The immune fraction of the tumor microenvironment has been proven to play a fundamental role in glioblastoma progression and therapeutic response. Here, we present a detailed magnetic-bead-enrichment-based protocol to isolate and analyze the composition of this fraction from mouse brain tumors. The protocol is optimized to achieve high yields of viable immune cells. We also detail characterization of the immune subtypes by FACS analysis. Our procedure is applicable for either lentiviral-induced tumors or transplant models in syngeneic immunocompetent mice.
Protocol
Isolation and characterization of the immune cell fraction from murine brain tumor microenvironment

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SUMMARY
The immune fraction of the tumor microenvironment has been proven to play a fundamental role in glioblastoma progression and therapeutic response. Here, we present a detailed magnetic-bead-enrichment-based protocol to isolate and analyze the composition of this fraction from mouse brain tumors. The protocol is optimized to achieve high yields of viable immune cells. We also detail characterization of the immune subtypes by FACS analysis. Our procedure is applicable for either lentiviral-induced tumors or transplant models in syngeneic immunocompetent mice.
For complete details on the use and execution of this protocol, please refer to Magod et al. (2021).

BEFORE YOU BEGIN
Initiation of brain tumors

© Timing: ~2 months

This protocol requires tumor tissue derived from mouse brain tumor models. Ethical approvals are required prior to starting this procedure. Animal experiments described in this protocol were approved by the animal care and use committee (IACUC) of Tel Aviv University (approval protocol no. 04-19-074 and 04-16-073) and conducted in accordance with NIH guidelines.

For a detailed protocol of lentiviral models of Brain Cancer, including design and cloning of lentivectors expressing oncogenes and shRNAs, production of lentiviral vectors and stereotaxic injection into the brain, refer to Friedmann-Morvinski and Singer (2013).

Preparation of myelin buffer

© Timing: 30 min

Refer to materials and equipment for buffer recipes.
### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Rat Anti-mouse CD45-FITC (Clone 13/2.3) Working dilution – 1:200 | BioLegend | Cat# 147709; RRID: AB_2563541 |
| Rat Anti-mouse CD11b-BV785 (Clone M1/70) Working dilution – 1:20 | BioLegend | Cat#101243; RRID: AB_2561373 |
| Armenian hamster Anti-mouse CD11c-BV605 (Clone N418) Working dilution – 1:20 | BioLegend | Cat# 117333; RRID:AB_11204262 |
| Rat Anti-mouse Ly-6C-APC (Clone HK1.4) Working dilution – 1:100 | BioLegend | Cat# 128015; RRID:AB_1732087 |
| Rat Anti-mouse Ly-6G-PE (Clone 1A8) Working dilution – 1:100 | BioLegend | Cat#127607; RRID: AB_11204262 |
| Rat Anti-mouse CD3-APC (Clone 17A2) Working dilution – 1:100 | BioLegend | Cat#100236; RRID: AB_2561455 |
| Rat Anti-mouse CD4-BV785 (Clone GK1.5) Working dilution – 1:100 | BioLegend | Cat#100453; RRID: AB_2565843 |
| Rat Anti-mouse CD8-FITC (Clone 53-6.7) Working dilution – 1:200 | BioLegend | Cat#100726; RRID: AB_312744 |
| Rat TruStainFcX™-Fc blocker CD16/32 (Clone 93) Working dilution – 1:100 | BioLegend | Cat#101320; RRID: AB_1574975 |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| H₂NaO₄P- Sodium phosphate monobasic monohydrate | Fisher BioReagents | Cat#7558-80-7 |
| NaCl | Fisher BioReagents | Cat#7647-14-5 |
| KCl | Fisher BioReagents | Cat#7447-40-7 |
| Glucose | Formedium LTD | Cat#GLU02 |
| HNa₂O₄P- Sodium phosphate dibasic dihydrate | Fisher BioReagents | Cat#7558-79-4 |
| FBS- Fetal bovine serum | Biological Industries | Cat#04-007-1A |
| PBS | Biological Industries | Cat#02-023-1A |
| EDTA | Fisher Chemical | Cat#6381-92-6 |
| Percol™ GE Healthcare | Cat#17-0891-02 |
| CLORKETAM® 1000 - Ketamine (as Hydrochloride) 1g/10mL | Vetoquinol | Cat#LA01250 |
| SEDAXYLAN—Xylazine (as Hydrochloride) 20mg/mL | Phibro Israel | Cat#6031-1-01/04 |
| **Critical commercial assays** |        |            |
| Neural Dissociation Kit | Miltenyi Biotec | Cat# 130-092-628 |
| CD45MicroBeads | Miltenyi Biotec | Cat#130-052-301 |
| **Experimental models: organisms/strains** |        |            |
| B6.Cg-Tg(Syn1-cre)671Jxm/J mice | The Jackson Laboratory | Cat# JAX:003966; RRID:IMSR_JAX:003966 |
| **Recombinant DNA** |        |            |
| pTomo-HRas-shp53 | Friedmann-Morvinski et al. (2012) | N/A |
| pTomo-PDGF-β-shp53 | Magod et al. (2021) | N/A |
| pTomo-mock | Marumoto et al. (2009) | RRID:Addgene_26291 |
| pMDLg/pRRE | Addgene | RRID:Addgene_12251 |
| pRSV-Rev | Addgene | RRID:Addgene_12253 |
| pCMV-VSV-G | Addgene | RRID:Addgene_8454 |
| **Software and algorithms** |        |            |
| Kaluza software v2.1 | Beckman Coulter | https://www.beckman.com/flow-cytometry/software/kaluza; RRID: SCR_016182 |
| GraphPad Prism Version 8.2.1 | GraphPad Software, La Jolla, California USA | https://www.graphpad.com:443/; RRID: SCR_002798 |

(Continued on next page)
Buffers

Myelin gradient buffer

| Reagent       | Final concentration | Amount |
|---------------|---------------------|--------|
| H$_2$NaO$_4$P | 6.5 mM              | 0.78 g |
| NaCl          | 14 mM               | 8 g    |
| KCl           | 5.3 mM              | 0.4 g  |
| Glucose       | 11.10 mM            | 2 g    |
| HNa$_2$O$_4$P | ~25 mM              | Until pH is adjusted to 7.4 (~3.56 g) |
| ddH$_2$O      | N/A                 | Adjust to 1 L |
| Total         | N/A                 | 1 L    |

**Note:** Dissolve the H$_2$NaO$_4$P (Sodium phosphate monobasic monohydrate) in 900 mL double distilled H$_2$O. Add NaCl, KCl and Glucose. Adjust pH to 7.4 with HNa$_2$O$_4$P (Sodium phosphate dibasic dihydrate). Adjust volume to 1 L and filter sterilize. Keep refrigerated at 4°C for up to 3 months.

FACS buffer

| Reagent | Final concentration | Amount |
|---------|---------------------|--------|
| FBS     | 2% (v/v)            | 1 mL   |
| EDTA    | 2 mM                | 2 mL (from 50 mM solution) |
| PBS     | N/A                 | 47 mL  |
| Total   | N/A                 | 50 mL  |

**Note:** Keep refrigerated at 4°C up to 1 week.

Equipment and reagent alternatives

- GentleMACS™Dissociator (Miltenyi Biotec, Cat#130-093-235) can be replaced simply by a set of 3 glass Pasteur Pipettes in steps 3d, 3f, 3i. The Pipette’s diameter must be adjusted manually and used as further detailed in the protocol.
- In the **key resources table**, we recommend using the listed antibodies. However, different antibodies of choice can replace the ones we mention. It is of vital importance to perform optimization and antibody titration for each panel in case of using antibodies with different properties (vendor, fluorophore, clonality).
The Attune NxT Flow Cytometer was used to perform acquisition of fluorescence. Any other flow cytometer that allows fluorescent acquisition at the required optical paths can be used. Please, note that the settings are specific for the brand and model of the flow cytometer and optimization of the procedure always needs to be performed.

A Miniature Peristaltic Pump BQ50-1J was used to perform the trans-cardiac perfusion. Any other perfusion will do the work.

**STEP-BY-STEP METHOD DETAILS**

**Tumor dissection and dissociation**

© Timing: 1 h 45 min

This step describes our approach to dissect and dissociate brain tumor tissue. The expected outcome after dissociation is a high yield of mixed cell subpopulations that comprise the tumor stroma and the tumor microenvironment (TME). The applications for the resultant cells (either with or without enrichment for a particular cell subpopulation) that we mostly use are: staining for FACS analysis, RNA extraction, and cell culture.

**Note:** Sterile conditions during the protocol depend on the final procedure to be executed with the cells. However, we do advise working in a biological hood, in order to keep all the reagents sterile for further use and applications.

1. Anesthetize mice at the desired time point.
   
   **Note:** We usually use a Ketamine (100mg/mL)/Xylazine (200mg/mL) combination by preparing the mix of 3:1 ratio, giving a dose of 70 µL per 10g of mouse.

   **Note:** The desired time point depends on the researcher’s questions, hypothesis and model. In Magod et al. 3 time points were used: 2, 5 and 8 weeks. The last one corresponds with the endpoint of our R53 model, by then the mice are already showing symptoms (hunched back, difficulty to walk, loss of body weight) and are close to death.

   **Note:** We usually work with mice aged 7–8 weeks at the time of the injection. No gender preferences were considered in this study. Mice species are detailed in key resources table.

2. Dissect brain tumors.
   a. Perfuse the mice with sterile ice-cold PBS.
   b. Isolate the brain and transfer it to sterile 10cm transparent plate.
   c. Dissect the brain tumors under fluorescent microscope.

   **Note:** Tumor tissue is easily identifiable in our model as GFP+ (Figure 1). For similar approach in the dissection a model with a fluorescent reporter is needed. The transfer vector used in our system contains HRasV12 oncogene, a hairpin targeting p53, and a GFP reporter (Figure 2) (Friedmann-Morvinski et al., 2012).

   d. Transfer the tumor to a well of a 6-well plate containing cold HBSS—/—.

   **Note:** Keep plate with tumors on ice until further use.

3. Dissociate tumor tissue using a Neural Dissociation Kit (Miltenyi Biotec) according to manufacturer’s instructions (https://www.miltenyibiotec.com/US-en/products/neural-tissue-dissociation-kits.html#grep).
**Note:** For this step, we use a gentleMACS™Dissociator (MiltenyiBiotec, Cat#130-093-235)

**Note:** This protocol describes the amounts necessary for 1 mouse. Increase the amounts by the number of mice to be processed.

a. Prepare enzyme 1 mix (1900 μL buffer x + 50 μL enzyme P) according to manufacturer’s instructions and warm up in C tubes for 15 min.

b. Aspirate the HBSS−/− and cut the tumor into small pieces (2–3 mm).

c. Add enzyme 1 mix on top of chopped dissected tissue, then transfer it back to C tube. Cut end of P1000 tip to transfer chopped tissue easily.

d. Run “m_brain_01” protocol on gentleMACSTM tissue dissociator (pre-programmed by manufacturer).

e. Incubate at 37°C for 15 min.

f. Run “m_brain_02” protocol on gentleMACSTM tissue dissociator (pre-programmed by manufacturer).

g. Prepare enzyme A mix according to manufacturer’s instructions (20 μL buffer Y + 10 μL enzyme A). Then add the mix to the C tube containing tissue homogenate.

h. Incubate at 37°C for 10 min.

i. Run “m_brain_03” protocol on gentleMACSTM tissue dissociator (pre-programmed by manufacturer).

j. Add 3 mL of cold HBSS +/+ into C tube in order to stop reaction.

k. Transfer the content from the C tube into a Falcon tube through a 70 μm cell strainer.

l. Add 3 mL of HBSS +/+ into C tube to wash and repeat step j.

m. Grind the remaining tissue pieces against the mesh. Use the rubber tip of a syringe and add HBSS +/+ to help (Usually 1 mL before you start grinding and 1 mL once you finish) (Figure 3 and Methods video S1).

**Note:** For a better myelin separation we recommend performing all the further spin steps at 4°C.

n. Spin the Falcon tube at 300 g for 10 min.

o. Prepare Percoll gradient mix.

| Reagent          | Final concentration | Amount   |
|------------------|---------------------|----------|
| Myelin gradient buffer | N/A                 | 19 mL    |
| Percoll™         | 22%                 | 5.5 mL   |
| NaCl (1.5M)      | 35.9 mM             | 600 μL   |

p. Resuspend the pellet in percoll gradient mix (25 mL). Add on top 5 mL of cold PBS SLOWLY!!!

q. Spin for 20 min at 950 g. NO BREAK.

**CRITICAL:** In this step it is critical to work in a no break regime in the centrifuge, for both acceleration and break. Gradient and further myelin layer formed in centrifugation are quite fragile.

r. Aspirate the myelin layer first, then the rest of the supernatant (both PBS and Percoll layers) without disturbing the pellet (Figure 4B).

**Note:** We recommend to vacuum the myelin layer first given it is not very compact and may get carried on as the rest of the supernatant is removed.

s. Resuspend in 0.5% BSA in 1× PBS. 10 mL for each brain.
Note: We advise to continue with the rest of the protocol as soon as possible. If needed, cells can be kept on ice. However, both time and temperature of storage have effects on cell viability, as seen in Figure 5.

Alternatives: This protocol can also be achieved without a gentleMACS™ tissue dissociator. Prepare a set of 3 glass Pasteur pipettes by changing their diameter using fire. These pipettes will be used to break down the tissue homogenates by pipetting several times, mocking the action of the different protocols from the gentleMACS™ tissue dissociator. Pipettes should be used in decreasing diameter order as you move forward in the steps. Special caution to bubble formation while resuspending the homogenate. While this is a good alternative, the use of the gentleMACS™ tissue dissociator is preferred. Higher yields are possible and the protocol is more accurate and less time consuming. Bordt et al. (2020) give a detailed and very illustrative instruction on how to use shear force of successively smaller Pasteur pipets for tissue dissociation.

Immune cell-subpopulation enrichment and staining for FACS analysis

@ Timing: 3 h 30 min

In this part of the protocol we describe how to isolate the immune cell population from the whole-tumor cell suspension obtained in the previous step. Here we also describe our staining protocol for further TME composition analysis.

Note: Sterile conditions are not required since cells will be stained for FACS analysis.

4. Proceed to immune cell enrichment using CD45 murine MicroBeads (MiltenyiBiotec, Cat#130-052-301). Follow manufacturer’s instructions according to the MS Columns protocol (https://www.miltenyibiotec.com/US-en/products/ms-columns.html#gref).

Note: This protocol describes procedure for one sample. Increase the amounts by the number of samples to be processed.

a. Count the cells and divide them according to the column’s capacity. Additionally, take 1 million cells to stain with CD45 to estimate % of total CD45+ cells in the tumor.

△ CRITICAL: Take into consideration column capacity, which is 10 million magnetically labeled cells from up to 20 million total cells. For the amount of CD45+ percentage we
expect in our type of samples we advise to take up to 20 million live cells from the whole-tumor cell suspension.

b. Transfer the cells to 15mL Falcon tubes.

Note: From this step all the spin steps could be done at room temperature (RT; 15°C–25°C).

c. Spin the cells at 300g for 10 min
d. Resuspend each pellet in 90µL of 0.5% BSA in PBS. Add 10µL of CD45microbeads.
e. Incubate 15 min at 4°C.
f. Wash with 1mL of 0.5% BSA, then centrifuge sample for 10min at 300g.
g. Aspirate the supernatant and then resuspend pellet in 500µL of 0.5% BSA.
h. Set up the MS columns against the magnet stand and a falcon tube beneath it to collect CD45-cell fractions.
i. Equilibrate the MS columns with 500µL of 0.5% BSA.

△ CRITICAL: Whenever using the MS columns avoid formation of air bubbles. Also, always wait until the column is empty before proceeding to add any more liquid.

△ CRITICAL: Clumps should be removed before adding cell suspension. Any type of filter should work.

j. Filter the cell suspension through 70µm mesh.

Note: In order to remove clumps and aggregates, we first wet the mesh of a FACS’ cup tube with 0.5% BSA (250µL should work). Then we run the whole-tumor-cell suspension through it.

k. Add the filtered cell suspension to the column.
l. Wash the column 3 times with 500µL of 0.5% BSA. Collect eluate in 15mL Falcon tube.

Note: The eluate contains tumor cells and non-immune cell populations from the TME such as endothelial cells (Rouso-Nooriet al., 2021) and astrocytes. Any of the further described procedures can be done with the CD45- fraction.

m. Take the MS Column out of the magnetic stand. Quickly add 1mL of 0.5% BSA into it and immediately flush out retained fraction into a new Falcon tube labeled CD45+.

Note: The eluate contains the enriched fraction of the immune compartment of the TME.

5. Stain the enriched cells for flow cytometry analysis in order to determinate TME’s myeloid and lymphoid composition.
a. Count the cells and divide them according to the amount of antibody panels in use. Make sure to keep some cells for unstained or other necessary controls.

Note: In this protocol, we work with two panels. One built to analyze the myeloid immune TME composition and another one to analyze the lymphoid immune TME component. We use from 0.5 to 1 million cells per panel.

b. Spin the cells down in Eppendorf centrifuge for 5 min at 400g.
c. Stain with eflour-405 Live/Dead Fixable dye (eBioscience) for live-cell population.
   i. Resuspend the cell pellet in 100 µL of FACS buffer.
   ii. Add 1µL of eflour-405 Live/Dead Fixable dye per million cells.
   iii. Incubate at 4°C for 30 min.
iv. Add 1mL of FACS buffer to wash and spin the samples down for 5 min at 400g.

d. Incubate with TruStainFcXTM-Fc blocker CD16/32 (Biolegend).
   i. Resuspend the cell pellet in 100 μL of FACS buffer.
   ii. Add 1μL of Fc blocker per million cells.
   iii. Incubate at room temperature for 15 min.

e. Stain the samples for 30 min at 4°C using different combinations of anti-mouse antibodies listed in key resources table according to the table below.

f. Add 1mL of FACS buffer to wash and spin the samples down for 5 min at 400g.

g. Resuspend in 300 μL of FACS buffer.

h. Read each sample on Attune NxT Flow Cytometer.

i. Perform analysis using Kaluza software v2.1 and determine the composition of the TME according to our gating strategy (Figure 6).

### EXPECTED OUTCOMES

Following this protocol, we expect to obtain a highly enriched cell population of immune (CD45+) cells from mouse brain tumors.

The amount of CD45+ cells present in the tumors depend on several factors, including the type of model you are working with and the time point chosen for the TME analysis.

For our R53 model, where the percentage of CD45+ cells at the endpoint average is 20%–25% (Figure 7A), we usually get between 4 to 5 million highly enriched (percentage of purity ~98%, (Figure 7B)) immune cells.

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**Figure 2. Schematic representation of the lentiviral vector used as an example in this protocol**

The schemes show the pTomo lentiviral vector constructs with HRasV12 (Friedmann-Morvinski et al., 2012) in both their “off” state and the activated state after the CRE-recombinase processing.

| Marker | Fluorophore | Company     | Final dilution | Volume (for 1 million cells in 100μL) |
|--------|-------------|-------------|----------------|---------------------------------------|
| *CD45  | FITC        | BioLegend   | 1:200          | 0.5 μL                                |
| *CD11b | BV785       | BioLegend   | 1:20           | 5 μL                                  |
| *CD11c | BV605       | BioLegend   | 1:20           | 5 μL                                  |
| *LY6G  | PE          | BioLegend   | 1:100          | 1 μL                                  |
| *LY6C  | APC         | BioLegend   | 1:100          | 1 μL                                  |
| **CD3  | APC         | BioLegend   | 1:100          | 1 μL                                  |
| **CD4  | BV785       | BioLegend   | 1:100          | 1 μL                                  |
| **CD8  | Alexa Fluor-488 | BioLegend | 1:200      | 0.5 μL                                |

*Myeloid Panel

**Lymphoid Panel
We developed gating strategies for analysis of myeloid and lymphoid cell composition in the TME, which can be visualized in (Figure 6).

Composition of TME varies within each model, with the time point being considered and with experimental conditions. Here, we merely illustrate results at the endpoint of our R53 model (Figure 8). Examples of said TME variations can be seen in Magod et al. (2021).

LIMITATIONS
This protocol was optimized to work with brain tissue and brain tumors of any kind. While the antibody panel was used to study the immune composition of other organs such as spleen and bone marrow, the dissociation and enrichment part of the protocol are restricted to brain tissue. Working with any other organs will require optimization of a new protocol.

Figure 3. Use of plunger rubber tip to finish grinding digested tissue against the mesh

Figure 4. Myelin separation
(A) Layering of PBS on top of Percoll cell-suspension.
(B) Myelin layer is clearly visible and easily removable after 20 min spin.
The panels were designed to distinguish most of the myeloid cell types in brain TME. For these cells, very reliable results are obtained and there is room for even further characterization by adding other antibodies that can work in the panel configuration. However, given the infiltration of cells from the lymphoid lineage is so poor, we are limited to deep characterization. Enrichment with CD3 magnetic beads and further pooling of the cell fractions obtained could be an alternative for a deeper lymphoid-related characterization.

Additionally, this protocol is time consuming and the number of samples processed at once depends on the expertise of the researcher. The estimated time describes in the protocol is based on the processing of 2 samples at once by one person.

**TROUBLESHOOTING**

**Problem 1**
Mice showing no symptoms of bearing brain tumors *(before you begin—initiation of brain tumors)* after the expected time period *(for H-RasV12-shp53 the latency is 8–10 weeks)*.

**Potential solution**
This means that very few or no cells were infected at all after injecting the virus. One possibility is that the needle was clogged and no virus was actually injected. Another problem could have been the virus titer was too low given a mistake or problem in the titer protocol. A solution to confirm if the cells were successfully infected, a representative mouse from the group of injected mice can be euthanized 7 days after the injection and the brain sections can be analyzed for the presence of GFP-positive cells *(or reporter present in the lentiviral vector)*. If no cells are infected we recommend checking if the needle is clean and working correctly, if that is the case we would proceed to make new virus from the starting point.

**Problem 2**
Low amounts of CD45 population in early time points *(end of step 4)*.

**Potential solution**
This problem is most likely due to low infiltration of immune cells into the small tumors present at early time points. In this case we recommend pooling CD45+ fractions from several animals in order to reach the necessary cell amounts to do the staining.
Problem 3
Low purity (i.e., <90%) of immune cells after enrichment (step 4).

Figure 6. Gating strategies for flow cytometry analysis for both panels
In the myeloid panel, some of the gating is happening in parallel from the same parental population, indicated by appearing next to each other. Subsequent gating is separated by an arrow and appears under the parental population.
Potential solution
There are several reasons why this could be happening, and usually they affect both the yield and purity of the immune fraction. First, if the cell count is underestimated (refer to step 4c in the step-by-step method details section), the number of cells added to the column may be over its capacity and may result in clogging. Second, excessive number of clumps may not only clog the column but also may bind CD45MicroBeads in a non-specific way. If there is still clumps after filtering through a 70 μm, a 40 μm filter could be used. Third, non-specific binding could have happened if the step 4e was performed for too long at a too high temperature. It is critical to be careful about temperature and time in this step.

Problem 4
Low viability of isolated cells (steps 4 and 5).

Potential solution
The timing is vital to keep high cell viability. Substantial delays (>3h) in sample processing can result in a diminished cell viability. Be cautious with temperature, pH and composition of buffers and work-solutions. Any big deviation, such as time taken for start staining and cell storage conditions (Figure 5), will affect cell viability.
Problem 5
No staining (step 5).

Potential solution
Problems with the staining could have several origins. We do recommend before working with the antibody panel to use single stains to find the fitting antibody dilution and the appropriate photo-multiplier tubes (PMT) voltage in the flow cytometer that gives the clearest separation. If there is any problem with the antibodies they should be detected at this stage. In that case using a new clone or a new antibody from other company is suggested. Another reason for lacking of staining could be related to epitope damage by digestion enzymes used on the protocol (step 3). A potential solution to this problem should involve changing the enzyme in use.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dinorah Friedmann-Morvinski (dino@tauex.tau.ac.il)

Materials availability
This study did not generate new unique reagents.
Data and code availability
This study did not generate/analyze datasets/code.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.101106.

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AUTHOR CONTRIBUTIONS
D.F.-M. and P.M. designed the research. I.M., D.S., and P.M. performed the experiments. I.M., D.S., P.M., and D.F.-M. analyzed the results. I.M., D.S., and D.F.-M. wrote the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES
Bordt, E.A., Block, C.L., Petrozziello, T., Sadri-Vakili, G., Smith, C.J., Edlow, A.G., and Bilbo, S.D. (2020). Isolation of microglia from mouse or human tissue. STAR Protoc. 1, 100035.

Friedmann-Morvinski, D., and Singer, O. (2013). Overexpression models: lentiviral modeling of brain cancer. Curr Protoc. Mouse Biol. 3, 121–139.

Friedmann-Morvinski, D., Bushong, E.A., Ke, E., Soda, Y., Marumoto, T., Singer, O., Ellisman, M.H., and Verma, I.M. (2012). Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science 338, 1080–1084.

Magod, P., Mastandrea, I., Rousso-Noori, L., Agemy, L., Shapira, G., Shimron, N., and Friedmann-Morvinski, D. (2021). Exploring the longitudinal glioma microenvironment landscape uncovers reprogrammed pro-tumorigenic neutrophils in the bone marrow. Cell Rep. 36, 109480.

Marumoto, T., Tashiro, A., Friedmann-Morvinski, D., Scadeng, M., Soda, Y., Gage, F.H., and Verma, I.M. (2009). Development of a novel mouse glioma model using lentiviral vectors. Nat. Med. 15, 110–116.

Rousso-Noori, L., Mastandrea, I., Talmor, S., Waks, T., Globerson Levin, A., Haugas, M., Teesalu, T., Alvarez-Vallina, L., Eshhar, Z., and Friedmann-Morvinski, D. (2021). P32-specific CAR T cells with dual antitumor and antiangiogenic therapeutic potential in gliomas. Nat. Commun. 12, 3515.