**Supplemental Table 1. List of antibodies used in this study.**

| Antibodies | Source | Clone No. | Catalog No. |
|---|---|---|---|
| Anti-mouse CD3 | BioXCell | 145-2C11 | BE0001-1 |
| Anti-mouse CD28 | BioXCell | 37.51 | BE0015-1 |
| Anti-mouse IL-4 | BioXCell | 11B11 | BE0045 |
| Anti-mouse IFN-γ | BioXCell | XMG1.2 | BE0055 |
| Anti-mouse CD4 | BioXCell | GK1.5 | BE0003-1 |
| Anti-mouse FoxO4 | Santa Cruz | N/A | sc-5221 |
| Anti-mouse DKK3 | Proteintech | N/A | 10365-1-AP |
| APC Anti-mouse CD4 | BD Biosciences | RM4.5 | 553051 |
| PerCP-Cyanine5.5 Anti-mouse CD4 | eBioscience | RM4.5 | 45-0042-82 |
| PE Anti-mouse CD8 | eBioscience | 53-6.7 | 12-0081-82 |
| PE-Cyanine7 Anti-mouse CD25 | eBioscience | PC61.5 | 25-0251-82 |
| eFluor™ 450 Anti-mouse CD44 | eBioscience | IM7 | 48-0441-82 |
| APC-eFluor™ 780 Anti-mouse CD62L | eBioscience | MEL-14 | 47-0621-82 |
| PE Anti-mouse IFN-γ | Biolegend | XMG1.2 | 505808 |
| Alexa Fluor® 700 Anti-mouse IFN-γ | BD Biosciences | XMG1.2 | 557998 |
| PE Anti-mouse IL-4 | Biolegend | 11B11 | 504104 |
| PE Anti-mouse IL-17A | BD Biosciences | TC11-18H10 | 559502 |
| FITC Anti-mouse Foxp3 | eBioscience | FJK-16s | 11-5773-82 |
| APC Anti-mouse B220 | BD Biosciences | RA3-6B2 | 553092 |
| Alexa Fluor® 700 Anti-mouse TCRβ | BD Biosciences | H57-597 | 560705 |
| FITC Anti-Mouse PD-1 | eBioscience | RMP1-30 | 11-9981-82 |
| Biotin Anti-Mouse CXCR5 | BD Biosciences | 2G8 | 551960 |

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|---|---|---|---|
| PE Anti-Mouse CD95 | BD Biosciences | Jo2 | 554258 |
| eFluor™ 450 Anti-Mouse GL-7 | eBioscience | GL7 | 48-5902-82 |

**Supplemental Table 2. Primers for Real-Time qPCR and ChIP-qPCR.**

| Name | Forward (5'→3') | Reverse (5'→3') |
|---|---|---|
| *Gapdh* | GACAACTTTGGCATTGTGG | ATGCAGGGATGATGTTCTG |
| *Ifng* | GATGCATTCATGAGTATTGCCAAGT | GTGGACCACTCGGATGAGCTC |
| *Tbx21* | CAACAACCCCTTTGCCAAAG | TCCCCCAAGCAGTTGACAGT |
| *Stat4* | TGGCAACAATTCTGCTTCAAAAC | GAGGTCCCTGGATAGGCATGT |
| *Runx3* | CAGGTTCAACGACCTTCGATT | GTGGTAGGTAGCCACTTGGG |
| *Gata3* | AGGGACATCCTGCGCGAACTGT | CATCTTCCGGTTTCGGGTCTGG |
| *Il4* | AGATCATCGGCATTTTGAACG | TTTGGCACATCCATCTCCG |
| *Il5* | CGCTCACCGAGCTCTGTTG | CCAATGCATAGCTGGTGATTTTT |
| *Rorc* | GACCCACACCTCACAAATTGA | AGTAGGCCACATTACACTGCT |
| *Il17a* | CTGGAGGATAACACTGTGAGAGT | TGCTGAATGGCGACGGAGTTC |
| *Foxp3* | CCCATCCCCAGGAGTCTTG | ACCATGACTAGGGGCACTGTA |
| *Il3* | GGGATACCCACCGTTTAACCA | AGGTTTACTCTCCGAAAGCTCTT |
| *Dkk3* | CTCGGGGGTATTTTGCTGTGT | TCCTCCTGAGGGTAGTTGAGA |
| *Csf1* | ATGAGCAGGAGTATTGCCAAGG | TCCATTCCCAATCATGTGGCTA |
| *Csf2* | GGCCTTGGAAGCATGTAGAGG | GGAGAACTCGTTAGAGACGACTT |
| *Csf3* | ATGGCTCAACTTTCTGCCCAG | CTGACAGTGACCAGGGGAAC |
| *Bcl6* | CCGGCACGCTAGTGATGTT | TGTCTTATGGGCTCTAAACTGCT |
| *Ccr6* | CCTGGGCAACATTATGGTGGT | CAGAACGGTAGGGTGAGGACA |
| *Il23r* | TTCAGATGGGCATGAATGTTTCT | CCAAATCCGAGCTGTTGTTCTAT |
| *Tcf4* | CGAAAAGTTCCTCCGGGTTTG | CGTAGCCGGGCTGATTCAT |
| *Tcf7* | AGCTTTCTCCACTCTACGAACA | AATCCAGAGAGATCGGGGGTC |
| *Lef1* | TGTTTATCCCATCACGGGTGG | CATGGAAGTGTCGCCTGACAG |

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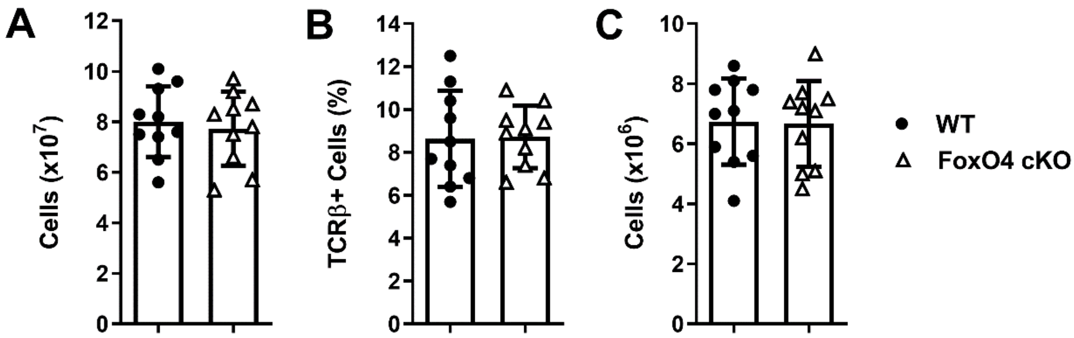
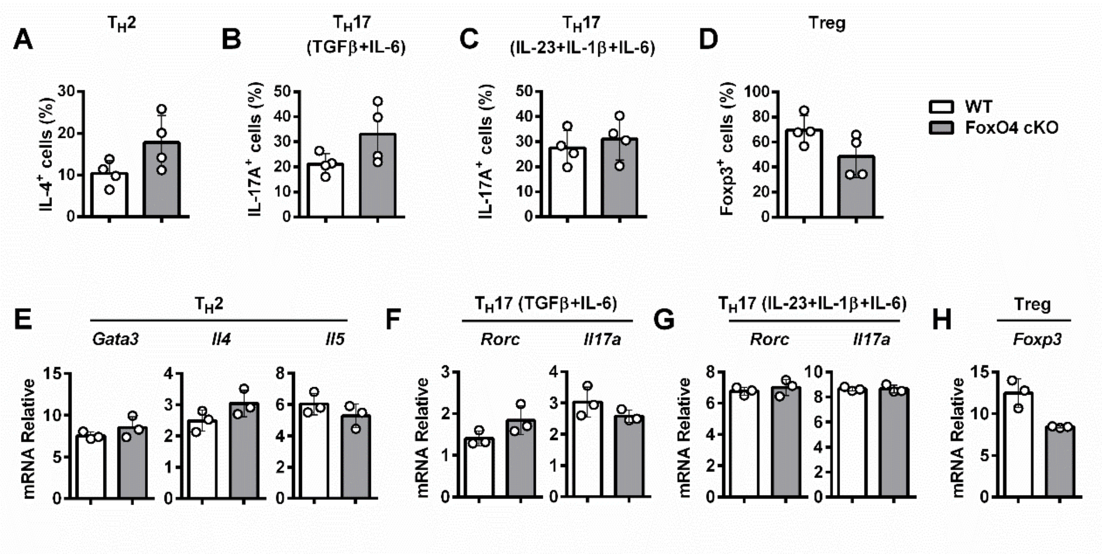
|---|---|---|
| *Myc* | ATGCCCCTCAACGTGAACTTC | CGCAACATAGGATGGAGAGCA |
| *H19* | GCATGGTCCTCAAATTCTGCA | GCATCTGAACGCCCCAATTA |
| *Ifng*CNS1 | CACTTCTGTGCAACCCTTGA | AAGCACTCACTGGGTCATTG |
| *Ifng*CNS2 | AACTGGAAAATGGCAGGCTA | CCCGAGATAAATTCCATCCA |
| *Ifng*CNS22 | ATGACAAAATGCAGGGCTTC | CCCACACTAGATGATATATGATTTTCC |
| *Ifng*CNS34 | AAAAGAGTCCAAGATATGAAAGCAA | GGCTTTGGGAATTCTACCTTG |
| *Ifng*CNS55 | TGTCTCGGTGACACATCCTT | GGGAGGCAGGAGGAACTTTA |
| *Ifng*Promoter | CCCCACCTATCTGTCACCAT | CACCTCTCTGGCTTCCAGTT |
| *Eomes*FBS1 | CGGGGTTTGTTTTCTTGCG | GATTGTAGGTGCCCCTTTCCT |
| *Eomes*FBS2 | AAGATCAAGGTCTGGGAACCCG | GTGGGGAGTGTAAACAAGCCG |
| *Eomes*FBS3 | CACCGATAAACAAGCCTCCATT | GGGTTCCCAGACCTTGATCTTTAT |

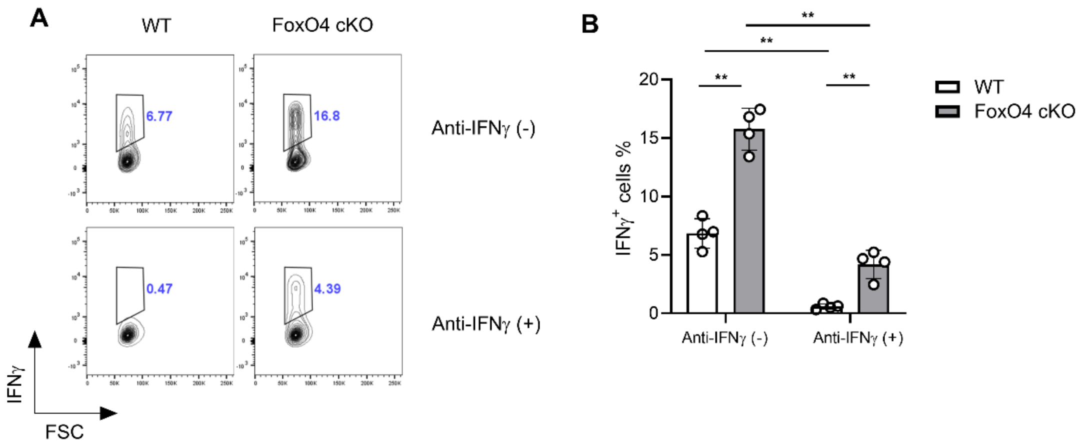
**Supplemental Figures**



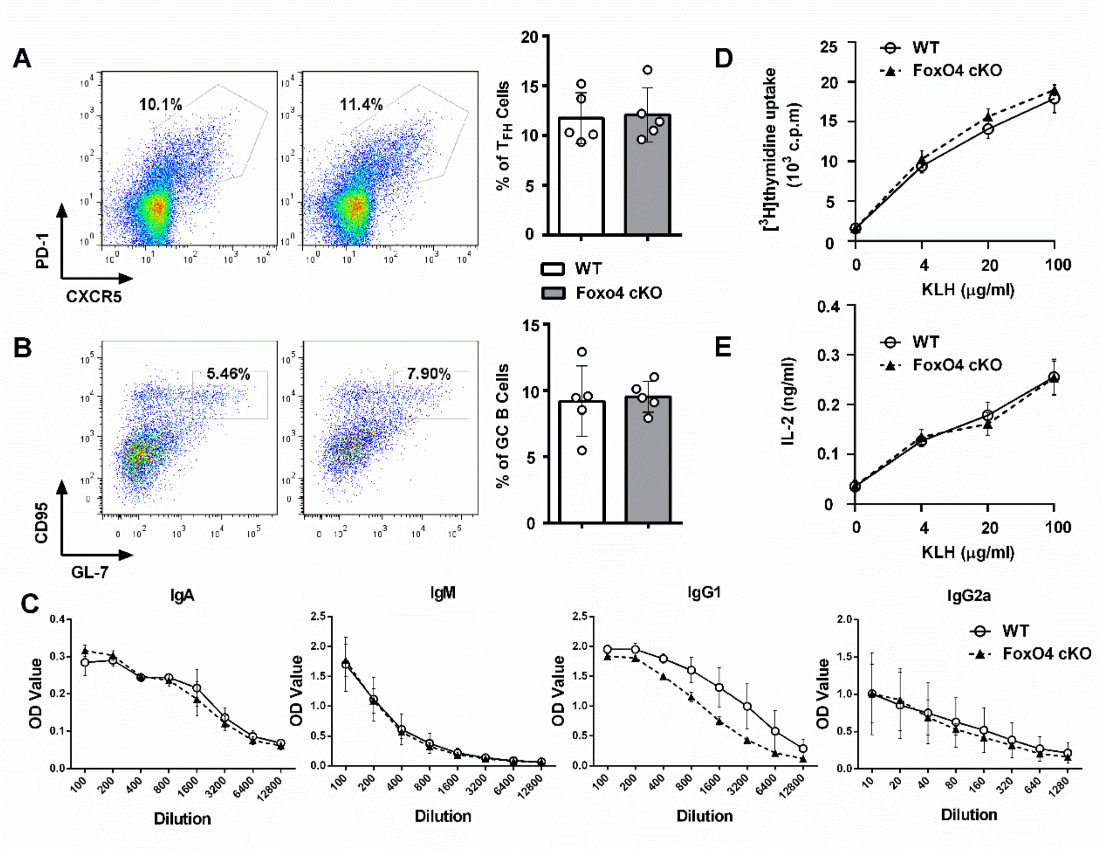

**Supplemental Figure 1. Numbers of mature T lymphocytes in thymus are normal in *FoxO4* cKO mice.** (**A**) Absolute thymocyte numbers in WT and *FoxO4* cKO mice ($n$=10). (**B** and **C**) Percentages (B) and absolute cell numbers (C) of TCR$\beta^+$ T lymphocytes in thymus in WT and *FoxO4* cKO mice ($n$=10). Each symbol (A-C) represents an individual mouse. ns=not statistically significant, unpaired Student's $t$-test. Data are one representative of three independent experiments with similar results (means and SD in A-C).



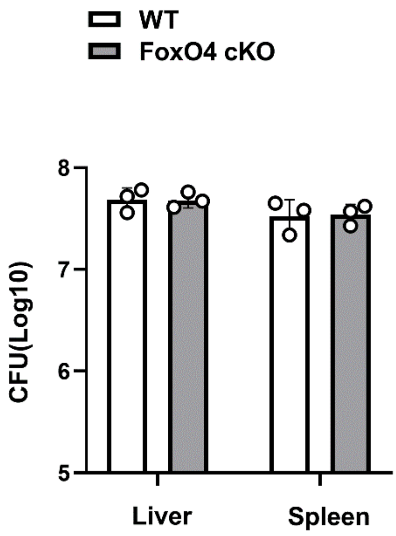

**Supplemental Figure 2. FoxO4 does not affect differentiation of $T_H2$, $T_H17$, and iTreg subsets. (A-D)** Percentage of IL-4 (A), IL-17A (B and C), Foxp3 (D) expressing WT and *FoxO4* cKO $CD4^+$ T cells, which were differentiated from naïve T cells in various polarizing conditions for 3 days (n=4). (**E-H**) Real-time qPCR analysis of signature genes of $T_H2$ (E), $T_H17$ (induced by TGFβ/IL-6, F), $T_H17$ (induced by IL-23/IL-1β/IL-6, G) and iTreg (H) from WT and *FoxO4* cKO mice, by stimulating naive $CD4^+$ T cells for 3 days with plate-bound anti-CD3 and anti-CD28 in various polarizing conditions, and assessing after re-stimulation by plate-bound anti-CD3 for 5 hours (n=3). Results were presented relative to the expression *Gapdh* mRNA. ns=not statistically significant, unpaired Student's *t*-test. Data are one representative of two independent experiments with similar results (means and SD in A-H).



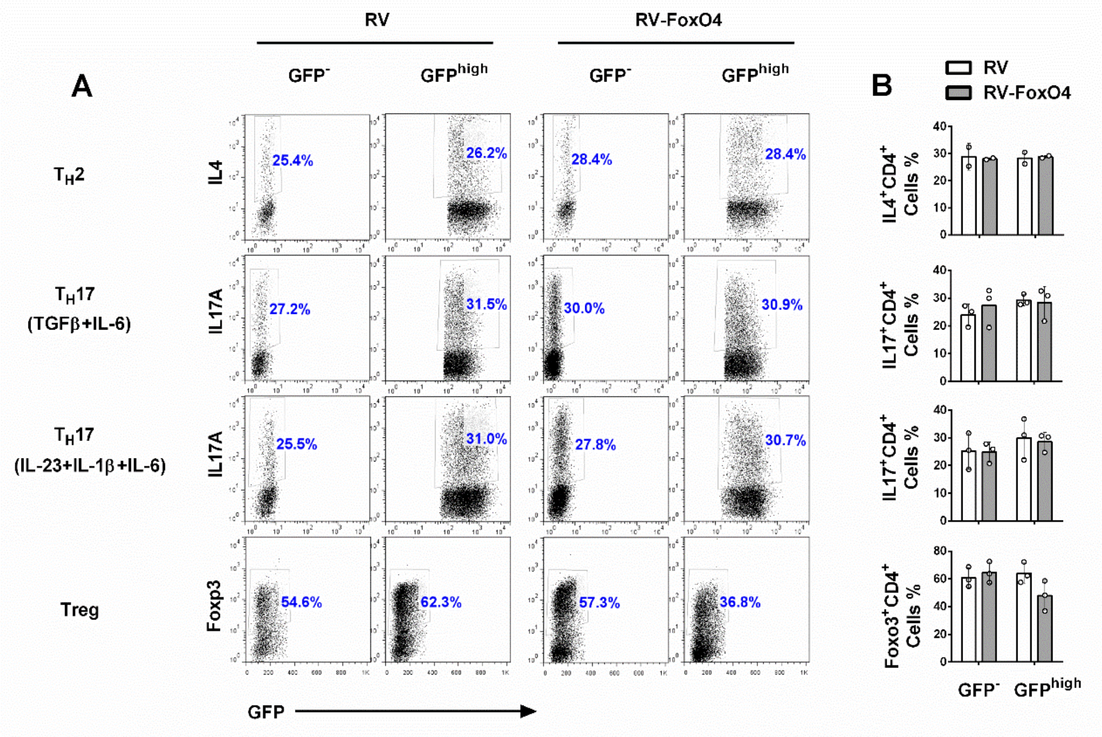

**Supplemental Figure 3. FoxO4 negatively regulates IFN-γ expression in $T_H1$ polarizing condition with or without anti-IFN-γ.** (A) Flow cytometry of WT and FoxO4 cKO Naïve CD4+ T cells after 2 days of polarization toward the $T_H1$ lineage, with or without anti-IFN-γ (n=4). (B) Frequencies of IFN-γ-expressing cells as in (A) (n=4). **P<0.01; ***P<0.001, by two-way ANOVA with Tukey's multiple-comparison test (B). Data are one representative of two independent experiments (means and SD in B).



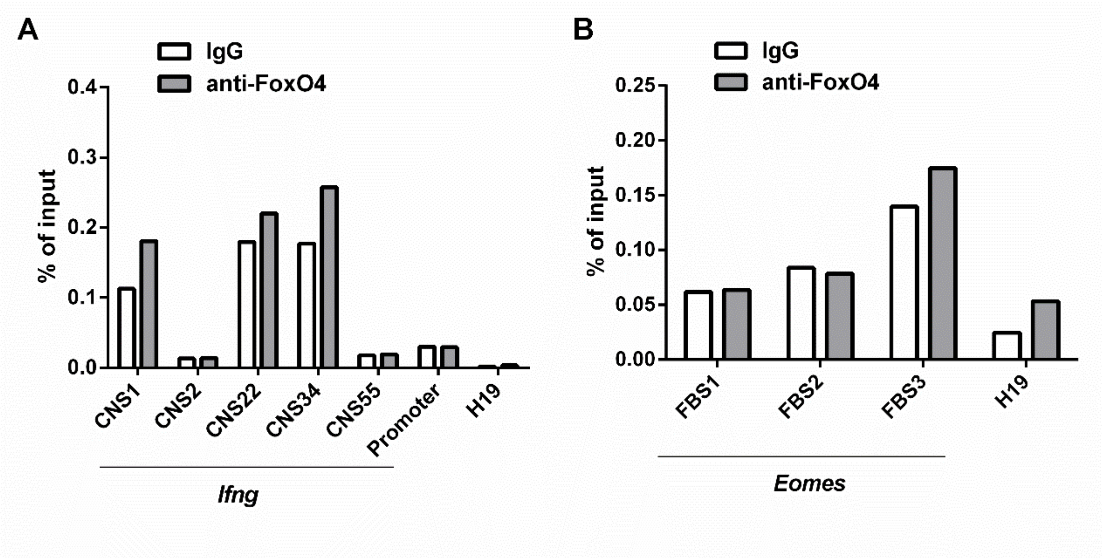

**Supplemental Figure 4. *FoxO4* cKO mice have normal germinal center responses and antigen specific Ig production.** (**A** and **B**) Flow cytometry analysis (left) and quantification (right) of $T_{FH}$ cells (left in A, PD-1$^+$CXCR5$^+$, gating on CD4$^+$CD44$^{hi}$) and germinal center B cells (left in B, GL-7$^+$CD95$^+$, gating on B220$^+$) in dLNs from WT and *FoxO4* cKO mice which received subcutaneous KLH/CFA immunization for 7 days (n=5). (C) ELISA analysis was performed to determine the concentrations of KLH specific IgA, IgM, IgG1 and IgG2a in serum from WT and *FoxO4* cKO mice which were immunized as in A and B. Cells from draining lymph nodes were isolated 7 days later and restimulated with KLH (n=5). Proliferation was measured by [3H] thymidine incorporation assay (D) and IL-2 expression was determined by ELISA (E) (n=5). ns=not statistically significant, unpaired Student's *t*-test. Data are one representative of three independent experiments with similar results (means and SD in A-E).



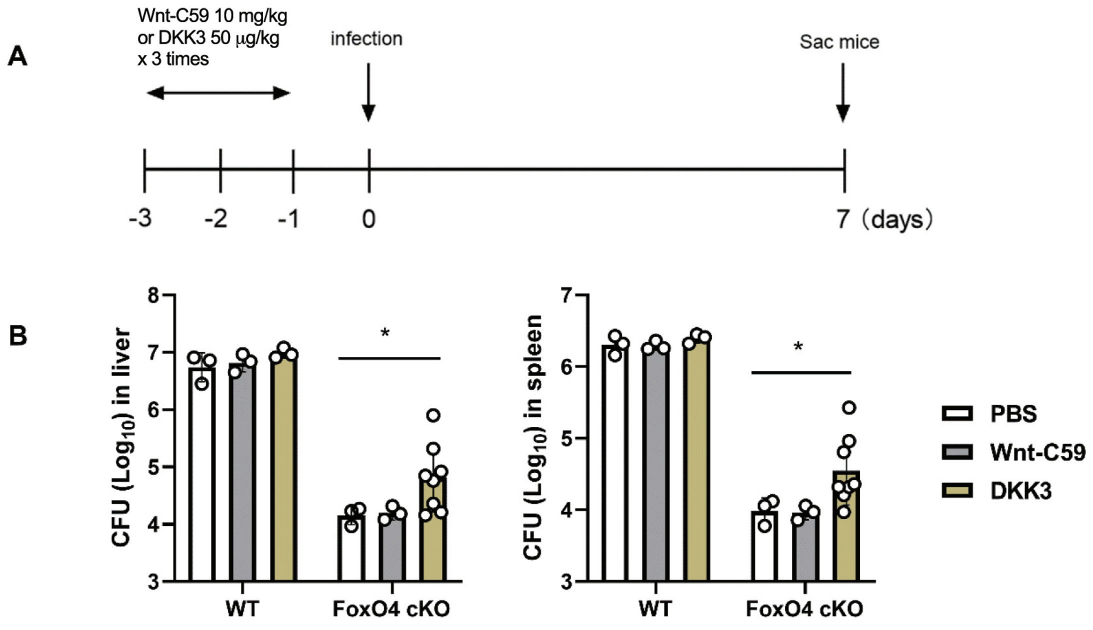

**Supplemental Figure 5. FoxO4 deficiency in CD4$^{+}$ T cells provides protection from listeria infection.** Infected mice were treated with anti-CD4 antibody (GK1.5, BioXcell) intraperitoneally at day -1 and +1 respective to challenge listeria infection. Livers and spleens were harvested at day 4 post infection for enumeration of bacterial burdens (n=3). ns=not statistically significant, unpaired Student's *t*-test. Data are one representative of two independent experiments with similar results (means and SD).




**Supplemental Figure 6. Ectopic expression of FoxO4 doesn't affect differentiation of $T_H2$, $T_H17$, and iTreg subsets.** (**A**) Flow cytometry of various polarized $CD4^+$ T cells, which were transduced with empty vector (RV) or vector encoding FoxO4 (RV-FoxO4). Data were analyzed in $GFP^-$ and $GFP^{high}$ populations, respectively (n=3). (**B**) Frequency of specific marker-expressing cells as in A (n=3). ns=not statistically significant, unpaired Student's *t*-test. Data are one representative of three independent experiments with similar results (means and SD in B).




**Supplemental Figure 7. FoxO4 doesn't bind to *Ifng* and *Eomes* gene loci.** (**A** and **B**) Chromatin immunoprecipitation analysis of FoxO4 binding to *Ifng* (A) and *Eomes* (B) gene loci in purified CD4$^+$ T cells stimulated for 24 hours with plate-bound anti-CD3. Results were expressed as percentage of input. Data are one representative of two independent experiments with similar results.




**Supplemental Figure 8. FoxO4-DKK3 axis functions in anti-listeria immunity.** (A) Schematic of animal experimental setup. (B) L. monocytogenes titers in the spleens and livers of WT and *FoxO4* cKO mice infected with Lm-OVA for 7 days, with treatment of PBS, Wnt-C59 and DKK3, respectively, showed as CFUs (n=3-8). *P<0.05, by two-way ANOVA with Tukey's multiple-comparison test. Data are one representative of two independent experiments with similar results (means and SD in B).

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| Alexa Fluor® 700 Anti-mouse TCRβ | BD Biosciences | H57-597 | 560705 |
| FITC Anti-Mouse PD-1 | eBioscience | RMP1-30 | 11-9981-82 |
| Biotin Anti-Mouse CXCR5 | BD Biosciences | 2G8 | 551960 |
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| *Runx3* | CAGGTTCAACGACCTTCGATT | GTGGTAGGTAGCCACTTGGG |
| *Gata3* | AGGGACATCCTGCGCGAACTGT | CATCTTCCGGTTTCGGGTCTGG |
| *Il4* | AGATCATCGGCATTTTGAACG | TTTGGCACATCCATCTCCG |
| *Il5* | CGCTCACCGAGCTCTGTTG | CCAATGCATAGCTGGTGATTTTT |
| *Rorc* | GACCCACACCTCACAAATTGA | AGTAGGCCACATTACACTGCT |
| *Il17a* | CTGGAGGATAACACTGTGAGAGT | TGCTGAATGGCGACGGAGTTC |
| *Foxp3* | CCCATCCCCAGGAGTCTTG | ACCATGACTAGGGGCACTGTA |
| *Il3* | GGGATACCCACCGTTTAACCA | AGGTTTACTCTCCGAAAGCTCTT |
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| *Csf1* | ATGAGCAGGAGTATTGCCAAGG | TCCATTCCCAATCATGTGGCTA |
| *Csf2* | GGCCTTGGAAGCATGTAGAGG | GGAGAACTCGTTAGAGACGACTT |
| *Csf3* | ATGGCTCAACTTTCTGCCCAG | CTGACAGTGACCAGGGGAAC |
| *Bcl6* | CCGGCACGCTAGTGATGTT | TGTCTTATGGGCTCTAAACTGCT |

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|---|---|---|
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| *Il23r* | TTCAGATGGGCATGAATGTTTCT | CCAAATCCGAGCTGTTGTTCTAT |
| *Tcf4* | CGAAAAGTTCCTCCGGGTTTG | CGTAGCCGGGCTGATTCAT |
| *Tcf7* | AGCTTTCTCCACTCTACGAACA | AATCCAGAGAGATCGGGGGTC |
| *Lef1* | TGTTTATCCCATCACGGGTGG | CATGGAAGTGTCGCCTGACAG |
| *Myc* | ATGCCCCTCAACGTGAACTTC | CGCAACATAGGATGGAGAGCA |
| *H19* | GCATGGTCCTCAAATTCTGCA | GCATCTGAACGCCCCAATTA |
| *Ifng*CNS1 | CACTTCTGTGCAACCCTTGA | AAGCACTCACTGGGTCATTG |
| *Ifng*CNS2 | AACTGGAAAATGGCAGGCTA | CCCGAGATAAATTCCATCCA |
| *Ifng*CNS22 | ATGACAAAATGCAGGGCTTC | CCCACACTAGATGATATATGATTTTCC |
| *Ifng*CNS34 | AAAAGAGTCCAAGATATGAAAGCAA | GGCTTTGGGAATTCTACCTTG |
| *Ifng*CNS55 | TGTCTCGGTGACACATCCTT | GGGAGGCAGGAGGAACTTTA |
| *Ifng*Promoter | CCCCACCTATCTGTCACCAT | CACCTCTCTGGCTTCCAGTT |
| *Eomes*FBS1 | CGGGGTTTGTTTTCTTGCG | GATTGTAGGTGCCCCTTTCCT |
| *Eomes*FBS2 | AAGATCAAGGTCTGGGAACCCG | GTGGGGAGTGTAAACAAGCCG |
| *Eomes*FBS3 | CACCGATAAACAAGCCTCCATT | GGGTTCCCAGACCTTGATCTTTAT |