

Oligodendrocyte transcription factor 2 orchestrates glioblastoma immune evasion by suppressing CXCL10 and CD8⁺ T cell activation

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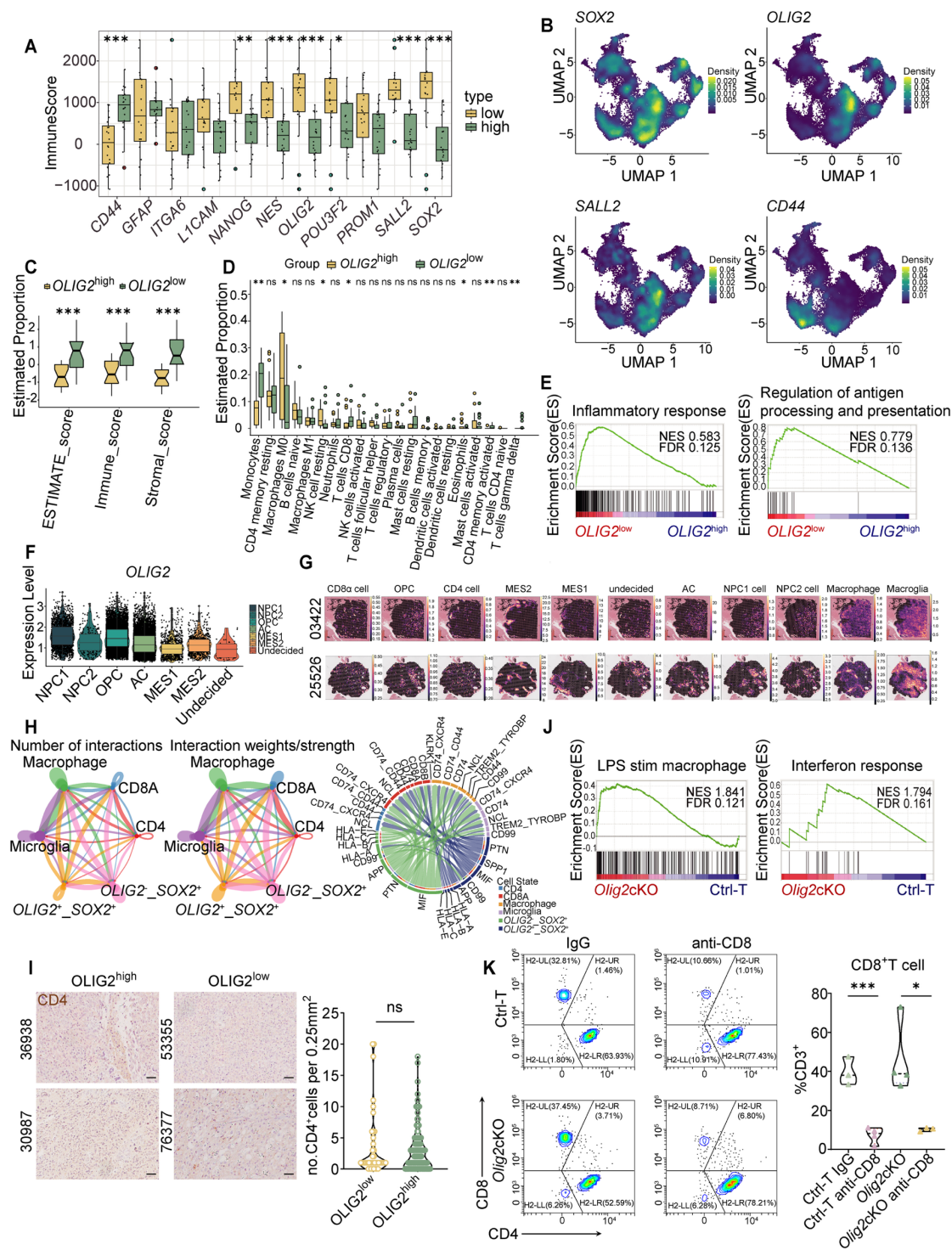
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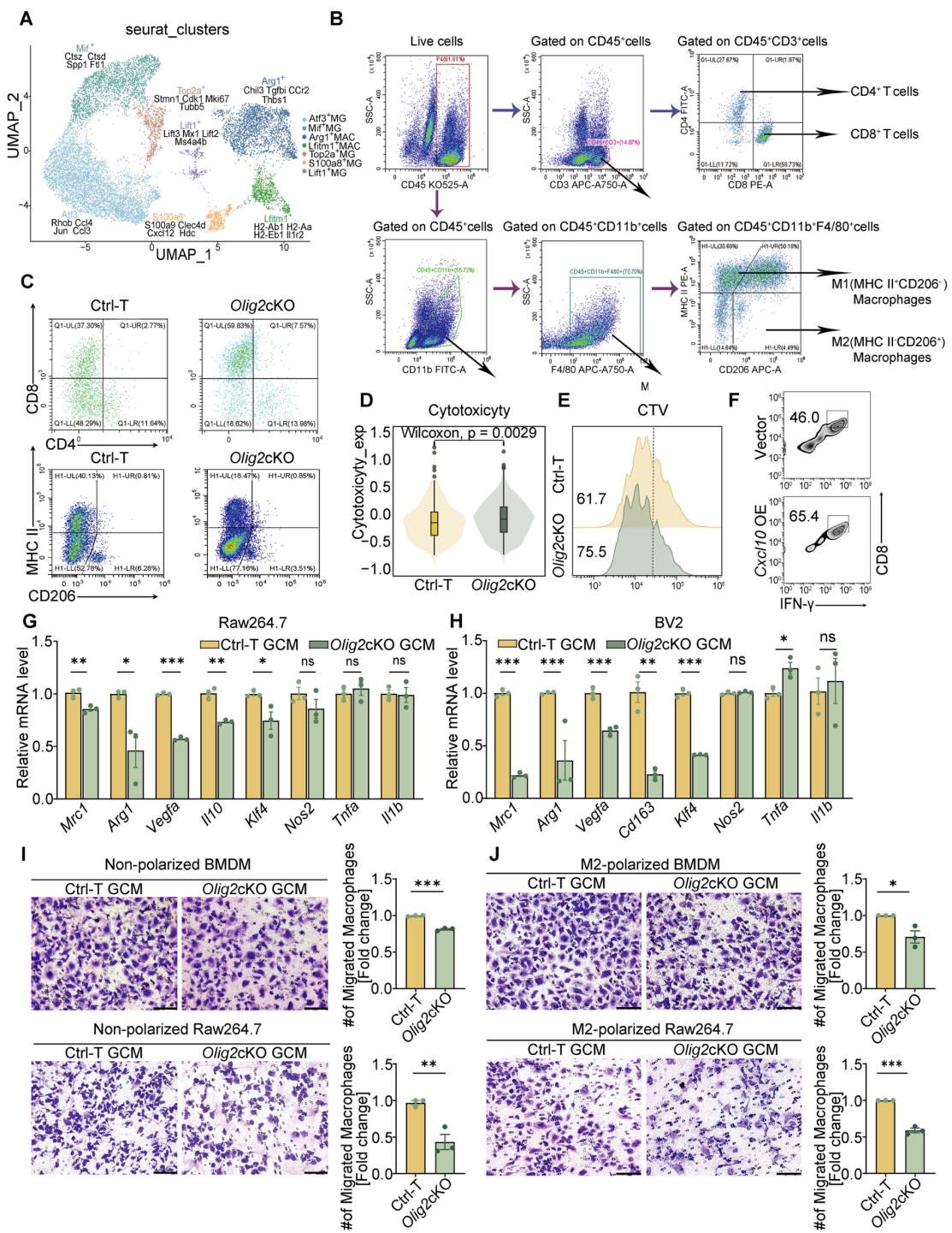
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Supplementary figure 1



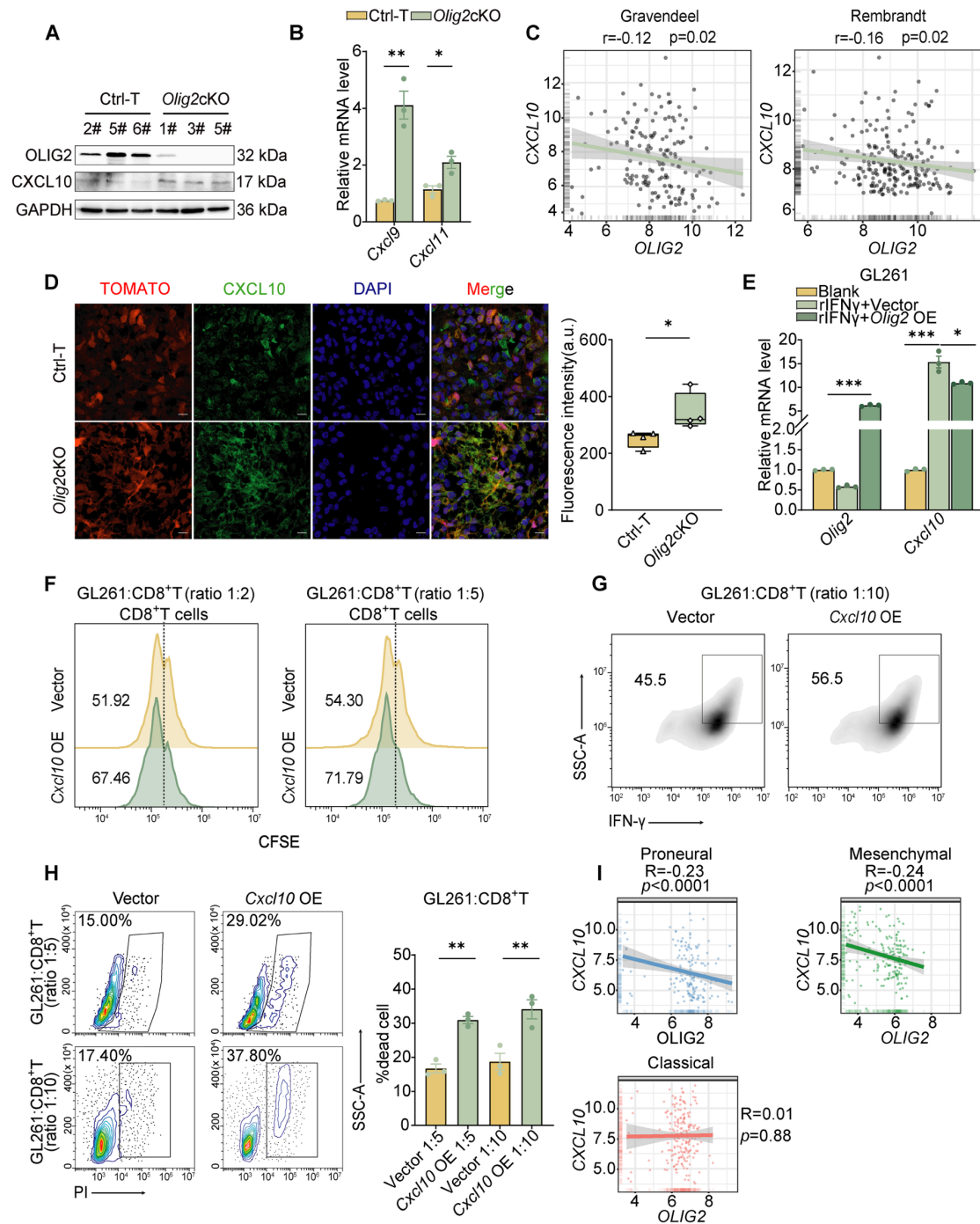
Supplementary Figure 1. High OLIG2 expression positively correlates with the pro-tumor immune microenvironment in GBM

(A) ESTIMATE analysis of immune infiltrations in GBM patients from TCGA database with high or low expression of GSC markers ($n=40$). (B) UMAP visualization of the specific GSC markers distribution in GBM cells (GSE182109). (C) ESTIMATE analysis of immune infiltrations in GBM patients from TCGA database with high or low *OLIG2* expression ($n=40$). (D) Characterization of the immune cell composition in GBM from TCGA databases by CIBERSORT analysis ($n=40$). (E) GSEA showing the enrichment of signature gene sets of immunoregulation in *OLIG2*^{low} GBM cases compared with *OLIG2*^{high} specimen ($n=40$). (F) The *OLIG2* expression levels in different subtypes of tumor cells (GSE182109). (G) Spatial feature plots showing the scores of cell types in GBM slides (GSE276841). (H) Cell-cell communications among *OLIG2*⁺*SOX2*⁺ GSC, *OLIG2*⁻*SOX2*⁺ GSC and immune cells from GBM scRNA-seq dataset (GSE182109) by CellChat. (I) Representative staining and quantification for CD4 ($n=164$) in GBM cases with high or low *OLIG2* expression. Scale bar: 50 μ m. Each plot represents the number of CD4 per area (0.25 mm²). (J) GSEA showing the enrichment of signature gene sets of LPS-stimulated macrophage and Interferon response in GBM of *Olig2*cKO mice compared with Ctrl-T mice. (K) The efficiency of CD8⁺ T cells depletion in mouse spleen ($n=3-4$ mice/group). Unpaired Student's *t*-test in C, D, H and J. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.



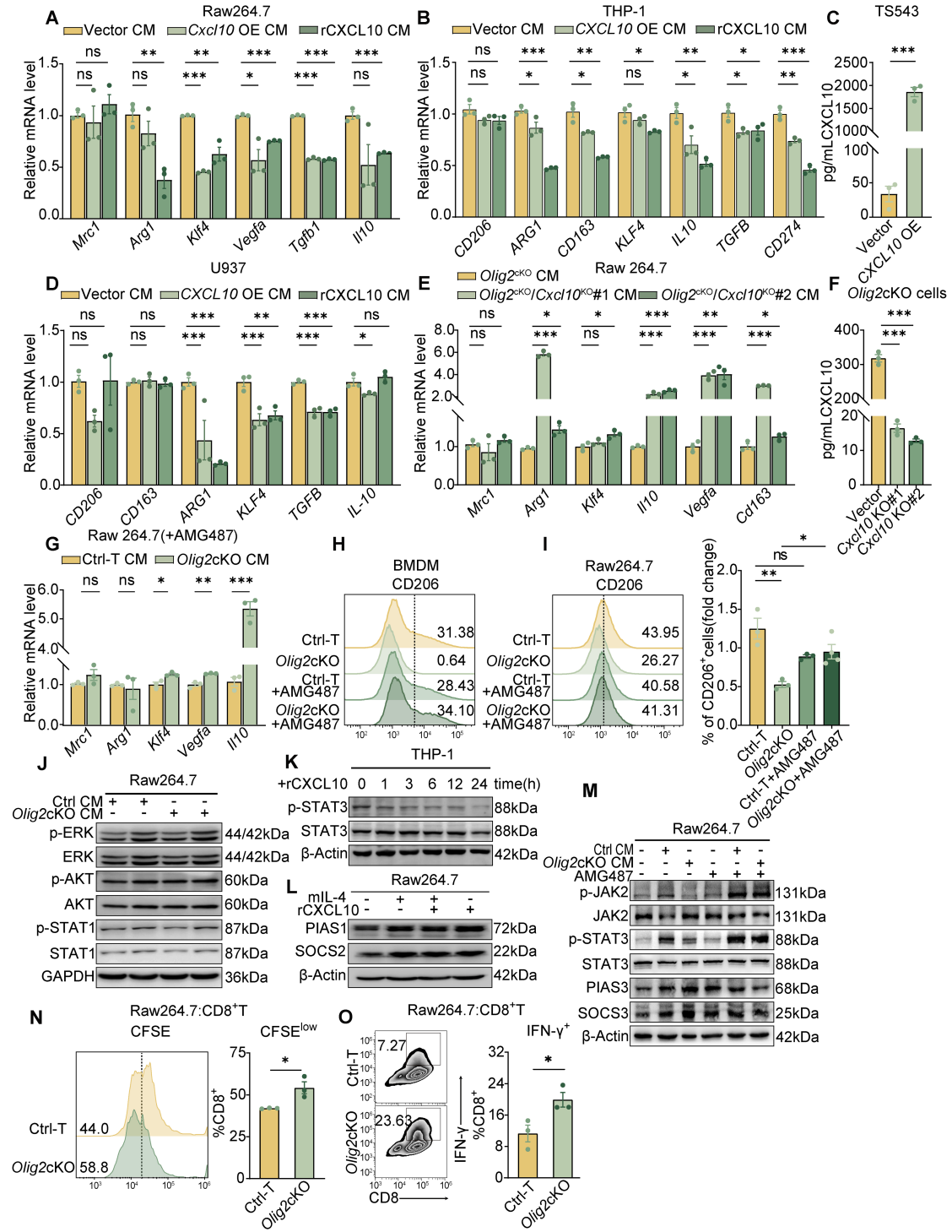
Supplementary Figure 2. OLIG2 deletion enhances T cells activation and inhibits protumor TAMs in GBM

(A) UMAP of scRNAseq data from TAMs and signature genes for each TAM cluster were labeled. (B) Gating strategy for CD8⁺ T cells, CD4⁺ T cells, MHC II⁻CD206⁺ TAMs, and MHC II⁺CD206⁻ TAMs in mouse GBM. (C) Representative flow cytometry scatter plots of the CD8⁺ T cells, CD4⁺ T cells, MHC II⁻CD206⁺ TAMs, and MHC II⁺CD206⁻ TAMs in Ctrl-T and *Olig2*cKO mice. (D) Violin-box plots depicting the cytotoxicity score of the CD8⁺ T cells using scRNA seq data from Ctrl-T and *Olig2*cKO groups. Two-tailed unpaired Wilcoxon rank-sum test. (E) Representative histograms for CellTrace Violet proliferation assay of CD8⁺ T cells treated with CM from Ctrl-T and *Olig2*cKO tumor cells for 72h (*n*=3). (F) Representative plots for IFN- γ expression of CD8⁺ T cells treated with CM from Ctrl-T and *Olig2*cKO tumor cells (*n*=3). (G, H) qPCR for mRNA expression of anti-tumor and pro-tumor markers in Raw264.7 and BV2 cells treated with Ctrl-T and *Olig2*cKO CM (*n*=3). (I, J) Representative images and quantifications of migrated non- or M2-polarized Raw264.7 and BMDMs toward Ctrl-T CM or *Olig2*cKO CM by Transwell assay (*n*=3). Scale bar: 100 μ m. Unpaired Student's *t*-test in G-J. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, ns, not significant.



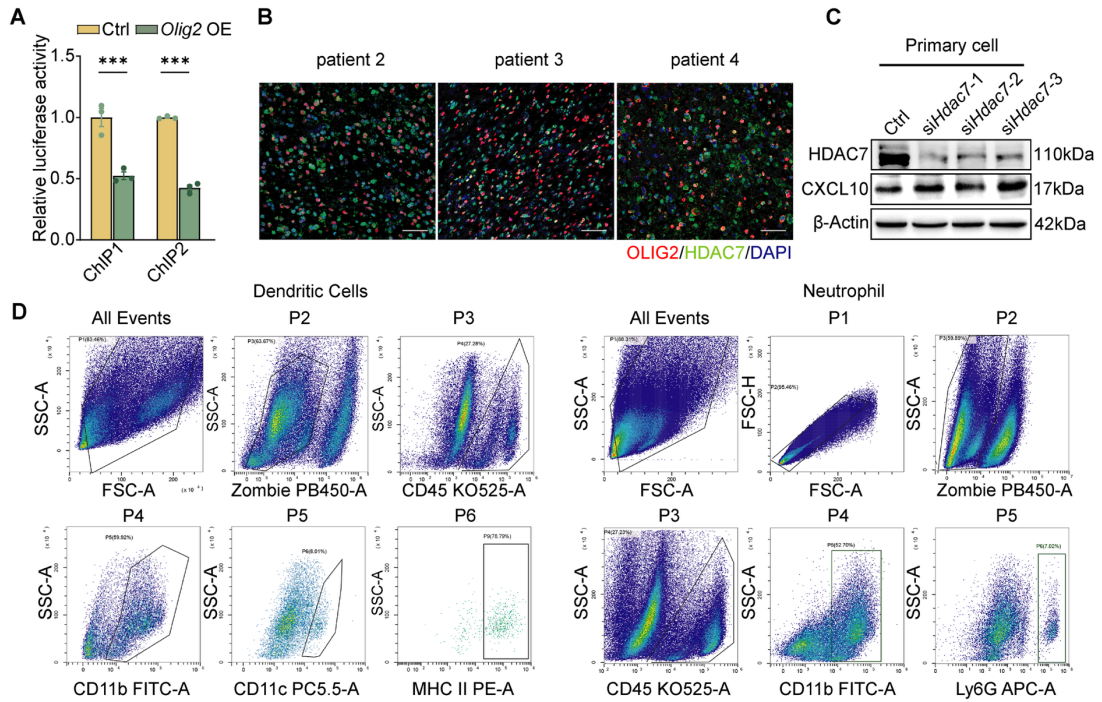
Supplementary Figure 3. OLIG2 regulates CXCL10 expression to modulate T Cell proliferation and IFN- γ secretion

(A) Western blot for CXCL10 protein expression in Ctrl-T and *Olig2*cKO GBM cells. (B) qPCR for mRNA expression levels of *Cxcl9/11* in Ctrl-T and *Olig2*cKO GBM primary cells ($n=3$). (C) Correlation between *OLIG2* expression and *CXCL10* expression in Gravendeel and Rembrandt database. (D) Representative images and quantification of CXCL10 staining from Ctrl-T and *Olig2*cKO mice. Scale bar: 10 μ m. (E) qPCR for mRNA expression of *Cxcl10* in 20ng/mL IFN- γ stimulated GL261 cells upon *Olig2* overexpression ($n=3$). (F) Representative histograms for CFSE proliferation assay of CD8⁺ T cells co-cultured with GL261-Ctrl and GL261-*Cxcl10* OE cells at the indicated ratio for 72h ($n=3$). (G) Representative plots for IFN- γ expression of CD8⁺ T cells treated with CM from GL261-Ctrl and GL261-*Cxcl10* OE cells for 24h ($n=3$). (H) Representative plots and quantification for the PI-positive tumor cells in GL261-Ctrl and GL261-*Cxcl10* OE groups cocultured with CD8⁺ T cell at the indicated ratio for 72h ($n=3$). (I) Correlation between *OLIG2* and *CXCL10* expressions in TCGA GBM dataset. Unpaired Student's *t*-test in B, One-way ANOVA in E, H. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.



Supplementary Figure 4. CXCL10 attenuates M2 macrophage polarization through STAT3 signaling suppression

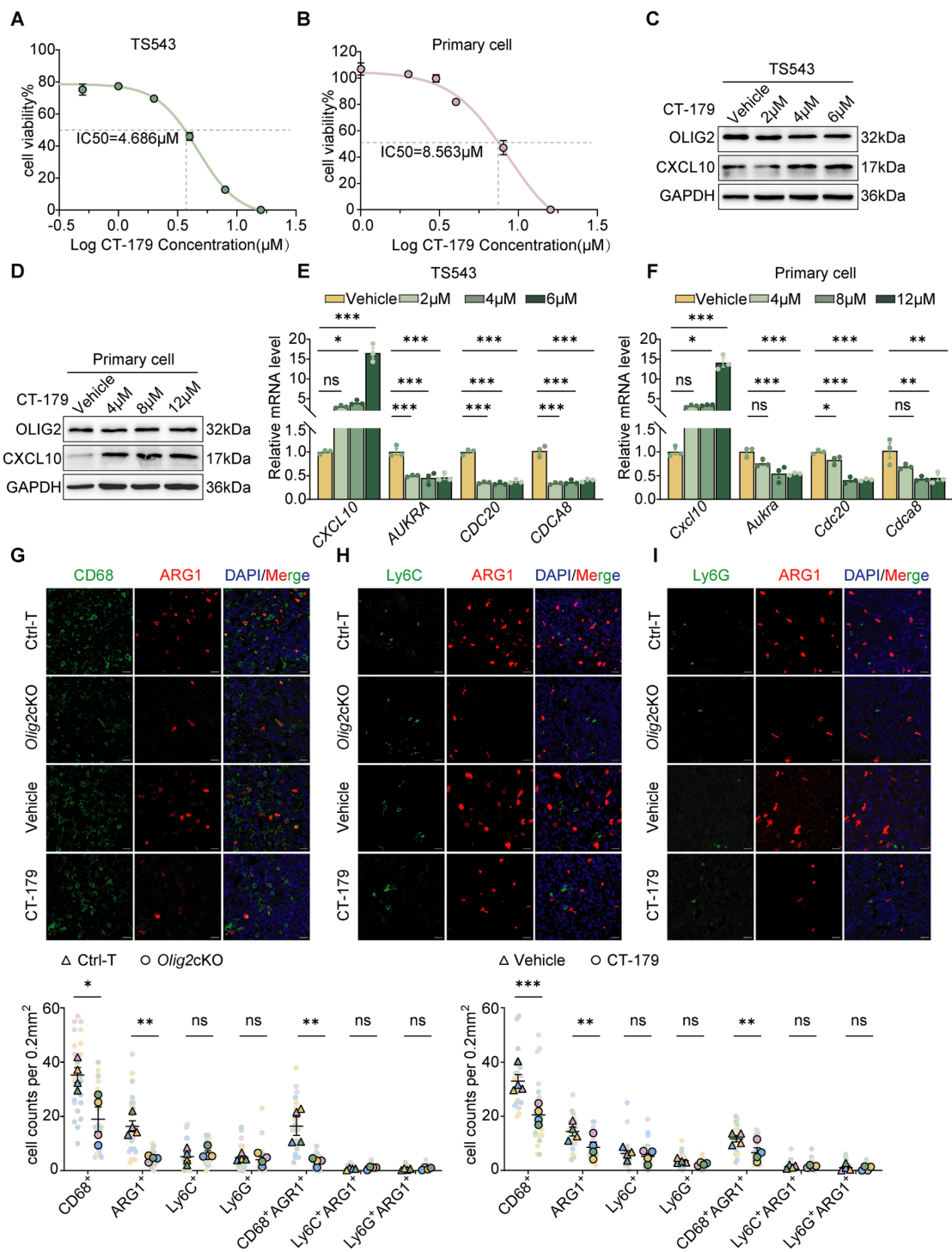
(A) qPCR for mRNA expression of anti-inflammatory markers in Raw264.7 cells treated with CM from the indicated groups for 48h ($n=3$). (B) qPCR for mRNA expression of anti-inflammatory markers in THP-1 cells treated with CM from the indicated groups for 48h ($n=3$). (C) ELISA for CXCL10 secretion levels in the supernatant of TS543-Vector and TS543-*CXCL10* OE cells ($n=3$). (D) qPCR for mRNA expression of anti-inflammatory markers in U937 cells treated with CM from the indicated groups for 48h ($n=3$). (E) qPCR for mRNA expression of anti-inflammatory markers in Raw264.7 cells treated with CM from the indicated groups for 48h ($n=3$). (F) ELISA for CXCL10 secretion levels in the supernatant of *Olig2cKO* and *Olig2cKO-Cxcl10* KO cells ($n=3$). (G) qPCR for mRNA expression of anti-inflammatory markers in Raw264.7 cells treated with Ctrl-T and *Olig2cKO* CM containing 1 μ M AMG487 for 48h ($n=3$). (H) Representative histograms for the CD206 expression of BMDMs treated with Ctrl-T and *Olig2cKO* CM containing AMG487 for 24h ($n=3$). (I) Representative histograms and quantification for the CD206 expression of Raw264.7 cells treated with Ctrl-T and *Olig2cKO* CM containing AMG487 for 24h ($n=3$). (J) Western blot for the protein levels in Raw264.7 cells treated with Ctrl-T and *Olig2cKO* CM. (K) Western blot for the protein levels in THP-1 cells treated with 100 ng/mL rCXCL10 proteins at indicated time points. (L) Western blot for the protein levels in Raw264.7 cells treated with mIL-4 and rCXCL10 proteins. (M) Western blot for the protein levels in Raw264.7 cells co-cultured with Ctrl-T and *Olig2cKO* CM containing AMG487. (N) Representative histograms for CFSE proliferation assay of the CD8⁺ T cells co-cultured with BMDMs pre-incubated with Ctrl-T and *Olig2cKO* CM ($n=3$). (O) Representative flow cytometry for the IFN- γ expression of CD8⁺ T cells co-cultured with BMDMs pre-incubated with Ctrl-T and *Olig2cKO* CM ($n=3$). One-way ANOVA in F, I, Unpaired Student's *t*-test in A-E, G, N, O. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.



Supplementary Figure 5. OLIG2 recruits HDAC7 to epigenetically silence *CXCL10* expression

(A) Dual-luciferase reporter assay for *Cxcl10* enhancer activity in control or *Olig2* overexpressed 293T cells ($n=3$). (B) Representative immunofluorescence images showing OLIG2 (red) and HDAC7 (green) signals from three GBM patient specimen. Scale bars = 50 μ m. (C) Western blot for protein levels of HDAC7 and CXCL10 in Ctrl-T tumor cells transfected with siHdac7. (D) Gating strategy for CD11b⁺CD11c⁺MHC II⁺ dendritic cells and CD11b⁺Ly6G⁺ neutrophils in mouse GBM for Figure 2B. Unpaired Student's *t*-test in A. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

Supplementary figure 6



Supplementary Figure 6. CT-179 suppresses OLIG2 transcriptional activity and reduces ARG1⁺ TAMs in mouse GBM

(A, B) Cell viability assays for TS543 cells and mouse primary GBM cells exposed to CT-179 for 48h. (C, D) Western blot for OLIG2 and CXCL10 protein expressions in TS543 cells and mouse primary GBM cells treated with CT-179 for 48h. (E, F) qPCR for mRNA levels of the classical target genes of OLIG2 in TS543 GBM cells and mouse primary GBM cells treated with Vehicle or CT-179 for 48 hours ($n=3$). (G-I) (Top) Representative immunofluorescence images of tumor sections from four experimental groups: Ctrl-T, *Olig2*cKO, Vehicle, and CT-179 (20 mg kg⁻¹). CD68⁺ macrophages (green), ARG1⁺ cells (red) in G; Ly6C⁺ monocytes (green), ARG1⁺ cells (red) in H; Ly6G⁺ neutrophils (green), ARG1⁺ cells (red) in I. (Bottom) Quantitative analysis of immunosuppressive myeloid cell densities. Scale bars = 20 μ m; $n = 4$ mice/group. Data was presented as mean $\pm$ SEM. One-way ANOVA in E, F. Unpaired Student's *t*-test in G-I, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns, not significant.

Supplementary Table 1

Name	Sequence
Primers for qPCR	
q-m <i>Il1b</i> -F	GCAACTGTTCTGAACTCAACT
q-m <i>Il1b</i> -R	ATCTTTTGGGGTCCGTCAACT
q-m <i>Tnfa</i> -F	GACGTGGAAGTGGCAGAAGAG
q-m <i>Tnfa</i> -R	TTGGTGGTTTGTGAGTGTGAG
q-m <i>Arg1</i> -F	CTCCAAGCCAAAGTCCTTAGAG
q-m <i>Arg1</i> -R	AGGAGCTGTCATTAGGGACATC
q-m <i>Il10</i> -F	GCTCTTACTGACTGGCATGAG
q-m <i>Il10</i> -R	CGCAGCTCTAGGAGCATGTG
q-m <i>Klf4</i> -F	GTGCCCCGACTAACCGTTG
q-m <i>Klf4</i> -R	GTCGTTGAACTCCTCGGTCT
q-m <i>Vegfa</i> -F	GCACATAGAGAGAATGAGCTTCC
q-m <i>Vegfa</i> -R	CTCCGCTCTGAACAAGGCT
q-m <i>Nos2</i> -F	GTTCTCAGCCCAACAATACAAGA
q-m <i>Nos2</i> -R	GTGGACGGGTCGATGTCAC
q-m <i>Cd163</i> -F	ATGGGTGGACACAGAATGGTT
q-m <i>Cd163</i> -R	CAGGAGCGTTAGTGACAGCAG
q-m <i>Tgfb1</i> -F	CTCCCGTGGCTTCTAGTGC
q-m <i>Tgfb1</i> -R	GCCTTAGTTTGGACAGGATCTG
q-m β - <i>Actin</i> -F	GGCTGTATTCCTCCATCG
q-m β - <i>Actin</i> -R	CCAGTTGGTAACAATGCCATGT
q-m <i>Mrc1</i> -F	CTCTGTTTCAGCTATTGGACGC
q-m <i>Mrc1</i> -R	CGGAATTTCTGGGATTCAGCTTC
q-m <i>CD274</i> -F	GCTCCAAAGGACTTGTACGTG
q-m <i>CD274</i> -R	TGATCTGAAGGGCAGCATTTTC
q-m <i>Vegfb</i> -F	GCCAGACAGGGTTGCCATAC
q-m <i>Vegfb</i> -R	GGAGTGGGATGGATGATGTCAG
q-m <i>Cxcl10</i> -F	CCAAGTGCTGCCGTCATTTTC
q-m <i>Cxcl10</i> -R	GGCTCGCAGGGATGATTTCAA
q-m <i>Cxcl9</i> -F	TCCTTTTGGGCATCATCTTCC
q-m <i>Cxcl9</i> -R	TTTGTAAGTGGATCGTGCCTCG
q-m <i>Cxcl11</i> -F	GGCTTCCTTATGTTCAAACAGGG
q-m <i>Cxcl11</i> -R	GCCGTTACTCGGGTAAATTACA
q-m <i>Tnc</i> -F	ACGGCTACCACAGAAGCTG
q-m <i>Tnc</i> -R	ATGGCTGTTGTTGCTATGGCA
q-m <i>Il17ra</i> -F	AGTGTTTCCTCTACCCAGCAC
q-m <i>Il17ra</i> -R	GAAAACCGCCACCGCTTAC
q-m <i>Sema3e</i> -F	AGGCTACGCCTGTCACATAAA
q-m <i>Sema3e</i> -R	CCGTTCTTGATACTCATCCAGC
q-m <i>Adm</i> -F	CACCCTGATGTTATTGGGTTCA

q-m <i>Adm</i> -R	TTAGCGCCCACTTATTCCACT
q-H- <i>ACTIN</i> -F	CATGTACGTTGCTATCCAGGC
q-H- <i>ACTIN</i> -R	CTCCTTAATGTCACGCACGAT
q-H- <i>OLIG2</i> -F	GCTCCTCAAATCGCATCCA
q-H- <i>OLIG2</i> -R	AAAGGTCATCGGGCTCTG
q-H- <i>CXCL10</i> -F	GTGGCATTCAAGGAGTACCTC
q-H- <i>CXCL10</i> -R	TGATGGCCTTCGATTCTGGATT
q-H- <i>IL-10</i> -F	TCACATGCGCCTTGATGTCTG
q-H- <i>IL-10</i> -R	GACTTTAAGGGTTACCTGGGTTG
q-H- <i>TNFA</i> -F	TCGAGAAGATGATCTGACTGCC
q-H- <i>TNFA</i> -R	GCTGCACTTTGGAGTGATCG
q-H- <i>CD206</i> -F	TTTCTTGTCTGTTGCCGTAGTT
q-H- <i>CD206</i> -R	GGGTTGCTATCACTCTCTATGC
q-H- <i>Arg1</i> -R	CCAGTCCGTCAACATCAAACT
q-H- <i>Arg1</i> -F	TGGACAGACTAGGAATTGGCA
q-H- <i>Klf4</i> -F	CGGACATCAACGACGTGAG
q-H- <i>Klf4</i> -R	GACGCCTTCAGCACGAAC
q-H- <i>TGFBI</i> -F	GGCCAGATCCTGTCCAAGC
q-H- <i>TGFBI</i> -R	GTGGGTTTCCACCATTAGCAC
q-H- <i>CD163</i> -F	TTTGTAACCTTGAGTCCCTTCAC
q-H- <i>CD163</i> -R	TCCCGCTACACTTGTTTTAC
q-H- <i>CXCR3</i> -F	TTTGACCGCTACCTGAACATAGT
q-H- <i>CXCR3</i> -R	GGGAAGTTGTATTGGCAGTGG
q-H- <i>HDAC7</i> -F	GGCGGCCCTAGAAAGAACAG
q-H- <i>HDAC7</i> -R	CTTGGGCTTATAGCGCAGCTT
sgRNA	
Crispr1-m <i>Cxcl10</i> F	CACCGGCGGACCGTCCTTGCGAGA
Crispr1-m <i>Cxcl10</i> R	AAACTCCTGCGAGGAACGGTCCGCC
Crispr2-m <i>Cxcl10</i> F	CACCGGGGAGGGACGTTTATCGGCC
Crispr2-m <i>Cxcl10</i> R	AAACGCCGATAAACGTCCCTCCCC
siRNA	
m-si- <i>Hdac7</i> -1-s	GGCUGGAAACAGAAACCCA
m-si- <i>Hdac7</i> -1-a	UGGGUUUCUGUUUCCAGCC
m-si- <i>Hdac7</i> -2-s	CGGUCAUGCUGAAGCACCA
m-si- <i>Hdac7</i> -2-a	UGGUGCUUCAGCAUGACCG
m-si- <i>Hdac7</i> -3-s	GGAUAGUCGUGAUGCCCAU
m-si- <i>Hdac7</i> -3-a	AUGGGCAUCACGACUAUCC
H-si- <i>HDAC7</i> -1-s	GCAGUGUGGUCAAGCAGAA
H-si- <i>HDAC7</i> -1-a	UUCUGCUUGACCACACUGC
H-si- <i>HDAC7</i> -2-s	GGCAGGCUUACACCAGCAA
H-si- <i>HDAC7</i> -2-a	UUGCUGGUGUAAGCCUGCC

H-si- <i>HDAC7</i> -3-s	CCUGAAGUUGCGCUACAAA
H-si- <i>HDAC7</i> -3-a	UUUGUAGCGCAACUUCAGG

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Supplementary Table 2

The information of antibody		
Antibodies	Source	Catalogue number
Anti-HLA-DMB	Abcam	#ab131273
Anti-Iba1	Abcam	#ab5076
Anti-Acetyl-Histone H3-K27	ABclonal	#A7253
Anti-HDAC7	ABclonal	#A13008
Anti-CXCL10	ABclonal	#A19138
Anti- β -Tubulin	ABclonal	#AC008
HRP-Mouse Anti-Rabbit IgG Light Chain	ABclonal	#AS061
PerCP/Cy5.5 anti-mouse CD8 α (clone 53-6.7)	BD Biosciences	#561109
BV510 anti-mouse CD45 (clone 30-F11)	BioLegend	#103138
FITC anti-mouse/human CD11b (clone M1/70)	BioLegend	#101205
APC-Cy7 anti-mouse F4/80 (clone BM8)	BioLegend	#123117
APC anti-mouse CD206 (clone C068C2)	BioLegend	#141707
APC-Cy7 anti-mouse CD3 (clone 17A2)	BioLegend	#100222
APC anti-mouse CD4 (clone GK1.5)	BioLegend	#100405
PE-Cy7 anti-mouse PD-1 (clone RMP1-30)	BioLegend	#109109
APC anti-mouse IFN- γ (clone XMGI.2)	BioLegend	#505810
PerCP/Cy5.5 anti-mouse CD11c (clone N418)	BioLegend	#117327
BV605 anti-mouse NK1.1 (clone PK136)	BioLegend	#108739
APC anti-mouse Ly-6G (clone 1A8)	BioLegend	#127613
Purified anti-mouse CD3 ϵ	BioLegend	#100340
Purified anti-mouse CD28	BioLegend	#117003
Zombie Violet	BioLegend	#423113
Anti-Ly6G	BioLegend	#127601
Anti-CD68	Bio-Rad	#MCA1957T
Anti-CD4	BOSTER	#A00344-2
Anti-CD8 α	CST	#D8A8Y
Anti-CD8 α	CST	#98941
Anti-p-ERK1/2	CST	#4370S
Anti-ERK	CST	#4695S
Anti-AKT	CST	#9272S
Anti-p-AKT	CST	#9271S
PE anti-mouse MHC II (clone M5/114.15.2)	Invitrogen	#12-5321-81
Rabbit IgG isotype control	Invitrogen	#02-6102
Donkey anti-Mouse IgG (H+L), Alexa Fluor™ Plus 488	Invitrogen	#A32766TR
Donkey anti-Mouse IgG (H+L), Alexa Fluor™ 555	Invitrogen	#A-31570
Donkey anti-Mouse IgG (H+L), Alexa Fluor™ Plus 647	Invitrogen	#A32787TR
Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ 555	Invitrogen	#A-31572
Donkey anti-Rabbit IgG (H+L), Alexa Fluor™ Plus 647	Invitrogen	#A32795TR
Donkey anti-Goat IgG (H+L), Alexa Fluor™ 488	Invitrogen	#A-11055

Donkey anti-Goat IgG (H+L), Alexa Fluor™ 555	Invitrogen	#A-21432
Donkey anti-Rat IgG (H+L), Alexa Fluor™ Plus 647	Invitrogen	#A48272TR
Anti-OLIG2	Millipore	#ab9610
Anti-CXCL10	Novus	#AF-466-NA
Anti-Iba1 Guinea pig pAb	Oasis Biofarm	#OB-PGP049
Donkey-anti-Guinea pig IgG, AF488	Oasis Biofarm	#D-GP488
Anti-Arginase-1	Proteintech	#16001-1-AP
Anti-STAT3	Proteintech	#91408
Anti-β-Actin	Proteintech	#66009-1-Ig
Anti-GAPDH	Proteintech	#60004-1-g
HRP-Goat Anti-Mouse IgG	Proteintech	#SA00001-1
HRP-Goat Anti-Rabbit IgG	Proteintech	#SA00001-2
HRP-Rabbit Anti-Goat IgG	Proteintech	#SA00001-4
Anti-Ly6C	Santa Cruz	#sc-271811
Anti-CD69	Santa Cruz	#sc-373799
Anti-mouse PD-L1-InVivo	Selleck	#A2115
Rat IgG2b isotype control-InVivo	Selleck	#A2116
Anti-mouse CD8α-InVivo	Selleck	#A2102
Anti-p-STAT3 (Tyr705)	ZenBio	#R381552
Anti-p-STAT1 (Ser727)	ZenBio	#R25797
Anti-STAT1	ZenBio	#R25799
Anti-JAK2	ZenBio	#R24775
Anti-p-JAK2 (Tyr1007/1008)	ZenBio	#R381556
Anti-PIAS1	ZenBio	#R383086
Anti-PIAS3	ZenBio	#370213
Anti-SOCS2	ZenBio	#R25765
Anti-SOCS3	ZenBio	#500694