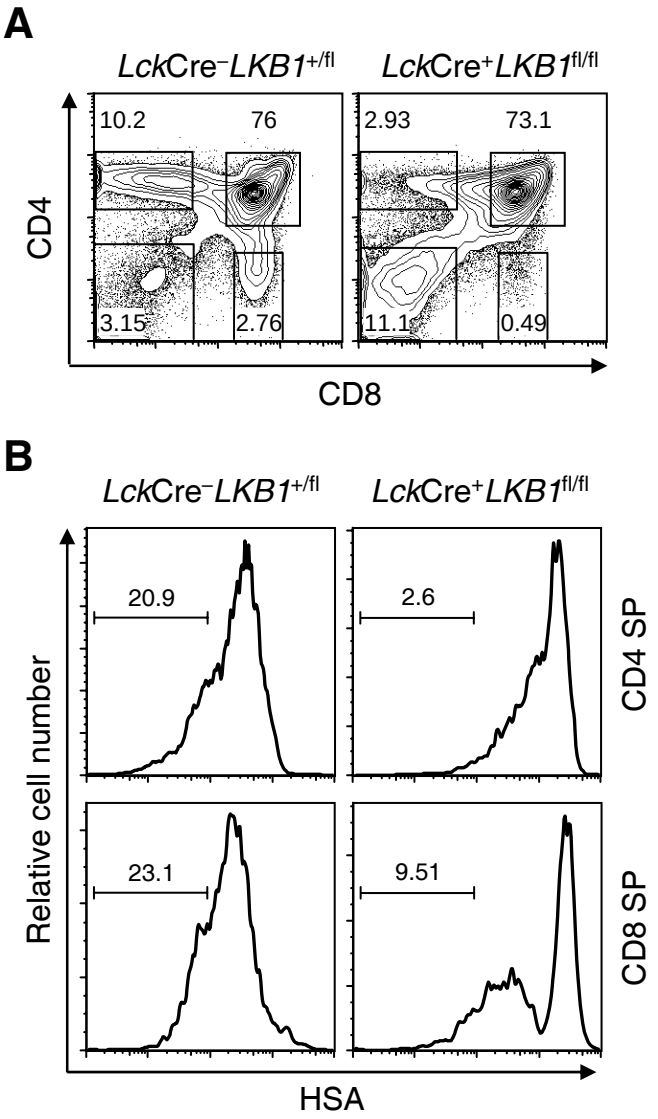
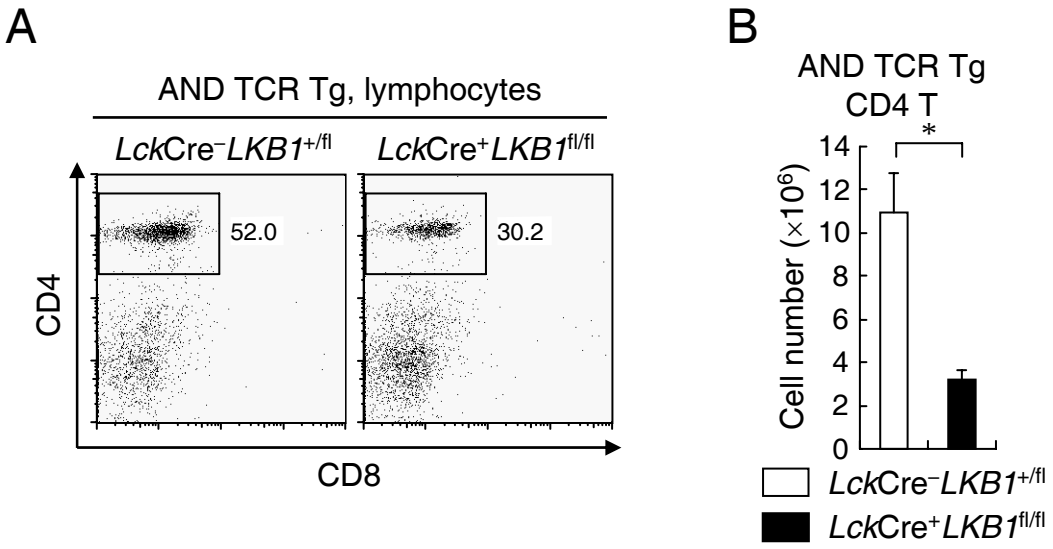
Supplementary Figure 1AB



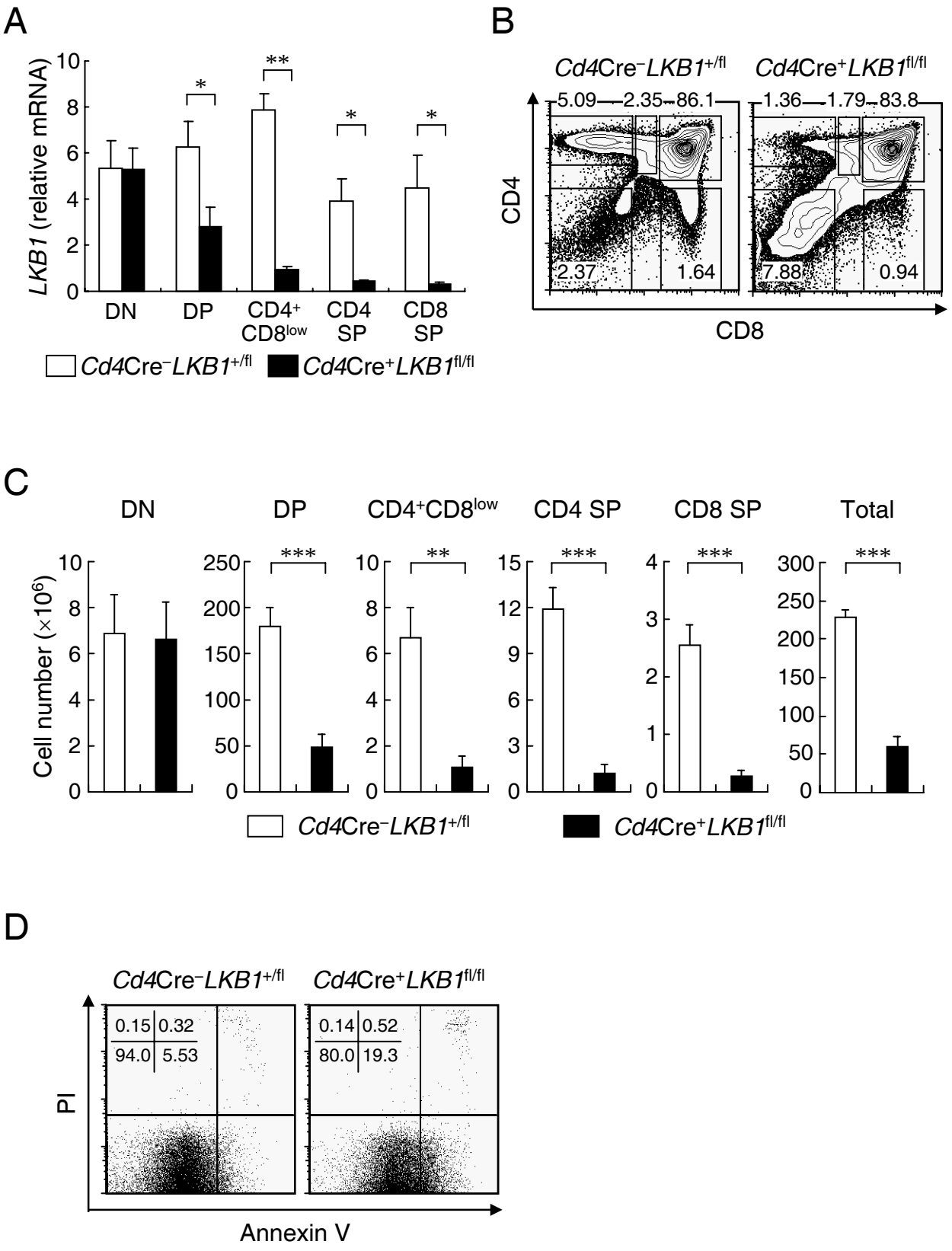
Supplementary Figure 1. Gating strategy for SP thymocytes. Thymocytes from *Lck*Cre^{-} *LKB1*$^{+/fl}$ or *Lck*Cre^{+}*LKB1*$^{fl/fl}$ mice were stained with antibodies specific for CD4, CD8 and HSA. (**A**) Cells are gated on viable cells using FSC and SSC, and then analyzed for CD4 and CD8 expression. (**B**) Histograms show the surface expression of HSA protein on gated CD4 SP and CD8 SP thymocytes, and the numbers above the brackets indicate the percentage of HSA^{low} cells.

Supplementary Figure 2AB

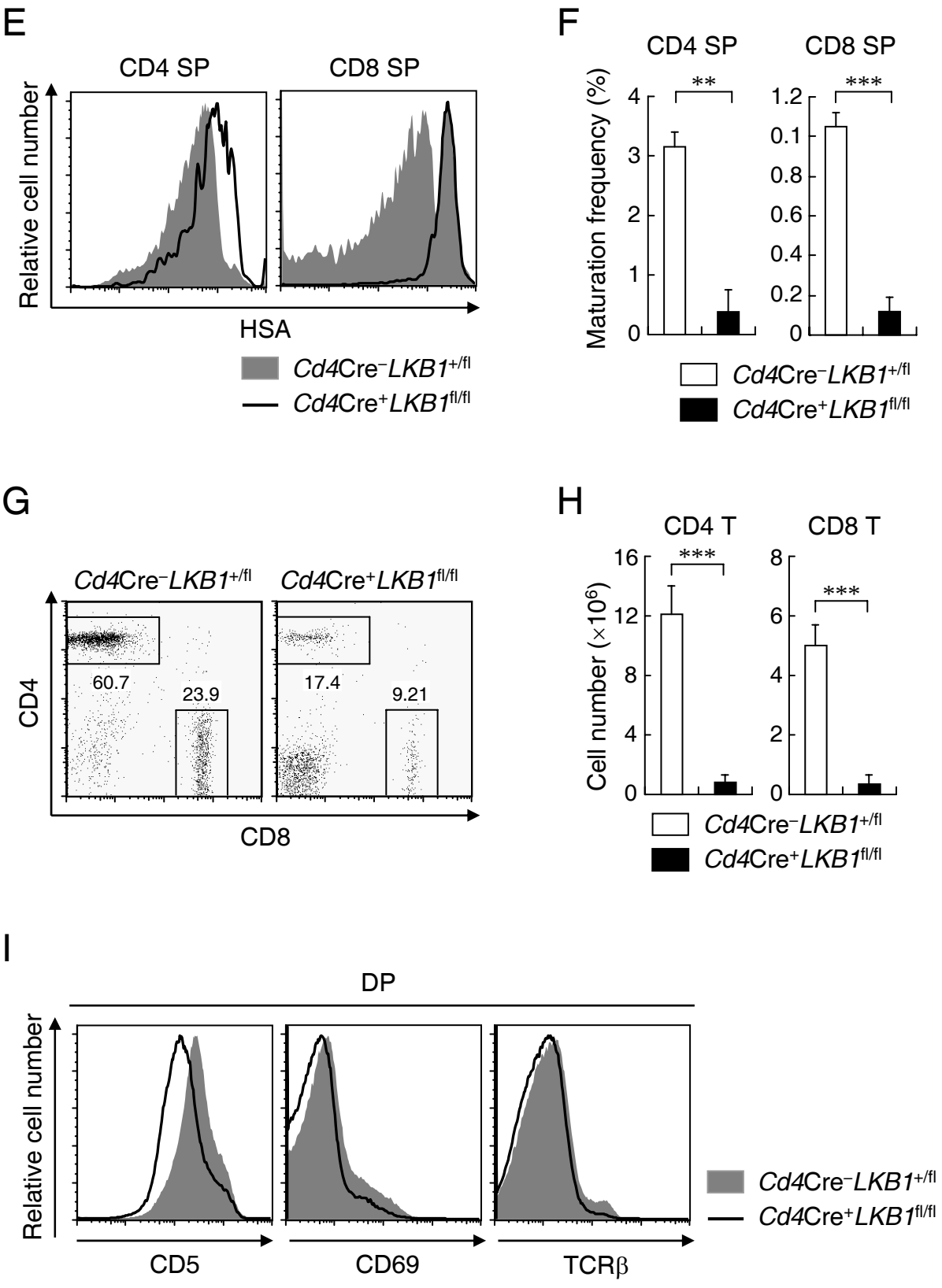


Supplementary Figure 2. Analysis of the lymphocyte subpopulations. (**A**) Total lymphocytes from AND TCR transgenic *Lck*Cre^{-}*LKB1*$^{+/fl}$ mice or *Lck*Cre^{+}*LKB1*$^{fl/fl}$ mice were analyzed for CD4 and CD8 expression by flow cytometry. The numbers next to boxes indicate the percentage of cells. (**B**) The absolute cell numbers of CD4 T cell subsets were quantified by multiplying viable cell numbers by the fraction of cells in that population. Data are expressed as mean ± SEM (n=3). *P < 0.05 (Student's t-test). Results are representative of three independent experiments.

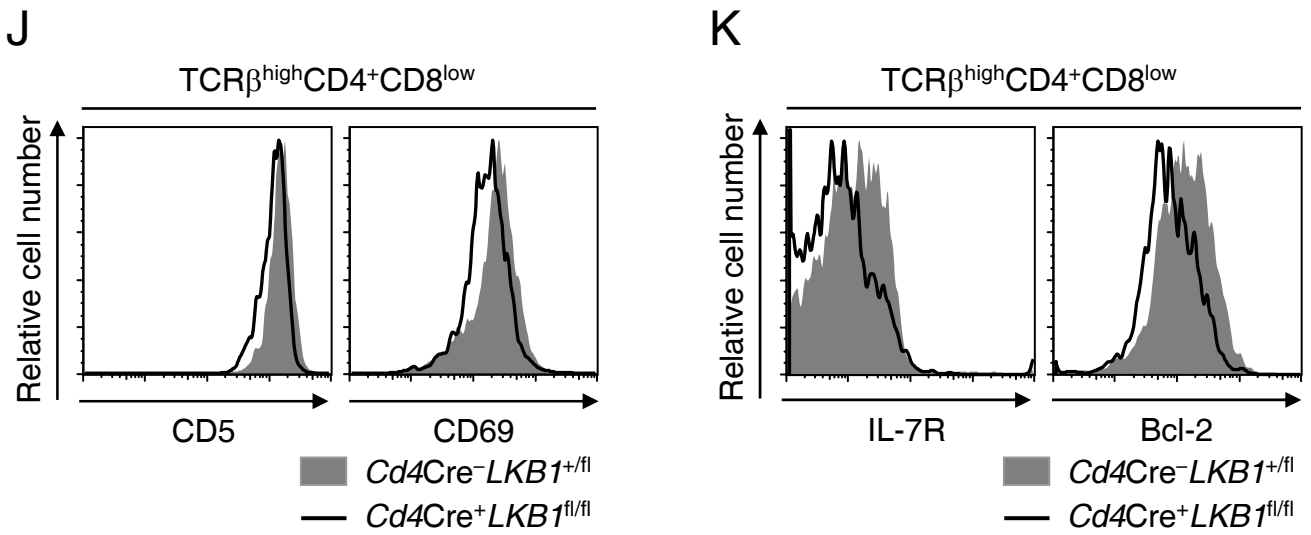
Supplementary Figure 3ABCD



Supplementary Figure 3EFGHI

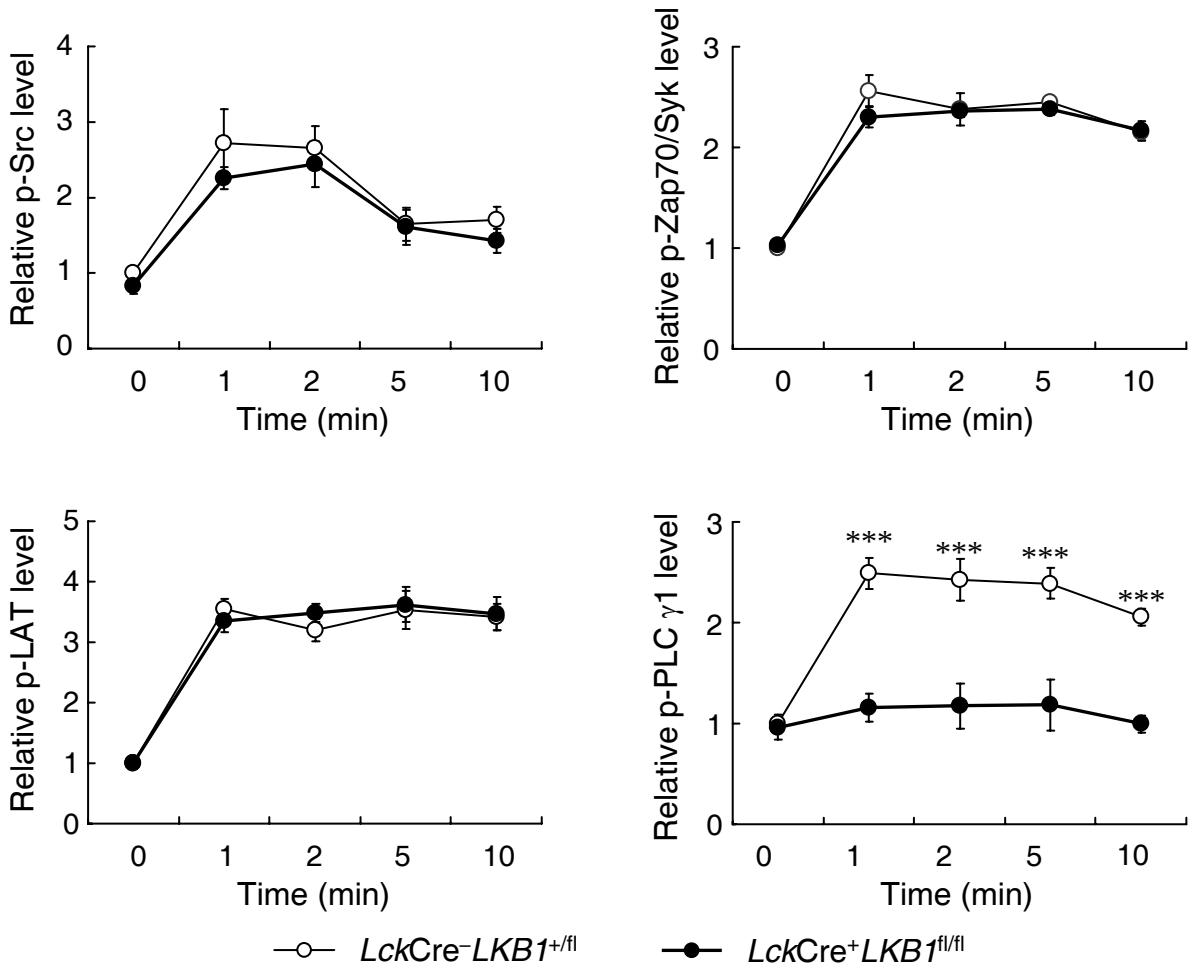


Supplementary Figure 3JK



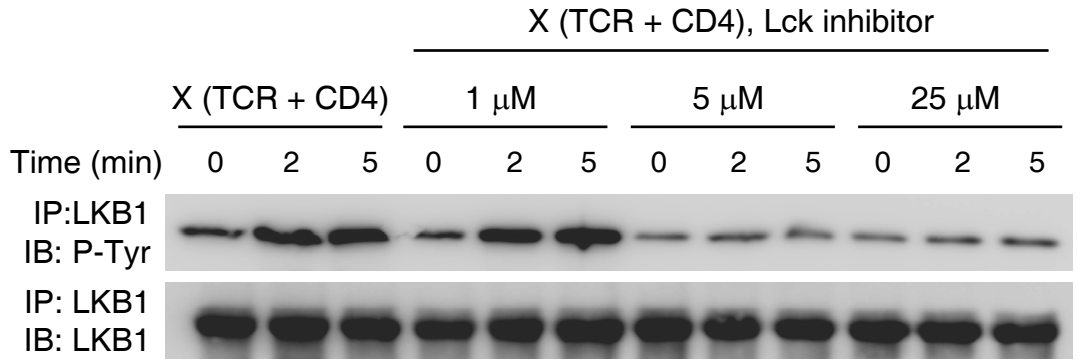
Supplementary Figure 3. Loss of LKB1 at the DP stage impairs the development of DP thymocytes to SP cels. (**A**) Quantification of *LKB1* mRNA expression. Sorted thymocyte subsets from *Cd4*Cre^{+}LKB1$^{fl/fl}$ mice and littermate controls were used for real-time RT-PCR analysis. The *LKB1* mRNA was normalized to *Hprt* mRNA according to the Ct value and primer efficiency. Data are expressed as mean ± SEM (*n*=3). (**B**) Total thymocytes from *Cd4*Cre^{+}LKB1$^{fl/fl}$ mice or littermate controls were analyzed for CD4 and CD8 expression by flow cytometry. Numbers next to or within boxes of the respective contour diagrams indicate cell percentages examined. (**C**) Cell numbers of thymic subpopulations were calculated by multiplying total live cell numbers by the fraction of cells in that population and the data expressed as mean ± SEM (*n*=4). (**D**) Total thymocytes from *Cd4*Cre^{-}*LKB1*$^{+/fl}$ mice or *Cd4*Cre^{+}*LKB1*$^{fl/fl}$ mice were stained with anti-CD4 and anti-CD8, followed by dual-labeling for Annexin V and PI, and then analyzed by flow cytometry. The percentages of cells within each quadrant are shown in the upper left quadrants. (**E**) Histograms showing HSA surface expression on CD4 and CD8 SP thymocytes from *Cd4*Cre^{+}*LKB1*$^{fl/fl}$ mice or littermate controls. (**F**) Maturation frequencies of CD4 and CD8 SP thymocytes were calculated as described above. Data are expressed as mean ± SEM (*n*=4). (**G**) Total lymphocytes from *Cd4*Cre^{-}*LKB1*$^{+/fl}$ mice or *Cd4*Cre^{+}*LKB1*$^{fl/fl}$ mice were analyzed for CD4 and CD8 expression by flow cytometry. The numbers next to boxes indicate the percentage of cells. (**H**) The absolute cell numbers of CD4 and CD8 T cell subsets were quantified by multiplying viable cell numbers by the fraction of cells in that population. (**I**) Thymocytes were stained with antibodies specific for CD4, CD8, and either CD5, CD69 or TCRβ. The expression levels of CD5, CD69 and TCRβ on gated DP thymocytes of the indicated genotypes are shown as histograms. (**J**, **K**) Thymocytes were stained with antibodies specific for CD4, CD8, TCRβ, and either CD5, CD69, IL-7R or Bcl-2. The expression levels of (**J**) CD5 and CD69 or (**K**) IL-7R and Bcl-2 of gated TCR^{high}CD4^{+}CD8^{low} thymocytes of the indicated genotypes are shown as histograms. Results are representative of three (**A**, **D**, **F**, **G**, **H**, **I**, **J**, **K**) or four (**B**, **C**, **E**, **F**) independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 (Student's *t*-test).

Supplementary Figure 4



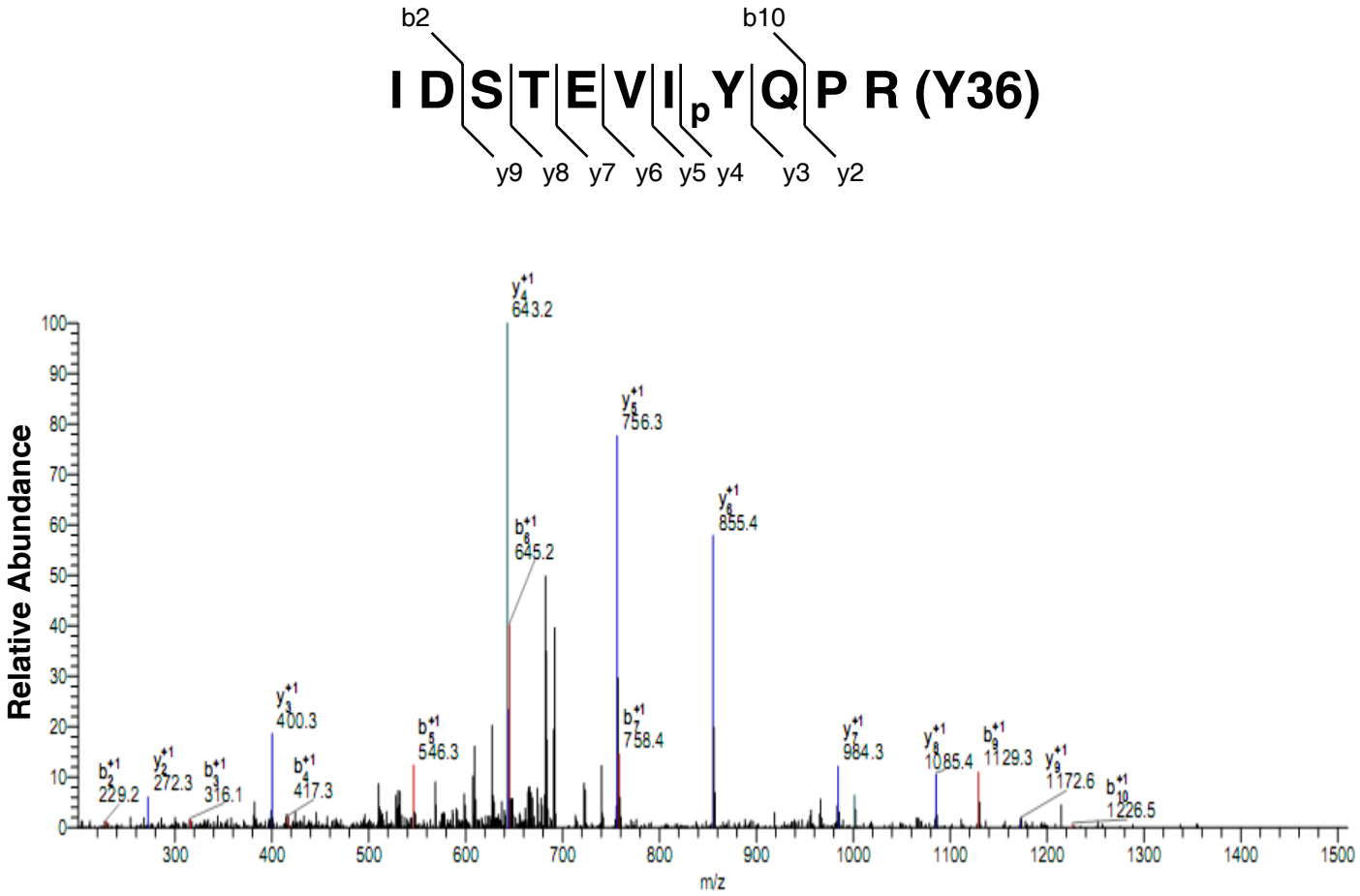
Supplementary Figure 4. The relative stimulation of phosphorylation of Src, Zap70, LAT and PLCγ1 protein levels after TCR crosslinking. Quantitative image analysis was performed with Multi-Gauge V3.0 software. The ratio of phosphorylated protein content to the total PLCγ1 content was defined as the phosphorylation level . And the relative phosphorylation level was calculated as the phosphorylation level (at the 1, 2, 5 and 10 min) relative to that of wild-type sample at the 0 min. Results are presented as mean ± SEM (n = 3). ***P < 0.001 (Student's *t*-test).

Supplementary Figure 5



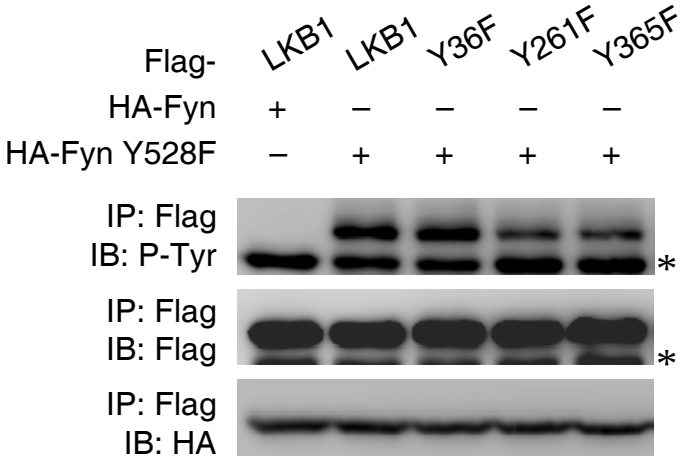
Supplementary Figure 5. Pilot experiments were used to identify an appropriate concentration of Lck inhibitor. Sorted DP thymocytes were treated without or with the indicated concentration of Lck inhibitor and were labeled with biotinylated anti-TCR and anti-CD4. Cells were then cross-linked with streptavidin for the indicated times or left untreated (0 min). LKB1 was subsequently precipitated by anti-LKB1 antibodies. The immunoprecipitated LKB1 protein was analyzed for tyrosine phosphorylation levels and precipitated LKB1 served as a loading control. Results are representative of three independent experiments.

Supplementary Figure 6



Supplementary Figure 6. The identification of tyrosine residue 36 as a new phosphorylation site of LKB1. Human 293T cells were transfected with plasmids encoding Flag-tagged LKB1 and HA-tagged Lck Y505F by calcium phosphate precipitation and harvested 36 h later. Lysates were precipitated with anti-Flag beads, and then the immunoprecipitated proteins were subjected to SDS-PAGE gel. The LKB1 protein band was excised and was in-gel enzymatically digested with trypsin. Then the peptides were enriched and analyzed by a LTQ VELOS Mass Spectrometer (Thermo), and the spectra were interpreted manually.

Supplementary Figure 7



Supplementary Figure 7. LKB1 tyrosine residues 36 is not phosphorylated by Fyn. 293T cells were transfected with plasmids encoding LKB1 or the indicated LKB1 mutants and co-transfected with Fyn or Fyn Y528F (constitutively active mutant) and harvested 36 h later. Lysates were precipitated with anti-Flag beads and immunoprecipitated proteins were immunoblotted with the indicated antibodies. The asterisk indicates the IgG heavy chain. Results are representative of three independent experiments.