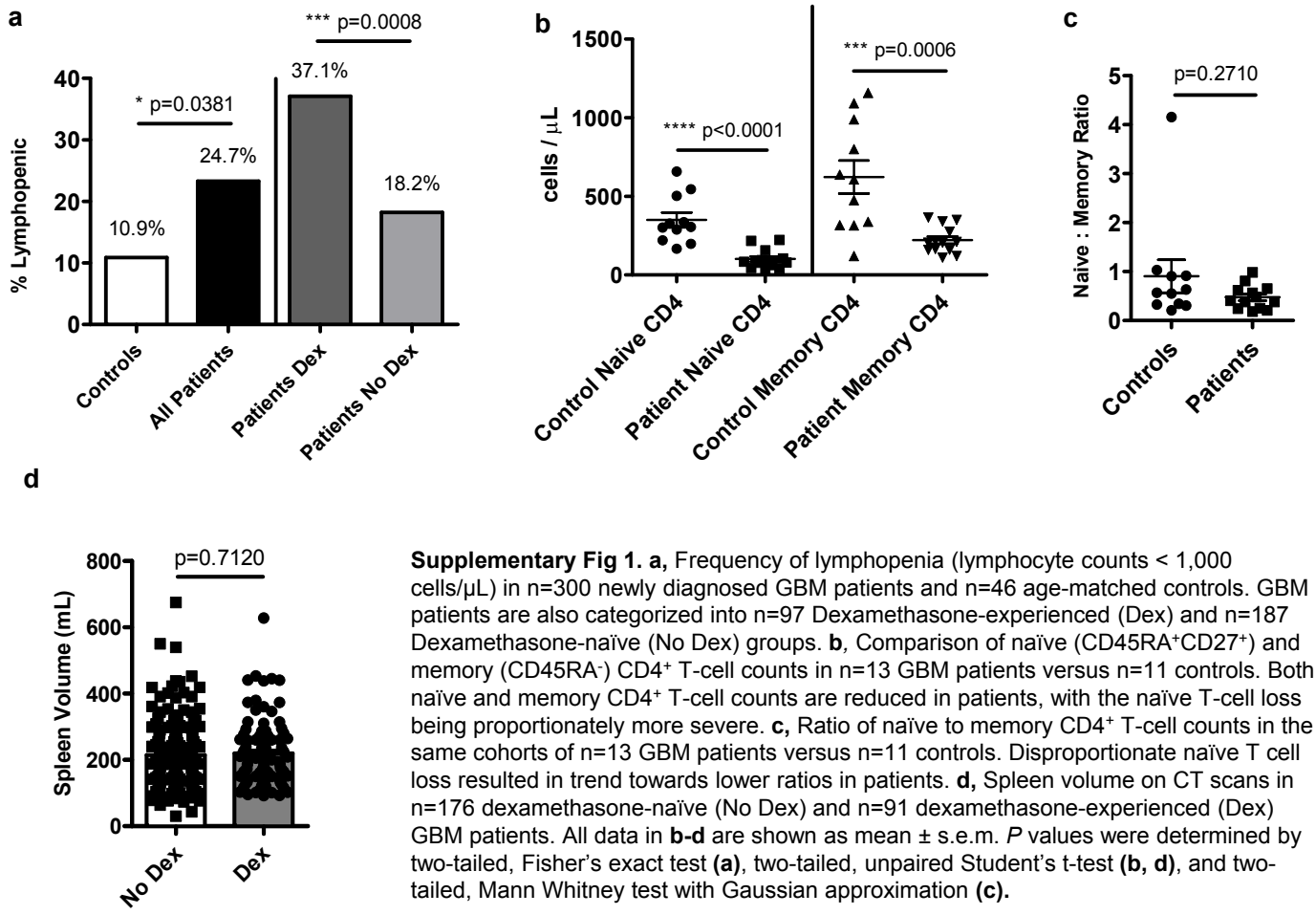
SUPPLEMENTARY INFORMATION

Table 1 | Retrospective Study: Patient and Control Characteristics

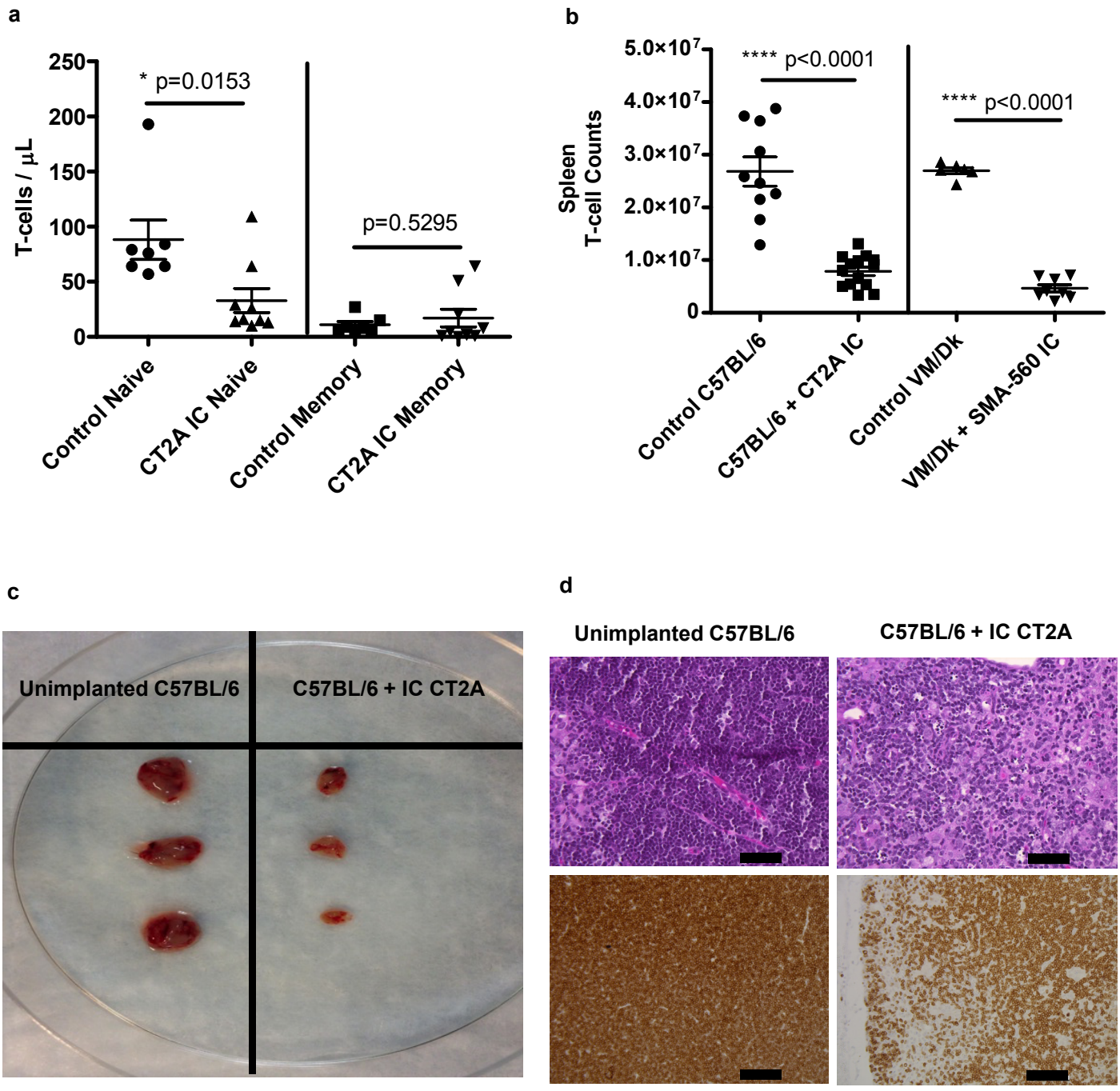
Characteristics	No. of controls (%) N = 46	No. of patients (%) N = 300
Age		
Median	62.5	66
Range	38-83	21-91
Sex		
Male	28 (61)	162 (54)
Female	18 (39)	138 (46)
Steroid status		
Naive	46 (100)	187 (63)
Experienced	0 (0)	97 (32)
Unknown	0 (0)	16 (5)

Table 2 | Prospective Study: Patient and Control Characteristics

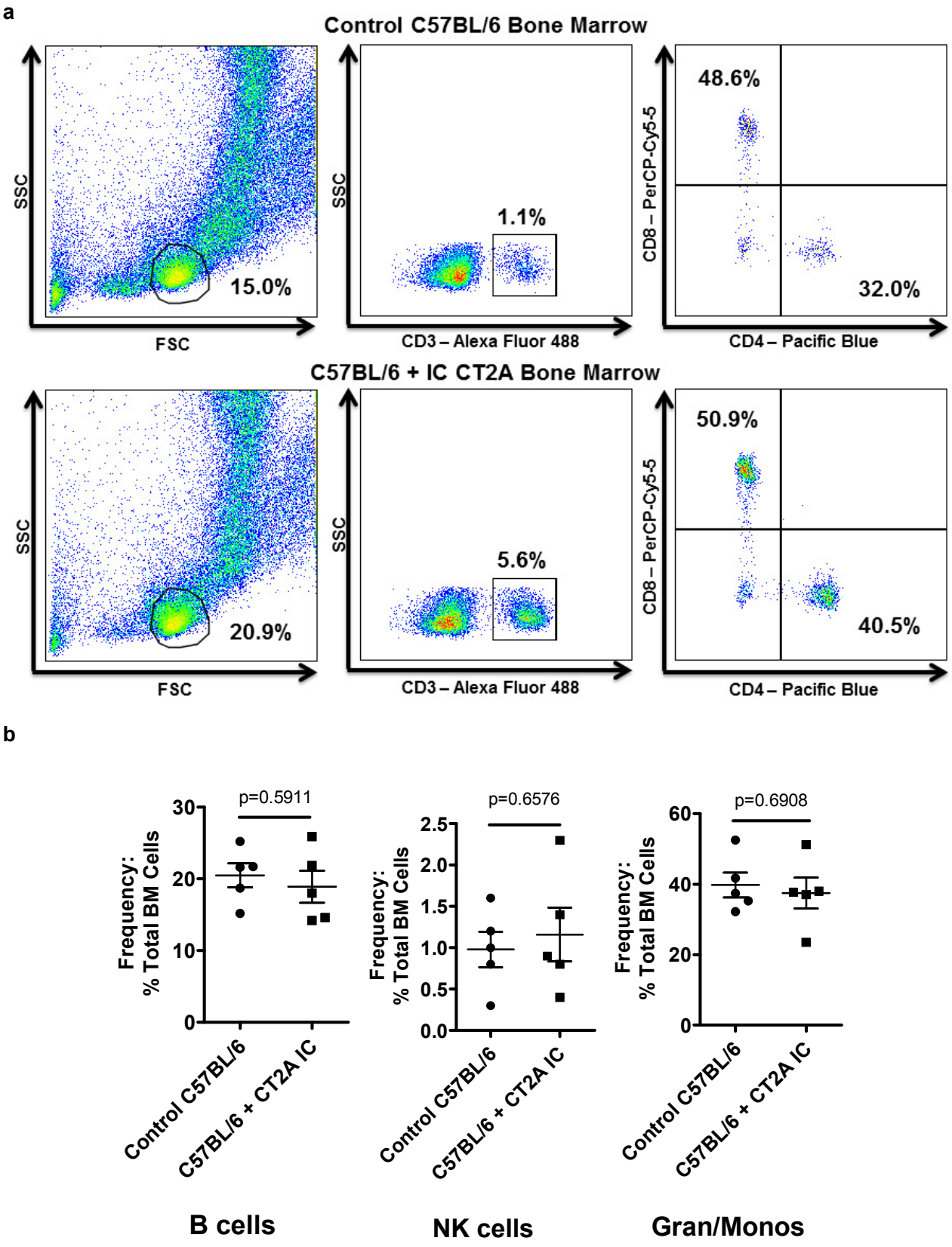
Characteristics	No. of controls (%) N = 13	No. of patients (%) N = 15
Age		
Median	68	56
Range	41-86	30-75
Sex		
Male	7 (54)	11 (73)
Female	6 (46)	4 (27)



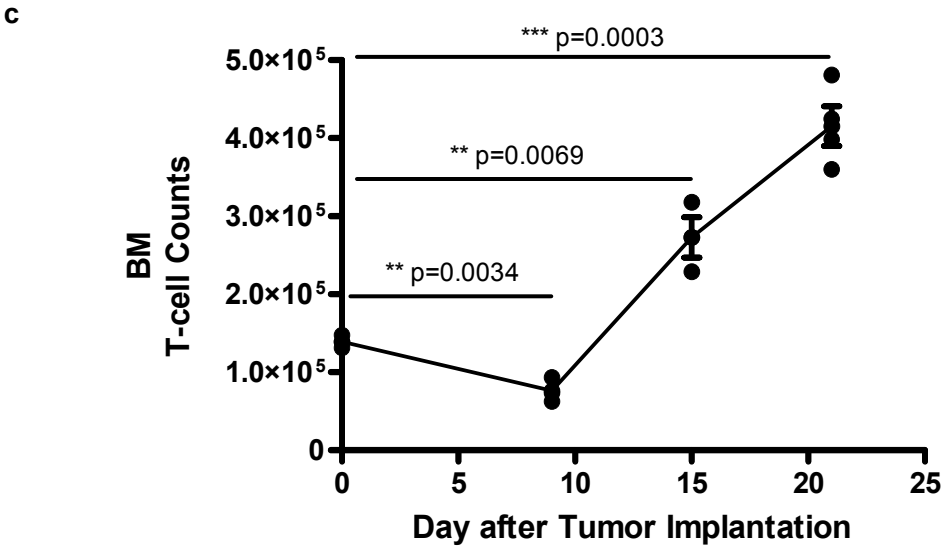
Supplementary Fig 1. a, Frequency of lymphopenia (lymphocyte counts < 1,000 cells/μL) in n=300 newly diagnosed GBM patients and n=46 age-matched controls. GBM patients are also categorized into n=97 Dexamethasone-experienced (Dex) and n=187 Dexamethasone-naïve (No Dex) groups. **b**, Comparison of naïve ($CD45RA^+CD27^+$) and memory ($CD45RA^-$) $CD4^+$ T-cell counts in n=13 GBM patients versus n=11 controls. Both naïve and memory $CD4^+$ T-cell counts are reduced in patients, with the naïve T-cell loss being proportionately more severe. **c,** Ratio of naïve to memory $CD4^+$ T-cell counts in the same cohorts of n=13 GBM patients versus n=11 controls. Disproportionate naïve T cell loss resulted in trend towards lower ratios in patients. **d,** Spleen volume on CT scans in n=176 dexamethasone-naïve (No Dex) and n=91 dexamethasone-experienced (Dex) GBM patients. All data in **b-d** are shown as mean ± s.e.m. *P* values were determined by two-tailed, Fisher's exact test **(a)**, two-tailed, unpaired Student's t-test **(b, d)**, and two-tailed, Mann Whitney test with Gaussian approximation **(c).**

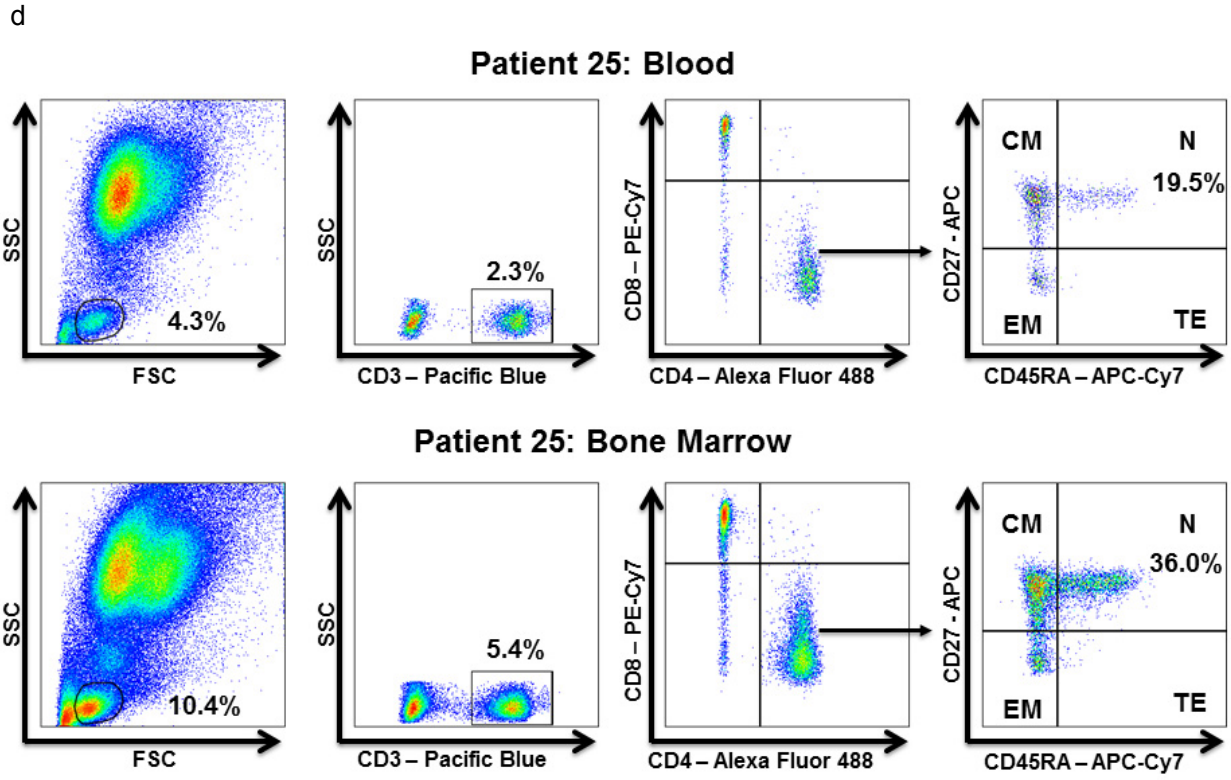


Supplementary Fig. 2. a, Blood naïve ($CD44^{-}CD62L^{+}$) and memory ($CD44^{+}$) $CD4^{+}$ T-cell counts depicted for n=7 control C57BL/6 and n=9 IC CT2A IC mice. **b,** Spleen T-cell counts in n=10 control C57BL/6 and n=6 control VM/Dk mice or n=14 IC CT2A glioma-bearing C57BL/6 and n=8 IC SMA-560 glioma-bearing VM/Dk mice. Data in **a-b** are shown as mean ± s.e.m. *P* values were determined by two-tailed, unpaired Student's t-test. **c,** Gross image depicting thymuses taken from unimplanted or IC CT2A glioma-bearing C57BL/6 mice. **d,** H&E staining (upper panel) or IHC for CD3 (lower panel) of FFPE thymus taken from unimplanted or IC CT2A glioma-bearing C57BL/6 mice. Histopathologic examination of thymus from IC CT2A mice showed loss of normal cortico-medullary architecture. These findings accompanied marked organ lymphopenia and lymphoid necrosis. IHC confirmed thymus of IC CT2A mice has marked T-cell lymphopenia, for H&E, scale bar = 50 µm; for IHC, scale bar = 200 µm. All data in **a-d** are representative findings from one of at least three independently repeated experiments with similar results. Both blood draw **(a)** and spleen/thumus harvest **(b-d)** were performed at 18 days following tumor implantation.

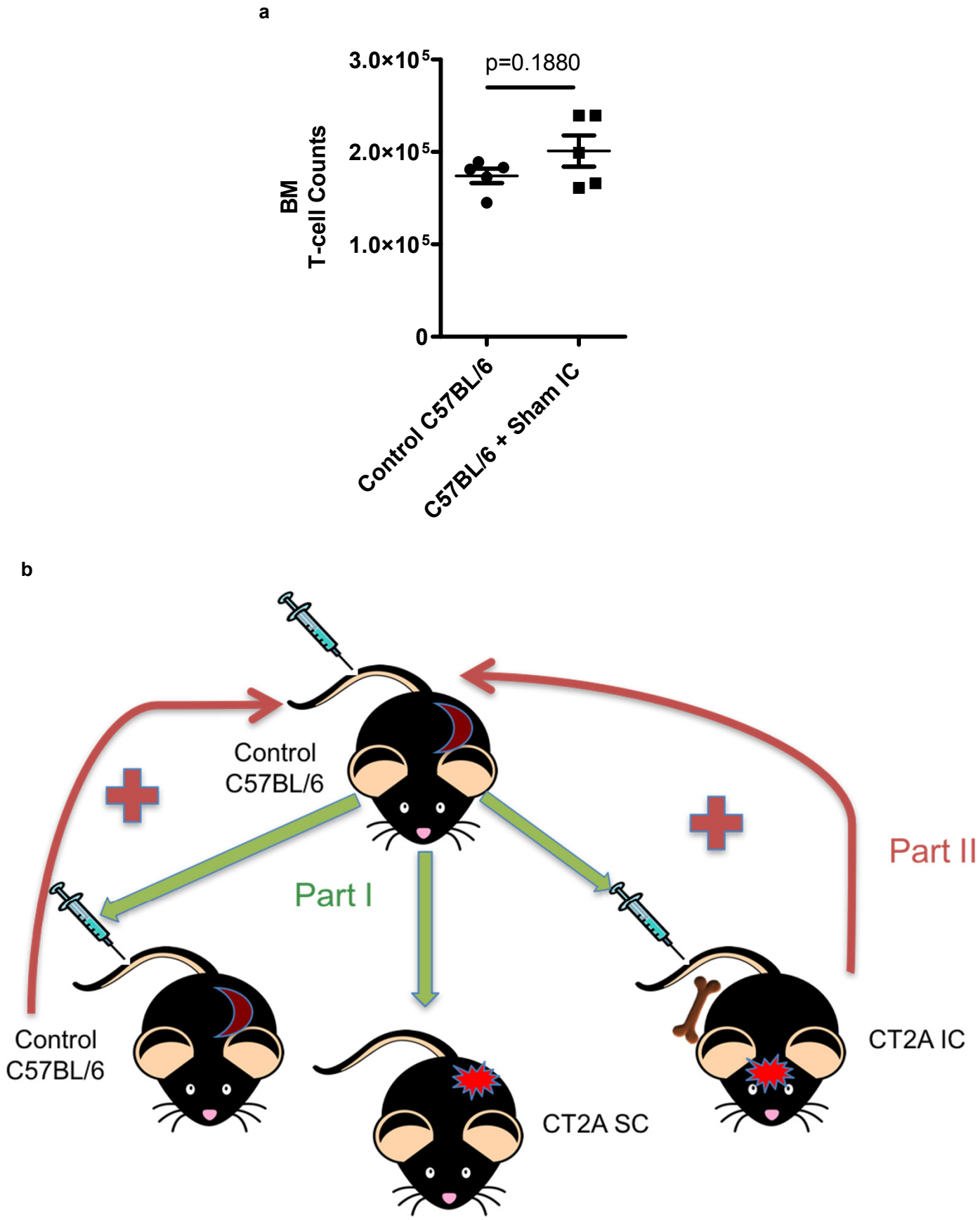


Supplementary Fig. 3. a, Sample flow cytometry plot examining bone marrow T-cells in control C57BL/6 mice (top), or the same mice bearing IC CT2A (bottom). **b,** Frequency of additional leukocyte populations in the bone marrow of n=5 control C57BL/6 or n=3 IC CT2A glioma-bearing C57BL/6 mice. Data in **a** are representative findings from one of at least three independently repeated experiments with similar results. Data in **b** are cumulative results from two experiments. Data in **b** are shown as mean ± s.e.m. *P* values were determined by two-tailed, unpaired Student's t-test.

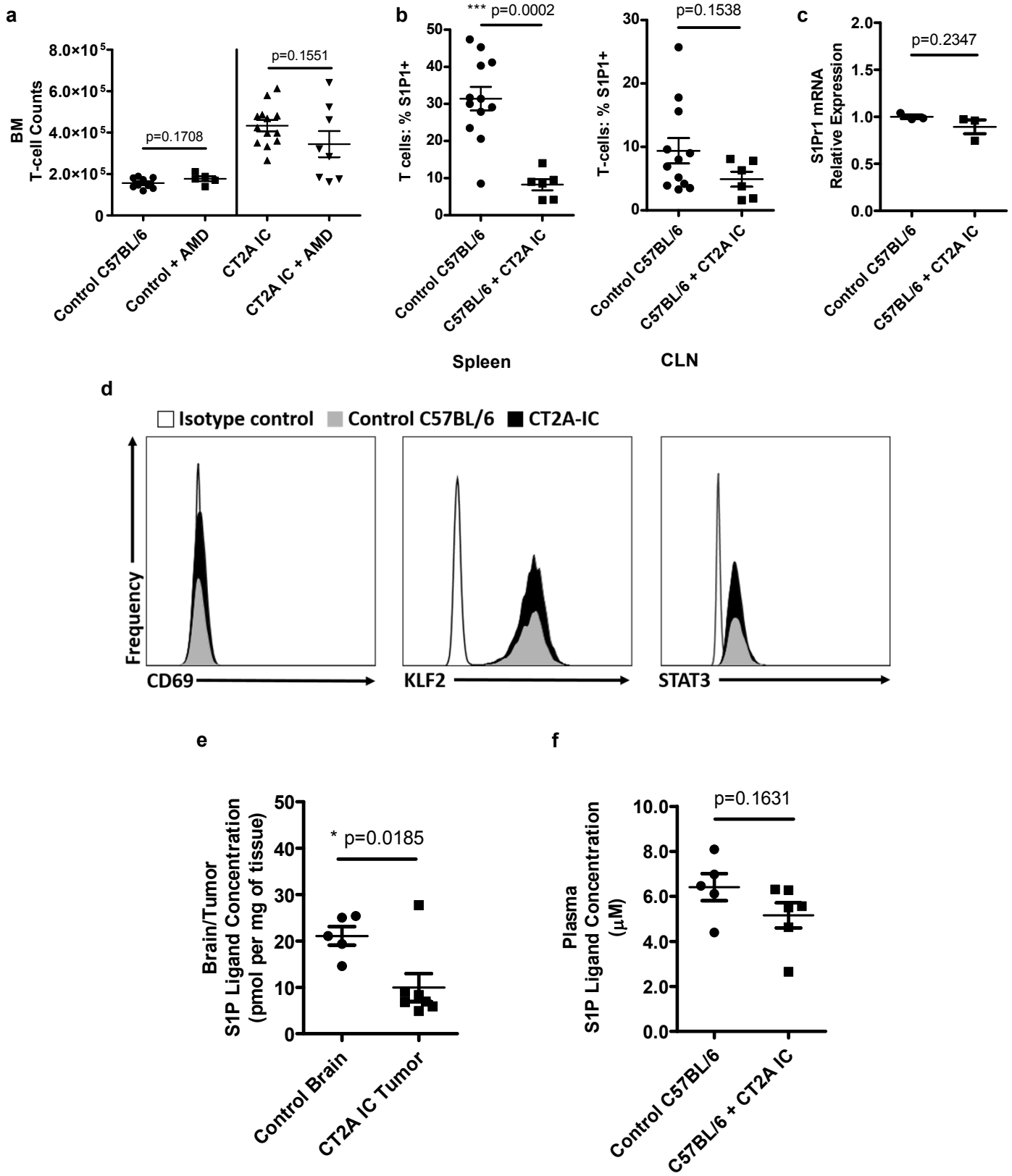




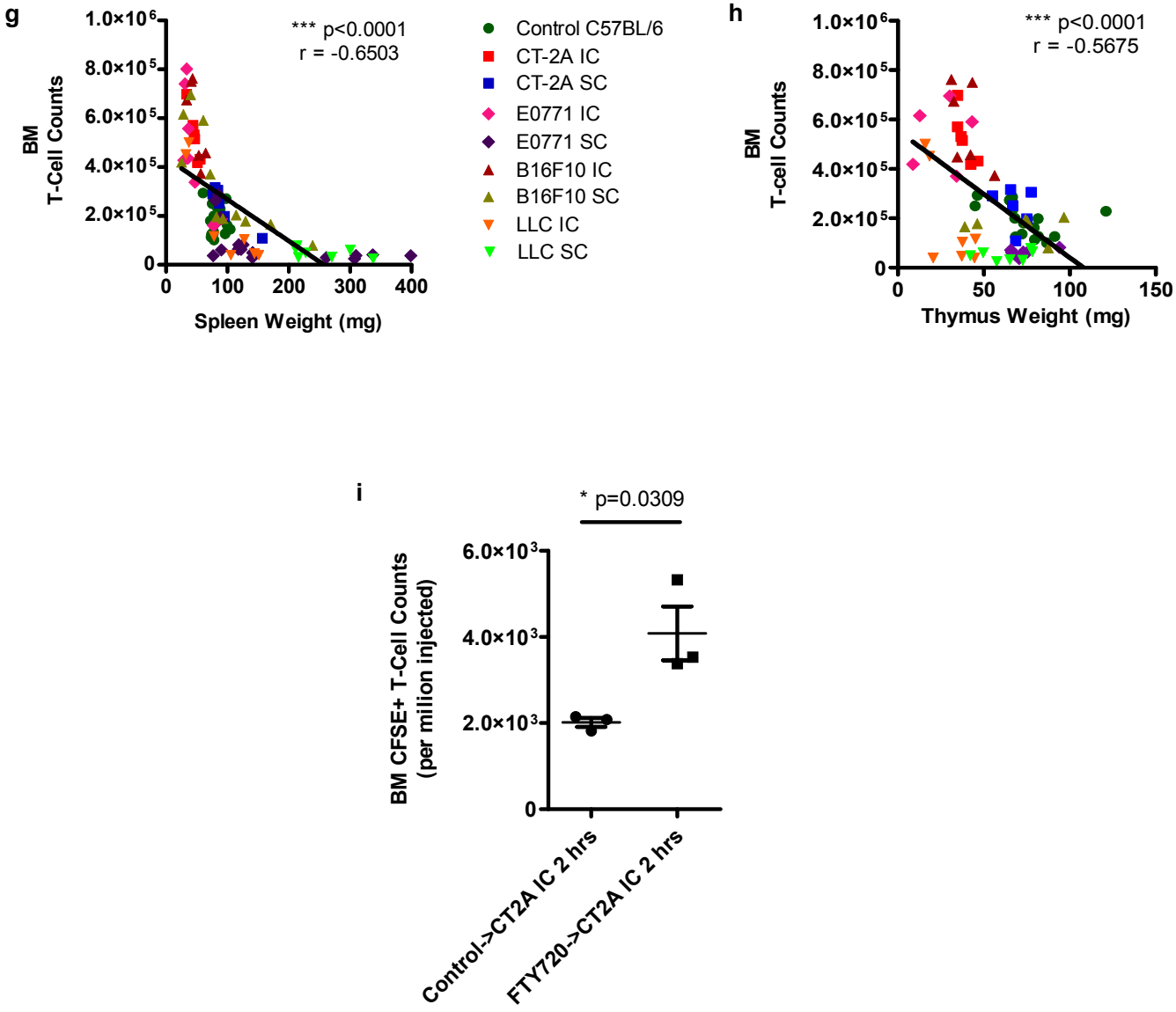
Supplementary Fig. 3. (Cont.) c, Time course of T-cell accumulation in the bone marrow of mice bearing IC CT2A after tumor implantation. Bone marrow were harvest from n=3 IC CT2A glioma mice on day 0, 9, 15 and n=4 IC CT2A glioma mice on day 21 after tumor implantation. Data in **c** are representative findings from one of at least three independently repeated experiments with similar results. Data in **c** are shown as mean ± s.e.m. *P* values were determined by two-tailed, unpaired Student's t-test. **d,** Sample flow cytometry plot examining bone marrow T-cells. The relative proportions of central memory (CM), naïve (N), effector memory (EM), and terminal effector (TE) populations in a patient blood (top) and bone marrow (bottom). $CD4^+$ T-cells are depicted. Similar results were obtained for $CD8^+$ T-cells.



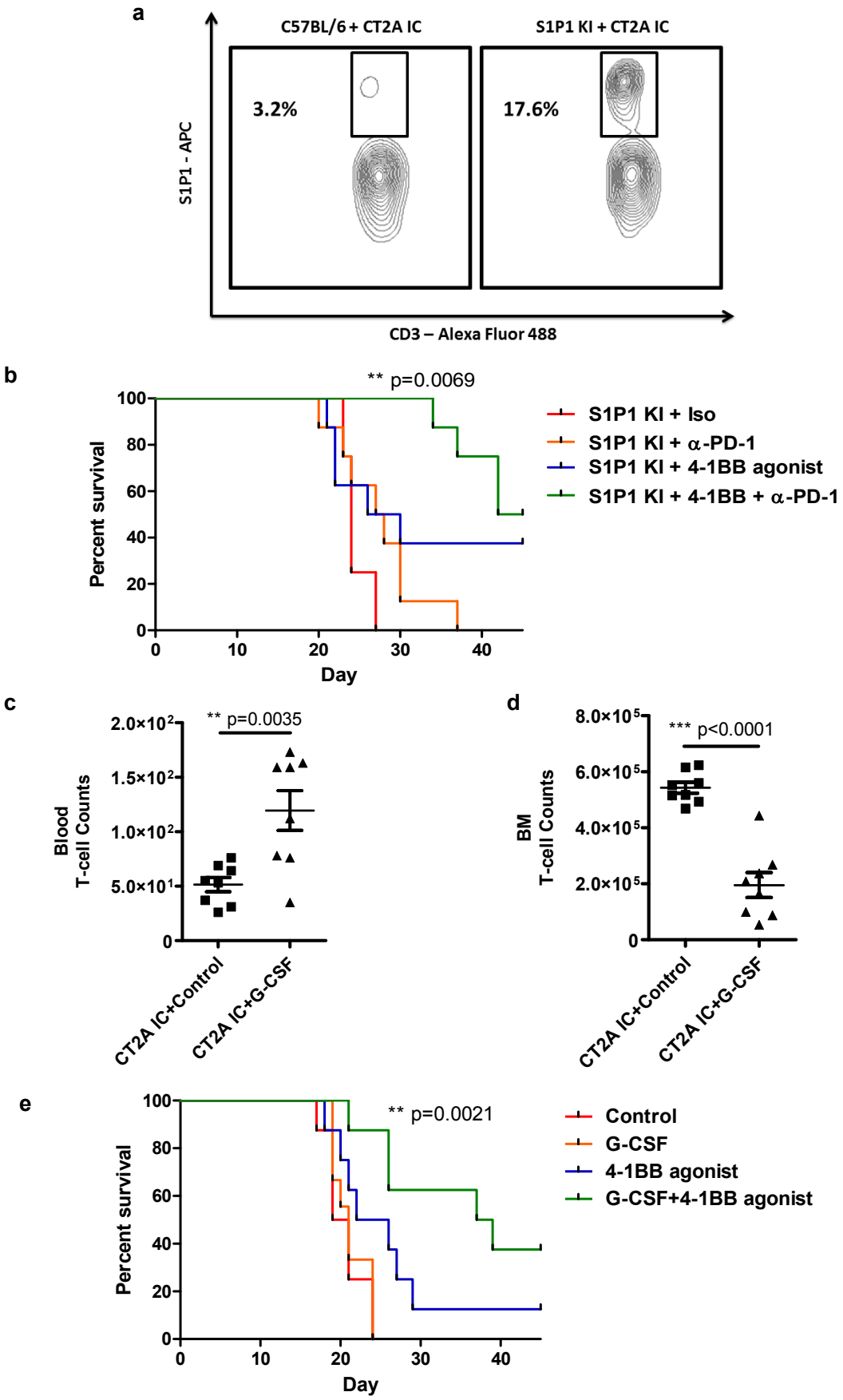
Supplementary Fig. 4. a, Bone marrow T-cell counts were compared among n=5 control un-operated C57BL/6 mice and n=5 C57BL/6 mice receiving sham IC injections of a saline/methylcellulose mixture. No difference in bone marrow T-cell counts was observed. Data depicted are from Day 18 post-injection. Data in **a** are representative findings from one of two independently repeated experiments with similar results. Data are shown as mean ± s.e.m. *P* values were determined by two-tailed, unpaired Student's t-test. **b,** Pictorial schematic for the experiments producing the data depicted in Figs 4 **d-f**



Supplementary Fig. 5. a. Bone marrow T-cell counts are shown for n=9 control tumor-naïve C57BL/6 and n=13 IC CT2A-bearing mice, or n=5 control tumor-naïve C57BL/6 and n=8 IC CT2A-bearing mice administered the CXCR4 antagonist AMD3100 (AMD). **b,** Frequency of S1P1^{+} T-cell populations in the spleen (left) and cervical lymph nodes (CLN) (right) of n=12 control C57BL/6 or n=6 CT2A IC mice. **c,** S1Pr1 mRNA expression levels in T-cells sorted from spleens of n=3 control C57BL/6 or n=3 CT2A IC mice assessed by qRT-PCR and normalized to GAPDH expression. **d,** Histograms showing expression levels of CD69, KLF2, and STAT3 in the T-cells of bone marrow of control C57BL/6 (gray) or CT2A IC (black) mice assessed by RNA prime flow. Data in **a-d** are representative findings from one of two independently repeated experiments with similar results. **e,** Concentration of S1P ligand in the plasma of n=5 control C57BL/6 or n=6 IC CT2A-bearing mice, as assessed by LC-MS/MS. **f,** Concentration of S1P ligand in the brain or brain tumor of n=5 control C57BL/6 or n=7 IC CT2A-bearing mice, as assessed by LC-MS/MS. Data in **f** are normalized to tissue weight. Data in **a-c, e, f** are shown as mean ± s.e.m. All *P* values were determined by two-tailed, unpaired Student's t-test.



Supplementary Fig. 5. (Cont.) g,h. Negative correlation between bone marrow T-cell counts and either spleen **(g)** or thymus **(h)** weight across IC and SC murine tumor models. Data in **g** were obtained from n=6 IC CT2A, n=9 IC E0771, n=6 IC B16F10, n=7 IC LLC, n=6 SC CT2A, n=10 SC E0771, n=11 SC B16F10, and n=7 SC LLC tumor-bearing mice. N=21 control C57BL/6 were also included. Data in **h** were obtained from n=6 IC CT2A, n=5 IC E0771, n=6 IC B16F10, n=7 IC LLC, n=6 SC CT2A, n=7 SC E0771, n=6 SC B16F10, and n=7 SC LLC tumor-bearing mice. N=21 control C57BL/6 were also included. Data in **g, h** are cumulative results from a minimum of two experiments with each tumor type. Two-tailed, *p* values and Pearson coefficients for **g, h** are depicted. **i,** Accumulation of adoptively transferred CFSE-labeled T-cells in the bone marrow of CT2A IC recipients that were treated with either vehicle control (n=3 recipient mice) or FTY720 (n=3 recipient mice) 2 hours prior to receiving transfers. Transferred cells were splenocytes from control C57BL/6 donors. Data in **i** are shown as mean ± s.e.m. The *p* value was determined by two-tailed, unpaired Student's t-test.



Supplementary Fig. 6. a, Representative flow cytometry plot depicting the frequency of S1P1 on the surface of T-cells in the bone marrow of C57BL/6 mice and S1P1 KI bearing IC CT2A tumor. **b**, S1P1 KI mice were implanted with IC CT2A tumors and treated with anti (α)-PD-1, 4-1BB agonist, or the combination of both (n=8 per group). Bone marrow **(c)** and blood **(d)** T-cell counts in n=8 control mice and n=8 IC CT2A-bearing mice administered control treatment or G-CSF intraperitoneally every 3 days following tumor implantation (Days 3-18). Counts were assessed 18 days following tumor implantation. **e,** IC CT2A IC mice were administered G-CSF, 4-1BB agonist, or the combination regimen of both drugs (n=8 per group). All data in **a-e** are representative findings from one of two independently repeated experiments with similar results. Data in **c, d** are shown as mean ± s.e.m. *P* values in **c, d** were determined by two-tailed, unpaired Student's t-test. Survivals in **b, e** were assessed by two-tailed generalized Wilcoxon test. *P* values for overall comparison are depicted.