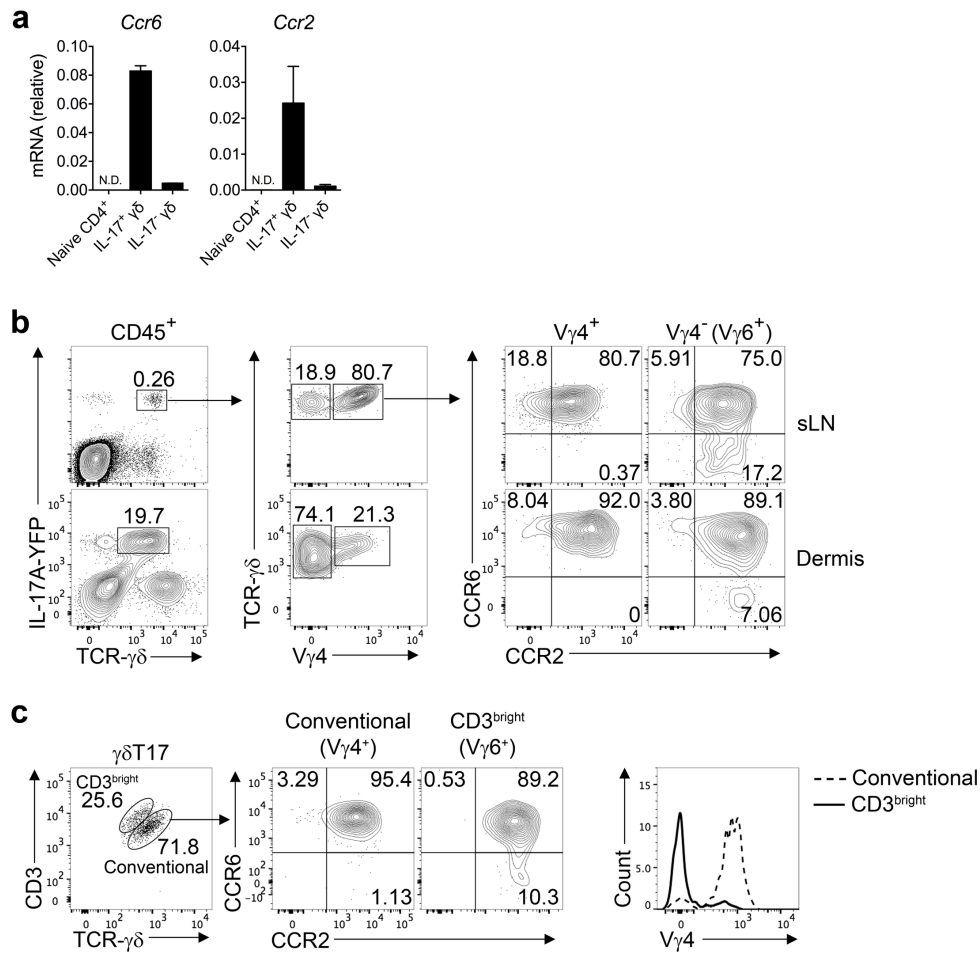
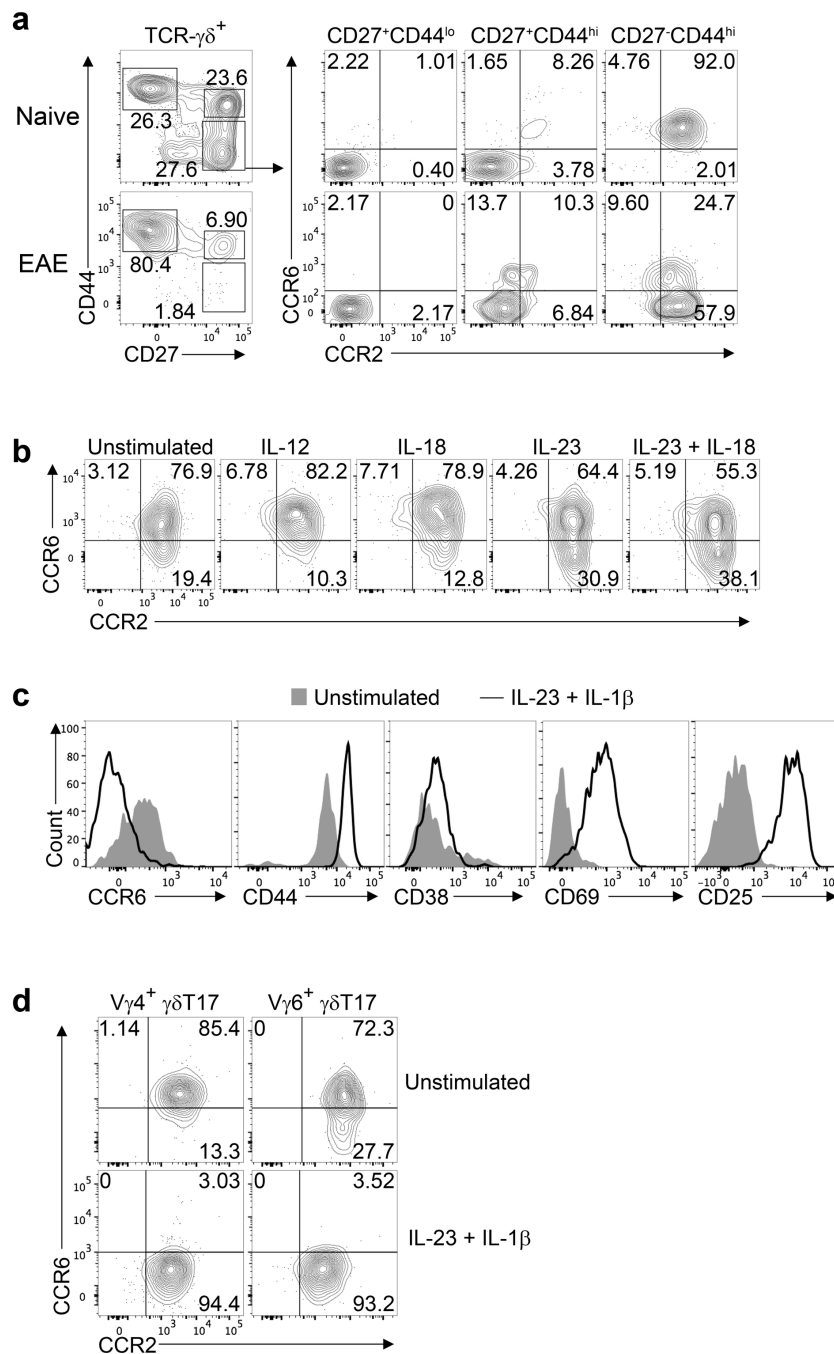


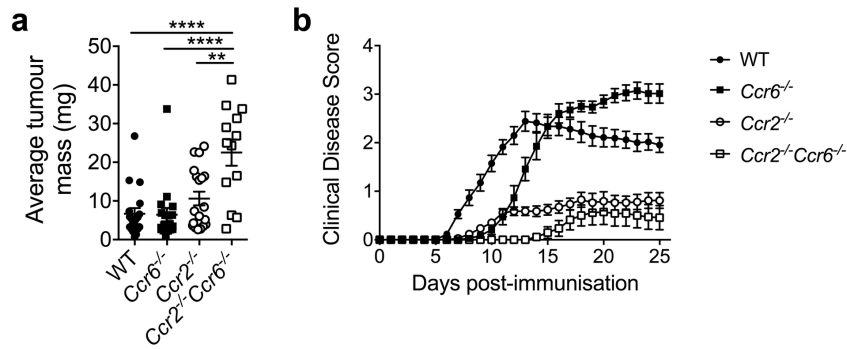
SUPPLEMENTARY INFORMATION



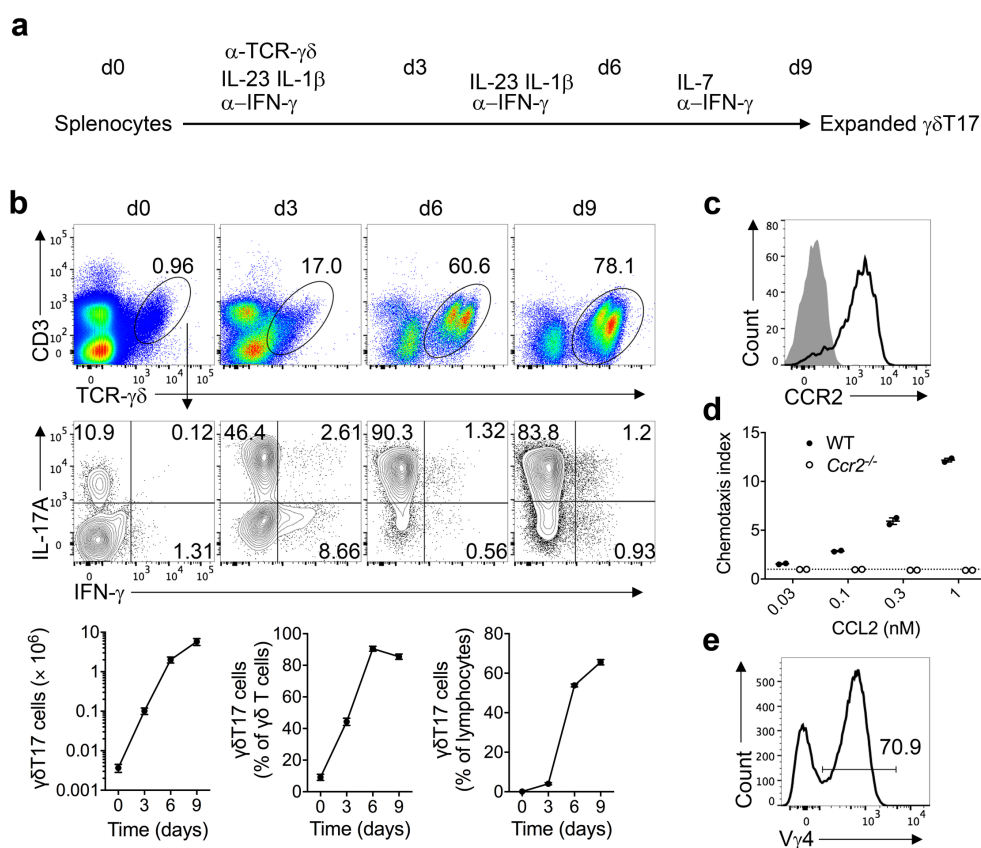
Supplementary Figure 1: CCR6 and CCR2 expression by resting $\gamma\delta$ T17 cells. **a)** *Ccr6* and *Ccr2* mRNA in sorted CD3⁺CD4⁺CD44^{lo}CD25⁻ naïve CD4 T cells and CD90⁺CD3⁺TCR- $\gamma\delta$ ⁺IL-17A-YFP⁺ $\gamma\delta$ T17 and YFP⁻ IL-17⁻ $\gamma\delta$ T cells from skin-draining lymph nodes (sLNs) and spleen of naïve *Il17a*^{Cre} \times *Rosa26*^{eYFP} mice (pooled from n=5). N.D.: not detected. **b)** Representative flow cytometry of CCR6/CCR2 expression by V γ 4⁺ and V γ 4⁻ (V γ 6⁺) $\gamma\delta$ T17 cells from sLNs and dermis of naïve *Il17a*^{Cre} \times *Rosa26*^{eYFP} mice (n=3). **c)** Representative flow cytometry of CCR6/CCR2 and V γ 4 expression by conventional (V γ 4⁺) and CD3^{bright} (V γ 6⁺) $\gamma\delta$ T17 cells from sLNs of naïve *Il17a*^{Cre} \times *Rosa26*^{eYFP} mice (n=3). Mean \pm SD. **b-c)** Representative of two experiments.



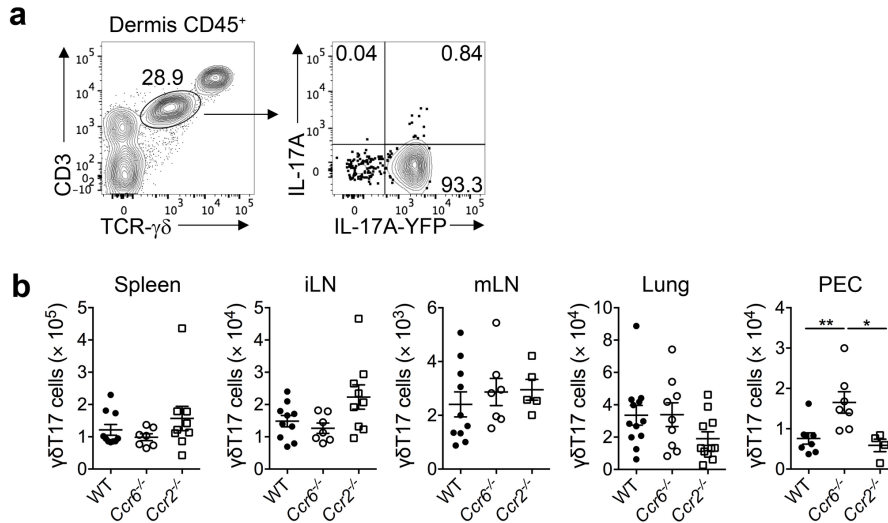
Supplementary Figure 2: Downregulation of CCR6 in activated $\gamma\delta$ T17 cells. **a)** Representative flow cytometry of CCR6/CCR2 expression by subsets of $\gamma\delta$ T cells distinguished by CD44 and CD27 expression in skin-draining lymph nodes of wildtype (WT) mice either naïve or at experimental autoimmune encephalomyelitis (EAE) onset (n=3/group). **b)** Representative flow cytometry of CCR6/CCR2 expression by $\gamma\delta$ T17 cells from *Il17a^{Cre} × Rosa26^{eYFP}* lymphocytes cultured with indicated stimuli for 72 hr (n=3). **c)** Representative flow cytometry of activation marker expression by $\gamma\delta$ T17 cells from *Il17a^{Cre} × Rosa26^{eYFP}* lymphocytes either unstimulated or IL-23/IL-1 β stimulated for 72 hr (n=3). **d)** Representative flow cytometry of CCR6/CCR2 expression by splenic conventional (V γ 4⁺) and CD3^{bright} (V γ 6⁺) $\gamma\delta$ T17 cells (gated as in Supplementary Figure 1c) from *Il17a^{Cre} × Rosa26^{eYFP}* mice either unstimulated or IL-23/IL-1 β stimulated for 72 hr (n=3). **a, d)** Representative of two experiments.



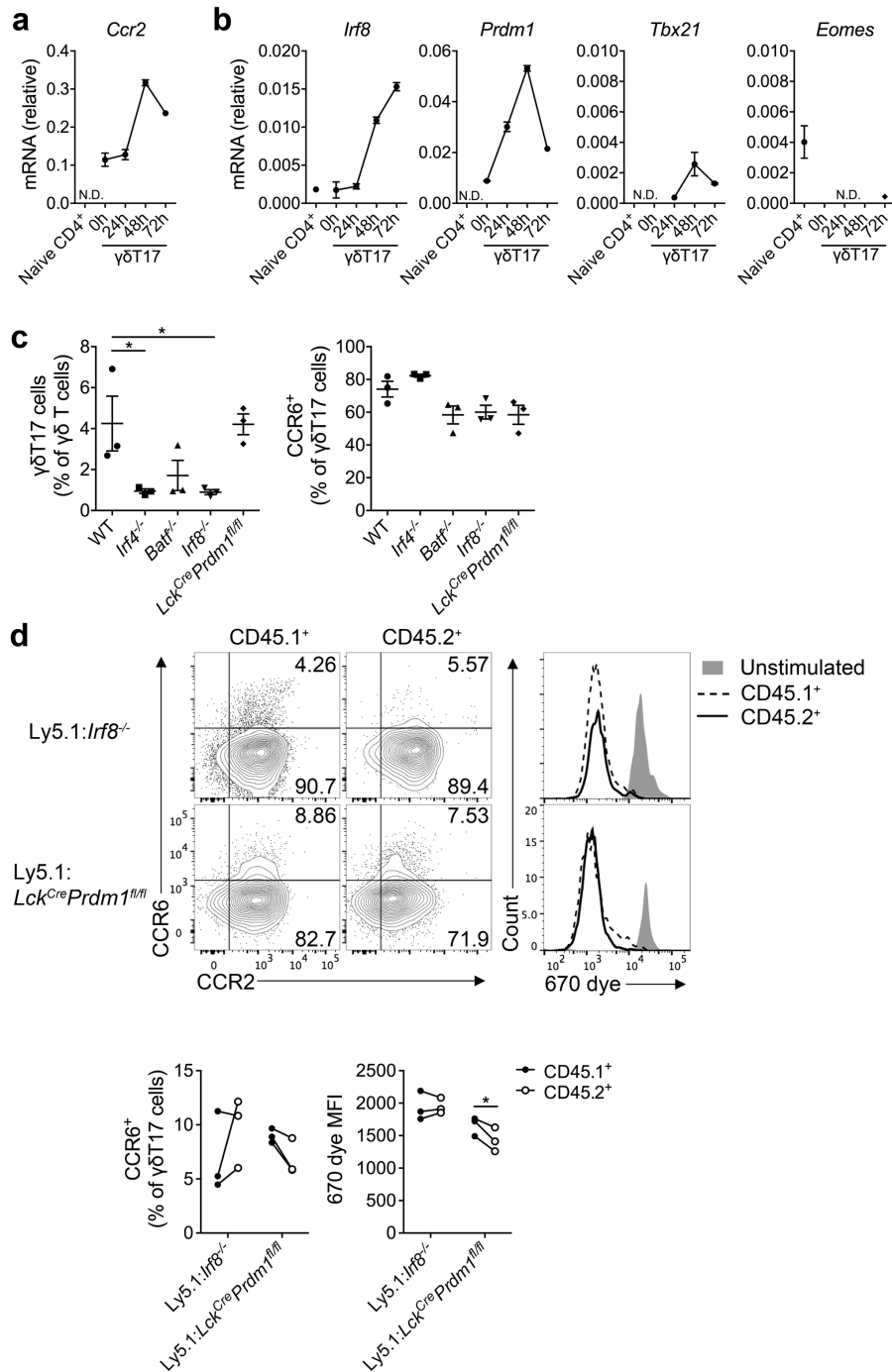
Supplementary Figure 3: B16 melanoma and EAE progression in chemokine receptor-deficient mice. a) Average mass (per mouse) of B16 melanomas 7d post-challenge in wildtype (WT) (n=19), *Ccr6*^{-/-} (n=18), *Ccr2*^{-/-} (n=19) and *Ccr2*^{-/-}*Ccr6*^{-/-} mice (n=13). **b)** Clinical disease scores of WT, *Ccr6*^{-/-}, *Ccr2*^{-/-} and *Ccr2*^{-/-}*Ccr6*^{-/-} mice given experimental autoimmune encephalomyelitis (EAE) (n=17/group). Mean±SEM. **a-b)** Pooled from two experiments. **a)** One-way ANOVA with Bonferroni's multiple comparisons test. ** p < 0.01, **** p < 0.0001.



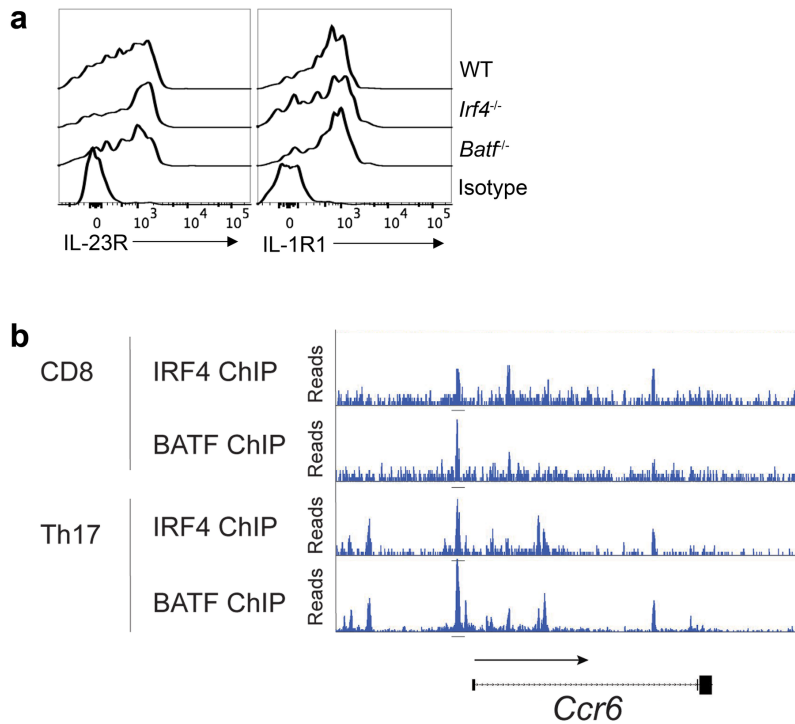
Supplementary Figure 4: *In vitro* expansion of $\gamma\delta$ T17 cells. **a)** Schematic of culture protocol. **b)** Representative flow cytometry and number and frequency of *in vitro*-expanded $\gamma\delta$ T17 cells at different stages of culture (n=3). **c)** Flow cytometry of CCR2 expression by *in vitro*-expanded $\gamma\delta$ T17 cells relative to isotype (grey). **d)** Transwell chemotaxis of *in vitro*-expanded $\gamma\delta$ T17 cells from wildtype (WT) and *Ccr2*^{-/-} mice to CCL2. **e)** Flow cytometry of V γ 4 expression by *in vitro*-expanded $\gamma\delta$ T17 cells. **b)** Mean \pm SEM, **d)** Mean \pm SD. **a-e)** Representative of two experiments.



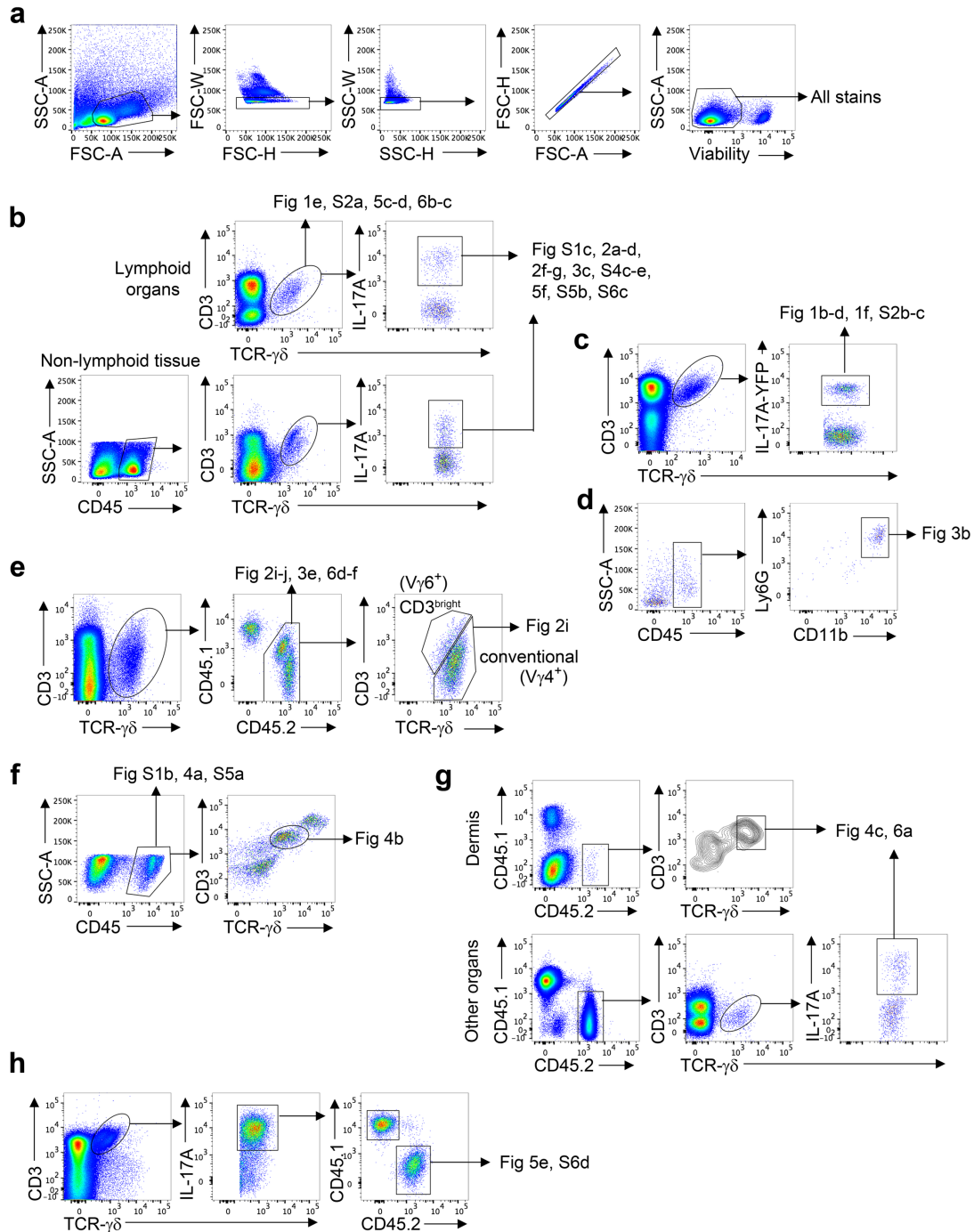
Supplementary Figure 5: CCR6 regulates homeostatic $\gamma\delta$ T17 cell positioning in the dermis. a) Representative flow cytometry of IL-17A-YFP and IL-17A expression by dermal $\gamma\delta$ T¹⁰ cells from ears of *Il17a*^{Cre}*xRosa26*^{eYFP} mice (n=3). **b)** Number of $\gamma\delta$ T17 cells in organs of unimmunized wildtype (WT), *Ccr6*^{-/-} and *Ccr2*^{-/-} mice (n=4-12/group). iLN: inguinal lymph node; mLN: mesenteric lymph node; PEC: peritoneal exudate cells. Mean \pm SEM. **a)** Representative of two experiments, **b)** pooled from two experiments. **b)** One-way ANOVA with Bonferroni's multiple comparisons test. * p < 0.05, ** p < 0.01.



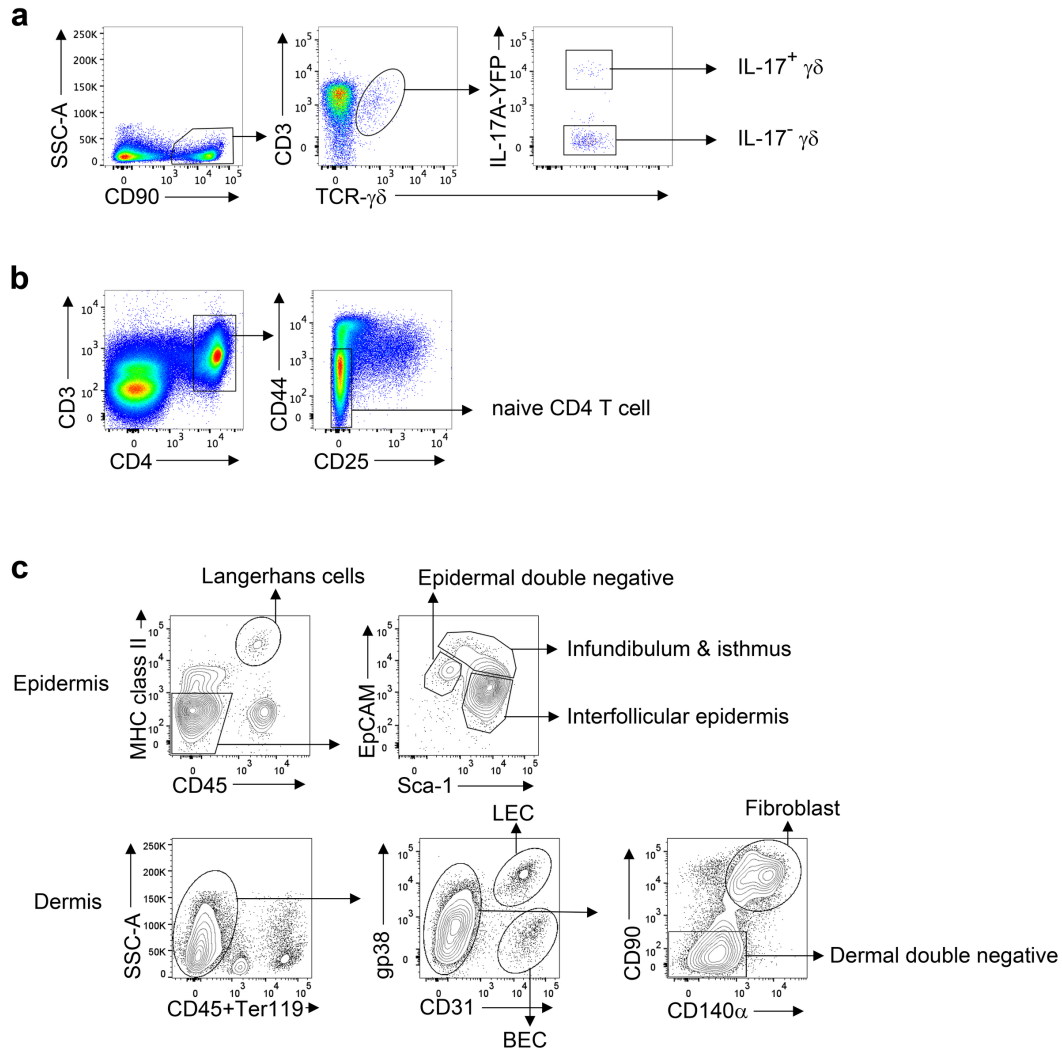
Supplementary Figure 6: IRF8 and Blimp1 do not regulate CCR6 expression in $\gamma\delta$ T17 cells. a) *Ccr2* and **b)** transcription factor mRNA in sorted $\gamma\delta$ T17 cells from *Il17a*^{Cre}*xRosa26*^{eYFP} lymphocytes fresh *ex vivo* or cultured with IL-23/IL-1 β for indicated times (pooled from 5-7 mice). N.D.: not detected. **c)** Frequency of total and CCR6⁺ $\gamma\delta$ T17 cells in spleens of wildtype (WT) and transcription factor-deficient mice (n=3/group). **d)** Splenocytes from *Ly5.1; Irf8*^{-/-} or *Lck*^{Cre}*Prdm1*^{fl/fl} mice were 670 dye-labelled, mixed 50:50 and stimulated with IL-23/IL-1 β for 72 hr. Representative flow cytometry and quantitation of CCR6 expression and proliferation in CD45.1⁺ or CD45.2⁺ $\gamma\delta$ T17 cells (n=3/group). **a-b)** Mean \pm SD, **c)** Mean \pm SEM. **a-b, d)** Representative of two similar experiments. **c)** One-way ANOVA with Dunnett's multiple comparisons test relative to WT control, **d)** paired two-tailed Student's *t*-test. * *p* < 0.05.



Supplementary Figure 7: IRF4 and BATF promote CCR6 downregulation in $\gamma\delta$ T17 cells. **a)** Representative flow cytometry of IL-23R and IL-1R1 expression by splenic CD44^{hi}CD27⁻ $\gamma\delta$ T cells (gated as in Supplementary Figure 2a) from wildtype (WT), *Irf4*^{-/-} and *Batf*^{-/-} mice (n=3/group). **b)** IRF4 and BATF ChIP-Seq data at the *Ccr6* locus from CD8⁺ T cells and T helper 17 (Th17) cells from published datasets. Line indicates binding site consistently detected for both transcription factors in both cell types. Datasets are from Kurachi *et al.* 2014 (BATF CD8⁺ T cells), Man *et al.* 2013 (IRF4 CD8⁺ T cells) and Ciofani *et al.* 2012 (IRF4/BATF Th17 cells) as referenced in results section.



Supplementary Figure 8: Flow cytometry gating strategies. Each panel denotes pre-gates to flow cytometry plots and/or data points in indicated figures. **a)** Lymphocyte gating, single cell discrimination and viability gating serves as pre-gate for all flow cytometry data and sorting strategies. **b)** Gating of $\gamma\delta$ T17 cells by IL-17A protein expression. **c)** Gating of $\gamma\delta$ T17 cells in *Ill7a^{Cre} × Rosa26^{eYFP}* mice. **d)** Gating of neutrophils from nasal wash. **e)** Gating of donor CD45.1⁺/CD45.2⁺ *in vitro*-expanded $\gamma\delta$ T17 cells in co-transfer trafficking experiments, including V γ 4/V γ 6 distinction by CD3^{bright} staining. **f)** Gating of dermal $\gamma\delta$ T¹⁰ cells. **g)** Gating of donor CD45.2⁺ $\gamma\delta$ T¹⁰/ $\gamma\delta$ T17 cells in skin-homing transfer experiments. **h)** Gating of CD45.1⁺ and CD45.2⁺ $\gamma\delta$ T17 cells in co-culture experiments.



Supplementary Figure 9: Flow sorting strategies. **a)** Sorting of $\gamma\delta$ T17 cells and IL-17 $^+$ $\gamma\delta$ T cells from pooled splenocytes and lymph node cells from *Il17a^{Cre} × Rosa26^{eYFP}* mice. **b)** Sorting of naïve CD4 $^+$ T cells from wildtype (WT) splenocytes. **c)** Sorting of indicated epidermal keratinocyte and dermal stromal populations from skin of WT mice. All strategies first gated on live single cells as in Supplementary Figure 8a.

Supplementary Table 1: Monoclonal antibodies and related reagents

Primary antibodies for flow cytometry, ELISA and cell culture				
Antigen	Conjugate	Clone	Company	Final concentration
BrdU	FITC	B44	BD	15µL/well
CCL2	Biotin (ELISA) Purified (ELISA)	Polyclonal	R&D R&D	200ng/ml 200ng/ml
CCR2	Purified	MC21	In house	5.6µg/ml
CCR6	Purified PE	MAB590 140706	R&D R&D	8.3µg/ml 7µL/well
CD3ε	Biotin BV510 FITC PECy7	17A2	eBioscience	2µg/ml
		145-2C11	BD	0.833µg/ml
			BD	2µg/ml
			eBioscience	2µg/ml
CD4	Alexa 647	RM4-5	BD	0.67µg/ml
CD11b	PECy7	M1/70	BD	0.67µg/ml
CD25	Biotin PE	7D4	BD	4.17µg/ml
				0.83µg/ml
CD27	PECy7	LG.3A10	Biolegend	0.67µg/ml
CD31	FITC	MEC 13.3	BD	4.17µg/ml
CD38	APC	90	eBioscience	0.83µg/ml
CD44	Biotin FITC V450	IM7	BD	1.67µg/ml
			BD	0.67µg/ml
			BD	1.67µg/ml
CD45	APC FITC PE	30-F11	BD	0.67µg/ml
			BD	1.67µg/ml
			BD	0.83µg/ml
CD45.1	APC Biotin PerCPCy5.5	A20	eBioscience	0.67µg/ml
			Biolegend	1.67µg/ml
			eBioscience	1.67µg/ml
CD45.2	Biotin FITC PE PECy7 PerCPCy5.5	104	BD	1.67µg/ml
			BD	1.67µg/ml
			BD	0.67µg/ml
			Biolegend	0.83µg/ml
			eBioscience	1.67µg/ml
CD69	PECy7	HI.2F3	BD	0.83µg/ml
CD90.2	APC	53-2.1	BD	0.56µg/ml
CD121α (IL-1R1)	PE	JAMA-147	Biolegend	3.33µg/ml
CD140α	BV421	APA5	BD	1.11µg/ml
Ep-CAM	Purified	G8.8	BD	2.08µg/ml
gp38	Biotin	eBio8.1.1	eBioscience	2.78µg/ml
I-A/I-E	Biotin	2G9	BD	2.08µg/ml
IFN-γ	Purified (cell culture) FITC	XMG1.2	BioXCell	10µg/ml
			BD	2.78µg/ml
IL-17A	BV421 BV510 PE	TC11- 18H10.1	Biolegend	0.28µg/ml
			Biolegend	1.33µg/ml
			BD	1.11µg/ml
IL-23R	APC	753317	R&D	20µL/well
Ly6G	FITC	1A8	BD	1.67µg/ml
RORγt	PerCPeFluor710	B2D	eBioscience	1.67µg/ml
Sca-1	FITC	D7	eBioscience	2.08µg/ml
TCR-γδ	Biotin BV421 PECy7 Purified (cell culture)	GL3	eBioscience	1.67µg/ml
			Biolegend	0.83µg/ml
			Biolegend	0.83µg/ml
			Biolegend	1µg/ml
Vγ4	PECy7	UC3-10A6	eBioscience	0.67µg/ml

Supplementary Table 1 (continued)				
Other flow cytometry reagents				
Reagent	Conjugate	Clone	Company	Final dilution
α -rat IgG	Alexa 647	Polyclonal	Life Technologies	10 μ g/ml
Proliferation dye	eFluor670	-	eBioscience	5 μ M
Live/Dead fixable dye	Near infrared	-	Life Technologies	1/1000
Streptavidin	Alexa 647	-	Jackson	2.5 μ g/ml
	BV510		ImmunoResearch	0.33 μ g/ml
	PerCPy5.5		BD	0.67 μ g/ml

Supplementary Table 2: Primers used in qPCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Rplp0</i>	TGC AGA TCG GGT ACC CAA CT	ACG CGC TTG TAC CCA TTG A
<i>Ccr2</i>	GTT CAT CCA CGG CAT ACT ATC AAC	GCC CCT TCA TCA AGC TCT TG
<i>Ccr6</i>	CCT GGG CAA CAT TAT GGT GGT	CAG AAC GGT AGG GTG AGG ACA
<i>Rorc</i>	CAG CCA ACA TGT GGA AAA GCT	GGG AAG GCG GCT TGG A
<i>Irf4</i>	CGG GCA AGC AGG ACT ACA AT	ACA ATG CCC AAG CCT TGA TG
<i>Irf8</i>	GCT GAT CTG GGA AAA TGA TGA GA	CAC CTC CTG ATT GTA ATC CTG CTT
<i>Prdm1</i>	TGG CAG AGA CTG GGA TCA TG	CTC GGC CTC TGT CCA CAA A
<i>Batf</i>	GTT CTG TTT CTC CAG GTC C	GAA GAA TCG CAT CGC TGC
<i>Tbx21</i>	GCC AGG GAA CCG CTT ATA TG	AAC TTC CTG GCG CAT CCA
<i>Eomes</i>	TGA GCT TCA ACA TAA ACG GAC TCA	CGG CCA GAA CCA CTT CCA