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Supplemental Information

Systemically Administered Reovirus-Induced Downregulation of Hypoxia Inducible Factor-1α in Subcutaneous Tumors Takuma Hotani, Hiroyuki Mizuguchi, and Fuminori Sakurai

Supplemental figures



Supplemental figure legends

Figure S1. Luciferase expression levels in H1299-5HRE-Luc cells under normoxic and hypoxic conditions.

H1299-5HRE-Luc cells were incubated under normoxic or hypoxic conditions for 24 hrs, then subjected to luciferase assay. The data represent the mean \pm S.D. (n = 4). The experiments were repeated at least twice.

Figure S2. Tumor growth following reovirus administration.

Reovirus (1x10⁸ PFU/mouse) or PBS was intravenously administered into H1299-5HRE-Luc tumor-bearing mice *via* the tail vain. Tumor volumes were measured following reovirus administration at the indicated time points. The data represent the mean \pm S.D. (n = 7-10). *p < 0.05 and ***p<0.001, compared with the PBS group. The experiments were repeated at least twice.

Figure S3. Pimonidazole-positive hypoxic area and reovirus capsid protein-positive area in the tumors following reovirus administration.

Tumor-bearing mice were treated as described in Figure 2. Immunohistochemical analysis using anti-pimonidazole (green), anti-reovirus capsid protein σ 3 (red) and DAPI (blue) was carried out using frozen sections of the tumor xenografts at 120 hrs following reovirus systemic administration. Scale bars, 50 µm. The experiments were repeated at least twice.

Supplemental table

S1 Table. Primer sequences

Gene symbol	Sequence (forward/reverse; 5' to 3')
SLC2A1	ATTGGCTCCGGTATCGTCAAC/GCTCAGATAGGACATCCAGGGTA
ABCB1	TTGCTGCTTACATTCAGGTTTCA/AGCCTATCTCCTGTCGCATTA
MMP2	GATACCCCTTTGACGGTAAGGA/CCTTCTCCCAAGGTCCATAGC
TWIST1	GTCCGCAGTCTTACGAGGAG/GCTTGAGGGTCTGAATCTTGCT
VEGFA	AGGGCAGAATCATCACGAAGT/AGGGTCTCGATTGGATGGCA
18S rRNA	GTAACCCGTTGAACCCCATT/CCATCCAATCGGTAGTAGCG