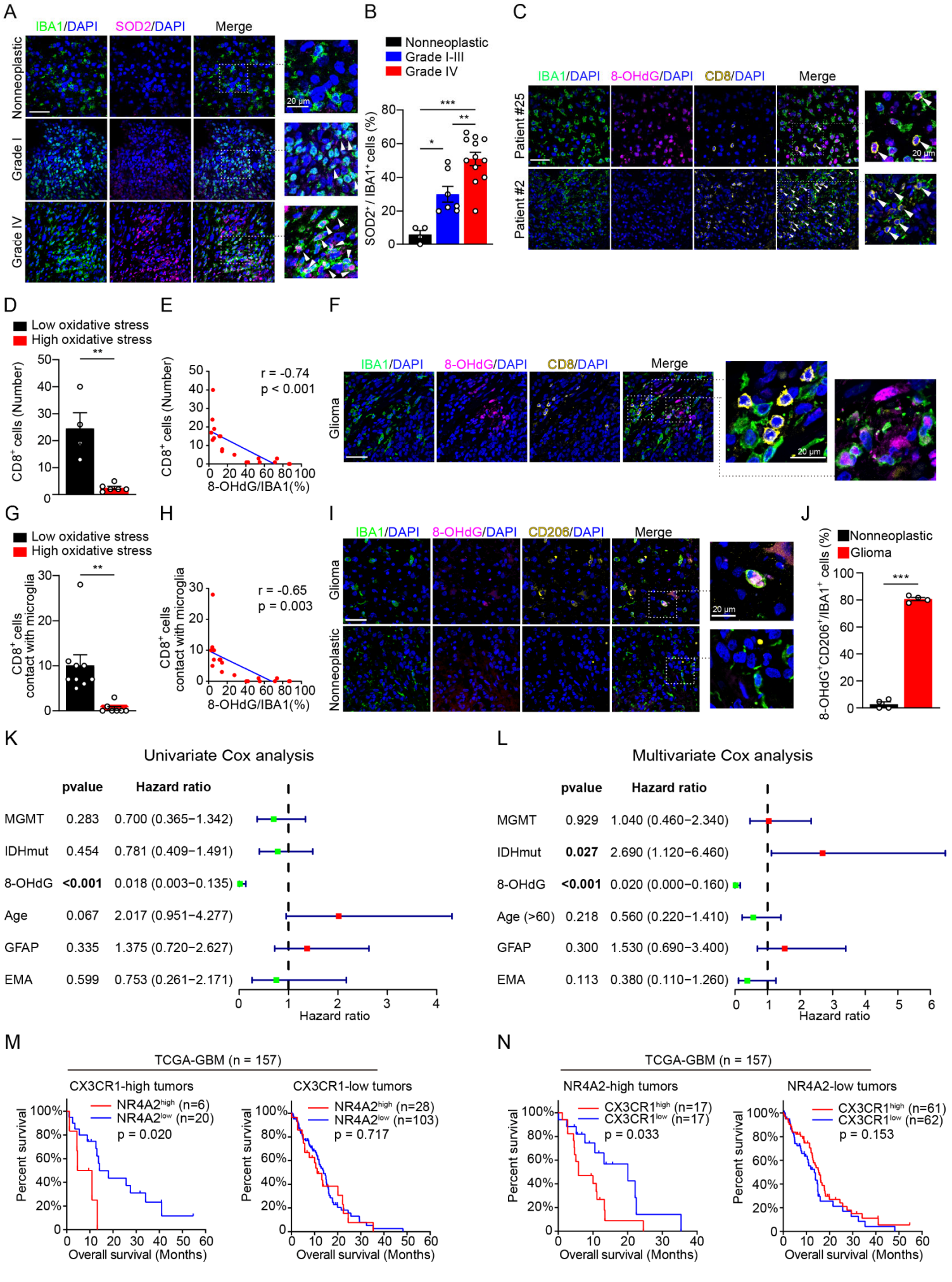
A
IBA1/DAPI
SOD2/DAPI
Merge
Nonneoplastic
Grade I
Grade IV
20 μm
B
Nonneoplastic
Grade I-III
Grade IV
SOD2+ / IBA1+ cells (%)
C
IBA1/DAPI
8-OHdG/DAPI
CD8/DAPI
Merge
Patient #25
Patient #2
D
Low oxidative stress
High oxidative stress
CD8+ cells (Number)
E
r = -0.74
p < 0.001
8-OHdG/IBA1(%)
F
Glioma
G
r = -0.65
p = 0.003
CD8+ cells contact with microglia
H
I
CD206/DAPI
J
8-OHdG+CD206+/IBA1+ cells (%)
K
Univariate Cox analysis
pvalue
Hazard ratio
MGMT 0.283 0.700 (0.365−1.342)
IDHmut 0.454 0.781 (0.409−1.491)
8-OHdG <0.001 0.018 (0.003−0.135)
Age 0.067 2.017 (0.951−4.277)
GFAP 0.335 1.375 (0.720−2.627)
EMA 0.599 0.753 (0.261−2.171)
Hazard ratio
L
Multivariate Cox analysis
MGMT 0.929 1.040 (0.460−2.340)
IDHmut 0.027 2.690 (1.120−6.460)
8-OHdG <0.001 0.020 (0.000−0.160)
Age (>60) 0.218 0.560 (0.220−1.410)
GFAP 0.300 1.530 (0.690−3.400)
EMA 0.113 0.380 (0.110−1.260)
M
TCGA-GBM (n = 157)
CX3CR1-high tumors
NR4A2high (n=6)
NR4A2low (n=20)
p = 0.020
CX3CR1-low tumors
NR4A2high (n=28)
NR4A2low (n=103)
p = 0.717
Percent survival
Overall survival (Months)
N
TCGA-GBM (n = 157)
NR4A2-high tumors
CX3CR1high (n=17)
CX3CR1low (n=17)
p = 0.033
NR4A2-low tumors
CX3CR1high (n=61)
CX3CR1low (n=62)
p = 0.153

**Supplementary Figure S7. Oxidative stress in microglia as an independent predictor for clinical outcome in glioma patients, related to Figure 7**

(A, B) Immunofluorescence staining for SOD2 and microglia maker (IBA1) in glioma tissues of different grades and nonneoplastic brain tissues. Scale bars, 50 μm (A). Quantification of $SOD2^+$ microglia levels in respective group (B).

(C) Immunofluorescence staining for microglia markers (IBA1), oxidative stress markers (8-OHdG) and $CD8^+$ T cells markers (CD8) in brain sections of glioma patients. Scale bars, 50 μm.

(D) Abundance of $CD8^+$ T cells in glioma patients with high (red) or low (black) oxidative stress in microglia.

(E) *Pearson* correlation scatter plots of $CD8^+$ T cells number and percentage of microglia with 8-$OHdG^+$ in each vision field of glioma patients brain sections (n=19 fields, glioma patients≥3).

(F) In the same field, immunofluorescence staining showed microglia with low oxidative stress was observed to contact with $CD8^+$ T cells more frequently in tumor beds. Scale bars, 50 μm.

(G) Abundance of $CD8^+$ T cells contacting with microglia in glioma patients with high or low oxidative stress in microglia.

(H) Correlation of number of $CD8^+$ T cells contacting with microglia and percentage of microglia with 8-$OHdG^+$ in each vision field of glioma patients brain sections (n=19 fields, glioma patients≥3).

(I, J) Immunofluorescence staining for IBA1, 8-OHdG and CD206 on tumor and nonneoplastic brain sections (I). Scale bars, 50 μm. Proportions of 8-$OHdG^+CD206^+$ cells in microglia were shown (J).

(K, L) Univariate and multivariate Cox regression analysis of six factors based on tissue microarray of 125 glioma patients. Statuses of age, GFAP (Glial fibrillary acidic protein) and EMA (epithelial membrane antigen) were provided by SHANGHAI OUTDO BIOTECH CO., LTD. According to immunofluorescence staining, oxidative stress marker (8-OHdG), IDHmut marker (IDH1-R132H) and MGMT expression were evaluated on glioma tissues microarray.

(M) Kaplan-Meier survival curve of GBM patients with NR4A2$^{high}$ VS NR4A2$^{low}$ in CX3CR1-high tumors, NR4A2$^{high}$ VS NR4A2$^{low}$ in CX3CR1-low tumors in TCGA-GBM database.

(N) Kaplan-Meier survival curve of GBM patients with CX3CR1$^{high}$ VS CX3CR1$^{low}$ in NR4A2-high tumors and CX3CR1$^{high}$ VS CX3CR1$^{low}$ in NR4A2-low tumors in TCGA-GBM database.

Data are shown as mean ± SEM. In (B), P value was calculated using one-way *ANOVA* analysis. In (D), (G) and (J), P value was calculated using the two-tailed Student's *t*-test. In (E) and (H), coefficient of determination (r) and statistical significance levels were determined by linear regression with linear model method. In (M) and (N), survival difference was calculated using log-rank test. *p < 0.05, **p < 0.01, ***p < 0.001.