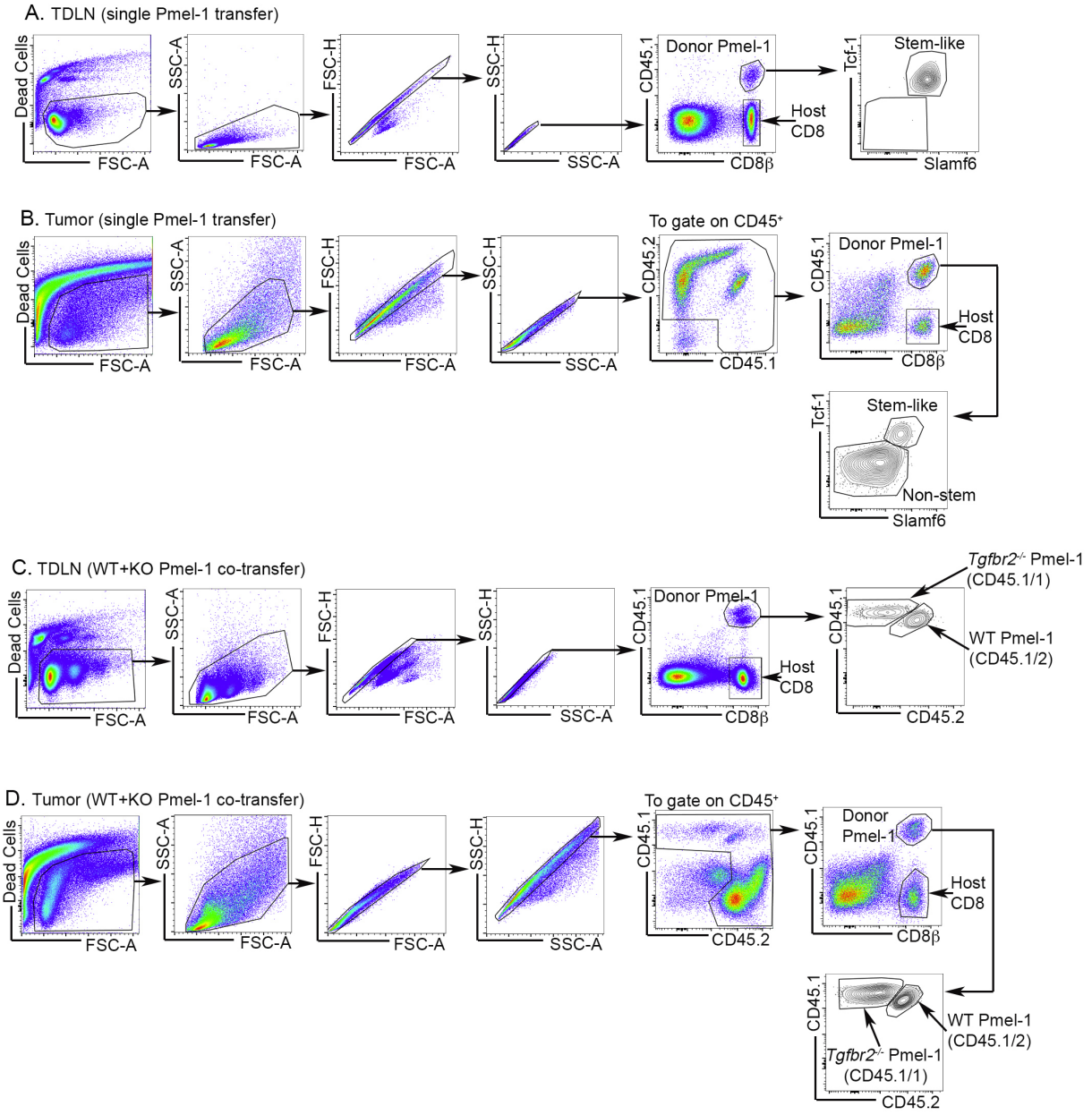
Supplementary Information

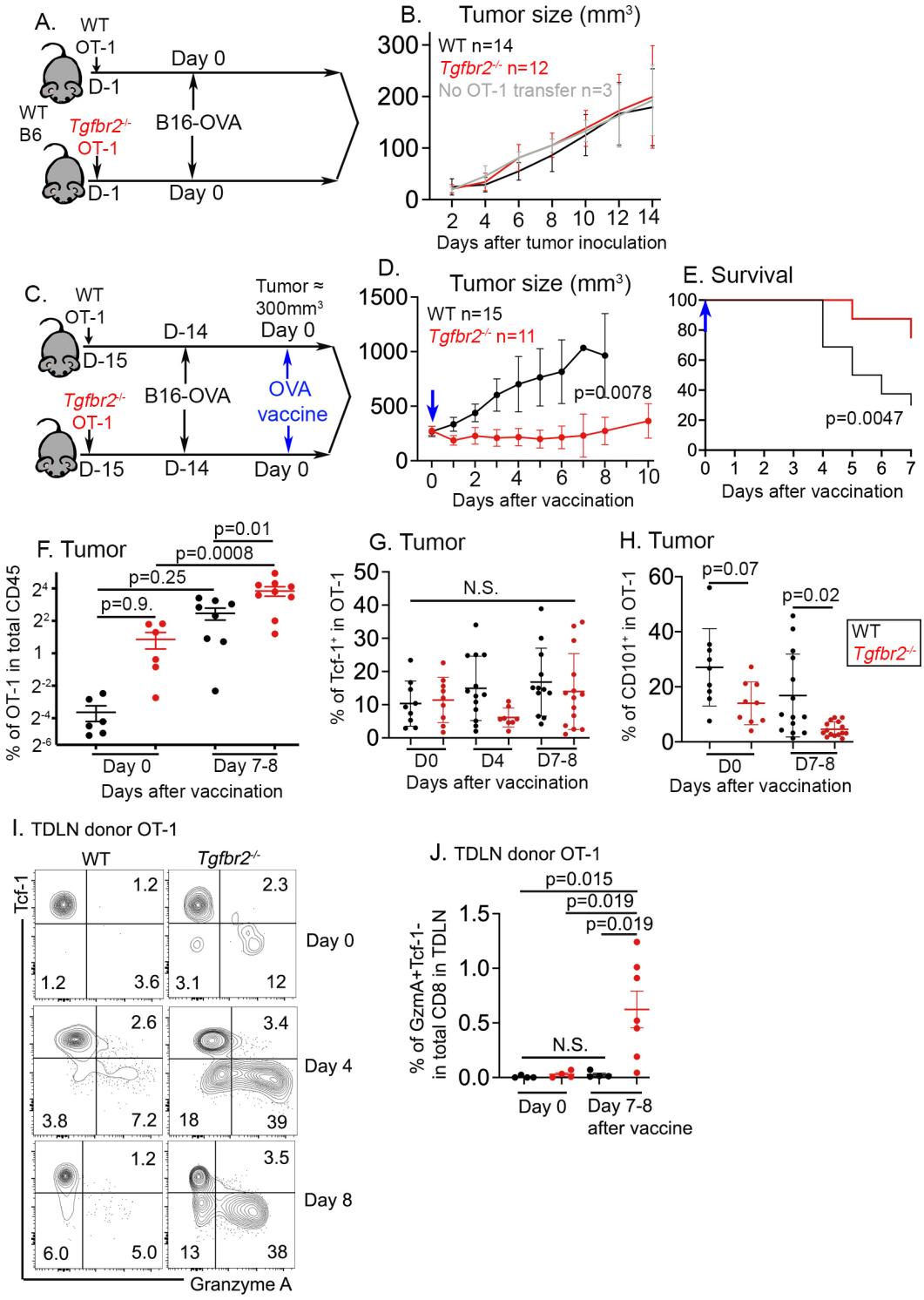
# TGF-β-dependent Lymphoid Tissue Residency of Stem-like T cells Limits Response to Tumor Vaccine

Guo Li[1,2,3,4], Saranya Srinivasan[1#], Liwen Wang[1,5#], Chaoyu Ma[1], Kai Guo[2,9], Wenhao Xiao[2], Wei Liao[1,6,10], Shruti Mishra[1,11], Xin Zhang[2,3,4,7], Yuanzheng Qiu[2,3,4,7], Qianjin Lu[6,8], Yong Liu[2,3,4,7*] and Nu Zhang[1*]

## Supplementary Figures

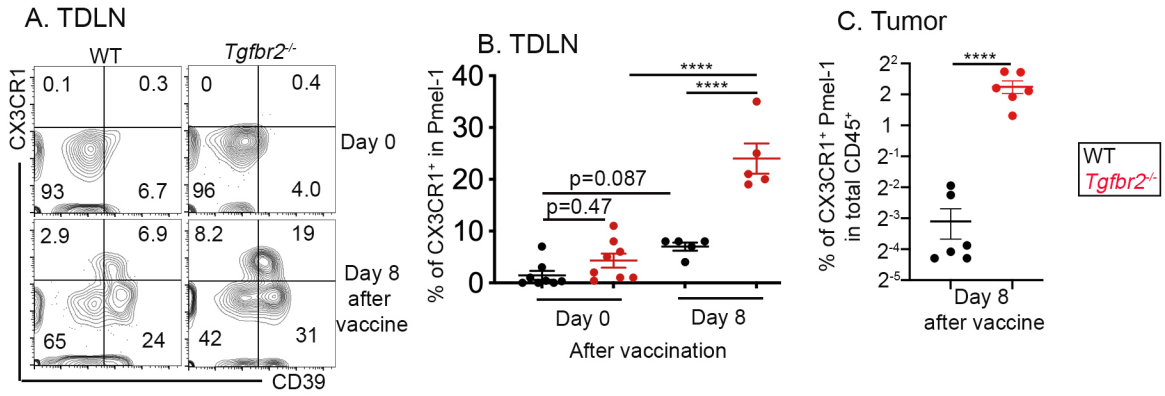



**Supplementary Figure 1. FACS gating strategies.** For single Pmel-1 donor T cell transfer experiments, gating strategies for TDLN (**A**, used in Fig. 3, 4, 5, 8, S2, S3, S4 and S7) and tumor (**B,** used in Fig. 2, 3, 4, 8, S2, S3 and S7) are shown. For WT (wild type) and *Tgfbr2*$^{-/-}$ Pmel-1 co-transfer experiments, gating strategies for TDLN (**C,** used in Fig. 5, 6, 7, S5 and S6) and tumor (**D,** used in Fig. 6, 7, S5 and S6) are shown.

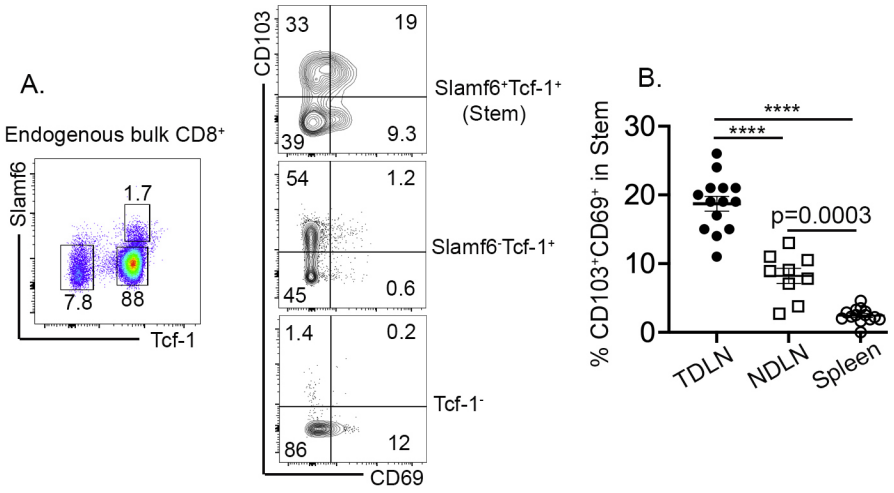



**Supplementary Figure 2. *Tgfbr2*$^{-/-}$ OT-1 T cells synergize with OVA peptide vaccination to control B16-OVA.** (**A**) and (**B**), OT-1 transfer alone or No OT-1 control in grey. (**A**) Schematics; (**B**) Tumor growth. (**C**) to (**E**), OT-1+OVA vaccine. (**C**) Schematics; (**D**) Tumor growth and (**E**) Survival curve after vaccination are shown. Black, WT OT-1 recipients, red, *Tgfbr2*$^{-/-}$ recipients and grey, no T cell transfer. For (D) and (E),

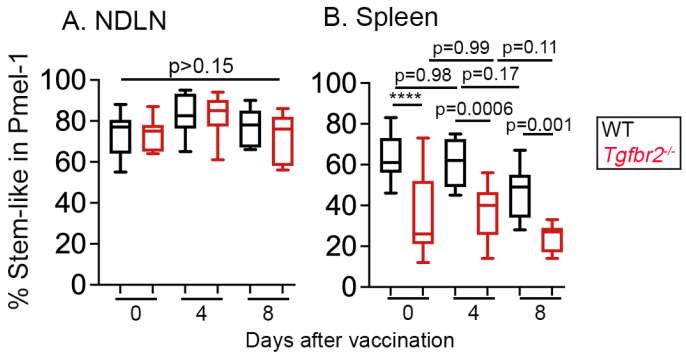
WT, n=15 and *Tgfbr2*$^{-/-}$, n=11. (**F**) The percentage of OT-1 T cells in total CD45$^{+}$ tumor infiltrating cells is shown (WT, n=6, *Tgfbr2*$^{-/-}$, n=6, WT+Vaccine, n=8 and *Tgfbr2*$^{-/-}$, n=9). (**G**) The percentage of Tcf-1$^{+}$ subset in tumor infiltrating OT-1 T cells is shown (WT d0, n=9, *Tgfbr2*$^{-/-}$ d0, n=9, WT d4, n=13, *Tgfbr2*$^{-/-}$ d4, n=8, WT d8, n=12 and *Tgfbr2*$^{-/-}$ d8, n=14). (**H**) The percentage of CD101$^{+}$ subset in tumor infiltrating OT-1 T cells is shown(WT, n=9, *Tgfbr2*$^{-/-}$, n=9, WT+Vaccine, n=14 and *Tgfbr2*$^{-/-}$, n=15). (**I**) Representative FACS profiles of TDLN OT-1 T cells are shown (WT, n=4, *Tgfbr2*$^{-/-}$, n=4, WT+Vaccine, n=4 and *Tgfbr2*$^{-/-}$, n=7). (**J**) The percentage of granzyme A$^{+}$Tcf-1$^{-}$ cells in TDLN OT-1 T cells are shown. Each symbol in (F to J) represents the results from an individual mouse. Black symbols, WT and red symbols, *Tgfbr2*$^{-/-}$. Data are presented as mean±SEM. Pooled results from 2-3 independent experiments are shown. N.S., not significant ($p>0.05$) and indicated p values are calculated by two tailed Wilcoxon test (D), Log-rank Mantel-Cox test (E) or Ordinary one-way ANOVA with Tukey's multi-comparison posttest (F to H, and J). Two sided tests were used. Source data are provided as a Source Data file.



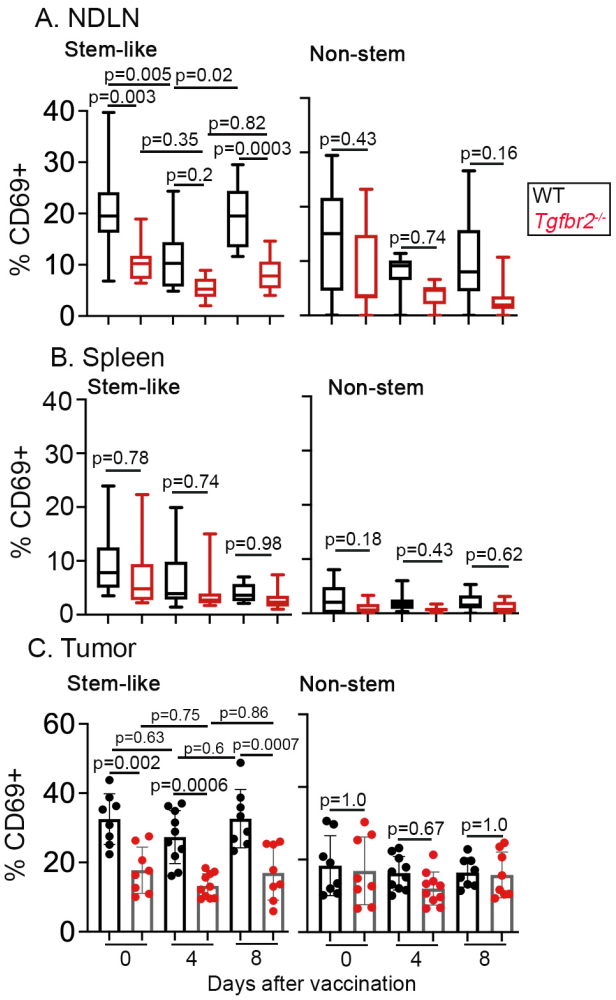

**Supplementary Figure 3. Tumor vaccine boosts the differentiation of CX3CR1$^+$ migratory effectors in TDLN and tumor.** Similar experimental setup as in Fig. 2A. (**A**) Representative FACS profiles of donor Pmel-1 T cells in TDLN before and after tumor vaccine are shown. (**B**) The percentage of CX3CR1$^+$ effectors in Pmel-1 T cells from TDLN is shown (WT, n=8, *Tgfbr2*$^{-/-}$, n=8, WT+Vaccine, n=5 and *Tgfbr2*$^{-/-}$, n=5). (**C**) The percentage of CX3CR1$^+$ Pmel-1 T cells in total CD45$^+$ tumor infiltrating cells after tumor vaccine is shown (n=6 for both WT and *Tgfbr2*$^{-/-}$). Pooled results from 2 independent experiments are shown. Each symbol in (B) and (C) represents the results from an individual mouse. Black symbols, WT and red, *Tgfbr2*$^{-/-}$. Data are presented as mean±SEM. Indicated p values and ****, p<0.0001 are calculated by Ordinary one-way ANOVA with Tukey's multi-comparison posttest (B) or two-tailed unpaired Student *t*-test (C). Two sided tests were used. Source data are provided as a Source Data file.



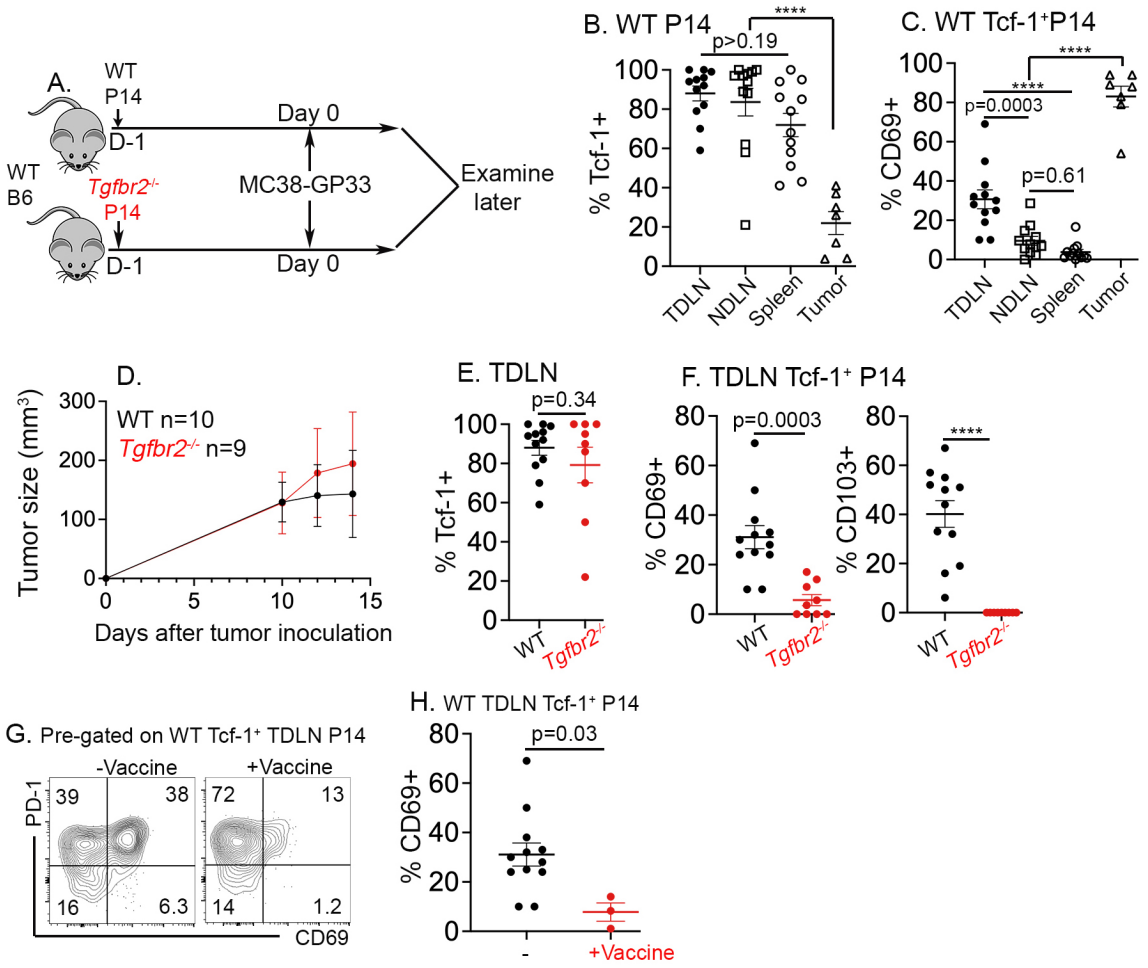

**Supplementary Figure 4. Stem-like endogenous polyclonal CD8$^+$ T cells differentiate into $T_{RM}$ in TDLN**. Same experimental setup as in Fig. 4a. (**A**) Left, representative gating strategy on endogenous bulk CD8$^+$ T cells isolated from TDLN; Right, representative FACS profiles of pre-gated endogenous CD8$^+$ T cells to show $T_{RM}$ phenotype. (**B**) The percentage of CD69$^+$CD103$^+$ subset in endogenous stem-like CD8$^+$ T cells isolated from different lymphoid organs (TDLN, n=14, NDLN, n=9 and spleen, n=14). Data are presented as mean±SEM. Each symbol in (B) represents the results from an individual mouse. Filled circle, TDLN, empty square, NDLN and empty circle, spleen. Pooled results from 3 independent experiments are shown. Indicated *p* value and ****, $p<0.0001$ are calculated by Ordinary one-way ANOVA with Tukey's multi-comparison posttest. Two sided tests were used. Source data are provided as a Source Data file.



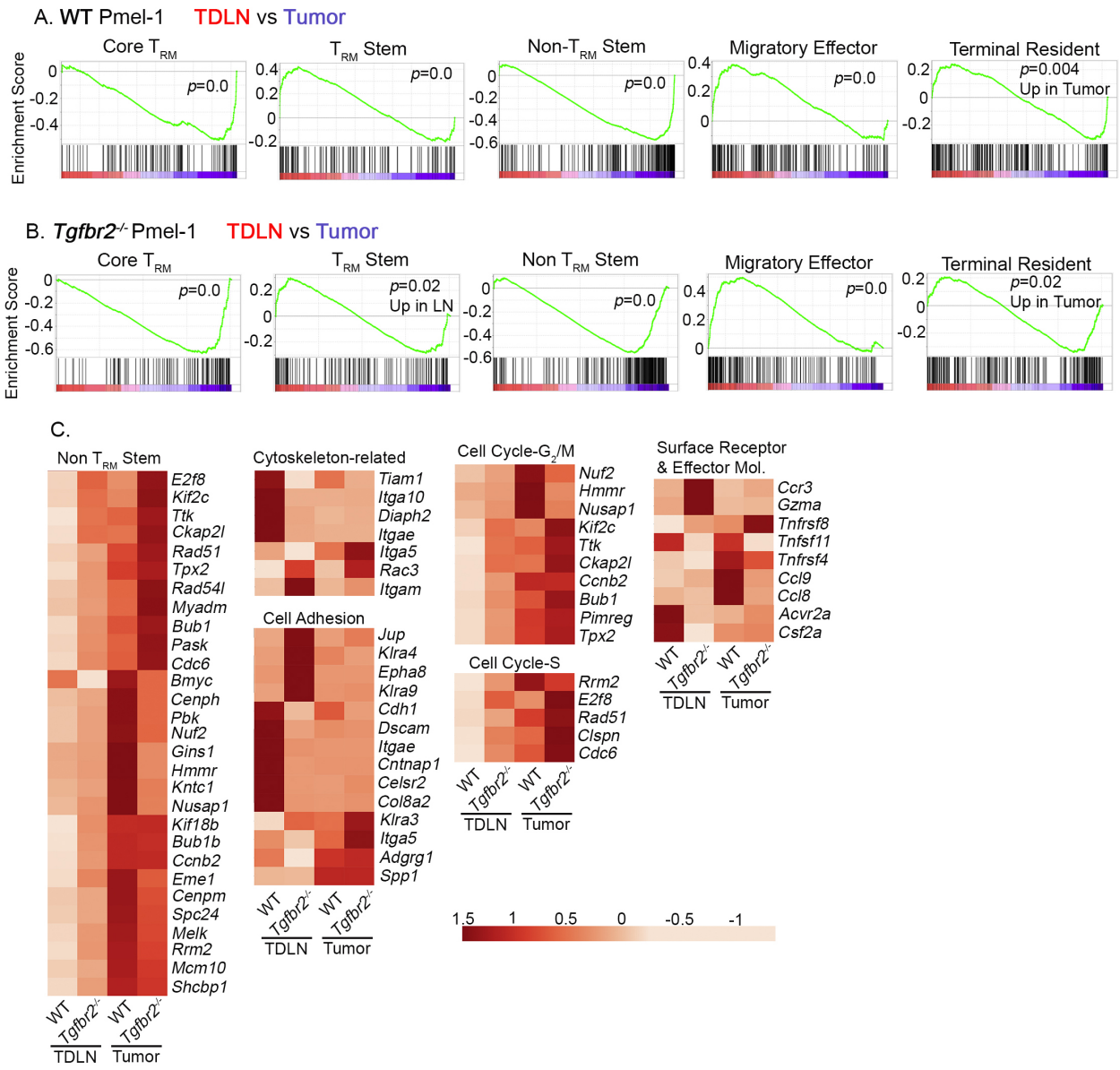

**Supplementary Figure 5. Stem-like T cell subset in other lymphoid organs after tumor vaccination**. Same experimental setup as in Fig. 7. The percentage of stem-like subset in donor Pmel-1 T cells isolated from NDLN (**A**) and spleen (**B**) at different time points after vaccination are shown. For (A), WT d0, n= 9, *Tgfbr2*$^{-/-}$ d0, n=9, WT d4, n= 10, *Tgfbr2*$^{-/-}$ d4, n=10, WT d8, n= 11 and *Tgfbr2*$^{-/-}$ d8, n=11. For (B), WT d0, n= 11, *Tgfbr2*$^{-/-}$ d0, n=11, WT d4, n= 13, *Tgfbr2*$^{-/-}$ d4, n=13, WT d8, n= 11 and *Tgfbr2*$^{-/-}$ d8, n=11. Data are presented as mean±SEM. Pooled results from 3 independent experiments are shown. Black, WT and red, *Tgfbr2*$^{-/-}$. Indicated *p* values and ****, $p<0.0001$ are calculated by Ordinary one-way ANOVA with Tukey's multi-comparison posttest. Two sided tests were used. Source data are provided as a Source Data file.




**Supplementary Figure 6. The alteration of tissue residency after tumor vaccination**. Same experimental setup as in Fig. 7. (**A**) The percentage of CD69$^+$ cells in stem-like (Left) and non-stem (Right) Pmel-1 T cells isolated from NDLN are shown. For stem-like, WT d0, n= 9, *Tgfbr2*$^{-/-}$ d0, n=9, WT d4, n= 10, *Tgfbr2*$^{-/-}$ d4, n=10, WT d8, n= 11 and *Tgfbr2*$^{-/-}$ d8, n=11. For non-stem, WT d0, n= 9, *Tgfbr2*$^{-/-}$ d0, n=9, WT d4, n= 10, *Tgfbr2*$^{-/-}$ d4, n=10, WT d8, n= 11 and *Tgfbr2*$^{-/-}$ d8, n=11. (**B**) The percentage of CD69$^+$ cells in stem-like (Left) and non-stem (Right) Pmel-1 T cells isolated from spleen are shown. For stem-like, WT d0, n= 11, *Tgfbr2*$^{-/-}$ d0, n=11, WT d4, n= 13, *Tgfbr2*$^{-/-}$ d4, n=13, WT d8, n= 11 and *Tgfbr2*$^{-/-}$ d8, n=11. For non-stem, WT d0, n= 11, *Tgfbr2*$^{-/-}$ d0, n=11, WT d4, n= 13, *Tgfbr2*$^{-/-}$ d4, n=13, WT d8, n= 11 and *Tgfbr2*$^{-/-}$ d8, n=11. (**C**) The percentage of CD69$^+$ cells in stem-like (Left) and non-stem (Right) Pmel-1 T cells isolated from tumors at different time points after vaccination are shown. For stem-like, WT d0, n= 8, *Tgfbr2*$^{-/-}$ d0, n=8, WT d4, n= 10, *Tgfbr2*$^{-/-}$ d4, n=10, WT d8, n= 8 and *Tgfbr2*$^{-/-}$ d8, n=8. For non-stem, WT d0, n= 8, *Tgfbr2*$^{-/-}$ d0, n=8, WT d4, n= 10, *Tgfbr2*$^{-/-}$ d4, n=10, WT d8, n= 8 and *Tgfbr2*$^{-/-}$ d8, n=8.Pooled results from 3 independent experiments are shown. Data are presented as mean±SEM. Each symbol in (C) represents the results from an individual recipient mouse. Black symbols, WT and red symbols, *Tgfbr2*$^{-/-}$. Indicated *p* values are calculated by Ordinary one-way ANOVA with Tukey's multi-comparison posttest. Two sided tests were used. Source data are provided as a Source Data file.




**Supplementary Figure 7. Colorectal tumor induces $T_{RM}$ stem-like CD8⁺ T cell differentiation in TDLN.** (**A**) Experimental design. (**B**) The percentage of Tcf-1⁺ cells in WT donor P14 T cells are shown. Filled circle, TDLN, empty square, NDLN, empty circle, spleen and empty triangle, tumor. (**C**) The percentage of CD69⁺ cells in WT Tcf-1⁺ P14 T cells are shown. For (B) and (C), TDLN, n=12, NDLN, n=12, Spleen, n=12 and Tumor, n=7. (**D**) Tumor growth (WT, n=10 and *Tgfbr2*$^{-/-}$, n=9). (**E**) The percentage of Tcf-1⁺ cells in WT and *Tgfbr2*$^{-/-}$ P14 T cells isolated from TDLN are shown (WT, n=12 and *Tgfbr2*$^{-/-}$, n=9). (**F**) The percentage of CD69⁺ (left) and CD103⁺ (right) in TDLN Tcf-1⁺ P14 T cells are shown (WT, n=12 and *Tgfbr2*$^{-/-}$, n=9). (**G**) Representative FACS of pre-gated TDLN Tcf-1⁺ P14 T cells before and 4 days after vaccination are shown. (**H**) The percentage of CD69⁺ cells in Tcf-1⁺ P14 T cells isolated from TDLN are shown (-Vaccine, n=12 and +Vaccine, n=3). Each symbol in (B to C) and (E, F, and H) represents the results from an individual mouse. For (D) to (F), black symbols, WT and red symbols, *Tgfbr2*$^{-/-}$. For (H), black symbols, no vaccine and red symbols, after vaccine. Data are presented as mean±SEM. Pooled results from 2 independent experiments are shown. Indicated p values and ****, p<0.0001 by Ordinary one-way ANOVA with Tukey's multi-comparison posttest. (B and C) or unpaired Student *t*-test (E to H). Two sided tests were used. Source data are provided as a Source Data file.




**Supplementary Figure 8. Enrichment of $T_{RM}$ signature genes in tumor.** GSEA results comparing TDLN (red) vs tumor (violet) samples. (**A**) WT and (**B**) *Tgfbr2*$^{-/-}$ samples (**C**) Heatmap of DEGs for selected signature genes.