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Supplemental Information

Intravenous Injections of a Rationally Selected Oncolytic Herpes Virus as a Potent Virotherapy for Hepatocellular Carcinoma

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Figure S1

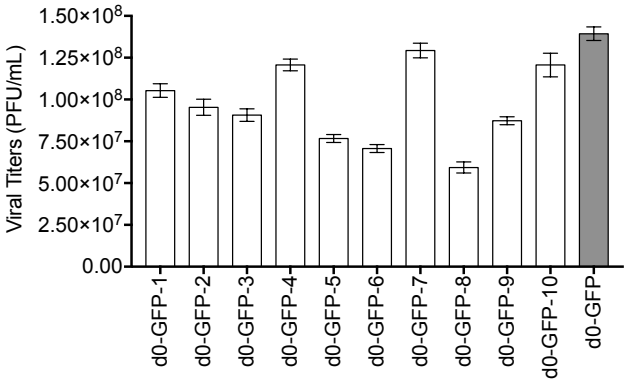

Figure S1. Assessing viral replication efficiency of fusogenic d0-GFP progenies. Viral titer was measured in various infected U-2 OS cell lines at 72 h after virus infection.

Figure S2

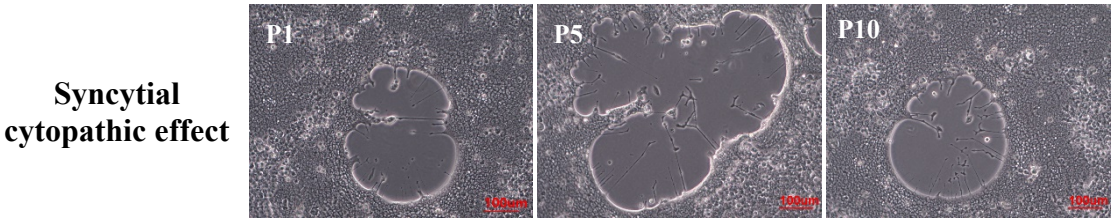

Figure S2. Syncytial cytopathic effect of Ld0-GFP progenies. Observation of Ld0-GFP plaque patterns were monitored at 48 h after virus infection (MOI=0.1 PFU/cell). P1 represents one time of passage, P5 represents five times of passage, P10 represents ten times of passage.

Figure S3

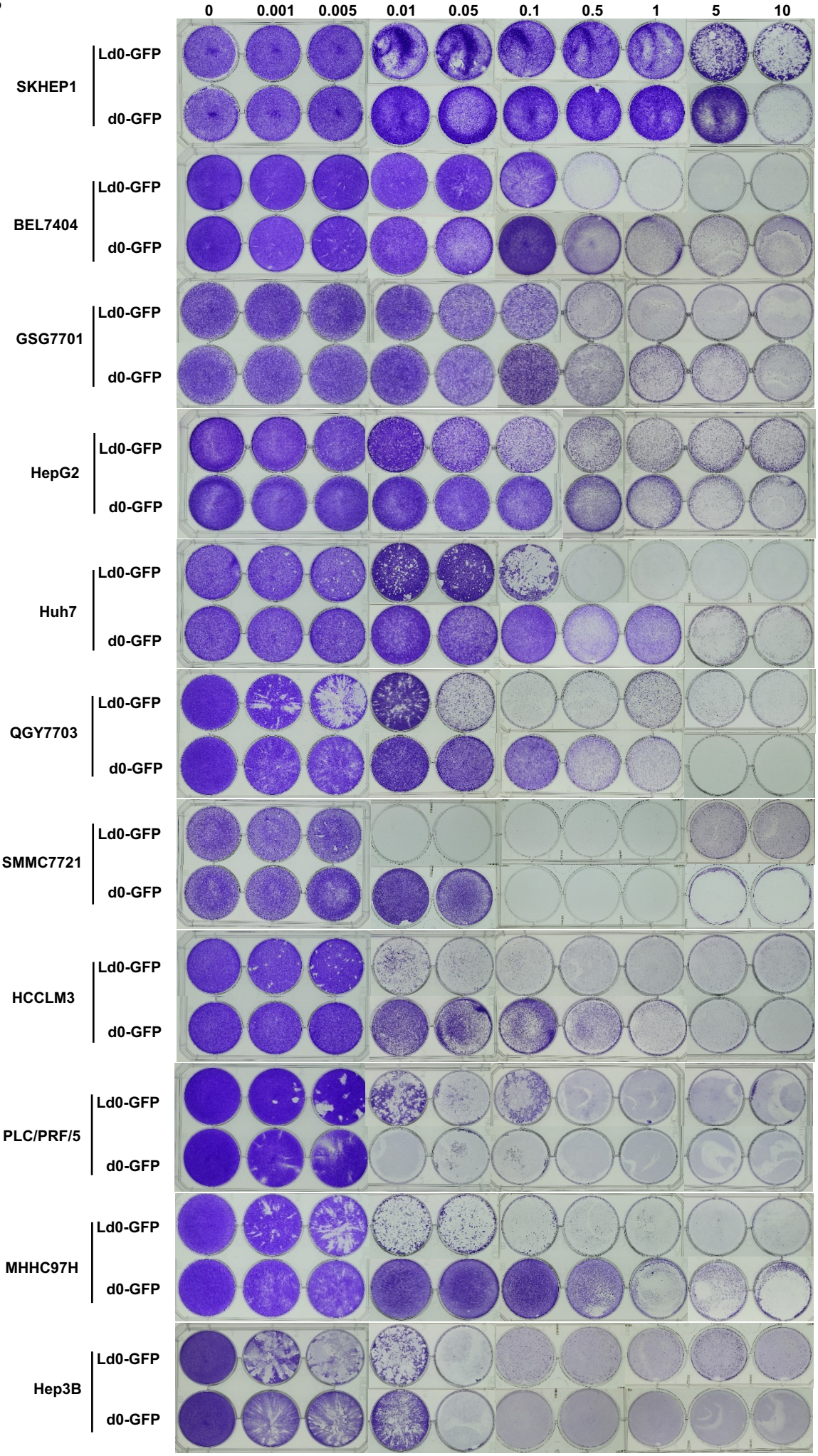
0
0.001
0.005
0.01
0.05
0.1
0.5
1
5
10
SKHEP1
Ld0-GFP
d0-GFP
BEL7404
Ld0-GFP
d0-GFP
GSG7701
Ld0-GFP
d0-GFP
HepG2
Ld0-GFP
d0-GFP
Huh7
Ld0-GFP
d0-GFP
QGY7703
Ld0-GFP
d0-GFP
SMMC7721
Ld0-GFP
d0-GFP
HCCLM3
Ld0-GFP
d0-GFP
PLC/PRF/5
Ld0-GFP
d0-GFP
MHHC97H
Ld0-GFP
d0-GFP
Hep3B
Ld0-GFP
d0-GFP

Figure S3. Oncolytic ability of Ld0-GFP and d0-GFP against different HCC cell lines. Cell viability assays were performed on a panel of HCC cell lines on 72 h post Ld0-GFP or d0-GFP infection (MOI=0.001~10 PFU/cell), respectively. Crystal violet staining was used to determine the remaining HCC cells after oncolysis.

Figure S4

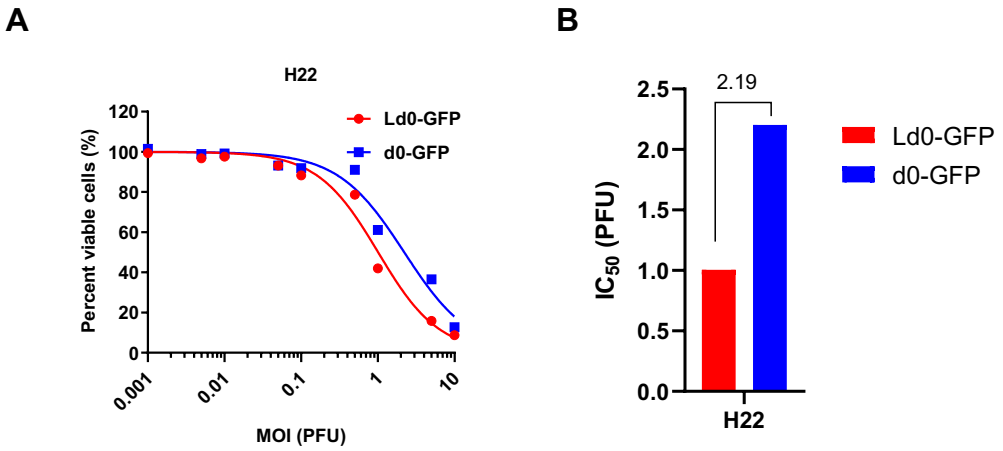

Figure S4. Oncolytic ability of Ld0-GFP and d0-GFP against murine H22 cell lines. (A) Cell viability assays were performed on murine H22 cell lines on 72 h post Ld0-GFP or d0-GFP infection (MOI=0.001~10 PFU/cell). (B) IC50 was calculated in various infected murine H22 cell lines.

Figure S5

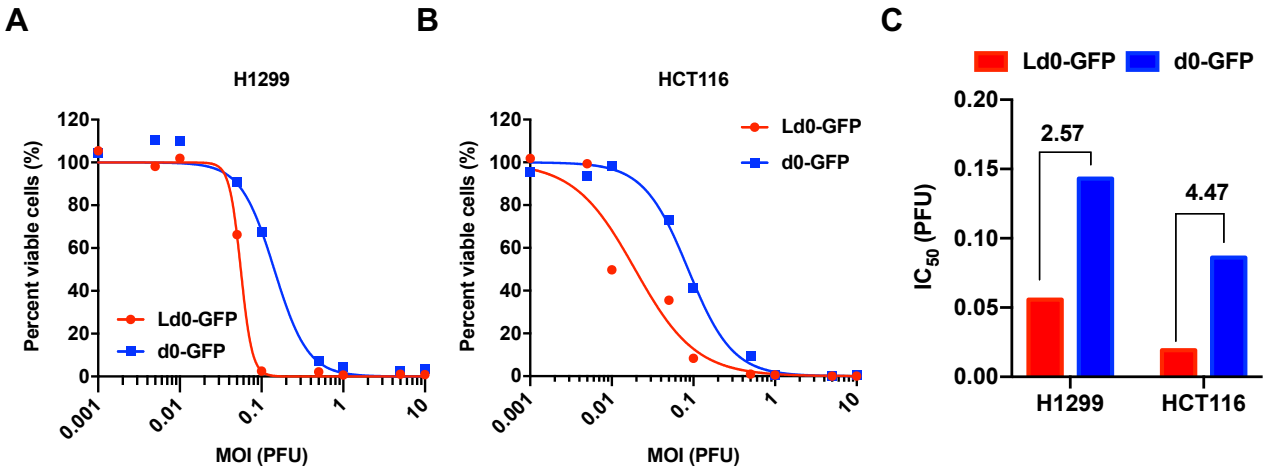

Figure S5. Oncolytic ability of Ld0-GFP and d0-GFP against non-HCC cell lines. (A) Cell viability assays were performed on the HCT116 and H1299 cell lines on 72 h post Ld0-GFP or d0-GFP infection (MOI=0.001~10 PFU/cell), respectively. (B) IC50 was calculated in various infected cancer cell lines.

Figure S6

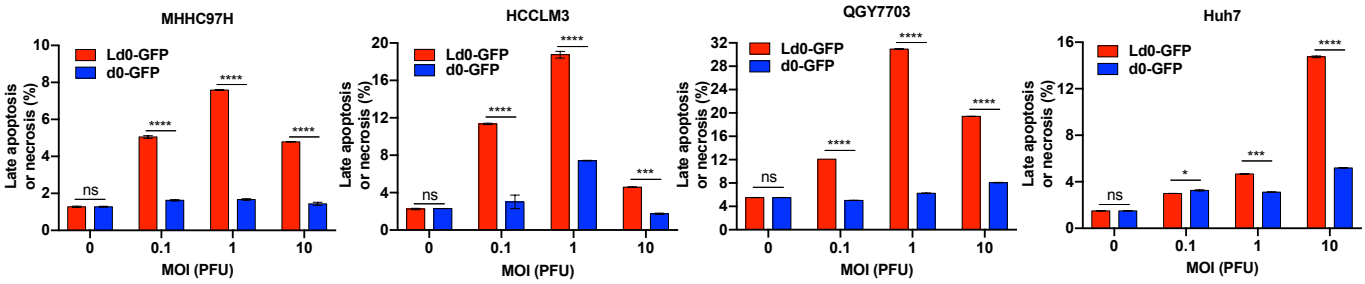

Figure S6. Ld0-GFP can induce stronger late apoptosis or necrosis in HCC cell lines. (A) Determination of levels of late apoptosis or necrosis in four HCC cell lines left uninfected or infected with Ld0-GFP or d0-GFP at MOIs of 0.1, 1 and 10 PFU/cell for 24 h by using annexin-V/PI-labeled flow cytometry.

Table S1. Amino acid sequence variation of Ld0-GFP relative to d0-GFP

ORF	Protein name	Function of gene product	Amino acid substitution	Possible impact of amino acid substitution on virus phenotype
UL9	UL9	Helicases; Required for HSV-1 DNA replication	A797P	Unknown
UL12	NUC	Alkaline nuclease; essential for viral replication.	A467T	Unknown
UL13	VPK	Virion (nuclear) protein kinase; substrates include ICP0, ICP22, etc.	D376E	Unknown
UL27	gB	Associate with membrane fusion.	E816D	Causes extensive virus-induced cell fusion.
UL53	gK	Glycoprotein required for efficient viral exocytosis; contains Syncytial-locus.	A40V	Display a reduced ability to block cell-cell fusion and endow fusogenicity.

The amino acids of all ORF in the virus genome were compared between d0-GFP and Ld0-GFP. Five genes, including UL53 (gK), UL27 (gB), UL9, UL12 (NUC) and UL13 (VPK), had amino acid changes unique to Ld0-GFP.