Mutual expression of Runx3 and ThPOK regulates intestinal CD4$^+$ T cell immunity

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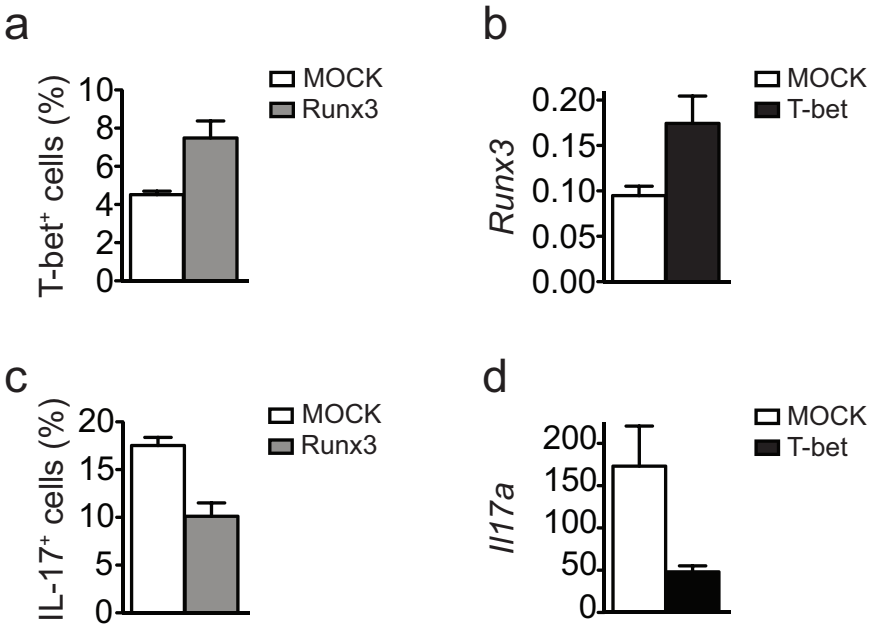
Supplementary figure 1



Supplementary figure 1. Runx3 and T-bet overexpression in T_H17 cells. (**a-d**) Sorted naïve CD4 T cells from WT mice were *in vitro* activated with plate-bound anti-CD3ε and soluble anti-CD28 for 36-48h, transduced with *Runx3*-IRES-GFP (**a,c**) and *Tbx21*-IRES-GFP (**b.d**) expressing retrovirus or with MOCK controls and differentiated towards T_H17 phenotype for additional 4.5 days. Intracellular T-bet (**a**) and IL-17A (**c**) expression by gated GFP^+CD4^+ T cells. Relative mRNA expression of long-form *Runx3* (**b**) and *Il17a* (**d**) from total $CD4^+$ T cells. Data are representative of two independent experiments (error bars, s.e.m. of duplicates).

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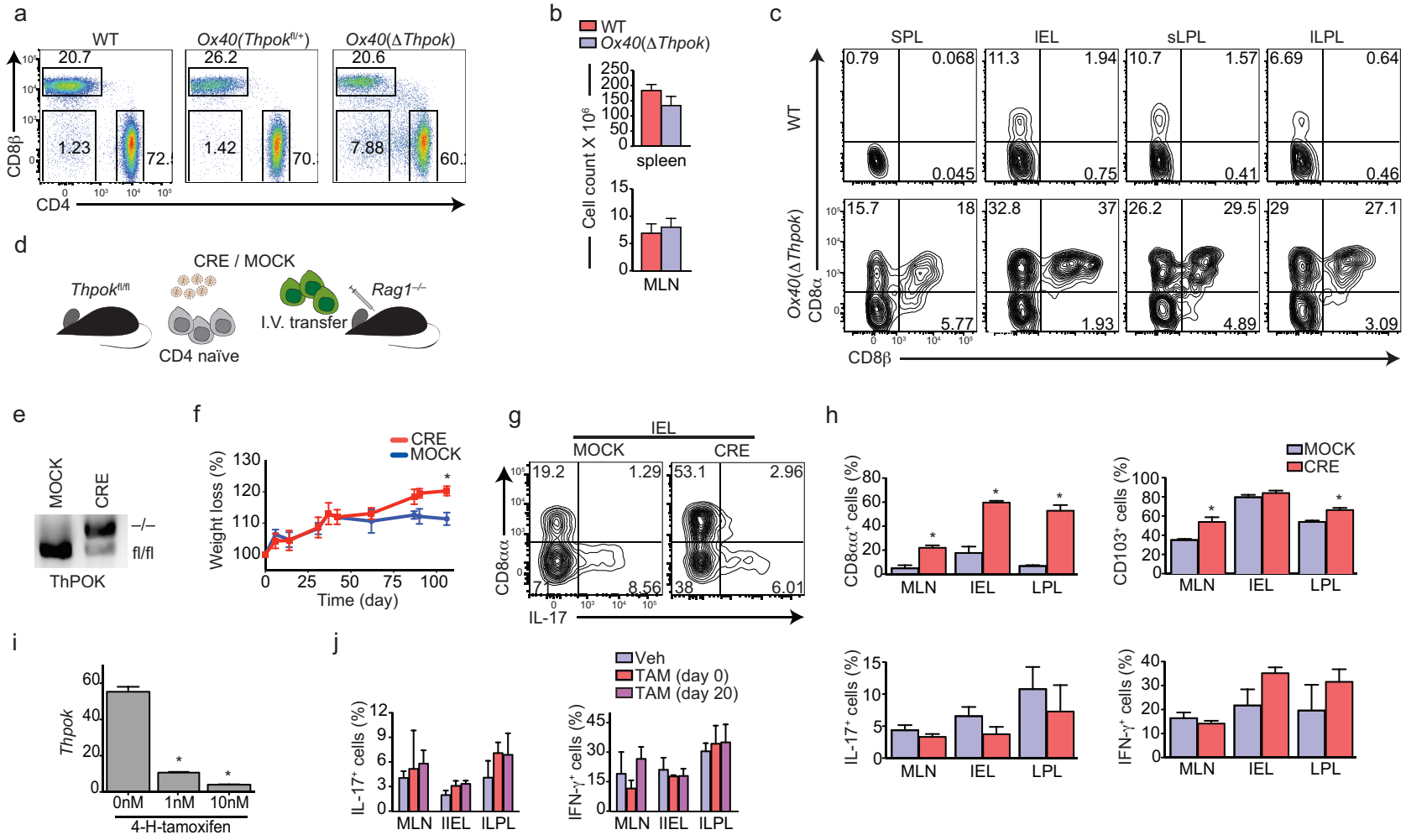
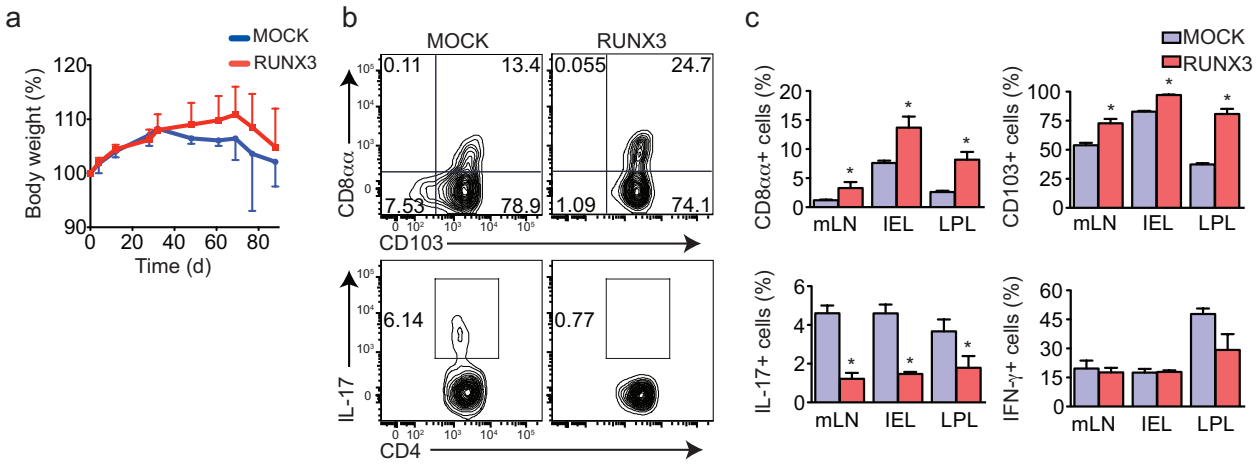
Supplementary figure 2



Supplementary figure 2. ThPOK loss by CD4$^+$ T cells hinders colitis development. (**a**) CD8β and CD4 expression by gated CD45$^+$TCRβ$^+$ cells isolated from the spleen of naive WT, *Ox40*(*Thpok*$^{fl/+}$) or *Ox40*(Δ*Thpok*) mice. Plots are representative of n=5 mice per group. (**b,c**) Sorted naïve CD4 T cells isolated from spleen of WT or *Ox40*(Δ*Thpok*) mice were adoptively transferred to *Rag1*$^{-/-}$ recipients and mice were analyzed 40 to 50 days later. (**b**) Total cell count of spleen (SPL) and mesenteric lymph nodes (MLN) recipient mice (error bars, s.e.m.). (**c**) CD8α and CD8β expression by CD45$^+$TCRβ$^+$CD4$^+$ cells isolated from the spleen, small intestine IEL, LPL and large intestine LPL of recipient mice. Data are representative of two independent experiments (n=3 to 6 per group). (**d-h**) Sorted naïve CD4 T cells from *Thpok*$^{fl/fl}$ mice were *in vitro* activated and transduced with MOCK- or Cre-GFP-containing retroviral constructs and GFP$^+$ cells were transferred to *Rag1*$^{-/-}$ recipients. (**d**) Schematic protocol. (**e**) PCR for intact or "floxed" ThPOK to confirm ThPOK deletion in GFP-positive cells. (**f**) Body weight of recipient mice (error bars, s.e.m.). (**g,h**) CD8α (CD8β$^-$), CD103 and intracellular IL-17 and IFN-γ expression by CD45$^+$TCRβ$^+$CD4$^+$ cells isolated from MLN and large intestine IEL and LPL of recipient mice. Graphs depict frequency of cells in the different tissues (error bars, s.e.m.). Data are representative of two independent experiments (n=3 per group). (**i**) Sorted naïve CD4 T cells isolated from spleen of *Rosa26*-Cre^{ERT2}–*Thpok*$^{fl/fl}$ (*Thpok*^{ERT2}) mice were cultured for 4.5 days with plate-bound anti-CD3ε and soluble anti-CD28 in the presence of the indicated concentration of tamoxifen (TAM). Data are representative of two independent experiments (error bars, s.e.m. of triplicates). (**j**) Sorted naïve CD4 T cells isolated from spleen of *Thpok*^{ERT2} mice were adoptively transferred to *Rag1*$^{-/-}$ recipients and mice were analyzed 40 days later. Recipient mice were administered TAM intraperitoneally three times, every three days, starting on day 0 or day 20 after T cell transfer. Graphs depict frequency of IL-17 and IFN-γ expressing cells among CD45$^+$TCRβ$^+$CD4$^+$ cells in the different tissues (error bars, s.e.m.). Data are representative of two independent experiments (n=3 per group). * $p<0.05$.

Supplementary figure 3



Supplementary figure 3. Runx3 overexpression hinders colitogenic potential and T_H17 differentiation of CD4$^+$ T cells *in vivo*. (**a-c**) Sorted naïve CD4 T cells isolated from spleen of WT mice were *in vitro* activated and transduced with a MOCK- or *Runx3*-IRES-GFP-containing retroviral construct. GFP$^+$ cells were sorted and adoptively transferred to *Rag1*$^{-/-}$ recipients. (**a**) Body weight of recipient mice (error bars, s.e.m.). (**b,c**) CD8α (CD8β$^-$), CD103 and intracellular IL-17 and IFN-γ expression by CD45$^+$TCRβ$^+$CD4$^+$ cells isolated from MLN and large intestine IEL and LPL of recipient mice. Graphs depict frequency of cells in the different tissues (error bars, s.e.m.). Data are representative of two independent experiments (n=3 per group).* $p<0.05$.