

- 1. Mock
- 2. Reovirus dsRNA genome
- 3. Reovirus
- 4. Purified reovirus

Supplemental Fig.1

Undetectable levels of virus protein expression following transfection with the reovirus dsRNA genome. H1299 cells were seeded on a 12-well plate at a density of $5x10^4$ cells/well. On the following day, cells were transfected with the reovirus dsRNA genome at 4 ng/mL using Lipofectamine RNAiMAX, and were infected with reovirus at a multiplicity of infection (MOI) of 50. Following a 24-h incubation, cells were recovered, followed by SDS-PAGE and silver staining of cellular proteins (A) and western blotting analysis of sigma 3 protein expression in the cells (B). The arrow at the right side indicates the bands predicted for sigma 3 protein.



Supplemental Fig.2

Cell viabilities following transfection with the reovirus dsRNA genome. H1299 cells were seeded on a 96-well plate at a density of 1×10^4 cells/well. On the following day, cells were transfected with the reovirus dsRNA genome at the indicated doses using Lipofectamine RNAiMAX. Following 24-h and 48-h incubation, cell viabilities were determined by a WST-8 assay. Data are expressed as means \pm S.D. (n=4).



Supplemental Fig.3

Reovirus-mediated down-regulation of HIF-1 α in a PKR-independent manner. H1299 cells were transfected with an siRNA against PKR at 50 nM. Following a 24-h incubation, cells were infected with reovirus at an MOI of 20. Cell lysates were prepared 24 h after infection, followed by western blotting analysis. Representative images of two independent experiments are shown.



Supplemental Fig.4

dsRNA-mediated down-regulation of HIF-1 α in a VHL-independent manner. H1299 cells were transfected with an siRNA against VHL at 50 nM. Following a 24-h incubation, cells were transfected with the reovirus dsRNA genome at 4 ng/mL. Real-time RT-PCR analysis was performed 24 h after siRNA transfection. Cell lysates were prepared 24 h after transfection with reovirus dsRNA genome, followed by western blotting analysis. Data are expressed as means \pm S.D. (n=4). The representative images from at least two independent experiments are shown.





Supplemental Fig.4

dsRNA-mediated down-regulation of HIF-1 α in a RACK1-independent manner. H1299 cells were transfected with an siRNA against RACK1 at 50 nM. Following a 24-h incubation, cells were transfected with the reovirus dsRNA genome at 4 ng/mL. Real-time RT-PCR analysis was performed 24 h after siRNA transfection. Cell lysates were prepared 24 h after transfection with the reovirus dsRNA genome, followed by western blotting analysis. Data are expressed as means \pm S.D. (n=4). The representative images from at least two independent experiments are shown.

HIF-1a





β-actin



Supplemental Fig.6

Raw image of western blots for HIF-1 α and β -actin in H1299 cells used in Figure 1B.



Supplemental Fig.7 Raw image of western blots for HIF-1 α , PKR, and β -actin in H1299 cells used in Figure 1E.



Supplemental Fig.8

Raw image of western blots for HIF-1 α , PKR, phosphorylated PKR (pPKR), and β -actin in H1299 cells used in Figure 1F.



Supplemental Fig.9

Raw image of western blots for HIF-1 α , PKR, phosphorylated PKR (pPKR), and β -actin in H1299 cells used in Figure 2A.



Supplemental Fig.10

Raw image of western blots for HIF-1 α and β -actin in H1299 cells used in Figure 2C.



Supplemental Fig.11

Raw image of western blots for HIF-1 α and β -actin in H1299 cells used in Figure 3A.



Supplemental Fig.12 Raw image of western blots for HIF-1 α and β -actin in H1299 cells used in Figure 3B.





Supplemental Fig.14

Raw image of western blots for HIF-1 α and β -actin in human cultured cells used in Figure 4A.



Supplemental Fig.15

Raw image of western blots for HIF-1 α and β -actin in H1299 cells used in Figure 4B.



Supplemental Fig.16

Raw image of western blots for reovirus sigma 3 and GAPDH in H1299 cells used in Supplemental Figure 1B.



Supplemental Fig.17

Raw image of western blots for HIF-1 α , PKR, and β -actin in H1299 cells used in Supplemental Figure 3.



Supplemental Fig.18

Raw image of western blots for HIF-1 α and β -actin in H1299 cells used in Supplemental Figure 4B.



Supplemental Fig.19

Raw image of western blots for HIF-1 α , RACK1, and β -actin in H1299 cells used in Supplemental Figure 5B.