**Supplementary information for EZH2 is crucial for both differentiation of**

regulatory T cells and T effector cell expansion

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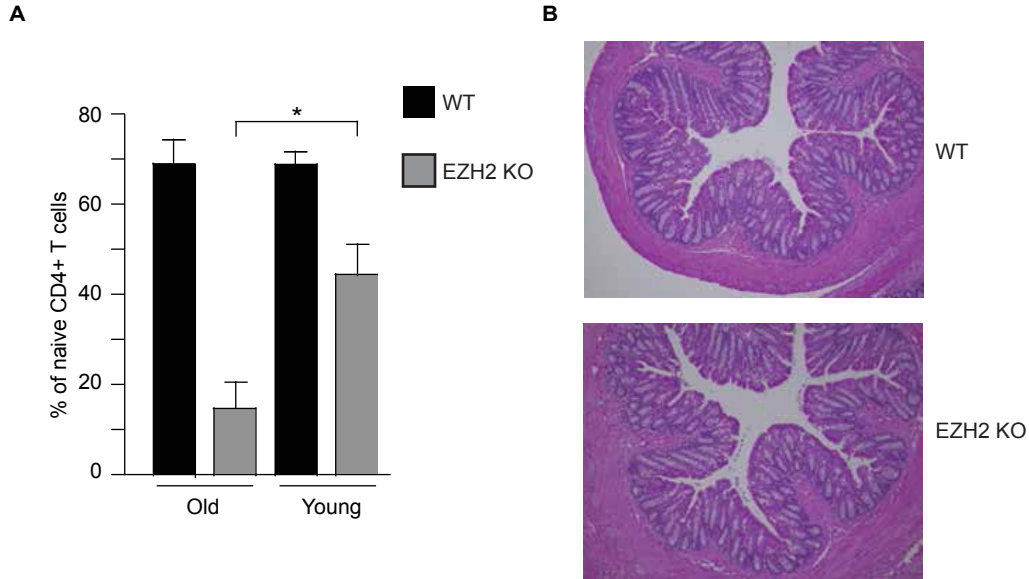
Ezh2-deficient mice have fewer naïve T cells in their periphery with age.

The spleen and mesenteric lymph nodes were isolated from Ezh2-deficient mice at age
6 weeks and 26 weeks, followed by red blood cell lysis. The isolated lymphocytes were
stained for cell surface marks of CD4, CD62L, and CD44. (A) Cumulative histogram showing
percentage of $CD4^+CD44^{low}CD62L^{high}$ and $CD4^+CD44^{high}CD62L^{low}$ in the spleen of Ezh2-deficient
mice at age 6 weeks (young) or 26 weeks (old). *P < 0.05 and **P < 0.005 (unpaired t-test).
(B) The histopathology staining of the colons of 6 month old control mice and Ezh2-deficient mice.
Mouse tissues were fixed in 10% formyl-saline followed by embedding in paraffin blocks and tissue
sections were stained in hematoxylin and eosin.
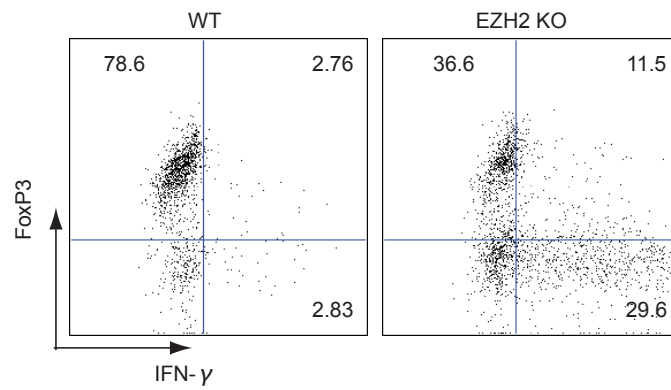
Destablization of nTreg cells in the absence of EZH2

Sorted splenic CD4$^+$CD25$^+$nrp$^+$ cells from WT mice or EZH2-deficient mice were stimulated with anti-CD3/CD28 (10 μg/ml) and IL-12 (10 ng/ml) for 3 days, expressions of FoxP3 and IFN-γ were determined by flow cytometry.
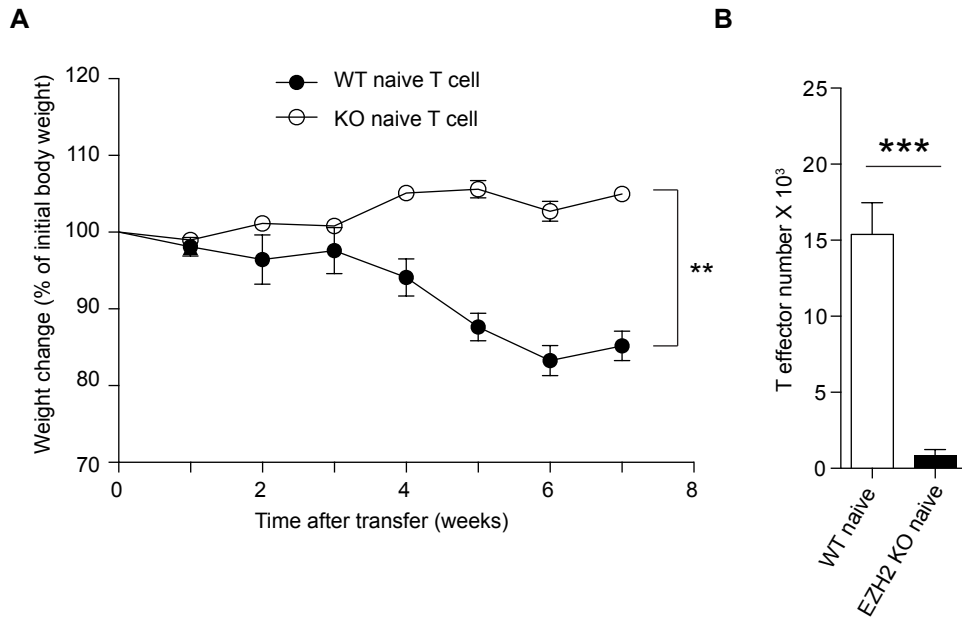
Ezh2-deficient T cells failed to induce colitis.

Sorted 4×10^5 CD4$^+$CD45RB^{hi}CD25$^-$ cells from CD45.1 control mice or sorted 4×10^5 CD4$^+$CD45RB^{hi}CD25$^-$ cells from Ezh2-deficeint mice were injected intravenously into 6-8 weeks Rag2-/- mice alone (n=5). Mice were monitored for evidence of colitis. (A) Weight loss (percentage of initial weight at day 0) was calculated for each mouse over 8 weeks. Data show mean (±SEM) weight changes for each group (n = 3–5 mice) (**P < 0.01). (B) Total lymphocyte cells were recovered from the lamina propria. **P < 0.01 (unpaired t-test).