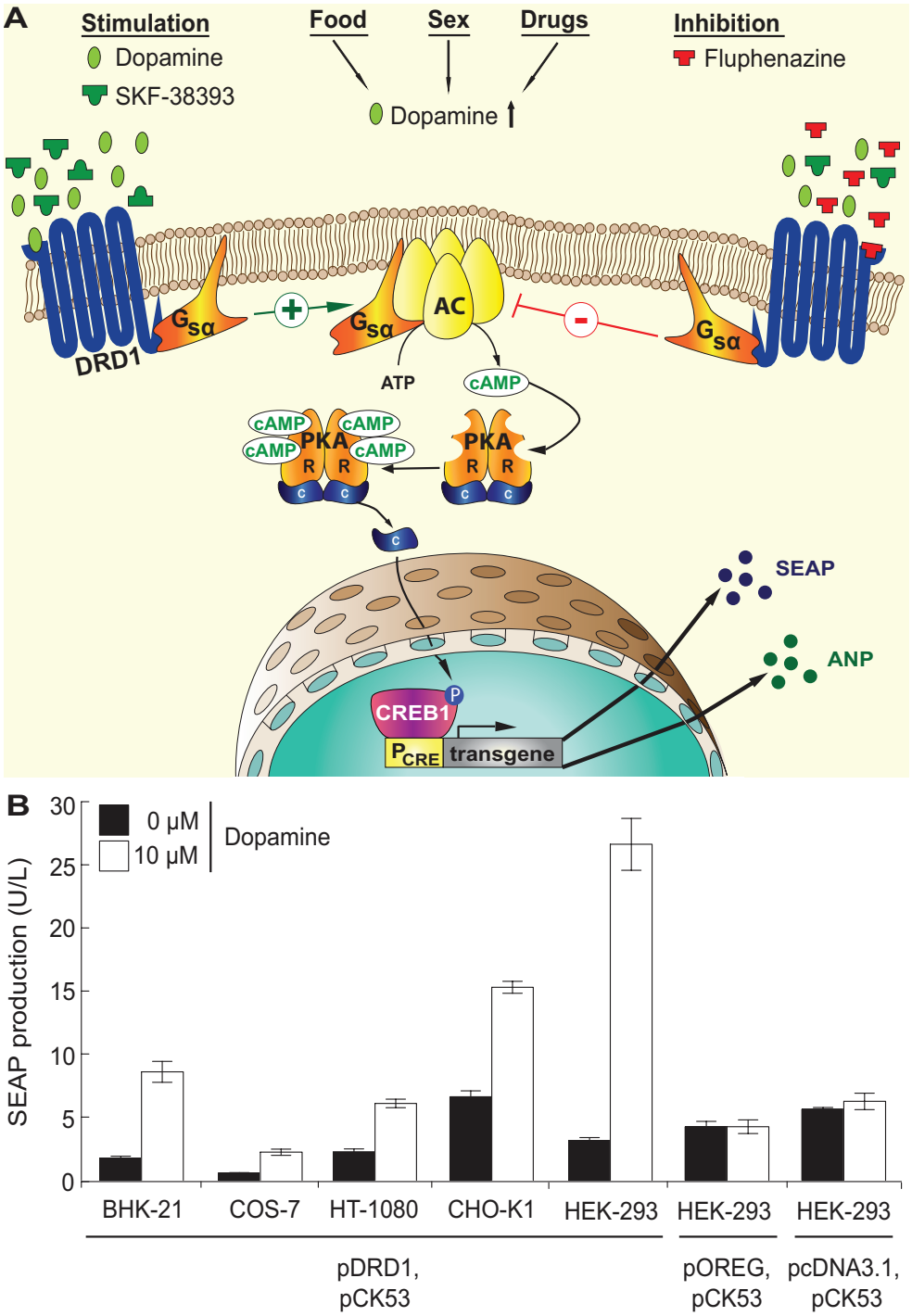

Fig. 1. Design and functionality of the synthetic dopamine-triggered signaling cascade. (**A**) Binding of dopamine to ectopically expressed human dopamine receptor 1 (DRD1) triggers the G protein $G_{s\alpha}$ to stimulate the adenylyl cyclase (AC) which catalyzes conversion of ATP to cyclic AMP (cAMP). This surge in the cAMP second messenger pool activates the protein kinase A (PKA) whose subunit C subsequently dissociates and translocates into the nucleus where it activates the cAMP-responsive element binding protein 1 (CREB1) by phosphorylation. Activated CREB1 binds to a synthetic promoter (P_{CRE}) containing consensus cAMP-response elements and triggers specific transgene expression. Rewiring of heterologous human DRD1 via endogenous control components to a chimeric promoter in a human cell produces an all-human synthetic signaling cascade that senses dopamine as well as related inducing (SKF-38393) and inhibitory (Fluphenazine) compounds and converts a transient trigger input to a sustained transcription response. (**B**) Dopamine-responsive expression of the human glycoprotein SEAP (placental secreted alkaline

phosphatase expression) in different mammalian cell lines (co-)transfected (1:1 ratio) with constitutive DRD1 (pKR22) and P_{CRE}-driven SEAP (pCK53) expression vectors and cultivated for 48h in the presence (10μM) or absence of the neurotransmitter dopamine. The parental vector (pcDNA3.1) or an isogenic expression plasmid encoding an unrelated GPCR (pOREG) were (co-)transfected with pCK53 and served as controls. Data are mean ± SD; n=4 independent experiments.

Characterization and Specificity of the Reward-based Biosensor. Sequential stable transfection and clonal selection of HEK-293 with pDRD1 (HEK_{DRD1}) and pCK53 resulted in a double-transgenic cell line HEK_{REWARD} that ectopically expresses DRD1 and SEAP in a dopamine-inducible manner. With a sensitivity as low as 1nM dopamine and maximum dopamine-triggered SEAP production up to 150-fold above basal expression the HEK_{REWARD} exhibited unprecedented dopamine responsiveness in the human-physiologic range [1ng/mL=6.5nM; (19)] (Fig. 2*A*), while showing excellent neurotransmitter dose-dependent adjustability (Fig. 2*A*), reversibility (Fig. 2*B*) and time-programmable induction kinetics (Fig. 2*C*). To confirm the specificity of the dopamine-sensor device we have exposed HEK_{REWARD} to compounds that are metabolically related to dopamine such as its precursor L-Dopa (Levodopa), its breakdown products Dopac and homovanillic acid as well as its hormone derivatives norepinephrine and epinephrine. Unlike dopamine and the DRD1-agonist SKF-38393, none of the metabolically related small molecules were able to trigger DRD1-driven SEAP expression, confirming the specificity of the dopamine-sensor device (Fig 2*D*). Also, HEK-293 cells express the human beta-2-adrenergic (25) and adenosine A_{2B} receptors (26), both of which activate the same G_s-protein-coupled cAMP-signaling cascade as the synthetic dopamine sensor device when triggered by (nor-)epinephrine and adenosine, respectively. However, neither of those unrelated GPCRs were able to activate P_{CRE}-driven SEAP expression at physiologic inducer concentrations (epinephrine/norepinephrine, 0-100nM; adenosine, 0-2 μM; Fig. S2).

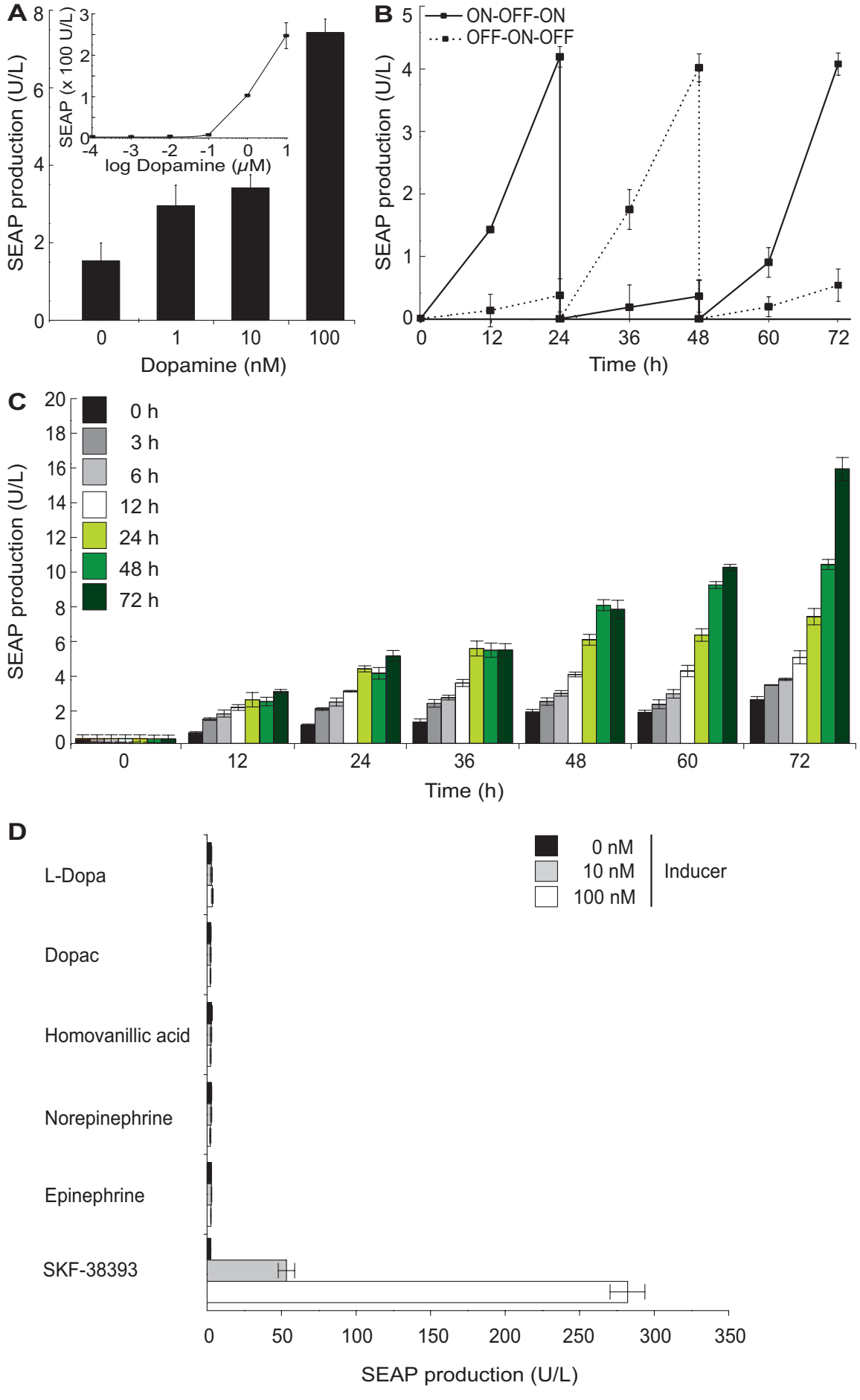
A
SEAP production (U/L)
Dopamine (nM)
SEAP (x 100 U/L)
log Dopamine (μM)
B
ON-OFF-ON
OFF-ON-OFF
SEAP production (U/L)
Time (h)
C
0 h
3 h
6 h
12 h
24 h
48 h
72 h
SEAP production (U/L)
Time (h)
D
L-Dopa
Dopac
Homovanillic acid
Norepinephrine
Epinephrine
SKF-38393
0 nM
10 nM
100 nM
Inducer
SEAP production (U/L)

Fig. 2. Characterization of the clonal HEK_{REWARD} cell line stably transgenic for dopamine-responsive SEAP expression. (**A**) Dose-dependent SEAP production profile of HEK_{REWARD} cultivated for 48h in the presence of increasing dopamine concentrations. (**B**) Reversibility of dopamine-responsive SEAP expression was assessed by cultivating $2.5x10^6$ HEK_{REWARD} for 72h while alternating the dopamine status of the culture (100nM, ON; 0nM, OFF) every 24h. (**C**) SEAP expression kinetics of HEK_{REWARD} exposed for different periods of time (color code) to 100nM dopamine. (**D**) Specificity of the dopamine-sensor circuit. HEK_{REWARD} were exposed for 48h to various concentrations of different dopamine-related compounds and corresponding SEAP production was profiled in the culture supernatant after 48h. Data are mean ± SD; n=4 independent experiments.

Dopamine- and Reward-based Transgene expression in Wild-Type Mice. Covering a wide range of dopamine concentrations with high sensitivity, the dopamine-sensor device provides a way to precisely score peripheral physiologic dopamine levels. To validate dopamine-triggered transgene expression in vivo, we implanted wild-type mice with HEK_{REWARD}, microencapsulated into clinically licensed alginate-poly-L-lysine-alginate capsules with a molecular cut-off of 72kDa (27) to insulate the sensor device from the mouse physiology and its immune system and treated the animals with injections of diverse concentrations of dopamine or the synthetic DRD1 agonist SKF-38393. The SEAP levels profiled in the bloodstream of treated animals confirmed trigger dose-dependent transgene expression in their peripheral circulation (Fig. 3*A* and *B*). Control treatment groups implanted with pCK53-engineered HEK-293 showed no variations of basal SEAP levels in the blood corroborating that HEK-293's endogenous DRD1 expression was insufficient and that ectopic DRD1 production was required to mediate dopamine responsiveness in vivo (Fig. 3*A* and *B*).

In the absence of a transfer across the blood-brain barrier and liver-based removal of catecholamines delivered from the gut via the portal vein, plasma dopamine was found to originate from the brain and be co-released as precursor of norepinephrine via sympathetic nerves enmeshing blood vessels throughout the body (17, 20). To test the potential direct correlation between brain activities and peripheral dopamine levels we subjected wild-type mice containing HEK_{REWARD} implants to different conditions that are known to stimulate the mammalian reward system by triggering the release of the neurotransmitter dopamine and profiled the resulting SEAP levels in the peripheral circulation. Sex, drugs and food, the three major mammalian reward stimuli, all activated the implanted dopamine-sensor device and resulted in increased SEAP levels in the peripheral circulation (Fig. 3*C*). Sexual arousal was stimulated by transferring a wild-type female mouse to a group of male mice containing a dopamine-sensor implant. The blood SEAP levels of the male mice were significantly higher compared to the same male mice kept without female company suggesting that SEAP expression was indeed triggered by sexual arousal controlling the

reward system rather than by male rivalry. Likewise, when female mice with dopamine sensor implants were offered water-diluted sugar syrup in an all-you-can-drink condition their blood SEAP levels were significantly higher compared to an identical treatment group kept on a standard water-drinking scheme. Interestingly, the higher the syrup content of the drinking water was the more SEAP could be detected in the blood of the mice, indicating that the animal's reward system could be dose-dependently stimulated by the quality of the food (Fig. 3*C*). Because drug addiction is linked with the stimulation of the mammalian reward system, we tested the impact of methamphetamine a major drug of abuse that has a long clinical history and a similar physiologic effect as today's lifestyle drug cocaine (28, 29). Administration of methamphetamine to mice containing dopamine-sensor implants resulted in dose-dependent increase in blood SEAP levels. In all situations, triggering the reward system – sex, drugs and food – the dopamine-sensor device was able to capture changes in peripheral dopamine levels and coordinate a dose-dependent response that precisely adjusted SEAP expression to reach desired levels in the bloodstream of the animals. In all reward situations the stimulated SEAP levels correlated with blood dopamine (Fig. 3*D*) and blood norepinephrine (Fig. 3*E*) levels suggesting that reward-relevant dopamine levels seem to replicate in the peripheral circulation and that this neuronal-peripheral dopamine balance is mediated via sympathetic neurons. Indeed, when blocking the activity of sympathetic nerves by the ganglionic blocker pentolinium tartrate in mice implanted with the dopamine-sensor device and treated with methamphetamine, SEAP, dopamine and norepinephrine were equally decreased to basal levels reached in the absence of the drug treatment (Fig. 3*C*-*E*).

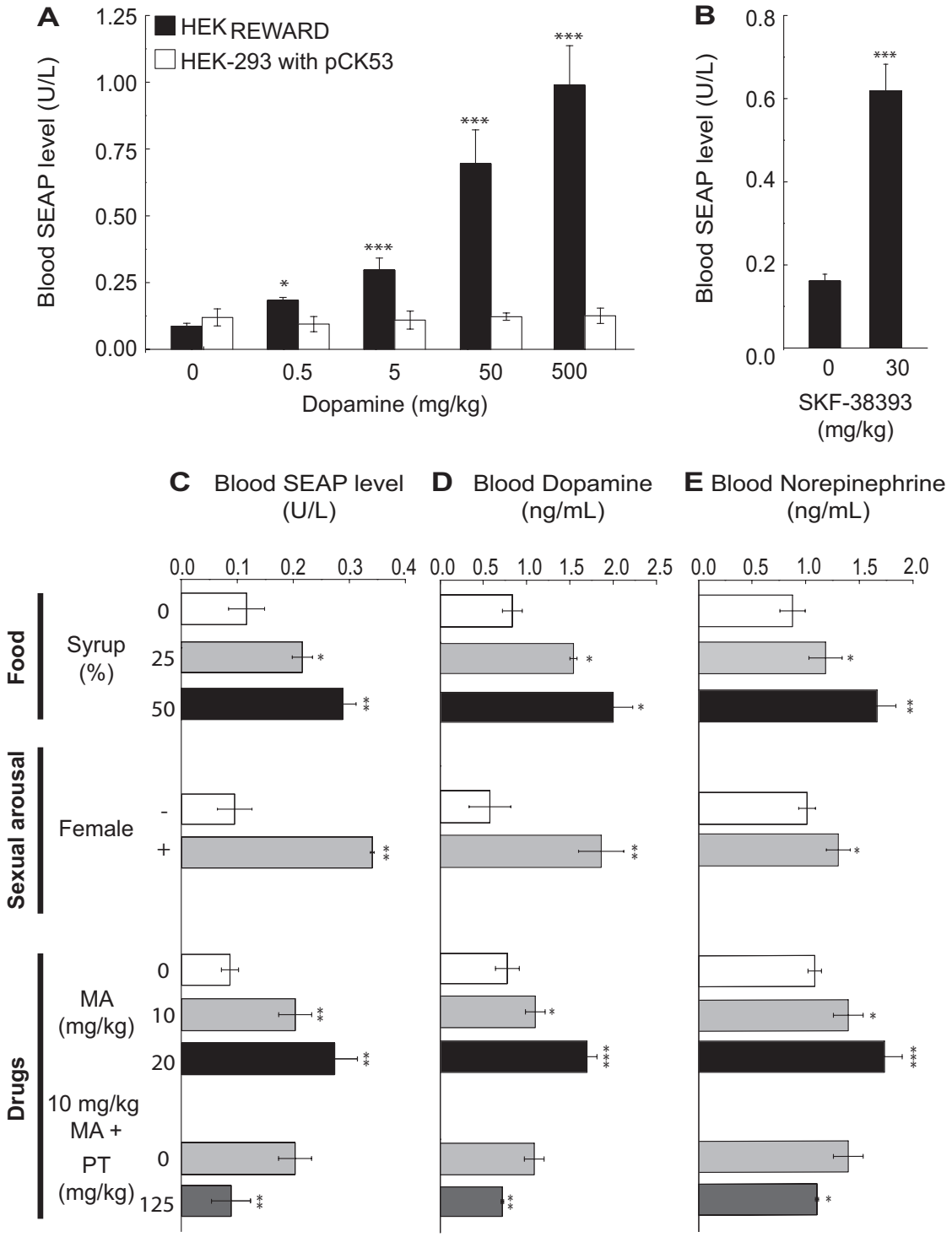

Fig. 3. Dopamine and reward-controlled transgene expression in mice. **(A, B)** Dopamine-responsive SEAP expression fine-tuning in mice. Wild-type mice implanted with microencapsulated HEK_{REWARD}, HEK-293 transfected with pCK53 (P_{CRE}-SEAP-pA) or non-engineered parental HEK-293 cells ($2x10^6$ cells/mouse) were injected with different doses of dopamine (**A**) or the synthetic DRD1 agonist SKF-38393 (**B**) and corresponding blood SEAP levels were profiled after 48h. (**C to E**) Reward-controlled SEAP expression in mice. Wild-type mice implanted with microencapsulated HEK_{REWARD} ($2x10^6$ cells/mouse) were exposed to different reward conditions including food (syrup/water mixtures), sexual arousal (treated male mice with (+) and without (-) female mouse company) and the addiction drug methamphetamine (MA) that was optionally co-administered with the ganglionic blocker pentolinium tartate (PT). SEAP **(C)**, dopamine **(D)** and norepinephrine **(E)**

levels were profiled in the blood of treated animals after 48h. Data are mean ± SEM; statistics by two-tailed t test; n = 8 mice. *P < 0.05, **P < 0.005, *** P < 0.0001.

Reward-based Attenuation of High blood Pressure in hypertensive Mice. To confirm dopamine-based reward-triggered transgene expression in a prototype therapeutic context we chose to link the synthetic dopamine-sensor device to expression of the half life-optimized (30) atrial natriuretic peptide (ANP; pKR147, P_{CRE}-ANP-pA), a powerful vasodilator attenuating high blood pressure (31-33). After validation of dopamine-triggered ANP expression in vitro (Fig. S3) we implanted microencapsulated pKR147-transgenic HEK_{DRD1} into male mice serving as an animal model for hypertension. When hypertensive male animals containing an implanted dopamine sensor-controlled ANP production device had their reward system triggered by sexual arousal mediated by female company, they showed significant increases in serum ANP (Fig. 4*A*), dopamine (Fig. 4*B*) and norepinephrine (Fig. 4*C*) levels compared to an equally treated male-only community. As a consequence of sexual arousal-triggered ANP expression, the hypertonic systolic blood pressure of treated animals reversed to normal levels matching those observed for dopamine treatment (positive control), whereas the blood pressure of isolated male groups and the treatment groups implanted with non-engineered parental cells (negative control) continued to show severe hypertonia (Fig. 4*D*).

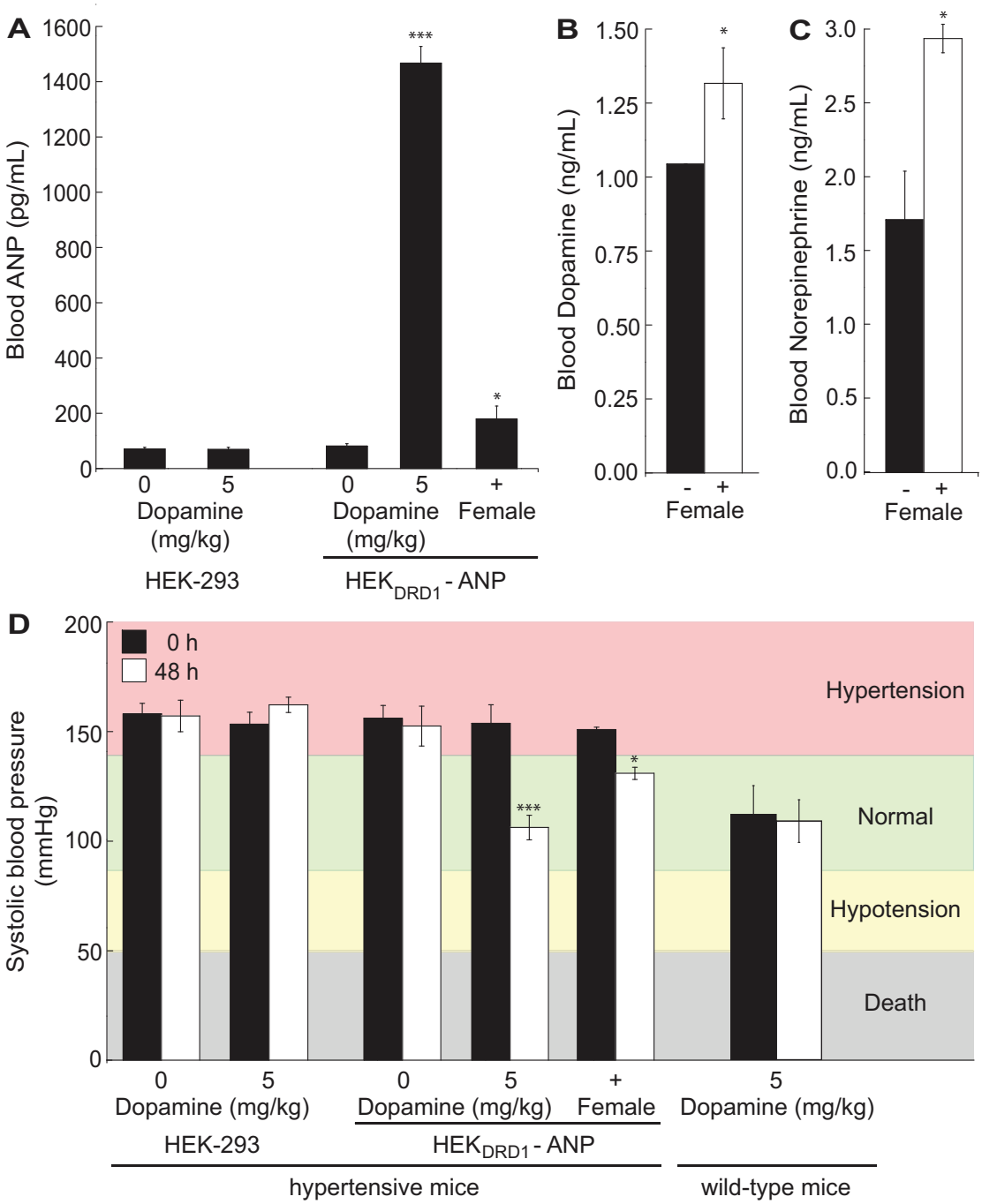

Fig. 4. Reward-controlled expression of the atrial natriuretic peptide (ANP) in hypertensive mice. (**A** to **C**) Hypertensive mice were implanted with pKR147 (P_{CRE}-ANP-pA)-transgenic HEK_{DRD1} (HEK_{DRD1}-ANP) or non-engineered parental HEK-293 and either treated with dopamine injections or exposed to sexual arousal (male with (+) female company). ANP (**A**), dopamine (**B**) and norepinephrine (**C**) levels were profiled in the blood of treated animals and their systolic blood pressure was scored (**D**) after 48h and compared to the blood pressure of wild-type mice. Data are mean ± SEM; statistics by two-tailed t test; n = 8 mice. *P < 0.05, **P < 0.005, *** P < 0.0001.

Discussion

Natural rewards, such as food and sexual arousal that can also be simulated by addictive drugs trigger a dopamine release in the brain (1, 3), which increases the central sympathetic outflow and results in accumulation of the neurotransmitter in the peripheral circulation (21). Capitalizing on this correlation between reward-related brain activities and dopamine levels in the blood we have designed a synthetic dopamine sensor-effector device that interfaces with peripheral dopamine, remotely wires into reward-associated behavior and converts transient reward stimuli into a dose-dependent systemic expression response. When operating in implanted designer cells self-connecting to the peripheral circulation (34-39) and insulated from the host immune system inside semi-permeable microcapsules (27), the synthetic dopamine sensor-effector device functionally interfaced with reward-associated brain activities and successfully managed antihypertensive treatment in an animal model by coordinating expression of the clinically licensed atrial natriuretic protein. Synthetic interfaces between brain activities and peripheral circulation may enable therapeutic interventions to be programmed by the autonomic nervous system and its quick-response sympathetic branch to deliver instantaneous therapeutic impact in acute situations. Also, functional interconnection of recurrent behavioral activities with production and systemic delivery of protein therapeutics will likely foster novel gene- and cell-based treatment strategies that require seamless and self-sufficient drug dosing at regular intervals. With the advent of personalized medicine, it may now be foreseen that autologous designer cells that are implanted into humans inside vascularizing semi-permeable microcontainers could be remote-controlled by brain activities to produce and systemically deliver protein therapeutics at the right time and dose (40-43). Provided that such designer cell implants are scalable to the patients' needs, could be replaced by ambulant interventions at patient-compliant intervals when becoming fibrotic, such brain-instructed peripheral designer cell implants could make the taking of pills in specified amounts and in precise doses a thing of the past (43-46).

Materials and Methods

Dopamine Sensor-Effector Components. Comprehensive design and construction details for all expression vectors are provided in supplementary **Table S1**. Key plasmids include: **pDRD1**, encodes constitutive expression of the human dopamine receptor D1 (DRD1; P_{hCMV}-DRD1-pA). **pCK53**, harbors a SEAP expression unit driven by a synthetic promoter (P_{CRE}) containing a CREB1 (cAMP response element-biding protein) -dependent cAMP-response element (CRE) [(P_{CRE}-SEAP-pA (23); GenBank no. KF528989]. **pKR147**, manages P_{CRE}-driven expression of a modified mouse atrial natriuretic peptide (P_{CRE}-ANP-pA; GenBank no. KF528988).

Cell culture, transfection. Human embryonic kidney cells (HEK-293, ATCC: CRL-11268), Baby hamster kidney cells (BHK-21, ATCC: CCL-10), African green monkey kidney cells (COS-7, ATCC: CRL-1651), and human fibrosarcoma cells (HT-1080, ATCC: CCL-121) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Basel, Switzerland) supplemented with 10% (v/v) fetal calf serum (FCS, PAN Biotech GmbH, Aidenbach, Germany, lot no. P251110) and 1% (v/v) penicillin/streptomycin solution (PAN Biotech). Wild-type Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultured in ChoMaster® HTS (Cell Culture Technologies GmbH, Gravesano, Switzerland) supplemented with 5% (v/v) FCS and 1% penicillin/streptomycin solution. All cell types were cultivated at 37°C in a humidified atmosphere containing 5% CO_2. For (co-)transfection of HEK-293 and CHO-K1, 60,000 cells seeded per well of a 24-well plate 12h prior to transfection were incubated for 4h with a DNA-$Ca_3(PO_4)_2$ precipitate (0.6µg total DNA; co-transfection, 0.5µg of receptor-encoding vector and 0.1µg of reporter plasmid), subjected to a glycerol shock (CHO-K1 only; 30s in ChoMaster® HTS containing 15% glycerol) and cultivated for indicated periods of time in 0.4mL DMEM or ChoMaster® HTS containing different concentrations of trigger compounds (37). Likewise, BHK-21, COS-7 and HT-1080 were (co-)transfected overnight using FuGENE® 6 (Roche Diagnostics AG, Basel, Switzerland).

Design and characterization of stable transgenic cell lines. HEK_{DRD1}, the stable transgenic HEK-293-derived cell line constitutively expressing the human dopamine receptor D1 (DRD1) was designed by (co-)transfection of pDRD1 (P_{hCMV}-DRD1-pA; 1.5µg) and pZeoSV2 (0.1µg), followed by selection of a polyclonal population for 10 days in DMEM containing 200µg/mL (w/v) zeocin (Invitrogen, San Diego, CA, USA), FACS-mediated single-cell cloning and clonal expansion. Stable transgenic cell clones were transfected with pCK53 (P_{CRE}-SEAP-pA) and profiled for dopamine responsiveness. The best-in-class cell line HEK_{DRD1} (cell clone no. 18 out of five randomly picked clones) was chosen for further experiments. The stable double-transgenic cell line HEK_{REWARD} (cell clone no. 12 out of five randomly picked clones) was constructed by co-transfecting HEK_{DRD1} with pCK53 (P_{CRE}-SEAP; 0.5µg) and pPUR (2.5µg), followed by selection

of a polyclonal population for 10 days in DMEM containing 200µg/mL (w/v) zeocin and 0.5µg/mL (w/v) puromycin (InvivoGen, San Diego, CA, USA), FACS-mediated single-cell cloning and clonal expansion. Dopamine-responsiveness of individual clones was profiled and HEK_{REWARD} was chosen for further experiments. For characterization of transgene induction kinetics and adjustability HEK_{REWARD} (120.000 cells/mL) were cultivated for up to 72h in DMEM containing different dopamine concentrations while continuously scoring SEAP expression levels. The reversibility of the dopamine control switch was validated by cultivating HEK_{REWARD} (120.000 cells/mL) for 72h while alternating the dopamine status of the culture (100nM, ON; 0nM, OFF) every 24h.

Analytical assays. **SEAP:** Human placental secreted alkaline phosphatase (SEAP) levels were profiled (i) in cell culture supernatants using a standard p-nitrophenylphosphate-based light absorbance time course (47) and (ii) in blood samples using a chemiluminescence-based assay (Roche Applied Science).

Norepinephrine and dopamine: Blood levels of these catecholamines were quantified using a TriCAT ELISA (IBL International GmbH, Hamburg, Germany).

ANP: Atrial natriuretic peptide was quantified (i) in cell culture supernatants via its Fc fusion tag using a mouse IgG ELISA (Immunology Consultant Laboratory, Inc., Portland, USA, cat. no. E90-G) and (ii) in blood samples using an ANP ELISA kit (Abcam, Cambridge, UK).

Semiquantitative RT-PCR: 1µg of total cellular RNA, isolated from HEK-293 and HEK_{DRD1} using the ZR RNA MicroPrep™ kit (Zymo Research Corporation, Irvine, CA), was reverse transcribed using SuperScript® II Reverse Transcriptase **(**Life Technologies, Carlsbad, USA). Specific RNA levels were quantified with the Mastercycler ep gradient/S system (Vaudaux-Eppendorf, Basel, Switzerland) and primers specific for human dopamine receptor D1 (OKR75, 5'-CCAGCCTCAGCAGGACATATG-3'; OKR76, 5'-CTTTCCGGTTGAGAACATTCG-3'; target size: 211bp) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (OKR500, 5'-CCATCACCATCTTCCAGGAG-3'; OKR501, 5'-CCTGCTTCACCACCTTCTTG-3').

Fluorescence microscopy: EGFP expression of pKR43-transfected HEK-293 was visualized using a Leica TCS-SP5 confocal microscope (Leica, Microsystems, Heerbrugg, Switzerland) equipped with a HCX PL APO 40.0x0,85 Dry objective, a 488 nm/509 nm laser for excitation, a 500/600 nm emission filter set and Leica Application Suite software installed (version 2.6.0.7266).

Viability assay: Individually treated non-transfected and pDRD1-transfected HEK-293 cells were washed and resuspend in PBS before mixing with Trypan Blue solution to determine viable cells using a hemacytometer.

Chemical compounds. Pentolinium tartrate (12.5g/L stock solution in water; Thermo Fisher Scientific Inc., Reinach, Switzerland). Adenosine, L-DOPA, DOPAC, the antipsychotic drug Fluphenazine, the PKA inhibitor H-89, homovanillic acid, epinephrine, norepinephrine (all 10mM stock solution in water; all Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), dopamine (*in vitro*, 10mM; *in vivo*, 8.3g/L stock solution in water), SKF-38393 (*in vitro*, 10mM; *in vivo*: 3g/L stock solution in water) and metamphetamine (*in vivo*, 2g/L stock solution in water) were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

Animal experiments. Intraperitoneal implants were produced by encapsulating HEK_{REWARD}, pKR147-transgenic HEK_{DRD1}, pCK53-engineered HEK-293 as well as native HEK-293 into coherent alginate-poly-(L-lysine)-alginate beads (400µm; 200 cells/capsule) using an Inotech Encapsulator Research Unit IE-50R (Buchi Labortechnik AG, Flawil, Switzerland) adjusted to the following settings: 200µm nozzle with a vibration frequency of 1023Hz and 900V for bead dispersion, 20mL syringe operated at a flow rate of 403 units. 400µL of serum-free DMEM containing $2x10^6$ microencapsulated transgenic cells (200 cells/capsule) were injected intraperitoneally into wild-type female or male OF1 (oncins France souche 1; Charles River Laboratories, Lyon France) or male hypertensive mice (BPH/2J, The Jackson Laboratory, Maine, USA). 1h after capsule implantation, the animals were treated intraperitoneally with chemicals (dopamine [0, 0.5, 5, 50, 500mg/kg], SKF-38393 [0, 30 mg/kg], methamphetamine [0, 10, 20mg/kg], methamphetamine [10mg/kg] plus pentolinium tartrate [125mg/kg]) or rewarded by either dopamine-free syrup (25, 50% [v/v in H_2O]; Goldsaft Zuckerruebensirup, Grafschafter Krautfabrik Josef Schmitz KG, Meckenheim, Germany**)** or sexual arousal (1 female mouse in a group 6 male mice). Control animals were either intraperitoneally implanted with non-engineered parental cells, received no chemicals or were not rewarded (water instead of syrup; same-sex groups). Blood levels of SEAP and ANP (serum isolation using microtainer SST tubes; Becton Dickinson, Plymouth, UK) as well as systolic blood pressure (tail-cuff plethysmography; NIBP LE-5002; Harvard Apparatus, Les Ulis, France) were profiled 48h after treatment or reward. All experiments involving animals were performed according to the European Parliament and European Union Directive 2010/63/EU, approved by the French Republic (no. 69266309), and carried out by Ghislaine Charpin-El Hamri at the Institut Universitaire de Technology, IUTA, F-69622 Villeurbanne Cedex, France.

Acknowledgments. We thank Tomonori Katsuyama for assistance with the microscopy and Pratik Saxena, Barbara Geering, Nadezda Masloboeva, and William Bacchus for critical comments on the

manuscript. This work was supported by an European Research Council (ERC) advanced grant (ProNet - No. 321381).

Reference

1. Bello EP, et al. (2011) Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat Neurosci* 14(8):1033-1038.
2. Schroeder MB & Riters LV (2006) Pharmacological manipulations of dopamine and opioids have differential effects on sexually motivated song in male European starlings. *Physiol Behav* 88(4-5):575-584.
3. Hull EM (2011) Sex, drugs and gluttony: How the brain controls motivated behaviors. *Physiol Behav* 104(1):173-177.
4. Bamford NS, et al. (2004) Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron* 42(4):653-663.
5. Beckstead MJ, Grandy DK, Wickman K, & Williams JT (2004) Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron* 42(6):939-946.
6. Leknes S & Tracey I (2008) A common neurobiology for pain and pleasure. *Nat Rev Neurosci* 9(4):314-320.
7. Volkow ND, Wang GJ, Fowler JS, Tomasi D, & Baler R (2011) Food and Drug Reward: Overlapping Circuits in Human Obesity and Addiction. *Curr Top Behav Neurosci* 11:1-24.
8. Blum K, *et al.* (2008) Activation instead of blocking mesolimbic dopaminergic reward circuitry is a preferred modality in the long term treatment of reward deficiency syndrome (RDS): a commentary. *Theor Biol Med Model* 5:24.
9. Stice E, Yokum S, Zald D, & Dagher A (2011) Dopamine-based reward circuitry responsivity, genetics, and overeating. *Curr Top Behav Neurosci* 6:81-93.
10. Ikemoto S (2007) Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* 56:27-78.
11. Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. *Nat Rev Neurosci* 10(3):211-223.
12. Rice ME, Patel JC, & Cragg SJ (2011) Dopamine release in the basal ganglia. *Neuroscience* 198:112-137.
13. Neve K & Seamans J (2004) Dopamine receptor signaling. *J Recept Signal Transduct Res* 24:165-205.
14. Missale C, Nash SR, Robinson SW, Jaber M, & Caron MG (1998) Dopamine receptors: from structure to function. *Physiol Rev* 78(1):189-225.
15. Pardal R, Ortega-Sáenz P, Durán R, & López-Barneo J (2007) Glia-like Stem Cells Sustain Physiologic Neurogenesis in the Adult Mammalian Carotid Body. *Cell* 131(2):364-377.
16. Biaggioni I, Hollister AS, & Robertson D (1987) Dopamine in dopamine-beta-hydroxylase deficiency. *N Engl J Med* 317(22):1415-1416.
17. Rubí B & Maechler P (2010) Minireview: new roles for peripheral dopamine on metabolic control and tumor growth: let's seek the balance. *Endocrinology* 151(12):5570-5581.
18. Volkow ND, Wang G-J, Fowler JS, & Telang F (2008) Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos Trans R Soc Lond B Biol Sci* 363(1507):3191-3200.
19. Goldstein D, *et al.* (1999) Sources and physiological significance of plasma dopamine sulfate. *J Clin Endocrinol Metab* 84(7):2523.
20. Goldstein D S & Holmes C (2008) Neuronal Source of Plasma Dopamine. *Clin Chem* 54:1864-1871.
21. Van Loon G (1983) Plasma dopamine: regulation and significance. *Fed Proc* 42:3008-3012.

22. LeBlanc J (2007) Plasma dopamine and noradrenaline variations in response to stress. *Physiol Behav* 91:208-211
23. Kemmer C*, et al.* (2010) A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. *J Control Release* 150:23-29.
24. Yu P*, et al.* (2004) D1 dopamine receptor signaling involves caveolin-2 in HEK-293 cells. *Kidney Int* 66(6):2167-2180.
25. Daaka Y, Luttrell LM, & Lefkowitz RJ (1997) Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 390(6655):88-91.
26. Cooper J, Hill SJ, & Alexander SP (1997) An endogenous A2B adenosine receptor coupled to cyclic AMP generation in human embryonic kidney (HEK 293) cells. *Br J Pharmacol* 122(3):546-550.
27. Zhang X, He H, Yen C, Ho W, & Lee LJ (2008) Biomaterials - A biodegradable, immunoprotective, dual nanoporous capsule for cell-based therapies. *Biomaterials* 29:4253-4259.
28. Tominaga GT, Garcia G, Dzierba A, & Wong J (2004) Toll of methamphetamine on the trauma system. *Archives of surgery* 139(8):844-847.
29. Shaner JW (2002) Caries associated with methamphetamine abuse. *J Mich Dent Assoc* 84(9):42-47.
30. Mezo AR*, et al.* (2012) Atrial natriuretic peptide-Fc, ANP-Fc, fusion proteins: semisynthesis, in vitro activity and pharmacokinetics in rats. *Bioconjug Chem* 23(3):518-526.
31. Schillinger KJ*, et al.* (2005) Regulatable atrial natriuretic peptide gene therapy for hypertension. *Proc Natl Acad Sci USA* 102(39):13789-13794.
32. Therrien J-P*, et al.* (2010) A gene therapy approach for long-term normalization of blood pressure in hypertensive mice by ANP-secreting human skin grafts. *Proc Natl Acad Sci USA* 107(3):1178-1183.
33. Lin KF, Chao J, & Chao L (1995) Human atrial natriuretic peptide gene delivery reduces blood pressure in hypertensive rats. *Hypertension* 26(6 Pt 1):847-853.
34. Ye H, Daoud-El Baba M, Peng R-W, & Fussenegger M (2011) A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science* 332(6037):1565-1568.
35. Ye H*, et al.* (2013) Pharmaceutically controlled designer circuit for the treatment of the metabolic syndrome. *Proc Natl Acad Sci USA* 110(1):141-146.
36. Weber W*, et al.* (2002) Macrolide-based transgene control in mammalian cells and mice. *Nat Biotechnol* 20(9):901-907.
37. Fussenegger M, Schlatter S, Dätwyler D, Mazur X, & Bailey JE (1998) Controlled proliferation by multigene metabolic engineering enhances the productivity of Chinese hamster ovary cells. Nat Biotechnol 16:468-472.
38. Ruder WC, Lu T, & Collins JJ (2011) Synthetic biology moving into the clinic. *Science* 333(6047):1248-1252.
39. Khalil AS & Collins JJ (2010) Synthetic biology: applications come of age. *Nat Rev Genet* 11(5):367-379.
40. Orive G*, et al.* (2004) History, challenges and perspectives of cell microencapsulation. *Trends Biotechnol* 22(2):87-92.
41. Catena R*, et al.* (2010) Improvement of the monitoring and biosafety of encapsulated cells using the SFGNESTGL triple reporter system. *J Control Release* 146(1):93-98.
42. Chang TMS (2005) Therapeutic applications of polymeric artificial cells. *Nat Rev Drug Discov* 4(3):221-235.

43. Jacobs-Tulleneers-Thevissen D*, et al.* (2013) Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient. *Diabetologia* 56(7):1605-1614.
44. Ausländer S, Wieland M, & Fussenegger M (2012) Smart medication through combination of synthetic biology and cell microencapsulation. *Metab Eng* 14(3):252-260.
45. Fischbach MA, Bluestone JA, & Lim WA (2013) Cell-based therapeutics: the next pillar of medicine. *Sci Transl Med* 5(179):179ps177.
46. Fussenegger M (2010) Synthetic biology: Synchronized bacterial clocks. Nature 463(7279): 301-302.
47. Schlatter S, Rimann M, Kelm J, & Fussenegger M (2002) SAMY, a novel mammalian reporter gene derived from Bacillus stearothermophilus alpha-amylase. *Gene* 282(1-2):19-31.
48. Baud O*, et al.* (2011) The mouse eugenol odorant receptor: structural and functional plasticity of a broadly tuned odorant binding pocket. *Biochemistry* 50(5):843-853.
49. Parsons GB*, et al.* (2007) Ectopic expression of glucagon-like peptide 1 for gene therapy of type II diabetes. *Gene therapy* 14(1):38-48.

Supplementary information

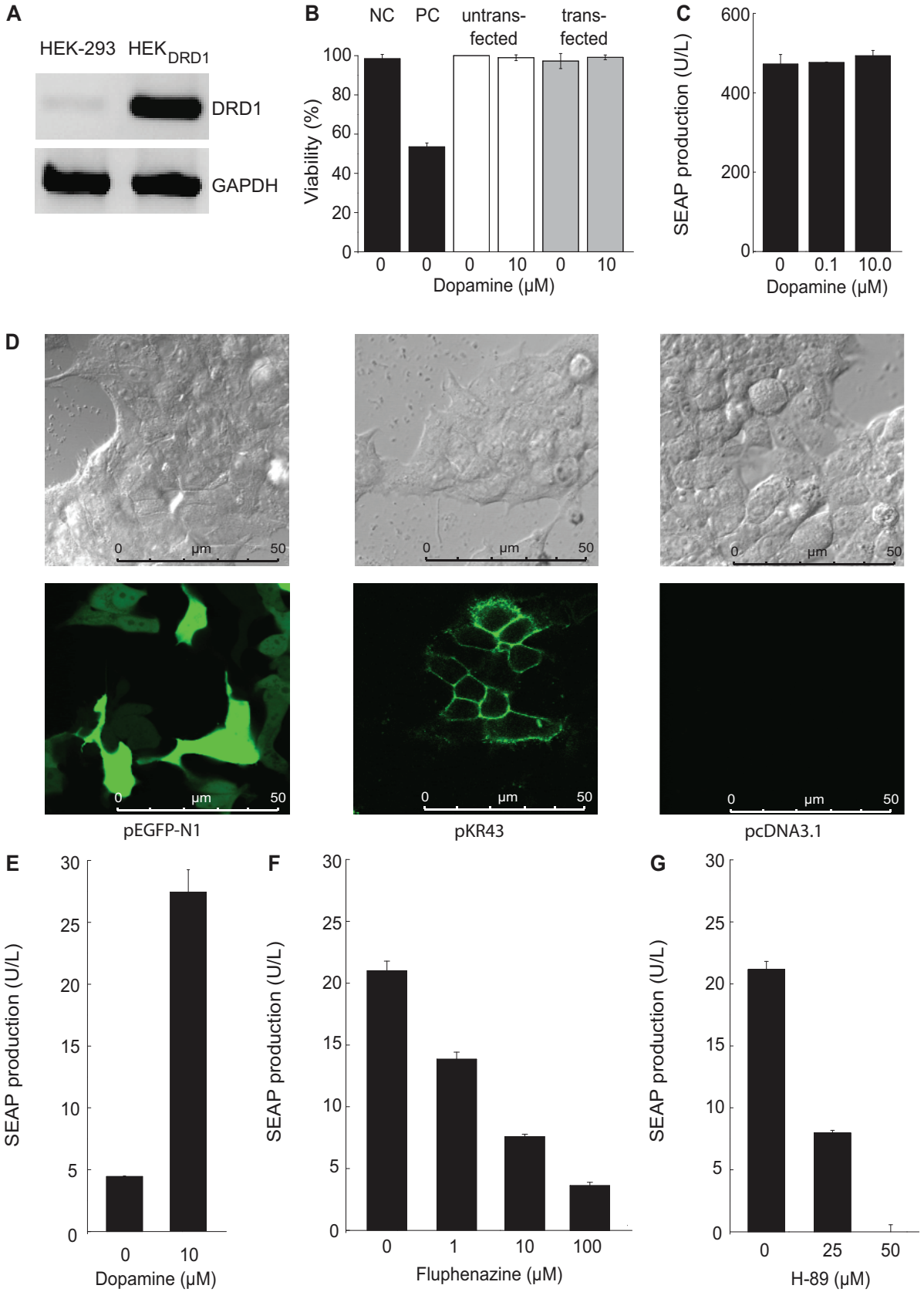

Fig. S1. Functionality of the synthetic dopamine signaling pathway. (*A*) DRD1 transcript-specific semiquantitative RT-PCR of HEK-293 and HEK$_{DRD1}$ using OKR75 (5'-CCAGCCTCAGCAGGA

CATATG-3') and OKR76 (5'-CTTTCCGGTTGAGAACATTCG-3'). GAPDH (OKR500 (5'-CCATCACCATCTTCCAGGAG-3'), OKR501 (5'-CCTGCTTCACCACCTTCTTG-3')) was used as loading control. (*B*) Viability assay with trypan blue staining. HEK-293 were either untreated (negative control NC), treated with 3% formaldehyde (positive control PC), treated with dopamine (0, 10 µM) or transfected with pDRD1 (P_{hCMV}-DRD1-pA) and cultured in the presence of dopamine (0, 10 µM). (*C*) Impact of regulation-effective dopamine concentrations on protein production capacity. HEK-293 were transiently transfected with pSEAP2-Control (P_{SV40}-SEAP-pA), cultivated in the presence of dopamine (0-10 µM) and SEAP levels were scored after 48h. (*D*) Fluorescence micrographs showing membrane-associated localization of GFP-tagged DRD1 in HEK-293 cells 24h after transfection with pKR43 (P_{hCMV}-DRD1-EGFP-pA) or the control vectors pcDNA3.1 (P_{hCMV}-MCS-pA) or pEGFP-N1 (P_{hCMV}-MCS-EGFP-pA). (*E*) Dopamine-inducible SEAP expression of HEK-293 cells 48h after (co-)transfection with pKR43 and pCK53 (P_{CRE}-SEAP-pA). (*F,G*) SEAP expression profile of HEK_{REWARD} grown in the presence of 10µM dopamine and different Fluphenazine (DRD1 antagonist) (*F*) or H-89 (PKA inhibitor) concentrations (*G*).

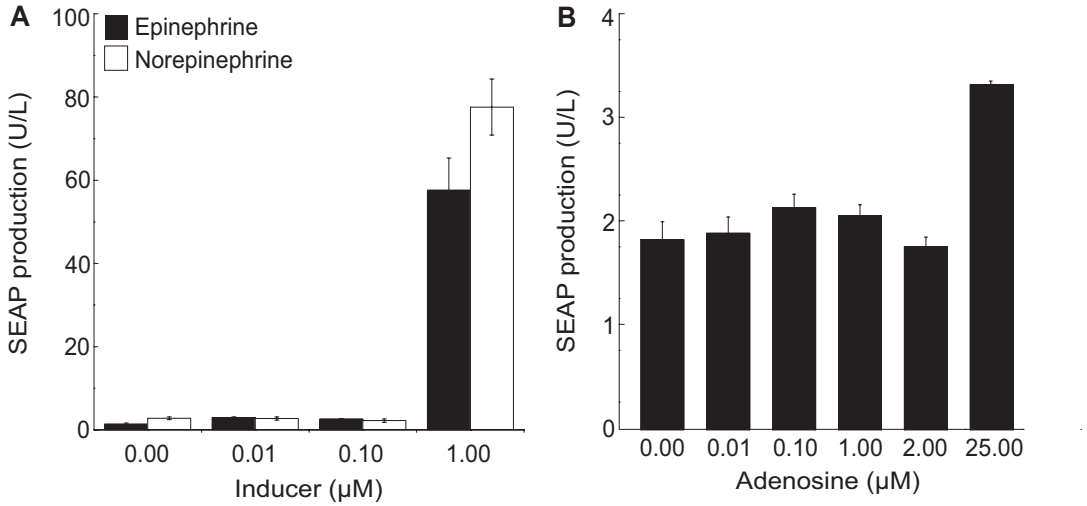

Fig. S2. Dopamine-specificity of the dopamine-sensor circuit. HEK_{REWARD} were exposed to various concentrations of epinephrine and norepinephrine (*A*) or adenosine (*B*) and corresponding SEAP production was profiled 48h after cultivation.

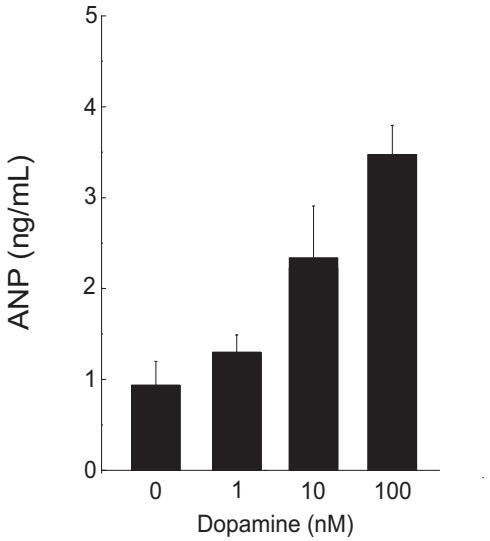

Fig. S3. Dopamine-responsive ANP expression after transfection of HEK_{DRD1} with pKR147 and cultivation for 48h in culture medium containing different concentrations of dopamine.

Table S1. Plasmids used and designed in this study.

Plasmid	Description and Cloning Strategy	Reference or Source
pcDNA3.1	Mammalian expression vector (P_{hCMV}-MCS-pA).	Life Technolo-gies, Carlsbad, CA, USA
pEGFP-N1	Constitutive mammalian EGFP expression vector (P_{hCMV}-MCS-EGFP-pA).	Clontech, Mountain View, CA,USA
pPUR	Mammalian expression vector conferring puromycin resistance (P_{SV40}-Puro-pA).	Clontech, Mountain View, CA,USA
pSEAP2-control	Constitutive mammalian SEAP expression vector (P_{SV40}-SEAP-pA).	Clontech, Mountain View, CA,USA
pZeoSV2	Mammalian expression vector conferring zeocin resistance (P_{SV40}-Zeo-pA).	Invitrogen, Basel, Switzerland
pOREG	Mammalian OR-EG expression vector (P_{SV40}-mOR-EG-pA).	Baud *et al*., 2010 (1)
pCK53	Mammalian expression vector encoding P_{CRE}-driven SEAP expression (P_{CRE}-SEAP-pA).	Kemmer et al., 2011 (2) (GenBank no. KF528989)
pDRD1	Constitutive pcDNA3.1-derived DRD1 expression vector (P_{hCMV}-DRD1-pA).	GenBank: BC096837; Image: 6519220
pKR43	Constitutive mammalian DRD1-EGFP expression vector (P_{hCMV}-DRD1-EGFP-pA). DRD1 was PCR-amplified from pDRD1 using oligonucleotides OKR45 (5'-ggggtaccccacc ATGAGGACTCTGAACACCTC-3') and OKR98 (5'-cttaaccgcggatccATGGTTGGGTG CTGACCGTTTTG-3'), restricted with *Kpn*I/*Bam*HI and cloned into the corresponding sites (*Kpn*I/*Bam*HI) of pEGFP-N1.	This work (GenBank no. KF528987)
pKR145	Custom-designed pUC57-derived vector encoding a modified mouse ANP that contains an Extendin-4 secretion signal and a furin cleavage site at its N-terminus (3) and a mouse IgG-Fc at its C-terminus (4).	This work
pKR147	P_{CRE}-driven ANP expression vector (P_{CRE}-ANP-pA). ANP was excised from pKR145 with *Hin*dIII/*Xba*I and cloned into the corresponding sites (*Hin*dIII/*Xba*I) of pCK53.	This work (GenBank no. KF528988)

Restriction endonuclease-specific sites are underlined in oligonucleotide sequences. Annealing base pairs contained in oligonucelotide sequences are shown in capital letters.

Abbreviations: **ANP**, mouse atrial natriuretic peptide; **DRD1**, human dopamine receptor D1; **EGFP**, enhanced green fluorescent protein; **MCS**, multiple cloning site; **mOR-EG**, mouse eugenol receptor; **pA**, SV40-derived polyadenylation site; $\mathbf{P_{CRE}}$, synthetic mammalian promoter containing a cAMP-response element; $\mathbf{P_{hCMV}}$, human cytomegalovirus immediate early promoter; $\mathbf{P_{SV40}}$, simian virus 40 promoter; **Puro**, puromycin resistance gene; **SEAP**, human placental secreted alkaline phosphatase; **Zeo**, zeocin resistance gene.

References

1. E. P. Bello *et al.*, Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors, *Nat. Neurosci.* **14**, 1033 (2011).
2. M. B. Schroeder, L. V. Riters, Pharmacological manipulations of dopamine and opioids have differential effects on sexually motivated song in male European starlings, *Physiol. Behav.* **88**, 575 (2006).
3. E. M. Hull, Sex, drugs and gluttony: How the brain controls motivated behaviors, *Physiol. Behav.* **104**, 173 (2011).
4. N. S. Bamford *et al.*, Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals, *Neuron* **42**, 653 (2004).
5. M. J. Beckstead, D. K. Grandy, K. Wickman, J. T. Williams, Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons, *Neuron* **42**, 939 (2004).
6. S. Leknes, I. Tracey, A common neurobiology for pain and pleasure, *Nat. Rev. Neurosci.* **9**, 314 (2008).
7. N. D. Volkow, G. J. Wang, J. S. Fowler, D. Tomasi, R. Baler, Food and drug reward: overlapping circuits in human obesity and addiction, *Curr. Top. Behav. Neurosci.* **11**, 1 (2012).
8. K. Blum *et al.*, Activation instead of blocking mesolimbic dopaminergic reward circuitry is a preferred modality in the long term treatment of reward deficiency syndrome (RDS): a commentary, *Theor. Biol. Med. Model.* **5**, 24 (2008).
9. E. Stice, S. Yokum, D. Zald, A. Dagher, Dopamine-based reward circuitry responsivity, genetics, and overeating, *Curr. Top. Behav. Neurosci.* **6**, 81 (2011).
10. S. Ikemoto, Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex, *Brain Res. Rev.* **56**, 27 (2007).
11. S. J. Sara, The locus coeruleus and noradrenergic modulation of cognition, *Nat. Rev. Neurosci.* **10**, 211 (2009).
12. M. E. Rice, J. C. Patel, S. J. Cragg, Dopamine release in the basal ganglia, *Neuroscience* **198**, 112 (2011).
13. K. Neve, J. Seamans, Dopamine receptor signaling, *J. Recept. Signal. Transduct. Res.* **24**, 165 (2004).
14. C. Missale, S. R. Nash, S. W. Robinson, M. Jaber, M. G. Caron, Dopamine receptors: from structure to function, *Physiol. Rev.* **78**, 189 (1998).
15. R. Pardal, P. Ortega-Sáenz, R. Durán, J. López-Barneo, Glia-like stem cells sustain physiologic neurogenesis in the adult mammalian carotid body, *Cell* **131**, 364 (2007).
16. I. Biaggioni, A. S. Hollister, D. Robertson, Dopamine in dopamine-beta-hydroxylase deficiency, *N. Engl. J. Med.* **317**, 1415 (1987).
17. B. Rubí, P. Maechler, Minireview: new roles for peripheral dopamine on metabolic control and tumor growth: let's seek the balance, *Endocrinology* **151**, 5570 (2010).
18. N. D. Volkow, G.-J. Wang, J. S. Fowler, F. Telang, Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 3191 (2008).
19. D. S. Goldstein, G. Eisenhofer, I. J. Kopin, Sources and significance of plasma levels of catechols and their metabolites in humans, *J. Pharmacol. Exp. Ther.* **305**, 800 (2003).
20. D. S. Goldstein, C. Holmes, Neuronal Source of Plasma Dopamine, *Clin. Chem.* **54**, 1864 (2008).
21. G. Van Loon, Plasma dopamine: regulation and significance, *Fed. Proc.* **42**, 3008 (1983).
22. J. LeBlanc, M. B. Ducharme, Plasma dopamine and noradrenaline variations in response to stress, *Physiol. Behav.* **91**, 208 (2007).

23. Materials and methods are available as supporting material on Science Online.
24. C. Kemmer *et al.*, A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms, *J. Control, Release* **150**, 23 (2011).
25. P. Yu *et al.*, D1 dopamine receptor signaling involves caveolin-2 in HEK-293 cells, *Kidney Int.* **66**, 2167 (2004).
26. X. Zhang *et al.*, Biomaterials - A biodegradable, immunoprotective, dual nanoporous capsule for cell-based therapies, *Biomaterials* **29**, 4253 (2008).
27. G. T. Tominaga, G. Garcia, A. Dzierba, J. Wong, Toll of methamphetamine on the rauma system, *Arch. Surg.* **139**, 844 (2004).
28. J. W. Shaner, Caries associated with methamphetamine abuse, *J. Mich. Dent. Assoc.* **84**, 42 (2002).
29. A. R. Mezo *et al.*, Atrial natriuretic peptide-Fc, ANP-Fc, fusion proteins: semisynthesis, in vitro activity and pharmacokinetics in rats, *Bioconjug. Chem.* **23**, 518 (2012).
30. K. J. Schillinger *et al.*, Regulatable atrial natriuretic peptide gene therapy for hypertension, *Proc. Natl. Acad. Sci. USA* **102**, 13789 (2005).
31. J. P. Therrien *et al.*, A gene therapy approach for long-term normalization of blood pressure in hypertensive mice by ANP-secreting human skin grafts, *Proc. Natl. Acad. Sci. USA* **107**, 1178 (2010).
32. K. F. Lin, J. Chao, L. Chao, Human atrial natriuretic peptide gene delivery reduces blood pressure in hypertensive rats, *Hypertension* **26**, 847 (1995).
33. H. Ye, M. Daoud-El Baba, R.-W. Peng, M. Fussenegger, A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice, *Science* **332**, 1565 (2011).
34. H. Ye *et al.*, Pharmaceutically controlled designer circuit for the treatment of the metabolic syndrome, *Proc. Natl. Acad. Sci. USA* **110**, 141 (2013).
35. W. Weber *et al.*, Macrolide-based transgene control in mammalian cells and mice, *Nat. Biotechnol.* **20**, 901 (2002).
36. M. Fussenegger, S. Schlatter, D. Dätwyler, X. Mazur, J. E. Bailey, Controlled proliferation by multigene metabolic engineering enhances the productivity of Chinese hamster ovary cells, *Nat. Biotechnol.* **16**, 468 (1998).
37. S. Schlatter, M. Rimann, J. Kelm, M. Fussenegger, SAMY, a novel mammalian reporter gene derived from Bacillus stearothermophilus [alpha]-amylase, *Gene* **282**, 19 (2002).
38. O. Baud *et al.*, The mouse eugenol odorant receptor: structural and functional plasticity of a broadly tuned odorant binding pocket, *Biochemistry* **50**, 843 (2011).
39. Parsons, G.B. *et al.* Ectopic expression of glucagon-like peptide 1 for gene therapy of type II diabetes. *Gene therapy* **14**, 38-48 (2007).

Acknowledgments. We thank Pratik Saxena, Barbara Geering, Nadezda Masloboeva and William Bacchus for critical comments on the manuscript. This work was supported by an European Research Council (ERC) advanced grant (ProNet - No. 321381)

CONCLUSION

Synthetic gene regulation networks are highly efficient tools for reprogramming metabolism, physiology, and the dynamic behavior of mammalian cells. Most recent control devices are highly advanced and thereby useful to address new clinical applications and treatment concepts including the ability to control diseases. As part of the host organism, cell-based therapeutic networks consisting of sensor and effector components, can monitor disease-relevant metabolites, response to varying disease states and control disease dispersion, which makes them attractive for future gene and cell-based therapies.

In this work we have developed three gene regulation circuits, which may foster new strategies for the treatment of metabolic diseases such as obesity or hypertension.

Focusing on obesity, one of the major health problems worldwide, we have designed the LSR sensor that shows excellent gene expression performance in response to several fatty acids and was able to monitor blood fat levels in mice. By combining the LSR with an effector compartment containing the anorectic peptide hormone pramlintide, diet-induced obesity mice implanted with this device produced controlled pramlintide expression levels, which resulted in reduced food intake, blood fat levels and hence loss of body weight. Additionally, pramlintide expression was not activated in wild-type mice with normal blood fat level and could be switched off at any time by supplying the apple metabolite phloretin as skin lotion, since LSR contains TtgR as DNA-binding component thereby providing a safety switch.

This approach demonstrates how synthetic cell-based therapies in the future could lead to novel treatment possibilities for obesity and related metabolic diseases, when conventional treatment strategies fail.

Bacterial repressors are widely used for the design of transcription-control devices, since they can easily adjust to changing microenvironmental condition inside the host and operate within the host's pathophysiological range. *Campylobacter jejunis'* repressor CmeR, which controls the expression of the CmeABC multidrug efflux pump, is responsible for the resistance to several antimicrobials as well as bile acids and supports the pathogen's colonization within the intestinal tract of the host. Hence, it represents an ideal candidate for the development of a synthetic gene regulation network. By fusing CmeR to a transactivator ($BEAR_{OFF}$) or transsilencer ($BEAR_{ON}$), we have developed two regulatory systems that could respond to pathophysiological bile acids concentrations. By performing several optimization steps of the $BEAR_{ON}$ system, such as by

varying the number of operator repeats, verifying the best performing promoter strength of the regulator, and exploring the best transfection ratio, as well as its combination with the best fitting cell line, we generated a highly sophisticated gene regulation network. Reduced leakiness, high target gene expression levels, excellent adjustability, rapid response kinetics and full reversibility of the optimized $BEAR_{ON}$ device in cells grown in culture or implanted into mice, qualifies this biosensor for the use in diagnostic or prosthetic networks to monitor and correct bile-acid dependent pathological diseases.

Additionally, this biosensor which response to one input signal and reacts in one output, provides several synthetic bio bricks that can be further combined with other gene regulation networks. With these parts a complex synthetic gene network such as an mammalian oscillator or biocomputer could be generated that responses to several input signals like different metabolic biomarkers and gives the possibility to produce more than 1 output signal such as various therapeutic proteins.

The brains' key neurotransmitter dopamine is released in response to pleasure-associated situations, such as food, sexual arousal or by stimulation of drug abuse. Dopamine is not able to cross the blood-brain barrier, but can be found in the blood, where it is predominantly derived from networks of the sympathetic nerves. By considering that dopamine, which is released into the brain by reward stimulation, has shown to correlate with peripheral dopamine levels, we have designed a dopamine biosensor device that converts reward-stimulated signals into a dose-dependent gene expression response. Operating with dopamine biosensor designer cells in the peripheral system of mice resulted in a functional reward-associated target gene expression response. Furthermore, rewiring of the biosensor with an effector component for the expression of the clinically licensed antihypertensive drug ANP resulted in the reduction of high blood pressure in hypertensive mice after reward-associated stimulation. Due to the quick response of the nervous system this synthetic device highly contributes to novel gene- and cell-based strategies to treat metabolic diseases such as hypertension by time-dependent delivery of therapeutic proteins in response to behavioral activities.

ACKNOWLEDGEMENT

This thesis was written during my work as a researcher at the Department of Biosystems Science and Engineering of the Swiss Federal Institute of Technology Zurich.

To this thesis, several people have contributed in different ways and I would like to thank all of them at this point.

My special thanks goes to Prof. Dr. Martin Fussenegger for the opportunity to conduct my research work in his laboratory, for the excellent care, support and above all things the freedom he has given me during the processing of the projects.

Furthermore, I want to thank Prof. Dr. Sven Panke for his work as a co-examiner.

A special thanks goes to Dr. William Bachhus, David Moore and Mingqi Xie, who helped me in revising of my manuscript.

I would also like to thank everyone participating in my projects, either by providing critical input on my work, a helping hand or general support.
Dr. Marie Daous-El Baba and Dr. Ghislaine Charpin-El Hamri helped me a lot with the mice experiments – thank you girls.

Many thanks to all members from the Fussenegger group (present and past), it was fun working with you.

A major thanks to all employees of the D-BSSE for the excellent working atmosphere that has contributed substantially to a motivating work.

To Alexandra, Aizhan, David M., Pratik, Sonja, Ina, Julia K. and Manuel I would like to give a huge hug for supporting me in- and outside the lab and for all the funny moments we shared.

Finally, my very special thanks are addressed to my sister Antje as well as to my entire Family for their permanent support, their help and love during all situations of life.

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Rössger, K., Charpin-El-Hamri, G., Fussenegger, M. 2013. A closed-loop synthetic gene circuit for the treatment of diet-induced obesity in mice. *Nat. Commun*. **4**, 2825.

Rössger, K., Charpin-El-Hamri, G., Fussenegger, M. 2013. Reward-based hypertension control by a synthetic brain-dopamine interface. *Proc. Natl. Acad. Sci. USA* **110**, 18150-5.

Rössger, K., Charpin-El-Hamri, G., Fussenegger, M. 2013. Bile acid-controlled transgene expression in mammalian cells and mice. *Metab. Eng.* **21**, 81-90.

PATENTS

Rössger, K. and Fussenegger, M. (2013). A Designer Circuit Controlling Diet-Induced Obesity. (*submitted*)