Supplementary Materials and Methods

Cell lines

The murine glioma cell lines GL261 (Tumor Bank Repository, National Cancer Institute, Frederick, MD), GL261-5 (a clone with slower *in vivo* growth kinetics) [1], and human HeLa and HEK293 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium with nutrient mixture F12 (DMEM/F12) (Corning). Murine GSC-005 glioma cells (kindly provided by I.M. Verma, The Salk Institute for Biological Studies, CA) [2] were maintained in DMEM/F12 supplemented with N2 (1x; Invitrogen), fibroblast growth factor-2 (20 ng/ml; PeproTech), epidermal growth factor (20 ng/ml; Promega), and heparin (50 μg/ml; Sigma). The murine melanoma cell line B16-F10 (ATCC) and murine breast cancer cell line 4T1.2 expressing the luciferase gene (kindly provided by C. Bartholomeusz, MD Anderson Cancer Center, TX) were both maintained in RPMI 1640 medium (Corning). The human glioma-like stem cell lines GSC11, GSC20, GSC7-2, GSC13, and GSC8-11 [3-5] were cultured in DMEM/F12 with B27 (1x; Invitrogen), antibiotic-antimitotic (1x; ThermoFisher Scientific), fibroblast growth factor-2 (20 ng/ml), and epidermal growth factor (20 ng/ml). Cultures, except for cultures of GSCs, were supplemented with 10% fetal bovine serum (HyClone Laboratories) and antibiotics (100 μg/ml penicillin, 100 μg/ml streptomycin; Corning). All cells were kept at 37˚C in a humidified atmosphere containing 5% CO_2.

Oncolytic adenoviruses

Two previously constructed oncolytic adenoviruses, Delta-24-RGD [6] and Delta-24-RGDOX [1], were propagated in human lung carcinoma A549 cells. Virions were collected and purified using the Adenopure kit (Puresyn, Inc.) following the manufacturer's instructions. Viral titers and replication were determined by measuring the plaque-forming units per ml (pfu/ml) using conventional methods. Briefly, HEK293 cells (2.5×10^5) were incubated in 24-well plates with serial dilutions of the viral stock. Forty-eight hours later, cultures were fixed with 100% ice-cold methanol for 10 min at –20°C. Cells were stained for hexon expression using an anti-adenovirus polyclonal antibody (Millipore Sigma, AB1056; 1 h, 37˚C) followed by secondary staining with a biotinylated anti-goat IgG antibody (H+L, Vector Biolabs, BA-5000; (1 h, 37˚C). The Vector Vectastain ABC kit (Vector Biolabs, PK-4000) and ImmPACT DAB peroxidase substrate kit (Vectro Biolabs, SK-4105) were utilized for the visualization of positive cells. Hexon-stained areas were counted under a light microscope (20× objective) in 10 individual fields per well. In wells with viral dilutions showing 5-50 positive cells/field, the viral titer was calculated using the following formula: pfu/ml = [(mean number of positive cells/field) × (fields/well)] / [volume virus (ml) × dilution factor]. The antibodies used are shown in Supplementary Table 1.

Viral replication assay

To determine the replication ability of Delta-24-RGDOX in the adherent cell lines used in this study, we seeded 1.5×10^5 cells/well in a 12-well plate and counted the cells after letting them attach overnight to calculate the appropriate amount of Delta-24-RGDOX needed to achieve specified multiplicities of infection (MOIs). Delta-24-RGDOX was diluted in PBS to achieve the specified MOIs and added dropwise to the attached cells, which were cultured in 200 μl of serum-free media for 20 min at 37˚C and shaken every 5 min to disperse the virus. The serum-free media was then removed, and the cells were cultured in 1 ml of complete medium. Non-adherent cells were cultured with diluted Delta-24-RGDOX in 200 μl of serum-free media for 20 min at 37˚C and shaken every 5 min to disperse the virus, and then 800 μl of complete medium was added. After a 48-h incubation period, adherent and non-

adherent cells and supernatant were collected, flash-frozen and thawed 3 times, and then centrifuged at 500 × g for 5 min. The supernatants were used for viral titration in HEK293 cells, as explained in the "Oncolytic adenoviruses" section above.

Viral-induced cytopathic ability of virus assay

To determine the virus-induced cytopathic ability of Delta-24-RGDOX, we seeded 1×10^4 cells/well in a 96-well plate (75 µl media/well). An additional 75 µl of media containing the appropriate treatment was then added to the cells. The virus-induced cytopathic ability was measured every 24 h for up to 168 h by quantifying ATP levels using the ViralToxGlo Assay (Promega) according to the manufacturer's protocol.

IDO inhibitors

The IDO inhibitors indoximod and 1-methyl-DL-tryptophan (1MT; Sigma-Aldrich) were suspended in PBS containing 3-mm glass balls (Thomas Scientific, #3000), which was rotated overnight to help re-suspend the drug. The clinical grade direct IDO enzyme inhibitor BGB-7204 (BeiGene; kindly provided by D. Wainwright, Northwestern University, IL), whose pharmacokinetic/pharmacodynamics properties have been reported previously [7], was suspended in Ora-Plus oral suspending vehicle (Perrigo). For *in vitro* use of these inhibitors, DMSO (Sigma) was used as the diluent.

***In vivo* studies**

For glioma implantations, 5×10^4 GL261-5 or GSC-005 cells/mouse or 1×10^3 B16-F10 cells/mouse were implanted into the caudate nucleus of 7- to 10-week-old male or female C57BL/6 mice using a guide-screw system as described previously [8]. The mice were then randomly assigned to experimental groups. Treatment began 7 days after tumor cell implantation. Delta-24-RGDOX adenoviruses (5×10^7 – 1×10^8 pfu/dose) were injected intratumorally on days 7, 9, and 11. For 4T1.2 breast tumor implantations, 1×10^4 4T1.2 cells/mouse were injected into the right mammary pads of 7-to 10-week-old female BALB/c mice; intratumoral Delta-24-RGDOX (1×10^8 pfu/dose) injections were administered on days 10, 14, 16, 18, and 21. Indoximod (275 mg/kg), 1MT (400 mg/kg), or BGB-7204 (100mg/kg) was administered twice daily, by oral gavage, 5 days/week, from days 7-35 after tumor implantation. Mice surviving 120 days were re-challenged with a new intracranial tumor injection using GL261-5 cells (5×10^4 cells/mouse) on the contralateral side of the initial tumor injection. For the depletion studies, anti-CD4 depletion antibodies, anti-CD25 depletion antibodies, or rat IgG2b isotype control antibodies were administered to mice intraperitoneally starting on day 4 after tumor implantation and every 4 days thereafter until day 36. C57BL/6 IDO-knockout (KO) mice (B6.129-Ido1tm1Alm/J; stock no. 005867) were purchased from The Jackson Laboratory and bred in MD Anderson Cancer Center's Research Animal Support Facility. The depletion antibodies used are shown in Supplementary Table 1. All experimental procedures involving the use of mice were done in accordance with protocols approved by the Animal Care and Use Committee of MD Anderson Cancer Center and National Institutes of Health and United States Department of Agriculture guidelines.

Preparation of single-cell suspensions from murine brains and spleens

Spleens and tumor-bearing brain hemispheres were collected from the mice. Initial suspensions were obtained by cutting the tissue or grinding the organs, filtering them through 100-µm cell strainers (Fisher Scientific), and then placing the cell suspension in RPMI 1640 medium (10 ml/sample). All tissues were pelleted by centrifugation (500 × g for 7 min at room temperature [RT]). The spleen-derived pellet was

re-suspended in Red Blood Cell Lysing Buffer Hybri-Max (Sigma-Aldrich) to lyse the red blood cells according to the manufacturer's instructions. Then, the cell suspension was brought up to 20 ml/sample with RPMI 1640 medium to stop the lysis reaction and washed once in 1x PBS. The brain-derived pellet was washed once with HBSS (Corning), and then the cells were incubated in 8 pg/ml Liberase TM (Millipore Sigma) for 15 min at 37°C, washed again with HBSS, and then resuspended in 40% Percoll (1.130 g/ml; GE Healthcare), which was overlaid on top of 80% Percoll at a 1:1 ratio. The cells were centrifuged for 20 min at 500 × g at RT with an acceleration of 1 and a deceleration of 0, and the lymphocyte gradient interphase was collected and washed once with PBS. The cells from the brains and spleens were pelleted by centrifugation at 500 × g for 7 min at RT and finally re-suspended in FACS buffer (PBS containing 10 mM HEPES, 2 mM EDTA, and 1% fetal bovine serum).

RNA sequencing and data analysis

Total RNA was extracted from flash-frozen tumor using the RNeasy Plus Mini Kit (Qiagen). Sequencing was performed by Novogene. RNA quality control was performed to measure quantification using Nanodrop, test RNA degradation or potential contamination using agarose gel electrophoresis, and check for RNA integrity using Agilent 2100 Bioanalyzer System. Library construction was developed from the mRNA of eukaryotic organisms, which was enriched using oligo(dT) beads. This eukaryotic mRNA was then fragmented randomly in fragmentation buffer, and then cDNA was synthesized using random hexamers and reverse transcriptase. After first-strand synthesis, a custom second-strand synthesis buffer (Illumina) was added with dNTPs, RNase H, and *Escherichia coli* polymerase I to generate the second strand by nick translation. The final cDNA library was ready after a round of purification, terminal repair, A-tailing, sequencing adapter ligation, size selection, and PCR enrichment. Sequencing was performed using HiSeq machines (Illumina). Key steps of the RNA-seq data analysis included the evaluation of the quality of the reads by fastqc (fastqc/0.11.8) followed by the removal of the sequencing adapters and unpaired reads by trimmomatic (trimmomatic/0.33) [9]. These trimmed FASTQ files were used to map reads to the mouse genome /ENSEMBL.mus_musculus.release-75 using the STAR aligner (star/2.6.0b) [10]. Feature counts were extracted from the resulting .bam files by subread (subread/1.6.3) [11]. Estimates of unwanted variations in the raw read counts across samples were determined using the remove unwanted variation (RUV) method on the Galaxy platform, which estimates the factors of unwanted variation using replicate samples [12]. The RUV method uses the empirical Bayes approach to estimate a moderated t-statistic. These estimates were used as batch factors in DESeq2 analyses to determine differentially expressed genes in the group comparisons [13]. For the preparation of heatmaps, the log2 normalized values for genes with significant adjusted P-values (<0.05) from the DESeq2 analyses were utilized; both rows and columns were hierarchically clustered using 1 – *r*, where *r* is the Pearson correlation, with average linkage. For gene ontology (GO) enrichment analysis, the log2 fold change (FC) values for genes with significant adjusted *P*-values (<0.05) from the DESeq2 PBS vs. RGDOX and PBS vs. RGD analyses were used; enrichment analyses were performed on http://www.pantherdb.org/, with false discovery rate correction. The cutoffs for the ingenuity pathway analyses (IPAs) were *P*-values ≤0.05 and log2 FCs of ±1; activation z-scores are plotted on graphs. An activation z-score of ±2 was considered significant. The expression levels of genes with significant FCs and *P*-values in the sample group comparisons analysis were overlaid onto the IDO1 network curated from the IPA knowledge base. For the prediction of immune cell compositions as inferred from RNA-seq data, we input transcripts per million for each sample to the seq-ImmuCC platform [14], and the predicted percentages of various immune cell populations in each sample were plotted in Excel. The gene set enrichment analyses were performed with GSEA_4.0.3 software, and the whole-genome expression profiles of PBS- and Delta-24-RGDOX–treated tumors were used as inputs. The Treg gene

set (Supplementary Data File 1) was curated from the IPA knowledge base, and the MSDC gene set (Supplementary Data File 2) was based on a previous publication [15].

Aryl-hydrocarbon-receptor activity assay

For the assessment of the activity of aryl-hydrocarbon-receptor (AhR) induced by Delta-24-RGDOX, cells were mock-infected or infected with Delta-24-RGDOX (50 MOIs) and incubated for 48 h. The transcriptional activity of AhR in cell supernatants was quantified using an AhR assay kit (Indigo Biosciences), which utilizes AhR reporter cells expressing a luciferase reporter gene functionally linked to an AhR-responsive promoter. The protocol was performed according to the manufacturer's instructions.

AhR immunofluorescence

HeLa cells were seeded in a 96-well black-sided, clear-bottom tissue culture plate (Corning) at a density of 1×10^4 cells per well. Cells were mock-treated, treated with kynurenine (150 µM; Santa Cruz Biotechnology), or treated with Delta-24-RGDOX (25 MOI) in complete media for 48 h. Then, immunofluorescence staining was performed by following the procedures provided by the antibody manufacturer. Briefly, cells were washed with PBS and fixed with 4% formaldehyde in PBS for 10 min at RT. After the wells were washed 3 times with wash buffer (2 mg/ml BSA in PBS), the cells were permeabilized with 0.2% Triton X-100 for 30 min at RT in a moist chamber. After permeabilization, the cells were washed 3 times and overlaid with the primary antibody against AhR (Santa Cruz Biotechnology) containing 2 mg/ml BSA. After overnight incubation, the cells were washed 3 times and incubated with the FITC-conjugated secondary antibody (Invitrogen, Alexa Fluor 488 goat anti-mouse) for 1 h at RT. The cells were washed 4 times and then stained with DAPI (Invitrogen; 1 µg/ml). The cells were then overlaid with mounting medium and imaged using an Axiovert 200 inverted microscope (Zeiss). The cellular and nuclear AhR intensities were quantified using ImageJ software (NIH). The antibodies and their working dilutions are shown in Supplementary Table 1.

Quantitative real-time polymerase chain reaction

For the detection of mRNA levels from cultured cells, Trizol reagent was used according to the established routine protocol. For the detection of mRNA levels from freshly dissected tissue samples, the RNeasy Plus Mini Kit (Qiagen) was used. One microgram of total RNA was reverse-transcribed into mRNA using the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on the 7500 Fast Real-Time PCR System (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems). Primers were purchased from Sigma Aldrich (human IDO, R: TGGAGGAACTGAGCAGCAT, F: TTCAGTGCTTTGACGTCCTG; mouse IDO, R: TTGCGGGGCAGCACCTTTCG, F: CCCACACTGAGCACGGACGG). The RT-PCRs were conducted in a 96-well plate with a holding stage of 10 min at 95˚C, followed by 40 cycles of 30 s at 95˚C, 30 s at 60˚C, and 45 s at 72˚C, followed by an infinite hold at 4˚C. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method by normalizing the threshold cycle (C_t) values of the gene of interest to the C_t values of the internal housekeeping gene.

Western blotting

For the detection of protein expression, cell lysates were prepared using RIPA lysis buffer (20 mM HEPES pH 7.0, 200 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 5 mM sodium pyrophosphate, 80 mM β-glycerophosphate, 50 mM NaF, and 0.1% SDS) plus freshly added protease

inhibitor cocktail (1x; Sigma-Aldrich), the proteasome inhibitor MG-132 (1 μM; Calbiochem), and phosphatase inhibitor cocktail 3 (2.5 mg/ml; Sigma-Aldrich). Cell lysates were flash-frozen in liquid nitrogen and thawed 3 times and centrifuged at 13,000 rpm for 10 min at 4°C. Supernatants were collected, and the concentration of protein was measured using Bradford reagent (Bio-Rad). DTT (50 mM; ThermoFisher) and NuPAGE LDS sample buffer (ThermoFisher) were added to 10-15 μg of total protein. The samples were heated at 95°C for 5 min, run on 4-20% Novex Tris-Glycine gels (Invitrogen), and then transferred to a PVDF membrane (ThermoFisher) and probed with the primary antibodies overnight at 4°C and then subjected to secondary antibody staining for 1 h at RT the next day. Protein bands were visualized using Western Lightning Plus-ECL enhanced chemiluminescence substrate (Perkin Elmer). The antibodies and working dilutions used are shown in Supplementary Table 1.

Liquid chromatography–mass spectrometry

For the detection of IDO activity, the kynurenine and tryptophan levels of flash-frozen brain tumors from mice in the different treatment groups were measured by the Mass Spectrometry Facility at the University of Texas Medical Branch, Galveston, TX. Liquid extraction was used to extract polar metabolites from the frozen brain tissue. Briefly, 400 μl of 80% methanol was added to the frozen tissue. Zirconia/silica beads (1-mm diameter) were added to the tube. The tissue was broken down using a bead beater by pulsing the tissue for 45 s. The resulting solution was kept on ice for 30 min to precipitate the proteins. Next, 400 μl of chloroform was added, and the tube was pulsed in the bead beater for an additional 45 s and kept on ice for 15 min. Then, 200 μl of water was added to induce phase separation. The tube was centrifuged, and the upper aqueous phase containing the amino acids/amines was carefully transferred to another tube. Of the 500-μl aqueous phase, 250 μl was dried under vacuum. The dried metabolites were resuspended in 40 μl of buffer used for the derivatization of the amino acids/amines. Of this, 10 μl was taken for further derivatization and analysis by liquid chromatography–mass spectrometry (LC-MS). Standard calibration curves of known concentrations of amino acids, including tryptophan and kynurenine, were made. Before analysis by LC-MS, 5 μM of stable isotope-labeled amino acids, including tryptophan and kynurenine, in 10 μl were added to each sample. The concentrations of kynurenine or tryptophan in the analysis sample were then calculated using linear regression on the standard concentrations by plotting the area ratio (the ratio of the peak area of analyte to the peak area of the internal standard) and the known concentrations. Concentrations of kynurenine or tryptophan were normalized to tumor mass.

Flow cytometry analysis

For the analysis of cell surface protein expression, single cell suspensions from murine brains and spleens in a 96-well round-bottom plate were first blocked in anti-mouse CD16/CD32 Fc Block diluted with FACS buffer and then washed once with 250 μl of cold PBS. The cells were then incubated in 100 μl of Fixable Viability Stain 780 (1:1000; BD Biosciences) at 4°C in the dark for 30 min and then washed once with 250 μl of cold PBS. Then, the cells were incubated in 100 μl of primary antibody solution diluted in FACS buffer. After incubation at 4°C in the dark for 30 min, the cells were washed once with 250 μl of cold PBS. For the analysis of intracellular proteins, cells were stained with the eBioscience FOXP3/Transcription Factor Staining Buffer Set (Invitrogen) following the manufacturer's instructions. The cells were finally re-suspended in 0.3 ml of FACS buffer containing 123count eBeads Counting Beads (Invitrogen) to acquire an accurate output for absolute cell count. The stained cells were analyzed using the FACSCelesta flow cytometer (BD Biosciences). FlowJo software, version 10 (FlowJo, LLC), was used for the analysis. To control for the technique and to arrange population gates

accurately, we generated fluorescence-minus-one samples for each antibody using pooled spleen cells from brain tumor–bearing mice in different treatment groups. The antibodies and working dilutions used are shown in Supplementary Table 1.

Analysis of splenocyte stimulation in co-cultures with target cells

Target cells were seeded and treated with the specified agents. Four hours later, mouse interferon gamma (IFNγ; 100 units/ml; ProSpec) was added to the cultures. Forty-eight hours after viral infection, the cells were detached with 2 mM EDTA in PBS, fixed with 1% paraformaldehyde, and cleaned with lysine (0.1 M) wash solution. A total of 2×10^4 fixed cells were seeded in 96-well round-bottom dishes. For immune cell activation, pre-fixed target cells were co-cultured with splenocytes (5×10^5/well) in RPMI 1640 medium containing 100 μg/ml penicillin (Corning), 100 μg/ml streptomycin (Corning), and 55 μM beta-mercaptoethanol (Gibco) for 48 h. Then, the concentration of IFNγ or interleukin-2 (IL-2) in the supernatant was assessed with standard ELISA (IFNγ or IL-2 DuoSet ELISA, R&D Systems) according to the manufacturer's instructions.

Histopathological staining

Tumor-bearing mouse brains were fixed in 10% buffered formalin for 24 h, transferred to 70% ethanol, and then embedded in paraffin for slide sectioning. Paraffin-embedded sections of the mouse brain tumors were deparaffinized at 60°C for 1 h and rehydrated with xylene and ethanol following conventional procedures. For hematoxylin and eosin staining, brain tumor sections were stained with Harris hematoxylin (Fisher Scientific) and Eosin-Y solution (Fisher Scientific) and mounted with Cytoseal 60 (Thermo Scientific). For CD3 immunohistochemistry, antigens were retrieved from the brain tumor sections by exposing the slides to 10 mM citric acid (pH 6.0) inside a steamer for 30 min. The slides were then incubated in 3% hydrogen peroxide in 100% methanol for 10 min at RT to quench endogenous peroxidases. The sections were then subjected to blocking with 5% goat serum in PBS for 1 h at RT followed by incubation with a primary rabbit monoclonal anti-CD3 antibody overnight and then incubation with a biotinylated anti-rabbit IgG secondary antibody diluted in 1% goat serum. The Vector Vectastain ABC kit (Vector Biolabs, PK-4000) and ImmPACT and DAB Peroxidase Substrate Kit (Vector Biolabs, SK-4105-Reagent 1) were utilized to visualize positive cells. Images were captured using an Aperio ScanScope slide scanner, which was also used to measure high-grade tumor areas. Specific antibody information is given in Supplementary Table 1.

Statistical analyses

Experiments involving the groups included in the quantitative analyses were performed at least in triplicate. GraphPad Prism 9 was used to perform all statistical analyses and generate all graphs for the *in vitro* and *in vivo* experiments. To determine statistical differences between 2 groups, we performed a two-tailed Student t-test; to determine statistical differences among 3 or more groups, we performed an ordinary one-way ANOVA. The statistical analyses of RNA sequencing data are described in the "RNA sequencing and data analysis" section above. *P*-values < 0.05 were considered significant.

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Supplementary Table 1: Antibody Information

Technique	Antibody	Company	Catalog #	Dilution/Dose
Hexon Titration	Anti-Adenovirus Antibody	Millipore	AB1056	1:500
Hexon Titration	Rabbit Anti-Goat IgG Antibody (H+L), Biotinylated	Vector Biolabs	BA-5000	1:500
Immunofluorescence	Anti-Ah Receptor Antibody (C-4)	Santa Cruz Biotech.	sc-74572	1:100
Immunofluorescence	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488	Invitrogen	A32723	1:100
Flow Cytometry	CD16/CD32 Monoclonal Antibody (93)	Invitrogen	14-0161-85	1:100
Flow Cytometry	APC-R700 Rat Anti-Mouse CD45 (30-F11)	BD Biosciences	565478	1:100
Flow Cytometry	PerCP-Cy™5.5 Hamster Anti-Mouse CD3e (145-2C11)	BD Biosciences	551163	1:100
Flow Cytometry	V450 Rat anti-Mouse CD4 (RM4-5)	BD Biosciences	560468	1:100
Flow Cytometry	BV510 Rat Anti-Mouse CD8a (53-6.7)	BD Biosciences	563068	1:100
Flow Cytometry	APC Rat Anti-Mouse CD25, PC61	BD Biosciences	557192	1:100
Flow Cytometry	FOXP3 Monoclonal Antibody eFluor 570	Invitrogen	11-5773-82	1:100
Flow Cytometry	FITC Rat Anti-Mouse Ly-6G and LY-6C (RB6-8C5)	BD Biosciences	553126	1:100
Flow Cytometry	APC Rat Anti-CD11b (M1/70)	BD Biosciences	553312	1:100
Immunohistochemistry	Anti-CD3 antibody (SP7)	abcam	ab16669	1:80
Immunohistochemistry	Goat Anti-Rabbit IgG Antibody (H+L), Biotinylated	Vector Biolabs	BA-1000	1:200
Western Blot	Adenovirus-2/5 E1A Antibody (M73)	Santa Cruz Biotech.	sc-25	1:25
Western Blot	α Tubulin Antibody (B-5-1-2)	Santa Cruz Biotech.	sc-23948	1:2,000
Western Blot	Donkey anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP	Invitrogen	SA1-100	1:10,000
Depletion	InVivoMAb anti-mouse CD4 (clone GK1.5)	BioXcell	BE0003-1	200 µg
Depletion	InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin	BioXcell	BE0090	200 µg
Depletion	InVivoMAb anti-mouse CD25 (IL-2Rα) (clone PC-61.5.3)	BioXcell	BE0012	200 µg

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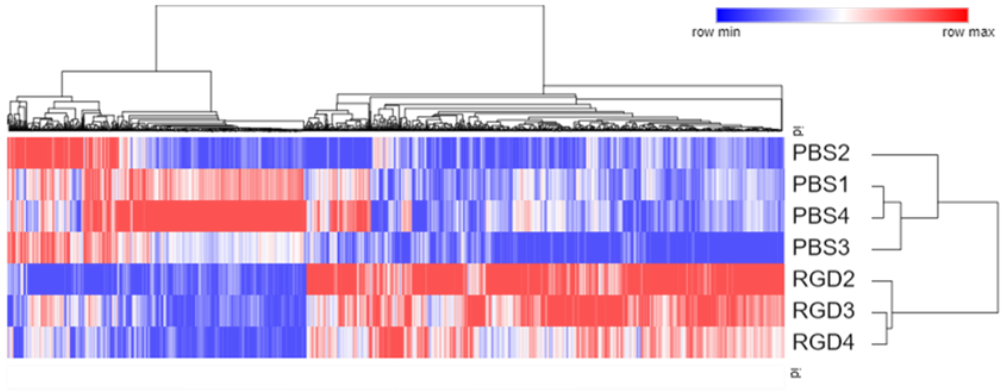
Supplementary Figure 1: The transcriptional signature derived from murine glioma treated with Delta-24-RGD shows reshaping of the tumor microenvironment.

A



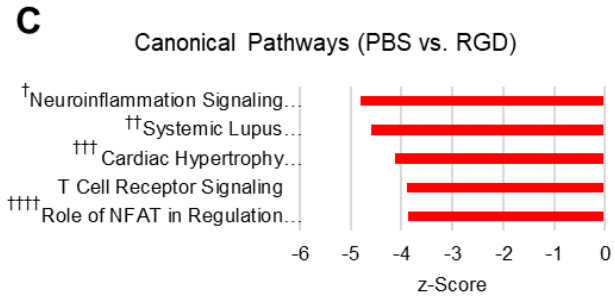
B

GO: Enrichment (PBS vs. RGD)

GO biological process complete	number	overUnder*	pvalue	FDR
immune response (GO:0006955)	348	-	0.00E+00	0.00E+00
inflammatory response (GO:0006954)	151	-	0.00E+00	0.00E+00
defense response (GO:0006952)	348	-	0.00E+00	0.00E+00
response to stress (GO:0006950)	598	-	0.00E+00	0.00E+00
response to cytokine (GO:0034097)	211	-	0.00E+00	0.00E+00
mitotic cell cycle process (GO:1903047)	102	+	0.00E+00	0.00E+00
chromosome segregation (GO:0007059)	66	+	0.00E+00	0.00E+00
cell cycle (GO:0007049)	193	+	0.00E+00	0.00E+00
microtubule-based process (GO:0007017)	95	+	0.00E+00	0.00E+00
cell division (GO:0051301)	97	+	0.00E+00	0.00E+00

* + : PBS; - : RGD

C



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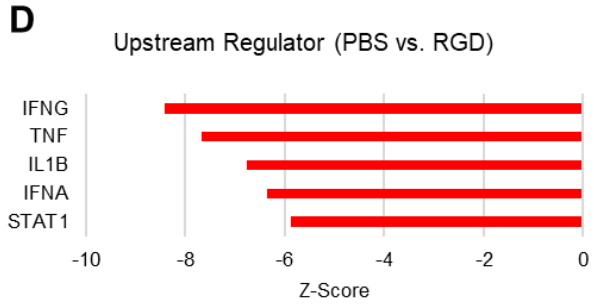
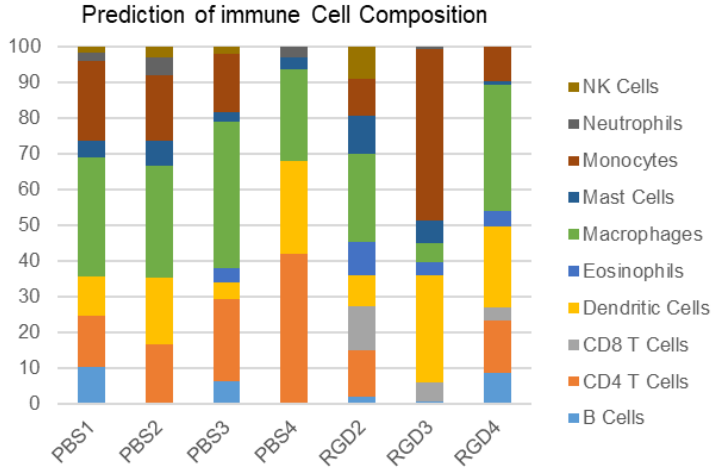


E



Supplementary Fig. 1. The transcriptional signature derived from murine glioma treated with Delta-24-RGD shows reshaping of the tumor microenvironment. Differentially expressed genes in tumors treated with PBS versus tumors treated with Delta-24-RGD were utilized for clustering, gene ontology (GO) biological process enrichment, and pathway analyses. (**A**) Heatmap comparing the transcription signature of GL261-5–derived brain tumors treated with PBS or Delta-24-RGD. The log2 normalized expression levels of genes with significant adjusted *P*-values (<0.05) across samples are shown. The color scale is shown above the heatmap. (**B**) GO biological process enrichment in tumors treated with PBS or Delta-24-RGD. The five most significant GO biological processes are shown. GO biological processes significantly associated with PBS-treated tumors are marked as '+', whereas those significantly associated with Delta-24-RGD–treated tumors are marked as '–'. (**C**) The five most significantly altered canonical pathways in tumors treated with PBS versus Delta-24-RGD. Activation z-scores are plotted in the graph. †Neuroinflammation signaling pathway; ††systemic lupus erythematosus in B cell signaling pathway; †††cardiac hypertrophy signaling (enhanced); ††††role of NFAT in regulation of the immune response. (**D**) The five most significantly altered upstream regulators in tumors treated with PBS versus Delta-24-RGD. Activation z-scores are plotted in the graph. The negative z-scores represent activation in Delta-24-RGD. (**E**) The prediction of immune cell composition in GL261-5–derived brain tumors treated with PBS or Delta-24-RGD. The percentages of various immune cell populations in each sample are presented in the graph. The color code for the various immune cell types is shown to the right of the graph.

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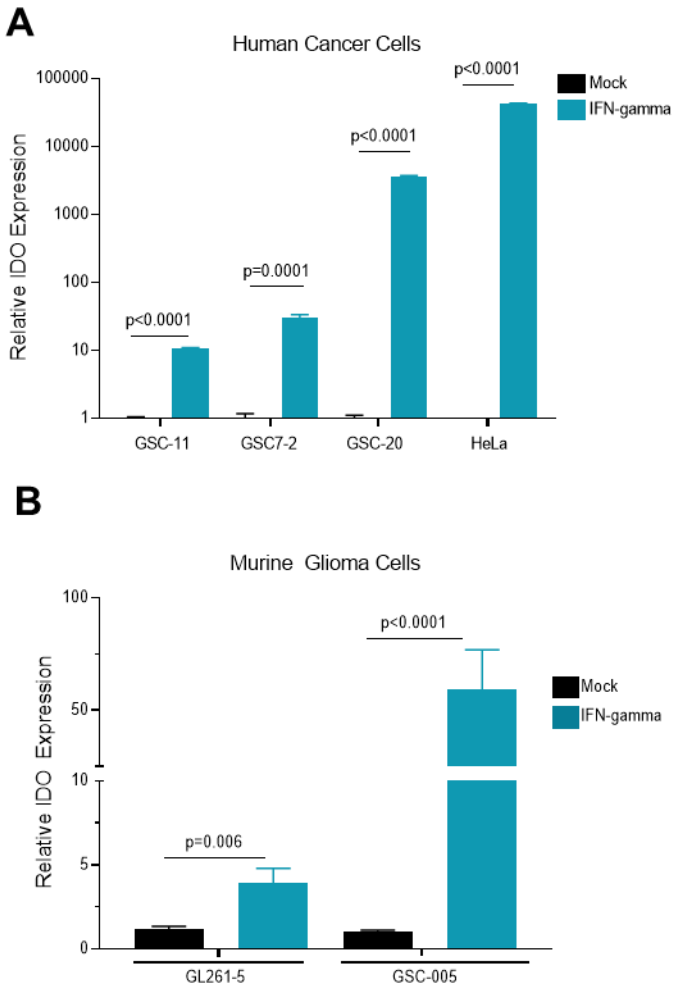
Supplementary Figure 2: IFN-γ induces IDO expression.



Supplementary Fig. 2. IFN-γ induces IDO expression. (**A** and **B**) Relative IDO expression of an array of human (A) and murine (B) cancer cells in response to IFN-γ. Cells were mock-treated or treated with IFN-γ (200 u/ml) for 24-48 h. RNA was extracted, and relative levels of IDO were measured using qRT-PCR; GAPDH or β-Actin was used as a housekeeping gene control. The column graphs show $2^{(Ct^{GAPDH\ or\ \beta\text{-}Actin}-Ct^{IDO})}$ normalized to the control. Data are means ± SDs. P-values were derived from a two-tailed Student t-test.

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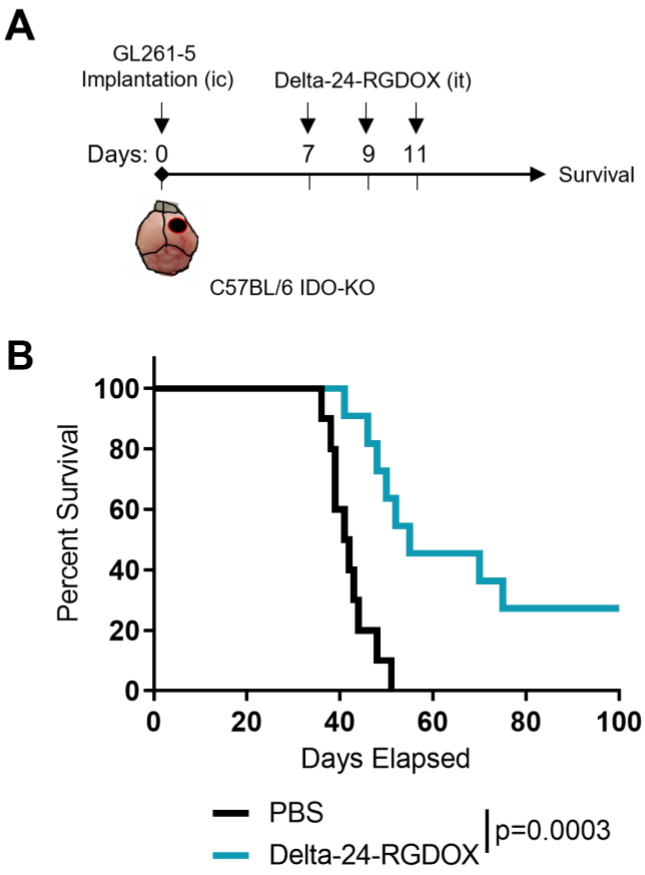
Supplementary Figure 3: Delta-24-RGDOX elicits anti-cancer effect in an IDO-KO genetic background glioma murine model.



Supplementary Fig. 3. Delta-24-RGDOX elicits anti-cancer effect in an IDO-KO genetic background glioma murine model. (**A**) Treatment schedule of intracranial GL261-5 tumor–bearing IDO-KO C57BL/6 mice receiving PBS or Delta-24-RGDOX (n=10 or 11 per group, respectively). Survival was monitored for up to 100 days. (**B**) Kaplan-Meier survival plot of IDO-KO GL261-5 tumor–bearing mice treated as explained in (A). P-value derived from a log-rank test.

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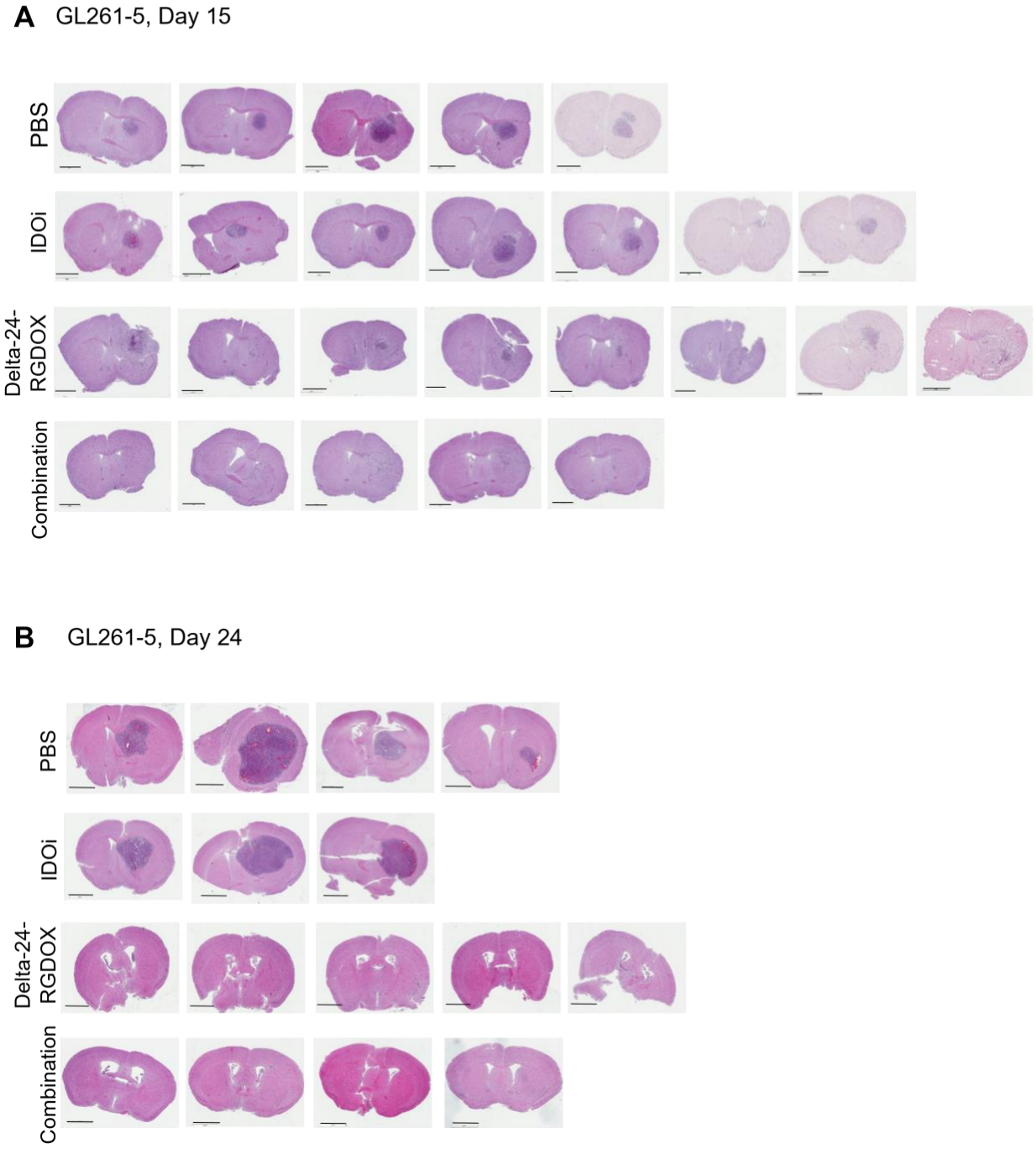
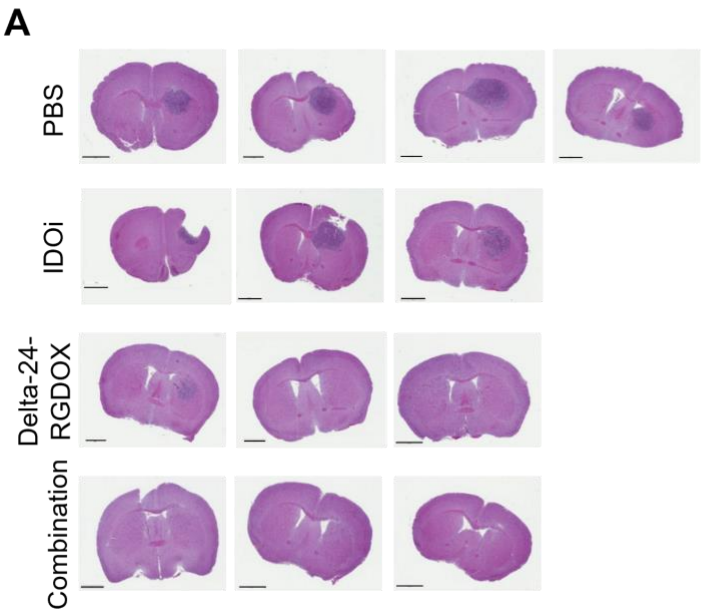
Supplementary Figure 4: Combined Delta-24-RGDOX infection and IDO inhibition induces anticancer effect in the intracranial GL261-5 glioma model.



Supplementary Fig. 4. Combined Delta-24-RGDOX infection and IDO inhibition induces anticancer effect in the intracranial GL261-5 glioma model. (**A** and **B**) Representative images of H&E-stained coronal brain sections of intracranial GL261-5 tumor–bearing C57BL/6 mice. Mice were implanted with GL261-5 cells and intratumorally treated with Delta-24-RGDOX or PBS with or without the IDO inhibitor (IDOi) indoximod. Brains were collected on day 15 (**A**) or day 24 (**B**). Coronal brain sections were subjected to H&E staining and scanned using Aperio ImageScope pathology slide viewing software. Brains from individual mice are shown. Scale bars, 2 mm.

Supplementary Figure 5: Combined Delta-24-RGDOX infection and IDO inhibition induces an anticancer effect in the intracranial GSC-005 glioma murine model.



Supplementary Fig. 5. Combined Delta-24-RGDOX infection and IDO inhibition induces an anti-cancer effect in the intracranial GSC-005 glioma murine model. (**A**) Representative images of H&E-stained brain sections from intracranial GSC-005 tumor–bearing mice receiving Delta-24-RGDOX alone or in combination with an IDO inhibitor (IDOi; BGB-7204). Mice were intracranially implanted with GSC-005 cells and randomly assigned to the indicated treatment groups 7 days later. On day 24, brains were collected, and brain sections were stained with H&E. Aperio ImageScope software was used to acquire images. Scale bars, 2 mm.

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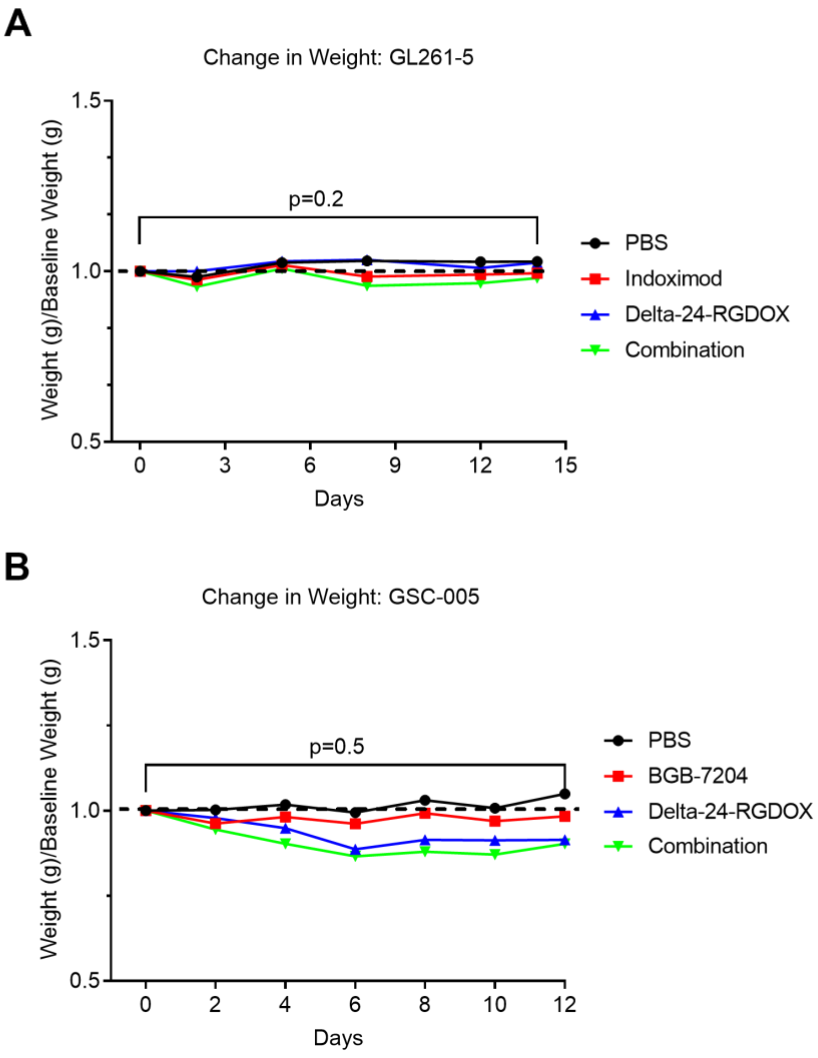
Supplementary Figure 6: Combined Delta-24-RGDOX and IDO inhibitor treatment did not cause significant weight loss in mice.



Supplementary Fig. 6. Combined Delta-24-RGDOX and IDO inhibitor treatment did not cause significant weight loss in mice. (**A** and **B**) XY plots showing the change in weight of mice bearing intracranial GL261-5 (A) or GSC-005 (B) tumors during indicated treatments over 12-15 days. Mice were implanted with GL261-5 or GSC-005 cells and administered the indicated treatments. (GL261-5 tumor–bearing mice received the IDO inhibitor [IDOi] indoximod; GSC-005 tumor–bearing mice received the IDOi BGB-7204). Weights of mice were measured on indicated day and were normalized to the baseline weight. P-values were derived from an ordinary one-way ANOVA.

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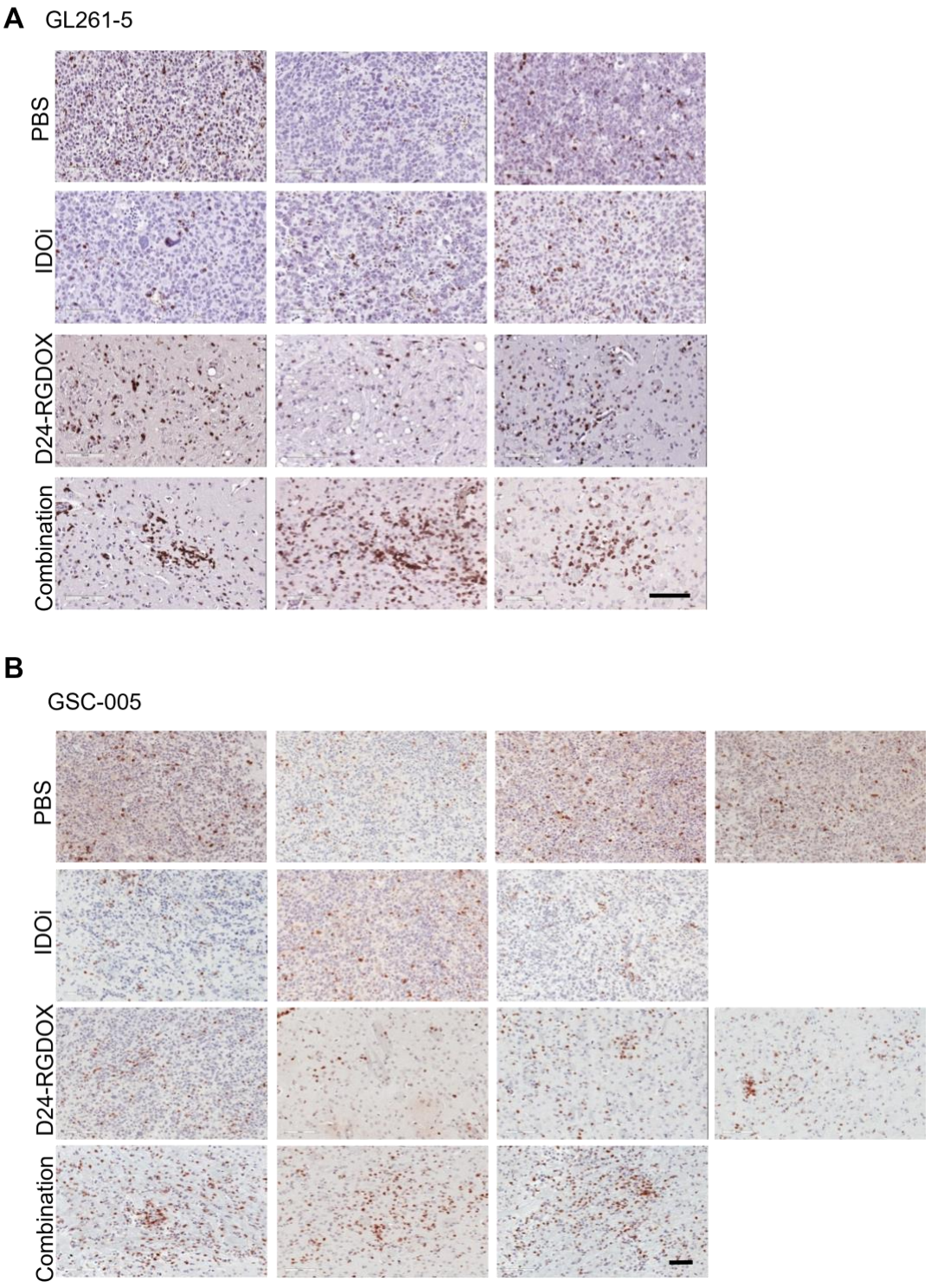
Supplementary Figure 7: CD3+ T-cell infiltration increases upon treatment with IDO inhibitors in combination with Delta-24-RGDOX.



Supplementary Fig. 7. $CD3^+$ T-cell infiltration increases upon treatment with IDO inhibitors in combination with Delta-24-RGDOX. (**A** and **B**) Representative images of CD3 immunohistochemistry of brain sections from individual mice bearing intracranial GL261-5 (A) or GSC-005 (B) tumors. Mice were implanted with GL261-5 or GSC-005 cells and administered the indicated treatments. (GL261-5 tumor–bearing mice received the IDO inhibitor [IDOi] indoximod; GSC-005 tumor–bearing mice received the IDOi BGB-7204). Mice were humanely killed on day 24. Slides of tumor-bearing coronal brain sections were stained for CD3 expression using immunohistochemistry. Slides were scanned and images were acquired using Aperio ImageScope pathology slide viewing software. Scale bars, 100 μm.

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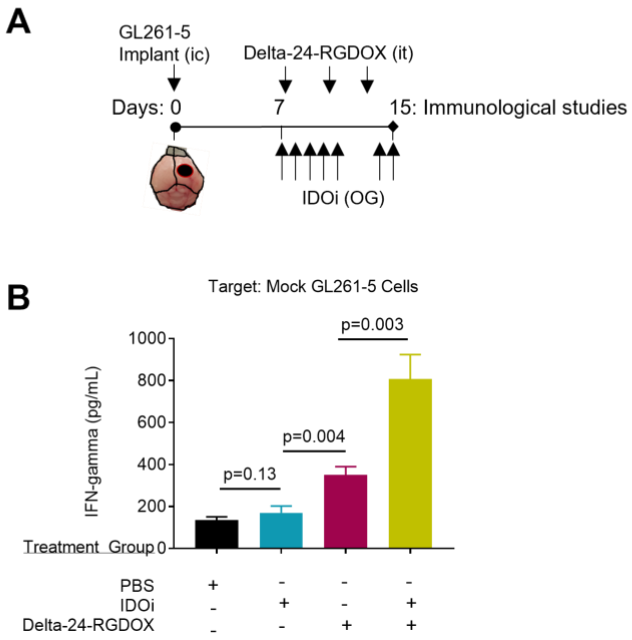
Supplementary Figure 8: Combined Delta-24-RGDOX infection and IDO inhibition results in anti-tumor T-cell activation.



Supplementary Fig. 8. Combined Delta-24-RGDOX infection and IDO inhibition results in anti-tumor T-cell activation. (**A**) Timeline of treatment for the functional splenocyte assays. C57BL/6 mice were intracranially (ic) implanted with GL261-5 cells and randomly assigned to receive PBS (control), the IDO inhibitor (IDOi) indoximod, Delta-24-RGDOX, or indoximod plus Delta-24-RGDOX. it, intratumorally; OG, oral gavage. (**B**) T-cell activity was assessed by co-culturing splenocytes from the treated GL261-5 glioma–bearing mice with prefixed GL261-5 cells for 48 h and then using ELISA to assess the concentration of secreted IFNγ. Column graphs show means ± SDs (n=3). P-values were derived from a two-tailed Student t-test.