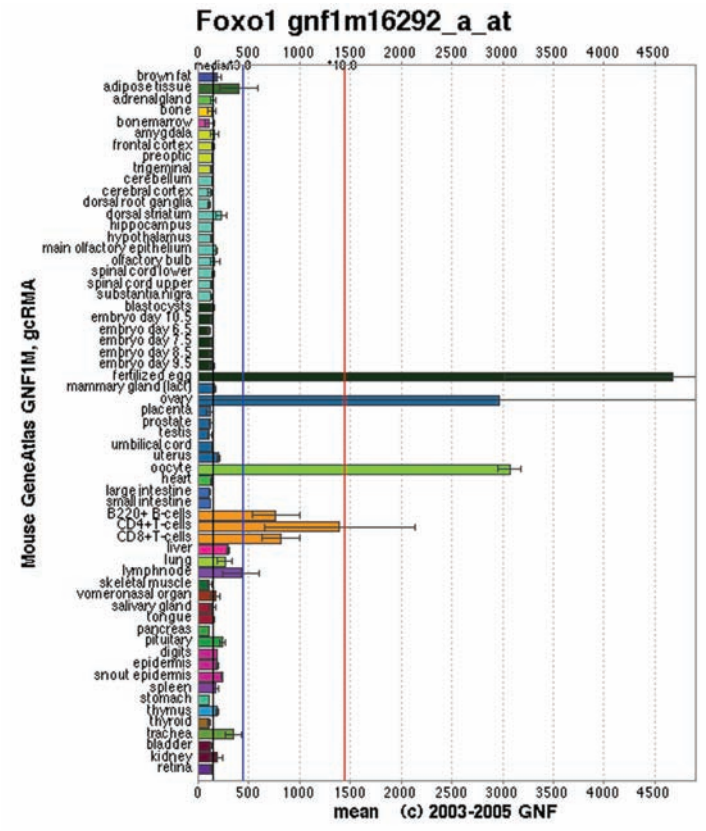
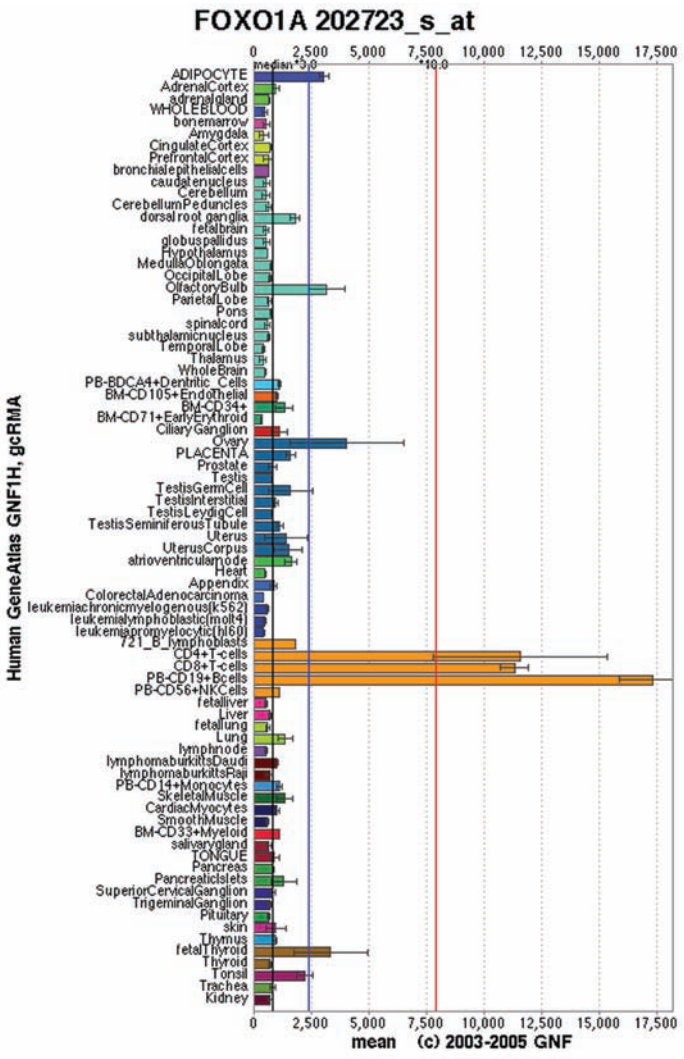
a

Mus musculus

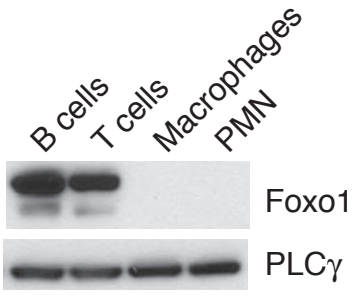


b

Homo sapiens



c



Supplementary Figure 1 Foxo1 expression pattern is conserved between mouse and human. **(a)** Expression profile of Foxo1 in mouse and **(b)** human tissues and cell subsets according to the Gene Atlas from the Genomics Institute of the Novartis Foundation (GNF) (http://symatlas.gnf.org/SymAtlas/). **(c)** Immunoblot analysis of Foxo1 expression in purified cells subsets. T and B cells were isolated from LN and spleen respectively. Macrophages and Polymorphonuclear cells (PMNs) were isolated from the peritoneal cavity of mice elicited with thioglycolate. Representative results from two independent experiments.

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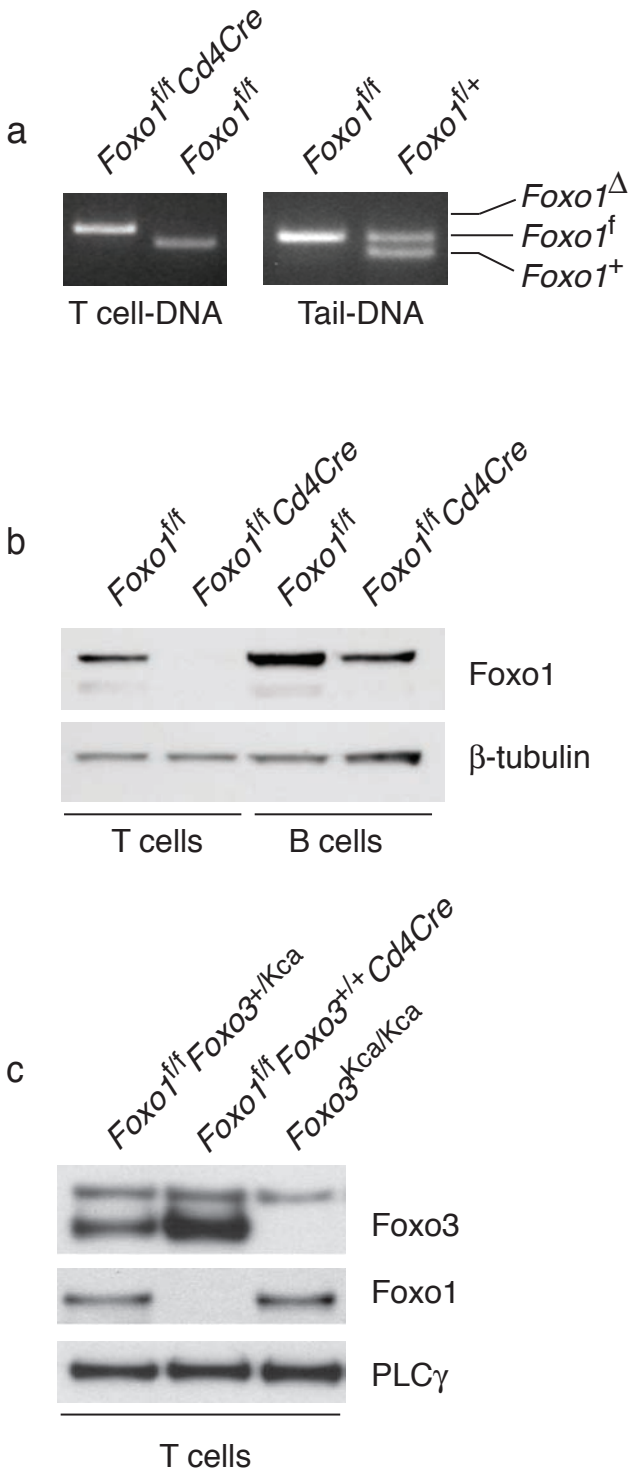
a
Foxo1f/f Cd4Cre
Foxo1f/f
Foxo1f/f
Foxo1f/+
Foxo1Δ
Foxo1f
Foxo1+
T cell-DNA
Tail-DNA
b
Foxo1f/f
Foxo1f/f Cd4Cre
Foxo1f/f
Foxo1f/f Cd4Cre
Foxo1
β-tubulin
T cells
B cells
c
Foxo1f/f Foxo3+/Kca
Foxo1f/f Foxo3+/+ Cd4Cre
Foxo3Kca/Kca
Foxo3
Foxo1
PLCγ
T cells

Supplementary Figure 2 Efficient deletion of Foxo1 in peripheral T cells of *Foxo1*$^{f/f}$*CD4Cre* mice. **(a)** PCR analysis of *Foxo1* genomic deletion in purified LN T cells and tail sample. **(b)** Immunoblot analysis of Foxo1 expression in purified LN T and B cells. **(c)** Immunblot analysis of Foxo1 and Foxo3 expression in purified LN T cells. Representative results from two to four experiments (**a-c**).

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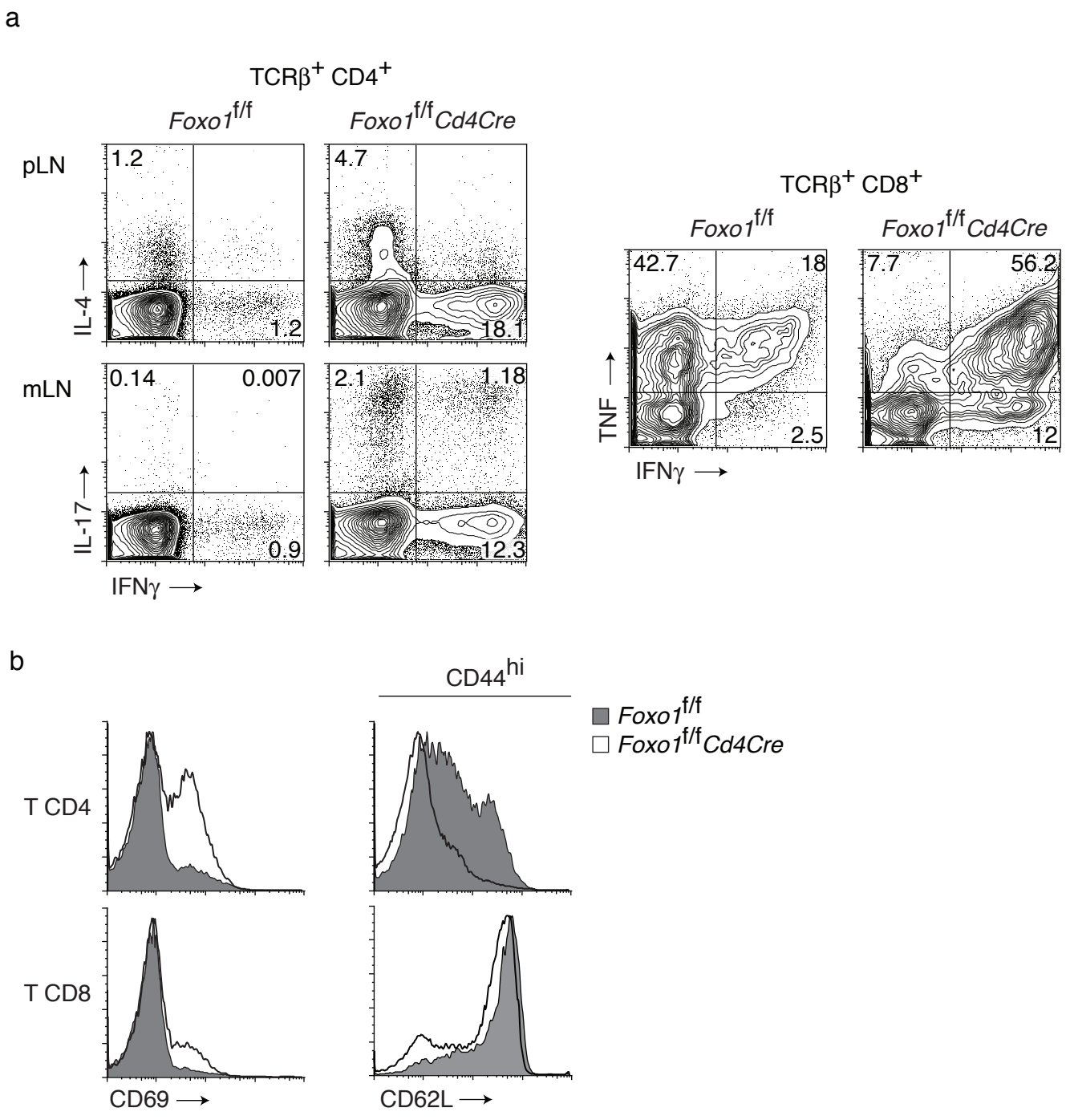
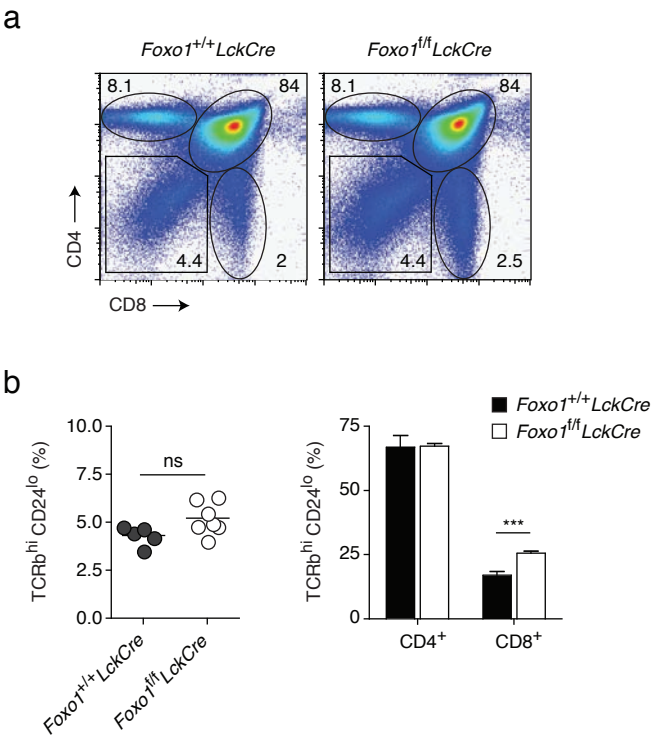

a
TCRβ+ CD4+
Foxo1f/f
Foxo1f/fCd4Cre
pLN
1.2
1.2
4.7
18.1
mLN
0.14
0.007
0.9
2.1
1.18
12.3
IL-4
IL-17
IFNγ
TCRβ+ CD8+
Foxo1f/f
Foxo1f/fCd4Cre
42.7
18
2.5
7.7
56.2
12
TNF
IFNγ
b
CD44hi
Foxo1f/f
Foxo1f/fCd4Cre
T CD4
T CD8
CD69
CD62L

Supplementary Figure 3 Foxo1 deletion in T cells induces the accumulation of activated and memory phenotype T cells in peripheral lymphoid organs. **(a)** LN cells from individual mice were stimulated for 4.5 h with PMA and ionomycin, in presence of Brefeldin A for the last 3 h. Stimulated cells were then surface stained, fixed, permeabilzed and intracellularly-stained for the indicated cytokines. (pLN: peripheral lymph nodes (inguinal, brachial, axillary), mLN: mesenteric lymph nodes). Representative results from *n* 6 mice per genotype analyzed in three independent experiments. **(b)** CD69 and CD62L expression on LN $CD44^{hi}TCR\beta^{+}$ $CD4^{+}$ and $TCR\beta^{+}$ $CD8^{+}$ cells of 8-12 week-old mice. Representative results for *n* 8 mice per staining and per genotype, analyzed in at least three independent experiments.

Nature Immunology: doi:10.1038/ni.1689


a
Foxo1+/+LckCre
Foxo1f/fLckCre
8.1
84
4.4
2
8.1
84
4.4
2.5
CD4
CD8
b
TCRbhi CD24lo (%)
10.0
7.5
5.0
2.5
0.0
ns
Foxo1+/+LckCre
Foxo1f/fLckCre
Foxo1+/+LckCre
Foxo1f/fLckCre
TCRbhi CD24lo (%)
75
50
25
0
***
CD4+
CD8+

Supplementary Figure 4 Foxo1 is dispensable for T cell development. **(a)** CD4 and CD8 expression and **(b)** proportion of mature T cells ($TCR\beta^{hi}HSA^{lo}$) and percentage of $CD4^+$ and $CD8^+$ single positive cells among $TCR\beta^{hi}HSA^{lo}$ cells (mean±s.e.m.) on thymocytes of 8-week-old mice. Each circle indicates one mouse. Data represent $n = 5$ *Foxo1*$^{+/+}$ and $n = 7$ *Foxo1*$^{f/f}$ mice, analyzed in two independent experiments (**a** and **b**) (***,$p<0.0001$; ns: not significant).

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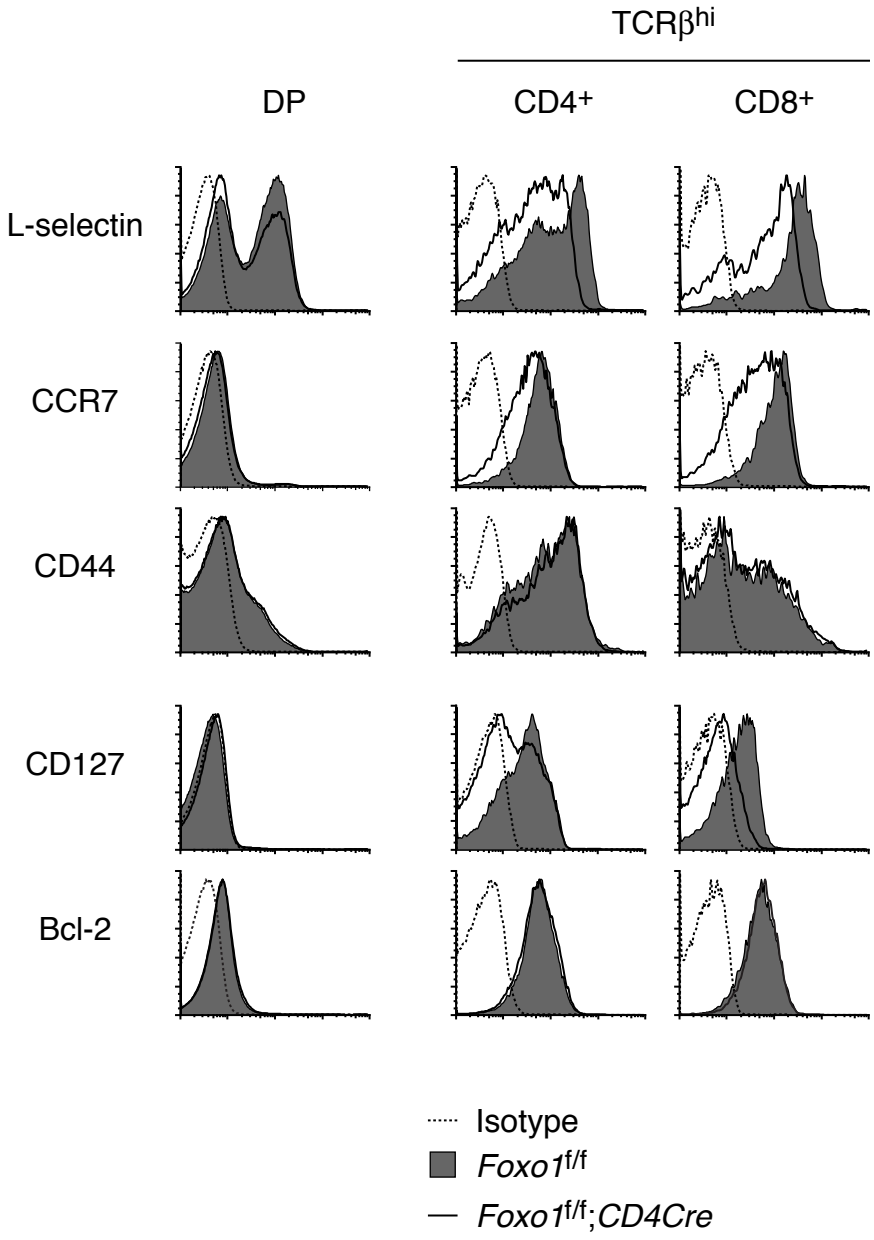
TCRβhi
DP
CD4+
CD8+
L-selectin
CCR7
CD44
CD127
Bcl-2
Isotype
Foxo1f/f
Foxo1f/f;CD4Cre

Supplementary Figure 5 Impaired expression of L-selectin, Ccr7 and IL-7Rα on thymic mature T cells. Representative results of *n* 6 mice per staining and per genotype, analyzed in at least two independent experiments.

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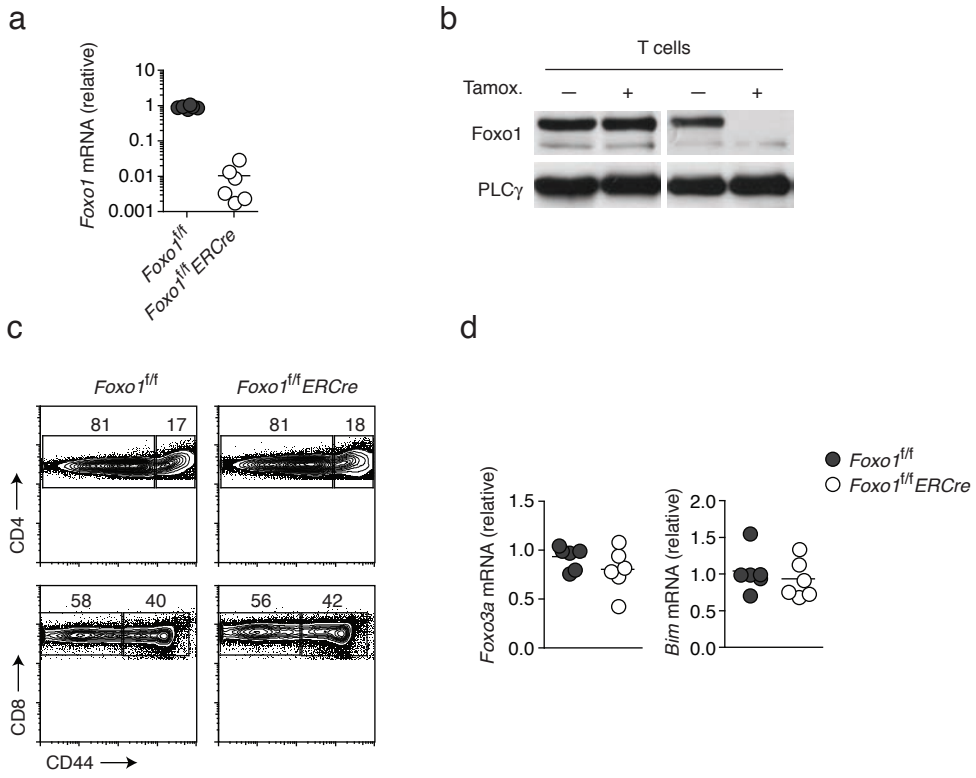
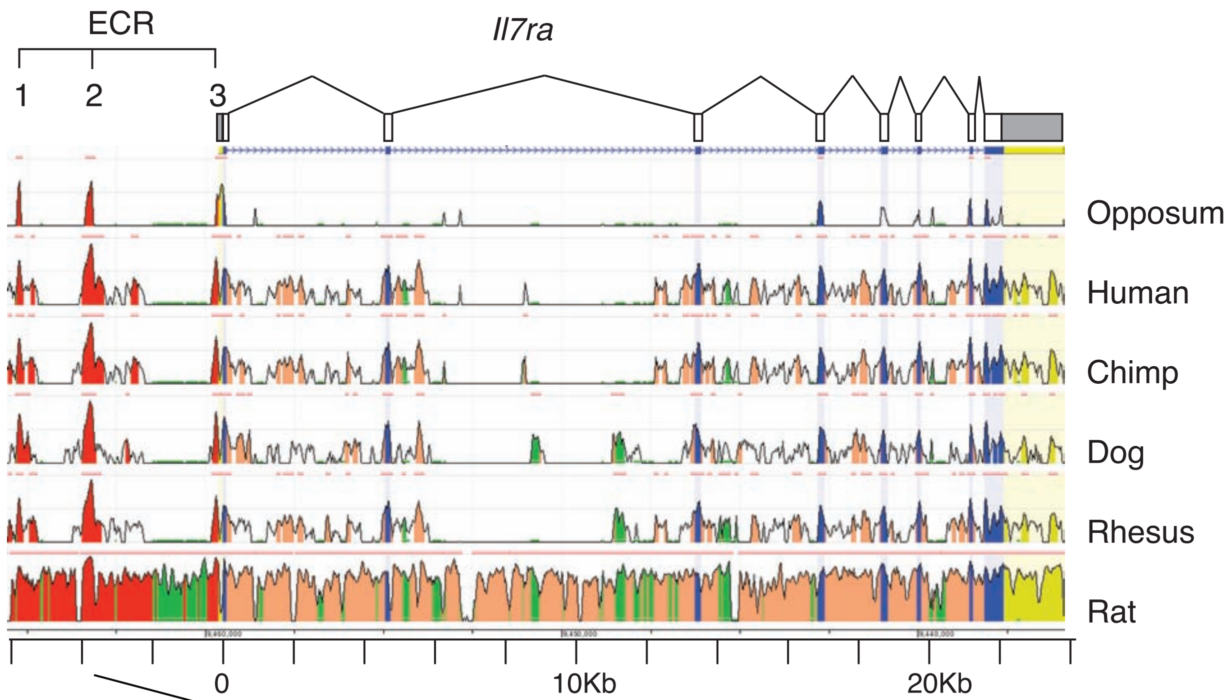

a
Foxo1 mRNA (relative)
10
1
0.1
0.01
0.001
Foxo1f/f
Foxo1f/fERCre
b
T cells
Tamox.
–
+
–
+
Foxo1
PLCγ
c
Foxo1f/f
Foxo1f/fERCre
81
17
81
18
CD4
58
40
56
42
CD8
CD44
d
Foxo1f/f
Foxo1f/fERCre
Foxo3a mRNA (relative)
1.5
1.0
0.5
0.0
Bim mRNA (relative)
2.0
1.5
1.0
0.5
0.0

Supplementary Figure 6 Short term tamoxifen treatment induces efficient deletion of Foxo1 and does not alter the ratio of naïve to activated-memory phenotype T cells in *Foxo1*$^{f/f}$*ERCre* mice. **(a-d)** *Foxo1*$^{f/f}$*ERCre* mice and littermates were treated for 5 days with tamoxifen and rested for 5 days. **(a)** QPCR anaysis of *Foxo1* mRNA expression, normalized to *Hprt* mRNA, in purified LN T cells. **(b)** Immunoblot analysis of Foxo1 expression in purified LN T cells. Representative results of three independent experiments. **(c)** CD44 expression by LN TCRβ^+ CD4$^+$ and TCRβ^+ CD8$^+$ cells. **(d)** QPCR analysis of *Foxo3* and *Bim* mRNA expression, normalized to *Hprt* mRNA, in purified LN T cells. Each circle indicates one mouse (**a** and **d**). Data represent *n* 6 mice analyzed in at least two independent experiments (**a, c** and **d**) (***,p<0.0001; ns: not significant).

Nature Immunology: doi:10.1038/ni.1689

a



b

```
Mouse    -------------------------------------------AATTGTATCATGTCTCT
Dog      AGGTGTCATGAATTGCT--CAGATTGTCACTCCATGGGTGAGTAATTGGGTTGTGTCTCT
Human    AAATCTCCTAAACTGCTAACAGATTGTCACTTCATGGGTGAGTAACTGGGTTATGTCTCT
Macaque  AGATCTCCTAAACGGCTAACAGATTGTCACTTCATGGGTGAGTAACTGGGTCATGTCTCT
Opossum  -------------------------------------------AATTGGGTAATGTCTCT
                                                            ** **  *  *******

                  FoxO
Mouse    TTAAATTCTCTTGTTTATCATTTAAGTGGACCATCATTTTGATTGTATCTCTTTCCTTTA
Dog      TTAAATTCTCTTGTTTATCATTTTAGTGGACTATCATTTTGATTGTATCTCTTTCCTTTA
Human    TTAAATTCTCTTGTTTATCATTTAAGTGGACTATCATTTTGATTGTATCTCTTTCCTTTA
Macaque  TTAAATTCTCTTGTTTATCATTTAAGTGGACTATCATTTTGATTGTATCTCTTTCCTTTA
Opossum  TTAAATTCTCTTGTTTATCACTTAAATGGACTATCATTTTGATTTTATCTCTTTCCTTCA
         ******************** ** * ***** ************ ************* *

                   GR
Mouse    GTTGTGAAGATGTAAAAGAACAAAGTTAAGACATAATATTTTGGTACAGCATGTGCTAGT
Dog      GTTGTGAAGATGTAAAAGAACAAAGCCAAGACACAATATTTTGGTACAGCATGTGCTAGT
Human    GTTGTGAAGATGTAAAAGAACAAAGCCAAGACACAATATTTTGGTACAGCATGTGCTGGT
Macaque  GTTGTGAAGATGTAAAAGAACAAAGCCAAGACACAATATTTTGGTACAGCATGTGCTGGT
Opossum  GATGTGAAG---CAAAAGAACAAAAA-AGGGCACAATATTTTGGTTCGGCTTGTGATCTT
         * *******    ***********   * * ** *********** * ** **** *  *

                 NF-κB                                 SP1
Mouse    TTGCTCAGGGGATTTCCACTGGTGTGCTTACCCCTTTTGGGTGGGG-CAGGAGATGCTGA
Dog      TTGCTCAGGGGATTTCCACTGGTGTACTTACTCTTTTTGGGTGGGG-CAGGAGATGCCGA
Human    TTGTTCTGGGGATTTCCACAGGGGTGCTTACTCT---TGGGTGGGG-CAGGAGATGCTGA
Macaque  TTGTTCTGGGGATTTCCACAGGGGTGCTTACTTTC--TGGGTGGGG-CAGGAGATGCTGA
Opossum  CAGCTCAGGGGATTTCCACTGGTATGTTCTCTTC----GGGTGGGGACAGGGGATGCTCT
           * ** ************ **  *  *  *       ******** **** *****

Mouse    A-AGGACATCAGAGAGAAATCTAAATAGAGTGCGGGAAGCTTAAAG--------------
Dog      A-GGACC--TAGAGAGAAACCTAGATATAGTGCGGGGAGCTCAAAGAAAA--GAGAAGTC
Human    A-AAGACCTCAGAGAGAAGCCTAAA----GTGTGGGGAGCTCAAAGAAAAATGACATGCG
Macaque  A-AAGACCTCAGAGAGAAGCCTAAA----GTGTGGGGAGCTCAAAGAAAAATGACATGCA
Opossum  CTGAGGAACCAGAGAGGAGCCCAAA------GAGAGCTGCCACAAG--------------
                   ****** *  * * *      * * *  **   ***
```

Supplementary Figure 7 Genomic sequence alignment of the *Il7ra* enhancer. **(a)** Comparative alignment of the *Il7ra* loci from NCBI decode.org (http://www.dcode.org/). **(b)** The aligned sequences from ECR2 showing the conserved Foxo, glucocorticoid receptor (GR), and NFκB binding sites.

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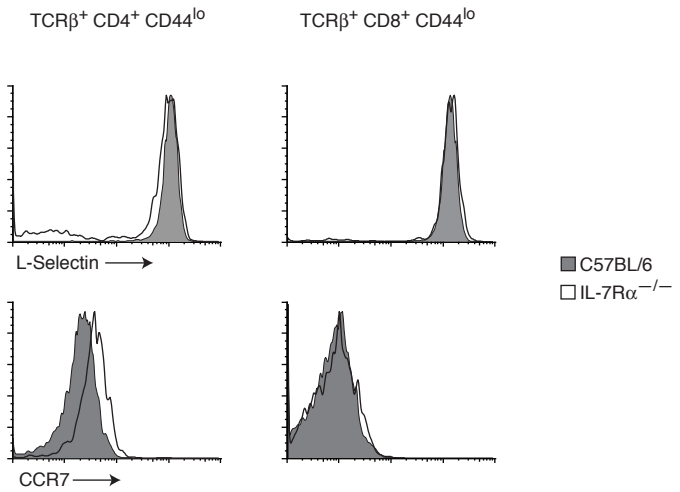
Kerdiles et al - Figure S8



Supplementary Figure 8 IL-7Rα is not required for L-selectin and Ccr7 expression on naïve T cells. L-selectin and Ccr7 expression on spleen $CD44^{lo}$ $CD4^{+}$ and $CD8^{+}$ T cells from wildtype and *Il7ra*$^{-/-}$ mice. Representative results of $n = 4$ mice per genotype.

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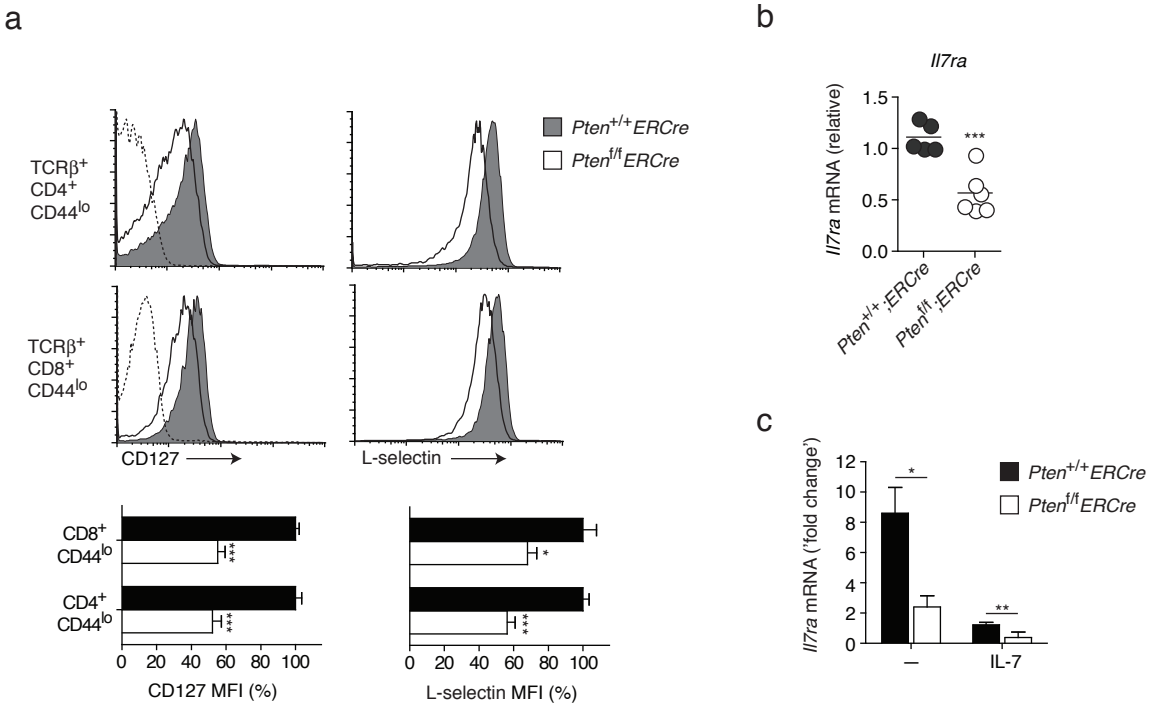
a
b
c
TCRβ+ CD4+ CD44lo
TCRβ+ CD8+ CD44lo
Pten+/+ERCre
Ptenf/fERCre
CD127
L-selectin
CD8+ CD44lo
CD4+ CD44lo
CD127 MFI (%)
L-selectin MFI (%)
Il7ra
Il7ra mRNA (relative)
Pten+/+;ERCre
Ptenf/f;ERCre
Il7ra mRNA (↑fold change)
—
IL-7

Supplementary Figure 9 PTEN-mediated control of IL-7Rα and L-selectin on naïve T cells. **(a-c)** Mice were treated with tamoxifen for 5 days and rested for 5 days. **(a)** Quantification of CD127 and L-selectin expression on LN $CD44^{lo}$ $TCR\beta^{+}$ $CD4^{+}$ and $TCR\beta^{+}$ $CD8^{+}$ cells (mean ± s.e.m.). Data represent n=7 $Pten^{+/+}$ and n=9 $Pten^{f/f}$ mice (CD127); and n=3 $PTEN^{+/+}$ and n=6 $PTEN^{f/f}$ mice (L-selectin), analyzed in two independent experiments. **(b)** QPCR analysis of *Il7ra* mRNA expression, normalized to *Hprt* mRNA, in purified LN T cells. Each circle indicates one mouse. **(c)** Purified LN T cells were cultured overnight in media supplemented or not with IL-7 (10 ng/mL) and *Il7ra* mRNA was quantified by QPCR. Results are presented as fold change (mean ± s.d. of triplicate culture) relative to the value obtained for freshly isolated T cells set to 1. Representative results of two independent experiments (*,$p<0.05$; **, $p<0.01$; ***,$p<0.0001$).

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Supplementary Table I – Antibodies		
Antibody specificity	Clone Identifier	Source
TCRbeta	H57-597	eBioscience
CD44	IM7	eBioscience
CD4	GK1.5	eBioscience
CD8	53-6.7	BioLegend
CD24	30-F1	BD Biosciences
L-selectin (CD62L)	MEL-14	eBioscience
CCR7	4B12	eBioscience
CD45.1	A20	eBioscience
CD45.2	104	eBioscience
Bcl-2	10C4	Santa Cruz Biotechnology
IL-7R (CD127)	A7R34	eBioscience
gC (CD132)	4G3	BD Biosciences
b-tubulin	AA2	Upstate-Millipore
PLCg	B-6-4	Santa Cruz Biotechnology
IL-4	11B11	eBioscience
IL-17	TC11-18H10	BD Biosciences
IFNg	XMG1.2	eBioscience
TNF	MP6-XT22	eBioscience
CD69	H1.2F3	eBioscience
Foxo1	76E10	Cell Signaling
Foxo3	Rabbit anti-mouse Foxo3	Gift, A. Brunet, Stanford U

Supplementary Table II Primers used to quantitate gene expression

Primer sequence	Gene
tcattatgccgaggatttgga	*Hprt* S
cagagggccacaatgtgatg	*Hprt* AS
tgtcaggctaagagttagtgagca	*Foxo1* S
gggtgaagggcatctttg	*Foxo1* AS
aagtggaaatgcccaggat	*Il7ra* S
ttgacttccatccacttcca	*Il7ra* AS
ggttactgaataccaagggaacttt	*IL2rg* S
tggcagaaccgttcactgta	*IL2rg* AS
ctaaaggcgcatctgcgta	*Klf2* S
tagtggcgggtaagctcgt	*Klf2* AS
tgatttctacagcccccaga	*Ccr7* S
gcacacctggaaaatgacaa	*Ccr7* AS
ccaagtgtgctttcaactgttc	*Sell* S
aaaggctcacactggaccac	*Sell* AS
ggagacgagttcaacgaaactt	*Bim* S
aacagttgtaagataaccatttgagg	*Bim* AS
aagcaggcctcatctcaaag	*Foxo3* S
cgtcagtttgagggtctgc	*Foxo3* AS

Supplementary Table III Primers used for Chromatin Immunoprecipitation (ChIP)

Primer sequence	*Il7ra* gene region
acctcatcagcctttcatgg	*Il7ra*-ECR2 S
atcccctgagcaaactagca	*Il7ra*-ECR2 AS
agcaaaaggattgctgctgt	*Il7ra*-ECR1 S
aagtgtggattttggcttg	*Il7ra*-ECR1 AS
tcttgggtgttgatgtggaa	*Il7ra*-ECR3 S
gtgccaggcttctttcata	*Il7ra*-ECR3 AS

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