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Supplemental Information

Immune Modulation by Telomerase-Specific Oncolytic Adenovirus Synergistically Enhances Antitumor Efficacy with Anti-PD1 Antibody

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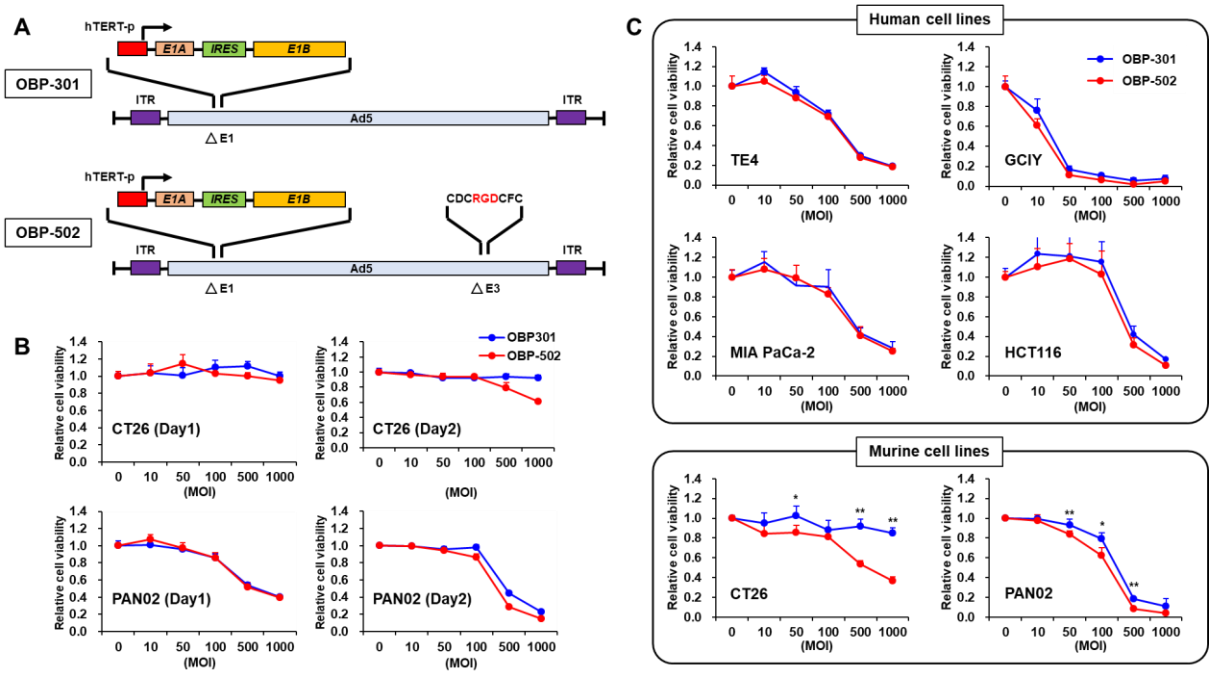

Figure S1. Comparison of OBP-301 and OBP-502

(A) OBP-301 and OBP-502 constructs. OBP-502 has the gene cassette expressing the RGD peptide in the E3 region. (B) Viability of CT26 and PAN02 cells was assessed using XTT assay 1 day or 2 days after OBP301 or OBP-502 treatment at the indicated doses (n=5). Percentage of viable cells relative to non-treated cells (0 MOI) was plotted. Error bars indicate 95% confidence intervals. (C) Viability of the following cells was assessed using XTT assay 3 days after OBP-301 or OBP-502 treatment (n=5). The human esophageal squamous cell carcinoma cell line TE4, the human scirrhus type gastric cancer cell line GCIY, the human pancreatic cancer cell line MIA PaCa-2, and the human colon cancer cell line HCT116 were used in addition to CT26 and PAN02. *, $P < 0.05$. **, $P < 0.01$.

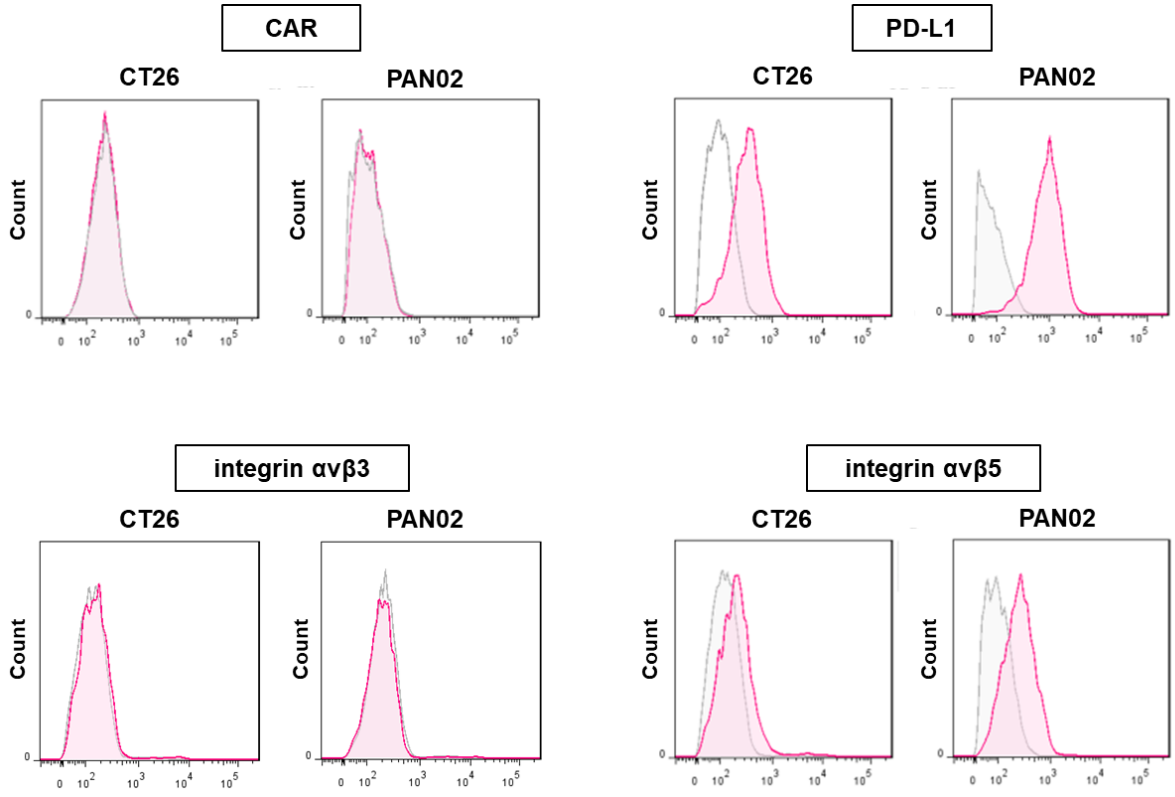

Figure S2. Characteristics of CT26 and PAN02 murine cell lines

Cells were incubated with antibodies to PD-L1 (Biolegend, San Diego, CA, USA), CAR (Millipore, Billerica, MA, USA), integrin αvβ3 (Bioss Antibodies, Woburn, MA, USA), and integrin αvβ5 (Bioss Antibodies) for 30 min on ice and analyzed using a FACS Array (BD Biosciences, San Jose, CA, USA).

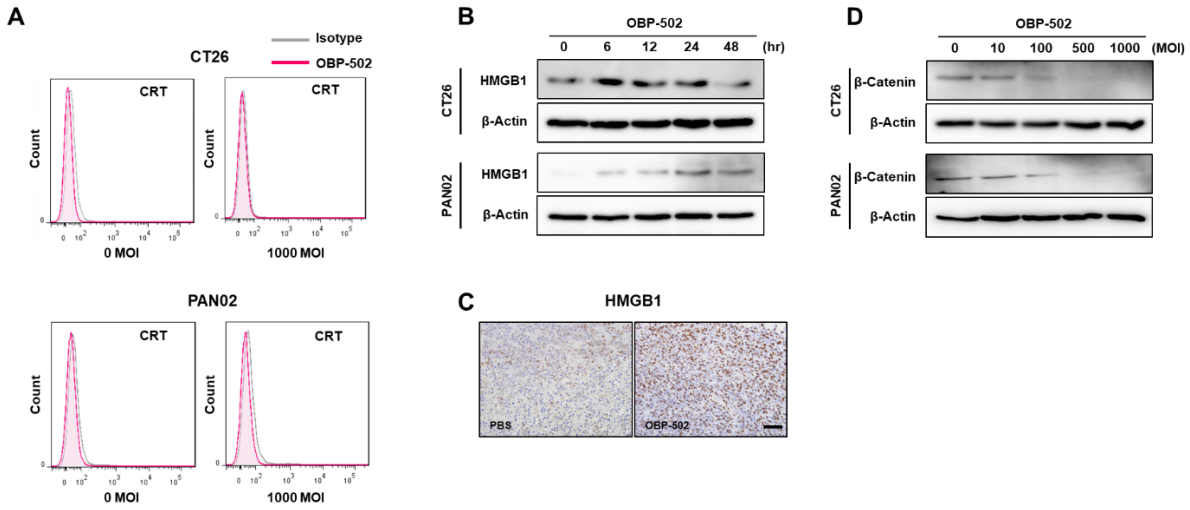

Figure S3. Effect of OBP-502 on CRT and HMGB1 expression

(A) CT26 and PAN02 cells treated with OBP-502 (1000 MOI) for 24 h were incubated with antibody to calreticulin (Abcam, Cambridge, MA, USA) and analyzed by flow cytometry. (B) Whole-cell lysates of CT26 and PAN02 cells collected 0, 6, 12, 24, and 48 h after OBP-502 treatment (1000 MOI) were subjected to Western blot analysis of HMGB1 expression. (C) Immunohistochemical staining for HMGB1 in CT26 tumor tissues harvested at 28 days after PBS or OBP-502 treatment (1×10^9 PFU). Scale bar, 100 μm. (D) Whole-cell lysates of CT26 and PAN02 cells collected 3 days after OBP-502 treatment (0, 10, 100, 500, and 1000 MOI) were subjected to Western blot analysis of β-catenin (Cell Signaling Technology, Danvers, MA, USA) and β-Actin expression.

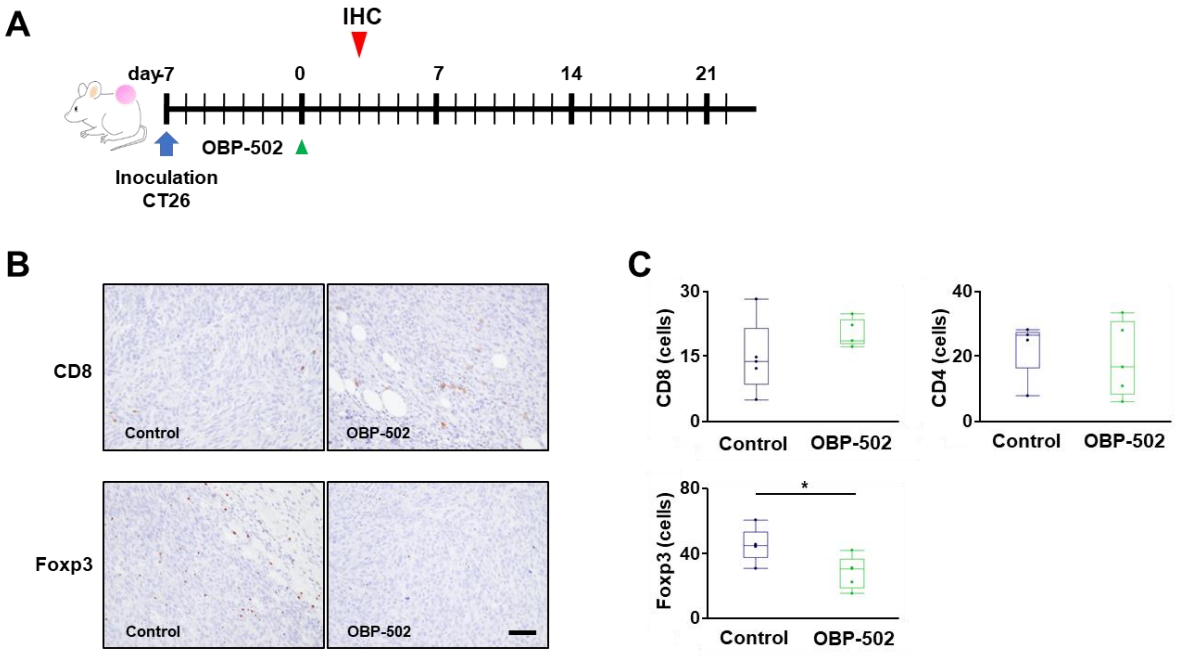

Figure S4. Rapid effect of OBP-502 on TIL recruitment

(A) Study protocol. Briefly, CT26 subcutaneous tumors were harvested 3 days after a single treatment with OBP-502 (1×10^9 PFU) or PBS for immunohistochemical staining. (B) Representative figures of immunohistochemical staining for CD8 and Foxp3 in CT26 tumor tissues. Scale bar, 100 μm. (C) Median number of TILs expressing CD8, CD4, and Foxp3 was statistically assessed from 5 selected fields. *, $P < 0.05$.

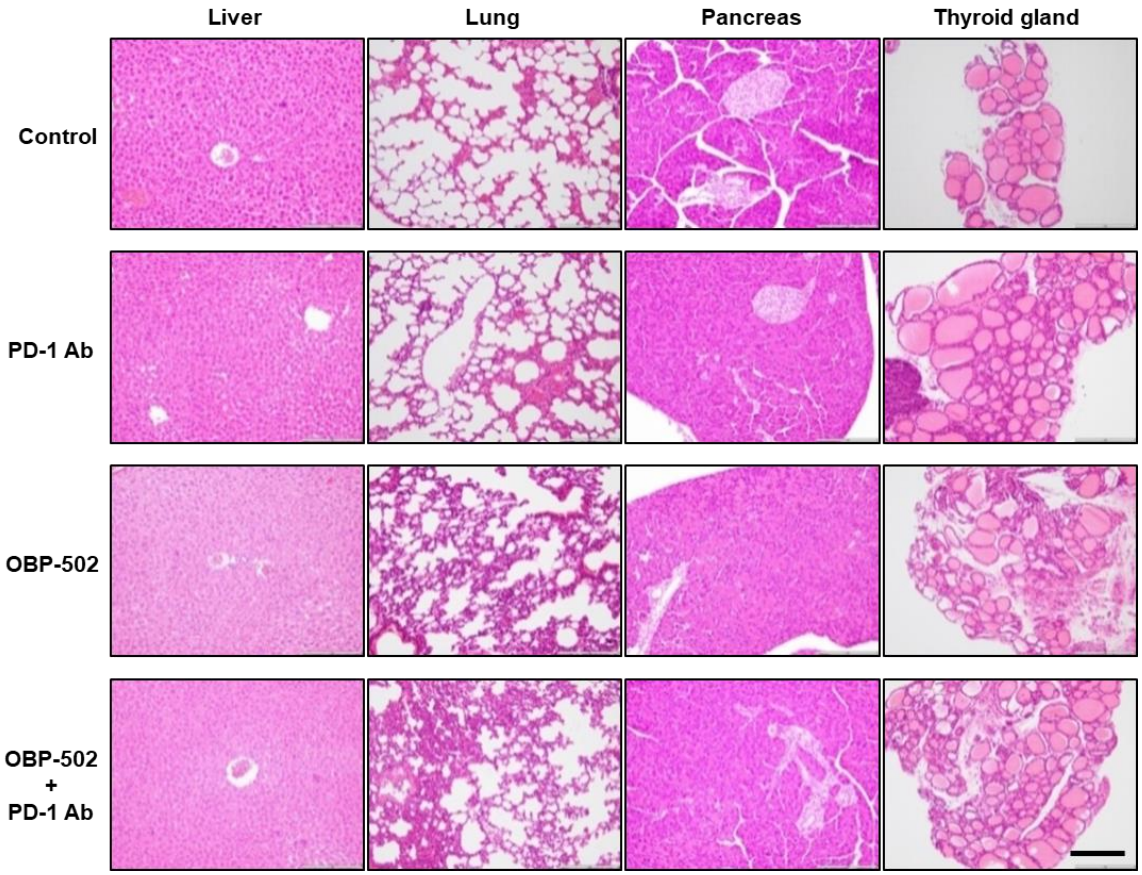

Figure S5. Toxicity in major organs after combination therapy *in vivo*

H&E staining of major organs harvested 28 days after initiation of treatment with PBS, monotherapy with OBP-502 or PD-1 Ab, or combination of both performed in the study setting indicated in Figure 4A on CT26 subcutaneous tumors. Scale bar, 20 μm.

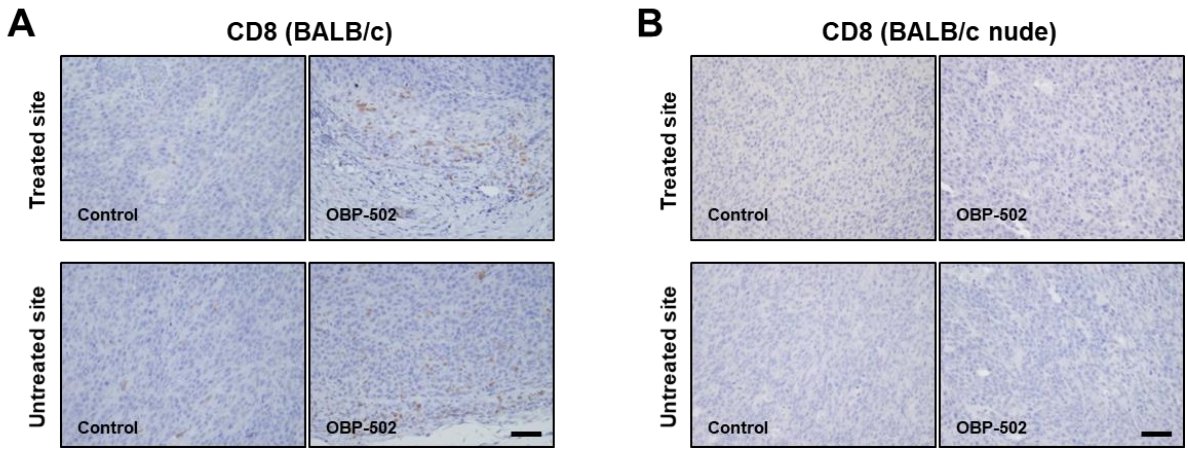

Figure S6. Difference between BALB/c and BALB/c nude mice in recruitment of CD8-positive TILs after OBP-502 treatment

Representative figures of immunohistochemical staining for CD8-positive TILs in OBP-502-treated tumor tissues and untreated tumor tissues harvested 28 days after initiation of treatment performed in the study setting shown in Figure 5A in the CT26 bilateral subcutaneous tumor model using BALB/c mice (A) and BALB/c nude mice (B). Scale bar, 100 μm.

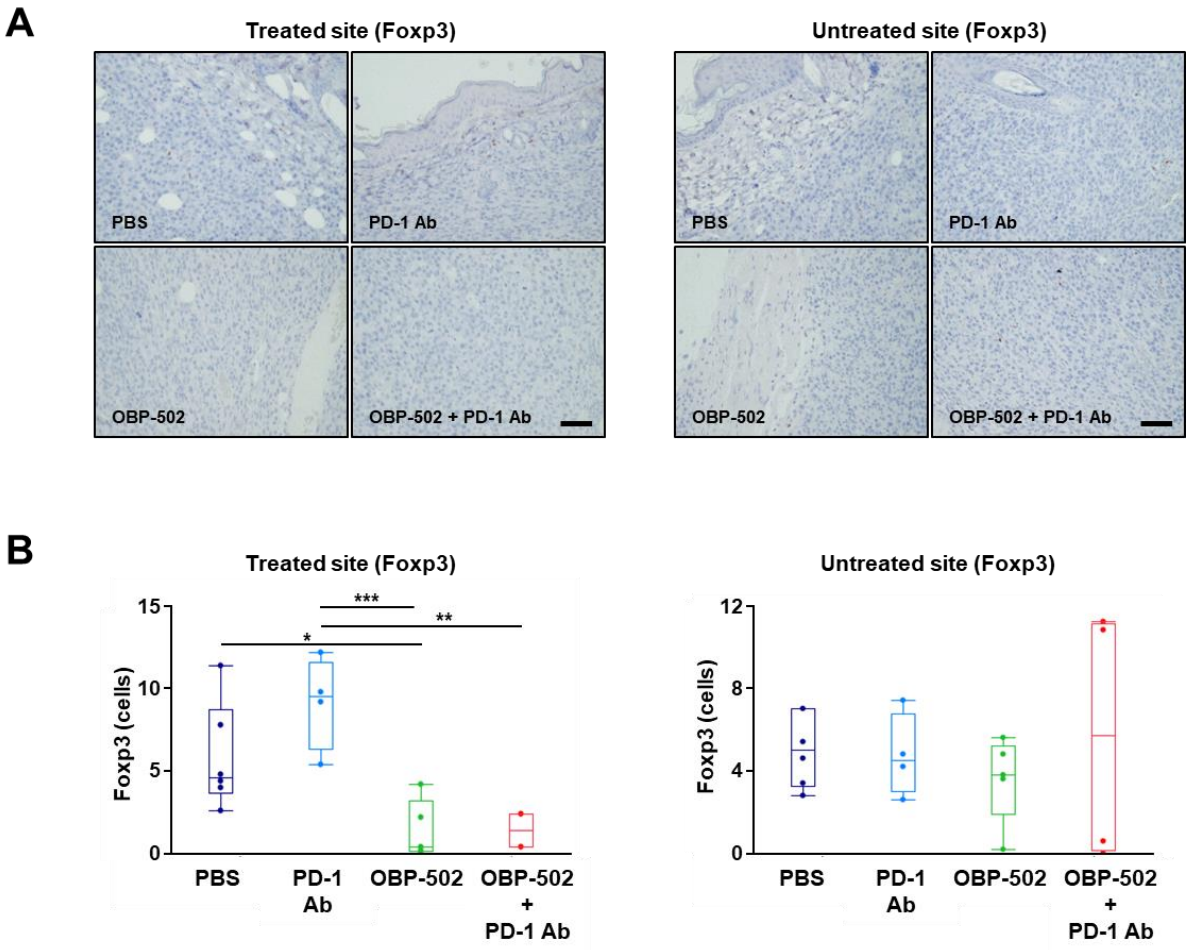

Figure S7. Effect of combination therapy on recruitment of Foxp3-positive TILs in a bilateral subcutaneous tumor model

(A) Representative figures for each treatment group of immunohistochemical staining for Foxp3-positive TILs in OBP-502-treated tumor tissues and untreated tumor tissues harvested 28 days after initiation of treatment performed in the study setting shown in Figure 5A. Scale bar, 100 μm. (B) Median number of Foxp3-positive TILs in OBP-502-treated tumor tissues and untreated tumor tissues was statistically assessed from 5 selected fields. *, $P< 0.05$. **, $P< 0.005$. ***, $P< 0.001$.