



**Supplementary information, Figure S3. Role of LKB1 in splenic and thymic DCs to restrain Tregs.** **a**, Flow cytometry analysis of active caspase-3 expression in Tregs in the spleen, PLN and thymus of WT and LKB1<sup>ADC</sup> mice. **b**, Flow cytometry analysis (left) and statistics (right) of Ki-67 expression in Tregs in the spleen, PLN and thymus of WT and LKB1<sup>ADC</sup> mice. **c**, Correlation between fold change of Treg frequency in the spleen of LKB1<sup>ADC</sup> vs WT mice and mouse age by linear regression analysis. In the plot,  $r$  and  $P$  indicate the Pearson correlation coefficient and the  $P$  value of significance, respectively. **d**, List of the top 30 upregulated (FDR < 0.02) pathways (out of 41) in splenic Tregs from LKB1<sup>ADC</sup> mice (compared to WT counterparts) profiled using gene expression, as revealed by gene set enrichment analysis (GSEA). **e**, Cytokine production by WT and LKB1-deficient splenic DCs stimulated with 100 ng/ml LPS for 2 days. U.D., undetermined. **f**, Statistics of IL-2 expression in splenic CD4<sup>+</sup> and CD8<sup>+</sup> T cells from WT and LKB1<sup>ADC</sup> mice. **g**, At day 0, CellTrace-labelled CD45.1<sup>+</sup> Tregs were transferred into WT and LKB1<sup>ADC</sup> mice that were treated with anti-IL-2 antibodies at days -1, 2, and 5. CellTrace dilution of transferred Tregs was analyzed seven days later. **h**, Relative thymidine incorporation of WT Tregs cultured with splenic DCs from WT and LKB1<sup>ADC</sup> mice in the presence or absence of anti-IL-7 or anti-IL-15 blocking antibodies (values cultured with WT thymic DCs without antibody treatment was set as 1). **i**, Real-time PCR analysis of *Stk11* mRNA expression in thymic DCs from WT and LKB1<sup>ADC</sup> mice. **j**, Statistics of cell frequencies and numbers of thymic DC populations from WT and LKB1<sup>ADC</sup> mice. Data in plots indicate the means  $\pm$  s.e.m; each symbol represents an individual mouse. Numbers in gates indicate percentage of cells. NS, not significant; \* $P$  < 0.05, \*\* $P$  < 0.01; \*\*\*\* $P$  < 0.0001; two-tailed Mann-Whitney test (b; f; j, frequency) or two-tailed unpaired Student's  $t$  test (e; h; j, cell number). Data are from three (a, e), four (b), fourteen (c), five (f), or two (g–j) independent experiments.