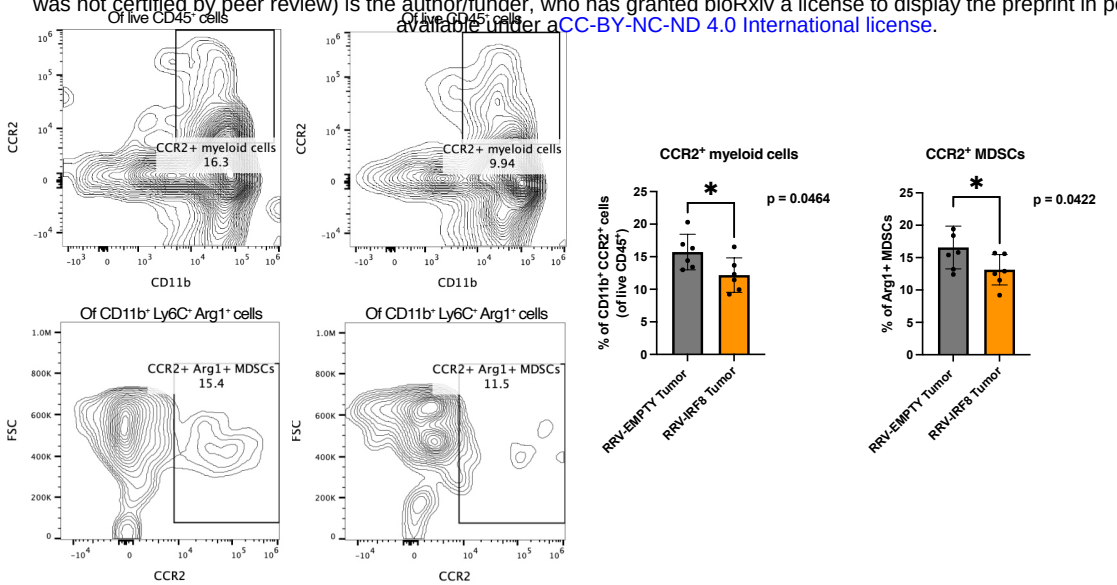
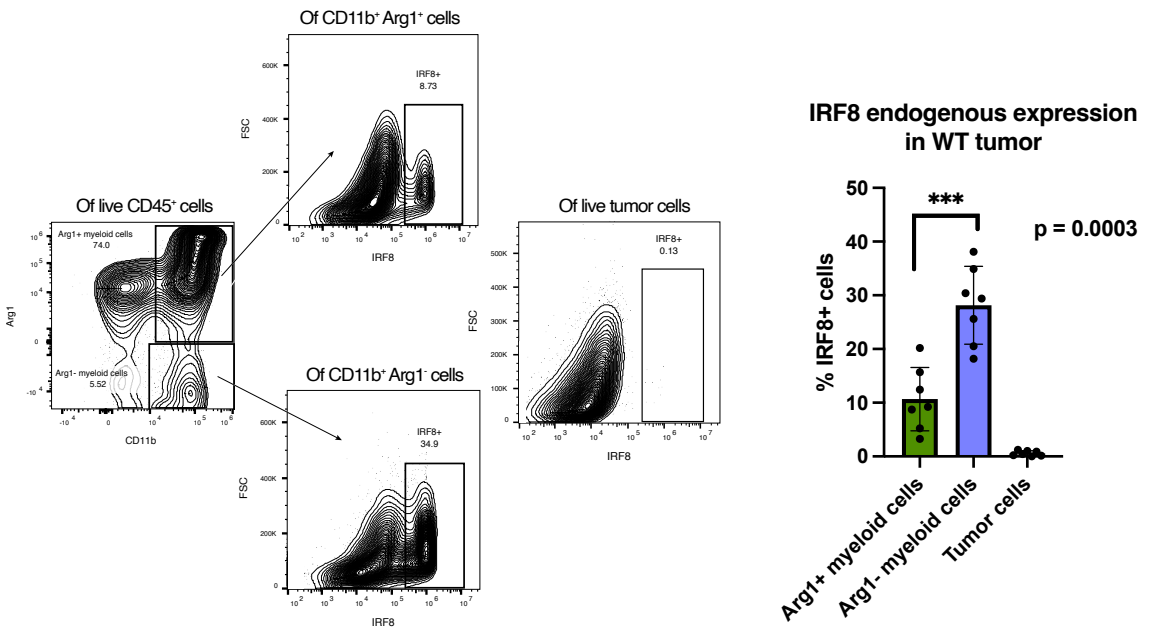
SUPPLEMENTARY FIGURE 1

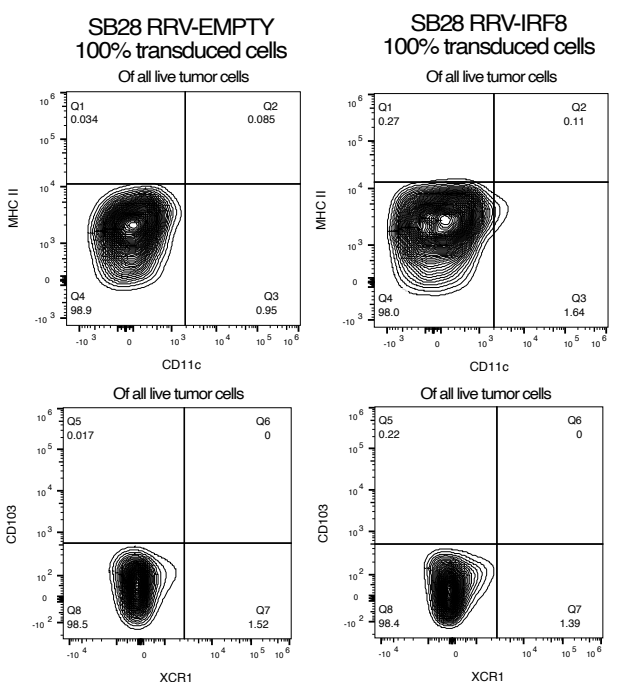
A

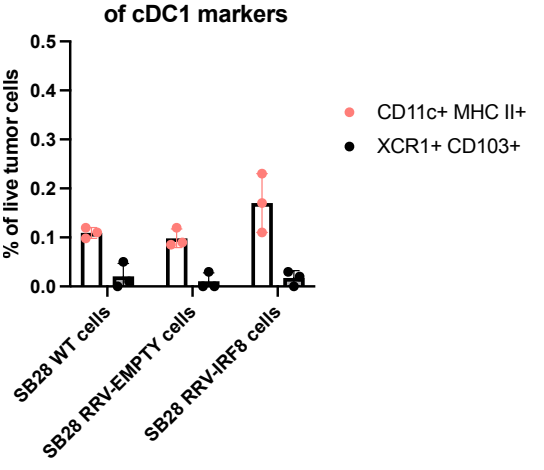


B



C

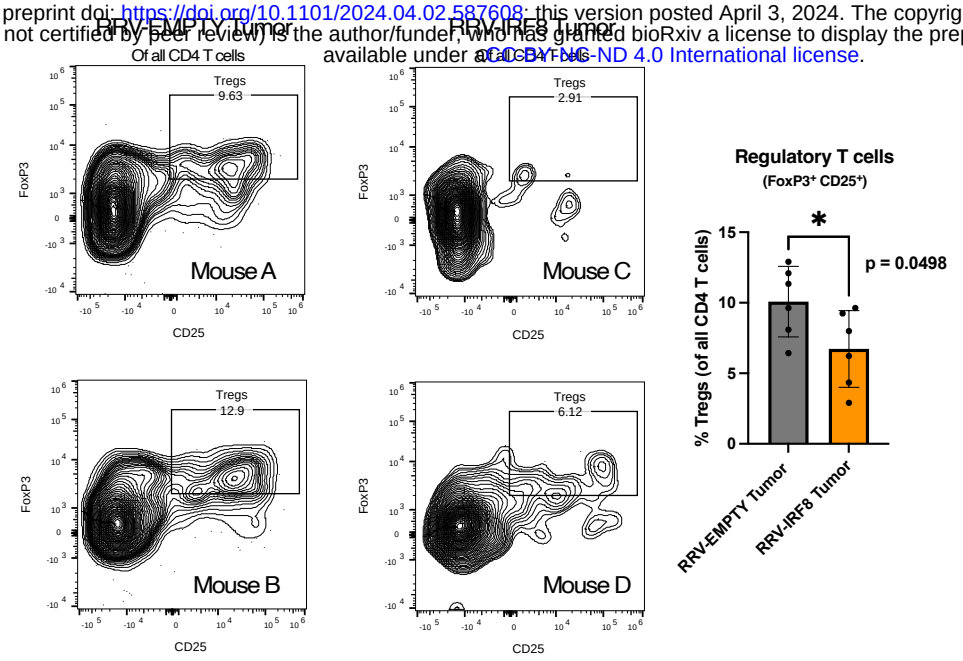




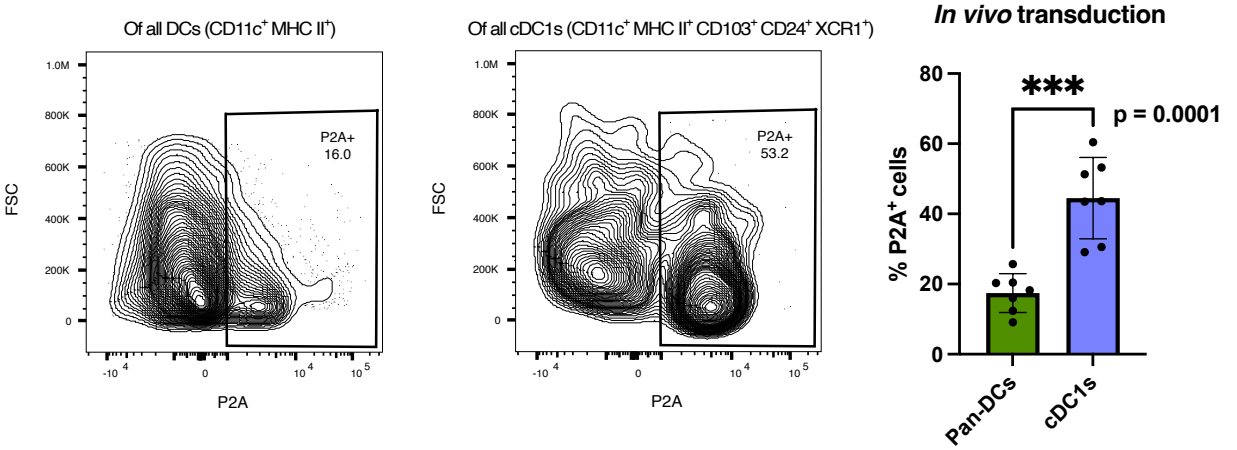
Supplementary Figure 1. (A) CCR2 expression in myeloid (CD11b+) cells and M-MDSCs (Ly6C+ Arg1+) from intracerebral SB28 RRV-EMPTY or RRV-IRF8 tumors. Bars represent the mean of 6 biological replicates **(B)** Endogenous IRF8 expression in CD11b+ Arg1+, CD11b+ Arg1-, and tumor cells in intracerebral SB28 WT tumors. Bars represent the mean of 6 biological replicates. **(C)** *In vitro* expression of cDC1-associated markers in SB28 WT, SB28 RRV-EMPTY 100% transduced, and SB28 RRV-IRF8 100% transduced cell lines. Bars represent the mean of 3 technical replicates.

SUPPLEMENTARY FIGURE 2

A



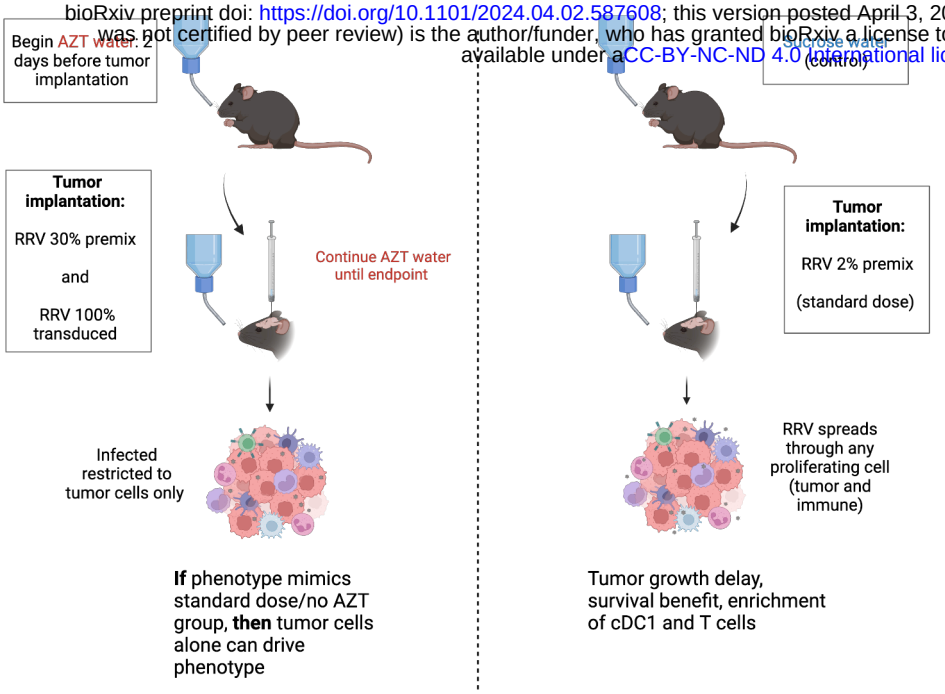
B



Supplementary Figure 2. (A) T regulatory cell infiltration in SB28 RRV-EMPTY or RRV-IRF8 tumors. Bars represent the mean of 6 biological replicates. **(B)** *In vivo* transduction efficiency of pan-DCs (CD11c+ MHC II+) or cDC1s (CD11c+ MHC II+ CD103+ CD24+ XCR1+). Bars represent the mean of 7 biological replicates.

SUPPLEMENTARY FIGURE 3

A



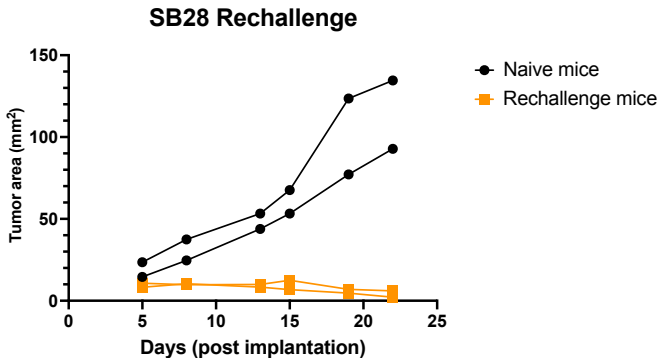
If phenotype mimics standard dose/no AZT group, **then** tumor cells alone can drive phenotype

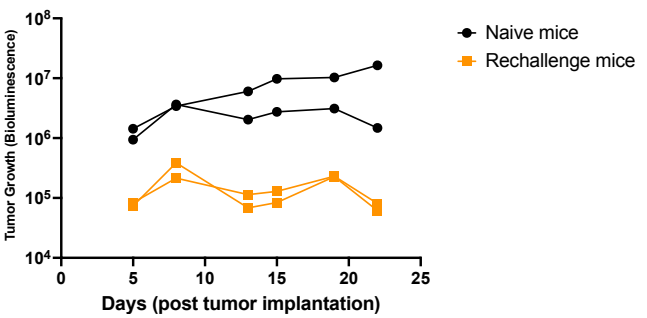
Tumor growth delay, survival benefit, enrichment of cDC1 and T cells

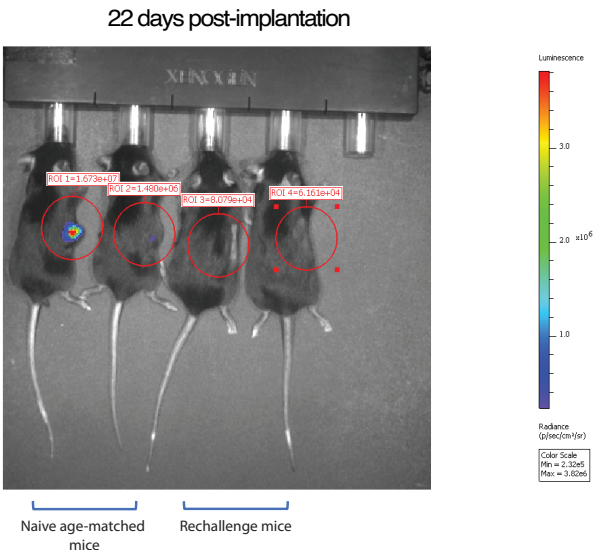
B

Group Comparison	P value
RRV-IRF8 2% vs. RRV-IRF8 100%	0.0005 ***
RRV-IRF8 2% vs. RRV-IRF8 30%	<0.0001 ****
RRV-IRF8 30% vs. RRV-IRF8 100%	0.0112 *
RRV-IRF8 2% vs. RRV-EMPTY 2%	<0.0001 ****
RRV-IRF8 30% vs. RRV-EMPTY 30%	0.0003 ***
RRV-IRF8 100% vs. RRV-EMPTY 100%	<0.0001 ****

C







Supplementary Figure 3. (A) Experimental schema for the data set presented in Fig. 5. **(B)** Group comparison statistics for Fig. 5e. **(C)** Long-term survivors from Fig. 5b-c were rechallenged with $4x10^5$ SB28 WT cells in the right flank on day 65 post-intracerebral tumor inoculation. Graphs represent tumor area (mm^2) and tumor growth bioluminescence until day 22 post-tumor inoculation.

Supplementary Table 1: Antibodies used in flow cytometry

Antigen	Clone	Fluorophore	Manufacturer	Item #
F480	BM8	BV605	Biolegend	123133
XCR1	ZET	BV650	Biolegend	148220
Ly6G	1A8	BV711	Biolegend	127643
MHC II	M5/114.15.2	BV785	Biolegend	107645
CD8a	S18018E	FITC	Biolegend	162313
CD11b	M1/70	PerCPCy5.5	Biolegend	101228
IRF8	V3GYWCH	PE	Invitrogen	12-9852-82
CD3	17A2	PE Dazzle 594	Biolegend	100246
CD11c	N418	PE Cy7	Biolegend	117318
CD45	30-F11	AF700	Biolegend	103128
Ly6C	HK1.4	APC Cy7	Biolegend	128026
IDO	2E2/IDO1	AF647	Biolegend	654003
Arg1	A1exF5	PE Cy7	Invitrogen	25-3697-82
CD4	GK1.5	APC	Biolegend	100411
CD103	2 E7	FITC	Biolegend	121419
CD24	M1/69	BV421	Biolegend	101825
P2A	3H4	APC	Novus Biologicals	NBP2-59627APC
CD25	3C7	PE Cy7	Biolegend	101915
FOXP3	MF-14	BV421	Biolegend	126419
Ki67	SolA15	PE	Invitrogen	12-5698-82
Cyclin A	E23.1	PE	Biolegend	644003
PCNA	PC10	AF647	Biolegend	307912
Phospho-Histone H3	11D8	AF488	Biolegend	650803
Human CD45	HI30	AF700	Biolegend	304023
Human Ki67	Ki-67	BV711	Biolegend	350515

Supplementary Table 2: Differentially expressed T cell pathway genes

Probe Label	Log2 fold change	std error (log2)	P-value	BY.p.value
Cd3g-mRNA	6.26	0.65	2.23E-06	0.00172
Ctla4-mRNA	5.53	0.597	3.21E-06	0.00206
Icos-mRNA	4.43	0.524	7.24E-06	0.00272
Il12rb1-mRNA	3.48	0.418	8.30E-06	0.00272
Cd3e-mRNA	5.48	0.66	8.48E-06	0.00272

Gzmb-mRNA	5.64	0.691	9.93E-06	0.00283
Cd3d-mRNA	4.7	0.579	1.03E-05	0.00283
Ccl5-mRNA	2.91	0.365	1.23E-05	0.00292
Zap70-mRNA	3.77	0.477	1.31E-05	0.00292
Pdcd1-mRNA	4.54	0.577	1.37E-05	0.00292
Lck-mRNA	4.15	0.55	1.96E-05	0.00378
Socs1-mRNA	1.29	0.183	3.42E-05	0.00599
Irf1-mRNA	1.12	0.161	3.82E-05	0.006
Il2ra-mRNA	2.99	0.438	4.52E-05	0.006
Tap1-mRNA	1.93	0.287	5.28E-05	0.00677
H2-K1-mRNA	1.62	0.244	5.85E-05	0.00704
H2-T23-mRNA	1.69	0.257	6.29E-05	0.00734
Cd74-mRNA	3.18	0.495	7.60E-05	0.00861
Il7r-mRNA	1.68	0.263	8.02E-05	0.00883
Fas-mRNA	1.23	0.197	9.31E-05	0.00969
Stat1-mRNA	1.81	0.301	0.00013	0.0125
H2-Aa-mRNA	2.99	0.501	0.000136	0.0128
H2-Ab1-mRNA	3.09	0.519	0.00014	0.0129
Cd247-mRNA	2.62	0.444	0.000149	0.0133
H2-D1-mRNA	1.24	0.21	0.000152	0.0133
Itgal-mRNA	1.65	0.295	0.00023	0.0188
Tnfsf13b-mRNA	2.34	0.422	0.000245	0.0197
Cd274-mRNA	1.49	0.277	0.000303	0.0233
Tnfrsf14-mRNA	1.58	0.302	0.000382	0.0283
Itgax-mRNA	1.2	0.23	0.000398	0.0284
Fcgr4-mRNA	1.69	0.33	0.000453	0.0306
Tigit-mRNA	1.82	0.359	0.000497	0.0322
Ccl19-mRNA	1.95	0.39	0.000546	0.0336
Cd40-mRNA	1.76	0.366	0.000718	0.0401
Flt3-mRNA	1.36	0.287	0.000801	0.0434
H2-DMa-mRNA	1.75	0.374	0.000851	0.0445
Jak2-mRNA	0.656	0.142	0.000935	0.0453
Ikzf2-mRNA	0.587	0.127	0.00094	0.0453
Il2rg-mRNA	1.31	0.293	0.0012	0.0549
Cd4-mRNA	2.23	0.511	0.0014	0.0635

Thy1-mRNA	0.663	0.152	0.00142	0.0635
H2-M3-mRNA	1.06	0.249	0.00172	0.0734
Psmb10-mRNA	0.992	0.234	0.00173	0.0734
Spn-mRNA	2.73	0.646	0.00177	0.074
Ptprc-mRNA	1.11	0.268	0.002	0.0795
Irf8-mRNA	1.04	0.251	0.00207	0.0799
Stat5b-mRNA	0.507	0.127	0.00251	0.0917
Xcl1-mRNA	2.63	0.673	0.00296	0.105
Socs3-mRNA	0.633	0.171	0.00405	0.137

Supplementary Table 3: Differentially expressed DC functions pathway genes

Probe Label	Log2 fold change	std error (log2)	P-value	BY.p.value
Ccl5-mRNA	2.91	0.365	1.23E-05	0.00292
Ccl19-mRNA	1.95	0.39	0.000546	0.0336
Cd40-mRNA	1.76	0.366	0.000718	0.0401
Ccr5-mRNA	1.27	0.356	0.00512	0.162
Cd86-mRNA	0.842	0.259	0.00868	0.237
Cxcr4-mRNA	0.695	0.237	0.015	0.333
Ccr1-mRNA	0.639	0.304	0.0622	0.881

Supplementary Table 4: Differentially expressed MHC pathway genes

Probe Label	Log2 fold change	std error (log2)	P-value	BY.p.value
Nlrc5-mRNA	1.88	0.263	3.16E-05	0.0058
Tap1-mRNA	1.93	0.287	5.28E-05	0.00677
H2-K1-mRNA	1.62	0.244	5.85E-05	0.00704
H2-T23-mRNA	1.69	0.257	6.29E-05	0.00734
Cd74-mRNA	3.18	0.495	7.60E-05	0.00861
H2-Aa-mRNA	2.99	0.501	0.000136	0.0128
H2-Ab1-mRNA	3.09	0.519	0.00014	0.0129
H2-D1-mRNA	1.24	0.21	0.000152	0.0133
H2-Eb1-mRNA	2.91	0.499	0.000165	0.0141
Tap2-mRNA	1.41	0.26	3.00E-04	0.0233
H2-DMb1-mRNA	2.05	0.406	0.000504	0.0322
Klrk1-mRNA	2.63	0.522	0.00051	0.0322
H2-DMa-mRNA	1.75	0.374	0.000851	0.0445

Tapbp-mRNA	0.897	0.208	0.00154	0.0666
H2-M3-mRNA	1.06	0.249	0.00172	0.0734

Supplementary Materials and Methods

Calcium phosphate transfection

293T cells were plated on Poly-L-Lysine coated dishes one day prior to transfection ddH_2O, plasmid DNA and 2.5 M $CaCl_2$ were mixed and added dropwise to 2X HBS (pH 7.12), while gently vortexing. The resulting DNA/$CaPO_4$ solution was added dropwise to cells and swirled gently. The following morning, media was replaced and supplemented with 20mM HEPES and 10mM Sodium Butyrate. 5-6 hours later, the media was replaced and supplemented with 10mM HEPES. The following day, the viral supernatant media was collected and filtered through 0.45 µM syringe filter, aliquoted, and frozen at -80C.

Media preparation

Complete RPMI (cRPMI) media was used for all cell culture: RPMI media with 10% FBS, 1% Sodium Pyruvate (final conc. 1mM), 1X MEM NEAA, 1X Glutamax, 1% HEPES (final conc. 0.01M), 1% Pen-Strep, and 0.1% Betamercaptoethanol.

Flow cytometry

Single-cell suspensions (0.5-1x10^6 cells/sample) of dissociated SB28 tumors were incubated with anti-CD16/CD32 Fc block (BioLegend, 156603) for 10 min, followed by viability staining (BioLegend, 423101) in PBS for 15 min. After washing, a cocktail of fluorophore-conjugated antibodies and monocyte blocker (BioLegend, 426101) was added to cells in a total volume of 100µL staining buffer (1X PBS, 0.5% BSA, 2mM EDTA) and incubated in the dark at 4ºC for 30 min, rocking. For intracellular staining (cytosolic and nuclear), cells were subsequently fixed and permeabilized following the

manufacturer's protocol (FoxP3/Transcription Factor Staining Buffer Set, Invitrogen, 00-5523-00). Fluorophore-conjugated intracellular antibodies were added and incubated in the dark for at least 30 min, rocking. Samples were washed twice and suspended in 100µL staining buffer. All flow cytometry experiments were performed on the Invitrogen Attune NxT (Thermo Fisher Scientific) flow cytometer and analyzed using FlowJo software (FLOWJO, LLC, Ashland, OR, USA). All antibodies used are listed in Supp. Table 1.

Orthotopic glioma model

Under anesthesia, mice received stereotactic tumor inoculation with $1x10^4$ cells in 2 µL HBSS (for SB28 OVA model: $2x10^4$ cells in 2 µL HBSS) at the following coordinates relative to bregma: mediolateral 2mm, dorsoventral -3mm. Mice were monitored daily and given post-operative care, per the approved IACUC protocol. Tumor growth was measured using bioluminescent imaging twice weekly: 3mg (100µL) D-Luciferin was injected intraperitoneally 10 minutes prior to image acquisition.

Preparation of SB28-premixed cells for intracerebral injection

For each premixed cell solution, two sets of cells were prepared, SB28 WT and SB28-RRV (EMPTY or IRF8). For RRV-transduced cells, previously frozen RRV stocks were added to low-passage SB28 WT cells and allowed to spread until 100% of cells were transduced. Transduction was measured using flow cytometry staining for P2A and/or IRF8. For intracerebral implantation, SB28 WT and SB28-RRV (EMPTY or IRF8) were counted and mixed at 98% SB28 WT and 2% SB28-RRV in cold HBSS.

Isolation of tumor-infiltrating cells

Tumor-bearing brain quadrant was dissected and manually dissociated into ~1mm^3 pieces. Tumor pieces were resuspended in 0.6-1 mL collagenase buffer (3.2 mg/ml Collagenase IV, 1 mg/ml Deoxyribonuclease I in PBS) and left shaking at 700 RPM at 37ºC for 45 min, pausing to mix thoroughly every 15 min. Resulting dissociated tumor suspensions were filtered through a 70 µm cell strainer and washed with excess PBS; red blood cells were lysed (Lonza, BP10-548E), and cell suspensions were stored at -80C in Bambanker (Bulldog Bio, BB01) or stained immediately for flow cytometry. Both human and mouse GBM tumors were prepared as above.

3'-Azido-3'-deoxythymidine (AZT) administration via drinking water

0.4 mg/mL AZT (Sigma, A2169) and 2% sucrose (Thermofisher, J65148.36) were dissolved in water and provided *ad lib* in a water bottle protected from light. As vehicle control, 2% sucrose only was used; fresh solutions were prepared weekly. To monitor water consumption, water bottles were weighed daily. Mice in the AZT/sucrose groups consumed water at the same rate as those in the control, sucrose-only groups.

Immunosuppression: Myeloid cell/T-cell co-culture

T-cells: T-cells were isolated from spleens of naïve non-tumor bearing C57BL/6J mice using a CD3 bead isolation kit (BioLegend, 480023). T-cells were resuspended in 0.5 mM CFSE dye (CellTrace CFSE Cell Proliferation Kit, Thermofisher, C34570) in PBS and incubated in the dark for 10 minutes. Cells were washed several times to remove any unbound CFSE dye and were resuspended in growth medium containing with

CD3/CD28 activating beads (Gibco, 11161D) and supplemented with 50 IU/mL hIL-2.

Myeloid cells: SB28 tumors were dissociated into single cells, as described above. Myeloid cells were isolated using a CD11b bead isolation kit (BioLegend, 480109) and resuspended in cRPMI. **Co-culture:** Myeloid cells and T-cells were combined at an effector: target ratio of 0.8:1. Cells were co-cultured in cRPMI for 4.5 days and stained for flow cytometry.

Antigen presentation: DC/CD8 T-cell co-culture

T-cells: T-cells were isolated from spleens of OT-1 transgenic (Jackson Laboratory, strain 003831) naïve non-tumor bearing mice using a CD8 bead isolation kit (BioLegend, 480007) and stained with CFSE dye (as above). Positive control T-cells were activated with CD3/CD28 beads, all T-cells were supplemented with 50 IU/mL

DCs: DCs were isolated from both tumors and lymph nodes. SB28 OVA RRV-EMPTY or RRV-IRF8 tumors were dissociated into single cells as described above. Cervical lymph nodes (cLNs) from the same tumor-bearing mice were incubated with collagenase buffer for 15 min at 37C, then mechanically dissociated through a 70 μm filter to generate a single cell suspension. DCs were isolated using a CD11c bead isolation kit (Milentyi, 130-100-875). **Co-culture:** $5x10^3$ DCs were combined with $1x10^5$ OT-1 T-cells in cRPMI a 96-well plate, incubated for 4 days, and stained for flow cytometry.