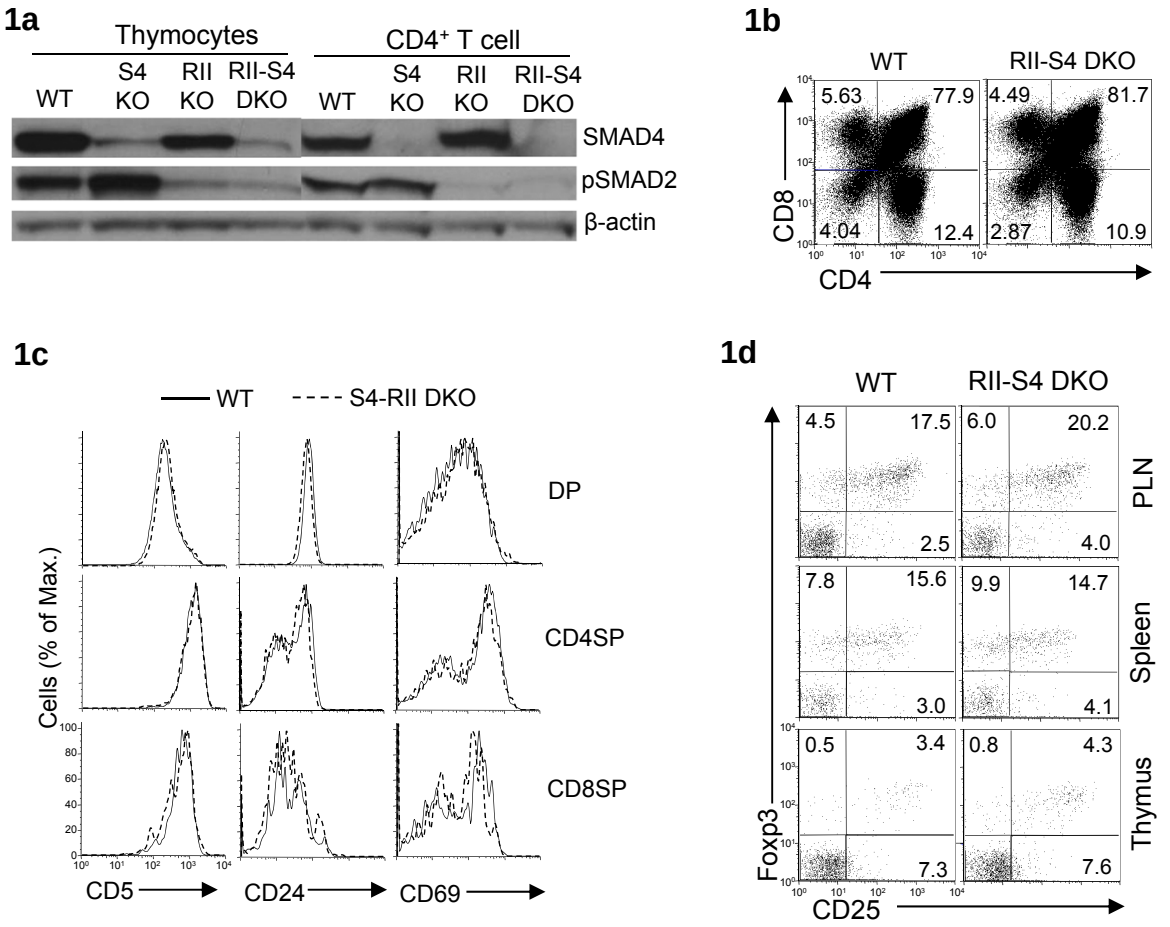
Supplemental Figures

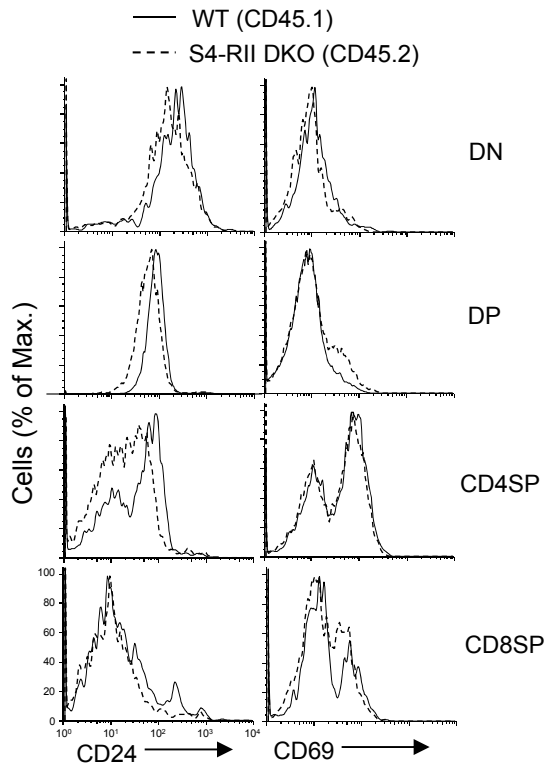
Supplemental Fig. 1



Supplemental Fig. 1. (**a**) Smad4 expression and TGF-β signaling activity assessed by immunoblotting for Smad4 and phosphorylated Smad2 in thymocytes and $CD4^+$ T cells isolated from wild-type, *Cd4-cre-Smad4*$^{fl/fl}$ (S4 KO), *Cd4-cre-Tgfbr2*$^{fl/fl}$ (RII KO) and Cd4-cre-*Tgfbr2*$^{fl/fl}$-*Smad4*$^{fl/fl}$ (RII-S4 DKO) mice. (**b**) T cell populations in the thymus of wild-type and RII-S4 DKO mice assessed by flow-cytometry. (**c**) The expression of thymocyte maturation markers CD5, CD24 and CD69 on $CD4^+CD8^+$ (DP) $CD4^+CD8^-$ (CD4SP) and $CD4^-CD8^+$ (CD8SP) thymocytes in wild-type (solid lines) and RII-S4 DKO (dashed lines) mice assessed by flow-cytometry. (**d**) Foxp3 and CD25 expression in $CD4^+CD8^-$ T cells in the thymus, spleen and peripheral lymph-nodes (PLN) of wild-type and RII-S4 DKO mice, assessed by flow-cytometry. Results are representative of at least three experiments.

(Related to Fig. 1)

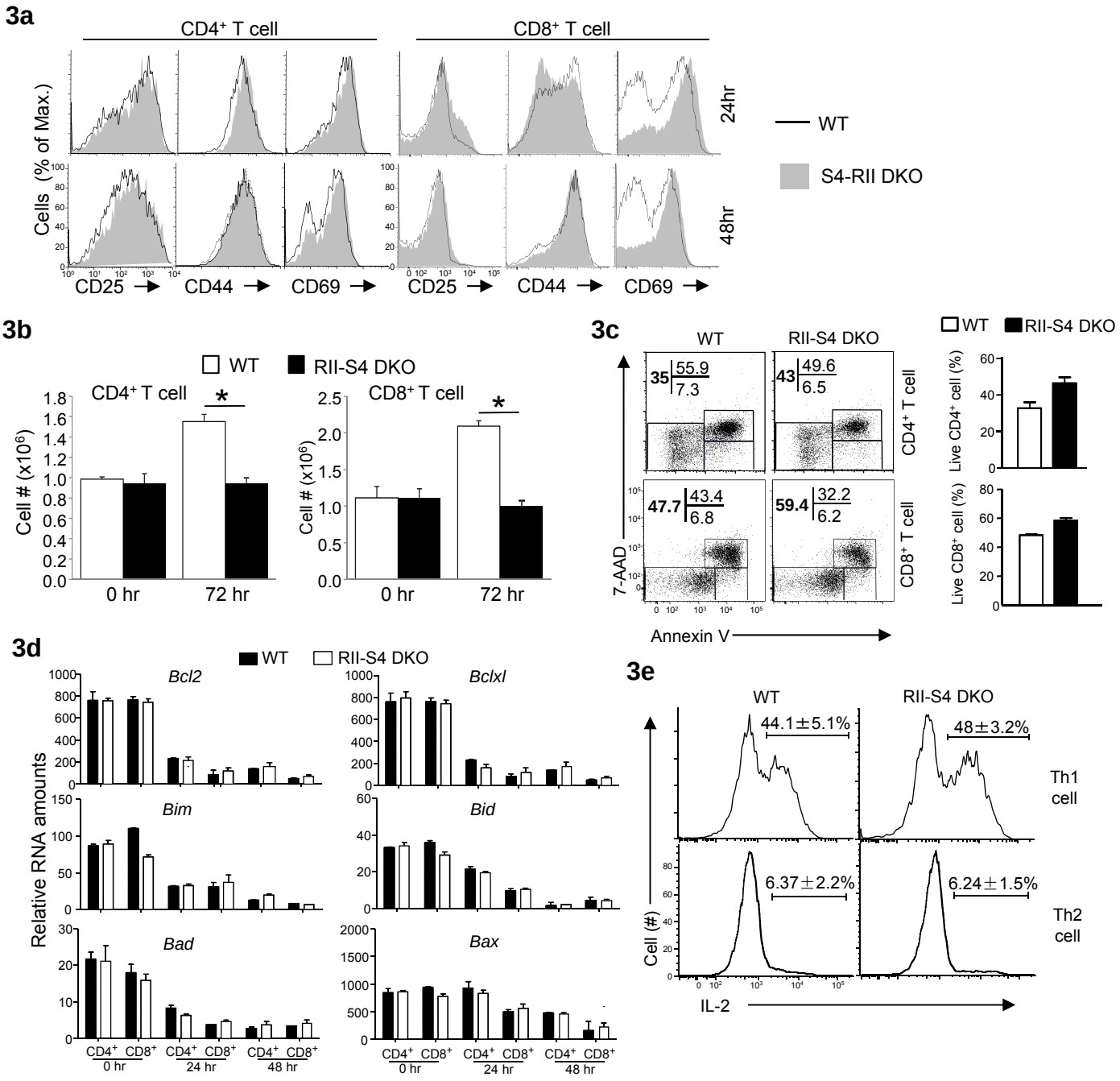
Supplemental Fig. 2



Supplemental Fig. 2. The expression of thymocyte maturation markers CD24 and CD69 on $CD4^-CD8^-$ (DN), $CD4^+CD8^+$ (DP), $CD4^+CD8^-$ (CD4SP), and $CD4^-CD8^+$ (CD8SP) thymocytes in mixed bone marrow chimeras reconstituted with equal numbers of bone marrow cells from wild-type (CD45.1) and *Cd4-Cre-Tgfbr2*$^{fl/fl}$-*Smad4*$^{fl/fl}$ (RII-S4 DKO, CD45.2) mice, assessed by flow-cytomery. Results are representative of at least three experiments.

(Related to Fig. 2)

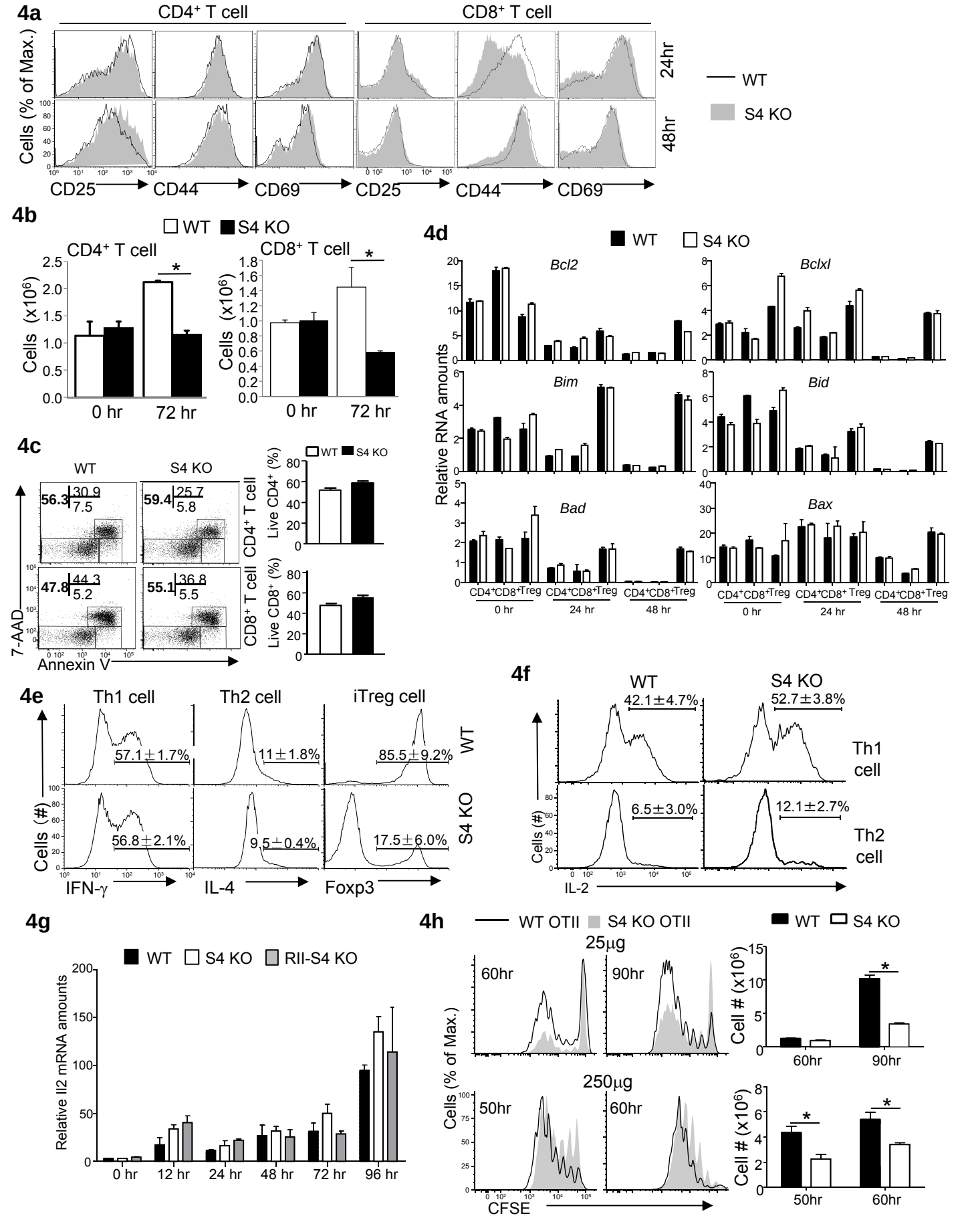
Supplemental Fig. 3



Supplemental Fig. 3. (**a**) The expression of activation makers CD25, CD44 and CD69 on wild-type and *Cd4-cre-Tgfbr2*$^{fl/fl}$-*Smad4*$^{fl/fl}$ (RII-S4 DKO) T cells at different time points after anti-CD3 and anti-CD28 stimulation, assessed by flow-cytomery. Results are representative of at least three experiments. (**b**) The numbers of recovered T cells 0 and 72 h after anti-CD3 and anti-CD28 stimulation. Means ± SD of three experiments are shown (*P<0.05). (**c**) The T cell survival 48 h after anti-CD3 and anti-CD28 stimulation assessed by 7-AAD and Annexin V staining and flow-cytometry. FACS plots (left) are representative of at least three experiments. Means ± SD of three experiments are also shown (right). **(d)** The mRNA expression of *Bcl2, Bclx, Bim, Bid, Bad* and *Bax* in CD4 and CD8 T cells at different time after activation was assessed by RT-PCR assays. Means ± SD of triplicates in one experiments of three were shown. **(e)** IL-2 production in wild-type and RII-S4 DKO CD4 T cells were assessed by flow-cytometry four days after Th1 and Th2 differentiation. Means ± SD of three experiments are shown.

(Related to Fig. 4)

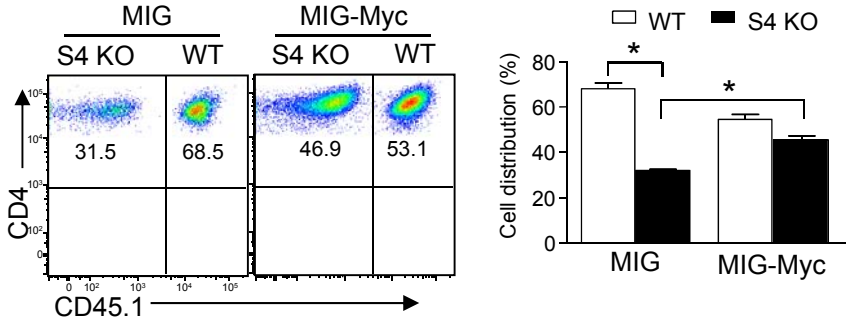
Supplemental Fig. 4



Supplemental Fig. 4. (**a**) The expression of activation makers CD25, CD44 and CD69 on wild-type and *Cd4-cre-Smad4*$^{fl/fl}$ (S4 KO) T cells at different time points after anti-CD3 and anti-CD28 stimulation, assessed by flow-cytomery. Results are representative of at least three experiments. (**b**) The numbers of recovered T cells 0 and 72 h after anti-CD3 and anti-CD28 stimulation. Means ± SD of three experiments are shown. (*P<0.05) (**c**) The T cell survival 48h after anti-CD3 and anti-CD28 stimulation assessed by 7-AAD and Annexin V staining and flow-cytometry. FACS plots (left) are representative of at least three experiments. Means ± SD of three experiments are also shown (right). (**d**) The mRNA expression of *Bcl2, Bclx, Bim, Bid, Bad* and *Bax* in CD4, CD8 and Treg ($CD4^+CD25^+$) cells at different time after activation was assessed by RT-PCR assays. Means ± SD of triplicates in one experiments of three were shown. **(e)** IFN-γ, IL-4 and Foxp3 expression in $CD4^+$ T cells was assessed by flow-cytometry after being differentiated under Th1, Th2 and iTreg conditions. **(f)** IL-2 expression in differentiated Th1 and Th2 cells were assessed by flow-cytometry. Means ± SD of three experiments are shown. (**g**) The mRNA expression of *Il2* in CD4 cells at different time points after TCR activation was assessed by qRT-PCR assays. Means ± SD of triplicates in one of two experiments are shown. (**h**) $CD4^+$ T cells were isolated from *Cd4-cre-Smad4*$^{fl/+}$-OTII (WT OTII, $CD45.1^+CD45.2^+$) and *Cd4-cre-Smad4*$^{fl/fl}$-OTII mice (S4 KO OTII, $CD45.2^+$) mice, labeled with CFSE, mixed at the ratio of 1 to 1, and then transferred into syngeneic wild-type mice ($CD45.1^+$). The proliferation and the numbers of transferred cells in the spleens were assessed at different time points after the recipient mice were injected with different doses of OVA proteins (as indicated). Representative flow-cytometry results of at least two experiments are shown. Means ± SD of the cell numbers of three mice in one of two experiment are shown. (* P<0.05)

(Related to Fig. 5)

Supplemental Fig. 5



Supplemental Fig. 5. CD4 T cells isolated from wild-type (CD45.1$^+$) and *Cd4-cre-Smad4*$^{fl/fl}$ (S4 KO) mice (CD45.2$^+$) that are in C57BL/6 background were mixed, activated and transduced with either MIG or MIG-Myc virus. Equal numbers of transduced T cells (GFP$^+$) were transferred into MHC-mismatched *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ recipient mice (Balb/c). The distribution of splenic GFP$^+$ CD4 T cells of different origins in the recipient mice were determined by flow-cytometry. Representative results (left) and means $\pm$ SD of three mice in one experiment of two are shown. (*P<0.05)

(Related to Fig. 6)