

Extended Data Materials

Extended Data Figures

Extended Data Figure 1. Age dependent T cell abundance in male and female GBM tumors

Extended Data Figure 2. Effects of androgen on GBM tumor growth

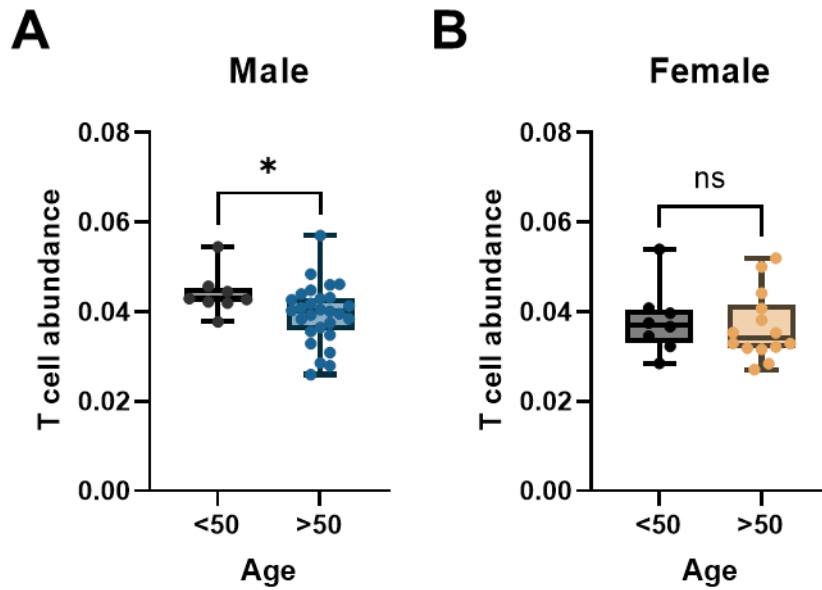
Extended Data Figure 3. Castration effect on anti-tumor immunity

Extended Data Figure 4. Serum levels of steroid hormones

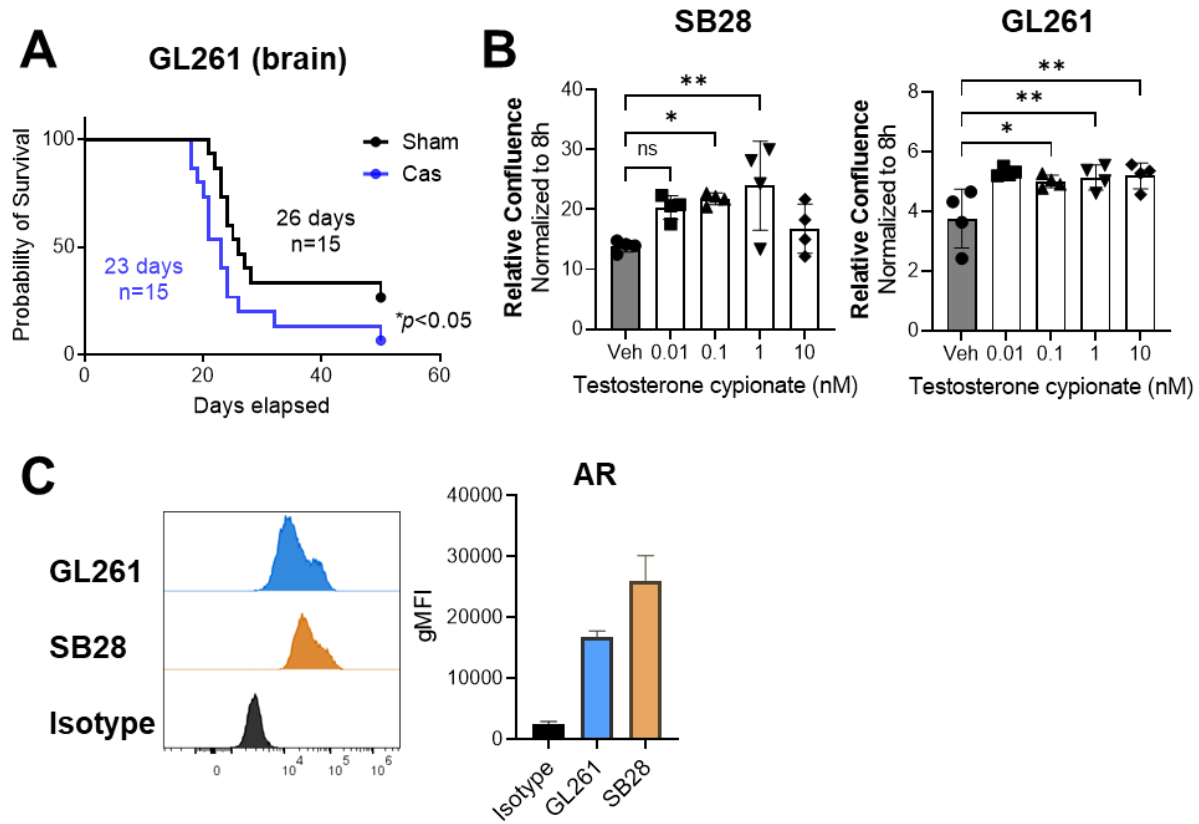
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Extended Data Table 1. Flow antibodies for immune cell subsets

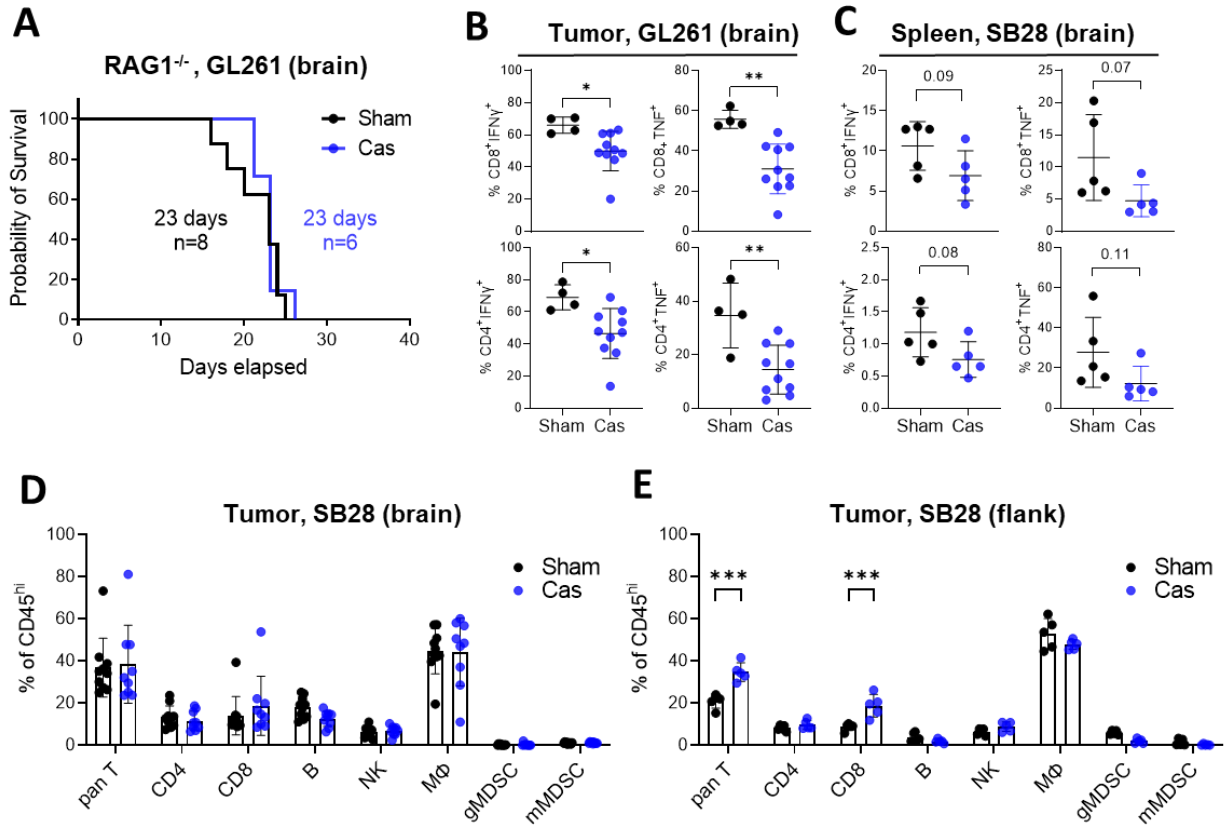
Extended Data Table 2. Flow antibodies for cytokine profiles



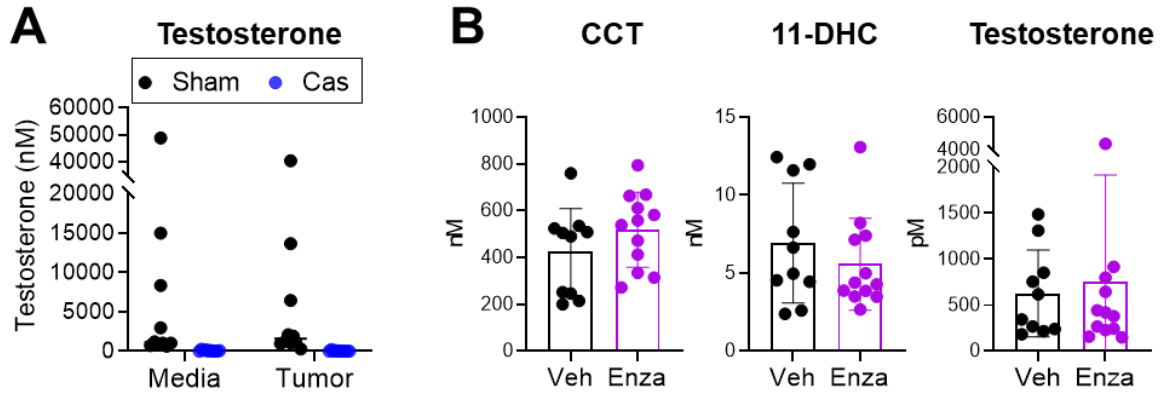
Extended Data Figure 1. Age dependent T cell abundance in male and female GBM tumors. T cell abundance was measured after deconvoluting bulk RNA-seq on tumor samples obtained using image-guided biopsy. Average T cell value was obtained for each patient. **A.** Male GBM patients (n=36 total), age <50 (n=8), age \geq 50 (n=28). **B.** Female GBM patients (n=22 total), age <50 (n=8), age \geq 50 (n=14). Unpaired student t-test was performed ($*p<0.05$).



Extended Data Figure 2. Effects of androgens on GBM tumor growth. **A.** Survival of GL261-bearing mice after sham or cas surgery. Median survival days and number of animals are indicated in the graph. Data combined from three independent experiments. Log-rank test was performed (* $p < 0.05$). **B.** SB28 or GL261 cells were cultured in the absence or presence of various concentrations of testosterone cypionate for 120h and 96h, respectively. Cell number was counted by Incucyte and normalized to 6h. One-way ANOVA with multiple comparisons (** $p < 0.01$, *** $p < 0.001$). **C.** Androgen receptor (AR) expression in murine GBM cells. AR expression was measured using flow cytometry. Data shown as representative histogram (left panel) and Mean \pm SD (n=3) (right panel).



Extended Data Figure 3. Castration effect on anti-tumor immunity. **A.** Survival of GL261-bearing RAG1^{-/-} mice after sham or cas surgery. Median survival days and number of animals are indicated in the graph. **B.** Intracellular expression of cytokines in T cells from GL261 brain tumor. n=4/sham, n=10/cas. **C.** Intracellular expression of cytokines in T cells from spleen of mice bearing SB28 brain tumor. n=5/sham, n=5/cas. Unpaired student *t*-test (**p*<0.05, ***p*<0.01). **D.** Frequency of immune cell subsets in the SB28 brain tumor measured by flow cytometry. n=10/sham, n=9/cas. **E.** Frequency of immune cell subsets in the SB28 flank tumor measured by flow cytometry. n=5/sham, n=5/cas. Two-way ANOVA with Tukey's multiple comparison test was performed (**p*<0.05, ****p*<0.001).



Extended Data Figure 4. Serum levels of steroid hormones. A. Mouse serum was collected 14 days after intracranial tumor implantation or media injection. Mass spectrometry analysis was performed to measure testosterone level. $n=10/\text{group}$. Two-way ANOVA analysis with Tukey's multiple comparison test was performed ($*p<0.05$, $**p<0.01$, $***p<0.001$). **B.** Serum levels of corticosterone (CCT), 11-dehydrocorticosterone (11-DHC), and testosterone by mass spectrometry from mice bearing SB28 brain tumor treated with vehicle ($n=10$) or enzalutamide ($n=12$).

Extended Data Table 1. Flow antibodies for immune cell subsets

Marker	Fluorophore	Clone	Vendor	Catalog number	Staining	Dilution
CD11b	BUV395	M1/70	BD Biosciences	563553	surface	1:250
CD69	BUV563	H1.2F3	BD Biosciences	741234	surface	1:250
CD11c	BUV737	HL3	BD Biosciences	612796	surface	1:250
CTLA4	BV421	UC10-4B9	BioLegend	106312	Intra	1:250
Ly6G	V450	1A8	BD Biosciences	560603	surface	1:250
PD1	BV510	29F.1A12	BioLegend	135241	surface	1:250
CD45R/B220	BV570	RA3-6B2	BioLegend	103237	surface	1:250
Ki67	BV605	16A8	BioLegend	652413	Intra	1:250
TIM3	BV711	RMT3-23	BioLegend	119727	Intra	1:100
CD3	BV786	145-2C11	BD Biosciences	564379	surface	1:250
I-A/I-E	FITC	M5/114.15.2	BioLegend	107606	surface	1:250
CD45	PerCP-Cy5.5	30-F11	BioLegend	103132	surface	1:250
Foxp3	PE	FJK-16s	eBioscience	2260608	Intra	1:250
LAG3	PE-dazzle 594	C9B7W	BioLegend	125224	surface	1:250
NK1.1	PE-Cy5	PK136	BioLegend	108716	surface	1:250
CD4	PE-Cy7	GK1.5	BioLegend	100422	surface	1:250
CD8	APC	52-6.7	BioLegend	100712	surface	1:250
CD206	Alexa Fluor 647	C068C2	BioLegend	141712	Intra	1:250
Ly6C	Alexa Fluor 700	HK1.4	BioLegend	128024	surface	1:250
CD68	APC-Cy7	FA-11	BioLegend	137024	intra	1:250
F4/80	APC-Cy7	BM8	BioLegend	123117	surface	1:250

Extended Data Table 2. Flow antibodies for cytokine profiles

Marker	Fluorophore	Clone	Vendor	Catalog number	Staining	Dilution
CD45	PerCP-Cy5.5	30-F11	BioLegend	103132	surface	1:500
CD3e	BV786	145-2C11	BD Biosciences	564379	surface	1:500
CD8a	PE-Cy5	53-6.7	BD Biosciences	553034	surface	1:500
CD4	V450	RM4-5	BD Biosciences	560468	surface	1:500
Foxp3	Alexa Fluor 488	FJK-16s	eBioscience	53-5773-82	Intra	1:250
CD44	BUV737	IM7	BD Biosciences	612799	surface	1:500
IFN- γ	PE-dazzle 594	XMG1.2	BioLegend	505846	Intra	1:250
TNF α	BV605	MP6-XT22	BioLegend	506329	Intra	1:250
Granzyme B	BV421	QA18A28	BioLegend	396414	Intra	1:250