Table 3 | GFP expression in target cells infected with VA-deleted AdVs

AdV	Type	Ratio	
		fluorescence	expressed mRNA
AxdV-4FVF-GFP	pre-vector	1	1
AxdV-4F-GFP	VA-deleted	0.97 ± 0.15	0.81 ± 0.16
AxdV-FVF-GFP	pre-vector	1	1
AxdV-F-GFP	VA-deleted	1.14 ± 0.31	1.18 ± 0.20

HeLa cells were infected with pre-vector and VA-deleted AdV at a transduction MOI of 10. Three days later, GFP fluorescence was measured using a fluorometer and the GFP mRNA was quantified using reverse transcriptase and qPCR.

target RNA and 18S-rRNA (correction standard) were quantified using reverse-transcription and real-time PCR (Applied Biosystems Prism 7000); the ratio of the target RNA to 18S-rRNA was then calculated. The linear correlation between the amount of infected vector and the Ct values was confirmed (Supplementary Fig. S2). To quantify the DNA amount of VA RNAs and the AdV genome, the infected total cell DNA was prepared from cells using a previously described method[33,34] or a DNA preparation kit (Macherey-Nagel, through TaKaRa Bio). Quantitative PCR was performed to detect the AdV genome using a probe for the pIX gene described above[10,12]. The amount of chromosomal DNA was simultaneously measured to correct the Ct values of the viral genome per cell, and the corrected Ct was shown throughout. The probes were derived from the sequence of the human β-actin gene for HeLa and HuH7[10]. The qPCR reaction was performed according to the manufacturer's protocol: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min (Applied BioSystems).

Southern blotting analysis. HuH-7 cells in a 6-cm dish were infected with VA-deleted AdV and its pre-vector, and three days later the total DNA was prepared from the dish. Before alkaline treatment, the agarose gel was exposed to 0.1-N HCl for partial depurination, causing the DNA fragmentation of several hundred base pairs to obtain the complete transfer to the membrane[33]. The DNA was then transferred to the nylon membrane Hybond-N (Amersham GE) using the capillary-transfer method. Specific DNA was detected using a DIG DNA Labeling and Detection Kit (Roche Diagnostics). The probe DNA fragment derived from the viral genome was labeled with digoxigenin-UTP, and specific DNA was detected using the chemiluminescence of CDP-Star (Roche Diagnostics); the bands were visualized using LAS-4000 (Fuji Film).

Northern blotting analysis. The cells indicated in the figure legends were infected with pre-vector and VA-deleted AdV, respectively, at a transduction MOI of 10 copies/mL. The total RNA of the infected cells was extracted, and 30 μg per lane was electrophoresed in the agarose gel with tris-acetate-EDTA buffer. The RNA was transferred to the nylon membrane Hybond-N$^+$ (Amersham GE) using the capillary-transfer method. Specific RNA was detected using a DIGDNA Labeling and Detection Kit (Roche Diagnostics).

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Author contributions

A.M. performed the experiments and contributed to the writing of the manuscript. Z.P., M.S., H.F., Y.O. and S.K. performed the experiments. I.S. discussed the data and wrote the manuscript. Y.K. designed the strategies and performed the experiments. All the authors discussed the results and commented on the manuscript.

Additional information

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