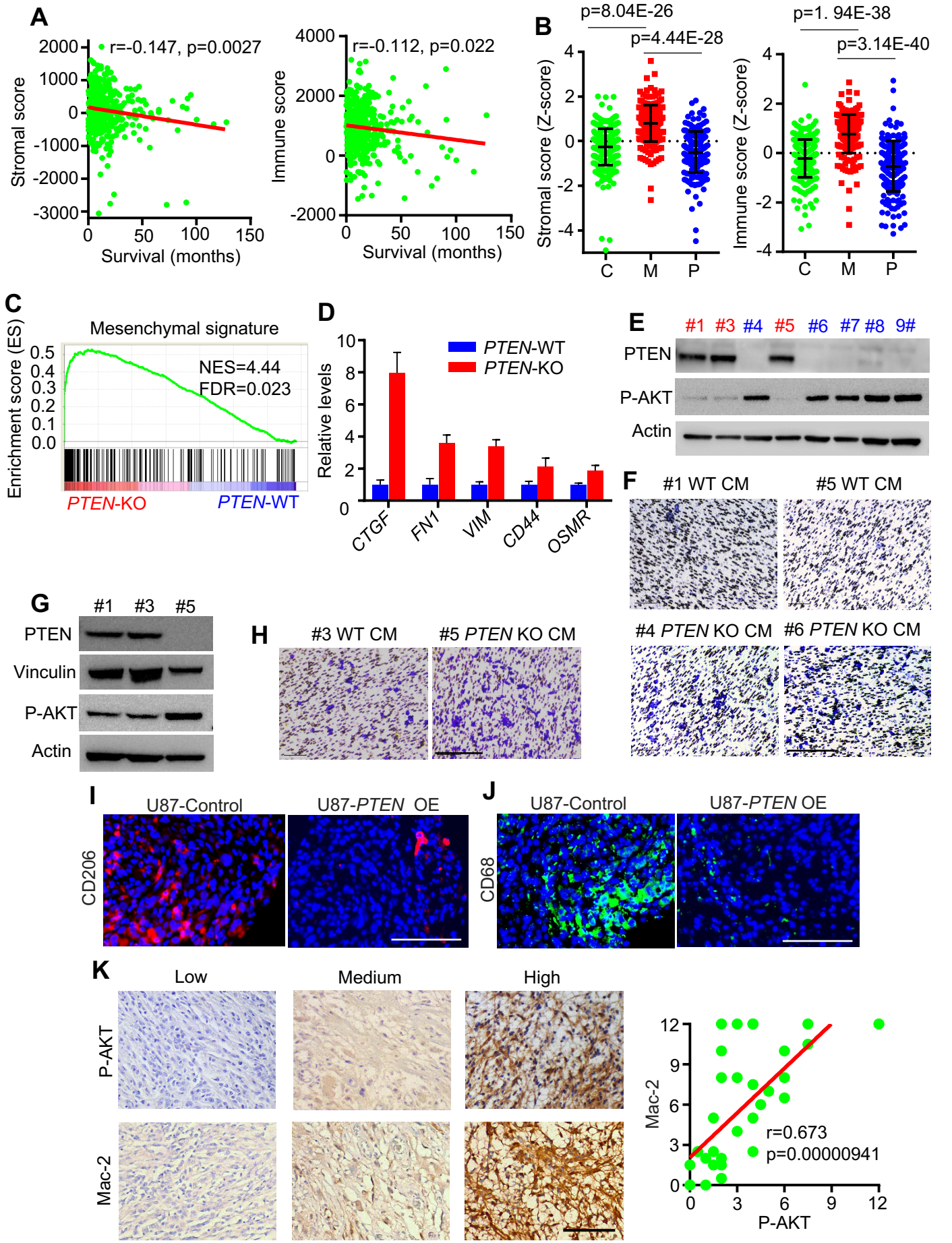

A
r=-0.147, p=0.0027
Stromal score
Survival (months)
r=-0.112, p=0.022
Immune score
Survival (months)
B
p=8.04E-26
p=4.44E-28
Stromal score (Z-score)
C M P
p=1. 94E-38
p=3.14E-40
Immune score (Z-score)
C M P
C
Mesenchymal signature
Enrichment score (ES)
NES=4.44
FDR=0.023
PTEN-KO
PTEN-WT
D
PTEN-WT
PTEN-KO
Relative levels
CTGF
FN1
VIM
CD44
OSMR
E
#1 #3 #4 #5 #6 #7 #8 9#
PTEN
P-AKT
Actin
F
#1 WT CM
#5 WT CM
#4 PTEN KO CM
#6 PTEN KO CM
G
#1 #3 #5
PTEN
Vinculin
P-AKT
Actin
H
#3 WT CM
#5 PTEN KO CM
I
U87-Control
U87-PTEN OE
CD206
J
U87-Control
U87-PTEN OE
CD68
K
Low
Medium
High
P-AKT
Mac-2
Mac-2
P-AKT
r=0.673
p=0.00000941

Figure S1. *PTEN* deletion/mutation induces glioma cell mesenchymal differentiation and promotes macrophage infiltration in GBM, related to Figure 1.

(A) The correlation analysis between stroma score (left panel) or immune score (right panel) and patient survival in TCGA GBM database. Pearson's correlation test.

(B) The stroma score (left panel) and immune score (right panel) in classical (C), mesenchymal (M) and proneural (P) patients in TCGA GBM database.

(C) GSEA analysis of mesenchymal gene signatures with alternatively expressed genes in *PTEN*-KO SF763 compared to *PTEN*-WT SF763 cells. NES, normalized enrichment score; FDR, false discovery rate.

(D) mRNA levels of *CTGF*, *FN1*, *VIM*, *CD44* and *OSMR* in *PTEN*-KO SF763 cells and *PTEN*-WT SF763 cells. n=3 biological replicates.

(E) Immunoblots for PTEN and phospho-Akt (P-AKT, Ser473) in lysates from SF763 *PTEN*-WT (#1, 3, 5) and CRISPR KO clones (#4, 6, 7, 8, 9).

(F) Representative transwell analysis of THP-1 macrophages following stimulation stimulated with conditioned media from *PTEN*-WT (#1 and #5) and *PTEN*-KO SF763 (#4 and #6) cells. Scale bar, 100 μm.

(G) Immunoblots for PTEN and P-AKT (Ser473) in lysates from LN229 *PTEN*-WT (#1 and #3) and CRISPR KO clones (#5).

(H) Representative transwell analysis of THP-1 macrophages upon stimulation with conditioned media from *PTEN*-WT (#3) and *PTEN*-KO (#5) LN229 cells. Scale bar, 100 μm.

(I, J) Representative images of CD206 (**I**) and CD68 (**J**) immunofluorescence staining in tumors established from U87 cells with or without *PTEN* overexpression (OE). Scale bar, 100 μm.

(K) Left panels, representative images showing the low, medium and high expression levels of P-AKT and Mac-2 in human GBM TMA (n=35). Scale bar, 100 μm. Right panel, correlation analysis between P-AKT and Mac-2 expression in TMA. Pearson's correlation test.

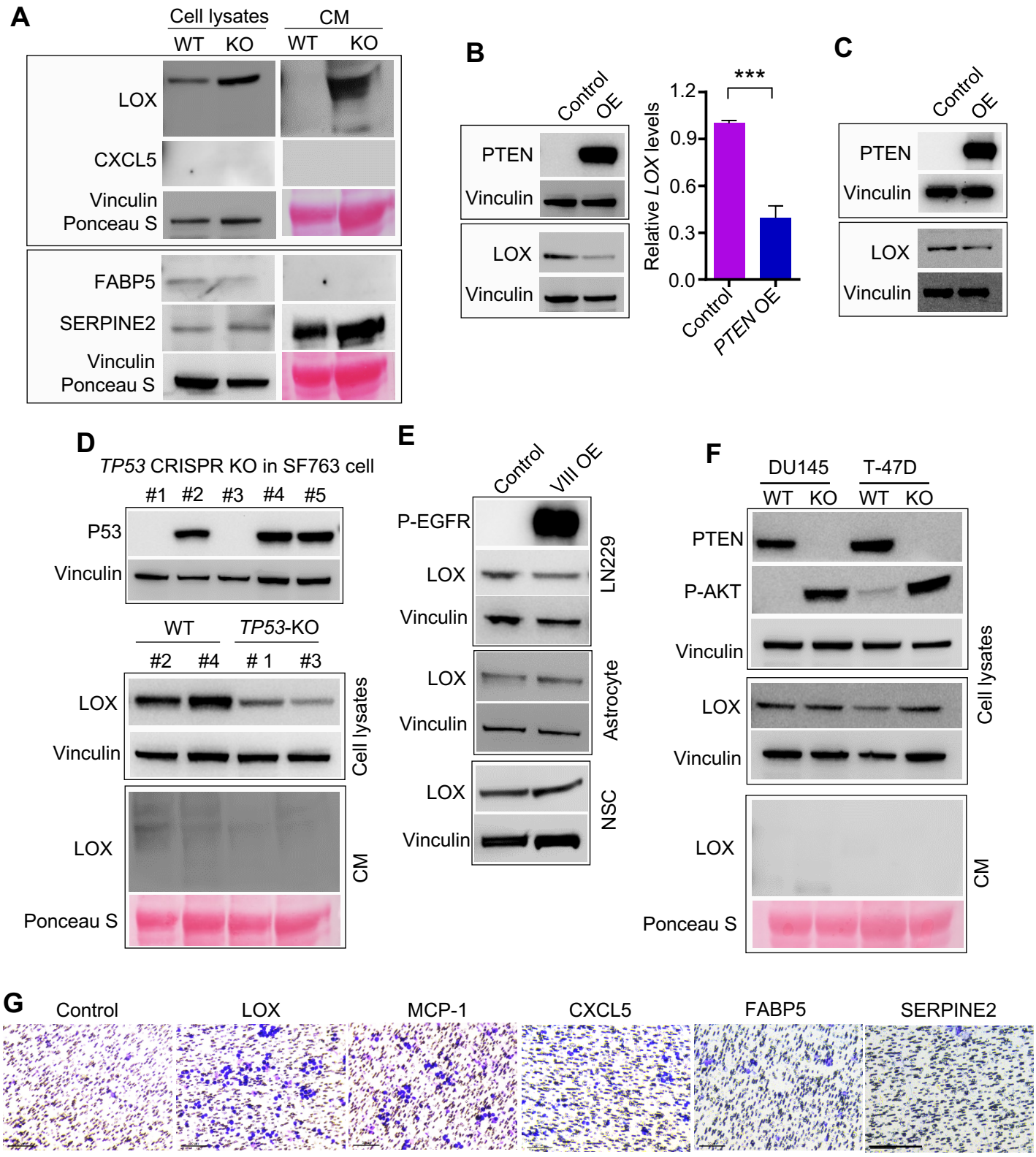
Data from multiple replicates are presented as mean. Error bars indicate mean ± SD. Student’s t test.

Table S1. Stroma and immune score is significantly correlated with the alteration of PTEN-PI3K pathway, but not other pathways in TCGA GBM patients, related to Figure 1.

Stromal or immune score	**Altered PTEN-PI3K pathway vs WT**	**Altered EGFR/PDGFRA-PI3K pathway vs WT**	**Altered NF1-RAS-BRAF pathway vs WT**	**Altered RB1 pathway vs WT**	**Altered P53 pathway vs WT**
Stromal score	Increased, p=0.000193	Decreased, p=0.016	No change, p=0.106	No change, p=0.111	No change, p=0.435
Immune score	Increased, p=0.005	No change, p=0.145	No change, p=0.441	Increased, p=0.037	No change, p=0.435

Table S2. *PTEN*, but not *TP53* and *EGFR*, genetic alteration promotes macrophage infiltration, related to Figure 1.

Gene sets	**WT vs *PTEN*-deficient patients**	***PTEN*-KO vs WT SF763 cells**	***TP53* WT vs mutation/deletion patients**	***EGFR* WT vs mutation/amplification patients**
Go regulation of macrophage chemotaxis	NES=-2.164; FDR<0.25	NES=2.192; FDR<0.25	NES=0.840; FDR>0.25	NES=-0.849; FDR>0.25
Go macrophage chemotaxis	NES=-1.386; FDR<0.25	NES=2.112; FDR<0.25	NES=1.056; FDR>0.25	NES=-0.974; FDR>0.25
Macrophage gene set 1	N/A	N/A	NES=1.61; FDR>0.25	NES=-0.95; FDR>0.25
Macrophage gene set 2	N/A	N/A	NES=1.19; FDR>0.25	NES=1.35; FDR>0.25
M1 macrophage	N/A	N/A	NES=1.51; FDR>0.25	NES=-1.5; FDR>0.25
M2 macrophages	N/A	N/A	NES=1.25; FDR>0.25	NES=0.99; FDR>0.25
DC	N/A	N/A	NES=1.24; FDR>0.25	NES=-0.84; FDR>0.25
Monocytes	N/A	N/A	NES=0.73; FDR>0.25	NES=1.52; FDR>0.25
Neutrophils	N/A	N/A	NES=0.82; FDR>0.25	NES=0.85; FDR>0.25
Naive T Cell	N/A	N/A	NES=-1.70; FDR<0.25	NES=0.88; FDR>0.25
Microglia	N/A	N/A	NES=0.78; FDR>0.25	NES=1.19; FDR>0.25
HSC	N/A	N/A	NES=0.69; FDR>0.25	NES=1.19; FDR>0.25
Nuc. erythrocytes	N/A	N/A	NES=0.77; FDR>0.25	NES=0.92; FDR>0.25
Eosinophils	N/A	N/A	NES=0.80; FDR>0.25	NES=-0.82; FDR>0.25
T CD8 naive	N/A	N/A	NES=0.51; FDR>0.25	NES=-0.84; FDR>0.25
T CD8 activated	N/A	N/A	NES=1.68; FDR>0.25	NES=1.28; FDR>0.25
B Cell	N/A	N/A	NES=0.54; FDR>0.25	NES=0.61; FDR>0.25
Granulocytes	N/A	N/A	NES=0.54; FDR>0.25	NES=1.15; FDR>0.25
iDC	N/A	N/A	NES=0.97; FDR>0.25	NES=-0.9; FDR>0.25
T CD4 naive	N/A	N/A	NES=0.83; FDR>0.25	NES=-0.75; FDR>0.25
Mast cell	N/A	N/A	NES=1.31; FDR>0.25	NES=0.79; FDR>0.25
NK cell	N/A	N/A	NES=0.67; FDR>0.25	NES=1.29; FDR>0.25


A
Cell lysates
CM
WT
KO
WT
KO
LOX
CXCL5
Vinculin
Ponceau S
FABP5
SERPINE2
Vinculin
Ponceau S
B
Control
OE
PTEN
Vinculin
LOX
Vinculin
***
Relative LOX levels
1.2
0.9
0.6
0.3
0.0
Control
PTEN OE
C
Control
OE
PTEN
Vinculin
LOX
Vinculin
D
TP53 CRISPR KO in SF763 cell
#1
#2
#3
#4
#5
P53
Vinculin
WT
TP53-KO
#2
#4
1
#3
LOX
Vinculin
Cell lysates
LOX
Ponceau S
CM
E
Control
VIII OE
P-EGFR
LOX
Vinculin
LN229
LOX
Vinculin
Astrocyte
LOX
Vinculin
NSC
F
DU145
T-47D
WT
KO
WT
KO
PTEN
P-AKT
Vinculin
LOX
Vinculin
Cell lysates
LOX
Ponceau S
CM
G
Control
LOX
MCP-1
CXCL5
FABP5
SERPINE2

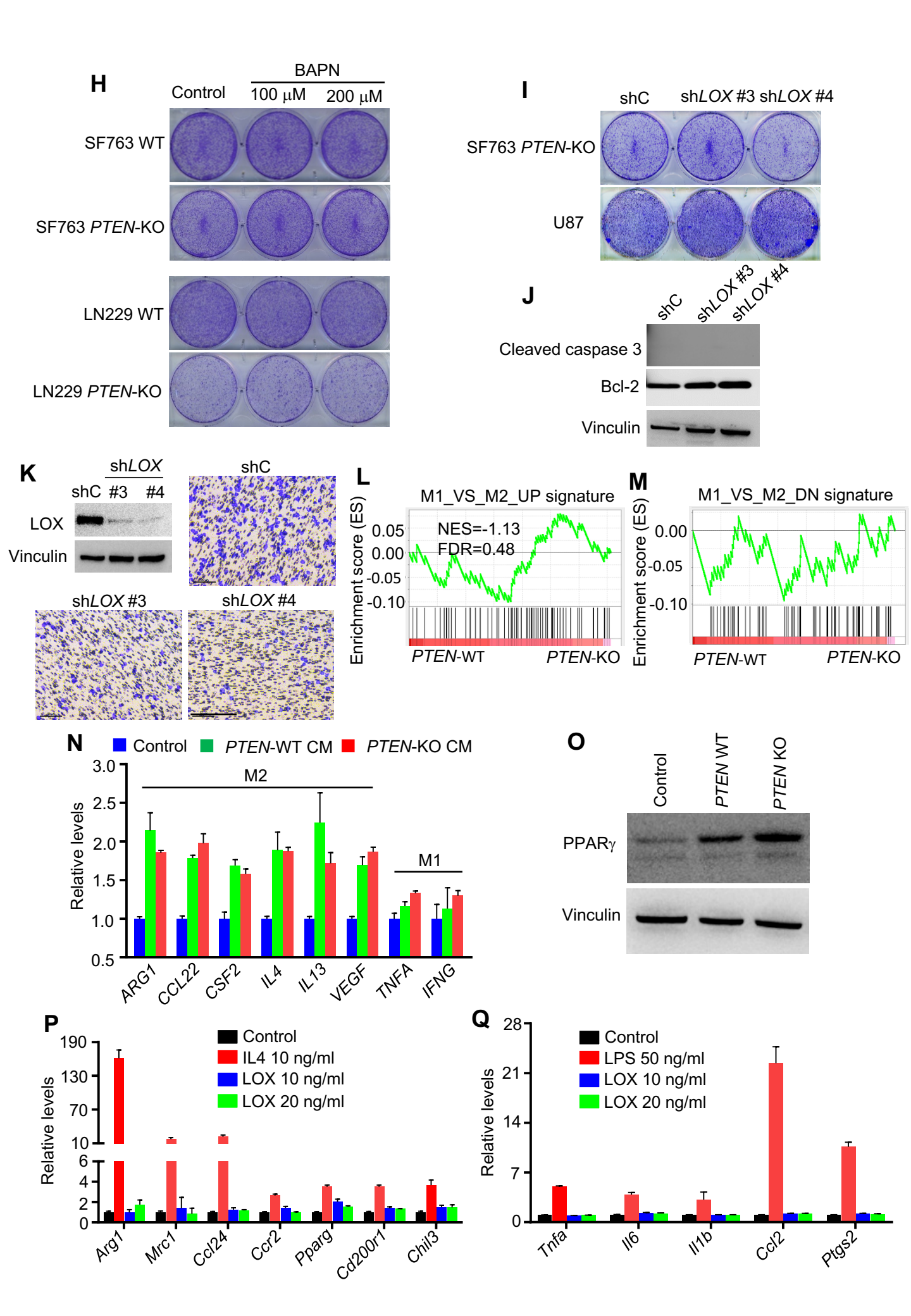

H
BAPN
Control
100 μM
200 μM
SF763 WT
SF763 PTEN-KO
LN229 WT
LN229 PTEN-KO
I
shC
shLOX #3
shLOX #4
SF763 PTEN-KO
U87
J
shC
shLOX #3
shLOX #4
Cleaved caspase 3
Bcl-2
Vinculin
K
shLOX
shC #3 #4
LOX
Vinculin
shC
shLOX #3
shLOX #4
L
M1_VS_M2_UP signature
NES=-1.13
FDR=0.48
Enrichment score (ES)
0.05
0.00
-0.05
-0.10
PTEN-WT
PTEN-KO
M
M1_VS_M2_DN signature
Enrichment score (ES)
0.00
-0.05
-0.10
PTEN-WT
PTEN-KO
N
Control
PTEN-WT CM
PTEN-KO CM
Relative levels
3.0
2.5
2.0
1.5
1.0
0.5
M2
M1
ARG1
CCL22
CSF2
IL4
IL13
VEGF
TNFA
IFNG
O
Control
PTEN WT
PTEN KO
PPARγ
Vinculin
P
Relative levels
190
130
70
10
6
4
2
0
Control
IL4 10 ng/ml
LOX 10 ng/ml
LOX 20 ng/ml
Arg1
Mrc1
Ccl24
Ccr2
Pparg
Cd200r1
Chil3
Q
Relative levels
28
21
14
7
0
Control
LPS 50 ng/ml
LOX 10 ng/ml
LOX 20 ng/ml
Tnfa
Il6
Il1b
Ccl2
Ptgs2

Figure S2. *PTEN*-regulated LOX promotes macrophage migration, but has no effect on glioma cell proliferation and macrophage polarization, related to Figure 2.

(A) Immunoblots for LOX, CXCL5, FABP5 and SERPINE2 in lysates and conditioned media of *PTEN*-WT and *PTEN*-KO LN229 cells.

(B) Immunoblots (left panel) and RT-qPCR (right panel) for LOX in control and *PTEN*-OE U251 cells. n=3 biological replicates.

(C) Immunoblots for LOX in control and *PTEN*-OE GSC23 cells.

(D) SF763 *TP53* CRISPR KO cell line establishment and immunoblots of LOX in SF763 *TP53*-WT (#2 and #4) and SF763 *TP53*-KO (#1 and #3) cells (top 2 panels) and their respective conditioned media (bottom 2 panels).

(E) *EGFRvIII* overexpression (VIII OE) in LN229 cells, astrocytes or NSCs and immunoblots of LOX in control and VIII overexpressing cells.

(F) Immunoblots of LOX in *PTEN*-WT and *PTEN* CRISPR KO prostate (DU145) and breast (T-47D) cells and their conditioned media (bottom 2 panels).

(G) Representative macrophage transwell analysis when THP-1 cells were stimulated with recombinant LOX, MCP-1, CXCL5, FABP5 and SERPINE2 proteins (10 ng/ml). Scale bar, 100 μm.

(H) Colony formation assay in SF763 and LN229 (*PTEN*-WT and *PTEN* CRIPSR KO) cells treated without or with the LOX inhibitor BAPN at indicated concentrations.

(I) Colony formation assay in SF763 *PTEN*-KO and U87 cells expressing shControl (shC) or *LOX* shRNA (sh*LOX*).

(J) Immunoblots for cleaved caspase 3 and Bcl-2 in U87 cells expressing shC or sh*LOX*.

(K) Representative shRNA knockdown efficiency and THP-1 macrophage transwell analysis of migrated macrophages following stimulation with conditioned media from SF763 *PTEN*-KO cells expressing shC or sh*LOX*. Scale bar, 100 μm.

(L, M) GSEA analysis for two gene sets related macrophage polarization in *PTEN*-WT GBM patients compared with *PTEN*-deficient GBM patients in TCGA GBM database.

(N) RT-qPCR for indicated M2 genes and M1 genes in PMA-differentiated macrophages treated with or without CM from parental SF763 and *PTEN*-KO SF763 cells.

(O) Immunoblots for PPARγ in PMA-differentiated macrophages treated with or without CM from parental SF763 and *PTEN*-KO SF763 cells.

(P) RT-qPCR for M2 genes in Raw264.7 macrophages treated with IL4 or LOX at indicated concentrations.

(Q) RT-qPCR for M1 genes in Raw264.7 macrophages treated with LPS or LOX at indicated concentrations.

Data from multiple replicates are presented as mean. Error bars indicate mean ± SD. ***p<0.001, Student's t test.

Table S3. A list of the secreted proteins differentially expressed by *PTEN*-KO SF763 cells relative to *PTEN*-WT SF763 cells (Fold change>2), related to Figure 2.

Gene Symbol	Fold Change (linear) (KO vs. WT)	ANOVA p-value (KO vs. WT)	FDR p-value (KO vs. WT)	Description	Chromosome
CXCL5	313.22	0.000141	0.116083	chemokine (C-X-C motif) ligand 5	chr4
SEMA3E	63.21	0.000339	0.136366	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E	chr7
DAB2	41.52	0.000293	0.132902	Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)	chr5
LOX	37.71	0.000922	0.177276	lysyl oxidase	chr5
SERPINE2	25.16	0.005712	0.29061	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	chr2
SERPINB2	19.24	0.05556	0.560525	serpin peptidase inhibitor, clade B (ovalbumin), member 2	chr18
SPP1	12.81	0.006714	0.308489	secreted phosphoprotein 1	chr4
FABP5	12.04	0.000047	0.088937	fatty acid binding protein 5 (psoriasis-associated)	chr8
VEGFC	9.67	0.004761	0.277937	vascular endothelial growth factor C	chr4
CTGF	7.94	0.014545	0.384372	connective tissue growth factor	chr6
TNC	7.39	0.007705	0.319189	tenascin C	chr9
CSF1	7.28	0.00454	0.27429	colony stimulating factor 1 (macrophage)	chr1
LCN2	5.9	0.009123	0.338539	lipocalin 2	chr9
GAL	4.57	0.007818	0.321112	galanin/GMAP prepropeptide	chr11
COL6A1	3.97	0.001605	0.206285	collagen, type VI, alpha 1	chr21
CCL5	3.43	0.028477	0.46741	chemokine (C-C motif) ligand 5	chr17
IGF2	2.6	0.047455	0.537892	insulin-like growth factor 2	chr11
IL6	2.55	0.054825	0.559328	interleukin 6	chr7
ADM	2.54	0.003047	0.245175	adrenomedullin	chr11
CKLF	2.37	0.000575	0.154231	chemokine-like factor; CKLF-CMTM1 readthrough	chr16

Table S4. Computational analysis demonstrates a key role of LOX for myeloid/leukocyte migration in TCGA GBM patients, related to Figure 2.

Top ten enriched pathways	Gene Ontology Enrichment Analysis			KEGG Enrichment Analysis
	Cell Compartment	Biological Process	Molecular Function	
#1	Extracellular matrix	Leukocyte migration	Cytokine activity	Cytokine-cytokine receptor interaction
#2	Proteinaceous extracellular matrix	Cell chemotaxis	Cytokine receptor binding	TNF signaling pathway
#3	Secretory granule	Leukocyte chemotaxis	G-protein coupled receptor binding	Chemokine signaling pathway
#4	Endoplasmic reticulum lumen	Myeloid leukocyte migration	Glycosaminoglycan binding	AGE-RAGE signaling pathway in diabetic complications
#5	Extracellular matrix component	Granulocyte migration	Sulfur compound binding	IL-17 signaling pathway
#6	Collagen trimer	Regulation of leukocyte migration	Heparin binding	Complement and coagulation cascades
#7	Vesicle lumen	Granulocyte chemotaxis	Chemokine receptor binding	Amoebiasis
#8	Cytoplasmic membrane-bounded vesicle lumen	Positive regulation of leukocyte migration	Chemokine activity	Rheumatoid arthritis
#9	Secretory granule lumen	Neutrophil migration	Serine-type endopeptidase activity	Protein digestion and absorption
#10	Complex of collagen trimer	Neutrophil chemotaxis	CCR chemokine receptor binding	Staphylococcus aureus infection

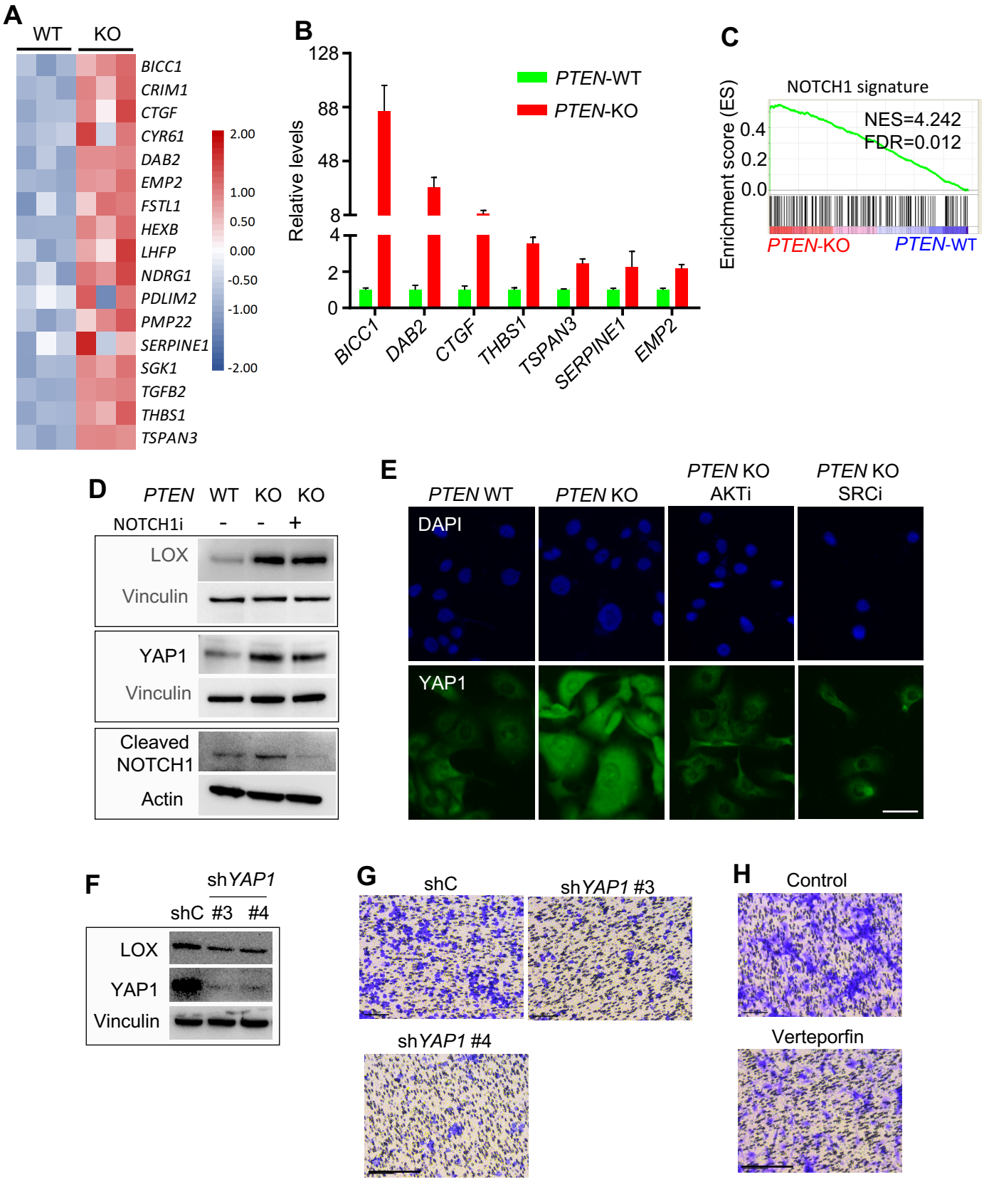

A
WT
KO
BICC1
CRIM1
CTGF
CYR61
DAB2
EMP2
FSTL1
HEXB
LHFP
NDRG1
PDLIM2
PMP22
SERPINE1
SGK1
TGFB2
THBS1
TSPAN3
2.00
1.00
0.50
0.00
-0.50
-1.00
-2.00
B
Relative levels
128
88
48
8
4
2
0
PTEN-WT
PTEN-KO
BICC1
DAB2
CTGF
THBS1
TSPAN3
SERPINE1
EMP2
C
NOTCH1 signature
Enrichment score (ES)
0.4
0.2
0.0
NES=4.242
FDR=0.012
PTEN-KO
PTEN-WT
D
PTEN WT KO KO
NOTCH1i - - +
LOX
Vinculin
YAP1
Vinculin
Cleaved NOTCH1
Actin
E
PTEN WT
PTEN KO
PTEN KO AKTi
PTEN KO SRCi
DAPI
YAP1
F
shYAP1
shC #3 #4
LOX
YAP1
Vinculin
G
shC
shYAP1 #3
shYAP1 #4
H
Control
Verteporfin

Figure S3. SRC/AKT-YAP1, but not NOTCH1, regulates LOX expression and macrophage migration in GBM, related to Figure 3.

(A) Heat map representation of the 17 most upregulated *YAP1* signature genes in SF763 *PTEN*-KO cells. Red signal indicates higher expression and blue signal denotes lower expression.

(B) RT-qPCR validation of key genes in *YAP1* signature in SF763 *PTEN*-WT and *PTEN* CRIPSR-KO cells. n=3 biological replicates. Error bars indicate mean ± SD.

(C) GSEA for NOTCH1 signature in *PTEN*-KO cells vs *PTEN*-WT SF763 cells.

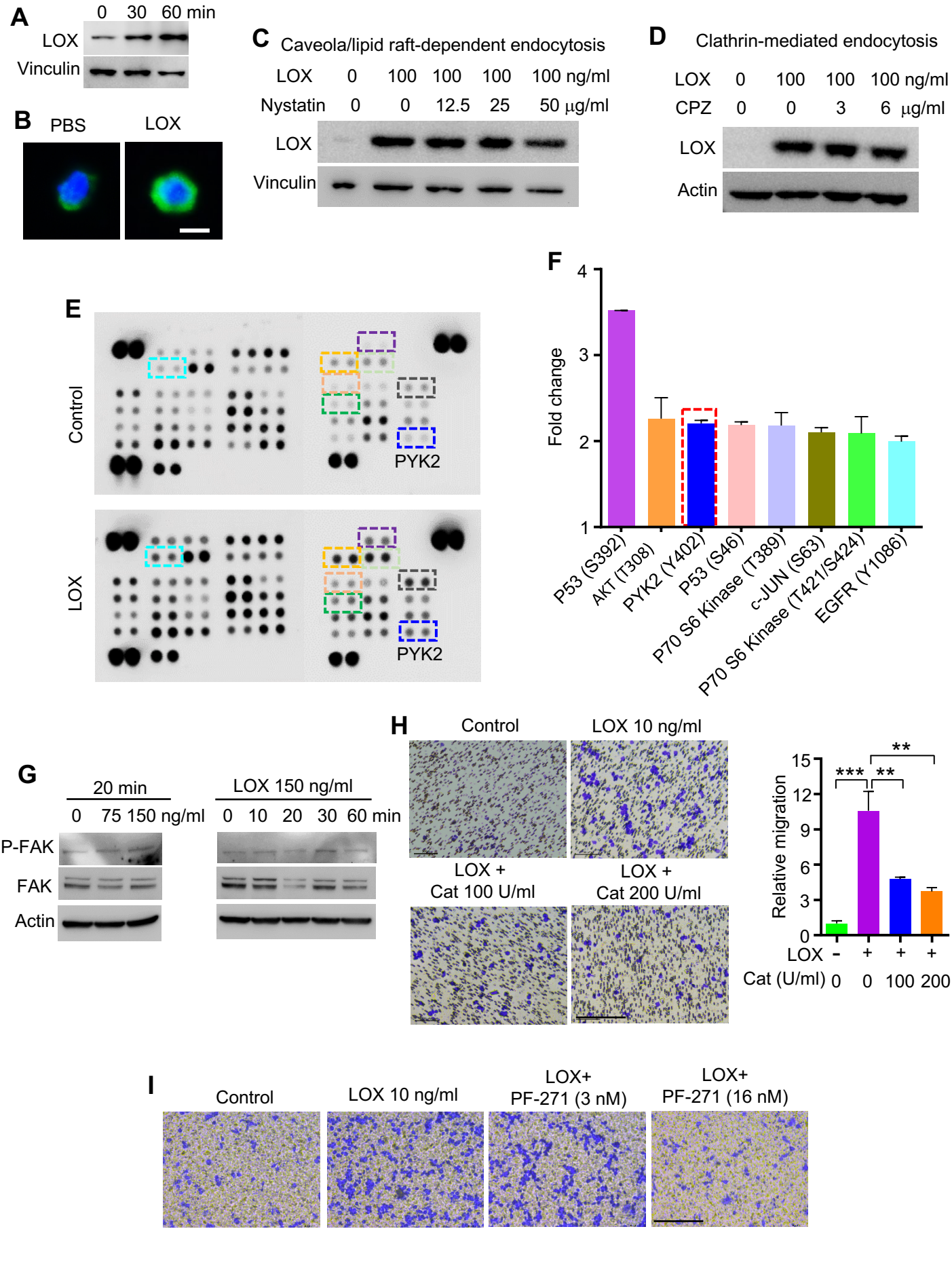
(D) Immunoblots of LOX, YAP1, cleaved NOTCH1 in *PTEN*-WT and *PTEN*-KO SF763 cells treated with or without NOTCH1 inhibitor (NOTCH1i) LY3039478 (1 μM).

(E) Immunofluorescence for YAP1 in *PTEN*-WT and *PTEN*-KO SF763 cells treated with or without SRC inhibitor (SRCi) KX2-391 (50 nM) and AKT inhibitor (AKTi) MK2206 (2 μM). Scale bar, 50 μm.

(F) Immunoblots of LOX and YAP1 in U251 *PTEN*-null GBM cell lines expressing shC or sh*YAP1*.

(G) Representative transwell analysis of THP-1 macrophages following stimulation with conditioned media from U343 cells expressing shC or sh*YAP1*. Scale bar, 100 μm.

(H) Representative transwell analysis of BMDMs following stimulation with conditioned media from SF763 *PTEN*-KO cells pretreated with verteporfin (1 μM). Scale bar, 100 μm.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0 30 60 min
LOX
Vinculin
B
PBS
LOX
C
Caveola/lipid raft-dependent endocytosis
LOX 0 100 100 100 100 ng/ml
Nystatin 0 0 12.5 25 50 μg/ml
LOX
Vinculin
D
Clathrin-mediated endocytosis
LOX 0 100 100 100 ng/ml
CPZ 0 0 3 6 μg/ml
LOX
Actin
E
Control
PYK2
LOX
PYK2
F
Fold change
4
3
2
1
P53 (S392)
AKT (T308)
PYK2 (Y402)
P53 (S46)
P70 S6 Kinase (T389)
c-JUN (S63)
P70 S6 Kinase (T421/S424)
EGFR (Y1086)
G
20 min
0 75 150 ng/ml
LOX 150 ng/ml
0 10 20 30 60 min
P-FAK
FAK
Actin
H
Control
LOX 10 ng/ml
LOX +
Cat 100 U/ml
LOX +
Cat 200 U/ml
Relative migration
15
12
9
6
3
0
***
**
**
LOX - + + +
Cat (U/ml) 0 0 100 200
I
Control
LOX 10 ng/ml
LOX+
PF-271 (3 nM)
LOX+
PF-271 (16 nM)

Figure S4. LOX can be internalized into macrophages and promote macrophage migration through PYK2 pathway, related to Figure 4.

(A) Immunoblots of LOX in J714 macrophages incubated with recombinant LOX (100 ng/ml) for indicated time. The cell surface binding of LOX was removed by washing with acid buffer.

(B) Representative immunofluorescence for LOX in J714 macrophages incubated with or without recombinant LOX (100 ng/ml) for 1 hr. Scale bar, 10 μm.

(C, D) Immunoblots of LOX in Raw264.7 macrophages incubated with recombinant LOX (100 ng/ml) for 1 hr pretreated with or without the inhibitors of the caveola/lipid raft pathway (nystatin, **C**) or the clathrin-dependent pathway (chlorpromazine, CPZ, **D**) at indicated concentrations. The cell surface binding of LOX was removed by washing with acid buffer.

(E) Representative images for human phospho-kinase array in human THP-1 macrophages treated with or without LOX protein (150 ng/ml). Kinases with more than a 2-fold change are marked.

(F) Relative levels (fold change of phosphorylation levels) of indicated signals in human THP-1 macrophages treated with or without human LOX protein (150 ng/ml). The cut-off was set as 2. Error bars indicate SD.

(G) Immunoblots for Phospho-FAK and FAK in Raw264.7 macrophages treated with LOX at indicated concentrations and time points.

(H) Representative THP-1 macrophage transwell analysis (left panels) and quantification (right panel) of migrated macrophages when they stimulated with LOX (10 ng/ml) pretreated with or without catalase (Cat) at indicated concentrations. Scale bar, 100 μm. n=3 biological replicates; Error bars indicate mean ± SD. $^{**}p<0.01$, $^{***}p<0.001$, Student's t test.

(I) Representative transwell analysis of Raw264.7 macrophage following stimulation with LOX (10 ng/ml) pretreated with or without PF-00562271 (PF-271) at indicated concentrations. Scale bar, 100 μm.

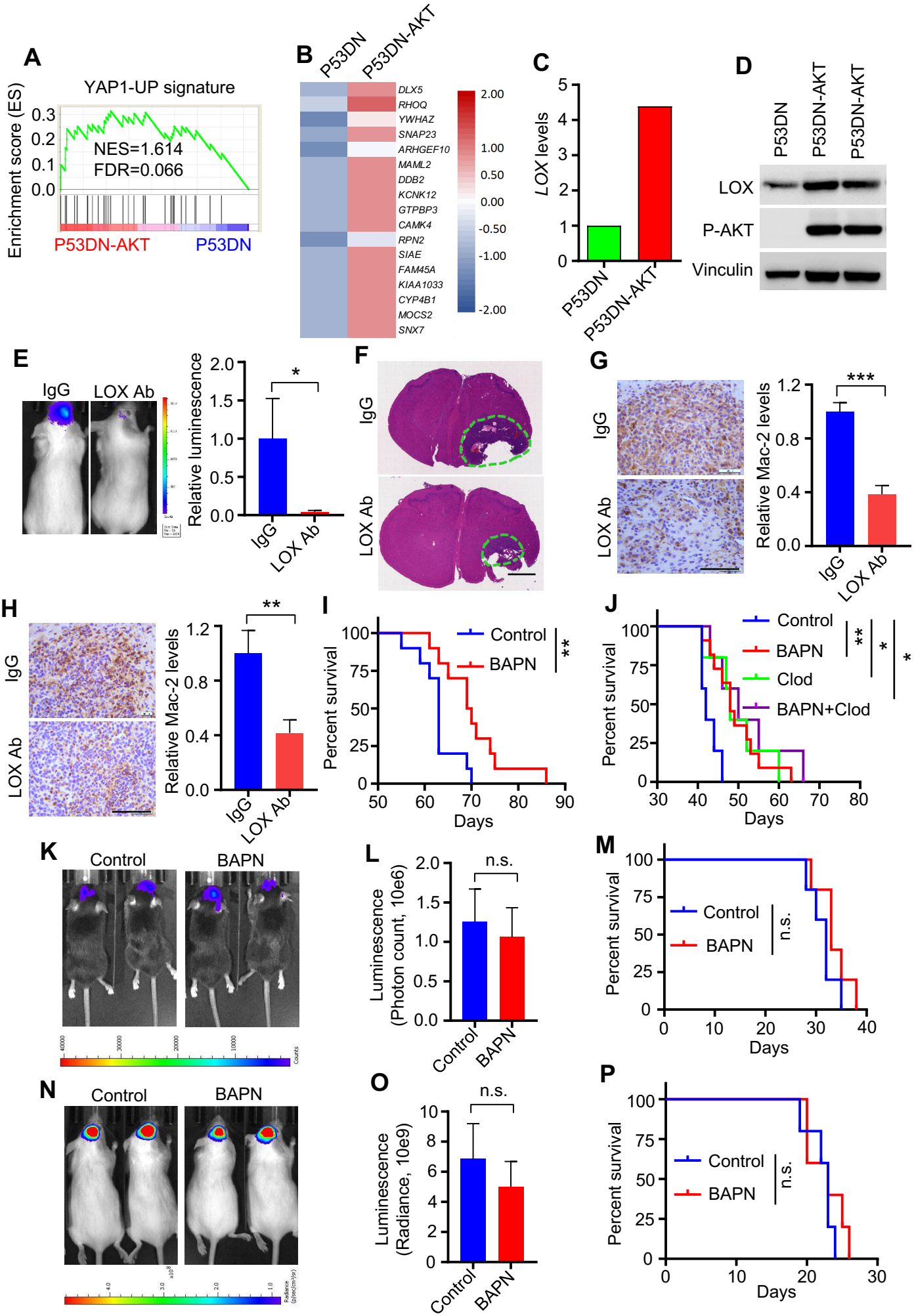
A
YAP1-UP signature
Enrichment score (ES)
NES=1.614
FDR=0.066
P53DN-AKT
P53DN
B
P53DN
P53DN-AKT
DLX5
RHOQ
YWHAZ
SNAP23
ARHGEF10
MAML2
DDB2
KCNK12
GTPBP3
CAMK4
RPN2
SIAE
FAM45A
KIAA1033
CYP4B1
MOCS2
SNX7
C
LOX levels
P53DN
P53DN-AKT
D
P53DN
P53DN-AKT
P53DN-AKT
LOX
P-AKT
Vinculin
E
IgG
LOX Ab
Relative luminescence
IgG
LOX Ab
F
IgG
LOX Ab
G
IgG
LOX Ab
Relative Mac-2 levels
IgG
LOX Ab
H
IgG
LOX Ab
Relative Mac-2 levels
IgG
LOX Ab
I
Control
BAPN
Percent survival
Days
J
Control
BAPN
Clod
BAPN+Clod
Percent survival
Days
K
Control
BAPN
L
Luminescence (Photon count, 10e6)
n.s.
Control
BAPN
M
Control
BAPN
n.s.
Percent survival
Days
N
Control
BAPN
O
Luminescence (Radiance, 10e9)
n.s.
Control
BAPN
P
Control
BAPN
n.s.
Percent survival
Days

Figure S5. LOX Inhibition impairs *PTEN*-null GBM growth and progression, related to Figure 5.

(A) GSEA for *YAP1* signature in P53DN-AKT-hNSCs comparing to P53DN-hNSCs.

(B) Heatmap of key gene expression of *YAP1* signature in P53DN-hNSCs and P53DN-AKT-hNSCs.

(C, D) *LOX* mRNA (**C**) and LOX protein expression (**D**) in P53DN-hNSCs and P53DN-AKT-hNSCs.

(E) Representative (left panels) and quantification (right panel) of *in vivo* bioluminescence-based images of SCID mice at day 17 post-orthotopic injection of U87 (5×10^5 cells). The mice were treated with IgG or LOX antibodies (20 mg/kg body weight, once every 4 days) starting at 4 days post-orthotopic injection of U87 cells.

(F) Representative images of H&E staining (Scale bar, 1000 μm) in tumors at 22 days post-orthotopic injection of 005 GSCs (2×10^4 cells). The mice were treated with IgG or LOX antibodies (20 mg/kg body weight, once every 4 days) starting at 4 days post-orthotopic injection of 005 GSC (n=5/group).

(G) IHC (left panel) and quantification (right panel) of macrophage marker Mac-2 in U87 tumors treated with IgG or LOX antibodies. Scale bar, 100 μm. n=3 biological replicates.

(H) IHC staining (left panel) and quantification (right panel) of Mac-2 in 005 GSC tumors treated with IgG or LOX antibodies. Scale bar, 100 μm. n=3 biological replicates.

(I) Survival curves of SCID mice implanted with GSC23 (1×10^5 cells). Mice were treated with or without LOX inhibitor BAPN (2 g/L in drinking water) starting at 7 days post-orthotopic injection of GSC23 (n=10/group).

(J) Survival curves of mice implanted with 005 GSCs (2×10^4 cells). Mice were treated with LOX inhibitor BAPN (2 g/L in drinking water), or clodronate liposomes (200 μl, once every 3 days) starting at 4 days post-orthotopic injection of 005 GSCs (n=5-10/group).

(K, L) *In vivo* bioluminescence-based images 11 days post-orthotopic injection of GL261 cells (5×10^4). The C57BL/6 mice treated with LOX inhibitor BAPN (2 g/L in drinking water) starting at 4 days post-orthotopic injection of Gl261 cells. Representative mice (**K**) and quantification of tumor volume based on bioluminescence (**L**) are shown (n=6/group).

(M) Survival curves of C57BL/6 mice implanted with GL261 cells (5×10^4). Mice were treated with or without LOX inhibitor BAPN (2 g/L in drinking water) starting at 4 days post-orthotopic injection of GL261 cells (n=5 per group).

(N, O) *In vivo* bioluminescence-based images 45 days post-orthotopic injection of *PTEN*-OE U87 cells (5×10^5). The SCID mice treated with LOX inhibitor BAPN (2 g/L in drinking water) starting at

4 days post-orthotopic injection of cells. Representative mice (**N**) and quantification of tumor volume based on bioluminescence (**O**) are shown (n=5/group) at 45 days post-orthotopic injection of cells.

(P) Survival curves of SCID mice implanted with *Inf4a/Arf*$^{-/-}$*EGFRviii* NSCs (5×10^4). Mice were treated with or without LOX inhibitor BAPN (2 g/L in drinking water) starting at 4 days postorthotopic injection of NSCs (n=5 per group).

Data from multiple replicates are presented as mean. Error bars indicate mean ± SD. *p<0.05, **p<0.01, ***p<0.001, n.s., no significant difference, Student's t test. In (I), (J), (M) and (P), *p<0.05, **p<0.01, n.s., no significant difference, log-rank test.

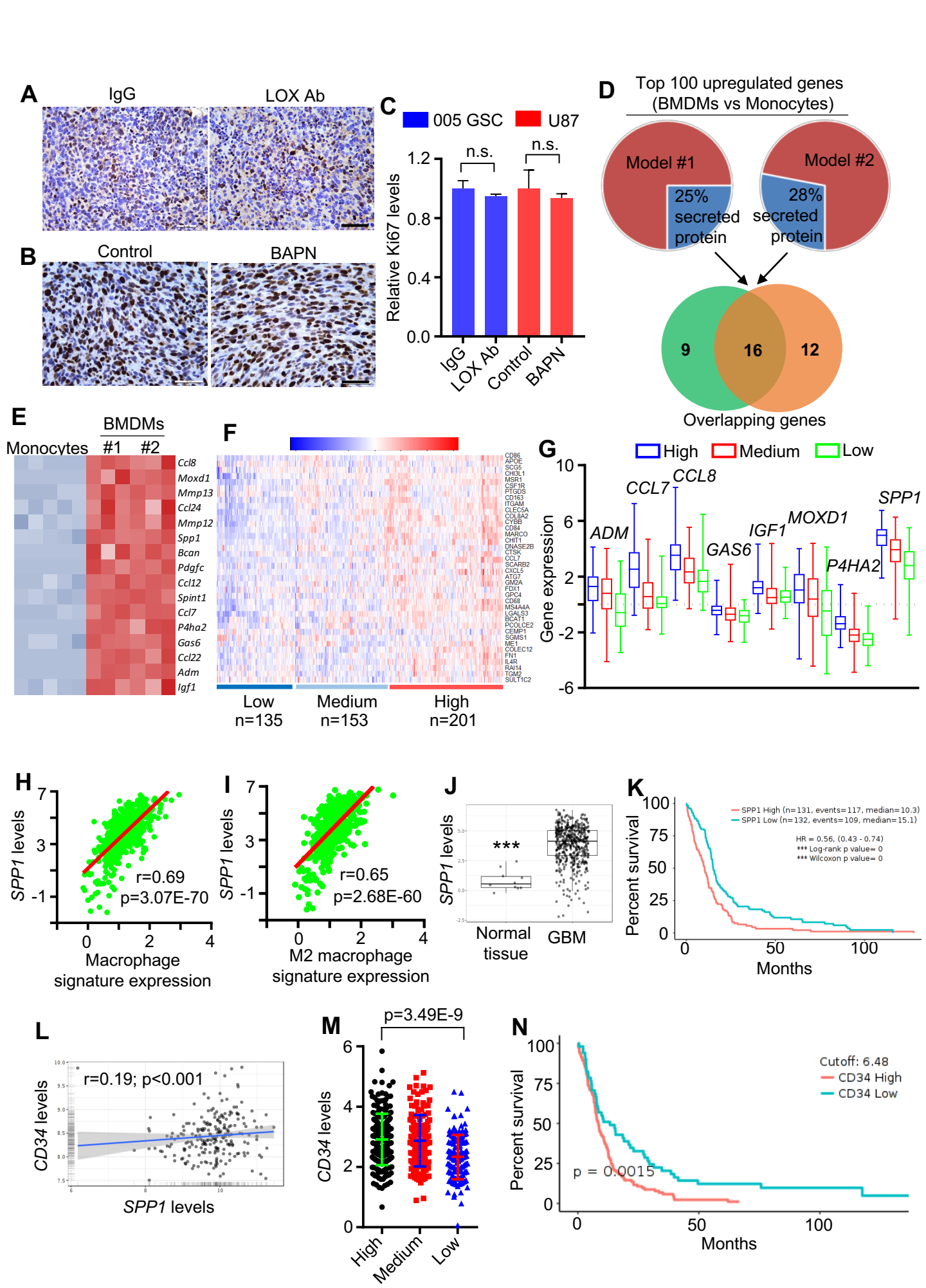
A
IgG
LOX Ab
B
Control
BAPN
C
005 GSC
U87
n.s.
n.s.
Relative Ki67 levels
1.2
0.8
0.4
0.0
IgG
LOX Ab
Control
BAPN
D
Top 100 upregulated genes
(BMDMs vs Monocytes)
Model #1
25%
secreted
protein
Model #2
28%
secreted
protein
9
16
12
Overlapping genes
E
BMDMs
Monocytes
#1
#2
Ccl8
Moxd1
Mmp13
Ccl24
Mmp12
Spp1
Bcan
Pdgfc
Ccl12
Spint1
Ccl7
P4ha2
Gas6
Ccl22
Adm
Igf1
F
Low
n=135
Medium
n=153
High
n=201
G
High
Medium
Low
Gene expression
10
6
2
-2
-6
ADM
CCL7
CCL8
GAS6
IGF1
MOXD1
P4HA2
SPP1
H
SPP1 levels
7
3
-1
r=0.69
p=3.07E-70
0
2
4
Macrophage
signature expression
I
SPP1 levels
7
3
-1
r=0.65
p=2.68E-60
0
2
4
M2 macrophage
signature expression
J
SPP1 levels
***
Normal
tissue
GBM
K
Percent survival
100
75
50
25
0
SPP1 High (n=131, events=117, median=10.3)
SPP1 Low (n=132, events=109, median=15.1)
HR = 0.56, (0.43 - 0.74)
*** Log-rank p value= 0
*** Wilcoxon p value= 0
0
50
100
Months
L
CD34 levels
r=0.19; p<0.001
SPP1 levels
M
p=3.49E-9
CD34 levels
6
4
2
0
High
Medium
Low
N
Percent survival
100
75
50
25
0
Cutoff: 6.48
CD34 High
CD34 Low
p = 0.0015
0
50
100
Months

Figure S6. LOX inhibition does not affect glioma cell proliferation and TAM-derived SPP1 promotes GBM progression and angiogenesis, related to Figure 6.

(A, B) IHC for Ki67 in 005 GSC mouse tumors treated with LOX antibodies (**A**), and in U87 tumors treated with LOX inhibitor BAPN (**B**). Scale bars, 50 μm.

(C) Quantification of relative Ki67 levels in 005 GSC mouse tumors treated with LOX antibodies and in U87 tumors treated with LOX inhibitor BAPN. n=3 biological replicates.

(D,E) The analysis of 16 common genes encoding secreted proteins from top 100 upregulated genes in glioma-associated BMDMs comparing to monocytes in two GBM mouse models (Bowman et al., 2016).

(F) Clustering of human TCGA GBM samples into Macrophage-high, -medium and -low groups using a 38-gene macrophages signature.

(G) The expression of *SPP1*, *P4HA2*, *MOXD1*, *IGF1*, *GAS6*, *CCL8*, *CCL7* and *ADM* in Macrophage-high, -medium and -low TCGA GBM patients.

(H,I) The correlation between total macrophage (**H**) and M2 macrophage (**I**) signatures and *SPP1* levels in TCGA GBM patients. r and p values are shown.

(J) The expression level of *SPP1* in normal brain tissues (n=10) and GBM tumor tissues (n=528) in TCGA GBM database.

(K) Kaplan-Meier survival curves of GBM patients relative to expression levels of *SPP1* in TCGA GBM database.

(L) The correlation between *SPP1* and *CD34* in patients of Rembrandt GBM database. r and p values are shown.

(M) The expression of *CD34* in Macrophage-high, -medium and -low TCGA GBM patients.

(N) Kaplan-Meier survival curves of GBM patients relative to expression levels of *CD34* in Gravendeel GBM database.

Data from multiple replicates are presented as mean. Error bars indicate mean ± SD. ***p<0.001, n.s., no significant difference, Student's t test. In (H), (I) and (L), pearson's correlation test.

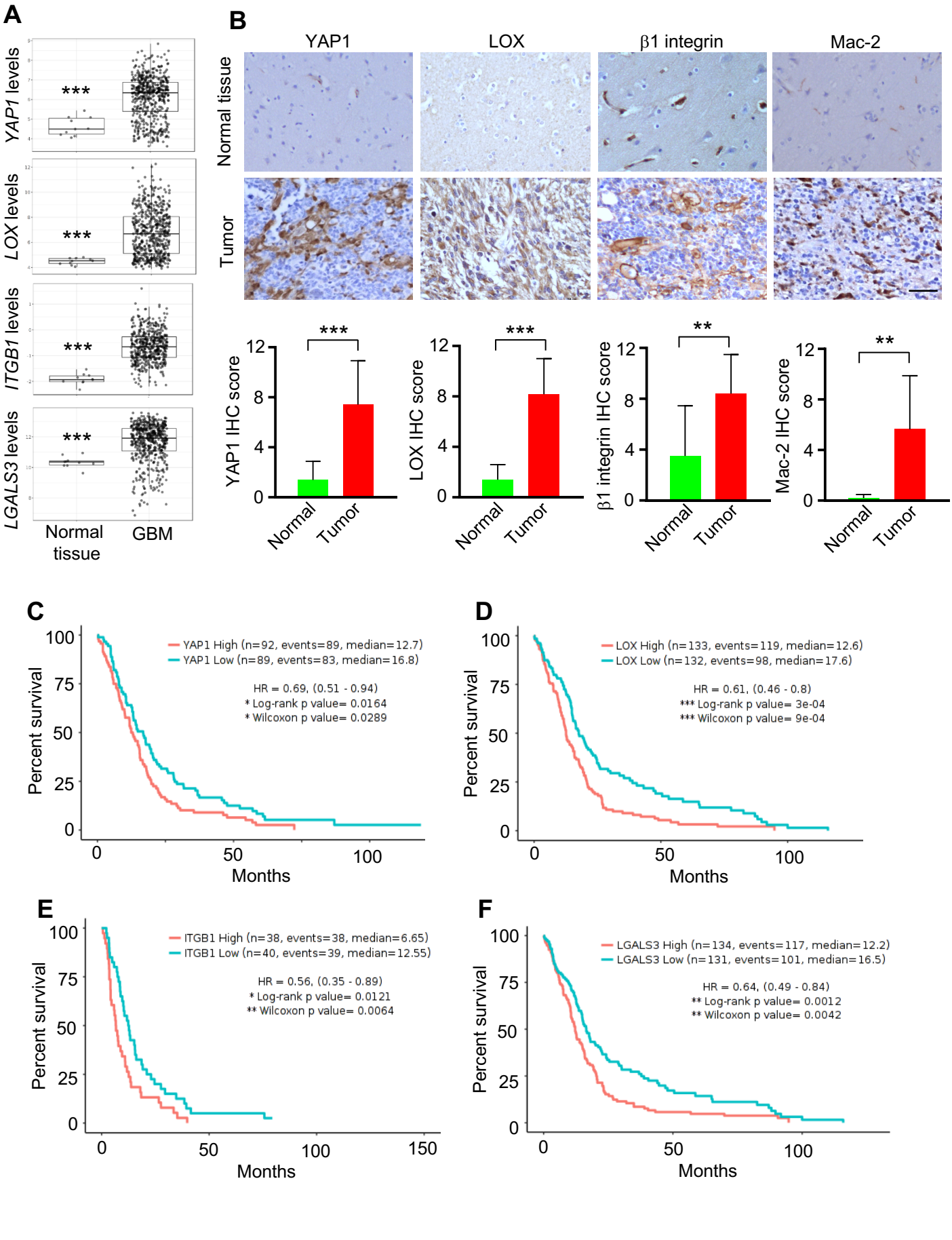
A
YAP1 levels
LOX levels
ITGB1 levels
LGALS3 levels
***
Normal tissue
GBM
B
YAP1
LOX
β1 integrin
Mac-2
Normal tissue
Tumor
YAP1 IHC score
LOX IHC score
β1 integrin IHC score
Mac-2 IHC score
***
**
Normal
Tumor
C
YAP1 High (n=92, events=89, median=12.7)
YAP1 Low (n=89, events=83, median=16.8)
HR = 0.69, (0.51 - 0.94)
* Log-rank p value= 0.0164
* Wilcoxon p value= 0.0289
Percent survival
Months
D
LOX High (n=133, events=119, median=12.6)
LOX Low (n=132, events=98, median=17.6)
HR = 0.61, (0.46 - 0.8)
*** Log-rank p value= 3e-04
*** Wilcoxon p value= 9e-04
E
ITGB1 High (n=38, events=38, median=6.65)
ITGB1 Low (n=40, events=39, median=12.55)
HR = 0.56, (0.35 - 0.89)
* Log-rank p value= 0.0121
** Wilcoxon p value= 0.0064
F
LGALS3 High (n=134, events=117, median=12.2)
LGALS3 Low (n=131, events=101, median=16.5)
HR = 0.64, (0.49 - 0.84)
** Log-rank p value= 0.0012
** Wilcoxon p value= 0.0042

Figure S7. YAP1-LOX-β1 integrin -macrophage axis in GBM progression, related to Figure 7.

(A) The mRNA expression levels of *YAP1*, *LOX*, *ITGB1* and *LGALS3* in normal brain tissues (n=10) and GBM tumor tissues (n=528) in TCGA GBM database. ****P*<0.001 vs normal brain tissue, Student's t test.

(B) Immunohistochemistry (IHC) for YAP1, LOX, β1 integrin and Mac-2 in human GBM tissue microarray which contains 5 normal brain tissues, 32 GBM and 3 anaplastic astrocytoma samples. The expression levels were quantified as the IHC score based on their expression level. Scale bar, 50 μm. Error bars indicate mean ± SD. ***P*<0.01, ****P*<0.001, Student's t test.

(C-F) Kaplan-Meier survival curves of GBM patients relative to expression levels of *YAP1* in Rembrandt GBM database (**C**), *LOX* in TCGA GBM database (**D**), *ITGB1* in Gravendeel GBM database (**E**), and *LGALS3* in TCGA GBM database (**F**).

Table S5. A list of primers used for RT-qPCR analysis, related to STAR Methods.

Gene name	Forward	Reverse
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
ADM	ATGAAGCTGGTTTCCGTCG	GACATCCGCAGTTCCCTCTT
ARG1	TGGACAGACTAGGAATTGGCA	CCAGTCCGTCAACATCAAAACT
BICC1	GGAAACAAATACGCAGATTGCTT	CTTCAGTGTGACTCGATTGCTT
CCL5	TCTGCGCTCCTGCATCTG	AGTGGGCGGGCAATGTAG
CCL22	ATCGCCTACAGACTGCACTC	GACGGTAACGGACGTAATCAC
CKLF	CGGCTGGCACTAACTGTGA	CGGTGACTTCAAATCCAGTGATA
COL6A1	ACAGTGACGAGGTGGAGATCA	GATAGCGCAGTCGGTGTAGG
CSF1	TGGCGAGCAGGAGTATCAC	AGGTCTCCATCTGACTGTCAAT
CSF2	TCCTGAACCTGAGTAGAGACAC	TGCTGCTTGTAGTGGCTGG
CTGF	CAAGGGCCTCTTCTGTGACT	ACGTGCACTGGTACTTGCAG
CXCL5	AGCTGCGTTGCGTTTGTTTAC	TGGCGAACACTTGCAGATTAC
DAB2	GTAGAAACAAGTGCAACCAATGG	GCCTTTGAACCTTGCTAAGAGA
EMP2	ATTCACGACAAAAACGCGAAAT	CAGTATCAGGTACATCATGCCG
FABP5	TGAAGGAGCTAGGAGTGGGAA	TGCACCATCTGTAAAGTTGCAG
GAL	CCGGCCAAGGAAAAACGAG	GAGGCCATTCTTGTCGCTGA
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
IFNG	GTCCAACGCAAAGCAATACA	ATATTGCAGGCAGGACAACC
IGF2	GTGGCATCGTTGAGGAGTG	CACGTCCCTCTCGGACTTG
IL4	CCAACTGCTTCCCCCTCTG	TCTGTTACGGTCAACTCGGTG
IL6	ATTCTGCGCAGCTTTAAGGA	ATCTGAGGTGCCCATGCTAC
IL13	CCTCATGGCGCTTTTGTTGAC	TCTGGTTCTGGGTGATGTTGA
LCN2	GACAACCAATTCCAGGGGAAG	GCATACATCTTTTGCGGGTCT
LOX	CGGCGGAGGAAAACTGTCT	TCGGCTGGGTAAGAAATCTGA
SEMA3E	GTTTGCTGGACTCTACAGTGAC	CTTTCAACAGACGCTCATCGT
SERPINB2	CAGCACCGAAGACCAGATGG	CCTGCAAAATCGCATCAGGATAA
SERPINE2	TGGTGATGAGATACGGCGTAA	GTTAGCCACTGTCACAATGTCTT
SPP1	GAAGTTTCGCAGACCTGACAT	GTATGCACCATTCAACTCCTCG
THBS1	AGACTCCGCATCGCAAAGG	TCACCACGTTGTTGTCAAGGG
TNC	TCCCAGTGTTCGGTGGATCT	TTGATGCGATGTGTGAAGACA
TNFA	CCTGTGAGGAGGACGAACAT	GGTTGAGGGTGTCTGAAGGA
TSPAN3	GAGTGTCCCTCTTAGCTGCTG	AGCTTCTTCACTACTAGAGCCTC
VEGF	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
VEGFC	GAGGAGCAGTTACGGTCTGTG	TCCTTTCCTTAGCTGACACTTGT
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Adm	CACCCTGATGTTATTGGGTTCA	CCACTTATTCCACTTCTTTCGGA
Arg1	TTGGGTGGATGCTCACACTG	GTACACGATGTCTTTGGCAGA
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ccl7	CCACATGCTGCTATGTCAAGA	ACACCGACTACTGGTGATCCT
Ccl8	TCTACGCAGTGCTTCTTTGCC	AAGGGGGATCTTCAGCTTTAGTA
Ccl24	ATTCTGTGACCATCCCCTCAT	TGTATGTGCCTCTGAACCCAC
Ccr2	ATCCACGGCATACTATCAACATC	CAAGGCTCACCATCATCGTAG
Cd200r1	AGGCATTTCCAGTATCACAAGG	CCAATGGCCGACAAAGTAAGG
Chil3	GTACAAGCTGGTCTGCTACTTC	ATGTGCTAAGCATGTTGTCGC
Gas6	CCGCGCCTACCAAGTCTTC	CGGGGTCGTTCTCGAACAC
Igf1	CACATCATGTCGTCTTCACACC	GGAAGCAACACTCATCCACAATG
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC

Moxd1	ACACACAGTGATCGAGTTTAGC	CGGGATCGTCATGGTGGTA
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
P4ha2	CACCTCCATTGGGCACATGA	GCTCTTAATCTTGGCGAGCTT
Pparg	GGAAGACCACTCGCATTCCTT	GTAATCAGCAACCATTGGGTCA
Ptgs2	TGAGCAACTATTCCAAACCAGC	GCACGTAGTCTTCGATCACTATC
Spp1	AGAGCGGTGAGTCTAAGGAGT	TGCCCTTTCCGTTGTTGTCC
Tnfa	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG