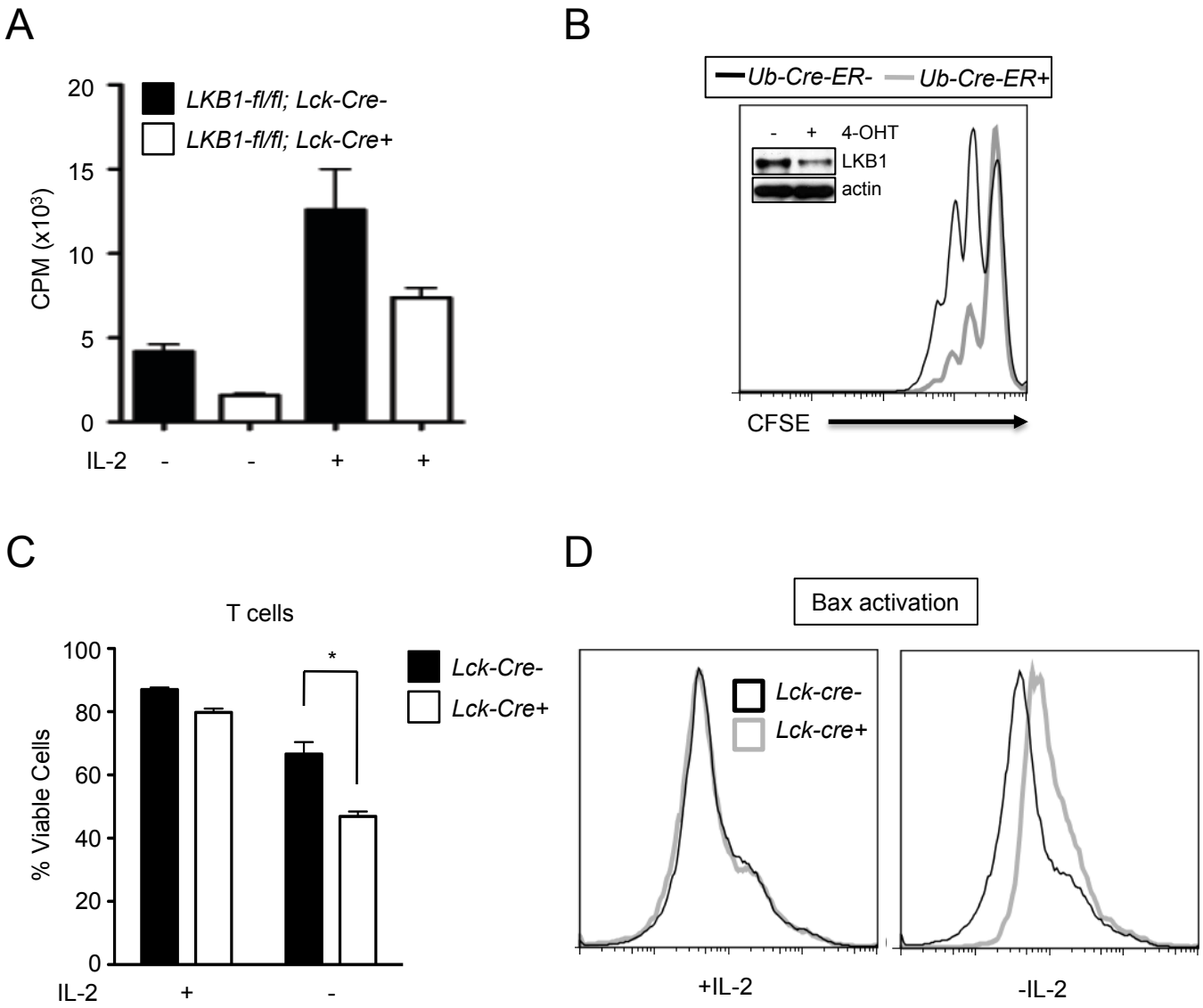
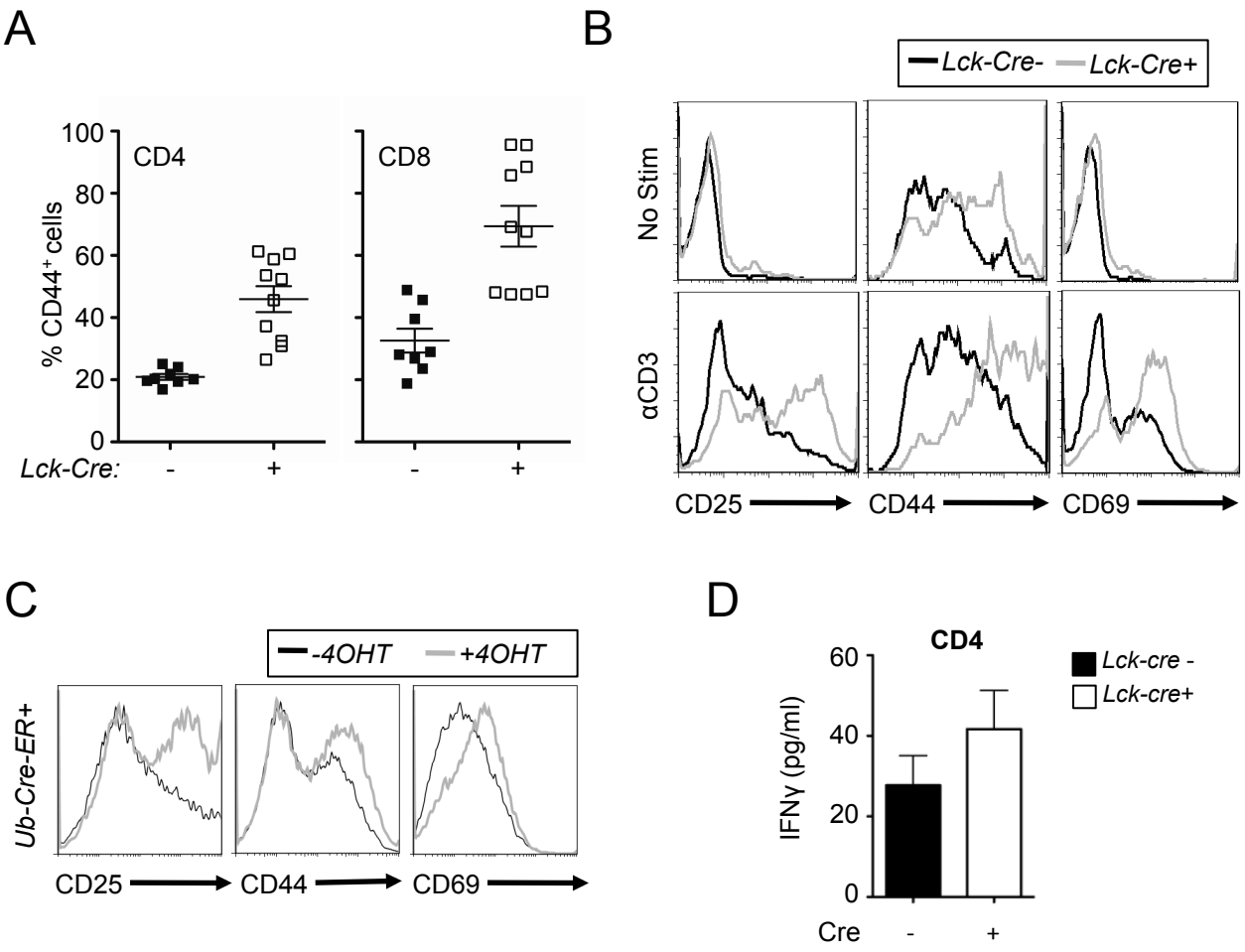
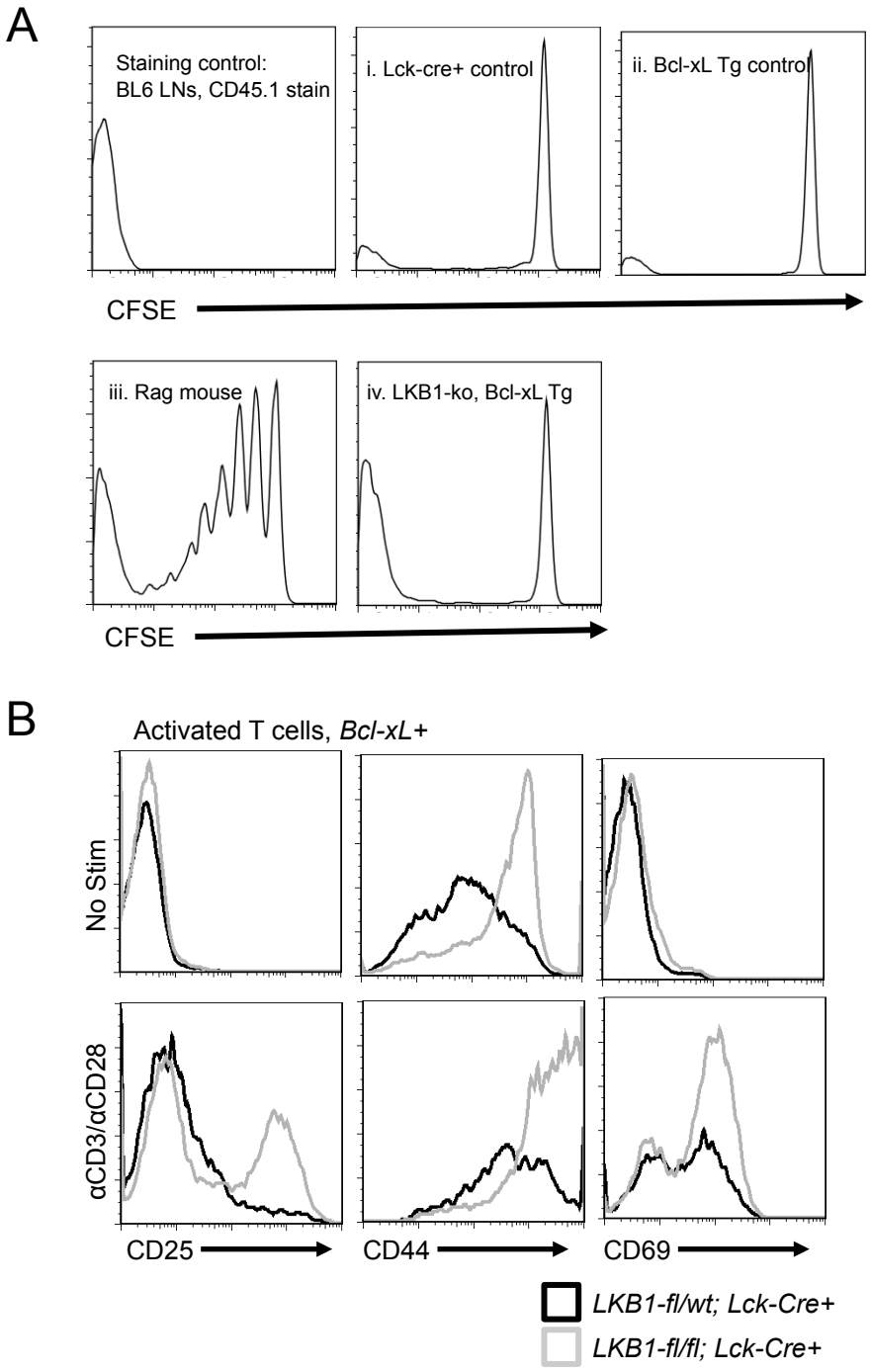


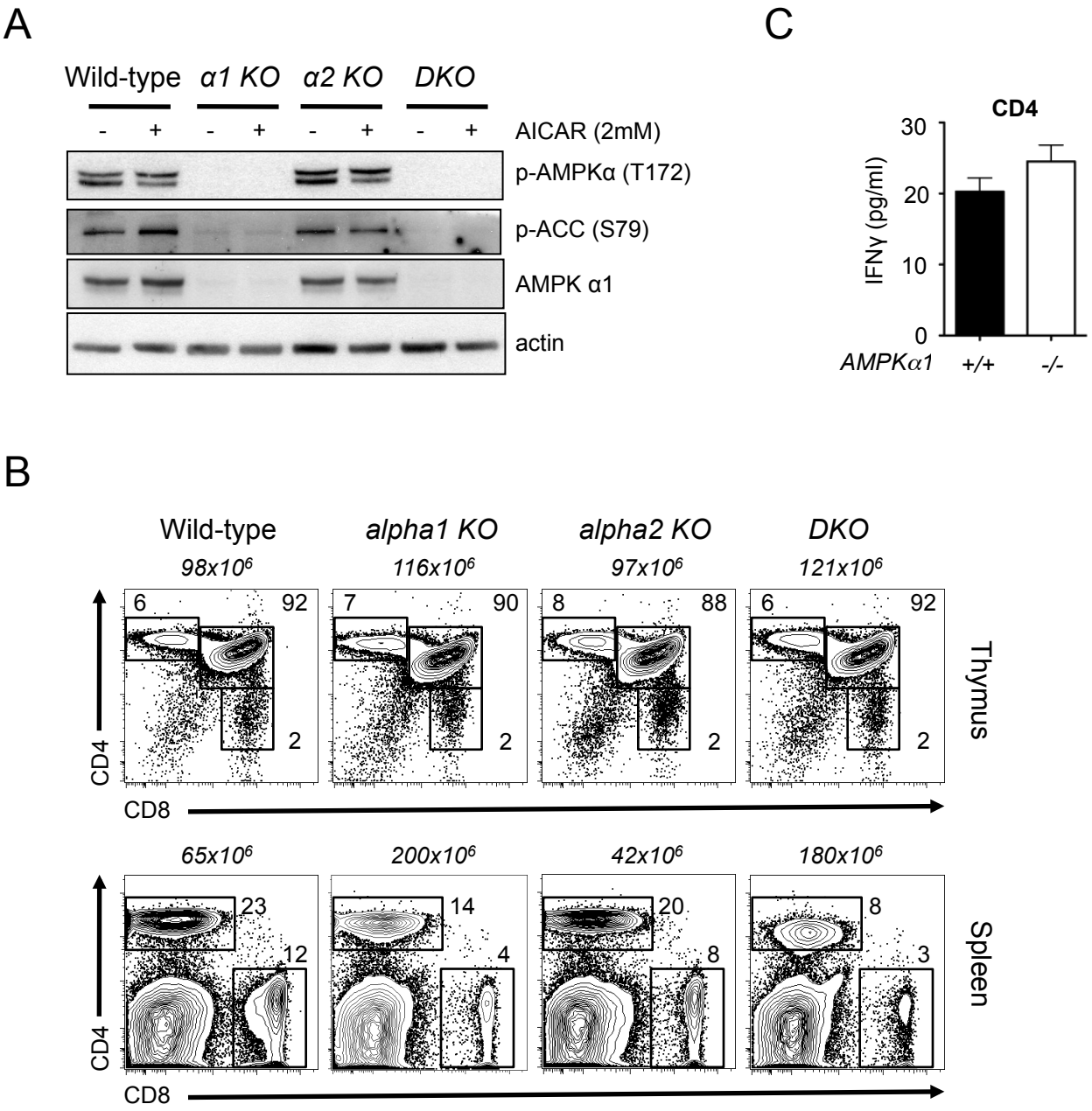
SUPPLEMENTAL FIGURE 1. LKB1-deficient T cells display defects in proliferation and viability. *A,* IL-2-dependent proliferation of LKB1-deficient T cells. Proliferation of control (black bars) or LKB1-deficient (open bars) T cells following anti-CD3/CD28 stimulation (1 mg/mL each) in the presence (+) or absence (-) of 50U/ml rIL-2. Data represent the mean ± s.d. for triplicate samples. *B,* Inducible LKB1 deletion results in decreased proliferation. T cells from an inducible LKB1 knockout mouse (*Ub-Cre-ER+, LKB1-fl/fl*) or control mouse (*Ub-Cre-ER-, LKB1-fl/fl*) were cultured with IL-7 in the presence of 4-OHT for 4 days to induce LKB1 knockout, after which they were activated with anti-CD3 and anti-CD28 antibodies for 48 hours, and proliferation was measured by CFSE dilution. Level of LKB1 depletion following 4-OHT treatment was assessed by Western Blot (inset). *C,* Comparison of T cell viability following IL-2 withdrawal. Activated control (black bars) or LKB1-deficient (open bars) T cells were cultured in the presence (+) or absence (-) of rIL-2, and cell viability measured 24 hours later. *D,* Activated control (black lines) or LKB1-deficient (grey lines) T cells were cultured in the presence (+) or absence (-) of rIL-2 for 24 hours and Bax activation was measured by flow cytometry.



SUPPLEMENTAL FIGURE 2. LKB1-deficient T cells display a hyperactivated phenotype. *A,* Percent CD44-expressing peripheral T cells from LKB1-expressing (*Lck-cre-*) or LKB1-deficient (*Lck-cre+*) mice. *B*, Expression of activation markers on LKB1-deficient T cells. LKB1-expressing (*LKB1-fl/fl, Lck-Cre-*) or LKB1-deficient (*LKB1-fl/fl, Lck-Cre+*) T cells were cultured in the presence or absence of anti-CD3 antibodies, and surface expression of CD25, CD44, and CD69 was analyzed by flow cytometry after 48 hours. *C,* Inducible LKB1 deletion results in increased activation marker expression. T cells from an inducible LKB1 knockout mouse (*Ub-Cre-ER+, LKB1-fl/fl*) were cultured with IL-7 in the presence or absence of 4-OHT for 4 days to induce LKB1 knockout, after which they were activated with anti-CD3 and anti-CD28 antibodies for 48 hours, and surface expression of CD25, CD44, and CD69 was analyzed by flow cytometry. *D*, IFNγ production by naïve CD4+ LKB1-deficient T cells. Naïve CD4+ T cells (CD25-CD44^{lo}) were isolated from spleen and lymph nodes of control (*LKB1-fl/fl, Lck-Cre-*) or T cell-specific LKB1-null animals (*LKB1-fl/fl, Lck-Cre+*) and stimulated with anti-CD3 and anti-CD28 antibodies. Culture supernatants were harvested 24 hours post-activation and analyzed for IFNγ production by ELISA.



SUPPLEMENTAL FIGURE 3. *A*, CD45.1+ T cells were CFSE labeled and adoptively transferred into (i) *Lck-cre+, LKB1-+/+* control mouse, (ii) LKB1-expressing (*Lck-cre+, LKB1-+/+*), Bcl-xL transgenic mouse, (iii) Rag-1 knockout mouse, and (iv) LKB1-knockout (*Lck-cre+, LKB1-fl/fl*), Bcl-xL transgenic mouse. After one week, cells were isolated from lymph nodes, and the adoptively transferred CD45.1+ T cells were identified flow-cytometry and analyzed for CFSE expression. A staining control from cells isolated from C57BL/6J lymph nodes is included. *B,* Loss of LKB1 results in increased T cell activation despite transgenic expression of Bcl-xL. T cells from LKB1-expressing (*LKB1-fl/+, Lck-cre+*; black lines) or LKB1-deficient (*LKB1-fl/fl, Lck-cre+*; grey lines) mice with expression of a Bcl-xL transgene were either unstimulated or activated with antibodies to CD3 and CD28, as indicated, for 48 hours, after which surface expression of CD25, CD44, and CD69 was analyzed by flow cytometry.



SUPPLEMENTAL FIGURE 4. *A*, Examination of AMPK expression and activity by Western blot. T cells were isolated from wildtype, AMPKα1 knockout, AMPKα2 knockout, and AMPK α1, α2 double knockout (DKO) mice and treated with or without 2mM AICAR, as indicated, for 60 min. Lysates were resolved by SDS-PAGE and immunoblotted for phosphorylated AMPKα or ACC, AMPKα, and actin. *B,* Representative analysis of CD4 and CD8 expression on total thymocytes and splenocytes isolated from mice of the indicated genotypes. Numbers within the plot represent the percentage of cells of the given population, while numbers above the plot represent cellularity of the organ. *C,* IFNγ production by naïve CD4+ AMPKα1-deficient T cells. Naïve CD4+ T cells (CD25-CD44^{lo}) were isolated from spleen and lymph nodes of control (*AMPKα1*+/+) or AMPKα1-null animals (*AMPKα1-/-*) and stimulated with anti-CD3 and anti-CD28 antibodies for 24 hours. Culture supernatants were analyzed for IFNγ production by ELISA.