**Supplementary Figure 1 Flow cytometry gating strategy.** (A) Gating strategy of CLP, ILCP, CHILP from bone marrow. (B) Gating strategy of NKT, ILC1, cNK from liver. (C) Gating strategy of ILC1s, ILC2s, ILC3s from LPL. (D) PLZF expression in ILC3 subsets was determined by flow cytometry.

**Supplementary Figure 2 Generation of PLZFCre-ERT2/EGFP knock-in mice.** (A) The sequence encoding a Cre-GFP-FRT-Neo-FRT cassette was inserted immediately after the start codon of Zbtb16 by homologous recombination. The following PCR primers were used to identify WT (311 base pairs) and knock-in (581 base pairs) alleles: 5′-AGTTGTTGTCACTTGCTCACCC-3′, 5′-GTGAACGAACCTGGTCGAAAT-3′, and5′-CTTGCTGGTGCAGGCTAGCA-3′. WT mice had only 311 base pairs product and *PLZF-/-* mice had only 581 base pairs product in genotyping test. (B) Western blot was performed on NKT cells from liver and ILC3 from the LPLs to assess PLZF expression in the WT and $PLZF^{-/-}$ mice. Data are representative of at least two independent experiments.

**Supplementary Figure 3 PLZF deficiency does not affect the number of total ILCs.** (A-B) Small intestine (SI) and large intestine (LI) LPLs were isolated from WT or *PLZF-/-* mice (n=4-6). (A) Representative flow cytometry plots for total ILCs (B) Frequencies of ILCs in $CD45^+$ lymphocytes and total numbers ILCs cells in SI and LI. (C) Representative flow cytometry plots for ILC3 subsets. (D-E) Large intestine LPLs were isolated from WT or *PLZF-/-* mice, and representative flow cytometry plots for Ki-67(D) and Caspase-3(E) in ILC3 were shown. (A-E) Data are representative of at least two independent experiments.

**Supplementary Figure 4 Expression of ILC3s activation makers and homing receptors.** (A-B) 6-8 weeks old WT or *PLZF-/-* mice were sacrificed, and LPLs were isolated for analysis(n=3-5). (A) Representative flow cytometry plots for CD69, MHCII, CCR9, and CCR6 expressed on ILC3 gated on $CD45.2^+Lin^-CD90.2^+$. (B) Frequencies of each activation marker on ILC3s in the WT and *PLZF-/-* mice. A-B Data are representative of at least two independent experiments. Data are represented as mean±SEM. Error bars show SEM. *P < 0.05; **P < 0.01.

**Supplementary Figure 5 The *Zbtb16* gene expression is downregulated in ILC3s under disease conditions.** (A-B) 6-8 weeks old WT mice were infected with *C. rodentium*(A) or induced DSS model with DSS(B), and ILC3s(Lin$^-$CD45.2$^{mid}$CD90.2$^{hi}$) was sorted from large intestine LPL. *Zbtb16* mRNA expression was analyzed by real-time RT-PCR(n=3). (C) ILC3s was sorted from the LPL of 6-8 weeks old WT mice, and ILC3s was stimulated with or without IL-23 for 4 hours. *Zbtb16* mRNA expression was analyzed by real-time RT-PCR(n=3). A-C Data are representative of at least two independent experiments. Data are represented as mean ± SEM. Error bars show SEM. *P < 0.05; **P < 0.01.

**Supplementary Figure 6 IL-22 expression regulated by PLZF is not dependent on Rorc, AhR, and Stat3.** (A) Expression of *Rorc* mRNA expression in ILC3 were analyzed by real-time RT-PCR(n=3). (B-C) Large intestinal LPLs from WT and *PLZF-/-* littermate mice were cultured in the presence of the Ahr antagonist (CAS 301326-22-7 Millipore) or STAT-3 inhibitor (STA-21) for 24 hours. Expression of IL-22 was measured by flow cytometry after gating on Lin$^-$CD45.2$^{mid}$CD90.2$^{hi}$ ILC3(n=3). A-C Data are representative of at least two independent experiments.

**Supplementary Figure 7 PLZF specific binding motif.**