Supplementary Figure Legends

Supplemental Figure 1. Analysis of IDO expression at the mRNA and protein expression levels. (A) $IDO^{-/-}$ mice were intracranially-injected (ic.) 4×10^5 V_c or IDO_{kd} cells. Brain tumors were removed at 2 weeks post-ic. and mRNA was isolated and reverse transcribed into cDNA (n=4). IDO expression was assayed using the cloning primers, specific for the full-length sequence for IDO. (B) RNA was isolated from the various GL261 cell lines and assayed for IDO expression using the cloning primers, specific for the full-length sequence for IDO. (C) Using an IDO1-specific antibody, a band at the predicted molecular weight for IDO was detected only in the GL261 cell line that was transfected with a plasmid specific for IDO overexpression (mIDO). V_c=GL261 cells transduced with vector control shRNA; IDO_{kd}=GL261 cells transduced with IDO knock down shRNA; Control (pEF6)=GL261 cells transfected with plasmid, alone; mIDO (pEF6)=GL261 cells transfected with a plasmid that overexpresses IDO.

Supplemental Figure 2. Correlation of IDO expression with overall survival, age at diagnosis and Karnofsky score for astrocytoma grades II, II and IV (GBM). Data was isolated from the Repository of Molecular Brain Neoplasia Data (REMBRANDT) and represents the analysis of 343 different glioma patients. The absolute level of IDO mRNA expression for astrocytoma grade(s) II (blue; squares), III (green; triangles) and IV (red; circles), correlated to (A) survival, (B) age at diagnosis and (C) Karnofsky score is shown. Lines of regression are included for each grade of glioma, as well as R-squared (R^2) and *p* values representing statistical significance.

Supplemental Figure 3. The presence or absence of brain tumor-derived IDO has no impact on Treg levels in the draining lymph nodes or spleen of wild-type (WT) mice. Wild-type mice were intracranially-injected (ic.) 4×10^5 GL261 cells transduced with -scrambled shRNA (vector control, V_c; white bars) or -shRNA specific to IDO (IDO knockdown, IDO_{kd}; black bars). The frequency and absolute numbers of $CD4^+FoxP3^+$ regulatory T cells isolated from the (A) dLNs and (B) spleen of naïve (grey bars)- or tumor- bearing mice at 1, 2 and 3 weeks post-injection. All T cell populations were initially identified by the expression of CD3 and CD4. Bar graphs in figures A - B are shown as mean ± SEM and are representative of two independent experiments (n = 4 – 5 mice/group).

Supplemental Figure 4. Proliferation and apoptosis in GL261 cells transduced with vector control (V_c) or IDO knockdown (IDO_{kd}) small hairpin RNA. (A) V_c and IDO_{kd} GL261 cells were pulsed with violet proliferation dye and plated for 24 or 72 hours and flow cytometrically analyzed. (B) V_c and IDO_{kd} GL261 cells were plated for 24 hours followed by flow cytometric analysis for Annexin V. As a positive control, IDO_{kd} GL261 cells were co-incubated with 138 mM sodium Azide (Na Azide) for 4.5 hours.