Supporting Information
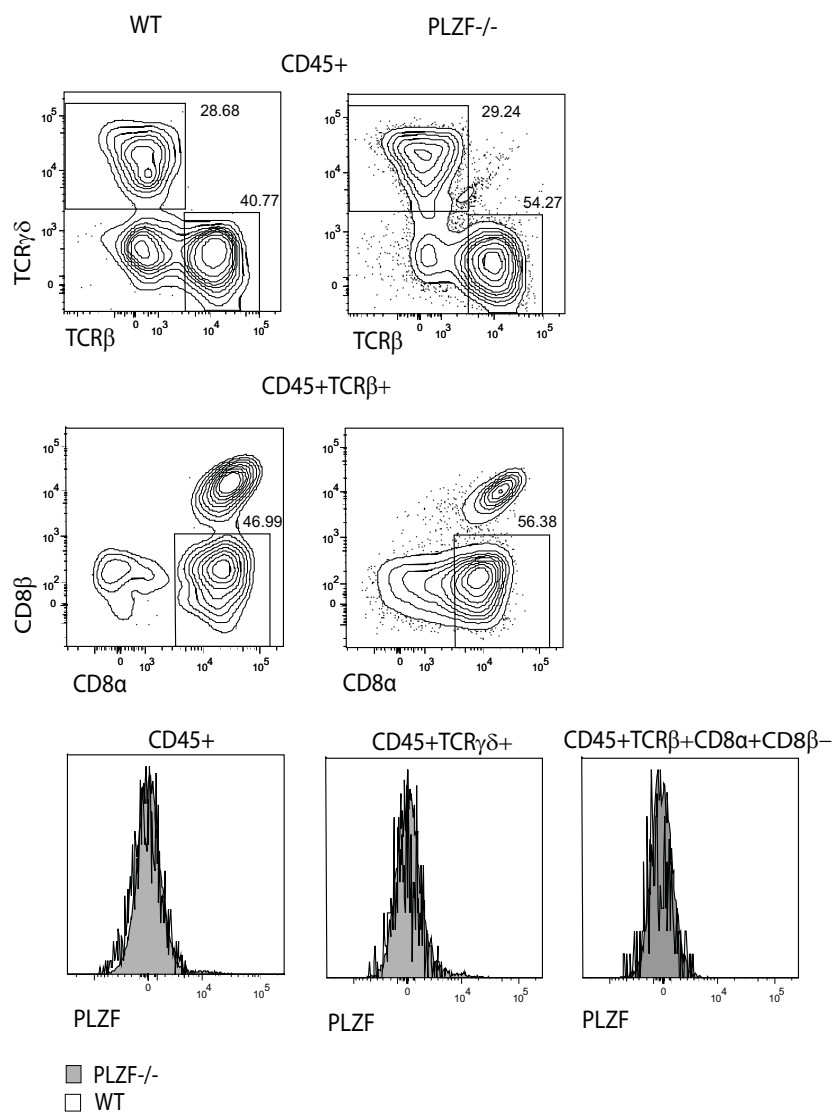
Kreslavsky et al. 10.1073/pnas.0903895106

Fig. S1. PLZF is not expressed by intestinal intraepithelial T cells. Intraepithelial lymphocytes were isolated from small intestine of WT and PLZF−/− mice, stained for surface expression of CD45, CD8a, CD8β, TCR-δ, TCRβ, intracellular expression of PLZF, and analyzed by FACS. Gates applied as indicated.

Fig. S2. Effects of PLZF deficiency on Vγ1+Vδ6.3+ cells are cell-intrinsic. Lethally irradiated CD45.1/CD45.2 heterozygous B6 mice were cotransferred with T-cell-depleted bone marrow from wt (CD45.1) and PLZF−/− (CD45.2) in 1:1 ratio. After 5 weeks, animals were killed and analyzed. (*A*) Thymocytes were stained *ex vivo* with CD45.1, CD45.2, TCRγδ, Vγ1, Vδ6.3, and PLZF antibodies and analyzed by FACS. Gated on wt (*Upper*) or PLZF−/− (*Lower*). Further gating is applied as indicated by arrows. (*B*) TCRγδ cells were sorted from 3 pooled thymi of the chimeras and stimulated with PMA/ionomycin as described in *Material and Methods* (*Center* and *Right*) or left unstimulated (*Left*). Surface expression of CD45.1, CD45.2, Vγ1, and Vδ6.3 and intracellular expression of IFNγ and IL-4 were analyzed. Gated on Vγ1+Vδ6.3+CD45.1+ (*Center*) or Vγ1+Vδ6.3+CD45.2+ (*Right*). (*C*) Thymocytes were stained *ex vivo* with CD45.1, CD45.2, TCRγδ, Vγ1, Vδ6.3, CD44, and CD62L antibodies and analyzed by FACS. Gated on wt (*Left*) or PLZF−/− (*Right*) Vγ1+ Vδ6.3+ cells. Representative FACS plots from 1 of 2 independent experiments are shown.

Fig. S3. Reduced numbers of Vγ1+Vδ6.3+ cells in SAP−/− mice. Cells from thymi, spleens, and livers of wt and SAP−/− mice were counted, stained for TCRγδ, Vγ1+, and Vδ6.3+, and analyzed by flow cytometry. Representative FACS plots showing frequency of Vγ1+Vδ6.3+ cells among total TCRγδ+ cells (*A*) and absolute numbers of Vγ1+Vδ6.3+ cells (*B*) are shown. ∗ and ∗∗∗ indicate statistical significance (*P* < 0.05 and *P* < 0.001, respectively) with an unpaired *t*-test.