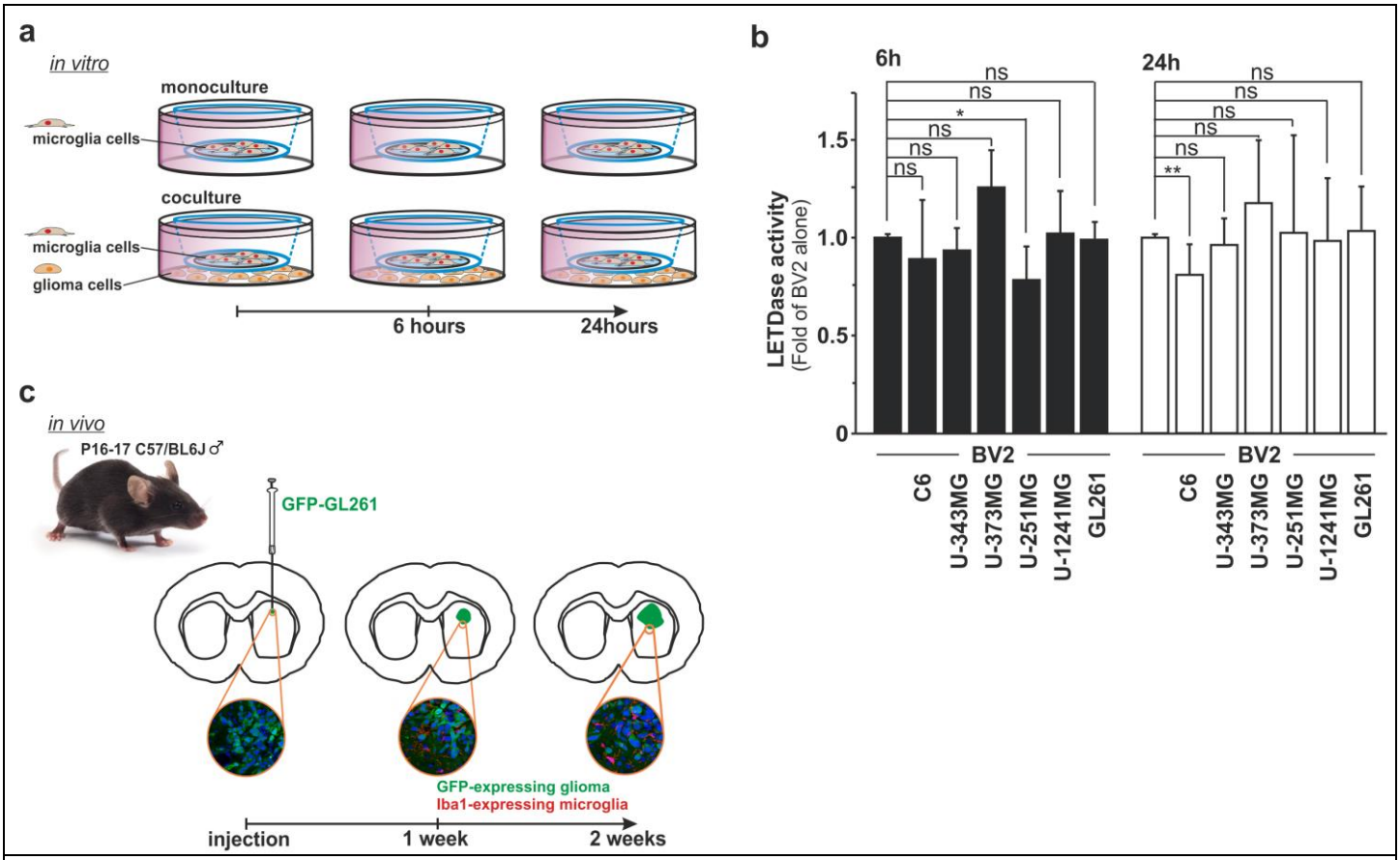
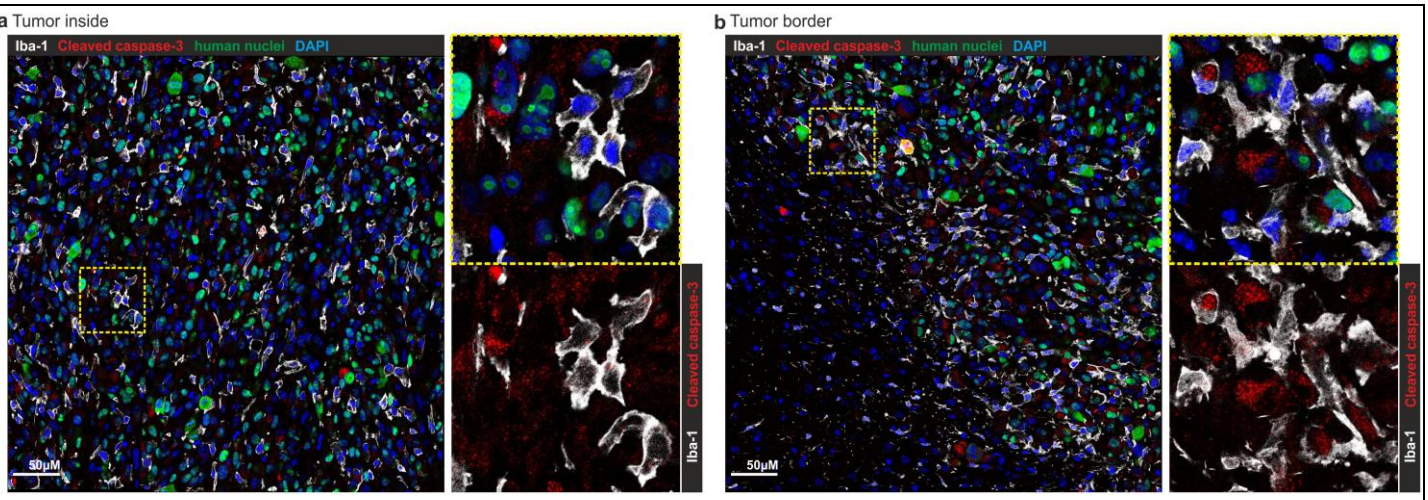


Supplementary Figure 1

In vitro and in vivo model systems used in these studies and in vitro caspase-8 activity measurement

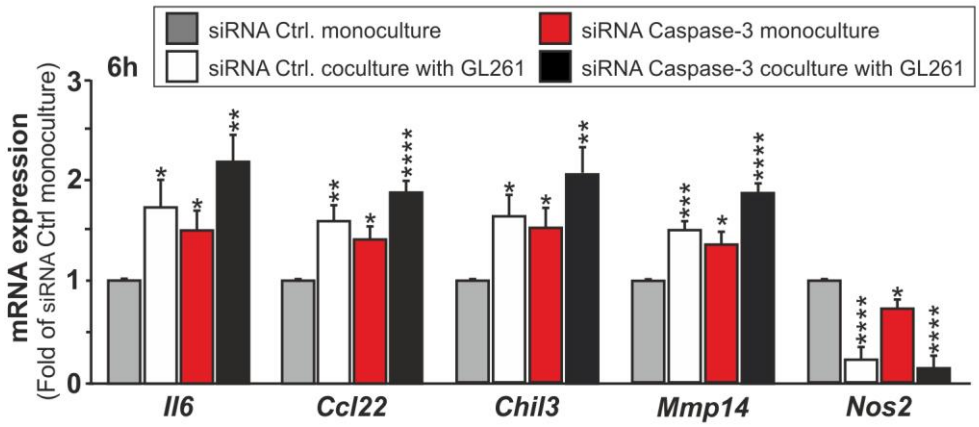
(**a**) Diagram illustrating a transwell segregated microglia-glioma coculture setup to study glioma's influence over microglia biology. (**b**) Measurement of LETDase (caspase-8) activity in BV2 microglia cells cultured in medium alone or segregated coculture with a panel of glioma cells at 6h (black bars) and 24h (white bars) after coculture. Results are represented relative to those of BV2 microglia cultured in medium alone, set as 1. (**c**) Illustration of how postnatal day 16-17 male C57/BL6/J mice were injected intrastriatally with syngeneic GFP-expressing GL261 glioma cells. One or two weeks after glioma injection, mice were sacrificed and brain tissue processed for analysis. Statistics and error bars: mean ± s.d. n=6 (b) of biological replicates. Data was analyzed using two-tailed Student's *t*-test. ns, not significant*; *P< 0.05* ; ***P< 0.01*.



Supplementary Figure 2

Microglial active caspase-3 expression in intracranial human glioblastoma xenografts

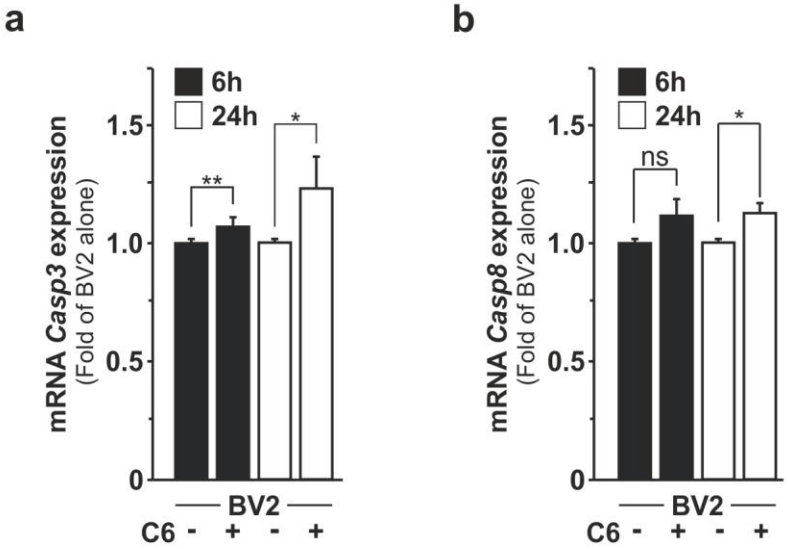
(**a, b**) Representative pictures of the immunohistochemical analysis of cleaved caspase-3 (red) in Iba-1 positive cells (microglia; white) inside (**a**) and in the border (**b**) of tumors formed 1 week after transplantation of Human U87-MG glioblastoma cells (green) injected into NOD.CB17-PrkcSCID/J mice brains (n=6/group).



Supplementary Figure 3

Microglial caspase-3 silencing effect over *Il6*, *Ccl22*, *Chil3, Mmp14* and *Nos2* expression in mono- or coculture microglia.

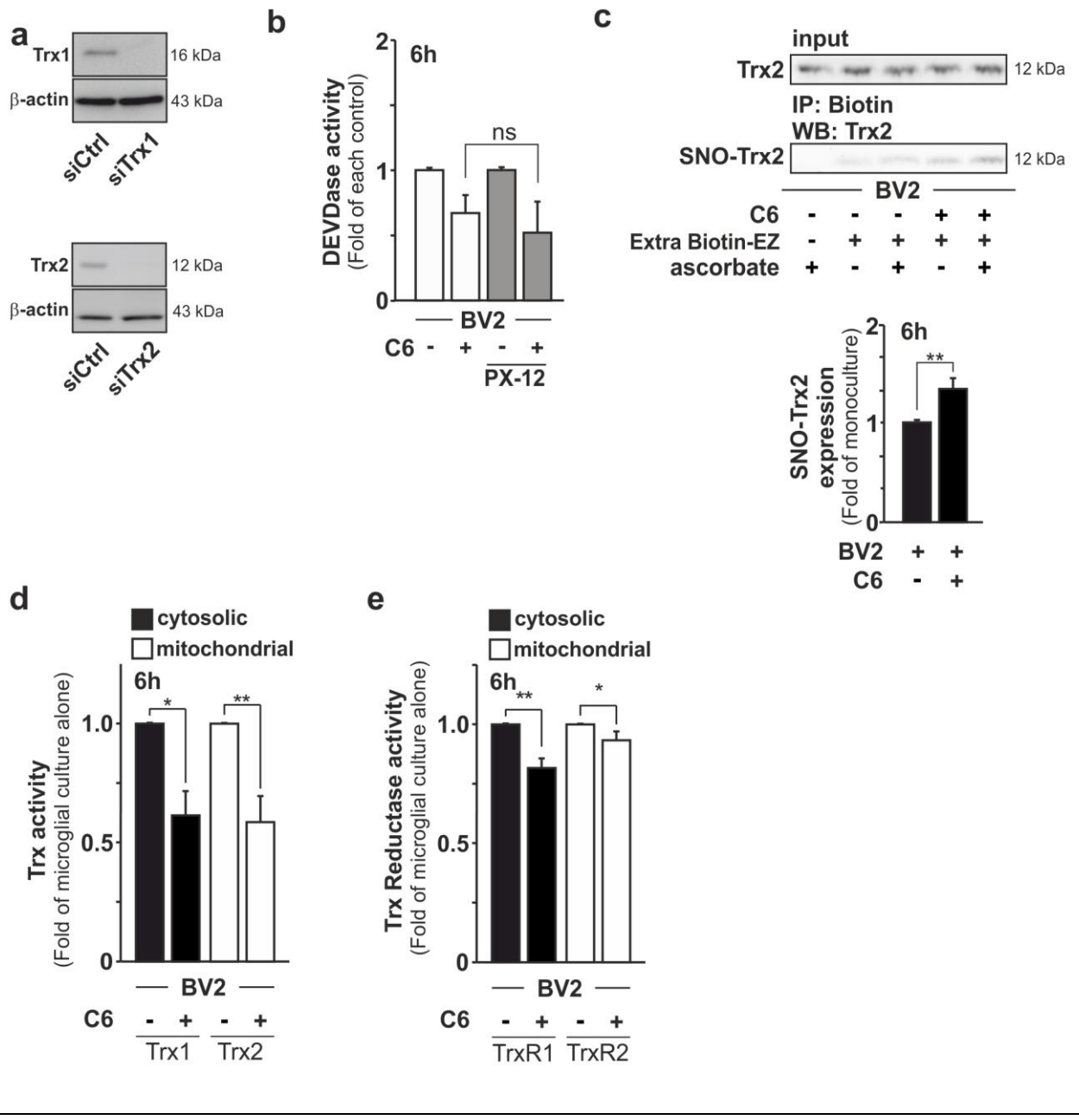
Analysis of the expression of different genes in microglia cells transfected with the indicated siRNA cultured in medium alone or segregated coculture for 6 hours with GL261 glioma cells. Results are represented relative to those of Ctrl siRNA transfected BV2 cells cultured alone, set as 1. Statistics and error bars: mean ± s.e.m. n=6 of biological replicates. Data were analyzed using two-tailed Student's *t*-test. **P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001.*



Supplementary Figure 4

Analysis of *Casp3* and *Casp8* expression in BV2 microglia cells after segregated coculture with C6 cells

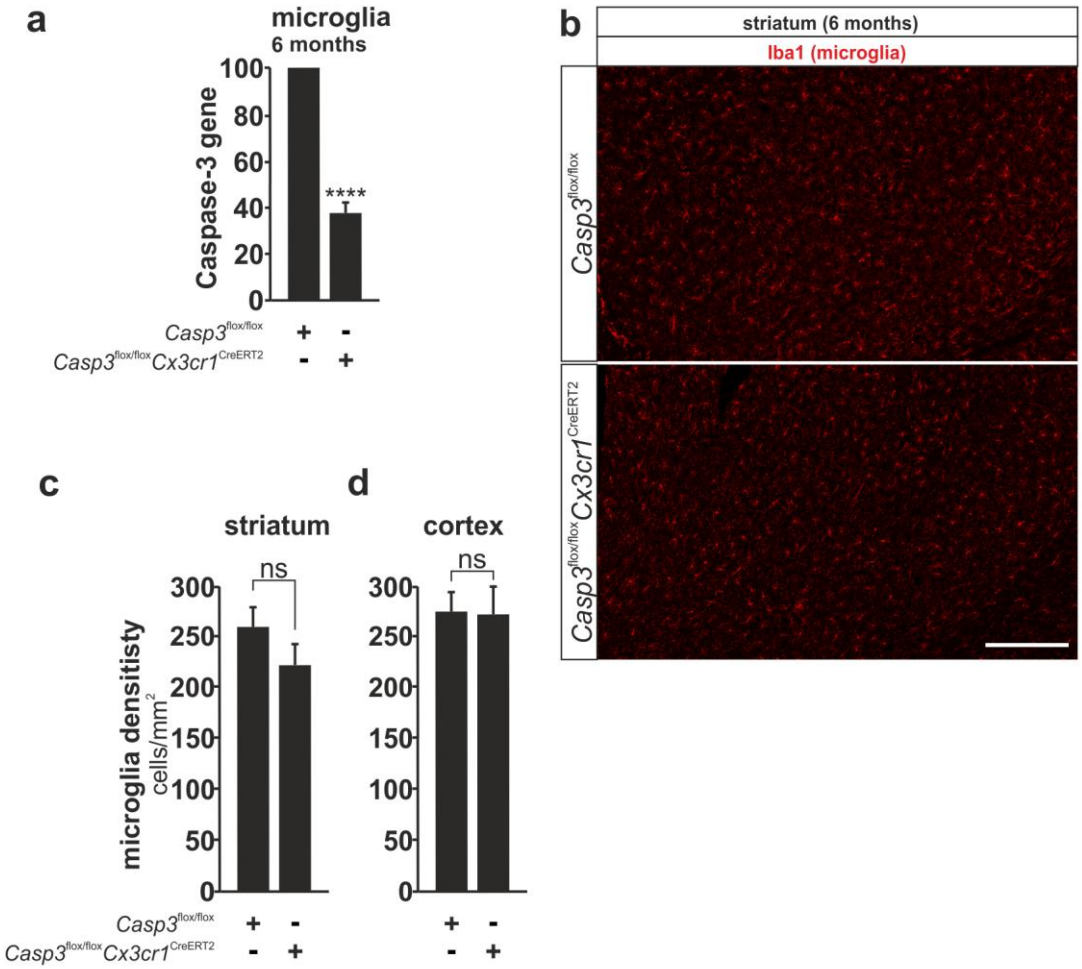
(**a,b**) Microglial mRNA expression level for *Casp3* (**a**) and *Casp8* (**b**) upon 6 (black bars) and 24h (white bars) segregated coculture with C6 glioma cells. Results are represented relative to those of BV2 microglia cultured in medium alone, set as 1. Statistics and error bars: mean ± s.d. *n=3* of biological replicates. Data was analyzed using two-tailed Student's *t*-test. *ns,* not significant ;**P< 0.05.*



Supplementary Figure 5

Role of thioredoxins in S-nitrosylation of caspase 3 after 6h of segregated coculture with C6 cells.

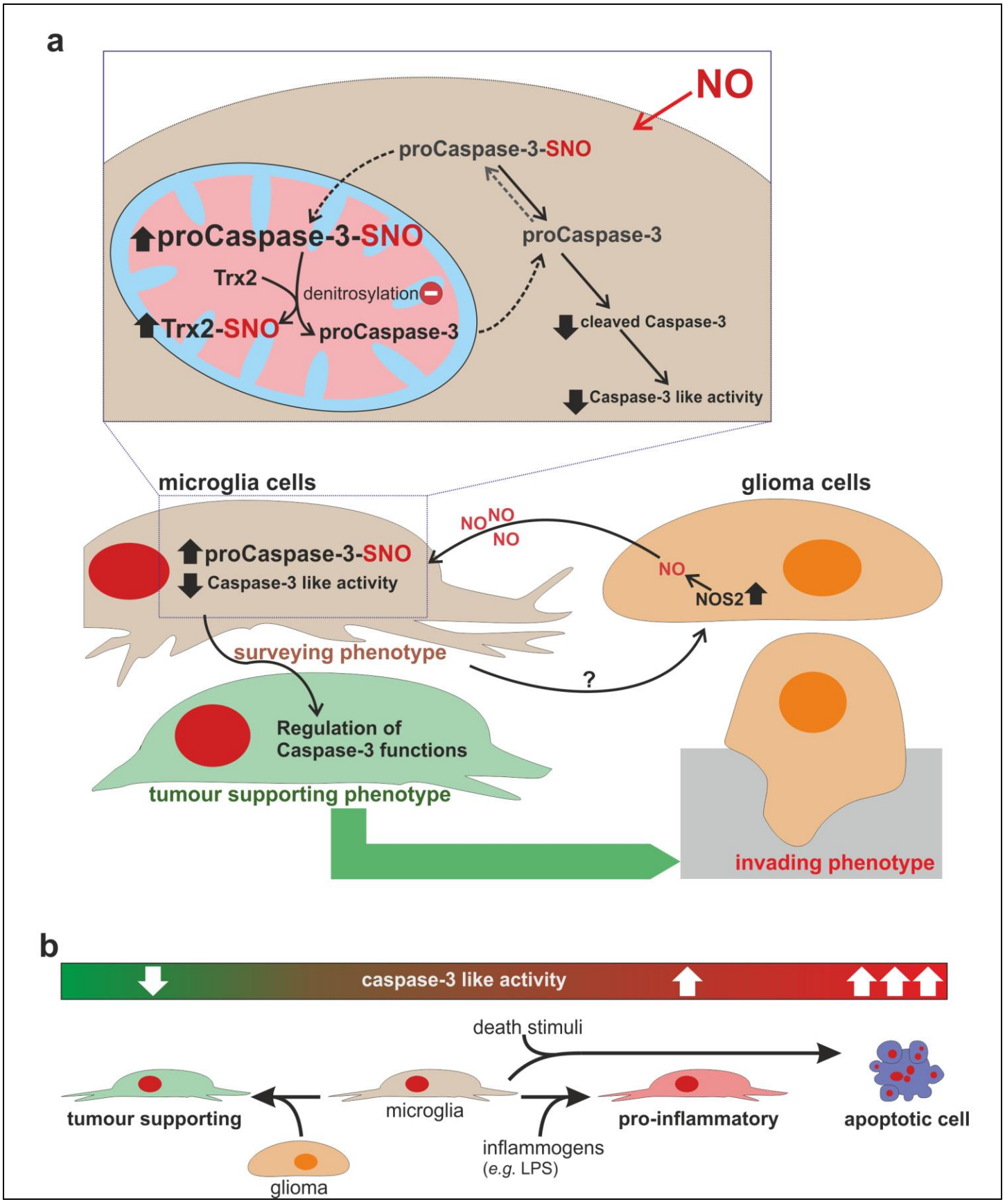
(**a**) Immunoblots showing knockdowns of Trx1 (top) and Trx2 (bottom) expression in BV2 microglia cells using different siRNA pools. (**b**) Effect of PX-12 (9μM), a specific Trx1 inhibitor, over microglial DEVDase activity upon glioma segregated coculture.(**c**) Immunoblots representing the levels of total Trx2 (top) and SNO-Trx2 (bottom) in BV2 cells upon segregated coculture with C6 cells using the biotin-switch method (results represented at the bottom of the panel). (**d,e**) Analysis of Trx1 and Trx2 (**d**) and Trx Reductase 1 and Trx Reductase 2 (**e**) activities in cytosolic (black bars) and mitochondrial (white bars) fractions in BV2 microglia cells after segregated coculture with C6 cells during 6h. Results in each of the panels are represented relative to those of BV2 cells cultured alone, set as 1. Statistics and error bars: mean ± s.d. except d, e which shows mean ± s.e.m. n=4 (b,d,e) and n= 3 (c) of biological replicates. Data was analyzed using two-tailed Student's *t*-test. ns, not significant;.*$P< 0.05$ and **$P< 0.01$.



Supplementary Figure 6

Analysis of microglia cell density 6 months after tamoxifen treatment in *Casp3*$^{flox/flox}$*Cx3cr1*^{CreERT2} mice

(**a**)Analysis of microglia caspase-3 expression 6 months after tamoxifen treatment in *Casp3*$^{flox/flox}$*Cre*$^{Cx3Cr1+/-}$ mice to induce the specific deletion of *Casp3* gene in microglia cells. *Casp3*$^{flox/flox}$Cre$^{Cx3Cr1-/-}$ mice were used as control. Tamoxifen was administered in 7 day-old-mice following standard procedures. The purity of the microglia preparation was evaluated by flow cytometry analysis using CD11b and CD45 antibodies. Genomic DNA was isolated and deletion of the *Casp3* floxed sequence was evaluated by qPCR analysis following an ABC primer strategy. Microglia from *Casp3*$^{flox/flox}$*Cre*$^{Cx3Cr1+/-}$ mice had 63 % *Casp3* gene deletion 6 months after inducing the deletion. Results are represented relative to *Casp3*$^{flox/flox}$Cre$^{Cx3Cr1-/-}$ mice, set as 100. (**b**) This panel shows a representative illustration of Iba1-labeled microglia in the striatum of *Casp3*$^{flox/flox}$*Cre*$^{Cx3Cr1+/-}$ and *Casp3*$^{flox/flox}$*Cre*$^{Cx3Cr1-/-}$ mice 6 months after tamoxifen treatment. (**c,d**) Quantification of the effect of *Casp3* gene deletion on microglia cell population in striatum and cortex. Statistics and error bars: mean ± s.d. n=3. Data were analyzed using two-tailed Student's *t*-test. ns, not significant; ****$P< 0.0001$. Scale bar= 200 μm.


a
NO
proCaspase-3-SNO
proCaspase-3-SNO
proCaspase-3
Trx2
denitrosylation
Trx2-SNO
proCaspase-3
cleaved Caspase-3
Caspase-3 like activity
microglia cells
glioma cells
NO
NO
NO
proCaspase-3-SNO
Caspase-3 like activity
NO
NOS2
surveying phenotype
?
Regulation of
Caspase-3 functions
tumour supporting phenotype
invading phenotype
b
caspase-3 like activity
death stimuli
tumour supporting
microglia
pro-inflammatory
apoptotic cell
inflammogens
(e.g. LPS)
glioma

Supplementary Figure 7
Proposed pathway
(**a**)Schematic representation of the suggested pathway of how glioma cells induce microglial caspase-3 S-nitrosylation, decreasing caspase-3 proteolytic activity and promoting their conversion towards a tumor-supporting activation state. (**b**) Illustration of how caspase-3 like (DEVDase) activity based on its degree of activation will regulate different microglial activation states (tumor-supporting versus pro-inflammatory phenotypes), or in some circumstances with very high activity levels, even cell death.