Supplemental material

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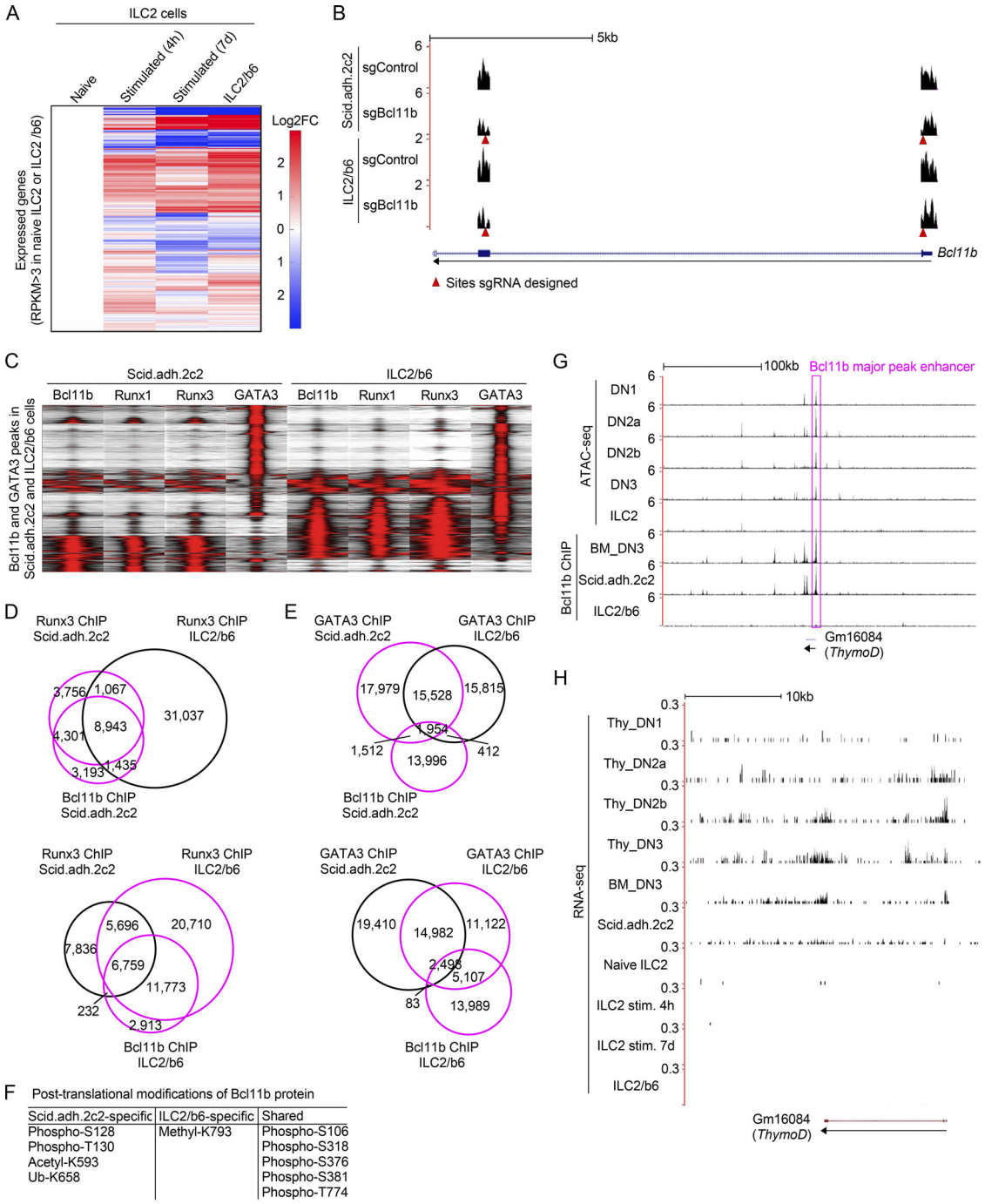

Figure S1. **Characterization of ILC2 and pro-T cell transcriptomes, Runx binding patterns, Bcl11b modifications, and activities in the *Bcl11b* enhancer region. (A)** Heat maps show hierarchical clustering analyses of the expression of all expressed genes, which have RPKM >3 in naive ILC2 cells or an ILC2 cell line, ILC2/b6 cells, in naive ILC2, stimulated ILC2 for 4 h or 7 d (Shih et al., 2016; Yagi et al., 2014), and ILC2/b6 cells. **(B)** Representative RNA-seq tracks are shown for *Bcl11b*-deficient Scid.adh.2c2 or ILC2/b6 cells at the *Bcl11b* locus (around exon1 and 2). Red arrowheads show sites against which sgRNA was designed. **(C)** Tag count distributions for Bcl11b, Runx1, Runx3, and GATA3 in Scid.adh.2c2 and ILC2/b6 cells are shown. All Bcl11b and GATA3 binding sites identified in the DN3 and ILC2/b6 cells were included in the analysis. **(D)** Venn diagrams show the number of Runx3 ChIP peaks in Scid.adh.2c2 and ILC2/b6 cells with Bcl11b ChIP peaks in Scid.adh.2c2 cells (top) or ILC2/b6 cells (bottom). **(E)**, Venn diagrams show the number of GATA3 ChIP peaks in Scid.adh.2c2 and ILC2/b6 cells with Bcl11b ChIP peaks in Scid.adh.2c2 cells (top) or ILC2/b6 cells (bottom). **(F)** Post-translational modifications of Bcl11b protein detected by mass spectrometry analysis are shown. **(G)** Representative ATAC-seq tracks for thymic DN subsets and mature small intestine ILC2 cells (Yoshida et al., 2019; downloaded from GEO accession no. GSE100738) and binding profiles of Bcl11b in DN3, Scid.adh.2c2, and ILC2/b6 cells are shown around the Bcl11b major peak enhancer (magenta rectangle) and *ThymoD* locus. The low activity state shown here in mature ILC2 cells contrasts with the state inferred to exist in ILC2 precursors, based on evidence in Fig. 5. **(H)** Representative RNA-seq tracks (mapped with STAR v2.4.0) at the *ThymoD* locus are shown for thymic DN subsets (Yoshida et al., 2019; GSE100738), in vitro cultured bone marrow precursor–derived DN3, Scid.adh.2c2, mature naive ILC2, mature ILC2 stimulated (stim.) for 4 h or 7 d (Shih et al., 2016; Yagi et al., 2014), and ILC2/b6 cells. Data from this study are based on two replicate RNA-seq results (A, B, and H) and are based on reproducible ChIP-seq peaks in two replicate samples (C–E and G).

Tables S1 and S2 are provided online as separate Excel files. Table S1 shows DEGs after Cas9-mediated disruption of Bcl11b gene in Scid.adh.2c2 and ILC2/b6 cells. Table S2 shows Bcl11b-interacting molecules in Scid.adh.2c2 and ILC2/b6 cells identified by LC-MS/MS.

References

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