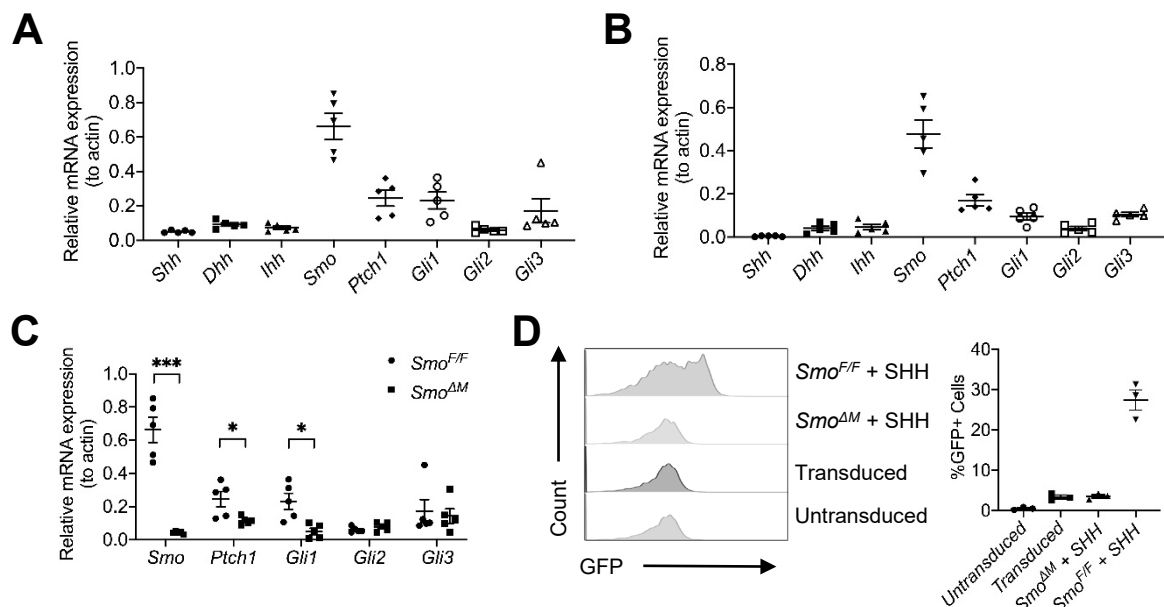
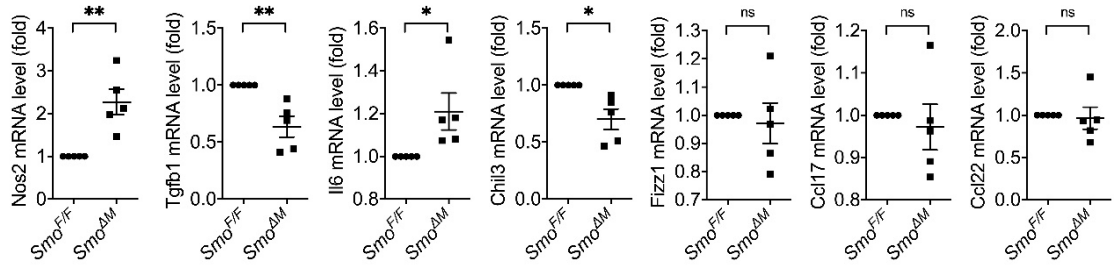
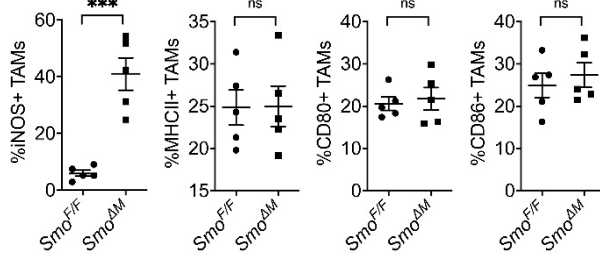
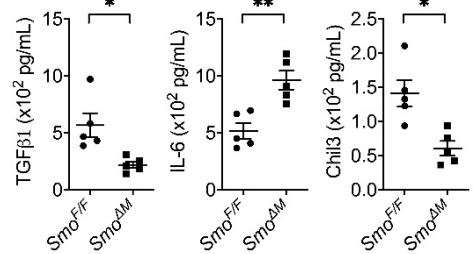


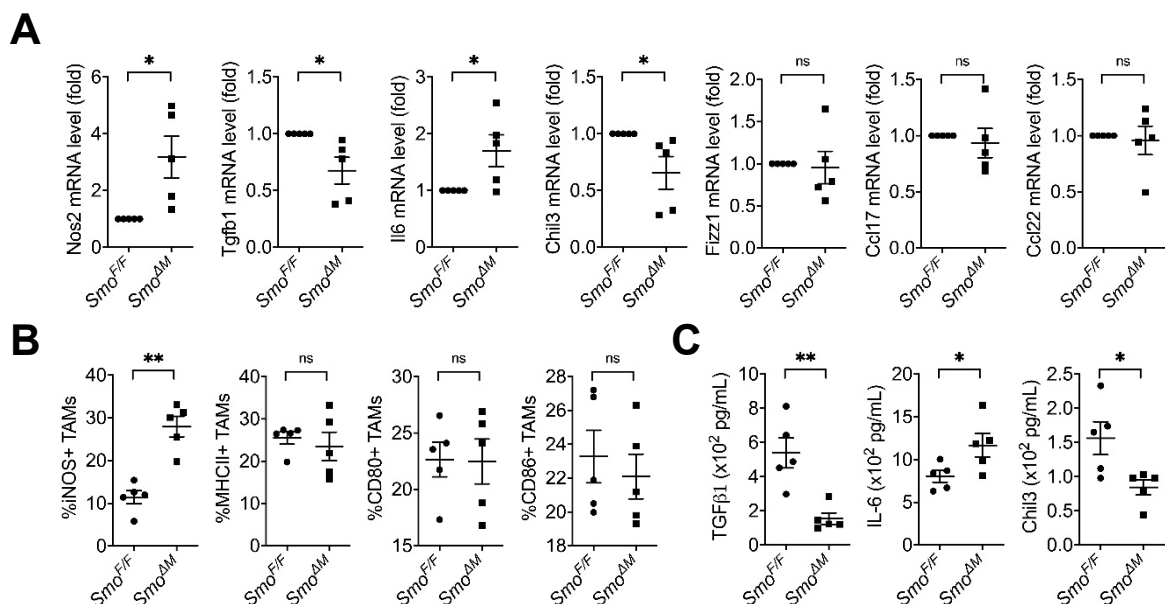
Supplemental Figure 1. Myeloid-specific *Smo* deletion does not impact myeloid cell numbers. (A) Number and percentage of F4/80⁺CD11b⁺ tumor-associated macrophages (TAMs) in Hepa1-6 tumor stroma were enumerated by FACS. (B) Percentages of F4/80⁺CD11b⁺ macrophages, Ly6G⁺CD11b⁺ neutrophils and Ly6C⁺CD11b⁺ monocytes in the TME were assessed using FACS. (C) Expressions of ICAM1, MHCII, FasL and TNFα in tumor-associated neutrophils (TANS) were measured with FACS. (D) Numbers of CD11b⁺Ly6C⁺ monocytes in circulation and the spleen and CD11c⁺MHCII⁺ dendritic cells (DCs) in the spleen of *Smo^{F/F}* and *Smo^{ΔM}* mice were measured by FACS. (E) Representative CFSE staining of OT1⁺ CD8⁺ T cells co-cultured with Ova-I peptide-pulsed DCs isolated from *Smo^{F/F}* and *Smo^{ΔM}* mice measured by FACS. Values are mean ± SEM of a minimum of two independent experiments. ns = no significance. n=5 biological replicates per group (A, B, D & E). N = 3 biological replicates per group (C). 2-tailed Student's t-test (A, C, D & E)



Supplemental Figure 2. Hedgehog signaling is active in macrophages. Expression levels of *Shh*, *Dhh*, *Ihh*, *Smo*, *Ptch1*, *Gli1*, *Gli2* and *Gli3* in TAMs (A) and macrophages (B) were measured by qRT-PCR and normalized to β -actin. (C) Expression levels of *Smo*, *Ptch1* and *Gli1* in *Smo*^{F/F} and *Smo*^{ΔM} TAMs measured by qRT-PCR. (D) Representative plot of GFP activity in macrophages untransduced or transduced with pGreenFire-Gli reporter. Data representative of a minimum of 2 independent experiments. *P<0.05. ***P<0.0005. Values are mean \pm SEM. n=5 biological replicates per group (A-C). n=3 technical replicates per group (D). 2-tailed Student's t-test (C).

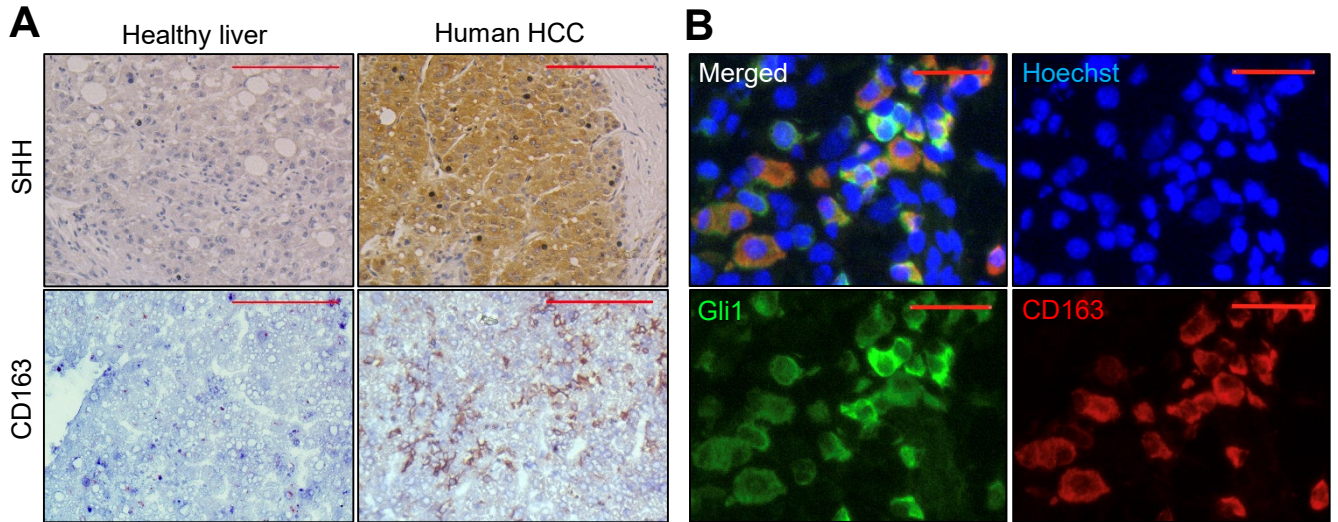
A**B****C**

Supplemental Figure 3. Loss of Hh signaling in myeloid cells interferes with M2-polarization of TAMs in subcutaneously implanted HCC. (A) Expression levels of *Nos2*, *Tgfb1*, *il6*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* in TAMs were measured by qRT-PCR. Expressions were normalized to β -actin and compared to *Smo^{F/F}* TAMs. **(B)** Expressions of iNOS, MHCII, CD80 and CD86 in TAMs were measured by FACS. **(C)** TGFβ1, IL-6 and Chil3 secretion from TAMs were measured by CBA and ELISA. Values are mean \pm SEM of a minimum of three independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=5 biological replicates per group **(A-C)**. 2-tailed Student's t-test **(A-C)**.

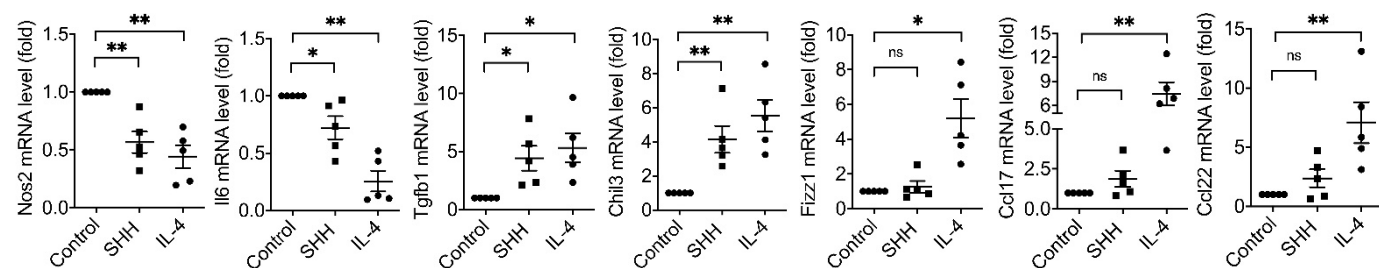
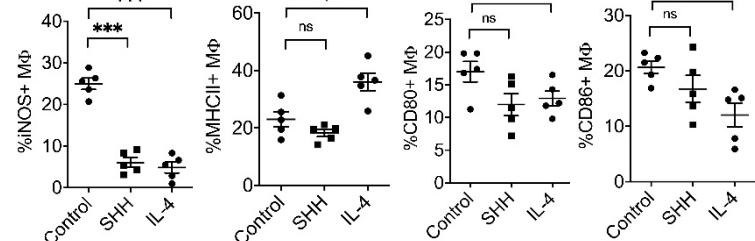
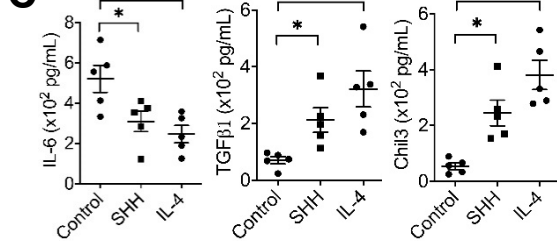


Supplemental Figure 4. Loss of Hh signaling in myeloid cells interferes with M2-polarization of TAMs in autochthonous HCC.

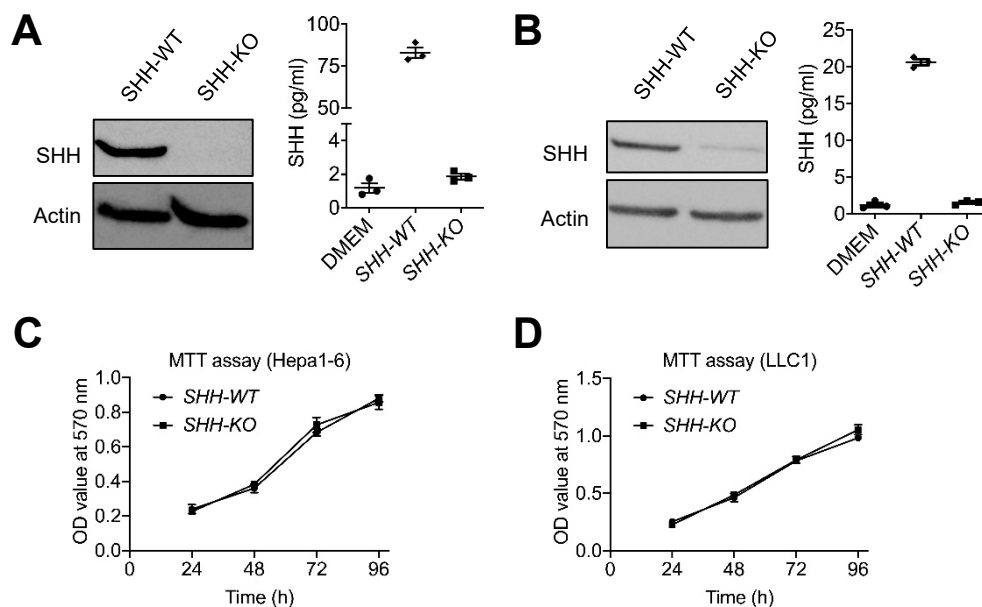
(A) Expression levels of *Nos2*, *Tgfb1*, *il6*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* in TAMs were measured by qRT-PCR. Expressions were normalized to β -actin and compared to *Smo^{F/F}MDR2^{-/-}* TAMs. **(B)** Expressions of CD206, iNOS, CD80 and CD86 in TAMs were measured by FACS. **(C)** TGFβ1, IL-6 and Chil3 secretion from TAMs were measured by CBA and ELISA. Values are mean \pm SEM of a minimum of three independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=5 biological replicates per group **(A-C)**. 2-tailed Student's t-test **(A-C)**.



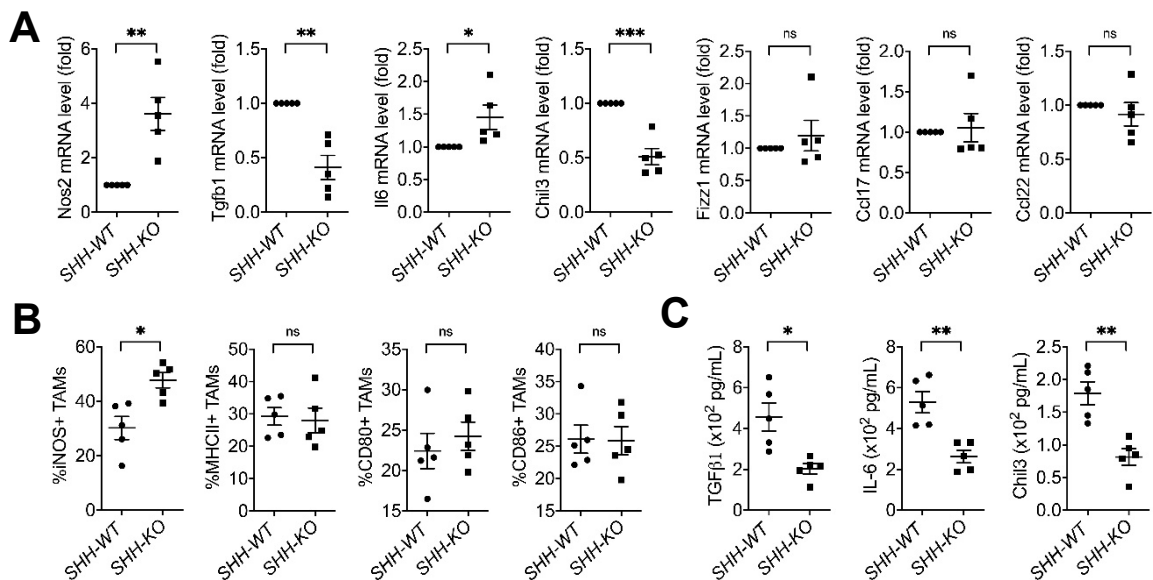
Supplemental Figure 5. Human M2 TAMs express Gli1 in HCC tumors with high SHH activity. (A) Representative immunohistochemical staining of SHH and CD163 in human HCC tumor samples with matched healthy liver controls. Scale bar = 100 μ m. (B) Representative immunofluorescent staining of Gli1 (green), CD163 (red) and nuclei (blue) with human HCC tumor samples. Scaled bar = 25 μ m. n = 3 biological replicates (A & B).

A**B****C**

Supplemental Figure 6. Effects of SHH treatment on macrophages *in vitro*. (A) *Nos2*, *Il6*, *Tgfb1*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* mRNA expression levels in Mφ were measured by qRT-PCR. Expressions were normalized to β -actin and compared to control. (B) Expressions of iNOS, MHCII, CD80 and CD86 in Mφ were measured by FACS. (C) IL-6, TGFβ1 and Chil3 secretions from Mφ were measured by CBA and ELISA. Values are mean \pm SEM of a minimum of three independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=5 biological replicates per group (A-C). One-way ANOVA (A-C).

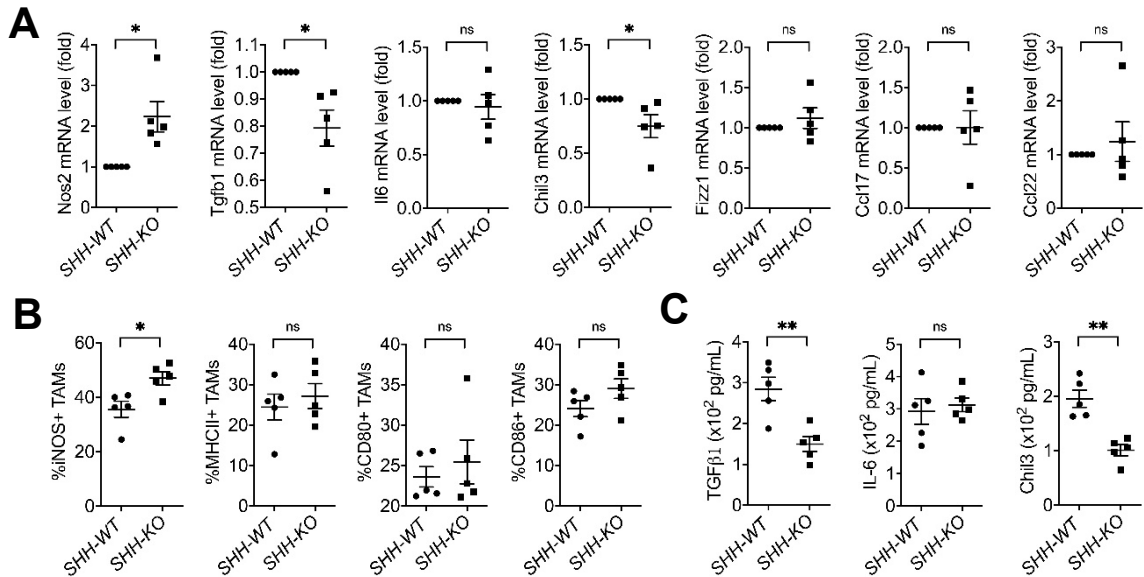


Supplemental Figure 7. Generation of SHH knocked-out tumor cell lines using the CRISPR-Cas9 technology. CRISPR-Cas9 knockout status of *SHH* in Hepa1-6 (**A**) and LLC1 (**B**) cells was confirmed with ELISA and Western blotting. Deletion of SHH in Hepa1-6 (**C**) and LLC1 (**D**) cells did not affect cell viability as shown by MTT assay. Values are mean \pm SEM of a minimum of two independent experiments. $n=3$ technical replicates per group (**A-D**).

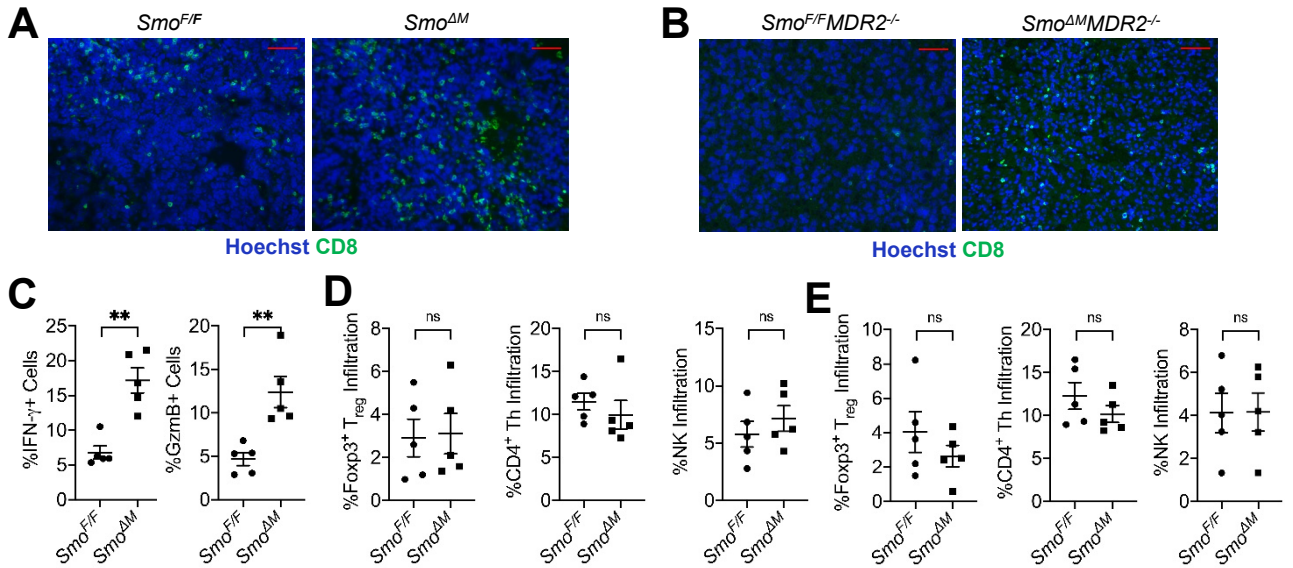


Supplemental Figure 8. Tumor-derived SHH from Hepa1-6 hepatoma promotes TAM M2-polarization *in vivo*. (A)

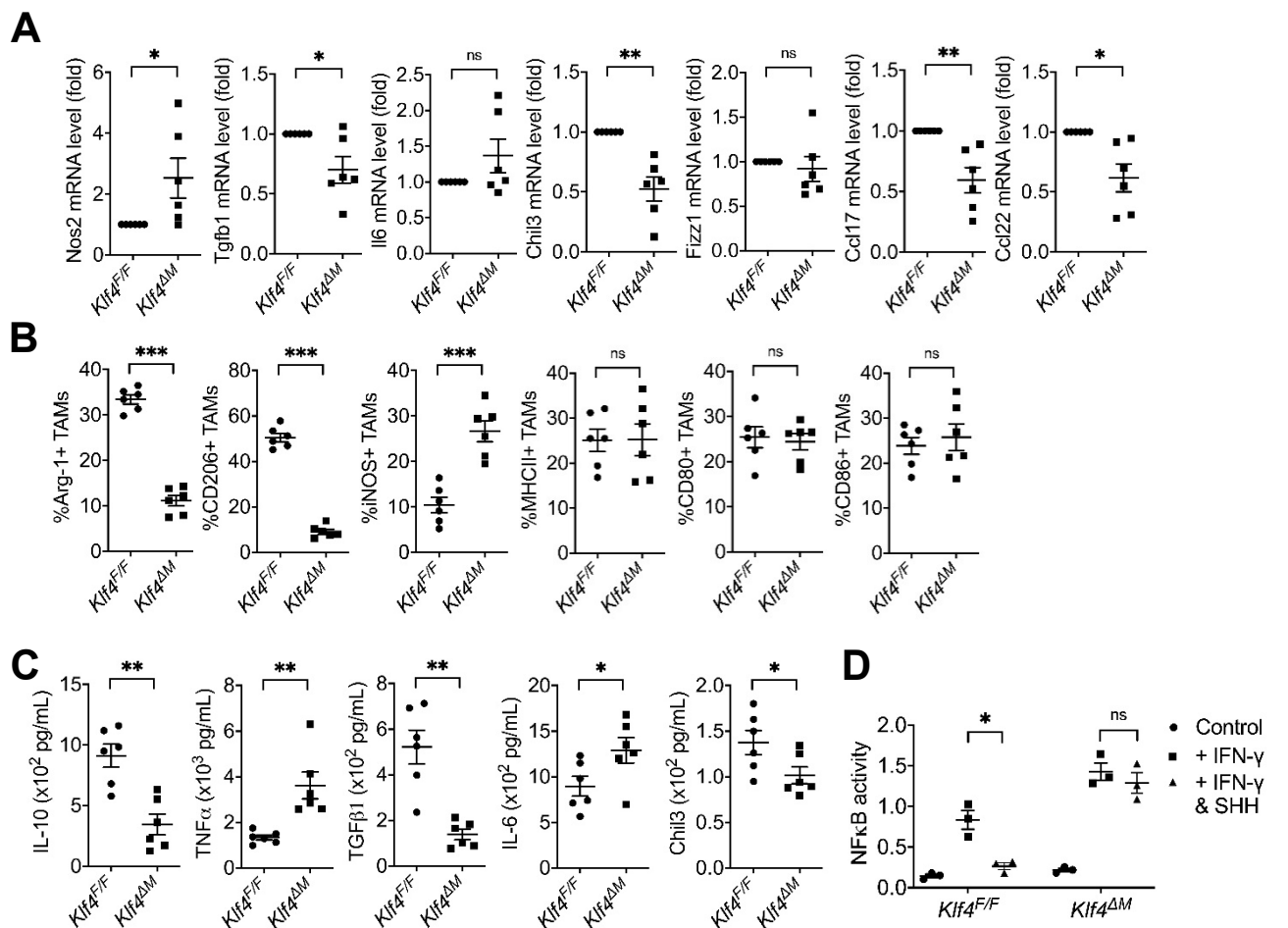
Expression levels of *Nos2*, *Tgfb1*, *il6*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* in TAMs purified from Hepa1-6 *SHH-WT* and Hepa1-6 *SHH-KO* tumor samples were measured by qRT-PCR. Expressions were normalized to β -actin and compared to *SHH-WT* TAMs. **(B)** Expressions of iNOS, MHCII, CD80 and CD86 in TAMs were measured by FACS. **(C)** TGFβ1, IL-6 and Chil3 secretion from TAMs were measured by CBA and ELISA. Values are mean ± SEM of a minimum of three independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=5 biological replicates per group **(A-C)**. 2-tailed Student's t-test **(A-C)**.



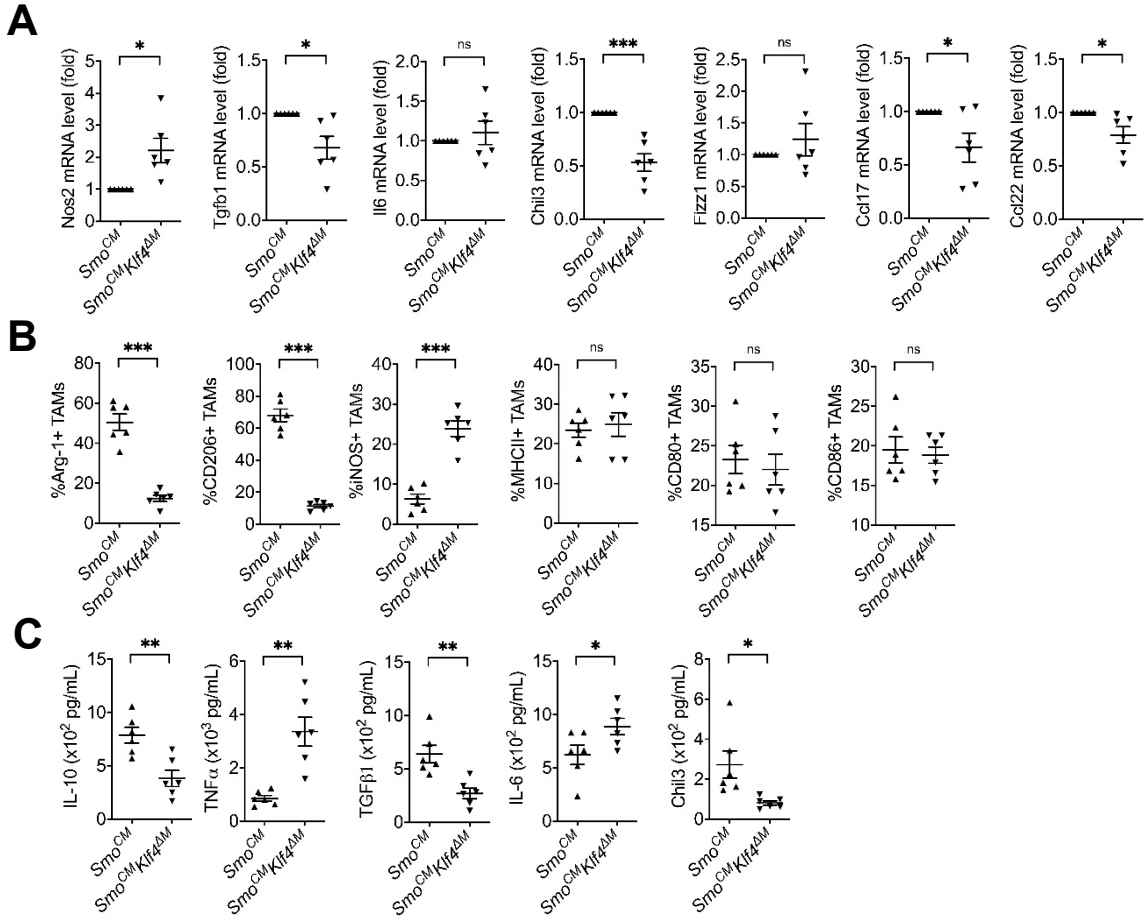
Supplemental Figure 9. Tumor-derived SHH from LLC1 Lewis lung carcinoma promotes TAM M2-polarization *in vivo*. (A) Expression levels of *Nos2*, *Tgfb1*, *il6*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* in TAMs purified from LLC1 *SHH-WT* and Hepa1-6 *SHH-KO* tumor samples were measured by qRT-PCR. Expressions were normalized to β -actin and compared to *SHH-WT* TAMs. (B) Expressions of iNOS, MHCII, CD80 and CD86 in TAMs were measured by FACS. (C) TGFβ1, IL-6 and Chil3 secretion from TAMs were measured by CBA and ELISA. Values are mean ± SEM of a minimum of three independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=5 biological replicates per group (A-C). 2-tailed Student's t-test (A-C).



Supplemental Figure 10. Characterizing the effects of *Smo* deletion in myeloid cells on other tumor-infiltrating immune cells. (A) Representative IF staining of CD8⁺ T cells in Hepa1-6 tumors inoculated in *Smo^{F/F}* and *Smo^{ΔM}* mice and (B) autochthonous HCC from *Smo^{F/F}MDR2^{-/-}* and *Smo^{ΔM}MDR2^{-/-}* mice. CD8⁺ T cells were stained with Alexa Fluor488 labeled anti-CD8a antibody (green) and nuclear Hoechst staining (blue). Scale bar = 100 μm. (C) Intracellular production of IFNγ and GranzymeB in *Smo^{F/F}* and *Smo^{ΔM}* TAMs were measured by FACS. (D) Percentages of CD4⁺Foxp3⁺ T_{reg}, CD4⁺ T cell and CD3⁺NK1.1⁺ NK cell in the stroma of subcutaneously inoculated Hepa1-6 tumor in *Smo^{F/F}* and *Smo^{ΔM}* mice. (E) Percentages of CD4⁺Foxp3⁺ T_{reg}, CD4⁺ T cell and CD3⁺NK1.1⁺ NK cell in the stroma of autochthonous HCC in *Smo^{F/F}MDR2^{-/-}* and *Smo^{ΔM}MDR2^{-/-}* mice. Values are mean ± SEM of a minimum of three independent experiments. ns = no significance. **P<0.005. n=5 biological replicates per group. 2-tailed Student's t-test (C-E).



Supplemental Figure 11. Klf4 is important for TAM M2-polarization *in vivo*. (A) Expression levels of *Nos2*, *Tgfb1*, *il6*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* in *Klf4^{F/F}* and *Klf4^{ΔM}* TAMs were measured by qRT-PCR. Expressions were normalized to β -actin and compared to *Klf4^{F/F}* TAMs. (B) Expressions of Arg-1, CD206, iNOS, MHCII, CD80 and CD86 in TAMs were measured by FACS. (C) IL-10, TNF α , TGF β 1, IL-6 and Chil3 secretion from TAMs were measured by ELISA. (D) NF κ B p65 activity in the nuclear extract of *Klf4^{F/F}* and *Klf4^{ΔM}* Mφ untreated (control) or treated with IFN- γ and IFN- γ + SHH was measured by ELISA. Values are mean \pm SEM of a minimum of two independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=6 biological replicates per group (A-C). n=3 technical replicates per group (D). 2-tailed Student's t-test (A-C). Two-way ANOVA (D).



Supplemental Figure 12. Myeloid-specific deletion of *Klf4* interferes with Hh-induced TAM M2-polarization *in vivo*.

(A) Expression levels of *Nos2*, *Tgfb1*, *il6*, *Chil3*, *Fizz1*, *Ccl17* and *Ccl22* in Smo^{CM} and Smo^{CM}Klf4^{ΔM} TAMs were measured by qRT-PCR. Expressions were normalized to β -actin and compared to Smo^{CM} TAMs. (B) Expressions of Arg-1, CD206, iNOS, MHCII, CD80 and CD86 in TAMs were measured by FACS. (C) IL-10, TNF α , TGF β 1, IL-6 and Chil3 secretion from TAMs were measured by ELISA. Values are mean \pm SEM of a minimum of three independent experiments. ns = no significance. *P<0.05. **P<0.005. ***P<0.0005. n=6 biological replicates per group (A-C). 2-tailed Student's t-test (A-C).

Supplemental Table 1 – Antibodies

Antigen	Fluorophore	Manufacturer	Clone
F4/80 (mouse)	APC-Cy7	BioLegend	BM8
CD11b (mouse)	PE-Cy5	BioLegend	M1/70
Ly6G (mouse)	PE	BioLegend	1A8
Ly6C (mouse)	PE-Cy7	BD Biosciences	AL-21
CD3 (mouse)	APC	BioLegend	17A2
CD4 (mouse)	PE	BioLegend	GK1.5
CD8a (mouse)	PE-Cy5	BioLegend	53-6.7
NK1.1 (mouse)	PE	BioLegend	PK136
Foxp3 (mouse)	FITC	BioLegend	MF-14
CD206 (mouse)	FITC	BioLegend	CD68C2
MHCII (mouse)	FITC	BioLegend	M5/114.15.2
CD80 (mouse)	PE	BD Biosciences	16-10A1
CD86 (mouse)	Biotin	BD Biosciences	PO3
iNOS (mouse)	FITC	BD Biosciences	Clone 6
Arg-1 (mouse)	APC	R&D Systems	N/A
SHH (human/mouse)	None	Cell Signaling Technology	C9C5
Gli1 (human/mouse)	None	Novus Biologicals	N/A
CD163 (human)	None	Abcam	EPR19518
IgG (rabbit)	Alex Fluor488	Invitrogen	N/A
Streptavidin	APC	BioLegend	N/A
IgG2a, κ	FITC	BioLegend	RTK2758
IgG2a, κ	APC	BioLegend	RTK2758
IgG	PE	BioLegend	HTK888

Supplemental Table 2 – qRT-PCR primers

Target	Direction	Sequence
Arg1	Forward	5'-CCACAGTCTGGCAGTTGGAAG-3'
	Reverse	5'-GGTTGTCAGGGGAGTGTTGATG-3'
Nos2	Forward	5'-CCAAGCCCTCACCTACTTCC-3'
	Reverse	5'-CTCTGAGGGCTGACACAAGG-3'
Mrc1	Forward	5'-AAGGCTATCCTGGTGGGAAGAA-3'
	Reverse	5'-AGGGAAGGGTCAGTCTGTGTT-3'
Il10	Forward	5'-GGTTGCCAAGCCTTATCGGA-3'
	Reverse	5'-ACCTGCTCCACTGCCTTGCT-3'
Tnf	Forward	5'-TCTCATGCACCACCATCAAGGACT-3'
	Reverse	5'-TGACCACTCTCCCTTGCAGAACT-3'
Il6	Forward	5'-TCCAGTTGCCTTCTTGGGAC-3'
	Reverse	5'-GTA CTCCAGAAGACCAGAGG-3'
Tgfb1	Forward	5'-AGCTGCTTATCCCAGATTCAGCCA-3'
	Reverse	5'-TATCGAGGCCAGCTTGTTTGAGGA-3'
Chil3	Forward	5'-GGGCATACCTTTATCCTGAG-3'
	Reverse	5'-CCACTGAAGTCATCCATGTC-3'
Fizz1	Forward	5'-CCTGCTGGGATGACTGCTA-3'
	Reverse	5'-TGGGTTCTCCACCTCTTCAT-3'
Ccl17	Forward	5'-TAAGACCTCAGTGGAGTGTC-3'
	Reverse	5'-AAATGCCTCAGCGGGAAGC-3'
Ccl22	Forward	5'-GTGCCGATCCCAGGCAGGTC-3'
	Reverse	5'-GGCGTCGTTGGCAAGGCTCT-3'
Shh	Forward	5'-CTGGCCAGATGTTTTCTGGT-3'
	Reverse	5'-TAAAGGGGTCAGCTTTTTGG-3'
Dhh	Forward	5'-GGGACCTCGTACCCA ACTAC-3'
	Reverse	5'-CTTTGCAACGCTCTGTCATC-3'
Ihh	Forward	5'-CCGAACCTTCATCTTGGTG-3'
	Reverse	5'-ACAGATGGAATGCGTG TGAA-3'
Smo	Forward	5'-GACTCCGTGAGTGGCATCTG-3'
	Reverse	5'-GTGGCAGCTGAAGGTGATGA-3'
Ptch1	Forward	5'-AGGCGCTAATGTTCTGACCA-3'
	Reverse	5'-CCTCCTGCCAATGCATATAC-3'
Gli1	Forward	5'-CCAAGCCA ACTTTATGTCAGGG-3'
	Reverse	5'-TCCTAAAGAAGGGCTCATGGTA-3'
Gli2	Forward	5'-CAACGCCTACTCTCCAGAC-3'
	Reverse	5'-GAGCCTTGATGTACTGTACCAC-3'
Gli3	Forward	5'-GAGCACTTGATGCTCCACAA-3'
	Reverse	5'-GGGTGCTCTTCAGCTTTGAG-3'
Ccl3	Forward	5'-ACTGCCTGCTGCTTCTCCTACA-3'
	Reverse	5'-AGGAAAATGACACCTGGCTGG-3'
Ccl4	Forward	5'-CCATGAAGCTCTGCGTGTCTG-3'
	Reverse	5'-GGCTTGGAGCAAAGACTGCTG-3'
Ccl5	Forward	5'-AGATCTCTGCAGCTGCCCTCA-3'
	Reverse	5'-GGAGCACTTGCTGCTGGTGTAG-3'
Cxcl9	Forward	5'-CCGAGGCACGATCCACTACA-3'
	Reverse	5'-CGAGTCCGGATCTAGGCAGGT-3'
Cxcl10	Forward	5'-GGATGGCTGTCCTAGCTCTG-3'
	Reverse	5'-TGAGCTAGGGAGGACAAGGA-3'
Klf2	Forward	5'-ACAGACTGCTATTTATTGGACCTTAG-3'
	Reverse	5'-CAGAACTGGTGGCAGAGTCATTT-3'

Target	Direction	Sequence
Klf4	Forward	5'-GGTGCAGCTTGCAGCAGTAA-3'
	Reverse	5'-AAAGTCTAGGTCCAGGAGGTCGTT-3'
Stat6	Forward	5'-TCTCCACGAGCTTCACATTG-3'
	Reverse	5'-GACCACCAAGGGCAGAGAC-3'
Cebpb	Forward	5'-GGTTTCGGGACTTGATGCA-3'
	Reverse	5'-CAACAACCCCGCAGGAAC-3'
Pparg	Forward	5'-CGGTTTCAGAAGTGCCTTG-3'
	Reverse	5'-GGTTCAGCTGGTCGATATCAC-3'
Actb	Forward	5'-GGTCCACACCCGCCACCAG-3'
	Reverse	5'-CACATGCCGGAGCCGTTGTC-3'