Supplementary Material

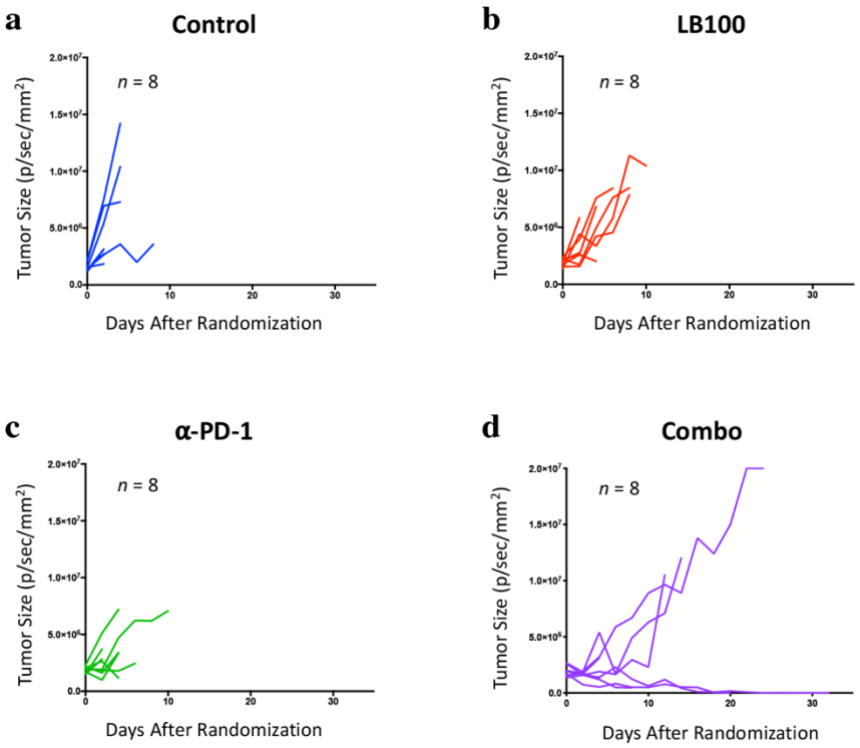
Antibody (flow)	Clone	Fluorophore	Dilution	Isotype
CD3e	145-2C11	PE	1:40	Armenian Hamster IgG
CD4	RM4-5	AF647	1:333	Rat IgG2ακ
CD8a	53-6.7	BUV737	1:40	Rat IgG2ακ
CD8b.2	53-5.8	FITC	1:100	Rat IgG1κ
CD11b	M1/70	BUV395	1:80	Rat IgG2βκ
CD11c	HL3	AF700	1:40	Armenian Hamster IgG1, λ2
CD11c	HL3	BUV737	1:40	Armenian Hamster IgG1, λ2
CD19	1D3	BB515	1:40	Rat IgG2ακ
CD25	PC61	BV605	1:160	Rat IgG1λ
CD44	IM7	BV421	1:80	Rat IgG2βκ
CD45	30-F11	BV785	1:160	Rat IgG2βκ
CD62L	MEL-14	BUV395	1:80	Rat IgG2ακ
CD161 (NK1.1)	PK136	BUV395	1:40	Mouse IgG2ακ
CD161 (NK1.1)	PK136	BV421	1:80	Mouse IgG2ακ
CD206 (mannose receptor)	MR5D3	AF647	1:80	Rat IgG2α
F4/80	BM8	PerCP/Cy5.5	1:200	Rat IgG2ακ
Foxp3	MF23	BV421	1:80	Rat IgG2β
IFN-y	XMG1.2	BV605	1:20	Rat IgG1κ
IFN-y	XMG1.2	PE	1:80	Rat IgG1κ
Ki-67	B56	PerCP/Cy5.5	1:40	Mouse IgG1κ
Ly-6C	AL-21	PerCP/Cy5.5	1:20	Rat IgMκ
Ly-6G	1A8	AF488	1:100	Rat IgG2ακ
LY-6G	1A8	BV605	1:80	Rat IgG2ακ
T-bet	4B10	AF700	1:80	Mouse IgG1κ
TNF-a	MP6-XT22	BV510	1:40	Rat IgG1

Blocking Antibody	Clone	Dilution
True-Stain Monocyte Blocker	n/a	1:20
CD16/32	93	Rat IgG2αλ

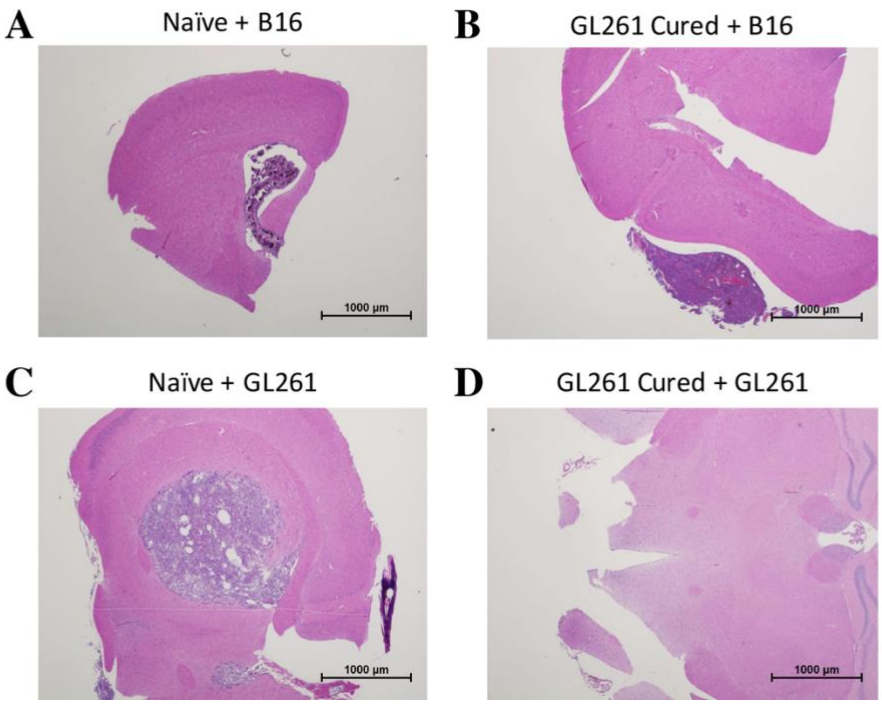
Theraputic Antibody	Clone	Dose
PD-1	RMP1-14	10 mg/kg
CD8	53.6.7	10 mg/kg
CD4	GK1.5	10 mg/kg

Antibodies bought from BD Bioscience, Biolegend, Invitrogen, eBiosicence, Bio X Cell and Novus

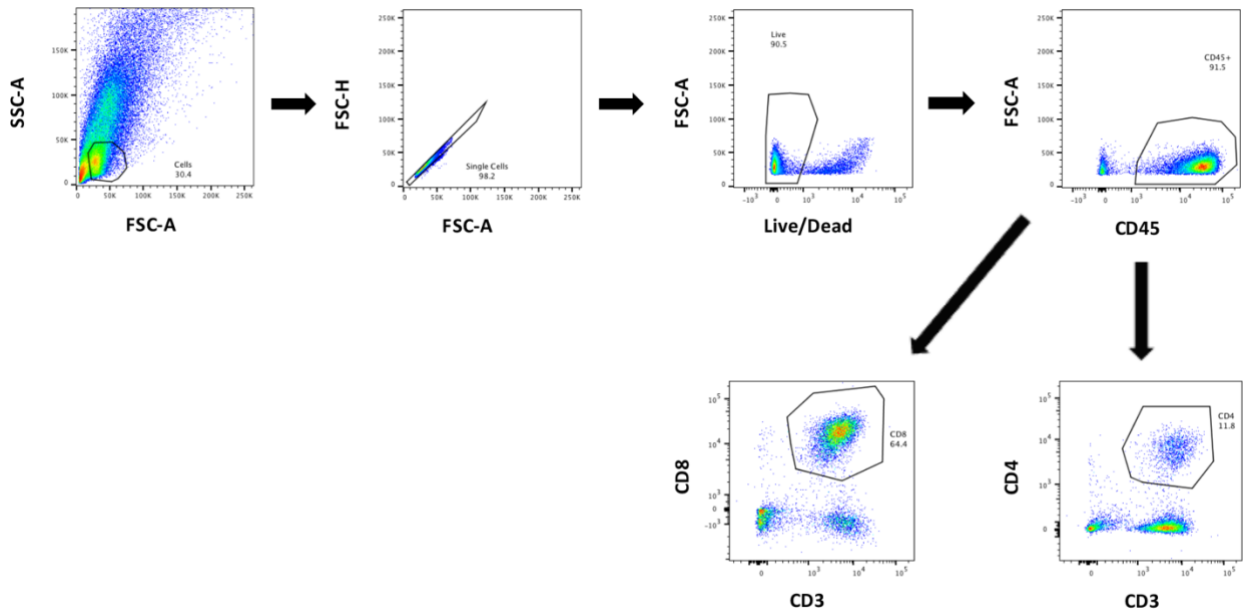
Supplementary Table 1. Antibodies. Antibodies used for flow cytometry (clones and dilutions) and therapeutics (clones and dosage)



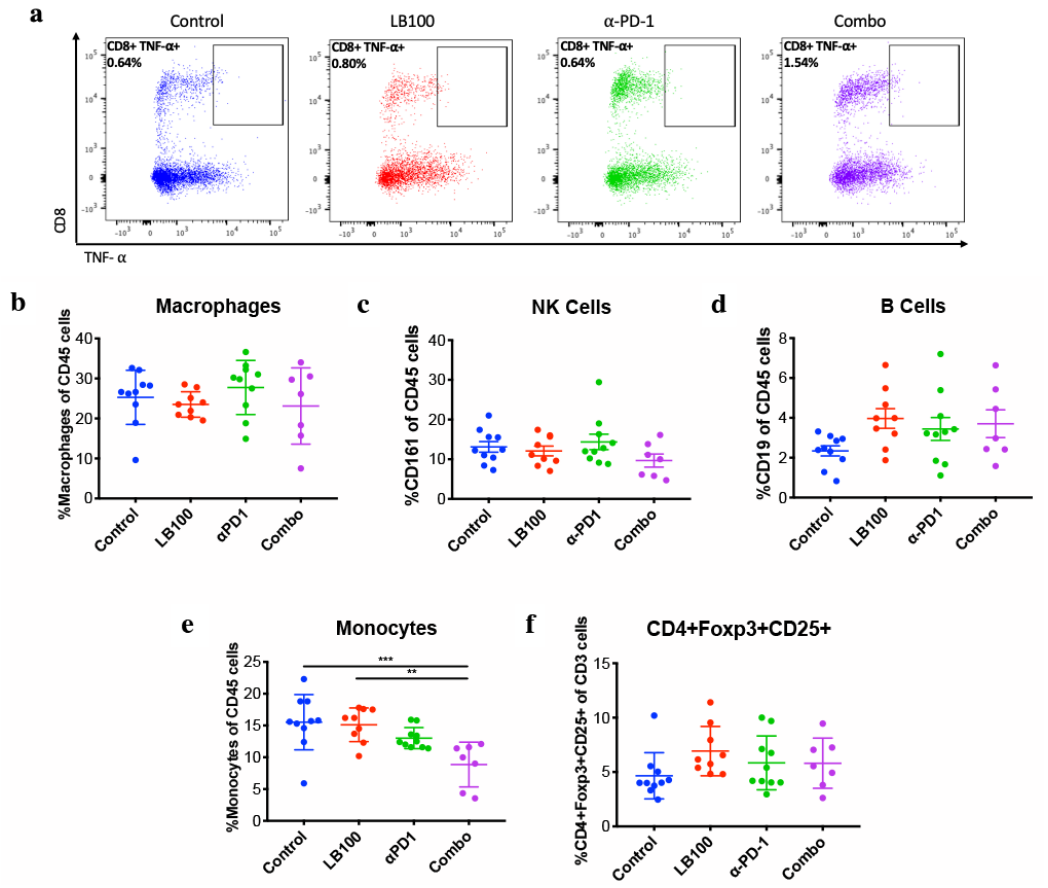
Supplementary Figure 1. PP2A inhibition and PD1 blockade synergistically elicit tumor rejection in GL261. Individual tumor growth measured in BLI of the ROI in (a) control (b) LB-100 (c) α-PD-1 and (d) combination groups plotted as days after randomization.



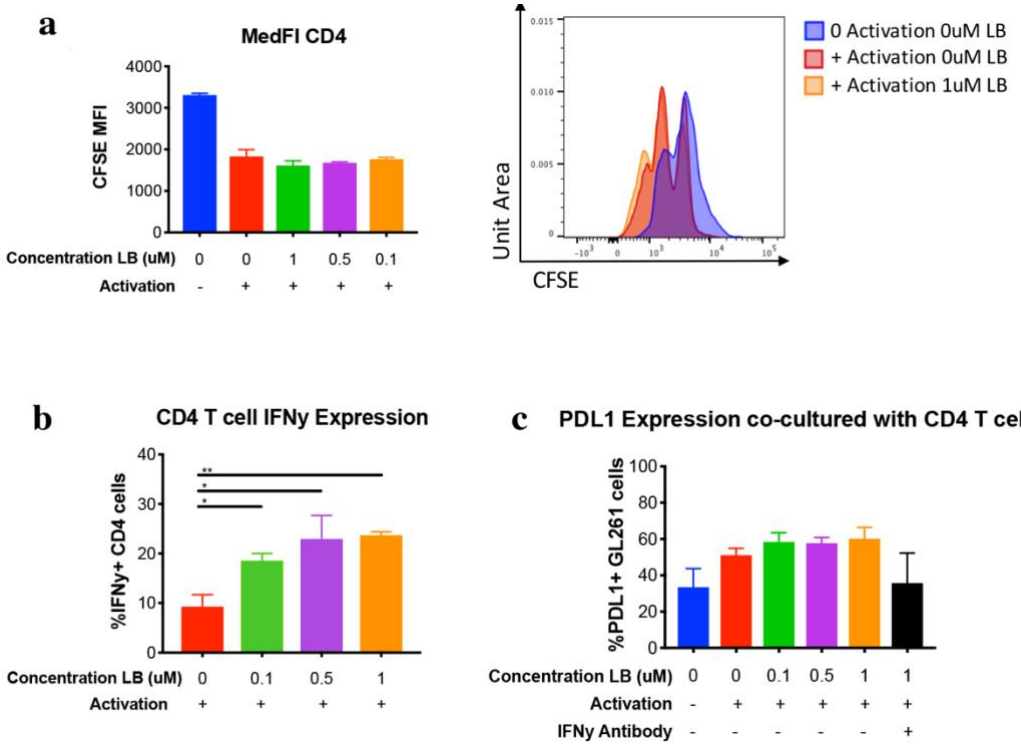
Supplementary Figure 2. Combination treatment result in a long-term antitumor antigen-specific memory of cured animals. Representative images of hematoxylin-and-eosin staining of brain specimens from (a) naïve mouse exposed to B16, (b) CR mouse exposed to B16, (c) naïve mouse exposed to GL261 and (d) cured mouse re-exposed to GL261. CR mice re-challenged with GL261 demonstrated no histologic evidence of tumor establishment.



Supplementary Figure 3. Gating strategy for flow cytometric analysis of tumor infiltrating lymphocytes. We first used SSC-FSC gate to exclude non-cellular debris, followed by exclusion of duplets by FSC-H-FSA-A gate. Fixable live-dead (L/D) stain was used to exclude dead cells. Live cells were then gated based on expression of CD45+ pan leukocyte marker. CD45- cells were considered as tumor cells. CD45+ cells were then phenotyped further based on CD3, CD8, CD4 expression. CD45+CD3+CD8+ cells were gated as CD8+ lymphocytes, while CD45+CD3+CD4+ cells were gated as CD4+ lymphocytes. Further, staining of the CD4+ and CD8+ subsets were then performed as indicated in the text.



Supplementary Figure 4. Immune profiling of tumor infiltrating leukocytes. Combination therapy enhanced CD8+ T cell function but did not alter tumor infiltration of macrophages, natural killer cells or B cells. Monocytes cells were decreased with combination treatment. (a) Representative FACS plots of TNF-α+ CD8+ as a percentage of CD45+ cells. (b) Macrophages (CD45+ CD3- CD11c^{low} CD11b+ Ly6G- Ly6C^{low}) expressed as a percentage of CD45+ cells were not significantly changed in any treatment group. (c) NK (CD45+ CD161+) cells expressed as a percentage of CD45+ cells were not significantly changed in any treatment group. (d) B cells (CD45+ CD19+) expressed as a percentage of CD45+ cells were not significantly changed in any treatment group. (e) Monocytes (CD45+ CD3- CD11c^{low} CD11b+ Ly6G- Ly6C^{high}) as a percentage of CD45+ cells were significantly decreased in combination compared to control or LB-100 alone. (f) Tregs (FoxP3+ CD25+ CD4+) expressed as a percentage of CD3+ cells were not significantly changed in any treatment group. *P<0.05 **P<0.01, ***P<0.001, ****P<0.0001 (one-way ANOVA with Tukey's multiple comparison test). Error bars depict SEM.



Supplementary Figure 5. LB-100 enhances IFN-gamma secretion of CD4+ T-cells in vitro. LB-100 did not alter CD4+ T cell proliferation, but increased CD4+ T cell production of IFN-gamma. There was a trend toward increase in tumor PD-L1 expression. (a) LB-100 did not increase proliferation of CD4+ T cells. Flow cytometry analyzing CFSE cytosolic dye as a marker of CD4+ T cell proliferation 72 hours after activation. Representative FACS histogram. (b) LB-100 increased secretion of IFN-gamma by CD4+ T cells. Flow cytometry analyzing expression of IFN-gamma in CD4+ T cells 72 hours after activation in the presence of LB-100 dose titration. (c) Flow cytometry analyzing PD-L1 expression in tumor cells co-cultured with CD4+ T cells exposed to a titration concentration of LB-100. There was a trend, but insignificant, increase in tumor PD-L1 expression with LB-100 treatment. *P<0.05 **P<0.01 (one-way ANOVA with Tukey's multiple comparison test). Error bars depict SEM.