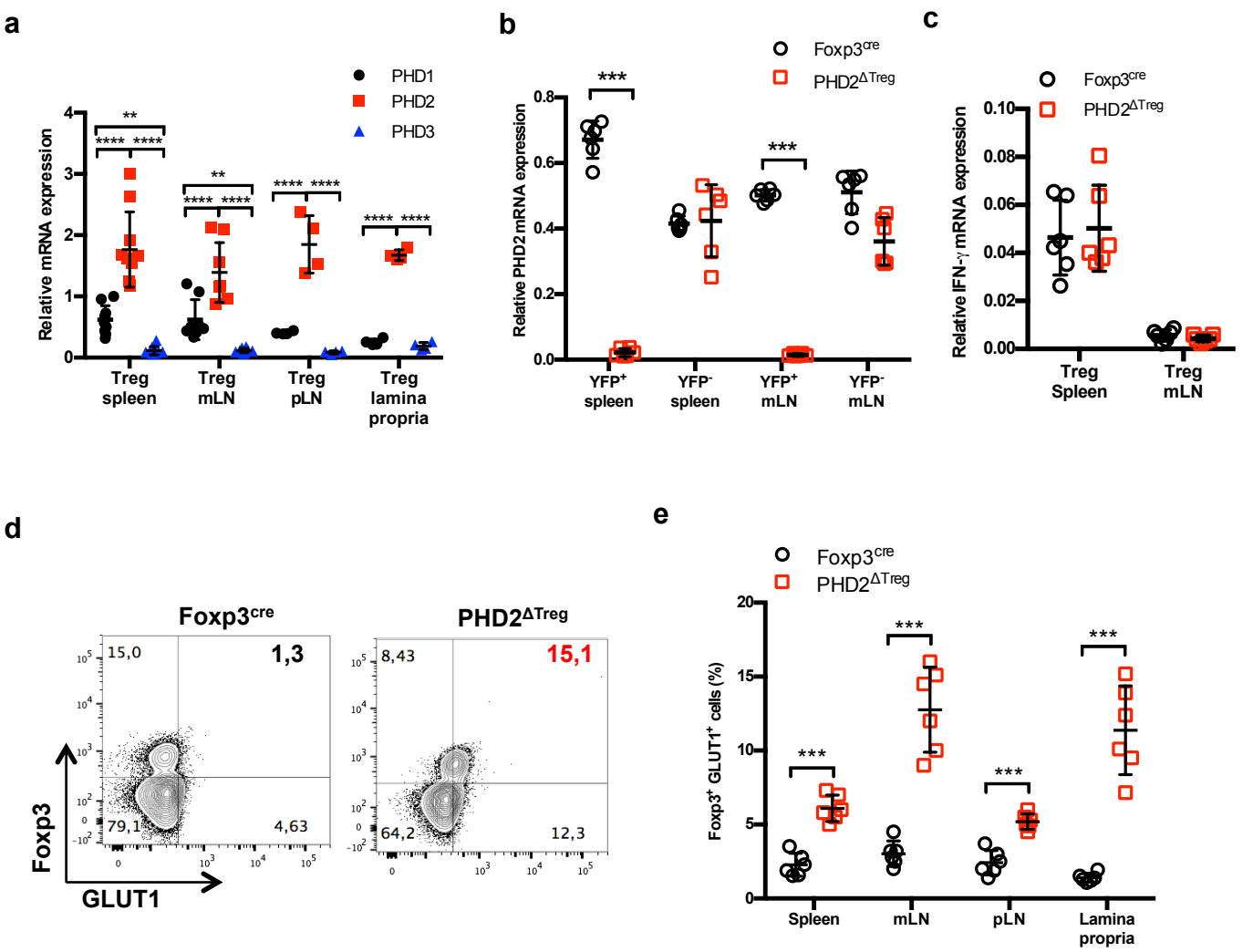


Fig. S1 Treg-restricted loss of PHD2 gene expression in PHD2$^{\Delta Treg}$ mice.
a Treg cells from Foxp3^{cre} mice were purified by cell sorting from spleen (n = 10), mesenteric (mLN) (n = 8), peripheral (pLN) lymph nodes (n = 4) or the small intestine lamina propria (n = 4) and expression of PHD1, PHD2 and PHD3 analyzed by qPCR. **b** YFP-positive (YFP^{+}) and YFP-negative (YFP^{-}) cells from PHD2-sufficient (Foxp3^{cre} mice) and PHD2-deficient (PHD2$^{\Delta Treg}$ mice) were purified by cell sorting from spleen and mesenteric lymph nodes (mLN) and expression of PHD2 analyzed by qPCR. The graph demonstrates selective loss of PHD2 gene expression in Tregs, but not in Tconvs purified from PHD2$^{\Delta Treg}$ mice. **c** IFN-γ gene expression relative to RPL32 by ex-vivo purified Tregs from spleen and mLN was determined by qPCR. **d** Representative flow cytometry expression profile of GLUT1, a specific HIF1α target gene in Foxp3 expressing or non-expressing cells from Foxp3^{cre} and PHD2$^{\Delta Treg}$ mice. **e** Frequency of GLUT1-expressing Tregs in lymphoid organs from Foxp3^{cre} and PHD2$^{\Delta Treg}$ mice. Data are representative of two independent experiments with n = 6 per group. Values are presented as the mean ± SD and were compared by two-tailed unpaired student's t-test. Only significant differences are indicated as follows: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001.

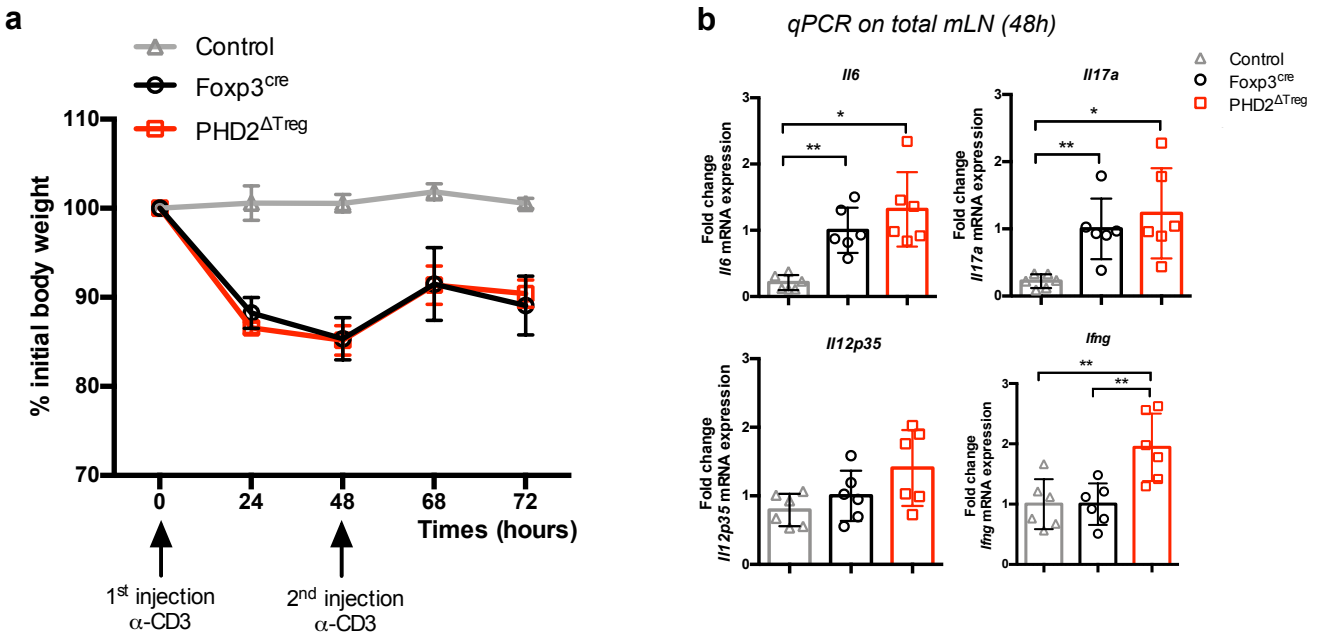


Fig. S2 PHD2$^{\Delta Treg}$ mice display a near-normal response to anti-CD3-induced enteritis.
Foxp3^{cre} and PHD2$^{\Delta Treg}$ mice were injected twice i.p. with anti-CD3 mAbs (20µg) at two days interval and weighted daily. **a** Weight loss was found similar in both mouse strains tested; **b** relative expression of inflammatory mediators evaluated by qPCR on whole, unfractionated mesenteric lymph nodes. A similar, Th17-like response was observed in both mouse strains. Data are representative of two independent experiments with n = 6 per group. Values are presented as the mean ± SD and were compared by one-way ANOVA with Tukey's multiple comparisons test. Only significant differences are indicated as follows: *: p<0.05, **: p<0.01.

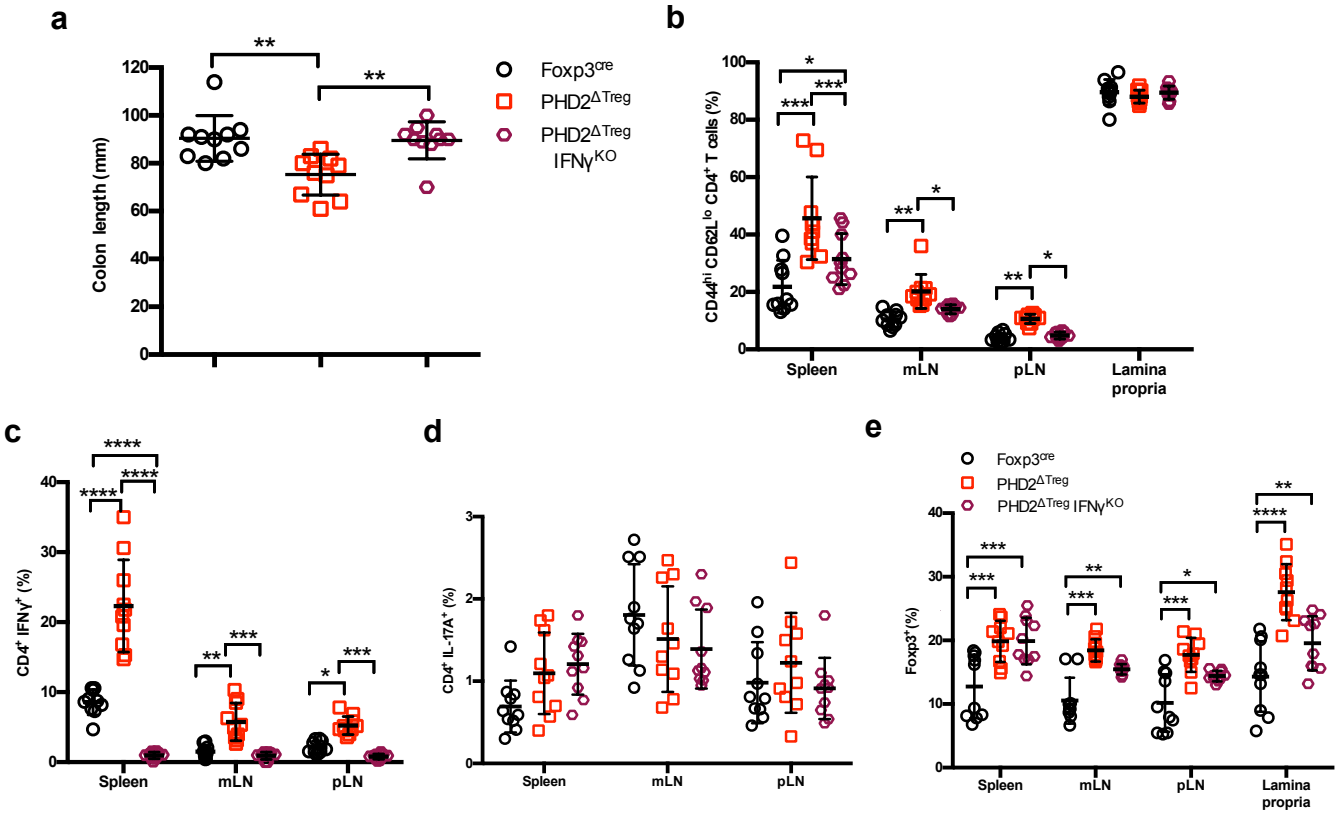


Fig. S3 Loss of IFN-γ gene expression attenuates the pro-inflammatory phenotype of PHD2$^{\Delta Treg}$ mice
PHD2$^{\Delta Treg}$ mice were crossed with IFN-γKO mice (PHD2$^{\Delta Treg}$ IFN-γ^{KO} mice) and were compared to Foxp3^{cre} and PHD2$^{\Delta Treg}$ mice and analyzed for **a** colon length; **b** frequency of effector-like (CD44^{hi} CD62L^{lo}) conventional T lymphocytes in the indicated lymphoid organs; **c** frequency of IFN-γ production after in vitro stimulation; **d** frequency of IL-17A-producing cells after in vitro stimulation and **e** frequency of Foxp3^{+} cells in the indicated lymphoid organs. Data are representative of three independent experiments with n = 10 per groups. Values are expressed as the mean ± SD and were compared by One-way ANOVA with Tukey's multiple comparisons test (**a**) or by Two-way ANOVA with Tukey's multiple comparisons test (**b-e**). Only significant differences are indicated as follows: *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001.

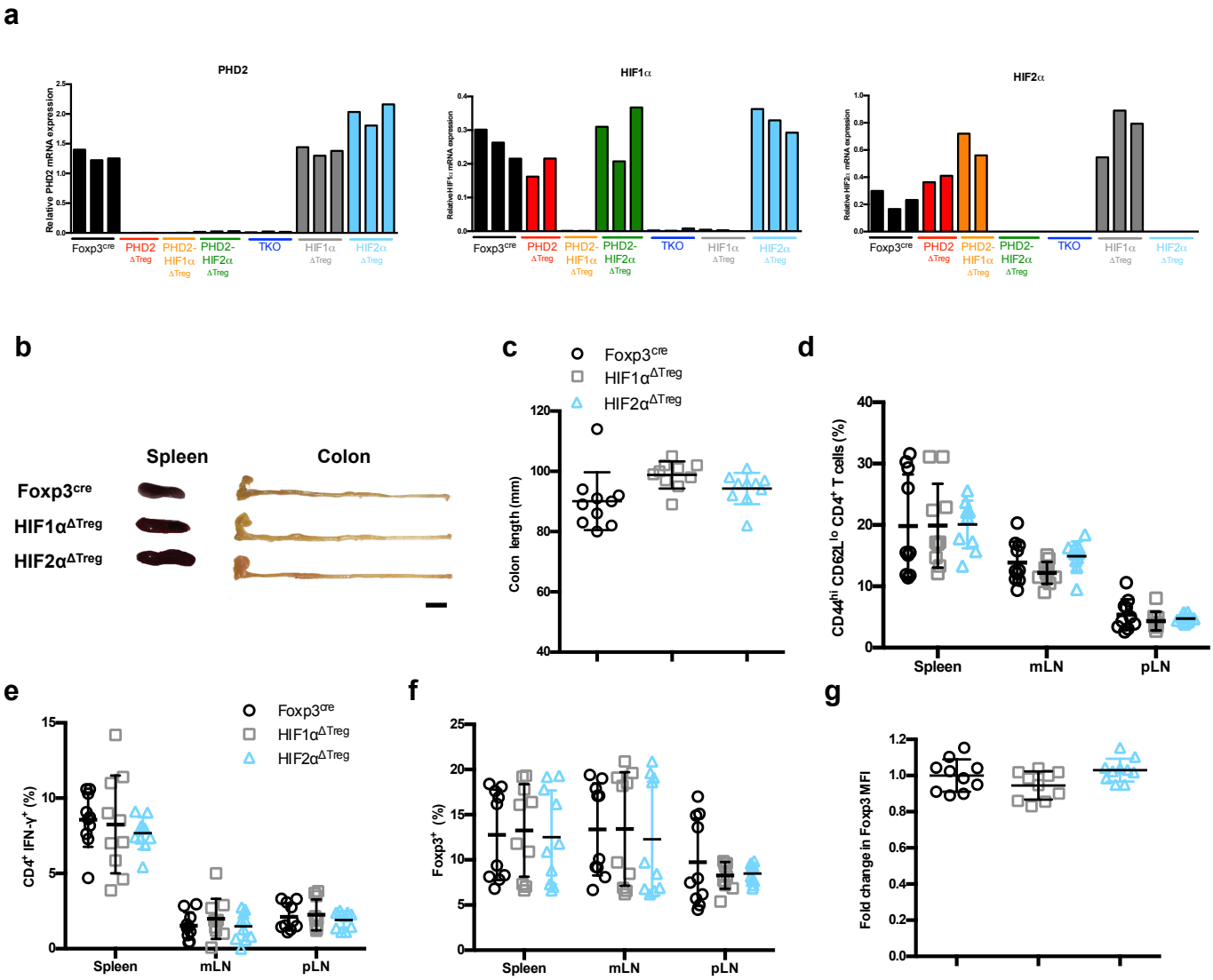
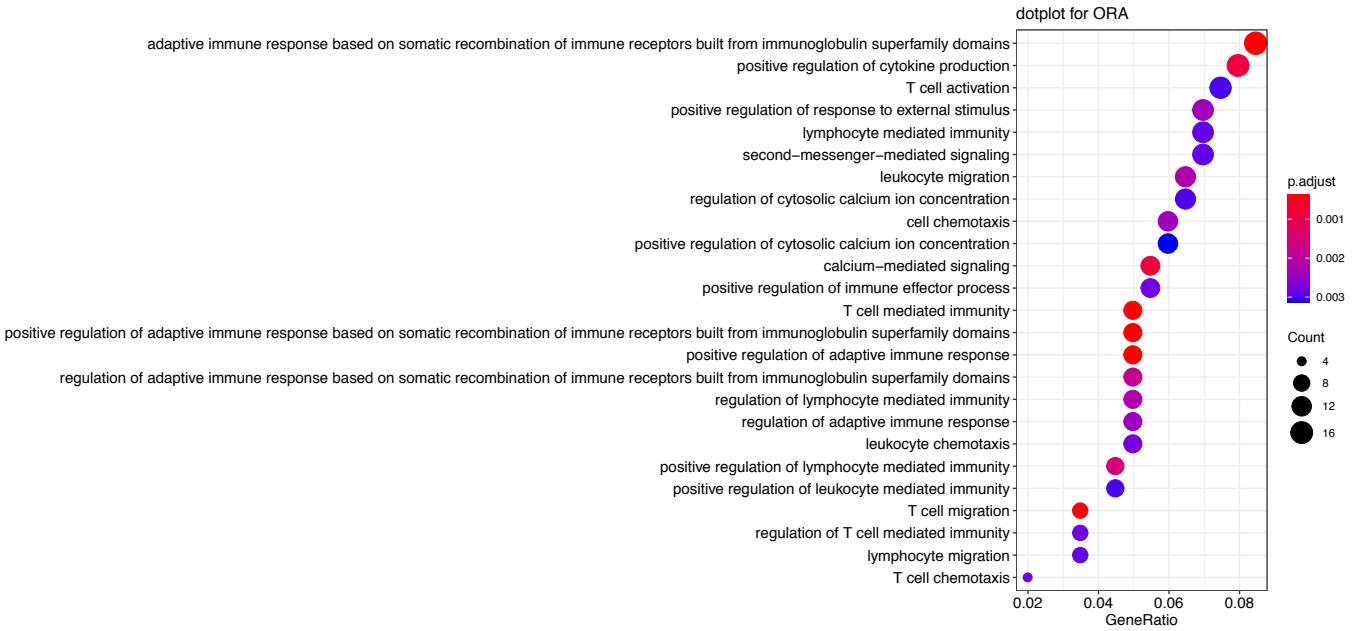


Fig. S4 Treg-selective HIF1α or HIF2α deficiency does not affect immune homeostasis in naive mice.
a Splenic Treg cells were purified by cell sorting from Foxp3^{cre} (n = 3), PHD2$^{\Delta Treg}$ (n = 2), PHD2-HIF1α$^{\Delta Treg}$ (n = 2), PHD2-HIF2α$^{\Delta Treg}$ (n = 3), PHD2-HIF1α-HIF2α$^{\Delta Treg}$ (TKO) (n = 3), HIF1α$^{\Delta Treg}$ (n = 3) and HIF2α$^{\Delta Treg}$ (n = 3) mice and their genotype verified by qPCR on the extracted total RNA fraction. **b** representative gross autopsy findings revealing normal spleen and colon size (summarized in panel **c**) in 12 weeks aged mice. **d** frequency of CD4^{+} Foxp3^{-} CD44^{hi} CD62L^{lo} cells in lymphoid organs; **e** frequency of CD4^{+} lymphocytes producing IFN-γ upon in vitro stimulation; **f** frequency of Foxp3^{+} cells in the indicated lymphoid organs; **g** Ratio of the Foxp3 MFI of HIF1αKO or HIF2αKO splenic Tregs to Foxp3^{cre} splenic Tregs. Data are representative of three independent experiments with n = 10. Values are presented as the mean ± SD and were compared by one-way ANOVA with Tukey's multiple comparisons test (**c, g**) or by Two-way ANOVA with Tukey's multiple comparisons test (**d-f**). No statistical difference were found between groups.

a **Downregulated pathways**



b **Upregulated pathways**

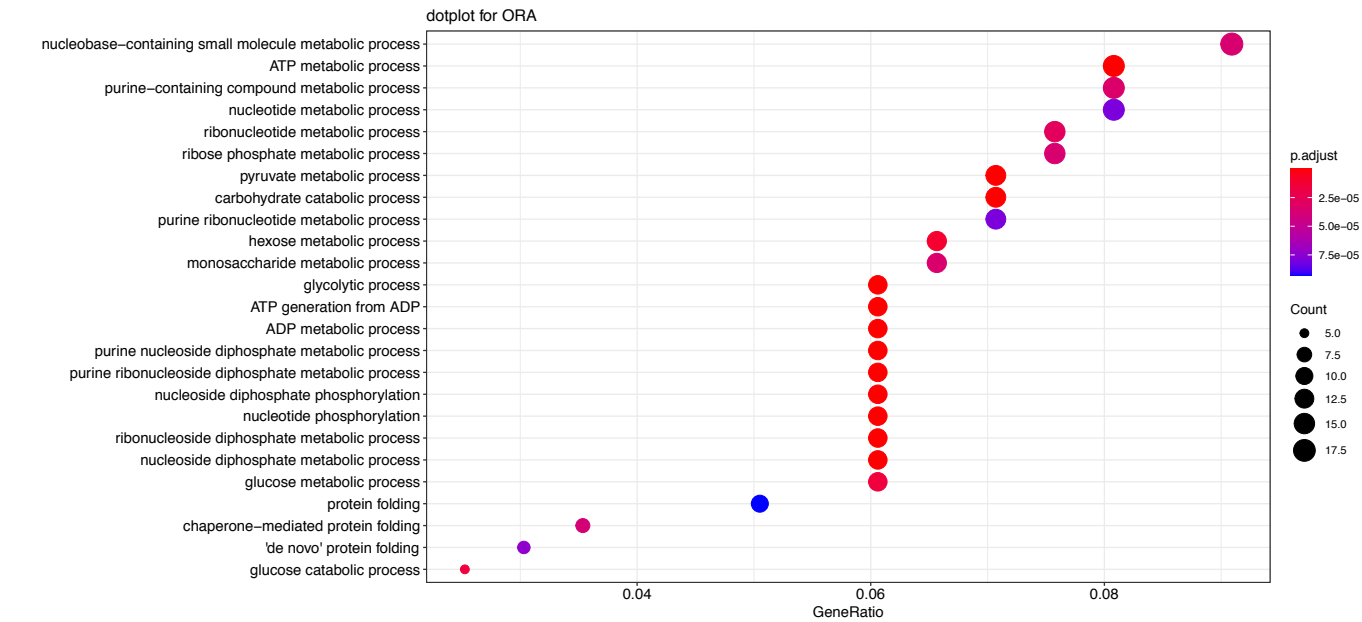


Fig. S5: Signaling pathways affected by loss of PHD2-expression in Treg.
a Top significantly downregulated pathways in PHD2-deficient Tregs compared to Tregs from Foxp3^{cre} mice **b** top significantly upregulated pathways in PHD2-deficient Tregs compared to Tregs from Foxp3^{cre} mice. Affected pathways were determined by over representation analysis (ORA analysis) in R program after Deseq2 analysis. Dots color and size represent respectively FDR (false discovery rate) and the number of genes affected in a given pathway.

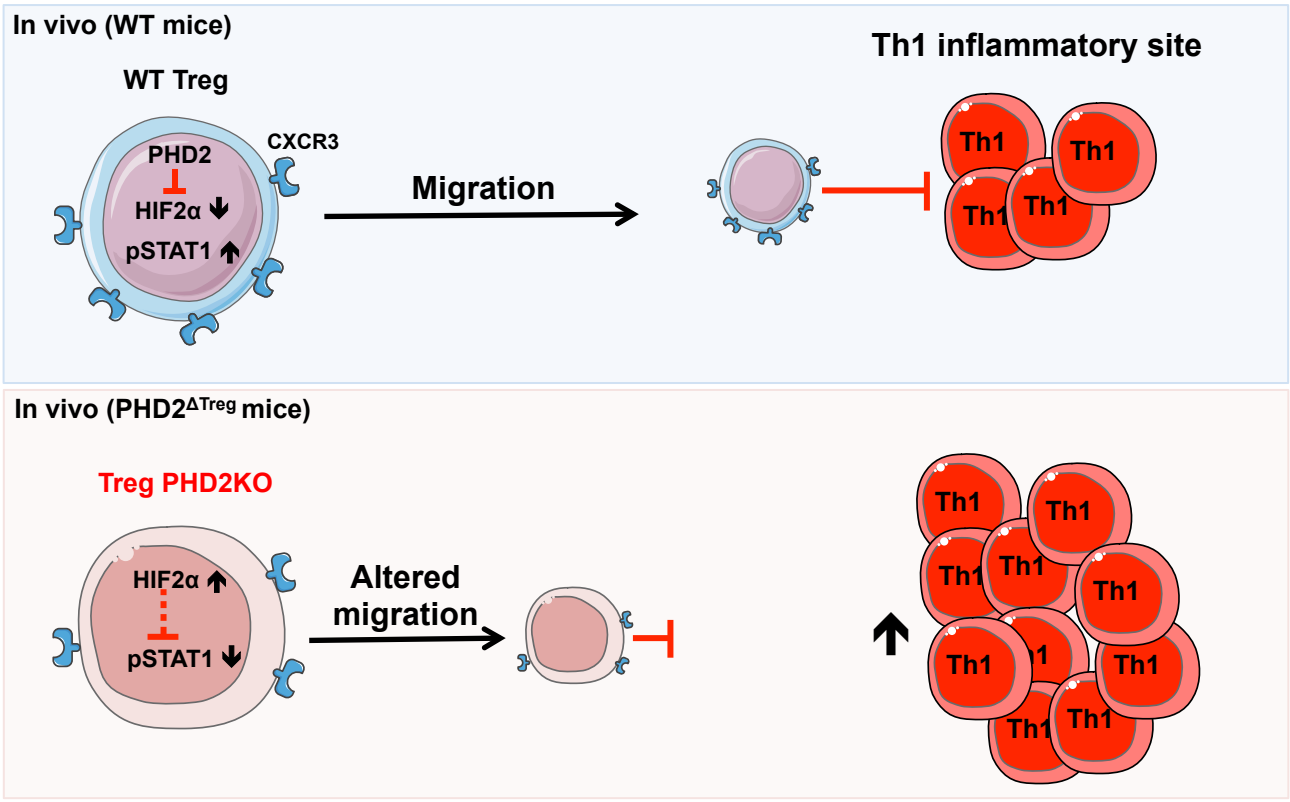


Fig. S6 Working hypothesis.
PHD2 expression allows adequate CXCR3-dependent signaling in Tregs by controlling expression of HIF2α, a putative, indirect inhibitor of the STAT1-dependent pathway. Lack of PHD2 expression leads to enhanced expression / activity of HIF2α, causing reduced expression of STAT1-dependent chemokine receptor expression and altered in vivo tissue localization.