**Supplementary Online Data**

# Opossum APOBEC1 is a DNA mutator with retrovirus and retroelement restriction activity

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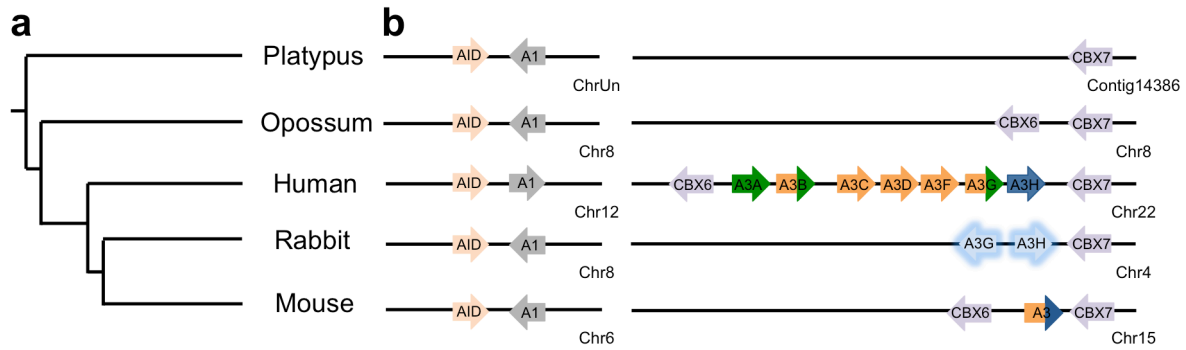


**Figure S1. No *A3* gene exists in monotremes and marsupials.** (**a**) Phylogenetic tree of selected monotreme (platypus), marsupial (opossum), and eutherian mammals (human, rabbit and mouse). (**b**) Genomic organization of *A1*, *AID*, and *A3* genes. A homology BLAST search was performed for all seven human *A3* genes (*A3A*, *A3B*, *A3C*, *A3D*, *A3F*, *A3G*, and *A3H*) in the genomes of platypus and opossum. Although all species have *A1* and *AID*, non-eutherian mammals did not show any evidence for homologous *A3* genes between the conserved *CBX6* and *CBX7* genes. We also found no evidence to suggest that a gene similar to one of human *A3* genes exists in a different locus in the opossum and platypus genomes. Green, orange, and blue colors indicate Z1, Z2, and Z3 domains of A3 enzymes. For rabbit, two *A3*-like genes (shown in glowing blue color) have been annotated. Based on the available sequences from Ensemble, the rabbit *A3G*-like gene (ensemble ID: ENSOCUG00000023532) appears to encode a single Z1 domain, and the rabbit *A3H*-like gene (ensemble ID: ENSOCUG00000023913) has signatures of Z2 and Z3 domains. However, the database sequences may not be complete or correctly annotated.

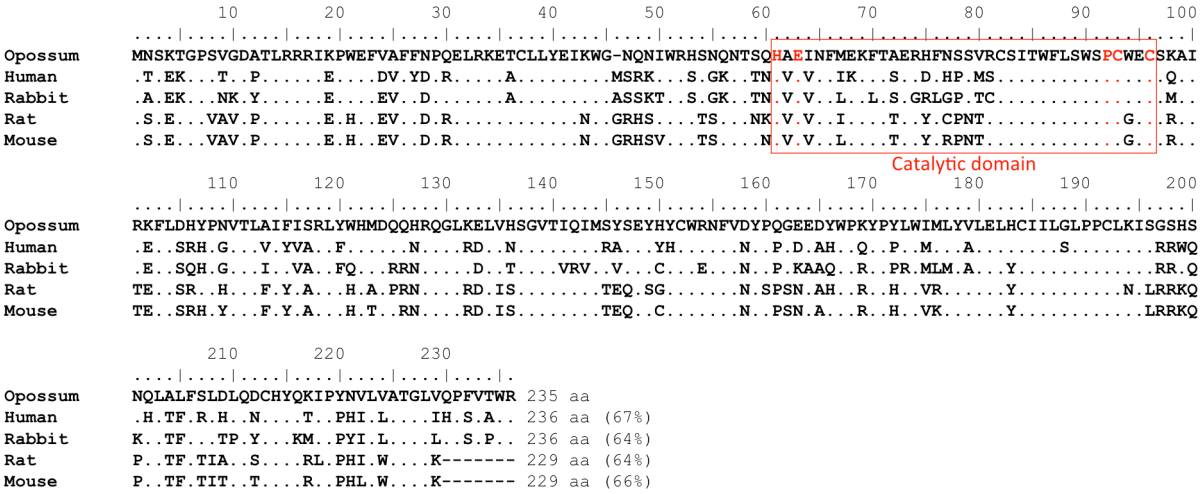


**Figure S2. Alignment of amino acid sequences of mammalian A1s.** Sequences of the predicted full-length mammalian A1 proteins were aligned with Clustal W software. The numbers above each amino acid sequences are the positions of amino acid residues. The identical amino acids to opossum A1 are indicated by a single dot. The amino acid positions that were not aligned are shown with a bar. The amino acid residues involved in the catalytic reaction are shown in red. The amino acid length of opossum A1 is 235 aa. The percent of amino acid identity of each eutherian A1 with opossum A1 is indicated in parentheses at the end of each sequence.

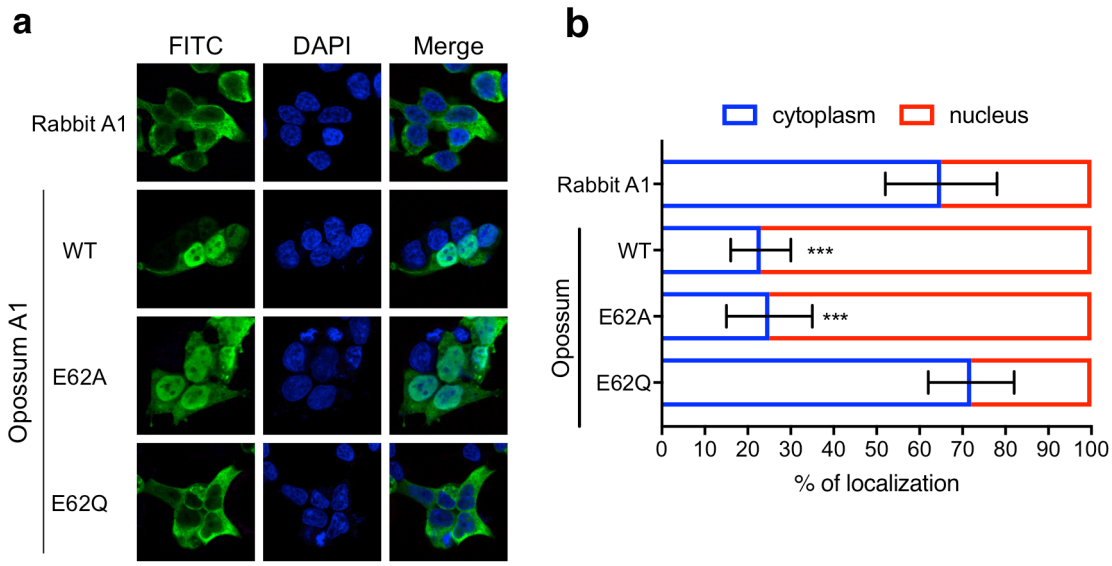


**Figure S3. Subcellular localisation of opossum A1 in 293T cells.** (**a**) Images show HA-tagged A1s stained with FITC (green) and nuclei stained with DAPI (blue). (**b**) Nuclear and cytoplasmic distribution levels of A1s. The percentage nuclear and cytoplasmic localisation is indicated as the graphs (n = 20, average +/- SD). *P* values represent comparisons with rabbit A1.