
Supplementary information

Androgen loss accelerates brain tumour growth via HPA axis activation

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Androgen loss accelerates brain tumor growth via HPA axis activation

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Supplementary information

This file contains Supplementary Discussion, Supplementary information for SEER dataset analysis, and Supplementary Figure 1-5.

Supplementary Discussion

The HPA axis response to stress exhibits sex differences, with females typically displaying a more pronounced activation of the stress response compared to males¹. These sex differences primarily arise from activating effects of circulating sex hormones or are patterned during *in utero* development². The inhibitory effect of androgens on the HPA axis has been demonstrated in various stress models³. In this study, we demonstrated concordant findings in our brain tumor models, emphasizing that crosstalk between gonadal and adrenal hormones can impact tumor outcomes. This interaction appears to be mediated by proinflammatory cytokines, such as IL-1 β and TNF, which can activate the HPA axis and trigger glucocorticoid production as a regulatory feedback mechanism in response to inflammation⁴. Our data suggest that androgen loss enhances the HPA axis activity, both by removing the inhibitory effect of androgens and by increasing proinflammatory cytokine expression.

In this study, we found that androgen signaling suppresses inflammasome activity in microglia. Although inflammasome expression and activation in GBM tumor cells^{5,6} and proinflammatory immune responses^{7,8} have been linked to tumor progression, the specific contribution of inflammasome activation in GBM-associated microglia/macrophages remains poorly defined. Our data indicate that androgens may shape the inflammatory state of the tumor microenvironment by modulating microglial inflammasome pathways, suggesting a previously unrecognized layer of sex-dependent immune regulation in GBM. The molecular mechanisms underlying androgen-mediated suppression of the inflammasome, and how this intersects with neuroinflammation and tumor-immune interactions in GBM, warrant deeper investigation.

Sex differences are well documented in neurological diseases, with females exhibiting higher incidence rates of multiple sclerosis and Alzheimer's disease and males have a higher prevalence of Parkinson's disease⁹. These differences are partly attributed to the immunomodulatory effects of sex steroid hormones. In our study, testosterone supplementation in females did not replicate the protective effects observed in males and instead accelerated tumor growth without altering ACTH production (**Extended Data Fig.6**). These findings point to complex, sex-specific interactions between sex hormones and stress pathways, possibly due to differences in AR expression, receptor sensitivity, or modulation by other sex hormones.

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Supplementary information for SEER dataset analysis

Limitations

While Medicare data is a powerful resource for research, there are several important limitations. All individuals included in this analysis are ≥ 65 years old, meaning that younger individuals are excluded. The median age of first diagnosis with glioblastoma is approximately 66 years old¹, and as a result this should capture $>50\%$ of the population with glioblastoma. While the vast majority of those ≥ 65 years old in the US are entitled to Medicare, there are several factors that affect whether claims are reported. Individuals in the workforce may retain employer-sponsored insurance as primary insurer and Medicare as secondary resulting in some claims being billed to the primary insurance. These data include only traditional Medicare, while many individuals select Medicare Advantage (MA) coverage through private insurance companies, which is paid for by Medicare but does not result in claims directly to CMS. While there are no substantial differences between those choosing traditional Medicare as opposed to MA², there may be significant differences between those with private insurance, MA, and those with traditional Medicare that would limit the generalizability of our findings. While SEER-Medicare contains nearly all claims billed through Medicare, previous research has shown that the information contained in the claims themselves is not always reliable or valid³⁻⁶. Threats to the validity of claims may include under/over-estimates prescribed, patient compliance with billed treatments, and inconsistent use of billing codes, among others. A major limitation of any analysis using insurance claims data is the inability to identify conditions or medications which do not have an associated billing code, such as participation in a clinical trial or imaging evidence of disease progression. As a result, we are not able to assess potential interactions with unapproved treatments such as immunotherapies. Finally, given this data was intended for billing purposes, we are unable to determine whether the person actually took the medication as intended. We simply know that they filled the prescription and were billed for the medication. Therefore, observational nature of our study precludes establishing causality between testosterone usage and improved glioblastoma survival.

Supplemental methods

The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program includes specially funded population-based cancer registries that collect data on newly diagnosed cancers within catchment area. For individuals that are enrolled in Medicare, existing SEER case data can be linked to Medicare claims. This linked dataset includes 15 SEER registries covering $\sim 24\%$ of the US population⁷.

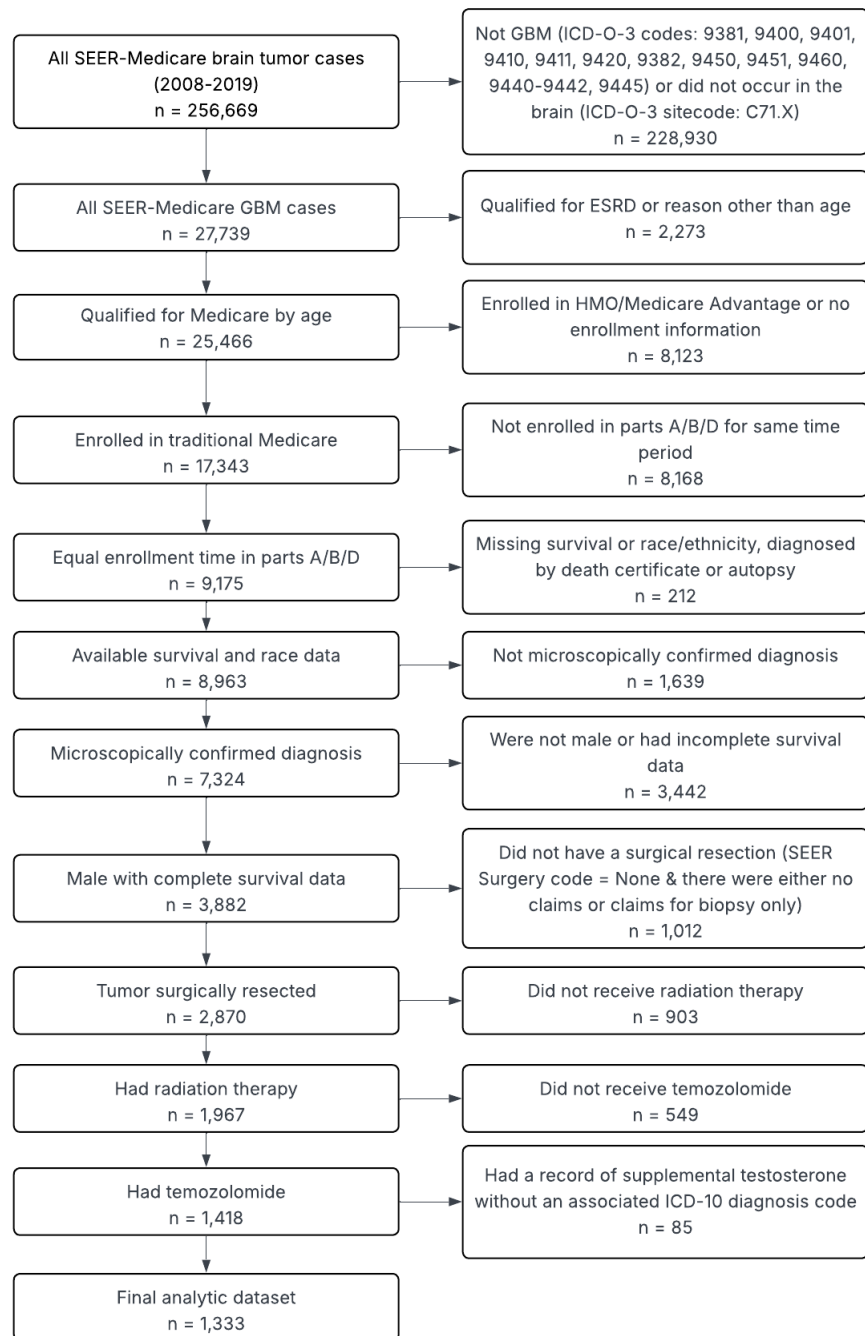
Male glioblastoma cases were identified using International Classification of Diseases, Oncology 3rd edition [ICD-O-3] morphology codes 9440-9442/3, and ICD-O-3 topography codes C71.0-C71.9. Individuals were excluded if: 1) qualified for Medicare for a reason other than age, including End-Stage Renal Disease and/or disability; 2) enrolled in a Health Maintenance Organization during the period of six months prior to twelve months after primary diagnosis with glioblastoma; 3) not continuously enrolled in Medicare Parts A, B, and D for the period of 6 months prior to diagnosis through the end of follow-up; 4) missing race, sex, or follow-up time (3,886 glioblastoma cases in males after these exclusions).

Primary site was categorized as supratentorial for primary sites C71.0-C71.4, infratentorial at primary sites C71.6-C71.7, and other for all other primary sites. Comorbidity score was estimated using the rule-out option of the SEER-Medicare SAS comorbidity macro (2021 version)⁸ using both ICD-9 and ICD-10 diagnosis codes. Comorbidity scores were calculated with the Charlson weight, and categorized as a score of 0-1, 2 or 3. Cancer treatment patterns extent of surgical resection, radiation, chemotherapy (temozolomide) of the primary site, were identified using SEER data and claims from the MEDPAR, NCH, OUTPAT, Part D Event, and Durable Medical Equipment files and treatments were only included if they occurred within 3 months of initial diagnosis. Extent of resection was identified using SEER-provided variable, RX_SUMM_SURG_PRIM_SITE, and claims with CPT and ICD-9 and ICD-10 procedure codes.

In SEER, having a surgical procedure is based on the variable RX_SUMM_SURG_PRIM_SITE_1998 or the presence of a Medicare claim. Extent of resection is further classified into no surgery, gross total resection (GTR), subtotal resection (STR), unknown surgery, and other surgery. Using the SEER variable, extent of resection was identified as no surgery if they had a value of 0, gross total resection (GTR) if they had a value of 30 or 55, subtotal resection (STR) if they had a value of 20-22 or 40, other surgery if they had a value of 10 or 90, and unknown surgery if they had a value of 99. When the SEER surgery variable is either 0 or 99, we further classified surgery type using the linked Medicare claims data. In the claims data, we are able to identify “biopsy only” claims based on *International Classification of Disease version 9 or version 10 (ICD-9, ICD-10)* procedure code or *Common Procedural Terminology (CPT)*. For all other neurosurgical resection *International Classification of Disease version 9 or version 10 (ICD-9, ICD-10)* procedure code or *Common Procedural Terminology (CPT)*, those were classified as other surgery, given we cannot distinguish from the procedure codes the extent of surgical resection.

Radiation therapy and chemotherapy were defined by ≥ 2 claims for the same code separated by at least one week started after the diagnosis date. Radiation therapy was identified using the SEER-provided radiation variable (RADIATION), with a value of '0' or '7' corresponding to no radiation, values 1-6 corresponding to some radiation, and all other values corresponding to missing data. When SEER radiation data were missing, the presence of radiation therapy in the linked Medicare claims data were used to identify radiation therapy. Temozolomide use was identified using FDA National Drug Classification (NDC) codes in the Medicare linked claims data.

After all exclusions, 1,333 total individuals remained in the analytic dataset. The process of exclusions is shown below.



Supplemental testosterone use was identified using both NDC codes from Part D pharmaceutical claims as well as CPT codes from the Durable Medical Equipment file. Use of supplemental testosterone was identified one of two ways:

1. Presence of ≥ 2 - codes associated with testosterone supplementation (Table 1), greater than or equal to 14 days apart
2. Presence of at least 1 CPT code for receiving an injection or implantation of testosterone replacement (Table 2), or at least 1 CPT code associated with receiving a non-specific injection (which may be used for supplemental testosterone) and at least 1 diagnosis code associated with the Medicare Reimbursement list (Table 3).

Individuals with no claims following date of diagnosis were excluded from the analysis.

Data processing and analyses were conducted in SAS 9.4 and R 4.3 using the following packages: using the following packages: gtsummary, patchwork, survival, survminer, and tidyverse suite.⁹⁻¹²

Table 1. FDA NDC Codes for identifying testosterone therapy use

Drug	NDC codes
Ando	10039010002, 10039010003, 10039004802, 10039003902
Andro	456100410, 456060410
Andro-Cyp	588507670, 588507770
Andro-Cypionate	361112270
AndroGel	51842501, 51842530, 54569533800, 54868479200, 68115080930, 51845001, 51845030, 16590071930, 21695011230, 35356037605, 54569533900, 54569533901, 54868481000, 51846212, 51846231, 51846201, 51846230, 51846233, 54569633700, 51848833, 51848888, 54868581400
Androderm	23599002, 23599060, 52544007654, 52544007660, 23599204, 23599230, 52544007730, 52544007754, 7315518, 52544046954, 52544046960, 54868370400, 62109913302, 7315613, 52544047030, 52544047054, 54868603200, 62109913402
Android-T	248355010
Androlan	527020855, 527019955, 527010655
Andronaq-La	131119105
Andryl	588507170
Anthatest	351004970
Aveed	67979051143
Axiron	2197561, 2197590, 54868640600
D-Tes	191005121, 191005221
Delatestyl	3032840, 54396032840, 54569541600, 54868501600, 67979050140, 3032816, 54396032816, 54569462000
Dep_Andro	456060310, 456101910
Depatesone	52604025506, 52604025606
Depo-Artest	49633098110, 49633098010
Depo-Testosterone	9008510, 9034701, 9034702, 35356005810, 54569530100, 54868079600, 9008601, 9008610, 9041701, 9041702, 9052001, 9052010, 54569141100, 54868021600, 54868021601, 55045302902, 55175500701
Depotest	314081570, 351411070, 314083570, 351411570
Duratest	43797001712, 43797001812
Durathate	43797026012
Ed-Test	485108410, 485125610
Everone	314065270
Fortesta	63481018316
Histerone	43797002112
Homogene-S	251121010
Jatenzo	69087015812, 69087019812, 69087023712
Malogen_Cyp	237407065
Malogen_La	237064065
Meditest	52349011510
Natesto	63481023901, 69654023901
Primotest_Depot	52083050810
Reposterone	537241170
Ru-Androspan	524011910
Russ-Test	50474001310
Shotest	47649012905
Shotest_La	47649012805
Striant	52244003060, 55056306001
Tes	191008821
Tesamone	217681208
Tesionate	551002310, 551002410
Tesone	52604008406
Testa_C	298683561

Testa_Span	684015210
Testaqua	351048270, 351048170
Testim	16590085330, 35356075830, 54569559500, 54868498900, 66887000101, 66887000105
Testo_Nate_100_La	276045010
Testoderm	17314460803, 17314460824, 54569394400, 17314283603, 17314460903, 17314460936, 54569394500
Testoderm_TTS	17314471703
Testoject-La	259031110, 259035810
Testone	50930084020, 50930084050
Testone_CIK	76420065001
Testone_La	298621561
Testopel	10116100101, 10116100102, 10116100103, 43773100102, 43773100103, 43773100104, 66887000401, 66887000410, 66887000420
Testosterone_Aqueous	463106910
Testra-D	647056810, 647056910
Testred_Cypionate_200	187020010
Testro_La	463107010
Testron_100	647056710
Testronate	32889035610
Theranate	826008710
Virilon_IM	76030110
Vogelxo	245087135, 245087189, 245087240, 245087242, 245087105, 245087165
Xyosted	54436020002, 54436020004, 54436025002, 54436025004, 54436027502, 54436027504
Testosterone	72627220301, 591321617, 591321630, 45802011665, 49884041848, 49884041872, 591321726, 591321730, 832112035, 832112089, 45802011639, 49884051063, 49884051072, 45802028103, 45802028139, 45802075403, 45802075439, 68382036212, 68382036214, 45802036602, 45802036665, 45802075402, 45802075465, 68382036213, 68382036215, 591236360, 603783188, 591292102, 591292118, 832112140, 832112142, 45802011602, 591292418, 16714096701, 24979007815, 43598030488, 45802075401, 68180094111, 68382036211, 69238101302, 591211481, 45802061001, 66993096389, 68180094311, 69097036344, 254101211, 254101263, 591352426, 591352430, 832112005, 832112065, 66993093430, 66993093454, 143615070, 144343014, 150087610, 150298910, 157025270, 182071463, 191004321, 223860010, 223860130, 228246460, 298613661, 304133556, 304133559, 314077170, 364660754, 364660756, 381008410, 381008430, 385103870, 402008410, 402008430, 418079141, 427065070, 524015210, 536950070, 536950075, 551004710, 574091610, 588506370, 677031021, 686008410, 719338687, 779760865, 781309370, 785801310, 814772340, 814772346, 839563230, 839563236, 853141070, 10719010242, 10974005110, 11289840008, 12071053079, 17022316303, 17022326303, 35470900604, 45985056110, 47202410201, 49727075210, 52406008410, 52406008430, 53638008410, 53638008430, 54274052762, 54569220500, 63353915, 143614570, 144342514, 150087510, 150298810, 157025170, 182026163, 182026166, 188818344, 191004521, 223859010, 223859130, 228246260, 298611961, 304054456, 304054459, 314008310, 314076870, 361108370, 364660654, 364660656, 381008310, 381008330, 385103770, 402008310, 402008330, 418078110, 427064970, 436024870, 536930075, 551004610, 574091510, 588506270, 677030821, 684012610, 686008310, 719338571, 719338587, 779760565, 781309270, 814772040, 814772046, 839563530, 853145070, 893007189, 904087510, 904087610, 10719010142, 10974007510, 11289839008, 12071052979, 17022314203, 17022324203, 25332007003, 35470900505, 35470913204, 47202414301, 49648054456, 49648054459, 49727075010, 52406008310, 52406008330, 53638008310, 53638008330, 54274052562, 395812543, 395812556, 395812562, 574046001, 11299001013, 11299001017, 17317056700, 17317056707, 38779004703, 38779004704, 38779004705, 38779259805, 38779259809, 49452765001, 49452765002, 49452765003, 49452765303, 49871082510, 49871082511, 51552002909, 51552056402, 51552056404, 51552056405, 51552056407, 51552128302, 51552128304, 51552128305, 51552128306, 51927102600, 53118021301, 53118021305, 53118021325, 62991267207, 63275989804, 63275989805, 63275989808, 63275989809, 63370097025, 63370097035, 63370098035, 63370098050
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Testosterone propionate	93039731, 93039743, 143616870, 157025670, 182119763, 191004421, 223866010, 223866130, 228246760, 298613861, 304054756, 304054759, 304182959, 314077270, 364668654, 364668656, 381038310, 381038330, 385104170, 402038310, 402038330, 418085110, 463107310, 522044730, 522044770, 524015610, 536890070, 536890075, 574091910, 588506870, 677030921, 686038310, 719338187, 779761465, 781310270, 814768840, 839563830, 839563836, 904086810, 10719011042, 10974005210, 12071053479, 35470750604, 43797029112, 45124039731, 45124039743, 47202411701, 49072071710, 49137038330, 49648054756, 49648054759, 50272038310, 51309043310, 52406038310, 52406038330, 52584038310, 53638038310, 53638038330, 54274053162, 54569236300, 55045204402, 72627210602, 455477000, 455477010, 455477020, 455477030, 574046101, 574046105, 574046125, 802395717, 802395719, 802395721, 17317056800, 17317056802, 17317056803, 17317056807, 17317056808, 38779005403, 38779005404, 38779016502, 38779016503, 38779016504, 38779016505, 38779016506

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Table 2. CPT Codes for identifying testosterone therapy use

CPT Code	Description
11980	SUBCUTANEOUS HORMONE PELLET IMPLANTATION (IMPLANTATION OF ESTRADIOL AND/OR TESTOSTERONE PELLETS BENEATH THE SKIN)
84410	TESTOSTERONE; BIOAVAILABLE, DIRECT MEASUREMENT (EG, DIFFERENTIAL PRECIPITATION)
96372	THERAPEUTIC, PROPHYLACTIC, OR DIAGNOSTIC INJECTION (SPECIFY SUBSTANCE OR DRUG); SUBCUTANEOUS OR INTRAMUSCULAR
J1071	INJECTION, TESTOSTERONE CYPIONATE, 1 MG
J3121	INJECTION, TESTOSTERONE ENANTHATE, 1 MG
J3145	INJECTION, TESTOSTERONE UNDECANOATE, 1 MG
J3490	UNCLASSIFIED DRUGS

Codes based on CMS reimbursement policy¹³

Table 3. ICD-10 Codes for identifying medical necessity of supplemental testosterone

ICD-10 Code	Description
D35.2	Benign neoplasm of pituitary gland
D44.3	Neoplasm of uncertain behavior of pituitary gland
E23.0	Hypopituitarism
E23.1	Drug-induced hypopituitarism
E23.3	Hypothalamic dysfunction, not elsewhere classified
E23.6	Other disorders of pituitary gland
E23.7	Disorder of pituitary gland, unspecified
E29.1	Testicular hypofunction
E29.8	Other testicular dysfunction
E30.0	Delayed puberty
E89.5	Postprocedural testicular hypofunction
N44.00	Torsion of testis, unspecified
N44.01	Extravaginal torsion of spermatic cord
N44.02	Intravaginal torsion of spermatic cord
N44.03	Torsion of appendix testis
N44.04	Torsion of appendix epididymis
N45.2	Orchitis
N50.89	Other specified disorders of the male genital organs
Q53.00	Ectopic testis, unspecified
Q53.01	Ectopic testis, unilateral
Q53.02	Ectopic testes, bilateral
Q53.10	Unspecified undescended testicle, unilateral
Q53.111	Unilateral intraabdominal testis
Q53.112	Unilateral inguinal testis
Q53.12	Ectopic perineal testis, unilateral
Q53.20	Undescended testicle, unspecified, bilateral
Q53.211	Bilateral intraabdominal testes
Q53.212	Bilateral inguinal testes

Q53.22	Ectopic perineal testis, bilateral
Q53.9	Undescended testicle, unspecified
Q55.0	Absence and aplasia of testis
Z87.890	Personal history of sex reassignment
Z90.79	Acquired absence of other genital organ(s)

Codes based on CMS reimbursement policy¹³

After all exclusions and identification of testosterone treatments, demographics of the two treatment groups included in the analysis are provided below in Table 4.

Table 4. Demographics of male GBM patients

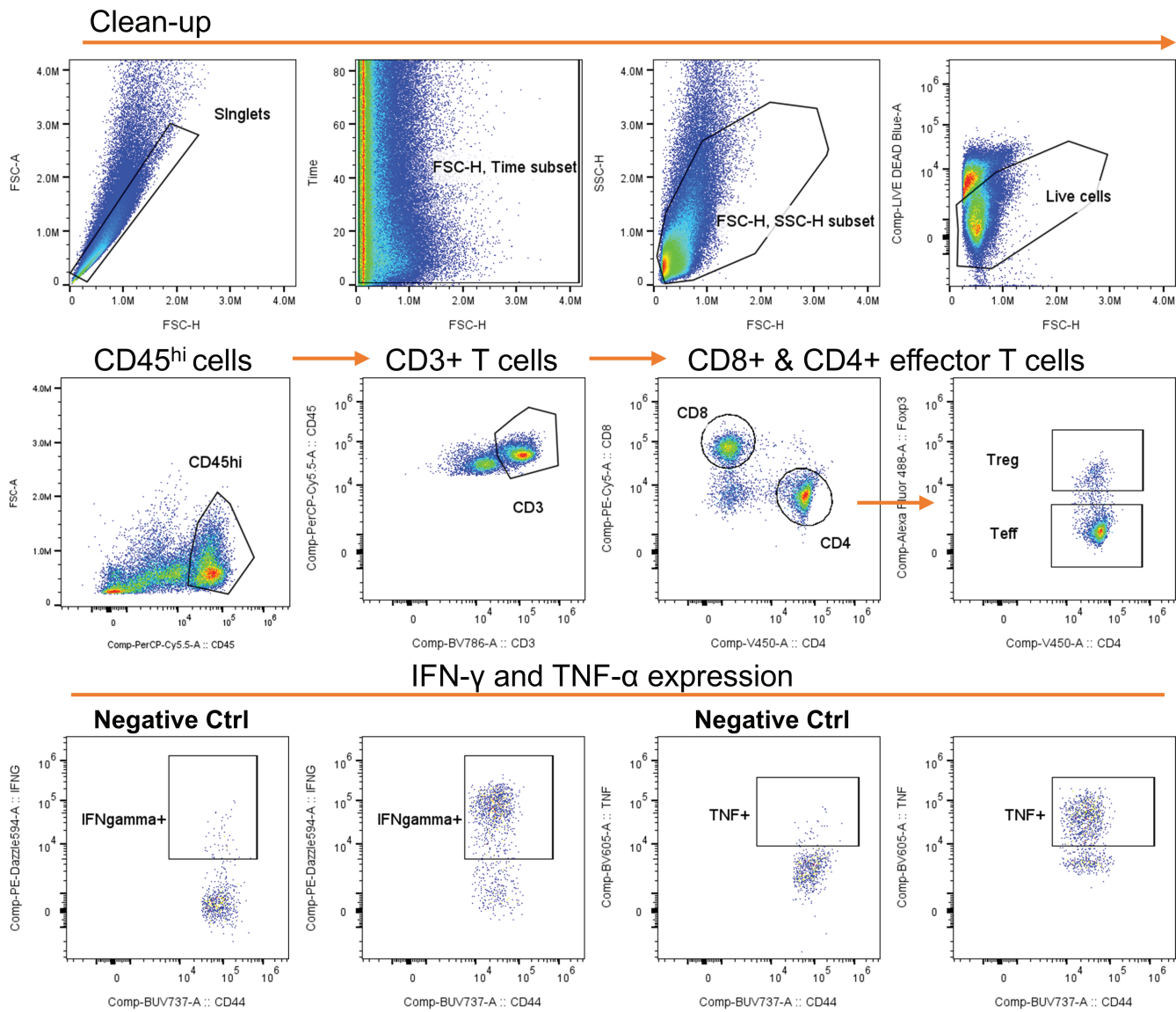
Characteristic	Temozolomide Only N = 1,272	Testosterone + Temozolomide N = 61
Patient Age	71 (68, 76)	71 (68, 74)
Race/Ethnicity		
Non-Hispanic White	1,159 (91%)	55 (90%)
Hispanic	62 (4.9%)	<11
Non-Hispanic Black	23 (1.8%)	<11
Non-Hispanic Other	27 (2.1%)	<11
Unknown	<11	<11
Extent of surgical resection		
Subtotal resection	478 (38%)	17 (28%)
Gross total resection	420 (33%)	21 (34%)
Other	374 (29%)	23 (38%)
Charlson Score		
0	684 (54%)	30 (49%)
1	240 (19%)	<11
2	192 (15%)	12 (20%)
3+	156 (12%)	< 11

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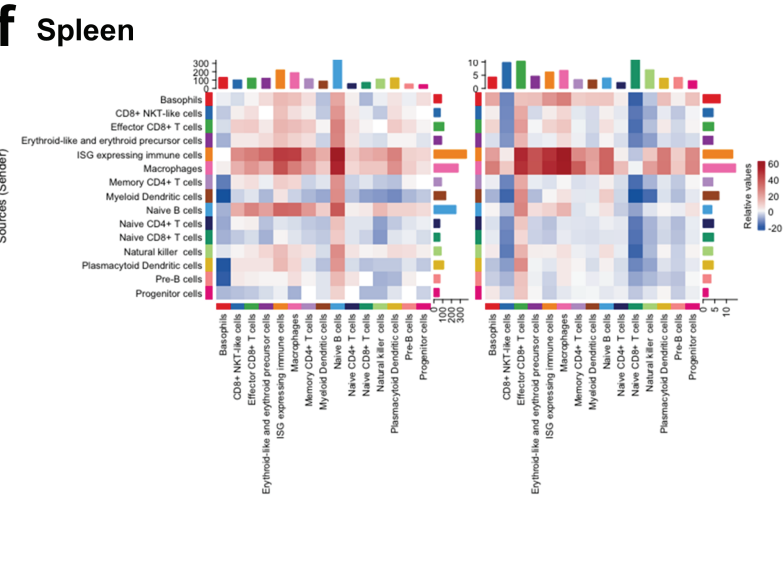
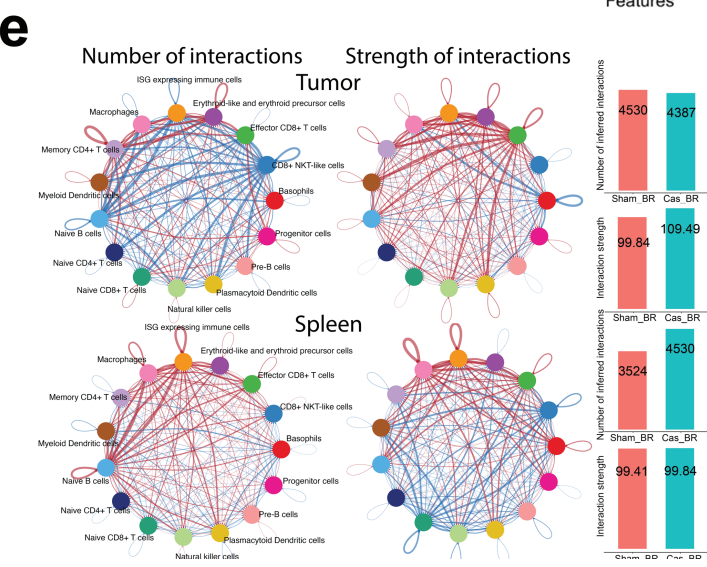
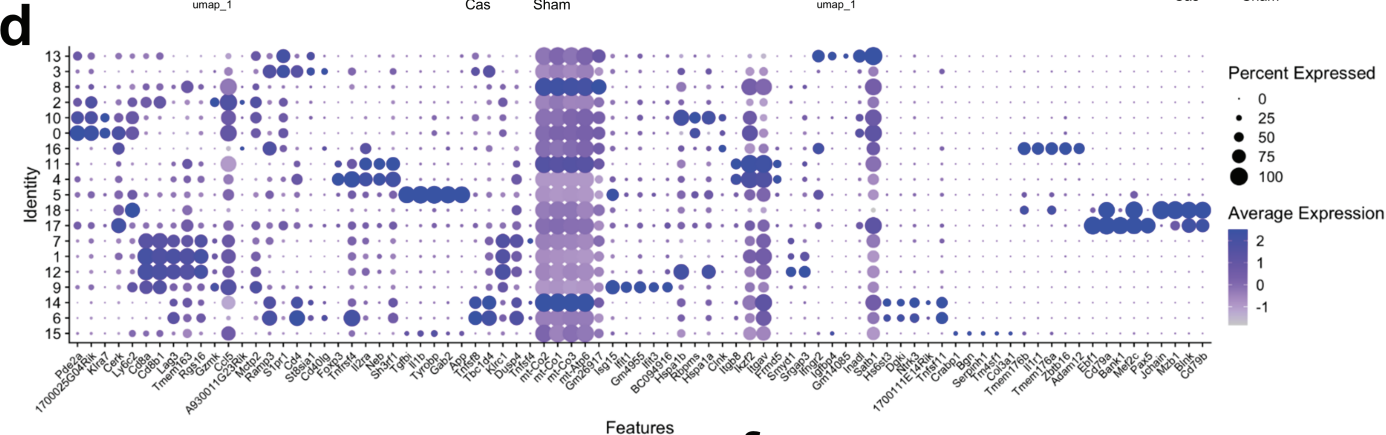
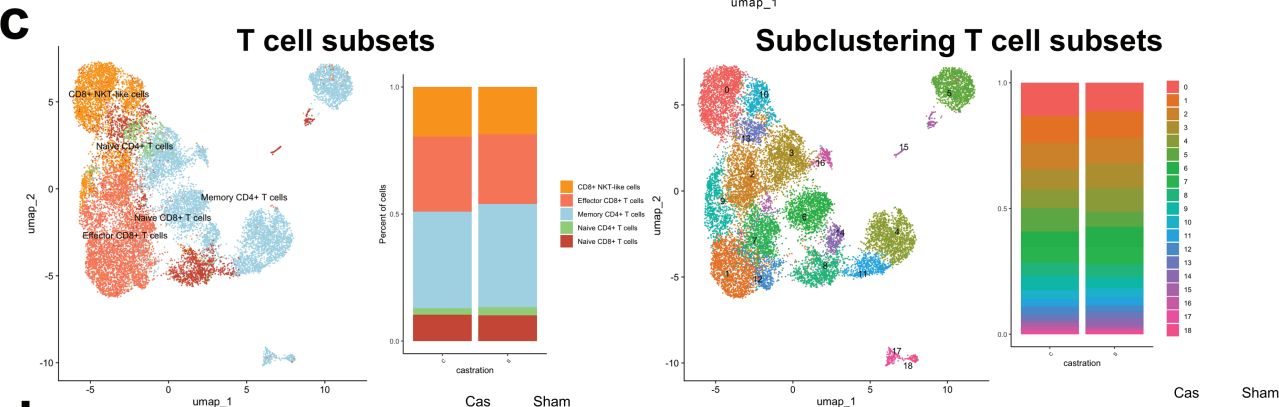
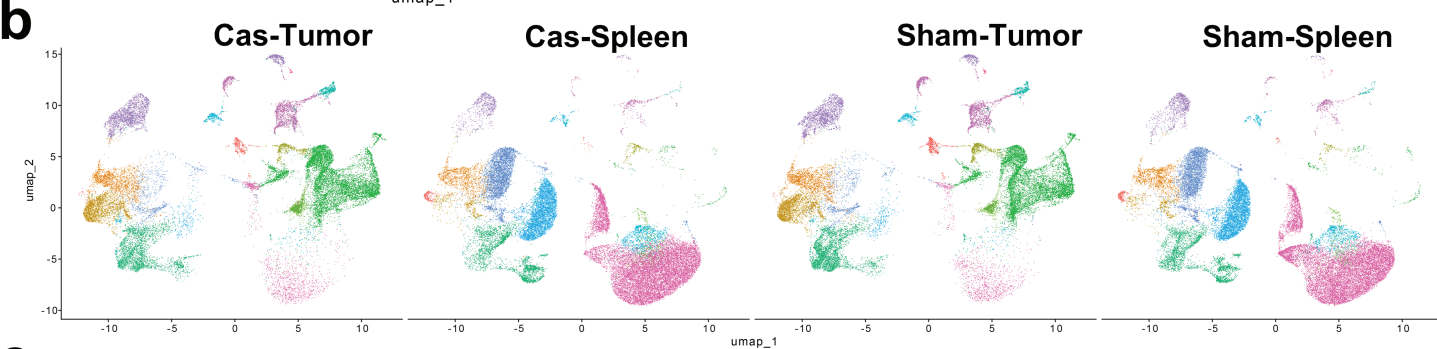
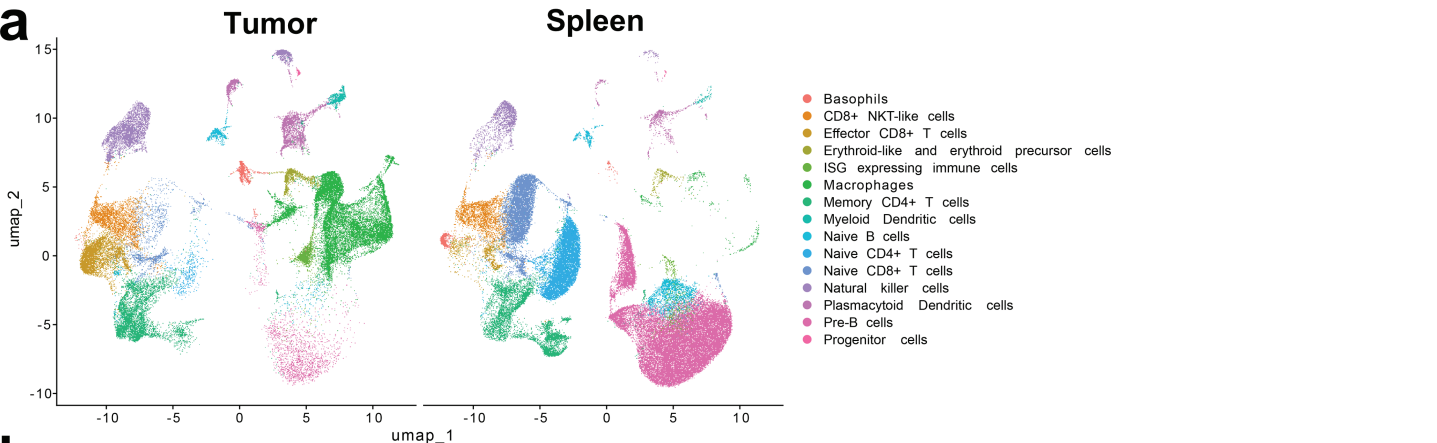
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Supplementary Fig 1.



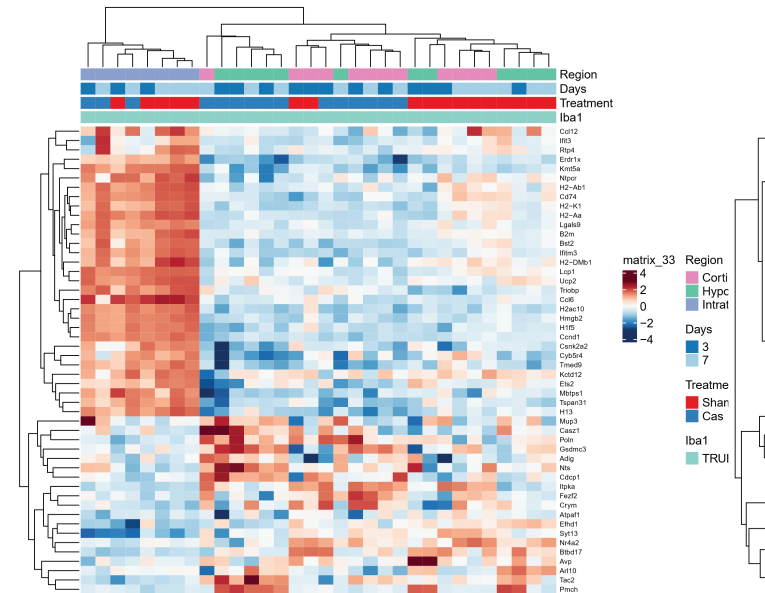
Supplementary Figure 1. A gating strategy of flow cytometry analysis. Cytokine expression in T cells. Samples without re-stimulation were used as a negative control for cytokine expression.

Supplementary Fig 2.

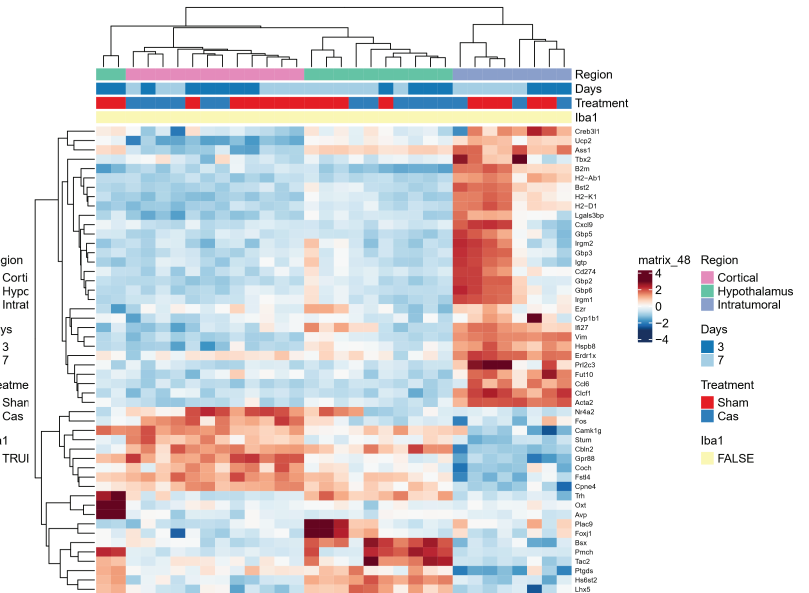


Supplementary Fig 3.

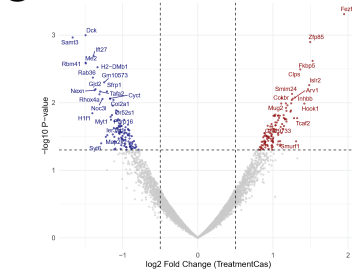
a Iba1+, day 3 & 7, all ROIs



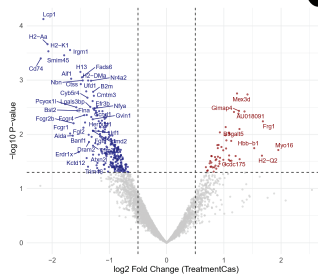
b Iba1-, day 3 & 7, all ROIs



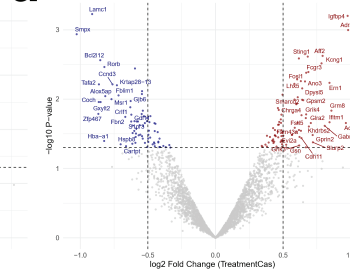
c Day 3 Iba1+ Cortical



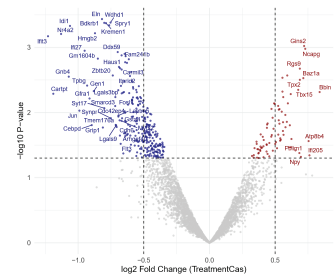
Day 7 Iba1+ Cortical



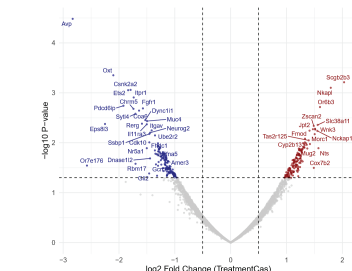
d Day 3 Iba1- Cortical



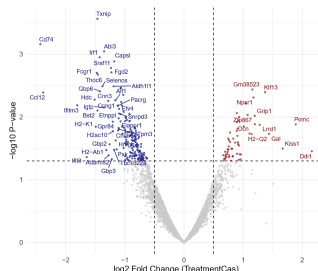
Day 7 Iba1- Cortical



Day 3 Iba1+ Hypothalamus



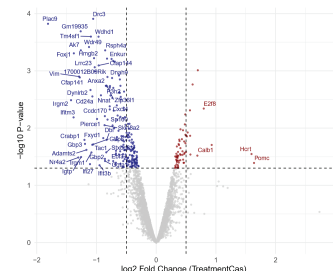
Day 7 Iba1+ Hypothalamus



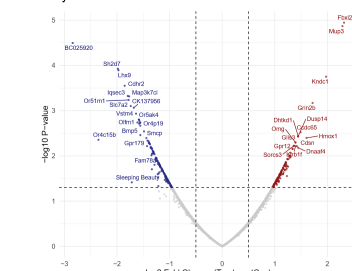
Day 3 Iba1- Hypothalamus



Day 7 Iba1- Hypothalamus



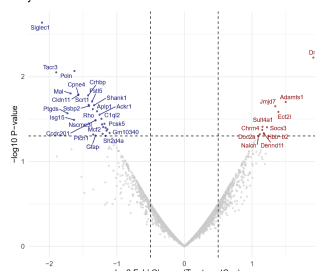
Day 3 Iba1+ Intratumoral



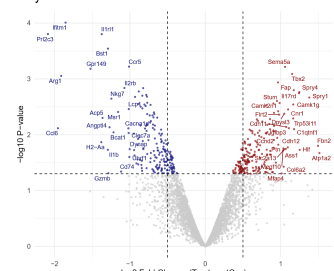
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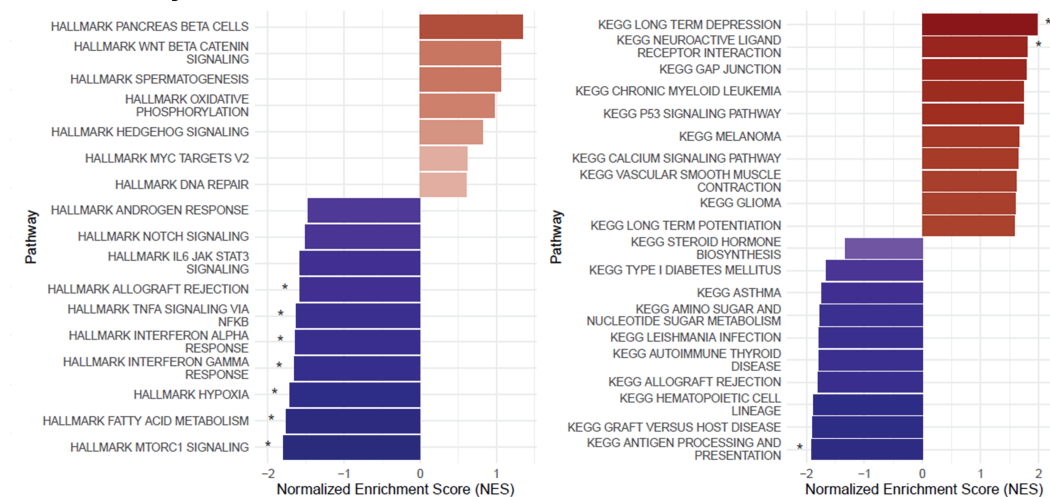
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Day 7 Iba1- Intratumoral

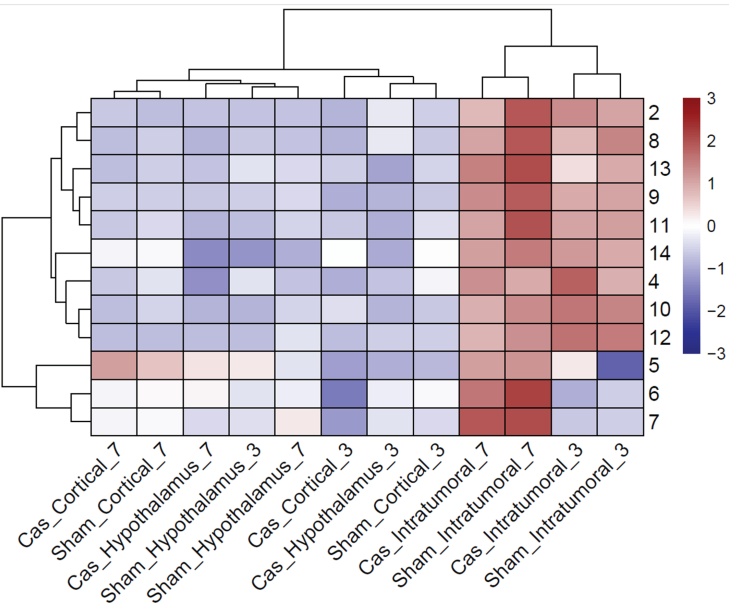


e Iba1-, day 3 & 7, all ROIs

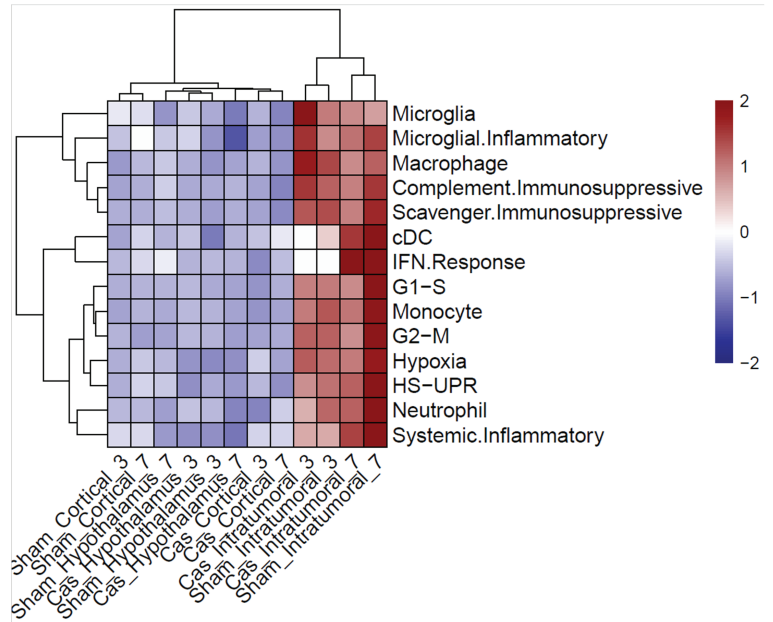


f Iba1+, day 3, intratumoral

Myeloid signature gene set
from Ruiz-Moreno *et al.*, 2025

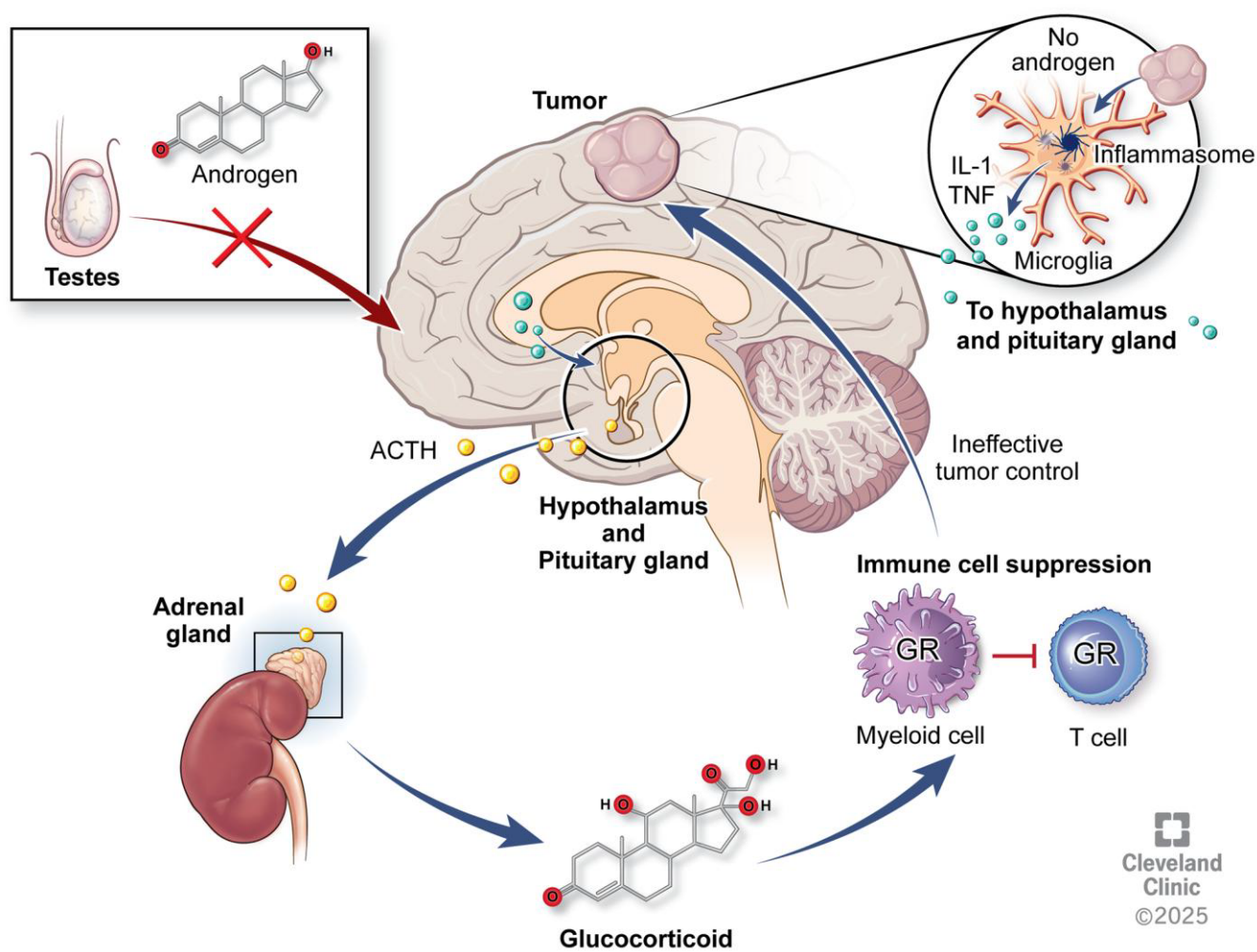


Myeloid signature gene set
from Miller *et al.*, 2025



Supplementary Figure 3. Spatial transcriptomics on mouse brain with brain tumors. Whole transcriptomics analysis was performed on brain tissues using Nanostring Digital Spatial Profiler platform. Samples were prepared from castrated or sham mice with brain tumors (SB28) on day 3 or 7 post-tumor implantation. n=3/group. Tissues were stained with Iba1 (myeloid marker) and cyto13 (nuclei). Regions of interest (ROIs) were selected on cortical, hypothalamic, and intratumoral areas. **a-b**, Heatmap of Iba1+ myeloid or Iba1- non-myeloid cell compartments. **c-d**, Volcano plots showing differentially expressed genes in cas vs sham in Iba1+ myeloid (**c**) and Iba1- non-myeloid (**d**) cell compartments. **e**, Pathway enrichment analysis on Iba1- non-myeloid cells. **f**, GBM-associated myeloid signature gene set analysis on Iba1+ myeloid cells from day 3 intratumoral regions.

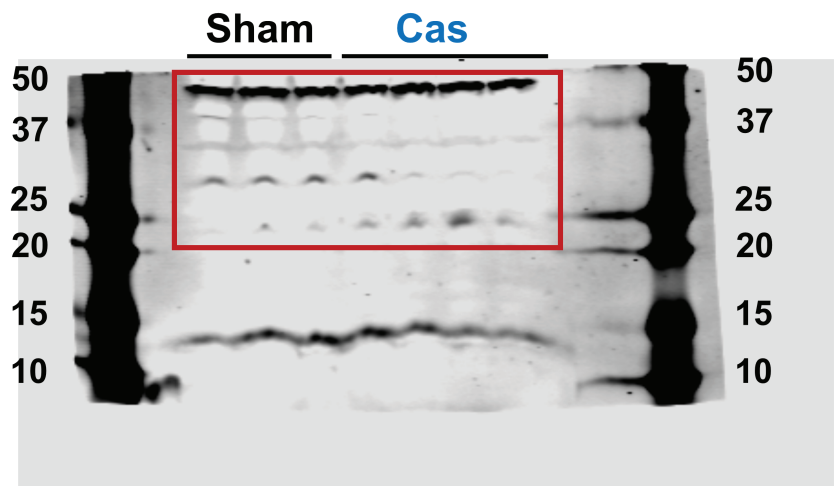
Supplementary Fig 4.



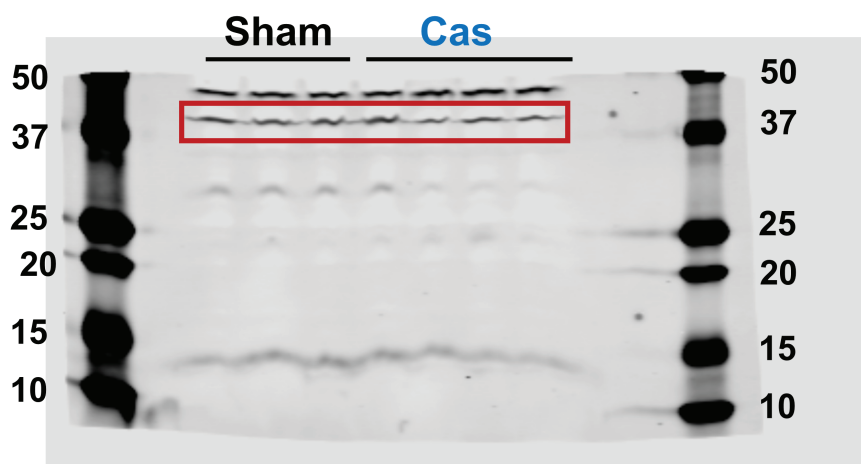
Supplementary Figure 4. Proposed model depicting how the loss of testosterone and the presence of a brain tumor synergistically induce heightened neuroinflammation, which activates the HPA axis and regulates anti-tumor immunity. Illustration reproduced with permission. Cleveland Clinic Foundation ©2026.

Supplementary Fig 5.

a Caspase-1 and cleaved caspase-1



b β -Actin



Supplementary Figure 5. Unprocessed immunoblot for Figure 6l-m. The blot was probed for caspase-1 and cleaved caspase-1, stripped, and then re-probed for β -actin.