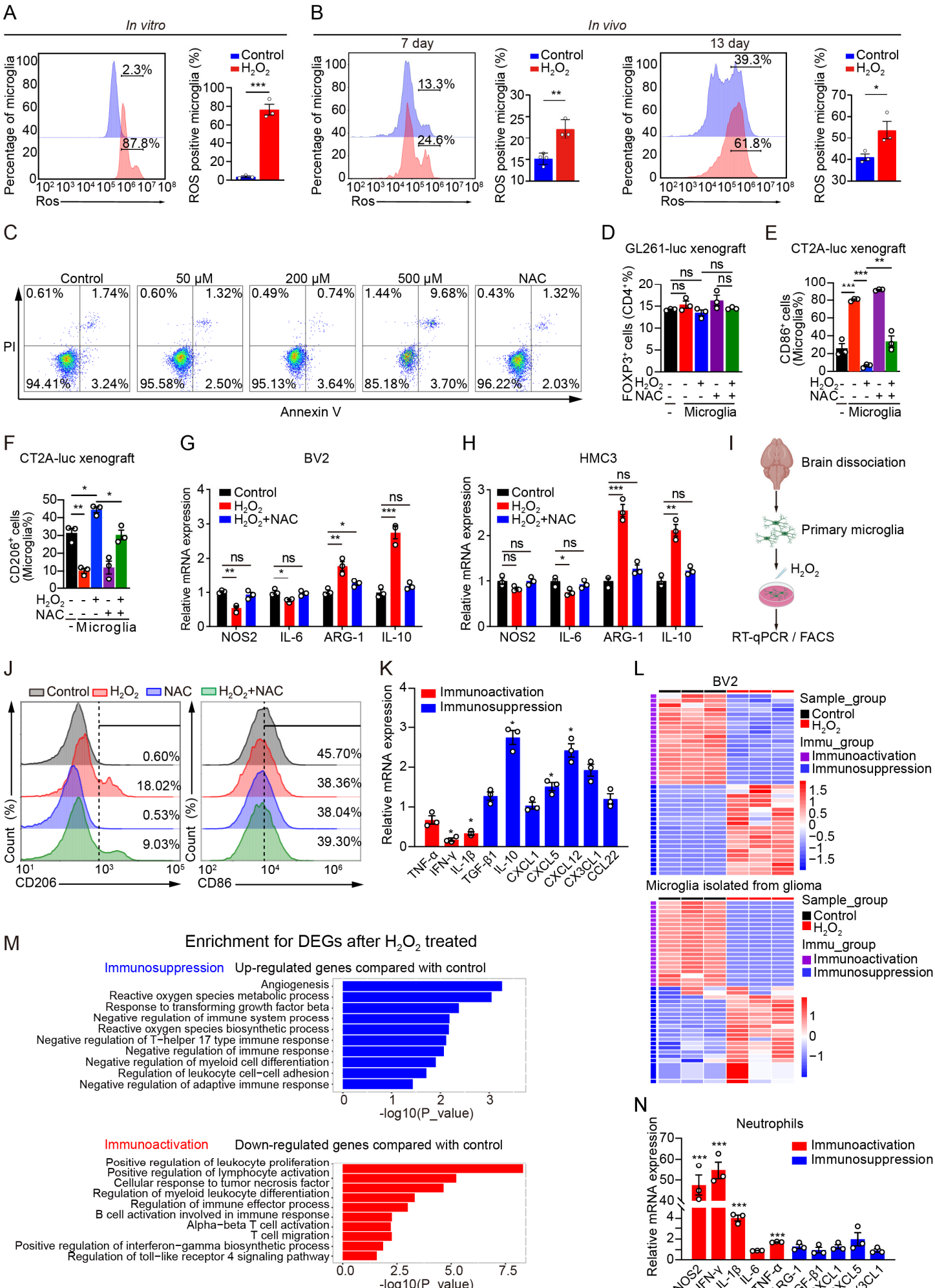
A
In vitro
Percentage of microglia
2.3%
87.8%
Ros
ROS positive microglia (%)
Control
H2O2
B
In vivo
7 day
13.3%
24.6%
13 day
39.3%
61.8%
C
Control
50 μM
200 μM
500 μM
NAC
0.61%
1.74%
94.41%
3.24%
0.60%
1.32%
95.58%
2.50%
0.49%
0.74%
95.13%
3.64%
1.44%
9.68%
85.18%
3.70%
0.43%
1.32%
96.22%
2.03%
PI
Annexin V
D
GL261-luc xenograft
FOXP3+ cells (CD4+%)
ns
H2O2
NAC
Microglia
E
CT2A-luc xenograft
CD86+ cells (Microglia%)
F
CT2A-luc xenograft
CD206+ cells (Microglia%)
G
BV2
Relative mRNA expression
Control
H2O2
H2O2+NAC
NOS2
IL-6
ARG-1
IL-10
H
HMC3
I
Brain dissociation
Primary microglia
H2O2
RT-qPCR / FACS
J
Control
H2O2
NAC
H2O2+NAC
Count (%)
0.60%
18.02%
0.53%
9.03%
45.70%
38.36%
38.04%
39.30%
CD206
CD86
K
Immunoactivation
Immunosuppression
Relative mRNA expression
TNF-α
IFN-γ
IL-1β
TGF-β1
IL-10
CXCL1
CXCL5
CXCL12
CX3CL1
CCL22
L
BV2
Sample_group
Control
H2O2
Immu_group
Immunoactivation
Immunosuppression
Microglia isolated from glioma
M
Enrichment for DEGs after H2O2 treated
Immunosuppression Up-regulated genes compared with control
Angiogenesis
Reactive oxygen species metabolic process
Response to transforming growth factor beta
Negative regulation of immune system process
Reactive oxygen species biosynthetic process
Negative regulation of T−helper 17 type immune response
Negative regulation of immune response
Negative regulation of myeloid cell differentiation
Regulation of leukocyte cell−cell adhesion
Negative regulation of adaptive immune response
-log10(P_value)
Immunoactivation Down-regulated genes compared with control
Positive regulation of leukocyte proliferation
Positive regulation of lymphocyte activation
Cellular response to tumor necrosis factor
Regulation of myeloid leukocyte differentiation
Regulation of immune effector process
B cell activation involved in immune response
Alpha−beta T cell activation
T cell migration
Positive regulation of interferon−gamma biosynthetic process
Regulation of toll−like receptor 4 signaling pathway
N
Neutrophils
Relative mRNA expression
Immunoactivation
Immunosuppression
NOS2
IFN-γ
IL-1β
IL-6
TNF-α
ARG-1
TGF-β1
CXCL1
CXCL5
CX3CL1

Supplementary Figure S2. Oxidative stress in microglia promotes the immunosuppressive functions of microglia, related to Figure 3

(A) Analysis of ROS levels in microglia treated by H_2O_2 (500 μM, 12h) *in vitro*.

(B) Primary microglia were pretreated by H_2O_2 (500 μM) for 12 hours. Glioma cells (CT2A-luc, 2×10^4 cells) incubated with conditional medium of pretreated primary microglia cells for 12 hours. Treated glioma cells and treated primary microglia (1:1) were inoculated together into the frontal region of cerebral cortex. Microglia was isolated from glioma tissues by FACS sorting at day 7 and day 13, respectively (B). FITC signals represent ROS production.

(C) Apoptosis assay of microglia treated by H_2O_2 (50, 200 and 500 μM; 12h) or NAC (5 mM; 12h) using flow cytometry after staining with FITC-annexin V/propidium iodide.

(D) Flow cytometry analysis of FOXP3 in $CD4^+$ T cells of glioma tissue in GL261-luc glioma-bearing mice.

(E, F) Flow cytometry analysis of CD86 (E) and CD206 (F) in microglia ($CD11b^+CD45^{low}$) of glioma tissue from CT2A-luc glioma-bearing mice.

(G-H) Expression levels of immune activation marker (*NOS2* and *IL-6*) and immunosuppression marker (*ARG-1* and *IL-10*) in BV2 cells (G) and HMC3 cells (H) after H_2O_2 (500 μM) treatment in absence or presence of NAC (5 mM).

(I-J) Primary microglia isolated from normal brain were treated by H_2O_2 (500 μM) in absence or presence of NAC (5 mM) for 12 hours (I). Flow cytometry analysis to test CD206 and CD86 levels of treated primary microglia in respective group (J).

(K) Relative expression levels of immune activation genes (*TNF-α, IFN-γ* and *IL-1β*) and immune suppression genes (*TGF-β1*, *IL-10*, *CXCL1*, *CXCL5*, *CXCL12*, *CX3CL1* and *CCL22*) in primary microglia after H_2O_2 (500 μM) treatment.

(L) Heatmap of immune activation and immune suppression genes in H_2O_2-pretreated BV2 cells (above) and H_2O_2-pretreated primary microglia isolated from glioma bearing mice (below).

(M) GO analysis of DEGs in BV2 cells treated by H_2O_2 (500 μM). The enriched GO terms were listed in terms for immune activation (red, down-regulated genes) and immune suppression (blue, up-regulated genes) based on p values.

(N) Relative expression levels of immune activation genes (*NOS2, IFN-γ, IL-1β, IL-6,* and *TNF-α*) and immune suppression genes (*ARG-1, TGF-β1, CXCL1, CXCL5,* and *CX3CL1*) in primary neutrophils after H_2O_2 (500 μM) treatment.

Data are shown as mean ± SEM. In (D), (E) and (F) P value was calculated using one-way *ANOVA* analysis. In (A), (B), (G), (H), (K) and (N), P value was calculated using the two-tailed Student's *t* test. *p < 0.05, **p < 0.01, ***p < 0.001.