Supplemental material

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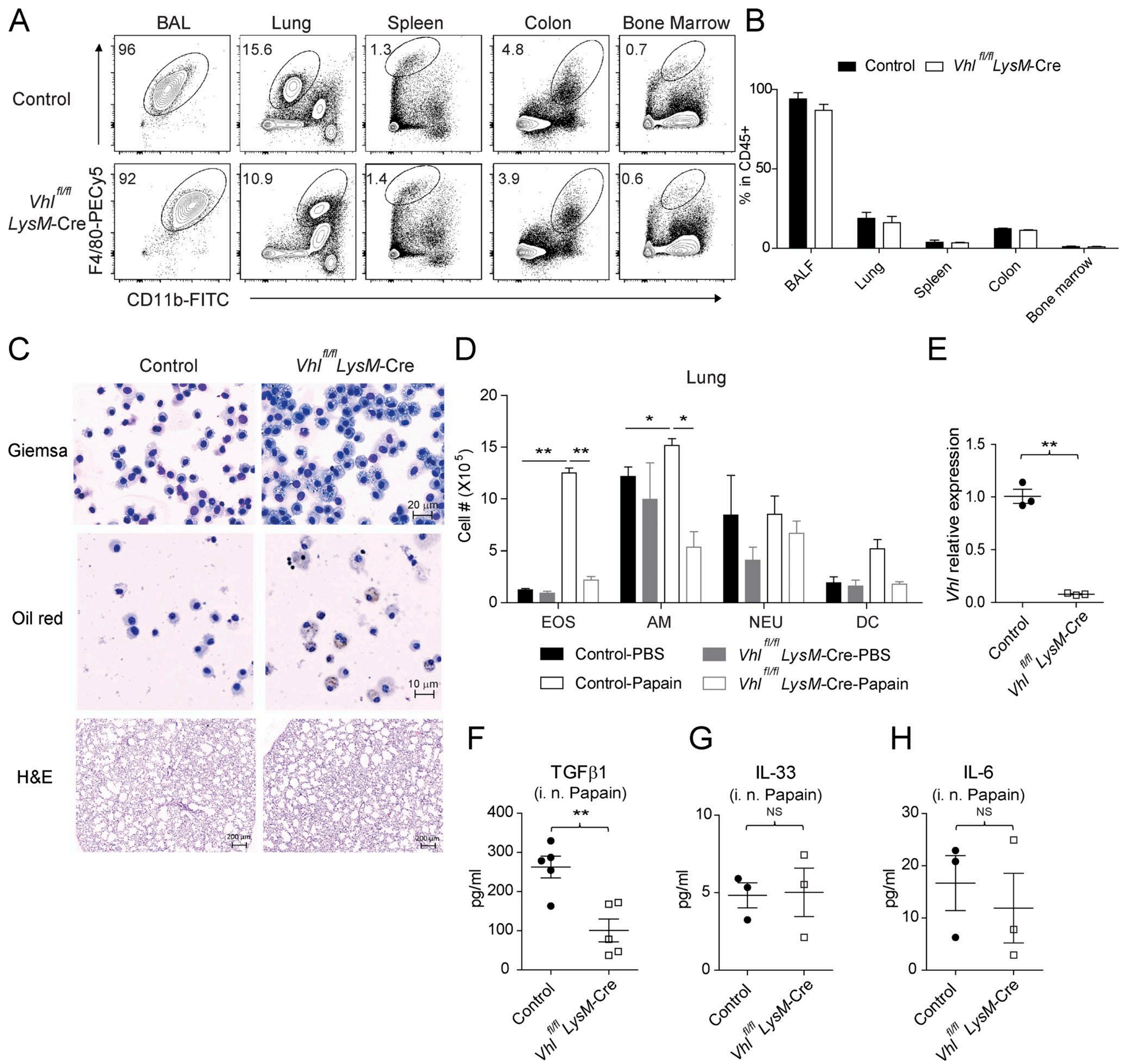

Figure S1. **VHL deficiency affects AMs.** Related to Fig. 1. **(A)** Expression of F4/80 and CD11b among $CD45^+$ cells in the indicated tissues. **(B)** Frequency of cells gated as shown in A. **(C)** Cytospin photograph of BAL cells stained with Giemsa or Oil Red (top and middle). Bars, 20 µm or 10 µm, respectively. Representative H&E staining of lung sections (bottom). Bar, 200 µm. **(D)** Absolute numbers of $CD11b^+$ $CD11c^-$ $SiglecF^+$ eosinophils (EOS), $CD11b^+$ $CD11c^+$ $SiglecF^+$ AMs, $CD11b^+$ $Ly6C^-$ $Ly6G^+$ neutrophils (NEU), and $CD11c^+$ $MHCII^{hi}$ dendritic cells (DC) in the lungs. **(E)** Expression of *Vhl* mRNA in sorted AMs. **(F–H)** Concentrations of TGFβ1, IL-33, and IL-6 in the BAL fluid. *, P < 0.05; **, P < 0.01 (Student's *t* test). Data are from one experiment representative of three independent experiments (mean and SEM of three to six mice per group).

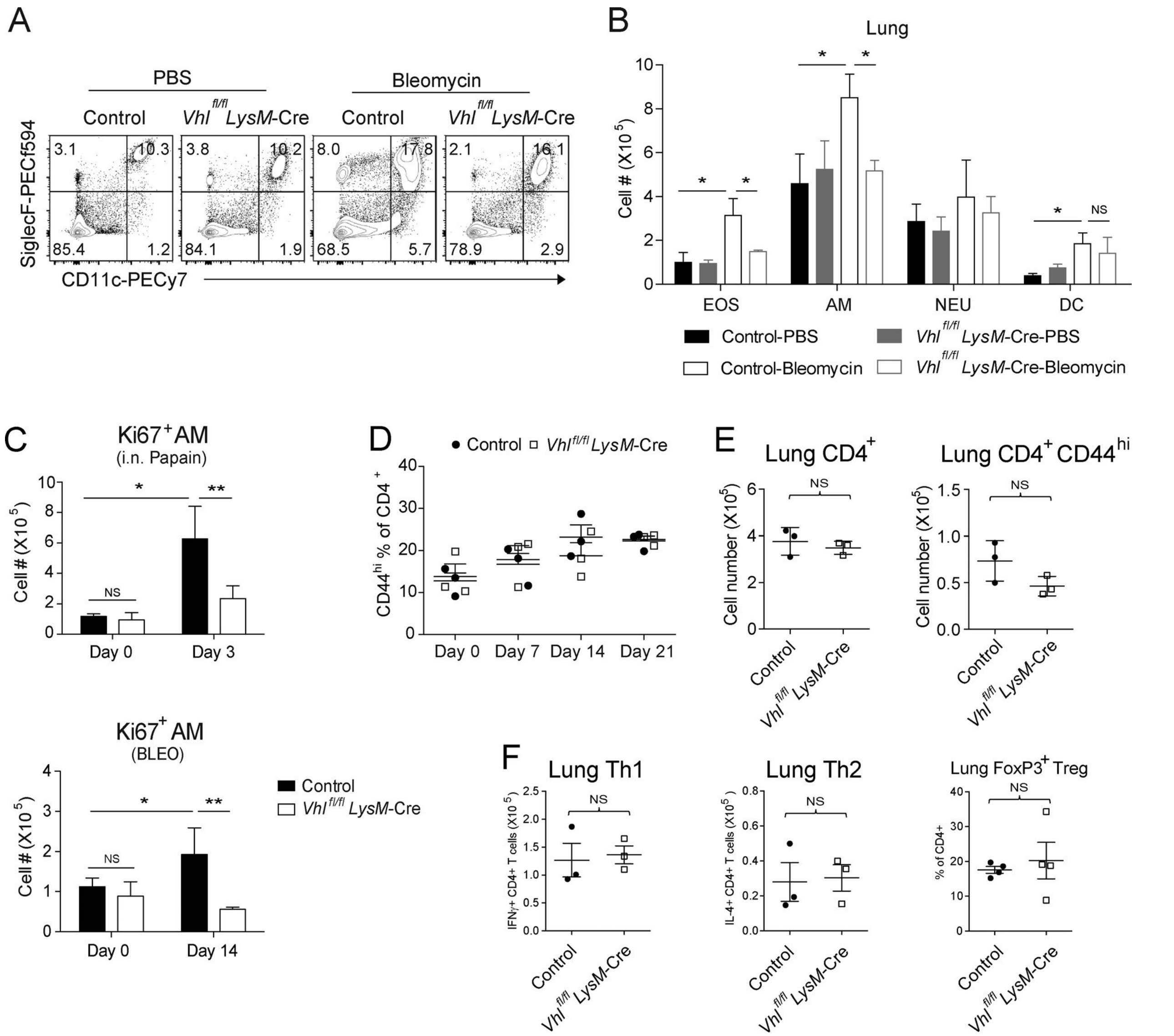

Figure S2. **Loss of myeloid-VHL ameliorates pulmonary fibrosis.** Related to Fig. 2. **(A)** Flow cytometry of CD45⁺ cells in lungs. **(B)** Absolute numbers of CD11b⁺ CD11c⁻ SiglecF⁺ eosinophils (EOS), CD11b⁺ CD11c⁺ SiglecF⁺ AMs, CD11b⁺ Ly6C⁻ Ly6G⁺ neutrophils (NEU), and CD11c⁺ MHCII^{hi} dendritic cells (DC) in the lungs. **(C)** Absolute numbers of Ki67⁺ AMs in papain or bleomycin-treated mice, assessed on indicated time. **(D)** Frequencies of CD4⁺ CD44⁺ T cells in bleomycin-challenged lungs, assessed on days 0, 7, 14, and 21. **(E)** Absolute numbers of lung CD4⁺ T cells or CD4⁺ CD44^{hi} T cells, assessed on day 14 after bleomycin challenge. **(F)** Absolute numbers of lung CD4⁺ IFNγ⁺ CD4⁺ T cells (Th1) and IL-4⁺ CD4⁺ T cells (Th2) and frequencies of lung CD4⁺ FoxP3⁺ regulatory T cells (Treg), assessed on day 14 after bleomycin challenge. *, $P < 0.05$; **, $P < 0.01$ (Student's *t* test). Data are from one experiment representative of three independent experiments (mean and SEM of three to six mice per group).

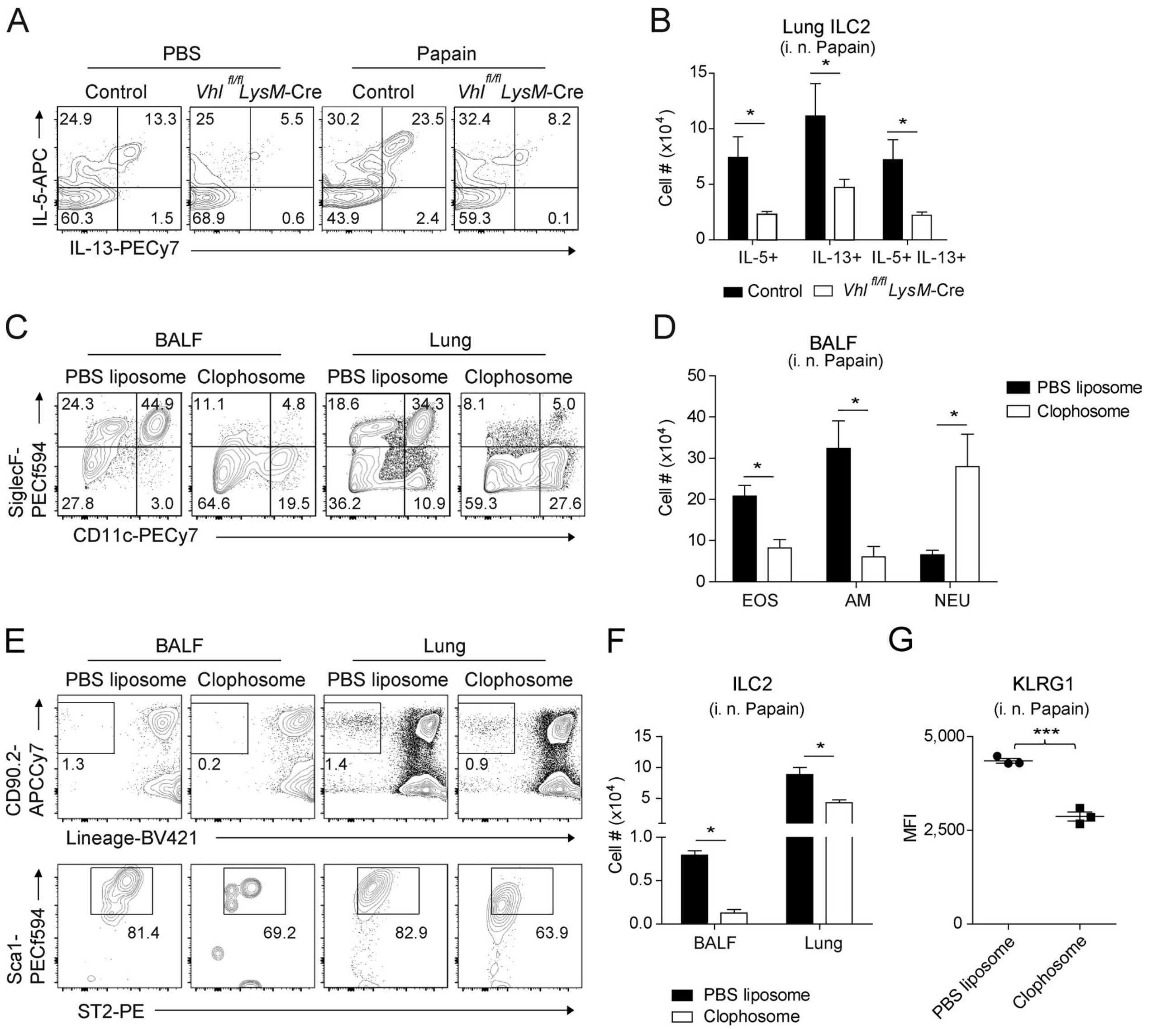

Figure S3. **VHL deficiency impairs lung ILC2 activation and function.** Related to Fig. 3. **(A)** IL-5 and IL-13 expression in CD45+ Lineage− CD90.2+ ST2+ Sca1+ lung ILC2s. **(B)** Absolute numbers of IL-5– or IL-13–producing lung ILC2s. **(C and D)** Flow cytometry and absolute numbers of myeloid cells in lungs of mice treated with control or clodronate liposomes (clophosome) followed by intranasal papain. **(E and F)** Flow cytometry and total numbers of ILC2s. BALF, BAL fluid. **(G)** MFI of KLRG1 in lung ILC2s. *, $P < 0.05$; ***, $P < 0.001$ (Student's *t* test). Data are from one experiment representative of three independent experiments (mean and SEM of three to six mice per group).

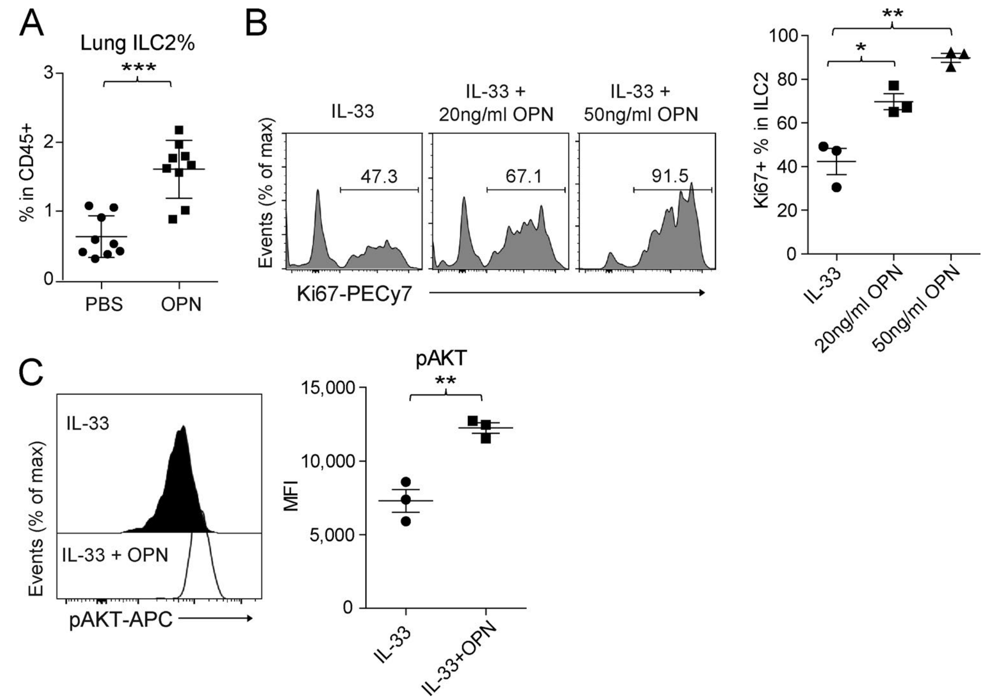

Figure S4. **Osteopontin supports ILC2 expansion.** Related to Fig. 4. **(A)** Frequencies of $CD45^+$ $Lineage^-$ $ST2^+$ $ICOS^+$ lung ILC2s from *Rag1*$^{-/-}$ mice treated with 200 ng OPN for 3 consecutive days. **(B)** Ki67 expression of sorted bone marrow ILC2s cultured in the presence of IL-33 and IL-7, treated without or with mouse recombinant osteopontin (OPN) for 5 d. **(C)** Histogram and MFI of phosphorylated AKT in ILC2s as shown in B. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Student's *t* test). Data are combined from three independent experiments (A) or from one experiment representative of two independent experiments (B and C; mean and SEM of cells from two mice per group).

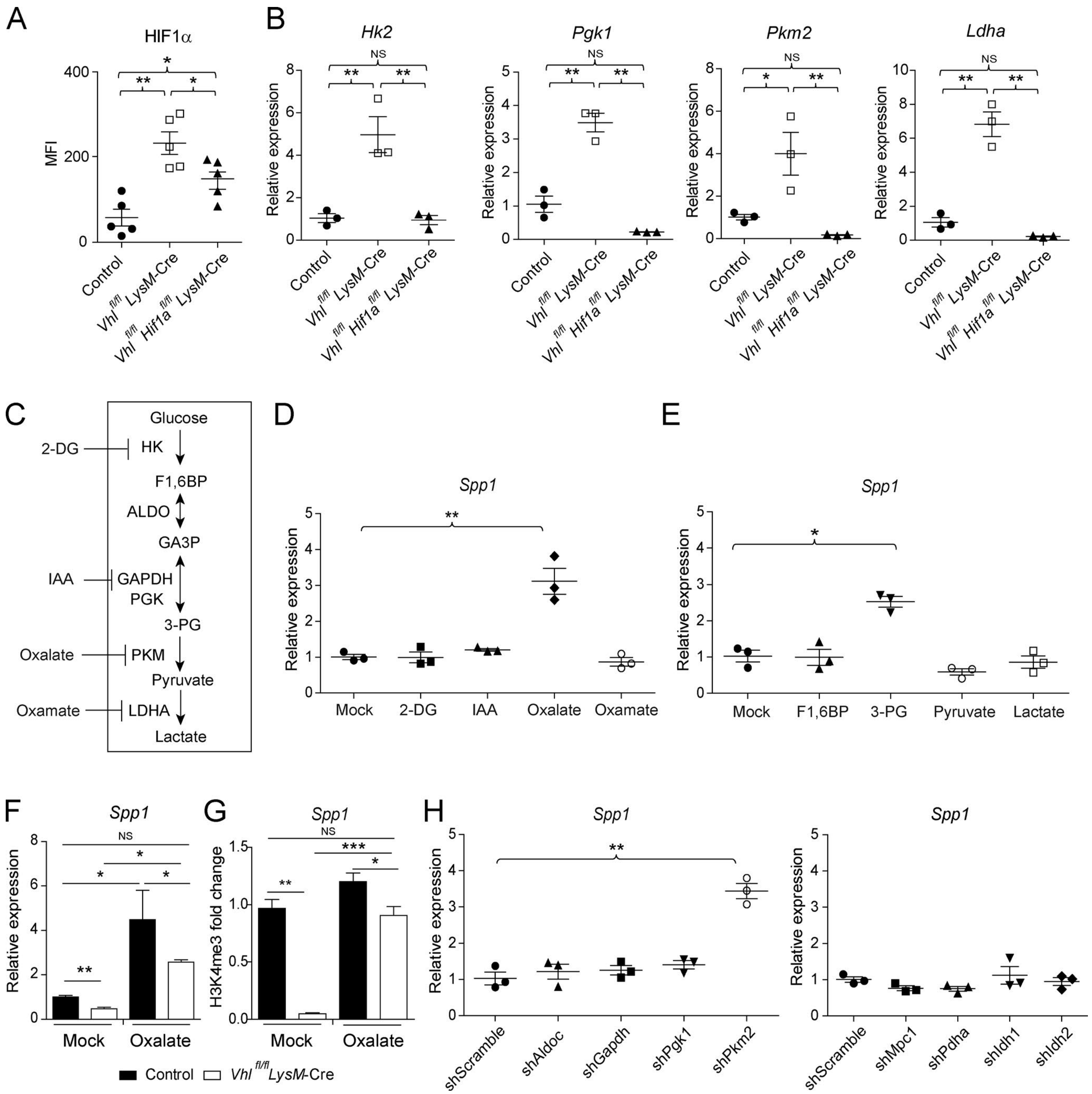

Figure S5. **Control of osteopontin expression by VHL–HIF1α–glycolysis pathway.** Related to Figs. 5 and 6. **(A)** HIF1α expression in CD45⁺ CD11c⁺ SiglecF⁺ AMs. **(B)** mRNA levels of genes encoding glycolytic enzymes in sorted AMs. **(C)** Illustration of the glycolytic pathway and the targets of the indicated glycolytic inhibitors. 2-DG, 2-deoxyglucose; IAA, iodoacetate. **(D and E)** *Spp1* mRNA levels in WT BMDMs treated without (mock) or with small molecular inhibitors targeting indicated glycolytic enzymes or indicated glucose intermediates for 24 h. **(F and G)** *Spp1* mRNA expression and H3K4me3 levels in control and VHL-deficient BMDMs treated without or with oxalate for 24 h. **(H)** *Spp1* mRNA levels in WT BMDMs transduced with retrovirus expressing scrambled shRNA (sh*Scramble*) or shRNA targeting indicated glycolytic enzymes or TCA cycle enzymes. *, P < 0.05; **, P < 0.01; ***, P < 0.001 (Student's *t* test). Data represent the mean and SEM from three independent experiments.

Table S1. **List of oligonucleotide sequences for shRNA knockdown**

Gene	shRNA oligos (5′→3′)
Scramble	TGCTGTTGACAGTGAGCGACCATAGATGTTACCCTTTATTTAGTGAAGCCACAGATGTAAATAAAGGGTAACATCTATGGCTGCCTACTGCCTCGGA
Aldoc	TGCTGTTGACAGTGAGCGCTCTCTCAACCTCAATGCCATCTAGTGAAGCCACAGATGTA GATGGCATTGAGGTTGAGAGAG TGCCTACTGCCTCGGA
Gapdh	TGCTGTTGACAGTGAGCGAACTGAGCATCTCCCTCACAATTAGTGAAGCCACAGATGTAATTGTGAGGGAGATGCTCAGTTTGCCTACTGCCTCGGA
Pgk1	TGCTGTTGACAGTGAGCGACTAGACAAAGTCAATGAGATGTAGTGAAGCCACAGATGTA CATCTCATTGACTTTGTCTAGT TGCCTACTGCCTCGGA
Pkm2	TGCTGTTGACAGTGAGCGCGCTTTGCATCTGATCCCATTCTAGTGAAGCCACAGATGTA GAATGGGATCAGATGCAAAGCG TGCCTACTGCCTCGGA
Mpc1	TGCTGTTGACAGTGAGCGCGCTATCAATGACATGAAGAAATAGTGAAGCCACAGATGTA TTTCTTCATGTCATTGATAGCG TGCCTACTGCCTCGGA
Pdha	TGCTGTTGACAGTGAGCGAGCGGATCAGCTGTATAAGCAGTAGTGAAGCCACAGATGTA CTGCTTATACAGCTGATCCGCT TGCCTACTGCCTCGGA
Idh1	TGCTGTTGACAGTGAGCGCTCTGACTACTTGAATACATTTTAGTGAAGCCACAGATGTA AAATGTATTCAAGTAGTCAGAG TGCCTACTGCCTCGGA
Idh2	TGCTGTTGACAGTGAGCGCCGTGGGAAGCTGGATGGGAACTAGTGAAGCCACAGATGTA GTTCCCATCCAGCTTCCCACGG TGCCTACTGCCTCGGA

Table S2. **Primers used for quantitative real-time PCR**

Name	Forward primer (5′→3′)	Reverse primer (5′→3′)
Vhl	AAGAGCACGCAGCTTAGGAG	TTTCTGAGTCCTGGGGATTG
Hk2	GGAGAGCACGTGTGACGAC	GATGCGACAGGCCACAGCA
Aldoc	GGAAAAGTGAGCTGTGCTGTG	GCTGCCTACGGACTCATCTG
Pgk1	GATTCAGGTTCACGTCACGCT	GACGGATTCTGTCGACAGAG
Pkm2	TTGCAGCTATTCGAGGAACTCCG	CACGATAATGGCCCCACTGC
Ldha	CACAAGCAGGTGGTGGACAG	AACTGCAGCTCCTTCTGGATTC
Spp1	GCCTGTTTGGCATTGCCTCCTC	CACAGCATTCTGTGGCGCAAGG
Tgfb1	AGCTGCGCTTGCAGAGATTA	AGCCCTGTATTCCGTCTCCT
Actb	GCTGTGCTGTCCCTGTATGCCTCT	CCTCTCAGCTGTGGTGGTGAAGC

Table S3. **Primers used for ChIP assay**

Name	Forward primer (5′→3′)	Reverse primer (5′→3′)
Spp1-I	TCCTTGCCTCTGCTGAAGAT	CCAGACTGCCTTTCAGTCCT
Spp1-II	CAGAGCAACAAGGTTCACGA	CAGTTGGGGCAACAGAAAGT
Spp1-III	TGATGCTCTTCCGGGATTCT	CAGGAGGTGGAGTGATGTGT
Spp1-IV	CTGTTTGGCATTGCCTCCTC	TGCGTGTGAGTGTGCTAAAG
Spp1-V	TGCACCCAGATCCTATAGCC	CTGTGGCGCAAGGAGATTC
Spp1-VI	TGCACCCAGATCCTATAGCC	CTGTGGCGCAAGGAGATTC
Spp1	TCTGAGGGTGAGTCCAGAGA	ACCTGCAATGTACCTGACCA
Tgfb1-promoter	TGCACGCAGATACCATCTACA	GCTTCACTGCTGTGCCATTA
Tgfb1-exon	GTGGCCAGATCCTGTCCAAACTAA	ATTAGCACGCGGGTGACCTCTTTA

Table S4. **Antibody list**

Antibody	Clone	Vendor
FACS		
Anti-CD3ε	145-2C11	BioLegend
Anti-CD4	GK1.5	BioLegend
Anti-CD8α	53-6.7	BioLegend
Anti-CD11b	M1/70	BioLegend
Anti-CD11c	N418	BioLegend
Anti-CD16/32	93	eBioscience
Anti-CD19	6D5	BioLegend
Anti-CD25	PC61.5	eBioscience
Anti-CD44	IM7	eBioscience
Anti-CD45	30-F11	BioLegend
Anti-CD62L	MEL-14	eBioscience
Anti-CD90.2	53-2.1	eBioscience
Anti-ICOS	C398.4A	eBioscience
Anti-F4/80	BM8	eBioscience
Anti-Ly6C	HK1.4	BioLegend
Anti-Ly6G	1A8	BioLegend
Anti-Sca1	D7	BD Biosciences
Anti-Gr1	RB6-8C5	BioLegend
Anti-MHCII	M5/114.15.2	BioLegend
Anti-SiglecF	E50-2440	BD Biosciences
Anti-ST2	RMST2-2	eBioscience
Anti–IL-7Rα	A7R34	eBioscience
Anti-B220	RA3-6B2	BioLegend
Anti-NK1.1	PK136	BioLegend
Anti-KLRG1	2F1	eBioscience
Anti-TCRβ	H57-597	BioLegend
Anti-TCRγδ	GL3	BioLegend
Anti-TER119	TER-119	BioLegend
Anti-FoxP3	FJK-16s	eBioscience
Anti-HIF1α	241812	R&D Systems
Anti-Ki67	SolA15	eBioscience
Anti-IFNγ	XMG1.2	eBioscience
Anti–IL-4	11B11	eBioscience
Anti–IL-5	TRFK5	BioLegend
Anti–IL-13	eBio13A	eBioscience
Anti-pAKT1	SDRNR	eBioscience
ChIP		
Anti-H3K4me3	ab8580	Abcam
Neutralization		
Anti-Osteopontin	AF808	R&D Systems

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