

1 **SUPPLEMENTARY INFORMATON**

2 ARS2/MAGL signaling in glioblastoma stem cells promotes self-renewal and
3 M2-like polarization of tumor-associated macrophages

4 Yin et al.

5 Contents

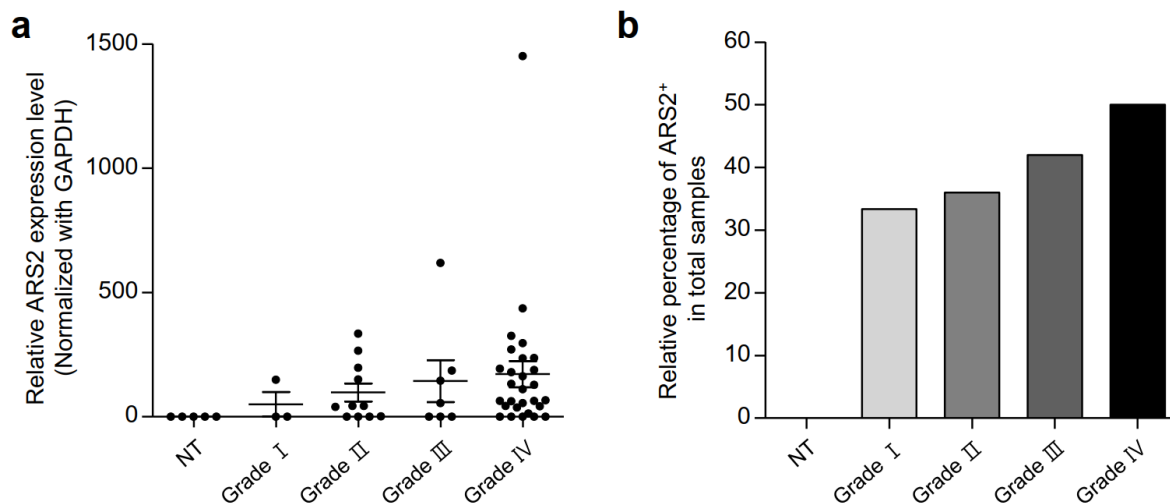
6 Supplementary Figures 1-8

7 Supplementary Tables 1-3

8

1 **SUPPLEMENTARY FIGURES 1-7**

2 **Supplementary Figure 1**



3

4 **Supplementary Figure 1. Comparison of ARS2 expression level according to brain**

5 **tumor grade. a,** The dot plot of relative ARS2 expression level according to brain tumor

6 grade in patient tissues from National Cancer Center (NCC), Republic of Korea. Expression

7 level normalized by loading control GAPDH. (n; NT=5, Grade I=3, II=11, III=6, IV=26). All

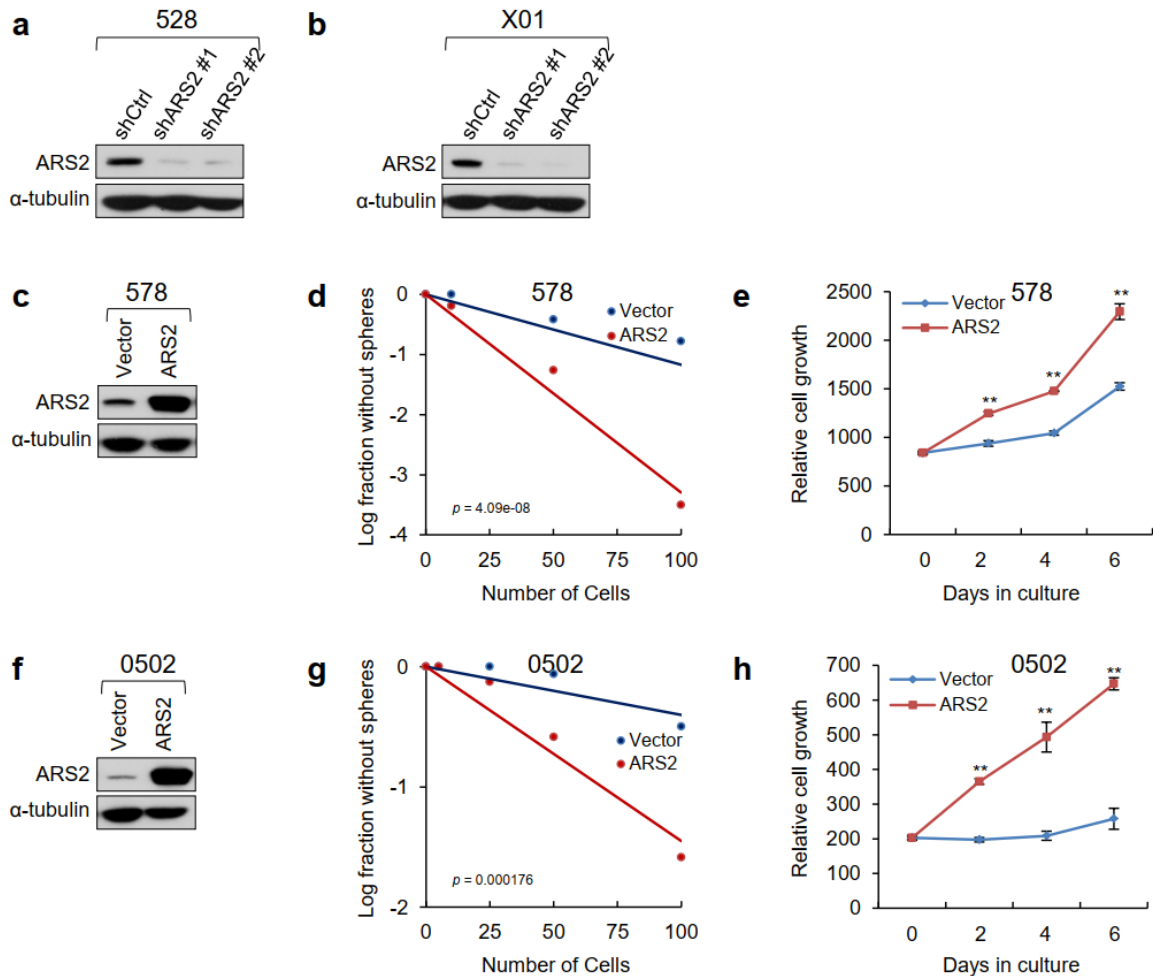
8 error bars represent mean \pm standard error of the mean (SEM). **b,** The bar graph of ARS2

9 positive percentage according to brain tumor grade in patient tissues from NCC. The index

10 score of y-axis are counted as percentages compared to total samples.

11

1 **Supplementary Figure 2**



2

3 **Supplementary Figure 2. Overexpression of ARS2 Promotes GSCs Self-renewal. a,b,**

4 Immunoblot (IB) analysis of ARS2 in GSCs (528 cells and X01 cells) infected with a

5 shARS2-expressing lentiviral or shCtrl construct. α -tubulin was used as a loading control. **c,**

6 IB analysis of ARS2 in GSCs (578 cells) infected with an ARS2-expressing lentiviral or

7 vector construct. α -tubulin was used as a loading control. **d,** Limiting Dilution Assays

8 (LDAs), performed in 578-Vector and 578- ARS2 cells. (n = 24, *t*-test). **e,** Cell proliferation

9 assays performed using 578-Vector and 578- ARS2 cells. All error bars represent mean \pm

10 SEM (n=3). ** $P < 0.01$. **f,** IB analysis of ARS2 in GSCs (0502 cells) infected with an ARS2-

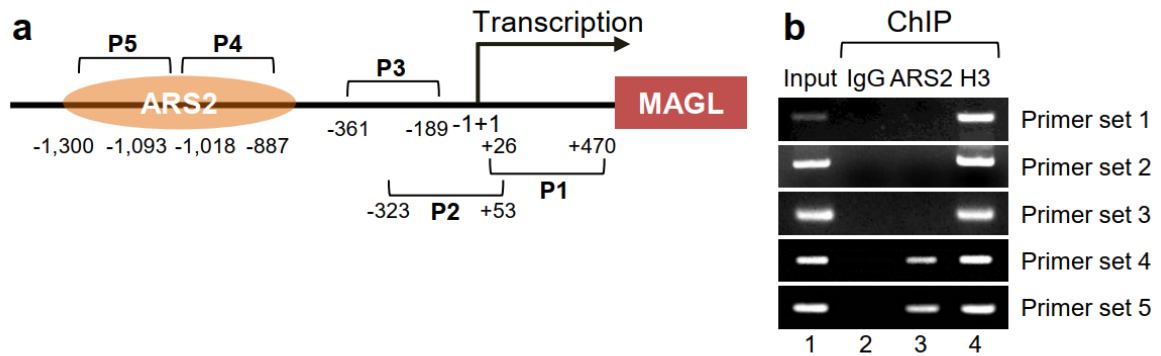
11 expressing lentiviral or vector construct. α -tubulin was used as a loading control. **g,** LDAs,

12 performed in 0502-Vector and 0502- ARS2 cells. (n = 24, *t*-test). **h,** Cell proliferation assays

13 performed using 0502-Vector and 0502-ARS2 cells. All error bars represent mean \pm SEM

14 (n=3). ** $P < 0.01$.

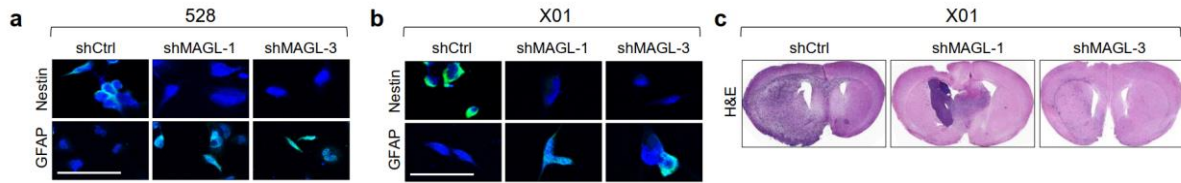
1 **Supplementary Figure 3**



2
3 **Supplementary Figure 3. ARS2 Directly Binding to MAGL Promoter.** **a**, Schematic
4 primer design for Chromatin immunoprecipitation (ChIP) analysis in MAGL promoter. **b**,
5 Immunoprecipitated chromatin was analyzed by PCR using primer specific for the promoter
6 region of the MAGL gene. An antibody against IgG was used as a nonspecific control and
7 histone H3 antibody as positive ChIP grade control.

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1 **Supplementary Figure 4**

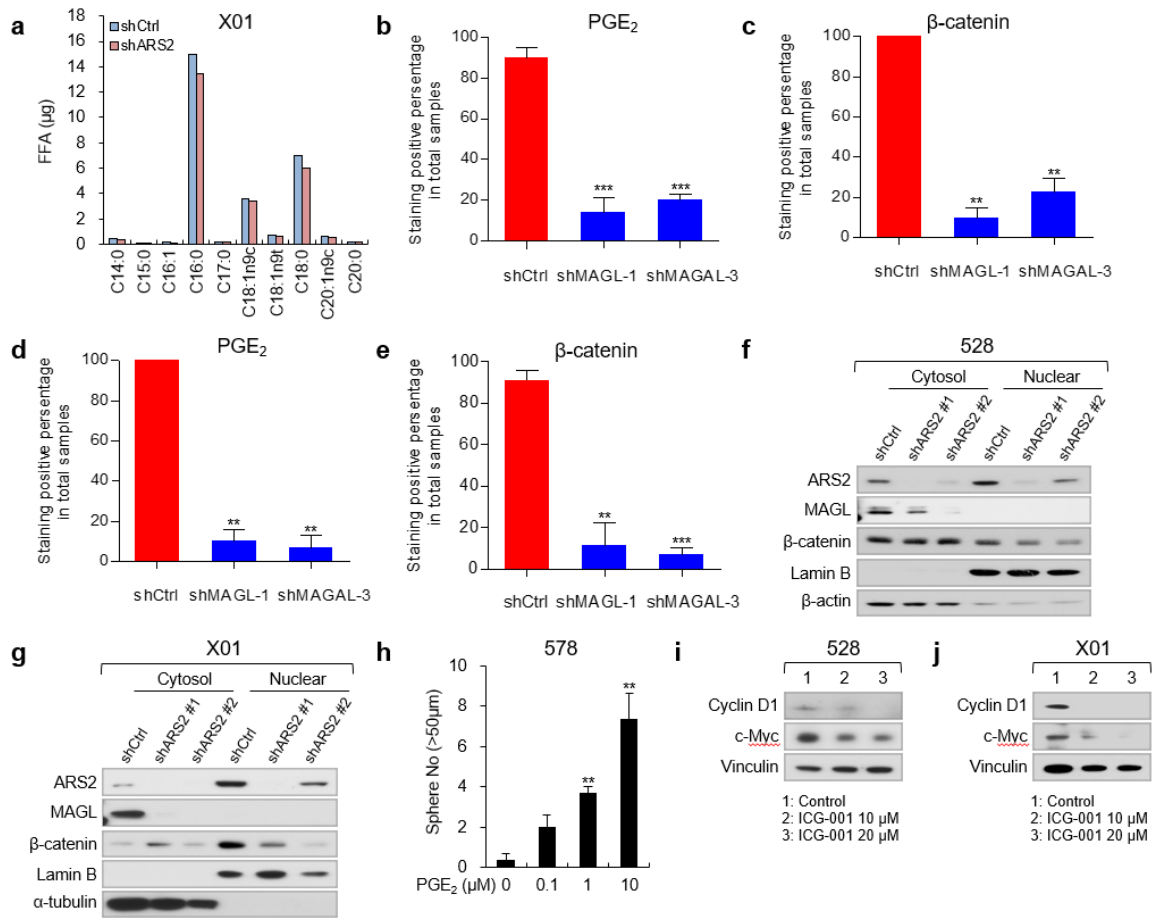


2

3 **Supplementary Figure 4. MAGL Regulates GSCs Stemness.** **a,b**, Representative
4 immunocytochemistry (ICC) images of Nestin and GFAP in GSCs (528 cells and X01 cells)
5 infected with a shMAGL-expressing lentiviral or shCtrl construct. Nuclei were
6 counterstained with DAPI (blue). Scale bar, 50 μ m. **c**, H&E staining of the whole brain in the
7 orthotopic xenograft mouse model of GSCs (X01 cells) infected with a shMAGL-expressing
8 lentiviral or shCtrl construct. The sample is extracted at 39 days after cell injection. Scale bar,
9 50 μ m.

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1 Supplementary Figure 5

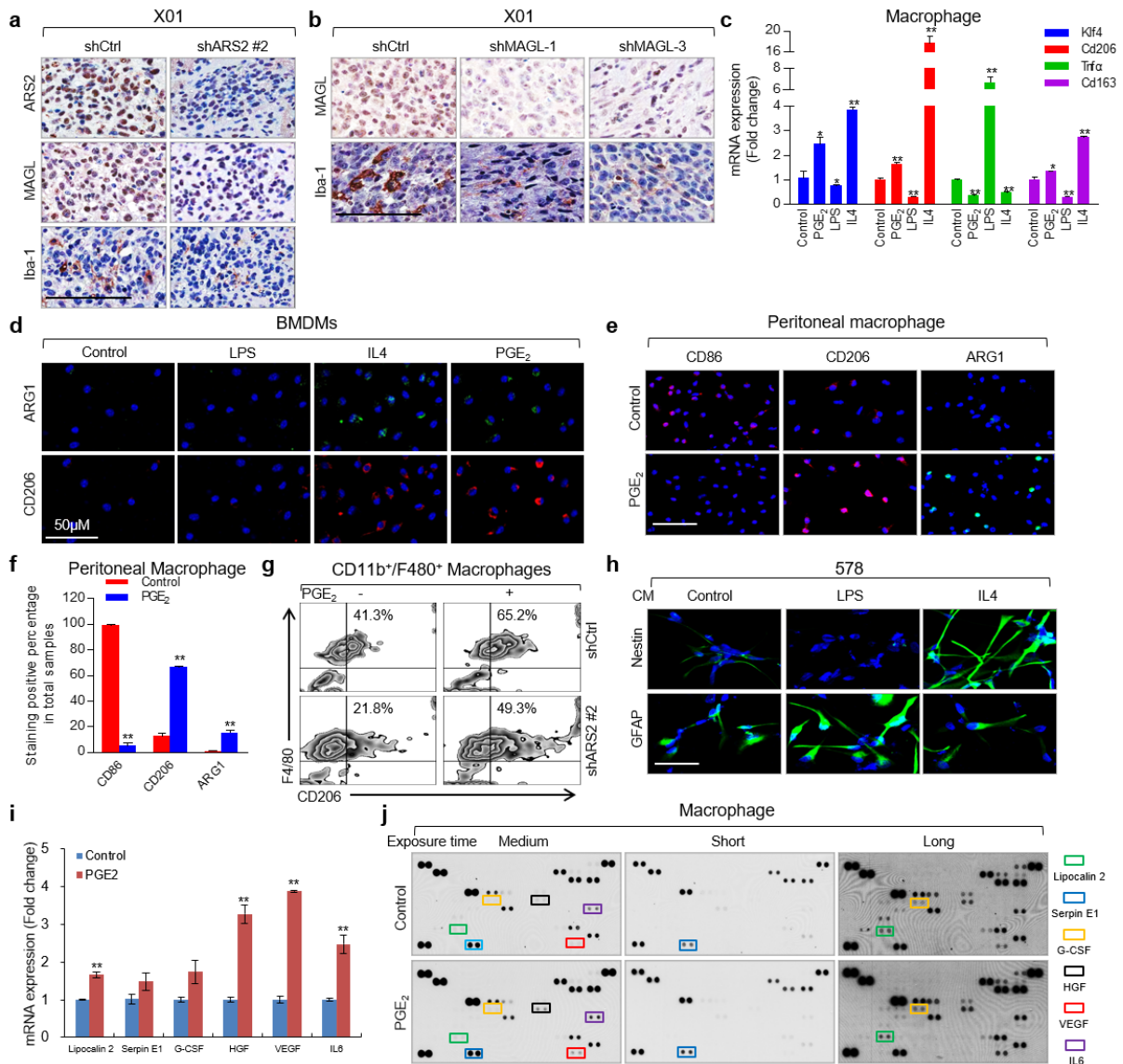


2

3 **Supplementary Figure 5. MAGL Regulates Stemness of GSCs by PGE₂.** **a**, Fatty Acid
 4 analysis performed using GSCs (X01 cells) infected with a shARS2-expressing lentiviral or
 5 shCtrl construct. (n = 2). **b,c**, The graph shows the average positive cells percentage of ICC
 6 analysis of PGE₂ and β-catenin in 528 cell infected with a shMAGL-expressing lentiviral or
 7 shCtrl construct. All error bars represent mean ± SEM (n=3). (** *P* < 0.01, *** *P* < 0.001, *t*-
 8 test). **d,e**, The graph shows the average positive cells percentage of ICC analysis of PGE₂ and
 9 β-catenin in X01 cell infected with a shMAGL-expressing lentiviral or shCtrl construct. All
 10 error bars represent mean ± SEM (n=3). (** *P* < 0.01, *** *P* < 0.001, *t*-test). **f,g**, Immunoblot
 11 (IB) analysis of ARS2, MAGL and β-catenin in fractionated nuclear or cytosolic lysates from
 12 528 cells and X01 cells infected with a shARS2-expressing lentiviral or shCtrl construct.
 13 Lamin B and β-actin were used as markers for nucleus and cytoplasm, respectively. **h**, Sphere
 14 formation assay performed using GSCs (578 cells) treated with different concentrations of
 15 PGE₂ (0.1, 1, 10µM) or control. The graph shows the average number of spheres greater than

1 50 μm in diameter. All error bars represent mean \pm SEM (n=3). (** $P < 0.01$, t -test). **i,j**, IB
2 analysis of Cyclin D1 and c-Myc in GSCs (528 and X01 cells) treated with different
3 concentrations of ICG-001 (10, 20 μM) or control. Vinculin was used as a loading control.
4

1 Supplementary Figure 6



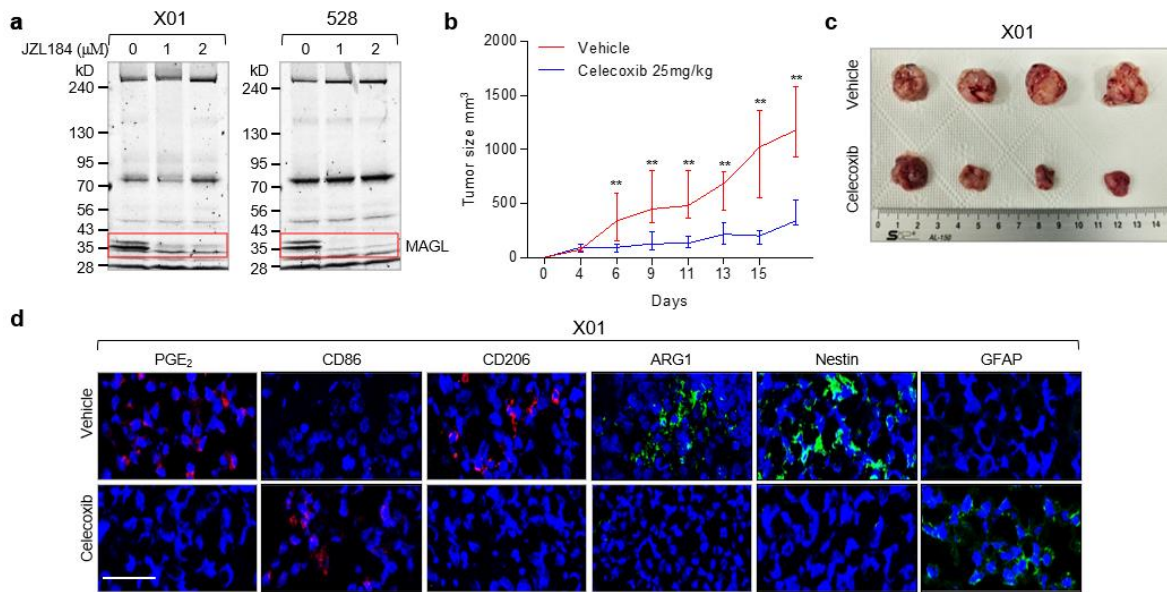
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3 **Supplementary Figure 6. ARS2/MAGL Expression Correlates with TAM Density.** **a**,
 4 Representative immunohistochemistry (IHC) images of ARS2, MAGL, and Iba-1 in the
 5 orthotopic xenograft mouse model of GSCs (X01 cells) infected with a shARS2-expressing
 6 lentiviral or shCtrl construct. Scale bar, 100 μm. **b**, Representative IHC images of MAGL and
 7 Iba-1 in the orthotopic xenograft mouse model of GSCs (X01 cells) infected with a
 8 shMAGL-expressing lentiviral or shCtrl construct. Scale bar, 100 μm. **c**, qRT-PCR analysis
 9 of Klf4, Cd206, Tnfα, and Cd163 in peritoneal mouse macrophages after induction with
 10 PGE₂ (10 μM), LPS or IL4, or treatment with control. All error bars represent mean ± SEM
 11 (n=3). (* *P* < 0.05, ** *P* < 0.01, *t*-test). **d**, Representative ICC images of ARG1 and CD206 in
 12 bone marrow derived-macrophages (BMDMs) after treatment with LPS, IL-4, PGE₂ (10 μM),

1 or control. Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm . **e, f,**
2 Representative ICC images (e) and corresponding quantification (f) of CD86, CD206, and
3 ARG1 in peritoneal mouse macrophages after treatment with PGE₂ (10 μM) or control. Scale
4 bar, 50 μm . All error bars represent mean \pm SEM (n=3). (** P < 0.01, *t*-test). **g,** Expression of
5 CD206 in macrophages treated with PGE₂ or vehicle control for 24hrs by flow cytometry.
6 CD11b and F4/80 was co-stained for macrophagic population verification. **h,** Representative
7 ICC images of Nestin and GFAP in GSCs (578 cells) cultured in conditioned media (CM)
8 from peritoneal mouse macrophages after induction with LPS or IL4, or treatment with
9 control. Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm . **i,** qRT-PCR analysis
10 of Lipocalin 2, Serpin E1, G-CSF, HGF, VEGF and IL6 in peritoneal mouse macrophages
11 after induction with PGE₂ (10 μM). All error bars represent mean \pm SEM (n=3). (** P < 0.01,
12 *t*-test). **j,** Cytokine array performed using peritoneal mouse macrophages after induction with
13 PGE₂ (10 μM).

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1 Supplementary Figure 7

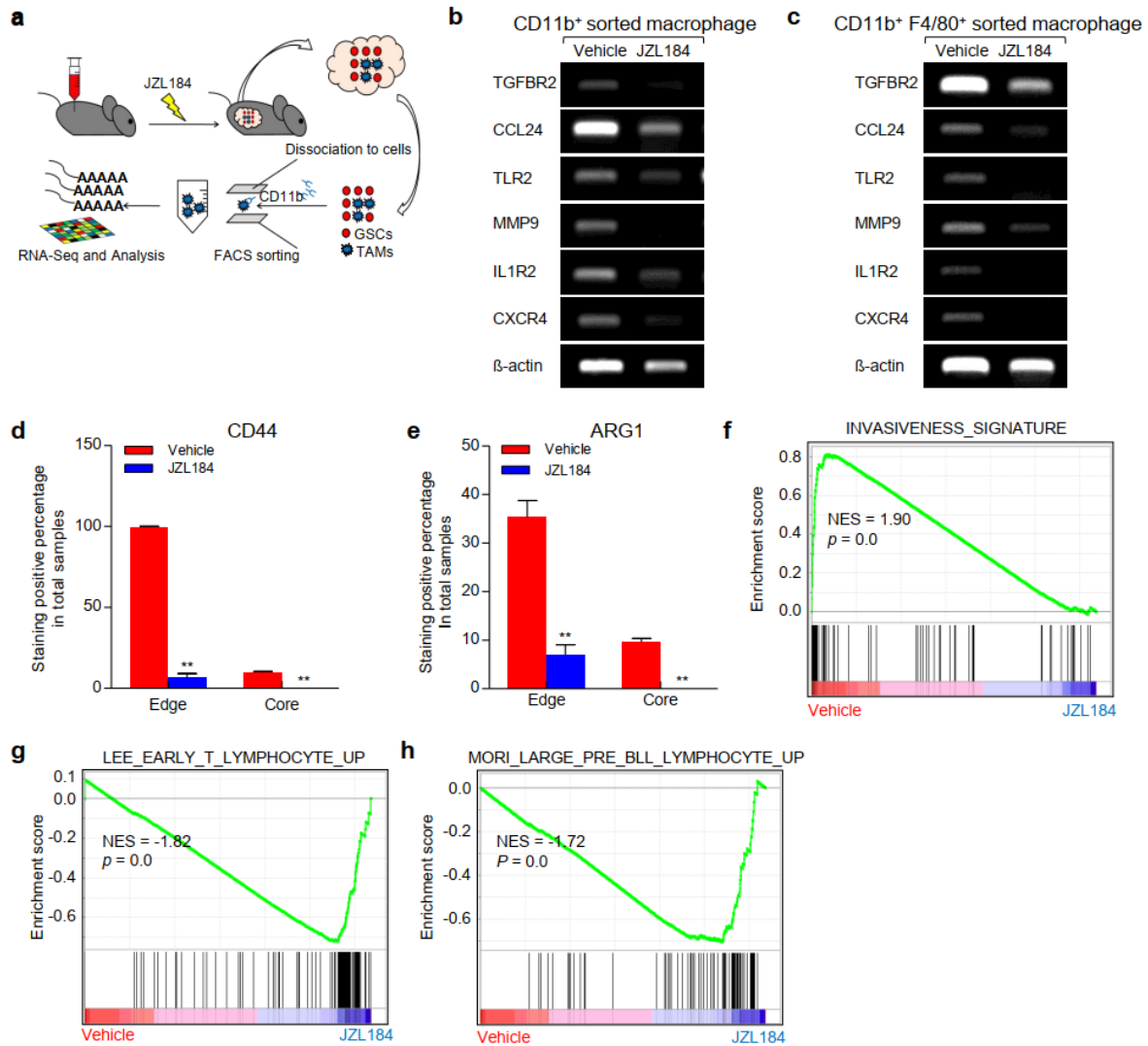


2

3 **Supplementary Figure 7. Celecoxib inhibits GSC self-renewal and tumorigenicity. a,**
4 **Immunoblot (IB) analysis of MAGL hydrolase activity in GSCs (X01, 528 cells) treated with**
5 **JZL184 (0, 1, 2 μ M). **b, Comparative analysis of subcutaneous tumor size of mice implanted**
6 **with X01 treated with Celecoxib 25mg/kg or vehicle. All error bars in the graph represent**
7 **mean \pm SD. (n=4, 3×10^6 cells injected per mouse). ** $P < 0.01$, t -test. **c, Images of tumor**
8 **tissues from mice treated with vehicle or celecoxib. **d, Representative IF images of PGE₂,**
9 **CD86, CD206, ARG1, Nestin and GFAP in a celecoxib-treated subcutaneous mouse model.**
10 **Sale bar, 50 μ M.********

11

1 Supplementary Figure 8



2

3 **Supplementary Figure 8. JZL184 downregulates M2-like TAM markers expression. a,**
 4 Schematic representation of the fluorescence-activated cell sorting (FACS) of CD11b⁺
 5 macrophages from the subcutaneous mouse models of GSC X01. **b,c,** RT-PCR analysis of
 6 M2-like TAMs markers in CD11b⁺ (b) and CD11b⁺/F4/80⁺ (c) sorted macrophages treated
 7 with JZL184 or vehicle. β-actin was used as a loading control. **d,e,** The graph shows the
 8 staining positive percentage of IHC and IF analysis of CD44 (d), ARG1 (e) at region of tumor
 9 edge or core of brain slices treated with JZL184 or vehicle. All error bars represent mean ±
 10 SEM (n=3). (n = 3, ** P < 0.01, t-test). **f** Gene set enrichment assay (GSEA) plot for
 11 invasiveness gene signature in comparison of TAMs from vehicle- vs. JZL184-treated
 12 subcutaneous mouse model. **g,h,** GSEA plot for two types of lymphocyte gene signature in
 13 comparison of TAMs from vehicle- vs. JZL184-treated subcutaneous mouse model.

1 **SUPPLEMENTARY TABLES 1-3**

2 Supplementary Table 1. Oligonucleotides used in PCR-based cloning of various plasmid
3 constructs.

4 * S, sense; A, antisense

Plasmid constructs	Sequence of oligonucleotides
pcDNA3-ARS2-FLAG	S: 5'- CGG GAT CCG CCA CCA TGG GTG ACA GTG ATG ACG -3' A: 5'- GCT CTA GAA AGA AAT CAA CAT CGT CTG GG -3'
pLenti6-ARS2-FLAG	S: 5'- AGT GTG GTG GAA TTC GGA TCC GCC ACC ATG GG -3' A: 5'- CCC TCT AGA CTC GAG GGC CCT ACT TGT CAT CGT CG - 3'
pLenti6-MAGL	S: 5'- CGG GAT CCG CCA CCA TGC CAG AGG AAA GTT CCC -3' A: 5'- GGA ATT CAC CGG CCA ATG CAT TCA G -3'
pGreen-MAGL-pro.	S: 5'- TTT TAT CGA TGA ATT CCG GTG CAC TTA GCA TGT C -3' A: 5'- TAC ACG CCT AAC TAG TCA TCG GAA ATG CCG CTG G-3'

5

6

1 Supplementary Table 2. Oligonucleotides used in shRNA expressing lentivirus constructs.

2 * S, sense; A, antisense

Plasmid constructs	Sequence of oligonucleotides
pLKO.1puro-shARS2- #1	S: 5'- CCG GGC TGA GAA TGA CAG TTC TAA TCT CGA GAT TAG AAC TGT CAT TCT CAG CTT TTT G -3' A: 5'- AAT TCA AAA AGC TGA GAA TGA CAG TTC TAA TCT CGA GAT TAG AAC TGT CAT TCT CAG C -3'
pLKO.1puro-shARS2- #2	S: 5'- CCG GGC CAT TGT CAA GAT GCT GGA TCT CGA GAT CCA GCA TCT TGA CAA TGG CTT TTT G -3' A: 5'- AAT TCA AAA AGC CAT TGT CAA GAT GCT GGA TCT CGA GAT CCA GCA TCT TGA CAA TGG-3'
pLKO.1puro-shMGLL- #1	S: 5'- CCG GCA ACT CCG TCT TCC ATG AAA TCT CGAGAT TTC ATG GAA GAC GGA GTT GTT TTT G-3' A: 5'- AAT TCA AAA ACA ACT CCG TCT TCC ATG AAA TCT CGA GAT TTC ATG GAA GAC GGA GTT G -3'
pLKO.1puro-shMGLL- #2	S: 5'- CCG GCC AGG ACA AGA CTC TCA AGA TCT CGA GAT CTT GAG AGT CTT GTC CTG GTT TTT G-3' A: 5'- AAT TCA AAA ACC AGG ACA AGA CTC TCA AGA TCT CGA GAT CTT GAG AGT CTT GTC CTG G -3'

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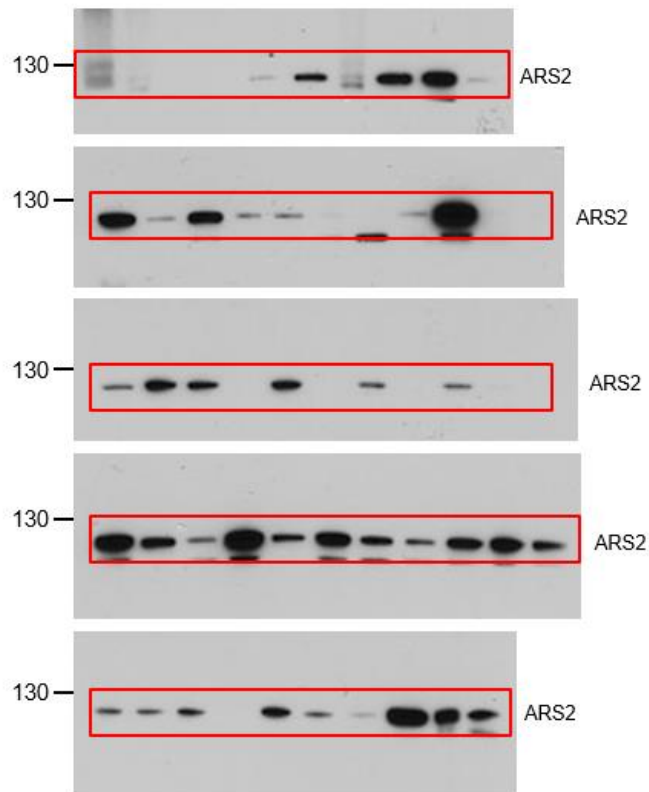
1 Supplementary Table 3. Oligonucleotides used in ChIP and semi-quantitative PCR analysis.

2 * S, sense; A, antisense

Genes	Sequence of oligonucleotides
Primer set 1	S: 5'- CCGGCCCAGGGATAAAGTGG -3' A: 5'- TCGGAAATGCCGCTGGGAAA -3'
Primer set 2	S: 5'-TACACGTGTGGTGAGTGTGC-3' A: 5'-CTGCGCCGCCACTTTATC-3'
Primer set 3	S: 5'-CCTGCGTGCAGTGTAGTGAC-3' A: 5'-GAACTGAGCTGGGTTCATGG-3'
Primer set 4	S: 5'- AGC GGA GGA GCT AAT GTT CA -3' A: 5'- GCT GTG ACC CCC AGA TAA AA -3'
Primer set 5	S: 5'- CAG TGG GTA AGT CAC GCT CA -3' A: 5'- CCA GTG GAG TGT CCC TGT CT -3'

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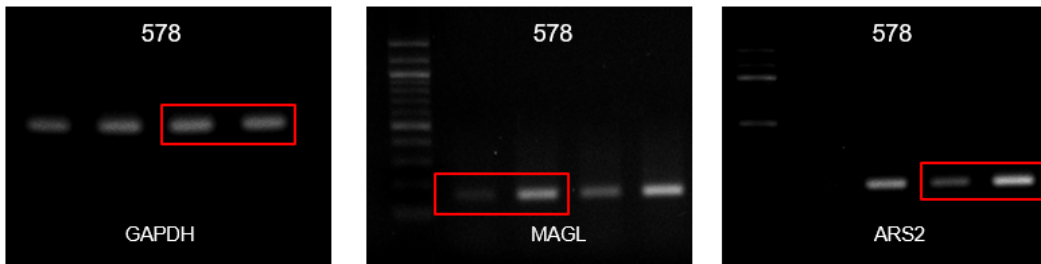
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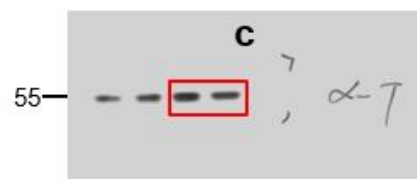
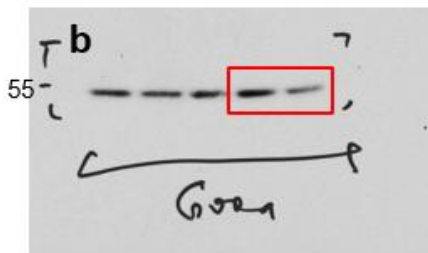
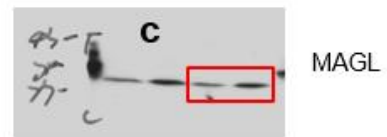
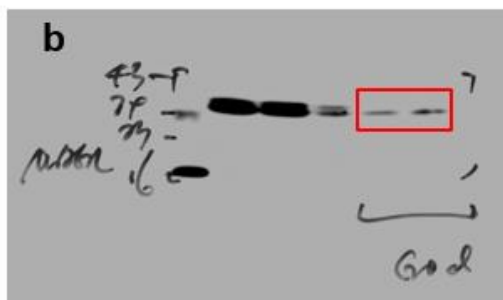
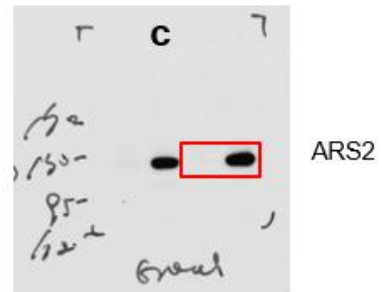
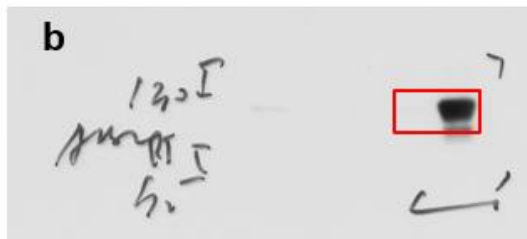
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2 **Original immunoblot for Fig. 1f.**

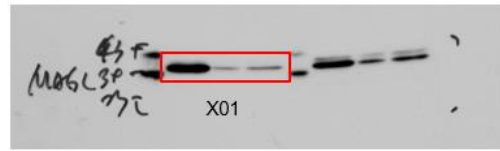
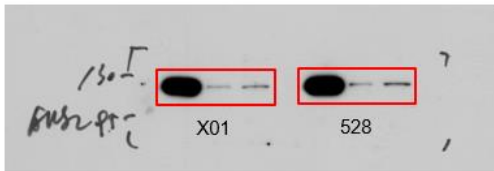
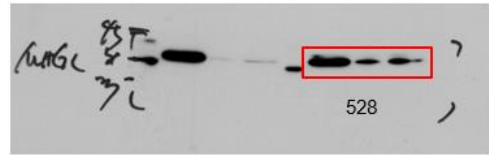
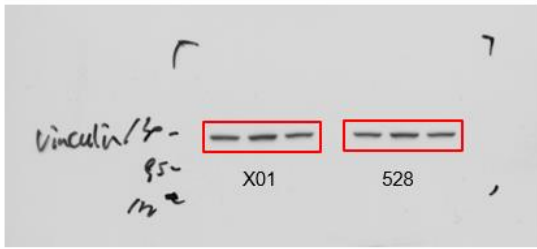
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1
2 Original RT-PCR for Fig. 3b,c.

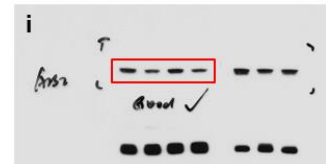
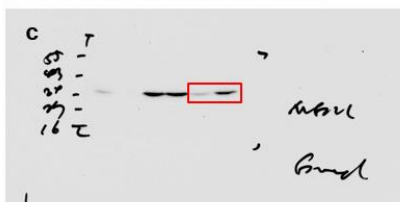
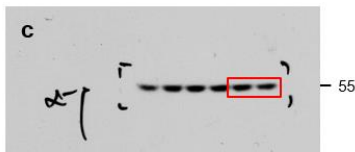
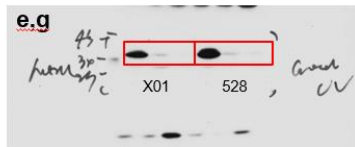
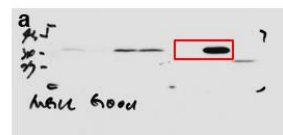


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4 Original immunoblot for Fig. 3b,c.



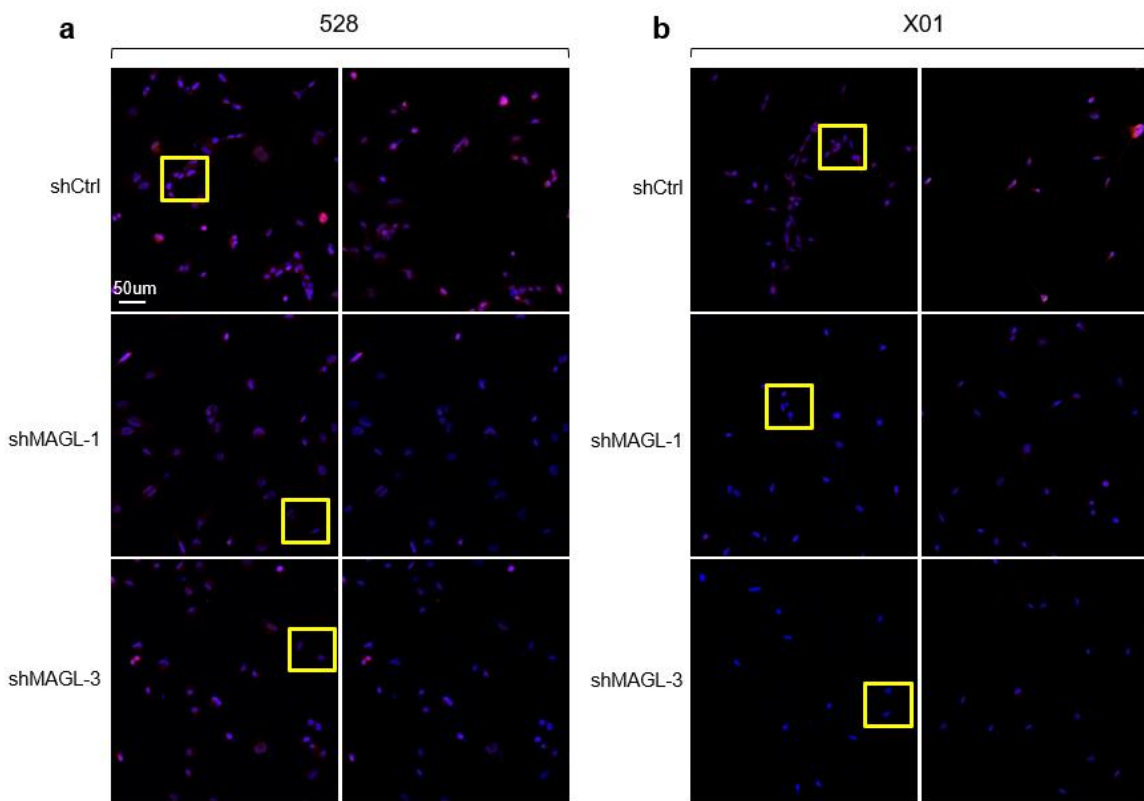
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Original immunoblot for Fig. 3d,e.

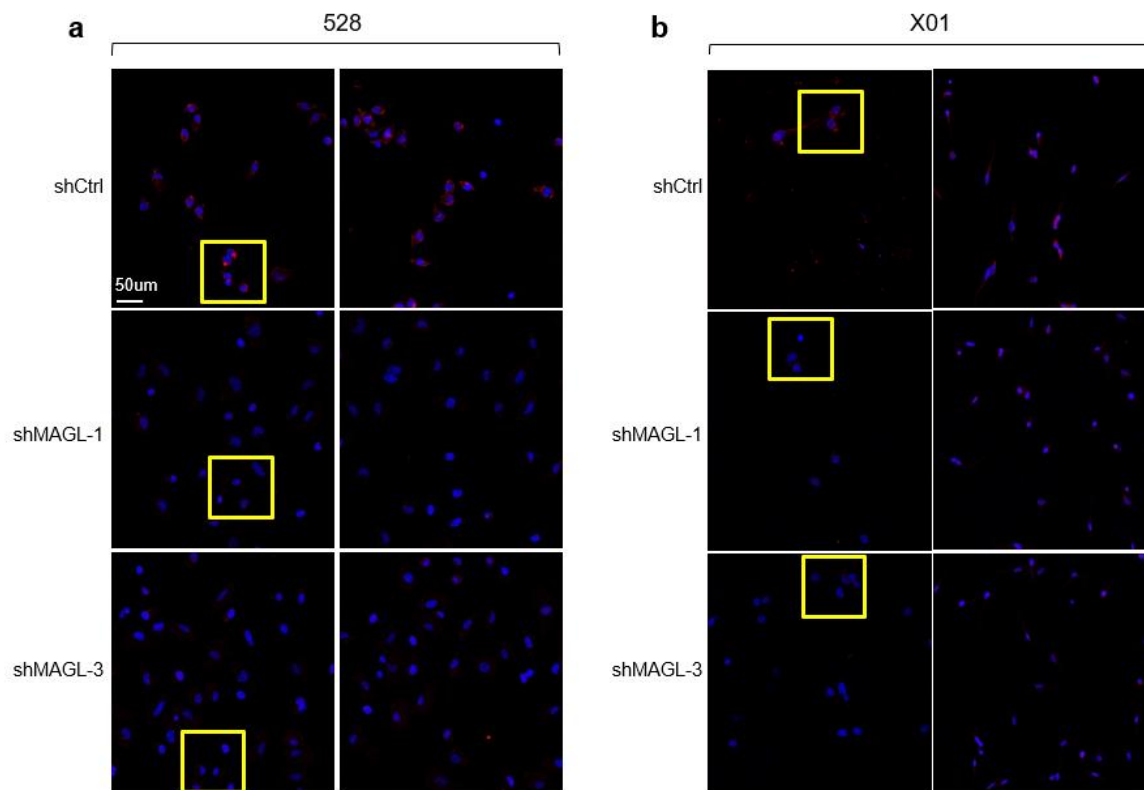


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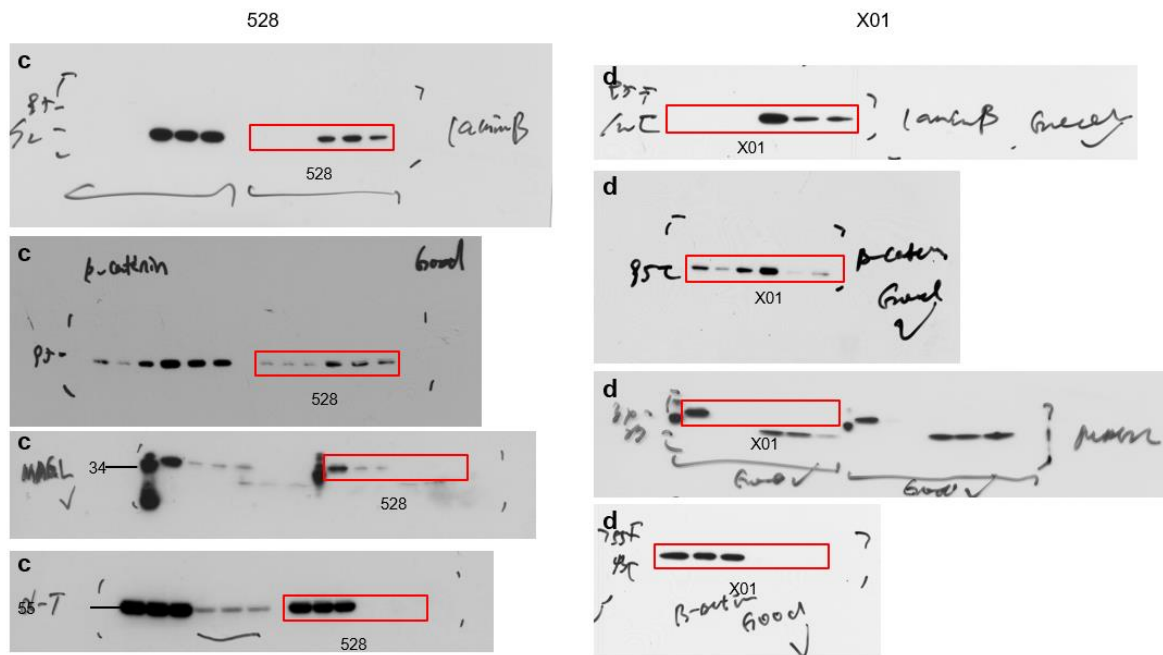
Original immunoblot for Fig. 4a,c,e,g,i.



1
2 **Original ICC staining (PGE₂) for Fig. 5a,b.**

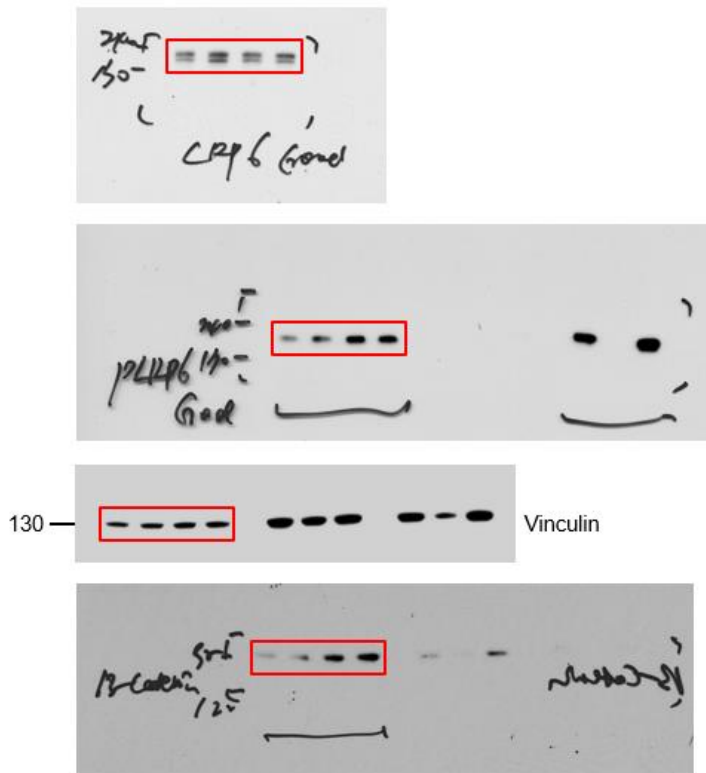


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4 **Original ICC staining (β-Catenin) for Fig. 5a,b.**



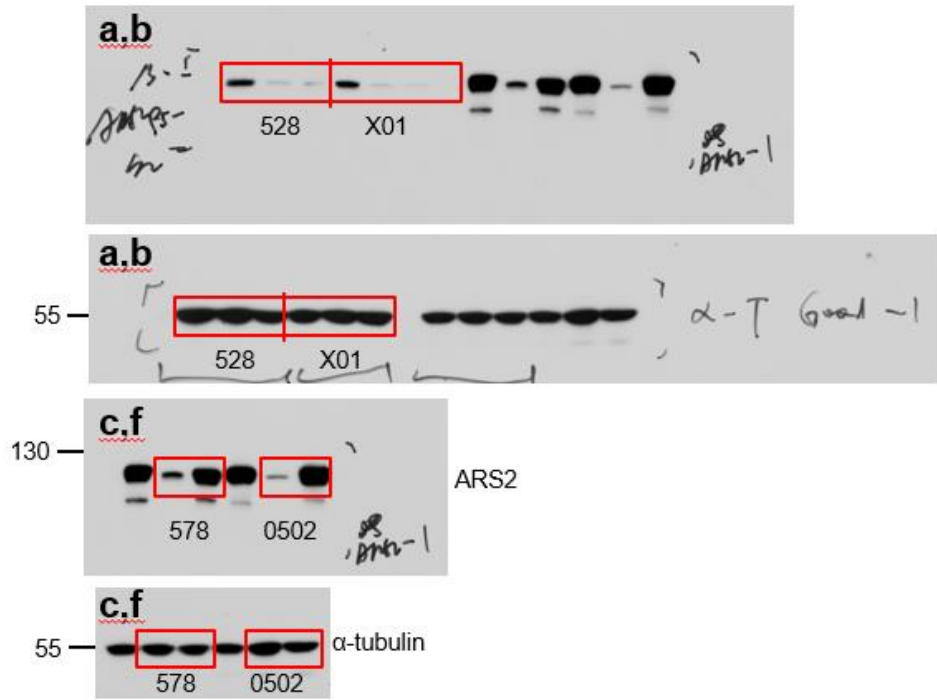
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Original immunoblot for Fig. 5c,d.



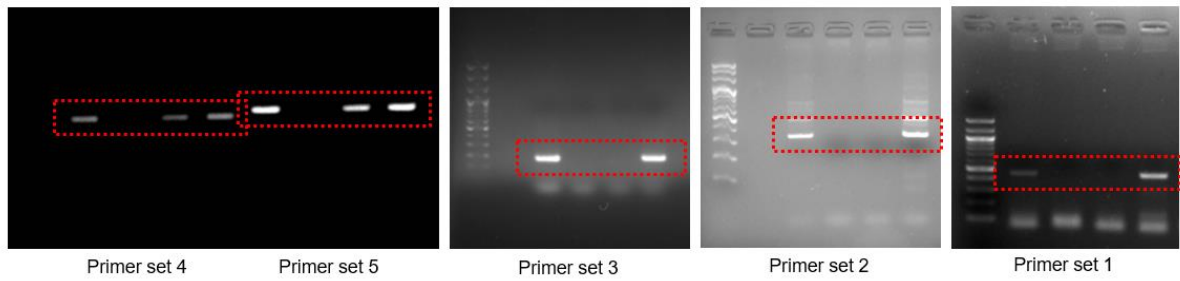
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Original immunoblot for Fig. 5e.



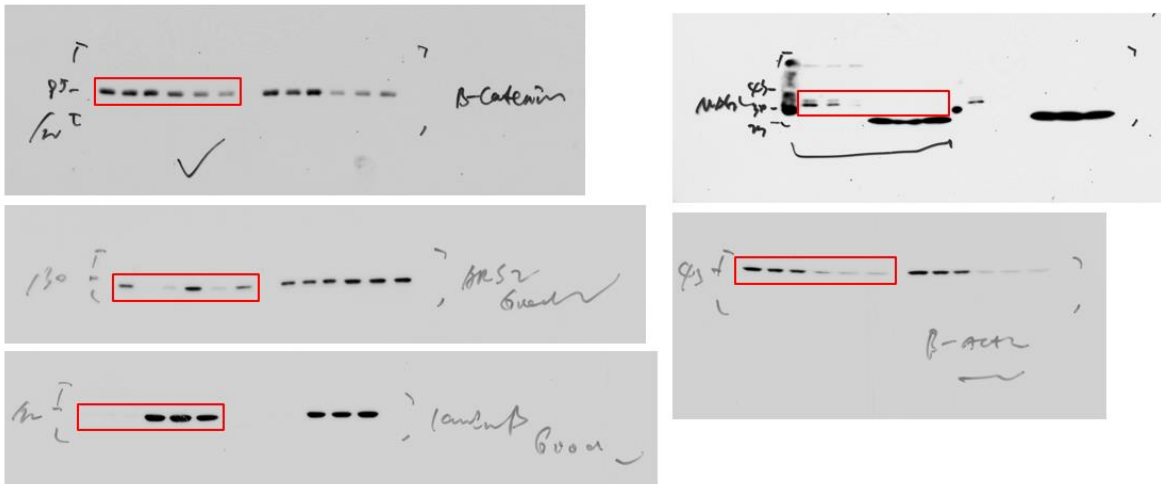
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2 **Original immunoblot for Supplementary Fig. 2a,b,c,f.**



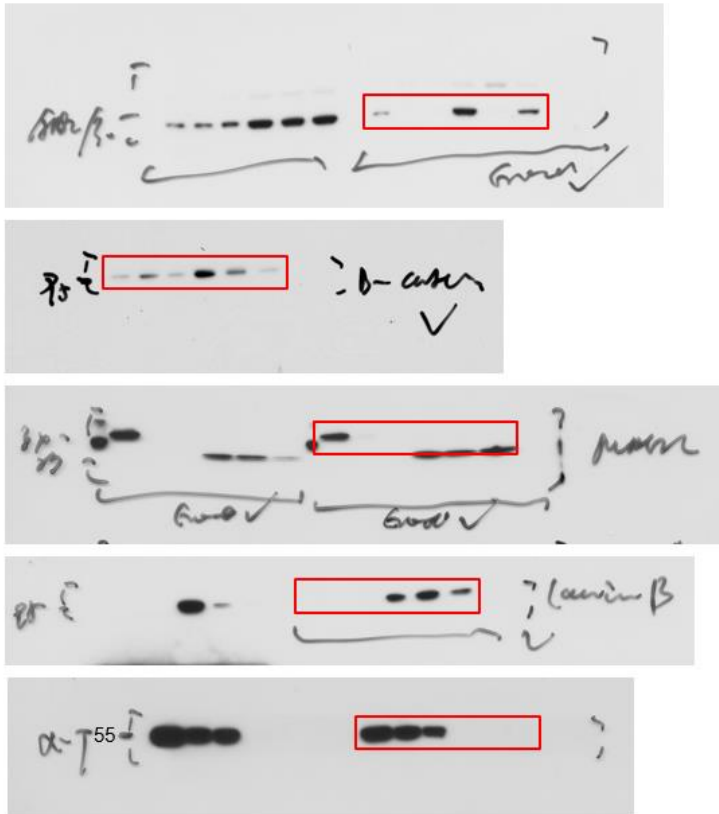
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4 **Original RT-PCR for Supplementary Fig. 3b.**



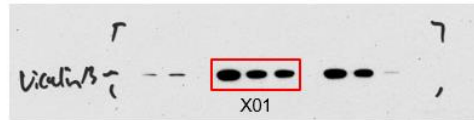
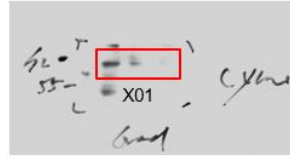
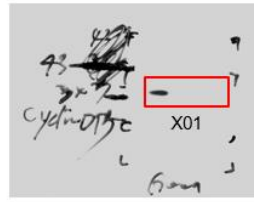
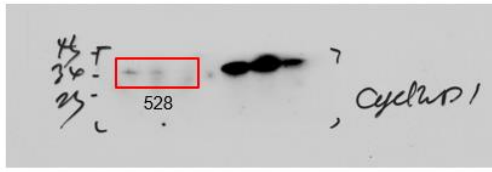
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Original immunoblot for Supplementary Fig. 5f.



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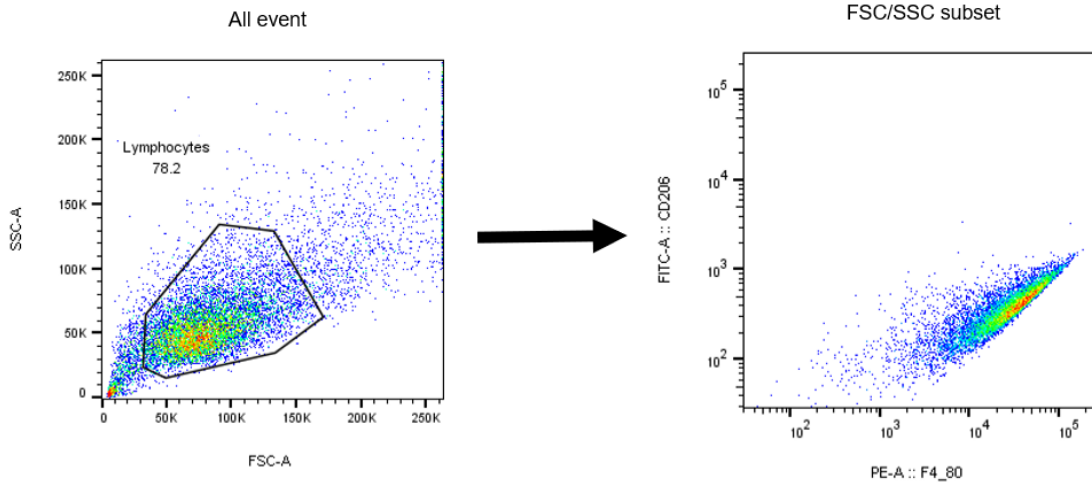
Original immunoblot for Supplementary Fig. 5g.



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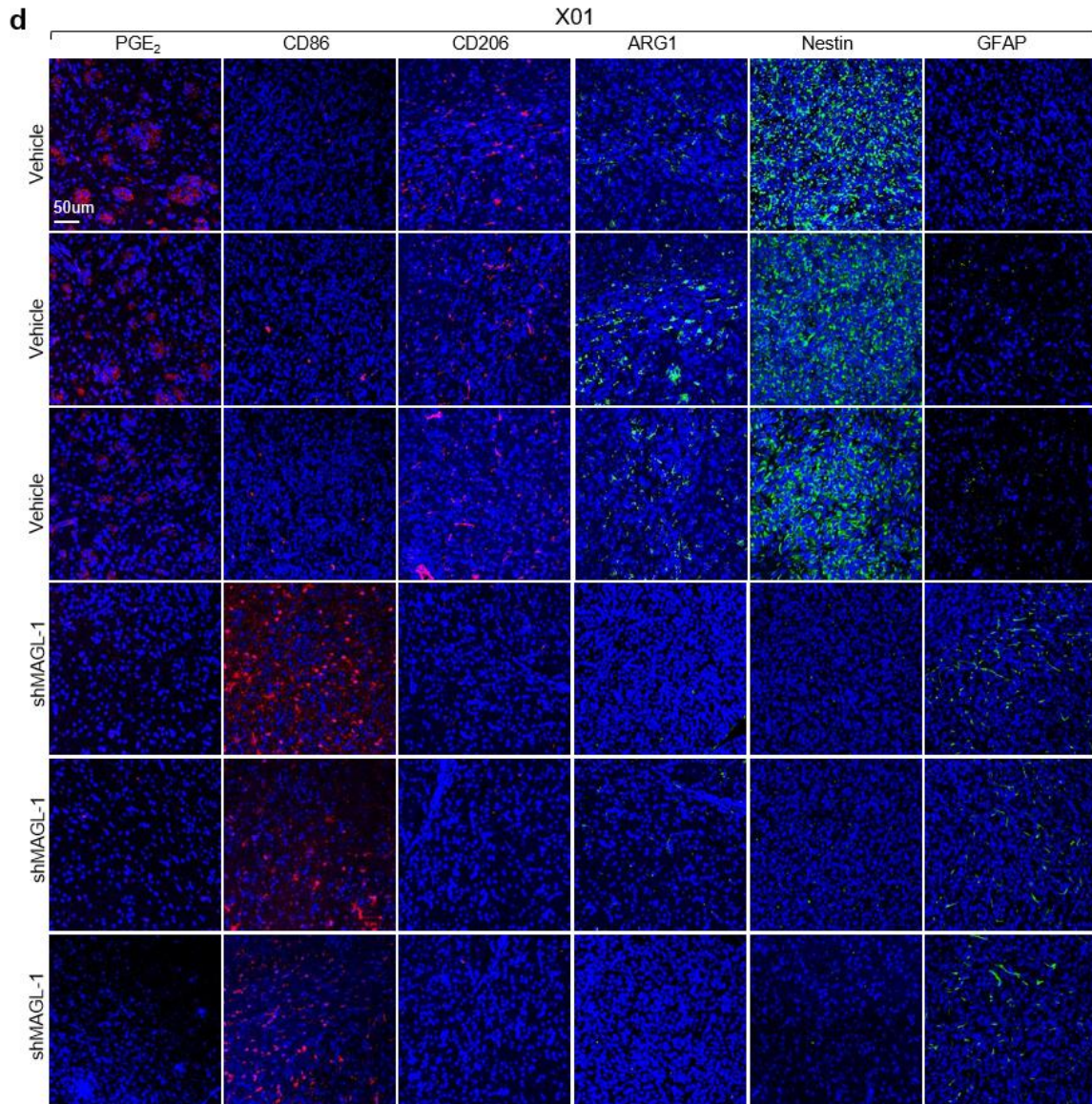
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Original immunoblot for Supplementary Fig. 5i,j.

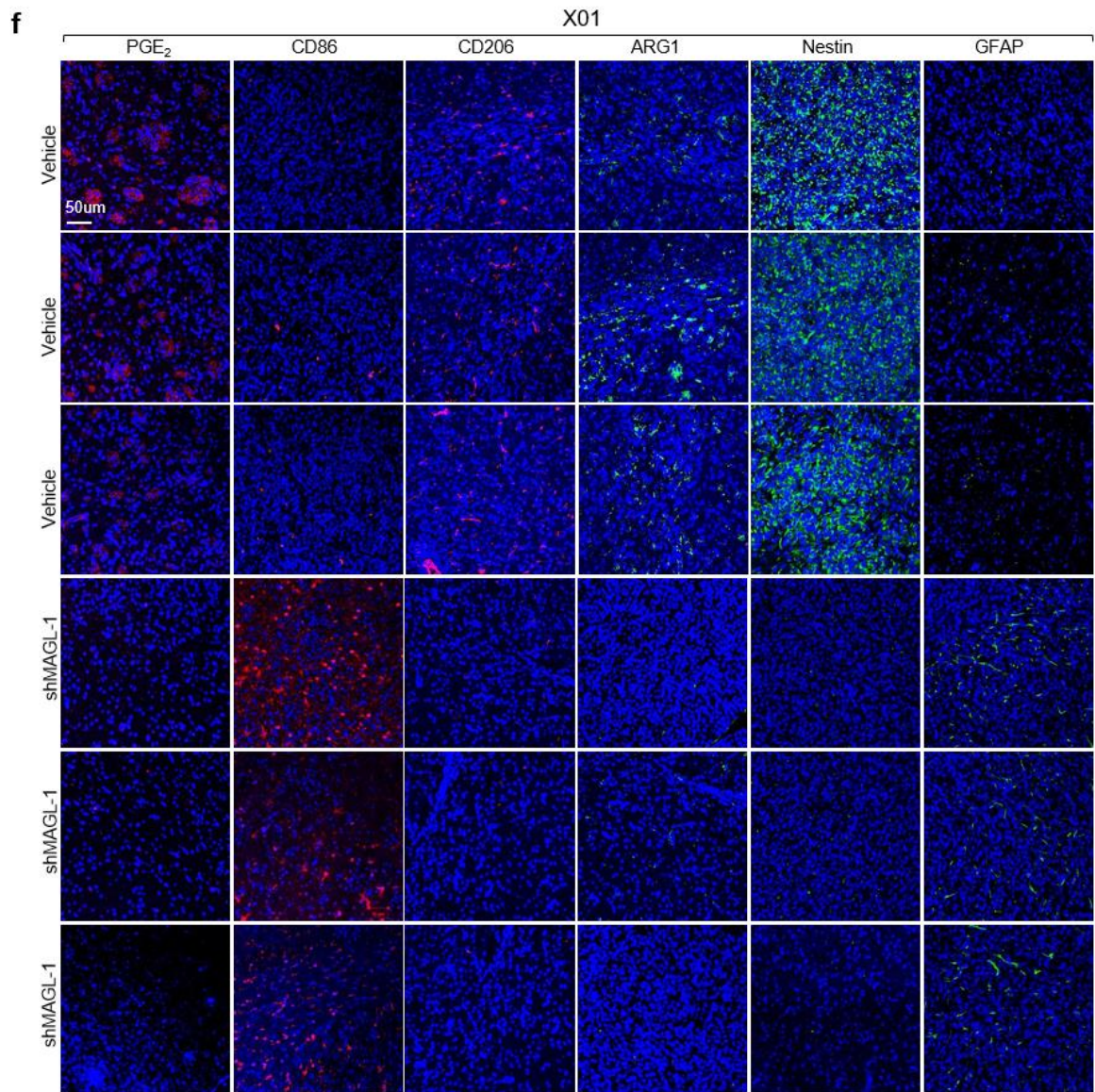


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Original FACS gating method for Supplementary Fig. 6c,d.

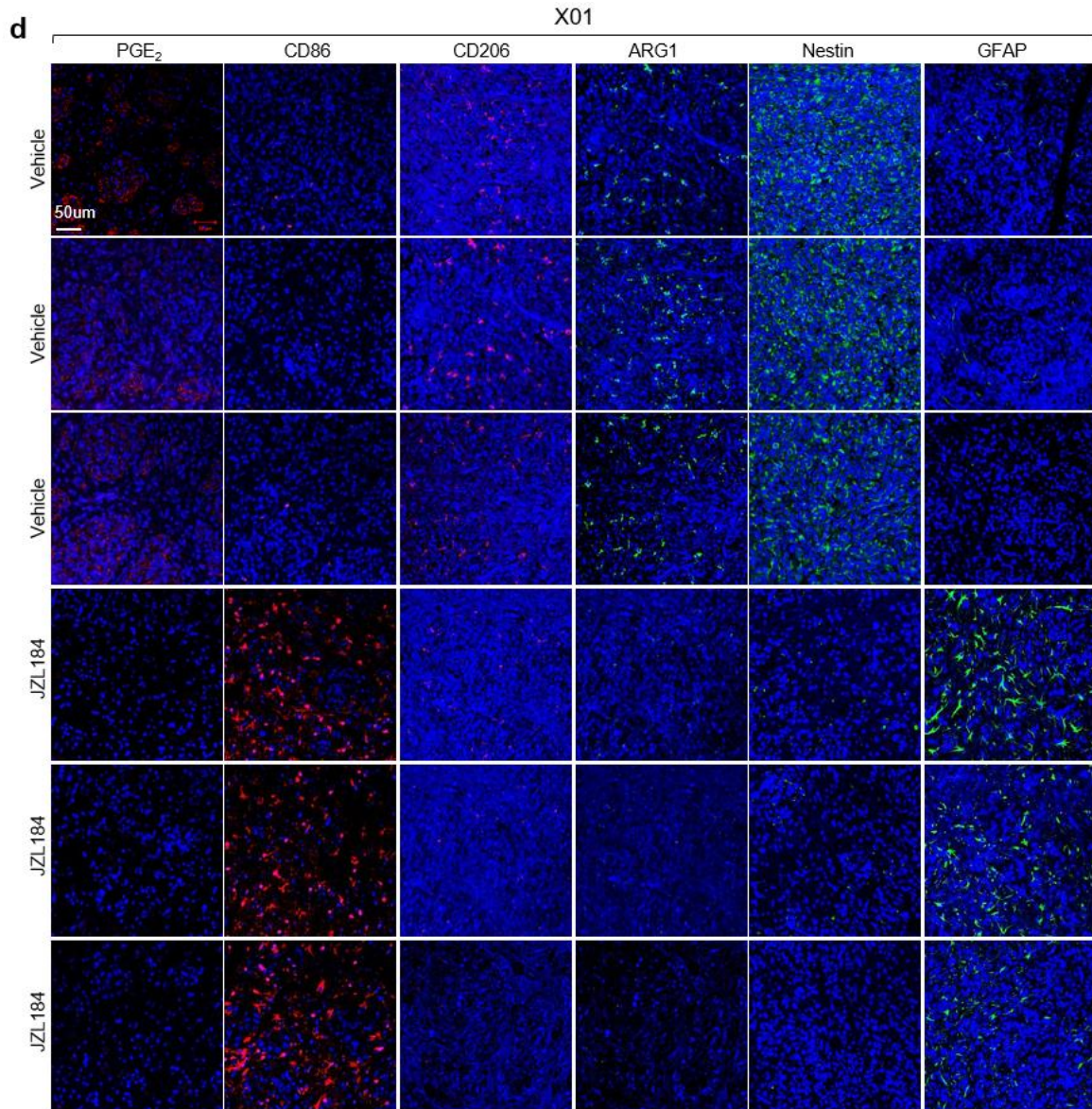


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2 **Original ICC staining for Fig. 6d.**



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Original ICC staining for Fig. 6f.



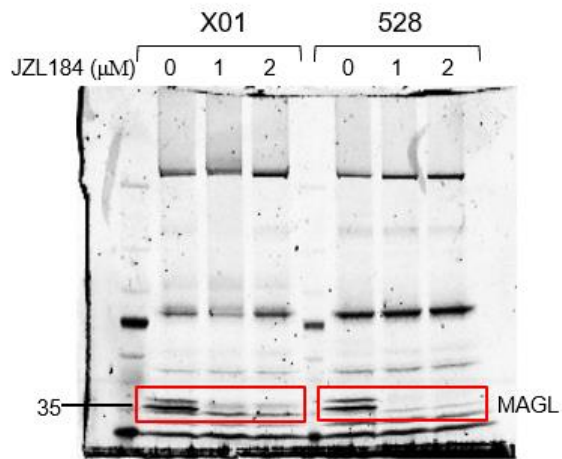
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Original ICC staining for Fig. 7d.



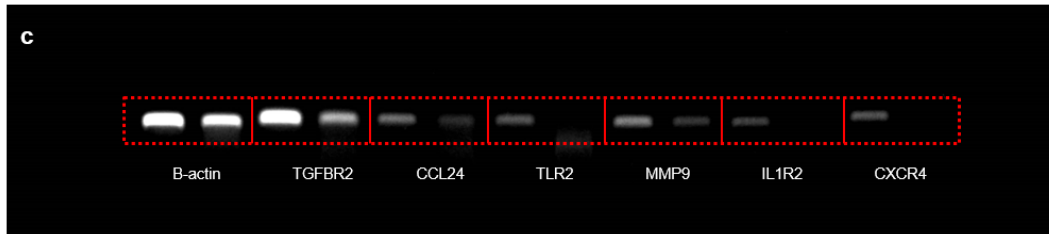
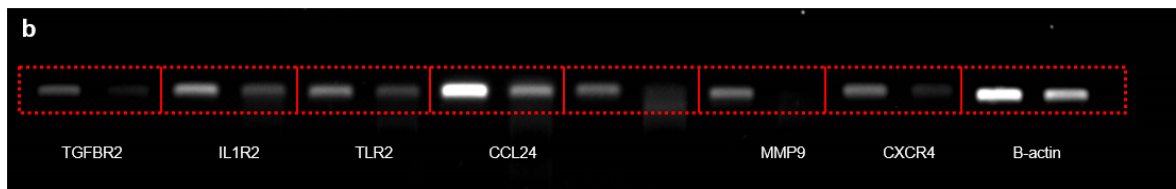
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Original image for Supplementary Fig. 6i .



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2 **Original gel image for Supplementary Fig. 7a.**



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4 **Original RT-PCR for Supplementary Fig. 8b, c.**