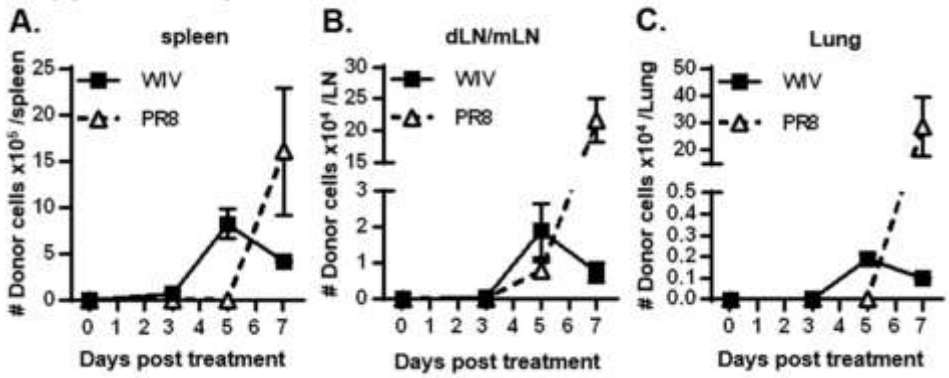
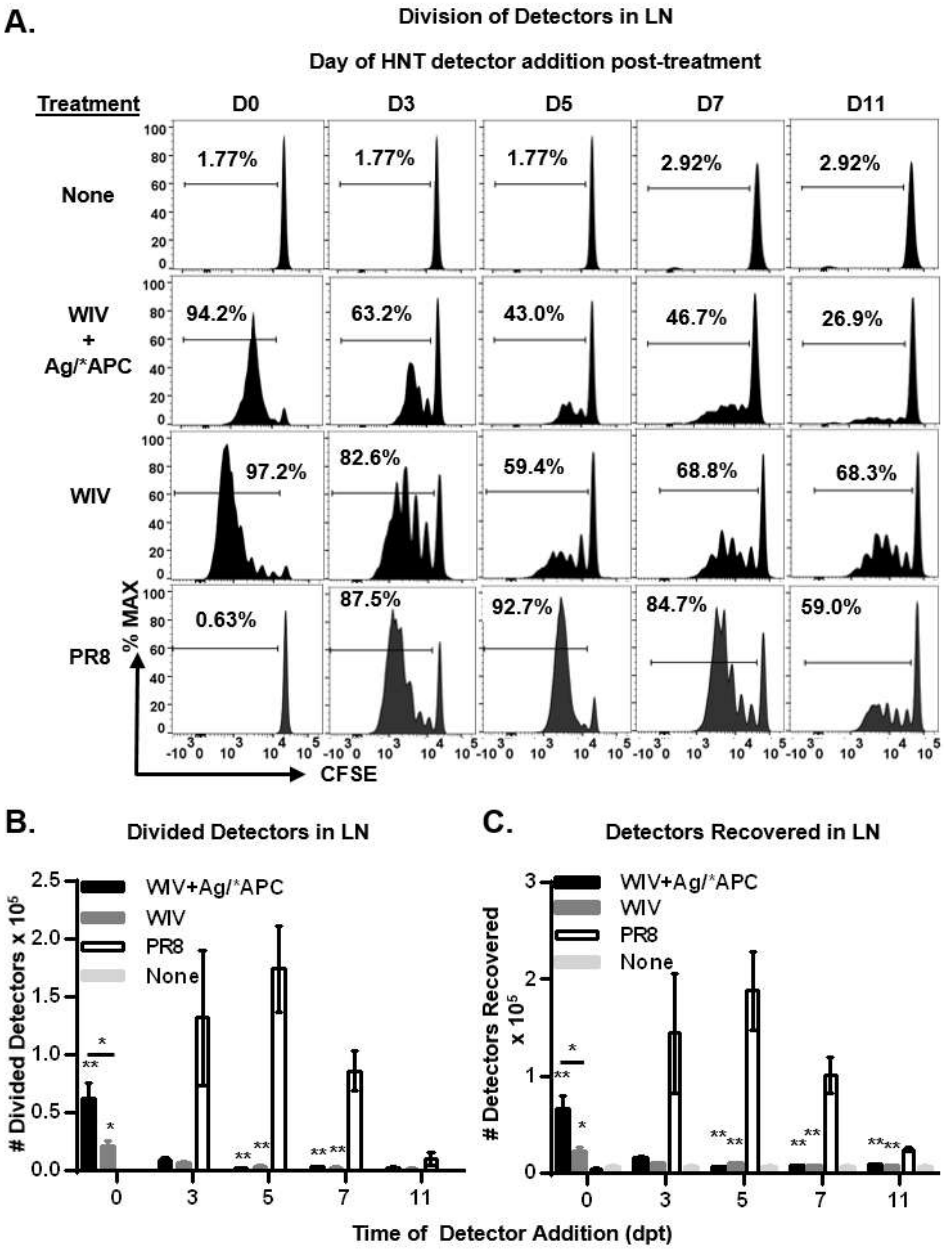
Supplementary FIGURE 1.



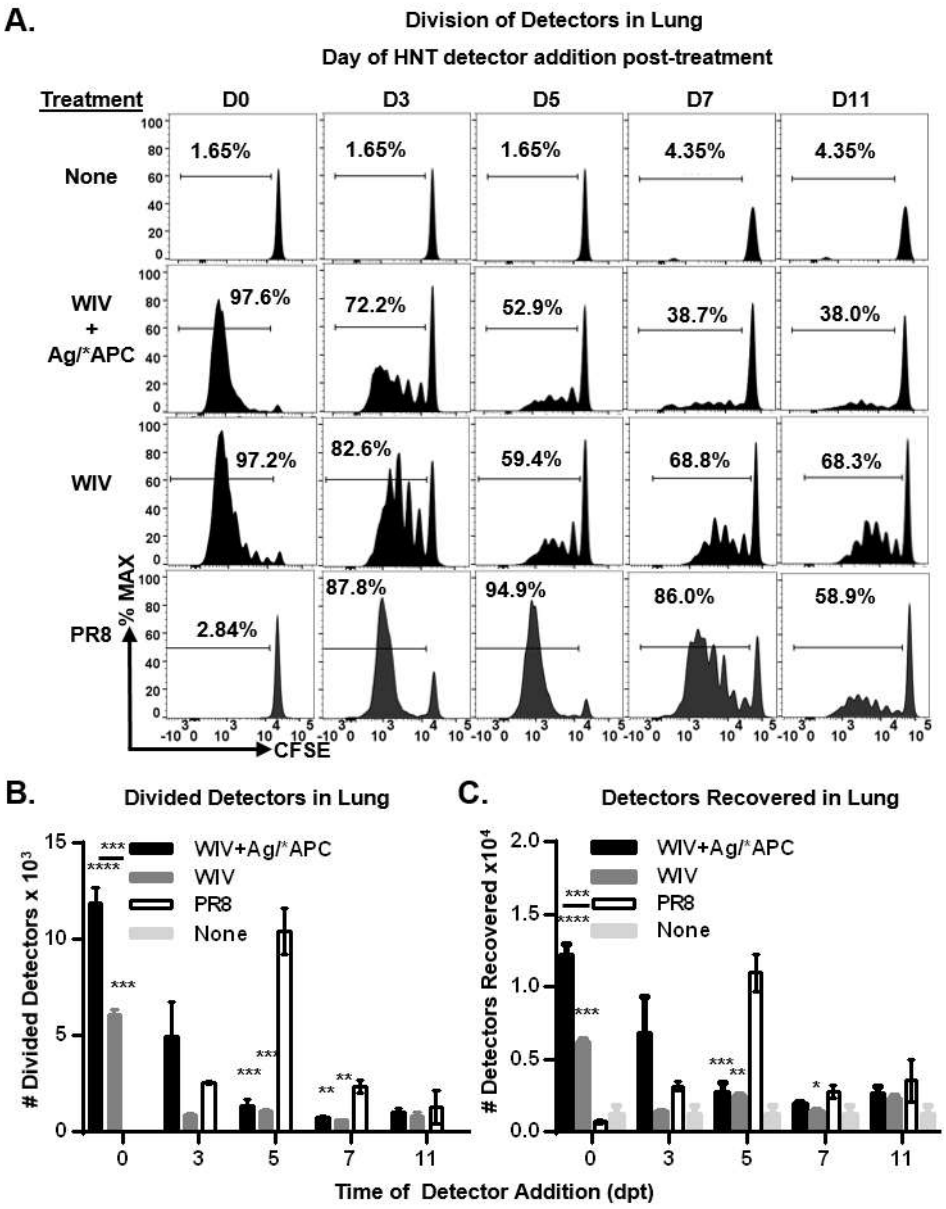
Supplementary Figure 1 Kinetics of the CD4 T cell expansion in response to WIV immunization. C57BL/6.FluNP.Thy1.1 TcR Tg naïve CD4 T cells isolated from spleen and 10^5 were transferred to each B6 host, 1 day before the treatments. The FluNP TCR recognizes the influenza $NP_{311-325}$ peptide epitope. C57BL/6 hosts were treated with WIV (100 μg) via i.v. injection or PR8 infection via i.n. instillation at a sublethal dose ($0.3LD_{50}$) on day 0. Following WIV immunization (solid line, filled square) or PR8 infection (dashed line, empty triangle), the donor $Thy1.1^+$ $CD4^+$ T cells were enumerated in spleen (A), dLN (draining LN) for infection and mLN (mediastinal LN) for immunization (B) and lung (C) at indicated time points: 0, 3, 5, 7 days post-treatment (dpt) using FACS analysis (Thy1.1). Mice (n=3) in each group at each indicated time points. The exact experiment was repeated twice. Data from one of the experiments is shown. Similar results were seen in other 2 experiments.

Supplementary FIGURE 2.



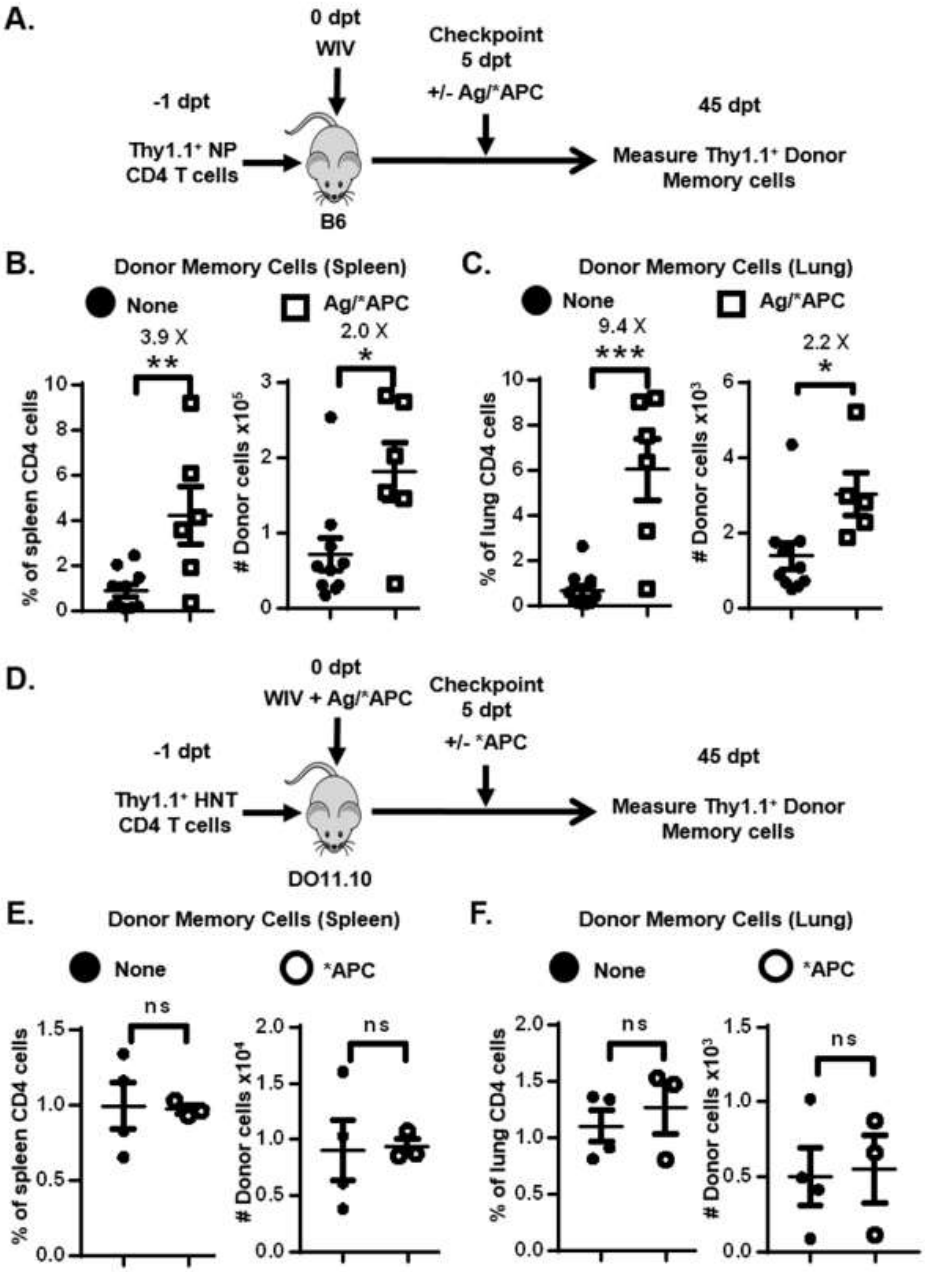
Supplementary Figure 2. The kinetics of Ag presentation in LN. A). LN data from the same experiment as in Fig 2. Detector cells from pooled dLN or mLN were analyzed for CFSE by FACS. Representative histograms showing CFSE staining in detector cells harvested from LN of the individual immunized mice (WIV+Ag/*APC or WIV), PR8 infected (PR8) and control (None) mice. The gate shows the percentage of the divided detector cells. B) The total number of the divided donor cells in LN from hosts that were: PR8-infected (empty), WIV+Ag/*APC immunized (filled black), WIV (filled dark grey) immunized and no treatment (filled light grey) mice. C) The total number of the donor cells recovered in LN from each group of hosts. Mice (n=3) in each group at each indicated time points. Experiment repeated twice. Data from one of the experiments is shown. For statistics, the data of the immunized groups was compared to that of the PR8 infected group and the data of WIV immunized groups was compared to that of WIV+Ag/*APC immunized group.

Supplementary FIGURE 3.



Supplementary Figure 3. The kinetics of Ag presentation in the lung. A. Lung data from the same experiment shown in Figure 2. A) Detector cells from lung were analyzed for CFSE by FACS. Representative histograms of CFSE in detector cells harvested from lungs of the individual immunized (WIV+Ag/*APC or WIV), PR8 infected (PR8) and control (None) mice. The gate shows the percentage of the divided detector cells. B) The number of divided donor cells in lungs from hosts that were: PR8-infected (empty), WIV+Ag/*APC immunized (filled black), WIV immunized (filled dark grey) and no treatment [None] (filled light grey) mice. C) The number of the total donor cells recovered in lung from each group of hosts. Mice (n=3) in each group at each indicated time point. Experiment repeated twice. Data from one of the experiments is shown. For statistics, the data of the immunized groups was compared to that of the PR8 infected group and the data of WIV immunized groups was compared to that of WIV+Ag/*APC immunized group.

Supplementary FIGURE 4.



Supplementary Figure 4. Impact of checkpoint Ag/*APC addition on donor CD4 memory generation.. As in supplementary Fig 1, naïve NP.Thy1.1 TcR Tg CD4 T cells were transferred into C57BL/6 hosts 1 day before immunization with WIV immunization alone. Ag/*APC were i.v. injected (empty squares) or not injected (filled circles) into the immunized mice hosts to provide checkpoint Ag. At 45 dpt, Thy1.1$^+$ CD4$^+$ donor NP memory cells were detected by FACS. A) Schematic diagram of the experiment. B-C) The enumeration of Thy1.1$^+$ CD4$^+$ donor memory cells in spleen (B) and lung (C). The graphs show the frequency of Thy1.1$^+$ CD4$^+$ donor memory cells on 45 dpt (left panel) as a % of spleen or lung CD4 T cells, and the total number recovered per organ (right panel). The results of 2 experiments were pooled. For each experiment, n$\geqslant$3 mice were used in each group. D-F) **Requirement for Ag on the *APC at the checkpoint on memory induction.** As in Fig 3A, naïve HNT.Thy1.1 TcR Tg CD4 T cells were transferred into DO11.10 BALB/c hosts 1 day before immunization with WIV+Ag/*APC. On 5 dpt, *APC were i.v. injected (empty circles) or not injected (filled circles) into the immunized mice hosts. At 35 dpt, Thy1.1$^+$ CD4$^+$ donor HNT memory cells were enumerated by FACS. D) Schematic diagram of the experiment. E-F) The enumeration of Thy1.1$^+$ CD4$^+$ donor memory cells in spleen (E) and lung (F). The exact experiment was repeated twice. n$\geqslant$3 mice were used in each group. Data from one of the experiments is shown. Similar results were seen in other 2 experiments.