Supplementary Figure 1. Analysis of peripheral T cells from c-Cbl mutant mice.

(A) c-Cbl(C379A) knockin mice have reduced numbers of peripheral T cells. Mesenteric lymph node cells from c-Cbl +/+, -/-, A/A or A/- mice were labeled with anti-CD4-PE and anti-CD8-FITC antibodies and analyzed by flow cytometry. The percentages of CD8+ and CD4+ cells are indicated in the respective quadrants.

(B) Reduced CD3ε, but not TCRβ, levels on mature T cells from c-Cbl mutant mice. Mesenteric lymph node cells from c-Cbl +/+ (shaded histogram), A/- (bold) and -/- (dashed) mice were labeled with anti-CD3ε, anti-TCRβ or anti-CD19 antibodies and analyzed by flow cytometry. Identical decreases in CD3 levels were found on peripheral T cells from both c-Cbl-/- and RING finger mutant mice compared to wt T cells, a phenomenon also observed in mice mutated in the c-Cbl tyrosine kinase binding domain (Thien et al, 2003). We hypothesized that this reduction of CD3 on mature T cells is a selection process to counter enhanced signaling strength in c-Cbl mutant mice (Thien et al, 2003). The percentages of CD19 positive cells were: +/+ 40%, A/- 69% and -/- 43%.

(C) Expression of CD44, CD25 and CD62L on CD4+ lymph node T cells from c-Cbl +/- (shaded histogram), A/- (bold) and -/- (dashed) mice. c-Cbl A/- mutant mice show increased proportions of high expressing CD44 and CD25 CD4+ T cells (upper panel), however the absolute numbers of these cells were less in c-Cbl A/- mice (lower panel). Equivalent changes to levels of CD62L expression on CD4+ T cells was observed in both c-Cbl-/- and A/- mutant mice relative to wild-type mice.