Appendix- SUPPLEMENTARY MATERIALS

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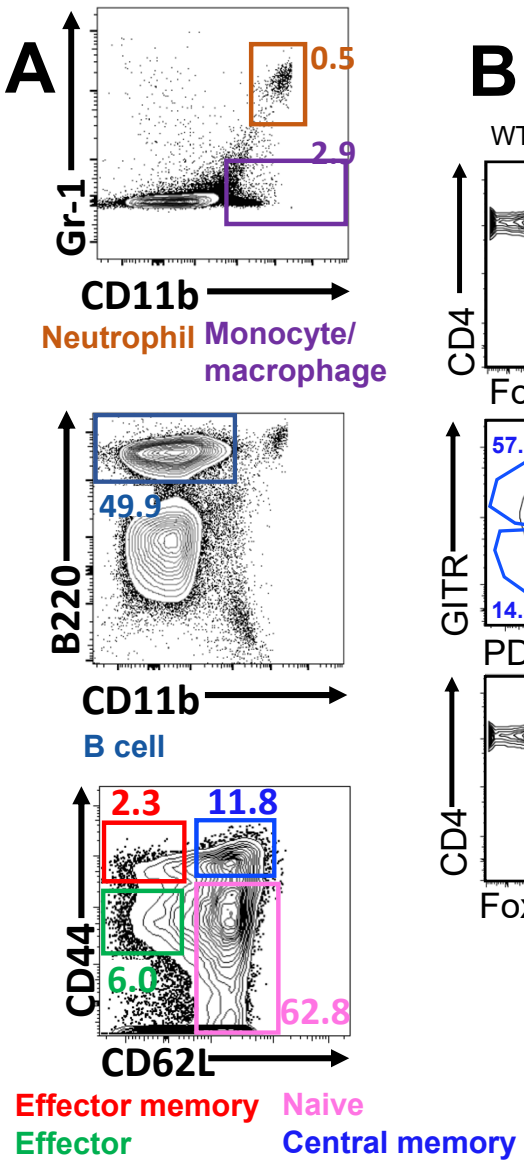
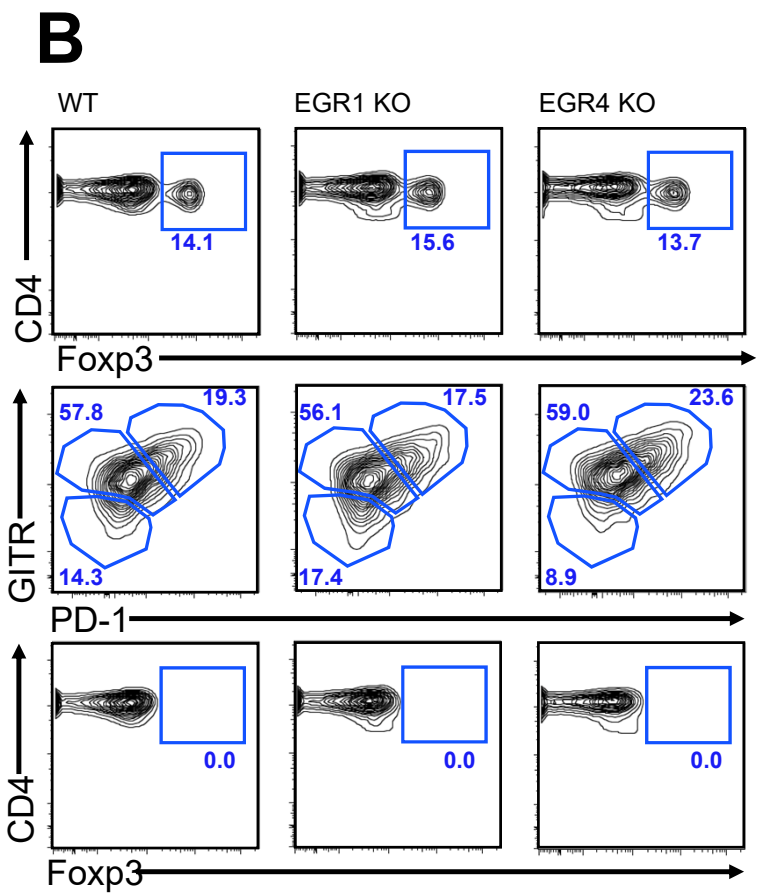
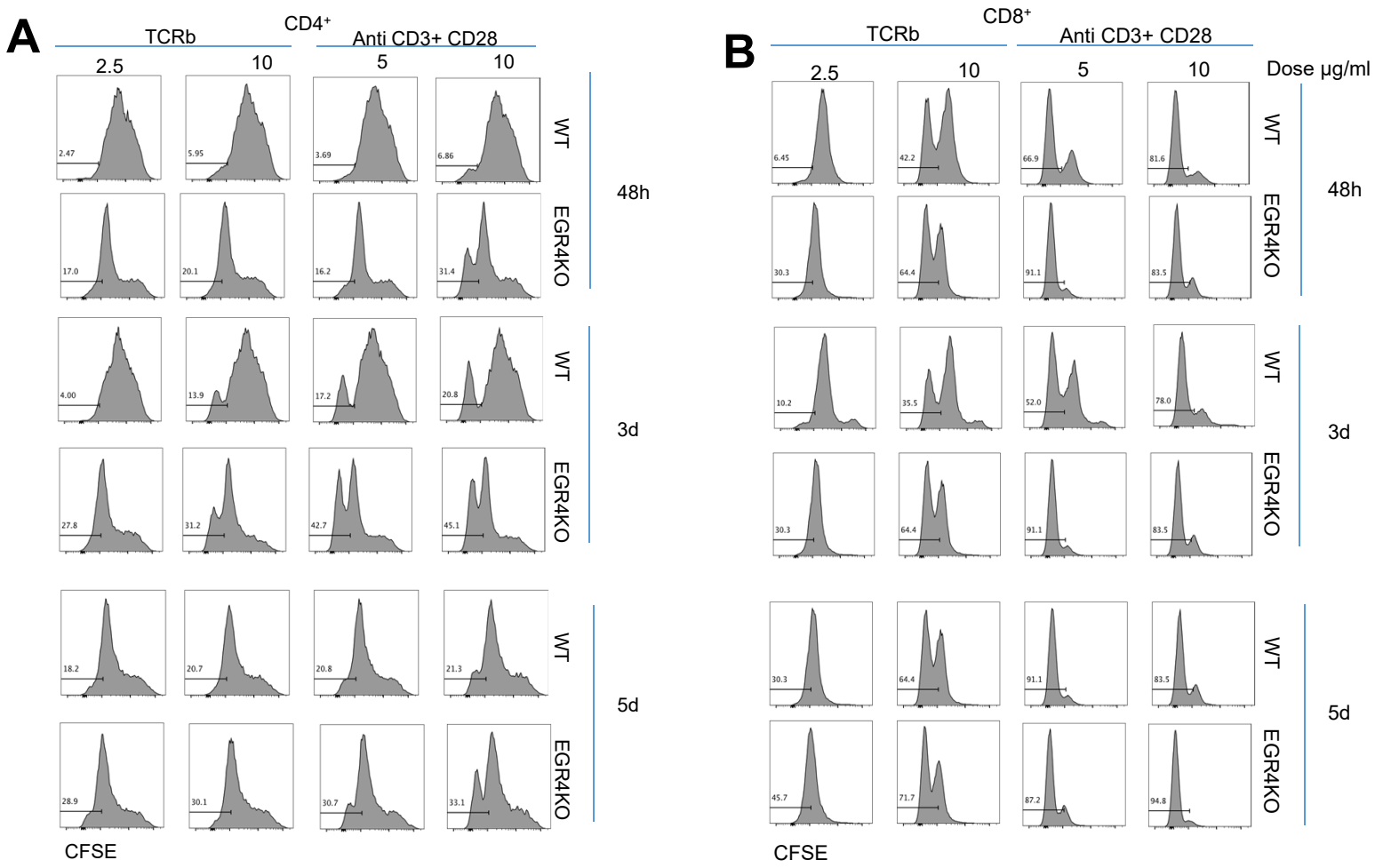
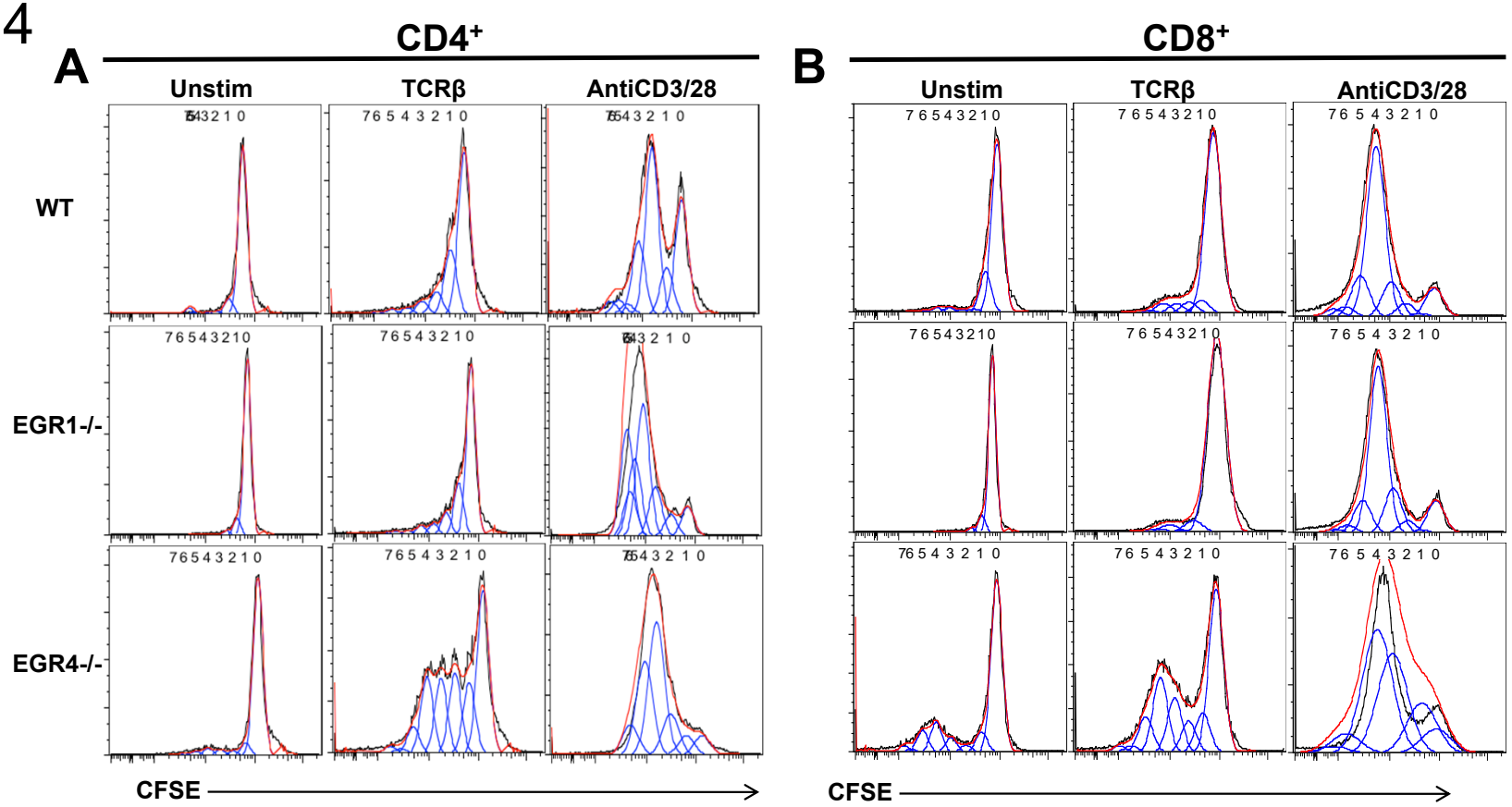




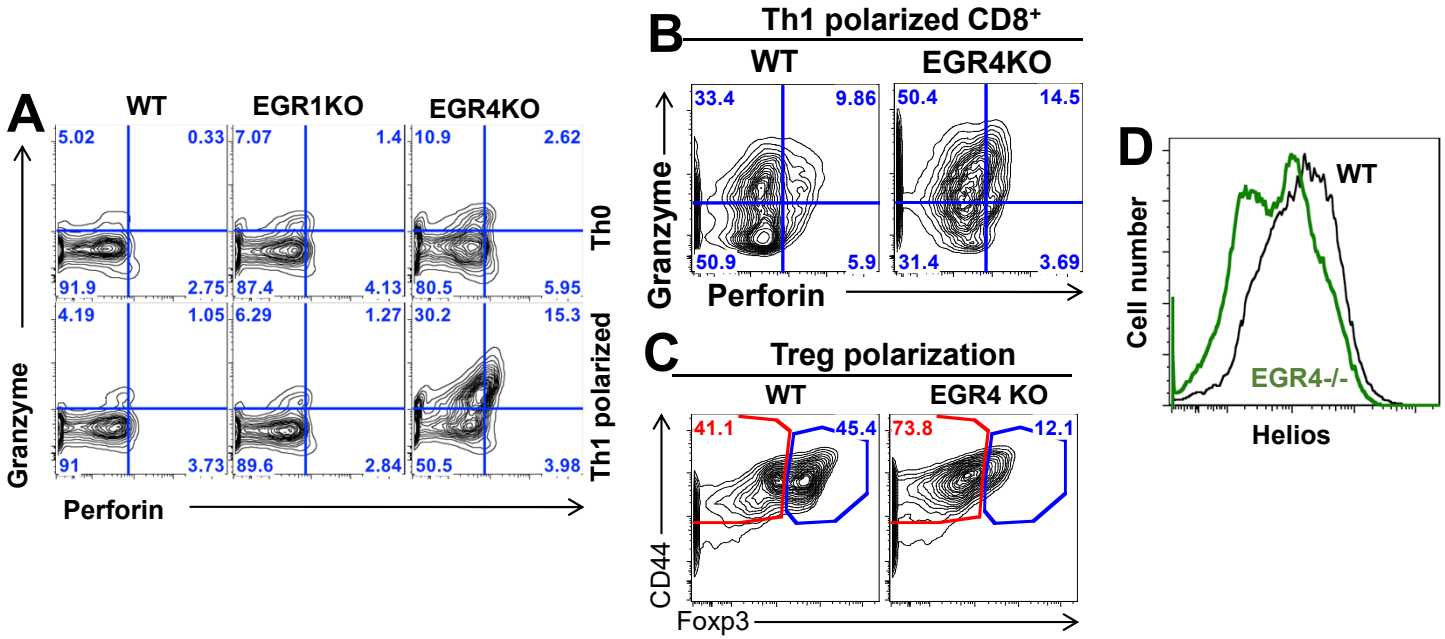
Appendix Fig S1. Gating schemes and staining controls for experiments performed in figure 1. (A) Gating scheme for neutrophils, macrophages, and B cells (as fractions of total splenocytes), or naïve, central memory, effector, and effector memory CD4⁺ T cell subsets (as fractions of total T cells), as applied in Fig. 1B. Gates are colored as indicated at right. Data are for wt mice. **(B)** Representative FACS plots depicting Foxp3, GITR and PD-1 staining for total T cells (top row), or gated Foxp3⁺ cells (2nd row), for wt, EGR1 KO, and EGR4 KO mice, as indicated. Bottom row shows FMO (fluorescence minus one) controls for Foxp3 staining. FMO controls contain all the fluorochromes in a panel, except for Foxp3.



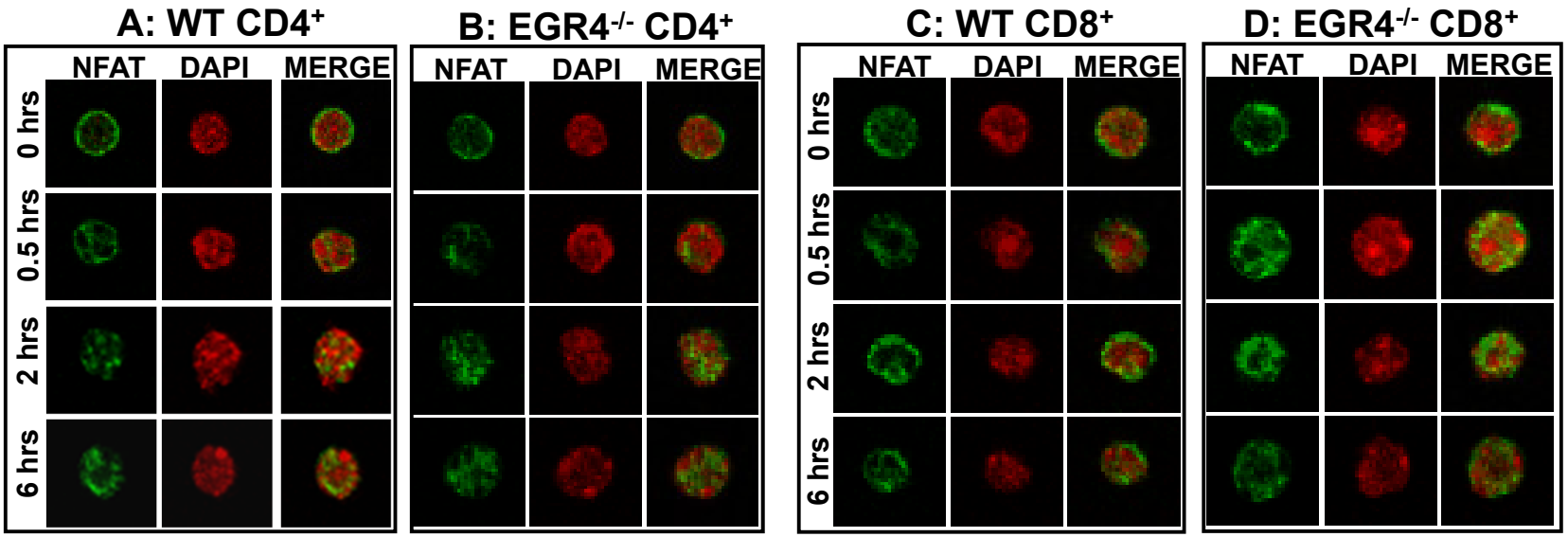
Appendix Fig S2. Raw plots of time course of proliferation data. Representative histograms of CFSE stained CD4⁺ (**A**) and CD8⁺ (**B**) T cells as depicted in figure 2A,B.



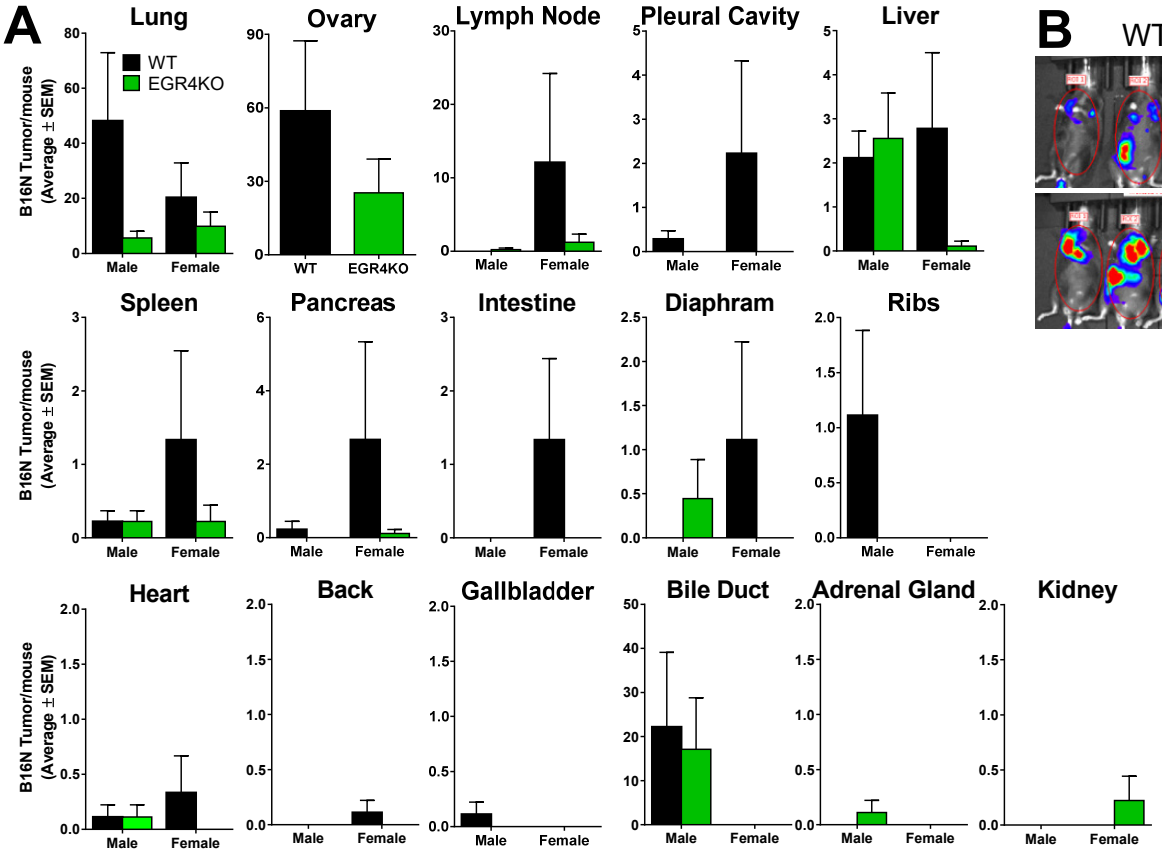
Appendix Fig S3. Constitutive hyper-proliferation in EGR4KO T cells. WT, EGR1-/- and EGR4-/- CD4$^+$ **(A)** and CD8$^+$ **(B)** T cells were isolated from the spleen by cell sorting and stained with CFSE. Cells were incubated under unstimulated conditions, or stimulated with either anti-TCRβ or anti-CD3/CD28 antibodies. After 4 days, cells were collected for FACS analysis. Generation analysis is depicted in figure 2 **(C,D)**.

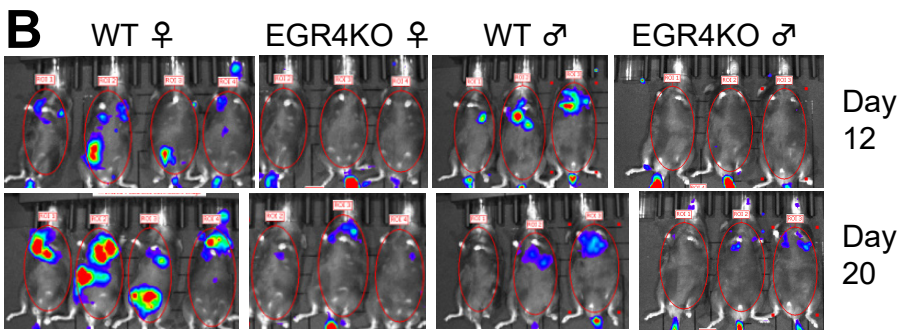


Appendix Fig S4. Differences in CTL and Treg generation. (A) WT, EGR1KO or EGR4KO CD4$^+$ T cells were incubated under Th0 or Th1 polarizing conditions for 5 days before intracellular staining for Granzyme B and Perforin followed by FACS analysis. **(B)** WT or EGR4KO CD8$^+$ T cells were incubated for 5 days under Th1 conditions before intracellular staining for Granzyme B and Perforin followed by FACS analysis. **(C)** WT or EGR4KO CD4$^+$ T cells were incubated under Treg polarizing conditions before FACS analysis as depicted. **(D)** Helios expression was determined in WT and EGR4-/- FOXP3$^+$ CD44$^+$ T cells. Data shown are representative of at least 2 independent experiments.

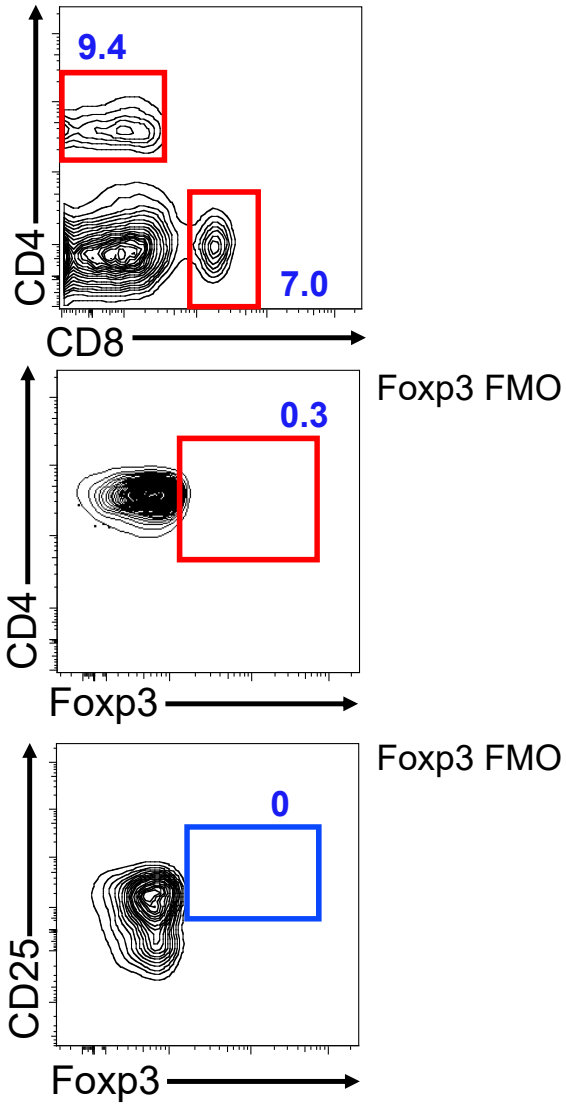


Appendix Fig S5. Representative Images of NFAT localization in T cells. NFAT nuclear localization was measured was measured by immunocytochemistry in $CD4^+$ and $CD8^+$ T cells isolated by negative selection from WT, EGR1KO and EGR4KO mice. Cells were stimulated with anti-CD3/CD28 antibodies for the indicated time periods before fixation and staining. Representative images of $CD4^+$ **(A,B)** and $CD8^+$ **(C,D)** T cells from WT **(A,C)** and EGR4-/- **(B,D)** mice showing the localization of NFATc1 and the nucleus (DAPI staining) at 0 and 6 hrs of CD3/CD28 stimulation.

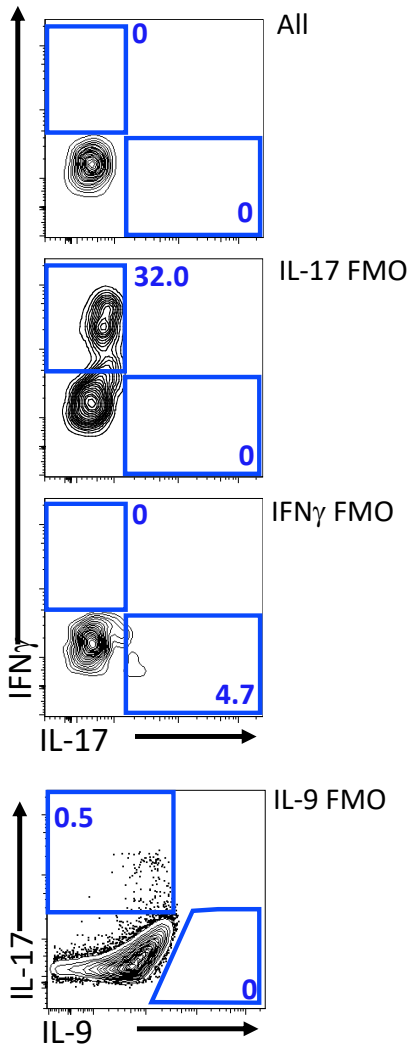




Appendix Fig S6. Melanoma lung colonization and metastasis in WT vs. EGR4-/- mice. (A) B16N melanoma cells were injected into syngeneic WT and EGR4-/- mice by tail vein injection. Mice were sacrificed on day 20 and the numbers of tumors at different locations were counted. **(B)** Luciferase expression was monitored by IVIS imaging at the indicated timepoints after luciferin injections. Sample images of tumor formation are shown from days 12 and 20.



Appendix Fig S7. Staining controls for experiments performed in figure 7G. Representative FACS plots depicting CD4/CD8 gating and FMO (fluorescence minus one) control for Foxp3 staining of gated $CD4^+$ T cells. FMO controls contain all the fluorochromes in a panel, except for Foxp3. Staining is shown for a wt mouse.



Appendix Fig S8. Staining controls for experiments performed in figure 8B,C. Representative FACS plots depicting FMO (fluorescence minus one) cytokine controls. FMO controls contain all the fluorochromes in a panel, except for the one indicated at right. EGR4-/- spleen T cells from tumor-bearing mice.