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.
Kerdiles et al - Figure S2

(a) Foxo1^{f/f} Cd4Cre
Foxo1^{f/f} 
Foxo1^{f/+} 

T cell-DNA
Tail-DNA

(b) Foxo1^{f/f} 
Foxo1^{f/f} Cd4Cre 
Foxo1^{f/f} 
Foxo1^{f/+} Cd4Cre

Foxo1

T cells
B cells

β-tubulin

(c) Foxo1^{f/f} Foxo3^{+/-} Kca
Foxo1^{f/f} Foxo3^{+/-} Cd4Cre
Foxo3^{+/-} Kca

Foxo3
Foxo1
PLCγ

T cells

Nature Immunology: doi:10.1038/ni.1689
Supplementary Figure 2 Efficient deletion of Foxo1 in peripheral T cells of Foxo1^{fl/fl}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) Immunoblot analysis of Foxo1 and Foxo3 expression in purified LN T cells. Representative results from two to four experiments (a-c).
**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, permeabilized 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.
**a**

`Foxo1+/+ LckCre` vs `Foxo1-/- LckCre`

**b**

TCRβhi CD24lo (%)

- `Foxo1+/+ LckCre`
- `Foxo1-/- LckCre`

CD4+ CD8+ TCRβhi CD24lo (%)

- CD4+
- CD8+

Kerdiles et al - Figure S4

Nature Immunology: doi:10.1038/ni.1689
Supplementary Figure 4 Foxo1 is dispensable for T cell development. (a) CD4 and CD8 expression and (b) proportion of mature T cells (TCRβ^hi^HSA^lo^) and percentage of CD4^+^ and CD8^+^ single positive cells among TCRβ^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^flo^ mice, analyzed in two independent experiments (a and b) (***,p<0.0001; ns: not significant).
Kerdiles et al - Figure S5

Nature Immunology: doi:10.1038/ni.1689
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.
**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<sup>f/f</sup> ERCre mice. (a-d) Foxo1<sup>f/f</sup> ERCre mice and littermates were treated for 5 days with tamoxifen and rested for 5 days. (a) QPCR analysis 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<sup>+</sup> CD4<sup>+</sup> and TCRβ<sup>+</sup> CD8<sup>+</sup> 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).
**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.
TCRβ⁺ CD4⁺ CD44lo 

L-Selectin

CCR7

TCRβ⁺ CD8⁺ CD44lo 

C57BL/6

IL-7Rα−/−
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 CD44lo CD4+ and CD8+ T cells from wildtype and Il7ra−/− mice. Representative results of n = 4 mice per genotype.
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 CD44lo TCRβ+ CD4+ and TCRβ+ CD8+ cells (mean ± s.e.m.). Data represent n=7 Pten+/+ and n=9 Pten+/− mice (CD127); and n=3 PTEN+/+ and n=6 PTEN+/− 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).
### Supplementary Table I – Antibodies

<table>
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<tr>
<th>Antibody specificity</th>
<th>Clone Identifier</th>
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<td>CD44</td>
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<td>Foxo3</td>
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<td>Gift, A. Brunet, Stanford U</td>
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**Supplementary Table II Primers used to quantitate gene expression**

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**Supplementary Table III Primers used for Chromatin Immunoprecipitation (ChIP)**

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