SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure S1. Summary of the anti-L1 activity of AID and A3A. A histogram reporting the mean relative transposition frequency of L1 in the presence of vector, human AID, human A3A or $A3A_{E72A}$ as indicated. Transposition was measured as the percentage of GFP^+ cells in the culture 5 days post-transfection. Bars represent the mean of 18 independent cultures from 6 different experiments. For each experiment, the mean of vector alone was normalized to 1. Error bars indicate the standard deviation. *P*-values were calculated for each construct using the Student's *t*-test and were as follows: AID, $p = 1.7 \times 10^{-21}$; A3A, $p = 1.0 \times 10^{-22}$; $A3A_{E82A}$, $p = 7.5 \times 10^{-8}$.

Supplementary Figure S2. Summary of AID activity in *E. coli.* (**A**) A histogram reporting the averages and standard deviations of the relative median mutation frequencies of the indicated *AID* expression constructs for two independent experiments, each with a minimum of six independent cultures for each construct (*e.g.*, Figure 1C). Vector represents the background level of mutation in *ung*-deficient *E. coli*. *P*-values were calculated for each construct using the Student's *t*-test and were as follows: human, p = 0.0018; pig, p = 0.00065; mouse, p = 0.027; rat, p = 0.0049; chicken, p = 0.18; catfish, p = 0.00081; zebrafish, p = 0.0016; pufferfish, p = 0.82. We note that chicken and pufferfish did not display significant activity. However, the *E. coli* mutation assay only provides an indication of activity and not absolute values, and these data do not impact the overall result that AID can inhibit L1. (**B**) A histogram reporting the mean of the relative median mutation frequencies of the indicated *AID* expression constructs. For

vector and WT AID, n=7. For the other constructs, n=2 or 3. A minimum of 6 to 8 independent cultures were assayed for each AID variant in each independent experiment. The errors represent one standard deviation from the mean. *P*-values were calculated for each construct using the Student's *t*-test and were as follows: wild-type, 1.2×10^{-8}; E58Q, 0.31; AIDΔC, 2.3×10^{-9}; W84A, 0.18; R24E, 0.20; R112E, 0.23; R24E/R112E, 0.42; C87A, 0.032; C90A, 0.30.

Supplementary Figure S3. Effect of AID and A3 proteins on the expression of GFP from a co-transfected plasmid. (**A**) The percentage of puromycin-resistant cells that were GFP-positive five days after transfection with pEGFP-N3 and an AID or A3 expression plasmid, or empty vector. Bars represent the mean of three independent cultures and error bars indicate the standard deviation from the mean. (**B**) Western blot showing expression of the HA-tagged proteins from a representative experiment from panel A. Tubulin is a loading control.

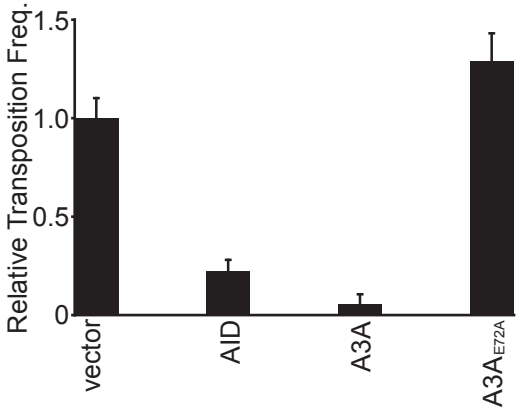
Supplementary Figure S4. Inhibition of L1 retrotransposition in HeLa cells. **(A)** Percentage of puromycin-resistant HeLa cells that were GFP-positive five days after transfection with the L1 and indicated AID variants. A3A and $A3A_{E72A}$ were included as controls. Bars represent the mean of three independent cultures. Error bars indicate the standard deviation from the mean. (**B**) Western blot showing expression of the HA-tagged proteins from a representative experiment from panel A. The A3A-containing lysates were diluted 1/10, as indicated. Tubulin is a loading control.

Supplementary Figure S5. Summary of the anti-MusD activity of human AID. A histogram reporting the mean relative transposition frequency of MusD in the presence of vector or human AID. Transposition was measured as the number of neomycin-resistant colonies divided by the transfection efficiency. Bars represent the mean of 12 independent cultures from 4 different experiments. For each experiment, the mean of vector alone was normalized to 1. Error bars indicate the standard deviation. P = 1.83 x 10^{-8} (Student's *t*-test).

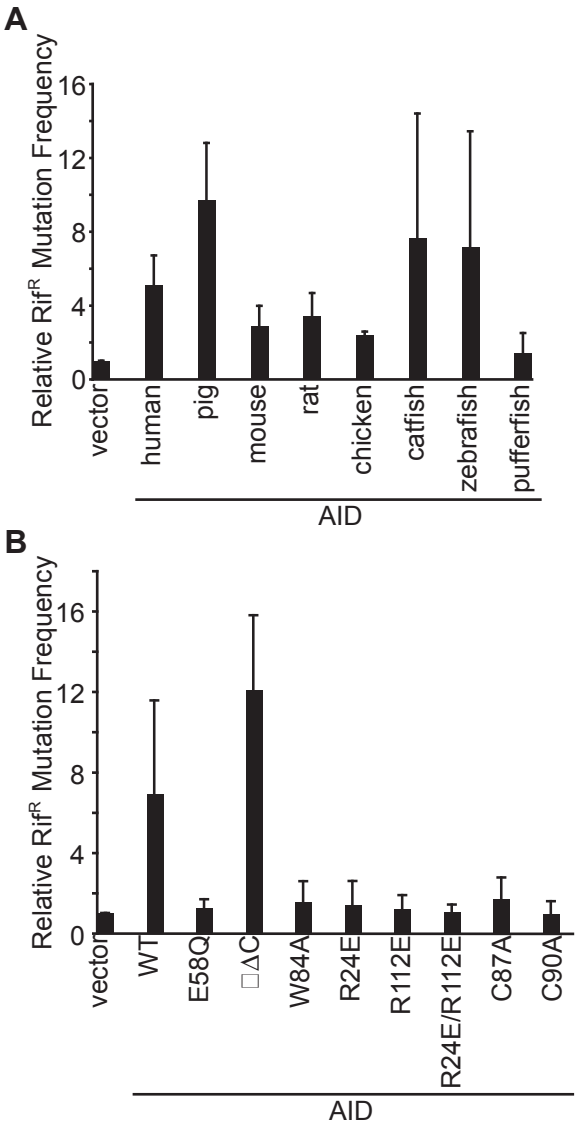
Supplementary Figure S6. AID DNA or RNA is not sufficient to inhibit L1 retrotransposition. Percentage of puromycin-resistant cells that were GFP-positive five days after transfection with the L1 and indicated AID variants. A3A and $A3A_{E72A}$ were included as controls. Histogram bars represent the mean of three independent cultures, and the standard deviation is shown. WT = wild-type.

Supplemental Figure S7. A model for the cytoplasmic restriction of L1 inhibition by AID. (**A**) AID (red ovals) is depicted engaging the L1 ribonucleoprotein complexes (RNPs) and interfering with RNP nuclear import by sequestering them in high molecular mass complexes in the cytoplasm. The cytoplasmic interaction between AID and the RNPs may be direct, by binding to L1 ORF1 or ORF2 (green and blue circles, respectively), or indirect, by binding an AID-interacting protein (AIP; orange circles). Alternatively, as suggested by Kinomoto *et al*. (31), the nuclear localization signal of ORF2 could transport AID (or A3) proteins bound to an RNP complex into the nuclear

compartment, thereby enabling AID to block a step of target-primed reverse transcription (TPRT), such as reverse transcription itself, 2nd strand DNA synthesis or integration.



MacDuff et al. Supp. Figure S1



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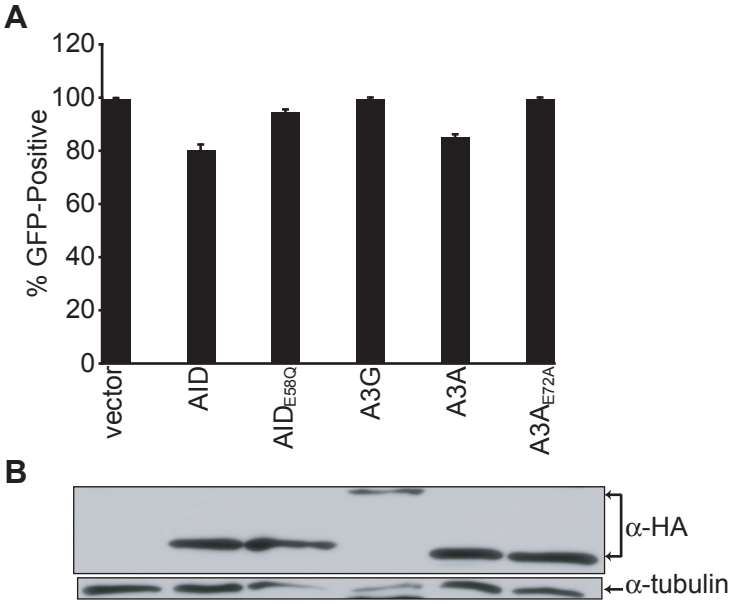

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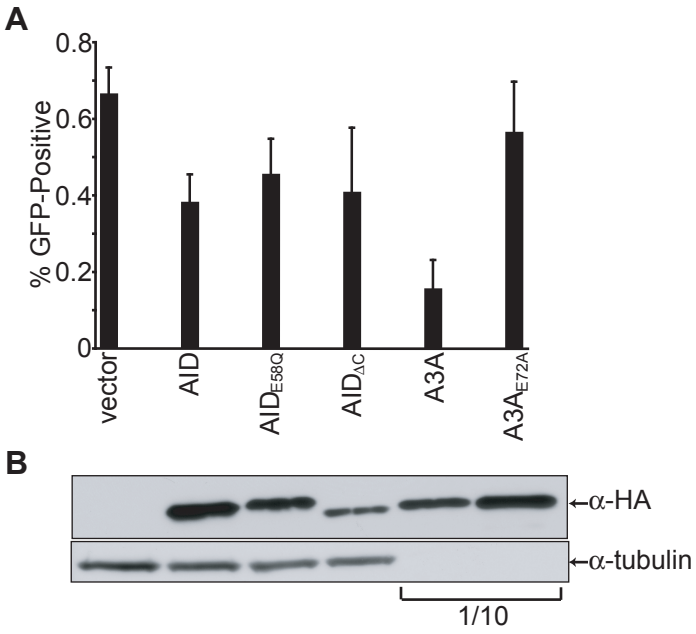

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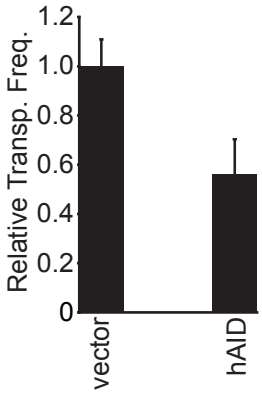

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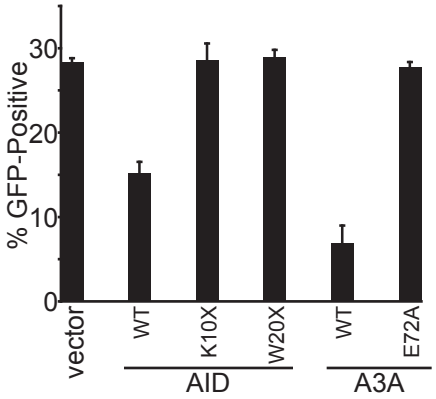
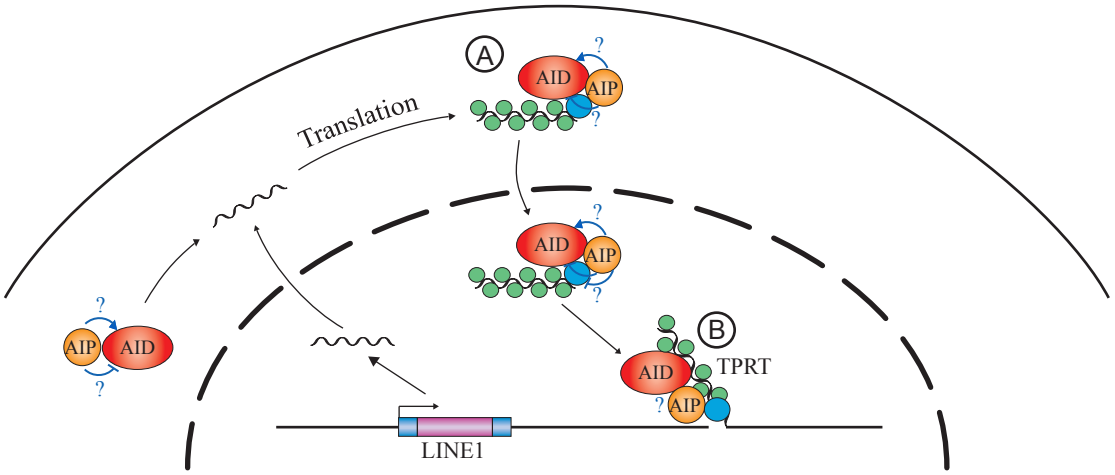


MacDuff et al. Supp. Figure S6



MacDuff et al. Supp. Figure S7