AID up-mutants isolated using a high-throughput screen highlight the immunity/cancer balance limiting DNA deaminase activity

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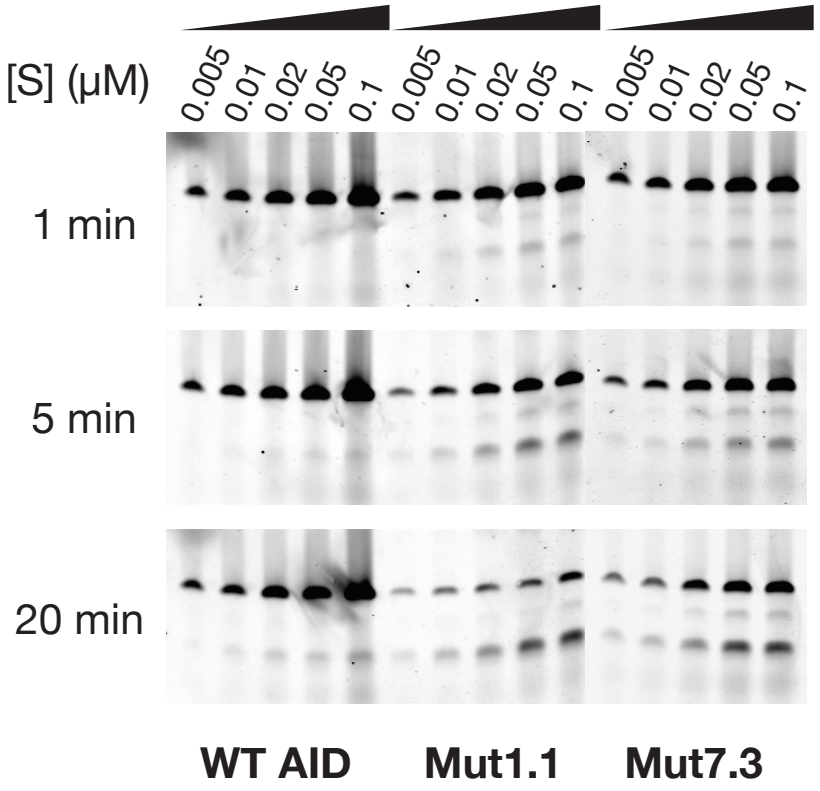
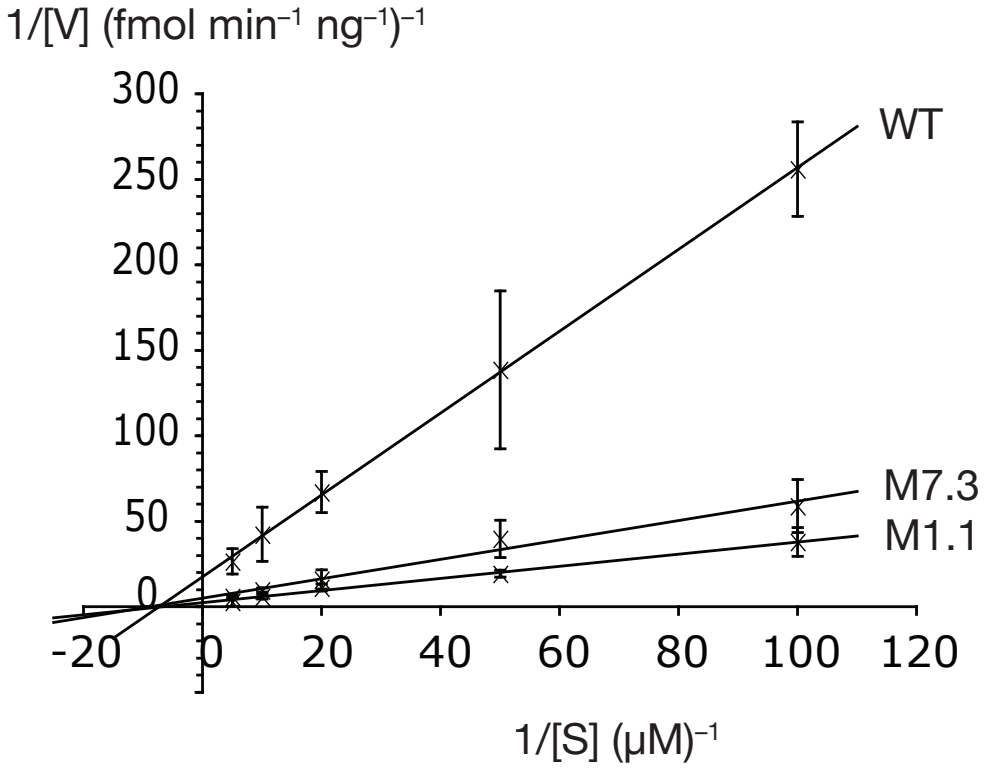
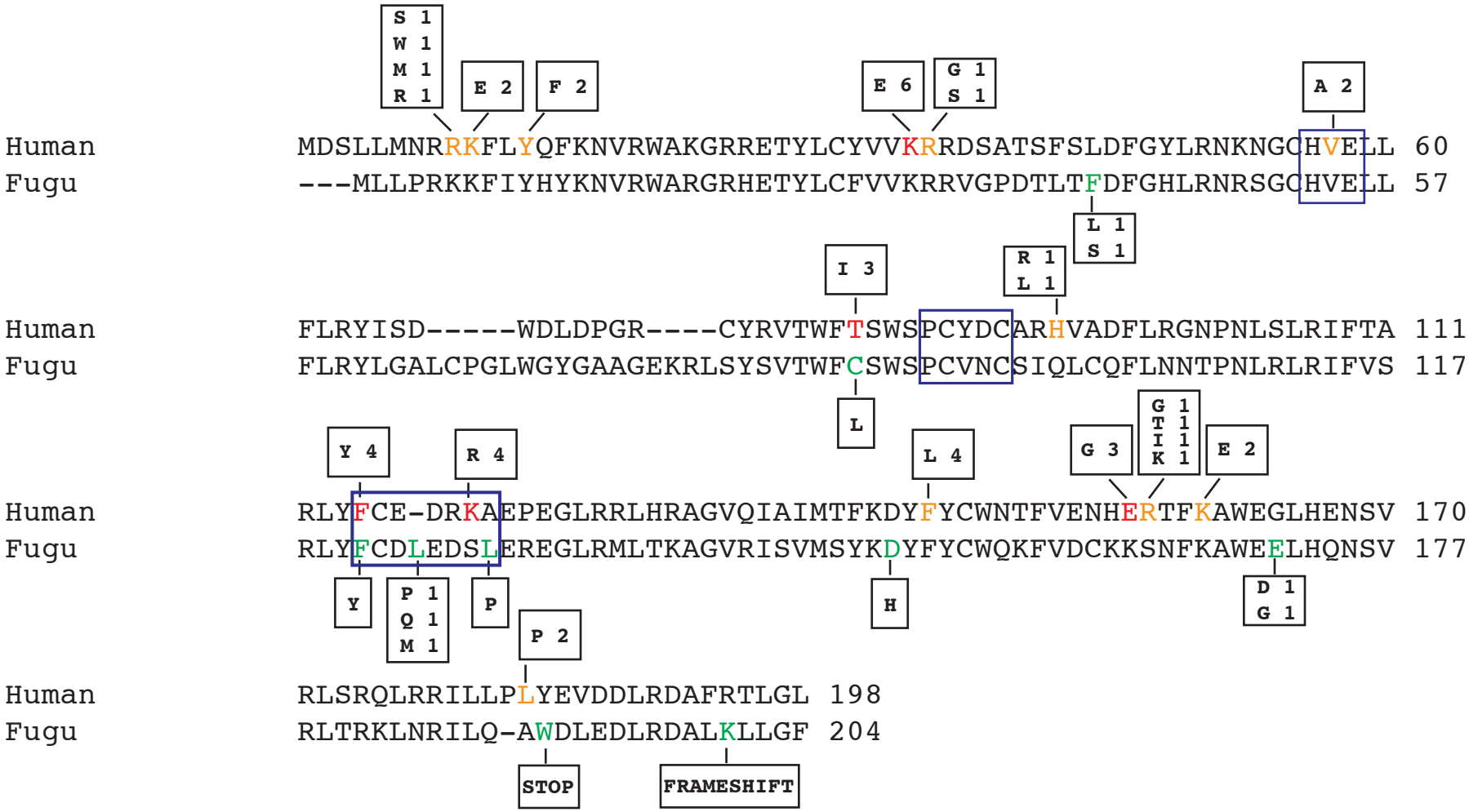
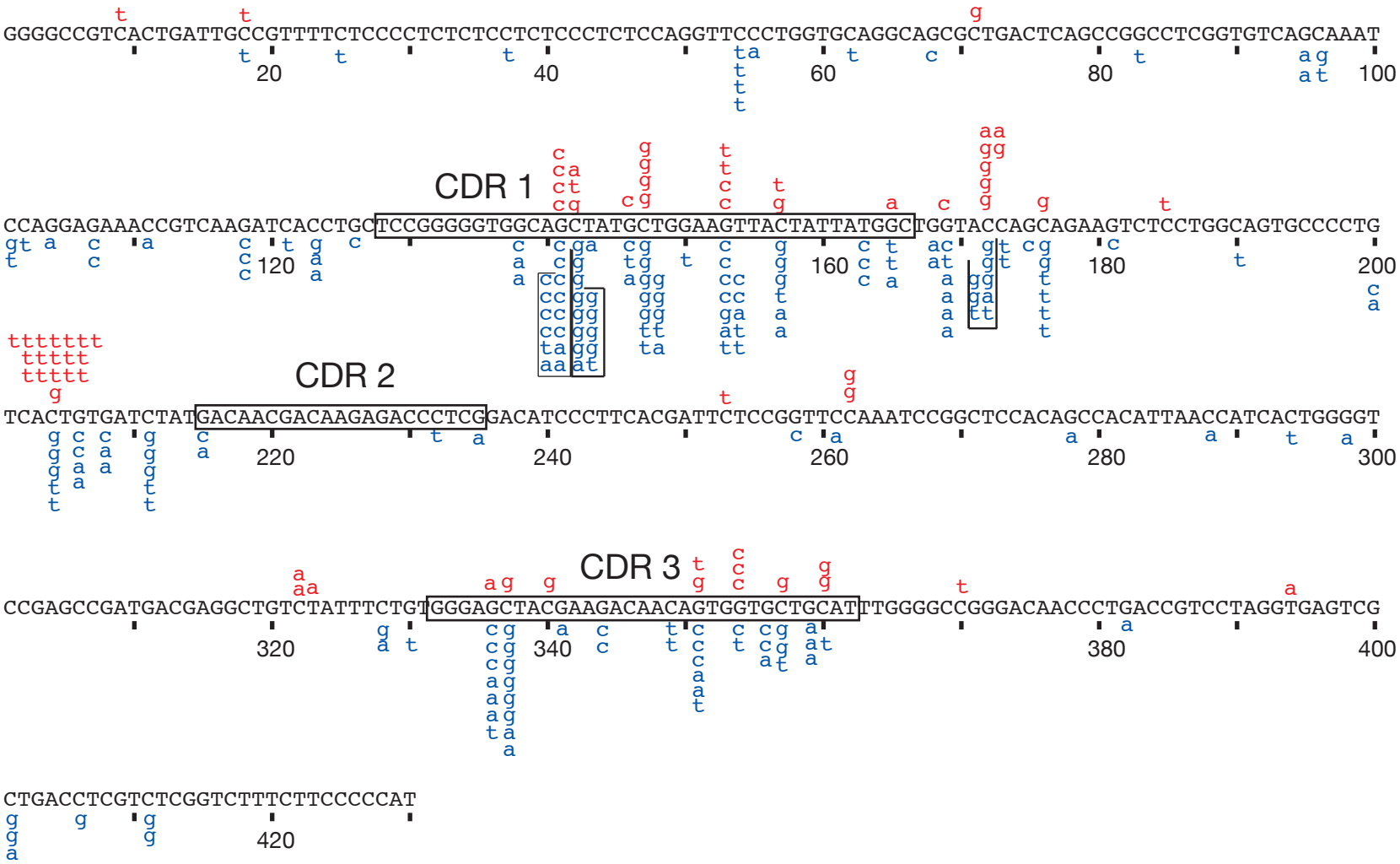




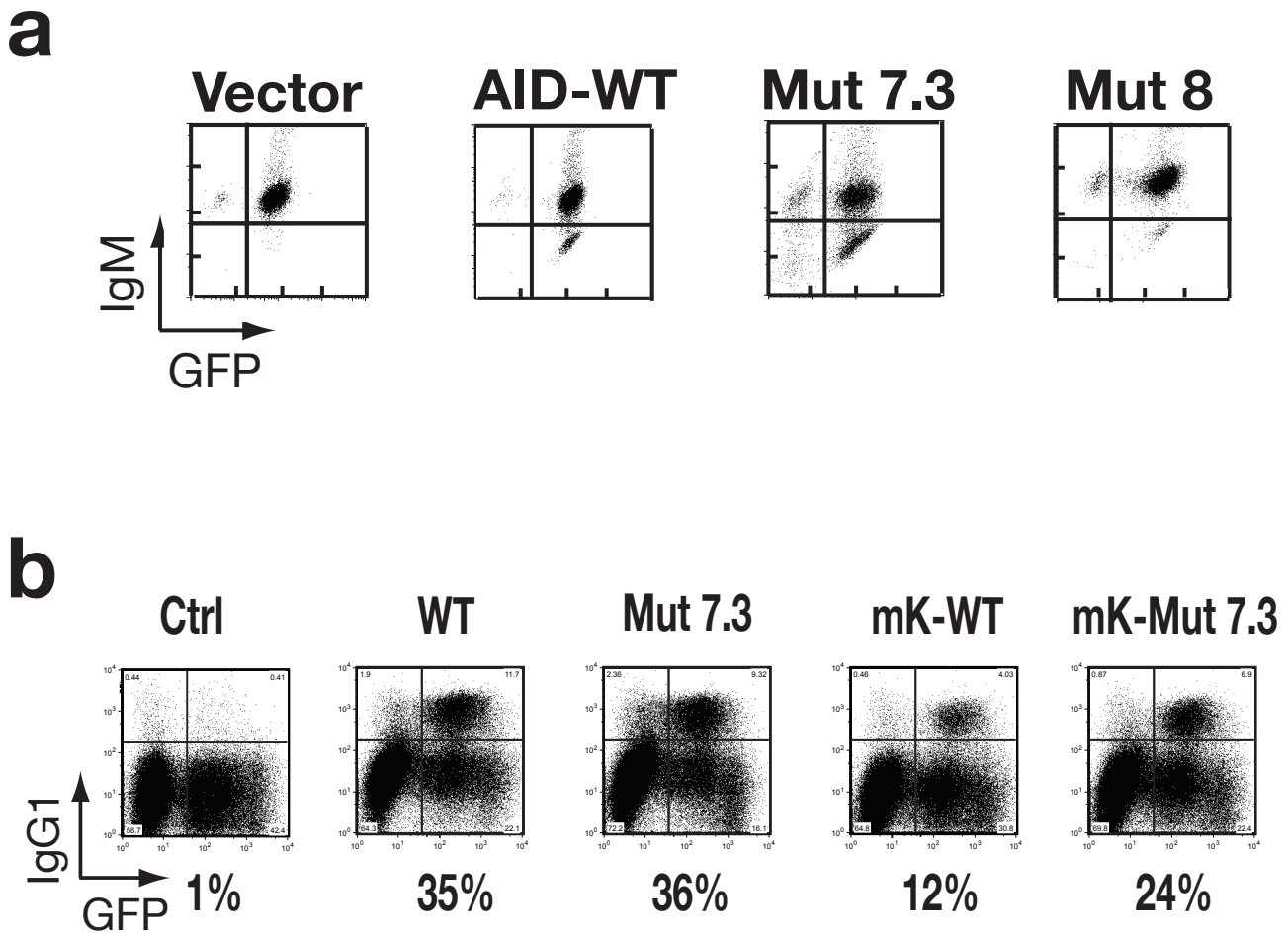
Supplementary Figure 1 Kinetic analysis of human GST-AID upmutants. Deaminase activity of GST-fusions of wild type AID and of upmutants Mut1.1 and Mut7.3 was assayed on various concentrations of the single-stranded oligodeoxy-ribonucleotide substrate as indicated and as described under Methods. Michaelis-Menten kinetic analysis was performed using Graphpad Prism software. The deduced values of K_M for the wild type, Mut1.1 and Mut7.3 fusion proteins were 80, 80 and 100 nM respectively.



Supplementary Figure 2 Comparison of human and fugu AID upmutations.
Human and fugu AID primary sequences are aligned using ClustalW2 (www.ebi.ac.uk/Tools/clustalw2/). The human AID upmutations are highlighted in red and orange as described in Figure 3a. The fugu AID upmutations are in green, having been identified either because they constitute the sole mutation in a fugu upmutant or because the residue was mutated in multiple fugu upmutants. The nature of the substitutions are indicated in boxes above or below the highlighted residues as in Figure 3a. The zinc-coordination motifs (HVE, PCYDC) and regions of suggested polynucleotide contact (FCEDRKA) are highlighted by a blue box.



Supplementary Figure 3 Distribution of mutations in IgVλ sequences determined from AID-WT and AID-Mut7.3 DT40 transfectants. The consensus IgVλ sequence was determined from parental AID$^{-/-}$ ψV$^{-/-}$ sIgM^{+} DT40 cells. Complementarity determining region (CDR) sequences are highlighted with a box. Lower case letters above and below the consensus sequence show the nucleotide substitutions in IgVλ sequences from sIgM^{-} sorted cells, with those from AID-WT transfectants in red and AID-Mut7.3 in blue. For both AID-WT and AID-Mut7.3 transfectants, the Vλ sequences presented are in each case a compilation of sequences obtained from two separate pools of sIgM^{-} sorted cells. Mutated sequences from individual pools of cells that contained identical sets of mutations were removed from the analysis both here and in the Pie chart in Fig. 5b to avoid repeat counting of the same mutation.



Supplementary Figure 4 Flow cytometry plots of the activity of AID upmutants in antibody diversification. (**a**) Representative plots analysing the surface IgM loss of AID$^{-/-}$ ψV$^{-/-}$ sIgM^{+} DT40 cells stably transfected with constructs co-expressing the indicated AID mutants together with GFP. (**b**) Representative plots showing switching to IgG1 by AID-deficient B cells that have been transduced with GFP-(WT/Mut7.3) and cultured in LPS+IL4. 'mK' indicates a mutated Kozak sequence preceding the coding sequence of AID in the vector, so as to reduce the extent of AID overexpression.

Supplementary Table 1. Mammalian *A3* and *AID* sequences used to generate Figure 7.

Species	Common name	Protein	Accession number/Ensembl ID
Homo sapiens	Human	A3A (A3Z1)	NM_145699
Homo sapiens	Human	A3B (A3Z2-Z1)	NM_004900
Homo sapiens	Human	A3C (A3Z2)	NM_014508
Homo sapiens	Human	A3DE (A3Z2-Z2)	NM_152426
Homo sapiens	Human	A3F (A3Z2-Z2)	NM_145298
Homo sapiens	Human	A3G (A3Z2-Z1)	NM_021822
Homo sapiens	Human	A3H (A3Z3)	NM_181773
Macaca mulatta	Macaque	A3A (A3Z1)	ENSMMUG00000019046
Macaca mulatta	Macaque	A3B (Z2Z1)	XM_001117049
			XM_001117028
Macaca mulatta	Macaque	A3C (A3Z2)	NM_001114359
Macaca mulatta	Macaque	A3DE (A3Z2-Z2)	XM_001094328
Macaca mulatta	Macaque	A3F (A3Z2-Z2)	NM_001042373
Macaca mulatta	Macaque	A3G (A3Z2-Z1)	XM_001094452
Macaca mulatta	Macaque	A3H (A3Z3)	XM_001096739
Bos taurus	Cow	A3Z1	EU864534
Bos taurus	Cow	A3Z2	EU864535
Bos taurus	Cow	A3Z3	EU864536
Ovis aries	Sheep	A3Z1	EU864541
Ovis aries	Sheep	A3Z2	EU864542
Ovis aries	Sheep	A3Z3	EU864543
Sus scrofa	Pig	A3Z2	EU864539
Sus scrofa	Pig	A3Z3	EU864540
Tayssu tajacu	Peccary	A3Z2-Z3	EU864537
Equus caballus	Horse	A3Z1	XM_001499871
Equus caballus	Horse	A3Z2	XM_001501833
Equus caballus	Horse	A3Z3	XM_001501833
Felis catus	Cat	A3Z2-Z3	EF173021
Canis lupus	Dog	A3Z1	XM_847690
Canis lupus	Dog	A3Z2	AACN010393938
Canis lupus	Dog	A3Z3	XM_538369
Mus musculus	Mouse	A3Z2-Z3	NM_030255
Rattus norvegicus	Rat	A3Z2-Z3	NM_001033703
Homo sapiens	Human	AID	NM_020661
Macaca mulatta	Macaque	AID	XM_001113641
Bos taurus	Cow	AID	NM_001038682
Ovis aries	Sheep	AID	EE793762
Sus scrofa	Pig	AID	BP157753
Tayssu tajacu	Peccary	AID	EU864538
Equus caballus	Horse	AID	XM_001493186
Felis catus	Cat	AID	ENSFCAG00000006052
Canis lupus	Dog	AID	NM_001003380
Mus musculus	Mouse	AID	NM_009645
Rattus norvegicus	Rat	AID	XM_001060382

Macaque A3A and Cat AID use Ensembl IDs.