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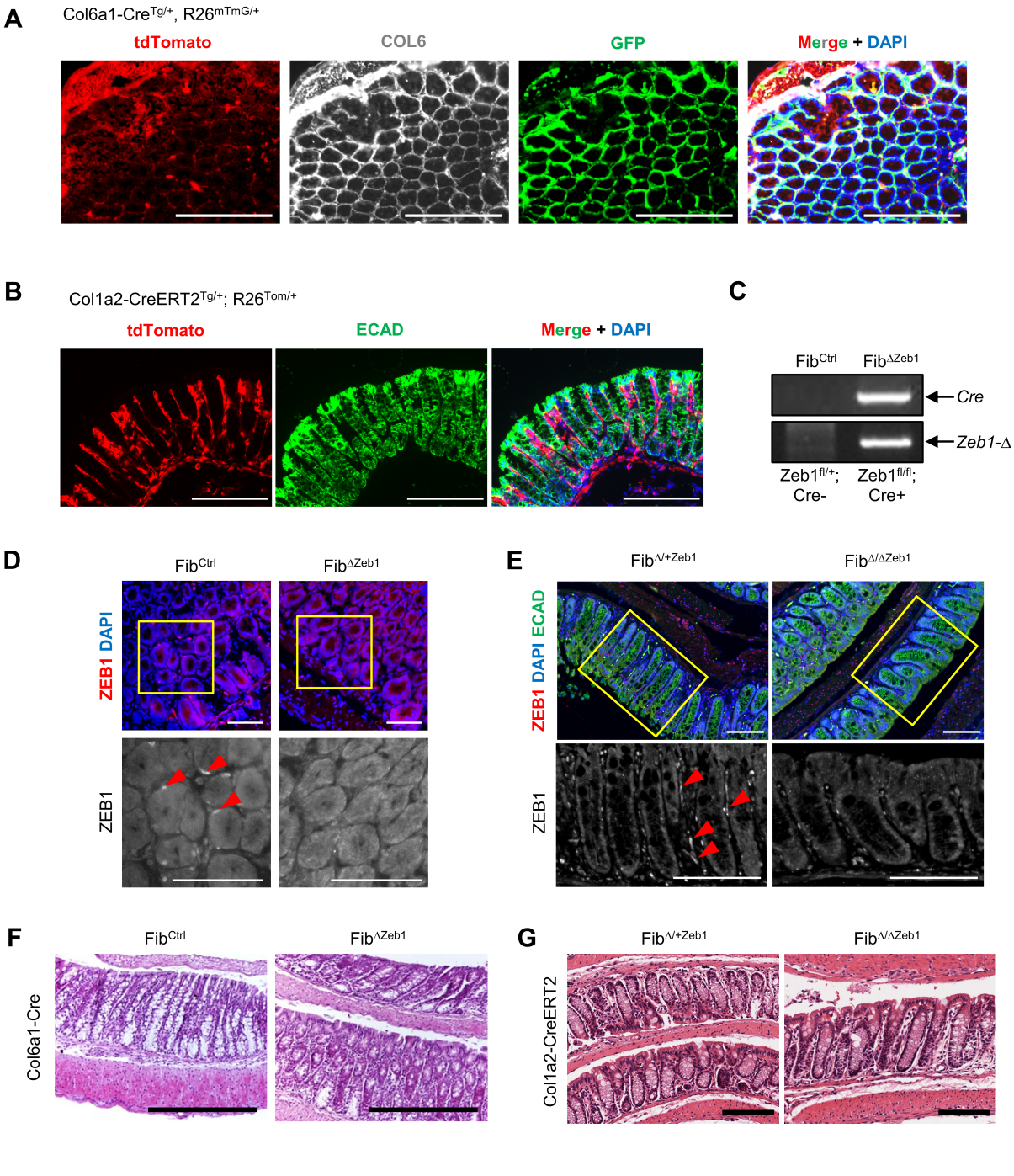

A
Col6a1-Cre^Tg/+; R26^mTmG/+
tdTomato
COL6
GFP
Merge + DAPI
B
Col1a2-CreERT2^Tg/+; R26^Tom/+
tdTomato
ECAD
Merge + DAPI
C
Fib^Ctrl
Fib^ΔZeb1
Cre
Zeb1-Δ
Zeb1^fl/+; Cre-
Zeb1^fl/fl; Cre+
D
Fib^Ctrl
Fib^ΔZeb1
ZEB1 DAPI
ZEB1
E
Fib^Δ/+Zeb1
Fib^Δ/ΔZeb1
ZEB1 DAPI ECAD
ZEB1
F
Fib^Ctrl
Fib^ΔZeb1
Col6a1-Cre
G
Fib^Δ/+Zeb1
Fib^Δ/ΔZeb1
Col1a2-CreERT2

Appendix Fig. S1. Loss of *Zeb1* in fibroblasts cells does not affect intestinal homeostasis.

(**A**) Col6 immunofluorescence (IF) and DAPI staining on colon cryosections of Col6a1-Cre$^{Tg/+}$;Rosa26-mTmG$^{Tg/+}$ mice. Overlay with tdTomato and GFP fluorescence. Scale bar: 200 µm.

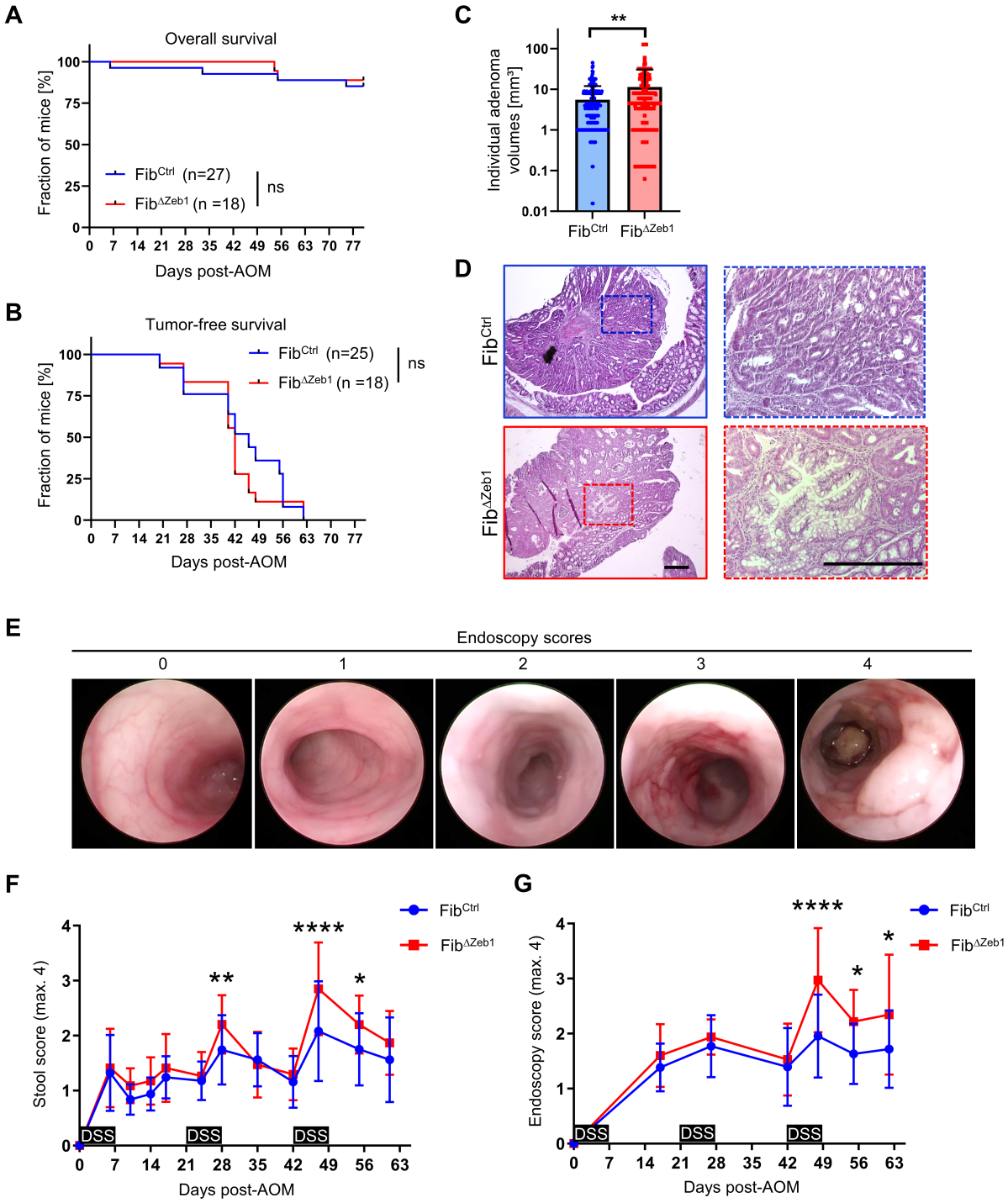
(**B**) IF of tdTomato, E-cadherin and DAPI on colon sections of Col1a2-CreERT$^{Tg/+}$;Rosa26-tdTomato$^{Tg/+}$ mice. Scale bar: 200 µm.

(**C**) Genotyping of colon DNA confirms presence of Cre transgene and Col6a1-Cre induced recombination of *Zeb1* in indicated mouse genotypes.

(**D, E**) ZEB1 IF and DAPI staining on colon sections of Fib^{Ctrl} (*Zeb1$^{fl/fl}$*;Col6a1-Cre$^{+/+}$) and Fib$^{\Delta Zeb1}$ (*Zeb1$^{fl/fl}$*;Col6a1-Cre$^{Tg/+}$) mice (**D**) and ZEB1/E-cadherin IF and DAPI staining on colon sections of heterozygous Fib$^{\Delta/+Zeb1}$ (*Zeb1$^{fl/+}$*;Col1a2-CreERT2$^{Tg/+}$) and Fib$^{\Delta Zeb1}$ (*Zeb1$^{fl/fl}$*;Col1a2-CreERT2$^{Tg/+}$) tamoxifen-fed mice (**E**). Bottom rows show magnification of ZEB1 of the marked areas in grayscale. Red arrowheads indicate ZEB1-positive stromal cells, which are absent in Fib$^{\Delta Zeb1}$. Scale bars: 100 µm.

(**F, G**) H&E stainings of colon sections from Fib^{Ctrl} and Fib$^{\Delta Zeb1}$ mice (**F**), as well as of Fib$^{+/\Delta Zeb1}$ and Fib$^{\Delta Zeb1}$ tamoxifen-treated mice (**G**). Scale bars: 200 µm.

Data information: Scale bars represent 200 µm (A-B,F-G) or 100 µm (D-E).


A
Overall survival
Fraction of mice [%]
100
75
50
25
0
Fib^Ctrl (n=27)
Fib^ΔZeb1 (n =18)
ns
0 7 14 21 28 35 42 49 56 63 70 77
Days post-AOM
B
Tumor-free survival
Fraction of mice [%]
Fib^Ctrl (n=25)
Fib^ΔZeb1 (n =18)
ns
0 7 14 21 28 35 42 49 56 63 70 77
Days post-AOM
C
**
Individual adenoma volumes [mm³]
100
10
1
0.1
0.01
Fib^Ctrl
Fib^ΔZeb1
D
Fib^Ctrl
Fib^ΔZeb1
E
Endoscopy scores
0
1
2
3
4
F
Fib^Ctrl
Fib^ΔZeb1
Stool score (max. 4)
**
****
*
DSS
DSS
DSS
0 7 14 21 28 35 42 49 56 63
Days post-AOM
G
Fib^Ctrl
Fib^ΔZeb1
Endoscopy score (max. 4)
****
*
*
DSS
DSS
DSS
0 7 14 21 28 35 42 49 56 63
Days post-AOM

Appendix Fig. S2. ZEB1 in fibroblasts moderately exacerbates AOM/DSS-induced colitis.

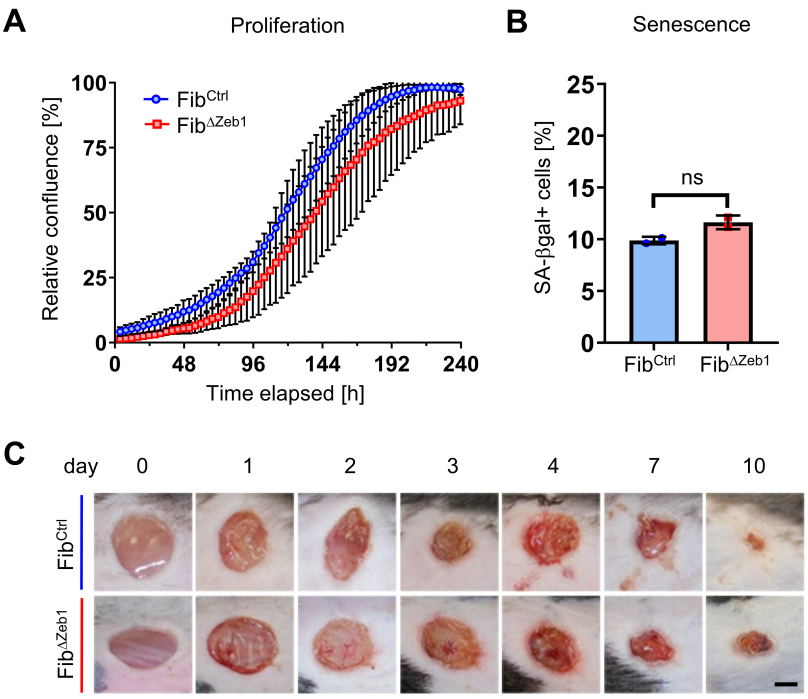
(**A, B**) Kaplan-Meier plots showing overall survival (**A**) and tumor-free survival (**B**) of Fib^{Ctrl} and $Fib^{\Delta Zeb1}$ mice during AOM/DSS tumorigenesis. Numbers of experimental mice are indicated (Overall: p=0.7151, Tumor-free: p=0.3613, Mantel-Cox test).

(**C**) Quantification of volumes of individual adenomas after AOM/DSS tumorigenesis (n=204/207 for Fib^{Ctrl}/$Fib^{\Delta Zeb1}$ depicted on logarithmic scale and derived from 23/16 mice, p=0.0015, Mann-Whitney test).

(**D**) Representative images of H&E stainings of colon adenoma sections from Fib^{Ctrl} and $Fib^{\Delta Zeb1}$ mice. Higher magnification of the indicated region is shown on the right.

(**E-G**) Representative endoscopic images (**E**), and associated stool (**F**) and endoscopy scores (**G**). Quantification of intestinal inflammation based on stool scoring (**F**) and on endoscopy scoring (**G**, also refer to images in E). n=25/17 (**F**) and n=25/18 (**G**) mice for Fib^{Ctrl}/$Fib^{\Delta Zeb1}$, Stool day 28, 47, 55: p= 0.0070, <00001, 0.0127, pUncorrected Fisher's LSD, Endoscopy day 48, 55, 62: p=<0.0001, 0.0343, 0.0195, Šídák's multiple comparisons test).

Data information: Data are presented as mean ± SD (C,F-G). Scale bars represent 300 µm (D).



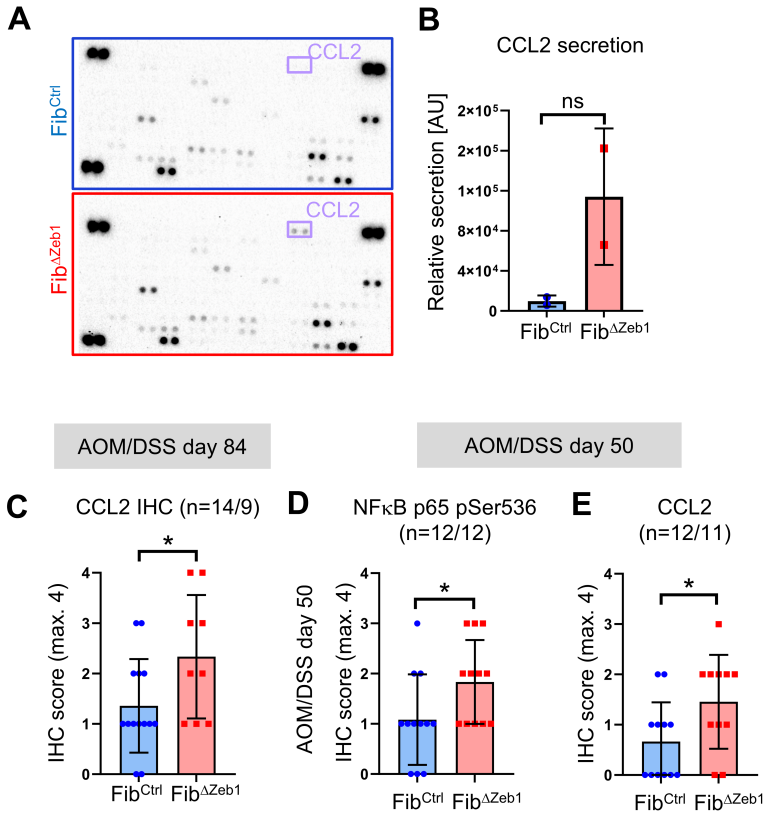
Appendix Fig. S3. Effects of *Zeb1* loss in fibroblasts on proliferation, senescence and wound healing.

(**A**) Proliferation assay by live-cell monitoring of cell confluence in Fib^{Ctrl} and $Fib^{\Delta Zeb1}$ fibroblast cultures using IncuCyte (n=2/2 for Fib^{Ctrl}/$Fib^{\Delta Zeb1}$).

(**B**) Senescence-associated β-galactosidase (SA-β-gal) assay in cultured Fib^{Ctrl} and $Fib^{\Delta Zeb1}$ fibroblasts (n=2/2 for Fib^{Ctrl}/$Fib^{\Delta Zeb1}$, p=0.0819, student's t-test).

(**C**) Representative images of the *in vivo* wound healing assay in Fib^{Ctrl} and $Fib^{\Delta Zeb1}$ mice (n=16/22).

Data information: Data are presented as mean ± SD (A-B). Scale bars represent 2 mm (C).



Appendix Fig. S4. Increased CCL2 secretion in *Zeb1* deficient CAFs and IHC staining during AOM/DSS tumorigenesis.

(**A, B**) Secretome analysis of Fib^{Ctrl} and $Fib^{\Delta Zeb1}$ CAFs isolated from AOM/DSS tumors using a secretome array showing one representative exposure of an arrayed membrane (**A**) and quantification using two independent pairs of CAFs (p=0.1639, student's t-test) (**B**).

(**C**) IHC-based quantification of stromal CCL2 in tumors 84 days after AOM/DSS tumorigenesis. Numbers of experimental mice per genotype are indicated (p=0.0414, student's t-test)

(**D, E**) IHC-based quantification of phospho-NFκB p65 (Ser536) (**D**) and stromal CCL2 (**E**) in colons 50 days after AOM/DSS tumorigenesis. Numbers of experimental mice per genotype are indicated (p65: p=0.0459, Ccl2: p=0.0388, student's t-test).

Data information: Data are presented as mean ± SD (B-E).

Appendix Table S1. Genotyping Primer sequences

Target gene	Primer direction	Primer sequence
Zeb1 floxed	forward	CGTGATGGAGCCAGAATCTGACCCC
Zeb1 floxed	reverse	GGCCTGTCTTTCTCAGCAGTGTGG
Zeb1 deleted	reverse	GCCATCTCACCAGCCCTTACTGTGC
Cre	forward	TCCCCGCAGAACCTGAAGATGTTCG
Cre	reverse	GCCAGATTACGTATATCCTGGCAGC
Col1a2-Cre	forward	CAGGAGGTTTCGACTAAGTTGG
Col1a2-Cre	reverse	CATGTCCATCAGGTTCTTGC
Col6a1-Cre	forward	ACACACCGTAGCAACAGGAAGTC
Col6a1-Cre	reverse	TAGCTGGCCCAAATGTTGCT
R26-mTmG	forward	GTACTTGGCATATGATACACTTGATGTAC
R26-mTmG	reverse	AAAGTCGCTCTGAGTTGTTAT
p53FRT	forward	TCCCTTCCCTTATCCCTGTC
p53FRT	reverse	GGGTCACAGTATCCACAGAG
FLP	forward	GAGCCTGCAGTTCAAGTACAAGAC
FLP	reverse	GTTGTAAGGGATGATGGTGAACTC
Gabra1 (internal DNA control)	forward	AACACACACTGGAGGACTGGCTAGG
Gabra1 (internal DNA control)	reverse	CAATGGTAGGCTCACTCTGGGAGATGATA

Appendix Table S2. Antibodies

Target antigen	used for	labeled	company	clone	catalogue number	working concentration	RRIDs
cl. CASP3	IHC	unconjugated	Cell Signaling	rabbit polyclonal	9661S	1:200 dilution	Cell Signaling Technology Cat# 9661 (also NYUIHC-314, 9661S, 9661L), RRID:AB_2341188
KI67	IHC	unconjugated	abcam	SP6	ab16667	1:300 dilution	Abcam Cat# ab16667, RRID:AB_302459
CD4	IHC	unconjugated	Sino Biol	Clone #1	50134-R001	1:500 dilution	Sino Biological Cat# 50134-R001, RRID:AB_2860490
CD8α	IHC	unconjugated	Sino Biol	rabbit polyclonal	50389-T26	1:500 dilution	-
FOXP3	IHC	unconjugated	eBiosciences	FJK-16s	14-5773-82	1:200 dilution	Thermo Fisher Scientific Cat# 14-5773-82, RRID:AB_467576
PD-L1	IHC	unconjugated	LSBio	rabbit polyclonal	LS-C19686	1:300 dilution	-
B220	IHC	unconjugated	Thermo Fisher Scientific	RA3-6B2	14-0452-82	1:800 dilution	Thermo Fisher Scientific Cat# 14-0452-82, RRID:AB_467254
MCP1 (CCL2)	IHC	unconjugated	Thermo Fisher Scientific	rabbit polyclonal	PA5-115555	1:200 dilution	Thermo Fisher Scientific Cat# PA5-115555, RRID:AB_2893318
RELA/NFkB p-p65 (p-Ser536)	IHC	unconjugated	Novus	rabbit polyclonal	NB100-82088	1:100 dilution	Novus Cat# NB100-82088, RRID:AB_1144569
ZEB1	IHC	unconjugated	Novus	rabbit polyclonal	NBP1-05987	1:250 dilution	Novus Cat# NBP1-05987, RRID:AB_1556166
CD4	IHC	unconjugated	Cell Signaling	D7D2Z	25229	1:100 dilution	Cell Signaling Technology Cat# 25229, RRID:AB_2798898
FOXP3	IHC	unconjugated	R&D Systems	1054C	MAB8214	1:500 dilution	R and D Systems Cat# MAB8214, RRID:AB_2929004
PD-L1	IHC	unconjugated	Cell Signaling	E1L3N	13684S	1:200 dilution	-
CD8	IHC	unconjugated	Cell Signaling	D4W2Z	98941	1:500 dilution	Cell Signaling Technology Cat# 98941, RRID:AB_2756376
F4/80	IHC	unconjugated	Life Technologies	BM8	MF48000	1:300 dilution	Thermo Fisher Scientific Cat# MF48000, RRID:AB_10376289
F4/80	IHC	unconjugated	Biorad	CI:A3-1	MCA497	1:200 dilution	Bio-Rad Cat# MCA497, RRID:AB_2098196
anti-rabbit-polymer	IHC	HRP	DAKO		K4003		Agilent Cat# K4003 (also K4002), RRID:AB_2630375
anti-rat-HRP	IHC	HRP	Life Technologies	rabbit polyclonal	A18915		Thermo Fisher Scientific Cat# A18915, RRID:AB_2535690
ZEB1	IF	unconjugated	Sigma	rabbit polyclonal	HPA027524	1:200 dilution	Sigma-Aldrich Cat# HPA027524, RRID:AB_1844977
COL6	IF	unconjugated	abcam	EPR17072	ab182744	1:500 dilution	Abcam Cat# ab182744, RRID:AB_2847919
ZEB1	IF	unconjugated	Bethyl	rabbit polyclonal	IHC-00419	1:200 dilution	Bethyl Cat# IHC-00419 (also IHC-00419-T), RRID:AB_1659852
E-cadherin	IF	unconjugated	BD Biosciences	36	610181	1:200 dilution	BD Biosciences Cat# 610181, RRID:AB_397580
αSMA	IF	unconjugated	Thermo Fisher Scientific	1A4	14-9760-82	1:200 dilution	Thermo Fisher Scientific Cat# 14-9760-82 (also 14-9760), RRID:AB_2572996
anti-mouse	IF	Alexa Fluor 488	Thermo Fisher Scientific	donkey polyclonal	A21202	1:500 dilution	Thermo Fisher Scientific Cat# A-11055, RRID:AB_2534102

anti-rabbit	IF	Alexa Fluor 594	Thermo Fisher Scientific	donkey polyclonal	A21207	1:500 dilution	Thermo Fisher Scientific Cat# A-21207, RRID:AB_141637
anti-rabbit	IF	Alexa Fluor 647	Thermo Fisher Scientific	donkey polyclonal	A31573	1:500 dilution	Thermo Fisher Scientific Cat# A-31571, RRID:AB_162542
IκBα	Western blot	unconjugated	Cell Signaling	rabbit polyclonal	CS9242	1:5000 dilution	Cell Signaling Technology Cat# 9242, RRID:AB_331623
NFκB p65	Western blot	unconjugated	Cell Signaling	D14E12	CS8242	1:2000 dilution	Cell Signaling Technology Cat# 8242, RRID:AB_10859369
p-IκBα Ser32	Western blot	unconjugated	Cell Signaling	14D4	CS2859	1:1000 dilution	Cell Signaling Technology Cat# 2859 (also 2859S, 2859P, 2859L), RRID:AB_561111
p-NFκB p65 Ser536	Western blot	unconjugated	Cell Signaling	7F1	CS3036	1:1000 dilution	Cell Signaling Technology Cat# 3036, RRID:AB_331281
Zeb1	Western blot	unconjugated	Sigma	rabbit poyclonal	HPA027524	1:2000 dilution	Sigma-Aldrich Cat# HPA027524, RRID:AB_1844977
β-actin	Western blot	unconjugated	Sigma	AC-15	A5441	1:20000 dilution	Sigma-Aldrich Cat# A5441, RRID:AB_476744
anti-rabbit IgG-HRP	Western blot	unconjugated	Dianova	goat polyclonal	111-035-144	1:10000 dilution	Jackson ImmunoResearch Labs Cat# 111-035-144, RRID:AB_2307391
anti-mouse IgG-HRP	Western blot	unconjugated	Dianova	goat polyclonal	115-035-146	1:10000 dilution	Jackson ImmunoResearch Labs Cat# 115-035-146, RRID:AB_2307392
EPCAM	Phenoptics	unconjugated	Cell Signaling	E6V8Y	93790S	1:500 dilution	Cell Signaling Technology Cat# 93790, RRID:AB_2800214
VIM	Phenoptics	unconjugated	abcam	EPR3776	ab92547	1:100 dilution	Abcam Cat# ab92547, RRID:AB_10562134
αSMA	Phenoptics	unconjugated	Sigma	1A4	F3777	1:5000 dilution	Sigma-Aldrich Cat# F3777, RRID:AB_476977
C3	Phenoptics	unconjugated	abcam	EPR19394	ab200999	1:2000 dilution	Abcam Cat# ab200999, RRID:AB_2924273
MHCII	Phenoptics	unconjugated	Invitrogen	M5/114.15.2	14-5321-82	1:400 dilution	Thermo Fisher Scientific Cat# 14-5321-82, RRID:AB_467561
CD45	Phenoptics	unconjugated	Cell Signaling	D3F8Q	70257	1:500 dilution	Cell Signaling Technology Cat# 70257, RRID:AB_2799780
CD16/CD32	FACS	unconjugated	BD Biosciences	2.4G2	553142	1:100 dilution	BD Biosciences Cat# 553141, RRID:AB_394656
CD45	FACS	PE/Cy7	BioLegend	30-F11	103113	1:100 dilution	BioLegend Cat# 103114 (also 103113), RRID:AB_312979
CD31	FACS	PE	Thermo Fisher Scientific	390	12-0311-82	1:100 dilution	Thermo Fisher Scientific Cat# 12-0311-82, RRID:AB_465632
EPCAM	FACS	eFluor450	Thermo Fisher Scientific	G8.8	48-5791-82	1:100 dilution	Thermo Fisher Scientific Cat# 48-5791-82, RRID:AB_10717090
CD140a	FACS	APC	Thermo Fisher Scientific	APA5	17-1401-81	1:100 dilution	Thermo Fisher Scientific Cat# 17-1401-81, RRID:AB_529482

Appendix Table S3. qRT-PCR Primer sequences

Target mRNA	primer direction	primer sequence	UPL
Zeb1	forward	AGGTGATCCAGCCAAACG	93
Zeb1	reverse	AGGCCTGACATGTAGTCTTGTG	93
Acta2	forward	CTCTCTTCCAGCCATCTTTCAT	58
Acta2	reverse	TATAGGTGGTTTCGTGGATGC	58
Gapdh	forward	GGGTTCCTATAAATACGGACTGC	52
Gapdh	reverse	CCATTTTGTCTACGGGACGA	52
Gapdh #2	forward	AGCTTGTCATCAACGGGAAG	9
Gapdh #2	reverse	TTTGATGTTAGTGGGGTCTCG	9
Pdgfrb	forward	TCAAGCTGCAGGTCAATGTC	67
Pdgfrb	reverse	CCATTGGCAGGGTGACTC	67
Ccl2	forward	CCAATGAGTAGGCTGGAGAGC	62
Ccl2	reverse	CCCATTCCTTCTTGGGGTCA	62
Cxcl1	forward	GACTCCAGCCACACTCCAAC	83
Cxcl1	reverse	TGACAGCGCAGCTCATTG	83
Hprt	forward	AAGCTTGCTGGTGAAAAGGA	-
Hprt	reverse	TTGCGCTCATCTTAGGCTTT	-
Tagln	forward	CGGCCTTTAAACCCCTCACC	-
Tagln	reverse	GACTGCACTTCTCGGCTCAT	-
Acta2 #2	forward	AGGGCTGTTTTCCCATCCAT	-
Acta2 #2	reverse	GGCCCATTCCAACCATTACTC	-
Ccl2 #2	forward	AGCCTACTCATTGGGATCATCTTG	-
Ccl2 #2	reverse	CAGCCAGATGCAGTTAACGC	-
Cxcl1 #2	forward	ACTGCACCCAAACCGAAGTC	-
Cxcl1 #2	reverse	TGGGGACACCTTTTAGCATCTT	-