Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/23/eaaz6105/DC1

Supplementary Materials for

Reprogramming of tumor-associated macrophages by targeting β-catenin/FOSL2/ARID5A signaling: A potential treatment of lung cancer

Poonam Sarode, Xiang Zheng, Georgia A. Giotopoulou, Andreas Weigert, Carste Kuenne, Stefan Günther, Aleksandra Friedrich, Stefan Gattenlöhner, Thorsten Stiewe, Bernhard Brüne, Friedrich Grimminger, Georgios T. Stathopoulos, Soni Savai Pullamsetti, Werner Seeger, Rajkumar Savai*

*Corresponding author. Email: rajkumar.savai@mpi-bn.mpg.de

Published 5 June 2020, *Sci. Adv.* **6**, eaaz6105 (2020)
DOI: 10.1126/sciadv.aaz6105

This PDF file includes:

Fig. S1

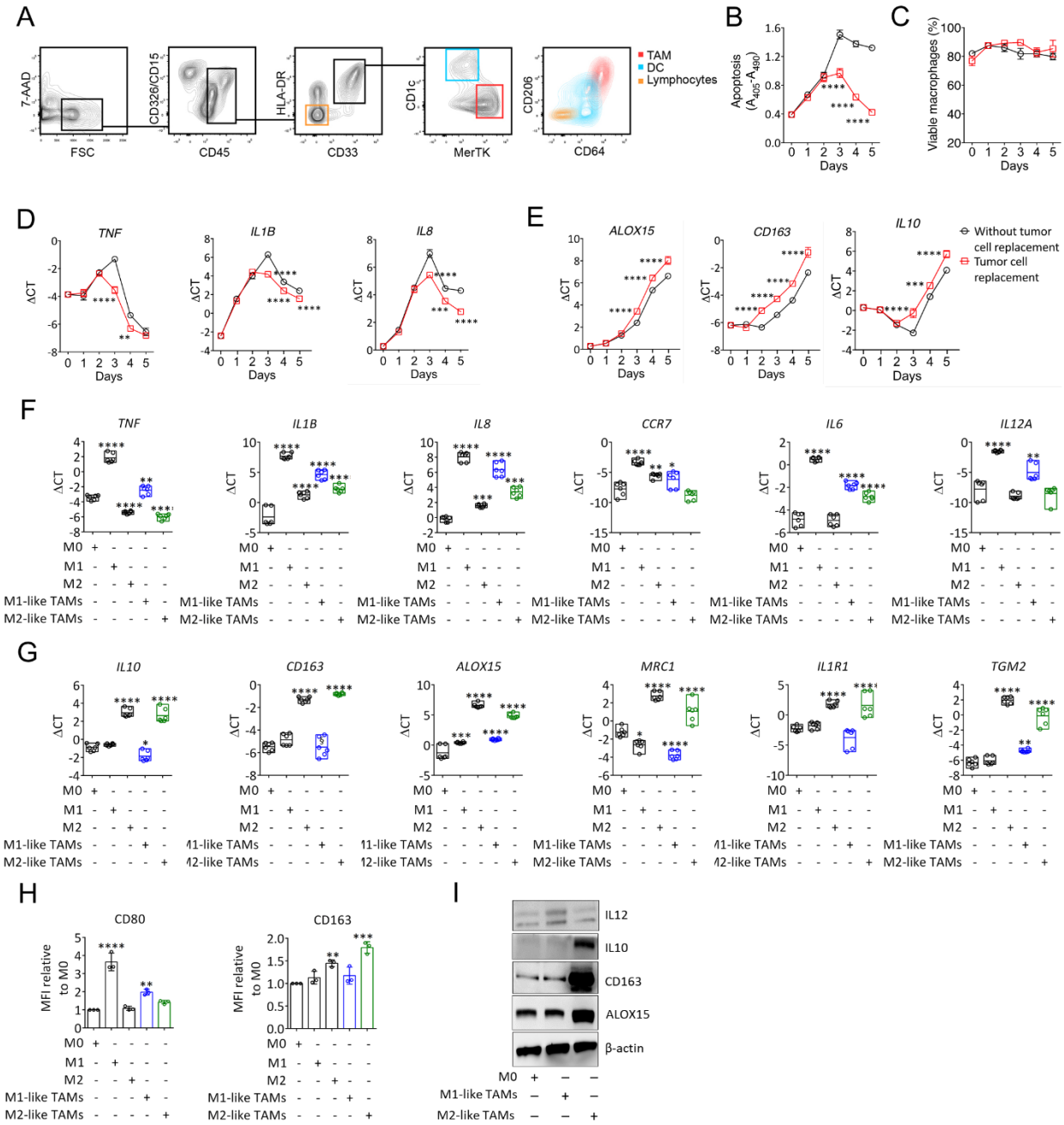

Fig. S1. Generation of *in vitro* trained M1-like and M2-like TAMs: (A) Representative FACS plot demonstrating macrophages ($CD15^+$, $CD45^+$, $CD33^+$, $HLA\text{-}DR^+$, $CD1c^+$, $MerTK^+$, $CD64^+$, $CD206^+$, and $CD326^-$) isolated from human lung cancer tissues (n=12) Macrophages and A549 tumor cells cultured together for 1–5 days without the addition of new A549 cells (black line) and cultured together for 5 days with the replacement of new tumor cells on all days (red line). **(B)** Apoptosis of cocultured tumor cells from each time point, n=12, **/***/****P < 0.01/0.001/0.0001 versus day 0 **(C)** Viable cocultured macrophages from each time point; n=4 biological replicates. Relative mRNA quantification of **(C)** M1 macrophage markers (*TNF*, *IL1B*, and *IL8*) and **(D)** M2 macrophage markers (*IL10*, *CD163*, and *ALOX15*) in cocultured macrophages at each time point, n=4. mRNA expression of **(E)** *TNF, IL1B*, *IL8*, *CCR7*, *IL6*, *IL12A* **(F)** *IL10, CD163*, *ALOX15*, *MRC1*, *IL1R1*, *TGM2* **(H)** FACS analysis of CD80, CD163 in M0, M1, M2, A549-trained M1-like, M2-like TAMs, n=3, */**/***/****P <

0.05/0.01/0.001/0.0001 versus M0 **(I)** Western blot of IL12, IL10, CD163, ALOX15, CCL18 in M0 and A549-trained M1-like, M2-like TAMs.

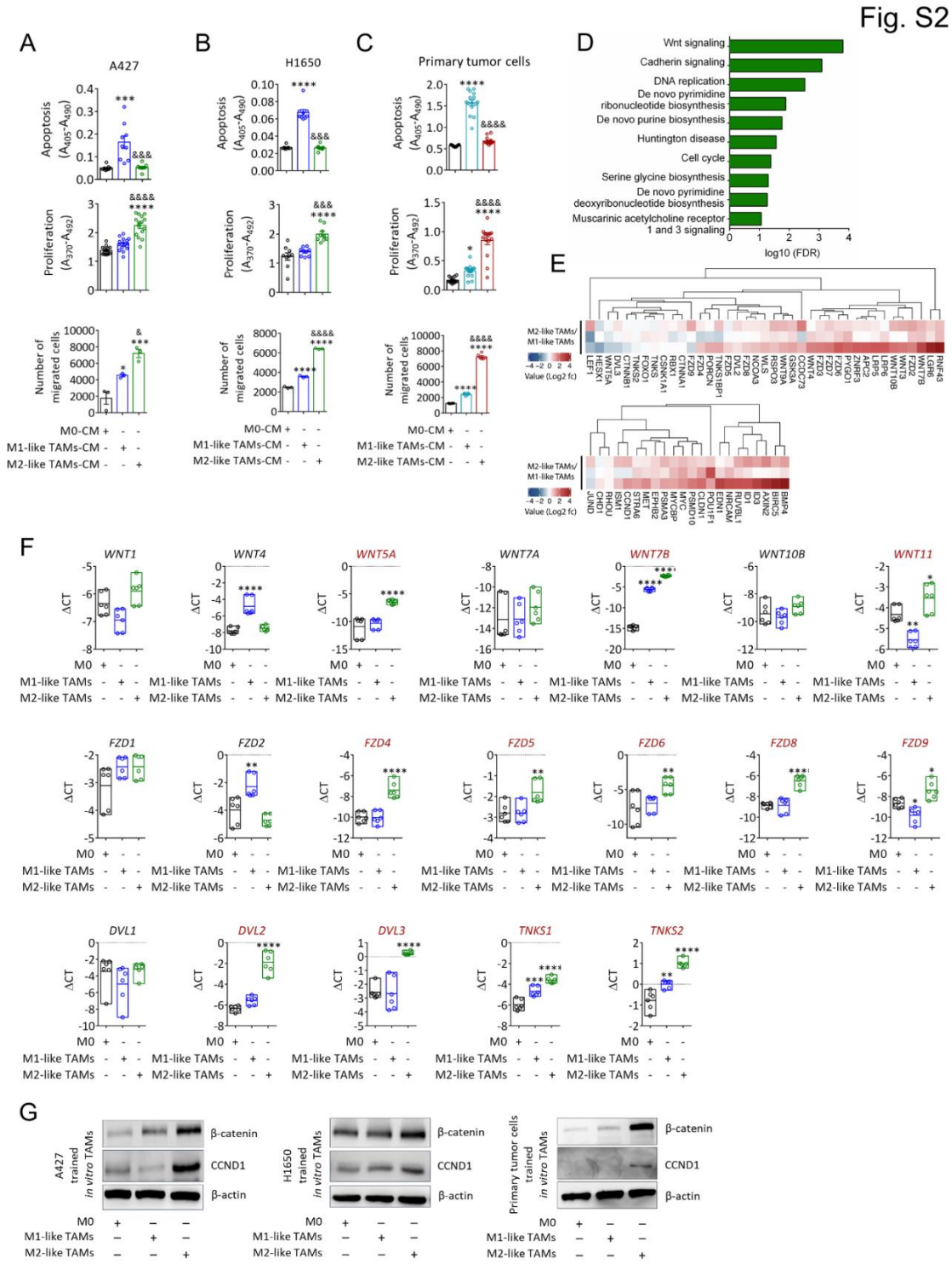

Fig. S2. M2-like TAMs induce tumorigenicity in lung tumor cell lines and primary tumor cells and showed upregulation of Wnt/β-catenin signaling: Apoptosis, proliferation, and migration of **(A)** A427 **(B)** H1650 **(C)** primary tumor cells in presence of CM from M0 and representative tumor cell-trained M1-like, M2-like TAMs, n=9, ***/****P < 0.001/0.0001 versus M0-CM, &&&/&&&&P < 0.001/0.0001 versus M1-like TAMs-CM **(D)** Top 10 panther pathways in M2-like TAMs-upregulated-DEGs **(E)** Heatmaps display Wnt/β-catenin pathway

genes and target genes expression in M1-like, M2-like TAMs, n=3 **(F)** mRNA expression of WNT ligands (*WNT 1, 4, 5A, 7A, 7B, 10B, 11*), frizzled receptors (*FZD1, 2,4, 5, 6, 8, 9*), disheveled (*DVL 1, 2, 3*), and tankyrases (*TNKS 1, 2*) in M0, A549-trained M1-like, M2-like TAMs, n=6, */**/***/****P < 0.05/0.01/0.001/0.0001 versus M0 **(G)** Western blot of β-catenin, CCND1 in M0 and A427, H1650, primary tumor cell-trained M1-like, M2-like TAMs.

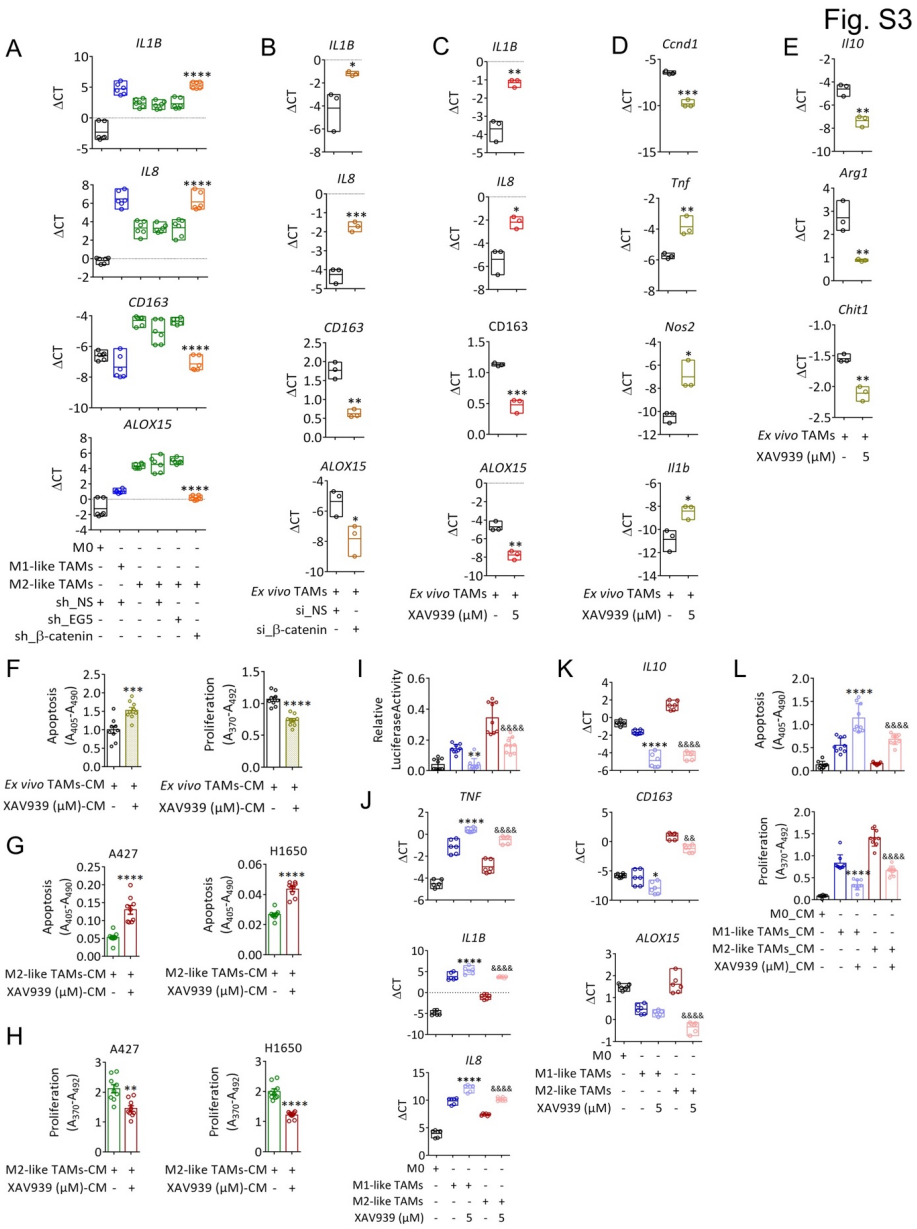

Fig. S3. Inhibition of β-catenin in different *in vitro* models of M2-like TAMs: mRNA expression of macrophage markers in **(A)** M0, M1-like, M2-like TAMs with sh_NS, sh_EG5, sh_β-catenin, n=6, ****P < 0.0001 versus sh_NS **(B)** *ex vivo* TAMs with si_NS, si_β-catenin **(C)** XAV939-treated-*human-ex-vivo*-TAMs, n=6, */**/***P < 0.05/0.01/0.001 versus si_NS or *ex vivo* TAMs. mRNA expression of **(D, E)** *Ccnd1*, macrophage markers in XAV939-treated-*mouse-ex-vivo*-TAMs, n=3, */**/***P < 0.05/0.01/0.001 versus *ex vivo* TAMs **(F)** Apoptosis and proliferation of LLC1 in XAV939-treated-*mouse-ex-vivo*-TAMs_CM, n=6, **/***P < 0.01/0.001 versus *ex vivo* TAM-CM. Apoptosis and proliferation of **(G)** A427 **(H)** H1650 in A427, H1650-

trained M2-like TAMs treated with 5 µM-XAV939_CM, n=9, ***/****P < 0.001/0.0001 versus M2-like TAMs **(I)** Relative TCF/LEF luciferase activity, mRNA expression of **(J, K)** macrophage markers in M0, M1-like, M2-like TAMs treated with 5 µM-XAV939, n=6, **/****P < 0.01/0.0001-M1-like TAMs with M1-like TAM_5 µM XAV939, $^{\&\&/\&\&\&\&}$P < 0.001/0.0001- M2-like TAMs with M2-like TAM_5 µM XAV939 **(L)** Apoptosis and proliferation of A549 in M0, M1-like, M2-like TAMs treated with 5 µM-XAV939_CM, n=9, ****P < 0.0001-CM_M1-like TAMs with CM_M1-like TAM_5 µM XAV939, $^{\&\&\&\&}$P < 0.0001-CM_M2-like TAMs with CM_M2-like TAM_5 µM XAV939.

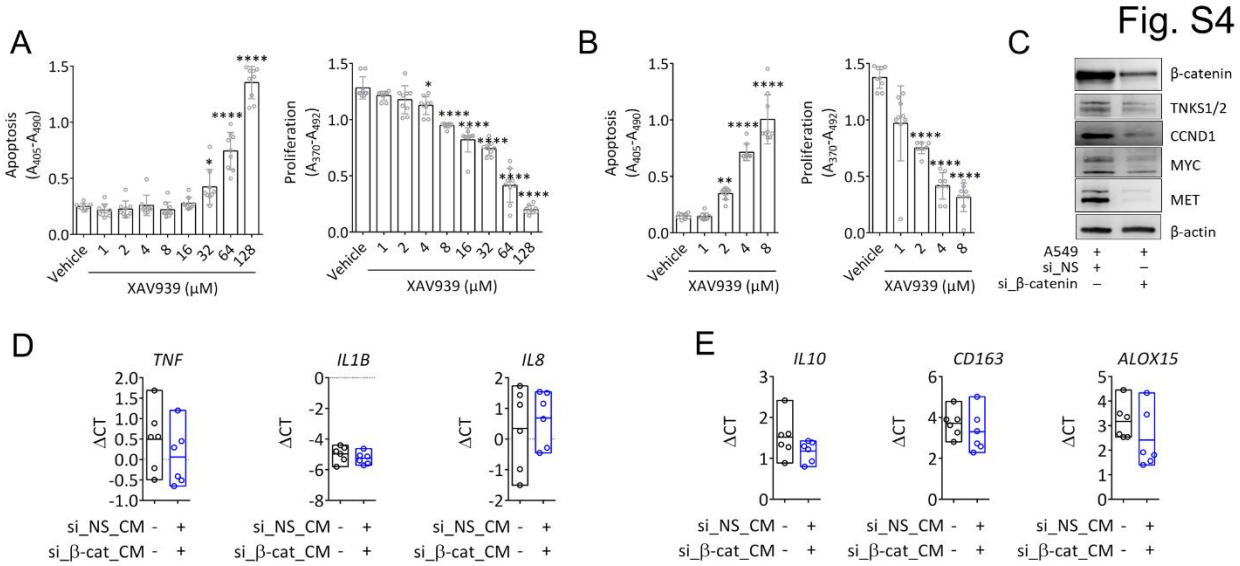

Fig. S4. Low dose of XAV939 required to induce antitumor response of M1-like TAMs compared with direct treatment: Apoptosis and proliferation of A549 treated **(A)** directly with XAV939-treated (1, 2, 4, 8, 16, 32, 64, and 128 µM) and **(B)** with CM from XAV939-treated (1, 2, 4, and 8 µM) M2-like TAMs, n=9, */**/****P < 0.5/0.01/0.0001 versus vehicle (**C**) Western blot of Wnt/β-catenin signaling genes in A549 transfected with si_NS, si_β-catenin for 24 h. mRNA expressions of **(D)** *TNF*, *IL1B*, *IL8* **(E)** *IL10*, *CD163*, *IL10* in M0 macrophages treated with CM from A549-transfected si_NS and si_β-catenin for 24 h, n=6.

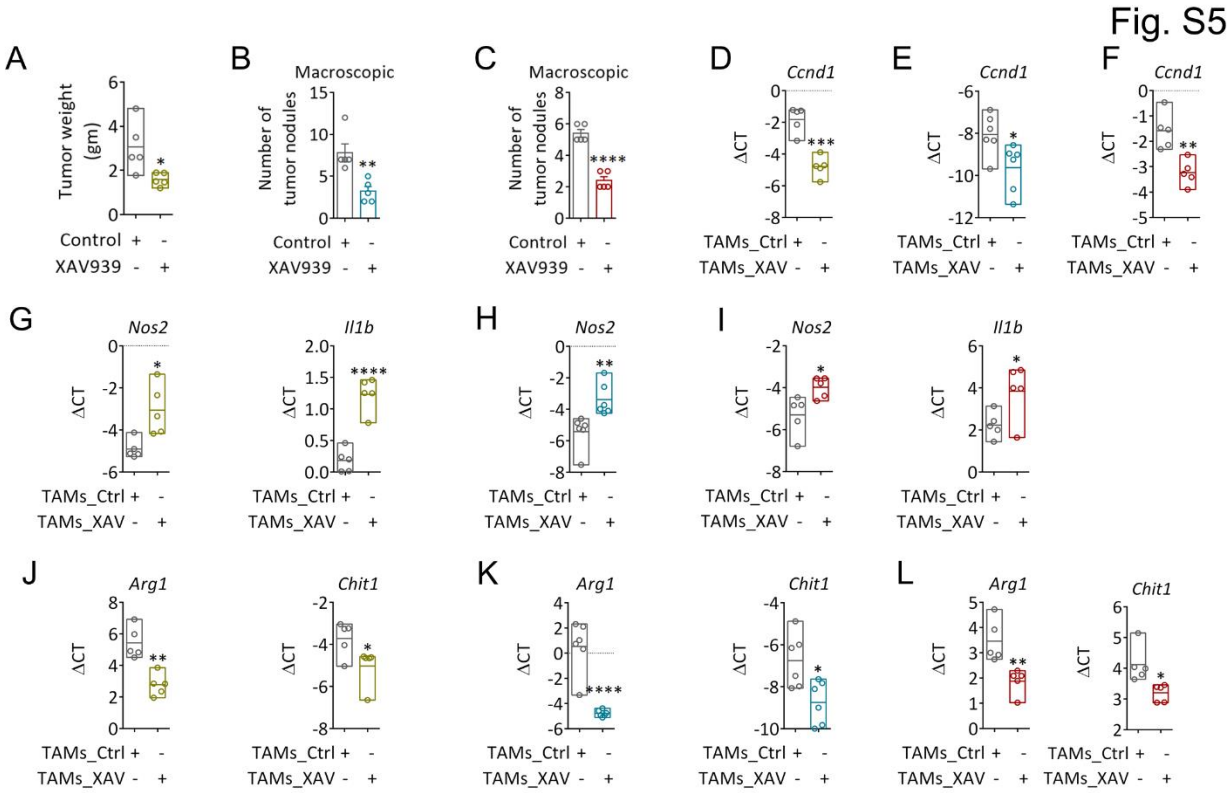

Fig. S5. Pharmacological ablation of β-catenin restricted tumor growth and infiltration of M2-like TAM phenotype switch in TME: Subcutaneous (s.c.), carcinogen-induced, metastatic lung tumor mice were treated with XAV939, n=5 **(A)** Tumor weight of s.c., macroscopic lung tumor nodules in **(B)** carcinogen-induced **(C)** metastatic lung tumor models, */**/****P < 0.05/0.01/0.0001 versus control **(D–F)** mRNA expression of *Ccnd1* in TAMs from tumor tissues obtained from mice treated with control (DMSO; TAM_Ctrl) and XAV939 (TAM_XAV) in **(D)** s.c., **(E)** carcinogen-induced **(F)** metastatic lung tumor models, n=5 **(G–I)** mRNA expression of *Nos2*, *Il1b* in **(G)** s.c., **(H)** carcinogen-induced **(I)** metastatic lung tumor models **(J–L)** *Arg1, Chit1* in TAM_Ctrl, TAM_XAV in **(J)** s.c., **(K)** carcinogen-induced **(L)** metastatic lung tumor models, n=5, */**/***/****P < 0.05/0.01/0.001/0.0001 versus TAM_Ctrl.

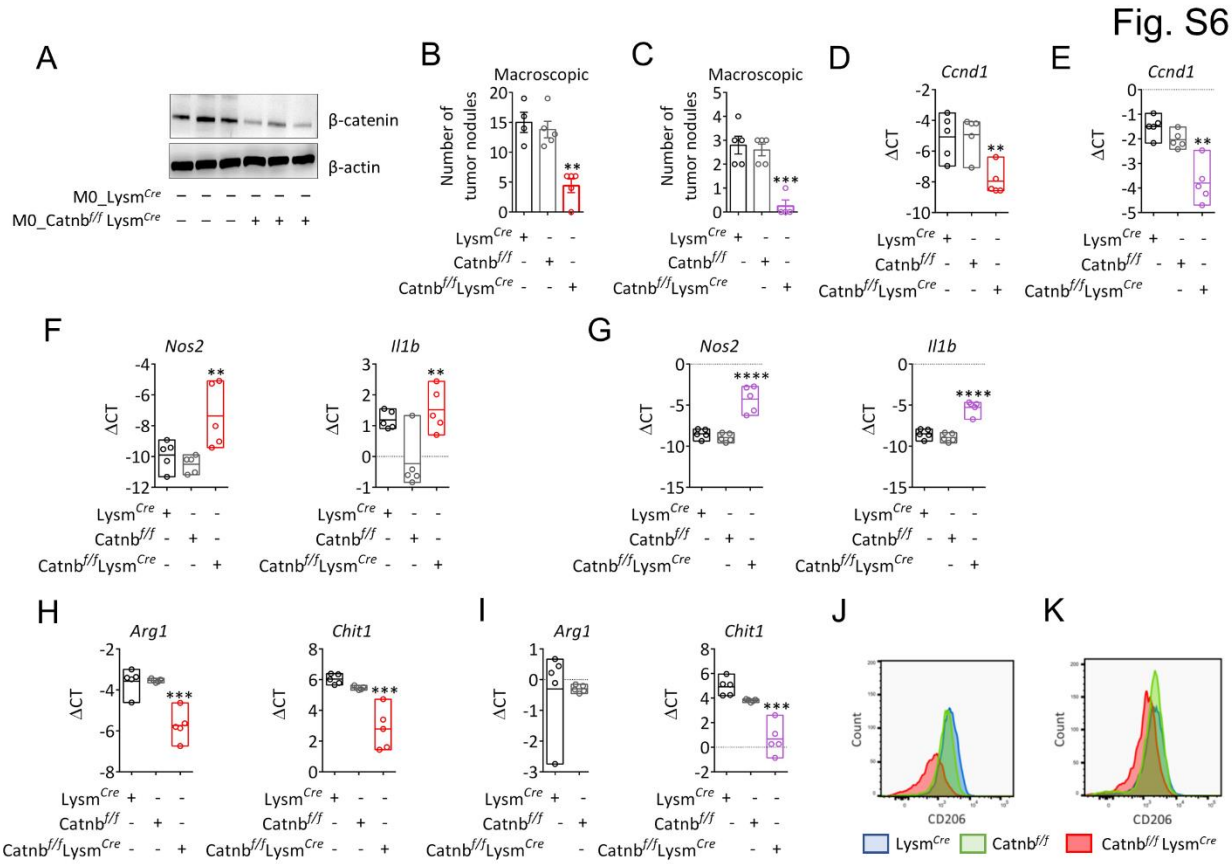

Fig. S6. Macrophage-specific genetic ablation of β-catenin reduced the development of lung tumor by phenotypically switching M2-like TAMs to M1-like TAMs: (A) Western blot of β-catenin in BM M0 macrophages from Lysm^{Cre}, Catnb$^{f/f}$ Lysm^{Cre} mice, n=3. Quantification of macroscopic lung tumor nodules in **(B)** carcinogen-induced **(C)** BMT lung tumor models, n=5, **P < 0.01 versus Catnb$^{f/f}$. mRNA expression of *Ccnd1* in TAMs from macrophage-specific-β-catenin deficient tumors (Catnb$^{f/f}$Lysm^{Cre}) and wild-type-tumors (Lysm^{Cre}, Catnb$^{f/f}$) in **(D)** carcinogen-induced **(E)** BMT lung tumor models. mRNA expression of *Nos2*, *Il1b* in Lysm^{Cre}, Catnb$^{f/f}$, Catnb$^{f/f}$Lysm^{Cre} from **(F)** carcinogen-induced **(G)** BMT lung tumor models. mRNA expression of *Arg1*, *Chit1* in Lysm^{Cre}, Catnb$^{f/f}$, Catnb$^{f/f}$Lysm^{Cre} from **(H)** carcinogen-induced **(I)** BMT lung tumor models, n=5, */**/***/****P < 0.05/0.01/0.001/0.0001 versus Catnb$^{f/f}$. FACS histograms indicate the mean-fluorescence-intensity of CD206^{+} macrophages in macrophage-specific-β-catenin-deficient tumors (Catnb$^{f/f}$Lysm^{Cre}) and wild-type-tumors (Lysm^{Cre} Catnb$^{f/f}$) from **(J)** carcinogen-induced **(K)** BMT lung tumor models, n=5.

Fig. S7

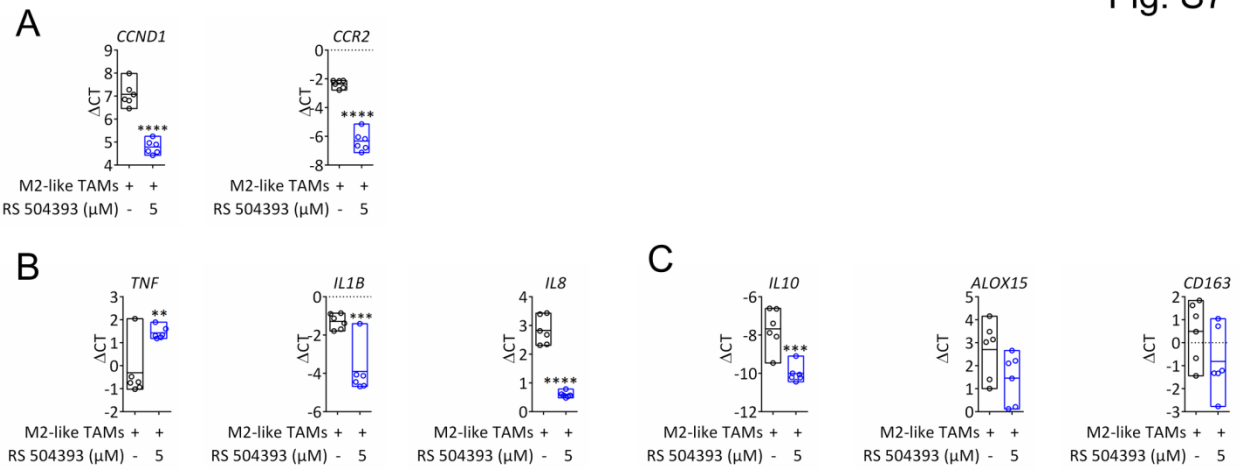

Fig. S7. A positive feedback loop of the β-catenin/CCR2 axis does not regulate β-catenin-driven macrophage polarization: mRNA expressions of **(A)** *CCND1*, *CCR2* **(B)** *TNF*, *IL1B*, *IL8* **(C)** *IL10*, *CD163*, *ALOX15* in M2-like TAMs treated with control (DMSO) or RS 504393 (5 μM), n=6, **/***/****P < 0.01/0.001/0.0001 versus M2-like TAMs

Fig. S8

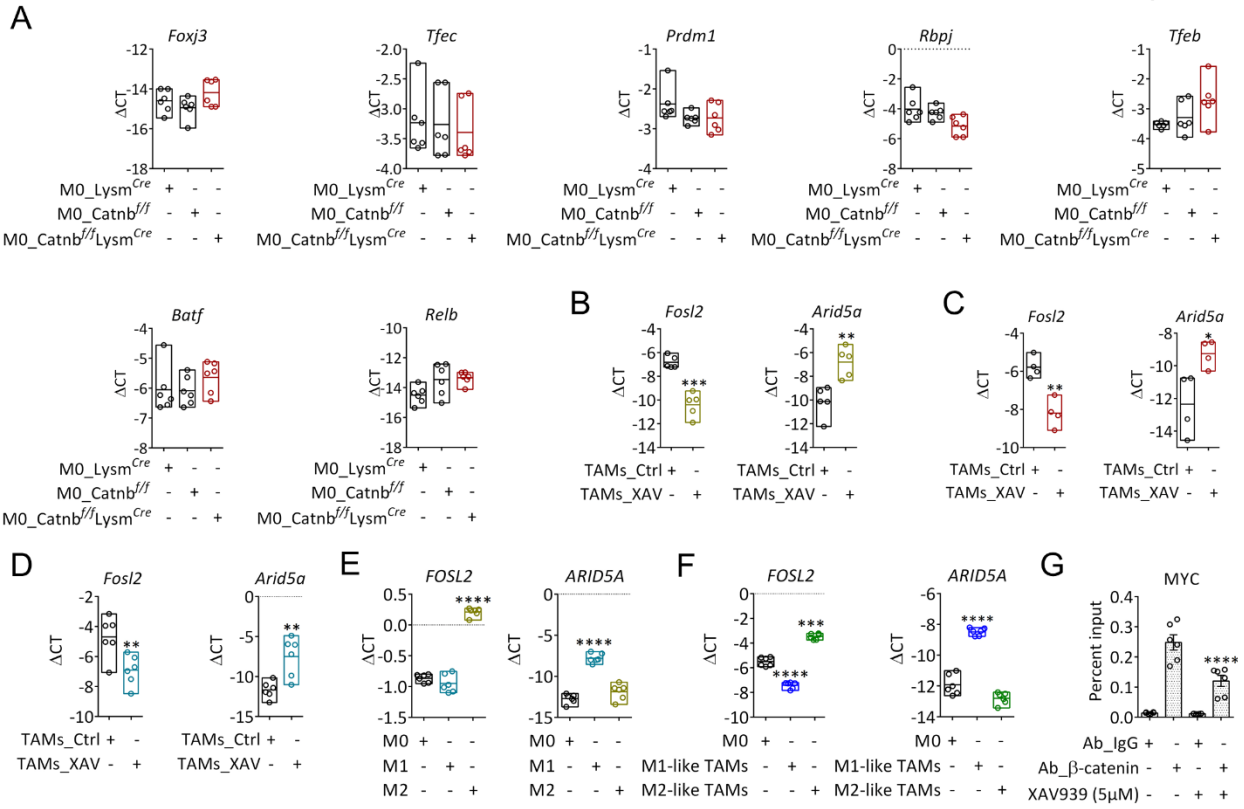

Fig. S8. β-catenin directly bound to the promoter region of FOSL2 and ARID5A: (A) mRNA expressions of *Foxj3*, *Tfec*, *Prdm1*, *Rbpj*, *Tfeb*, *Relb*, *and Batf* in undifferentiated BMDM (M0) from Lysm^{Cre}, Catnb$^{f/f}$, Catnb$^{f/f}$Lysm^{Cre} mice, n=6. mRNA expressions of *Fosl2* and *Arid5a* in TAMs from tumor tissues obtained from mice treated with control (DMSO; TAM_Ctrl) and XAV939 (TAM_XAV) in **(B)** s.c., **(C)** carcinogen-induced **(D)** metastatic lung tumor models, n=5, */**/***P < 0.05/0.01/0.001 versus TAM_Ctrl. mRNA expressions of *FOSL2* and *ARID5A* in **(E)** M0, M1, M2 **(F)** M0, M1-like, M2-like TAMs, n=6, ****P < 0.0001 versus M0 **(G)** Real-time PCR of MYC in β-catenin ChIP assays performed in THP1-derived M2 macrophages treated with control (DMSO) and XAV939 (5 μM) for 24 h, n=6, ****P <0.0001 versus Ab_β-catenin.

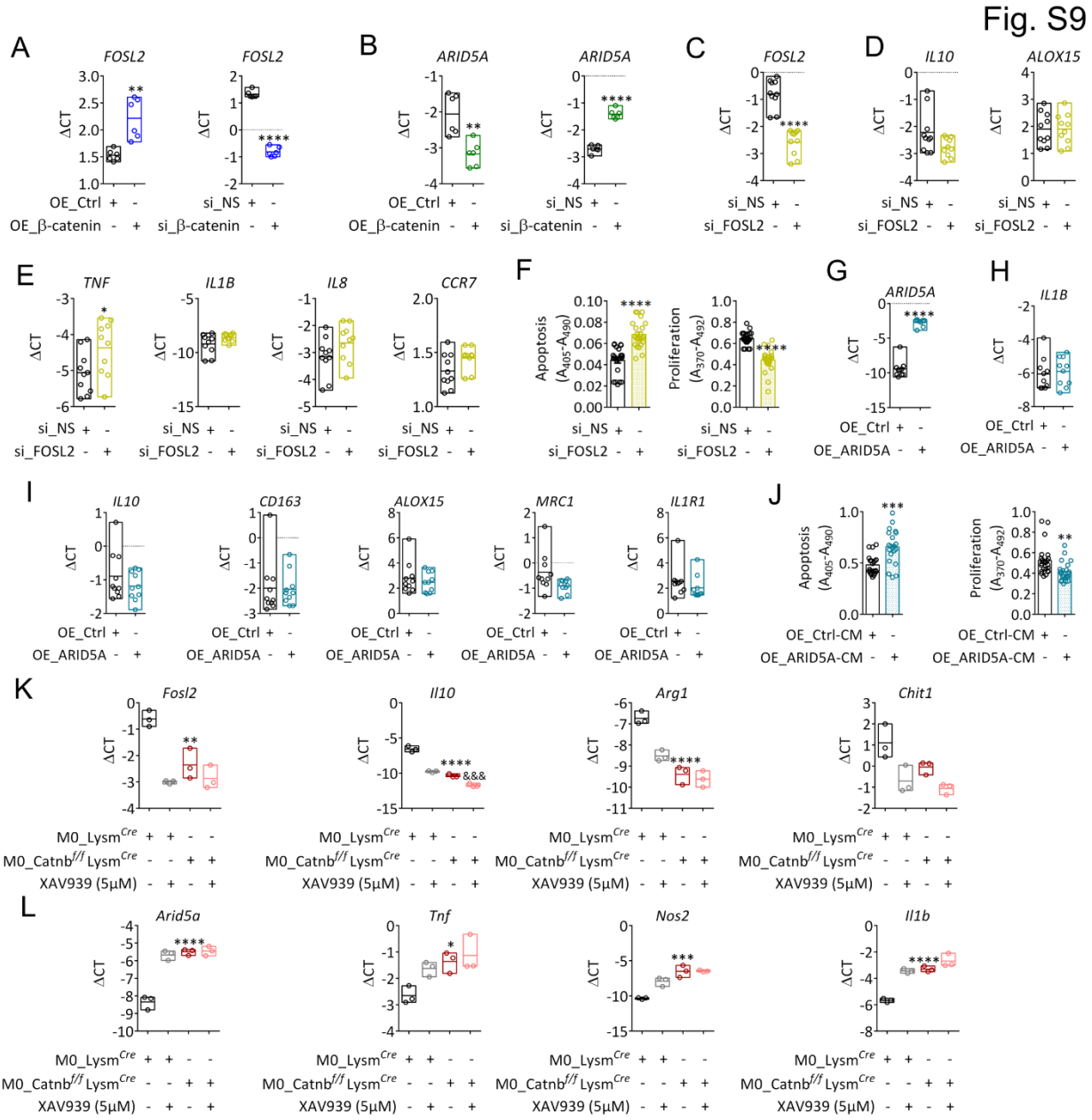

Fig. S9. β-catenin acts as a transcriptional activator and repressor of FOSL2 and ARID5A, respectively, in M2-like TAMs: mRNA expressions of **(A)** *FOSL2* **(B)** *ARID5A* in M2-like TAMs with OE_NS, OE_β-catenin, si_NS, si_β-catenin, n=6, **/****P < 0.01/0.0001versus si_NS or OE_NS. mRNA expressions of **(C)** *FOSL2* **(D)** *IL10*, *ALOX15* **(E)** *TNF*, *IL1B*, *IL8*, *CCR7* in M2-like TAMs with si_NS and si_FOSL2, n=10, ***P < 0.001 versus si_NS **(F)** Apoptosis and proliferation of A549 in presence of CM from M2-like TAMs transfected with si_NS, si_FOSL2 for 24 h, n=15, ***/****P < 0.001/0.0001 versus si_NS-CM. mRNA expressions of **(G)** *ARID5A* **(H)** *IL1B* **(I)** *IL10*, *CD163*, *ALOX15*, *MRC1*, *IL1R1* in M2-like TAMs transfected with OE_NS, OE_ARID5A for 24 h, n=10, ***P < 0.001 versus OE_NS **(J)** Apoptosis and proliferation of A549 in presence of CM from M2-like TAMs transfected with OE_NS, OE_ARID5A, n=10, ***/****P < 0.001/0.0001 versus OE_NS-CM. mRNA expressions of **(K)** *Fosl2*, *Il10*, *Arg1*, *Chit1* **(L)** *Arid5a*, *Tnf*, *Nos2*, *Il1b* in BMDM from Lysm^{Cre}, Catnb$^{f/f}$ Lysm^{Cre} mice treated with control (DMSO) and 5 μM-XAV939, n=3, */**/***/****P < 0.05/0.01/0.001/0.0001 versus M0_Lysm^{Cre}.

Table S1: List of human and mouse primers

Human primers

Gene	Sequence (5`–3`)		Accession No.
HPRT	FP	TGACACTGGCAAAACAATGCA	NM_000194.3
	RP	GGTCCTTTTCACCAGCAAGCT	
WNT1	FP	GCGTCTGATACGCCAAAATC	NM_005430
	RP	GGATTCGATGGAACCTTCTG	
WNT 4	FP	CCTTCGTGTACGCCATCTCT	NM_030761
	RP	GCCTCATTGTTGTGGAGGTT	
WNT5A	FP	CCACATGCAGTACATCGGAG	NM_003392
	RP	CACTCTCGTAGGAGCCCTTG	
WNT7A	FP	AGTACAACGAGGCCGTTCAC	NM_00462
	RP	GCACGTGTTGCACTTGACAT	
WNT7B	FP	AAGCTCGGAGCACTGTCATC	NM_058238
	RP	CCCTCGGCTTGGTTGTAGTA	
WNT10B	FP	GCAAGAGTTTCCCCCACTCT	NM_003394
	RP	GATTGCGGTTGTGGGTATC	
WNT11	FP	TTGCTTGACCTGGAGAGAGG	NM_004626
	RP	GACGAGTTCCGAGTCCTTCA	
FZD1	FP	GTGAGCCGACCAAGGTGTAT	NM_003505
	RP	CAGCCGGACAAGAAGATGAT	
FZD2	FP	GCGTCTTCTCCGTGCTCTAC	NM_001466
	RP	CTGTTGGTGAGGCGAGTGTA	
FZD4	FP	AACCTCGGCTACAACGTGAC	NM_012193
	RP	GTTGTGGTCGTTCTGTGGTG	
FZD5	FP	CTTGTTTCCAAAGTCCAATCAAGTG	NM_003468
	RP	GCCTACTCTTCACCCTTCTTTAACG	
FZD6	FP	ATTTTGGTGTCCAAGGCATC	NM_003506
	RP	TATTGCAGGCTGTGCTATCG	
FZD8	FP	TCTTGTCGCTCACATGGTTC	NM_031866
	RP	GTAGAGCACGGTGAACAGG	
FZD9	FP	CGCTGGTCTTCCTACTGCTC	NM_003508
	RP	AGAAGACCCCGATCTTGACC	
DVL1	FP	GCTGACGGTGAAGAGTGA	NM_001330311.2
	RP	GCATTGGCGATGGTGAT	
DVL2	FP	GCCTATCCAGGTTCCTCCTC	NM_004422.3
	RP	AGAGCCAGTCAACCACATCC	
DVL3	FP	CACAGCGAAGGCAGTCGG	NM_004423.4
	RP	TGCTCACATCACATCCACAAAG	
TNKS1	FP	ATGCCCCCAGAGGCCTTAC	NM_003747.3
	RP	GGTGGATGCTGGTGAGATCA	
TNKS2	FP	ATCTGCTCTGCCCTCTTGTTACAA	NM_025235.4
	RP	GCTAAAATCTACTCCTGGAACCTC	
CCND1	FP	TATTGCGCTGCTACCGTTGA	NM_053056.2
	RP	CCAATAGCAGCAAACAATGTGAAA	
TNFα	FP	GAGGCCAAGCCCTGGTATG	NM_000594.4

	RP	CGGGCCGATTGATCTCAGC	
IL1B	FP	CTAAACAGATGAAGTGCTCC	NM_000576.2
	RP	GGTCATTCTCCTGGAAGG	
IL8	FP	ACAGCAGAGCACACAAGCTTC	NM_000584.4
	RP	ATCAGGAAGGCTGCCAAGAG	
CCR7	FP	GCTGGTGGTGGCTCTCCTT	NM_001838.4
	RP	GTAATCGTCCGTGACCTCATCTT	
ALOX15	FP	CTTCAAGCTTATAATTCCCCAC	NM_001140.4
	RP	GATTCCTTCCACATACCGATAG	
IL10	FP	GAGGCTACGGCGCTGTCA	NM_000572.3
	RP	TCCACGGCCTTGCTCTTG	
IL1R1	FP	CCTGCTATGATTTTCTCCCAATAAA	NM_000877.4
	RP	CACAAAAATATCACAGTCAGAGGTAGAC	
CD163	FP	AGCATGGAAGCGGTCTCTGTGATT	NM_203416.3
	RP	AGCTGACTCATTCCCACGACAAGA	
CD206	FP	ACAACAAAAGCTGACACAAGGA	NM_002438.4
	RP	AGGACAGACCAGTACAATTCAG	
TGFB1	FP	GCAGCACGTGGAGCTGTA	NM_000660
	RP	CAGCCGGTTGCTGAGGTA	
FOSL2	FP	GCCCAGTGTGCAAGATTAGC	NM_005253.4
	RP	GGGCTCCTGTTTCACCACTA	
ARID5A	FP	GTCTTGGGCCAGTAAGGAGTG	NM_001319092.1
	RP	AGGACCAGCCTCTCGTAGT	
CCR2	FP	TGTCCACATCTCGTTCTCGGT	NM_001123396.3
	RP	CCGCTCTCGTTGGTATTTCTGA	

CHIP primers

CCND1	FP	CCTCCCGCTCCCATTCTCTGT
	RP	CAAAACTCCCCTGTAGTCCGTG
Myc	FP	AGGCAACCTCCCTCTCGCCTA
	RP	AGCAGCAGATACCGCCCCTCCT
IL10	FP	AGTCTTGGGTATTCATCCCAGGT
	RP	GAGCTCCTCCTTCTCTAACCTC
FOSL2	FP	GGCCGGAATGTCTTGACTGG
	RP	GGCTGGCCTGCCTATTTTTC
ARID5A	FP	GCACAGGGCCACTTTCAAATC
	RP	AGGCAAAACTAGAGCCTTGGA

Mouse primers

Gene	Sequence (5`–3`)		Accession No.
HPRT	FP	GCTGACCTGCTGGATTACAT	NM_013556.2
	RP	TTGGGGCTGTACTGCTTAAC	
CCND1	FP	GGGCAGCCCCAACAACTTCC	NM_007631.2
	RP	TCCTCAGTGGCCTTGGGGTC	
TNFα	FP	CATCTTCTCAAAATTCGAGTGACAA	NM_013693.3
	RP	TGGGAGTAGACAAGGTACAACCC	
IL1B	FP	ACCCCAAAAGATGAAGGGCTG	NM_008361.4
	RP	TACTGCCTGCCTGAAGCTCT	

iNOS	FP	CACCAAGCTGAACTTGAGCG	NM_001313922.1
	RP	CCATAGGAAAAGACTGCACCG	
IL10	FP	CAGAGAAGCATGGCCCAGA	NM_010548.2
	RP	TGCTCCACTGCCTTGCTCTTA	
Arginase1	FP	GGTTCTGGGAGGCCTATCTT	NM_007482.3
	RP	CACCTCCTCTGCTGTCTTCC	
Chitinase 1	FP	CCCTGGGTCTCGAGGAAGCCC	NM_009892.3
	RP	GCAGCCTTGGAATGTCTTTCTCCAC	
FOSL2	FP	CCAGCAGAAGTTCCGGGTAG	NM_008037.4
	RP	GTAGGGATGTGAGCGTGGATA	
ARID5A	FP	CAGCACCTCCGGCCAAA	NM_001290726.1
	RP	CTTGAAGCCAAGATGGGGCA	
FOXJ3	FP	GCGGCCCCGGATGTT	NM_172699.3
	RP	GGAGTTGAGGCCCGTTCTAC	
TFEC	FP	AGGTTATGAGACGAGGGGCT	NM_031198.3
	RP	CCTGGACCAGCACTGATTGG	
PRDM1	FP	TGCTTATCCCAGCACCCC	NM_007548.4
	RP	CTTCAGGTTGGAGAGCTGACC	
RBPJ	FP	ATCCATCTCTTGGACGACGAC	NM_001359152.1
	RP	CTGCATGTCACACCTGCACT	
TFEB	FP	GCAGAAGAAAGACAATCACAA	NM_001161723.1
	RP	GCCTTGGGGATCAGCATT	
RELB	FP	CTTTGCCTATGATCCTTCTGC	NM_001290457.1
	RP	GAGTCCAGTGATAGGGGCTCT	
BATF	FP	CTGGCAAACAGGACTCATCTG	NM_016767.2
	RP	GGGTGTCGGCTTTCTGTGTC	
CCR2	FP	TCCTTGGGAATGAGTAACTGTGT	NM_009915.2
	RP	TGGAGAGATACCTTCGGAACTT	

siRNA sequences

Gene	Catalog number	Target sequence
β-catenin	SI04379662	CAGGATGAATCCTAGCTATCGT
FOSL2	SI02780379	GCGGATCATGTACCAGGATTA
TNF	SI00012453	TAGGGTCGGAACCCAAGCTTA

Plasmid details

Gene	Catalog number	Company
β-catenin	EX-I4822-M03	GeneCopoeia
ARID5A	EX-Y5502-M03	GeneCopoeia
NS	EX-NEG-M03	GeneCopoeia
β-catenin	RHS4430	GE Dharmacon
EG5	RHS4480	GE Dharmacon
NS	RHS4346	GE Dharmacon

Table S2: List of primary and secondary antibodies

Antibody	Host	Catalog number	Company	Application	Dilution
ACTB\β-actin	Mouse	8227	Abcam	WB	1:5000
CTNNB1 \ β-catenin	Rabbit	9582	Cell signalling	WB	1:1000
	Rabbit	71-2700	Invitrogen	CHIP	5µg per 500µg protein
	Rabbit	06-734	Millipore	IF	1:100
TNKS1\2	Rabbit	Sc-8337	Santa Cruz	WB	1:500
GSK3	Mouse	Sc-7297	Santa Cruz	WB	1:500
CCND1	Rabbit	2978	Cell signalling	WB	1:1000
MYC	Rabbit	5605	Cell signalling	WB	1:1000
p-GSK3^{S9}	Rabbit	9336	Cell signalling	WB	1:1000
MET	Rabbit	8198	Cell signalling	WB	1:1000
CD68	Mouse	Ab-955	Abcam	IF	1:100
ARID5A	Rabbit	HPA023879	Sigma	WB	1:1000
FOSL2	Rabbit	HPA004817	Sigma	WB	1:1000
CCR2	Rabbit	ab32144	Abcam	WB	1:500
Anti-mouse IgG, HRP-linked Antibody	Anti-Mouse	W4018	Promega	WB	1:2000
Anti-rabbit IgG, HRP-linked Antibody	Anti-Rabbit	W4028	Promega	WB	1:2000
Anti-goat IgG, HRP-linked Antibody	Anti-Goat	sc-2378	Santa cruz	WB	1:1000
Alexa Fluor 488 goat anti-rabbit IgG	Anti-Rabbit	A11008	Life technologies	IF	1:1000
Alexa Fluor 555 goat anti-mouse IgG	Anti-Mouse	A21422	Life technologies	IF	1:1000
Rabbit isotype IgG	Rabbit	ab171870	Abcam	CHIP	5µg per 500µg protein

WB= Western blot; IF= Immunofluorescence; CHIP= Chromatin immunoprecipitation

Table S3: Patients characteristics

Samples	**Primary tissue**	**Histology-reduced (WHO categories based on diagnosis reported in surgical pathology report)**	**Sex**	**Age at Surgery (Years)**	**Tumor Stage**
1	Lung	Squamous cell carcinoma G3	M	65	pM1 R0
2	Lung	Squamous cell carcinoma G3	F	78	pT3, N0(0/13) L0 V1 R0
3	Lung	Squamous cell carcinoma G3	F	64	pT4 N0(0/21) L0 V0 R0
4	Lung	Squamous cell carcinoma G2	M	76	pT2a N0(0/13) L0 V0 R0
5	Lung	Squamous cell carcinoma G2	M	60	pM1 (PUL, LYM) L0 V0
6	Lung	Bronchopulmonary adenocarcinoma G3	F	64	pT2b N0(0/29) L1 V0 Rx
7	Lung	Bronchopulmonary adenocarcinoma G3	M	63	pT3 n1 (1/16) LX V0 R0
8	Lung	Bronchopulmonary adenocarcinoma G2	F	75	pT2a N0 (0/19) L0 V0 R0
9	Lung	Squamous cell carcinoma G2	F	73	pT2b N0 (0/21) L0 V0 R0
10	Lung	Squamous cell carcinoma G3	M	75	pT2a N3 (6/6) LX V0 R0
11	Lung	Bronchopulmonary adenocarcinoma G2	M	61	pT3 N1 (5/5) L0 V0 R0
12	Lung	Squamous cell carcinoma G2	F	74	pT2a N0(0/20) L0 V0 R0
13	Lung	Squamous cell carcinoma G2	M	76	pT2a N0(0/13) L0 V0 R0
14	Lung	Squamous cell carcinoma G3	M	75	pT2a N3 (6/6) LX V0 R0
15	Lung	Squamous cell carcinoma G2	M	60	pM1 (PUL, LYM) L0 V0 (oral cavity metastasis)