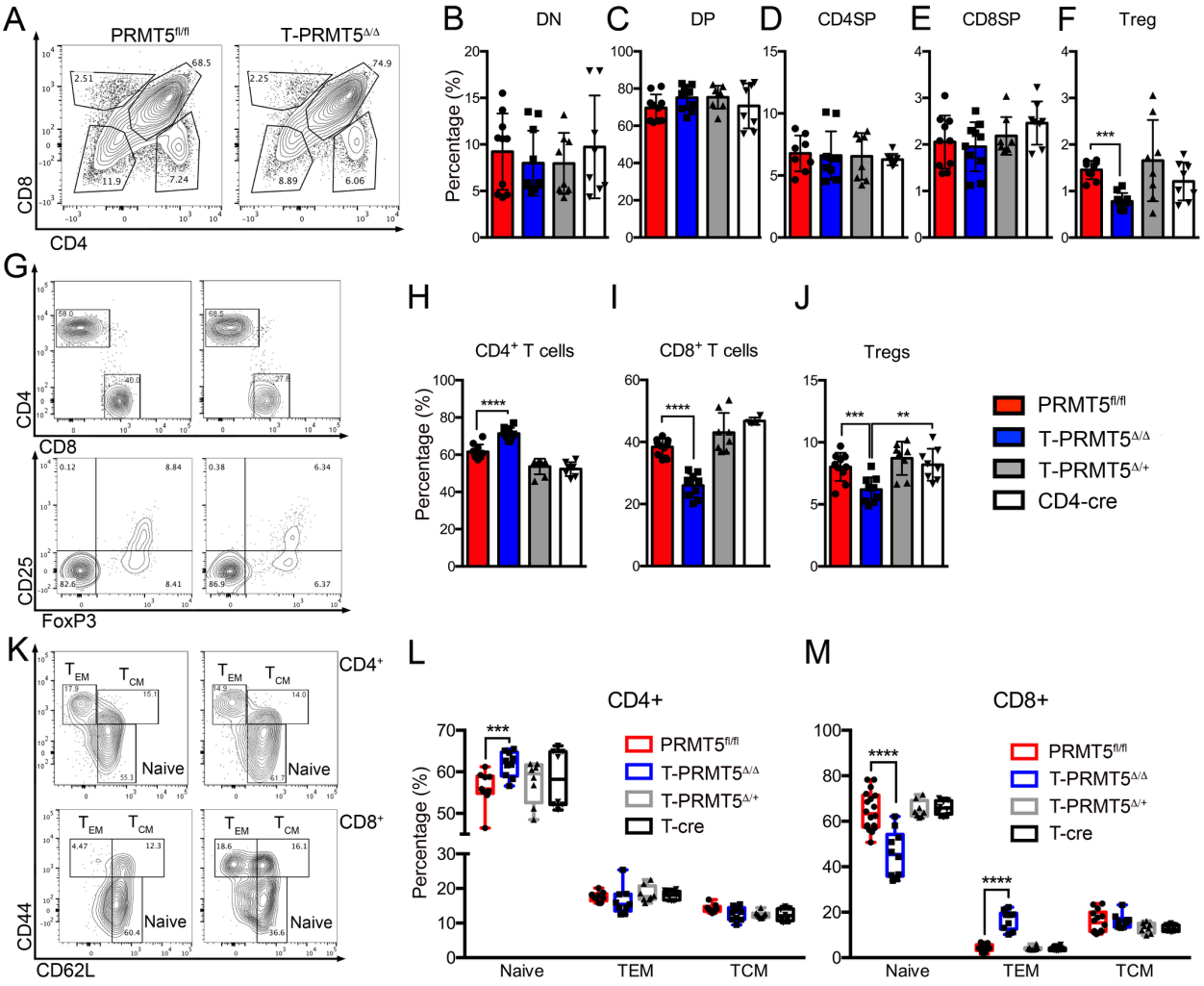
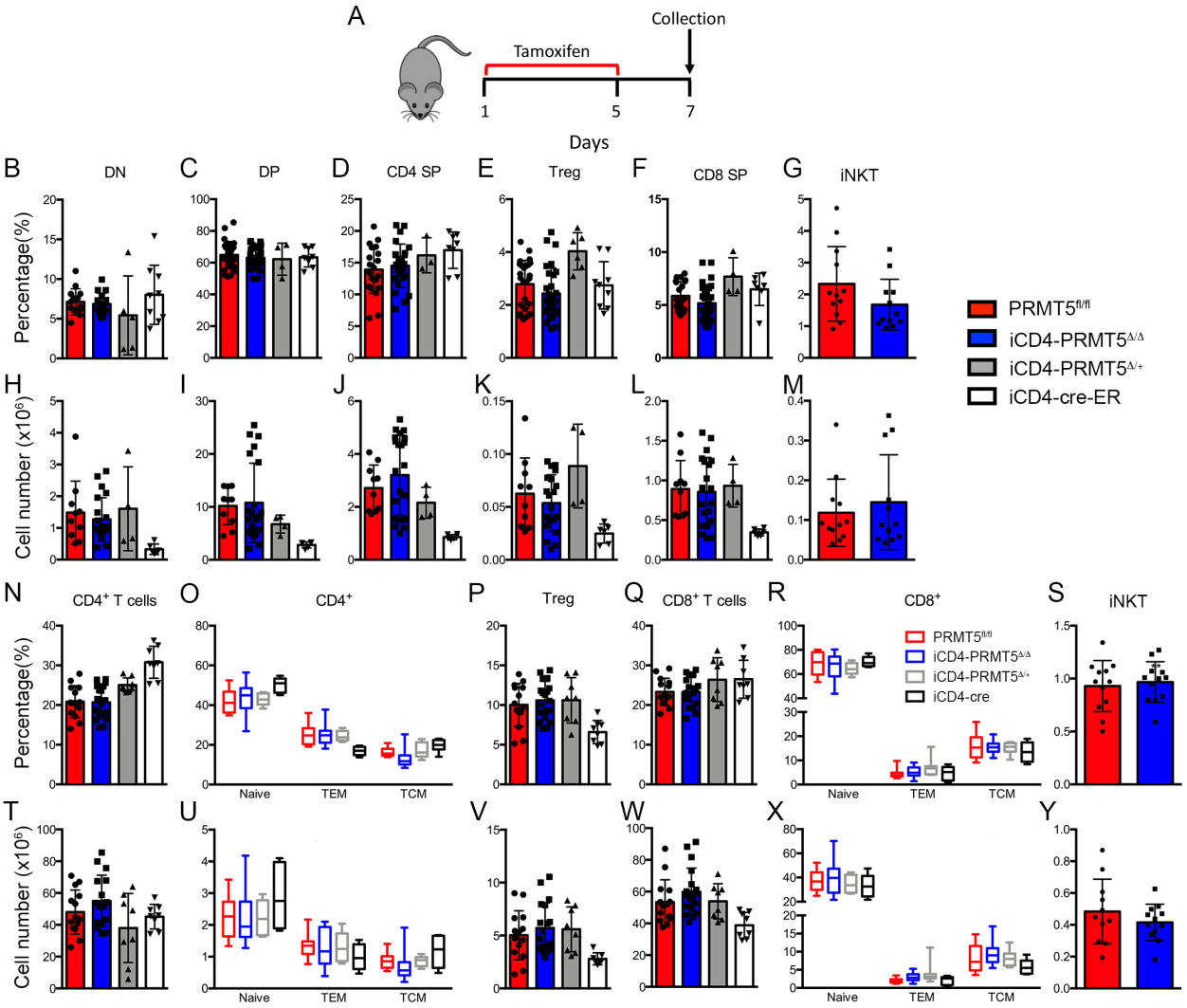


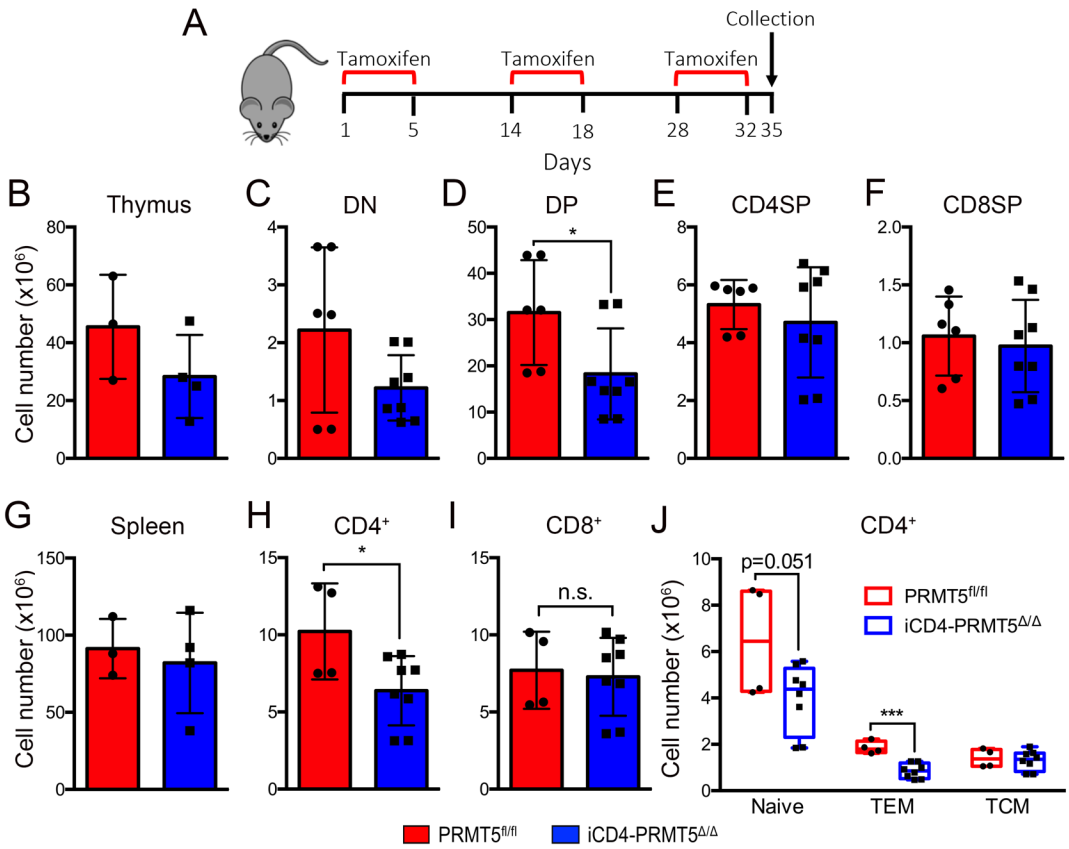
Supplemental Figure 1. Impact of T-PRMT5$^{\Delta/\Delta}$ on thymic and peripheral immune cell frequencies.

(**A-F**) Thymocytes from T-PRMT5$^{\Delta/\Delta}$ and appropriate control mice were analyzed by flow cytometry. (**A**) Representative DN, DP, CD4SP and CD8SP thymocyte profile. A representative Treg plot is shown in main text **Fig. 2**. (**B-F**) Frequencies of (**B**) DN, (**C**) DP, (**D**) CD4SP, (**E**) CD8SP and (**F**) Treg cell populations. (**G-M**) Splenocytes from T-PRMT5$^{\Delta/\Delta}$ and appropriate control mice were analyzed by flow cytometry. (**G**) Representative CD4/CD8 and Treg splenocyte flow plots. (**H-J**) Percentages of (**H**) CD4$^+$, (**I**) CD8$^+$ and (**J**) Tregs. (**K**) Representative T_{EM}, T_{CM}, and naive (based on CD62L/CD44 staining) CD4 and CD8 T cell populations flow plots. (**L-M**) Percentage of (**L**) T_{EM}, T_{CM}, and naive CD4$^+$ T cell subsets and (**M**) T_{EM}, T_{CM}, and naive CD8$^+$ T cell subsets. Data are pooled from 2 independent experiments (shown n=4-5). (**B-F, H-J, L-M**) One-way ANOVA, followed by Dunnett's multiple comparison test was done within each T cell population. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bar graphs display mean +/- SD. Box and whiskers plots display box from 25th to 75th percentiles, all points shown, whiskers extend from min to max, line represents median.

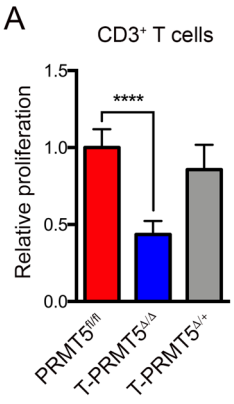


Supplemental Figure 2. Acute PRMT5 knockout in CD4⁺ T cells does not affect thymic development or peripheral immune cell compartments.

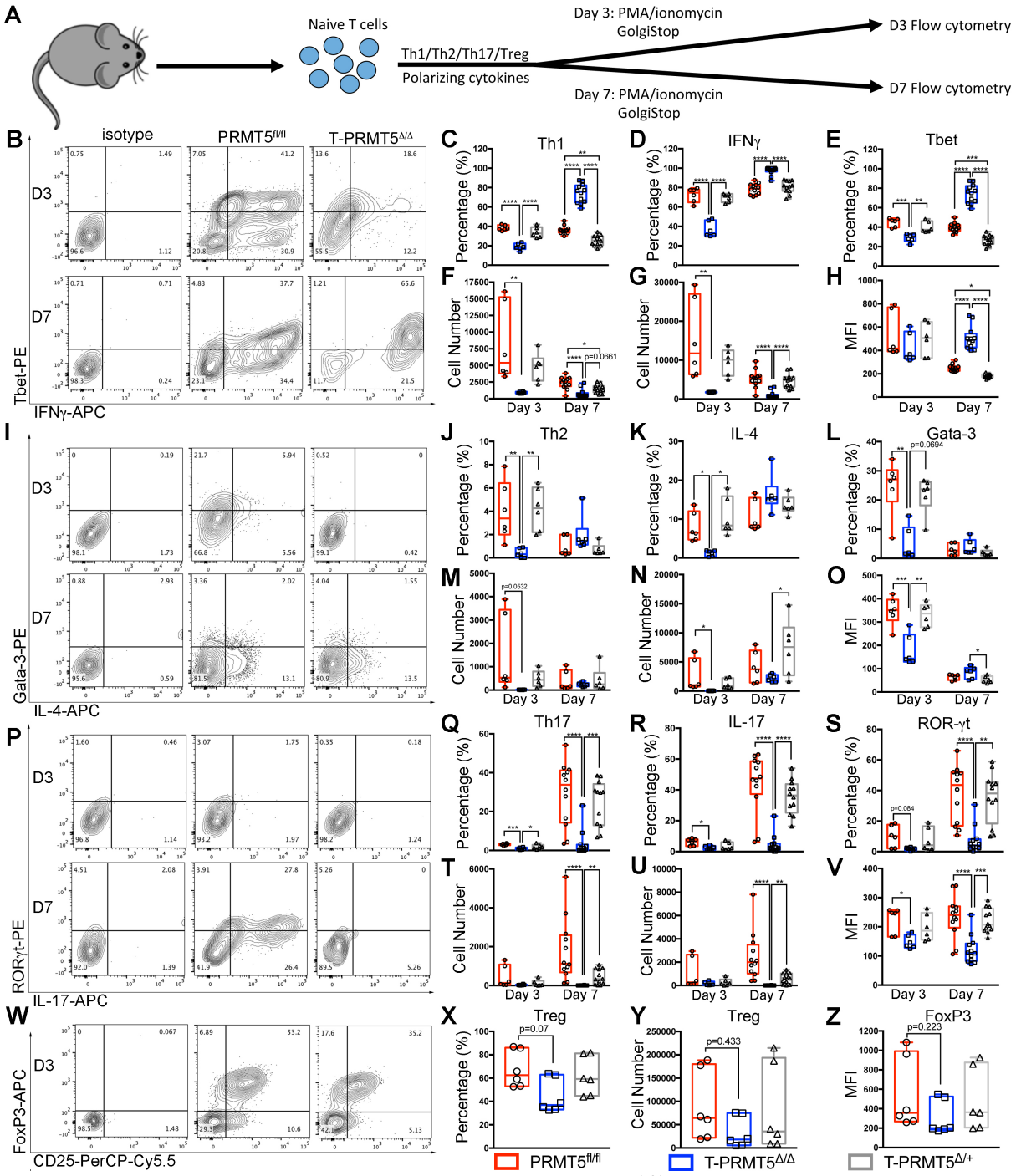
(**A**) Schematic of tamoxifen treatment experimental design and collection for direct ex-vivo flow cytometry analyses. (**B-M**) Thymocytes from iCD4-PRMT5$^{\Delta/\Delta}$ and appropriate control mice treated with tamoxifen for one week to induce acute peripheral CD4 T cell PRMT5 deletion were analyzed by flow cytometry. (**B-G**) Frequency and (**H-M**) cell number of thymic (**B, H**) CD4$^-$CD8$^-$ DN, (**C, I**) CD4$^+$CD8$^+$ DP, (**D, J**) CD4SP, (**E, K**) Treg, (**F, L**) CD8SP and (**G, M**) iNK T cell populations. Splenocytes were analyzed by flow cytometry for (**N-S**) percentages and (**T-Y**) cell numbers of (**N, T**) CD4$^+$, (**O, U**) CD4$^+$ T_{EM}, T_{CM}, and naive, (**P, V**) Tregs, (**Q, W**) CD8$^+$, (**R, X**) CD8$^+$ T_{EM}, T_{CM}, and naive and (**S, Y**) iNK T cell populations. Data are pooled from at least 4 independent experiments (shown n=6-8). One-way ANOVA, followed by Dunnett's multiple comparison test (**B-R, T-X**) or Student's t-test (**S, Y**). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bar graphs display mean +/- SD. Box and whiskers plots display box from 25th to 75th percentiles, whiskers extend from min to max, line represents median. DN: double negative, DP: double positive, SP: single positive.



Supplemental Figure 3. Impact of extended *Prmt5* deficiency in iCD4-PRMT5$^{\Delta/\Delta}$ mice.
(**A**) Schematic of experimental design for long-term tamoxifen treatment of iCD4-PRMT5$^{\Delta/\Delta}$ and PRMT5$^{fl/fl}$ mice and collection for direct ex-vivo flow cytometry analyses. (**B-F**) Thymi were processed and (**B**) total cell numbers were counted. Flow cytometric analysis was performed and cell numbers were calculated for (**C**) DN, (**D**) DP, (**E**) CD4SP and (**F**) CD8SP compartments. (**G-J**) Splenocytes were isolated and (**G**) total cell numbers were counted. Flow cytometric analysis was performed and cell numbers were calculated for (**H**) CD4$^+$, (**I**) CD8$^+$ and (**J**) CD4$^+$ T_{EM}, T_{CM}, and naive, cell compartments. Data are pooled from 3-4 independent mice. Student's *t* test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bar graphs display mean +/- SD. Box and whiskers plots display box from 25th to 75th percentiles, all points shown, whiskers extend from min to max, line represents median. DN: double negative, DP: double positive, SP: single positive.



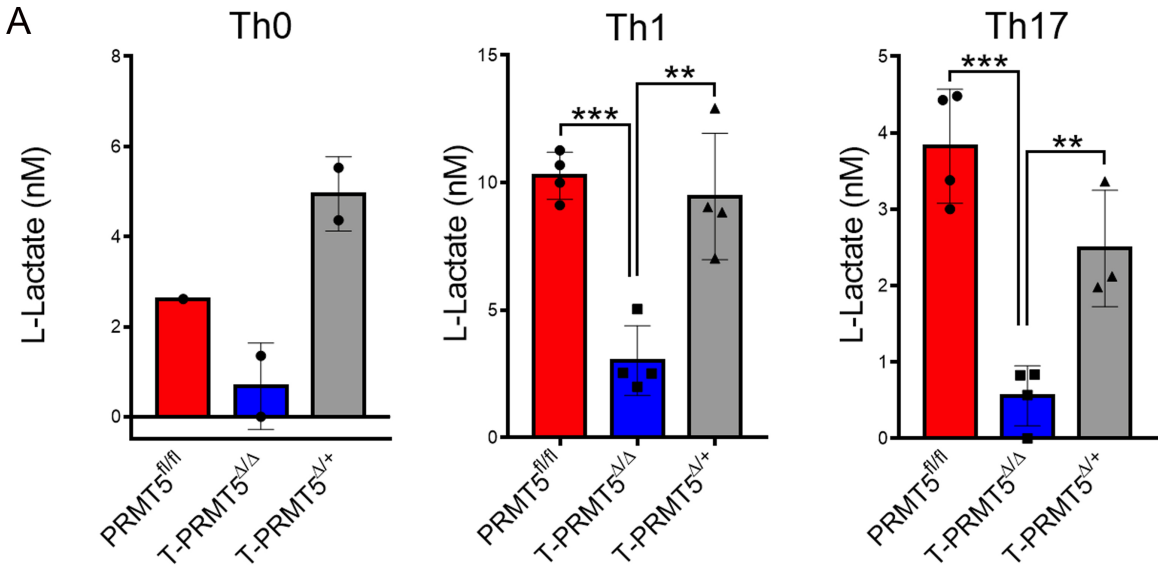
Supplemental Figure 4. Impact of *Prmt5* deficiency on CD3$^+$ T cell proliferation.
(**A**) CD3$^+$ T cells were isolated from T-PRMT5$^{\Delta/\Delta}$ and control mice and activated on anti-CD3/CD28 for 48 hours. Proliferation was monitored by ^{3}H-thymidine incorporation. Data are pooled from 2 independent experiments (shown n = 3-4). One-way ANOVA, followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bar graph displays mean +/- SD.



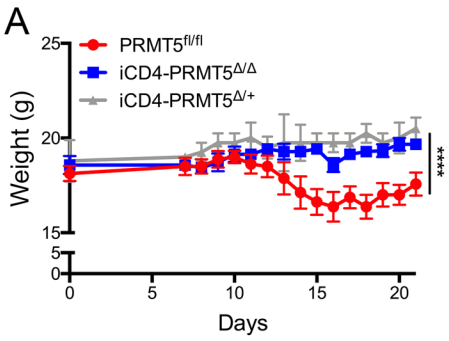
Supplemental Figure 5. Th cell differentiation in T-PRMT5$^{\Delta/\Delta}$ mice T cells.

(**A**) Experimental design for Th cell differentiation in T-PRMT5$^{\Delta/\Delta}$ mice. Naive CD4$^+$ T cells isolated from T-PRMT5$^{\Delta/\Delta}$ mice were polarized into (**B-H**) Th1, (**I-O**) Th2, (**P-V**) Th17 or (**W-Z**) Tregs and assessed by flow cytometry. Cells shown are gated on live (LiveDead Dye$^-$) CD44$^+$ cells. Th1 cells were assessed by Tbet$^+$IFNγ$^+$ cell (**C**) % and (**F**) number, IFNγ$^+$ cell (**D**) % and (**G**) number, T-bet$^+$ (**E**) cell % and (**H**) mean fluorescence intensity (MFI) by flow cytometry. Th2 cells were assessed by GATA-3$^+$IL-4$^+$ cell (**J**) % and (**M**) number, IL-4$^+$ cell (**K**) % and (**N**) number, GATA-3$^+$ (**L**) cell % and (**O**) MFI by flow cytometry. Th17 cells were assessed by RORγt$^+$IL-17$^+$ cell (**Q**) % and (**T**) number,

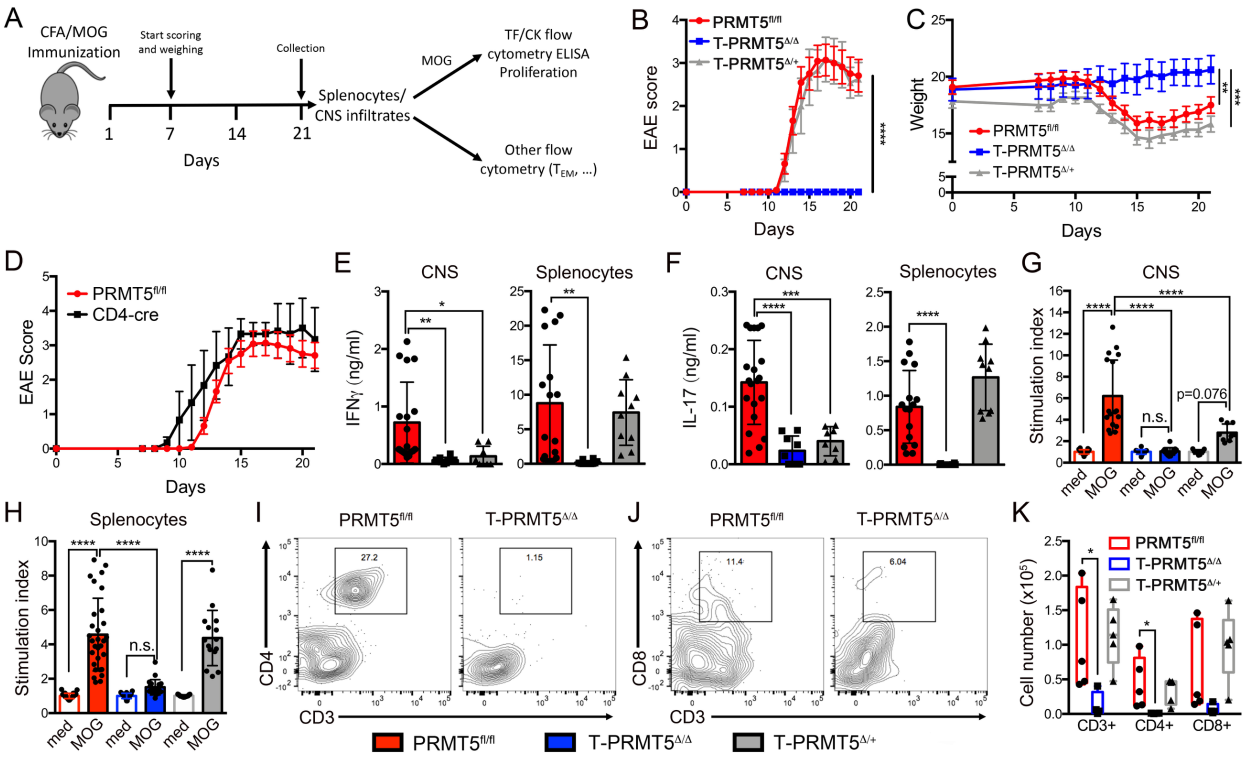
IL-17^{+} cell (**R**) % and (**U**) number, RORγt^{+} (**S**) cell % and (**V**) MFI by flow cytometry. Tregs were assessed by Foxp3^{+}CD25^{+} (**X**) cell % and (**Y**) number, and (**Z**) Foxp3 MFI. Data pooled 3 independent experiments, n=6/group. One-way ANOVA, followed by Tukey's multiple comparison test or Kruskal-Wallis followed by Dunn's multiple comparison test was used as appropriate. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Graphs are box and whiskers plots (box extends from 25th to 75th percentiles, all points shown, whiskers extend from min to max, line represents median).



Supplemental Figure 6. Impact of PRMT5 deficiency on lactate metabolism.
(**A**) Naïve CD4^{+} T cells were isolated from T-PRMT5$^{\Delta/\Delta}$ and indicated control mice and activated on anti-CD3/CD28 in Th0, Th1 and Th17 conditions for 72 hours. Supernatants were collected and analyzed for lactate levels, as a measure of glycolytic metabolism activity. Data are pooled from 1-4 independent mice. One-way ANOVA, followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bar graph displays mean +/- SD.



Supplemental Figure 7. T cell specific *Prmt5* deficiency prevents induction of EAE autoimmunity (**A**) iCD4-PRMT5$^{\Delta/\Delta}$ and appropriate control mice were immunized with CFA/MOG and weights of EAE mice were monitored daily. Data are pooled from four independent experiments (shown n = 6-10). Student's t test was performed for EAE weight analysis comparing PRMT5$^{fl/fl}$ and iCD4-PRMT5$^{\Delta/\Delta}$; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplemental Figure 8. T cell specific *Prmt5* deficiency prevents induction of EAE autoimmunity.

(**A**) Schematic of EAE experimental design and downstream analyses. (**B**) EAE score in T-PRMT5$^{\Delta/\Delta}$ and indicated controls after MOG$_{35-55}$/CFA immunization. (**C**) Weights of EAE mice were monitored daily. (**D**) Scores of PRMT5$^{fl/fl}$ (n=10) and additional CD4-cre control (n=3) mice were monitored daily. (**E-H**) Splenocytes and infiltrating CNS cells were isolated at day 21 after MOG$_{35-55}$/CFA immunization, and reactivated with MOG to measure (**E**) IFNγ and (**F**) IL-17 production by ELISA and (**G-H**) proliferation by ^{3}H-thymidine incorporation. (**I-K**) Flow cytometric analysis of *ex vivo* infiltrating CNS cells quantifying (**K**) CD3^{+}, (**I, K**) CD3^{+}CD4^{+}, and (**J, K**) CD3^{+}CD8^{+} populations at day 21. Data are pooled from four independent experiments, n=6-10 mice. Mann-Whitney was performed for EAE score analysis comparing PRMT5$^{fl/fl}$ and iCD4-PRMT5$^{\Delta/\Delta}$ (**B**) or comparing PRMT5$^{fl/fl}$ and CD4-cre (**D**); for other analyses, one-way ANOVA, followed by Dunnett's (**C, E, F, K**) or Sidak's (**G, H**) multiple comparison test were performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **B**, **C**, **D** display mean +/- SEM. Bar graphs display mean +/- SD. Box and whiskers plots display box from 25th to 75th percentiles, all points shown, whiskers extend from min to max, line represents median.