Supplementary Materials for

G-CSF secreted by mutant IDH1 glioma stem cells abolishes myeloid cell immunosuppression and enhances the efficacy of immunotherapy

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Materials and Methods

Experimental model and subject details

6-8 weeks old wild type C57BL/6 female mice (Taconic) were housed under standard specific pathogen-free conditions. All animal procedures were carried out in accordance with the University of Michigan’s Institutional Animal Care and Use Committee. All animals were housed in an AAALAC accredited animal facility.

Genetically engineered murine glioma models

A murine model of \textit{mIDH1} glioma and their cognate controls were created using the Sleeping Beauty (SB) Transposon system which allows the integration of genetic lesions into the genome of neonatal mice \cite{19,21}. Tumors then develop intracranially \textit{de novo} from neural progenitor cells. Plasmids encoding these genetic lesions are (i) SB transposase and luciferase (pT2C-LucPGK-SB100X, henceforth referred to as SB/Luc) \cite{20,21}, (ii) a short hairpin against p53 (pT2-shp53-GFP4, henceforth referred to as shp53), (iii) an NRAS-G12V activating mutation (pT2CAG-NRASV12, henceforth referred to as NRAS), (iv) a short hairpin against ATRX (pT2-shATRX53-GFP4, henceforth referred to as shATRX) \cite{19-21}, and (v) mutant IDH1-R132H (pKT-IDH1(R132H)-IRES-Katushka, henceforth referred to as mIDH1) \cite{19}, Supplementary Table S6. SB/Luc, shp53, and NRAS plasmids were the generous gift of Dr. John Ohlfest (University of Minnesota). The genotype of the genetically engineered mice involved these combinations: (i) shp53, NRAS, and shATRX (wt-IDH1), (ii) shp53, NRAS, shATRX, and IDH1-R132H (\textit{mIDH1}). Mice were injected according to a previously described protocol \cite{21}. Briefly, plasmids were mixed in mass ratios of 1:2:2; 1:2:2:2, or 1:2:2:2:2 (20 µg plasmid in a total of 40 µL plasmid mixture) with \textit{in vivo}-jetPEI reagent (Polyplus Transfection) (2.8 µL per 40 µL plasmid mixture) and dextrose (5% total). The transfection mixture was incubated for 20 min at room temperature and then maintained in ice before injection. The lateral ventricle (1.5 mm AP, 0.7 mm lateral, and 1.5 mm deep from lambda) of neonatal mice (P01) was injected with 0.75 µL plasmid mixture (0.5 µL/min) that included: (1) SB/Luc, (2) NRAS, (3) shp53, (4) with or without shATRX, and (5) with or without IDH1-R132H \cite{19}.

Generation of primary mouse glioma neurospheres (NS)

Mouse glioma neurospheres were generated by harvesting brain tumors at the time of euthanasia by transcardial perfusion with Tyrode’s solution only. The brains were excised, and the tumors generated using the SB Transposon
System were identified by GFP and Katushka expression under an epifluorescence microscope at the time of resection (Fig 1a). The tumor mass was dissociated using non-enzymatic cell dissociation buffer (BioLegend, Accutase, 423201), filtered through a 70 µm strainer and maintained in neural stem cell medium [DMEM/F12 with L-Glutamine (Gibco, 11320-033), B-27 supplement (1X) (Gibco, 12587-010), N-2 supplement (1X) (Gibco, 17502-048), penicillin-streptomycin (100 U/mL) (Corning, 30-001-CI), and Normocin (1X) (InvivoGen, ant-nr-1)] at 37 °C, 5% CO2. FGF and EGF (Shenandoah Biotech, 100-26, 100-146) were added twice weekly at 1 µL (20 ng/µL each stock, 1000x formulation) per 1 mL medium. Cells expressing GFP and Katushka were sorted by flow cytometry.

Generation of a mouse mIDH1 glioma model independent of RAS activating mutations

Mice: Previously characterized Ntv-a/Ink4a-Arf−/−/Ptenfl/fl and Ntv-a/Ptenfl/fl mice in the age range of 4-8 weeks were used. Ntv-a/Ink4a-Arf−/−/Ptenfl/fl was in a mixed genetic background, whereas Ntv-a/Ptenfl/fl was in C57BL/6 background, and they were generated by 10 generations of crosses (19, 27).

RCAS transfections: Tumors were generated by injection of DF-1 (ATCC, CRL-12203) cells transfected with a combination of RCAS plasmids (RCAS PDGFB-HA, RCAS shp53-RFP, and RCAS IDH1 wildtype or RCAS IDH1 R132H) using the Fugene 6 transfection kit (Roche, 11814443001) according to the manufacturer’s protocol and as previously described (11, 27).

Intracranial injections of transfected DF-1 cells: Injections were performed using a stereotactic fixation device (Stoelting). Mice were first anesthetized with i.p. injections of ketamine (0.1 mg/g) and xylazine (0.01 mg/g). A local injection of bupivacaine was used as an analgesic. One microliter of 4x10^4 transfected DF-1 cells in suspension was delivered using a 30-gauge needle attached to a Hamilton syringe. The location of injection was AP 1.7 mm from bregma, Lat 0.5 mm, and depth 2 mm from the dural surface and was determined according to the coordinates in the mouse brain atlas to target the right frontal striatum. Buprenorphine was administered to mice for pain relief post-injection. They were continually monitored and sacrificed when they displayed endpoint symptoms (lethargy, scruffy coat, hunched posture).
Tissue processing and generation of primary neurospheres: Upon development of terminal symptoms, mice were anesthetized with ip injections of ketamine (0.1 mg/g) and xylazine (0.01 mg/g) and perfused with cold, sterile Ringer’s solution (Sigma-Aldrich, 96724). The brains were then extracted and placed in NeuroCult basal medium (Stemcell Technologies, 05700). The tumor mass was dissociated mechanically by using a sterile pestle and moving it in up and down motion 20 times under laminar flow hood. Then 1 ml non-enzymatic cell dissociation buffer (BioLegend, Accutase, 423201) was added, and the mixture was incubated for 5 min at 37 °C. Subsequently, the suspension was filtered through a 70 µm strainer and maintained in neural stem cell medium [DMEM/F12 with L-Glutamine (Gibco, 11320-033), B-27 supplement (1X) (Gibco, 12587-010), N-2 supplement (1X) (Gibco, 17502-048), penicillin-streptomycin (100 U/mL) (Corning, 30-001-CI), and Normocin (1X) (InvivoGen, ant-nr-1)]. To collect the neurospheres, the cell suspension was centrifuged at 500 × g, the supernatant was discarded, and neurospheres were re-suspended into 6 mL of NS media, plated into a T-25 (Thermofisher scientific) culture flask and maintained at 37 °C (5% CO2). FGF and EGF (Shenandoah Biotech, 100-26, 100-146) were added twice weekly at 1 µL (20 ng/µL each stock, 1000x formulation) per 1 mL medium (I9). After 3 days in culture a mixed population of cells can be identified morphologically as: (i) adherent cells, (ii) dead cells, and (iii) glioma neurospheres. Glioma neurospheres population can be identified by the floating cell clusters, and they were isolated and plated in a new T-25 culture flask.

Mouse glioma cells stably transfected to knock down ATRX: Neurospheres were generated as detailed above from both the wtIDH1 and mIDH1 glioma models in the context of PDGFB/shP53/Ink4a/Arf−/−, which do not encode RAS activating mutations. Neurospheres were transfected with pT2-shATRX-GFP (Addgene plasmid number 124259), or pMax-GFP™ vector (Lonza) using the P3 primary cell 4D-Nucleofector™ X kit (Lonza, Allendale, NJ). The procedure was as follows: neurospheres from 80–90% confluent T75 flask were centrifuged at 500 × g, and the supernatant was discarded by decantation. Neurospheres were dissociated by using 1 mL of Accutase® cell detachment solution (Biolegend, 423201), incubated for 90 sec at 37 °C, then the accutase was neutralized with 9 mL HBSS. Neurospheres were centrifuged again at 500 × g to separate the supernatant. Neurospheres were resuspended again in 10 ml HBSS, counted, and 200,000 cells were transferred into another tube. Each tube was spun at 90 × g for 10min at room temperature. Neurospheres were re-suspended with 20µl of transfection solution (4D-Nucleofector solution), and
500ng of either pT2-shATRX-GFP or pMax-GFP™ vector were added to each tube in duplicate. Each tube was mixed gently by pipetting and incubated for 5 min at room temperature. The mixture was transferred into Nucleocuvette™ Vessel, placed into the retainer of the 4D-Nucleofector™ X Unit, and ran using pulse code: EN138. Successful transfection was confirmed, and neurospheres were sorted for at least 3 passages (based on GFP expression) before they were used for implantation.

Intracranial Implantation: Tumor implantation was done as described before (55). Briefly, mice are anesthetized using ketamine and dexmedetomidine prior to stereotactic implantation with $5 \times 10^4$ PDGFB/shP53/shATRX/Ink4a/Arf$^{-/-}$ wtIDH1 or mIDH1 neurospheres in the right striatum. The coordinates for implantation are 0.5 mm anterior and 2.0 mm lateral from the bregma and 3.0 mm ventral from the dura. Neurospheres were injected at a rate of 1 μL/min. Mice were given a combination of buprenorphine (0.1mg/kg) and carprofen (5mg/kg) for analgesia. At symptomatic stage, tumors were isolated and immune cells were characterized as described in the flow cytometry section (see below).

Human glioma cells stably transfected to express IDH1$^{R132H}$

SJGBM2 pediatric glioma cells are ATRX mutant (original female donor) and they were a kind gift by the Children’s Oncology Group (COG) Repository, Health Science Center, Texas Tech University; these cells were cultured in IMDM medium with L-glutamine (0.3 mg/mL) (Gibco,12440-053), 20% FBS (Gibco,10437-028), and antibiotic-antimycotic (1X) (Gibco, 15240-062) at 37 °C, 5% CO2. SJGBM2 cells (1.5 x 105 each per well) were seeded in a 6-well plate, and after 24 hours they were transfected with p-CMV-IDH1-R132H-Entry plasmid (Origene, RC400096) using jetPRIME transfection system (Polyplus, 114-07) according to the manufacturer’s instructions and as described before (19). One day after transfection, the medium was replaced by a selection medium containing G418 (Geneticin; Gibco, 10131-035) at a concentration of 800 μg/mL cells. On day 15 of selection, individual cell colonies were taken from the well using autoclaved filter paper (Whatman, 1001-185) squares (2x2 mm approx.) previously embedded in HyQTase Cell Detachment Solution (GE Healthcare Life Science, HyClone, SV30030.01) and placed in wells of 24-well plates with the appropriate cell culture medium. After 24 hours, the medium was replaced with selection medium.

TK/Flt3L gene therapy and Ly6G Depletion
For the TK/Flt3L gene therapy, mice received $5 \times 10^8$ pfu of Ad-Flt3L and $2 \times 10^8$ pfu of Ad-TK in 1.5 μl volume in three locations at 3.5 mm, 3.0 mm and 2.5 mm ventral from the dura followed by Ganciclovir (GCV; TSZ Chemicals) administration twice a day for 7 days. Ly6G depletion was performed by i.p. administration of 250μg InVivoPlus anti-mouse Ly6G (BioXcell, BP0075-1). Due to the differences in tumor progression between the wtIDH1 and mIDH1 groups, the TK/Flt3L and anti-mouse Ly6G were administered at different times so that they are given at the same stage of tumor development (tumor size). The wtIDH1 tumor grows faster, and it has MS=21 days; whereas, the mIDH1 tumor bearing mice have a MS=33 days (see Supp Figure S1B). For survival studies, gene therapy was administered 7 days and 16 days post wtIDH1 and mIDH1 tumor implantation, respectively, as described before (12, 56). The anti-mouse Ly6G was administered twice, on days 12 and 17 post wtIDH1 tumor implantation and days 22 and 27 post mIDH1 tumor implantation, as described before (12). As the Ly6G depletion was done in combination with TK/Flt3L gene therapy, the anti-mouse Ly6G administrations started days 5 and 6 days after TK/Flt3L gene therapy in wtIDH1 and mIDH1 glioma harboring mice, respectively. The peak of antibody depletion takes 48-72 hours after administration (12), which results in a peak depletion at days 7-8 after gene therapy. For functional analysis of T cells studies, gene therapy was administered 7 days and 12 days post wtIDH1 and mIDH1 tumor implantation, respectively. The anti-mouse Ly6G was administered 4 days following gene therapy, which results in a peak depletion at day 7 after gene therapy (12). TK/Flt3L completely eradicates the mIDH1 tumors in all tumor-bearing mice and elicits a memory response. Therefore, to investigate the change in the mIDH1 TME in response to TK/Flt3L, we terminated the experiment at an earlier time point, day 21 post implantation, for the mIDH1 glioma mouse models, which is before the tumor eradication. This time point was determined to match with the maximum efficacy of TK/Flt3L gene therapy.

**In vivo imaging**

*In vivo* imaging was performed as described previously (12, 20). Briefly, *in vivo* bioluminescence was measured using an IVIS Spectrum (Perkin Elmer, 124262) imaging system. For the IVIS spectrum, the following settings were used: automatic exposure, large binning, and aperture f = 1. To monitor the SB plasmid uptake in neonatal pups, 30 μL of luciferin (30 mg/mL) was injected subcutaneously into each pup 24 hours after plasmid injection. For *in vivo* imaging of tumor formation and progression in adult mice, 100 μL of luciferin solution was injected ip, and mice were then
anesthetized with oxygen/isoflurane (1.5-2.5% isoflurane). To score luminescence, Living Image Software Version 4.3.1 (Caliper Life Sciences) was used. A region of interest (ROI) was defined as a circle over the head, and luminescence intensity was measured using the calibrated units photons/s/cm²/sr. Multiple images were taken over 15 minutes following injection, and maximal intensity was reported. For survival studies, animals were monitored daily for signs of morbidity, including ataxia, impaired mobility, hunched posture, seizures, and scruffy fur. Animals displaying symptoms of morbidity were euthanized.

**Flow cytometry**

For isolation and characterization of tumor-infiltrating immune cells, mice were euthanized and tumors were extracted. Tumor mass within the brain was carefully dissected and homogenized using Tenbroeck (Corning) homogenizer in DMEM media containing 10% FBS. Then, the tumor was placed onto a 70 μm strainer attached to a 50 mL conical tube filled with 10 mL of DMEM media supplemented with 10% FBS. Using the plunger end of a syringe, isolated tumor was carefully mashed, and cells were forced through the 70 μm strainer (Alkali Scientific Inc.) on ice. The strainer was washed two times with 10 mL complete media to force cells through and to minimize cell loss. Then, tumor-infiltrating immune cells were enriched using with 30% / 70% Percoll (GE Lifesciences) density gradient. Briefly, cells were centrifuged, and the supernatant was discarded. Then, cells were resuspended in 7 mL of complete media (in 15 mL Falcon tube), and 3 ml of 90% stock isotonic percoll™ (SIP, GE Healthcare) were added and mixed well by pipetting up and down 3–5 times. To layer the 70% percoll™ under the 30% percoll™ gradient, 1 mL serological pipette was filled with 1 mL of 70% percoll™ and pushed to the bottom of the 15 mL falcon tube, and the 70% percoll™ was slowly released making sure a clear interface is formed between the two gradients. Finally, the solution was spun at 800 × g for 20 min at room temperature, and the tumor-infiltrating immune cells were collected by carefully isolating the white band that formed at the interface between the two gradients. For splenocytes isolation, freshly isolated spleens were mashed on the surface of 70μm sterile cell strainer (Alkali Scientific Inc.,) using a syringe plunger. Cells were flushed with 20ml of DMEM+10% FBS to force cells through the strainer and to minimize cell loss. Blood was isolated from anesthetized mice by decapitation and was directly added to an EDTA-coated tubes (Fisher Scientific). Bone marrow cells were extracted by flushing freshly isolated tibia and femur with DMEM+10% FBS using a syringe with a 30G needle until the bone is clear. All cells were then centrifuged, and RBCs were lysed.
using 2 ml RBCs lysis buffer (Biolegend), after which they were washed with DPBS and prepared for antibody staining.

For antibody staining, cells were resuspended in PBS containing 2% FBS and non-specific antibody binding was blocked with FC block (CD16/CD32). All of the staining was performed at 4°C to minimize cellular metabolic activity and marker expression changes during the staining procedure. Dendritic cells were labeled with CD45, CD11c, CD11b, and MHCII antibodies. Macrophages were labeled with CD45, F4/80, CD11b, and CD206 antibodies. T-cells were labeled with CD45, CD3, and CD8 or CD4 for CD8 T-cells and CD4 T-cells, respectively. Cells were first stained with viability dye (Amcyan or Alexa Fluor® 780, Fisher Scientific) to label the dead cells, after which cells were blocked with either fluorescence conjugated CD16/32 or FC block according to the experimental design for 10 min at 4°C. Cells were then stained with fluorescence conjugated antibodies cocktail for 30 min at 4°C; after which, they were washed 2X with BD FACS Flow Sheath Fluid (Becton Dickinson). The flow buffer contains a small percentage of FBS (2%).

For intracellular molecule detection, the cells were fixed and permeabilized before intracellular staining. Fixation and permeabilization were performed using BD Fixation/Permeabilization Solution Kit (BD biosciences, Cat. No.554714). The procedure was done following the manufacturer protocol. Briefly, cells were fixed with the fixation buffer for 20-30 min, followed by washing 2 times with 1X perm/wash buffer. Cells were then stained with the intracellular antibody cocktail for 30 min, after which they were washed 2X with the BD FACS Flow Sheath Fluid. All flow data were acquired on FACSAria II flow cytometer (BD Biosciences) and analyzed using FlowJo version 10 (Treestar Inc.). All primary fluorescence-labeled antibodies used for flow cytometry staining are listed in Supplementary Table S2. The expression of immunosuppressive/ co-stimulatory molecules was calculated using the geometric mean by the FlowJo software after performing appropriate gating and data normalization to the matching antibody isotype control. All gating strategies used to identify the immune cells are listed in Supplementary Table S3. The gating was done based on the fluorescence minus one (FMO) controls. For the hematopoietic stem cells (HSCs) and myeloid progenitors (MPs), cells from BM or spleens were extracted as described above, counted, and were first stained with CD16/CD32 (FcyRII/III) for 20 mins on ice to prevent non-specific binding of antibodies to the FC receptors. Cells were then centrifuged at 300 x g at 4°C for 5 mins, followed by staining with the following antibodies cocktail: lineage cocktail, c-Kit, Sca-1, CD34, and IL7Rα antibodies. 5–6 × 10^6 cells were used for staining from each sample to be able to acquire a sufficient number of stem cells and myeloid progenitor populations. HSCs were identified as Lin^-, Thy1.1^low^, c-Kit^+, and Sca-1^+, and MPs were identified as Lin^-, Thy1.1^low^, c-Kit^+, and Sca-1^+.
The Granulocyte-macrophages progenitors (GMP) were gated from MPs population and were identified using Lin<sup>-</sup>, IL-7Rα<sup>-</sup>, c-Kit<sup>+</sup>, Sca-1<sup>-</sup>, CD34<sup>+</sup>, FcyRII/III<sub>high</sub>, whereas common lymphocyte progenitors (CLPs) population were determined as c-Kit<sup>low</sup>, Sca-1<sup>low</sup>, Lin<sup>-</sup>, IL-7Rα<sup>+</sup>.

**Mass cytometry**

Mass cytometry was performed in collaboration with Indiana University Flow Cytometry Core using Cytof2 system. All antibodies were titrated and validated for overlapping using Maxpar Panel Designer tool (Fluidigm). For titration, all antibodies were separated into two panels to avoid overlapping spectrum. After the two panels were created, each antibody was diluted to a concentration of 5µg/ml in Maxpar cell staining buffer (Cat no. 201068). Then, serial dilutions of each panel were performed to obtain the following dilutions 2.5µg/ml, 1.25µg/ml, 0.6 µg/ml, 0.32µg/ml, and 0.15µg/ml. Splenocytes isolated from a naïve wild type mouse were used to perform the antibody staining for each tube (see staining protocol in the next paragraph), and the optimal concentration for each antibody was determined based on the ideal separation between the positive and negative population.

The staining was performed following the manufacturer protocol as follows: single-cell suspensions were extracted from brain, blood, spleen, and bone marrow as described in the flow cytometry section (see above). First, up to 1×10<sup>7</sup> cells were cleaned by removing debris and dead cells using dead cell removal kit (Miltenyi Biotec). Next, cells were stained with cisplatin 1.67µM (Cell-ID Cisplatin; Fluidigm) for 5 min at room temperature to label dead cells. After quenching Cisplatin reaction with 5X volume of MaxPar® Cell Staining Buffer, cells were counted and centrifuged at 300 × g for 5 minutes. Then, 2-3×10<sup>6</sup> cells were stained with a cocktail of pre-titrated metal-conjugated surface antibodies which were added at the appropriate concentration (determined by the antibodies titration) to each sample for 30min. Samples were washed 2X with MaxPar® Cell Staining Buffer. Samples were fixed and permeabilized with 1.6% of freshly prepared formaldehyde and incubated for 10 min at room temperature. After being washed twice in 1ml MaxPar® Fix and Perm Buffer, they were stained with an intracellular antibody cocktail and incubated for 30 min at room temperature. After being washed once with 2 ml MaxPar® Cell Staining Buffer, cells were re-suspended in 2 ml cell intercalation solution (125 nM Cell-ID Intercalator-Ir in Maxpar Fix and Perm Buffer) for 24 hrs at 4°C to label the nucleated cells and shipped to Indiana University Simon Cancer Center Flow Cytometry for CyTOF2 Mass Cytometer analysis. Before loading the samples, cells were washed with Maxpar® water, counted, and diluted.
to the appropriate concentration. The output signals were normalized using EQ™ Four Element Calibration beads. Cytobank software was used to perform population gating and advanced data analysis.

Mass cytometry data analysis

FCS files were analyzed using Premium CytoBank Software (cytobank.org). Data were checked for quality of staining and normalized by the use of internal bead standards. First, cells were separated from the beads by Ir191 DNA Intercalator which labeled nucleated cells. Then, singlets live cells were identified based on the event length cells and Pt195 Cisplatin stain intensity. Finally, immune cells were gated based on CD45<sup>high</sup>, and downstream gating was performed as described in the flow cytometry section (see above) and in the gating strategy in Supplementary Table S3. Unbiased identification of cellular subpopulations was performed in parallel using advanced analysis in cytobank including viSNE (25), SPADE (58), and CITRUS (26). SPADE clustering parameters were as follows: ArcSinh transformation with a factor of 5, target number of nodes= 100 for the minimum spanning tree graph, downsampling to 10 percent, and using symmetric scaling. For viSNE visualization, cells were gated on live CD45<sup>+</sup> CD11b<sup>+</sup> Ly6G<sup>+</sup> population, and only myeloid-specific markers were selected by performing viSNE clustering in both wtIDH1 and mIDH1 TME. The comparisons of cell populations in the TME of wtIDH1 glioma and mIDH1 glioma were tested for significant differences using $\chi^2$ test ($p < 0.0001$). For heatmap visualization of myeloid markers of clusters C1, C2, and C3, the transformed median marker intensities for each cluster were exported and visualized using Morpheus software, (https://software.broadinstitute.org/morpheus). CITRUS was also used for cluster identification, characterization, and comparison of the cell populations in wtIDH1 and mIDH1 tumors. SAM abundance was chosen for analyzing the differences in abundance between wtIDH1 and mIDH1 CD45<sup>high</sup>. Population sizes (clusters) with a minimum size of 5%, FDR 0.01. All antibodies used for mass cytometry are listed in Supplementary Table S1.

Total RNA sequencing

RNA-sequencing was performed in collaboration with the University of Michigan Sequencing Core. Total RNA was isolated from sorted tumor-infiltrating CD45<sup>high</sup>/CD11b<sup>+</sup>/Gr-1<sup>+</sup> cells using RNeasy Plus Mini Kit (Qiagen, 74134), and 100 ng samples of purified RNA were sent for analysis. RNA quality was assessed using a TapeStation (Agilent Technologies) following the manufacturer's recommended protocol. Samples with RINs (RNA Integrity Numbers) of
8 or greater were prepared using the Illumina TruSeq mRNA Sample Prep Kit v2 (Illumina, RS-122-2001, RS-122-2002). An amount of 0.1-3 μg of total RNA was converted to mRNA using polyA purification. The mRNA was then fragmented and copied into first-strand cDNA using reverse transcriptase and random primers. The 3’ ends of the cDNA were then adenylated and ligated to adapters. Each ligated adapter had a unique 6 nucleotide barcode to identify each sample, enabling multiple samples to be sequenced from each lane of a HiSeq flow cell (Illumina). The products were purified and enriched by PCR to create the final cDNA library. Libraries were checked for quality and quantity by TapeStation (Agilent Technologies) and qPCR using Kapa’s library quantification kit for Illumina Sequencing platforms (Kapa Biosystems, KK4835) using the manufacturer's recommended protocols. They were clustered on the cBot (Illumina) and sequenced 4 samples per lane on a 50-cycle single end on a HiSeq 4000 (Illumina) in High Output mode using version 4 reagents according to manufacturer's recommended protocols. Results were obtained from three biological replicates.

**Total RNA sequencing analysis**

All sequencing was performed by the University of Michigan DNA Sequencing Core, using the Illumina HiSeq 4000 platform. Fastq files were processed using the Tuxedo Suite for alignment and differential expression analysis. TopHat version 2.0.13 and Bowtie version 2.2.1 were used for alignment, based on the UCSC mm10 genome build. Cufflinks/CuffDiff (version 2.1.1) was used for quantification and differential expression. Genes and transcripts were identified as differentially expressed based on three criteria: test status = “OK”, FDR \( \leq 0.05 \) and fold change \( \geq \pm 1.5 \). Hierarchical clustering, using Ward’s method, was implemented in R using the `cluster` package (version 2.0.5), and heatmaps were produced using the `gplots` package (version 3.0.1).

Gene Set Enrichment Analysis was done as previously described (59). Briefly, differentially expressed genes were ranked according to \( \log_2 \) (fold change). We then performed gene set enrichment analysis (GSEA) using this fold-change ranking. We downloaded the gmt file of all GO sets from the Broad Institute website (http://software.broadinstitute.org/gsea/index.jsp) and used it for GSEA analysis. Then, we used the Cytoscape enrichment map plugin and the rank and gmt files along with the positive/negative GSEA report files set to a nominal \( p < 0.05 \) and false discovery rate (FDR) at 0.5 to view enriched GO groups with \( P < 0.05 \).

**T cell proliferation assays**
Granulocytes were purified from freshly isolated brain or spleen of symptomatic \textit{wtIDH1} and \textit{mIDH1} tumor-bearing mice by flow sorting using the specific markers CD45$^+$, CD11b$^+$ and Ly6G$^+$. The inhibitory potential of these cells was assessed by co-culturing with Carboxyfluorescein succinimidyl ester (CFSE, Fisher scientific) labeled total splenocytes from \textit{Rag2 Knockout/Transgenic OT-I T cell receptor} mice (Taconic) at various ratios. Total splenocytes were used, not only isolated T cells, since the dendritic cells and other antigen presenting cells are needed for OVA peptide (SIINFEKL) presentation to stimulate T cell proliferation (12, 57). The procedure is as follow: OT-1 \textit{Rag2} knockout mouse was euthanized by injecting intraperitoneally (i.p.) 400 μL of anesthetics containing Ketamine (75 mg/Kg, Pfizer, NY) and dexmedetomidine (1 mg/kg). Spleen was carefully dissected, mashed through 70μm strainer using the plunger end of a syringe, and washed with 20 ml of Roswell Park Memorial Institute (RPMI, Gibco) media supplemented with 10% FBS, 100 unit/mL penicillin, and 55 μM 2-mercaptoethanol. Isolated cells were centrifuged 500 × g for 5 min, RBCs were lysed by adding 2 ml of RBC lysis buffer (Biolegend), and cells were incubated at 4 °C for 2 min. Lysis buffer was neutralized, and cells were washed 2x with DPBS (Gibco). Finally, OT-1 cells were counted, checked for viability, and resuspended in DPBS (Gibco) at a concentration of 1.0 × 10^6 cells/mL. Cells were then labeled with CFSE by adding 1ml of freshly prepared CSFE solution (5 μM) to obtain a final concentration of 2.5 μM, and cells were incubated for 5 min at 37 °C with occasional shaking. The labeling was quenched by adding 2 mL of pure FBS (Gibco), and splenocytes were washed 2x with RPMI, counted, and plated at a concentration of 100,000 cells/ 50 μL. Tumor-infiltrating granulocytes were sorted from percoll™ purified immune cells as described in the flow cytometry section. Cells were first labeled with viability dye (Alexa Fluor 780) to label dead cells, and granulocytes were FACS sorted using the following antibodies: CD45, CD11b, and Ly6G. Purified granulocytes were counted and diluted to 100,000 cells/50 μl in RPMI media containing 2-mercaptoethanol and were added to the cultured OT-1 splenocytes in the following ratios (1:1, 1:2, and 1:4). Finally, 100μl of 200 nM SIINFEKL peptide (AnaSpec Inc.) were added to the mixture to stimulate the OT-1 splenocytes proliferation for 4 days. After 4 days, cells were stained with anti-mouse CD3 and CD8 antibodies (listed in Supplementary Table S2) diluted in BD FACSDirect Flow Sheath Fluid, and T-cell proliferation was analyzed by CFSE dye dilution. In conditions where SIINFEKL was not added, all cells were at resting state (100% resting T-cells, 0% proliferating T-cells). In all conditions where SIINFEKL was added, the percentage of proliferating T cells were determined as \([{(Fluorescence\ \text{intensity\ of\ proliferating\ cells/Fluorescence\ intensity\ of\ the\ resting\ T-cells})\ \times\ 100}]\).
Single cell-RNA sequencing

Tumors from SB mice harboring either *wtIDH1* or *mIDH1* were harvested and kept in Roswell Park Memorial Institute media (RPMI, Gibco, NH). They were cut into small pieces (1 mm³). The whole solution containing the tumor pieces was then transferred into a 50 ml tube and centrifuged at 1500 RPMI for 5 minutes. Using large pore pipette tips, the pellet was re-suspended in 1ml StemPro Accutase® cell dissociation reagent (Gibco, NH) and incubated for 5-10 min at 37°C (all tumor pieces are dissociated at that point). The Accutase® was neutralized with 9 mL RPMI media and mixed by pipetting. Cells were then passed through 70 µm strainer (Alkali Inc.,) attached to a 50 ml conical tube, and centrifuged at 1500 RPM for 5 minutes. Debris and dead cells were removed using the dead cells removal column (Miltenyi Biotec). After that, cells were washed twice with PBS, counted, and resuspended at a concentration of 1000 cells/µL. The cell viability was determined by Countess automated cell counter to be > 90%.

For human samples, freshly isolated primary tumor tissue was kept in DMEM/F12 media (Gibco). Tissue was cut into small pieces until a homogenous solution is obtained. Cells were dissociated using StemPro Accutase® cell dissociation reagent (Gibco) in two-cycles of 5 min each until single-cell suspension was obtained. The cell suspension was passed through 70 µm strainer to remove debris and connective tissue, lysed with RBC’s lysis buffer (Biolegend), and passed through dead cells removal column (Miltenyi Biotec). A yield of at least > 90% live cells was considered adequate for single-cell RNA-sequencing.

Single cell suspensions were subjected to final cell counting on the Countess II Automated Cell Counter (ThermoFisher) and diluted to a concentration of 700 -1000 cells/ul. 3’ single cell libraries were generated using the 10X Genomics Chromium Controller and following the manufacturer’s protocol for 3’ V3.1 chemistry with NextGEM Chip G reagents (10X Genomics). Final library quality was assessed using the Tapestation 4200 (Agilent), and libraries were quantified by Kapa qPCR (Roche). Pooled libraries were subjected to 150 bp paired-end sequencing according to the manufacturer’s protocol (Illumina NovaSeq 6000). Bcl2fastq2 Conversion Software (Illumina) was used to generate de-multiplexed Fastq files and CellRanger Pipeline (10X Genomics) was used to align reads and generate count matrices. Two biological replicates for each condition (*wtIDH1* and *mIDH1*) were analyzed. An average sequencing depth of 20,000 reads/cell was recovered for each sample we processed.

Single-cell RNA sequencing analysis
Raw sequencing data files were converted to fastq files and aligned to the mouse/human reference genome (mm10 and hg38, respectively) \[\text{https://cf.10xgenomics.com/supp/cell-exp/refdata-cellranger-GRCh38-3.0.0.tar.gz}\] using the Cell Ranger Pipeline version 3.1.0. Filtered digital gene expression matrix files (DGE) containing the number of unique molecular identifiers (UMI) counts per gene per cell were analyzed using the Seurat R package version 3.1.4. Matrix files were loaded using the Seurat \textit{Read10X} function, and Seurat objects created retaining cells with a default minimum of 300 features (genes) and retaining features present in at least three cells. We merged DGE matrices from samples belonging to each condition (\textit{wtIDH1} or \textit{mIDH1}), yielding two DGE matrices. Possible doublet cells with more than 3000 unique expressed genes, or cells with more than 10% mitochondrial genome content, were filtered. Size normalization (natural-log transformed) for each cell was performed using the Seurat \textit{NormalizeData} function with the default scale factor of 10,000. Feature selection was performed using \textit{FindVariableFeatures} (‘vst’ selection method) to identify 4000 highly variable features in each condition. Next, \textit{ScaleData} was used to center the expression vectors and scale the mean and variance of each feature across cells. We then performed unsupervised clustering analysis following the procedure described in the Seurat3 package. First, the high-dimensional expression space was reduced using PCA. The number of informative components of each dataset was inferred from a jackstraw plot of each principal component. The component space was utilized to construct a neighborhood graph of cells using the \textit{FindNeighbors} function with the default k of 20, followed by the Seurat Louvain-based \textit{FindClusters} method with a resolution of 0.4 (60). Cell clusters were embedded in 2D and visualized using the \textit{RunUMAP} or \textit{RunTSNE} function. Cluster-specific genes were obtained using the function \textit{FindAllMarkers}. Clusters were annotated based on the feature markers expressed in each cluster.

For data integration, data from \textit{wtIDH1} and \textit{mIDH1} were processed and normalized as described above. We then identified anchors in each dataset—cells with common expression profiles—using the \textit{FindIntegrationAnchors} function with the following settings: \texttt{dims = 1:30}, \texttt{k.anchor = 5}, \texttt{k.filter = 200}, \texttt{k.score = 30}, and \texttt{anchor.features = 2000}. The two datasets were then integrated into a single Seurat object using the \textit{IntegrateData} function. The integrated data were then scaled and PCA ran as described above. Finally, after switching to the ‘RNA’ assay in Seurat, we ran \textit{FindConservedMarkers} to identify 50 cell type marker genes that are conserved across conditions.

For human samples, we merged DGE matrices from samples belonging to each condition (\textit{wtIDH1} or \textit{mIDH1}), filtered, normalized, and scaled data as described above. We then used the function \textit{AddModuleScore} to obtain a score for each cluster of the following PMN-MDSCs gene signature (\textit{S100A8}, \textit{S100A9}, \textit{TGFß}, \textit{Arg2}, \textit{IL1ß}) across the
combined samples. A positive number indicates that the selection of genes is expressed higher in a (cluster/cell) than the expected average expression. PMN-MDSCs cellular fraction was determined by dividing the total number of cells with positive scores by the total immune cells (cells expressing PTPRC).

Native ChIP

A protocol established in our lab was followed (19). Briefly, for each IP performed, 2x10^6 neurosphere cells were collected, washed in HBSS, and pelleted in low binding Corning Costar microtubes. For each cell type (NPA, NPAI), ChIP for 5 histone marks, plus isotype Igg control and Input, were performed, thus we used 7*(2x10^6=1.4x10^7) for each cell type were used. 1.4x10^7 NS were separated with Accutase treatment, washed twice, and resuspended in 5 ml nuclei isolation buffer (10% sucrose, 15 mM NaCl, 60 mM KCl, 15 mM HEPES, 0.5% Triton, supplemented with 0.5mM PMSF, 5 mM Na butyrate and Halt Protease Inhibitor Cocktail (100X) (Thermo Fisher Cat# 78430). Nuclei were incubated for 10 minutes at 4°C with rotation and loaded in a sucrose cushion (2 g sucrose in 20 ml of lysis buffer) with centrifugation of 3270 x g for 20 minutes at 4°C. After removing the supernatant, nuclei were washed twice with cold PBS and resuspended in 700 ul of MNase digestion buffer (50 mM Tris-HCl, 1 mM CaCl2, 0.2% Triton X-100, pH 8.0) supplemented with 0.5mM PMSF, 5 mM Na butyrate and Halt Protease Inhibitor Cocktail (100X) (Thermo Fisher Cat# 78430). The amount of chromatin was quantified by absorbance at 260 nm, and 50 U of Micrococcal Nuclease (Thermo Fisher Scientific, Affymetrix, AAJ70196ZCR) were used per 0.5 mg of DNA. MNase digestion was performed for 5 min at 37 °C, and stopped by the addition of 10X stopping buffer (110 mM Tris-HCl, 55 mM EDTA, pH 8). The lysate was then diluted with an equal volume of x2 RIPA buffer (280 mM NaCl, 1.8% Triton X-100, 0.2% SDS, 0.2% sodium deoxycholate, 5 mM EGTA, supplemented with 0.5mM PMSF, 5 mM Na butyrate and Halt Protease Inhibitor Cocktail (100X) (Thermo Fisher Cat# 78430). The digested chromatin was centrifuged at (8000 x g, 5 min), and low size nucleosomes were collected from the supernatant. Nucleosomes were then precleared with 10 μL of Dynabeads (1:1 mixture of Protein A and protein G, Thermo Fisher Scientific, Novex by Life Technologies, catalog numbers 10001D and 10003D, respectively) and pre-washed 5 times with RIPA buffer (10 mM Tris-HCl, 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate, pH 8). Samples were then incubated overnight at 4 °C with 5 μg of H3K36me3 (Diagenode, C15410192), H3K4me1 (Diagenode, C15410194), H3K4me3 (Diagenode, C15410003), H3K27me3 (Diagenode, C15410195), H3K27ac (Diagenode, C15410196) or IgG Normal Rabbit IgG. Antibodies were validated prior to use with Active Motif's
MODified Histone Peptide Array (13001, 13006). Immunoprecipitation was performed the following day by incubating for 3 hours with 10 μL of Dynabeads (1:1 mixture of Protein A and protein G) in 1x RIPA, followed by wash steps in x1 RIPA (5 times, supplemented with 1:1000 dilution of protease inhibitor), LiCl buffer (1 time, 250 mM LiCl, 10 mM Tris-HCl, 1 mM EDTA, 0.5% NP-40, 0.5 % sodium deoxycholate, pH 8, supplemented with 1:1000 dilution of protease inhibitor), and finally TE buffer (1 time, protease inhibitor-free). IP chromatin was freed from histones and Dynabeads by a 1-hour incubation at 37°C with proteinase K (Sigma-Aldrich, P2308) at a final concentration of 0.50 mg/mL. Chromatin samples were then purified by the Qiagen QIAquick PCR Purification Kit (28104) and eluted with 50 μL of the EB buffer provided in the kit.

**MDSCs and glioma neurospheres Co-culture system**

Bone marrow (BM) cells were obtained from the femur and tibia of wild type mice as described in the flow cytometry section (see above). First, a C57/BL6 mouse was euthanized by injecting intraperitoneally (i.p.) 400 μL of anesthetics containing Ketamine (75 mg/Kg, Pfizer, NY) and dexmedetomidine (1 mg/kg). Bone marrow cells were extracted by flushing freshly isolated tibia and femur with DMEM+10% FBS using a syringe with a 30G needle until the bone is clear. Cells were then centrifuged, and RBCs were lysed using 2 ml RBC lysis buffer (90 sec at 4°C) after which they were washed with DPBS, counted and seeded at 2 x 10^5 cells per well in a 48 well plate and 0.2 mL media (RPMI + 10% FCS + Pen/Strep + β-mercaptoethanol). Neurospheres from wtIDH1 or mIDH1 were added in a ratio of 2:1 (BM cells: neurospheres). Cells were supplemented with 100 μL of culture media every other day to maintain cell viability. Cultures were maintained for 7 days after which they were stained with CD45, CD11b, Ly6G, iNOS, Arg1, PD-L1, and CD80. The procedure for antibody staining and gating is the same as described in the flow cytometry section.

**α-GCSF neutralization**

Mice were injected i.p. with five doses (100 μg each dose diluted in PBS) of α-GCSF (R&D system; Clone: 67604) or isotype every 4-5 days after tumor implantation. Blood was collected from animals one day after α-GCSF treatment, and serum level of G-CSF was quantified by quantitative ELISA (see details below).

**TCGA and CGGA survival analysis**
Glioma TCGA dataset were downloaded from Broad Institute portal [http://firebrowse.org/?cohort=GBMLGG&download_dialog=true#](http://firebrowse.org/?cohort=GBMLGG&download_dialog=true#). Astrocytoma patients expressing high vs low CSF3 were stratified based on the median cut off expression value. All CGGA data were downloaded from the Chinese Glioma Genome Atlas [http://www.cgga.org.cn/](http://www.cgga.org.cn/) and stratified in a way similar to TCGA data.

**Quantitative ELISA**

Conditioned media from mouse neurospheres and from human glioma cells (wtIDH1-SJGBM2 or mIDH1-SJGBM2) were harvested after culturing of $2 \times 10^5$ cells/1mL for 48 hours in appropriate neurospheres culture media (IMDM medium with L-glutamine (0.3 mg/mL), 20% FBS, and antibiotic-antimycotic (1X)). Quantitation of G-CSF was determined by ELISA (Duosets, R&D Systems, Minneapolis, MN) using the manufacturer’s suggested protocol with few modifications. Briefly, diluted coating Ab was added to ELISA microplates (Greiner Bio-One, Monroe, NC) and incubated overnight. Assay plates were then washed, blocked, and samples and standards added and incubated overnight at 4° C. Diluted secondary Ab was added after washing and incubated for 3 hours at room temperature, followed by washing and HRP incubation for 90 minutes. Following a final series of washes, plates were developed with TMBX substrate (Surmodics, Eden Prairie, MN) and stopped by the addition of an equal volume of 0.4% NaF. Absorbances were obtained at 620 nm and sample concentrations determined by comparison to the G-CSF standards using a 4-parameter curve fit (Synergy HT & Gen5 Software, BioTek Instruments, Winooski, VT).

**Cytokine array**

Proteome Profiler Mouse Cytokine Array Kit, Panel A (R&D Systems) was used to analyze cytokine secretion from the conditioned media of tumor derived-neurospheres according to the manufacturer instructions. Total $1 \times 10^6$ neurospheres were cultured in 1 mL of media for 24 hrs. The conditioned media from this culture was collected, diluted with 0.5 mL of DMEM/F12 media, and incubated with pre-activated membranes according to the manufacturer protocol. The membranes were washed 2X with washing buffer (R and D, ARY006) before they were incubated with HRP conjugated antibodies mix. The spot pixel area for each cytokine was calculated using Image Lab software (Bio-rad) and was subtracted from the pixel intensity of the background spot pixel (generated by measuring the pixel intensity of the negative control). Each cytokine was analyzed in duplicate, and the average intensity was plotted.
**Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)**

Two-step QRT-PCR was performed following the manufacturer’s instructions. Total RNA was isolated using the RNeasy Plus Mini Kit (Qiagen), and 2 μg RNA was reverse transcribed to complementary DNA with the AffinityScript QPCR cDNA Synthesis Kit (Agilent Technologies). Quantitative real-time PCR (QPCR) analysis was performed using the Fast SYBR™ Green Master Mix (Thermo-Fisher Scientific) in a ViiA™ 7 Real-Time PCR System (Thermo-Fisher Scientific). Mouse Csf3 mRNA was amplified using primers 5’-ATCCCGAAGGCTTCCCTGAGTG-3’ and 5’-AGGAGACCTTGAGTAGGAGGAGA-3’, and mouse Tbp mRNA was amplified using primers 5’-ACCCTTCACCAATGACTCTATG-3’ and 5’-ATGATGACTGCAGCAAATCGC-3’. The relative expression level of Csf3 mRNA was calculated using the $2^{-\Delta\Delta C_t}$ method, normalized to the housekeeping gene: TATA-Box Binding Protein (Tbp). The qPCRs were performed in three biological triplicates.

**ChIP-seq**

ChIP-seq data from Nunez et al. 2019 was used to examine epigenetic enrichment of G-CSF in neurospheres from wtIDH1 or mIDH1 (NCBI's Gene Expression Omnibus with identifier GSE99806). Immunoprecipitation, quality control, read alignment, and peak annotation were performed as described before (19).

**Data and Material availability**

All data associated with this study are in the figures and/or the Supplementary Materials. The RNA-seq and sc-RNA-seq datasets have been deposited in NCBI's Gene Expression Omnibus with identifiers GSE152277. CyTOF data are deposited in Immport (accession number: SDY1774). The materials generated in this study will be provided upon request to M.G.C.; all plasmids described in this study have been deposited in Addgene (Supplementary Table S6).
Fig. S1: Enhanced response to TK/Flt3L immune stimulatory gene therapy in \textit{mIDH1} glioma. (A) Experimental design of SB neurospheres generation, growing, and intracranial implantation in animals. (B) Kaplan-Meier survival curves for implanted mice bearing \textit{mIDH1} and \textit{wtIDH1}. (C) Schematic illustrates the treatment strategy of the TK+Flt3L gene therapy in combination with Ly6G depletion in \textit{wtIDH1-Ova} or \textit{mIDH1-Ova} tumor-bearing mice. (D, E) Flow cytometry analysis of CD8+ T-cells infiltrating the \textit{wtIDH1-Ova} or \textit{mIDH1-Ova} tumors receiving vehicle or TK/Flt3L gene therapy. (F, G) Flow analysis of the activation status (represented by GzB expression) of CD8 T-cells within the TME of \textit{wtIDH1-Ova} or \textit{mIDH1-Ova} tumors. *$P<0.05$, **$P<0.01$, ***$P<0.005$, One-way ANOVA.
**Fig S2. mIDH1 glioma model has high expansion of granulocytic myeloid cells**

(CD45$^{\text{high}}$/CD11b$^+$/Ly6G$^+$). (A) Feature plot generated by Significance Analysis of Microarrays (SAM) abundance in CITRUS with a minimum population size of 5%. Red nodes indicate a significant difference in abundance between wtIDH1 and mIDH1 tumor infiltrating CD45$^{\text{high}}$ cells. Blue nodes indicate no difference in abundance. (B) Marker plot represents Ly6G expression in each cluster. Clusters 1-10 express Ly6G$^+$. (C-K) Quantitation of the relative abundance of between wtIDH1 and mIDH1 tumor infiltrating CD45$^{\text{high}}$ in cluster 1-9. The TME from mIDH1 tumors has a higher abundance in most of the granulocytic clusters. *P<0.05, **P<0.01, ***P<0.005, One-way ANOVA.
Fig. S3

A: Abundance

B: F4/80 expression

C-G: Cluster analysis for various clusters (12-20)

H-M: Cluster analysis for various clusters (21-27)
Fig S3. *mIDH1* glioma model has lower frequency of macrophages. (A) Feature plot generated by SAM abundance in CITRUS with a minimum population size of 5%. Red nodes indicate a significant difference in abundance between *wtIDH1* and *mIDH1* tumor infiltrating CD45\(^{\text{high}}\). Blue nodes indicate no difference in abundance. (B) Marker plot represents F4/80 expression in each cluster. (C-M) Quantitation of the relative abundance of Clusters 12, 17-25, and 27 between *wtIDH1* and *mIDH1* tumor infiltrating CD45\(^{\text{high}}\). The TME from *wtIDH1* tumors has a higher abundance in most of the macrophages clusters. *P<0.05*, **P<0.01**, ***P<0.005*, One-way ANOVA.
Fig. S4. Characterization of infiltrating immune cells in normal (N), SB induced *wtIDH1* (NPA), or SB induced *mIDH1* tumors (NPAI). Mass cytometry analysis of the percentage of (A) Granulocytic (Ly6G⁺) vs monocytic (Ly6C⁺) MDSCs (gated from CD45⁺ CD11b⁺), (B) Total macrophages (CD11b⁺ F4/80⁺), (C) Conventional DC (CD11b⁺ CD11c⁺), (D) Lymphoid CD3⁺ population, (E) Natural killer (NK1.1⁺), (F) B-cells (CD45R⁺ CD38⁺), (G) CD8⁺ vs CD4⁺ T-cells (gated from CD45⁺CD3⁺), and (H) Total frequency of CD45⁺. Frequency was calculated as a percentage of live CD45⁺ unless otherwise indicated. (N=3-5 animals) * P<0.05, ** P<0.01, *** P<0.005, One-way ANOVA
Immune cell populations within the Blood of wtIDH1 and mIDH1 GEMM (NPA/NPAi)

A  Ly6G vs Ly6C

B  Total Mac

C  cDC

D  Lymphoid CD3

E  NK cells

F  B cells

G  CD8 T vs CD4 T cells

H  CD45

Fig. S5
Fig. S5. Immune cells characterization of blood from normal (N), SB induced *wtIDH1*, or SB induced *mIDH1* tumor. Mass cytometry analysis of the percentage of (A) Granulocytic (Ly6G+) vs monocytic (Ly6C+) MDSCs (gated from CD45+ CD11b+), (B) Total macrophages (CD11b+ F4/80+), (C) Conventional DC (CD11b+ CD11c+), (D) Lymphoid CD3+ population, (E) Natural killer (NK1.1+), (F) B-cells (CD45R+ CD38+), (G) CD8+ vs CD4+ T-cells (gated from CD45+CD3+), and (H) Total frequency of CD45+. Frequency was calculated as a percentage of live CD45+ unless otherwise indicated. (N=3-5 animals) * P<0.05, ** P<0.01, *** P<0.005. One-way ANOVA
Immune cell populations within the Spleen of \textit{wtIDH1} and \textit{mIDH1} GEMM (NPA/NPAI)

**A** Ly6G vs Ly6C
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**B** Total Mac
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**C** cDC
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**D** Lymphoid CD3
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**E** NK cells
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**F** B cells
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**G** CD8 T vs CD4 T cells
- Normal
- WT-IDH1 (NPA)
- \textit{mIDH1} (NPAI)

**H** CD45
- Normal
- WT- \textit{mIDH1}

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**Legend:**
- **CD45**
- **CD3**
- **CD4**
- **CD8**
- **NK**
- **B-cells**
- **CD19**
- **CD68**
- **Ly6G**
- **Ly6C**
- **cDC**
- **CD11b**
- **CD11c**
- **CD45R**
- **CD38**
- **NOS**

* = statistically significant
ns = not significant
Fig. S6: Immune cell characterization of spleen from normal (N), SB induced wtIDH1, or SB induced mIDH1 tumor. Mass cytometry analysis of the percentage of (A) Granulocytic (Ly6G+) vs monocytic (Ly6C+) MDSCs (gated from CD45+ CD11b+), (B) Total macrophages (CD11b+ F4/80+), (C) Conventional DC (CD11b+ CD11c+), (D) Lymphoid CD3+ population, (E) Natural killer (NK1.1+), (F) B-cells (CD45R+ CD38+), (G) CD8+ vs CD4+ T-cells (gated from CD45+CD3+), and (H) Total frequency of CD45+. Frequency was calculated as a percentage of live CD45+ unless otherwise indicated. (N=3-5 animals) * P<0.05, ** P<0.01, *** P<0.005. One-way ANOVA
Myeloid cells in the TME: **PDGFβ/shP53/shATRX/Ink4a/Arf<sup>−/−</sup> ± miDH1 (RCAS)**

A. Normal  wtiDH1  miDH1

B. $\times 10^3$

C. % CD11b+ Gr1+

D. Normal  wtiDH1  miDH1

E. % CD11b+ Ly6C or CD11b+Ly6G+CD45

F. Normal  wtiDH1  miDH1

G. % CD11b+ Gr1+

H. % CD11b+ Gr1+

I. Normal  wtiDH1  miDH1

J. % CD11b+ Ly6C or CD11b+Ly6G+CD45

Myeloid cells in the TME: **CDKN2A<sup>−/−</sup> shP53/ shATRX/ ± miDH1 (CPA/CPAl)**

F. Normal  wtiDH1  miDH1

G. % CD11b+ Gr1+

H. % CD11b+ Gr1+

I. Normal  wtiDH1  miDH1

J. % CD11b+ Ly6C or CD11b+Ly6G+CD45
Fig. S7. *mIDH1* tumors have expansion in granulocytic myeloid cells. (A-C) Flow cytometry analysis of CD11b⁺ Gr-1⁺ cell frequency in normal mice and mice implanted with *wtIDH1* or *mIDH1* neurospheres developed using the RCAS system. *mIDH1* group showed a higher frequency of CD11b⁺ Gr-1⁺ compared to the *wtIDH1* and normal mice. (D, E) Phenotypic characterization of myeloid cells in normal mice and mice implanted with *wtIDH1* or *mIDH1* neurospheres developed using the RCAS system. (F-H) Flow cytometry analysis of CD11b⁺ Gr-1⁺ cell frequency in normal mice and mice implanted with *wtIDH1* or *mIDH1* neurospheres developed using the SB system (CPA/CPAI). (I, J) Phenotypic characterization of myeloid cells in normal mice and mice implanted with *wtIDH1* or *mIDH1* neurospheres developed using the SB system (CPA/CPAI). In both models, the expanded CD11b⁺ Gr-1⁺ in *mIDH1* were mainly Ly6G⁺ cells. * P<0.05, ** P<0.01, *** P<0.005. One-way ANOVA
Mouse: *PDGFB/shP53/shATRX/Ink4a/Arf*^+/−^ ± *miDH1* (T-cell proliferation)

**A**

- CD11b^+^ CD45^+^ → Ly6G → SIINFEKL → CFSE labeled OT-1 splenocytes
- Cell proliferation by flow cytometry
- 4d

**B**

- T-Only
- T+ pep
- WT-Idh1 Ly6G
- MiDH1 Ly6G

**C**

- % of proliferating T cells
- T-only
- T+SIIN
- MiDH1
- T-cells: TME CD11b^+^ Ly6G^+^

Mouse: *CDKN2A*^−/−^/shP53/shATRX/ ± *miDH1* (CPA/CPAl) (T-cell proliferation)

**D**

- T-Only
- T+ pep
- WT-Idh1 Ly6G
- MiDH1 Ly6G

**E**

- % of proliferating T cells
- T-only
- T+SIIN
- MiDH1
- T-cells: TME CD11b^+^ Ly6G^+^
Fig. S8. Granulocytes infiltrating mIDH1 glioma are not immunosuppressive. (A) Schematic of the in vitro T-cell proliferation assay to analyze immune suppressive properties of CD45\textsuperscript{high}/CD11b\textsuperscript{+}/Ly6G\textsuperscript{+} cells. CD45\textsuperscript{high}/CD11b\textsuperscript{+}/Ly6G\textsuperscript{+} cells were co-cultured with CFSE-labeled splenocytes from Rag2/OT-1 transgenic mouse. Cultures were stimulated with 100 nM SIINFEKL peptide for 4 days, after which proliferation was analyzed by flow cytometry. (B, D) Representative flow plots showing CFSE staining of unstimulated splenocytes (T only), splenocytes undergoing rapid proliferation in response to SIINFEKL (T+ SIIN), and the effect of SIINFEKL-induced T-cell proliferation in the presence of CD45\textsuperscript{high}/CD11b\textsuperscript{+}/Ly6G\textsuperscript{+} cells isolated from the TME of wtIDH1 or mIDH1 tumors developed by the RCAS model (B) or the CPA/CPAI model (D). (C, E) Flow analysis of the inhibitory potential of CD45\textsuperscript{high}/CD11b\textsuperscript{+}/Ly6G\textsuperscript{+} cells isolated from TME of wtIDH1 or mIDH1 tumors developed by the RCAS model (C) or the CPA/CPAI model (E). In both models, CD45\textsuperscript{high}/CD11b\textsuperscript{+}/Ly6G\textsuperscript{+} cells from TME-wtIDH1 tumors are immunosuppressive, whereas CD45\textsuperscript{high}/CD11b\textsuperscript{+}/Ly6G\textsuperscript{+} cells from TME of mIDH1 tumors did not suppress T-cell proliferation. * P<0.05, ** P<0.01, *** P<0.005. One-way ANOVA
**Antigen-induced T cell proliferation**

**A**

- **CD45**
- **Ly6G**
- **CD11b**

- **Splenocytes from wtlDH1 or mIDH1**

- **SIINFEKEL**
  - 4d
  - CFSE labeled OT-1 splenocytes

- **Cell proliferation by flow cytometry**

**B**

- **Spleen-wtlDH1**
- **Spleen-mIDH1**

- **% of proliferating T cells**

| Condition          | % Proliferating T Cells |
|--------------------|-------------------------|
| T                  | 100                     |
| T+ (1:1)           | 75                      |
| (2:1)              | 50                      |
| (4:1)              | 25                      |
| only Pep           |                         |

- **T-cells: myeloid cells**

- **ns**
Fig S9. Granulocytic myeloid cells from spleen of *wtIDH1* and *mIDH1* glioma bearing mice are not immunosuppressive. (A) Schematic figure of the *in vitro* T-cell proliferation assay. (B) Flow analysis of the inhibitory potential of CD45⁺/CD11b⁺/Ly6G⁺ cells from spleen of *wtIDH1* or *mIDH1* tumor bearing mice at different co-culturing ratios: (1:1), (2:1), and (4:1). CD45⁺/CD11b⁺/Ly6G⁺ cells from the spleen of both *wtIDH1* and *mIDH1* tumor bearing mice did not inhibit antigen induced T cell proliferation. One-way ANOVA
**T cells activation (CD3 and CD28 beads)**

### A
Tumor Free (WT)
- C57BL/6 → Spleen → T-cells
- CD3 CD28 Beads
- T-cells activation (CD69)

### B
- wtLDH1
  - No beads
  - +Beads
  - 1:1
  - CD69
  - 1:2
- mLDH1
  - No beads
  - +Beads
  - 1:1
  - CD69
  - 1:2

### C
- wtLDH1
- mLDH1

### D
- wtLDH1
  - % CD69+CD8+ T-cells
  - No Beads
  - +Beads
  - 1:1
  - 1:2

### E
- mLDH1
  - % CD69+CD8+ T-cells
  - No Beads
  - +Beads
  - 1:1
  - 1:2

**Fig. S10**
Fig S10. Granulocytic myeloid cells from TME of wtIDH1 tumors suppress T-cell activation. (A) Schematic figure of the in vitro T-cell activation assay. (B, C) Representative flow plots of the CD69 expression in T-cells in controls (No Beads, +Beads) and T-cells co-cultured at different ratios with CD45+/CD11b+/Ly6G+ cells isolated from TME of wtIDH1 or mIDH1 tumors. (D, E) Quantitative analysis of %CD69+ CD8 T-cells in the different conditions in (B) and (C). * P<0.05, ** P<0.01, *** P<0.005. One-way ANOVA
Fig S11. Granulocytes derived from *ex vivo* co-culture with *mIDH1* neurospheres are not inhibitory. (A) Schematic figure of the *ex vivo* differentiation assay using the neurospheres coculturing system. (B) Flow cytometry plots and analysis show the frequency of CD11b⁺/Ly6G⁺ in BM cultured in FBS media, or co-cultured with *wtIDH1* or *mIDH1* neurospheres for 7 days. Expression of (C) Arg1, (D) PD-L1, and (E) CD80 in BM-derived CD11b⁺/Ly6G⁺ cells after being cultured in FBS media, or co-cultured with *wtIDH1* or *mIDH1* neurospheres. (F) Schematic figure of the *in vitro* T-cell proliferation assay. (G) T-cell proliferation assay to assess the inhibitory potential of CD11b⁺/Ly6G⁺ cells derived from *ex vivo* co-culture. * P<0.05, ** P<0.01, *** P<0.005. One-way ANOVA
Fig. S12. Single-cell sequencing analysis of immune cells isolated from TME of SB induced wtIDH1 or mIDH1 tumors. (A, B) Combined Seurat analysis of immune cells from the TME of wtIDH1 and mIDH1 tumors results in various distinct clusters. Granulocytes were identified in one cluster (C7) in the wtIDH1, whereas the mIDH1 granulocytic population was composed of three granulocytic clusters (C1, C2, C3), (N=2). (C, D) Heat map showing the top 10 differentially expressed genes in each cell cluster within (C) wtIDH1 or (D) mIDH1 tumor samples.
A Mouse TME wtIDH1+mlDH1 integrative analysis

- 0=Granulocytes C2
- 1=Macrophages (APOE, Arg1)
- 2=Neuronal/glial (VEGFA)
- 3=Ly6C+ monocyte (CCR2)
- 4=Microglia-C1 (IBA1, CTSS)
- 5=Granulocytes C1
- 6=Tumor cells (Ribosomes, NRAS)
- 7=Microglia-C2
- 8=CDB T cells
- 9=Granulocytes C3
- 10=DC (MHC, CD74)
- 11=Neuronal Progenitor cells
- 12=Epithelial (Cola7, MAa4)
- 13=NK (Kirb, Kfra)
- 14=DC (CST3, FSCN1, ETV3)
- 15= Astrocyte (GFAP)
- 16=B cells (Igk, Ly6d)

B wtIDH1-TME

C mlDH1-TME

D Fraction of clusters/Total

- wtIDH1
- mlDH1

% Cluster/Total

C1 C2 C3
Fig. S13. Granulocytes infiltrating the *wtIDH1* tumors are similar to C1 granulocytes infiltrating the *mIDH1* tumors. (A) Seurat integrative analysis of immune cells from TME of *wtIDH1* and *mIDH1* genetically engineered mouse tumors results in various distinct clusters of TME CD45+ cells. (B, C) Split view of Seurat integrative analysis of immune cells isolated from *wtIDH1* (B) and *mIDH1* (C) tumors. The majority of granulocytes from *wtIDH1* are clustered with C1 in the *mIDH1*. (D) Quantification of the total granulocytes in cluster C1, C2, or C3 infiltrating the *wtIDH1* or *mIDH1*. *P*<0.05. One-way ANOVA
Gating Strategy for isolation Cluster C1, C2 and C3 from miDH1 glioma TME

Cells

Singlets_1

Singlets_2

CD11b⁺ Ly6G⁺

CD11b⁺ Ly6G⁺

Live CD45⁺

Viability

PDL-1

Ly6G

CD11b

CD45

Fig. S14
Fig. S14. Gating Strategy for sorting Cluster C1, C2 and C3 from mIDH1 glioma TME.

Percoll® purified immune cells from the TME of mIDH1 were first stained with viability dye (Amcyan), then cells were stained with CD45, CD11b, Ly6G, and PD-L1 fluorescence tagged antibodies (Table S2). C1, C2, and C3 were FACS sorted based on PD-L1 expression.
Quantitative cytokine analysis of WT-IDH1, and mIDH1 mouse neurospheres (NPA/ NPAI)

- **A**: IL-33 (pg/ml)
- **B**: CXCL10 (pg/ml)
- **C**: IL-6 (pg/ml)
- **D**: M-CSF (pg/ml)
- **E**: GM-CSF (pg/ml)
- **F**: RANTES (pg/ml)
- **G**: SCF (pg/ml)

Quantitative cytokine analysis of WT-IDH1, and mIDH1 mouse neurospheres (CPA/ CPAI)

- **H**: IL-33 (pg/ml)
- **I**: CXCL10 (pg/ml)
- **J**: M-CSF (pg/ml)
- **K**: GM-CSF (pg/ml)
- **L**: RANTES (pg/ml)

Quantitative cytokine analysis of WT-IDH1, mIDH1 Human SJ-GBM2

- **M**: IL-33 (pg/ml)
- **N**: CXCL10 (pg/ml)
- **O**: IL-6 (pg/ml)
- **P**: M-CSF (pg/ml)
- **Q**: GM-CSF (pg/ml)
- **R**: RANTES (pg/ml)
- **S**: SCF (pg/ml)
- **T**: CXCL12 (pg/ml)
Fig. S15. Quantitative ELISA analysis of cytokines from conditioned media of cultured wtIDH1 and mIDH1 neurospheres. (A-G) Quantitative ELISA analysis of the differentially secreted cytokines and chemokines in CM of cultured mouse wtIDH1 and mIDH1 neurospheres (NPA/NPAI). (H-L) Quantitative ELISA analysis of the differentially secreted cytokines and chemokines in CM of cultured mouse wtIDH1 and mIDH1 neurospheres (CPA/CPAI). (M-S) Quantitative ELISA analysis of the differentially secreted cytokines and chemokines from CM of SJ-GBM wtIDH1 and SJ-GBM mIDH1 cell cultures. * P<0.05, ** P<0.01, *** P<0.005. Student’s t-test
Fig. S16. FBS-containing media does not alter G-CSF expression between wtIDH1 and mIDH1 cultured mouse neurospheres. Quantitative ELISA of the G-CSF level in conditioned media collected from mouse NS cultured in either F12 media or FBS containing media for 7 days.
Fig. S17. Kaplan-Meier survival analysis of the different types of tumors in the TCGA data according to CSF3 expression (high vs low). LGG is the sole tumor within the TCGA database in which patients who express a high level of CSF3 have a favorable prognosis compared to patients with low CSF3 expression.
TCGA-LGG-Astrocytoma

A

CSF1

- <18.44 (n=261)
- >=18.44 (n=264)

P=0.91

B

IL4

- <9.89 (n=231)
- >=9.89 (n=232)

P=0.86

C

IL1B

- <14.7 (n=263)
- >=14.7 (n=263)

P=0.69

D

TNF

- <14.09 (n=264)
- >=14.09 (n=262)

P=0.1149

E

IL6

- <10.01 (n=263)
- >=10.01 (n=263)

P=0.075

CCGA-Secondary Astrocytoma

G

CSF1

- <6.0 (n=11)
- >=6.0 (n=10)

P=0.86

H

IL4

- <3.9 (n=12)
- >=3.9 (n=9)

P=0.49

I

IL1B

- <5.9 (n=10)
- >=5.9 (n=10)

P=0.423

J

TNF

- <2.44 (n=9)
- >=2.44 (n=11)

P=0.32

K

IL6

- <4.73 (n=10)
- >=4.73 (n=9)

P=0.066
Fig. S18. Kaplan-Meier survival analysis of the TCGA-LGG-Astrocytoma and CGGA-Secondary Astrocytoma according to cytokine expression levels (high vs low). (A-E) Kaplan-Meier survival analysis of TCGA-LGG Astrocytoma patients according to high vs low expression of (A) CSF1 (B) IL4 (C) IL1B (D) TNF and (E) IL6. (G-K) Kaplan-Meier survival analysis of CGGA-secondary Astrocytoma patients according to high vs low expression of (G) CSF1 (H) IL4 (I) IL1B (J) TNF and (K) IL6.
Fig. S19. Characterization of infiltrating immune cells in \textit{wtIDH1} tumor bearing mice treated with vehicle or rGCSF. (A) Schematic showing experimental design of recombinant G-CSF (rG-CSF) or vehicle administration in \textit{wtIDH1} tumor-bearing animals. Mass cytometry analysis of the percentage of (B) Granulocytic (Ly6G$^+$) vs monocytic (Ly6C$^+$) MDSCs (gated from CD45$^+$ CD11b$^+$), (C) Total macrophages (CD11b$^+$ F4/80$^+$), (D) Conventional DC (CD11b$^+$ CD11c$^+$), (E) Lymphoid CD3$^+$ population, (F) Natural killer (NK1.1$^+$), (G) B-cells (CD45R$^+$ CD38$^+$), (H) CD8$^+$ vs CD4$^+$ T-cells (gated from CD45$^+$CD3$^+$), and (I) Total frequency of CD45$^+$. Frequency was calculated as a percentage of live CD45$^+$ unless otherwise indicated. (N=3-5 animals) * $P<0.05$, ** $P<0.01$, *** $P<0.005$. One-way ANOVA
Fig S20. Csf3 expressing neurospheres resulted in global expansion of granulocytes in wtIDH1 tumor-bearing animals. (A) Schematic shows experimental design for implantation of G-CSF-expressing wtIDH1 neurospheres using Lentivector. (B) RT-qPCR for Csf3 in wtIDH1 neurospheres transduced with lentivirus expressing empty vector (red) or with Lentivirus encoding for Csf3. (C) Quantitative ELISA measuring G-CSF secretion in conditioned media from cultured mouse neurospheres with wtIDH1 transduced with the empty vector (red) or the lentivirus encoding Csf3 (blue). Representative flow plots and flow analysis show the frequency of granulocytes in (D) Tumors, (E) Spleen, and (F) BM of implanted animals with wtIDH1+Lenti-vector or wtIDH1+Lenti-GCSF. Animals implanted with wtIDH1+Lenti-GCSF had an expansion of granulocytes in the tumor, spleen, and BM compared to wtIDH1+Lenti-vector. * P<0.05. Student t-test
**Supplementary Table S1. Metal conjugated antibodies used in this study**

| Label | Target      | Clone          |
|-------|-------------|----------------|
| 143Nd | CD11a       | H155-78        |
| 147Sm | CD48        | HM48-1         |
| 148Nd | CD16/32     | 93             |
| 149Sm | Ly-6B       | 7/4            |
| 151Eu | CD103       | QA17A24        |
| 155Gd | CxCR2       | SA044G4        |
| 158Gd | Ly-6A/E (Sca-1) | D7    |
| 161Dy | CD90.2/Thy-1.2 | 30-H12 |
| 163Dy | CD162       | 4RA10          |
| 173Yb | CD43        | S11            |
| 176Nd | Ly-6G       | IA8            |
| 142Nd | CD11c       | N418           |
| 144Nd | CD115       | AFS98          |
| 145Nd | CD4         | RM4-5          |
| 146Nd | F4/80       | BM8            |
| 150Nd | CD24        | M1/69          |
| 152Sm | CD3e        | 145-2C11       |
| 153Eu | CD274 (PD-L1) | 10F.9G2   |
| 154Sm | TER-119 (Glycophorin A) | TER-119 |
| 156Gd | CD14        | Sa14-2         |
| 159Tb | CD184 (CXCR4) | L276F12 |
| 160Gd | CD45R (B220) | RA3-6B2       |
| 162Dy | Ly-6C       | HK1.4          |
| 164Dy | CD62L (L-selectin) | MEL-14 |
| 165Ho | CD161 (NK1.1) | PK136         |
| 166Er | CD117 (c-Kit) | 2B8           |
| 167Er | CD335 (NKp46) | 29A1.4        |
| 168Er | CD8a        | 53-6.7         |
| 169Tm | CD206 (MMR)  | C068C2        |
| 171Yb | CD44        | IM7            |
| 172Yb | CD11b (Mac-1) | M1/70         |
| 174Yb | Ly-6G/C (Gr-1) | RB6-8C5     |
| 175Lu | CD38        | 90             |
| 176Yb | FcεR1a      | Mar-1          |
| 209Bi | I-A/I-E     | M5/114.15.2    |
| 89Y  | CD45        | 30-F11         |
**Supplementary Table S2.** Fluorescence-conjugated antibodies used in the study

| Antibody                                      | Manufacturer | Cat #   |
|-----------------------------------------------|--------------|---------|
| PerCP/Cy5.5 anti-mouse CD3e                   | Biolegend    | 100328  |
| PE anti-mouse CD4                             | Biolegend    | 100512  |
| APC anti-mouse CD8                            | Biolegend    | 100712  |
| Alexa fluor 700 anti-mouse CD45              | Biolegend    | 103112  |
| PE/Cy7 anti-mouse CD45                       | Biolegend    | 103114  |
| APC anti-mouse/human CD11b                   | Biolegend    | 101212  |
| Pacific BlueTM anti-mouse/human CD11b        | Biolegend    | 101224  |
| PE anti-mouse/human CD11b                    | Biolegend    | 101208  |
| PE-Dazzle Ly-6G/Ly-6C (Gr-1)                 | Biolegend    | 108452  |
| FITC anti-mouse Ly-6G/Ly-6C (Gr-1)           | Biolegend    | 108406  |
| PE anti-mouse Ly-6G                           | Biolegend    | 127608  |
| PerCP/Cy5.5 anti-mouse Ly-6C                 | Biolegend    | 128012  |
| APC anti-mouse F4/80                         | Biolegend    | 123116  |
| FITC anti-mouse I-A/I-E                      | Biolegend    | 107606  |
| APC anti-mouse CD80                          | Biolegend    | 104714  |
| PE anti-mouse CD86                           | Biolegend    | 105008  |
| PE anti-mouse/human CD45/R/B220              | Biolegend    | 103208  |
| APC anti-mouse CD117 (c-Kit)                 | Biolegend    | 105812  |
| APC-Cy7 anti-mouse Ly-6A/E (Sca-1)           | Biolegend    | 108126  |
| PE anti-mouse Lineage Cocktail with Isotype Ctrl | Biolegend    | 133303  |
| PE-Cy7 anti-mouse CD16/32                    | Biolegend    | 101302  |
| APC anti-mouse CD274 (Rat anti LyG25)        | Biolegend    | 124311  |
| PE anti-mouse CD124 (IL-4 Receptor alpha)    | Biolegend    | 144803  |
| Anti-Mouse CD16/32 (FC Block)                | Biolegend    | 101302  |
| Human/Mouse Arginase I/ARG1                  | R and D      | IC5868F |
| FITC Mouse Anti-iNOS/NOS Type II             | BD Biosciences | 610330 |
| MHC Class I Murine Tetramer–PE              | MBL international | TB-5001-4 |
| Mouse G-CSF R/CD114 APC-conjugated Antibody | R and D      | FAB60391A |
| PE/Cyanine7 anti-human/mouse Granzyme B      | Biolegend    | 372214  |
| PE anti-mouse CD69 Antibody                  | Biolegend    | 104508  |
### Supplementary Table S3. Gating strategies used to define immune cells

| Immune Cells       | Gating Strategy                                      |
|--------------------|------------------------------------------------------|
| CD4 T cells        | CD45<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>+</sup>   |
| CD8 T cells        | CD45<sup>+</sup>, CD3<sup>+</sup>, CD8<sup>+</sup>   |
| Monocytes          | CD45<sup>+</sup>, CD14<sup>+</sup>                   |
| Macrophages        | CD45<sup>+</sup>, CD11b<sup>+</sup>, F4/80<sup>+</sup>|
| M1 Macrophages     | CD45<sup>+</sup>, CD11b<sup>+</sup>, CD206<sup>+</sup>, MHCII<sup>+</sup> |
| M2 Macrophages     | CD45<sup>+</sup>, CD11b<sup>+</sup>, CD206<sup>+</sup>, Arginase<sup>+</sup> |
| PMN MDSCs          | CD45<sup>+</sup>, CD11b<sup>+</sup>, Ly6G<sup>+</sup>, Ly6C<sub>low</sub> |
| Monocytic MDSCs    | CD45<sup>+</sup>, CD11b<sup>+</sup>, Ly6G<sup>+</sup>, Ly6C<sup>+</sup> |
| MDSCs              | CD45<sup>+</sup>, CD11b<sup>+</sup>, Gr-1<sup>+</sup> |
| B cells            | CD45<sup>+</sup>, CD19<sup>+</sup>, CD38<sup>+</sup>  |
| NK Cells           | CD45<sup>+</sup>, CD11b<sup>int</sup>, NK1.1<sup>+</sup> |
| LSK                | Lin<sup>−</sup>, c-Kit<sup>+</sup>, Sca-1<sup>+</sup>, Thy1.1<sub>low</sub> |
| CLP                | c-Kit<sup>low</sup>, Sca-1<sup>low</sup>, Lin<sup>−</sup>, IL-7Rα<sup>+</sup> |
| MP                 | c-Kit<sup>+</sup>, Sca-1<sup>−</sup>, Lin<sup>−</sup>, Thy1.1<sub>low</sub> |
| GMP                | Lin<sup>−</sup>, IL-7Rα<sup>−</sup>, c-Kit<sup>+</sup>, Sca-1<sup>−</sup>, CD34<sup>+</sup>, FcγRII/III<sup>high</sup> |
## Supplementary Table S4. 50 differentially expressed genes in C1, C2, and C3 clusters within the TME of mIDH1

| gene         | logFC  | P_adj | gene         | logFC  | P_adj | gene         | logFC  | P_adj |
|--------------|--------|-------|--------------|--------|-------|--------------|--------|-------|
| Lrg1         | 1.94883107 | 0     | Cxcl2        | 1.846162 | 0     | Mmp9        | 1.419399743 | 3.3E-128 |
| Retnlg       | 1.909792087 | 7.428E-240 | Ccl3        | 1.721826 | 0     | Csf1        | 1.281502873 | 8.7E-152 |
| Cxcr2        | 1.897336531 | 0     | G0s2         | 1.620716 | 0     | II1rn       | 1.279716915 | 1.5E-165 |
| Cd274        | 1.714185811 | 1.1212E-255 | Ankrd33b    | 1.619792 | 0     | Ets1        | 1.232236427 | 5E-119 |
| Lcn2         | 1.711462357 | 7.9788E-252 | Clc4         | 1.587228 | 3.8467E-179 | 1700017B05Rik | 1.17100845 | 7E-104 |
| Wfdc21       | 1.63959635 | 7.2976E-222 | F3           | 1.541258 | 2.907E-197 | Inhba       | 1.170848495 | 2.68E-29 |
| Arg2         | 1.593947223 | 3.991E-268 | Slfn4        | 1.516581 | 2.6349E-250 | Ccl3        | 1.141027133 | 7.1E-156 |
| S100a9       | 1.586460208 | 4.4029E-290 | Gadd45b      | 1.495735 | 1.7636E-142 | Scl31a2     | 1.110267819 | 1.49E-69 |
| Il1b         | 1.549522169 | 0     | Ifrd1        | 1.4689   | 8.2335E-142 | Txnip       | 1.101194254 | 5.35E-25 |
| Ifitm1       | 1.523050065 | 1.6327E-293 | Acod1        | 1.447899 | 0     | E230032D23Rik | 1.083659997 | 1.4E-131 |
| Tnfaip2      | 1.431745973 | 1.6646E-239 | Cxcl3        | 1.431991 | 7.4072E-144 | Ninj1       | 1.052091205 | 3.6E-112 |
| Pglyrp1      | 1.418772486 | 6.3823E-213 | Tiparp       | 1.4247   | 1.6428E-191 | Creb5       | 1.048581408 | 5.33E-58 |
| Steap4       | 1.415864528 | 0     | Cdc20        | 1.397764 | 1.8565E-137 | Fnip2       | 1.039348973 | 1.58E-67 |
| Csf3r        | 1.408028802 | 0     | Plaur        | 1.389422 | 1.1848E-247 | Cd24a       | 1.014549692 | 6.9E-121 |
| Hdc          | 1.398649055 | 1.6327E-293 | Tra2a        | 1.377652 | 5.8361E-220 | Ddit3       | 1.013190918 | 4.98E-60 |
| Il1r2        | 1.349218246 | 6.1682E-273 | Neat1        | 1.341235 | 1.8817E-272 | Gm5483      | 0.996717776 | 5.58E-52 |
| Wfdc17       | 1.307492254 | 1.8017E-227 | Hcar2        | 1.332779 | 2.0478E-256 | Clec5a      | 0.994507067 | 1.28E-64 |
| Ifitm2       | 1.249819468 | 5.063E-251 | Ccl300lf     | 1.329005 | 0     | Clec4n      | 0.963115278 | 1.96E-71 |
| Tgfβ1        | 1.219128383 | 6.8235E-151 | S100a9       | 1.323944 | 0     | Gadd45a     | 0.958857214 | 2.59E-42 |
| Mmp9         | 1.202280783 | 2.1643E-278 | Upp1         | 1.319999 | 5.3995E-216 | AC110211.1  | 0.954539417 | 1.59E-76 |
| Ifit1        | 1.195165477 | 1.18079E-35 | Stfa211      | 1.310327 | 4.3497E-148 | Cstb        | 0.954262874 | 1.3E-113 |
| Stfa211      | 1.191091009 | 1.1897E-136 | Wfdc17       | 1.291159 | 5.7663E-224 | Cxcl3       | 0.943896301 | 3.89E-57 |
| Rnasel       | 1.136882682 | 1.72851E-98 | Hilpda       | 1.282142 | 1.3832E-101 | Cited2      | 0.936331164 | 7.99E-37 |
| Igfbp6       | 1.1311509 | 6.212E-203 | Hk2          | 1.277135 | 2.0861E-106 | Gadd45g     | 0.929277649 | 9.61E-09 |
| Trem1        | 1.1300247 | 1.5E-197 | Llr4b        | 1.27393 | 0     | Card19      | 0.927330    | 1.1E-70 |
| gene   | logFC   | P_adj  | gene   | logFC   | P_adj  | gene   | logFC   | P_adj  |
|--------|---------|--------|--------|---------|--------|--------|---------|--------|
| Irfl   | 1.128785899 | 2.9564E-113 | Csmp1  | 1.269308 | 2.7703E-213 | Frrs1  | 0.925797952 | 2.17E-56 |
| Mxd1   | 1.117137781 | 1.7274E-243 | Ccn1l  | 1.258025 | 1.6554E-280 | 1810058124Rik | 0.905807221 | 2.06E-55 |
| Cxcl10 | 1.112979121 | 1.71734E-20 | Nfkbia | 1.256375 | 2.4886E-161 | Gm31718 | 0.883115202 | 4.38E-94 |
| Isg15  | 1.101546723 | 1.05869E-92 | Hdc    | 1.252687 | 1.7171E-140 | Hilpda | 0.882920318 | 8E-51  |
| Gbp2   | 1.086590726 | 9.38349E-50 | Smox   | 1.241497 | 8.1918E-245 | Ifi207 | 0.87479373 | 3.21E-23 |
| Slpi   | 1.084960683 | 3.9576E-206 | S100a8 | 1.236624 | 0       | Slfn1  | 0.873661369 | 1.19E-52 |
| SoCs3  | 1.083072214 | 5.34E-145 | Ccr2   | 1.231244 | 1.2517E-86 | Gm19951 | 0.86454795 | 2.15E-08 |
| Sell   | 1.079760255 | 3.0043E-176 | Rhov   | 1.220059 | 3.8781E-108 | C3     | 0.839895172 | 2.19E-59 |
| Rsad2  | 1.070101046 | 1.37768E-32 | Ptafr  | 1.205572 | 1.9053E-219 | Egfr1  | 0.839531349 | 6.59E-56 |
| Nfam1  | 1.058239412 | 1.93286E-94 | Tnfrsf23 | 1.193123 | 8.1172E-172 | Rnflf19 | 0.819612083 | 1E-100 |
| Trim30b| 1.055360515 | 3.2571E-198 | Il1f9  | 1.190499 | 3.0619E-191 | B4galt1 | 0.781472383 | 5.26E-34 |
| Cd14   | 1.047274425 | 5.8418E-141 | Il1rn  | 1.18325 | 1.8349E-211 | Sqstm1 | 0.773180538 | 2.86E-21 |
| S100a11| 1.02727437 | 1.3091E-194 | Mxd1   | 1.155384 | 6.5648E-289 | Basp1  | 0.766589969 | 4.04E-89 |
| Alox5ap| 1.026015949 | 1.4895E-148 | Dusp1  | 1.150352 | 2.553E-196 | Eif5   | 0.763963836 | 4.47E-74 |
| Cdk2ap2| 1.020242163 | 3.39795E-47 | Dedd2  | 1.145724 | 1.7445E-124 | Slc7a11 | 0.75487226 | 1.1E-15 |
| Lmnbl  | 1.014834195 | 4.7949E-165 | Ptgs2  | 1.135402 | 3.2881E-145 | Cpeb4  | 0.751717803 | 8.86E-12 |
| CebpD  | 1.01079925 | 5.96237E-70 | Ppp1r3b | 1.134409 | 1.8476E-120 | Atp6vl1 | 0.75109375 | 2.55E-35 |
| Sorl1  | 1.009862068 | 1.6009E-166 | Fth1   | 1.125552 | 8.9873E-298 | E030030106Rik | 0.749119383 | 6.38E-55 |
| Acod1  | 0.994059935 | 5.1927E-151 | Lmnbl  | 1.119028 | 2.2426E-148 | Mdm2   | 0.729758619 | 0.016078 |
| Tnfrsf1a| 0.987108984 | 9.8264E-117 | Ets2   | 1.111059 | 8.7983E-200 | Atf4   | 0.729021386 | 2.94E-46 |
| Asprv1 | 0.98532413 | 3.1725E-116 | Samsn1 | 1.103516 | 5.8649E-210 | Mirt1  | 0.728578214 | 3.79E-55 |
| Pde4b  | 0.984165737 | 2.76333E-83 | Mccl   | 1.095119 | 4.5964E-209 | Fkbpl5 | 0.717329775 | 1.63E-15 |
| Trib1  | 0.982696741 | 7.7856E-144 | Nlrp3  | 1.089935 | 1.10939E-37 | Hipk1  | 0.714673902 | 4.74E-05 |
| Btg2   | 0.972912421 | 5.41411E-92 | Rab7   | 1.089611 | 3.1865E-249 | Klf6   | 0.713155159 | 2.46E-35 |
| Slfn5  | 0.947427628 | 3.02133E-40 | Ppp1r15a | 1.08055 | 4.0553E-127 | Ccl2   | 0.70803389 | 2.23E-47 |
**Supplementary Table S5. Clinical characteristics of glioma patients**

| Sample ID | Patient ID | Gender | Age | Diagnosis | IDH status | 1p/19q status | ATRX | Other known mutations | scRNA-seq | Serum |
|-----------|------------|--------|-----|-----------|------------|---------------|------|-----------------------|-----------|-------|
| 126698    | 56895      | M      | 68  | diffuse LGG | wtIDH      | retained      | TERT pos, MGMT neg | yes |
| 126703    | 56895      | M      | 68  | diffuse LGG | wtIDH      | retained      | TERT pos, MGMT neg | yes |
| 141345    | 66366      | F      | 67  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 141346    | 66366      | F      | 67  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 141347    | 66366      | F      | 67  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 141348    | 66366      | F      | 67  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 138490    | 64636      | M      | 60  | GBM       | mIDH       | retained      | MGMT pos          | yes |
| 138491    | 64636      | M      | 60  | GBM       | mIDH       | retained      | MGMT pos          | yes |
| 138492    | 64636      | M      | 60  | GBM       | mIDH       | retained      | MGMT pos          | yes |
| 138493    | 64636      | M      | 60  | GBM       | mIDH       | retained      | MGMT pos          | yes |
| 135644    | 62785      | M      | 55  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 135645    | 62785      | M      | 55  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 135646    | 62785      | M      | 55  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 135647    | 62785      | M      | 55  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 127441    | 57328      | F      | 50  | GBM       | mIDH       | neg           | retained          | yes |
| 127442    | 57328      | F      | 50  | GBM       | mIDH       | neg           | retained          | yes |
| 134553    | 62137      | M      | 49  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 134554    | 62137      | M      | 49  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 135710    | 62918      | F      | 37  | GBM       | mIDH       | neg           | retained          | yes |
| 135711    | 62918      | F      | 37  | GBM       | mIDH       | neg           | retained          | yes |
| 135712    | 62918      | F      | 37  | GBM       | mIDH       | neg           | retained          | yes |
| 135741    | 62918      | F      | 37  | GBM       | mIDH       | neg           | retained          | yes |
| 129886    | 59376      | M      | 22  | GBM       | mIDH (R132c) | neg           | lost          | EGFR mutation neg | yes |
| 129887    | 59376      | M      | 22  | GBM       | mIDH (R132c) | neg           | lost          | EGFR mutation neg | yes |
| 168145    | 53556      | F      | 64  | GBM       | wtIDH      | retained      | MGMT neg          | yes |
| 168452    | 41465      | M      | 59  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 184523    | 65209      | M      | 60  | GBM       | wtIDH      | retained      | MGMT neg          | yes |
| 148546    | 59393      | M      | 23  | GBM       | wtIDH      | retained      | BRAF V600E neg   | yes |
| 126586    | 31236      | M      | 44  | GBM       | wtIDH      | retained      | BRAF V600E neg, MGMT neg | yes |
| 196524    | 72955      | M      | 51  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
| 174586    | 57819      | M      | 48  | GBM       | mIDH (R132L) | neg           | lost          | MGMT neg, | yes |
| 168542    | 45713      | F      | 27  | GBM       | mIDH       | neg           | lost          | yes |
| 186574    | 60274      | M      | 43  | GBM       | mIDH       | lost          | MGMT pos          | yes |
| 158457    | 45378      | F      | 26  | GBM       | mIDH (R132c) | neg           | lost          | GFAP positive, H3k27Me3 retained | yes |
| 173652    | 52277      | M      | 72  | GBM       | wtIDH      | retained      | MGMT pos          | yes |
Supplementary Table S6. Expressing vectors used in this study

| Plasmid number | Repository/source        | Plasmid Name                                      | Insert        |
|----------------|-------------------------|--------------------------------------------------|---------------|
| 124259         | Addgene                 | pT2-shATRX-GFP                                   | shATRX        |
| 124261         | Addgene                 | pT2-shP53                                        | shP53         |
| 124257         | Addgene                 | pKT-IDH1(R132H)-IRES-Katushka                    | IDH1          |
| LV455868       | Applied Biological      | pLenti-GIII-CMV-RFP-2A-Puro                       | CSF3          |
|                | Materials               |                                                  |               |
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