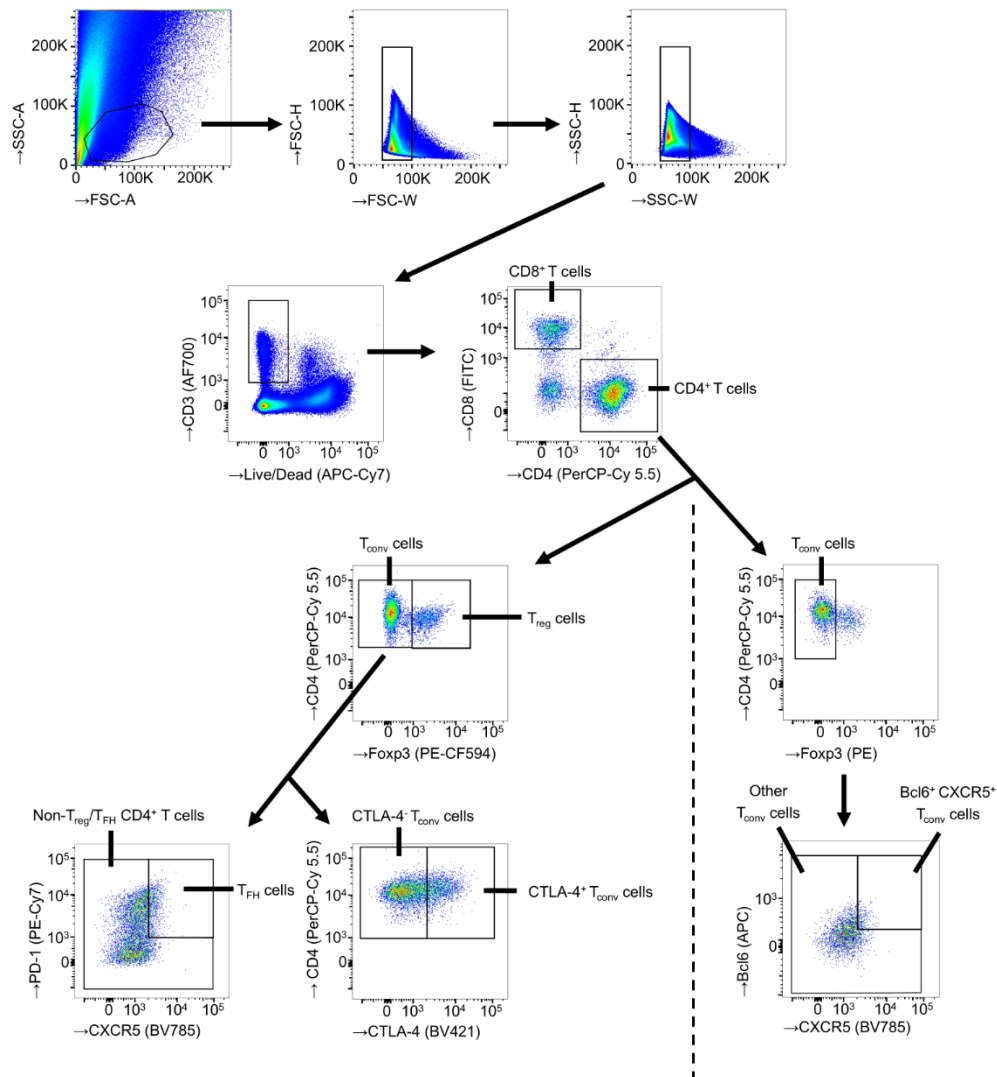
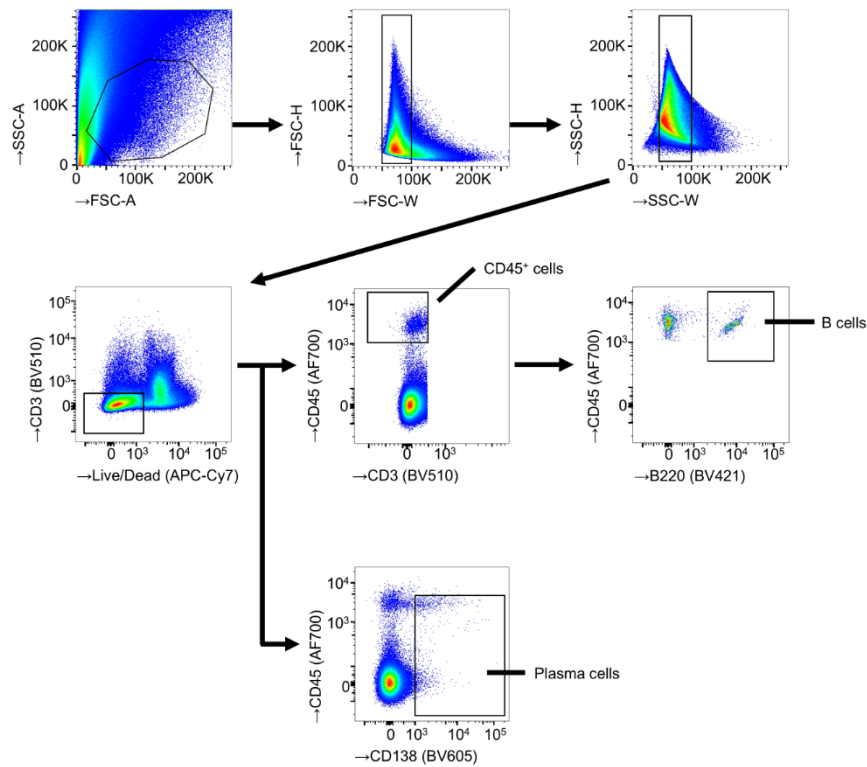


Supplementary Figure S1



Supplementary Fig. S1. Gating strategy for T cells in flow cytometry analysis
 T_{reg} cells, T_{conv} cells, and T_{FH} cells were gated on $Foxp3^+CD4^+$ T cells, $Foxp3^-CD4^+$ T cells, and $PD-1^+CXCR5^+Foxp3^-CD4^+$ T cells, respectively.

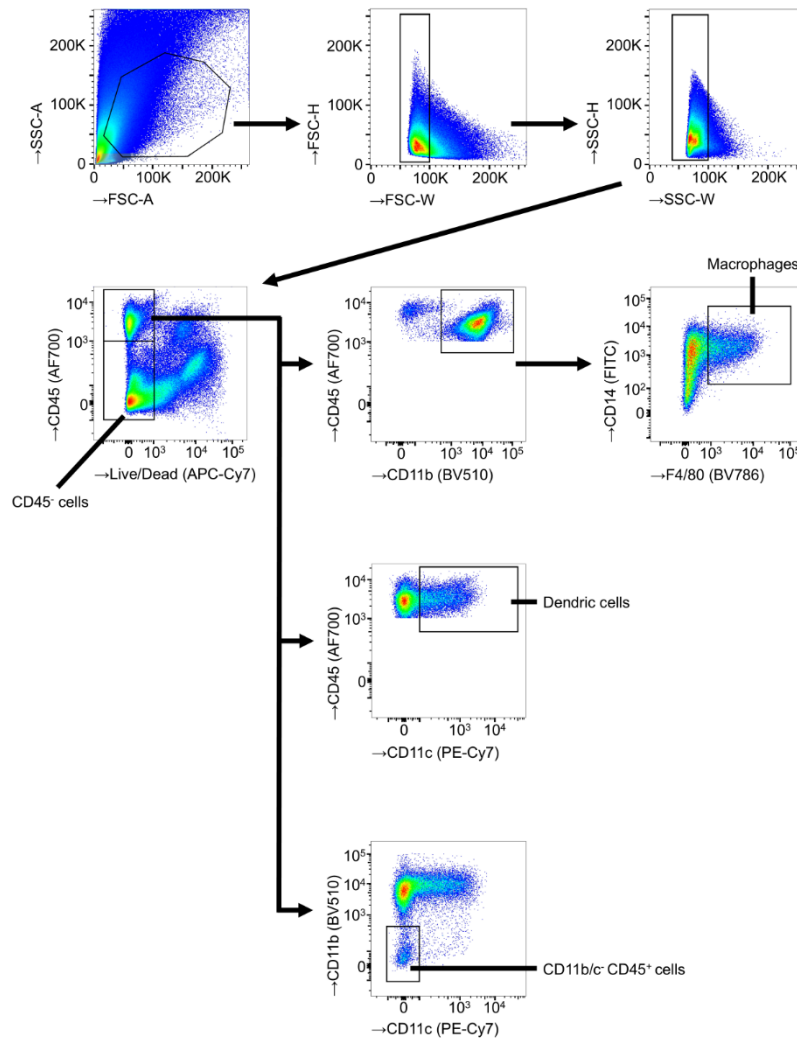
Supplementary Figure S2



Supplementary Fig. S2. Gating strategy for B cells and plasma cells in flow cytometry analysis

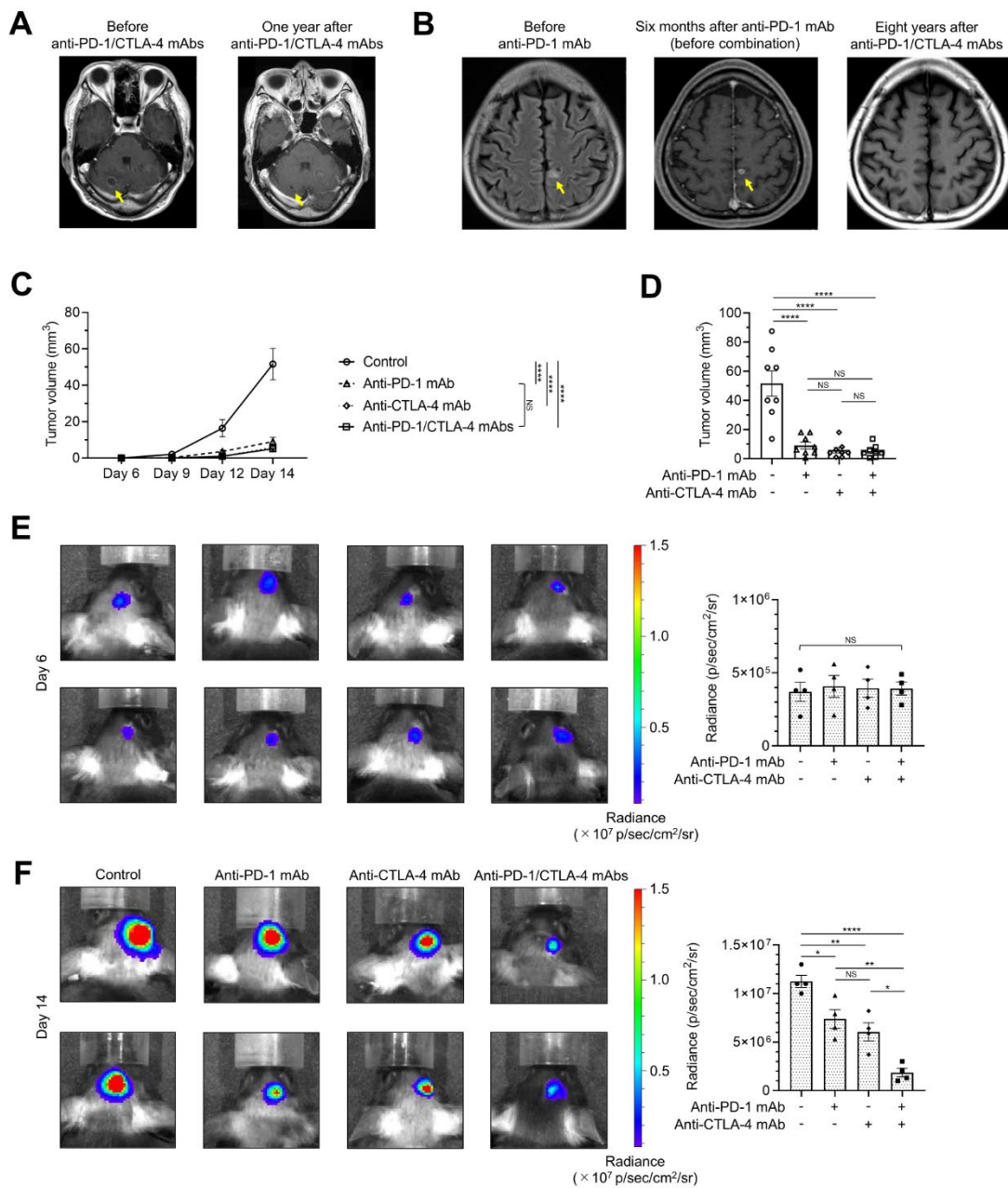
B cells and plasma cells were gated on $B220^+CD3^-CD45^+$ cells and $CD138^+CD3^-CD45^-$ cells, respectively.

Supplementary Figure S3



Supplementary Fig. S3. Gating strategy for myeloid cells in flow cytometry analysis. Macrophages and dendritic cells were gated on CD14⁺F4/80⁺CD11b⁺CD45⁺ cells and CD11c⁺CD45⁺ cells, respectively.

Supplementary Figure S4



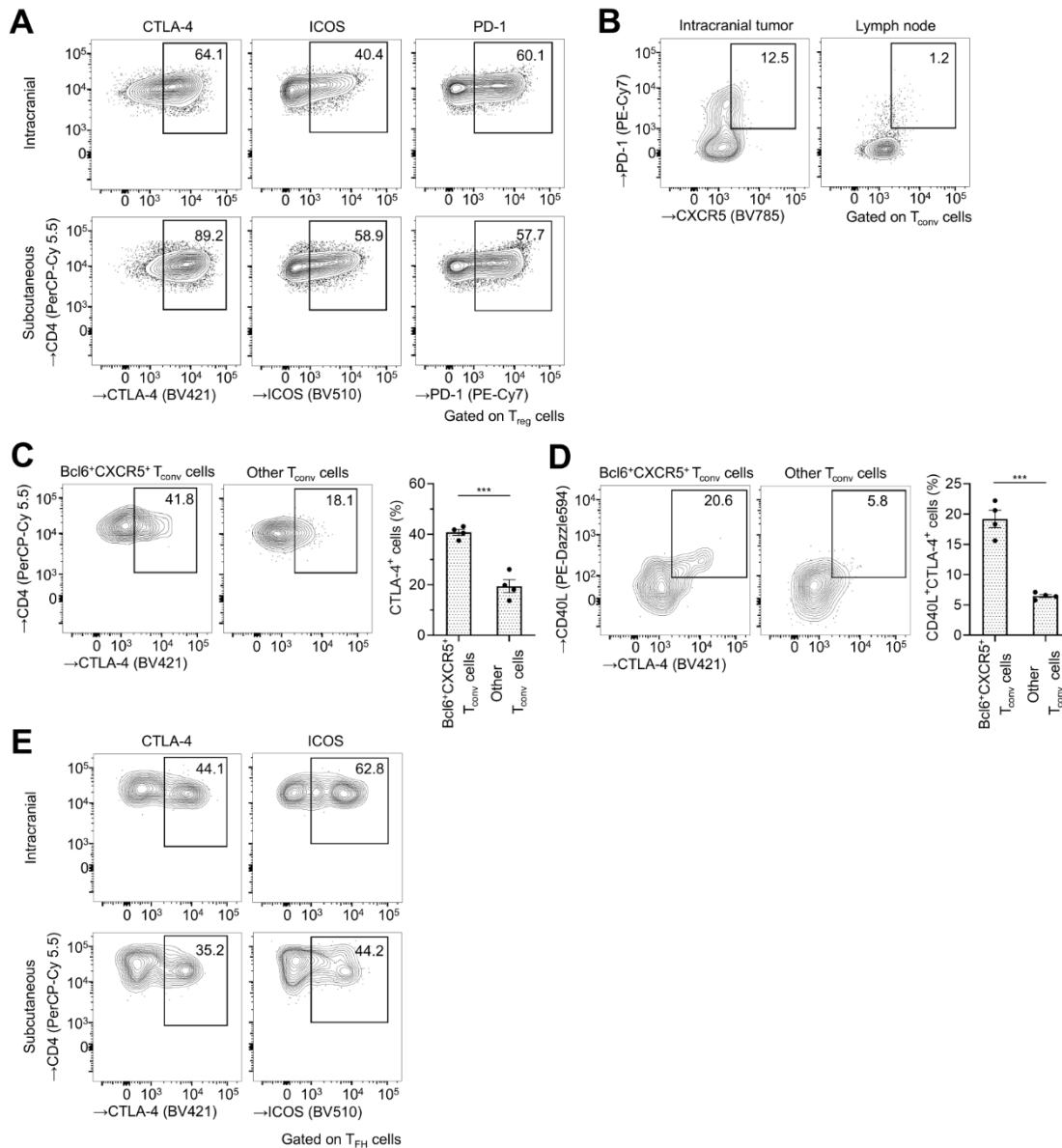
Supplementary Fig. S4. Clinical courses and additional *in vivo* efficacy data

(A) Brain MRI in case 1. Case 1 (male in middle 70s) had brain metastasis (BM) of non-small cell lung cancer at diagnosis (left). He received nivolumab and ipilimumab as first-line therapy and BM improved. One year later, shrinkage was maintained (right). (B) Brain MRI in case 2. Case 2 (female in early 50s) had a BM from melanoma after surgery (left). She received nivolumab monotherapy, and a slight shrinkage of the BM was observed 6 months later (middle). However, the lung metastases worsened and the patient received ipilimumab in addition to nivolumab. Two months later, BM shrank dramatically and she achieved a complete response for more than 8 years (right). (C) and (D) *In vivo* efficacy of PD-1 and/or CTLA-4 blockade in subcutaneous B16F10 tumors. *In vivo* experiments were performed as described in Figure 1A. The mean tumor volume was used to generate tumor growth curves. Tumor growth curves (C) and

summary on day 14 (D) are shown. **(E)** and **(F)** *In vivo* efficacy of PD-1 and/or CTLA-4 blockade against intracranial B16F10 tumors on days 6 (E) and 14 (F). *In vivo* experiments were performed as described in Figure 1A. Representative images (left) and summaries (right) are shown.

All *in vivo* experiments were performed at least twice and similar results were obtained. Two-way ANOVA with Bonferroni corrections was used in (C), and one-way ANOVA with Bonferroni corrections were used in (D), (E) and (F), respectively. Means and SEMs are shown. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$, NS, not significant.

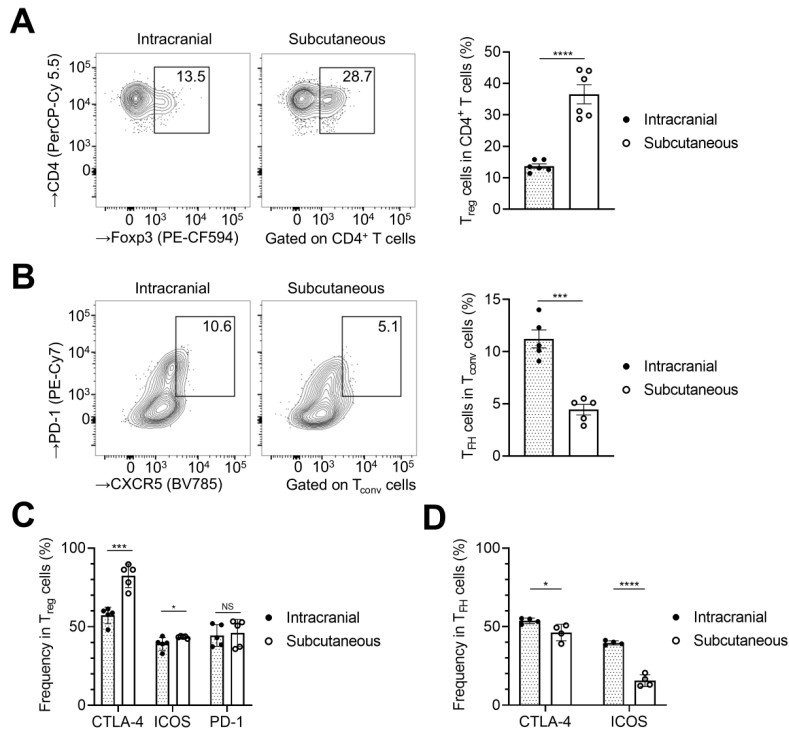
Supplementary Figure S5



Supplementary Fig. S5. Additional LL/2 TIL data related to CTLA-4⁺ T cell subsets. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. **(A)** CTLA-4, ICOS, and PD-1 expression in T_{reg} cells of intracranial and subcutaneous LL/2 TILs. Representative flow cytometric staining are shown. **(B)** CXCR5 and PD-1 expression in T_{conv} cells of intracranial LL/2 TILs and lymph node. Representative flow cytometric staining are shown. **(C)** and **(D)** CTLA-4 (C) and CD40L (D) expression in $Bcl6^+CXCR5^+T_{conv}$ cell and other T_{conv} cell subsets of intracranial LL/2 TILs. Representative flow cytometric staining (left) and summary (right) are shown. **(E)** CTLA-4 and ICOS expression in the T_{FH} cells of intracranial and subcutaneous LL/2 TILs. Representative flow cytometric staining are shown.

All *in vivo* experiments were performed at least twice and similar results were obtained. T-tests were used in (C) and (D). Means and SEMs are shown. ***, $P < 0.001$.

Supplementary Figure S6

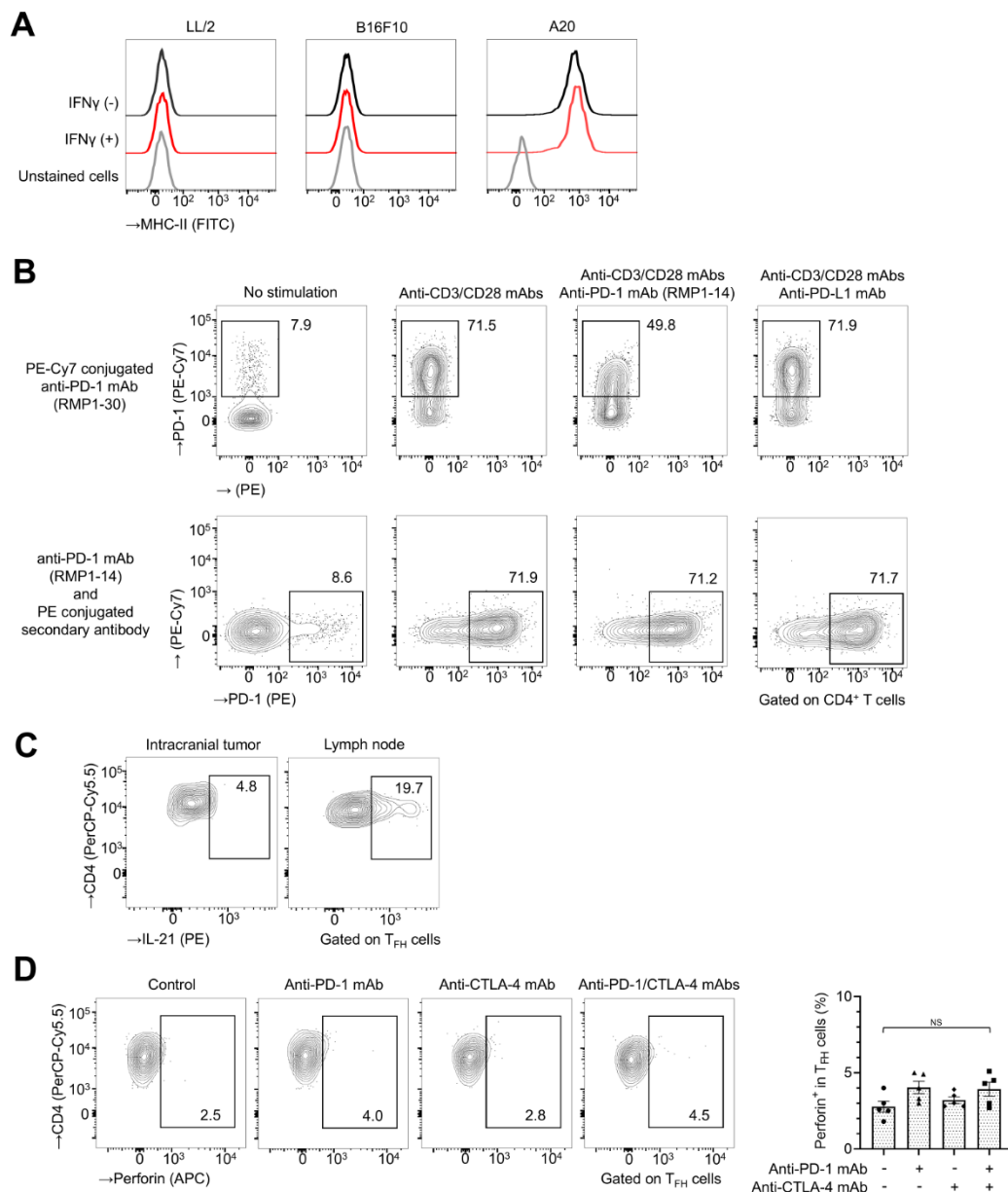


Supplementary Fig. S6. B16F10 TIL data related to CTLA-4⁺ T cell subsets

In vivo experiments were performed as described in Figure 1A using untreated control tumors. **(A)** and **(B)** Frequency of T_{reg} (A) and T_{FH} (B) cells in intracranial and subcutaneous B16F10 TILs. Representative flow cytometric staining (left) and summary (right) are shown. **(C)** CTLA-4, ICOS, and PD-1 expression in T_{reg} cells of intracranial and subcutaneous B16F10 TILs. Summary is shown. **(D)** CTLA-4 and ICOS expression in T_{FH} cells of intracranial and subcutaneous B16F10 TILs. Summary is shown.

All *in vivo* experiments were performed at least twice and similar results were obtained. T-tests were used in (A)-(D). Means and SEMs are shown. *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$, NS, not significant.

Supplementary Figure S7



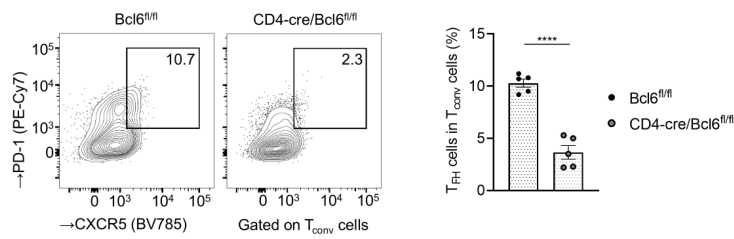
Supplementary Fig. S7. Additional data related the effects of anti-PD-1 and anti-CTLA-4 mAbs on TILs

(A) MHC-II expression in LL/2, B16F10 and A20 cells. The cells were cultured with IFN γ for 24 hours. Representative flow cytometric staining are shown. (B) Competition among anti-PD-1 mAb clones. CD4⁺ T cells isolated from mouse spleens were cultured in the presence or absence of anti-CD3e and CD28 mAbs, anti-PD-1 mAb (clone; RMP1-14) or anti-PD-L1 mAb. After 24 hours, the cells were stained with PE-Cy7-conjugated anti-PD-1 mAb (clone; RMP1-30) or anti-PD-1 mAb (clone; RMP1-14), followed by secondary staining with PE-conjugated anti-rat IgG2a. Representative flow cytometric staining are shown. (C) IL-21 expression in T_H cells of intracranial LL/2 TILs and lymph node. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Representative flow cytometric staining are shown. (D) Perforin expression in T_H cell of intracranial LL/2 TILs. *In vivo* experiments were

performed as described in Figure 1A. T_{FH} cells were gated on PD-1⁺CXCR5⁺Foxp3⁻CD4⁺ T cells. Representative flow cytometric staining (left) and summary (right) are shown.

All *in vivo* experiments were performed at least twice and similar results were obtained. One-way ANOVA with Bonferroni corrections were used in (D). Means and SEMs are shown. NS, not significant.

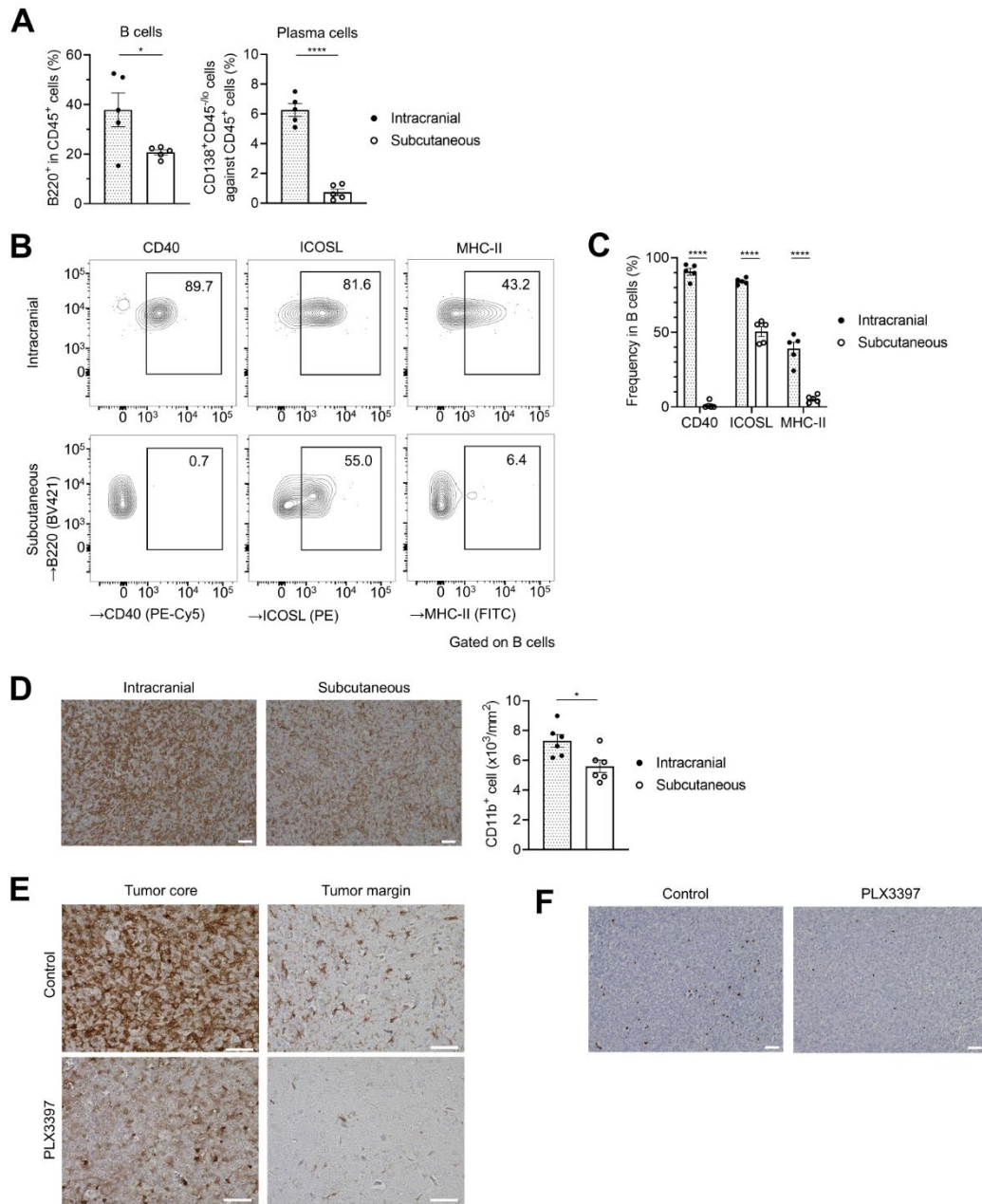
Supplementary Figure S8



Supplementary Fig. S8. T_H cells in intracranial TILs from CD4-cre/Bcl6^{fl/fl} mice
In vivo experiments were performed as described in Figure 1A using untreated control LL/2 tumors. T_H cells were gated on PD-1⁺CXCR5⁺Foxp3⁻CD4⁺ T cells. Representative flow cytometric staining (left) and summary (right) are shown.

All *in vivo* experiments were performed at least twice and similar results were obtained. A t-test was used. Means and SEMs are shown. ****, $P < 0.0001$.

Supplementary Figure S9

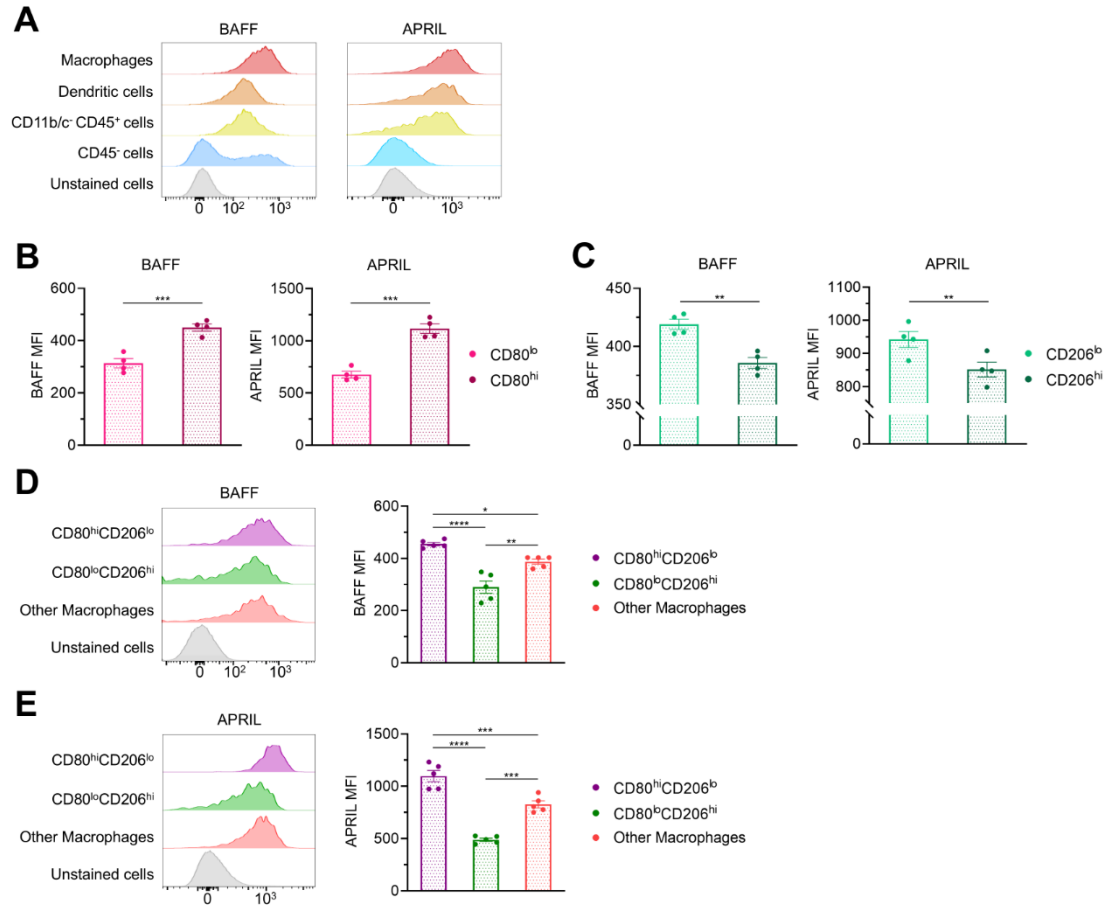


Supplementary Fig. S9. Additional TIL data related to myeloid-B-T_{FH} cell axis
(A) Frequency of B and plasma cells in intracranial and subcutaneous B16F10 TILs. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. B cells and plasma cells were gated on B220⁺CD3⁻CD45⁺ cells and CD138⁺CD3⁻CD45^{-/lo} cells, respectively. Summaries are shown. **(B)** and **(C)** Expression of CD40, ICOSL, and MHC-II in B cells isolated from intracranial and subcutaneous B16F10 TILs. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Representative flow cytometric staining **(B)** and summary **(C)** are shown. **(D)** CD11b⁺ myeloid cell infiltration in the intracranial and subcutaneous LL/2 tumors. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Representative immunohistochemical staining (left) and summary (right) are shown. Scale bar, 50 μ m. **(E)** CD11b⁺ myeloid cell infiltration

in intracranial LL/2 tumors treated with PLX3397. *In vivo* experiments were performed as described in Figure 5D and E. Representative immunohistochemical staining results are shown. Scale bar, 50 μm . **(F)** CD79a⁺ B and plasma cell infiltration in intracranial LL/2 tumors treated with PLX3397. *In vivo* experiments were performed as described in Figure 5E. Representative immunohistochemical staining are shown. Scale bar, 50 μm .

All *in vivo* experiments were performed at least twice and similar results were obtained. T-tests were used in (A), (C) and (D). Means and SEMs are shown. *, $P < 0.05$; ****, $P < 0.0001$.

Supplementary Figure S10

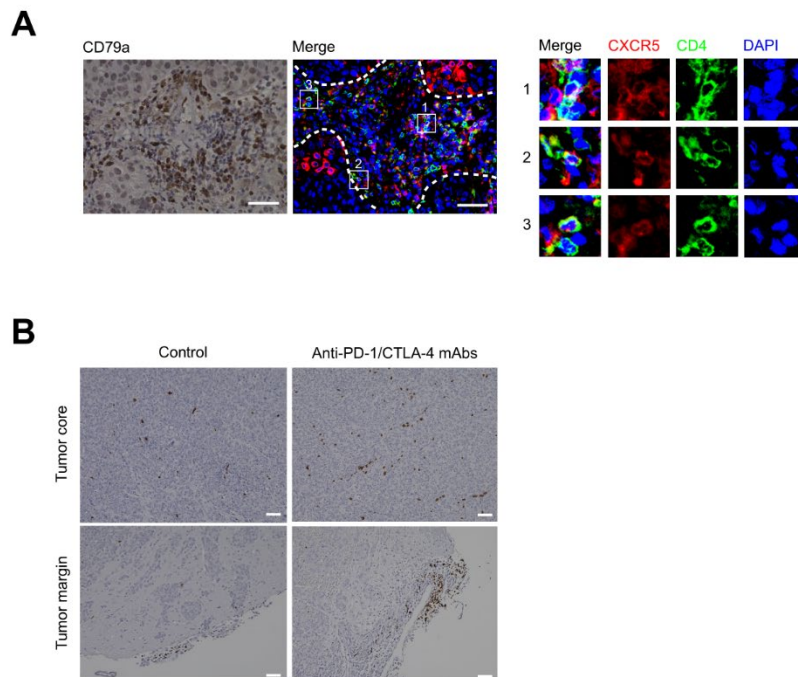


Supplementary Fig. S10. BAFF and APRIL in intracranial tumors

(A) BAFF and APRIL expression in macrophages, dendritic cells, other CD45⁺ cells and CD45⁻ cells of intracranial LL/2 tumors. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Macrophages and dendritic cells were gated on CD14⁺F4/80⁺CD11b⁺CD45⁺ and CD11c⁺CD45⁺ cells, respectively. Representative flow cytometric staining (left, BAFF; right, APRIL) are shown. **(B)** BAFF and APRIL expression in macrophages according to CD80 expression in intracranial LL/2 TILs. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Summaries (left, BAFF; right, APRIL) are shown. **(C)** BAFF and APRIL expression in macrophages according to CD206 expression in intracranial LL/2 TILs. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Summaries (left, BAFF; right, APRIL) are shown. **(D)** and **(E)** BAFF (D) and APRIL (E) expression in macrophages according to CD80 and CD206 expression in intracranial LL/2 TILs. *In vivo* experiments were performed as described in Figure 1A using untreated control tumors. Representative flow cytometric staining (left) and summaries (right) are shown.

All *in vivo* experiments were performed at least twice and similar results were obtained. T-tests were used in (B) and (C), one-way ANOVA with Bonferroni corrections was used in (D) and (E), respectively. Means and SEMs are shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Supplementary Figure S11



Supplementary Fig. S11. TLS in brain metastases of patients with NSCLC, and B and plasma cell cluster in the murine intracranial TME

(A) T_{FH} - and B-cell infiltration in brain metastases of patients with NSCLC. We stained CD79a to evaluate B and plasma cells, and CXCR5 combined with CD4 to evaluate T_{FH} cells in surgically resected brain metastasis samples. Representative immunohistochemical staining for CD79a and multicolor immunofluorescence staining for CXCR5 and CD4 are shown. White dotted lines indicate TLS boundaries. The areas including the T_{FH} cells are circled by white squares, which are magnified. Scale bar, 50 μ m. **(B)** B and plasma cell cluster in LL/2 tumor. *In vivo* experiments were performed as described in Figure 1A, and we stained CD79a to evaluate B and plasma cells. Representative immunohistochemical staining are shown. Scale bar, 50 μ m.

Supplementary Table S1. Antibodies for flow cytometry

Antibodies	Clone	Source	Identifier
Anti-mouse/human Bcl-6-APC	7D1	BioLegend	Cat# 358505; RRID: AB_2562471
Anti-mouse CD3e-Alexa Fluor 700	500A2	BD Biosciences	Cat# 557984; RRID: AB_396972
Anti-mouse CD3e-BV510	145-2C11	BD Biosciences	Cat# 563024; RRID: AB_2737959
Anti-mouse CD4-PerCP-Cy5.5	RM4-5	BD Biosciences	Cat# 561115; RRID: AB_393977
Anti-mouse CD4-FITC	GK1.5	BioLegend	Cat# 100405; RRID: AB_312690
Anti-mouse CD8a-FITC	53-6.7	BD Biosciences	Cat# 553031; RRID: AB_394568
Anti-mouse CD11b-BV510	M1/70	BD Biosciences	Cat# 562950; RRID: AB_2737913
Anti-mouse CD11c-PE-Cy7	N418	BioLegend	Cat# 117317; RRID: AB_493569
Anti-mouse CD14-FITC	Sa14-2	BioLegend	Cat# 123307; RRID: AB_940578
Anti-mouse CD40-PE-Cy5	3/23	BioLegend	Cat# 124617; RRID: AB_2075923
Anti-mouse CD45-Alexa Fluor 700	30-F11	BioLegend	Cat# 103128; RRID: AB_103128
Anti-mouse/human CD45R/B220-BV421	RA2-6B2	BioLegend	Cat# 103239; RRID: AB_10933424
Anti-mouse CD80-PerCP-Cy5.5	16-10A1	BioLegend	Cat# 104721; RRID: AB_893406
Anti-mouse CD95-PerCP-Cy5.5	SA367H8	BioLegend	Cat# 152609; RRID: AB_2632904
Anti-mouse CD138-BV605	281-2	BioLegend	Cat# 142515; RRID: AB_2562336
Anti-mouse CD152-BV421	UC10-4B9	BioLegend	Cat# 106311; RRID: AB_10901170
Anti-mouse CD154-PE-Dazzle594	SA047C3	BioLegend	Cat# 157015; RRID: AB_2922488
Anti-mouse CD185-BV785	L138D7	BioLegend	Cat# 145523; RRID: AB_2563981

Anti-mouse CD206-BV605	C068C2	BioLegend	Cat# 141721; RRID: AB_2562340
Anti-human/mouse CD256-APC	REA347	Miltenyi Biotec	Cat# 130-118-470 RRID: AB_2733360
Anti-mouse CD275-PE	HK5.3	BioLegend	Cat# 107405; RRID: AB_2248797
Anti-mouse CD278-BV480	7E.17G9	BD Biosciences	Cat# 746539; RRID: AB_2871650
Anti-mouse CD279-PE-Cy7	RMP1-30	BioLegend	Cat# 109110; RRID: AB_572017
Anti-mouse BAFF/TNFSF13B-PE	121808	R&D SYSTEMS	Cat# IC1357P; RRID: AB_2303642
Anti-mouse FoxP3-PE	MF-14	BioLegend	Cat# 126403; RRID: AB_1089118
Anti-mouse FoxP3-PE-CF594	R16-715	BD Biosciences	Cat# 567373; RRID: AB_2916572
Anti-mouse F4/80-BV785	BM8	BioLegend	Cat# 123141; RRID: AB_2563667
Anti-mouse I-A/I-E-FITC	M5/114.15.2	BioLegend	Cat# 107605; RRID: AB_313320
Anti-mouse IFN- γ -BV421	XMG1.2	BD Biosciences	Cat# 563376; RRID: AB_2738165
Anti-mouse Perforin-APC	S16009B	BioLegend	Cat# 154403; RRID: AB_2721464
Anti-mouse IL-21-PE	mhalx21	Thermo Fisher Science	Cat# 12-7213-82; RRID: AB_1834465
Anti-rat IgG2a-PE	MRG2a-83	BioLegend	Cat# 407507; RRID: AB_2564487

Supplementary Table S2. Characteristics of patients with non-small-cell lung cancer brain metastases

Patient	Gender	Age	Pathology	TLS	Chemotherapy prior to surgical resection	Duration from last chemotherapy to surgery
1	Male	Early 70s	Adenocarcinoma	+	Platinum doublet + Nivolumab + Ipilimumab	5 months
2	Male	Middle 70s	Adenocarcinoma	+	None	
3	Female	Early 60s	Adenocarcinoma	-	S-1	13 months
4	Female	Middle 60s	Adenocarcinoma	+	None	
5	Female	Early 70s	Adenocarcinoma	+	None	
6	Male	Middle 70s	Adenocarcinoma	+	Platinum doublet	3 months
7	Male	Middle 70s	Adenocarcinoma	-	None	
8	Male	Late 60s	Squamous cell carcinoma	-	Platinum doublet	18 months
9	Male	Middle 70s	Adenocarcinoma	-	None	
10	Female	Late 60s	Adenocarcinoma	+	Platinum doublet + Bevacizumab	27 months
11	Male	Middle 60s	Adenocarcinoma	-	S-1	1 month
12	Female	Early 60s	Adenocarcinoma	+	None	
13	Male	Late 60s	Squamous cell carcinoma	+	Bevacizumab	1 month
14	Female	Late 40s	Adenocarcinoma	-	Platinum doublet	14 months
15	Male	Late 60s	Adenocarcinoma	-	Platinum doublet	96 months
16	Male	Late 50s	Adenocarcinoma	-	Platinum doublet	54 months
17	Female	Late 50s	Adenocarcinoma	-	Platinum doublet	6 months
18	Male	Late 70s	Squamous cell carcinoma	-	None	

19	Male	Early 70s	Adenocarcinoma	-	None	
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TLS, tertiary lymphoid structure; S-1, tegafur, gimeracil, and oteracil potassium