

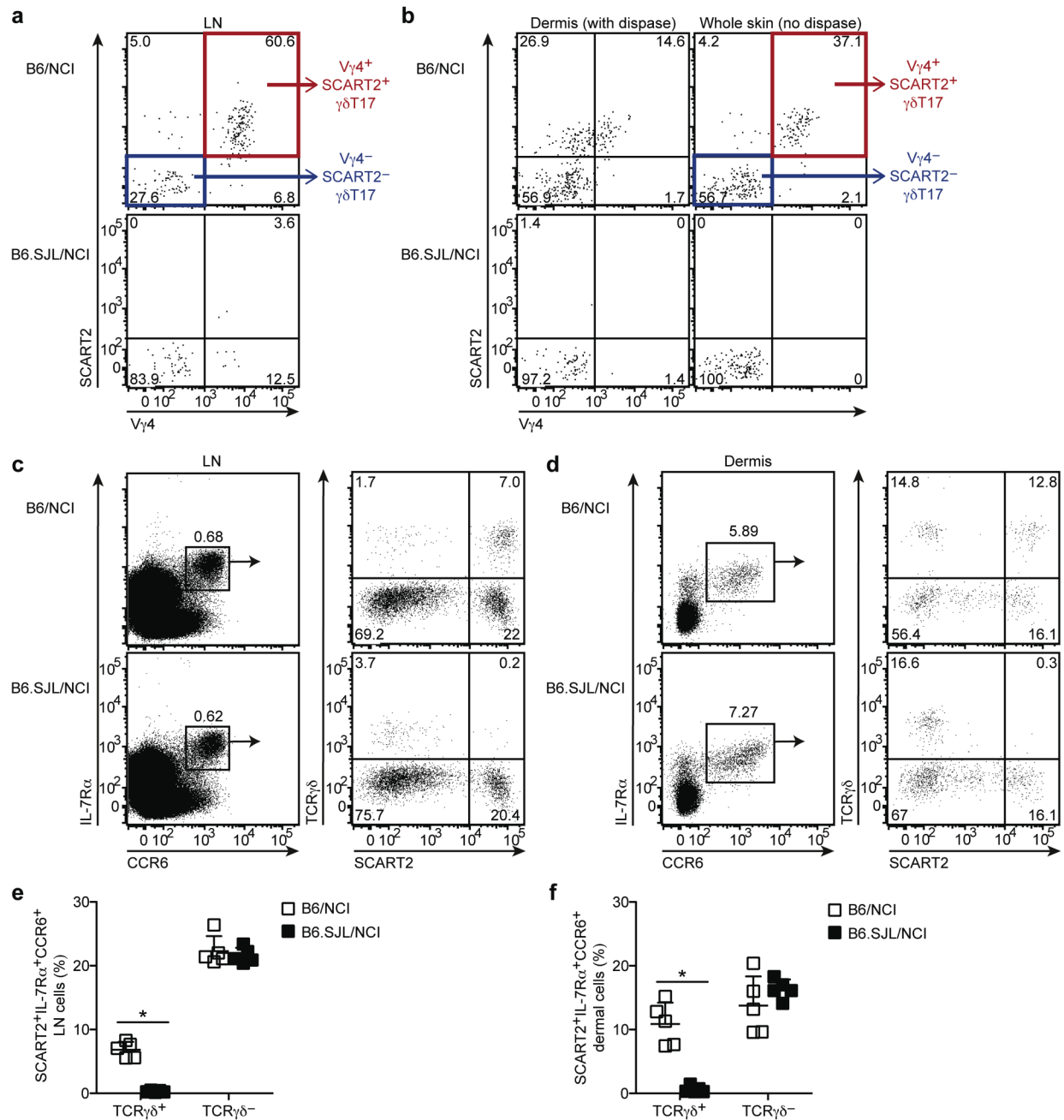
Supplementary Information

IL-17-committed V γ 4⁺ $\gamma\delta$ T cell deficiency in a spontaneous Sox13 mutant CD45.1 congenic mouse substrain protects from dermatitis

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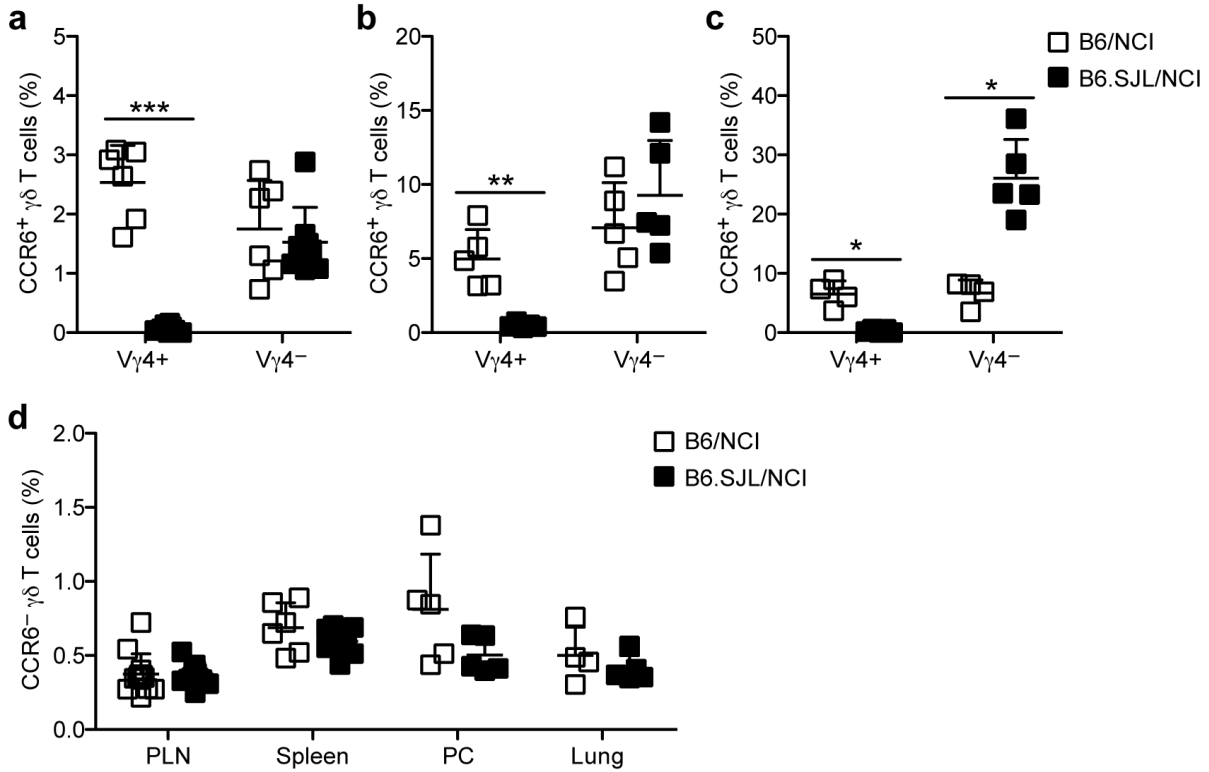
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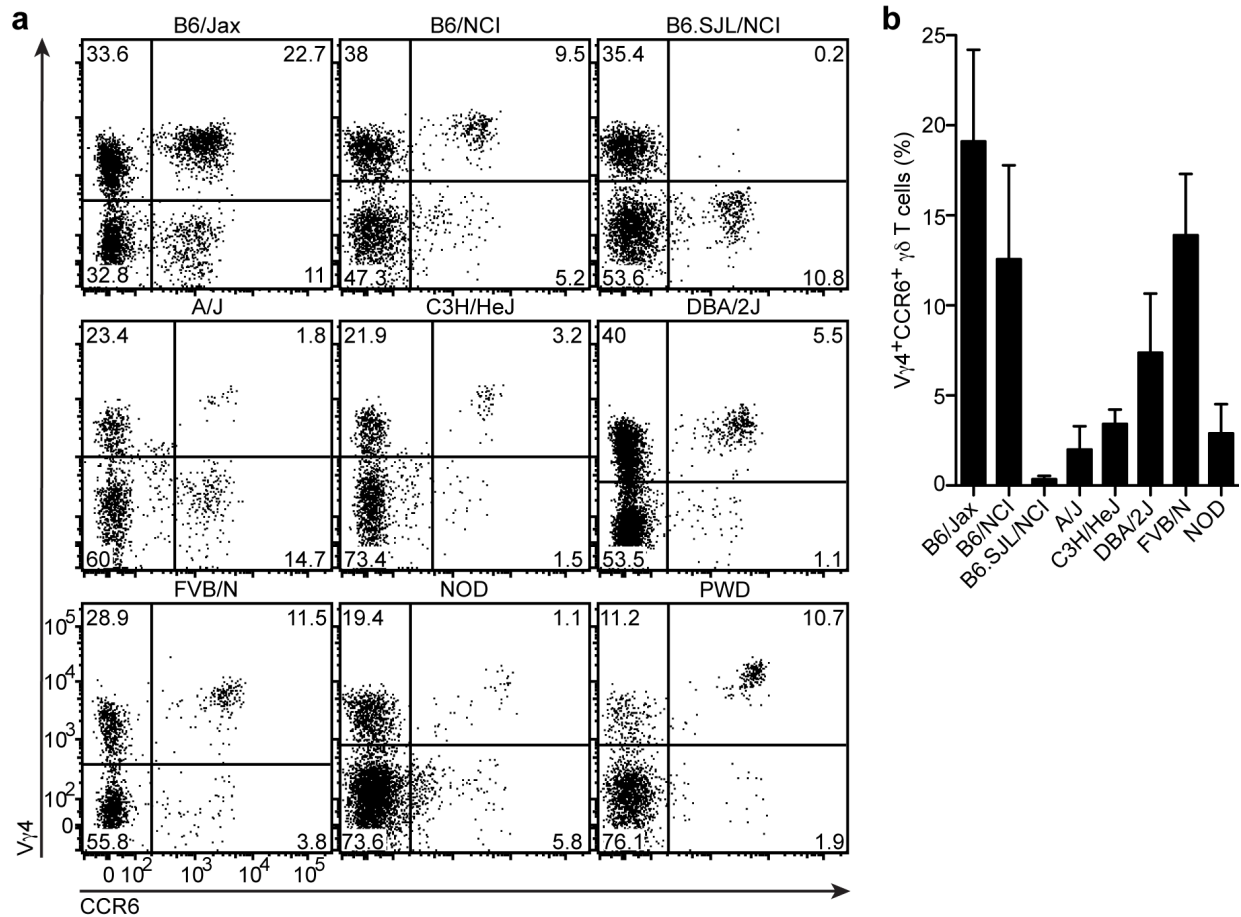


Supplementary Fig. 1 SCART2 and CCR6 expression by $\gamma\delta$ T and non- $\gamma\delta$ T cells in B6/NCI and B6.SJL/NCI mice. **(a,b)** Flow cytometric detection of V γ 4 and SCART2 on CCR6⁺ $\gamma\delta$ T cells in lymph node **(a)**, dermal, and whole ear skin **(b)** cell suspensions from B6/NCI (top panels) and B6.SJL/NCI (bottom panels) mice. Note that V γ 4 staining is sensitive to digestion with dispase (used to separate the dermal and epidermal layers of the ear skin). The two subsets of CCR6⁺ $\gamma\delta$ T17 cells are indicated in red (V γ 4⁺SCART2⁺) and blue (V γ 4⁻SCART2⁻). **(c,d)**

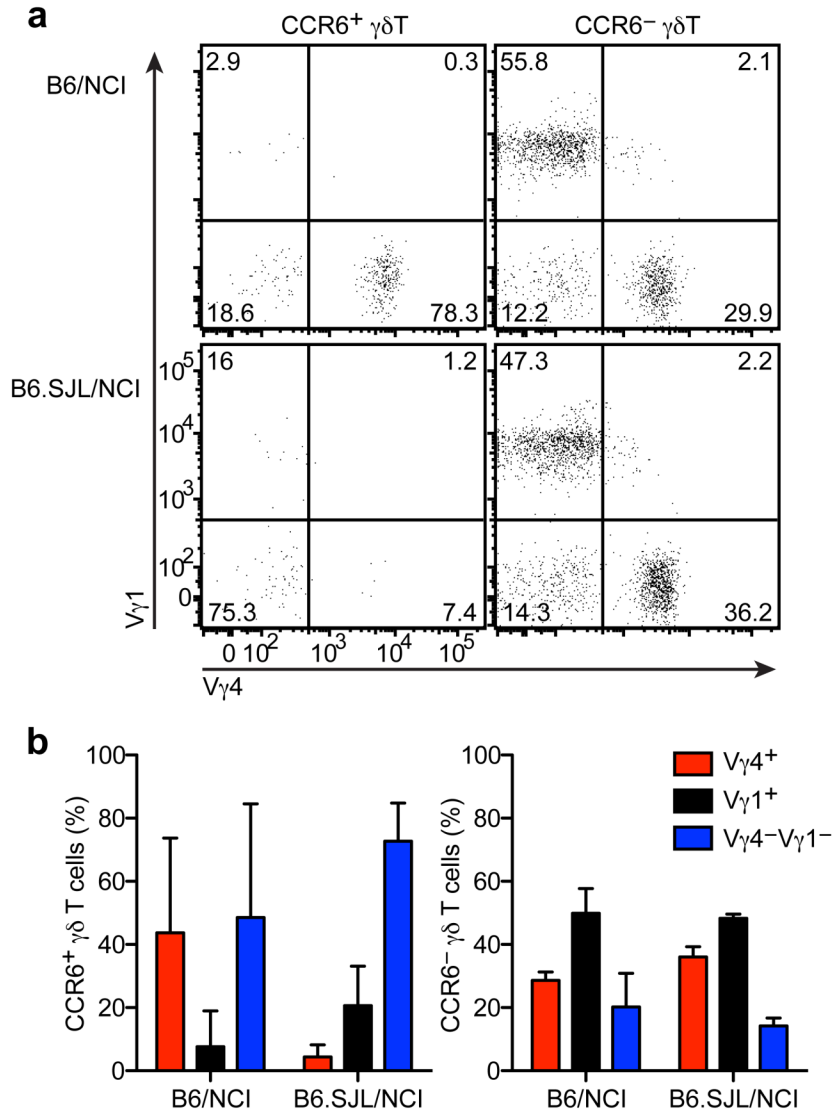
Flow cytometric detection of SCART2⁺IL-7R α ⁺CCR6⁺ IL-17-committed TCR γ δ ⁺ and TCR γ δ ⁻ cells in digested lymph node (**c**) and dermal (**d**) cell suspensions. (**e,f**) Quantification of SCART2⁺IL-7R α ⁺CCR6⁺ IL-17-committed TCR γ δ ⁺ and TCR γ δ ⁻ cell frequency in digested lymph node (**e**) and dermal (**f**) cell suspensions, plotted as percent of IL-7R α ⁺CCR6⁺ cells. Each symbol represents an individual mouse; horizontal and vertical bars represent the mean (\pm s.d.). * $P \leq 0.01$. Data are representative of at least two experiments with at least two mice.



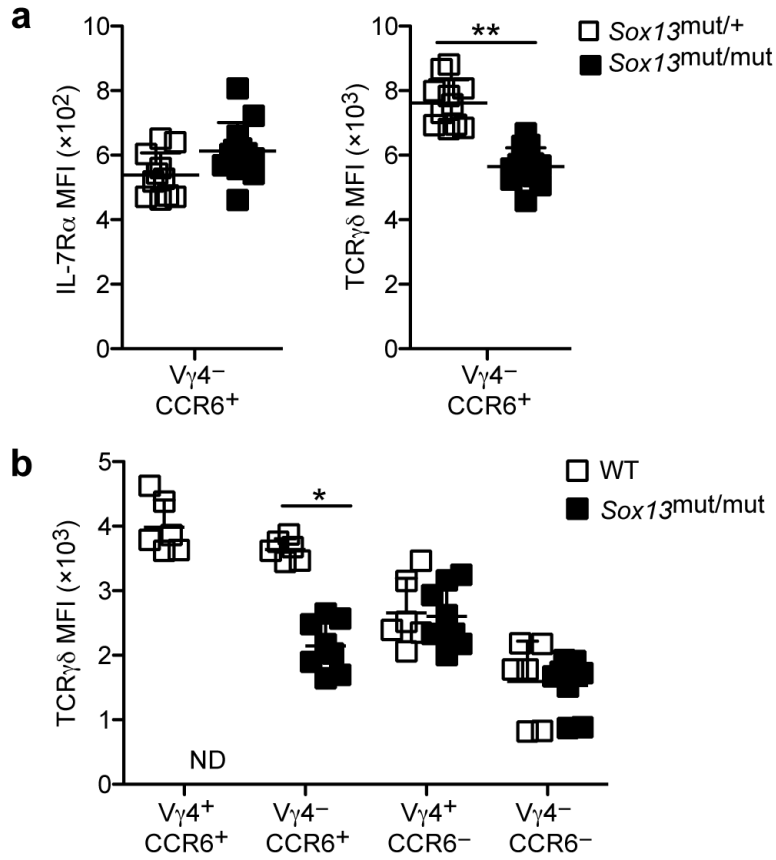
Supplementary Fig. 2 B6.SJL/NCI mice lack V γ 4⁺ γ δ T17 cells in multiple tissues. (**a-c**) Quantification of V γ 4⁺CCR6⁺ and V γ 4⁻CCR6⁺ γ δ T17 cell frequency in spleen (**a**), peritoneal lavage (**b**), and digested lung (**c**) cell suspensions in B6/NCI and B6.SJL/NCI mice gated as in **Fig. 1a**, plotted as % of total γ δ T cells. (**d**) Quantification of CCR6⁻ (non-IL-17-committed) γ δ T cell frequency in peripheral lymph node (PLN), spleen, peritoneal lavage, and digested lung cell suspensions in B6/NCI and B6.SJL/NCI mice, plotted as % of total live cells. Data are representative of at least two experiments. Each symbol represents an individual mouse; horizontal and vertical bars represent the mean (\pm s.d.). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Data are representative of at least two experiments with at least four mice.



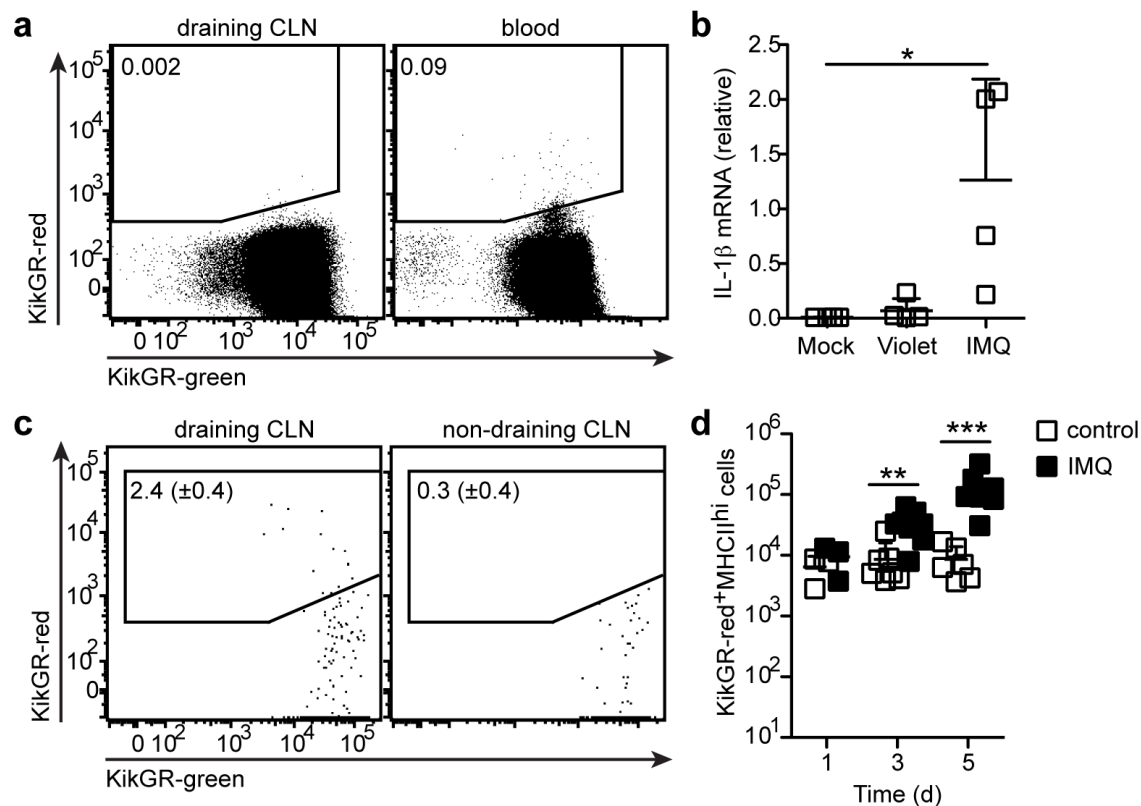
Supplementary Fig. 3 $V\gamma 4^+CCR6^+$ $\gamma\delta T17$ cell frequency in various inbred mouse strains. **(a)** Flow cytometric detection of $V\gamma 4^+CCR6^+$ $\gamma\delta T17$ cells in digested LN cell suspensions from mice of the indicated strain, gated on total $\gamma\delta$ T cells. Numbers indicate frequency of total $\gamma\delta$ T cells. **(b)** Frequency of $V\gamma 4^+CCR6^+$ $\gamma\delta T17$ cells (plotted as % of total $\gamma\delta$ T cells) in digested lymph node cell suspensions from mice of the indicated strain. Bars show mean (\pm s.d.). Data are representative of at least two mice of each strain, except the PWD strain in which only 1 mouse was analyzed. $V\gamma 4^+CCR6^+$ $\gamma\delta T17$ cells are present in all strains analyzed at frequencies significantly greater than B6.SJL/NCI mice (two-tailed, unpaired t-test). The frequency of $V\gamma 4^+CCR6^+$ $\gamma\delta$ T cells varied from 5% to more than 20% of total $\gamma\delta$ T cells both in wild-type mice raised in our colony as well as B6/NCI mice from NCI and B6/Jax mice from Jax. The frequency of $V\gamma 4^+CCR6^+$ $\gamma\delta T17$ cells is \sim 2-6-fold lower in A/J, C3H/HeJ, DBA/2J and NOD mice compared to B6/NCI mice. The basis for the intermediate frequency of cells in these strains is unknown, but is likely due to polymorphisms at multiple loci.



Supplementary Fig. 4 V γ 4 and V γ 1 expression by CCR6⁺ γ δ T17 and CCR6⁻ γ δ T cells. **(a)** Flow cytometric detection of V γ 4 and V γ 1 by CCR6⁺ (left panels) and CCR6⁻ (right panels) γ δ T cells in lymph node cell suspensions from B6/NCI (top panels) and B6.SJL/NCI (bottom panels) mice. Note that lymph nodes were not enzyme digested as V γ 1 staining was found to be sensitive to Liberase™. **(b)** Quantification of V γ 4⁺, V γ 1⁺, and V γ 1⁻V γ 4⁻ γ δ T cell frequency among CCR6⁺ (left panel) and CCR6⁻ (right panel) γ δ T cells in B6/NCI and B6.SJL/NCI mice. Bars show mean (\pm s.d.). Data are representative of four B6/NCI mice and three B6.SJL/NCI mice.



Supplementary Fig. 5 Expression of IL-7R α and TCR $\gamma\delta$ on V $\gamma 4^-$ $\gamma\delta$ T17 cells. **(a)** F₁ mice were backcrossed to *Sox13*^{mut/mut} (B6.SJL/NCI) mice to generate *Sox13*^{mut/+} or *Sox13*^{mut/mut} mice. IL-7R α (left panel) and TCR $\gamma\delta$ (right panel) mean fluorescence intensity on V $\gamma 4^-$ CCR6 $^+$ $\gamma\delta$ T17 cells from neonatal thymi harvested 0 or 5 days after birth. **(b)** TCR $\gamma\delta$ mean fluorescence intensity on indicated subsets of LN $\gamma\delta$ T cells from wild-type (B6/NCI) and *Sox13*^{mut/mut} (B6.SJL/NCI) mice. Each symbol represents an individual mouse; horizontal and vertical bars represent the mean (\pm s.d.). * $P \leq 0.001$, ** $P \leq 0.0001$. Data are representative of at least two experiments with at least six mice.



Supplementary Fig. 6 Detection of KikGR-red⁺ cells. **(a)** Flow cytometric detection of total KikGR-red⁺ and KikGR-green⁺ cells in draining cervical lymph node (CLN) and blood cell suspensions harvested immediately after ear skin exposure to violet light, gated on total live cells. **(b)** Quantitative RT-PCR analysis of IL-1β mRNA abundance relative to HPRT. Each point represents data for an individual mouse ear. **(c)** Flow cytometric detection of KikGR-red⁺ MHCII^{hi}CD11c⁺ dendritic cells in draining (left) and non-draining (right) CLNs harvested 24 hours after ear skin photoconversion, gated on total MHCII^{hi}CD11c⁺ dendritic cells. The mean (± s.d.) %KikGR-red⁺ cells from four independent experiments are indicated. **(d)** Number of KikGR-red⁺ MHCII^{hi}CD11c⁺ dendritic cells in control or imiquimod-treated draining lymph nodes harvested at the indicated day of imiquimod treatment and one day after photoconversion. Each symbol represents data for an individual CLN. Horizontal and vertical bars represent the mean (± s.d.) **(b,d)**. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Data are representative of at least two experiments with at least two mice.

Supplementary Table 1

Variation Location Left (bp)	Variation Location Right (bp)	C57BL/6 reference nucleotide sequence	B6.SJL/NCI nucleotide sequence	Gene	Change Type	C57BL/6 reference amino acid sequence	B6.SJL/NCI amino acid sequence	dbsnp	dbSNP in A/J, DBA2, FVB.N, NOD, or PWD
134963204	134963205	c	t	<i>Pik3c2b</i>	Non-Synonymous	P	S	rs32284602	DBA2
135184423	135184424	c	g	<i>Plekha6</i>	Non-Synonymous	T	S	rs3164254	A/J
135255656	135255657	a	t	<i>Ren1</i>	Non-Synonymous	E	D	rs3166350	DBA/2J
135260500	135260501	a	g	<i>Etnk2</i>	Non-Synonymous	S	G	rs33551174	DBA2
135273492	135273493	t	a	<i>Etnk2</i>	Non-Synonymous	V	E	rs33549639	DBA2, FVB.N
135273623	135273624	g	t	<i>Etnk2</i>	Non-Synonymous	A	S	rs33557119	DBA2, FVB.N
135518731	135518732	g	a	<i>Zc3h11a</i>	Non-Synonymous	P	S	rs31371115	DBA2, FVB.N
135521198	135521199	c	t	<i>Zc3h11a</i>	Non-Synonymous	V	I	rs32342388	DBA2, FVB.N
135576555	135576556	t	c	<i>Lax1</i>	Non-Synonymous	R	G	rs13472481	DBA2, FVB.N
135577146	135577147	t	c	<i>Lax1</i>	Non-Synonymous	N	D	rs32239291	DBA2, FVB.N
135580211	135580212	g	a	<i>Lax1</i>	Non-Synonymous	P	S	rs32582773	DBA2, FVB.N
135801747	135801748	c	g	<i>Optc</i>	Non-Synonymous	S	T	rs37647909	PWD
136085106	136085107	c	t	<i>Chi3l1</i>	Non-Synonymous	A	V	rs30876553	NOD
136195898	136195899	g	a	<i>Ppfia4</i>	Non-Synonymous	P	S	rs30872293	A/J
136304244	136304245	c	t	<i>Cyb5r1</i>	Non-Synonymous	R	W	rs30875255	A/J
136868744	136868745	c	t	<i>Ube2t</i>	Non-Synonymous	A	V	rs46095918	DBA2
136883665	136883666	c	t	<i>Lgr6</i>	Non-Synonymous	V	I	rs3661512	A/J
136884586	136884587	a	t	<i>Lgr6</i>	Non-Synonymous	S	T	rs30920584	DBA2, A/J
136887212	136887213	g	t	<i>Lgr6</i>	Non-Synonymous	H	N	rs50910866	DBA2
136900053	136900054	c	t	<i>Lgr6</i>	Non-Synonymous	S	N	rs30840049	DBA2, FVB.N, A/J
137001653	137001654	g	c	<i>Lgr6</i>	Non-Synonymous	A	G	rs50577640	DBA2, A/J
137005612	137005613	c	t	<i>Ptprv</i>	Non-Synonymous	G	S	rs51027693	DBA2, A/J
137005621	137005622	a	t	<i>Ptprv</i>	Non-Synonymous	Y	N	rs49112393	DBA2, A/J
137005975	137005976	g	c	<i>Ptprv</i>	Non-Synonymous	Q	E	rs45683311	A/J
137008017	137008018	t	c	<i>Ptprv</i>	Non-Synonymous	H	R		A/J, DBA/2J, NOD
137011210	137011211	g	c	<i>Ptprv</i>	Non-Synonymous	P	R	rs47516732	DBA2, A/J
137015837	137015838	t	c	<i>Ptprv</i>	Stop	*		rs30834255	DBA2, A/J
137016275	137016276	a	g	<i>Ptprv</i>	Non-Synonymous	V	A	rs30834254	DBA2, FVB.N, A/J

137016849	137016850	c	g	<i>Ptprv</i>	Non-Synonymous	V	L	rs47752571	DBA2
137016917	137016918	g	a	<i>Ptprv</i>	Non-Synonymous	T	I	rs32163068	DBA2, A/J
137020322	137020323	a	g	<i>Ptprv</i>	Non-Synonymous	V	A	rs30833331	A/J
137020329	137020330	t	c	<i>Ptprv</i>	Non-Synonymous	S	G	rs31336463	A/J
137020670	137020672	cg	aa	<i>Ptprv</i>	Non-Synonymous	A	V		A/J, DBA/2J, NOD
137021034	137021035	t	c	<i>Ptprv</i>	Non-Synonymous	I	V	rs32050402	DBA2, A/J
137021922	137021923	a	g	<i>Ptprv</i>	Non-Synonymous	Y	H	rs31629804	DBA2, A/J
137021931	137021932	c	t	<i>Ptprv</i>	Non-Synonymous	V	I	rs47978058	DBA2, A/J
137022875	137022876	c	t	<i>Ptprv</i>	Non-Synonymous	V	M	rs30791899	DBA2, A/J
137022894	137022895	t	a	<i>Ptprv</i>	Stop			rs32267008	DBA2, A/J
137023156	137023157	t	c	<i>Ptprv</i>	Non-Synonymous	E	G	rs33860307	DBA2, A/J
137031052	137031053	a	g	<i>Ptpn7</i>	Non-Synonymous	Q	R	rs107631253	A/J, DBA2, NOD
137039819	137039820	g	t	<i>Ptpn7</i>	Non-Synonymous	E	D	rs48605910	DBA2, A/J
137159673	137159674	c	t	<i>Rnpep</i>	Non-Synonymous	A	T	rs108794650	DBA2
137180206	137180207	c	g	<i>Rnpep</i>	Non-Synonymous	R	P	rs32740521	DBA2, A/J
137180554	137180555	g	c	<i>Rnpep</i>	Non-Synonymous	A	G	rs37563888	DBA2, A/J
137260650	137260651	c	t	<i>Lmod1</i>	Non-Synonymous	T	I	rs50126852	DBA2, A/J
137298827	137298828	a	g	<i>Ipo9</i>	Non-Synonymous	V	A	rs32645804	DBA2, FVB.N, A/J
137481304	137481305	a	t	<i>Nav1</i>	Non-Synonymous	D	E	rs13476124	DBA2
137723958	137723959	c	t	<i>Lad1</i>	Non-Synonymous	P	S	rs32494953	DBA2
137724600	137724601	c	t	<i>Lad1</i>	Non-Synonymous	R	C	rs31421022	DBA2
138015293	138015294	t	c	<i>Cacna1s</i>	Non-Synonymous	F	S	rs30889688	DBA2
138157287	138157288	g	a	<i>Gpr25</i>	Non-Synonymous	P	L		DBA2
138177892	138177893	c	t	<i>Camsap2</i>	Non-Synonymous	R	Q	rs30887353	DBA2
138313630	138313631	t	c	<i>Ddx59</i>	Non-Synonymous	V	A	rs31374585	DBA2
138364856	138364857	a	g	<i>Kif14</i>	Non-Synonymous	N	D	rs31343464	DBA2
138365552	138365553	a	g	<i>Kif14</i>	Non-Synonymous	K	E	rs31149596	DBA2
138374942	138374943	g	a	<i>Kif14</i>	Non-Synonymous	A	T	rs32330511	DBA2
138386909	138386910	a	g	<i>Kif14</i>	Non-Synonymous	S	G	rs33596371	DBA2
138400008	138400009	c	t	<i>Kif14</i>	Non-Synonymous	L	F	rs33591711	DBA2
138412538	138412539	t	c	<i>Kif14</i>	Non-Synonymous	F	L	rs32540095	DBA2
138422360	138422361	t	c	<i>Kif14</i>	Non-Synonymous	V	A	rs31477062	DBA2
138848702	138848703	c	a	<i>Nr5a2</i>	Non-Synonymous	R	L	rs31516099	DBA2

139996253	139996254	t	g	<i>Ptprc</i>	Non-Synonymous	N	T	rs33599178	FVB.N, NOD
140004400	140004402	ac	ga	<i>Ptprc</i>	Non-Synonymous	ES	DP		
140004414	140004415	a	g	<i>Ptprc</i>	Non-Synonymous	V	A	rs33604282	FVB.N, NOD
140008831	140008832	t	c	<i>Ptprc</i>	Non-Synonymous	K	E		NOD
141131356	141131357	t	c	<i>Crb1</i>	Non-Synonymous	M	V	rs30926079	DBA2, FVB.N, A/J
141134199	141134200	a	t	<i>Crb1</i>	Non-Synonymous	H	Q	rs30925219	DBA2, FVB.N, A/J
141137715	141137716	g	a	<i>Crb1</i>	Non-Synonymous	P	S	rs30932205	A/J, FVB.N
141139572	141139573	c	t	<i>Crb1</i>	Non-Synonymous	G	R	rs30930641	A/J, FVB.N
141139994	141139995	c	t	<i>Crb1</i>	Non-Synonymous	R	H	rs30930638	A/J, FVB.N
141370151	141370152	a	g	<i>Aspm</i>	Non-Synonymous	I	V	rs47988436	DBA2, FVB.N, A/J
141670651	141670652	g	c	<i>Gm4788</i>	Non-Synonymous	Q	E	rs50727546	DBA2, A/J
141710019	141710020	a	g	<i>Cfhr2</i>	Non-Synonymous	M	T	rs6233562	DBA2, FVB.N, A/J
141710036	141710037	a	c	<i>Cfhr2</i>	Non-Synonymous	N	K	rs6233591	DBA2, A/J
141727751	141727752	t	c	<i>Cfhr2</i>	Non-Synonymous	K	R	rs47778423	DBA2
142251124	142251125	g	a	<i>Kcnt2</i>	Non-Synonymous	S	N	rs32647250	A/J, FVB.N
145607144	145607145	c	t	<i>Trove2</i>	Non-Synonymous	V	I	rs30973564	A/J, FVB.N
145607164	145607165	t	c	<i>Trove2</i>	Non-Synonymous	D	G	rs30972582	PWD

Supplementary Table 1 List of coding variants identified by whole exome sequencing of B6.SJL/NCI mouse gDNA located within 3cM of *Ptprc*. 77 coding variants were either reported in the NIH dbSNP database (<http://www.ncbi.nlm.nih.gov/snp>) or Sanger mouse genomes project (<http://www.sanger.ac.uk/cgi-bin/modelorgs/mousegenomes/snps.pl>) in A/J, DBA2, C3H/HeJ, FVB.N, NOD and/or PWD strains (at least one strain in which the coding variant has been reported is listed). 1 coding variant is known to be present in *Ptprc^a* (CD45.2) alleles¹ (in bold).

Supplementary Table 2

Variation Location Right (bp)	C57BL/6 reference nucleotide sequence	B6.SJL/NCI nucleotide sequence	Gene	Change Type	C57BL/6 reference amino acid sequence	B6.SJL/NCI amino acid sequence	dbSNP in B6.SJL/Jax?
135283363		g	<i>Sox13</i>	Frameshift			Only B6.SJL/NCI
135811709	c	t	<i>Prelp</i>	Non-Synonymous	R	K	B6.SJL/Jax
136045973	aag	ggt	<i>Chit1</i>	Non-Synonymous	ER	EV	B6.SJL/Jax
136094058	c	t	<i>Mybph</i>	Non-Synonymous	R	C	B6.SJL/Jax
136437585	agtcat		<i>4931440L10Rik</i>	Non-Synonymous	DDY	D	B6.SJL/Jax
141671087	a	g	<i>Gm4788</i>	Non-Synonymous	V	A	B6.SJL/Jax
141727733	t	g	<i>Cfhr2</i>	Non-Synonymous	R	S	Not confirmed.
145586871	c	t	<i>Glrx2</i>	Non-Synonymous	A	V	B6.SJL/Jax

Supplementary Table 2 List of the coding variants identified by whole exome sequencing of B6.SJL/NCI mouse gDNA remaining after ruling out variants listed in **Supplementary Table 1**. Sanger sequencing confirmed that 6 coding variants were present in B6.SJL/Jax or SJL strains (right panel). One coding variant in *Cfhr2* was not confirmed to be present in B6.SJL/NCI or B6.SJL/Jax strains (“Not confirmed,” right panel). Sanger sequencing confirmed that the *Sox13* coding variant was present in B6.SJL/NCI and B6.SJL/Tac but absent in B6.SJL/Jax and B6.SJL/CR substrains.

Supplementary Table 3

Variation Location Left (bp)	Variation Location Right (bp)	C57BL/6 reference nucleotide sequence	B6.SJL/NCI nucleotide sequence	Gene	Forward (5' to 3')	Reverse (5' to 3')
135283363	135283363		g	<i>Sox13</i>	GAACGGAGAAAGGGAAGGAC	CCTCTTTGCTCTTGGCTCAC
135811708	135811709	c	t	<i>Prelp</i>	GCCTCAGCTGTTCCAAGTTC	ACCGACAAGACCAAACCTG
136045970	136045973	aag	ggt	<i>Chit1</i>	GGCAGTCCAGAACCATTGT	GCAGATGCTTTAGCCCTGAG
136094057	136094058	c	t	<i>Mybph</i>	AGCCAGTAGGGTGGGTTTCT	GAAGTAGGTCTGCGGCAAAG
136437579	136437585	agtcac		<i>4931440L10Rik</i>	CCCAGTGAGCAGGGTGAGTAG	ACAATGCACCAGCTTTACCC
141671086	141671087	a	g	<i>Gm4788</i>	ATGAAGAGCTGCTCCCAAAG	TGATATCCCCACAAACCAATG
141727732	141727733	t	g	<i>Cfhr2</i>	CATCTGTGCATAGTGAATAGGCTAA	TCTTTCTGATGAAGTTATTGTCAACT
145586870	145586871	c	t	<i>Glrx2</i>	GGGCGTGAGGAGCTCGGGAG	CCGGCTGGGTCCACACTAAC

Supplementary Table 3 List of primer pairs used for PCR amplification of gene segments containing the indicated coding variants. Sanger sequencing was used to confirm the presence or absence of these mutations in B6.SJL/NCI and B6.SJL/Jax or SJL mice.

Supplementary Table 4

Gene	Forward (5' to 3')	Reverse (5' to 3')	Ref.
<i>Il17a</i>	ATCCCTCAAAGCTCAGCGTGTC	GGGTCTTCATTGCGGTGGAGAG	²
<i>Il17f</i>	CAAACCAGGGCATTCTGT	ATGGTGCTGTCTTCCTGACC	³
<i>Defb3</i>	GTCTCCACCTGCAGCTTTTA	GACGAGTGTGCAATGCAC	n.a.
<i>Defb4</i>	TCTCCACTTGCAGCCTTTACC	AGCATATGGCTCCATTGGTCA	n.a.
<i>Cxcl2</i>	TGAACAAAGGCAAGGCTAACTG	CAGGTACGATCCAGGCTTCC	n.a.
<i>Sox13</i> (exon6-7)	GCTTTACCTATTCAGCCCAT	ACCTCTTCACCACAGGGG	n.a.
<i>Sox13</i> (exon12-13)	CAGGGTGCTCGCCAAAG	CCCAGGGAACAGGATA	n.a.
<i>Sox13</i> (exon1-2)	GATGCCACCAACGCTAAA	GAAGCAGCCTCGGAAGC	n.a.

Supplementary Table 4 List of primer pairs used for real-time PCR.

Supplementary References:

1. Zebedee, S.L., Barritt, D.S. & Raschke, W.C. Comparison of mouse Ly5a and Ly5b leucocyte common antigen alleles. *Dev Immunol* **1**, 243-254 (1991).
2. Smith, E. *et al.* IL-17A inhibits the expansion of IL-17A-producing T cells in mice through "short-loop" inhibition via IL-17 receptor. *J Immunol* **181**, 1357-1364 (2008).
3. Okamoto, K. *et al.* IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors. *Nature* **464**, 1381-1385 (2010).