*Supplementary Figures*

# A Novel Gene Delivery Vector of Agonistic Anti-Radioprotective 105 Expressed on Cell Membranes Shows Adjuvant Effect for DNA Immunization Against Influenza

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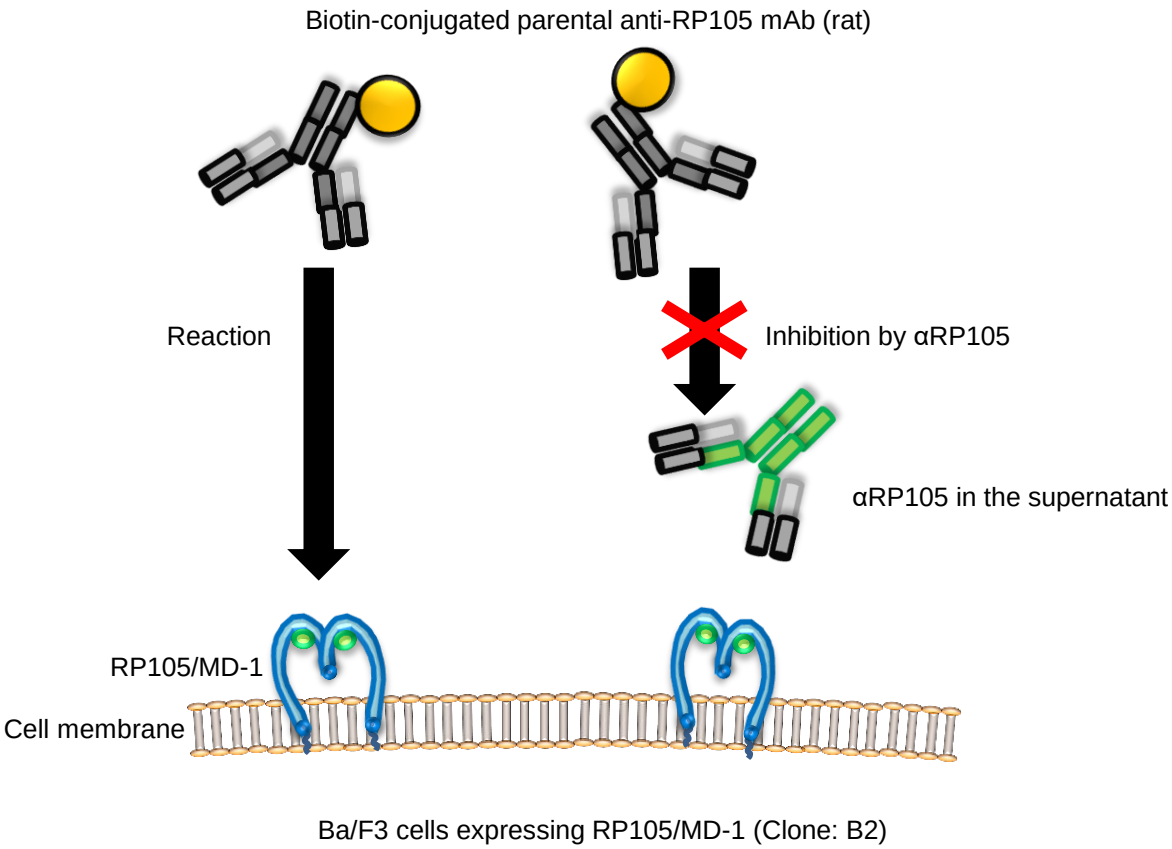
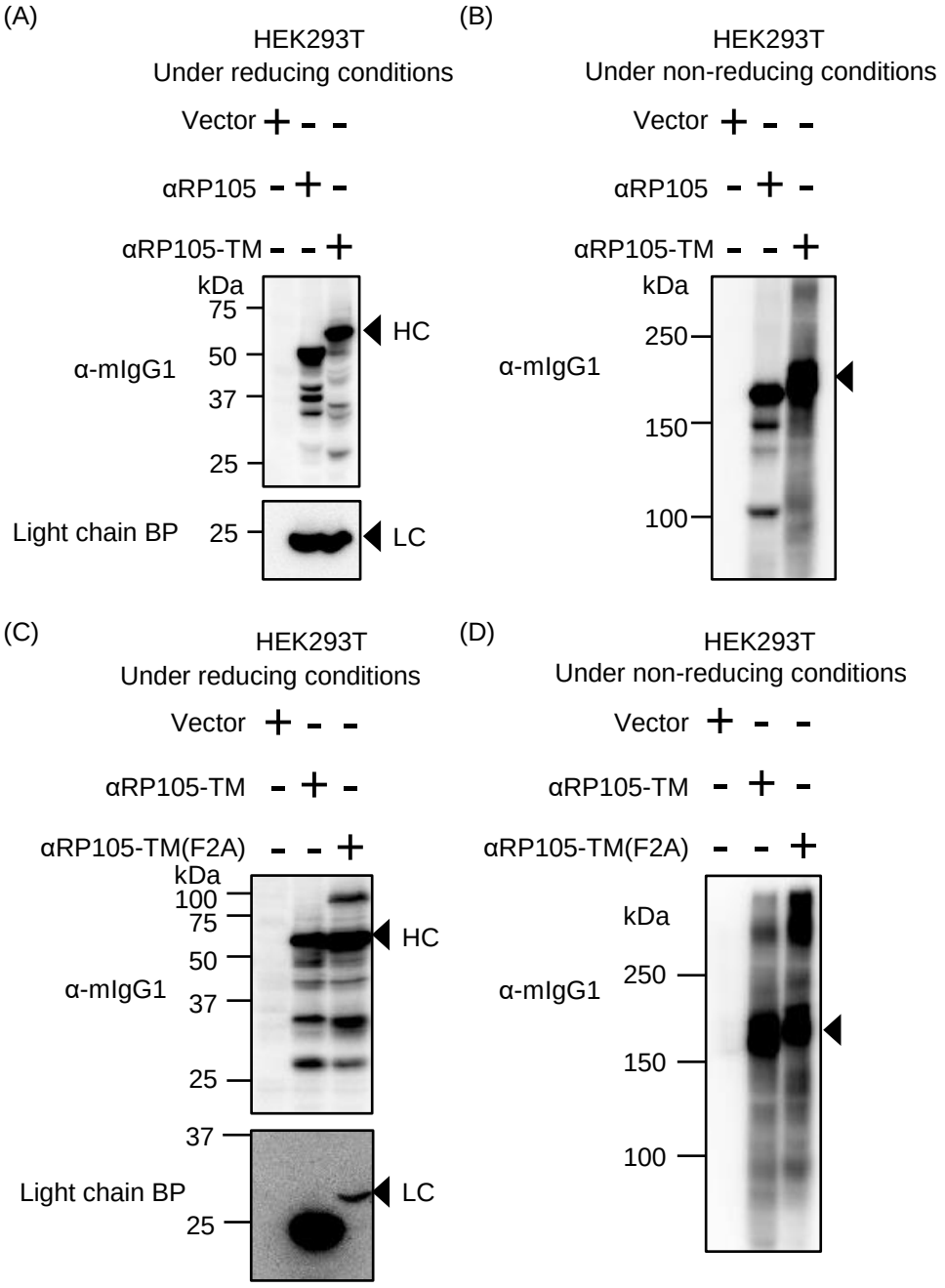
**Supplementary Figure 1**



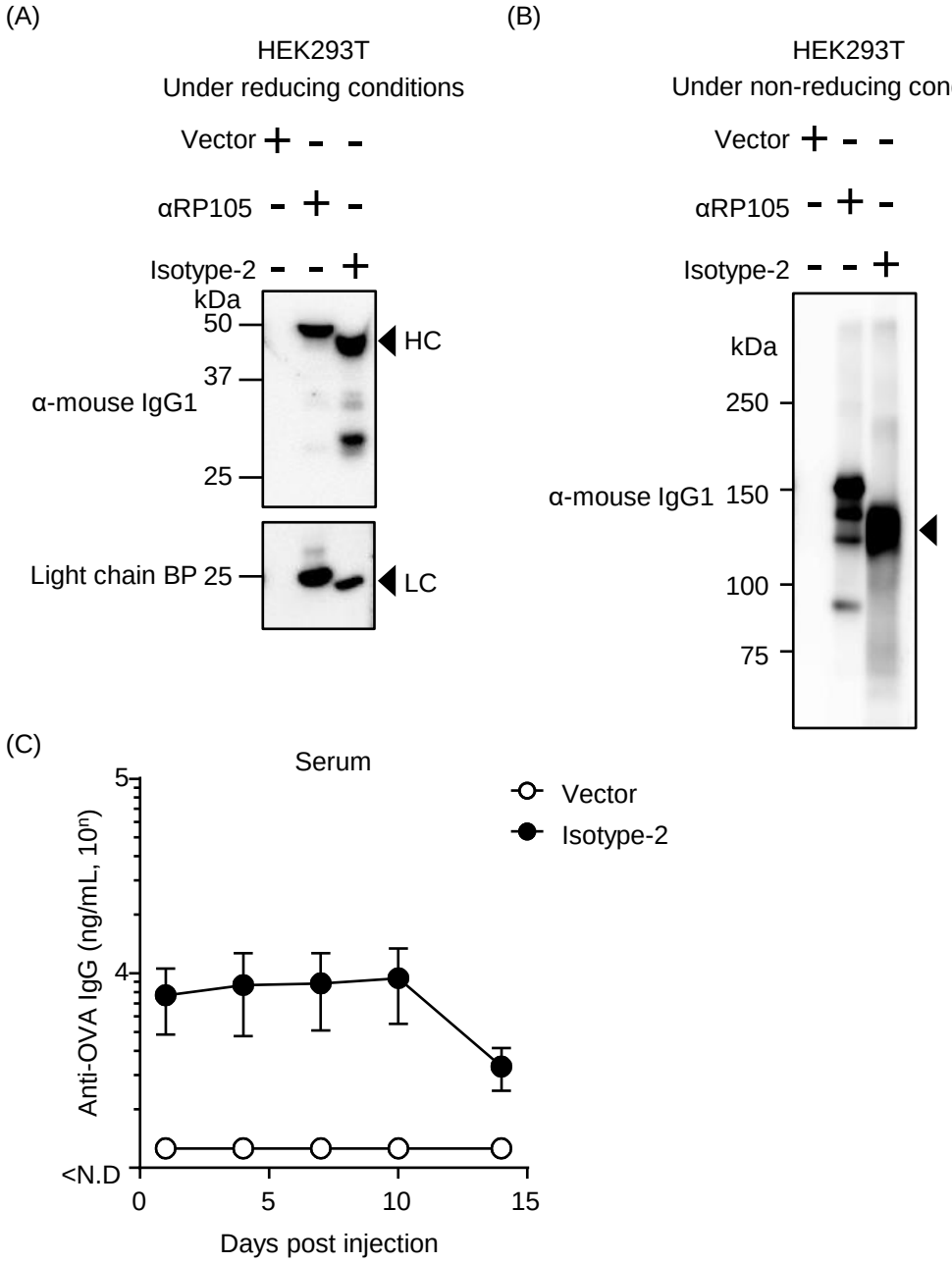

**Supplementary Figure 1| Scheme of the competitive binding assay method.** The Ba/F3 cells expressing RP105/MD-1 (Clone: B2) were incubated with the parental anti-RP105 mAb (rat) or the supernatants obtained from the HEK293T cells, followed by incubation with biotinylated parental anti-RP105 mAb (rat). The binding level was analyzed with geometric mean fluorescence (gMFI) using a BD LSRFortessa.

**Supplementary Figure 2**



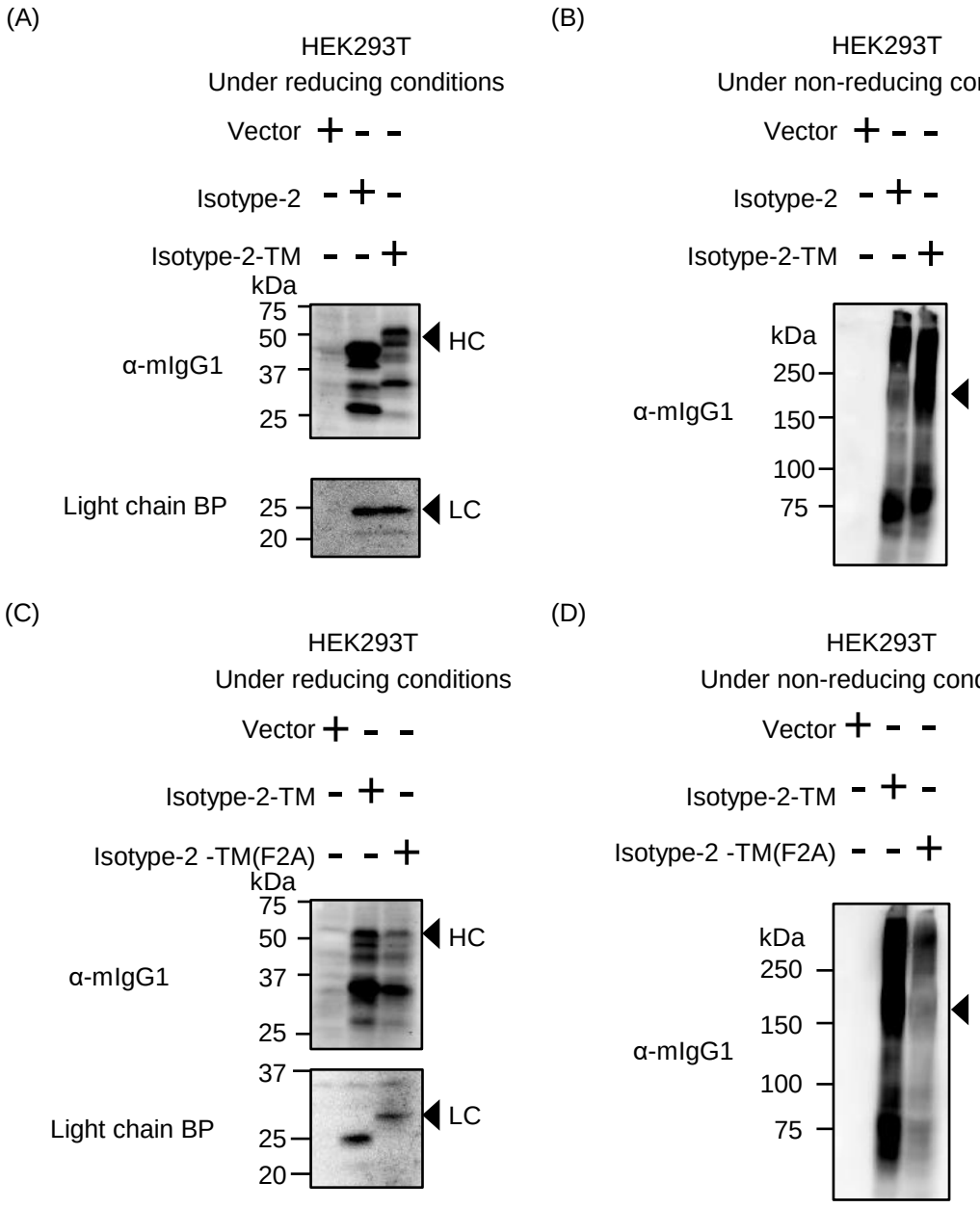

**Supplementary Figure 2| *In vitro* expression of αRP105-TM and αRP105-TM (F2A). (A, B)** HEK293T cells were transfected with pCADEST1-empty (Vector), pCADEST1-anti-RP105 mIgG1 and pCADEST1-anti-RP105 mkappa (αRP105), or pCADEST1-anti-RP105 mIgG1-TM and pCADEST1-anti-RP105 mkappa (αRP105-TM). Two days later, the cell lysates were collected and processed by western blotting under reducing **(A)** or non-reducing **(B)** conditions, followed by probing with HRP-conjugated goat anti-mouse IgG1 and HRP-conjugated light chain-binding protein (BP). **(C, D)** HEK293T cells were transfected with pCADEST1-empty (Vector), pCADEST1-anti-RP105 mIgG1-TM and pCADEST1-anti-RP105 mkappa (αRP105-TM), or pCADEST1-anti-RP105 kappa-F2A-anti-RP105 mIgG1-TM [αRP105-TM (F2A)]. Two days later, the cell lysates were collected and processed by western blotting under reducing **(C)** or non-reducing **(D)** conditions, followed by probing with HRP-conjugated goat anti-mouse IgG1 and HRP-conjugated light chain-BP. All indicated data are representative of two independent experiments.

**Supplementary Figure 3**



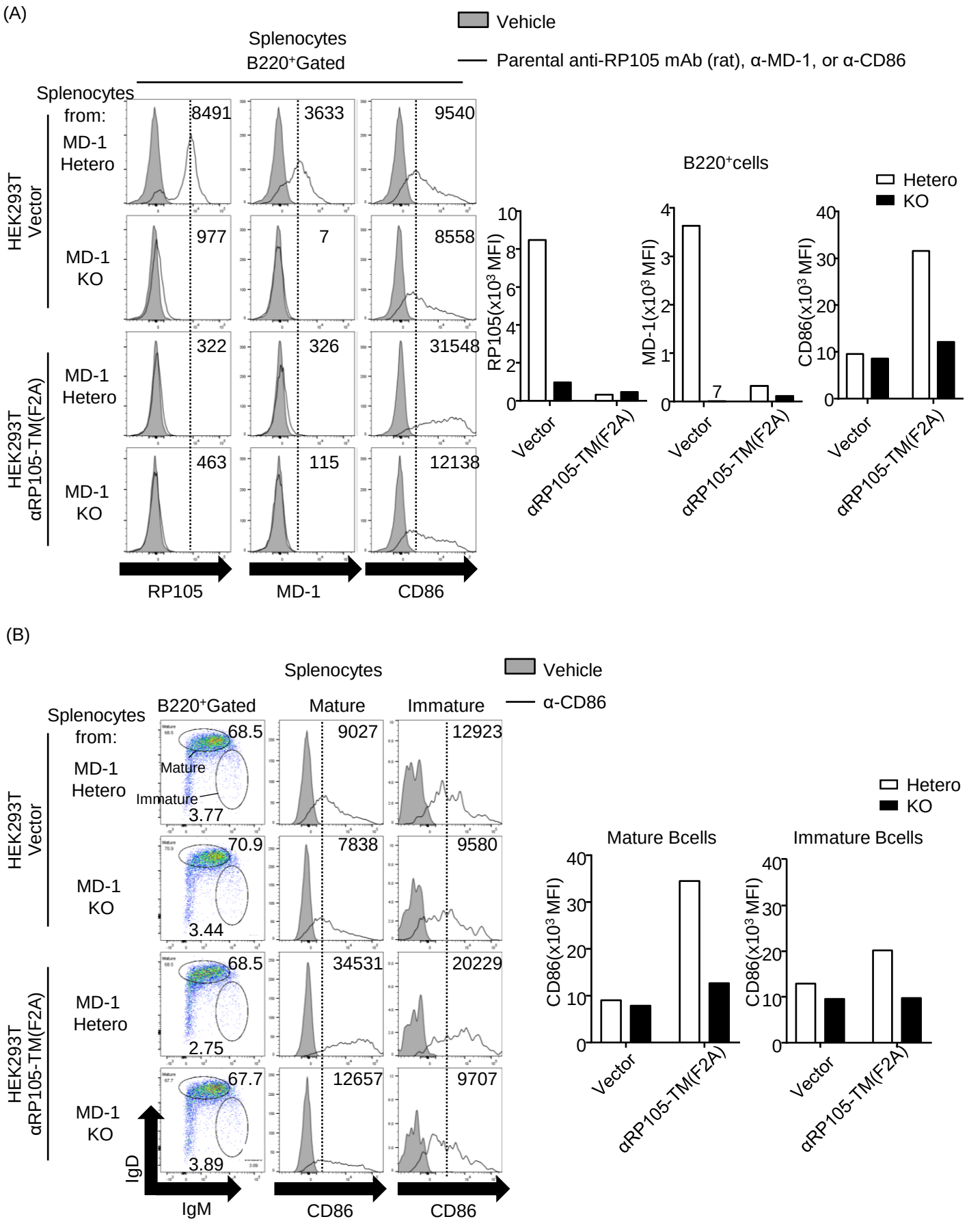

**Supplementary Figure 3| *In vitro* and *in vivo* expression of recombinant anti-OVA mIgG1 (Isotype-2). (A, B)** HEK293T cells were transfected with pCADEST1(Vector), pCADEST1-anti-RP105 mIgG1 and pCADEST1-anti-RP105 mkappa (αRP105), or pCADEST1-anti-OVA mIgG1 and pCADEST1 anti-OVA mkappa (Isotype-2). Seven days later, the supernatant was obtained and processed by western blotting under reducing **(A)** or non-reducing **(B)** conditions, followed by probing with HRP-conjugated goat anti-mouse IgG1 or HRP-conjugated light chain-binding protein (BP). **(C)** BALB/c mice (n = 5) were subjected to HD with the pCADEST1 (Vector) or pCADEST1-anti-OVA mIgG1 and pCADEST1 anti-OVA mkappa (Isotype-2). The serum was obtained at the indicated time, and the level of anti-OVA IgG was analyzed by quantitative ELISA. The detection limit was over 1230 ($=10^{3.09}$) ng/mL. Data are represented as mean ± S.D. All indicated data are representative of two independent experiments.

**Supplementary Figure 4**




**Supplementary Figure 4| *In vitro* expression of Isotype-2-TM. (A-D)** HEK293T cells were transfected with pCADEST1-empty (Vector), pCADEST1-anti-OVA mIgG1 and pCADEST1-anti-OVA mkappa (Isotype-2), pCADEST1-anti-OVA mIgG1-TM and pCADEST1-anti-OVA mkappa (Isotype-2-TM) **(A, B)**, or pCADEST1-anti-OVA kappa-F2A-anti-OVA mIgG1-TM [Isotype-2-TM (F2A)] **(C, D)** as indicated. Two days later, cells were lysed with lysis buffer containing 2% SDS and processed by western blotting under reducing **(A, C)** or non-reducing **(B, D)** conditions, followed by probing with HRP-conjugated goat anti-mouse IgG1 and HRP-conjugated light chain-binding protein (BP). All indicated data are representative of two independent experiments.

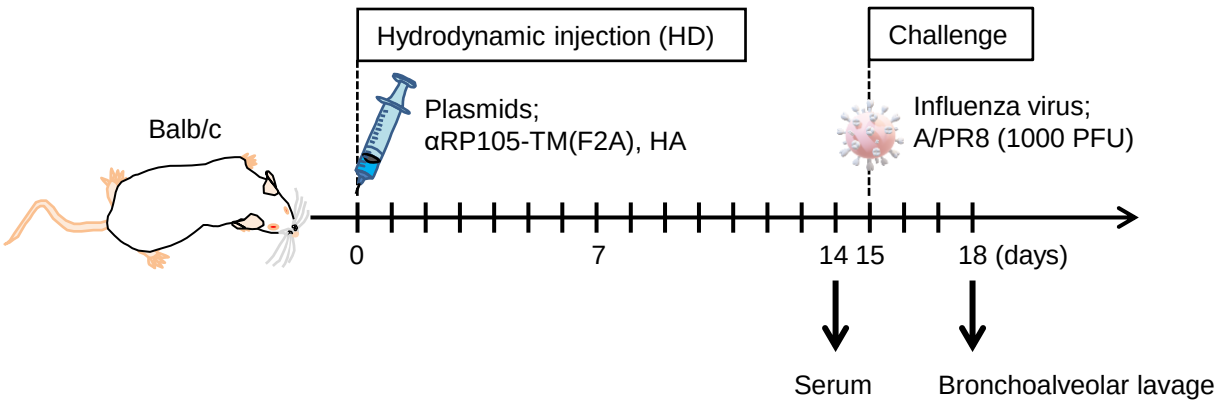
**Supplementary Figure 5**



**Supplementary Figure 5**

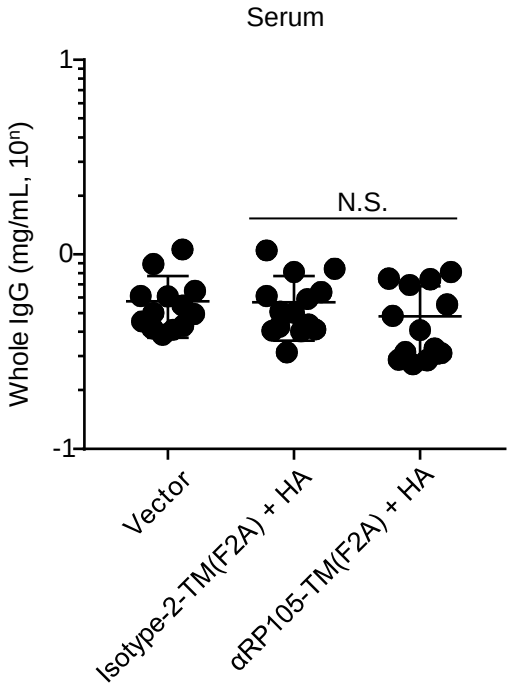
**Supplementary Figure 5| αRP105-TM can activate B cells that depends on RP105 expression on the cell membrane. (A, B)** HEK293T cells were transfected with pCADEST1-empty (Vector) or pCADEST1-anti-RP105 kappa-F2A-anti-RP105 mIgG1-TM [αRP105-TM (F2A)]. Two days later, the splenocytes obtained from MD-1-Hetero and MD-1-KO BALB/c mouse were co-cultured with HEK293T cells for two days. Then, the splenocytes, which were gated on B220 **(A)**, $IgM^{low}$ $IgD^{high}$ as mature B cells (Middle panel in **B**) or $IgM^{high}$ $IgD^{low}$ as immature B cells (Right panel in **B**), were respectively incubated with biotinylated parental anti-RP105 mAb (rat), anti-MD-1, or anti-CD86 antibodies as indicated, followed by incubation with PE-conjugated streptavidin. The indicated numbers on each gate (Left panel in **B**) respectively represent the percentage of cells of mature or immature B cells gated on $B220^{+}$ cells. The numbers in the histogram represent MFI. The expression level was analyzed using a BD LSRFortessa. All indicated data are representative of two independent experiments.

**Supplementary Figure 6**



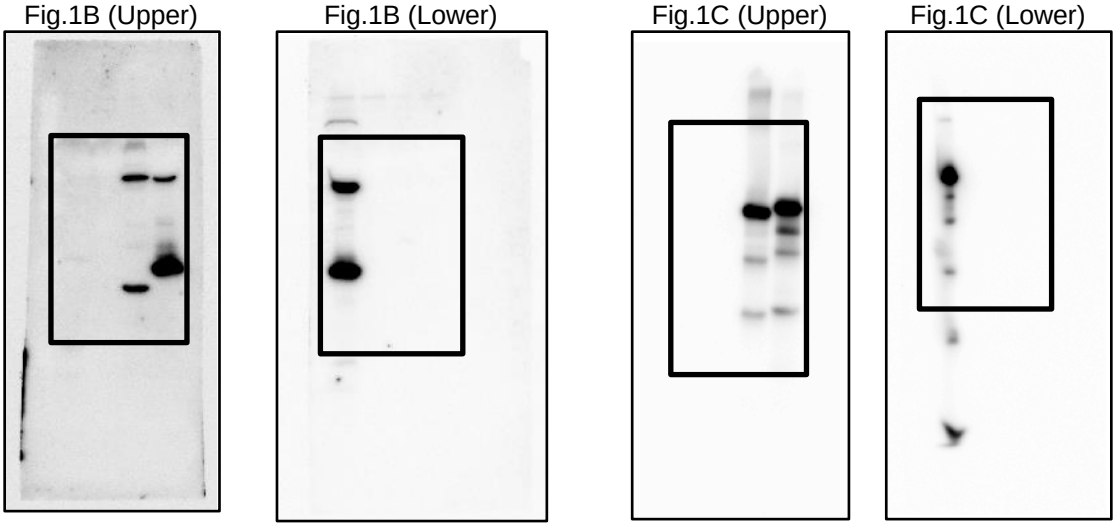

**Supplementary Figure 6|The schedule of the immunization and challenge of Figure 9.**

**Supplementary Figure 7**



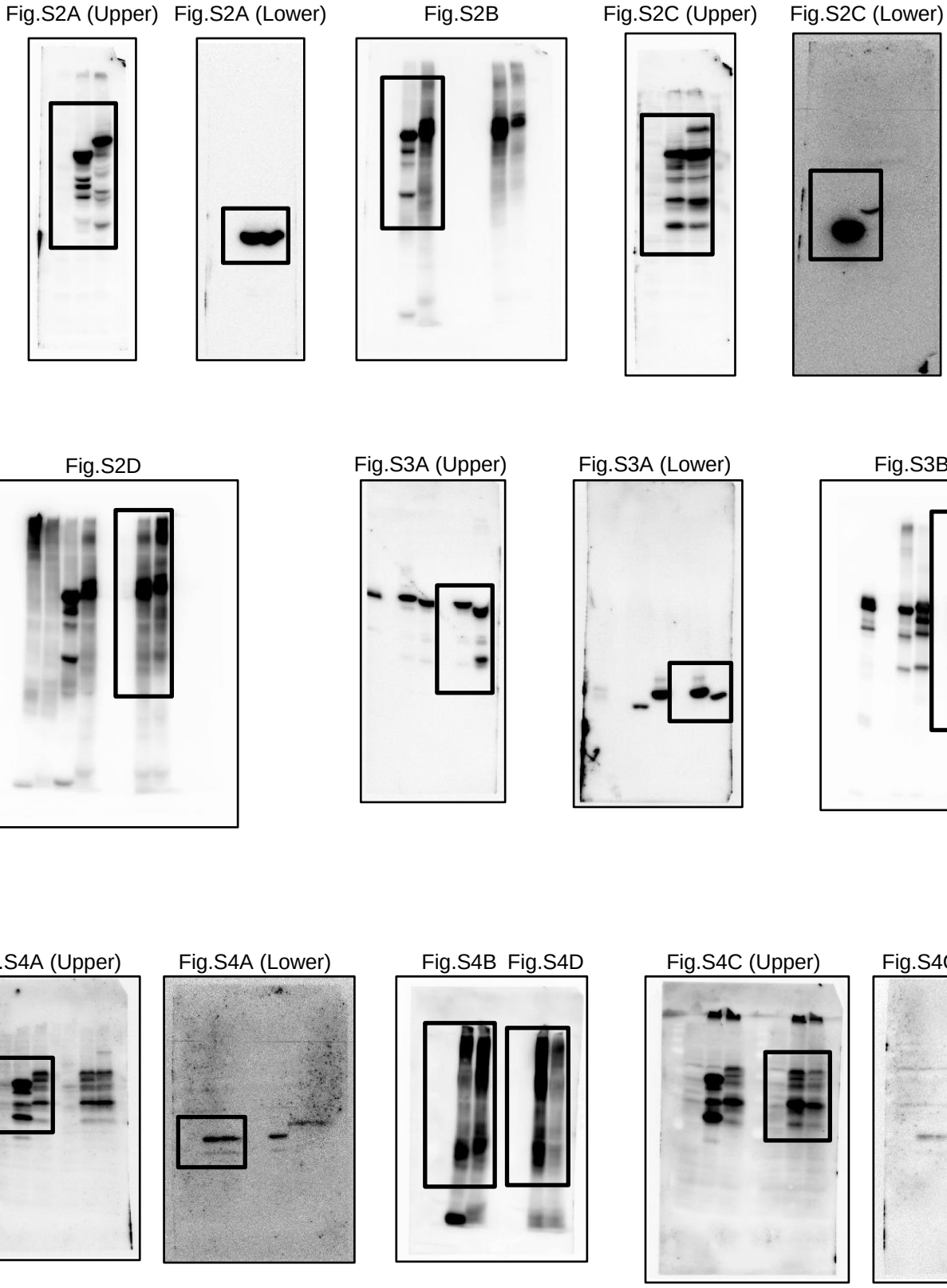

**Supplementary Figure 7| The whole IgG level is not significantly increased by αRP105-TM.** Whole IgG levels in the serum from **Figure 9** were analyzed by quantitative ELISA. The detection limit was over 0.0147 ($=10^{-1.832}$) mg/mL. The indicated data are combined from two independent experiments (n = 13–14) and indicated as the mean ± S.D. N.S., not significant (Mann-Whitney test).

**Supplementary Figure 8**




**Supplementary Figure 8| Full-length blots from the primary figures.** The cropped areas used in the current article are indicated in black boxes.

**Supplementary Figure 9**




**Supplementary Figure 9| Full-length blots from the supplementary figures.** The cropped areas used in the current article are indicated in black boxes.

**Supplementary Table 1 |** The following primers were used for cloning indicated constructions.

| Primer detail | Primer sequence | Construction |
|---|---|---|
| Anti-RP105 $V_H$ Forward | 5'-atGCGGCCGCATGGAACTGGGTCTTTTTTTCC-3' | pCADEST1-anti-RP105 mIgG1 |
| Mouse IgG1-overlapped anti-RP105 $V_H$ Reverse | 5'-GATGGGGGTGTCGTTTTGGCTGTTGTTTCAGCTGAGGAGA-3' | pCADEST1-anti-RP105 mIgG1 |
| Mouse IgG1 Forward | 5'-GCCAAAACGACACCCCCATC-3' | pCADEST1-anti-RP105 mIgG1 |
| Mouse IgG1 Reverse | 5'-aGGCGCGCCTCATTTACCAGGAGAGTGGG-3' | pCADEST1-anti-RP105 mIgG1 |
| Anti-RP105 $V_L$ Forward | 5'-aGCGGCCGCATGGAGACAGACAGACTCCT-3' | pCADEST1-anti-RP105 mkappa |
| Anti-RP105 $V_L$ Reverse | 5'-TTTCAACTCCAGCTTGGTGC-3' | pCADEST1-anti-RP105 mkappa |
| Anti-RP105 $V_L$-overlapped mouse kappa Forward | 5'-GCACCAAGCTGGAGTTGAAACGGGCTGATGCTGCACCAAC-3' | pCADEST1-anti-RP105 mkappa |
| Mouse kappa Reverse | 5'-aGGCGCGCCCTAACACTCATTCCTGTTGA-3' | pCADEST1-anti-RP105 mkappa |
| Anti-OVA $V_H$ Forward | 5'-atGCGGCCGCATGGCCGTACTCGGCCTCTT-3' | pCADEST1-anti-OVA mIgG1 |
| Mouse IgG1-overlapped anti-OVA $V_H$ Reverse | 5'-GATGGGGGTGTCGTTTTGGCAGACACTGTGACCAGTGTTC-3' | pCADEST1-anti-OVA mIgG1 |
| A/PR8-HA Forward | 5'-atGCGGCCGCATGAAGGCAAACCTACTGGT-3' | pCADEST1-HA |
| A/PR8-HA Reverse | 5'-aGGCGCGCCTCAGATGCATATTCTGCACT-3' | pCADEST1-HA |