Supplementary Materials and Methods

Cell viability assay. SaOS-2 and MNNG/HOS cells were seeded on 96-well plates at a density of 1 x 10^3 cells/well 24 h before viral replication. Cells were infected with Ad-p53 at the indicated doses for 5 days. Cell viability was determined using Cell Proliferation Kit II (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturer's protocol. To calculate the 50% inhibiting dose (ID_{50}) values of Ad-p53 in SaOS-2 and MNNG/HOS cells, cell viability data obtained on day 5 after Ad-p53 infection were used.

Western blot analysis. SaOS-2 cells were seeded in a 100-mm dish at a density of 1 x 10^5 cells/dish 24 h before infection and were infected with Ad-p53 at the indicated MOIs. The primary antibodies used were: mouse anti-p53 monoclonal antibody (mAb) (Calbiochem, Darmstadt, Germany), mouse anti-$p21^{WAF1}$ mAb (Calbiochem) and mouse b-actin mAb (Sigma-Aldrich, St. Louis, MO, USA). The secondary antibody used was horseradish peroxidase-conjugated antibody against mouse IgG (GE Healthcare, Buchinghamshire, UK). Immunoreactive bands on the blots were visualized using enhanced chemiluminescence substrates (ECL Plus; GE Healthcare).

Quantitative real-time PCR analysis. To compare the *E1A* copy number between OBP-702-and OBP-301-infected SaOS-2 cells, SaOS-2 cells, seeded on 6-well plates at a density of 5 x 10^5 cells/well 24 hr before viral replication, were infected with OBP-702 or OBP-301 at an MOI of 10 PFUs/cell. Genomic DNA was extracted from serially diluted viral stocks and tumor cells infected with OBP-702 or OBP-301 using the QIAmp DNA Mini kit (Qiagen, Valencia, CA, USA). *E1A* copy number was detremined using TaqMan real-time PCR systems (Applied Biosystems, Foster City, CA, USA).

Immunohistochemistry. Tumors were fixed in 10% neutralized formalin and embedded in paraffin blocks. Sections were prepared for immunohistochemical examination. After deparaffinization and rehydration, antigen retrieval was performed in 10 mM citrate buffer (pH 6.0). Tissue sections were incubated with rabbit anti-Ki67 mAb (Abcam, Cambridge, MA, USA). Immunoreactive signals were visualized by using 3,3'-diaminobenzidine tetrahydrochloride solution, and the nuclei were counterstained with hematoxylin.

Supplementary Figure S1. The structures of OBP-301 and OBP-702.

To induce exogenous *p53* gene expression by OBP-301, we generated OBP-702, in which the expression cassette of human wild-type *p53* gene derived by Egr-1 promoter was inserted at the E3 region, as previously reported (26).

Supplementary Figure S2. The ID_{50} values of OBP-702 and Ad-p53 in SaOS-2 and MNNG/HOS cells.

SaOS-2 and MNNG/HOS cells were seeded 24 h before viral infection. Cells were infected with Ad-p53 at multiplicity of infections (MOI) of 0, 0.1, 1, 10, 50, 100 plaque forming units (PFU)/cell. **A**, cell viability was examined on day 5 using the XTT assay. Cell viability was calculated relative to that of the mock-infected group, which was set at 1.0. Cell viability data are expressed as mean ± SD (n = 5). **B**, the 50% inhibiting doses (ID50) of OBP-702 and Ad-p53 were calculated using cell viability data on day 5 after infection.

Supplementary Figure S3. Expression of endogenous p53 protein in SaOS-2 and MNNG/HOS cells.

Endogenous p53 expression in SaOS-2 (p53 null) and MNNG/HOS (mutant p53) cells was analyzed by Western blot analysis. β-actin was assayed as a loading control.

Supplementary Figure S4. Quantitative measurement of viral DNA replication in SaOS-2 cells infected with OBP-702 or OBP-301.

The cells were infected with OBP-702 or OBP-301 at an MOI of 10 PFUs/cell, and *E1A* copy number was analyzed over the following 2 days by quantitative real-time PCR. The value of the *E1A* copy number at 2 h after infection was set at 1, and relative copy numbers were plotted. The data are expressed as mean values ± SD (n = 3).

Supplementary Figure S5. Chronological change of p21 expression in SaOS-2 cells after Ad-p53 infection.

SaOS-2 cells were infected with Ad-p53 at 10 and 100 MOIs, and infected cells were harvested at the indicated time points. Cell lysates were subjected to Western blot analysis for p21. β-actin was assayed as a loading control. The lower panel shows the intensity of each band of p21 determined by densitometric scanning using ImageJ software and normalized by dividing the b-actin signal.

Supplementary Figure S6. Immunohistochemistry of MNNG/HOS tumors.

Tumor sections were obtained 28 days after first treatment with PBS, Ad-p53, OBP-301 or OBP-702. Paraffin-embedded sections of MNNG/HOS tumors were immunostained with rabbit anti-Ki67 monoclonal antibody (Abcam, Cambridge, MA, USA). Scale bars; 100 mm.