Supplementary Material

Supplementary Methods

Bone marrow-derived macrophage isolation and culture

C57/BL6 mice 8-12 weeks of age were euthanized via CO_2 asphyxiation. The whole, intact femur and tibia were stripped of muscle and collected in sterile Dulbecco's phosphate-buffered saline (DPBS). Both ends of the bone were cut and the marrow was flushed into a clean petri dish using DPBS supplemented with 4% bovine serum albumin (BSA) (ThermoFisher, 15260037), heparin (STEMCELL, #07980), DNase I (Sigma-Aldrich, 11284932001), and penicillin/streptomycin (Fisher, SV30010). The resulting mixture was briefly triturated and passed through a 70 µm cell strainer. The cells were plated in a 15 cm non-cell culture-treated plate for a six-day differentiation in 15 ml of DMEM (ThermoFisher, 10569010) with 10% fetal bovine serum (FBS; HyClone, SH30396.03) and 40 ng/ml M-CSF (Peprotech, 315-02). An additional 15 ml of media with M-CSF was added after day three. The cells were harvested for experimentation via 10-minute incubation in DPBS with 5mM EDTA on ice. Cells were plated in 6-well plates in 2 ml of culturing media with macrophage colony-stimulating factor (M-CSF) at a concentration of 20 ng/ml for experimentation.

Microglia isolation and culture

Whole brains were extracted from p0-p3 C57/BL6 pups using sterile instruments, followed by three consecutive washes in DPBS. Brains were then digested in 0.5% trypsin (ThermoFisher, 15400054) and DNase I (Sigma-Aldrich, 11284932001) followed by mechanical dissociation and filtration through a 70 µm cell strainer. The filtrate was plated in flasks pre-coated with poly-D-lysine (Sigma-Aldrich, P6407) in DMEM (ThermoFisher, 10569010) with 10% FBS (HyClone, SH30396.03) and M-CSF (Peprotech, 315-02) at a concentration of 40 ng/ml. The cells grew until astrocytes reached confluence on the bottom of the flask and microglia were observed ballooning off the surface. During this time, additional M-CSF was periodically added. Microglia were collected by gently rinsing the back of the flask with culturing media and were plated in 6-well plates in 2 ml of the culturing media with M-CSF at a concentration of 40 ng/ml for experimentation.

Bone marrow-derived macrophage and microglia immunofluorescent validation

BMDM and MG were plated in 24-well plates on sterile coverslips and allowed to adhere overnight. The cells were then briefly fixed in 4% PFA, washed, and blocked. The cells were stained for IBA1 (Wako) and visualized with donkey anti-rabbit Alexa Fluor 647nm (Abcam, ab150075). Imaging was performed with Fluoview FV1000 confocal microscope (Olympus).

Bone marrow-derived macrophage and microglia flow validation

BMDM and MG were isolated from *Cx3cr1*$^{+/GFP}$/*Ccr2*$^{+/RFP}$ mice as described. Following isolation, an aliquot of cells was taken, and red blood cells were lysed in RBC lysis buffer (BioLegend, 420301). The remainder of the cells were differentiated as described. The aliquot of cells was then washed, blocked, and stained with CD11b PerCP-Cy5.5 (BioLegend, 101227). Cells were analyzed on an LSRII flow cytometer and CD11b, CCR2, and CX3CR1 positivity were quantified. This procedure was repeated with the remainder of the cells after culturing and differentiation.

Apoptosis assay

BMDM and MG were isolated and cultured as described. Cells were treated with vehicle solution, dexamethasone (Sigma-Aldrich, D4902), or lipopolysaccharide (LPS; Sigma Aldrich, L5293) to induce apoptosis. Following a six-hour incubation, cells were collected via trypsinization and allowed to rest in culturing media for thirty minutes. The cells were then assessed with a propidium iodide and annexin V apoptosis assay (ThermoFisher, V13242) according to the manufacturer's protocol.

Mice

Ntv-a/Cdkn2a$^{-/-}$ and *Ntv-a* mice in the age range of 8-16 weeks were used for experiments as previously described (Hambardzumyan *et al.*, 2009; Herting *et al.*, 2017). The former is a mixed genetic background, while the latter is a C57/BL6 background. All animals were housed in a climate controlled, pathogen-free facility with *ad libitum* food and water under a 12-hour light/dark cycle. Additionally, the previously-described *Il1r1*$^{-/-}$, *Il1b*$^{-/-}$, and *Il1a/b*$^{-/-}$ mice were crossed with the *Ntv-a* mice to generate *Ntv-a/Il1r1*$^{-/-}$ mice, *Ntv-a/Il1b*$^{-/-}$ mice, and *Ntv-a/Il1a/b*$^{-/-}$ mice (Glaccum *et al.*, 1997; Horai *et al.*, 1998). For validation of MG and BMDM cultures, *Cx3cr1*$^{+/GFP}$/*Ccr2*$^{+/RFP}$ mice were utilized as previously described (Chen *et al.*, 2017). Genotypes were confirmed by Transnetyx.

Virus production and tumour generation

DF-1 cells (ATCC, CRL-12203) were purchased and grown at 39°C according to supplier instructions. Cells were transfected with RCAS PDGFB-HA or RCAS shp53-RFP using a Fugene 6 transfection kit (Roche, 11814443001) according to the manufacturer's instructions. DF-1 cells ($4x10^4$) were stereotactically delivered with a Hamilton syringe equipped with a 30-gauge needle for tumour generation. For the *Ntv-a/Cdkn2a*$^{-/-}$ background mice, RCAS PDGFB-HA was delivered alone. RCAS PDGFB-HA and RCAS shp53-RFP were co-injected at a 1:1 mixture for tumour generation in *Ntv-a* background mice. The target coordinates were in the right-frontal striatum at AP-1.7mm and right-0.5mm from bregma; depth-2mm from the dural surface (Franklin and Paxinos, 2013). Prior to surgery, mice were anaesthetized with intraperitoneal injections of ketamine (McKesson, 494158) (0.1 mg/g) and xylazine (Akorn) (0.01 mg/g). A local injection of marcaine (McKesson, 57199) was delivered for pre-surgical analgesia followed by two post-operative administrations of

buprenorphine (McKesson, 1013922) within 24 hours of completion. Mice were continually monitored for signs of tumour burden and were sacrificed upon observation of endpoint symptoms including head tilt, lethargy, seizures, and excessive weight loss.

Immunohistochemistry

Prior to staining, 5 µm-thick sections were cut on a microtome (Leica). Sections were deparaffinized and antigen unmasking was performed with a citrate solution (Vector, H-3300) according to the manufacturer's instructions. CD31 (Dianova, DIA-310) was stained by hand, while IBA1 (Wako) was stained using an automated system (Ventana). Quantification of percent positive area and average vessel size was performed blinded to treatment condition using FIJI as previously described (Herting *et al.*, 2017).

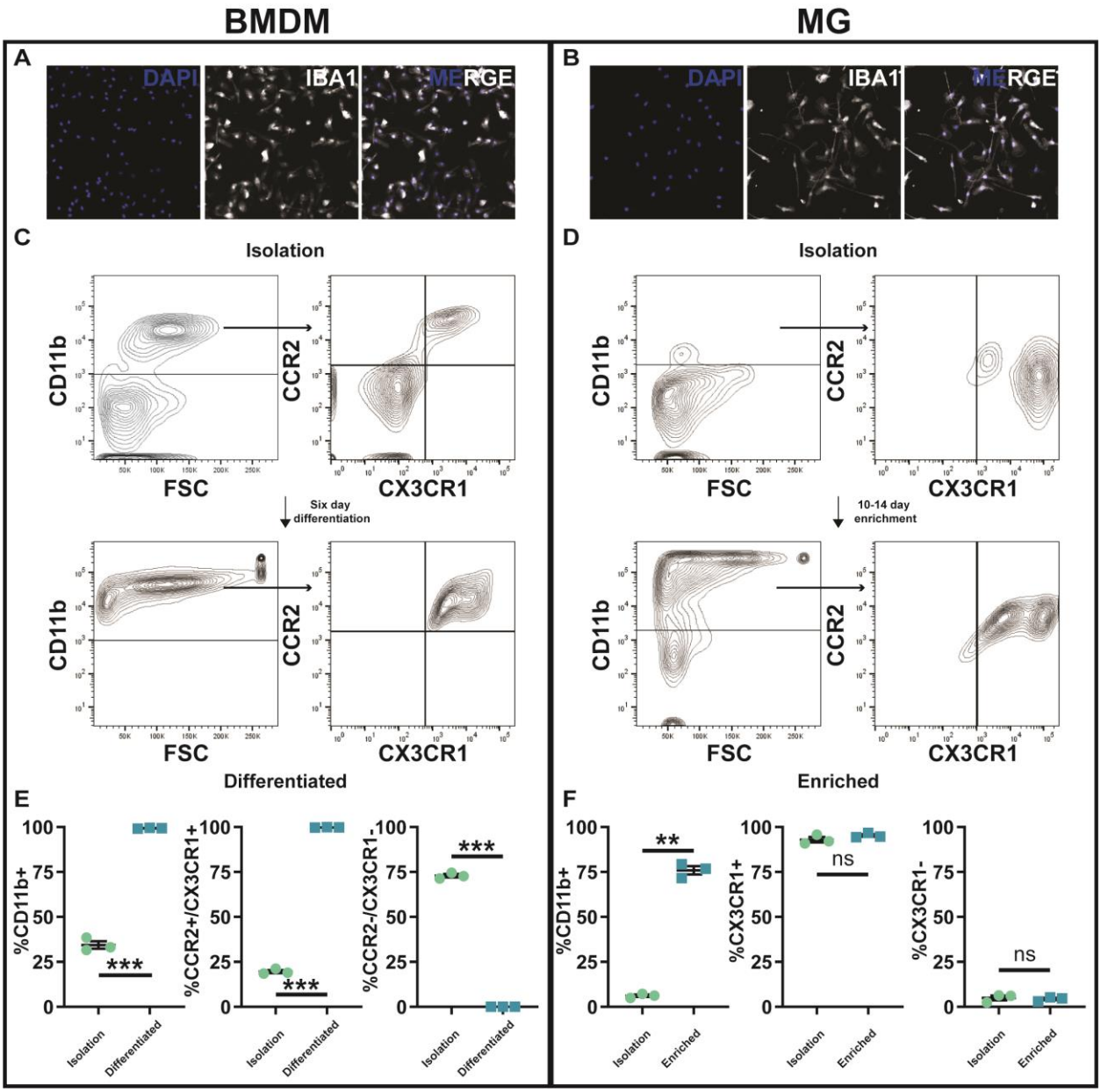
Hoechst dye leakage assay

Mice were anaesthetized with ketamine and xylazine prior to this procedure. Five minutes prior to euthanasia 50 µl of Hoechst 33342 (Sigma, #H6024) was injected intravenously through the retroorbital sinus. Animals were then perfused with 4% paraformaldehyde in PBS and the brains were extracted. The brains were embedded in O.C.T. (VWR, 25608-930) and cut into 30 µm sections on a cryostat (Leica). Sections were permeabilized with 0.3% Triton X-100 in PBS and stained with propidium iodide (ThermoFisher, P1304MP) for 30 min at 37 °C. Sections were then washed with PBS before mounting with 70% glycerol.

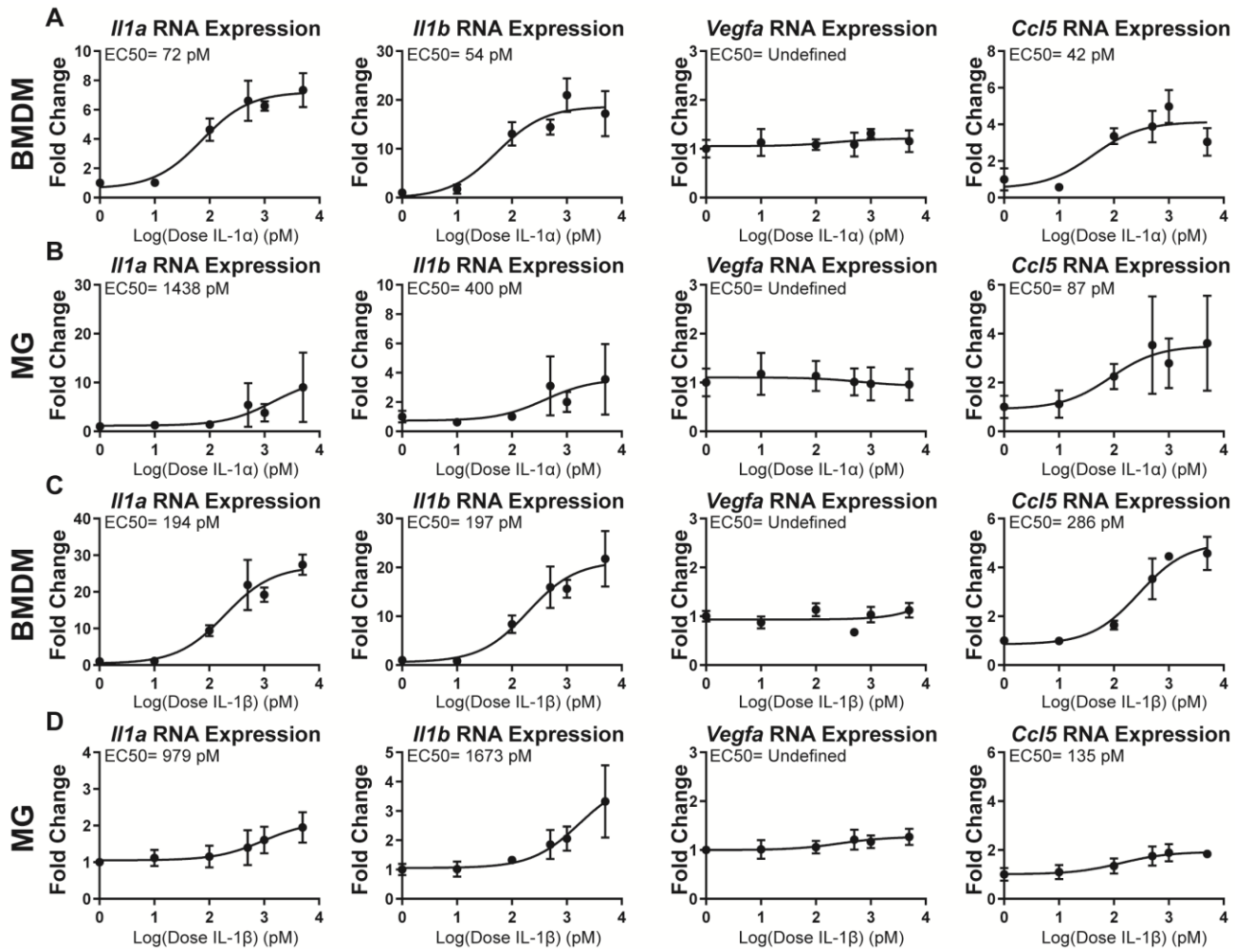
MRI image acquisition

We employed a 9.4 T, 20 cm horizontal bore Bruker magnet interfaced to an Avance console (Bruker) and equipped with an actively shielded gradient set. This set up has an inner diameter of 11 cm, a maximum gradient strength of 100 mT/m, and a rise time of 110 ms. A two-coil actively decoupled imaging set-up was employed with a 3 cm surface coil used to receive the signal generated by a 7.2 cm diameter volume transmission coil. The receiving coil was positioned over the cortical and subcortical areas of interest to maximize the signal-to-noise ratio. Mice were imaged while anaesthetized with 1.5-2% isoflurane (Baxter, 1001936060) and held in a custom-made cradle. Body temperature and respiration rate were continually monitored throughout the experiment with an animal physiological monitoring system (SA Instruments). The mouse head was imaged in the axial orientation using a T2-weighted fast spin-echo RARE sequence with a TR of 3.5 seconds, a TE of 50 ms, a RARE factor of 8, a NEX value of 24, a field of view of 3 x 2 cm, and a slice thickness of 0.7 mm generating an in-plane resolution of 117x156 mm. During the procedure, 14 slices were obtained.

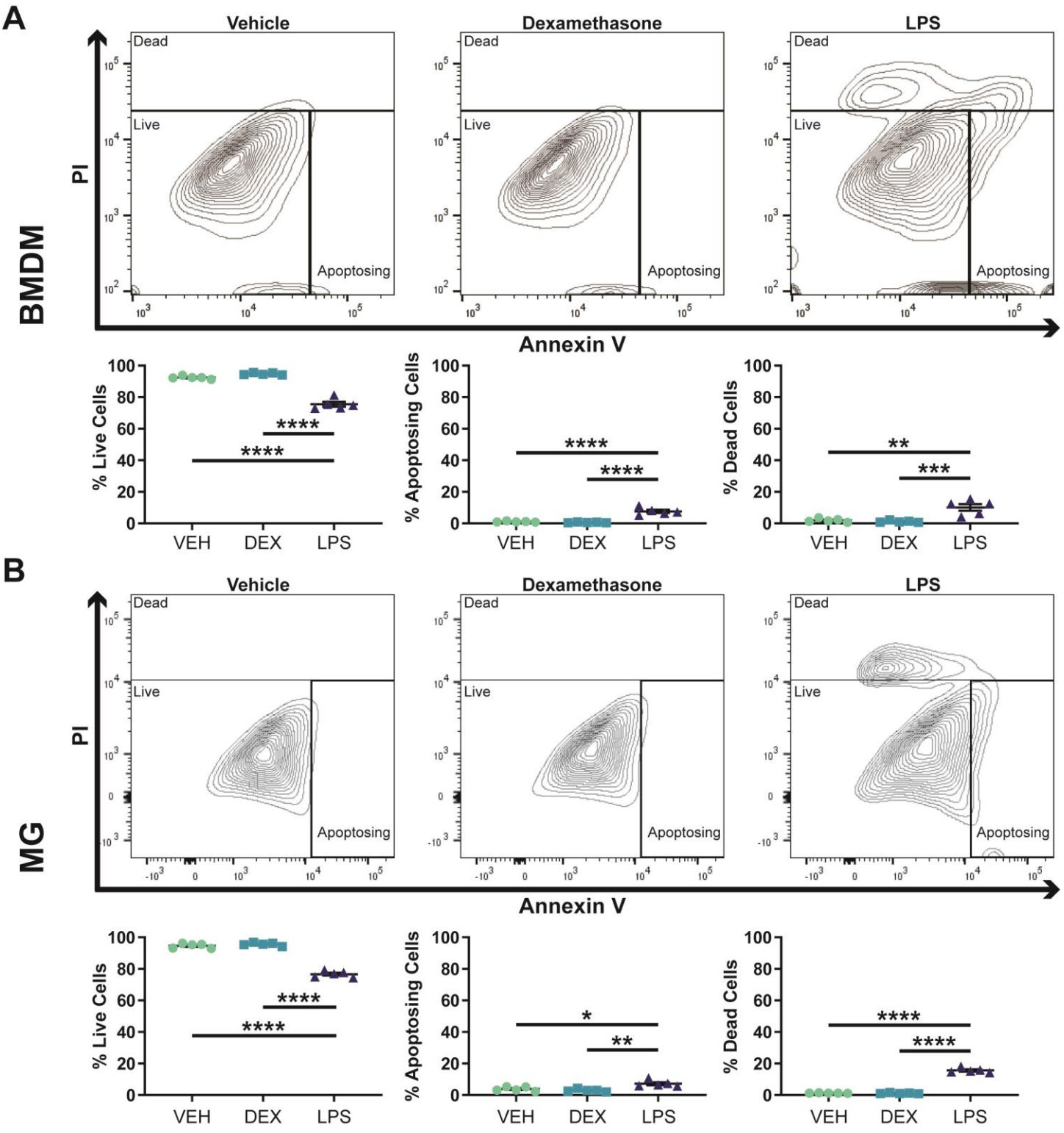
Supplementary Figures



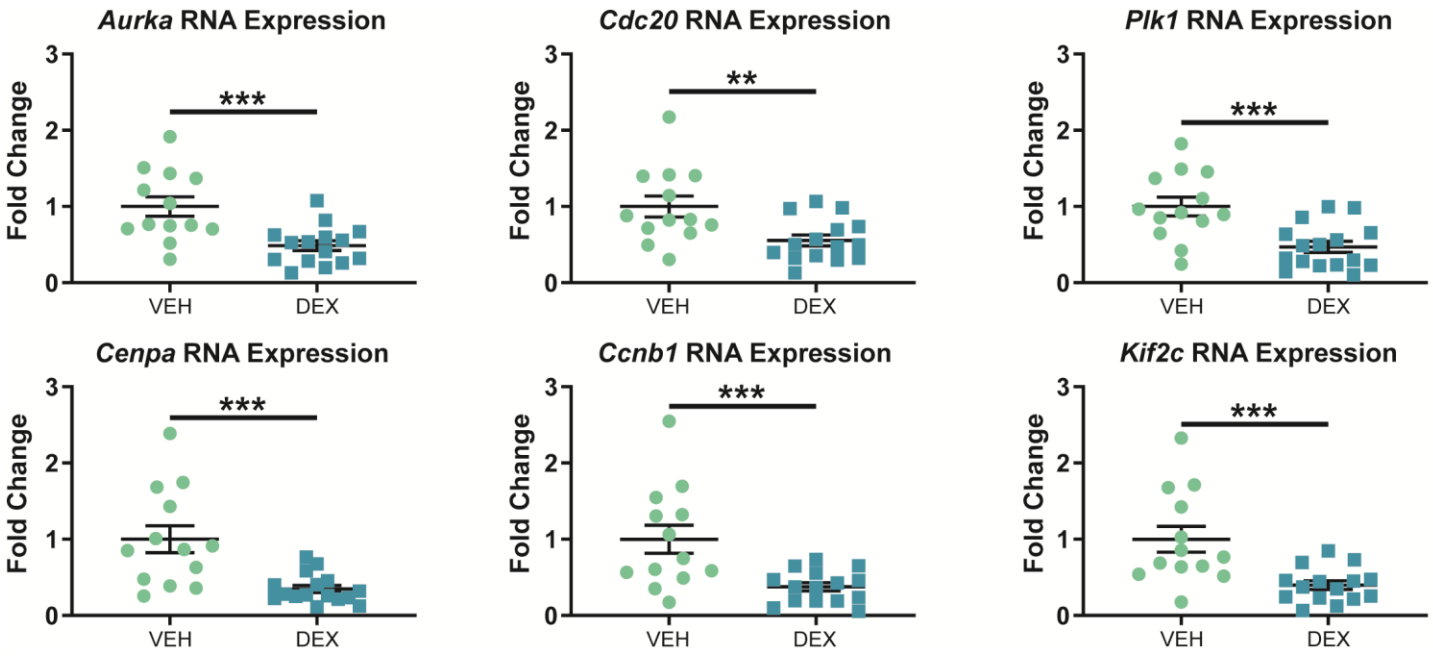
Supplementary Figure 1. Differentiation of primary cells in media supplemented with M-CSF produces pure BMDM and MG cultures. (A) Representative immunofluorescent staining of differentiated BMDM for the pan-macrophage marker IBA1. (B) Representative immunofluorescent staining of enriched MG for the pan-macrophage marker IBA1. (C) Representative flow cytometry plots of BMDM cultures from *Cx3cr1*$^{+/GFP}$*/Ccr2*$^{+/RFP}$ mice on the day of isolation (top) and terminal differentiation (bottom). (D) Representative flow cytometry plots of MG cultures from *Cx3cr1*$^{+/GFP}$*/Ccr2*$^{+/RFP}$ mice on the day of isolation (top) and following enrichment (bottom). (E) Quantification of the BMDM flow data (N=3). (F) Quantification of the MG flow data (N=3). Two-tailed Student's t-test, ns=not significant, ***P*<0.01, ****P*<0.001.



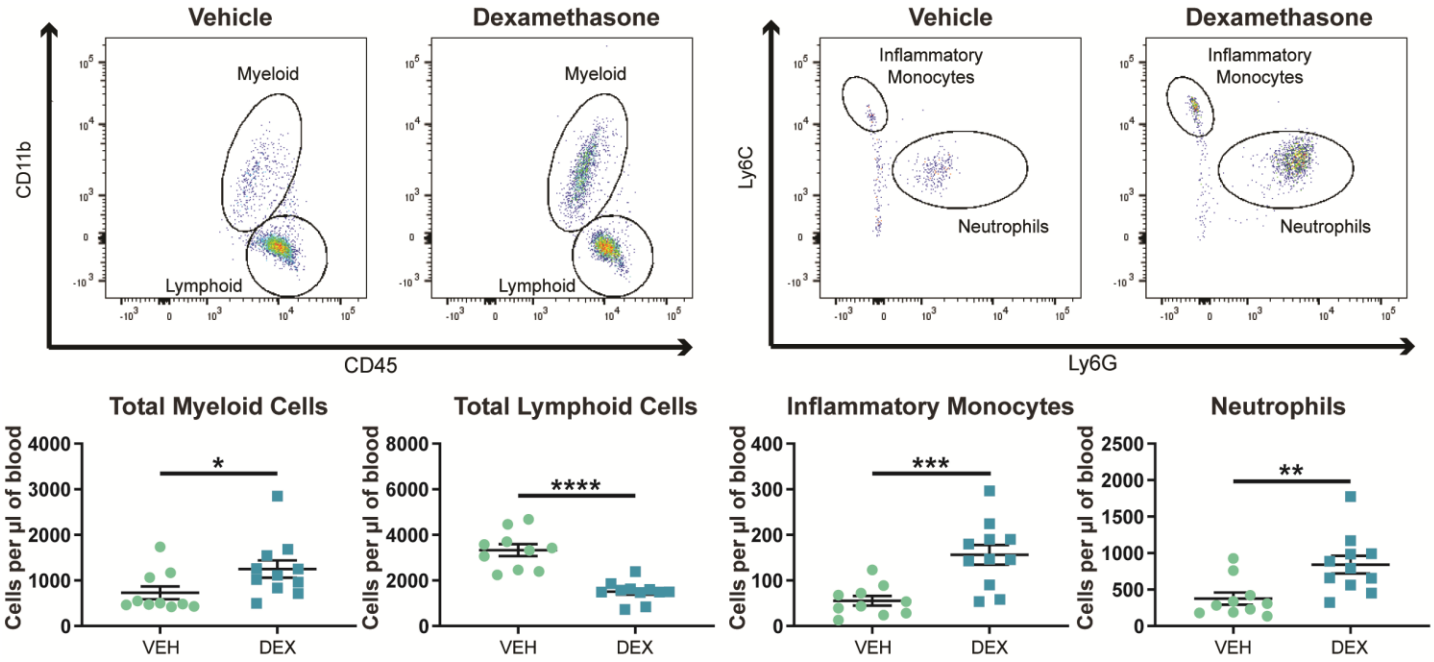
Supplementary Figure 2. IL-1α and IL-1β dose response experiments in primary BMDM and MG reveal effective doses for downstream analysis. Quantitative-PCR analysis of *Il1a*, *Il1b*, *Vegfa*, and *Ccl5* in BMDM stimulated with varying doses of IL-1α (A) or IL-1β (C) (N=3). Quantitative-PCR analysis of *Il1a*, *Il1b*, *Vegfa*, and *Ccl5* in MG stimulated with varying doses of IL-1α (B) or IL-1β (D) (N=3). EC50 values were determined via three parameter non-linear regression and resulting trendlines are illustrated.



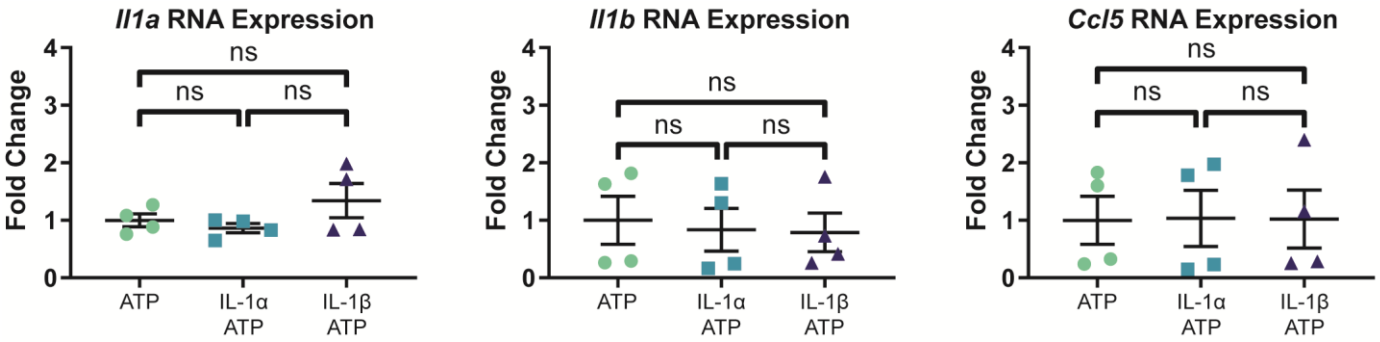
Supplementary Figure 3. Dexamethasone is non-toxic to primary murine BMDM and MG. (A) Representative flow cytometry plots and quantification for BMDM stimulated with vehicle, dexamethasone, or LPS and assessed with an annexin V and propidium iodide-based apoptosis assay (N=5). (B) Representative flow cytometry plots and quantification for MG stimulated with vehicle, dexamethasone, or LPS and assessed with an annexin V and propidium iodide-based apoptosis assay (N=5). One-way ANOVA, **P*<0.05, ***P* <0.01, ****P*<0.001, *****P*<0.0001.



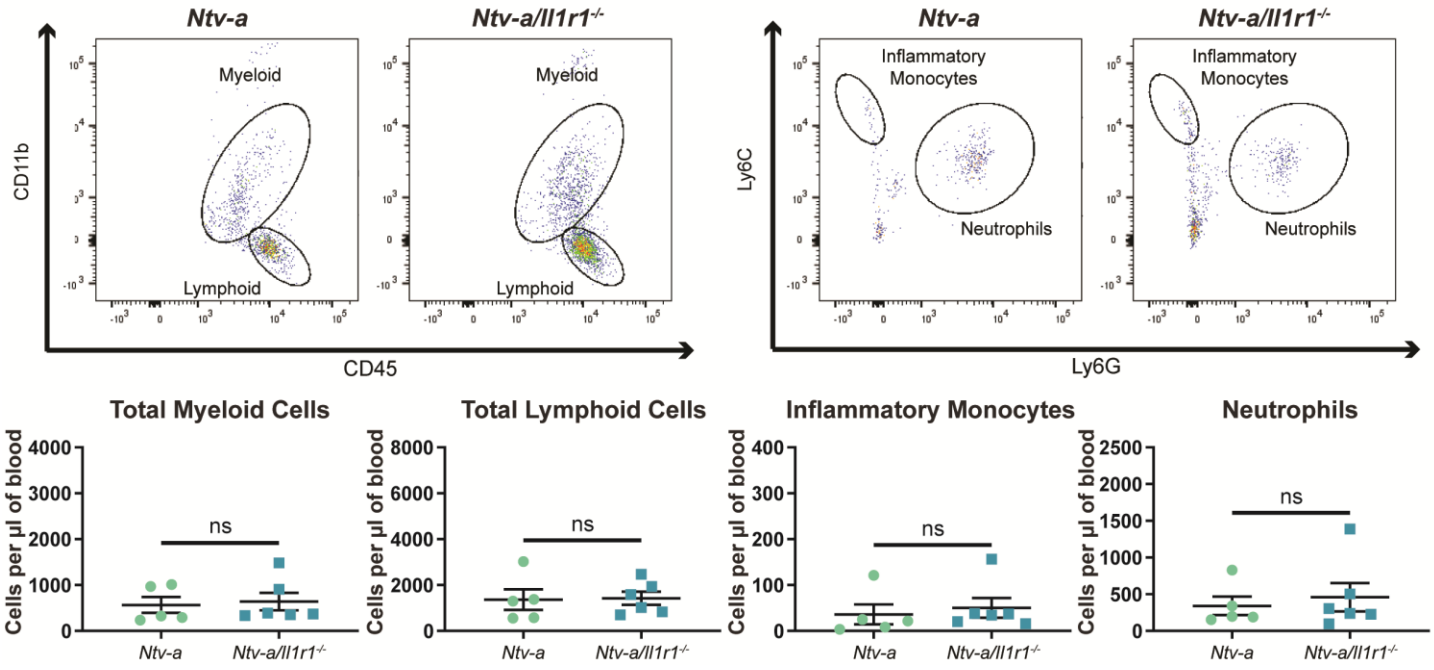
Supplementary Figure 4. Validation of the *in vivo* administration of dexamethasone by quantitative-PCR analysis of previously-reported markers. Quantitative-PCR for the markers *Aurka*, *Cdc20*, *Plk1*, *Cenpa*, *Ccnb1*, and *Kif2c* in tumour samples from mice following vehicle (N=13) or dexamethasone (N=15) treatment. Two-tailed Student's t-test, ***P*<0.01, ****P*<0.001.



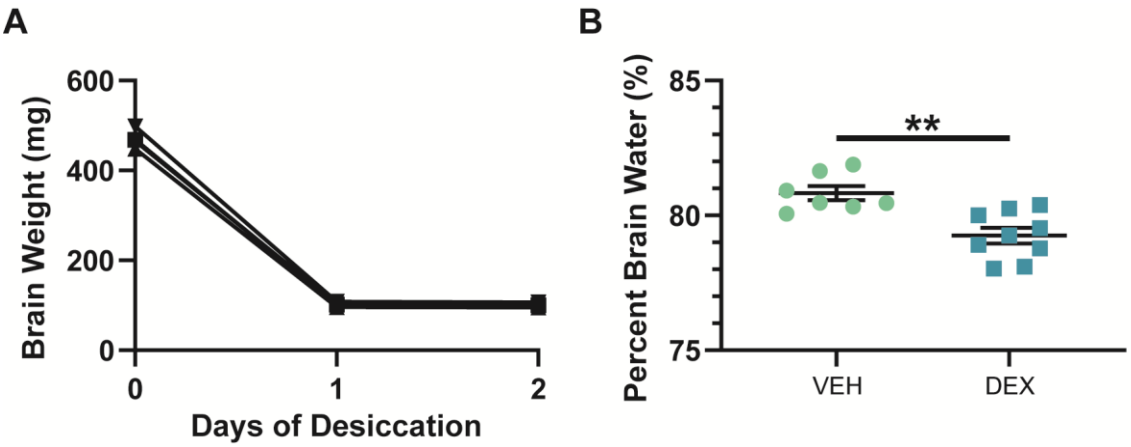
Supplementary Figure 5. Administration of dexamethasone to naïve C57/BL6 mice causes a significant reduction in circulating lymphoid cells and a significant increase in circulating myeloid cells. Representative flow cytometry plots for total circulating myeloid cells, lymphoid cells, inflammatory monocytes, and neutrophils in naïve C57/BL6 mice treated with vehicle (N=10) or dexamethasone (N=11). Two-tailed Student's t-test, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.



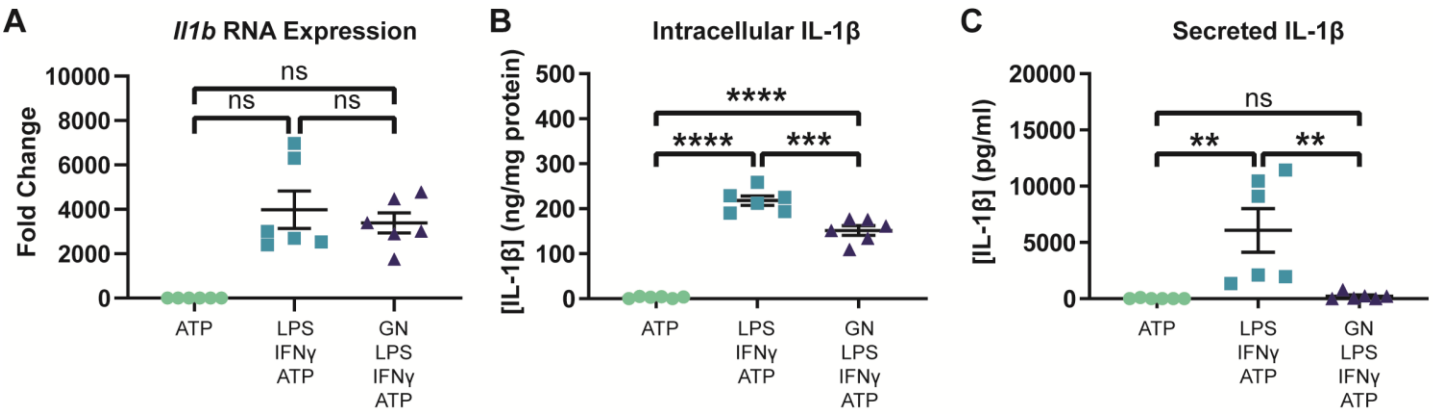
Supplementary Figure 6. Stimulation of *Il1r1*$^{-/-}$ BMDMs with IL-1α and IL-1β produces no change in the expression of the IL-1 responsive genes *Il1a*, *Il1b*, or *Ccl5*. BMDM were isolated from *Il1r1*$^{-/-}$ mice and stimulated with IL-1α and IL-1β (N=4). Quantitative-PCR was performed to assess the expression levels of the IL-1 responsive genes *Il1a*, *Il1b*, and *Ccl5*. One-way ANOVA, ns=not significant.



Supplementary Figure 7. Tumour-bearing *Ntv-a* and *Ntv-a/Il1r1*$^{-/-}$ mice display no differences in circulating immune cell profile. Representative flow cytometry plots and quantification of myeloid cells, lymphoid cells, inflammatory monocytes, and neutrophils in the blood of tumour-bearing *Ntv-a* (N=5) and *Ntv-a/Il1r1*$^{-/-}$ (N=6) mice at endpoint. Two-tailed Student's t-test, ns=not significant.



Supplementary Figure 8. Dexamethasone reduces oedema formation as assessed by a wet/dry tissue weight assay. (A) Brain weight of naïve C57/BL6 mice (N=5) after 24- and 48-hours of desiccation. (B) Comparison of percent brain water between vehicle- (N=7) and dexamethasone-treated (N=9) tumour-bearing *Ntv-a/Cdkn2a*$^{-/-}$ mice. Two-tailed student's t-test. ***P*<0.01.



Supplementary Figure 9. Gallium nitrate inhibits the synthesis and secretion of IL-1β protein from primary BMDM. (A) Quantitative-PCR for *Il1b*, (B) ELISA for intracellular IL-1β, and (C) ELISA for secreted IL-1β in BMDM stimulated with LPS and IFNγ with or without gallium nitrate pretreatment. One-way ANOVA, ns=not significant, ***P*<0.01. ****P*<0.001, *****P*<0.0001.