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## Supplemental Information

# Oncolytic Virus-Mediated Targeting of the ERK Signaling Pathway Inhibits Invasive Propensity in Human Pancreatic Cancer

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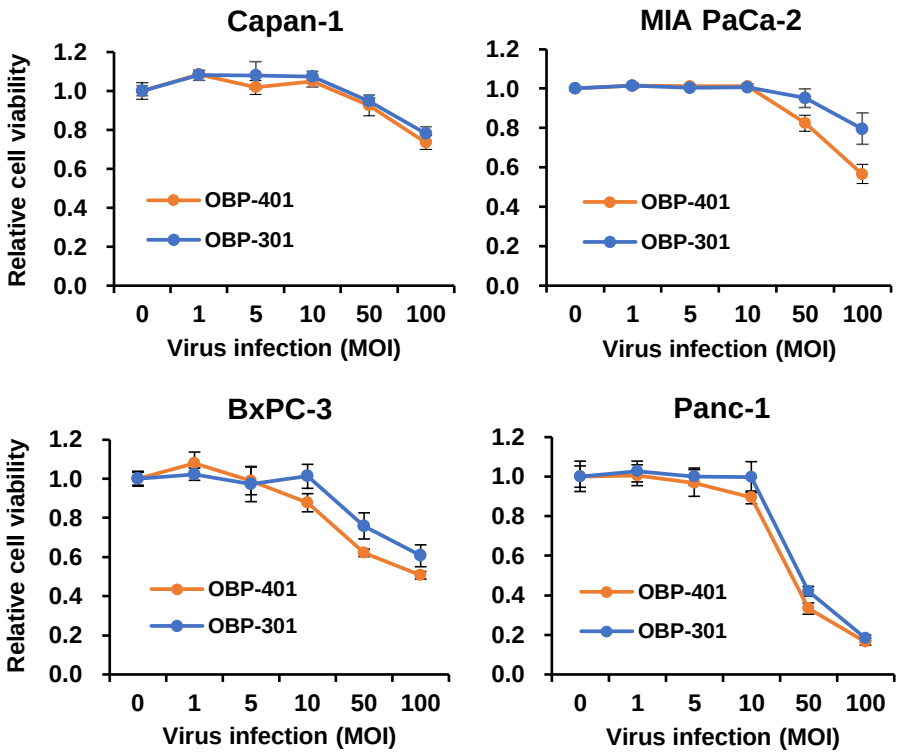
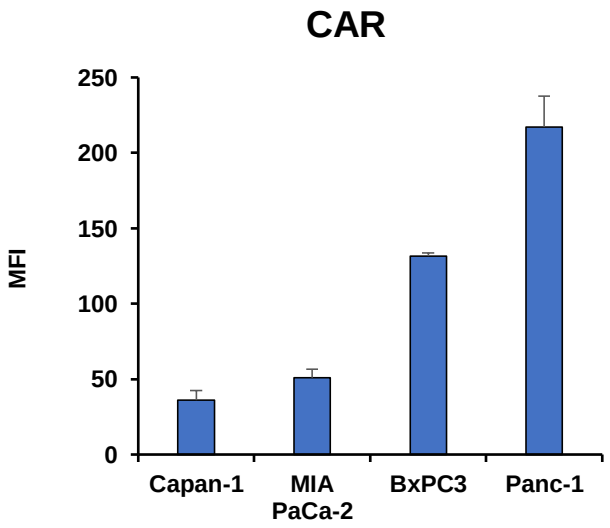


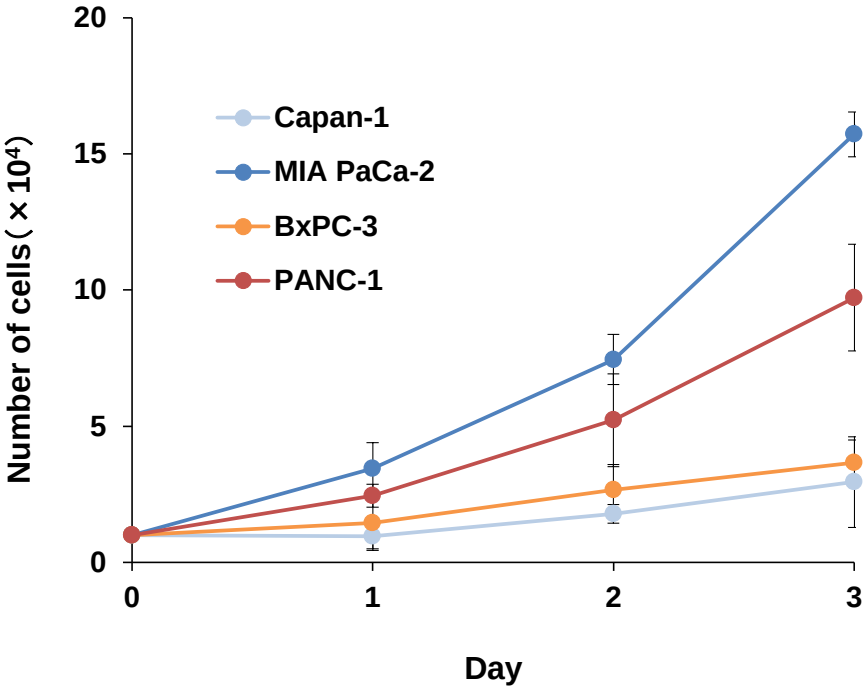

**Supplemental Figure S1**

The cytopathic effect of OBP-301 and OBP-401 in PDAC cells. Cell viability was determined 72 h after infection with OBP-301 or OBP-401 at the indicated MOI using an XTT assay. Cell viability was calculated relative to that of mock infected cells, whose viability was set at 1.0. Data are expressed as mean ± SD (n = 5).



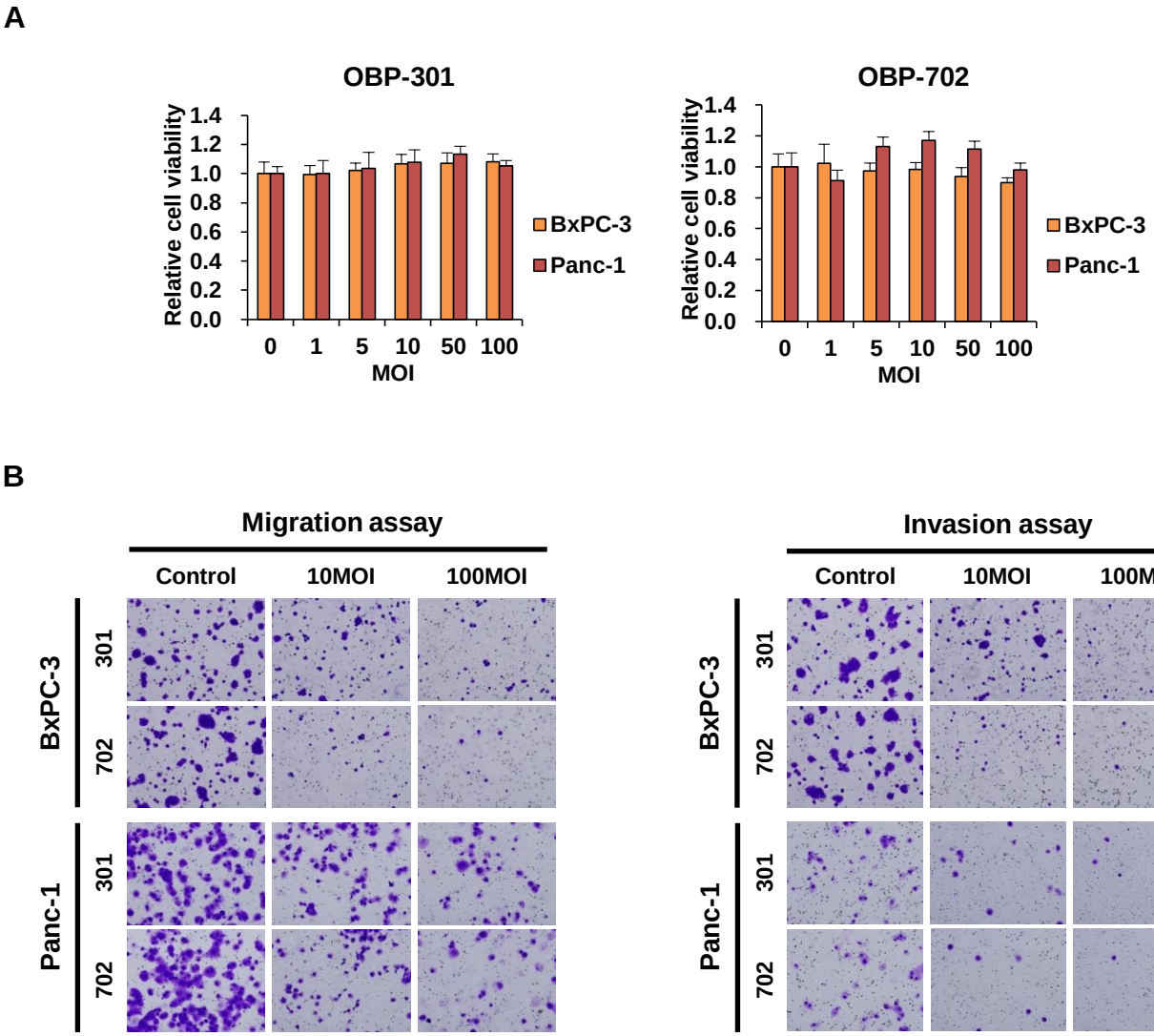

**Supplemental Figure S2**

Characterization of CAR expression in PDAC cells. The mean fluorescence intensity (MFI) of coxsackie and adenovirus receptor (CAR) expression was assessed by flow cytometric analysis. Data are expressed as mean values ± SD (n = 3).



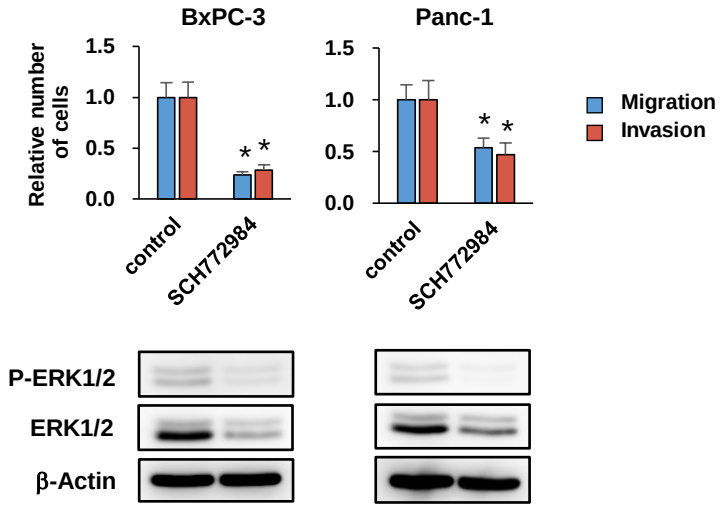

**Supplemental Figure S3**

Characterization of cell proliferation property in PDAC cells. PDAC cells (Capan-1, MIA PaCa-2, BxPC-3, Panc-1) were seeded at a density of $10^4$ cells in 24-well tissue culture plates. After 24 h, cells were counted every day for 3 days. Data are expressed as mean values ± SD (n = 3).



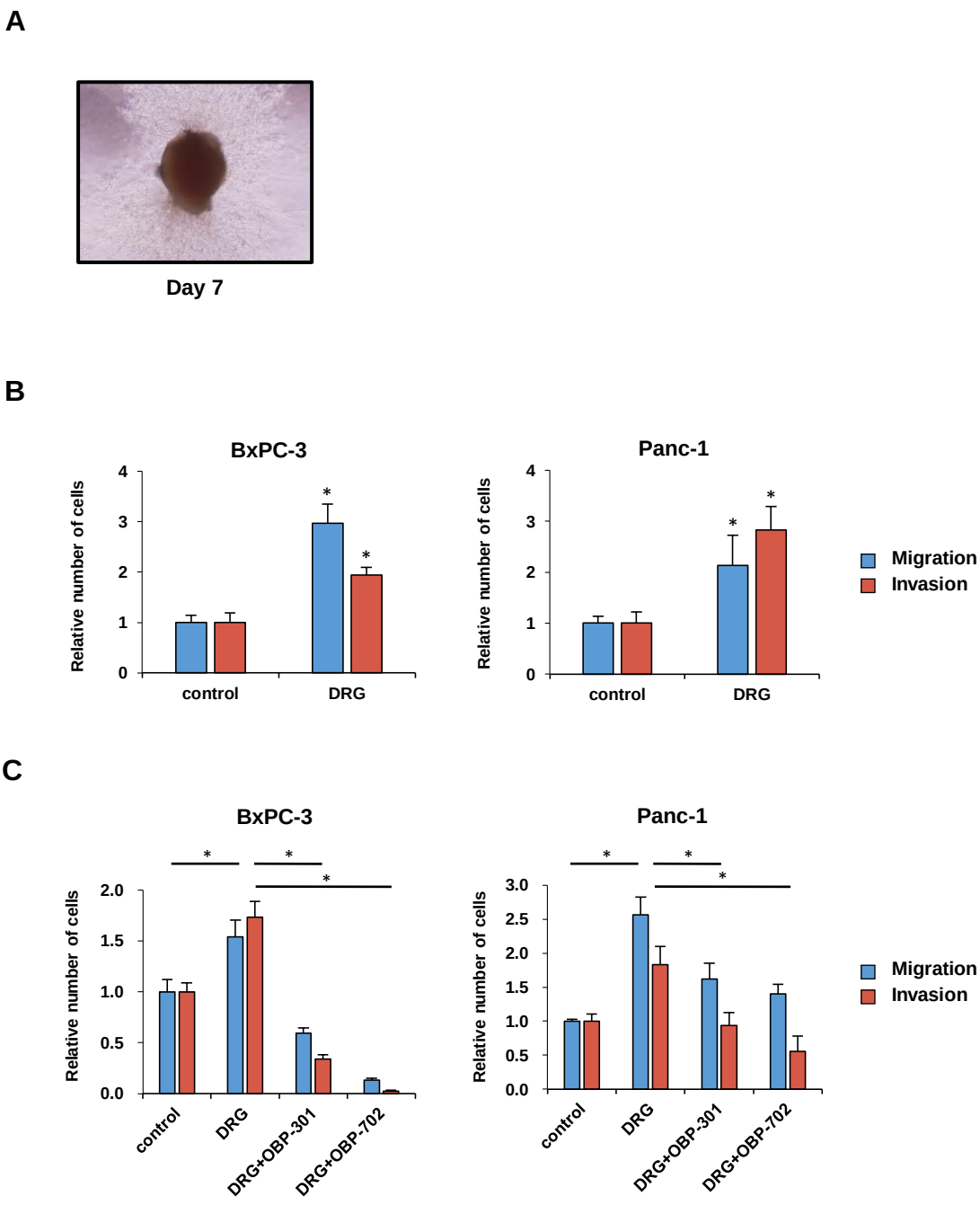

**Supplemental Figure S4**

OBP-301 and OBP-702 inhibit migration and invasion of high-invasive PDAC cells independent of cell viability. (A) Cell viability was determined 24 h after infection with OBP-301 or OBP-702 at the indicated MOIs using XTT assay. Cell viability was calculated relative to that of mock infected cells, whose viability was set at 1.0. Data are expressed as mean values ± SD (n = 5). (B) Representative photographs of migrating and invading cells stained with Crystal Violet. Original magnification: × 100.



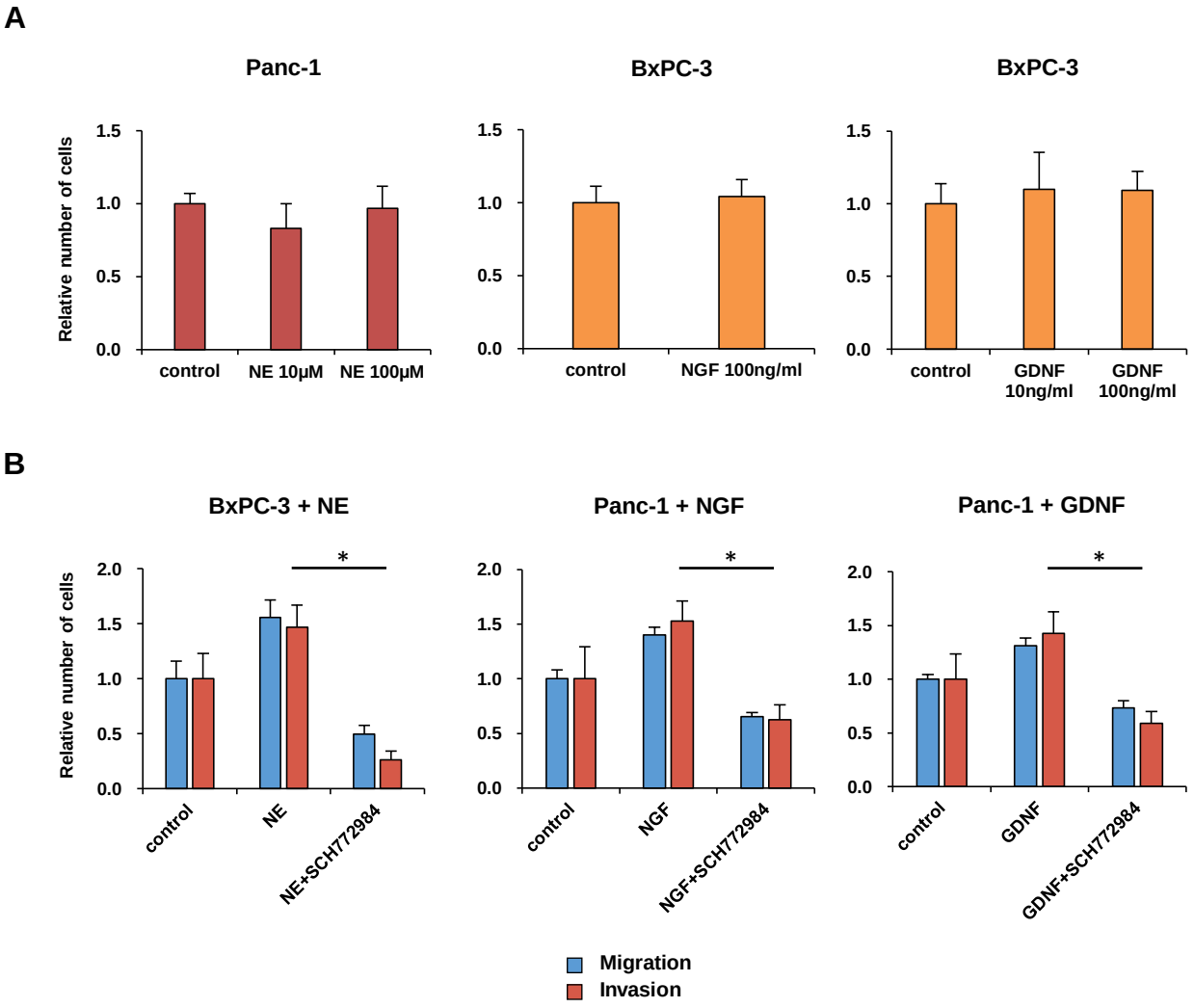

**Supplemental Figure S5**

SCH772984 inhibits migration and invasion of high-invasive PDAC cells by suppressing ERK1/2 expression. Migration and invasion assay using high-invasive PDAC cells treated with ERK1/2 inhibitor SCH772984 (500 nM) for 24 h. Data are expressed as mean values ± SD (n = 5). *: $p < 0.05$ (vs control). Expression of phosphor-ERK1/2 (P-ERK1/2) and ERK1/2 proteins in high-invasive PDAC cells treated with SCH772984 (500 nM) for 24 h. β-Actin was assayed as a loading control.




**Supplemental Figure S6**

OBP-301 and OBP-702 inhibit migration and invasion of high-invasive PDAC cells stimulated with dorsal root ganglion. (A) Representative photograph of mouse dorsal root ganglion (DRG), which was obtained from athymic nude mice and cultured in the matrigel-coated dish for 7 days. (B) Migration and invasion assay using high-invasive PDAC cells stimulated with DRG for 24h. *: $p < 0.05$ (vs control). (C) Migration and invasion assay using high-invasive PDAC cells stimulated with DRG for 24h in the presence of OBP-301 (100 MOI) or OBP-702 (100 MOI). The number of migrating and invading cells after treatment was calculated relative to that of non-treated cells, which was set at 1.0. Data are expressed as mean values ± SD (n = 5). *: $P < 0.05$ (vs DRG).




**Supplemental Figure S7**

SCH772984 inhibits migration and invasion properties of high-invasive PDAC cells stimulated with neurosecretory factors. (A) Migration assay using high-invasive PDAC cells stimulated with norepinephrine (NE), nerve growth factor (NGF) or glial cell line-derived neurotrophic factor (GDNF) at the indicated doses. (B) Migration and invasion assay using high-invasive PDAC cells stimulated with NE (100 μM), NGF (100 ng/ml) or GDNF (100 ng/ml) for 24h in the presence of SCH772984 (500 nM). The number of migrating and invading cells after treatment was calculated relative to that of non-treated cells, which was set at 1.0. Data are expressed as mean values ± SD (n = 5). *: $p < 0.05$ (vs NE, NGF, or GDNF).

## Supplemental Methods

### Flow Cytometric Analysis

Four human PDAC cell lines (Capan-1, MIA PaCa-2, BxPC-3, Panc-1) were labeled with mouse anti-coxsackie and adenovirus receptor (CAR) monoclonal antibody (RmcB; Upstate Biotechnology) or isotype control IgG for 60 min at 4°C. The cells were then incubated with Alexa Fluor 647-conjugated rabbit anti-mouse IgG second antibody (Invitrogen) for 30 min, and were analyzed using flow cytometry (FACS Lyric; Becton Dickinson). The mean fluorescence intensity (MFI) of CAR for each cell line was determined by calculating the difference between the MFI in antibody-treated and control IgG-treated cells from 3 independent experiments.

### Cell Proliferation Assay

Four human PDAC cell lines (Capan-1, MIA PaCa-2, BxPC-3, Panc-1) were seeded at a density of $10^4$ cells in 24-well tissue culture plates. After 24 h, cells were counted every day for 3 days. The average number of cells was determined at each time point in triplicate.

### Cell Viability Assay

High-invasive PDAC cells (BxPC-3 and Panc-1) were seeded on 96-well plates at a density of $10^3$ cells/well 24 h before virus infection. Cells were infected with OBP-301 or OBP-702 at a multiplicity of infection (MOI) of 0, 1, 5, 10, 50, 100 plaque-forming units (PFU)/cell. Cell viability was determined 24 h after virus infection using the Cell Proliferation Kit II (Roche, Indianapolis, IN, USA) according to the manufacturer's protocol.

### Reagents

Extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitor SCH772984 was purchased from CHEMIETEK (Indianapolis, IN, USA).

### Migration and Invasion Assay

Cell migration and invasion assay was conducted using 24-well Boyden chambers with 8 μm pore size filter membranes and 8 μm pore size filter membrane coated with matrigel, respectively (BD Biosciences). Then, 10% FBS-containing medium was placed in the lower chambers to be used as a chemoattractant. To assess the effect of ERK1/2 inhibitor SCH772984 and neurosecretory factors, $5 \times 10^4$ cells (BxPC-3) or $2.5 \times 10^4$ cells (Panc-1) were placed in the upper chambers for migration assay, and $10^5$ cells (BxPC-3) or $5 \times 10^4$ cells (Panc-1) were placed in the upper chambers for the invasion assay. Migrating or invading cells on the bottom surface of the membrane were stained with Crystal Violet (Sigma-Aldrich) and counted under a microscope ( × 100) in 5 randomly selected fields.

### Direct Co-Culture of PDAC Cells and Mouse Dorsal Root Ganglions

To evaluate the effect nerve tissues in the invasive phenotype of PDAC cells, we used mouse dorsal root ganglions (DRGs), which were obtained from athymic nude mice. At first, to confirm the viability of fresh DRGs, we cultured in the matrigel-coated dish for 7 days. Next, we co-cultured PDAC cells with fresh DRGs in migration and invasion assay. Moreover, to evaluate the effect of oncolytic adenoviruses in the DRG-enhanced migration and invasion of PDAC cells, we infected PDAC cells with OBP-301 (100 MOI) or OBP-702 (100 MOI) for 24h.