

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. (A) The relative expression of miR-124 as detected by TaqMan quantitative PCR in the CD11b+ GBM-infiltrating microglia/macrophages (n=4) is either very low or undetectable compared to normal brain tissues (n=3) ($P < 0.01$). (B) Summary dot plot demonstrating miR-124 expression is induced in gCSCs (n=8) upon neural differentiation ($P < 0.01$). Black filled circles: gCSCs; black filled squares: differentiated gCSCs. (C) Interleukin-8 (IL-8) ($P < 0.05$), galectin-3 ($P < 0.01$), and MIC-1 ($P < 0.05$) produced by gCSCs were reduced upon transfection with miR-124 compared with their levels in the scramble control.

Supplementary Figure 2. Schema demonstrating how miR-124 exerts target effects on the STAT3 pathway in gCSC cells. STAT3 can be activated by a variety of ligands such as IL-6 or EGFR. miR-124 can not only down-modulate SHC1, STAT3, P-STAT3 in all tested gCSC cell lines but also, occasionally, pMAPK1/3, probably in a contextual fashion when the IL-6R ligand is not present and the gCSC is dependent on EGFR signaling.

Supplementary Figure 3. miR-21 expression is higher in gCSCs and glioblastoma tumors than in normal brain and is inhibited by miR-124 overexpression. (A) Relative expression levels of miR-21 were detected by TaqMan quantitative PCR. There were significant differences in the relative expression levels of miR-21 between normal brain tissues, gCSCs and GBM specimens. (B) T-cell proliferation was detected by flow cytometry analysis with CFSE staining after 3 days of treatment with medium alone, gCSC-scramble-transfected conditioned medium, or gCSC-miR-21-transfected conditioned medium. miR-21 up regulation caused further inhibition in T-cell proliferation compared with the gCSC-scramble-transfected conditioned medium. (C) Forced overexpression of miR-124 in the gCSCs resulted in the down modulation of miR-21 expression.

Supplementary Figure 4. Ex vivo splenocytes from miR-124 treated intracranial GL261 mice have markedly increased cytotoxicity at 48 hours after coculture with GL261 cells compared to splenocytes from scramble control oligonucleotide-treated GL261-bearing mice. The ratio of splenocytes to GL261 cells is 100:1. Representative dot plots of dual PI- and CFSE- labeled GL261 cells with splenocytes and the associated gating strategy are shown to demonstrate how the target cells (GL261) are identified and quantified.

Supplementary Figure 5. Up regulation of miR-124 level and down regulation of p-STAT3 in peripheral blood T-cells and glioma- infiltrating T-cells after intravenous administration of miR-124. (A) The miR-124 expression level is below the limit of detection in CD3+ T-cells isolated from the blood of non-tumor bearing C57/BL6J mice (n = 3) and GL261-bearing mice (n = 3) ($P > 0.05$). Levels are shown relative to U6 snRNA. (B) miR-124 is detected in both CD3+ T-cells isolated from blood ($P < 0.05$ relative to scramble control; n=3) and gliomas ($P < 0.05$ relative to scramble control; n=3) 18 hours after the *in vivo* administration of miR-124. (C) Coinciding with the presence of miR-124, p-STAT3 expression levels were decreased in the T-cells from the peripheral blood and gliomas in the miR-124 treated mice. Representative histograms were shown. The p-STAT3 expression levels relative to isotype control in the peripheral blood T-cells in the miR-124 treated mice ($1.4 \pm 0.2\%$) was down-modulated compared to scramble treated mice ($17.9 \pm 1.9\%$) ($P = 0.007$; n=3) and glioma-infiltrating T-cells (miR-124: $4.3 \pm 0.1\%$; scramble: $18.2 \pm 4.3\%$; $P = 0.007$), but not within the splenic T-cells (miR-124: $16.8 \pm 0.9\%$; scramble: $12.9 \pm 2.7\%$; $P = 0.07$).

Supplementary Figure 6. miR-124 modulates Th1, Th17, and inducible Treg differentiation. CD4+CD45RA+CD45RO-naïve T-cells were isolated from healthy donor PBMCs and stimulated with plate-bound anti-CD3 and soluble anti-CD28 under Th1, Th17, and inducible

Treg polarization conditions before miR-124 transfection. After 3 rounds of T-cell stimulation and polarization, cytokine production in the different Th populations was quantified by intracellular cytokine FACS. One representative set of data was shown, but similar results were obtained from two additional independent experiments.