Doctoral Thesis

Reconstructing de novo silencing of an active retrotransposon in arabidopsis

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CORRIGENDUM

Arturo Marí-Ordóñez, the first author who performed the experiments and assembled all figures in the published manuscript, has worked on addressing, in the best possible way, the original allegations from PubPeer. Unfortunately, raw phosphorimager files from some figures were generated in Strasbourg and are no longer available. Therefore, the same membranes were retrieved and re-probed with the same subset of probes as used in the study. Although cases 5 and 6 do not constitute cases of errors, it was considered appropriate to include additional explanations. Possible corrections or additional information regarding the affected figures are also proposed in this document.

CASE 1: Figure 5h.

- **Issue:** Similar background patterns of lanes 5 and 6 of the upper panel of figure 5h (LTR probe). A similar pixel patterns is indeed possibly apparent between the two lanes.
- **Raw data:** The original phosphorimager row file was no longer available to assess the occurrence of an image manipulation. Hence, the original membrane was retrieved and re-hybridized with the same LTR probe as used in the paper. The results *(blot 1.1)* confirmed that the two tracks contained blank signals for the LTR probe. Therefore, the re-probing of the original blots shows that any background pixel duplication would not have affected the interpretation of the data and the conclusions drawn from the results. In order to prove that the same original membrane used for the manuscript was used for the re-hybridization shown here, the same membrane was afterwards re-hybridized with GAG *(blot 1.2)* and tasiRNAs 255 *(blot 1.3)*. These displayed the same band pattern as in the published Figure 5h, proving that the re-hybridizations have been performed on the same RNA blots.
- **Proposed correction:** The proposed correction to the published panel h on figure 5 includes a new panel presenting the re-hybridized membrane against EVD LTR.

CASE 2: Figure 6a (1).

- **Issue:** Similar background patterns of lanes 1 and 2 as well as in lanes 4 and 5 of the upper panel of figure 6a (LTR probe). A similar pixel patterns is indeed possibly apparent between lanes 1 and 2 and between lanes 4 and 5.
- **Raw data:** The original phosphorimager row file was no longer available to assess the occurrence of an image manipulation. Hence, the original membrane was retrieved and re-hybridized with the same LTR probe as used in the paper. The results *(blot 2.1)* confirmed that the two tracks contain blank signals for the LTR probe. Therefore, the re-probing of the original blot shows that any background pixel duplication would not have affected the interpretation of the data and the conclusions drawn from the results. In order to prove that the same original membrane used for the manuscript was used for the re-hybridization shown here, the same membrane was afterwards re-hybridized with GAG *(blot 2.2).*
These displayed the same band pattern as in the published Figure 5h, proving that the re-hybridizations have been performed on the same RNA blots.

- **Proposed correction:** Because the following case (Case 3) concerns the same figure panel, all the potential issues in Figure 6a are proposed to be corrected simultaneously (see below).

**CASE 3: Figure 6a (2).**

- **Issue:** Similar background patterns of lanes 6 and 7 of the second last panel of figure 6a (EVD probe). A similar pixel patterns is indeed possibly visible between lanes 6 and 7.
- **Raw data:** The original phosphorimager row file was not longer available to validate image manipulation. Hence, the original membrane was retrieved and re-hybridized with the same EVD probe as used in the paper. The results (blot 3.1) confirmed that the two tracks contained background signals for the EVD probe. Therefore, the re-probing of the original blot shows that any background pixel duplication would not have affected the interpretation of the data and the conclusions drawn from the results.
- **Proposed correction:** The proposed correction to the published Figure 6a (incorporating both case 2 and 3) entails the replacement of the LTR and GAG panels found in the original paper by the new re-hybridizations presented here.

**CASE 4: Figure 2c.**

- **Issue:** Similar band patterns are observed in the U6 loading control from panel c and miR171 from AGO1 IP siRNAs in panel f.
- **Raw data:** The original data from Figure 2 panels c and f was retrieved confirming that the original image belongs to panel f (blot 6.3a) but was inadvertently duplicated in panel c during figure assembly. The blot probed against 3’GAG (blot 4.1) is shown to display the blot that was used, from which only the 3 first lanes were used in Figure 2c (blot 4.2). The cognate, original, phosphorimager image of the same membrane probed against U6 as loading control was retrieved (blot 4.3). Altered brightness and contrast was applied to that image to reveal the membrane boundaries (blot 4.3), proving that the U6 hybridization had been performed on the same membrane as the one hybridized with the 3’GAG probe, and is, therefore the cognate loading control for Figure 2c. Inspection fo this panel (blot 4.3) confirms that there is no major loading difference between the samples (lanes) used in the figure.
- **Proposed correction:** The proposed correction to Figure 2c (entails the replacement of the duplicated panel by the cognate U6 loading control belonging to the membrane used for the rest of the panel c.

**CASE 5: Figure 2h.**

- **Issue:** Alleged differences in the pixilation between the area surrounding bands and the background in Figure 2h (upper panel ) has raised criticisms that the bands had been copied and pasted from another blot into a blank background.
- **Raw data:** The original scan of the autoradiography was retrieved (*blot 5.1*). The image used to assemble Figure 5a corresponded to the same image after uniform correction using high contrast to better display differences in signal intensity between samples (*blot 5.2*). This had led to compression artefacts during the conversion of the image for publication.

- **Proposed correction:** it is argued that no correction is required in this case.

**CASE 6: Figure 2f and 5a.**

- **Issue:** Similar band patterns and background are observed for the 3’GAG, miR171 and U6 signals between the total RNA blots in Figure 2f and Figure 5a.

- **Raw data:** The alleged duplication is legitimate since both figures represent RNA blots displaying RNA extracted from immunoprecipitated (IP) Argonaute proteins (AGO) from the same WT, epi15 F8, F11 and F14 sample lysates. These samples share, therefore, the same input control (Total RNA). IPs of AGO1, AGO2 and AGO4 were performed in parallel and the Total RNA, AGO1, AGO2 and AGO4 IP RNA were blotted and probed in parallel as well (original images used for the figures are provided, *blots 6.1 to 6.4*). In Figure 2f, only the first three lanes corresponding to WT (Col-0, c), epi15 F8 (F8) and epi15 F11 (F11) were used from Total RNA, AGO1 and AGO2 IPs, while for Figure 5a the four lanes (including epi15 F14, F14) were used from Total RNA and AGO4 IP. Blots hybridized against 3’GAG were obtained by autoradiography (*blot 6.1*) and using a phosphorimager (*blot 6.2*) to compensate for the strong signal intensity obtained in total RNA probed against 3’GAG. 6.1b was used for 3’GAG siRNA levels in input samples in both figures. Similarly, miR171 (*blot 6.3a*) and U6 loading control (*blot 6.4a*). were used in both figures as they correspond to the input (total RNA) control for the AGO1/2/4 IPs. Hence, the duplication is legitimate because the total RNA control samples are the same for both figures. Nonetheless, this was not specified in the corresponding figure legend.

- **Proposed correction:** A correction to the legend of Figure 5a is proposed, so as to specify that Total RNA (input controls) used in both figures are the same since the IPs were performed in parallel from the same sample lysates. Therefore, Figure 5a’s legend should read (added text underlined):

  (a) RNA analysis of LTR- and 3’ gag–derived siRNAs in AGO4 immunoprecipitations. siR1003 (AGO4) and miR171 (AGO1) were probed to validate specific sRNA enrichment with AGO4. U6, control for RNA carryover from cleared immunoprecipitation extracts. Total RNA blots correspond to those on Figure 2c, as AGO1, AGO2 and AGO4 immunoprecipitations were performed in parallel from the same plant lysates. Protein blot analysis of input and immunoprecipitation fractions is shown (the arrowhead indicates the AGO4 band in input samples), as is Coomassie staining of input protein.
Case 1: Figure 5h

- Similar background pixel patterns suggesting duplication of lanes 5 and 6 of the upper panel of Figure 5h.

1.1) Original membrane re-probed against LTR siRNAs (phosphorimager)

1.2) Original membrane re-probed against GAG siRNAs (phosphorimager)

1.3) Original membrane re-probed against tasiRNA 255 (phosphorimager)
Proposed corrected Figure 5h

Substitute panel h on Figure 5 by a new one containing the original membrane rehybridized with the LTR probe.
Case 2 and 3: Figure 6a

- CASE 2: Similar background pixel patterns suggesting duplication of lanes 1 and 2 as well as lanes 4 and 5 of the upper panel.

- CASE 3: Similar background pixel patterns suggesting duplication of lanes 6 and 7 of the second last panel.

2.1) Original membrane re-probed against LTR siRNAs (phosphorimager)

2.2) Original membrane re-probed against GAG siRNAs (phosphorimager)

3.1) Original membrane (HMW-NB) re-probed against EVD mRNA (phosphorimager)
Proposed corrected Figure 6a

Substitute panel a on Figure 5 by a new one containing the original membrane rehybridized with the LTR probe for the siRNAs, and with the EVD probe for the mRNA.
Case 4: Figure 2c

- Missused miR171 blot from Figure 2f as U6 loading control for Figure 2c instead of its cognate U6 control.

4.1) Original phosphorimager scan of membrane used for Figure 2c (probed against 3’GAG)

4.2) Only 3 first lanes are used for Figure 2c

4.3) Membrane probed against U6

4.4) Membrane probed against U6 with modified brightness and contrast allowing to see membrane boundaries
Proposed corrected Figure 2c

Substitute panel c on Figure 2 by a new one containing the corresponding U6 loading control.
Case 5: Figure 2h

- Compression artefacts that appeared due to the high contrast of the image raised concerns about bands being pasted into a blank background.

5.1) Scan of original film

5.2) After enhanced contrast

Used for figure
Case 6: Figures 2f and 5a

- IPs of AGO1, AGO2 and AGO4 were performed on the same WT, epi15 F8, epi15 F11 and epi15 F14 samples and therefore the same Total RNA used as input was used for both Figure 2f and 5a. This was not indicated in the figure legend as has been interpreted as malevolent duplications.

6.1) Original scan of autoradiography of:
input (total RNA, 1a) and RNA extracted from AGO1 (1b), AGO2 (1c) and AGO4 (1d) IPs hybridized against 3’Gag.

6.2) Original phosphorimager image of:
input (total RNA, 2a) and RNA extracted from AGO1 (2b), AGO2 (2c) and AGO4 (2d) IPs hybridized against 3’Gag.
Proposed corrected Figure 5a

We suggest to modify Figure 5a legend so it should read:

(a) RNA analysis of LTR- and 3' gag–derived siRNAs in AGO4 immunoprecipitations. siR1003 (AGO4) and miR171 (AGO1) were probed to validate specific siRNA enrichment with AGO4. U6, control for RNA carryover from cleared immunoprecipitation extracts. Total RNA blots correspond to those on Figure 2c, as AGO1, AGO2 and AGO4 immunoprecipitations were performed in parallel from the same plant lysates. Protein blot analysis of input and immunoprecipitation fractions is shown (the arrowhead indicates the AGO4 band in input samples), as is Coomassie staining of input protein.