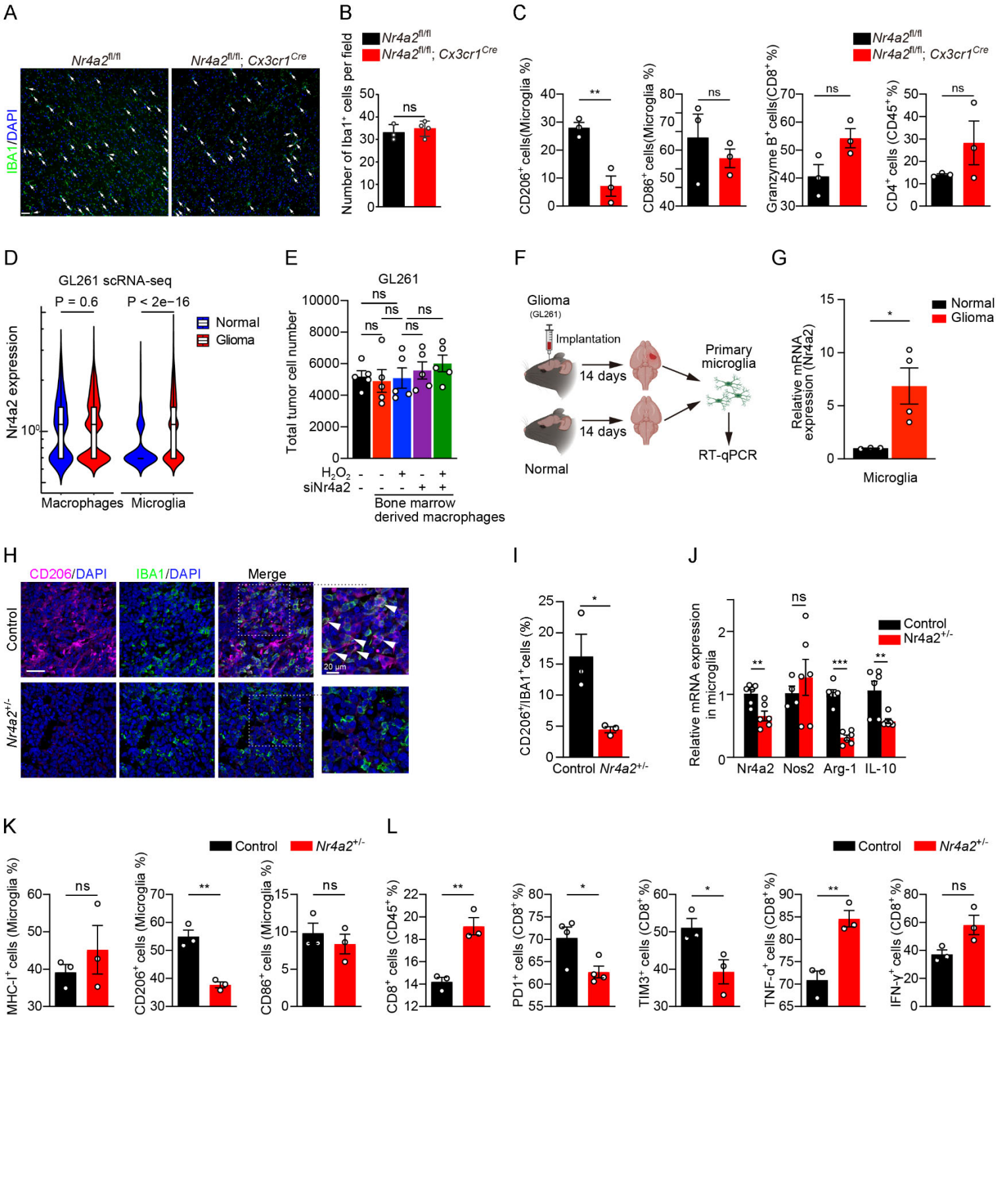
A
Nr4a2fl/fl
Nr4a2fl/fl; Cx3cr1Cre
IBA1/DAPI
B
Nr4a2fl/fl
Nr4a2fl/fl; Cx3cr1Cre
Number of Iba1+ cells per field
ns
C
Nr4a2fl/fl
Nr4a2fl/fl; Cx3cr1Cre
CD206+ cells(Microglia %)
CD86+ cells(Microglia %)
Granzyme B+ cells(CD8+ %)
CD4+ cells (CD45+ %)
D
GL261 scRNA-seq
P = 0.6
P < 2e−16
Normal
Glioma
Nr4a2 expression
Macrophages
Microglia
E
GL261
Total tumor cell number
H2O2
siNr4a2
Bone marrow derived macrophages
F
Glioma
(GL261)
Implantation
14 days
14 days
Primary microglia
RT-qPCR
Normal
G
Normal
Glioma
Relative mRNA expression (Nr4a2)
Microglia
H
CD206/DAPI
IBA1/DAPI
Merge
Control
Nr4a2+/-
20 μm
I
CD206+/IBA1+ cells (%)
Control
Nr4a2+/-
J
Control
Nr4a2+/-
Relative mRNA expression in microglia
Nr4a2
Nos2
Arg-1
IL-10
K
Control
Nr4a2+/-
MHC-I+ cells (Microglia %)
CD206+ cells (Microglia %)
CD86+ cells (Microglia %)
L
Control
Nr4a2+/-
CD8+ cells (CD45+ %)
PD1+ cells (CD8+ %)
TIM3+ cells (CD8+ %)
TNF-α+ cells (CD8+ %)
IFN-γ+ cells (CD8+ %)

Supplementary Figure S5. Genetic knockout of *Nr4a2* in microglia reverses immunosuppressive state, related to Figure 6

(A-B) Immunofluorescence staining for microglia (IBA1) on brain tissue of *Nr4a2*$^{fl/fl}$ and *Nr4a2*$^{fl/fl}$*Cx3cr1*^{cre} mice. Scale bars, 50 μm (A). Number of IBA1$^+$ cells per field was shown (B).

(C) Flow cytometry analysis to examine the proportion of CD206$^+$ and CD86$^+$ cells in microglia, proportion of granzyme B$^+$CD8$^+$ T cells and proportion of CD4$^+$ T cells in CD45$^+$ cells from glioma tissue of *Nr4a2*$^{fl/fl}$ and *Nr4a2*$^{fl/fl}$*Cx3cr1*^{cre} glioma-bearing mice.

(D) Violin plot comparing Nr4a2 expression in macrophages and microglia from normal brain and glioma-bearing brain in our GL261 scRNA-seq data, respectively.

(E) NR4A2-knockdown of macrophages had no effect on proliferation of glioma. Bone marrow derived macrophages transfected with siNr4a2 for 48 hours were treated by H_2O_2 (500 μM) for 12 hours. Conditional medium from treated macrophages was used to incubate with glioma cells (GL261) for 12 hours. Cell proliferation of glioma cells were assessed by cell counts.

(F) A schematic model to evaluate the Nr4a2 expression of microglia isolated from tumor tissues of GL261 glioma-bearing mice and the cortex of control mice.

(G) Relative expression of Nr4a2 in microglia isolated from glioma tissue compared with microglia from cortex of control mice.

(H, I) Immunofluorescence staining for CD206 of microglia on brain sections from *Nr4a2*$^{+/-}$ and control glioma-bearing mice (H). Scale bars, 50 μm. Proportions of CD206$^+$IBA1$^+$ cells (I) were shown.

(J) Relative expression levels of immunoactivation marker (*Nos2*) and immunosuppression marker (*Arg-1* and *IL-10*) in primary microglia isolated from *Nr4a2*$^{+/-}$ mice and control glioma-bearing mice.

(K) Flow cytometry analysis of CD206, CD86 and antigen presentation maker (MHC-I) of microglia in respective group.

(L) Flow cytometry analysis to test immune checkpoint and cytotoxic functions of CD8$^+$ T cells in respective group.

Data are shown as mean ± SEM. In (E), P value was calculated using one-way *ANOVA*

analysis. In (B), (C), (D), (G), (I), (J), (K) and (L), P value was calculated using the two-tailed Student's t test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.