Figure S1. Related to Figure 1

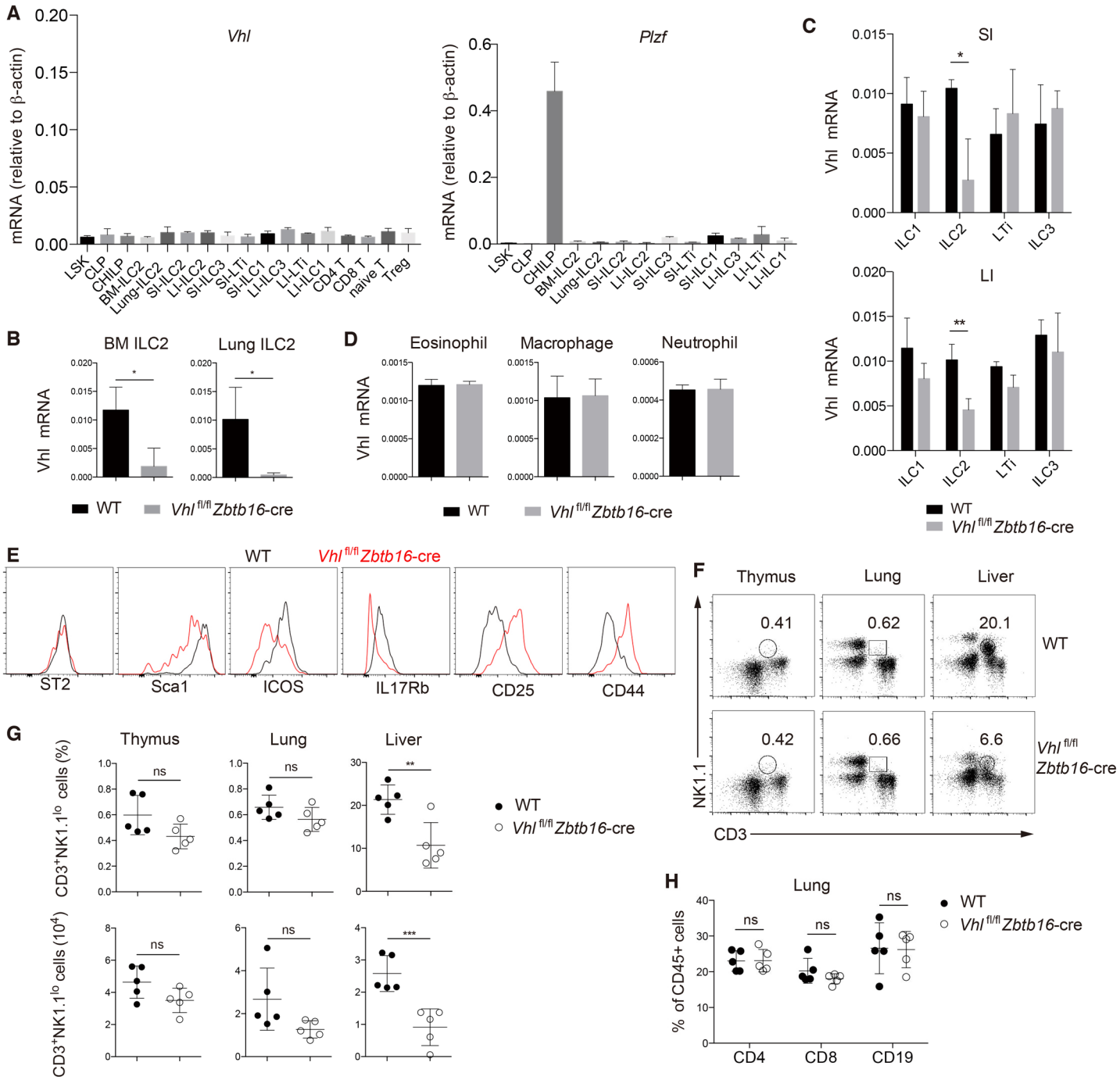


Figure S2. Related to Figure 2

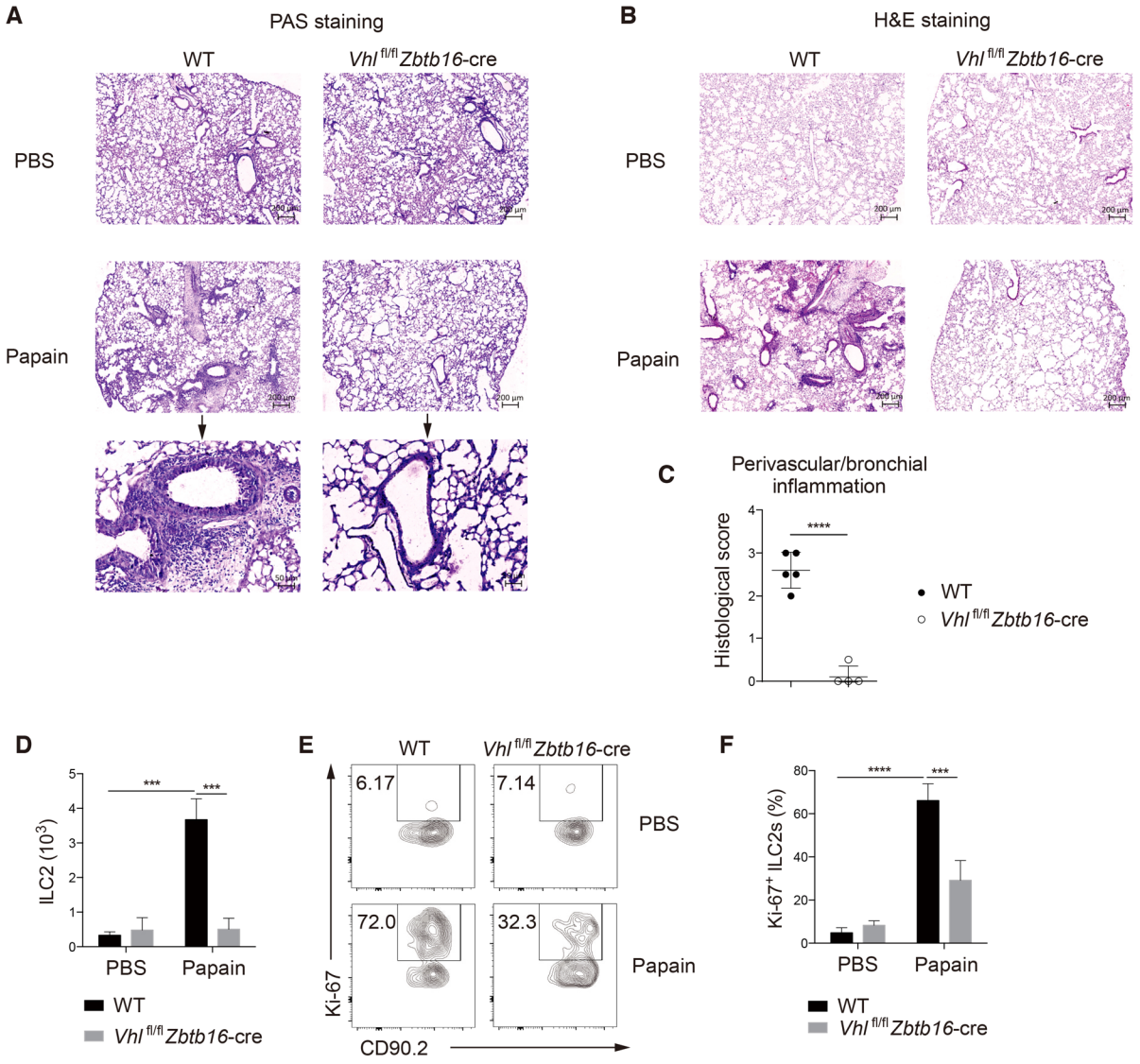


Figure S3. Related to Figure 3

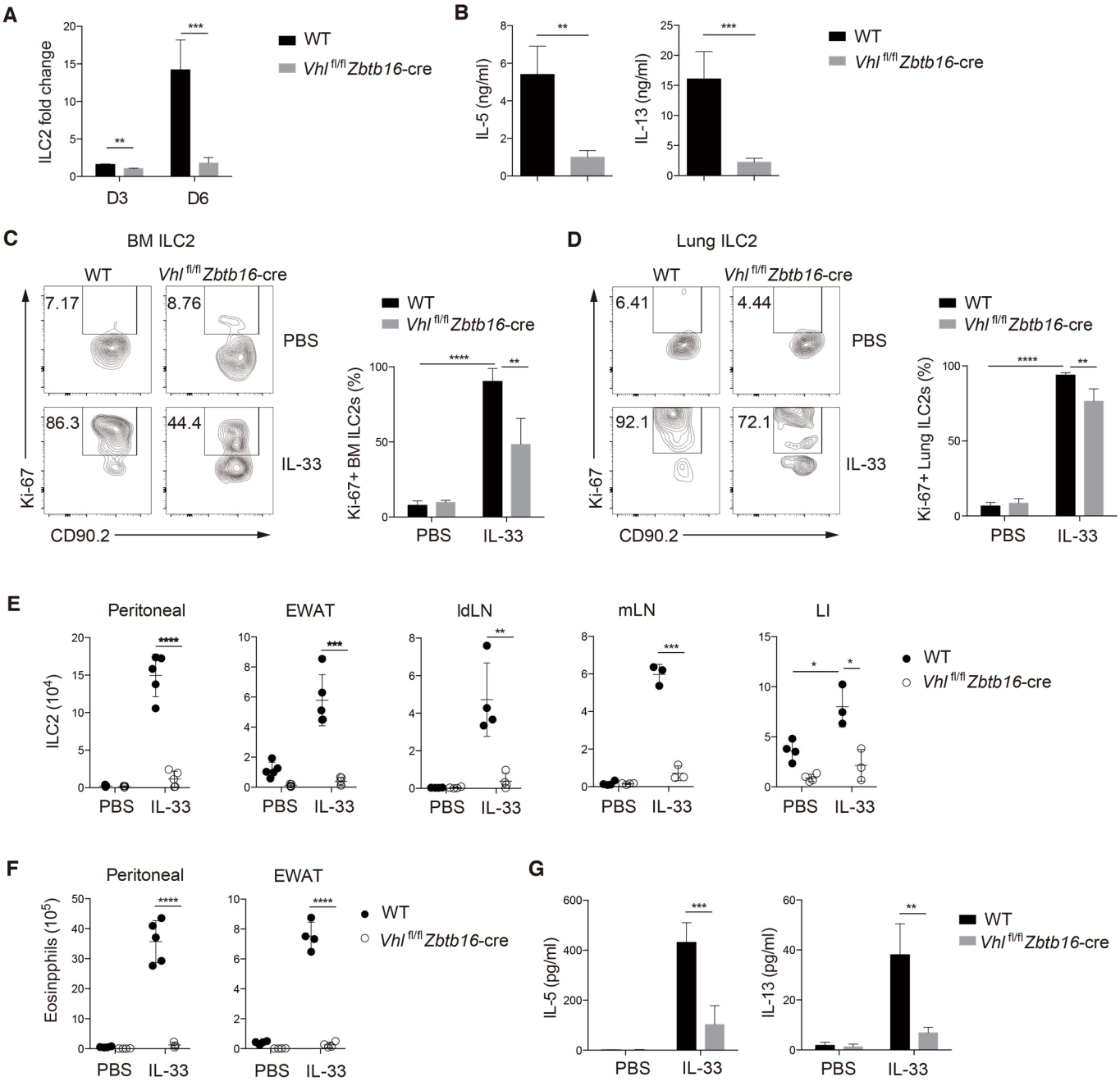


Figure S4. Related to Figure 5

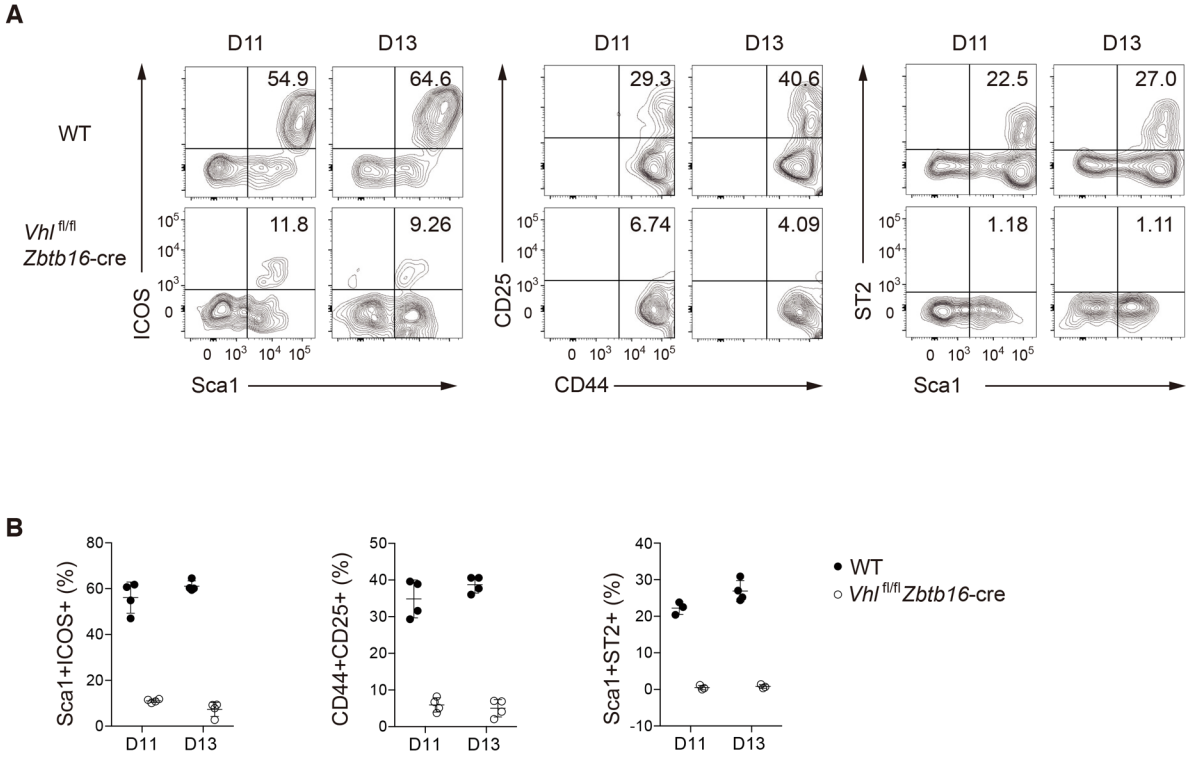


Figure S5. Related to Figure 6

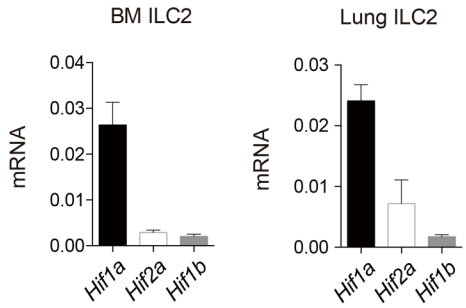
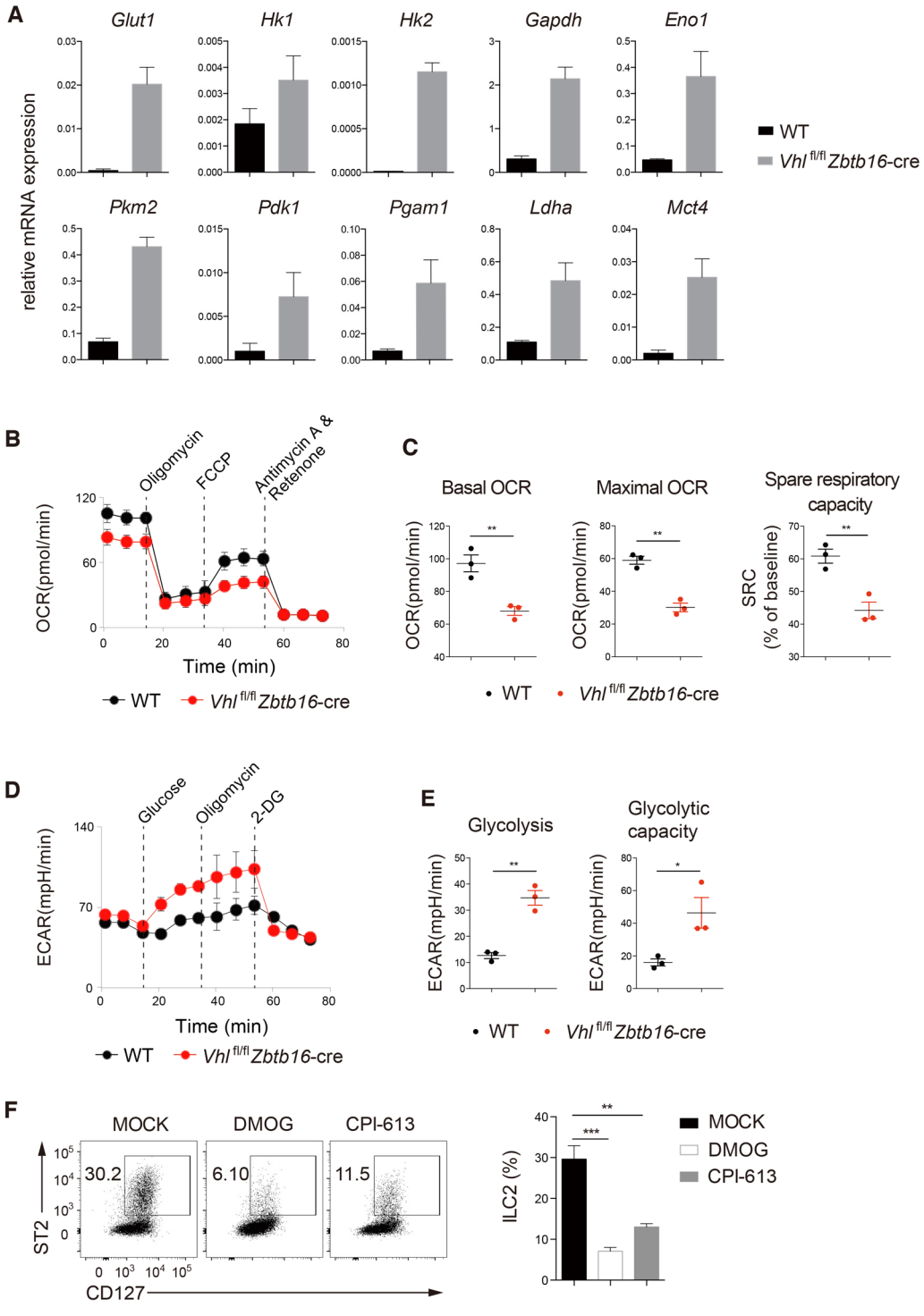


Figure S6. Related to Figure 7



SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Related to Figure 1. *Vhl* deletion efficiency in ILC2s and other cells.

(A) Expression of genes encoding VHL and PLZF in indicated cell subsets by quantitative RT-PCR. Cells were FACS sorted by following markers: LSK ($Lin^-CD127^-Sca1^+c\text{-}kit^+$), CLP ($Lin^-CD127^+Sca1^{lo}c\text{-}kit^{lo}Flt3^+$), CHILP ($Lin^-CD127^+Flt3^-CD25^-\alpha4\beta7^+$), BM ILC2 ($Lin^-CD90.2^+CD127^+ST2^+$), lung ILC2 ($CD45^+Lin^-CD90.2^+Sca1^{hi}ST2^+$), SI and LI ILC2 ($CD45^+Lin^-CD90.2^+Sca1^{hi}KLRG1^+$), ILC3 ($CD45^+Lin^-CD90.2^+CD127^+Sca1^-KLRG1^-NK1.1^-CD4^-$), LTi ($CD45^+Lin^-CD90.2^+CD127^+Sca1^-KLRG1^-NK1.1^-CD4^+$), ILC1 ($CD45^+Lin^-CD90.2^+CD127^+KLRG1^-NK1.1^+NKp46^+$), Treg ($CD4^+CD25^+$). For LSK, CLP, CHILP, and ILC2, Lin includes markers for CD3, CD5, CD4, CD8, TCRβ, TCRγδ, NK1.1, CD11b, B220, TER119, Gr-1 and CD11c; for ILC3, LTi, and ILC1, Lin includes markers for CD3, CD5, CD8, TCRβ, TCRγδ, CD11b, B220, TER119, Gr-1 and CD11c. Cells were pooled from 3-10 mice. (B) *Vhl* mRNA expression in sorted BM (left) and lung (right) ILC2s from wild-type (WT) and $Vhl^{fl/fl}$ *Zbtb16*-cre mice (pool of 3 mice per group). (C) *Vhl* mRNA expression in ILC1s, ILC2s, LTi cells, and ILC3s from the SI (top) and LI (bottom) of WT and $Vhl^{fl/fl}$ *Zbtb16*-cre mice. (D) *Vhl* mRNA expression in lung eosinophils, macrophages and neutrophils from WT and $Vhl^{fl/fl}$ *Zbtb16*-cre mice. (E) Flow cytometry analyzing the expression of ST2, Sca1, ICOS, IL17Rb, CD25, and CD44 on BM ILC2s from WT (black) and $Vhl^{fl/fl}$ *Zbtb16*-cre (red) mice. (F) Flow cytometric analysis of $CD3^+NK1.1^{lo}$ NKT cells from the thymus, lungs, and liver of WT and $Vhl^{fl/fl}$ *Zbtb16*-cre mice (n = 5 per group). (G) Frequencies (top) and total numbers (bottom) of NKT cells as assessed in F. (H) Frequencies of $CD4^+$, $CD8^+$, and $CD19^+$ lymphocytes in the lungs of WT and $Vhl^{fl/fl}$ *Zbtb16*-cre mice (n = 5 per group). mRNA expression was normalized to those of β-actin. Data are represented as mean ± SD. ns, not significant; $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ (two-tailed unpaired t-test). Data are pooled from or representative of two to three independent experiments.

Figure S2. Related to Figure 2. Loss of VHL attenuated lung inflammation upon papain challenge.

(A) PAS and (B) H&E staining of lung sections from WT and $Vhl^{fl/fl}$ *Zbtb16*-cre mice treated with PBS or papain. Scale bars, 200 μm (upper and middle panel in A, and B) or 50 μm (lower panel in A). (C) Histological scores of perivascular/bronchial inflammation in B. (D) Total numbers of ILC2s ($Lin^-CD90.2^+Sca1^{hi}ST2^+$) in lung draining lymph nodes from WT or $Vhl^{fl/fl}$ *Zbtb16*-cre mice treated with PBS or papain (n = 3-5 per group). (E) Flow cytometric analysis of Ki-67 expression in lung ILC2s from

WT or *Vhl*$^{fl/fl}$ *Zbtb16*-cre mice treated with PBS or papain (*n* = 4-5 per group). (F) Frequencies of Ki-67^{+} lung ILC2s as assessed in E. Data are represented as mean ± SD. ***P < 0.001, and ****P < 0.0001 (two-tailed unpaired t-test). Data are pooled from or representative of two to three independent experiments.

Figure S3. Related to Figure 3. Loss of VHL attenuated proliferation and responsiveness of ILC2s to IL-33.

(A,B) Freshly isolated BM ILC2s (2,000 cells/well) from WT or *Vhl*$^{fl/fl}$ *Zbtb16*-cre mice were cultured with 10 ng/ml IL-7 and 10 ng/ml IL-33. (A) Fold changes of total ILC2 numbers were calculated on day 3 (D3) and day 6 (D6) of culture. (B) Concentrations of IL-5 and IL-13 in the supernatants on day 3 of culture. (C-G) WT or *Vhl*$^{fl/fl}$ *Zbtb16*-cre mice were intraperitoneally injected with 500 ng of IL-33 or PBS on day 0, 1 and 3, and assessed on day 4. (C) Flow cytometric analysis of Ki-67 expression in BM ILC2s (left) and frequencies of Ki-67^{+} BM ILC2s (right) from WT or *Vhl*$^{fl/fl}$ *Zbtb16*-cre mice treated with PBS or IL-33 (*n* = 4-5 per group). (D) Analysis of lung ILC2s as in C. (E) Total numbers of ILC2s in the peritoneal cavity, EWAT, lung draining lymph nodes (ldLN), mesenteric LN (mLN) and LI from WT or *Vhl*$^{fl/fl}$ *Zbtb16*-cre mice treated with PBS or IL-33 (*n* = 3-5 per group). (F) Quantification of total eosinophils in peritoneal cavity and EWAT. (G) Concentrations of IL-5 and IL-13 in peritoneal cavity fluid. Data are represented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 (two-tailed unpaired t-test). Data are pooled from or representative of three independent experiments.

Figure S4. Related to Figure 5. VHL is essential for ILC2 development from PD-1^{hi} progenitors *in vitro*.

(A) Flow cytometric profiles of cells derived from sorted PD-1^{hi} progenitors (Lin^{-}CD127^{+}Flt3^{-}CD25^{-}PD-1^{hi}) of WT and *Vhl*$^{fl/fl}$ *Zbtb16*-cre mice cultured on OP9-DL4 stromal cells in the presence of IL-7 and IL-33, and analyzed for the percentage of ILC2s on day 11 (D11) or 13 (D13) of culture by the specific markers, including Sca1^{+}ICOS^{+}, CD44^{+}CD25^{+}, and Sca1^{+}ST2^{+}, pre-gated with Lin^{-} cells. Numbers indicate the frequency of flow cytometric events. (B) Total frequencies of PD-1^{hi} progenitor-derived cells as assessed in A. Data are from one experiment with four biological replicates and representative of two independent experiments with similar results.

Figure S5. Related to Figure 6. HIF expression in bone marrow and lung ILC2s.

mRNA expression of *Hif1a*, *Hif2a*, and *Hif1b* in BM (left) and lung (right) ILC2s by qRT-PCR (pool of 3 mice). Results were normalized to β-actin. Data are representative of two to three independent experiments with similar results.

Figure S6. Related to Figure 7. VHL deficiency upregulates glycolytic metabolism in ILC2s.

(A) Quantitative RT-PCR analysis of the abundance of transcripts encoding glycolytic enzymes in bone marrow ILC2s from WT and $Vhl^{fl/fl}$ *Zbtb16*-cre mice (pool of 3 mice per group). Results were normalized to those of β-actin mRNA. (B) OCR of ILC2s from WT or $Vhl^{fl/fl}$ *Zbtb16*-cre mice cultured with IL-7 and IL-33 for 7days. (C) Summarized metabolic measures of basal OCR (left), maximal OCR (middle) and spare respiratory capacity (right) of WT or VHL deficient ILC2s calculated from B. (D) ECAR of ILC2s from WT or $Vhl^{fl/fl}$ *Zbtb16*-cre mice. (E) Summarized metabolic measures of glycolysis (left) and glycolytic capacity (right) of WT or VHL deficient ILC2s calculated from D. (F) Flow cytometric analysis of $CD127^{+}ST2^{+}$ ILC2s derived from CHILPs of WT mice cultured on OP9-DL4 stromal cells with IL-7 and IL-33, in the presence or absence (MOCK) of 100 μM DMOG or 100 μM CPI-613, pre-gated with Lin^{-} cells (left). Frequencies of $CD127^{+}ST2^{+}$ ILC2s among Lin^{-} cells were assessed (right). Data are represented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ (two-tailed unpaired t-test). Data are representative of two to three independent experiments with similar results.

Table S1. Related to key resources table. Mouse primers for qRT-PCR and ChIP assay.

Gene name	Forward primer	Reverse primer
Vhl	CAGCTACCGAGGTCATCTTTG	CTGTCCATCGACATTGAGGGA
Plzf	CTGGGACTTTGTGCGATGTG	CGGTGGAAGAGGATCTCAAACA
Hif1a	ACCTTCATCGGAAACTCCAAAG	ACTGTTAGGCTCAGGTGAACT
Hif2a	CTGAGGAAGGAGAAATCCCGT	TGTGTCCGAAGGAAGCTGATG
Hif1b	GACAGACCACAGGACAGTTCC	AGCATGGACAGCATTTCTTGAA
Glut1	CAGTTCGGCTATAACACTGGTG	GCCCCCGACAGAGAAGATG
Hk1	CGGAATGGGGAGCCTTTGG	GCCTTCCTTATCCGTTTCAATGG
Hk2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
Gapdh	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGA
Eno1	TGCGTCCACTGGCATCTAC	CAGAGCAGGCGCAATAGTTTTA
Pkm2	TTGCAGCTATTCGAGGAACTCCG	CACGATAATGGCCCCACTGC
Pdk1	GGACTTCGGGTCAGTGAATGC	TCCTGAGAAGATTGTCGGGGA
Pgam1	TCTGTGCAGAAGAGAGCAATCC	CTGTCAGACCGCCATAGTGT
Ldha	TGTCTCCAGCAAAGACTACTGT	GACTGTACTTGACAATGTTGGA
Mct4	TCACGGGTTTCTCCTACGC	GCCAAAGCGGTTCACACAC
β-*actin*	GCTGTGCTGTCCCTGTATGCCTCT	CCTCTCAGCTGTGGTGGTGAAGC
Il1rl1 U1	GTATTTGGCAACTTTCGCTTG	TCATTTCAAAGTGAACGAGCA
*Il1rl1*p	TGGGGGAACAGTCTTGGA	CCAGTACTCAVAGATAGGCATCA
Il1rl1 int.4	TGGAGAGCTGTGAACAGTGG	TCAATATATCCATGACAAAGACGTG
Il1rl1 int.7	AATTCCCTTGCTTGGTCCTT	ATCGGATCAGGTTGGTCATC
*Gata3*p	TGCCTATGATAATGGCCCATTC	CTGCTCCTGGTGCCTACAAAG
Il5 int.1	TGGCCTTGAACTCACAGAGA	TTGGCCTTTAATCCCAGAAC
*Il5*p	AAGTCTAGCTACCGCCAATA	AGCAAAGGTGAGTTCAATCT
Il5 U1	CGGCAGATTTGGGGAGATA	TTGTGAGTTTCACATCATGCAC
Il5 U2	CATGGTGTTGAATCACTCTGAAA	TCAGGGGAATTGCCAAGTAA
*Id2*p	TTTTGGAGTCAAAGCCATCC	AGACCTTGCCAAAGCAAAAG