Supplementary Material

Intrinsic restriction activity by apolipoprotein B mRNA editing enzyme APOBEC1 against the mobility of autonomous retrotransposons

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Supplementary Figure Legends

Supplementary Figure S1. (A) Inhibition of L1 retrotransposition by A1 proteins. 293T cells were cotransfected with 0.5 μg of the expression plasmids for APOBEC family proteins and 1.5 μg of L1 retrotransposition indicator construct $pL1_{RP}$-EGFP. After 24 h, cells were subjected to puromycin (1.0 μg/ml) selection. After 7-9 days of puromycin selection, GFP expression within the transfected 293T cells were analyzed on flow cytometry. For subsequent comparative analysis, a window was drawn encompassing all GFP-positive cells, and the percentage of these cells was calculated. The level of GFP MFI detected within the GFP-positive windows are also indicated. The data are representative of those from three independent experiments. (B) Effect of rat and rabbit A1 subcellular localization mutants on L1 retrotransposition. 293T cells were cotransfected with 0.5 μg of the expression plasmids for HA-tagged rat and rabbit A1 (wild-type) or their subcellular localization mutants (P29T) along with 1.5 μg of $pL1_{RP}$-EGFP. EGFP-based L1 retrotransposition assay was performed as described

above, except for that the amounts of rat A1 wild-type and P29T plasmids was varied as indicated. The data is representative of those from three independent experiments.

Supplementary Figure S2. Sequence analysis of HIV-1 genomic RNA in the presence of rabbit A1 WT or N57A mutant. The 293T cells were transfected with HIV-1 *Δvif* pNL4-3 Luc E^-R^- together with one of the expression vectors encoding rabbit A1 WT (A) or N57A mutant (B) using Effectene® (Qiagen)**.** The cells were harvested 48 h posttransfection, and the total RNA was prepared using QIAamp RNA Mini Kit (Qiagen) and the viral genomic RNA was converted to cDNA *in vitro* using a High Capacity cDNA Archive kit with random primers, subsequent to the treatment with DNase. A 408-bp of the *Δvif* NL-Luc *pol* region was amplified with the high-fidelity DNA polymerase (Takara) and amplified fragments were subsequently gel purified, then cloned into pCR-Blunt vector (Invitrogen), and sequenced. Clone designation is shwn to the left. Sequences are aligned with respect to the parental sequence, and only differences are shown. Hyphens denote gaps.

Supplementary Figure S3. Inhibition of autonemous retrotransposition by APOBEC family proteins. HeLa cells were cotransfected with the expression plasmids for APOBEC family proteins (hA3A, hA3B, hA3G, A1s from human, ferret, rabbit, hamster, rat, mouse, rabbit A1 E63Q, N57A, P29T and rat A1 P29T mutants) and the neomycin-resistant (*neo*^{r})-based human L1 pCEP4/L1mneoI/ColE1 (A), murine L1 pCMV L1Md-Gf21neoTET (B), or murine MusD pCMV Mus-6DneoTNF (C) along with pIRES-EGFP. Cells were subjected in G418 and resistant colonies counted 17 days

after transfection. These experiments are representative of the data compiled in Figure 8. Rabbit, hamster, rat and mouse A1 catalytic mutants E63A and E63Q were furthere constructed and their inhibitory activity against retrotransposition of human L1 pCEP4/L1mneoI/ColE1, or murine MusD pCMV Mus-6DneoTNF was examined (D, E).

Supplementary Figure S4. (A) A1 catalytic mutants E63A and E63Q were furthere constructed and their inhibitory activity against human L1 pCEP4/L1mneoI/ColE1 and murine MusD pCMV Mus-6DneoTNF retrotransposition indicators were verified using *neo*^{r}-based retrotransposition assay described in Supplementary Figure S3. Respective A1-catalytic mutants were amplified using oligonucletide primers as below. Hamster A1 E63A, forward; 5'- AGC AGA CAC GTG GCG ATC AAC TTC-3'and reverse; 5'-GAA GTT GAT CGC CAC GTG TCT GCT-3'. Hamster A1 E63Q, forward; 5'-AGC AGA CAC GTG CAG ATC AAC TTC A-3' and reverse; 5'-TGA AGT TGA TCT GCA CGT GTC TGC T-3'. Rat A1 E63A, forward; 5'-AAC AAA CAC GTT GCA GTC AAT TTC ATA GAA-3' and reverse; 5'-TTC TAT GAA ATT GAC TGC AAC GTG TTT GTT-3'. Rat A1 E63Q, forward; 5'-ACC AAC AAA CAC GTT CAA GTC AAT TTC ATA-3' and reverse; 5'-TAT GAA ATT GAC TTG AAC GTG TTT GTT GGT-3'. Mouse A1 E63A, forward; 5'-AGC AAC CAC GTT GCA GTC AAC TTC TTA-3' and reverse; 5'-TAA GAA GTT GAC TGC AAC GTG GTT GCT-3'. Mouse A1 E63Q, forward; 5'-AGC AAC CAC GTT CAA GTC AAC TTC TTA GAA-3' and reverse; 5'-TTC TAA GAA GTT GAC TTG AAC GTG GTT GCT-3'. The amplified products were cloned into pCAGGS vector. HeLa cells were

cotransfected with 0.5 μg of the expression plasmids for APOBEC family proteins and 1.5 μg of human L1 pCEP4/L1mneoI/ColE1 or murine MusD pCMV Mus-6DneoTNF. Retrotransposition frequency was calculated as the number of *neo*^{r} clones/transfection efficiencies (persent of GFP^{+} cells). Relative retrotransposition frequency in the absence of APOBEC proteins (vector) was set as 1.0. The histogram bars represent the mean of three independent cultures, and the standard deviation is shown.

(B) Western blot analysis was performed by using extracts from HeLa cells transfected by the expression plasmids for the indicated APOBEC family proteins and detected by using antibodies specific for the epitopes present in the test proteins.

Supplementary Figure S5. Hyperediting of MusD genome by rat and rabbit A1s. HeLa cells were cotransfected with 1.5 μg of MusD-neo^{R} and 0.5 μg of respective pCAGGS-APOBEC expression plasmids. 72h later, total cellular DNA was isolated from the transfected HeLa cells and then amplified by nested PCR with primers designed for spliced neo^{R} gene (461bp) using Ex taq (TAKARA). PCR reaction was 94°C for 1 min, followed by 30 cycles (98°C for 10 sec, 68°C for 30 sec) and finally for 7min at 68°C. At the same time, the second PCR products from the MusD neo^{R} genes were subjected to 3D-PCR amplification. The PCR parameter was 94°C for 1 min, followed by 30 cycles (90°C for 10 sec, 68°C for 30 sec) and finally for 7min at 68°C. The primer sequences are as follows. Outer primer set: forward, neo F3; 5'-ATT GAA CAA GAT GGA TTG CAC GCA-3' and reverse, neo R1; 5'-CAG TTC GGC TGG CGC GAG CC-3'. Inner primer set: forward, neo F4; 5'-CGC TTG GGT GGA GAG GCT ATT C-3' and reverse, neo R2; 5'-GCG CGA GCC CCT GAT GCT-3'. The

amplified products were cloned into pGEM-T Easy vector (Peomega) and sequenced.

Supplementary Figure S6. Sequence analysis of *de novo* L1 insertions. HeLa cells were cotransfected with 1.5 μg of L1-neo^R and 0.5 μg of respective pCAGGS-APOBEC expression plasmids. 72h later, total cellular DNA was isolated from the transfected HeLa cells and then amplified by nested PCR with primers designed for spliced neo^R gene (461bp) as described above. PCR analyses were performed at 98°C. The amplified products were cloned and sequenced.

Supplementary Figure S7. PCR detection of A1 in different mouse and rabbit tissues. Primary tissues had been removed aseptically from euthanatized mouse (C57BL/6) and rabbit (Kbt: NZW). Total RNA was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA was then treated with DNase I (Takara, Japan) according to the manufacturer's instructions. The synthesis of the first strand cDNA was carried out with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) using a random primer. For the detection of A1 and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) in mouse and rabbit tissues, conventional PCR was carried out using ExTaq (Takara, Japan) according to the manufacturer's instructions, 200 ng of the cDNA was mixed with 5.0 μL of 10x PCR buffer, 200 μM of each dNTP, 1.25 U of Takara Taq. PCRs were performed on cDNA samples using 200 nM of oligonucleotide primer (Rabbit APOBEC1 376F; 5` ACCCGGGTGTGACTCTGATAATTTTTG 3`, Rabbit APOBEC1 607R; 5` GGGTGGGAGACCTAGAATGATGCAG 3`, Rabbit GAPDH 736F; 5`-GCTGAACGGGAAACTCACTGG-3`, Rabbit GAPDH 1045R; 5`-

GTCCACCACCCTGTTGCTGTA-3`, Mouse APOBEC1 652F; 5` AGCTTCGGAAAGAGACCTGTCTGC 3`, Mouse APOBEC1 994R; 5` GTCATGATCTGGATAGTCACACCGC 3`, Mouse GAPDH 570F; 5`-ACCACAGTCCATGCCATCAC-3`, Mouse GAPDH 1021R; 5`-TCCACCACCCTGTTGCTGTA-3`). PCR conditions were adjusted at 98°C for 1 min followed by 40 cycles of 94°C for 10 sec, 68°C for 1 min followed by 68°C for 7 min. In case of GAPDH only 30 cycles were used. PCR products were detected on 2% agarose gel electrophoresis. After staining with ethidium bromide, the gel was photographed.

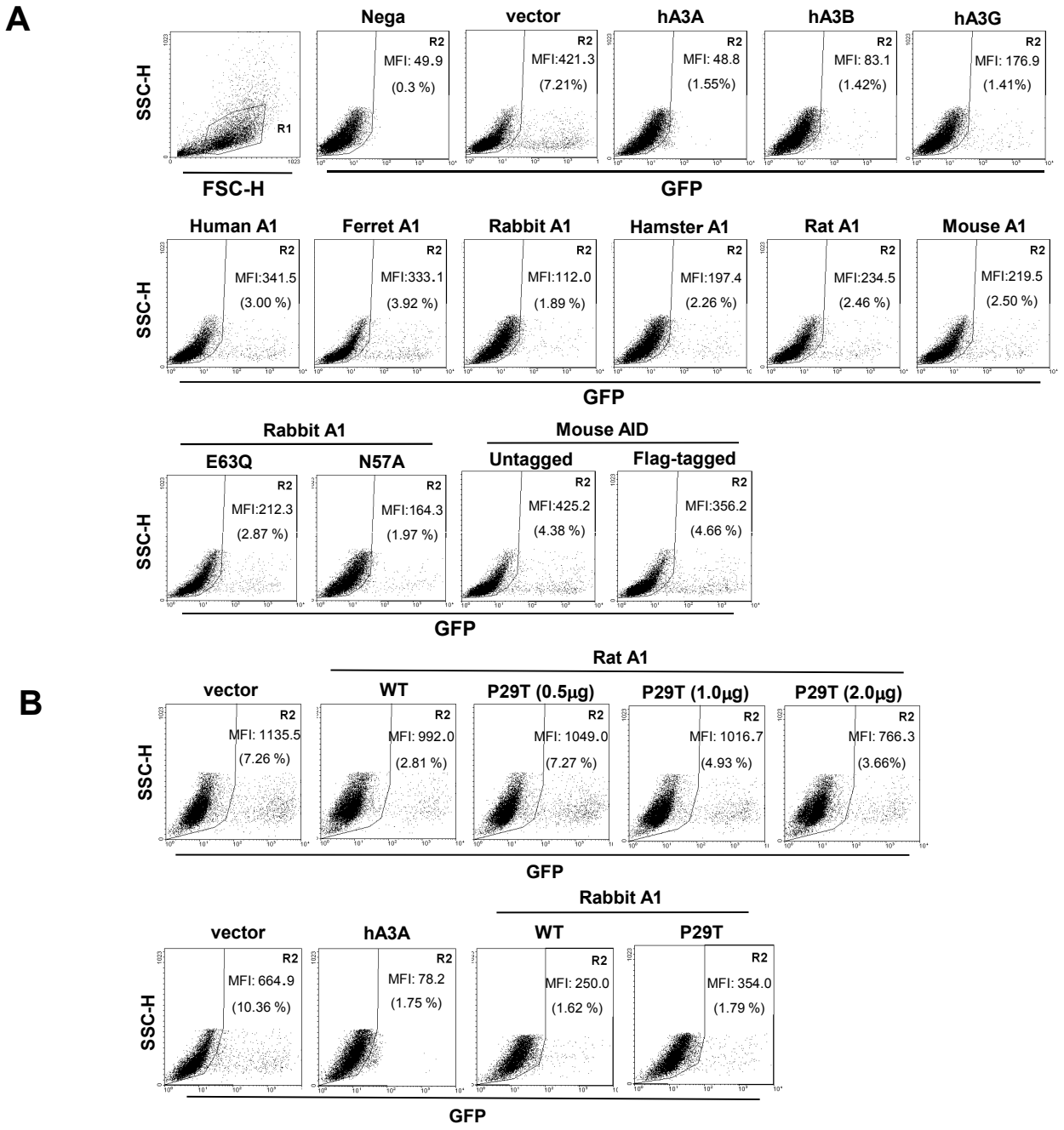
Ikeda et al. Supplementary Table S1

Rabbit A1 N57A mutant partially retained RNA editing activity

	Rabbit A1	
	WT	N57A
Clones sequenced	11	10
Total bp sequenced	4488	4080
Clones with C to T	11	5
# of C to T mutations	127	11
# of C to T mutations per 1 kp	28.30	2.70
# of Other mutations	0	0
# of deletions	0	3
# of insertions	0	0

293T cells were cotransfected with NL-luc *Δvif* and rabbit A1 wild-type (WT) or N57A mutant. Total RNAs were isolated from the 293T cells, and NL-luc *Δvif pol* gene was amplified at 85°C and sequenced.

Ikeda et al. Supplementary Figure S1

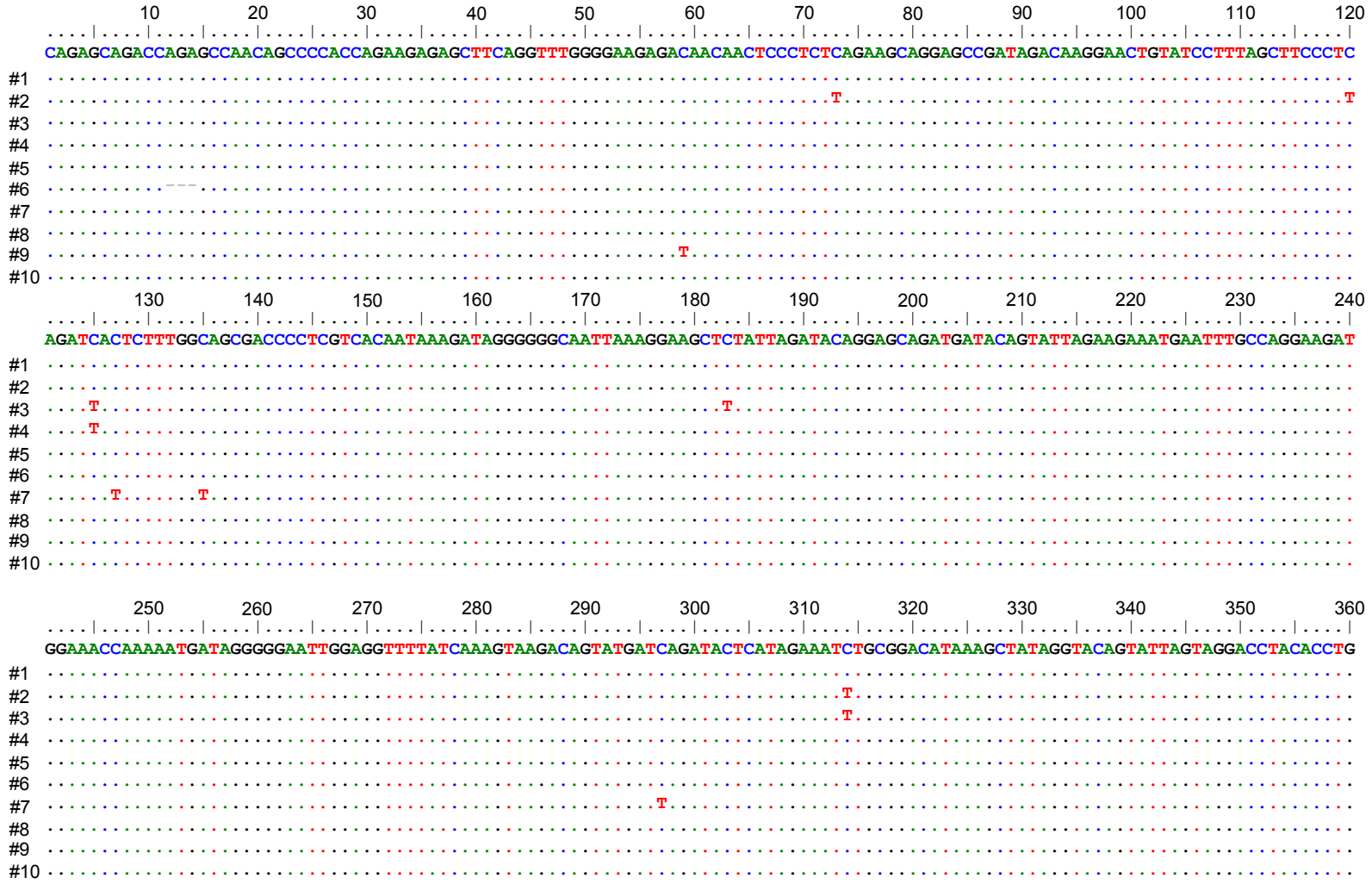


Ikeda et al. Supplementary Figure S2

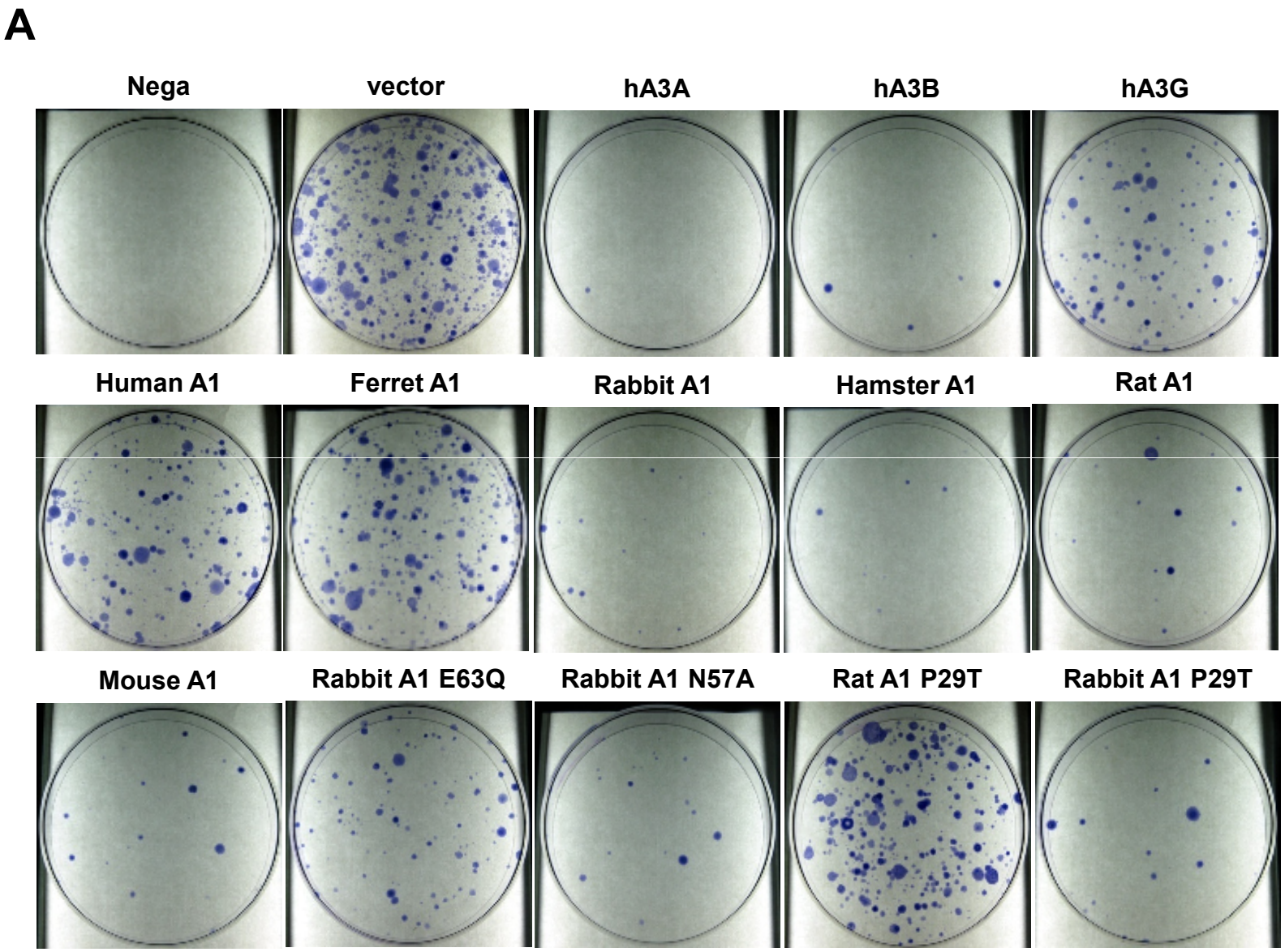
A

Ikeda et al. Supplementary Figure S2

B

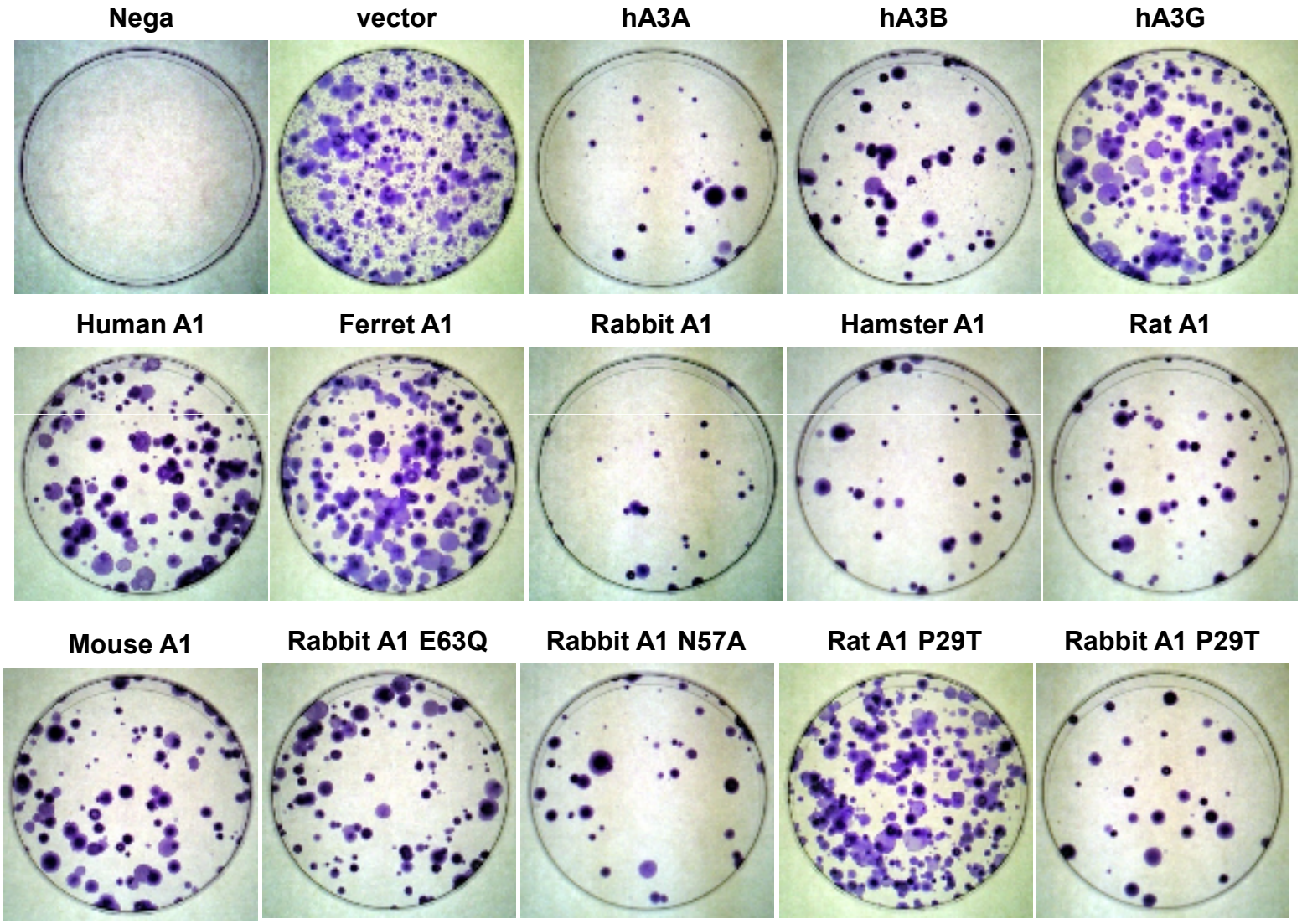


Ikeda et al. Supplementary Figure S3



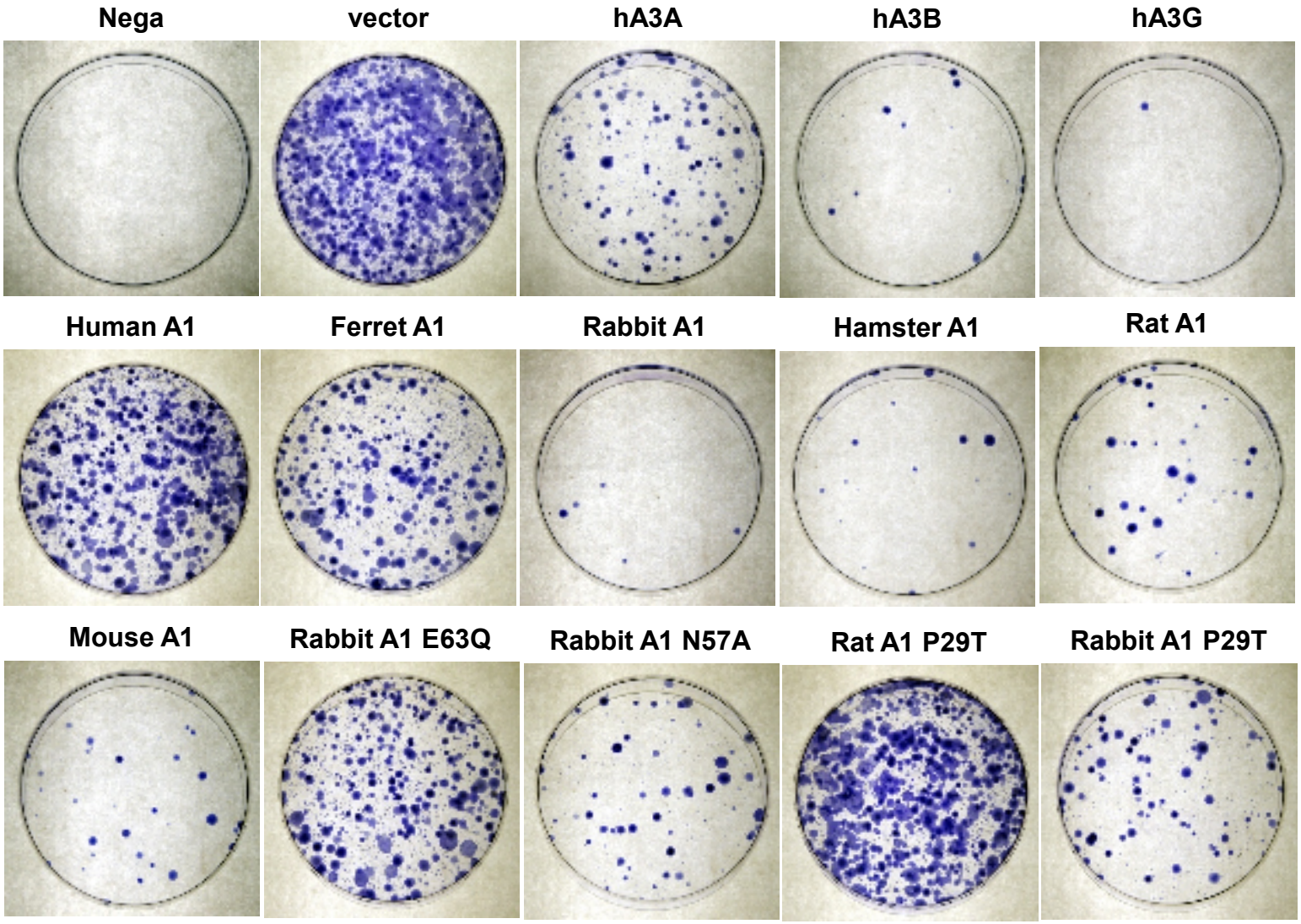
Ikeda et al. Supplementary Figure S3

B



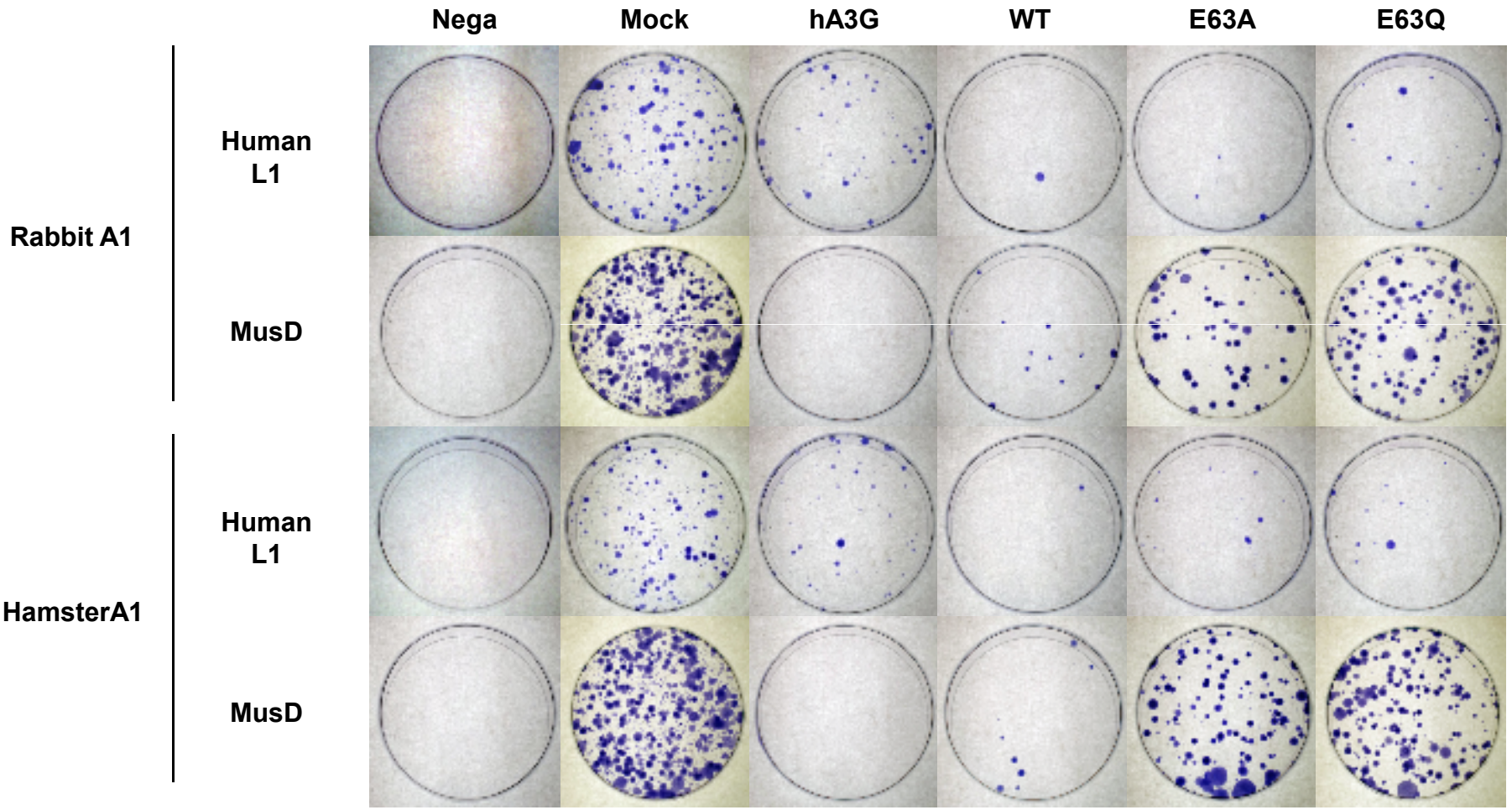
Ikeda et al. Supplementary Figure S3

C



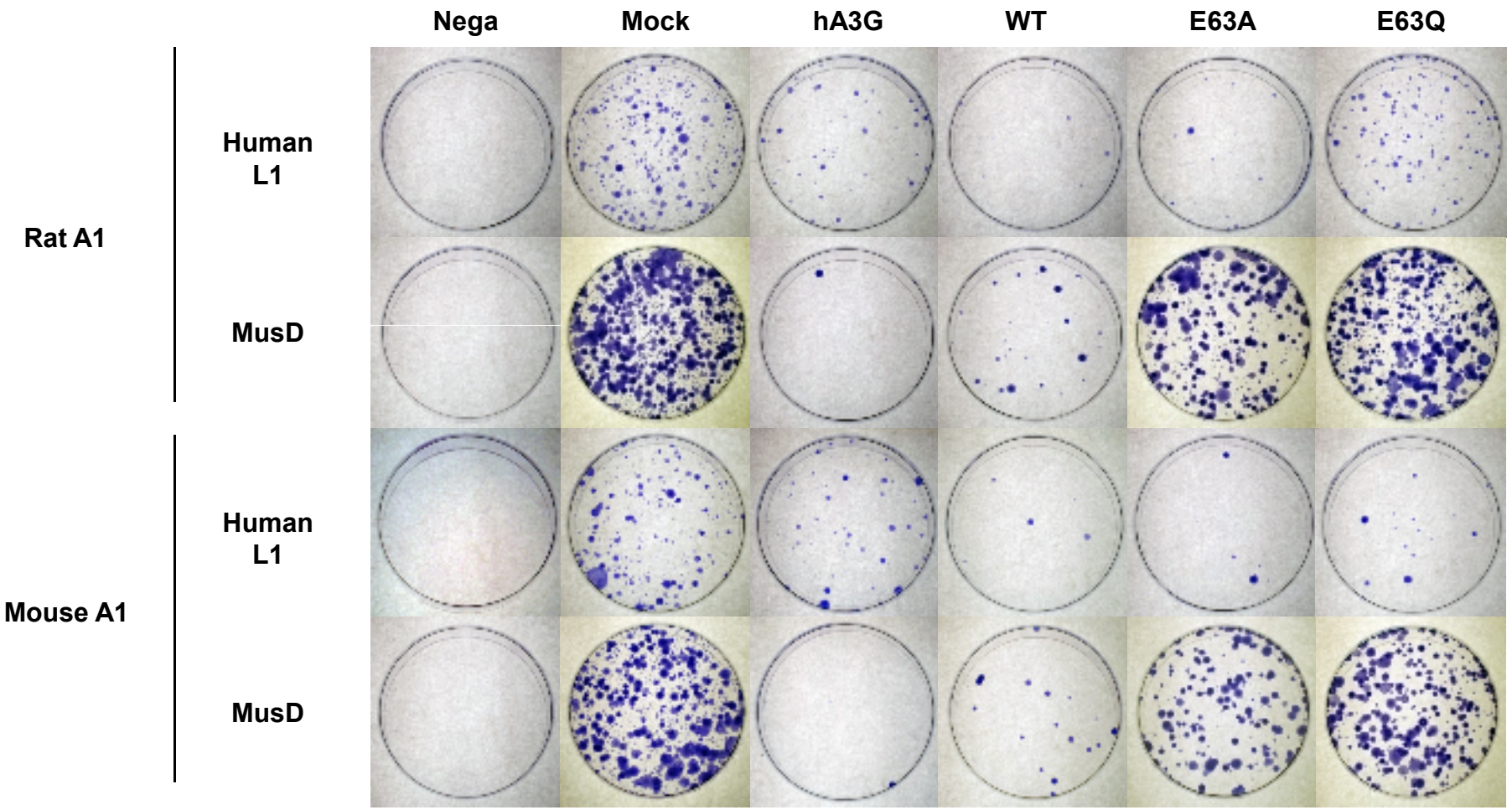
Ikeda et al. Supplementary Figure S3

D

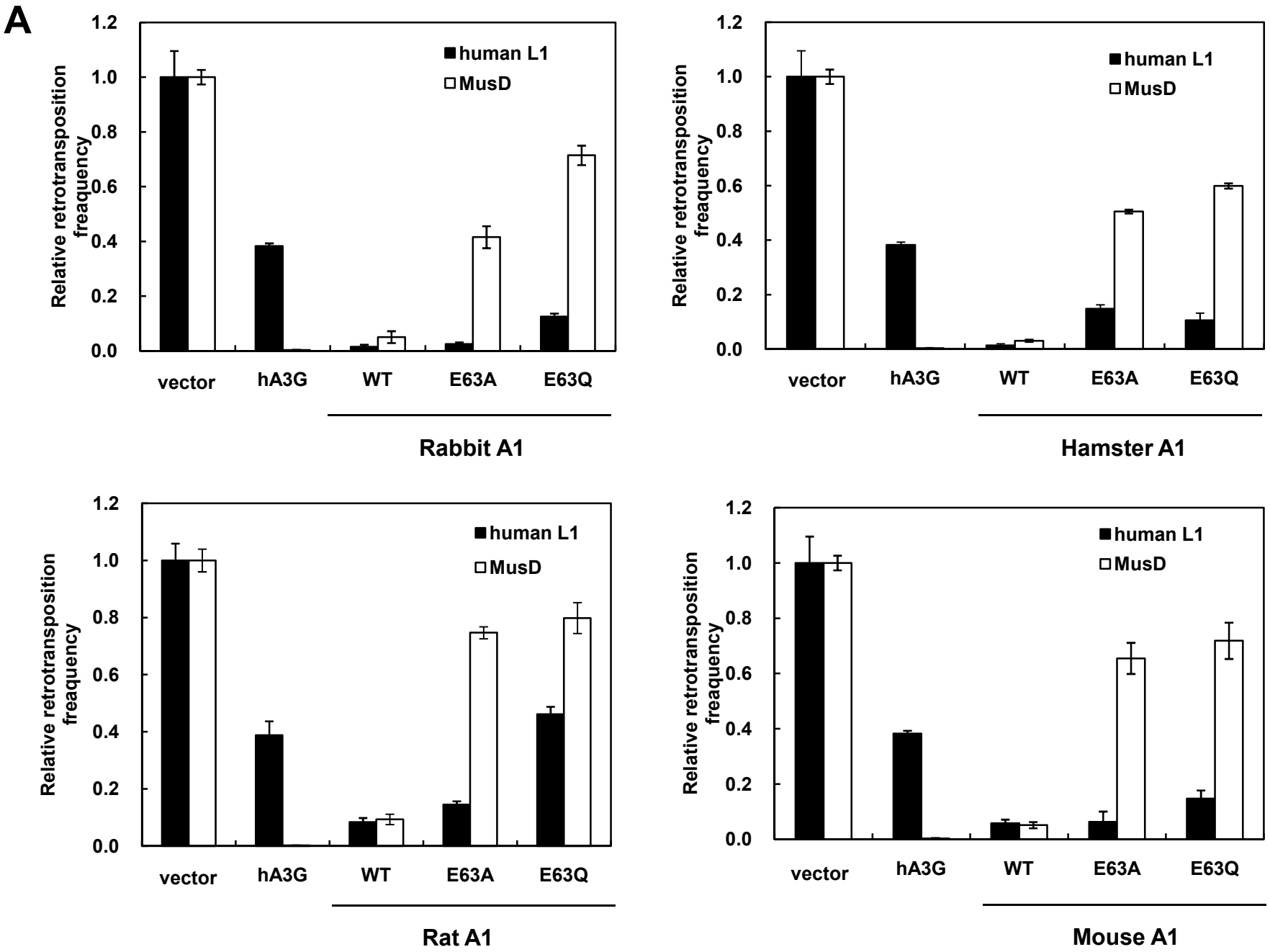


Ikeda et al. Supplementary Figure S3

E



Ikeda et al. Supplementary Figure S4



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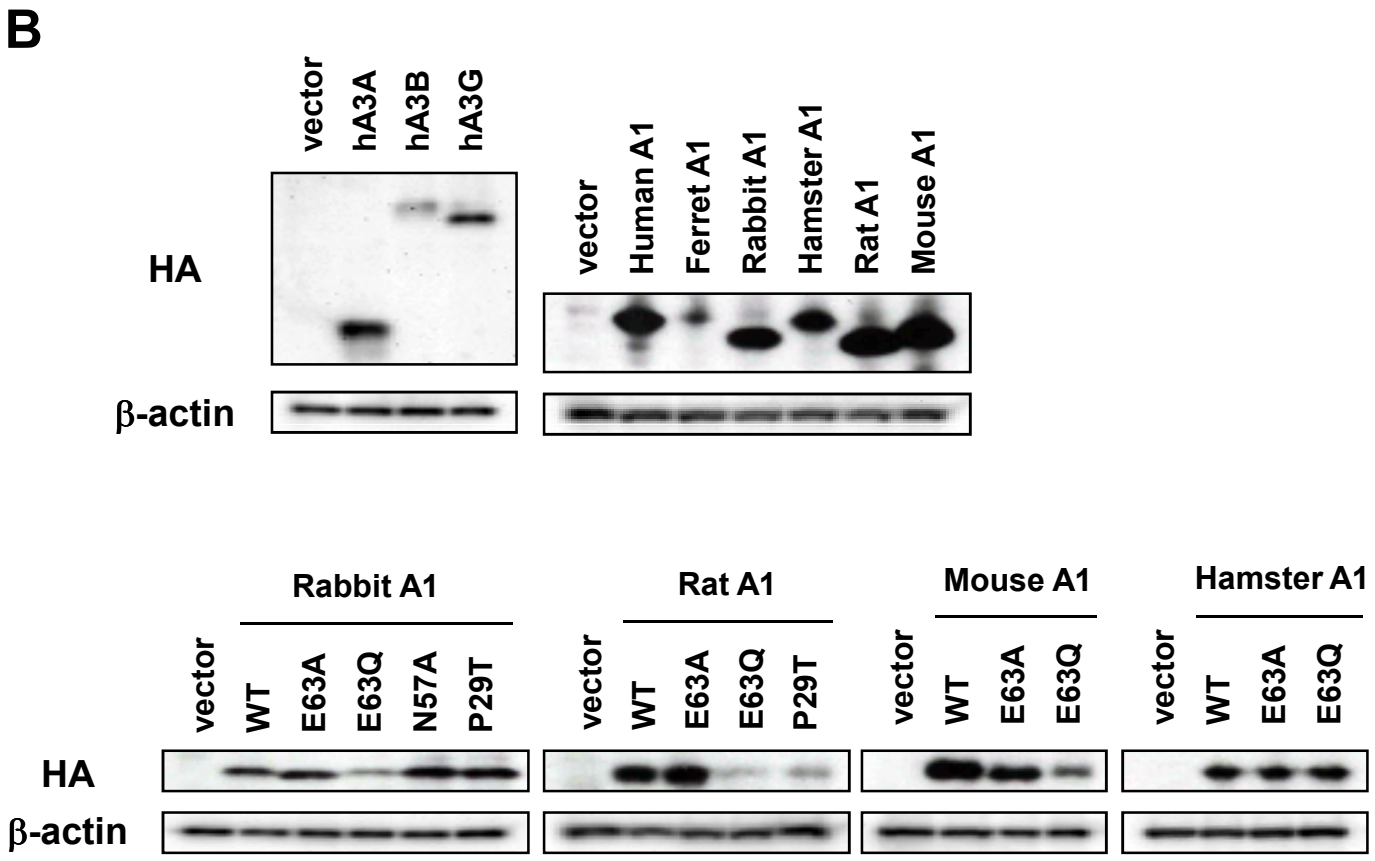
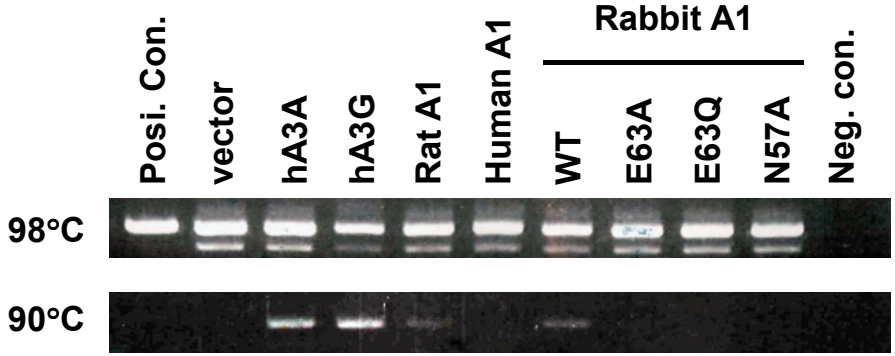


Ikeda et al. Supplementary Figure S5



hA3A

From \ To	A	C	G	T
A		0	2	0
C	0		0	**1**
G	**420**	0		0
T	0	3	0	

5532bp (n=12)

hA3G

From \ To	A	C	G	T
A		0	2	0
C	0		0	**2**
G	**483**	0		0
T	0	1	1	

5532bp (n=12)

Rat A1

From \ To	A	C	G	T
A		0	3	0
C	0		0	**1**
G	**420**	0		0
T	0	0	0	

4610bp (n=10)

Rabbit A1

From \ To	A	C	G	T
A		0	11	0
C	0		0	**13**
G	**1037**	0		3
T	0	4	1	

13830bp (n=30)

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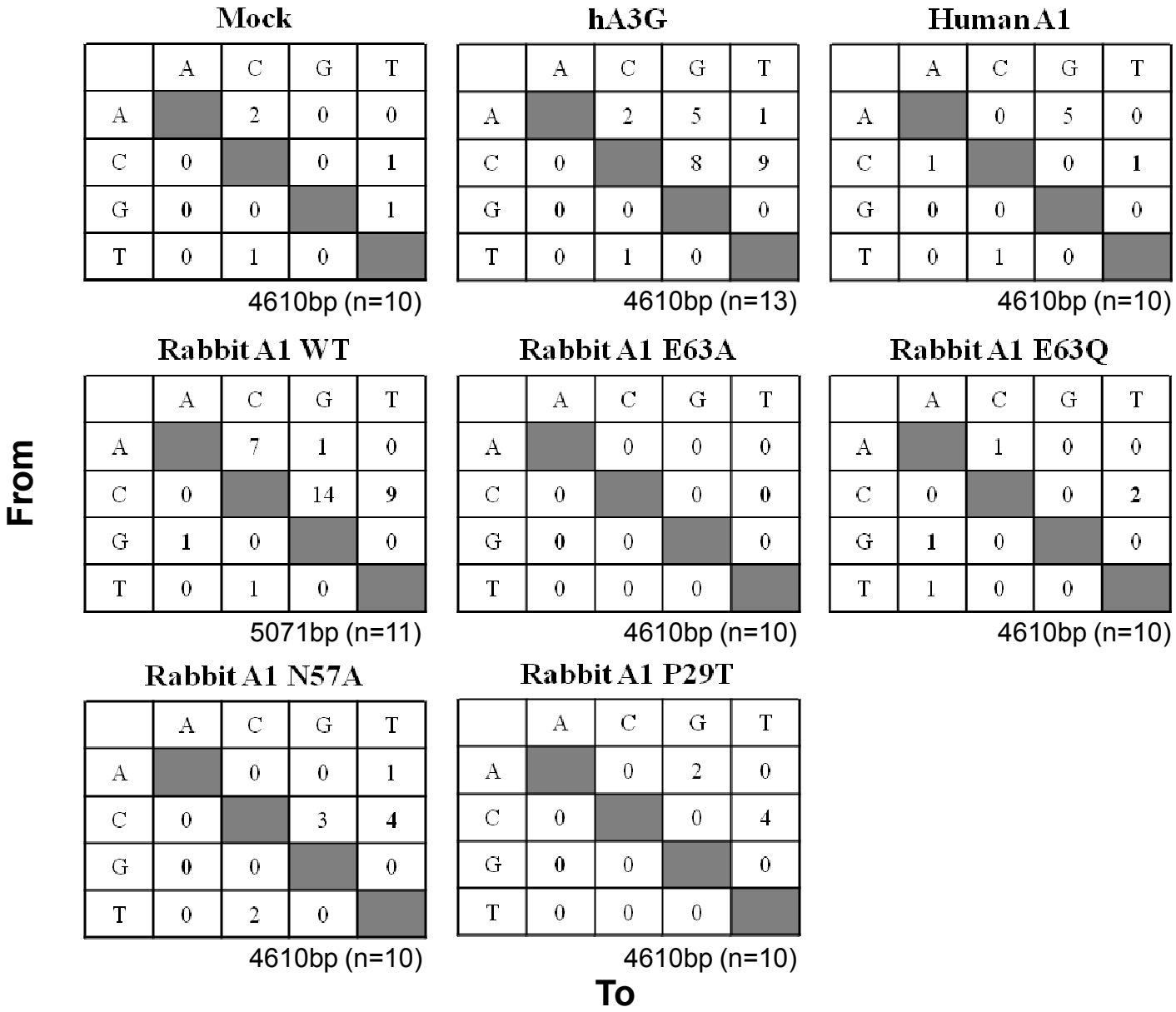

Ikeda et al. Supplementary Figure S7