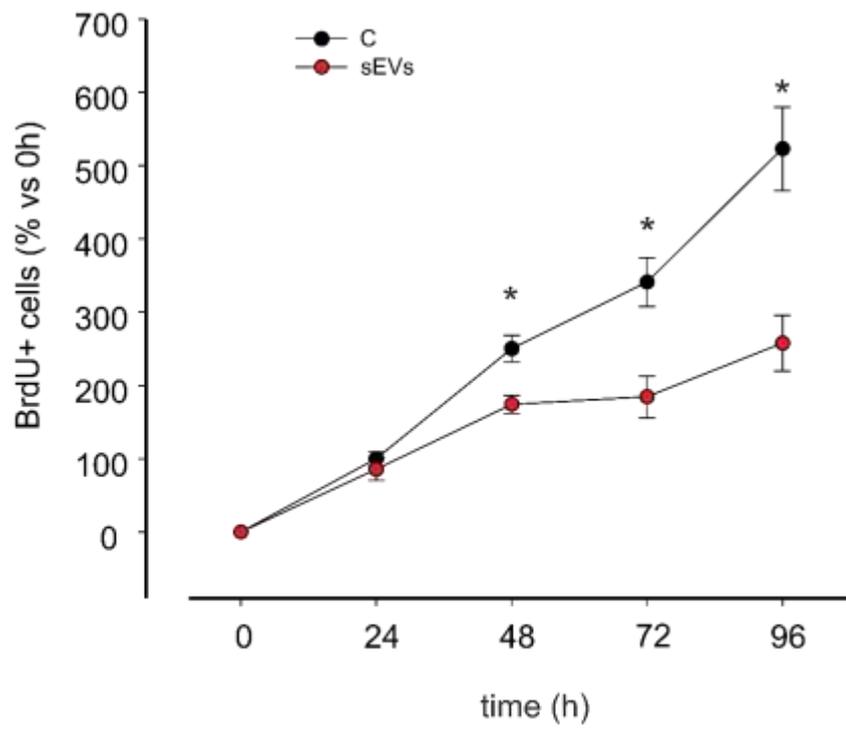
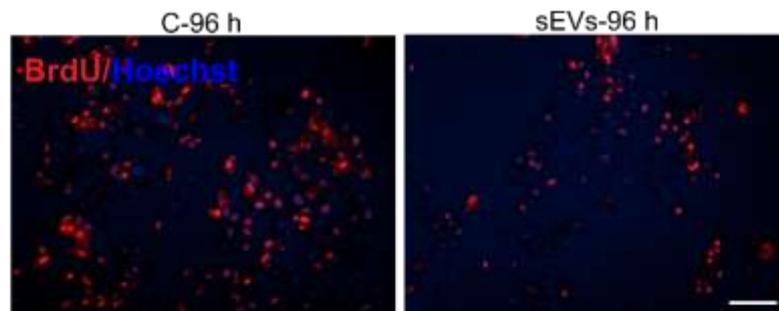


Figure S1: Expression analysis of pro-inflammatory/anti-tumoral-related genes in microglia-derived sEVs and anti-inflammatory/pro-tumoral genes in sEVs-treated microglia. (A-B) qPCR of *Il1-b* and *Tnfα* genes expressed in sEVs derived from untreated (C) or INFγ/LPS- or IL4-treated primary microglial cells. Data are the mean ± SE of fold increase vs C, normalized on *Gapdh* (used as housekeeping gene), N=3, * $p < 0.05$ vs C, Student's *t*-test. (C) Pro-tumoral anti-inflammatory related genes (*Arg1*, *Cd163*, *Cd206*, *Ym1* and *Fizz1*) in CD11b⁺ cells extracted from brains of GL261-bearing mice treated with vehicle or BV2-derived sEVs. Data are the mean ± SE of fold increase respect to contralateral cerebral hemispheres of each animal (C) and all genes expression was normalized vs *Gapdh*, N = 5, * $p < 0.05$ vs C, # $p < 0.05$ vs vehicle treated mice, Student's *t*-test. (D) Pro-tumoral anti-inflammatory related genes (*Cd163*, *Cd206*, and *Fizz1*) in primary microglial cells exposed to unconditioned (C) or GL261 conditioned medium (gcm), upon BV2-derived sEVs treatment. Data are the mean ± SE of fold increase respect to (C) and all genes expression was normalized vs *Gapdh*, N = 3, * $p < 0.05$ vs C, Student's *t*-test. (E) Pro-tumoral anti-inflammatory related genes (*Arg1*, *Cd163*, *Cd206*, *Ym1* and *Fizz1*) in primary microglial cells untreated (C), exposed to IL4 or IL4 plus BV2-derived sEVs. Data are the mean ± SE of fold increase respect to (C) and all genes expression was normalized vs *Gapdh*, N = 3, * $p < 0.05$ vs C, Student's *t*-test.

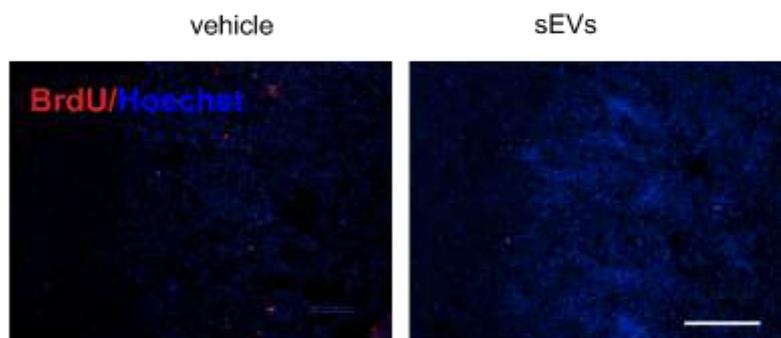
A



B



C



D

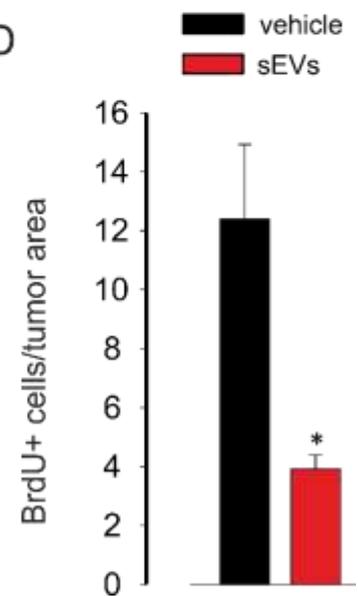


Figure S2: Microglia-derived sEV effects on glioma cell proliferation. (A) *In vitro* cell proliferation measured as immunofluorescence analysis of BrdU⁺ GL261 untreated (C) or treated with BV2-derived sEVs for 0, 24, 48, 72 and 96 h. Data are reported as % ± SE, N = 3, * $p < 0.05$ vs time 0 (0 h), Student's *t*-test. **(B)** Representative immunofluorescence of time point 96 h of (A), BrdU in red, Hoechst in blue, scale bars, 100 μm. **(C)** *In vivo* glioma cell proliferation measured as immunofluorescence analysis of BrdU⁺ cells in the tumor core of coronal brain sections of GL261-bearing mice vehicle treated (on the left) or treated with BV2-derived sEVs (on the right); BrdU in red, Hoechst in blue, scale bars, 20 μm. **(D)** Quantification of (C), data are expressed as the signal coverage area of BrdU normalized on tumor area (BrdU⁺/tumor area) ± SE, N = 3, * $p < 0.05$ vs vehicle, Student's *t*-test.

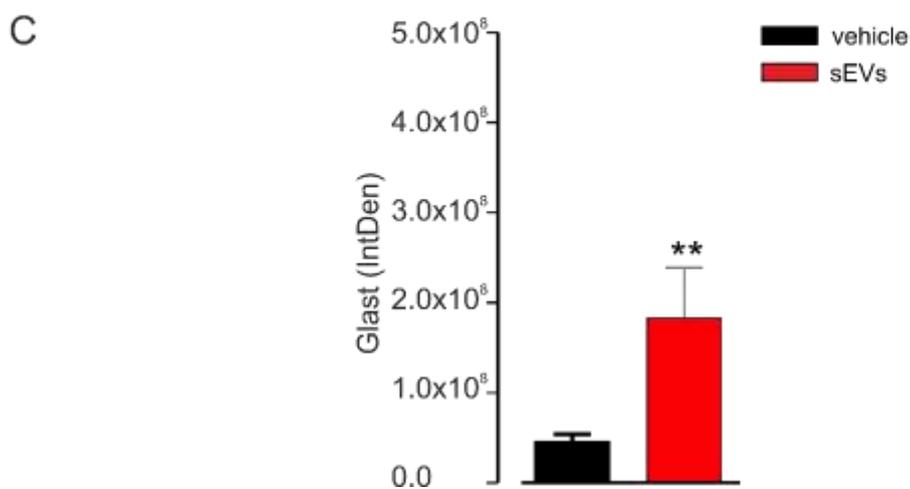
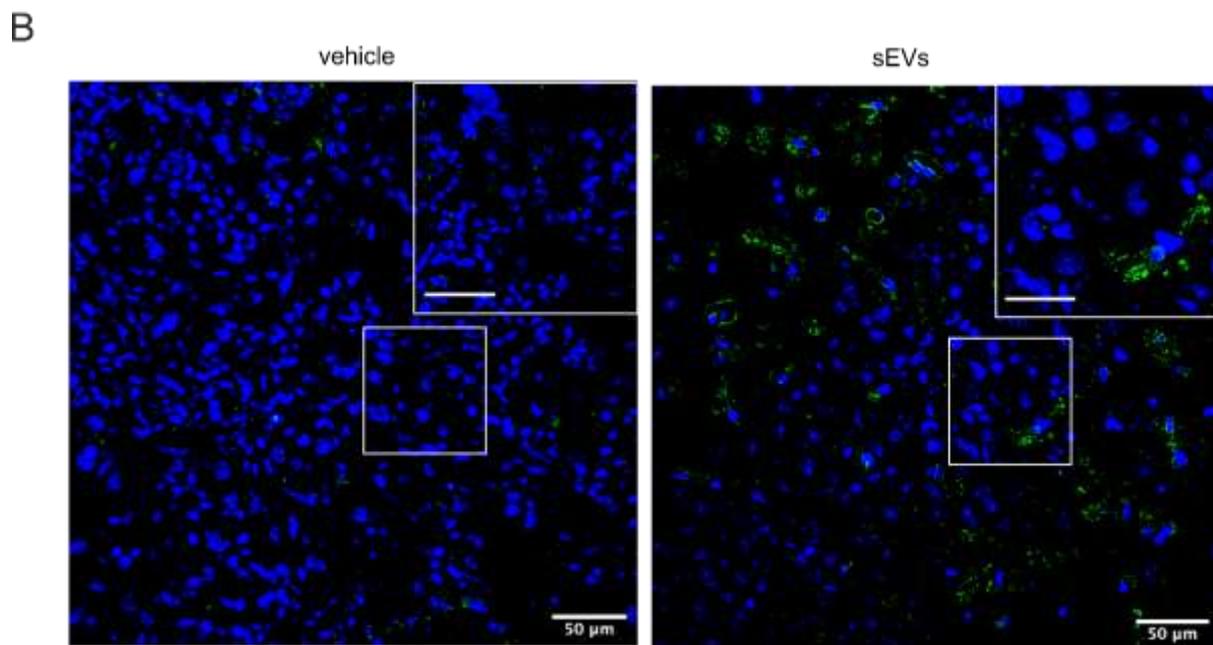
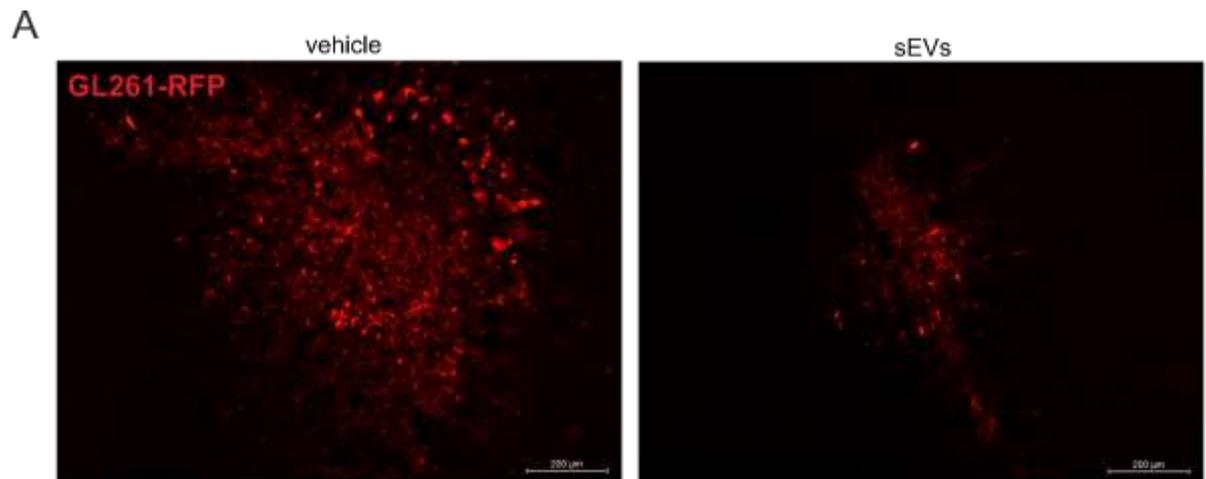


Figure S3: (A) Tumor visualization of GL261-RFP-bearing mice. Representative tumor area (GL261 in red) of coronal brain sections of GL261-bearing mice vehicle-treated (on the left) or treated with BV2-derived sEVs (on the right), scale bar, 200 μ m. **B) Glast expression in tumor area of GL261-bearing mice.** Representative immunofluorescence analysis for Glast (in green, Hoechst in blue) in the tumor core of coronal brain sections of GL261-bearing mice vehicle-treated (on the left) or treated with BV2-derived sEVs (on the right), scale bars, 50 μ m. In the inset an enlargement is shown, scale

bars, 25 μ m. (C) Quantification of (B), data are expressed as IntDen mean \pm SE, N = 3, ** $p \leq 0.001$ vs vehicle, Student's t -test.

Table S1: Expression analysis of inflammation related genes on sEVs derived from microglia. mRNAs isolated from sEVs released by untreated microglia (control) or microglia treated with LPS/IFN γ or microglia treated with IL4 were analysed by NanoString., N = 4, data are expressed as fold increase respect to *gapdh* used as housekeeping gene, * $p < 0.05$ vs control, Mann-Whitney Rank Sum test.

Gene	Control	LPS/IFN γ	IL4
<i>Ccl2</i>	5,99 \pm 2,5	128,08 \pm 26,9 *	7,8 \pm 2,5
<i>Ccl3</i>	29,93 \pm 16,34	441,21 \pm 72,5	10,74 \pm 3,6
<i>Ccl5</i>	66,12 \pm 36,3	5938,56 \pm 1612,4*	50,09 \pm 10,8
<i>Ccl7</i>	1,31 \pm 0,2	108,50 \pm 10,2*	6,33 \pm 1,9
<i>Cebpb</i>	11,19 \pm 5,5	118,94 \pm 15,4*	15,14 \pm 5,2
<i>Cxcl9</i>	1,31 \pm 0,2	476,99 \pm 186,5*	1,06 \pm 0,2
<i>Hif1a</i>	22,29 \pm 11,9	214,48 \pm 18,2*	21,02 \pm 7,4
<i>Il1b</i>	12,6 \pm 6,32	703,55 \pm 239*	1,06 \pm 0,2*
<i>Ptgs2</i>	1,31 \pm 0,2	779,24 \pm 354,34*	1,06 \pm 0,1
<i>Rapgef2</i>	15,35 \pm 7,9	72,50 \pm 10,1*	5,6 \pm 1,2*
<i>Tnfa</i>	12,69 \pm 5,5	262,12 \pm 56,19*	4,86 \pm 1,0*