

## **Supplementary Information**

### **Telomerase-specific oncolytic immunotherapy for promoting efficacy of PD-1 blockade in osteosarcoma**

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#### **Supplementary Figure S1**

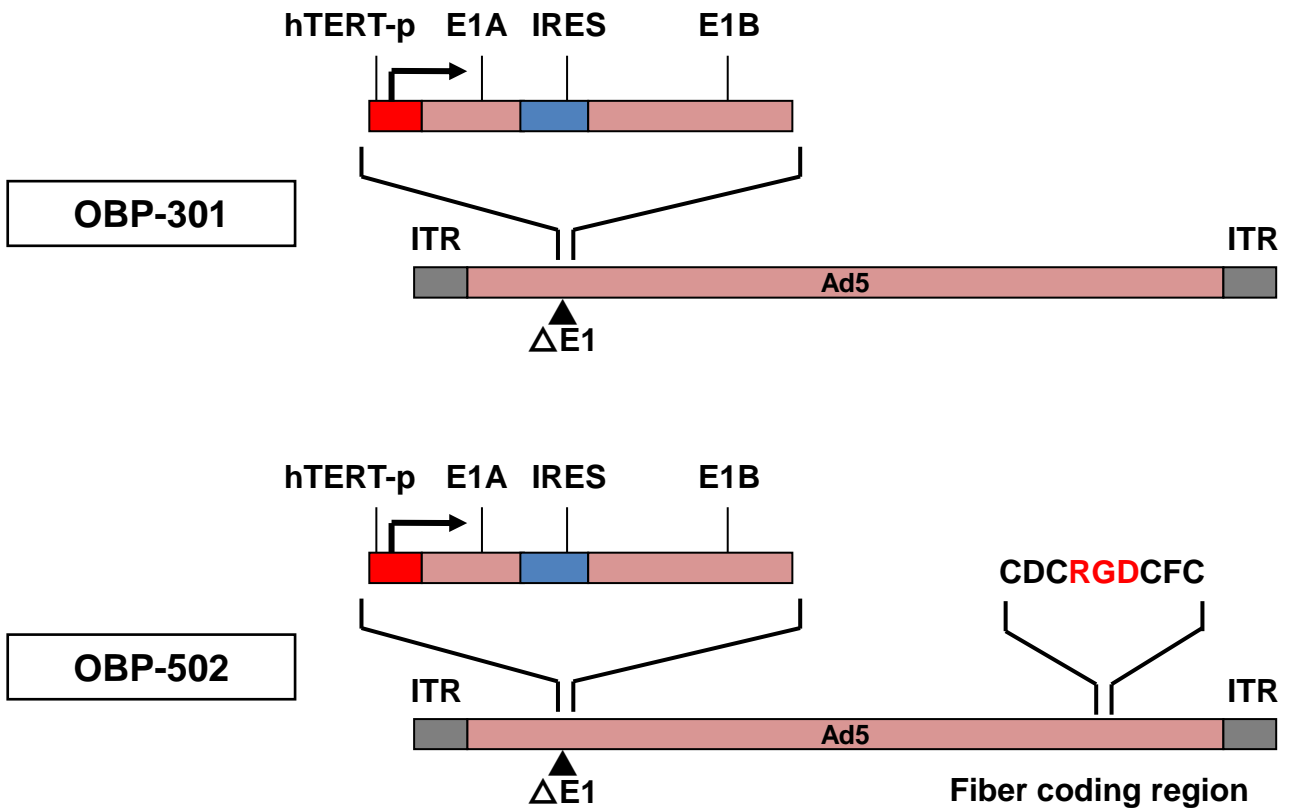
Schematic diagrams of OBP-301 and OBP-502 structures.

#### **Supplementary Figure S2**

Number of CD8+ T cells in K7M2 tumors  
treated with PD-1 blockade and OBP-502.

#### **Supplementary Figure S3**

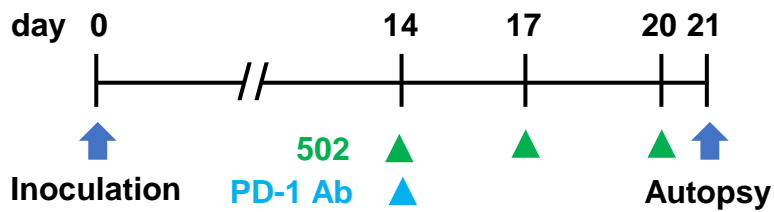
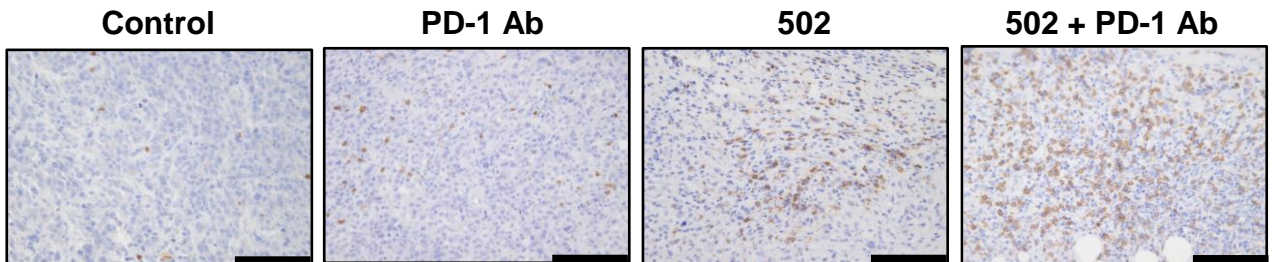
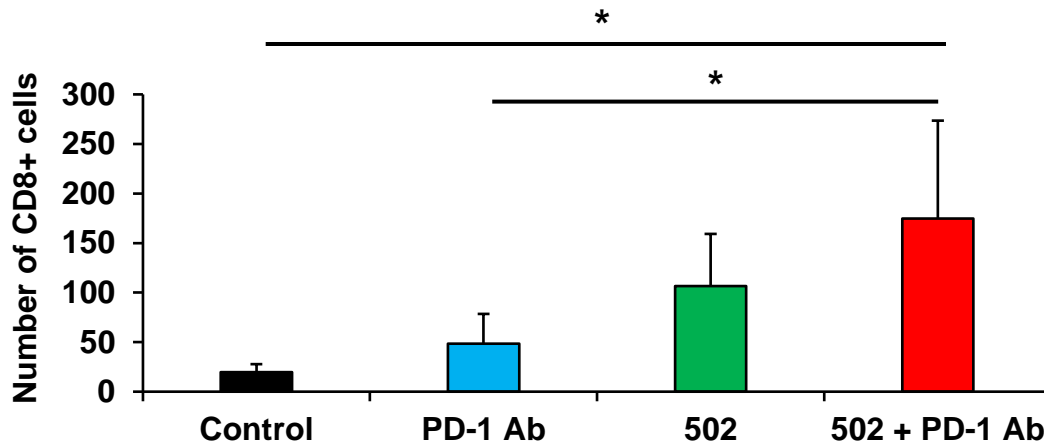
Number of Foxp3+ and CD4+ T cells in K7M2 and NHOS tumors  
treated with PD-1 blockade and OBP-502.



## Supplementary Figure S1

### Schematic diagrams of OBP-301 and OBP-502 structures.

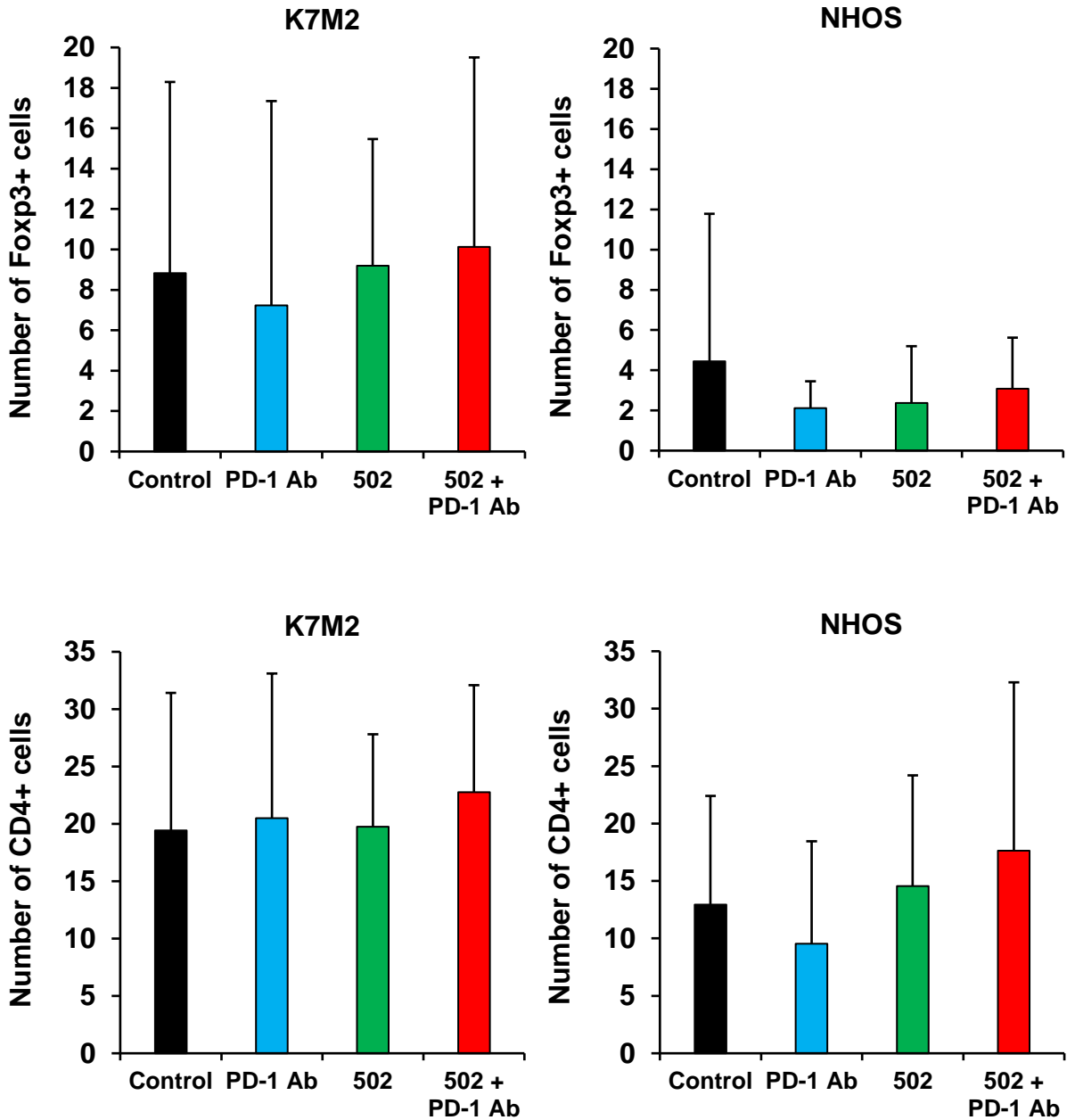
OBP-301 is a telomerase-specific replication-competent oncolytic adenovirus, in which the hTERT promoter drives expression of E1A and E1B genes that are linked with an IRES. In contrast, OBP-502 is a fiber-modified OBP-301, in which the RGD peptide is inserted into the fiber coding region.

**A****B**

### Supplementary Figure S2

#### Number of CD8+ T cells in K7M2 tumors treated with PD-1 blockade and OBP-502.

**A**, K7M2 cells ( $5 \times 10^6$  cells/site) were inoculated into the flank of BALB/c mice. OBP-502 ( $1 \times 10^9$  PFUs) was intratumorally injected once a week for three cycles ( $n = 3$  to  $4$ ; green arrow heads). PBS was used as a control. Anti-PD-1 Ab was intraperitoneally administered once a week for one cycle (blue arrow heads). **B**, Number of CD8+ T cells was calculated from five different randomly selected fields. Data was expressed as mean value  $\pm$  SD ( $n = 3$  to  $4$  in each group; \*,  $P < 0.05$ ). Representative photographs of immunohistochemical staining for CD8+ T cells in each group. Scale bar,  $100 \mu\text{m}$ .



### Supplementary Figure S3

#### Number of Foxp3+ and CD4+ T cells in K7M2 and NHOS tumors treated with PD-1 blockade and OBP-502.

Number of Foxp3+ and CD4+ T cells was calculated from five different randomly selected fields. Data was expressed as mean value  $\pm$  SD (n = 7 to 9 in each group).