

measure mIFN- β cDNA levels. mGAPDH normalized mIFN- β levels in the exo-AAV-GFAP- mIFN- β treated mice were compared to levels in mice treated with the control vector (exo-AAV-GFAP-null).

Supplementary Methods

m-IFN- β mRNA expression in tumor and brain punches. Brain tissues extracted from each mouse (C57BL/6) injected with exo-AAV9-GFAP-mIFN- β or exo-AAV9-GFAP-null (control), snap frozen and

stored at -80°C. Coronal sections were made to localize the tumor. Tumor biopsies from the tumor core, the margin of the tumor core (approximately 1mm depth from the outer margin of the tumor) or normal brain tissue outside the tumor were excised using microforceps and microscissors. The collected tissue was immediately processed using a tissue homogenizer and RNA was isolated using a “Quick-RNA Miniprep” kit (Zymo Research, Irvine CA), followed by reverse transcription and real-time PCR using QuantStudio 3 Real-Time PCR system (AppliedBiosystems). The following primer sequences for m-IFN- β were used: mIFNb Fwd, AGCTGAACGGCAAGATCAAT; mIFNb Rev, GCCTTCCTCCTGTTTTCC

Cells: Primary murine astrocytes and RAW 264.7 cells. Mixed primary glial culture was prepared from P1 neonatal mouse pups (C57BL/6 strain). The cells were seeded in T75 flasks coated with 10 μ g/ml Poly-D-lysine (Sigma, P7280), cultured for 7 days in high glucose Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Grand Island, NY) supplemented with 20% fetal bovine serum (Sigma, St Louis, MO), 100 U/ml penicillin, and 100 μ g/ml streptomycin (Life Technologies) in a humidified atmosphere supplemented with 5% CO₂ at 37°C. Enriched astrocyte culture was obtained by shaking the cells at 240 r.p.m. (LabGenius, Digital Orbital Shaker) at 37°C for overnight to remove microglial cells and oligodendrocytes. RAW 274.7 macrophage cells were purchased from ATCC and cultured in high glucose Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Sigma, St Louis, MO), 100 U/ml penicillin, and 100 μ g/ml streptomycin (Life Technologies) in a humidified atmosphere supplemented with 5% CO₂ at 37°C.

murine IFN- β ELISA. Raw 264.7 murine macrophage line was transduced with exo-AAV9-5NF-mIFN- β or exo-AAV9-5NF-GFP (control) using 10⁷ gc/cell in a 96 well plate. Three day’s post-transduction media was harvested from cells. Primary murine astrocytes were activated by treated for 24 h with cytokines IL-1 α (3 ng/ml, Sigma, I3901), TNF (30 ng/ml, Cell Signaling Technology, 8902SF) and C1q (400 ng/ml, MyBioSource, MBS143105) as described⁵⁵. Astrocytes were transduced with 10⁶gc/cell of either exo-AAV9-GFAP-mIFN- β or exo-AAV9-GFAP-null (control) and 7 days later, media harvested. Next, we performed an ELISA to detect mIFN- β using the Verikine-HS™ Mouse IFN β Serum ELISA Kit (PBL assay Science, Piscataway Township, NJ) per the manufacturer’s instructions, using undiluted media as well as a range of dilutions to ensure the OD values fell within the standard curve. The detection limit of the ELISA kit is 0.94 ng/ml of mIFN- β .

Quantitation of GFP signal in RAW cells transduced by AAV9-5NF-GFP.

The GFP signal in RAW cells was quantified by ImageJ software²⁸. The image was converted to an 8-bit grey scale image and adjusted to an equivalent threshold range across all the images. The thresholding creates a binary image, which was used as a reference to measure integrated density and mean values from the original image. The GFP signal for each image was graphed as the mean integrated density +/- SD of triplicate wells for each sample.