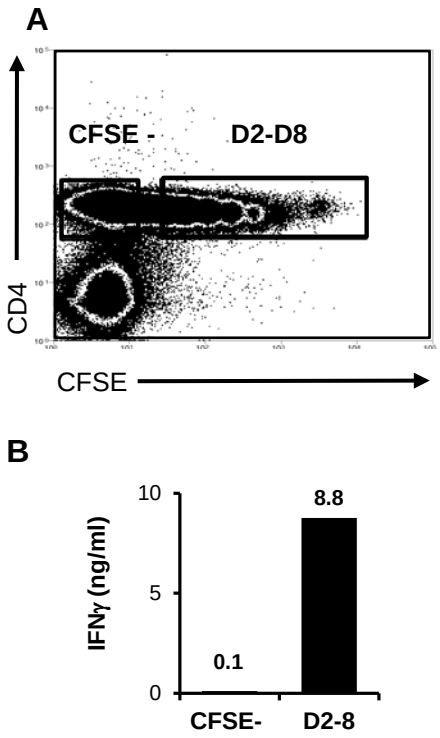
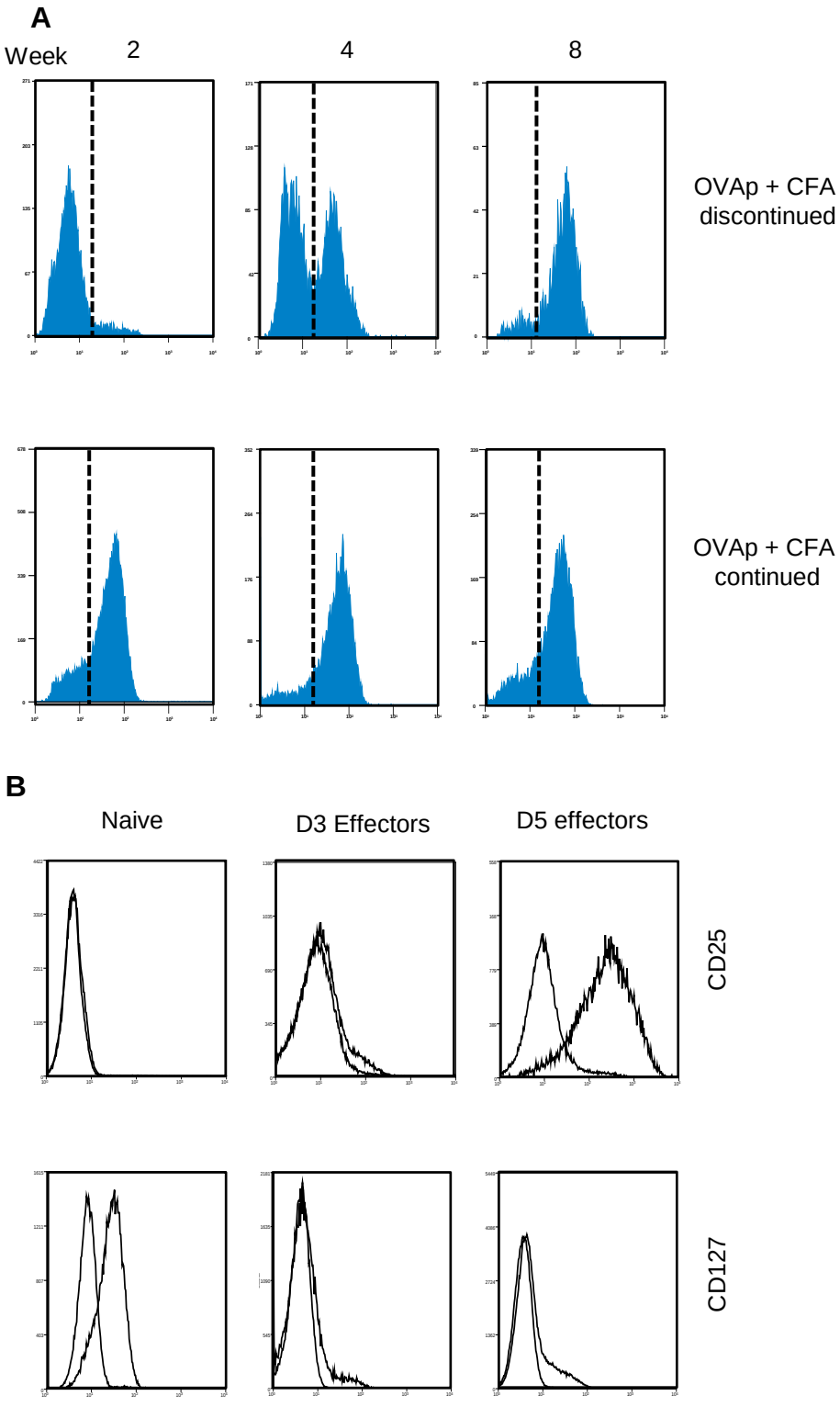
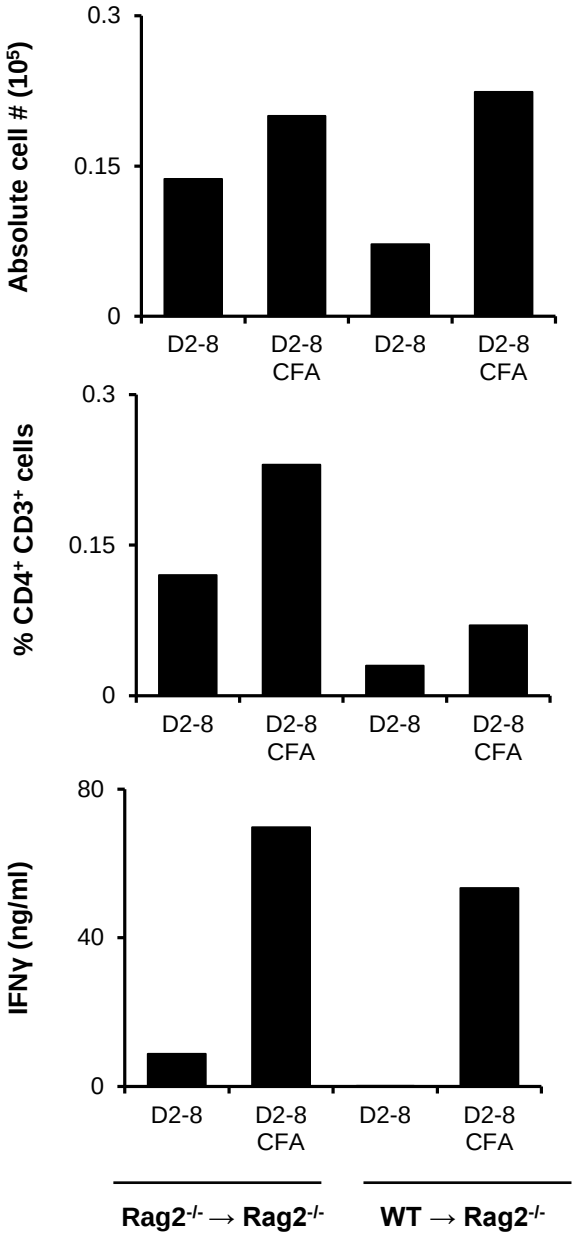
Guloglu et al. Figure S1



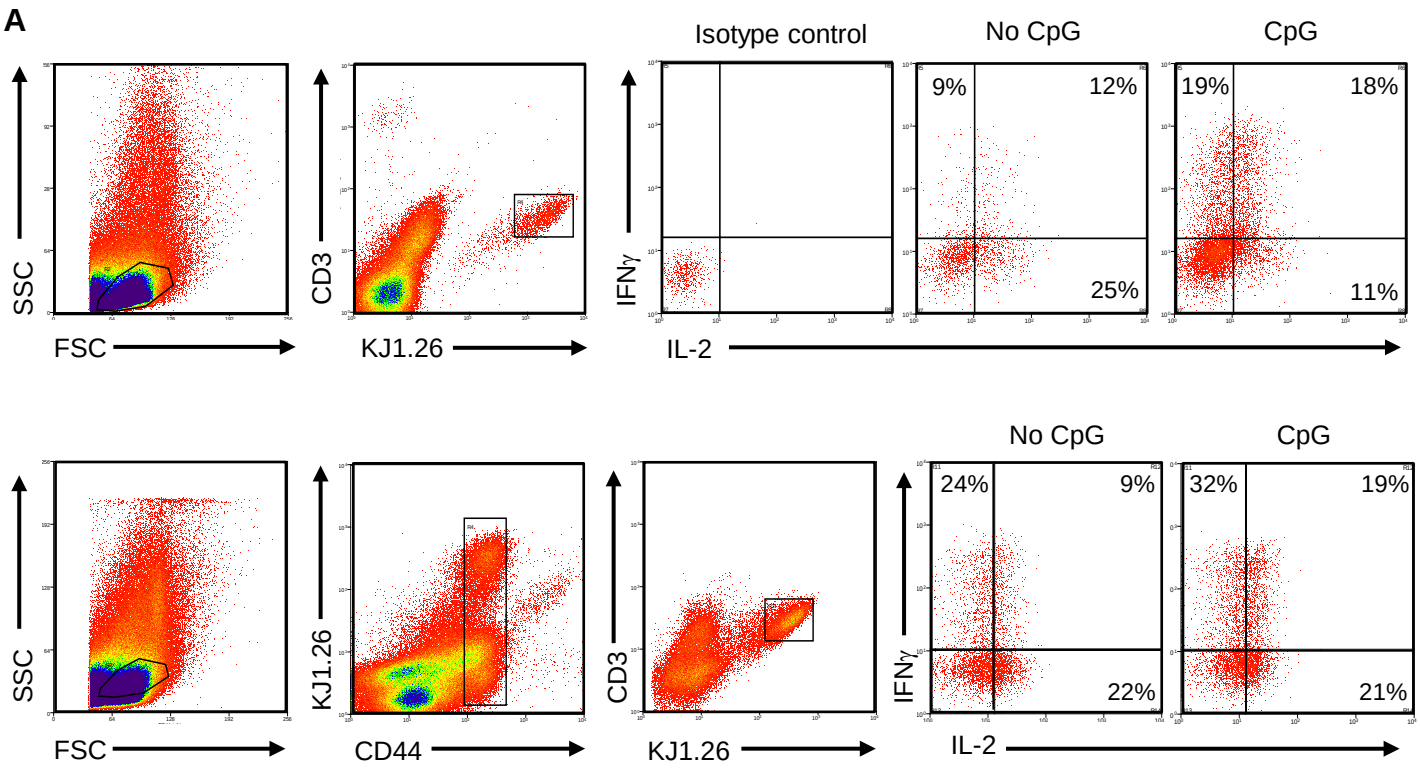
Guloglu et al. Figure S2



Guloglu et al. Figure S3



Guloglu et al. Figure S4



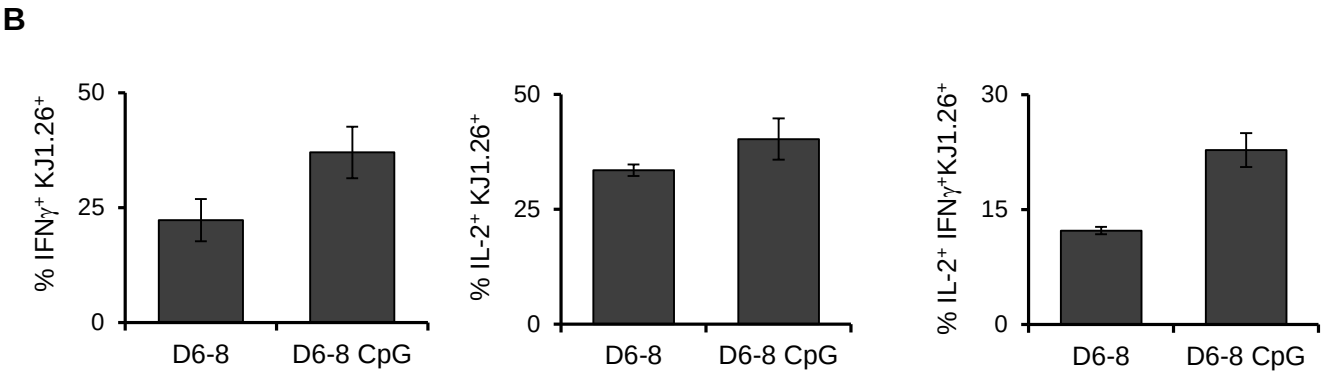


Figure S1. Effectors that have undergone more than 8 divisions (D>8) have less potential to transit to memory. CFSE-labeled naïve DO11.10 T cells were transferred into $Rag2^{-/-}$ mice and the hosts were immunized with OVAp in CFA. Eight days later the $CD4^{+}CFSE^{+}$ (D2-8) and $CD4^{+}CFSE^{-}$ ($CFSE^{-}$) LN effectors were sorted as indicated in (A) and transferred into $Rag2^{-/-}$ parking hosts. Seven weeks later, the SP cells were harvested and the $CD4^{+}KJ1\text{-}26^{+}$ cells were analyzed for IFNγ production (B). The results show that highly activated (D>8) effectors are less likely to transit to memory.

Figure S2. Re-expression of IL-7R during transition to memory may be dependent on IL-2 signaling. BALB/c mice recipient of CFSE-labeled naïve T cells were immunized with 125µg OVA/CFA and D2-8 effector T cells were isolated and transferred into $Rag2^{-/-}$ mice to generate CFA discontinued animals (OVAp+CFA discontinued). $Rag2^{-/-}$ mice, recipient of CFSE-labeled naïve T cells, were immunized with 125µg OVA/CFA and used as CFA continued (OVA+CFA continued) mice. (A) Shows IL-7R expression on $KJ1.26^{+}CD4^{+}$ splenic T cells harvested after 2, 4 and 8 weeks of parking. (B) Shows IL-2R (CD25) and IL-7R (CD127) expression on naïve T cells and on D2-8 effector T cells 3 and 5 days after immunization with OVAp+CFA. The cells were gated on KJ1.26 and CD4 and analyzed for CD25 and CD127 expression in comparison with isotype control (dotted lines) by flow cytometry. The results indicate that IL-2R (CD25) expression precedes IL-7R re-expression, perhaps suggesting that signaling through IL-2R is required for IL-7R expression and transition to memory .

Figure S3. Effectors primed in $Rag2^{-/-}$ and wild type (WT) BALB/c hosts show similar responses to antigen-free adjuvant. CFSE-labeled naïve DO11.10 T cells were transferred into $Rag2^{-/-}$ or WT BALB/c mice and the hosts were immunized with OVAp in CFA. Eight days later the $CFSE^{+}CD4^{+}$ LN D2-8 effectors were sorted from each group and transferred into $Rag2^{-/-}$

hosts recipient of effectors generated in $Rag2^{-/-}$ ($Rag2^{-/-} \rightarrow Rag2^{-/-}$) or WT ($WT \rightarrow Rag2^{-/-}$) mice. Next, each group was divided into 2 subgroups, one of which was given CFA (D2-8/CFA) and the other was not (D2-8). After 7 weeks of parking, the SP cells were harvested and the percentage and absolute number of surviving $CD4^{+}KJ1\text{-}26^{+}$ cells were determined by flow cytometry and their IFNγ responses were measured by ELISA.

Figure S4. CpG increases the percentage of IL-2-producing $IFN\gamma^{+}KJ1.26^{+}$ T cells. Late (D6-8) effectors were isolated from BALB/c mice 3 days after immunization with OVAp/CFA and transferred into $Rag2^{-/-}$ mice. On day 1, 4, 7 and 10 after cell transfer, the hosts were given CpG TLR9 ligand (50μg/injection) i.p. or left untreated. The splenic cells were harvested after 4 months of parking and analyzed for IL-2 and IFNγ production by flow cytometry. (A) Shows the gating strategy for CD3 and KJ1.26 (upper panel) or CD44, KJ1.26 and CD3 (lower panel), followed by analysis of IL-2 and IFNγ production by $CD3^{+}KJ1.26^{+}$ and $CD44^{+}CD3^{+}KJ1.26^{+}$ T cells from the 4-month parking experiments. (B) Shows compiled results for the percentage of $KJ1.26^{+}$ T cells producing IFNγ ($KJ1.26^{+}IFN\gamma^{+}$), IL-2 ($KJ1.26^{+}IL2^{+}$) or both IFNγ and IL-2 ($KJ1.26^{+}IFN\gamma^{+}IL\text{-}2^{+}$). Each bar represents the mean ± SD of four independent experiments. The results indicate that CpG treatment increases the frequency of cytokine-producing memory cells.