Supplementary Materials

Inhibition of PP2A with LB-100 Enhances Efficacy of CAR-T Cell Therapy Against Glioblastoma

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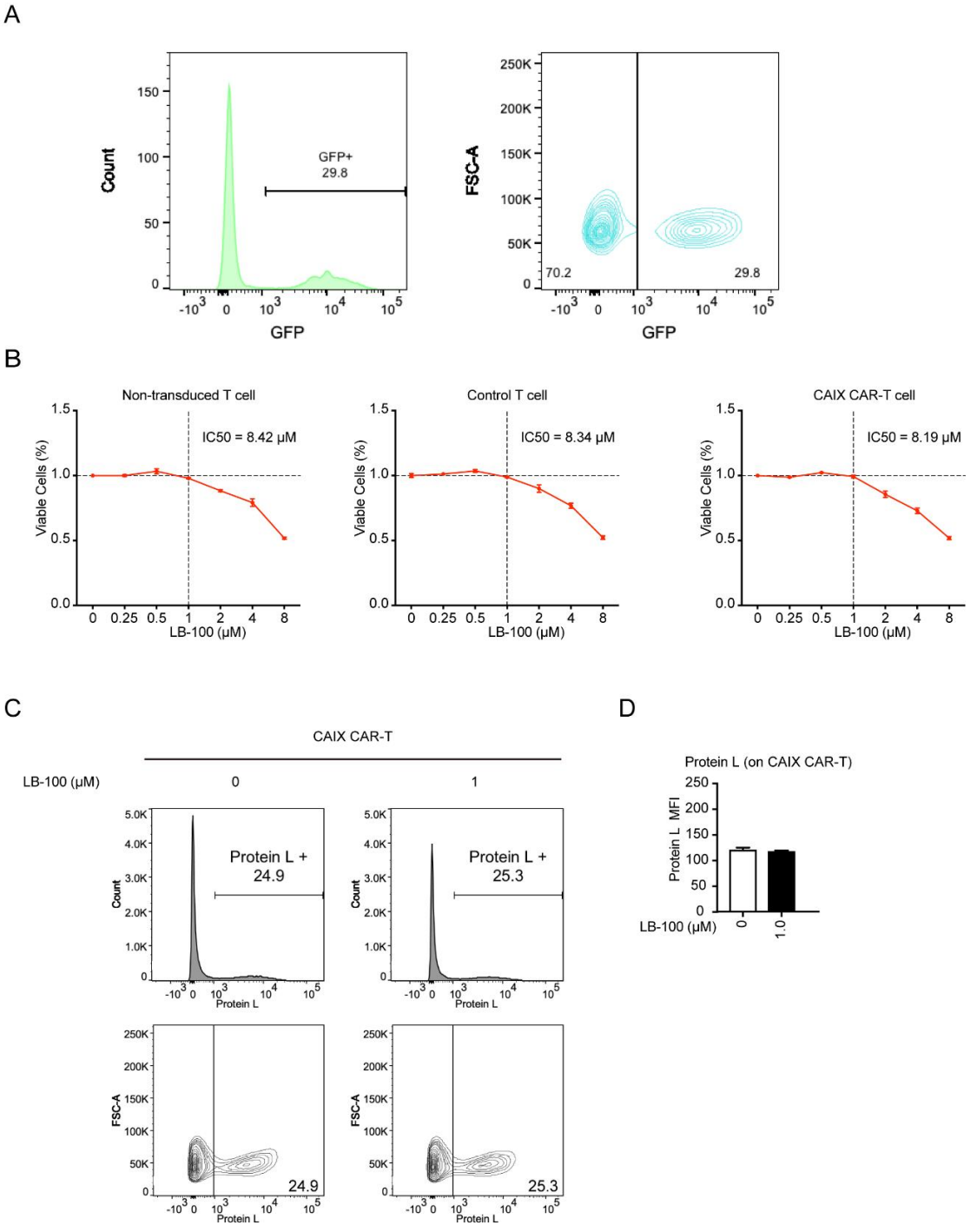
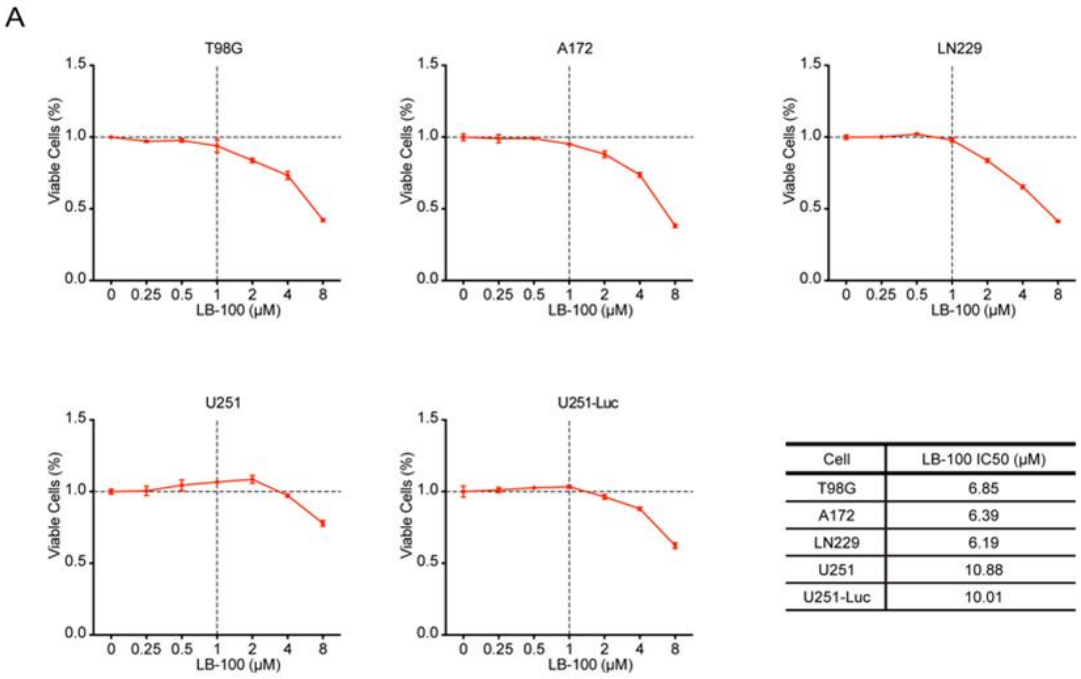


Figure S1. Effect of LB-100 on anti-CAIX CAR-T cells. (**A**) Transduction efficiency was detected by green fluorescent protein (GFP) expression in mock T cells (using GFP instead of CAIX CAR scFv) on day 4 post-transduction using flow cytometry. The transduction efficiency was around 30%. (**B)**

CCK-8 results showed LB-100 dose-response curve of non-transduced T, control T (empty vector transduced), and anti-CAIX CAR-T cells for 48 hours. IC50 of each cell line was calculated and listed as indicated. (**C**) Flow cytometry analyzing protein L (CAR scFv) expression on control T cells and anti-CAIX CAR-T cells in presence of 1 μM LB-100. (**D**) LB-100 has no effect on CAR expression.



Cell	LB-100 IC50 (μM)
T98G	6.85
A172	6.39
LN229	6.19
U251	10.88
U251-Luc	10.01

Figure S2. Effect of LB-100 on glioblastoma cells. (**A**) CCK-8 assay results showed the LB-100 dose-response curve of glioblastoma cells (T98G, A172, LN229, U251 and U251-Luc) for 48 hours. IC50 of each cell line was calculated and listed as indicated.

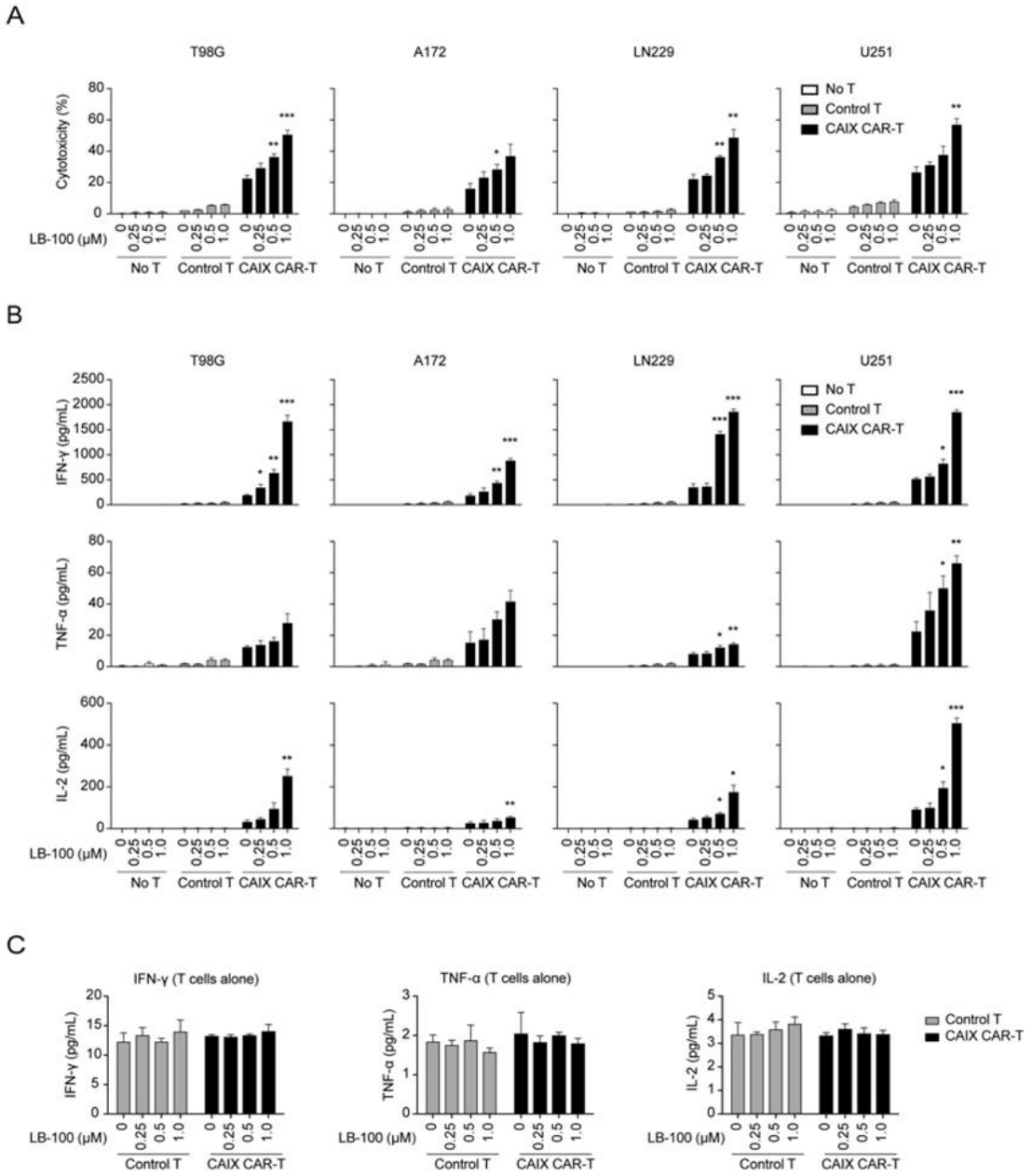


Figure S3. LB-100 enhances cytotoxicity of anti-CAIX CAR-T cells against glioblastoma in vitro. (**A**) Control T cells (empty vector transduced T cells) or anti-CAIX CAR-T cells were cocultured with glioblastoma cells (T98G, A172, LN229, and U251) with titration concentration of LB-100 as indicated (1 μM) at an E/T ratio at 4 for 48 hours. Cytotoxicity was determined by LDH releasing assay. $n = 3$ for each group. (**B**) Cytokine (IFN-γ, TNF-α, and IL-2) secretion in the supernatant obtained from the cocultured system was analyzed by ELISA. The bar graphs represent a significant increase in cytokine release in anti-CAIX CAR-T treated groups. A combination of LB-100 further enhanced cytokine release. (**C**) Cytokine (IFN-γ, TNF-α, and IL-2) secretion in the supernatant obtained from control T cells or anti-CAIX CAR-T cells cultured alone in the presence of LB-100 was analyzed by ELISA. Levels of cytokines were comparable in anti-CAIX CAR-T treated groups and in the combination groups. All data are shown as the mean ± SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by Student's *t*-test, anti-CAIX CAR-T combined with LB-100 groups vs. anti-CAIX CAR-T group.

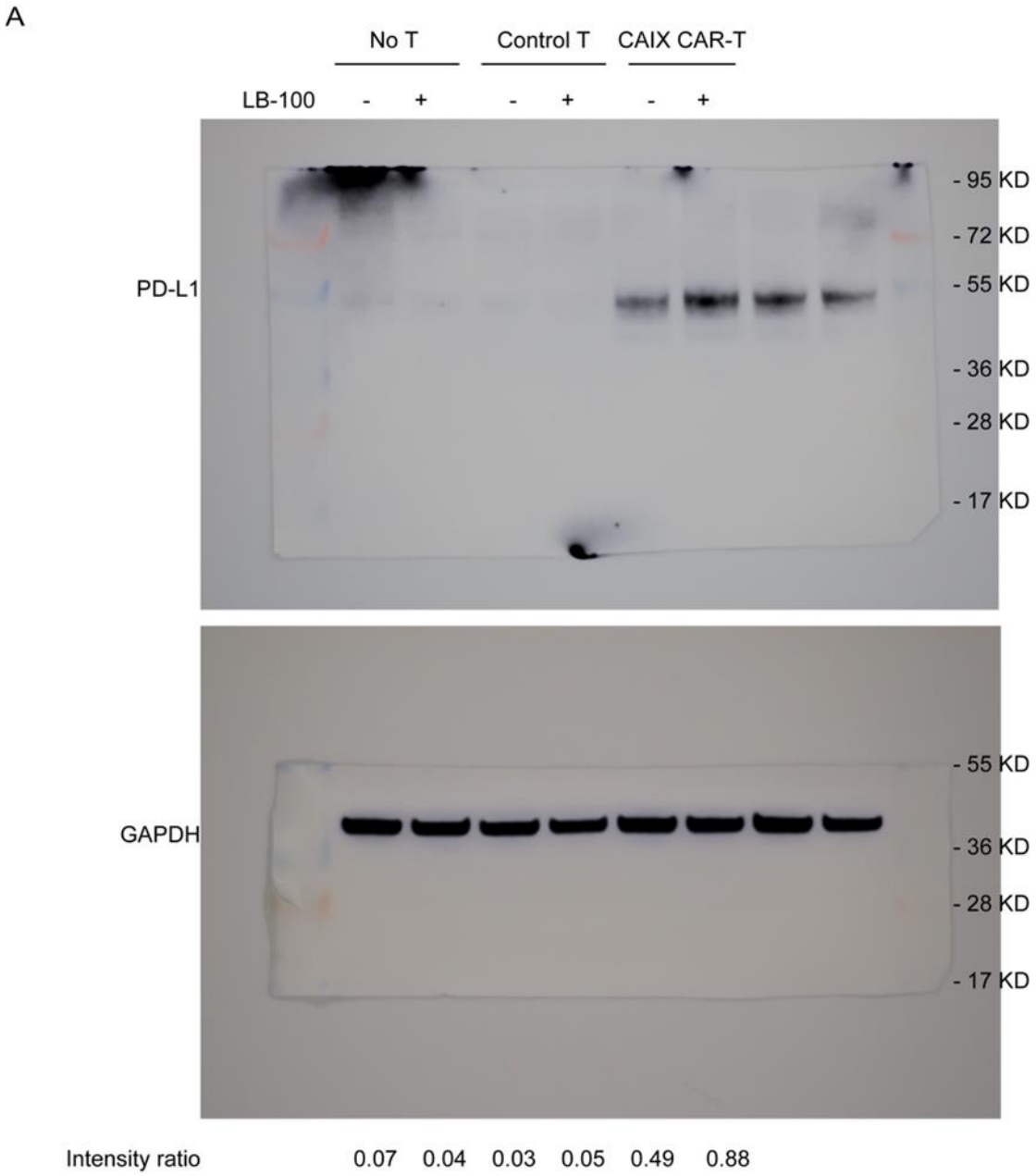


Figure S4. Original uncut gels of the cropped gels in Figure 1E.

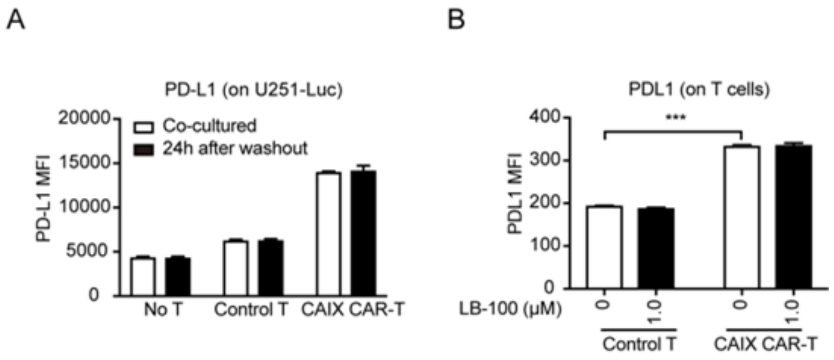


Figure S5. Anti-CAIX CAR-T cells increase stable ligand programmed death-1 (PD-L1) expression on tumor cells and CAR-T cells. (**A**) U251-Luc cells were treated with control-T or anti-CAIX CAR-T cells at an E/T ratio of 4 for 48 hours, followed by washout for 24 hours; un-treated U251-Luc cells

served as control. PD-L1 expression on U251-Luc cells in each group was determined by flow cytometry as indicated. There is no significant difference of mean fluorescence intensity (MFI) of PD-L1 positive cells in CAR-T cell treated U251-Luc cells before and after CAR-T cell washout ($n = 3$). (**B**) Control T cells (empty vector transduced T cells) or anti-CAIX CAR-T cells were co-cultured with U251-Luc cells with 1 μM LB-100 at an E/T ratio at 4 for 48 hours ($n = 3$). Flow cytometry analyzing PD-L1 expression on control-T or anti-CAIX CAR-T cells from co-cultured system. There is a significant increase in MFI of PD-L1 positive cells in anti-CAIX CAR-T cells compared with control T cells, but no further increase in the combination groups. All data are shown as the mean ± SEM. *** $p < 0.001$ by Student's *t*-test; anti-CAIX CAR-T groups vs. control T groups.