Supplemental Information

let-7 MicroRNAs Regulate Microglial

Function and Suppress Glioma

Growth through Toll-Like Receptor 7

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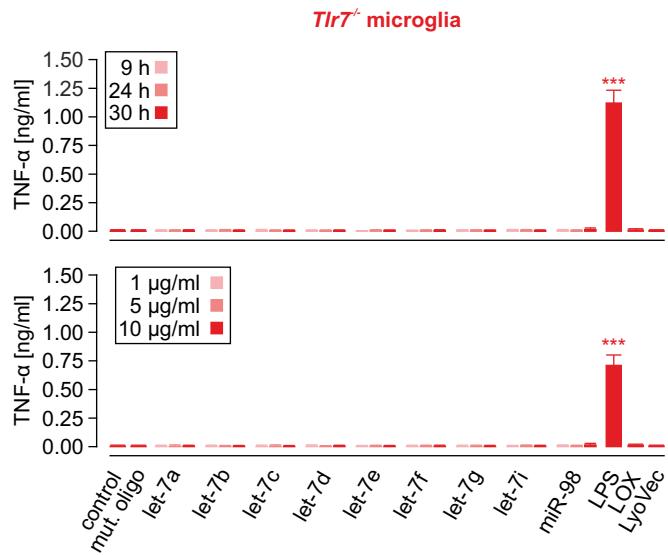
Figure S1. TLR7-deficient microglia do not release TNF-α in response to extracellularly delivered *let-7* miRNAs, Related to Figure 1.

Primary neonatal microglia from *Tlr7*^{-/-} mice were stimulated with 5 µg/*ml let-7a-let-7i* and *miR-98* oligoribonucleotides for 9, 24 or 30 h (top) or with 1, 5, or 10 µg/ml *let-7* miRNA for 24 h (bottom). TNF-α release was determined by ELISA. LPS (100 ng/ml) and loxoribine (LOX; 1 mM) and mutant oligoribonucleotide (5 µg/ml) and LyoVec were used as positive and negative controls, respectively. $n = 5$. Data are represented as mean ± SEM. Kruskal-Wallis followed by Dunn's multiple comparison post hoc test. ****P < 0.001 vs.* control.
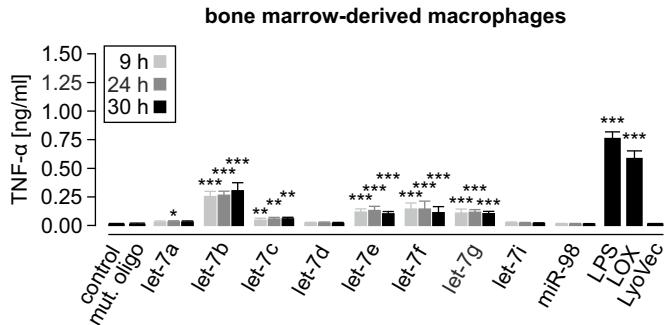
Figure S2. Bone marrow-derived macrophages release TNF-α after incubation with *let-7* miRNAs for 9 h, 24 h and 30 h, Related to Figure 2.

Primary cultured bone marrow-derived macrophages from WT mice were stimulated with 5 µg/ml *let-7a-let-7i* or *miR-98* oligoribonucleotides for 9, 24, or 30 h. LPS (100 ng/ml) and LOX (1 mM) and mutant oligoribonucleotide (mut. oligo; 5 µg/ml) and LyoVec were used as positive and negative controls, respectively. $n = 4$. Data are represented as mean ± SEM. Kruskal-Wallis test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs.* control.
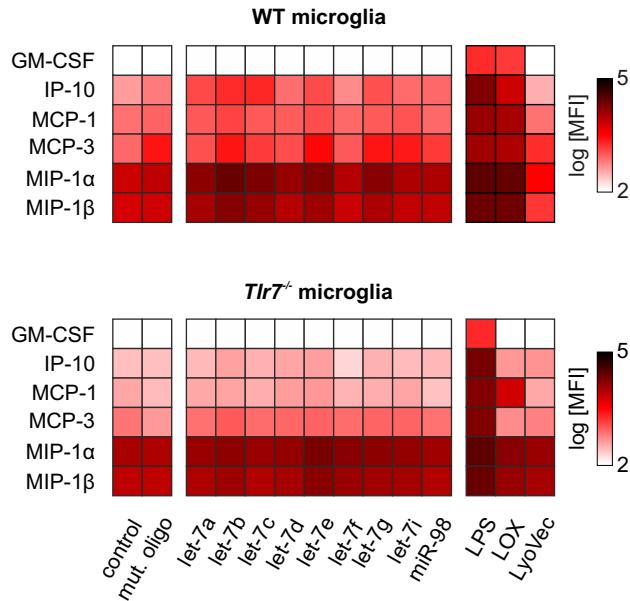
Figure S3. Microglia incubated with extracellularly delivered *let-7* miRNAs secrete a distinct pattern of inflammatory molecules, Related to Figure 3A.

Multiplex immunoassay showing the inflammatory response in WT (top) and *Tlr7⁻ᐟ⁻* (bottom) microglia after incubation with various *let-7* oligoribonucleotides, as indicated, using the supernatant collected for TNF-α analysis as described in Figure 4. Data are shown in a heatmap representing cytokine release expressed in logarithmic of mean fluorescence intensity (MFI). LPS (100 ng/ml), LOX (1 mM) and mutant oligoribonucleotide (mut. oligo; 5 µg/ml) as well as LyoVec were used as positive and negative controls, respectively. *n* = 3. For *P*-values yielded by Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test please refer to Table S3.

A

Single cell gating

CD45⁺/CD11b^high

MHC I⁺

B

let-7b **let-7d** **let-7e**

MHC I

CD54

MHC II

CD11b CD11b CD11b

- - - - unstained
——— WT control
——— WT incubated with let-7 oligo
——— Tlr7⁻/⁻ incubated with let-7 oligo
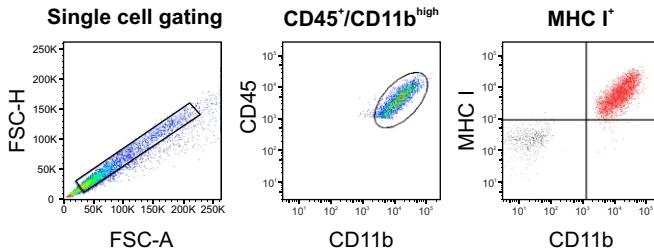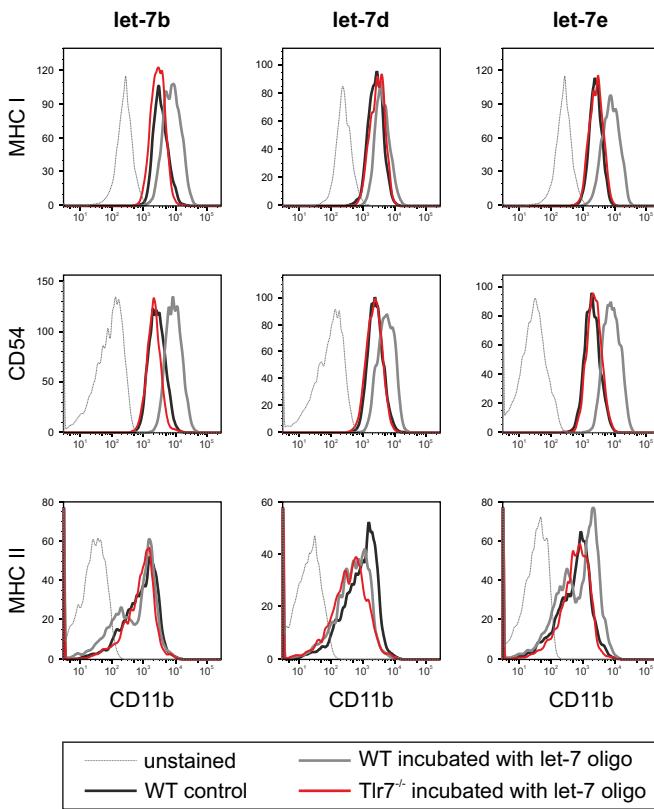
Figure S4. FACS gating strategy and representative histogram plots for MHC I, MHC II and CD54 expression in *let-7* miRNA-stimulated WT and TLR7-deficient microglia, Related to Figure 3B.

Primary neonatal microglia were prepared from WT and *Tlr7*^{-/-} mice and stimulated *with let-7b, let-7d* or *let-7e* to investigate the expression profile of MHC I, MHC II, and CD54. (**A**) Representative flow cytometry gating strategy for identification of CD45^{+}/CD11b^{high} microglia cell population and MHCI^{+} cell subsets. Forward scatter height (FSC-H) *vs.* forward scatter area (FSC-A) density plots were used to determine single cell populations and to exclude inaccurate cell clumps from the analysis. CD45-Pacific Blue and CD11b-PE Cy7 markers were applied to specify microglia cell population, and a subset of microglia cells such as the MHC I^{+} population is shown as an example. (**B**) After gating the CD45^{+}/CD11b^{high} microglia population, mean fluorescent intensity (MFI) of MHC I, MHC II, and CD54 was measured. Representative FACS histograms displaying MHC I, MHC II and CD54 expression in WT microglia under control conditions (black) as well as WT (dark grey) and *Tlr7*^{-/-} (red) microglia stimulated with *let-7b, let-7d* or *let-7e vs.* unstained cells (light grey) are shown.
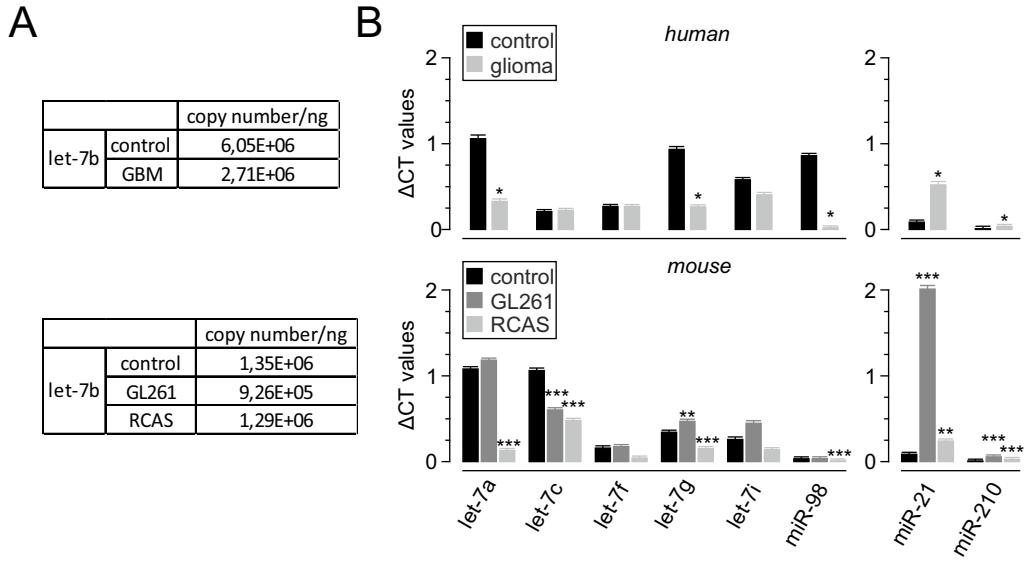
Figure S5. Expression of *let-7a, let-7c, let-7f, let-7g, let-7i* and *miR-98* in human and murine glioma, Related to Figure 6.

(**A**) Human glioblastoma tissue (GBM) and control tissue (top) as well as murine glioma tissue and healthy brain tissue (bottom) were assayed by Taqman PCR using primers specific for *let-7b,* and copy numbers per ng RNA were assessed by normalization to the standard of synthetic *let-7b* oligoribonucleotide. For expression analysis of human glioblastoma, tissue from patients with epilepsy served as control, while for expression analysis of the murine glioma models GL261 and RCAS-hPDGFb healthy murine WT brain tissue served as control. (**B**) Relative expression of *let-7a, let-7c, let-7f, let-7g, let-7i* and *miR-98*, as well as *miR-21* and *miR-210*, in human (top) and murine (bottom) glioma and respective control brain tissue was analyzed by Taqman PCR. *miR-16* was used as housekeeping control. $n = 5$ for human tissue samples and $n = 5-8$ for mouse tissue samples. Data are represented as mean ± SEM. Human data were analyzed by Mann Whitney *U* test. Mouse data were analyzed by One way ANOVA followed by Dunnett's multiple comparison post hoc test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs*. control.
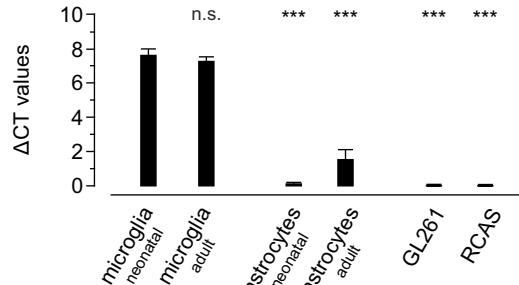
Figure S6. TLR7 is abundantly expressed in microglia, but not in GL261 or RCAS tumor cells, Related to Figure 7.

TLR7 was quantified by quantitative PCR in microglia and astrocytes freshly isolated from P14- and 12-week-old animals as well as in GL261 and RCAS glioma cells. TATA-binding protein (TBP) was used as a housekeeping gene. $n = 4$. Data are represented as mean ± SEM. One way ANOVA followed by Dunnett's multiple comparison post hoc test. *$***P < 0.001$ vs.* neonatal microglia.

Table S1, Related to Figure 1 and 5.

Sequences of *let-7* miRNA family members, control mutant oligoribonucleotide, and mutant oligoribonucleotides originated for *in silico* sequence prediction.

let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU
miR-98	UGAGGUAGUAAGUUGUAUUGUU
Mut. Oligo	UGAGGUAGAAGGAUAUAAGGAU
let-7d-mut-N	AGAGGUAGUAGGUUGUAUAGUU
let-7e-mut-N	UGAGGUAGGAGGAUGUAUAGUU
let-7e-mut-CS	AUGAGGAGGAGGUUGUAUAGUU

WT microglia

	mut. oligo	let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-98	LPS	LOX	LyoVec
TNF-α	ns (0,9867)	** (0,0029)	*** (<0,0001)	*** (0,0005)	ns (>0,9999)	*** (<0,0001)	** (0,0016)	** (0,001)	ns (>0,9999)	ns (>0,9999)	*** (<0,0001)	*** (<0,0001)	ns (>0,9999)
IL-6	ns (>0,9999)	ns (0,0778)	*** (<0,0001)	* (0,0421)	ns (>0,9999)	*** (<0,0001)	*** (<0,0001)	ns (0,0928)	ns (>0,9999)	ns (>0,9999)	*** (<0,0001)	*** (0,0005)	ns (>0,9999)
IL-10	ns (>0,9999)	ns (0,0882)	*** (<0,0001)	ns (0,1047)	ns (0,1248)	*** (<0,0001)	*** (<0,0001)	* (0,0335)	ns (>0,9999)	ns (>0,9999)	*** (<0,0001)	*** (0,0009)	ns (>0,9999)
IL-1β	ns (>0,9999)	ns (0,1286)	*** (0,0007)	ns (0,4764)	ns (>0,9999)	** (0,002)	** (0,002)	ns (0,2414)	ns (>0,9999)	ns (>0,9999)	**** (<0,0001)	*** (<0,0001)	ns (0,8846)
GRO-α	ns (>0,9999)	ns (0,4335)	*** (<0,0001)	ns (0,0894)	ns (>0,9999)	*** (0,0006)	** (0,0012)	ns (0,6062)	ns (>0,9999)	ns (>0,9999)	*** (0,0002)	*** (<0,0001)	ns (>0,9999)
MIP-2	ns (>0,9999)	ns (0,0989)	*** (<0,0001)	* (0,0286)	ns (0,4558)	*** (0,0008)	*** (<0,0001)	ns (0,4834)	ns (>0,9999)	ns (>0,9999)	*** (<0,0001)	*** (<0,0001)	ns (>0,9999)
RANTES	ns (0,6337)	ns (0,333)	*** (0,0001)	ns (0,4114)	ns (0,1709)	** (0,0016)	*** (<0,0001)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	*** (<0,0001)	*** (0,0008)	ns (>0,9999)

Tlr7 $^{-/-}$ microglia

	mut. oligo	let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-98	LPS	LOX	LyoVec
TNF-α	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	*** (0,0009)	ns (0,4386)	ns (>0,9999)
IL-6	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	** (0,0022)	ns (>0,9999)	ns (>0,9999)
IL-10	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	** (0,0027)	ns (>0,9999)	ns (>0,9999)
IL-1β	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	** (0,0068)	ns (>0,9999)	ns (>0,9999)
GRO-α	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	** (0,001)	ns (>0,9999)	ns (>0,9999)
MIP-2	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	* (0,0188)	ns (>0,9999)	ns (>0,9999)
RANTES	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,051)	ns (>0,9999)	ns (>0,9999)

Table S3, Related to Figure 3A.

P-values yielded by Kruskal-Wallis followed by Dunn's multiple comparison post hoc test analyzing WT (top) and *Tlr7* $^{-/-}$ (bottom) microglia incubated with various *let-7* miRNAs and control agents as indicated, for GM-CSF, IP-10, MCP-1, MCP-3, MIP-1α and MIP-1β.

WT microglia

	mut. oligo	let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-98	LPS	LOX	LyoVec
GM-CSF	ns (>0,9999)	ns (>0,9999)	ns (0,7187)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,1884)	ns (>0,9999)	ns (>0,9999)	*** (0,0001)	*** (0,0001)	ns (>0,9999)
IP-10	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	* (0,0194)	ns (>0,9999)	ns (>0,9999)
MCP-1	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,0516)	ns (0,6028)	ns (>0,9999)
MCP-3	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,1173)	ns (>0,9999)	ns (>0,9999)
MIP-1α	ns (>0,9999)	ns (>0,9999)	ns (0,31)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	* (0,0146)	ns (0,0924)	ns (>0,9999)
MIP-1β	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,0665)	ns (0,1481)	ns (>0,9999)

Tlr7 $^{-/-}$ microglia

	mut. oligo	let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-98	LPS	LOX	LyoVec
GM-CSF	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	* (0,0348)	ns (0,7995)	ns (>0,9999)
IP-10	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,1055)	ns (>0,9999)
MCP-1	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,3164)	ns (>0,9999)
MCP-3	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,592)	ns (>0,9999)
MIP-1α	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,1566)	ns (>0,9999)
MIP-1β	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (>0,9999)	ns (0,1084)	ns (>0,9999)