Supplementary information

HIF-2α-Dependent induction of miR-29a Restrains T_H1 Activity during T cell Dependent Colitis

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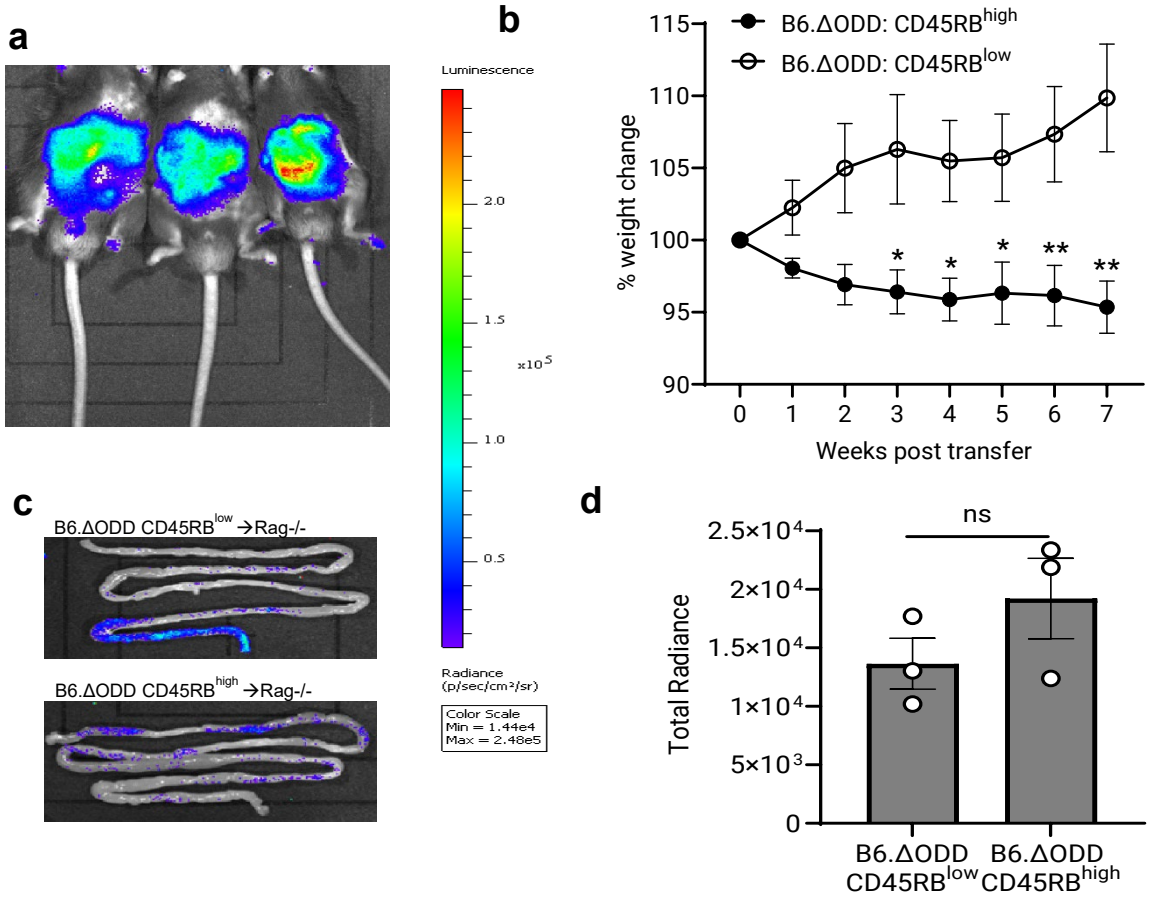
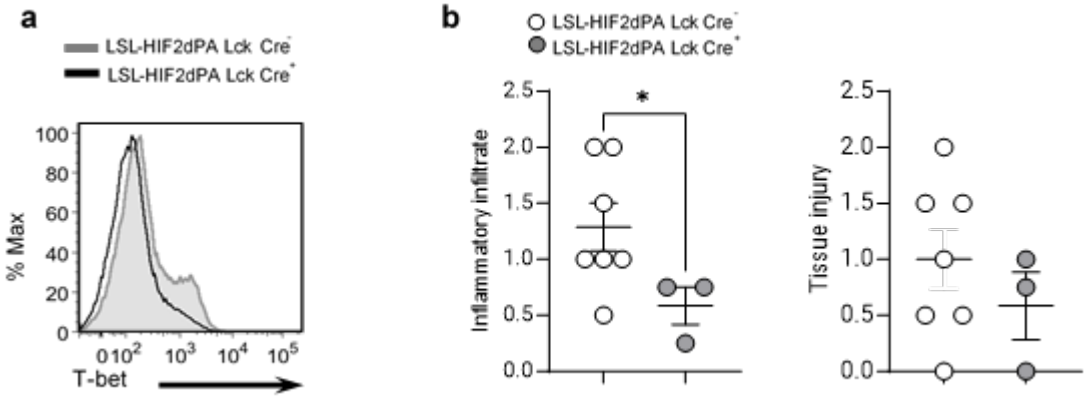
Supplementary Figure 1, related to Figure 1



T-cell intrinsic hypoxia in models of colitis.

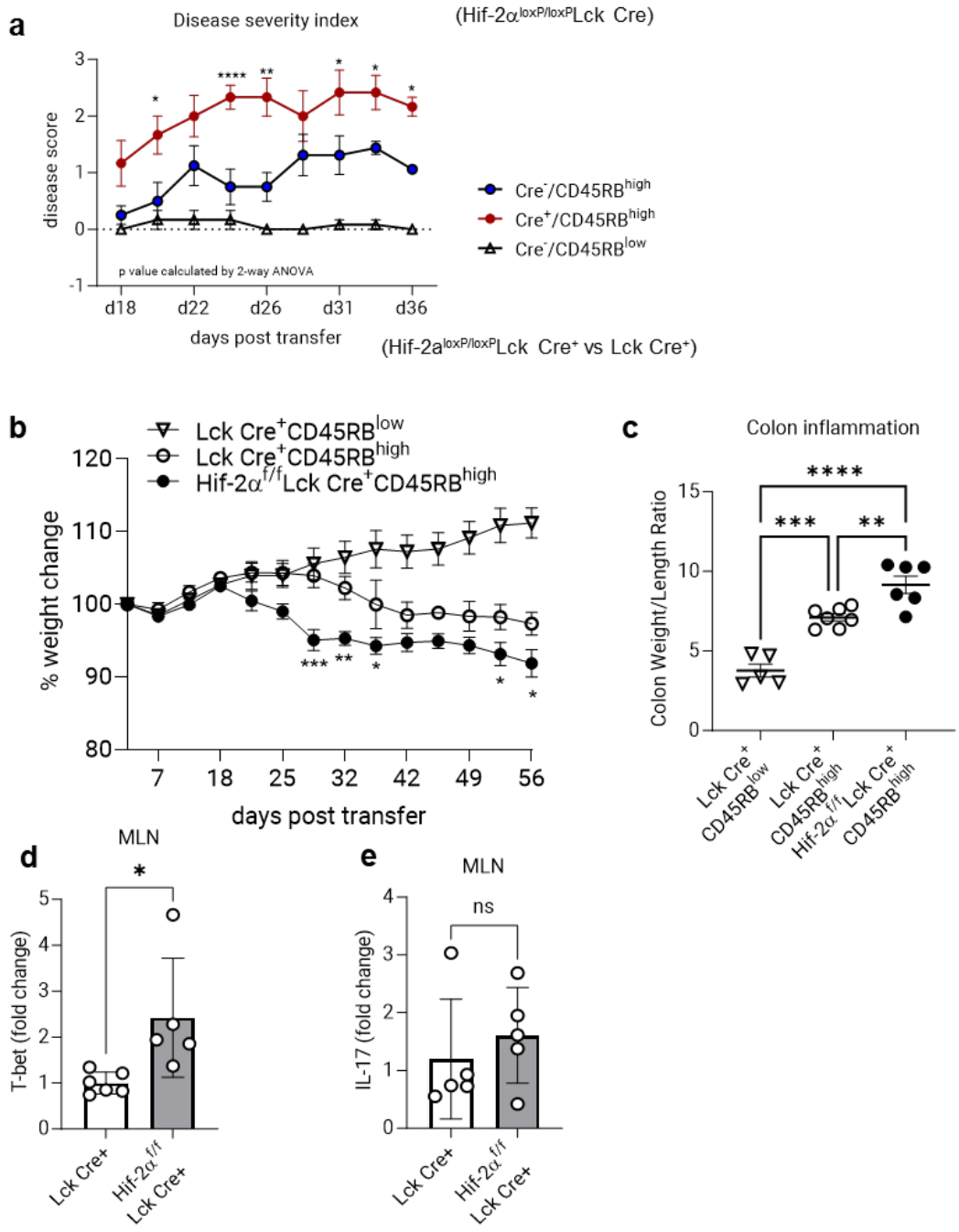
Chronic T cell-mediated colitis was induced in B6.RAG1$^{-/-}$ mice by intraperitoneal injection ($0.5x10^6$) of naïve CD4$^+$ CD45RB^{high} or CD45RB^{low} T cells derived from B6.ΔODD.luc mice. **a**. Representative IVIS imaging of hypoxic colons in live animals under anesthesia at fulminant disease. **b**. Weight change in adoptively transferred mice, n=5/group, 2-way ANOVA. **c.** Representative images of small intestines from B6.ΔODD.luc T cells-transferred mice were obtained using IVIS. **d**. Total radiance measurements in small intestines in T cells transfer mice are not significantly different between groups, n=3/group. Data expressed as Mean ± S.E.M. *p<0.05, **p<0.05 vs. indicated.

Supplementary Figure 2, related to Figure 4



Tbet expression in CD4$^+$ T_H1 T cells is specifically repressed by HIF-2α. T_H1 colitis was induced in LSL-HIF2dPA Lck Cre$^+$ mice and controls by epicutaneous skin sensitization followed by rectal gavage with TNBS as per Materials & Methods. Tbet protein expression in gated CD4$^+$ T-cells from lamina propria was measured by flow cytometry. **a.** Representative histogram from LSL-HIF2dPA Lck Cre$^+$ or Cre$^-$ littermate controls. **b**. Inflammatory and tissue injury scores from LSL-HIF2dPA Lck Cre$^+$ or Cre$^-$ littermate controls following TNBS trial; quantitated from histological slides. Data expressed as Mean from 2 pooled independent experiments (n=3-7/group, one-tailed T test)., *p<0.05, vs. indicated.

Supplementary Figure 3, related to Figure 5

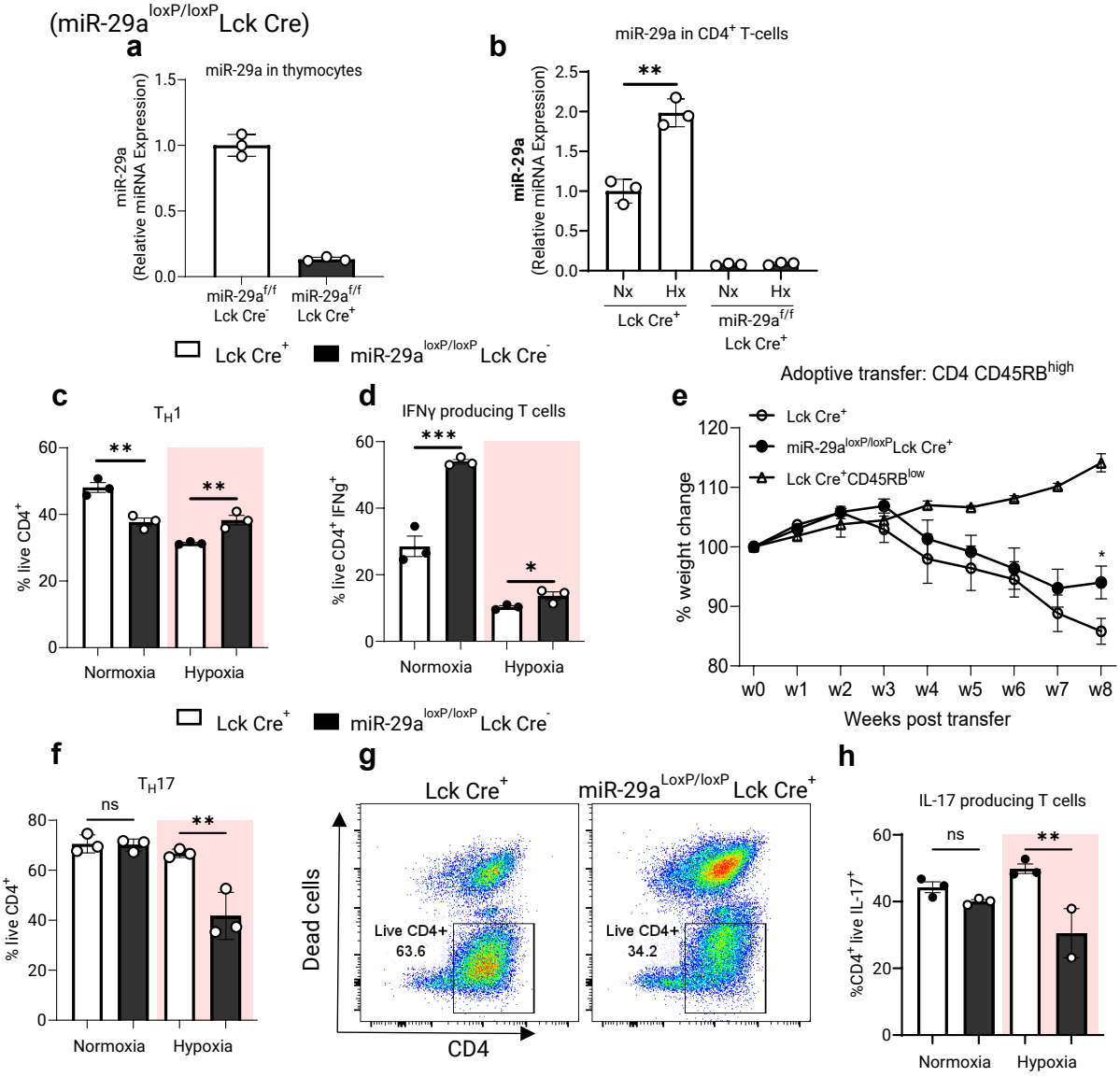


T cell-intrinsic HIF-2α regulates T-cell function in Colitis.

Chronic T cell-mediated colitis was induced in B6.RAG1$^{-/-}$ mice by intraperitoneal injection ($0.5x10^6$) of naïve CD4$^+$ CD45RB^{high} or CD45RB^{low} T cells derived from *Hif-2α$^{loxP/loxP}$* Lck Cre$^+$ or Cre$^-$ animals. **a.** Disease severity index as measured by the stool consistency and

presence/absence of rectal prolapse was assayed every two days from day 18 until day 36 post-injection in each group of transfer recipients, (n=6-7 mice/group, two-way ANOVA). Rag1-/- mice were adoptively transferred with $0.5*10^6$ FACS-sorted $CD4^+CD45RB^{high}$ or $CD4^+CD45RB^{low}$ T cells. **b**. Weight change in mice receiving donor cells from Lck Cre^+ or *Hif-2α$^{loxP/loxP}$* Lck Cre^+ mice (5-8 mice/group, two-way ANOVA). **c**. Colon inflammation in transfer recipient mice at conclusion of trial (n=5-8 mice/group, one-way-ANOVA). **e**. Tbet expression in T cells of MLN of the transfer recipient mice (n=5-6 mice/group, two-tailed T test). **e**. IL-17A expression in T cells of MLN in transfer recipient mice (n=5 mice/group, two-tailed T test). Data expressed as Mean± S.E.M, Hif2$\alpha^{loxP/loxP}$ abbreviated as Hif2$\alpha^{f/f}$ for clarity in some graphs, *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 4, related to Figure 6



T cell-intrinsic miR-29a regulates T-cell function in vitro and in vivo.

Thymocytes or naïve CD4⁺ T cells were isolated from *miR-29a$^{loxP/loxP}$* Lck Cre⁺ or Cre⁻ littermates.

a. Expression of miR-29a in thymocytes (n=3).

Purified naïve CD4⁺ T cells from *miR-29a$^{loxP/loxP}$* Lck Cre⁺ or Lck Cre⁺ were cultured for 3 days under T_H0 or T_H1 skewing conditions in the presence of IL-2 and anti-CD3/CD28 under normoxia

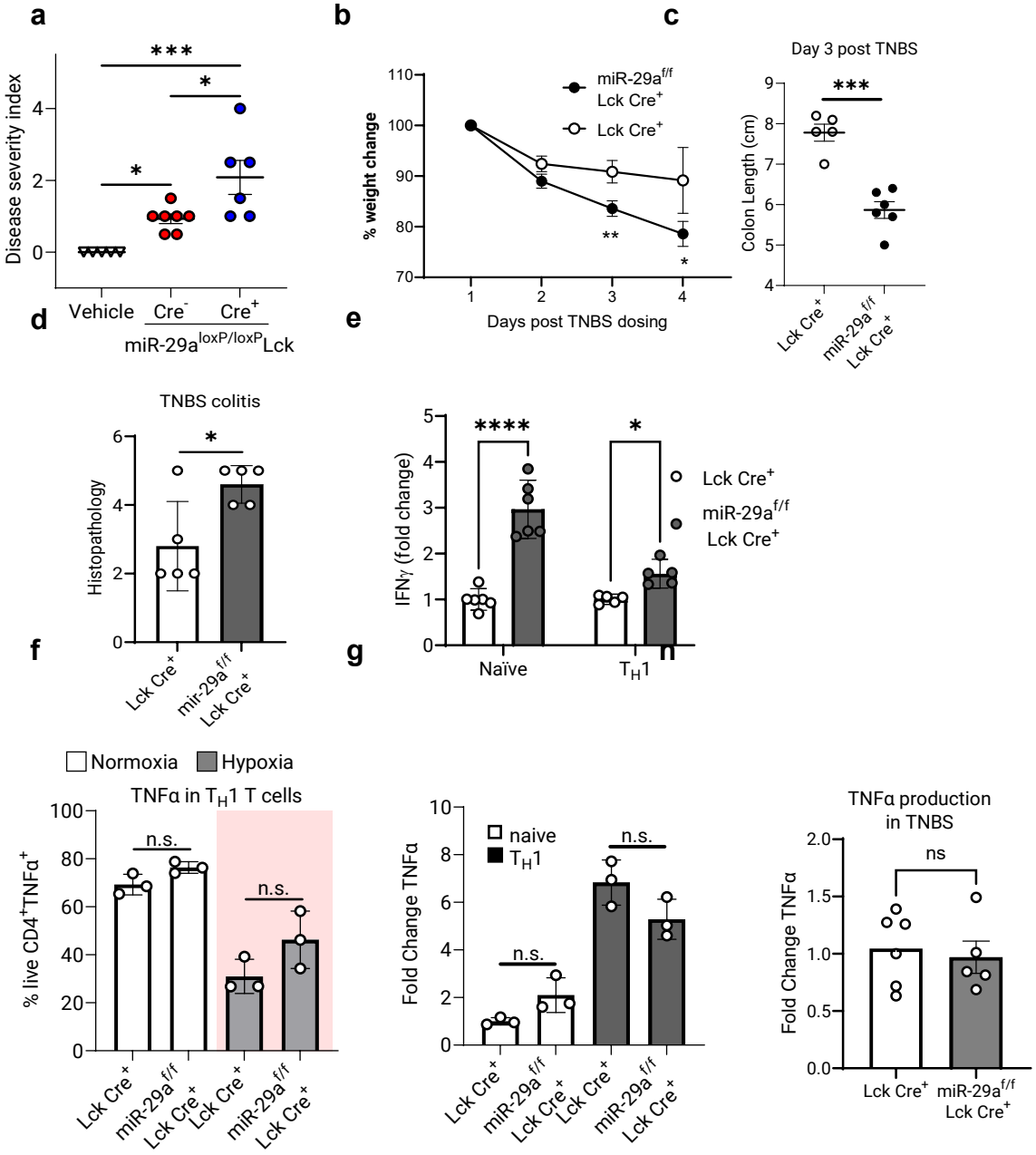
or hypoxia. **b.** Expression of miR-29a in T_H1-skewed T cells activated in vitro, (n=3, two-tailed T test).

Day 3 T_H1 skewed cells were re-stimulated with anti CD3/CD28 and grown under normoxia or hypoxia for 48h. **c**. Percentage of live $CD4^+$ T cells was determined using flow cytometry in each condition. **d**. Intracellular staining was used to determine levels of IFNγ in live $CD4^+$ T cells, (c, d: n=3, repeated 2 times, one-tailed T test).

e. 8-week-old Rag1-/- mice were adoptively transferred with naïve $CD4^+$ $CD45RB^{high}$ T cells at $0.5*10^6$ per mouse and weight change was recorded for the duration of the study (n=3-6 mice/group).

Purified naïve $CD4^+$ T cells from *miR-29a*$^{loxP/loxP}$ Lck Cre^+ or Lck Cre^+ were cultured for 3 days under T_H17 skewing conditions in the presence of IL-2 and anti-CD3/CD28 under normoxia or hypoxia. **f**. Percentage of live $CD4^+$ T cells was determined using flow cytometry in each condition. **g**. Representative flow cytometry plots showing decreased survival of miR-29a deficient T_H17-skewed T cells under hypoxia. **h**. Intracellular staining was used to determine levels of IL-17A in live $CD4^+$ T cells (n=2-3, repeated 2 times, one-way ANOVA). Data expressed as Mean± S.E.M, miR-29a$^{loxP/loxP}$ abbreviated as miR-29a$^{f/f}$ for clarity in some graphs, *p<0.05, **p<0.01, ***p<0.001, for all repeated trials see Source Data file.

Supplementary Figure 5, related to Figure 6



T cell-intrinsic miR-29a regulates T-cell function during inflammation. T_H1 colitis was induced in *miR-29a$^{loxP/loxP}$* Lck Cre$^+$ or Cre- littermate control mice by the epicutaneous skin sensitization and subsequent rectal gavage with TNBS. **a.** Disease scores were determined based on stool

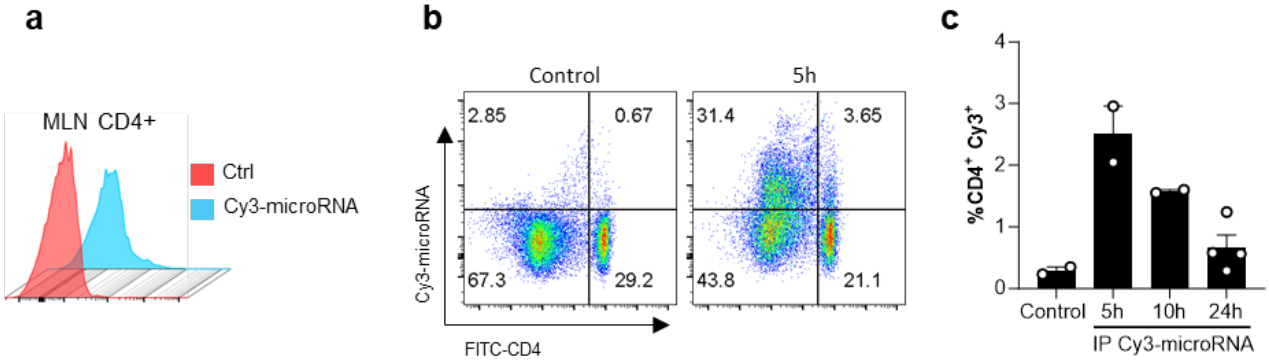
consistency and bleeding, as per Materials and Methods (n=5-7, pooled data from 2 experiments, one-way ANOVA).

TNBS colitis was induced in *miR-29a*$^{loxP/loxP}$ Lck Cre^{+} or Lck Cre^{+} control mice (non-littermate). **b.** Weight change in the course of TNBS colitis (n=8-9, pooled data from 2 experiments, one-way ANOVA). **c.** Colon length was measured at fulminant disease (n=5-6, pooled data, two-tailed T test). **d.** Histology scores were determined by scientists blinded to the genotype of mice (n=5, pooled data, two-tailed T test).

Naïve and T_H1-skewed CD4^{+} T cells were prepared from *miR-29a*$^{loxP/loxP}$ Lck Cre^{+} or Lck Cre^{+} mice. **e.** Production of IFNγ upon 18h re-stimulation was measured in both types of T cells.

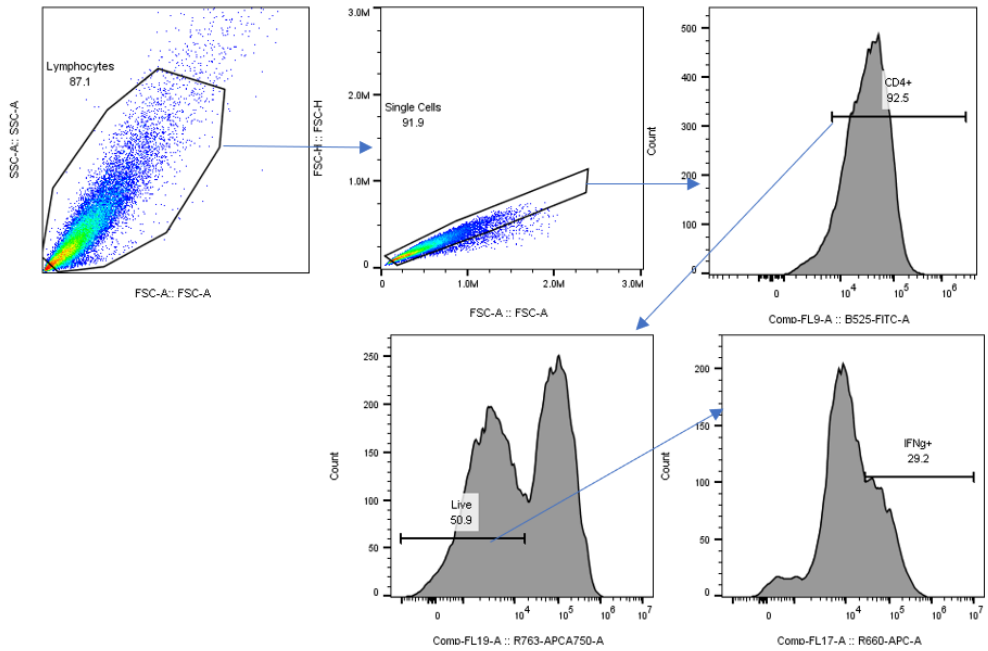
CD4+ T cells sufficient and deficient in miR-29a were purified from spleen suspensions and cultured under T_H1 conditions for 3 days then re-stimulated with anti CD3/CD28 for 48h under normoxia or hypoxia (n=3, one-way ANOVA). **f**. Numbers of live CD4^{+} T cells producing TNFα were determined using flow cytometry and intracellular staining (n=3, one way ANOVA). **g**. Q-PCR normalized to 18s was used to measure TNFα production in naïve and T_H1 (day 3) skewed CD4+ T cells re-stimulated for 18h with anti-CD3/CD28 antibodies (n=3, one-way ANOVA). **h**. Colon biopsies from TNBS-treated mice were used to extract total RNA and measure TNFα production (n=3, two-tailed T test). Data expressed as Mean± S.E.M, miR-29a$^{loxP/loxP}$ abbreviated as miR-29a$^{f/f}$ for clarity in some graphs, *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 6, related to Figure 7



Nanoparticle-mediated delivery of microRNA mimetic to colitic T cells. C57BL/6J mice that were previously challenged with TNBS (disease day 2 or 3) were subsequently intraperitoneally injected with Cy3-labeled and DOPC-packaged control microRNA. Mesenteric lymph nodes were isolated at 5h, 10h and 24h post mimic-injection from the mesenteric lymph nodes and assayed by flow cytometry. **a**. Representative histogram of Cy3-positve $CD4^+$ T cells. **b.** Representative FACS plots of Cy3 positive fraction of $CD4^+$ T cells. **c.** Percentages of $Cy3^+$ $CD4^+$ T cells as a function of time post injection of liposome-packaged mimetics. Data expressed as Mean± S.E.M, n=2-4 mice/group/time point in **c**.

Supplementary Figure 7, flow cytometry gating strategy



Example of Flow Cytometry gating strategy used for T cells

Supplementary Table 1.

Patient Characteristics, related to Figure 2

Phenotype	n	Age (yrs.; mean)	Disease duration (yrs.; mean)	Current IBD Medications		
				Prednisone	AZA/6MP	Anti-TNF
Healthy Controls	10	51.4	n/a	0%	0%	0%
Inactive UC	6	47.8	16.8	33%	50%	0%
Active UC	6	51.5	8.8	50%	33%	0%
Inactive Crohn's	4	47.5	22.0	0%	17%	33%
Active Crohn's	6	36.7	5.0	33%	0%	0%