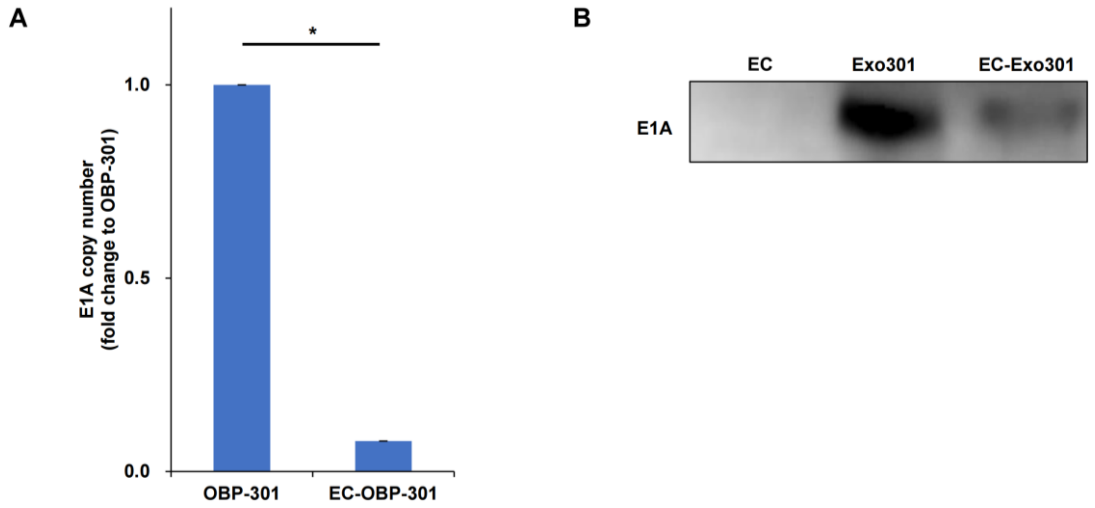
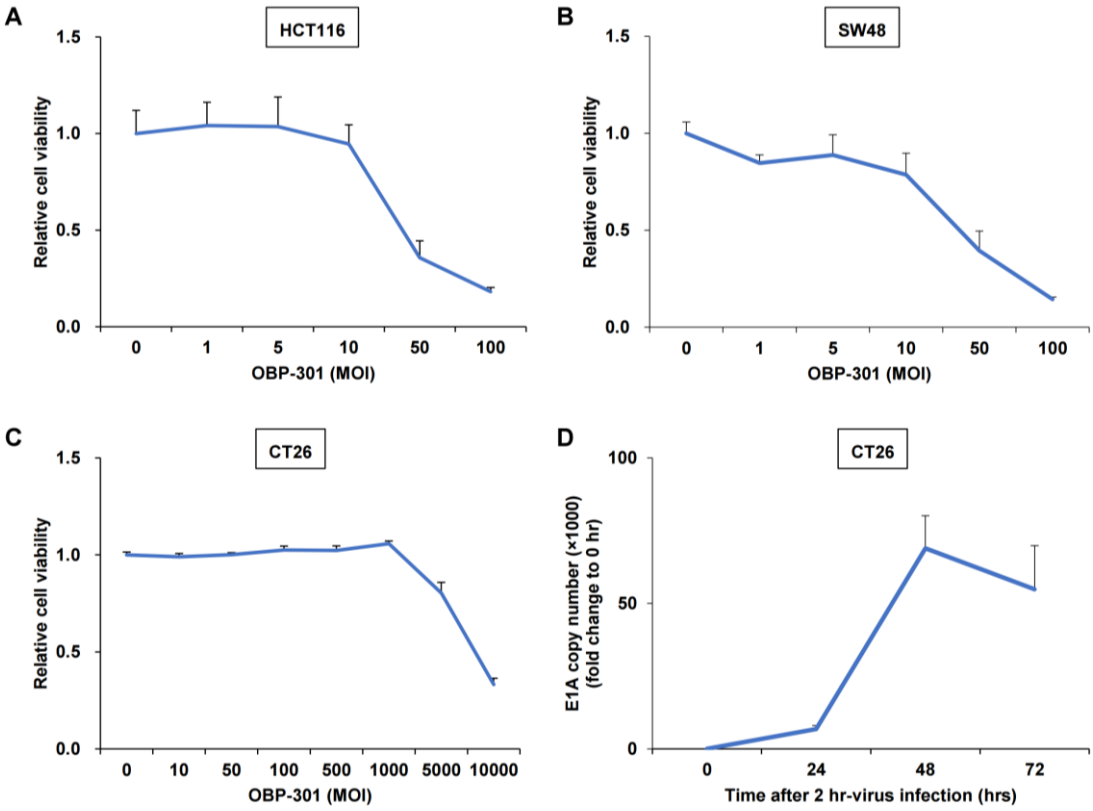


Supplemental Figure S1. ExoCap® isolation of OBP-301 and Exo301

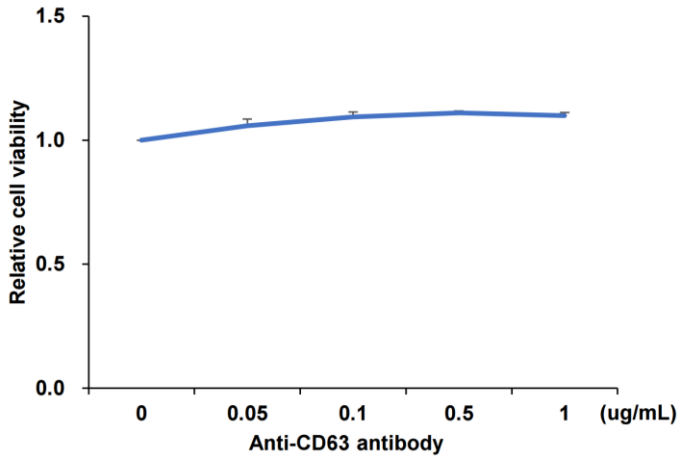
(A) DNAs extracted from OBP-301 or OBP-301 extracted by ExoCap® (EC-OBP-301) were subjected to qRT-PCR for the adenovirus E1A gene (n=3). E1A copy numbers are described as fold change relative to OBP-301. * $p<0.001$.

(B) Western blot of E1A in EC, Exo301, and EC-Exo301 isolated from HCT116 cells.



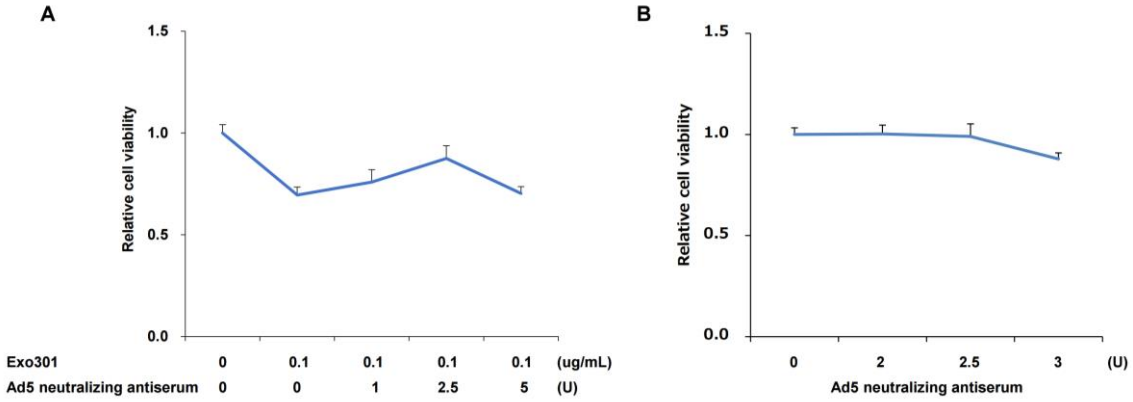
Supplemental Figure S2. *In vitro* cytotoxic effects of OBP-301 on HCT116, SW48, and CT26 cells

Viability of HCT116 (A), SW48 (B), and CT26 (C) cells treated with OBP-301 at the indicated concentrations were assessed using the XTT assay 3 days after treatment (n=5). The percentage of viable cells relative to untreated cells (0 MOI) is plotted. (D) CT26 cells were treated with OBP-301 (1000 MOI) for 2 h and were harvested at the indicated time points after removing the treatments. The extracted DNA was subjected to qRT-PCR analysis of adenovirus E1A gene levels (n=3). E1A copy numbers are described as fold change relative to time = 0 h.



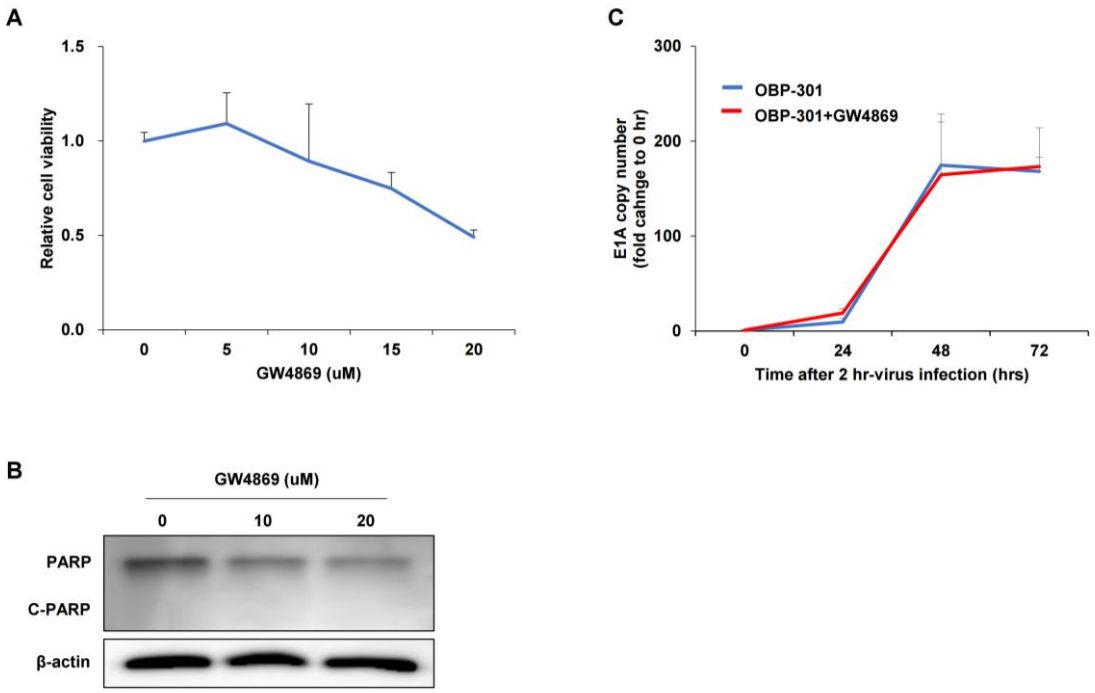
Supplemental Figure S3. *In vitro* cytotoxic effects of anti-CD63 antibody

Viability of HCT116 cells treated with anti-CD63 antibody at the indicated concentrations were assessed using the XTT assay 3 days after treatment (n=5). The percentage of viable cells relative to untreated cells (0 μg/mL) is plotted.



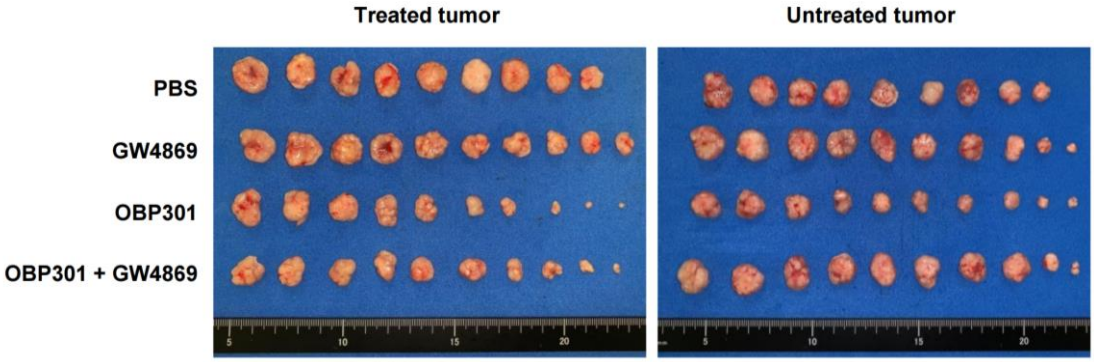
Supplemental Figure S4. Inhibition of Exo301 by Ad5 neutralizing antiserum

(A) Neutralizing antiserum to adenovirus type 5 (Ad5) (cat. 301253; DENKA SEIKEN, Japan) was added to the culture medium at the indicated concentrations together with Exo301 to neutralize free OBP-301 or OBP-301 attached on the surface of EVs, and the viability of HCT116 cells was subsequently assessed using the XTT assay 3 days after treatment (n=5). The percentage of viable cells relative to untreated cells (Exo301: 0 μg/mL, Ad5 neutralizing antiserum: 0 unit) is plotted. (B) Viability of HCT116 cells treated with Ad5 neutralizing antiserum at the indicated concentrations were assessed using the XTT assay 3 days after treatment (n=5). The percentage of viable cells relative to untreated cells (0 unit) is plotted.



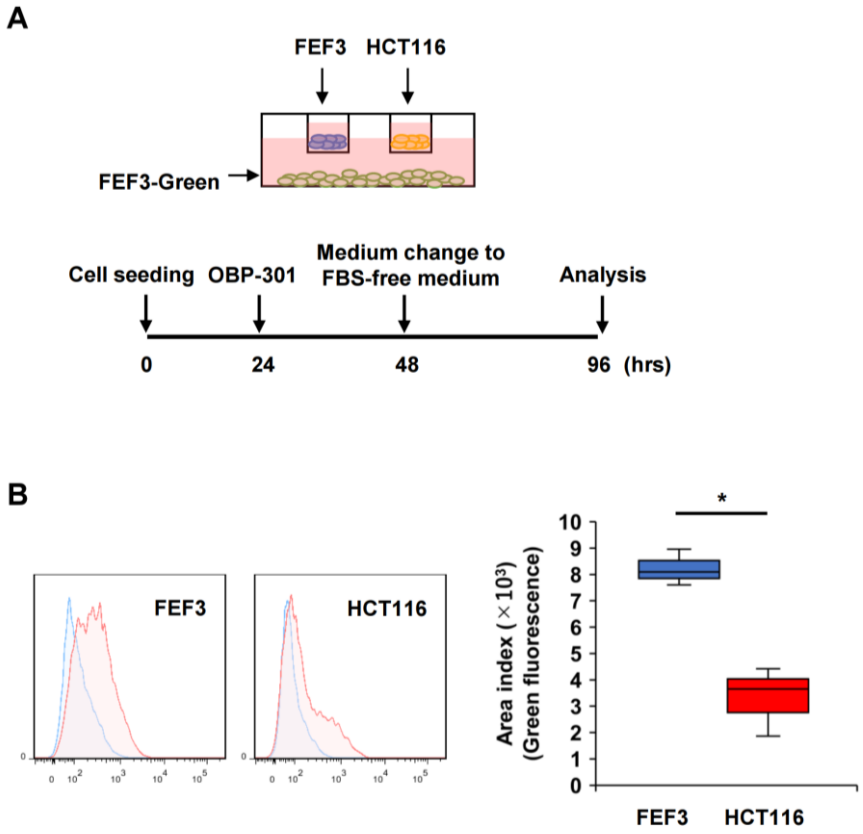
Supplemental Figure S5. Influence of GW4869 on cell viability and viral replication

(A) Viability of HCT116 cells treated with GW4869 at the indicated concentrations were assessed using the XTT assay 3 days after treatment (n=5). The percentage of viable cells relative to untreated cells (0 μg/mL) is plotted. (B) HCT116 cells were harvested 72 h after treatment with the indicated doses of GW4869, and whole cell HCT116 lysates were subjected to western blot analysis for PARP and β-actin. (C) HCT116 cells were treated with OBP-301 or EC-Exo301 + GW4869 for 2 h and were harvested at the indicated time points after removing the treatments. GW4869 was added to the culture medium at 6 h prior to OBP-301 treatment to block exosome secretion. The extracted DNA was subjected to qRT-PCR analysis of adenovirus E1A gene levels (n=3). E1A copy numbers are described as fold change relative to time = 0 h.



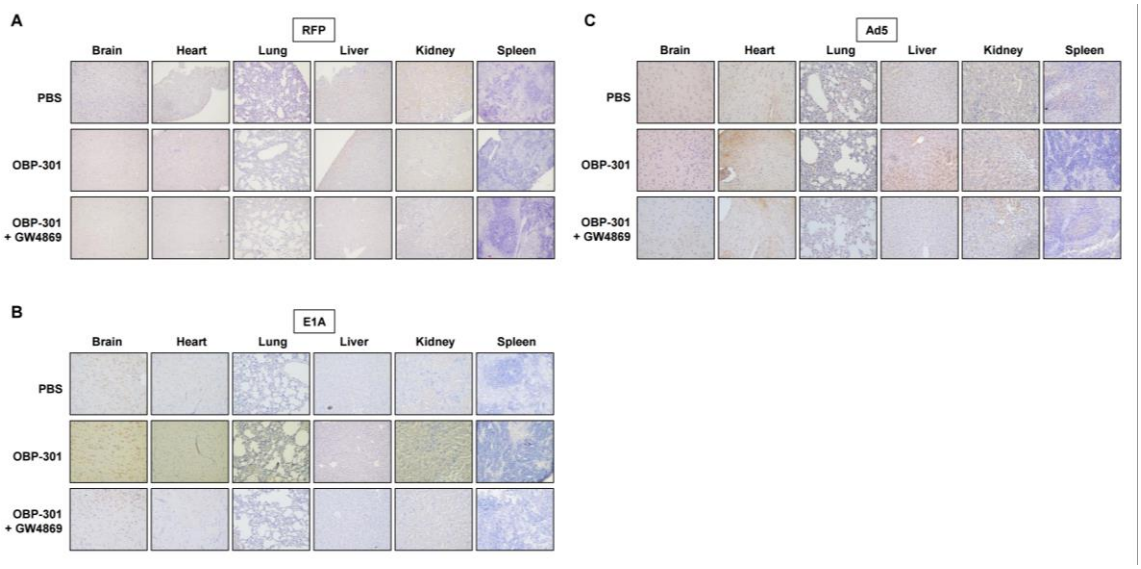
Supplemental Figure S6. Comparison of tumor sizes of treated- and untreated-tumors

Macroscopic pictures of all tumors harvested 28 days after initiation of treatment (PBS, GW4869, OBP-301, and OBP-301 + GW4869) in a HCT116 bilateral subcutaneous tumor model using BALB/c nu/nu mice.



Supplemental Figure S7. *In vitro* cell tropism of fibroblast-derived EVs

(A) Experimental design of a triple co-culture model. Briefly, FEF3 cells stained green (FEF3-Green) were seeded in the lower chamber, and FEF3 cells and HCT116 cells were individually seeded in the upper chambers. FEF3-Green cells were treated with OBP-301 (100 MOI) and the culture medium was changed to FBS-free medium 24 h after treatment. Cells were incubated for another 48 h and then harvested for analysis. (B) FEF3 cells and HCT116 cells in the upper chamber were subjected to flow cytometry for uptake of green fluorescence (n=3). Representative data for each cell line are shown on the left, and statistical assessment on green fluorescence amounts in each cell line is shown on the right. * $p<0.005$.



Supplemental Figure S8. RFP biodistribution after OBP-301 treatment in an orthotopic rectal tumor model

In an orthotopic model of rectal tumors (HCT116-RFP) with liver metastasis (HCT116-Luc) using BALB/c nu/nu mice, HCT116-RFP tumors were treated with intratumoral administration of PBS or OBP-301 (1×10^8 PFU) and/or intraperitoneal administration of GW4869 (2.5 μg/g) three times every two days, and primary rectal tumors and metastatic liver tumors along with major organs (brain, heart, lung, liver, kidney and spleen) were harvested 7 days after treatment initiation (n=4-5). Representative images of IHC staining for RFP (A), E1A (B), and Ad5 (C) in major organs such as brain, heart, lung, liver, kidney, and spleen in each treatment are shown.