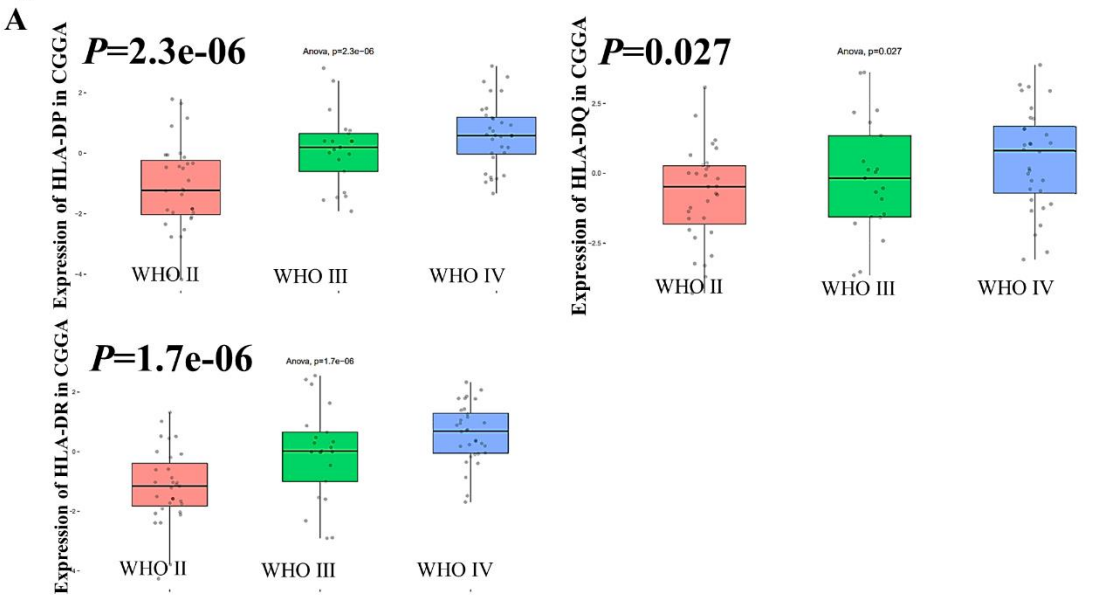
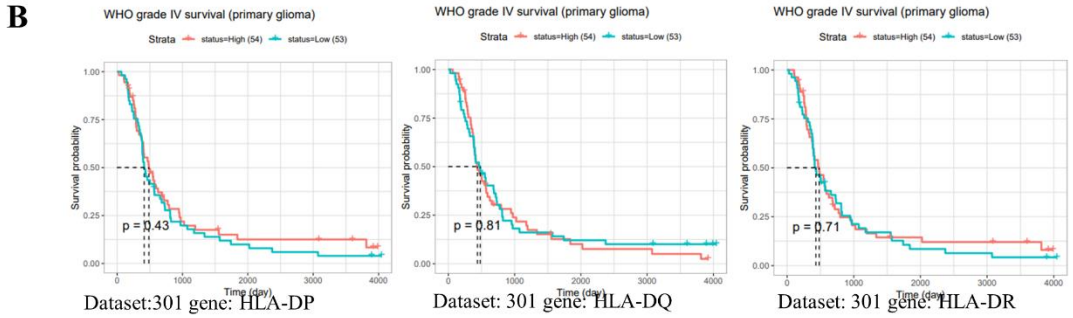
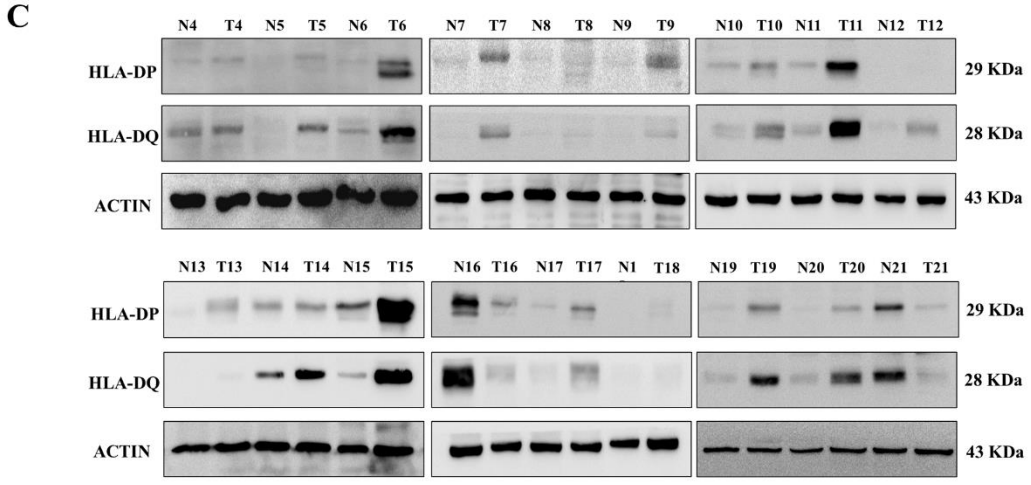
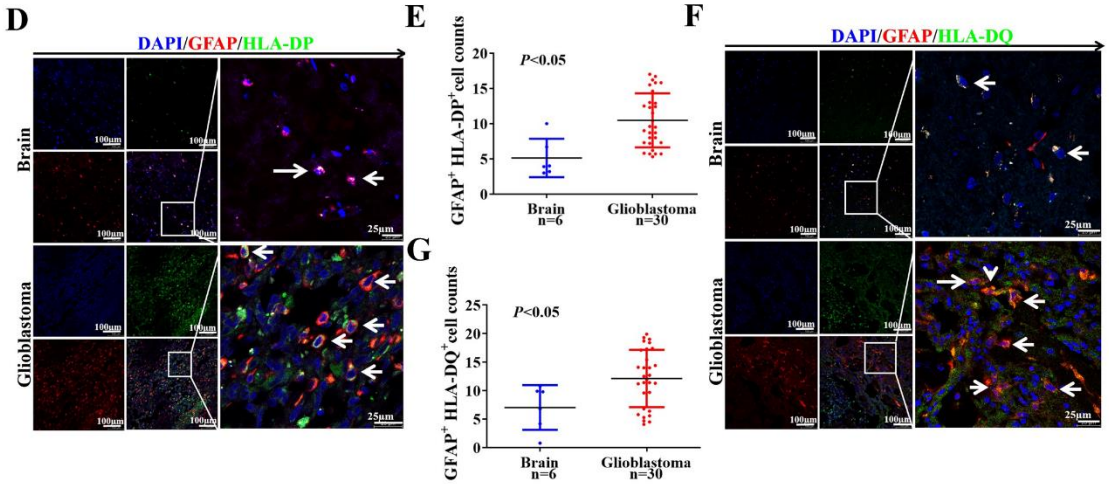
Fig1


A
P=2.3e-06
Anova, p=2.3e-06
Expression of HLA-DP in CGGA
WHO II
WHO III
WHO IV
P=0.027
Anova, p=0.027
Expression of HLA-DQ in CGGA
P=1.7e-06
Anova, p=1.7e-06
Expression of HLA-DR in CGGA

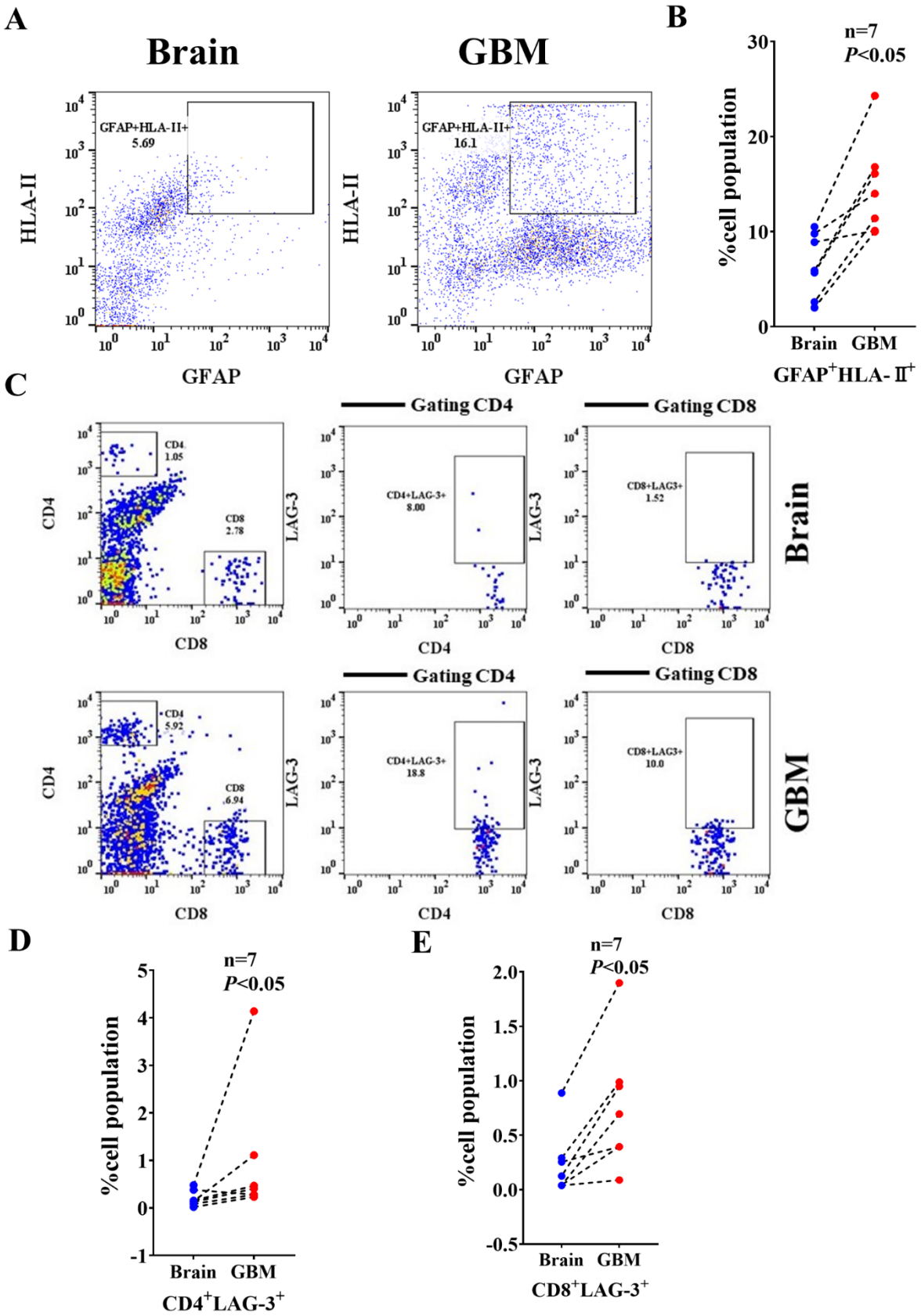

B
WHO grade IV survival (primary glioma)
Strata status=High (54) status=Low (53)
Survival probability
Time (day)
p = 0.43
p = 0.81
p = 0.71
Dataset:301 gene: HLA-DP
Dataset: 301 gene: HLA-DQ
Dataset: 301 gene: HLA-DR


C
N4 T4 N5 T5 N6 T6
N7 T7 N8 T8 N9 T9
N10 T10 N11 T11 N12 T12
HLA-DP
HLA-DQ
ACTIN
29 KDa
28 KDa
43 KDa
N13 T13 N14 T14 N15 T15
N16 T16 N17 T17 N1 T18
N19 T19 N20 T20 N21 T21


D
DAPI/GFAP/HLA-DP
Brain
Glioblastoma
100μm
25μm
E
GFAP+ HLA-DP+ cell counts
P<0.05
Brain n=6
Glioblastoma n=30
F
DAPI/GFAP/HLA-DQ
G
GFAP+ HLA-DQ+ cell counts
P<0.05
Brain n=6
Glioblastoma n=30

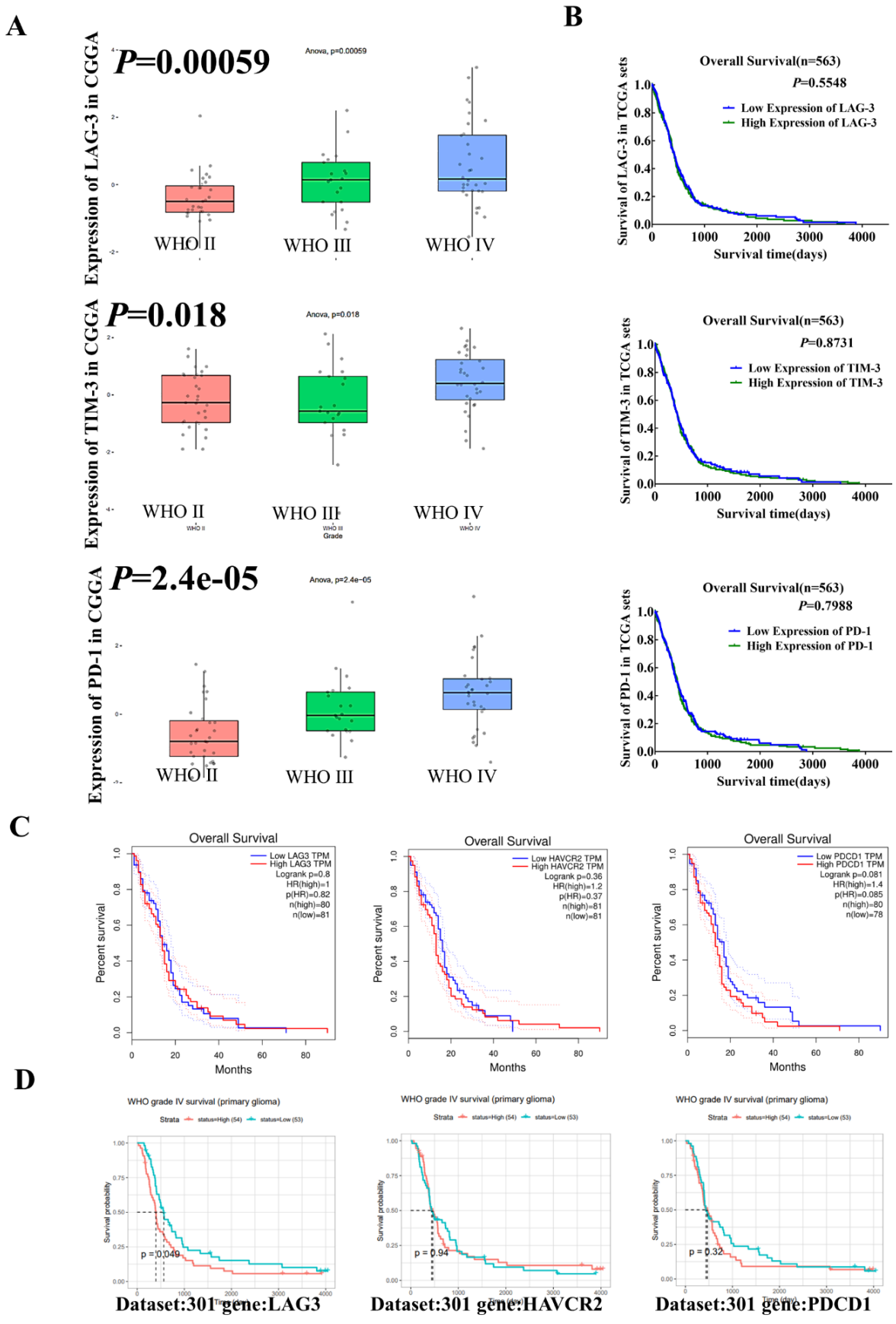
Supplementary Figure 1. Evaluation of HLA-Ⅱ expression in GBM samples. (A) The expression of HLA-II in GBM was shown from the Chinese Glioma Genome Atlas (CGGA). (B) Association between HLA-DP, HLA-DQ, and HLA-DR expression and survival follow-up time in CGGA datasets. (C) Fresh tumor tissues and peritumoral brain tissues were collected, and the expression of HLA-II was measured by western blot. (D, F) 30 cases of frozen GBM and 6 cases of control samples were collected, representative immunofluorescence images indicate HLA-II expression in $GFAP^+$ GBM cells. White arrows point to the colocalized cells. (E, G) The comparison of $HLA\text{-}DP^+GFAP^+$ and $HLA\text{-}DQ^+GFAP^+$ between GBM and control tissues was shown.

Fig2


A
Brain
GBM
GFAP+HLA-II+
5.69
GFAP+HLA-II+
16.1
HLA-II
GFAP
B
n=7
P<0.05
%cell population
Brain GBM
GFAP+HLA-II+
C
Gating CD4
Gating CD8
CD4
1.05
CD8
2.78
CD4+LAG-3+
8.00
CD8+LAG3+
1.52
Brain
CD4
5.92
CD8
6.94
CD4+LAG-3+
18.8
CD8+LAG3+
10.0
GBM
CD4
CD8
LAG-3
D
n=7
P<0.05
%cell population
Brain GBM
CD4+LAG-3+
E
n=7
P<0.05
%cell population
Brain GBM
CD8+LAG-3+

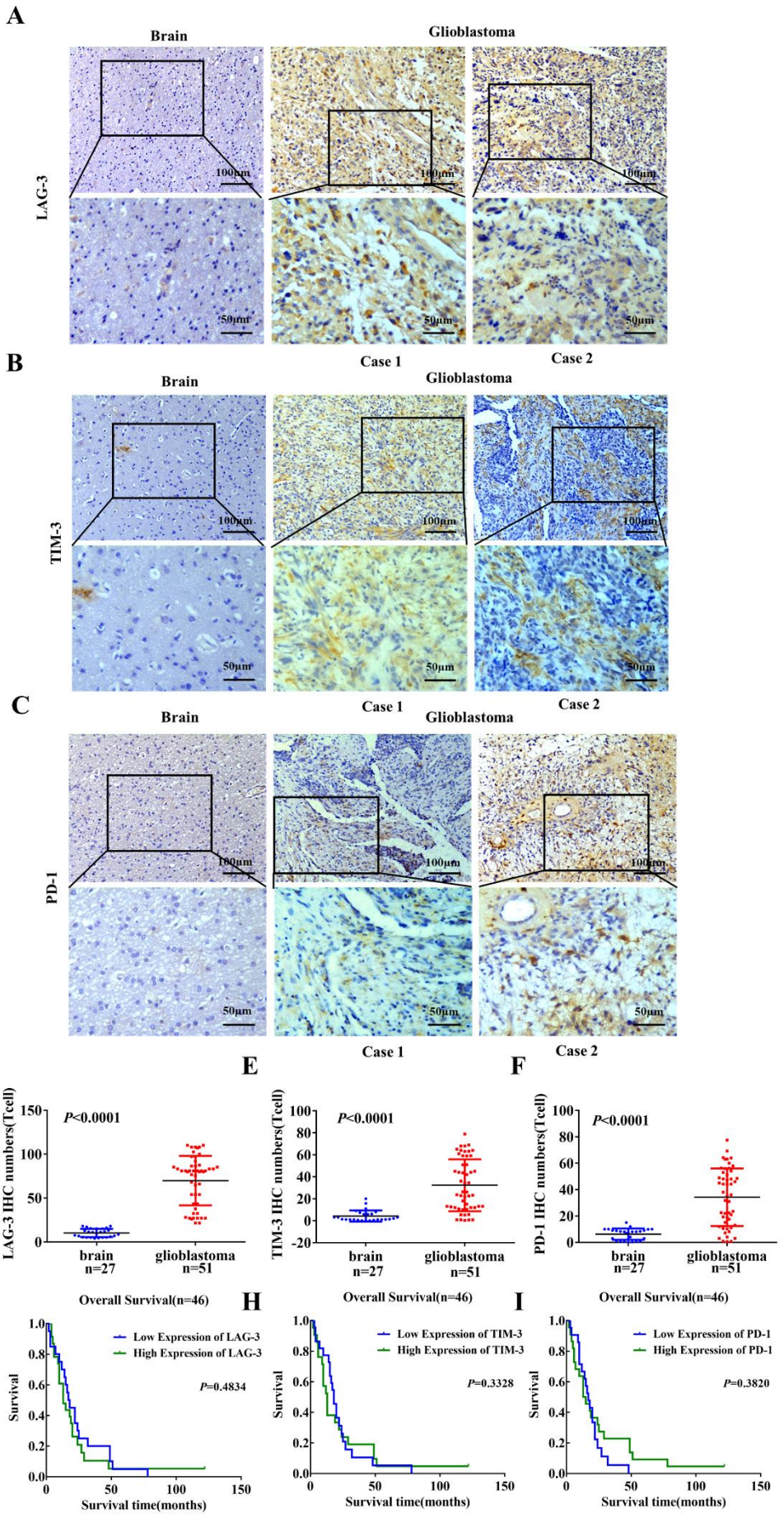
Supplementary Figure 2. FCM analysis of HLA-II$^+$GFAP$^+$ population, LAG-3$^+$CD4$^+$ and LAG-3$^+$CD8$^+$ T cells in human GBM. 7 pairs of fresh GBM and adjacent brain samples were processed for FCM analysis. (A) FCM analysis shows the percentage of HLA-II$^+$GFAP$^+$ cells in human GBM and adjacent brain tissues. (B) The percentage of HLA-II$^+$GFAP$^+$ cells in human GBM is higher than that in adjacent brain tissues. (C) FCM analysis shows the population of CD4$^+$, CD8$^+$, LAG-3$^+$CD4$^+$ and LAG$^+$CD8$^+$ T cells in human GBM and adjacent brain tissues. The percentage of LAG-3$^+$CD4$^+$ cells (D) and LAG-3$^+$CD8$^+$ (E) in human GBM is higher than that in adjacent brain.

Fig3


A
B
C
D
P=0.00059
Anova, p=0.00059
Expression of LAG-3 in CGGA
WHO II
WHO III
WHO IV
P=0.018
Anova, p=0.018
Expression of TIM-3 in CGGA
WHO II
WHO III
WHO IV
Grade
P=2.4e-05
Anova, p=2.4e-05
Expression of PD-1 in CGGA
WHO II
WHO III
WHO IV
Overall Survival(n=563)
P=0.5548
Low Expression of LAG-3
High Expression of LAG-3
Survival of LAG-3 in TCGA sets
Survival time(days)
Overall Survival(n=563)
P=0.8731
Low Expression of TIM-3
High Expression of TIM-3
Survival of TIM-3 in TCGA sets
Survival time(days)
Overall Survival(n=563)
P=0.7988
Low Expression of PD-1
High Expression of PD-1
Survival of PD-1 in TCGA sets
Survival time(days)
Overall Survival
Low LAG3 TPM
High LAG3 TPM
Logrank p=0.8
HR(high)=1
p(HR)=0.82
n(high)=80
n(low)=81
Percent survival
Months
Overall Survival
Low HAVCR2 TPM
High HAVCR2 TPM
Logrank p=0.36
HR(high)=1.2
p(HR)=0.37
n(high)=81
n(low)=81
Percent survival
Months
Overall Survival
Low PDCD1 TPM
High PDCD1 TPM
Logrank p=0.081
HR(high)=1.4
p(HR)=0.085
n(high)=80
n(low)=78
Percent survival
Months
WHO grade IV survival (primary glioma)
Strata
status=High (54)
status=Low (53)
Survival probability
p = 0.049
Dataset:301 gene:LAG3
WHO grade IV survival (primary glioma)
Strata
status=High (54)
status=Low (53)
Survival probability
p = 0.94
Dataset:301 gene:HAVCR2
WHO grade IV survival (primary glioma)
Strata
status=High (54)
status=Low (53)
Survival probability
p = 0.32
Dataset:301 gene:PDCD1

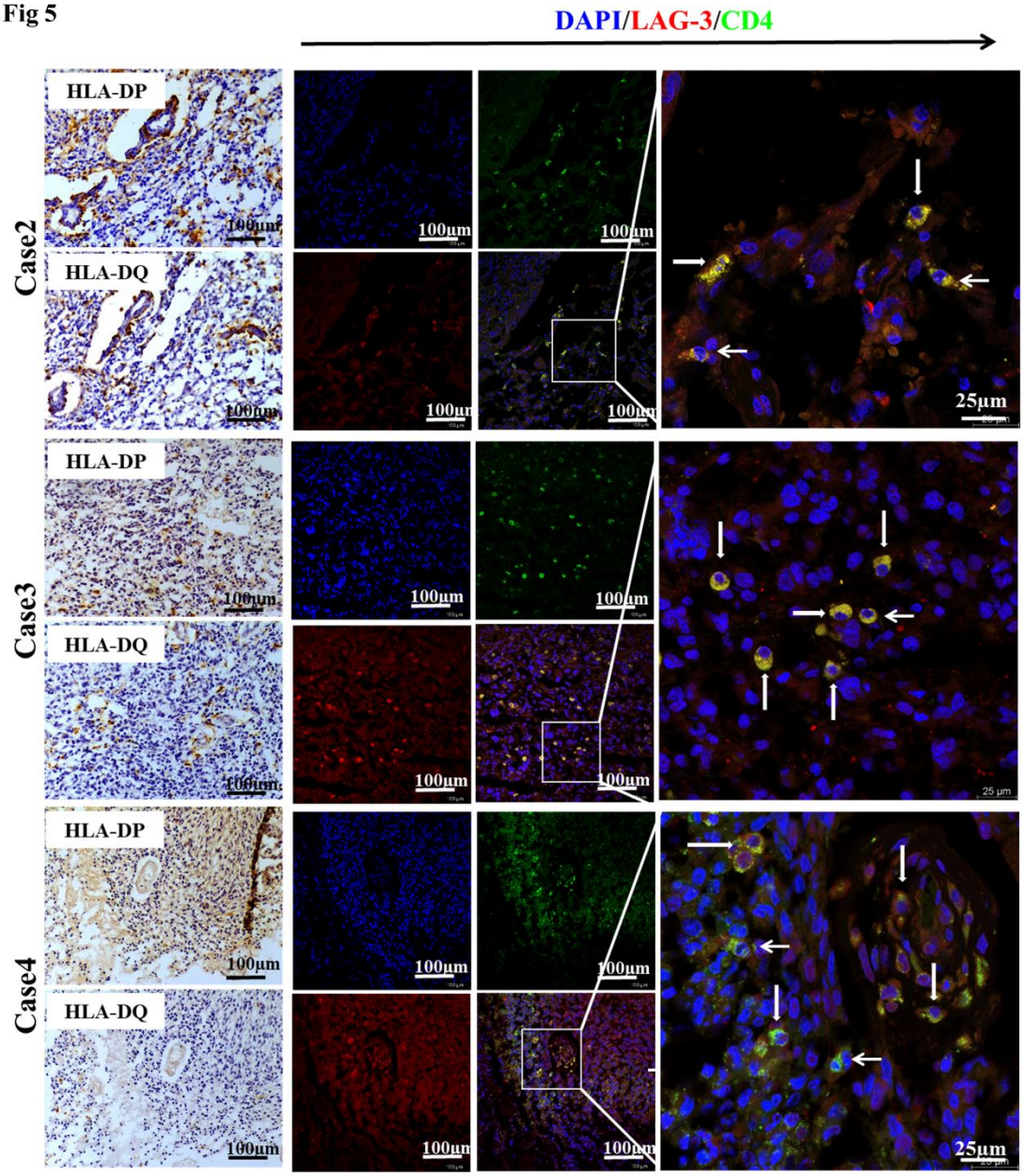
Supplementary Figure 3. Relationship between immune checkpoint molecules and patient survival outcomes in the TCGA and CGGA datasets. (A) The expression of LAG-3, TIM-3, and PD-1 **in GBM from CGGA was shown.** Oncomine (B) and GEPIA (C) database from TCGA and the data from CGGA (D) showed Kaplan-Meier curves for overall survival, according to the expression of LAG-3, TIM-3, and PD-1 at mRNA level in GBM.

Fig4


A
Brain
Glioblastoma
LAG-3
100μm
50μm
Case 1
Case 2
B
Brain
Glioblastoma
TIM-3
100μm
50μm
Case 1
Case 2
C
Brain
Glioblastoma
PD-1
100μm
50μm
Case 1
Case 2
D
LAG-3 IHC numbers(Tcell)
P<0.0001
brain n=27
glioblastoma n=51
E
TIM-3 IHC numbers(Tcell)
P<0.0001
brain n=27
glioblastoma n=51
F
PD-1 IHC numbers(Tcell)
P<0.0001
brain n=27
glioblastoma n=51
G
Overall Survival(n=46)
Low Expression of LAG-3
High Expression of LAG-3
P=0.4834
Survival
Survival time(months)
H
Overall Survival(n=46)
Low Expression of TIM-3
High Expression of TIM-3
P=0.3328
Survival
Survival time(months)
I
Overall Survival(n=46)
Low Expression of PD-1
High Expression of PD-1
P=0.3820
Survival
Survival time(months)

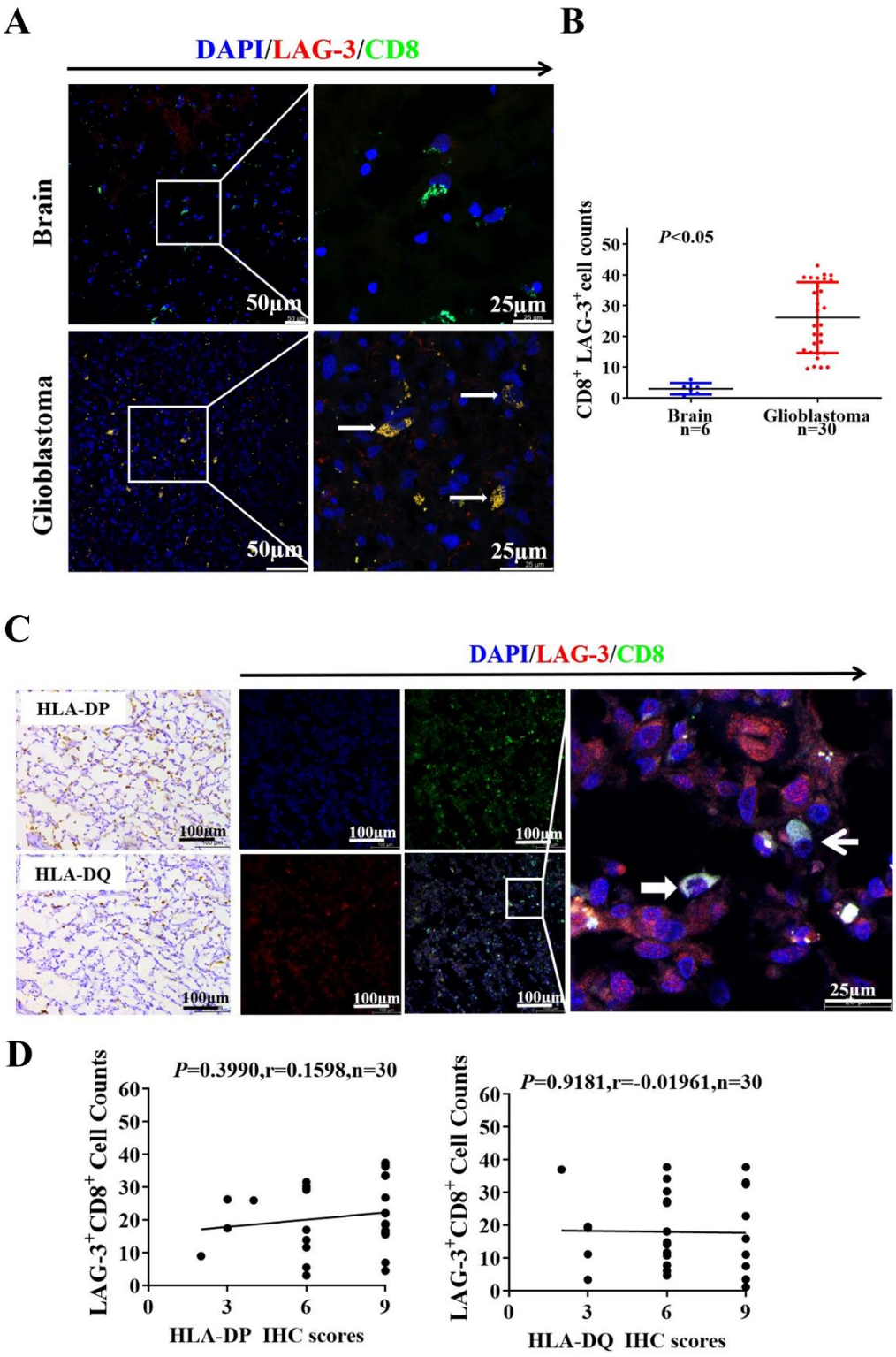
Supplementary Figure 4. Overall survival analysis according to the expression of LAG-3, TIM-3, and PD-1 in GBM. (A-C) Representative IHC images of LAG-3, TIM-3, and PD-1 staining in paraffin GBM specimens are shown (51 cases of GBM and 27 control brain tissues). (D-F) Infiltrated cells number of LAG-3^+, TIM-3^+, and PD-1^+ cells was shown (n=51). (G-I) The median of LAG-3, TIM-3, and PD-1 positive cells was used as cutoff point to split the GBM into high/low groups, and OS of patients was shown according to the expression of LAG-3, TIM-3, and PD-1 paraffin GBM specimens (n=46).

Fig 5



Supplementary Figure 5. Immunofluorescence staining images indicated LAG-3^{+}CD4^{+} cells infiltration in the tumor stroma, which is associated with HLA-DP and HLA-DQ expression. White arrows point to the colocalized cells.

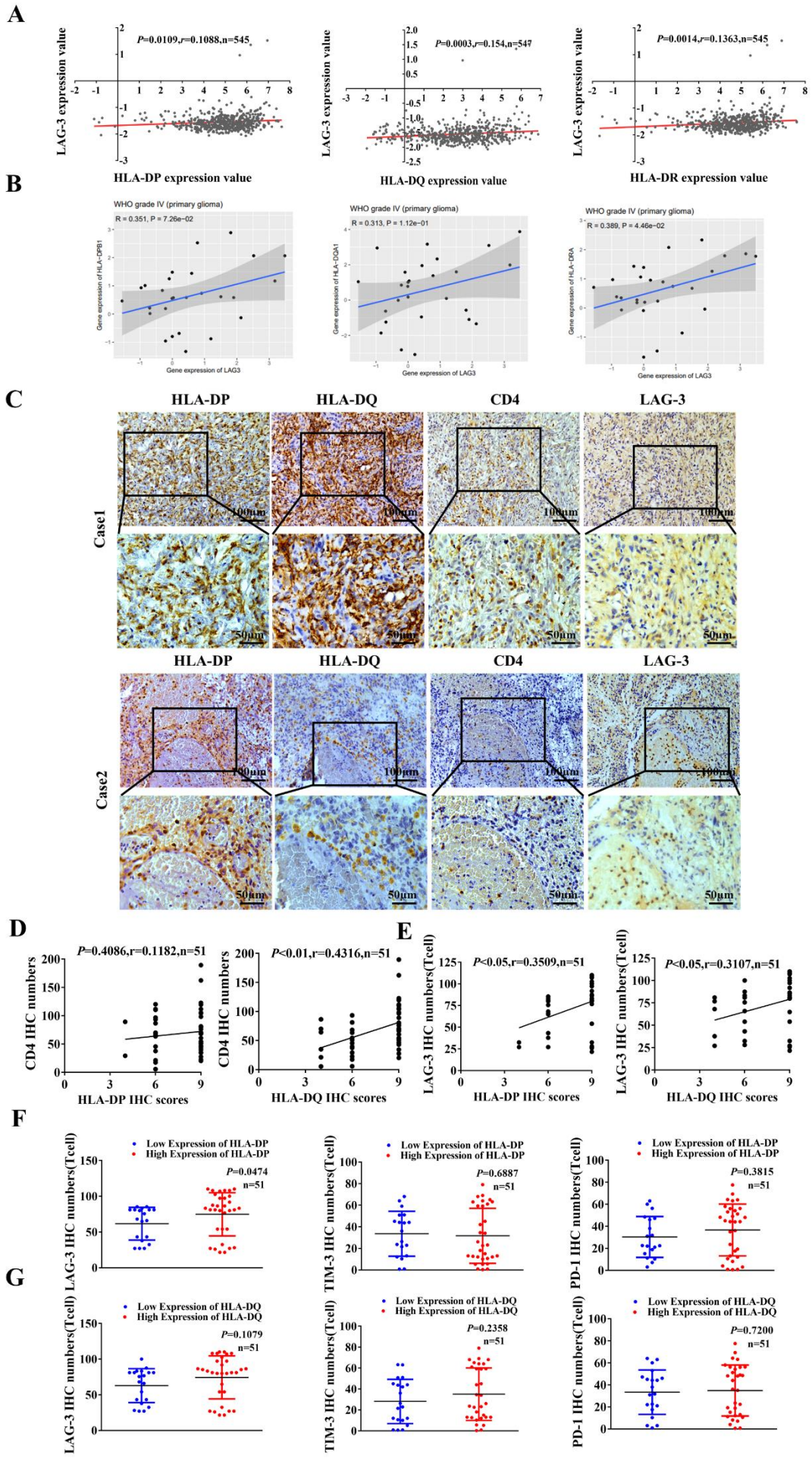
Fig6



Supplementary Figure 6. Immunofluorescence staining indicated the infiltration of CD8$^+$LAG-3$^+$ T cells in GBM. (A) Representative immunofluorescence images indicate CD8$^+$LAG-3$^+$ T cells infiltration. White arrows point to the colocalized cells.

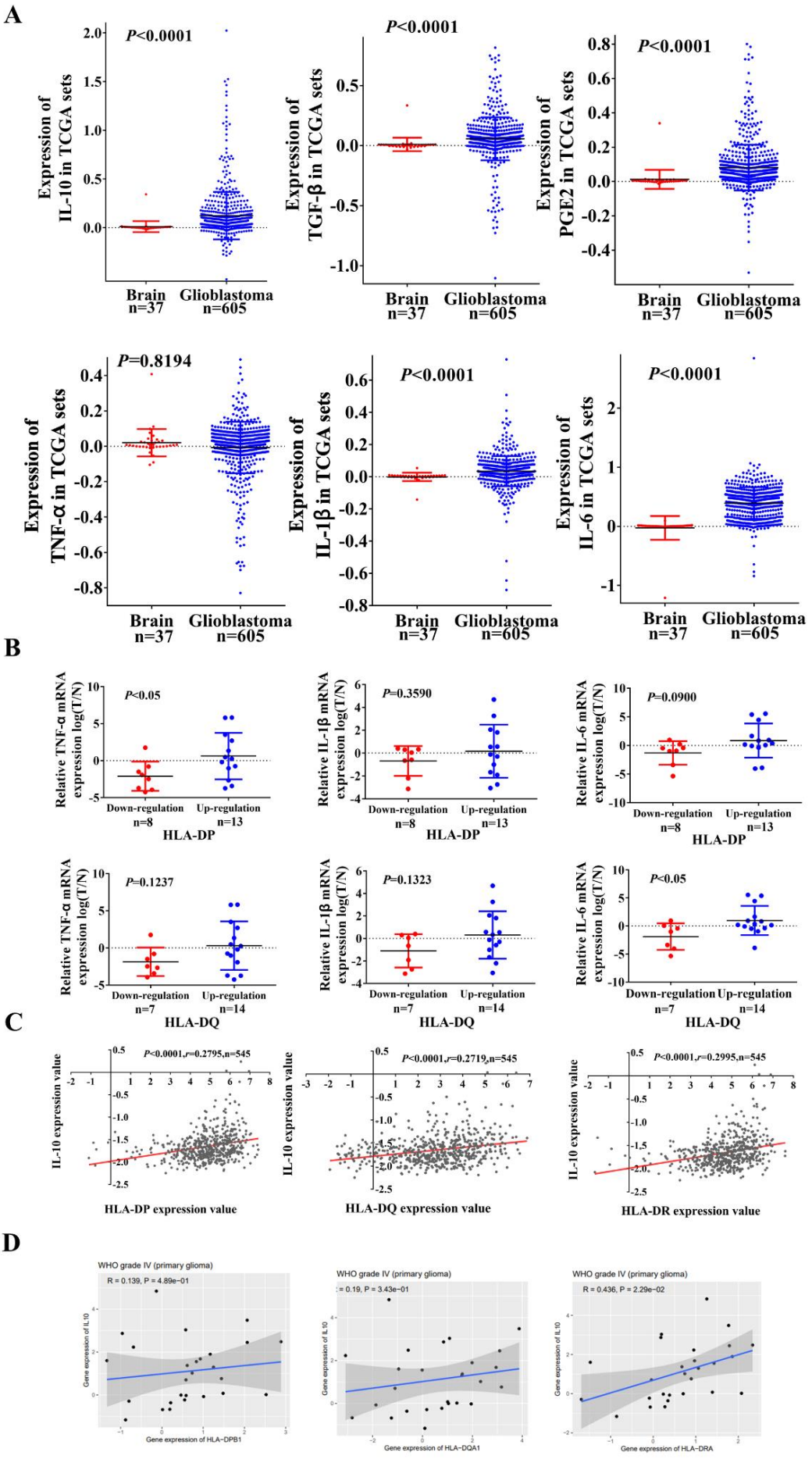
(B) The comparison of $CD8^+LAG\text{-}3^+$ T cells between GBM and control tissues was shown. (C) Immunofluorescence images indicated $CD8^+LAG\text{-}3^+$ cells infiltration in the tumor stroma, which is associated with HLA-DP and HLA-DQ expression. White arrows point to the colocalized cells. (D) The correlation between HLA-II expression and the number of $CD8^+LAG\text{-}3^+$ T cells was shown.

Fig 7


A
LAG-3 expression value
P=0.0109,r=0.1088,n=545
HLA-DP expression value
P=0.0003,r=0.154,n=547
HLA-DQ expression value
P=0.0014,r=0.1363,n=545
HLA-DR expression value
B
WHO grade IV (primary glioma)
R = 0.351, P = 7.26e−02
Gene expression of HLA-DPB1
Gene expression of LAG3
R = 0.313, P = 1.12e−01
Gene expression of HLA-DQA1
R = 0.389, P = 4.46e−02
Gene expression of HLA-DRA
C
HLA-DP
HLA-DQ
CD4
LAG-3
Case1
Case2
100μm
50μm
D
P=0.4086,r=0.1182,n=51
CD4 IHC numbers
HLA-DP IHC scores
P<0.01,r=0.4316,n=51
HLA-DQ IHC scores
E
P<0.05,r=0.3509,n=51
LAG-3 IHC numbers(Tcell)
P<0.05,r=0.3107,n=51
F
Low Expression of HLA-DP
High Expression of HLA-DP
P=0.0474
n=51
LAG-3 IHC numbers(Tcell)
P=0.6887
TIM-3 IHC numbers(Tcell)
P=0.3815
PD-1 IHC numbers(Tcell)
G
Low Expression of HLA-DQ
High Expression of HLA-DQ
P=0.1079
P=0.2358
P=0.7200

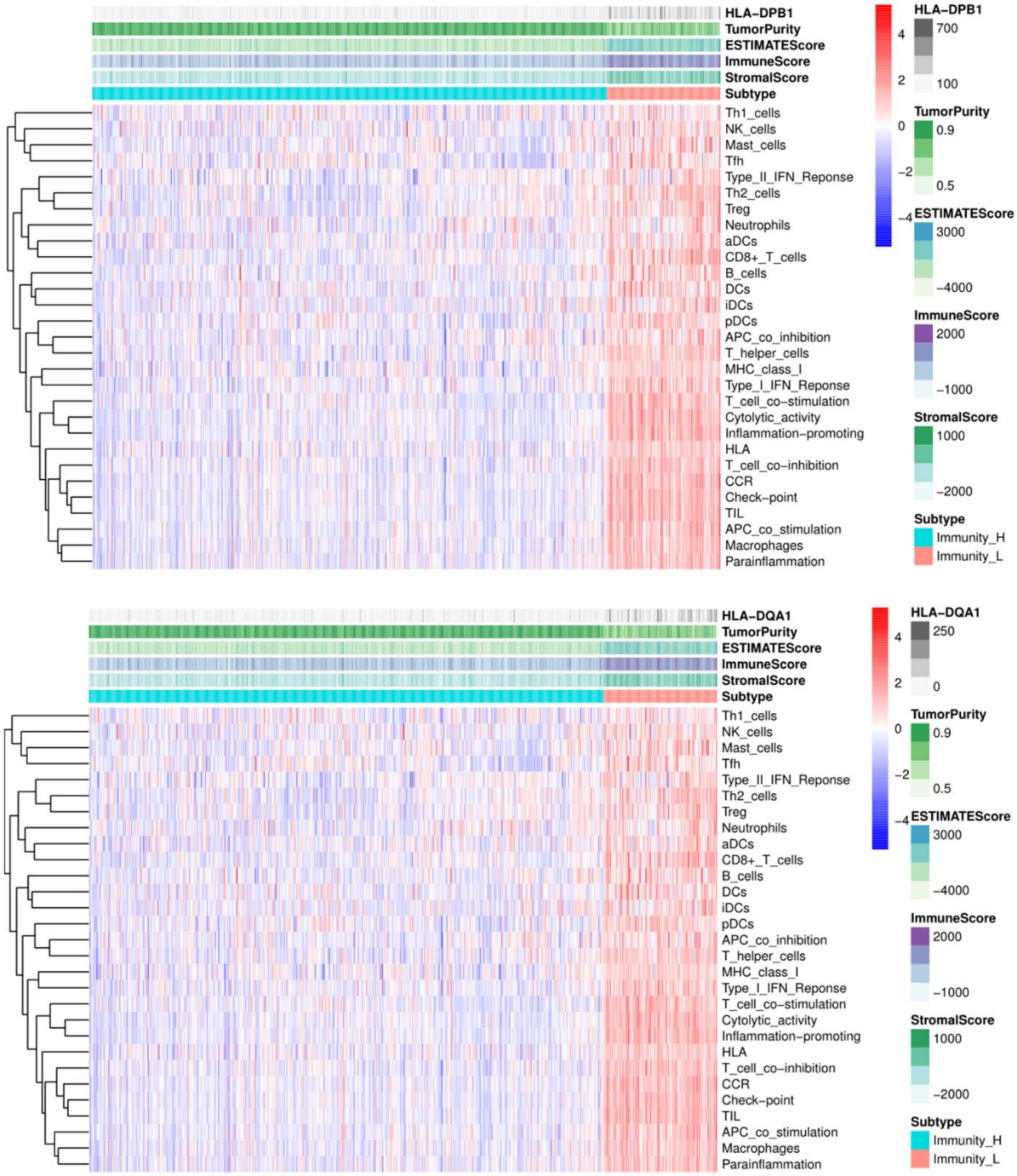
Supplementary Figure 7. Relationship between HLA-II expression status and the number of immune checkpoint positive cells. Data from TCGA (A) and CGGA (B) showed the correlation between HLA-DP/DQ and LAG-3 expression in GBM. (C) Representative IHC images indicate the expression of HLA-DP and DQ, CD4, and LAG-3 in the same field of GBM. (D) The correlation between HLA-DP/DQ and $CD4^+$ T cell numbers in paraffin GBM specimens was shown (n=51). (E) The correlation between HLA-DP/DQ and $LAG\text{-}3^+$ T cell numbers in paraffin GBM specimens was shown (n=51). (F) Based on IHC staining in paraffin GBM specimens, the number of LAG-3, TIM3, and PD-1 positive cells was compared between HLA-DP^{high} and HLA-DP^{low} groups (n=51). (G) The number of LAG-3, TIM3, and PD-1 positive cells was compared between HLA-DQ^{high} and HLA-DQ^{low} GBM samples (n=51).

Fig8


A
Expression of IL-10 in TCGA sets
P<0.0001
Brain n=37
Glioblastoma n=605
Expression of TGF-β in TCGA sets
P<0.0001
Expression of PGE2 in TCGA sets
P<0.0001
Expression of TNF-α in TCGA sets
P=0.8194
Expression of IL-1β in TCGA sets
P<0.0001
Expression of IL-6 in TCGA sets
P<0.0001
B
Relative TNF-α mRNA expression log(T/N)
P<0.05
Down-regulation n=8
Up-regulation n=13
HLA-DP
Relative IL-1β mRNA expression log(T/N)
P=0.3590
Relative IL-6 mRNA expression log(T/N)
P=0.0900
Relative TNF-α mRNA expression log(T/N)
P=0.1237
Down-regulation n=7
Up-regulation n=14
HLA-DQ
Relative IL-1β mRNA expression log(T/N)
P=0.1323
Relative IL-6 mRNA expression log(T/N)
P<0.05
C
IL-10 expression value
P<0.0001,r=0.2795,n=545
HLA-DP expression value
P<0.0001,r=0.2719,n=545
HLA-DQ expression value
P<0.0001,r=0.2995,n=545
HLA-DR expression value
D
WHO grade IV (primary glioma)
R = 0.139, P = 4.89e−01
Gene expression of IL10
Gene expression of HLA−DPB1
R = 0.19, P = 3.43e−01
Gene expression of HLA−DQA1
R = 0.436, P = 2.29e−02
Gene expression of HLA−DRA

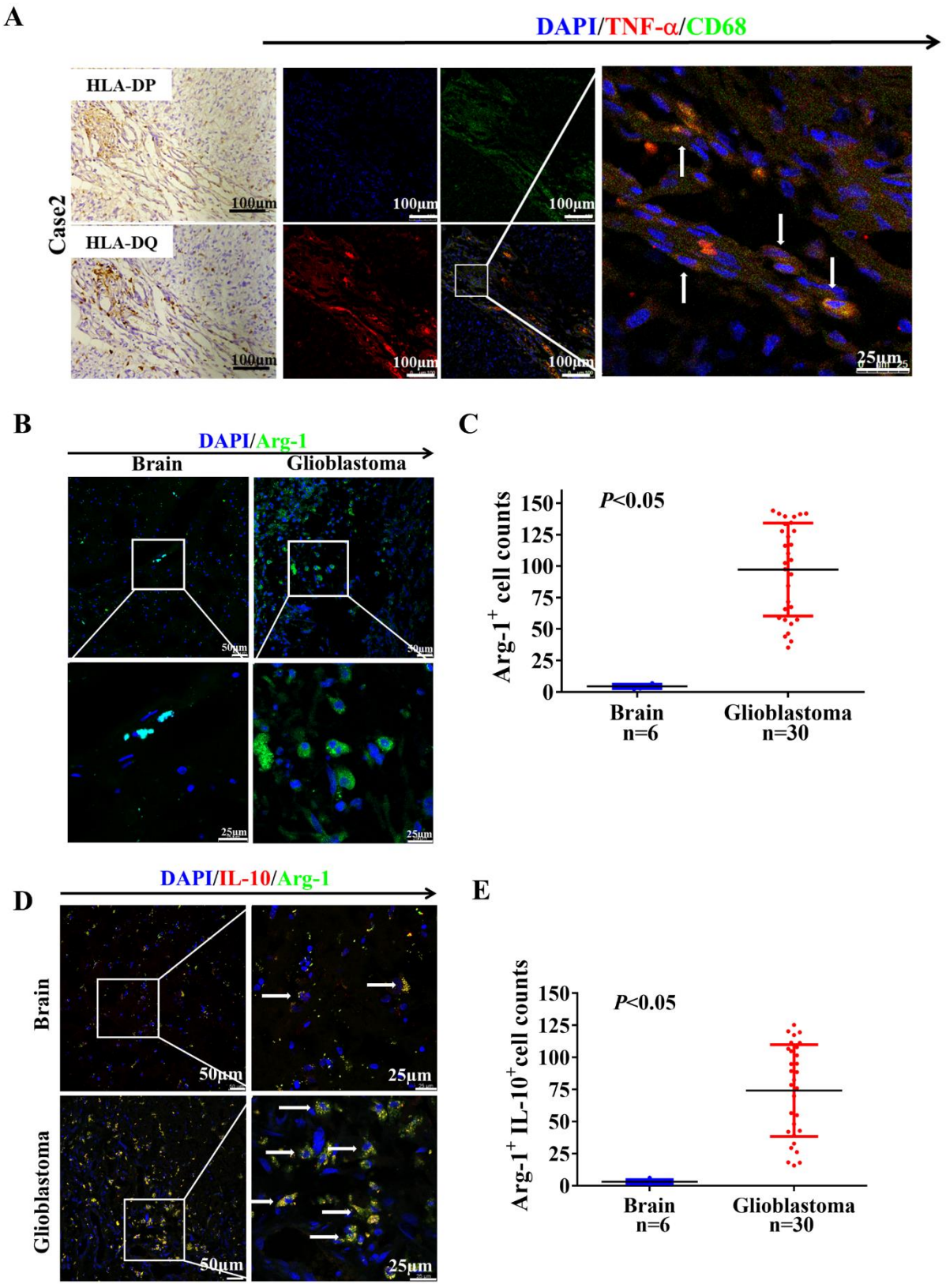
Supplementary Figure 8. The expression of cytokines in human GBM tissues. (A) The expression of cytokines was shown from the TCGA. (B) Based on the RT-PCR results of 21 pairs of fresh tumor tissues, the GBM tissues were divided into HLA-II upregulation and HLA-II downregulation groups. The expression of IL-1β, TNF-α, and IL-6 was compared between those two groups (p<0.05). Data from TCGA (C) and CGGA (D) showed the correlation between HLA-DP/DQ and IL-10 expression in GBM.

Fig9



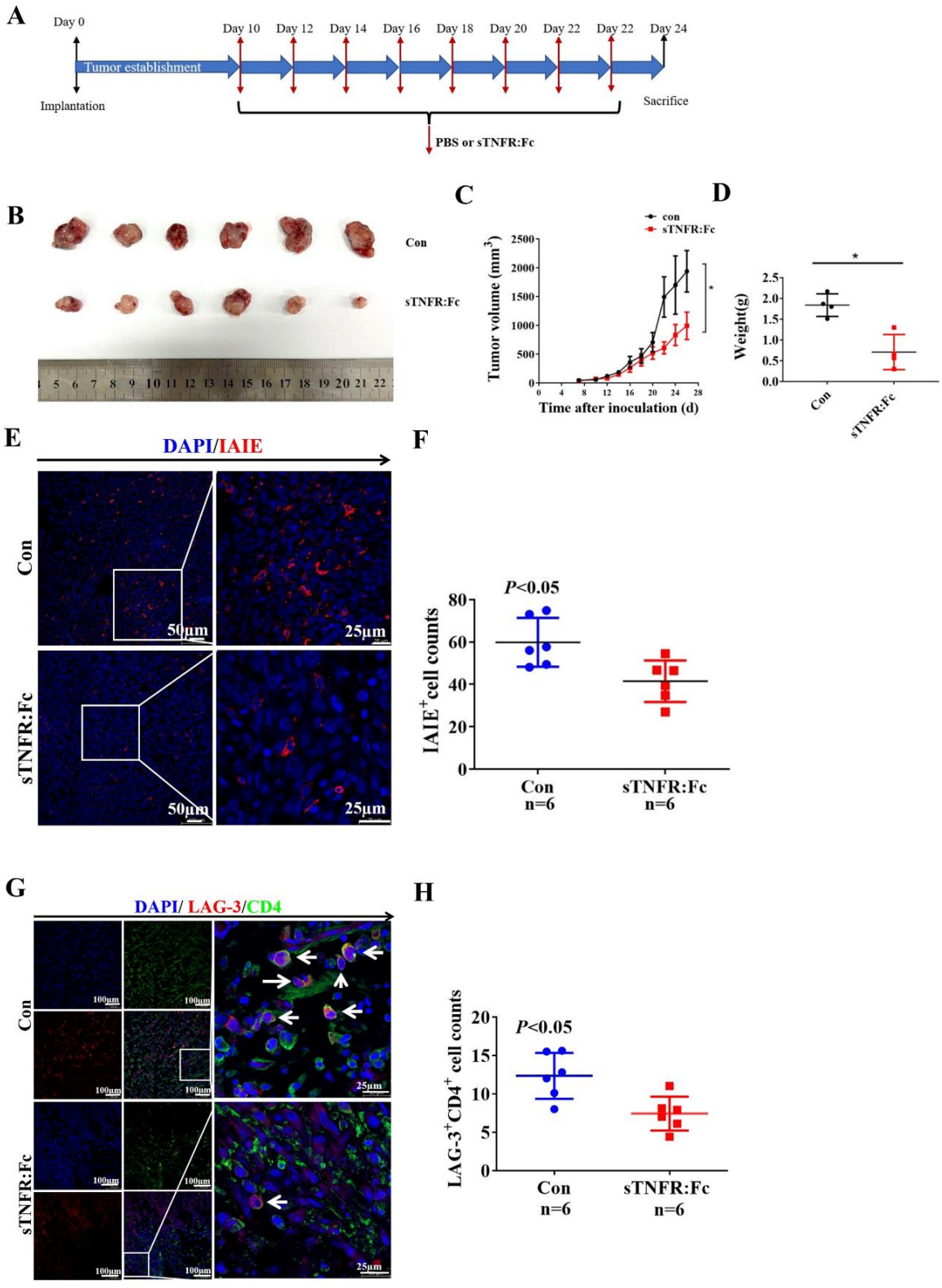
Supplementary Figure 9. The immune infiltration patterns based on low or high expression of HLA-II was analyzed by ssGSEA methods in GBM samples from the TCGA.

Fig10


A
DAPI/TNF-α/CD68
HLA-DP
HLA-DQ
Case2
100μm
25μm
B
DAPI/Arg-1
Brain
Glioblastoma
50μm
25μm
C
P<0.05
Arg-1+ cell counts
150
125
100
75
50
25
0
Brain
n=6
Glioblastoma
n=30
D
DAPI/IL-10/Arg-1
Brain
Glioblastoma
50μm
25μm
E
P<0.05
Arg-1+ IL-10+cell counts
150
125
100
75
50
25
0
Brain
n=6
Glioblastoma
n=30

Supplementary Figure 10. (A) Immunofluorescence staining showed that TNF-α and CD68 were colocalized in the tumor stroma, which is associated with HLA-DP and HLA-DQ expression in the same field. White arrows point to the colocalized cells. (B, C) Immunofluorescence staining showed the infiltration of Arg-1^+ cells in GBM, and the number of Arg-1^+ cells was compared between GBM tissues and control brain tissues (p<0.05). (D, E) Immunofluorescence staining shows that IL-10 and Arg-1 are colocalized in GBM samples, and the number of IL-10^+Arg-1^+ cells was compared between GBM and control brain samples (p<0.05). White arrows point to the colocalized cells.

Fig11



Supplementary Figure 11. Blocking TNF-α inhibits GBM progression in mice model. (A) Schematic of experimental design is shown. (B) Images of tumors from

control and *sTNFR:Fc* treatment group are shown at day 22 after injection. (C) Tumor volumes were measured every 2 days from day 12 to 22. Data were shown as mean ± SD (n=6, **p*<0.05). (D) Tumor weights were measured at day 22. Data were shown as mean ± SD (n =6, **p*<0.05). (E, F) Immunofluorescence image indicate MHC-II expression in the tumor tissues. Data were shown as mean ± SD (n =6, **p*<0.05). (G, H) Immunofluorescence image indicate that $CD4^+LAG\text{-}3^+$ cells are infiltrated in the tumor tissues. Data were shown as mean ± SD (n =6, **p*<0.05).