OMTO, Volume *13*

Supplemental Information

lncRNA UCA1-Mediated Cdc42 Signaling Promotes Oncolytic Vaccinia Virus Cell-to-Cell Spread in Ovarian Cancer

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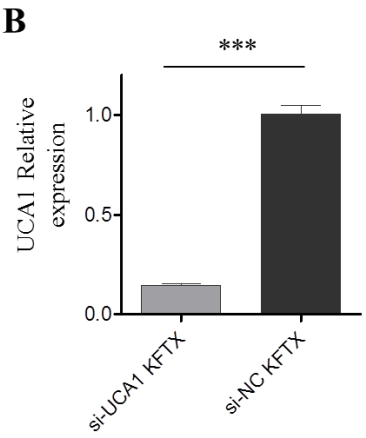
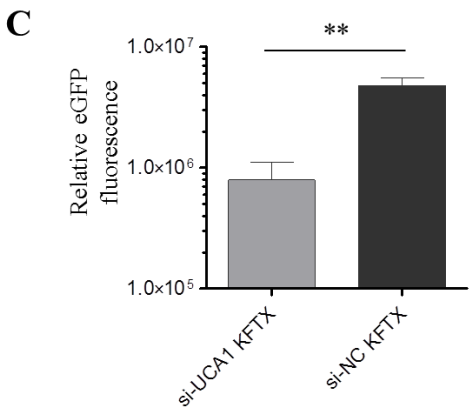
A

siRNA	Sequence(5'-3')	Length (mer)
si-UCA1	Sense: GAGCCGAUCAGACAAACAAdTdT Antisense: UUGUUUGUCUGAUCGGCUCdTdT	21





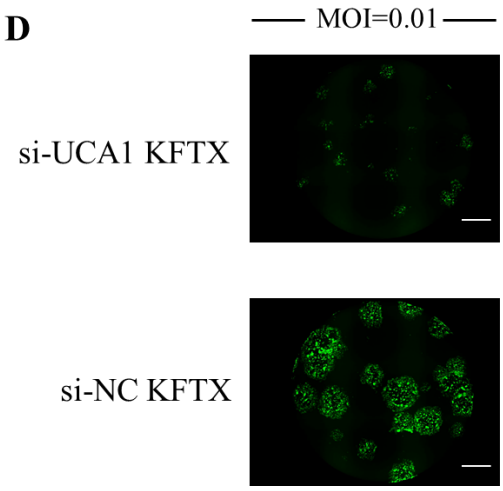


Fig. S1. UCA1 depletion impairs oncolytic vaccinia virus (OVV)-LG spread. (A) si-UCA1 and si-NC construction. **(B)** si-UCA1 or si-NC was transfected into KFTX cells with Lipofectamine™ RNAiMAX Transfection Reagent according to the manufacturer's protocol. After 36 hours, RNA was collected and UCA1 expression was confirmed by qRT-PCR; n = 3 **(C)** Cells were plated at 6×10^3/well in a 96-well plate and transfected with si-RNA. EGFP images are shown after infection with OVV-LG (MOI = 0.01) for 48 hours. Scale bar: 1000 μm. **(D)** The intensity and area of viral EGFP brightness was measured using a Keyence BZ-X700 fluorescence microscope; n = 3. Data with error bars represent mean ± s.e.m. An un-paired t-test was used for (B,C). $^{**}p < 0.01$, $^{***}p < 0.001$.

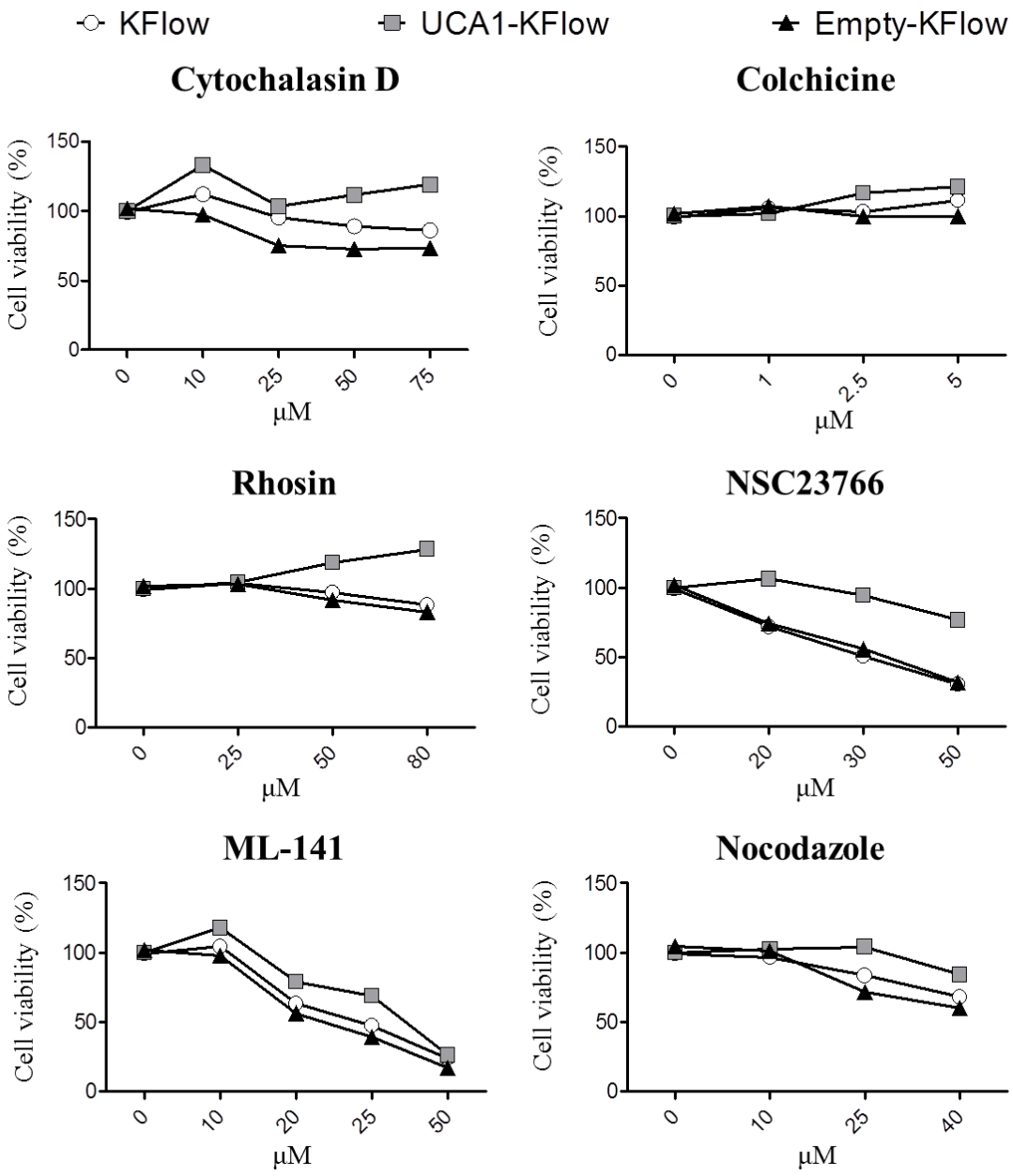


Fig. S2. Cytotoxicity of the inhibitor against intracellular transport, cell-to-cell spread and the Rho family of small GTPases. KFlow, UCA1-KFlow, and Empty-KFlow cells were plated at 6×10^3/well in 96-well plates. Cell viability was determined 48 hours after treatment with an inhibitor using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI).; n = 3. Data with error bars represent mean ± s.e.m.

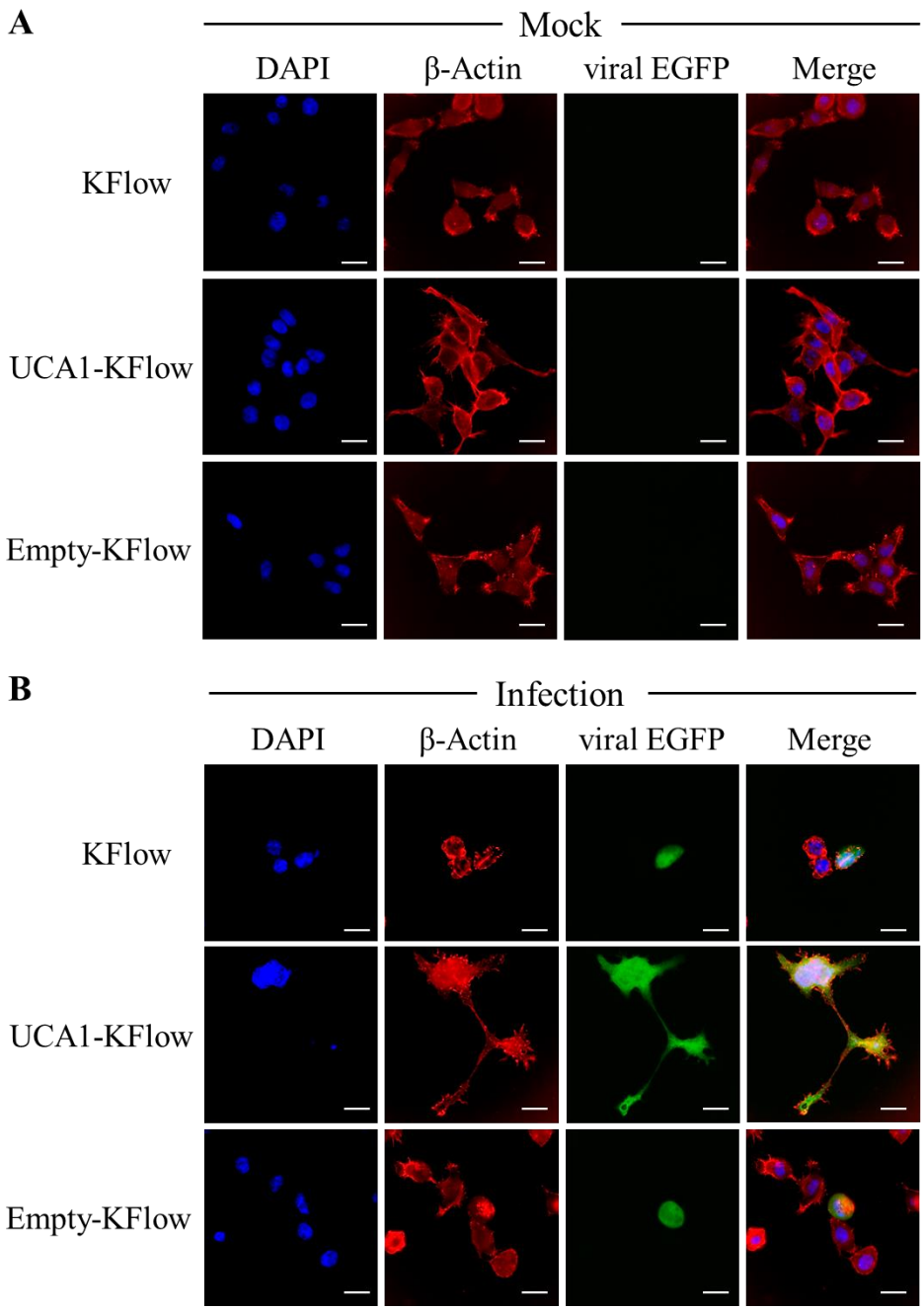


Fig. S3. UCA1 expression enhances filopodia formation. Cells were plated at 1.3×10^4/well in 8-well chambers and cultured with 10% FBS. After 24 hours, cells were infected with oncolytic vaccinia virus (OVV)-LG (MOI=1) for 12 hours. **(A)** Mock and **(B)** infected cells were fixed and stained. Images were captured under an immunofluorescence microscope with DAPI in blue, β-actin (13E5, Cell Signaling Technology, MA, USA) in red, viral EGFP in green, and merged pictures. Scale bar: 20 μm.

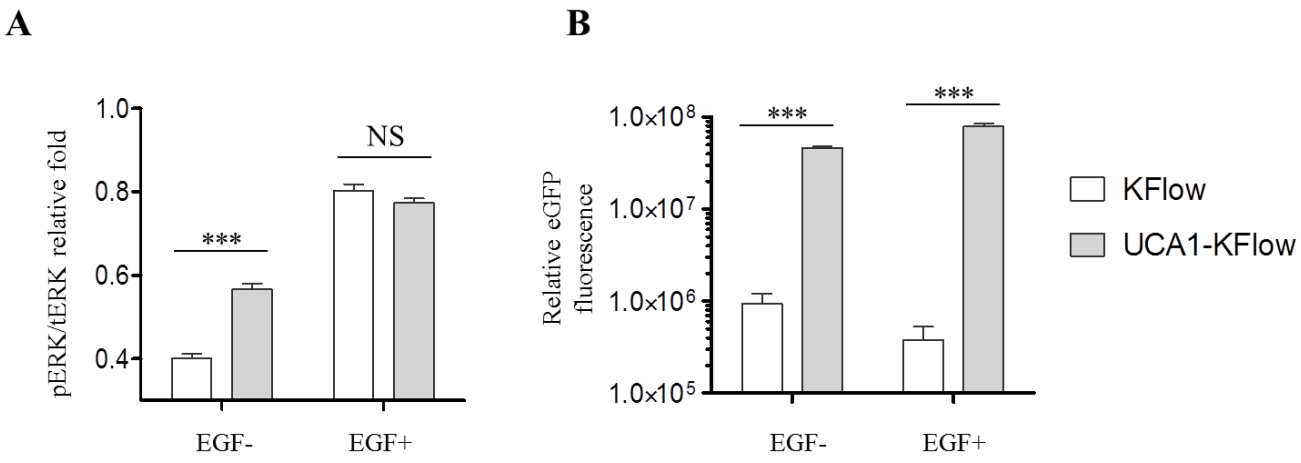


Fig. S4. Activation of ERK with high UCA1 expression had no effect on oncolytic vaccinia virus (OVV) spread. (A) Cells were plated at 6×10^3/well in 96-well plates and cultured with 10% FBS. After 36 hours, cells were treated with epidermal growth factor (EGF) for 30 minutes and tERK and pERK signals were detected using an In-Cell ELISA Kit according to the manufacturer's protocol (Thermo Fisher Scientific, Rochester, NY); n = 3. **(B)** After 30 minutes of EGF stimulation, KFlow and UCA1-KFlow cells were infected with OVV-LG (MOI = 0.01) for 72 hours. Intensity and area of viral EGFP brightness were measured using a Keyence BZ-X700 fluorescence microscope; n = 3. Data with error bars represent mean ± s.e.m. Two-way ANOVA was used for (A,B). ***$p < 0.001$.