Supplementary Material
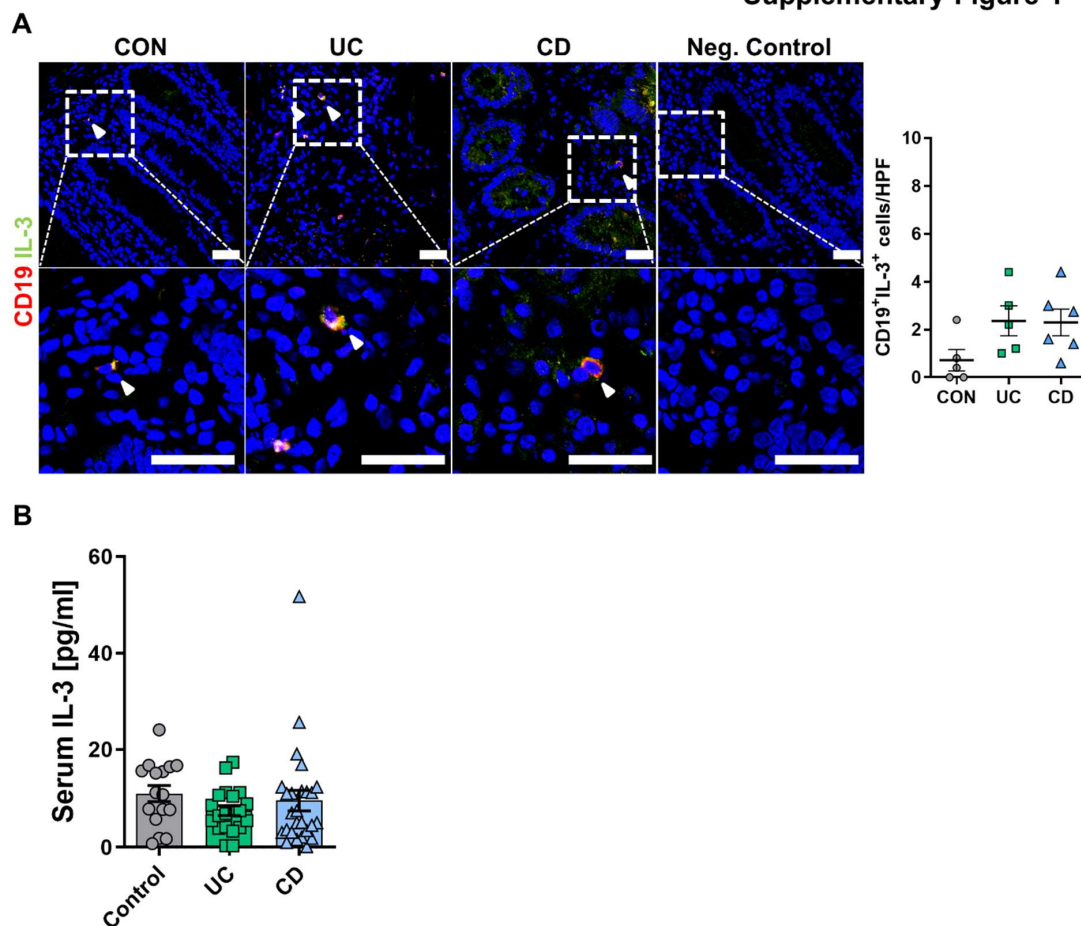
Supplementary Table 1: Characteristics of patients providing biopsies for the study.

		control	CD	UC
Number		34	139	129
Age (Ø, range)		63 (26-85)	37 (16-75)	39 (19-76)
Female [%]		41.2	49.6	37.2
Active disease [%]			56.1	59.7
Therapy [%]	**Mesalazine**		11.5	54.3
	Steroids		36.7	41.1
	Immunosuppressants		17.3	26.4
	anti-TNF		56.8	31.0
	Vedolizumab		3.6	17.8
	Ustekinumab		1.4	1.6
Disease localization [%]			**L1:** 29.5	**E1:** 14.0
			L2: 17.3	**E2:** 29.5
			L3: 52.5	**E3:** 56.6
			L4: 0.7	
			L4+: 25.9	
Biopsy collection side [%]			**Colon:** 74.1	**Colon:** 100
			Ileum: 25.9	

Supplementary Table 2: Characteristics of patients providing peripheral blood for the study.

			control	CD	UC
Number			40	24	19
Age (Ø, range)			27 (20-39)	36 (21-69)	40 (19-69)
Female [%]			70.0	50.0	73.7
Active disease [%]				29.2	15.8
Therapy [%]		**Mesalazine**		16.7	68.4
		Steroids		16.7	36.8
		Immunosuppressants		8.3	0.0
		anti-TNF		58.3	52.6
		Vedolizumab		25.0	47.4
		Ustekinumab		4.2	0.0
Disease localization [%]				**L1:** 16.7	**E1:** 15.8
				L2: 4.2	**E2:** 26.3
				L3: 79.3	**E3:** 57.9
				L4: 0.0	
				L4+: 4.2	

Supplemental material

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Supplementary Figure 1
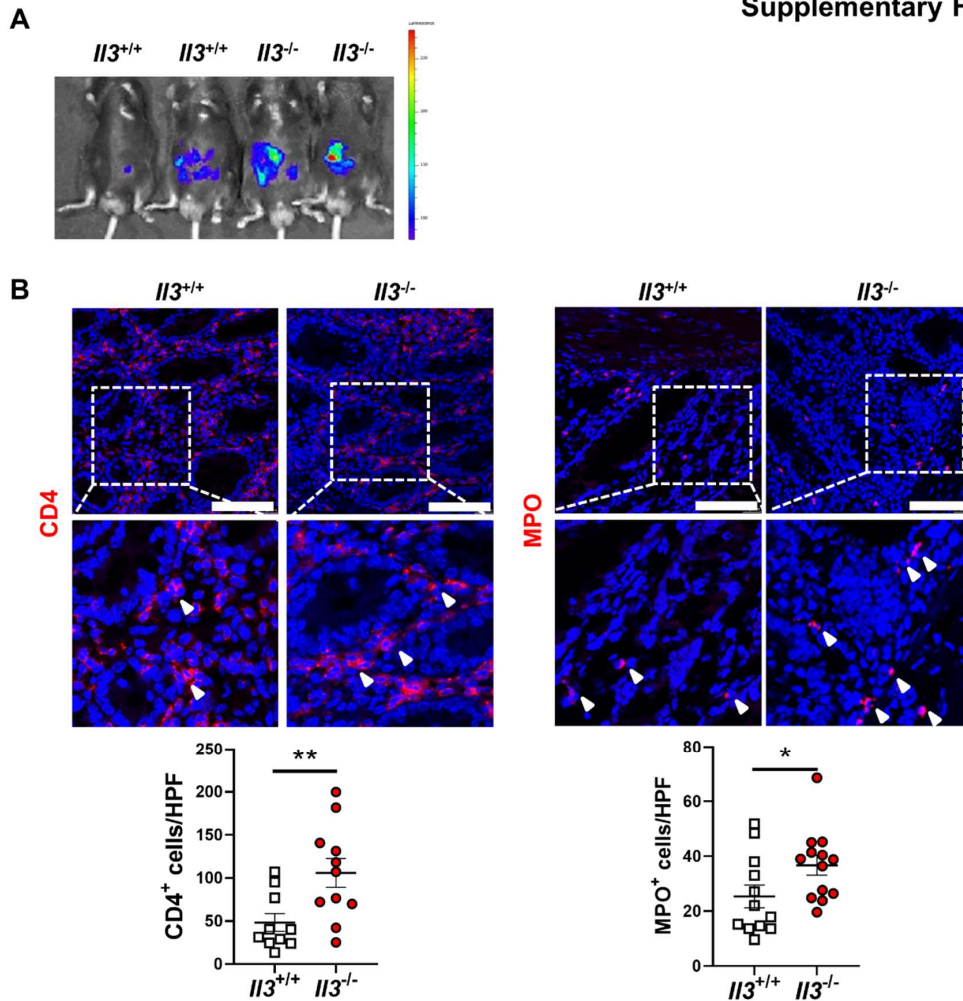

Supplementary Figure 1:
(**A**) Immunofluorescence of cryosections from patients with CD (*n* = 6), UC (*n* = 5) and CON (*n* = 5) for IL-3 (green) and CD19 (red); counterstaining with Hoechst (blue). Left: representative images, white arrowheads highlight double positive cells, scale bars – 25µm; right: quantification of double positive cells per high power field (HPF); One-way ANOVA with Tukey's multiple comparisons post-hoc test.
(**B**) Concentration of IL-3 in serum of patients with CD (*n* = 26), UC (*n* = 20) and CON (*n* = 16) as determined by ELISA.
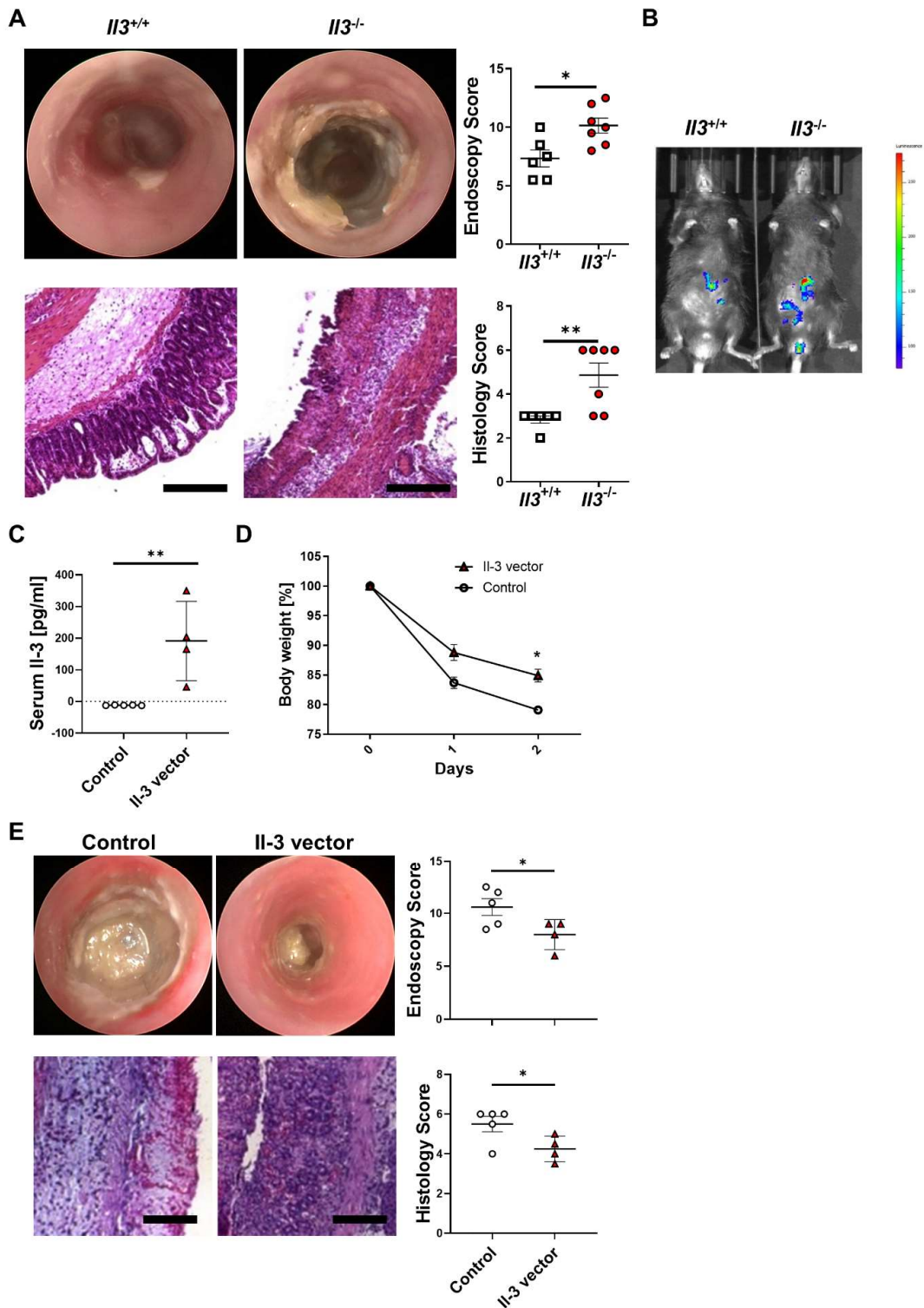
Supplementary Figure 2

Supplementary Figure 2:
(A) Representative *in vivo* IVIS luminescence imaging of reactive oxygen species after i.p. injection of L-012 in *Rag1*$^{-/-}$ mice after transfer of naïve CD4$^+$ splenocytes from *Il3*$^{+/+}$ and *Il3*$^{-/-}$ mice.
(B) Immunofluorescence staining for CD4 (left, red) and MPO (right, red) in colon tissue counterstained with Hoechst (blue) of *Rag1*$^{-/-}$ mice after transfer of naïve CD4$^+$ splenocytes from *Il3*$^{-/-}$ or *Il3*$^{+/+}$ mice. Upper panels: Representative images, white arrowheads highlight CD4$^+$ or MPO$^+$ cells. Lower panels: Quantification of CD4$^+$ and MPO$^+$ cells per high power field (HPF). *n* = 10-13 per group, unpaired t-test; scale bars – 75 µm.
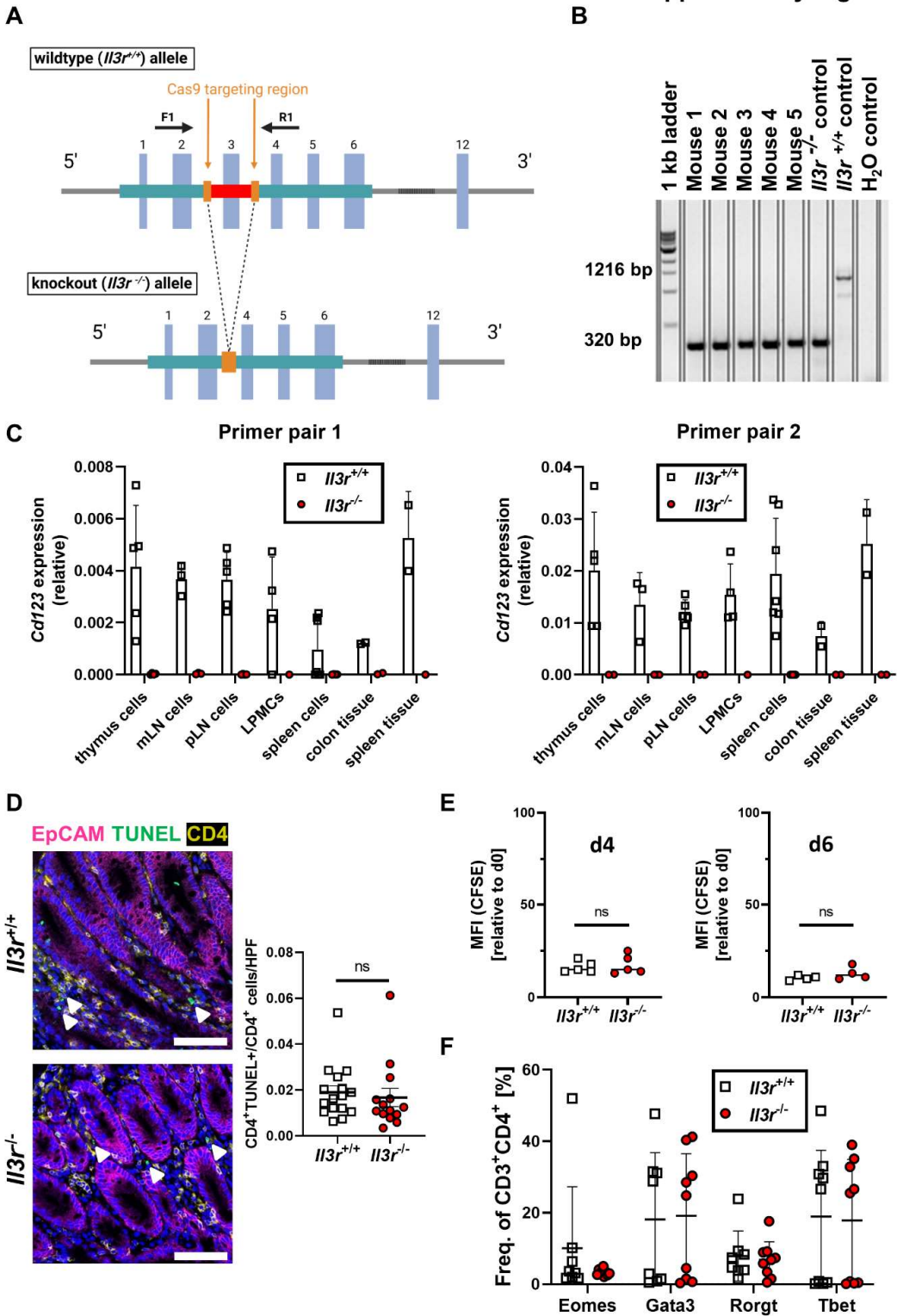
Supplementary Figure 3

Supplementary Figure 3:

(A, B) Oxazolone colitis with *Il3*$^{-/-}$ and *Il3*$^{+/+}$ mice. **(A)** Mini-endoscopy (top) and histology of colon tissue (bottom). Left: representative images (scale bars – 12.5 µm), right: quantitative endoscopic and histological scores of disease severity. *n* = 6-7 per group, unpaired t-test. **(B)** Representative *in vivo* IVIS luminescence imaging of reactive oxygen species after i.p. injection of L-012.

(C-E) Oxazolone colitis with C57BL/6 mice following hydrodynamic tail vein injection of a minicircle vector for Il-3 overexpression (mock vector for control mice) **(C)** Concentration of Il-3 in the serum as determined by ELISA. One-way ANOVA with Tukey's multiple comparisons post-hoc test. **(D)** Weight curve as well as **(E)** representative and quantitative endoscopic and histologic (scale bars – 12.5 µm) disease activity on day 2 after oxazolone application.

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Supplementary Figure 4

Supplementary Figure 4:

(A) Schematic representation of the generation of *Il3r^{-/-}* mice using the CripsR/Cas9-system; homology arm (turquoise blue), Cas9-cutting sides (orange), knockout-region (red) and exons 1-6 and 12 (light blue) are highlighted; figure drawn with licensed BioRender software.

(B) Representative genotyping PCR gel electrophoresis of *Il3r^{-/-}* mice along with *Il3r^{-/-}*, *Il3r^{+/+}* and water controls.

(C) *Cd123* mRNA expression in cells and tissue from various organs of *Il3r^{-/-}* and *Il3r^{+/+}* mice as determined by qPCR using two different primer pairs; n = 1-7 per group.

(D) Immunofluorescence staining for EpCAM (magenta), TUNEL (green) and CD4 (yellow) in colon tissue counterstained with Hoechst (blue). Upper panel: Representative images, white arrowheads highlight CD4^+TUNEL^+ cells. Lower panel: Quantification of CD4^+ TUNEL^+ cells per total CD4^+ cells per HPF. n = 14-16 per group, Mann-Whitney test. Scale bars – 50 µm.
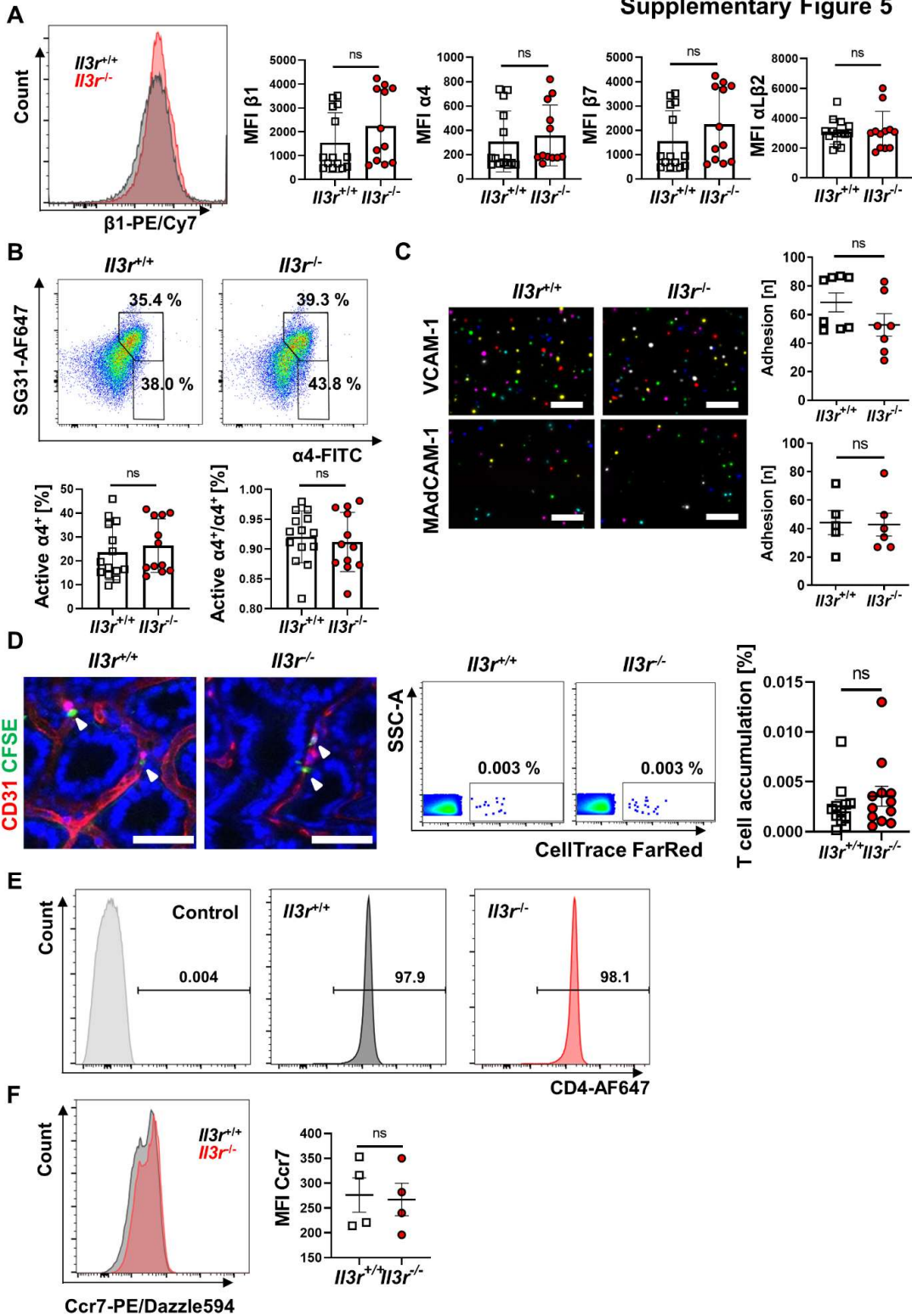
(E) Proliferation of *Il3r^{+/+}* and *Il3r^{-/-}* thymocytes as determined by CFSE dilution on flow cytometry. Representative histograms of the CFSE signal (left). Quantitative analysis of the MFI of the CFSE signal (right) on day 4 (upper) and day 6 (lower panel); n = 4-5 per group, students t-test.

(F) Quantitative flow cytometry of T cell lineage-specific transcription factors on CD3^+CD4^+ lamina propria cells from mice with transfer colitis after transfer of *Il3r^{+/+}* or *Il3r^{-/-}* T cells; n = 6-9 per group, 2-way ANOVA.

CFSE, carboxyfluorescein succinimidyl ester; MFI, mean fluorescence intensity; mLN, mesenteric lymph node; pLN, peripheral lymph node; UA, unit area.

Supplementary Figure 5

Supplementary Figure 5:

(A) Expression of gut homing integrins on CD3$^+$CD4$^+$ splenocytes as determined by flow cytometry. Left panel: Representative histograms of β1 expression. Right panel: Quantification of mean fluorescence intensity; *n* = 12-14 per group, Mann-Whitney test.

(B) Activation status of α4 integrin on CD3$^+$CD4$^+$ splenocytes as determined by flow cytometry with a conformation-specific antibody (SG31). Upper panels: Representative flow cytometry of active α4 and total α4 expression. Lower panels: Quantitative analysis of the frequency of active α4 (left) as well as active α4 per total α4 (right); *n* = 12-14 per group, Mann-Whitney test (active α4) and unpaired t-test (ratio).
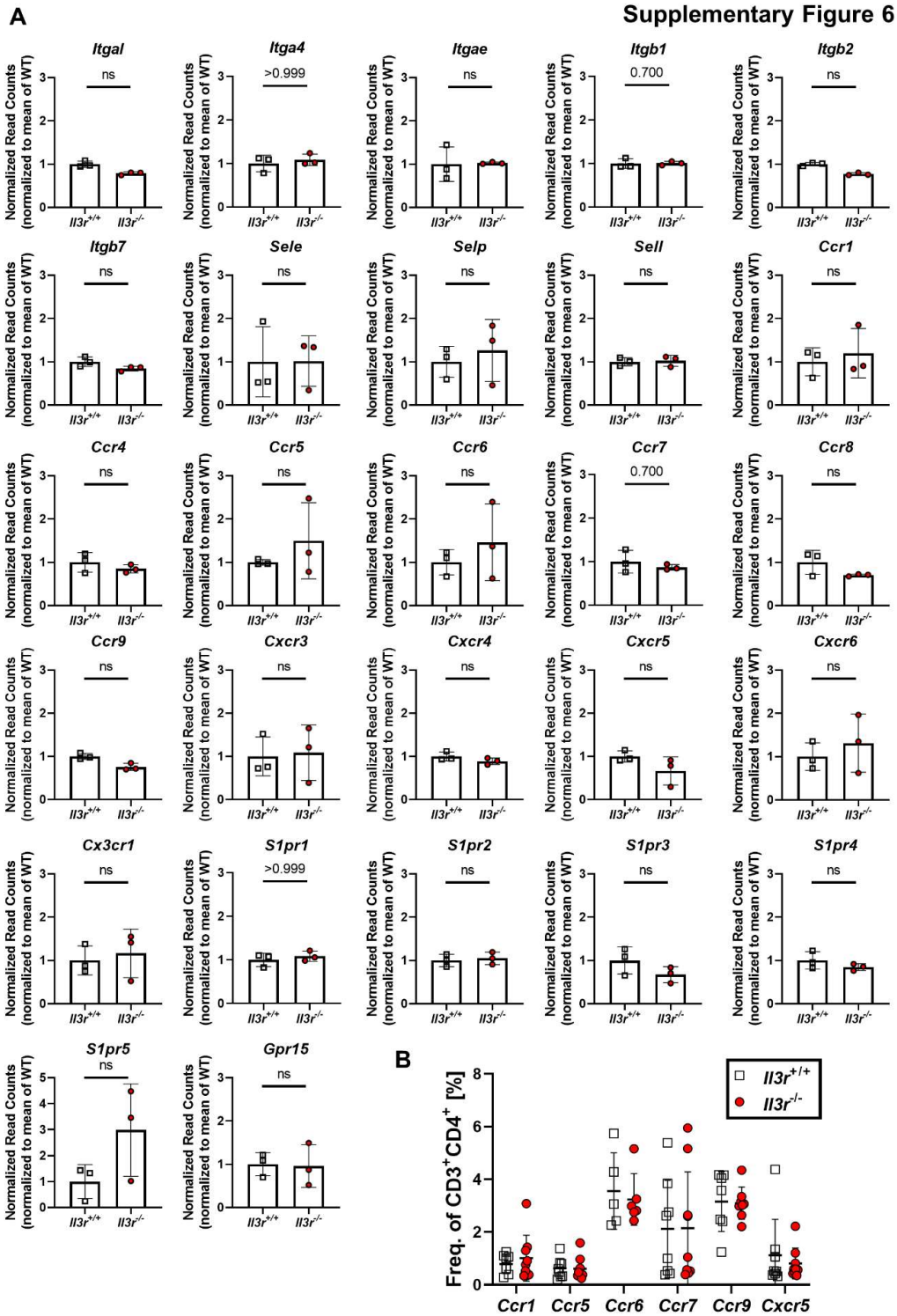
(C) Dynamic adhesion of CD4$^+$ *Il3r$^{-/-}$* or *Il3r$^{+/+}$* splenocytes to glass capillaries coated with rm VCAM-1 or rm MAdCAM-1. Representative overlays of seven microscopy pictures (left) and quantitative adhesion in eight pictures (right); *n* = 5-8 per group, Mann-Whitney test.

(D) Homing of CFSE-stained CD4$^+$ *Il3r$^{-/-}$* or *Il3r$^{+/+}$* splenocytes into the inflamed gut tissue in an *in vivo* homing assay. Representative intravital confocal microscopy (left) showing transferred cells (green), blood vessels (anti-CD31, red) and Hoechst staining (blue); white arrowheads highlight homed cells. Representative flow cytometry of isolated lamina propria mononuclear cells (middle) and quantification of CFSE$^+$ events (right); *n* = 12 per group, Mann-Whitney test; scale bars – 50 µm.

(E) Representative histograms of CD4 expression on *Il3r$^{-/-}$* or *Il3r$^{+/+}$* thymocytes as determined by flow cytometry compared to unstained control.

(F) Ccr7 expression on CD3$^+$CD4$^+$ *Il3r$^{-/-}$* or *Il3r$^{+/+}$* thymocytes as determined by flow cytometry. Representative histograms (upper panel) and quantification of mean fluorescence intensity (MFI, lower panel); *n* = 4 per group, unpaired t-test.

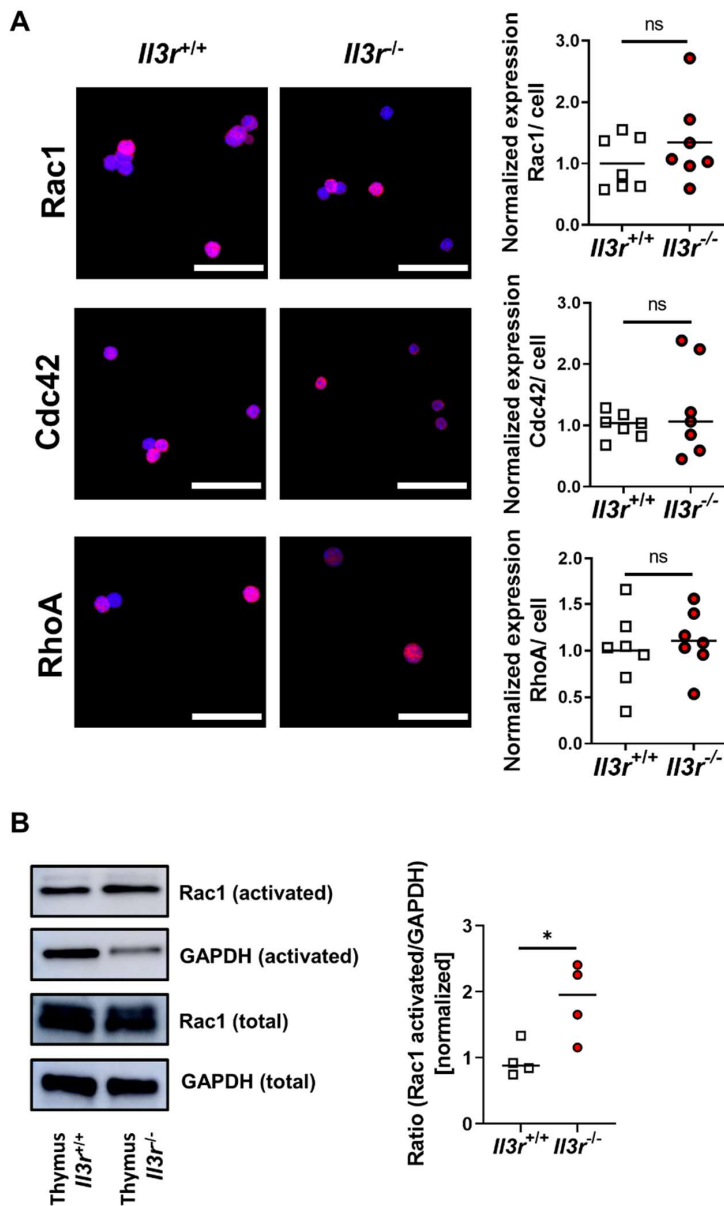CFSE, carboxyfluorescein succinimidyl ester; MFI, mean fluorescence intensity.

A

Supplementary Figure 6

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Supplementary Figure 6:
(**A**) Comparison of the expression of trafficking-associated genes in RNA sequencing data of *Il3r$^{-/-}$* or *Il3r$^{+/+}$* thymus T cells (*n* = 3 per group); Mann-Whitney test.
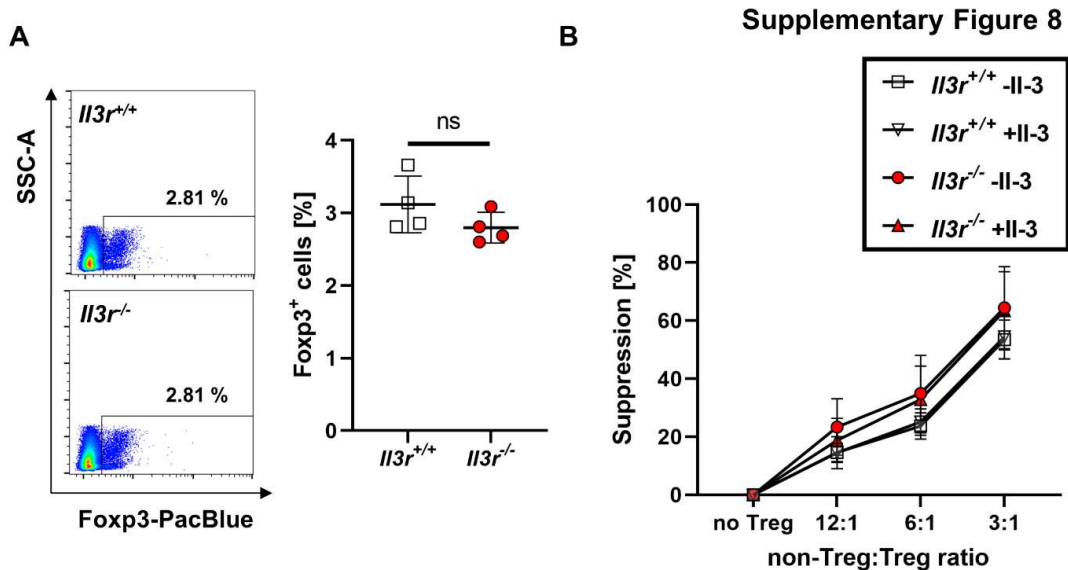(**B**) Quantitative flow cytometry of the expression of cell migration-associated chemokine receptors on CD3$^+$CD4$^+$ lamina propria cells from mice with transfer colitis after transfer of *Il3r$^{+/+}$* or *Il3r$^{-/-}$* T cells; *n* = 6-9 per group, 2-way ANOVA.

Supplementary Figure 7

Supplementary Figure 7:
(A) Representative fluorescence microscopy (left) and quantitative analysis (right) of Rac1-Cy3, Cdc42-Cy3 and RhoA-Cy3 (each magenta) staining of *Il3r*^-/- or *Il3r*^+/+ thymocytes counterstained with Hoechst (blue); *n* = 7 per group, unpaired t-test, scale bars – 25µm.
(B) Representative western blot with activated or total protein (upper panel) and quantification (lower panel) of Rac1 Activation Assays of *Il3r*^-/- or *Il3r*^+/+ thymocytes, *n* = 4 per group, unpaired t-test.
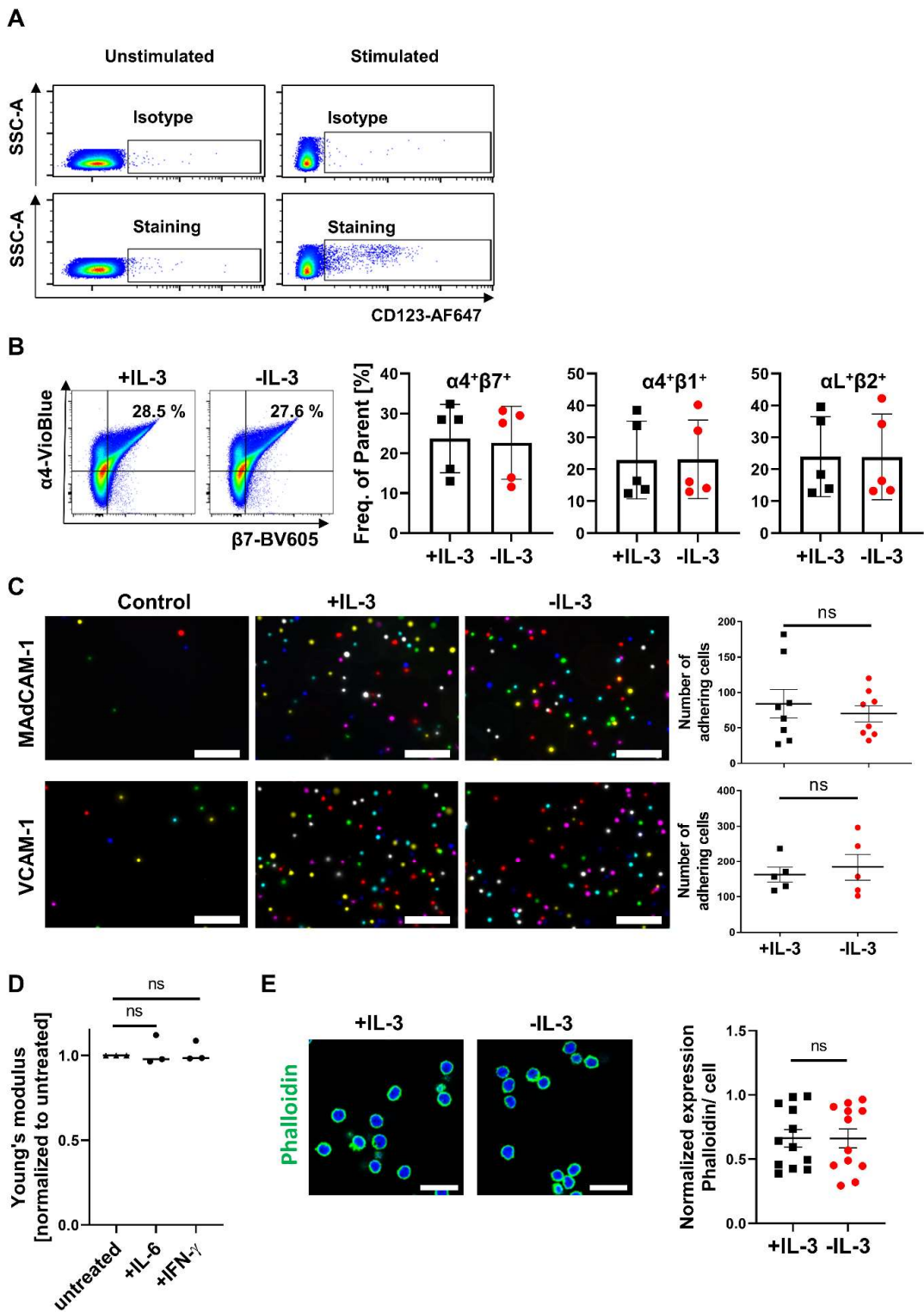
Supplementary Figure 8:
(A) Representative flow cytometry (left) and quantitative analysis (right) of the Foxp3 expression of *Il3r*^{+/+} and *Il3r*^{-/-} CD4^{+} splenocytes cultured under Treg-polarizing conditions; *n* = 4 per group, students t-test.
(B) Quantitative flow cytometry showing the suppression of *Il3r*^{+/+} Teffs by *Il3r*^{+/+} or *Il3r*^{-/-} Tregs co-cultured in the indicated ratio and additionally treated with or without Il-3 for 45h. Suppression was determined by CFSE dilution and calculated as difference to control normalized to control; *n* = 4-5 per group, 2way ANOVA.

Supplementary Figure 9

Supplementary Figure 9:

(A) Representative flow cytometry of CD123 expression on CD3⁺CD4⁺ T cells gated from peripheral blood mononuclear cells stimulated with anti-CD3/CD28 antibodies or not as indicated.

(B) Expression of gut homing integrins on CD3⁺CD4⁺ human peripheral blood T cells cells stimulated with anti-CD3/CD28 antibodies and treated with or without rh IL-3 as determined by flow cytometry. Left panels: Representative flow cytometry of α4β7 expression. Right panels: Quantification of integrin expression as indicated; n = 5 per group, paired t-test.
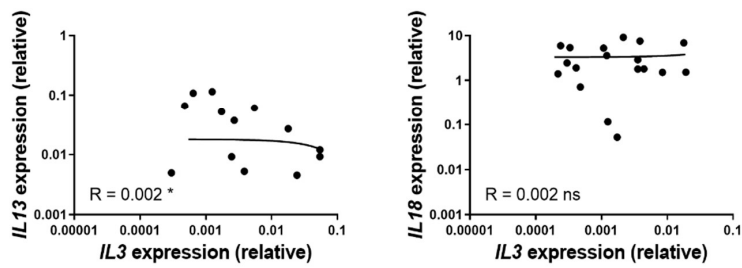
(C) Dynamic adhesion of CD4⁺ peripheral blood T cells stimulated with anti-CD3/CD28 antibodies and treated with or without rh IL-3 to glass capillaries coated with rh MAdCAM-1 or rh VCAM-1. Representative overlays of seven microscopy pictures (left panels) and quantitative adhesion in eight pictures (right panel); n = 5 per group, paired t-test.

(D) Relative Young's modulus of CD4⁺ human peripheral blood T cells stimulated with anti-CD3/CD28 antibodies and treated with or without rh IL-6 or IFN-γ as determined by RT-DC; n = 3 per group, repeated measures one-way ANOVA; effect size: Cohen's f = 0.009.

(E) Representative fluorescence microscopy (left) and quantitative analysis (right) of phalloidin-AF488 staining (green) in CD4⁺ human peripheral blood T cells stimulated with anti-CD3/CD28 antibodies and treated with or without rh IL-3 counterstained with Hoechst (blue); n = 12 per group, paired t-test, scale bars – 25 µm.

Supplementary Figure 10

Supplementary Figure 10:
Correlation of *IL3* mRNA with *IL13* (left) and *IL18* (right) mRNA expression as determined by qPCR in colon tissue from patients with IBD (*n* = 33 each). Spearman's R, significance levels and a regression line are indicated.