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Supplemental information

Enhanced therapeutic efficacy for glioblastoma immunotherapy with an oncolytic herpes simplex virus armed with anti-PD-1 antibody and IL-12

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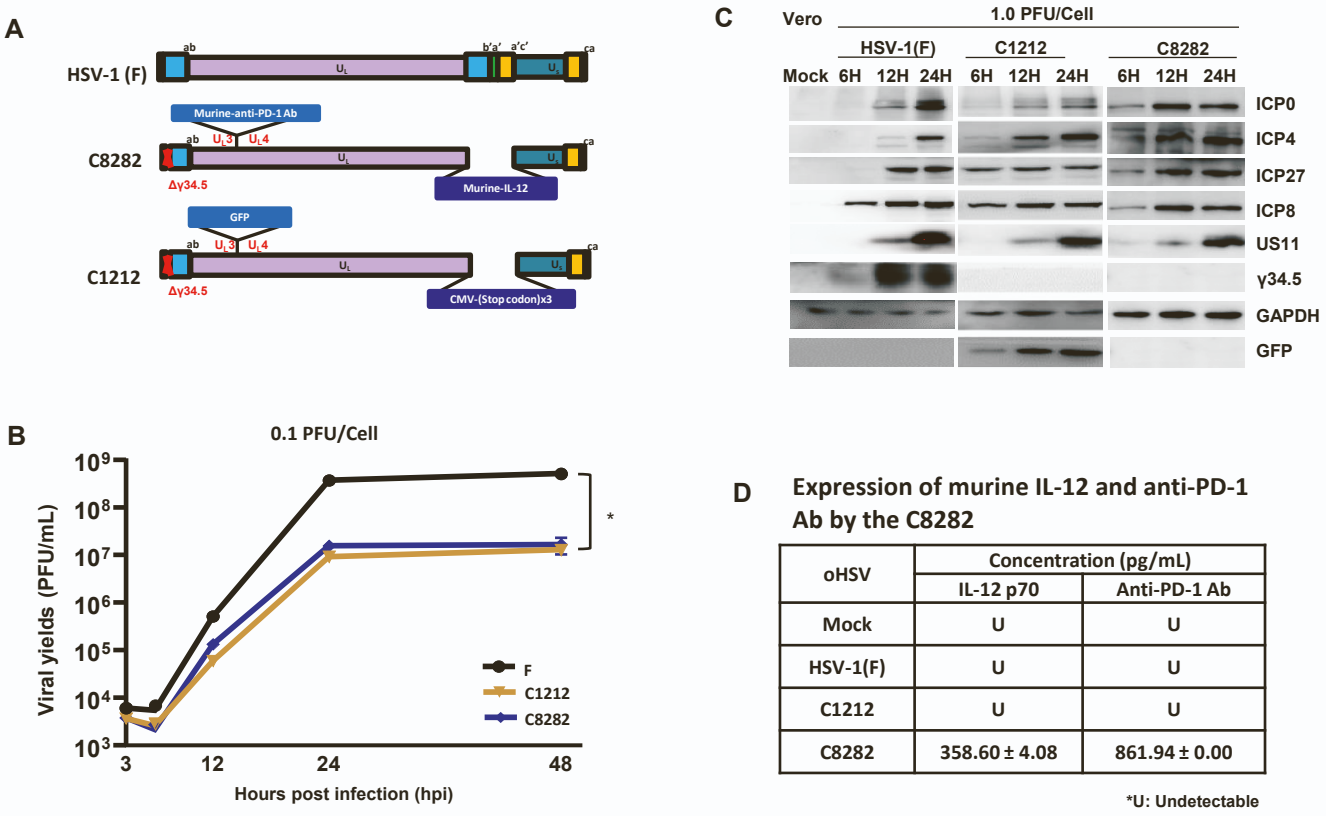
Figure S1



oHSV	Concentration (pg/mL)	
	IL-12 p70	Anti-PD-1 Ab
Mock	U	U
HSV-1(F)	U	U
C1212	U	U
C8282	358.60 ± 4.08	861.94 ± 0.00

*U: Undetectable

Figure S1. Construction and Identification of C8282. (A). Virus genome schematic. Schematic representation of the virus genome, including wild-type HSV-1(F), C1212 (control), and C8282 (murine version of C5252). C8282 contains an expression cassette for the murine IL-12 heterodimer and an antigen-binding fragment (Fab) of anti-murine PD-1Ab, with the IR region replaced. **(B). Growth Curves.** Vero cells exposed to 0.1 PFU of HSV-1(F), C1212, or C8282 per cell. Virus progeny collected at various time points (3, 6, 12, 24, and 48 h) and titered using Vero cells (*$p < 0.05$)**. (C). Accumulation of Viral Protein.** Vero cells infected with 1.0 PFU of HSV-1(F), C1212, or C8282 per cell for 6, 12, and 24 h. Cell samples collected at specified hours post-infection. Proteins separated on 10% denaturing gels and analyzed via immunoblotting with antibodies against specific viral and cellular proteins**. (D). Expression of Murine IL-12 p70 and Anti-PD-1 Ab.**

Figure S2

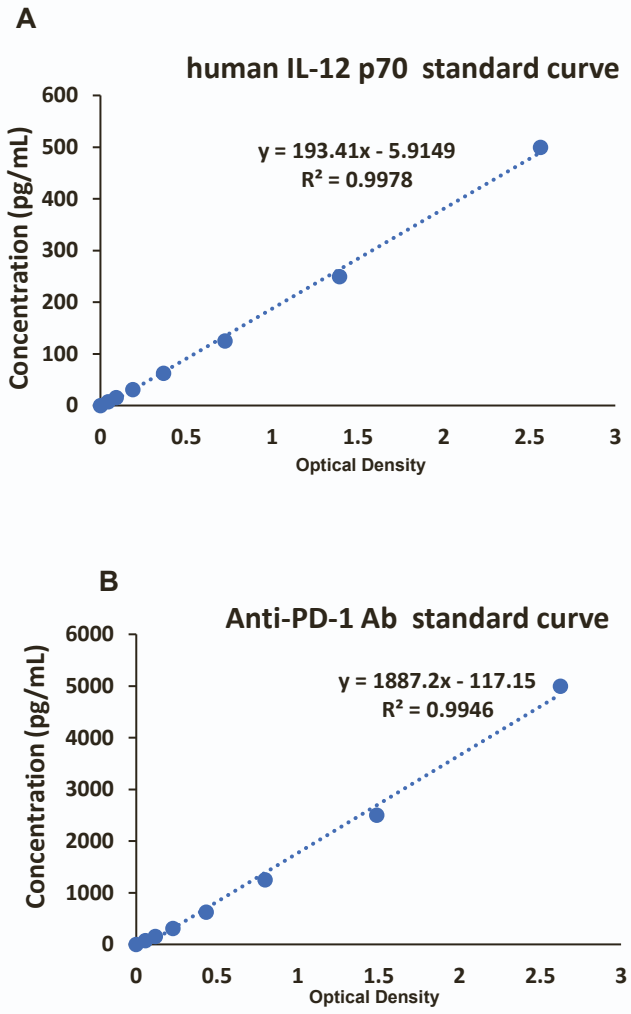


Figure S2. Standard Curves for IL-12 p70 and Anti-PD-1 Antibody.

Figure S3

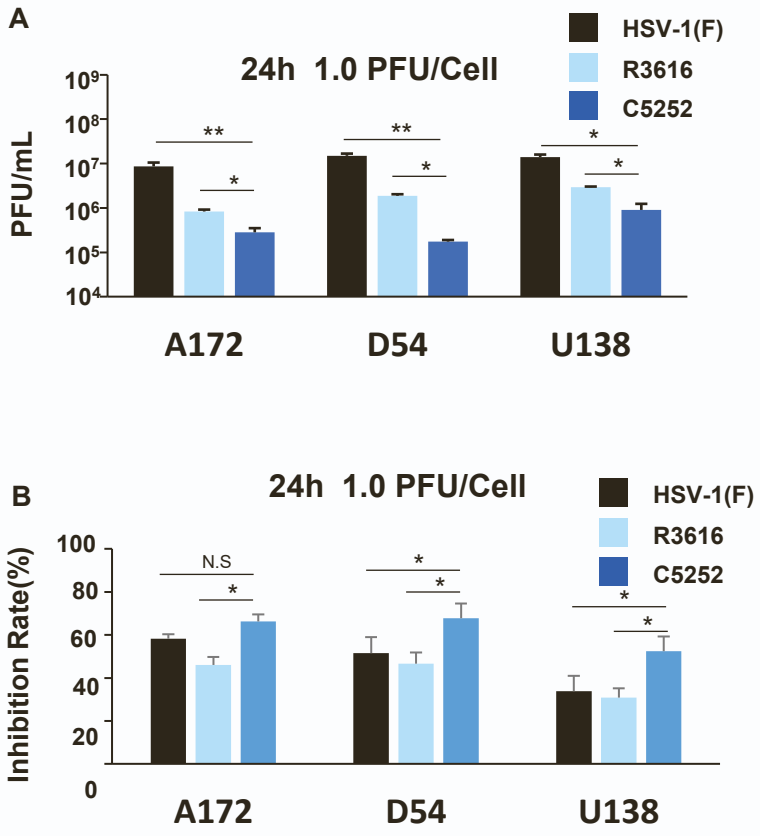


Figure S3. Virus replication and cytotoxicity in glioblastoma cells. (A). Viral Yields in Glioblastoma Cells. A172, D54, and U138 glioblastoma cells were exposed to 1.0 PFU of HSV-1(F), R3616, or C5252 per cell. After 2 hours, the inoculum was replaced with fresh medium. Virus progeny was harvested at 24 hpi and quantified using Vero cells. Data presented as mean±SD. **(B). Virus Cytotoxicity in Glioblastoma Cells.** Cytotoxicity of HSV-1(F), R3616, and C5252 in A172, D54, and U138 glioblastoma cells assessed using a CCK8 assay. Cells infected with 1.0 PFU/cell, and cell inhibition rates were measured at 24 hpi. Assays conducted in triplicate, and data represented as mean ± SD. Statistical differences analyzed with a two-tailed Student's t-test (*$p < 0.05$, **$p < 0.01$, $^{N.S}$ $p > 0.05$).

Figure S4

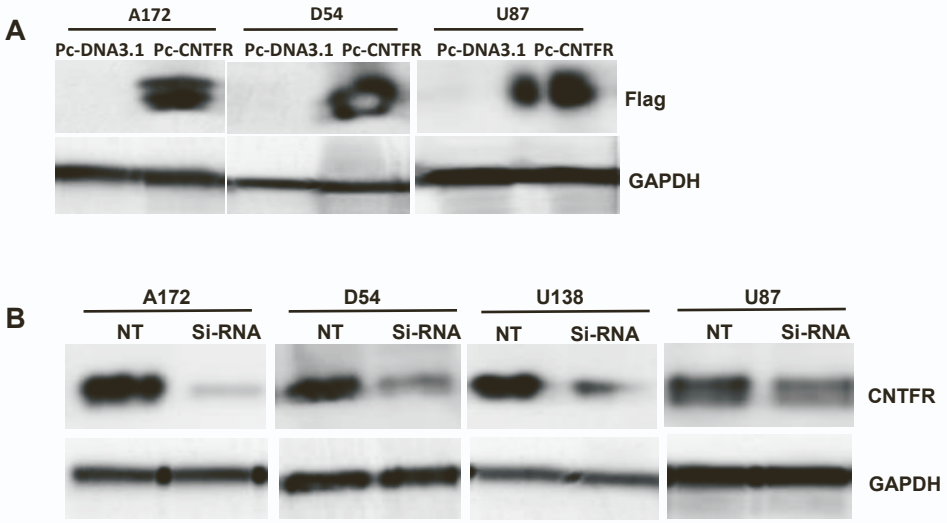


Figure S4. (A). Upregulation of CNTFRα. A172, D54, and U87 cells were transfected with Pc-CNTFRα with a Flag tag and Pc-DNA3.1 plasmid for 48 hours. Cell samples collected at specified hours post-infection. Proteins separated on 10% denaturing gels and analyzed via immunoblotting with antibodies against Flag or GAPDH. **(B). Downregulation of CNTFRα.** A172, D54, U138, and U87 cells were transfected with si-CNTFRα and si-NT for 72 hours. Cell samples collected at specified hours post-infection. Proteins separated on 10% denaturing gels and analyzed via immunoblotting with antibodies against CNTFRα or GAPDH.

Figure S5

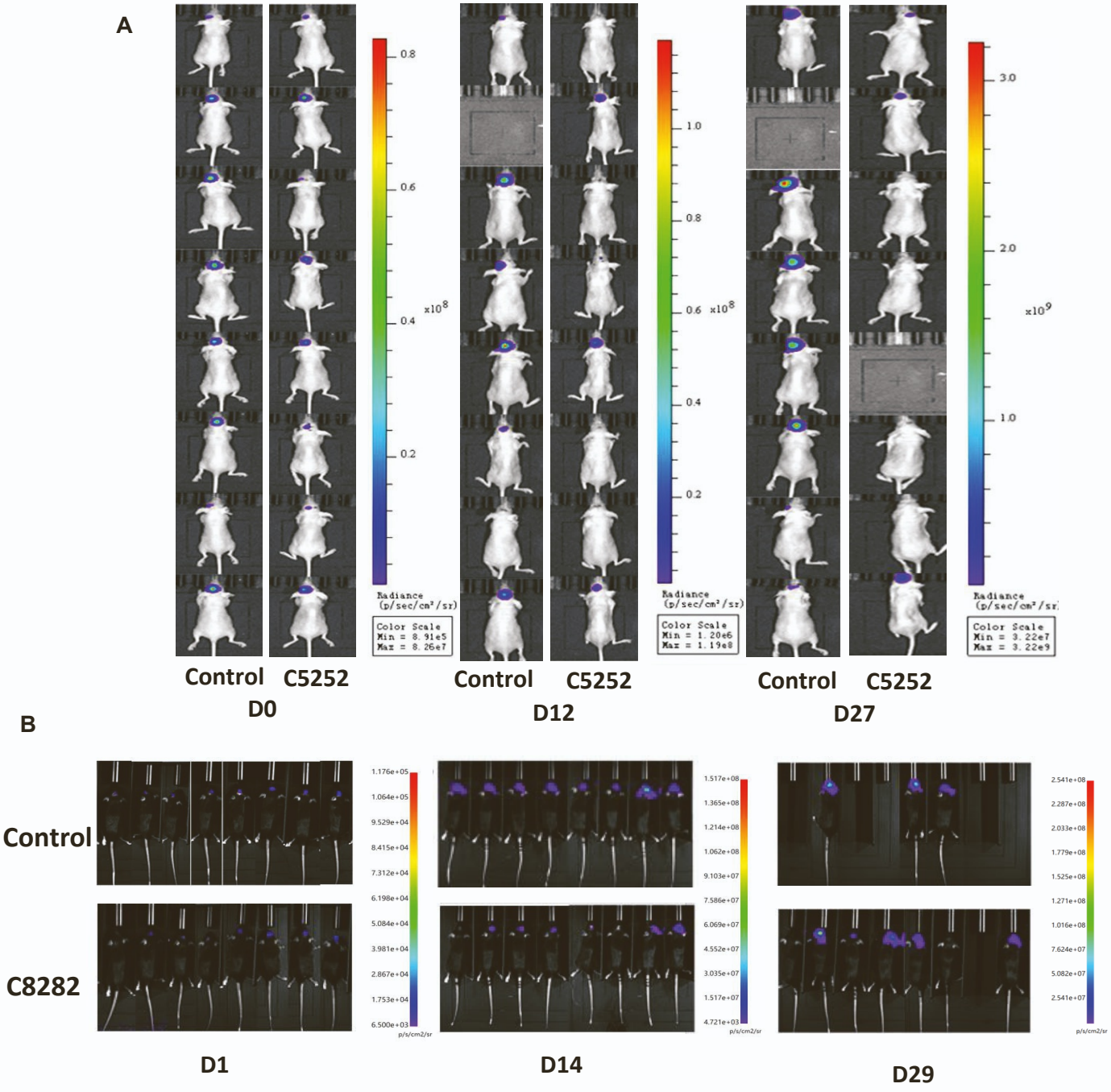


Figure S5. (A). The Luciferase (Luc) Image in the U-87MG-Luc Orthotopic Tumor Model. In U-87MG-Luc tumor model, luciferase (Luc) imaging was performed to visualize and monitor tumor growth. The emitted bioluminescent signal from luciferase-expressing tumor cells was captured and presented in the image. **(B). The Luciferase (Luc) Image in the CT-2A-GFP-Luc Orthotopic Tumor Model.** In the CT-2A-GFP-Luc tumor model, luciferase (Luc) imaging was conducted to visualize and monitor tumor progression. The emitted bioluminescent signal from luciferase-expressing tumor cells was captured and depicted in the image, providing insights into tumor growth dynamics.

Figure S6

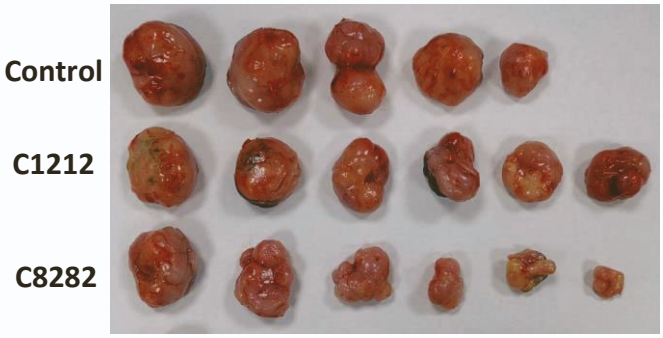


Figure S6. Dissection of CT-2A Murine Model. After the conclusion of the murine tumor model experiment, mice were euthanized, and tumor dissections were carried out. Tumor samples were carefully collected.