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Supplemental information

Intracellular delivery of oncolytic viruses with engineered Salmonella causes viral replication and cell death

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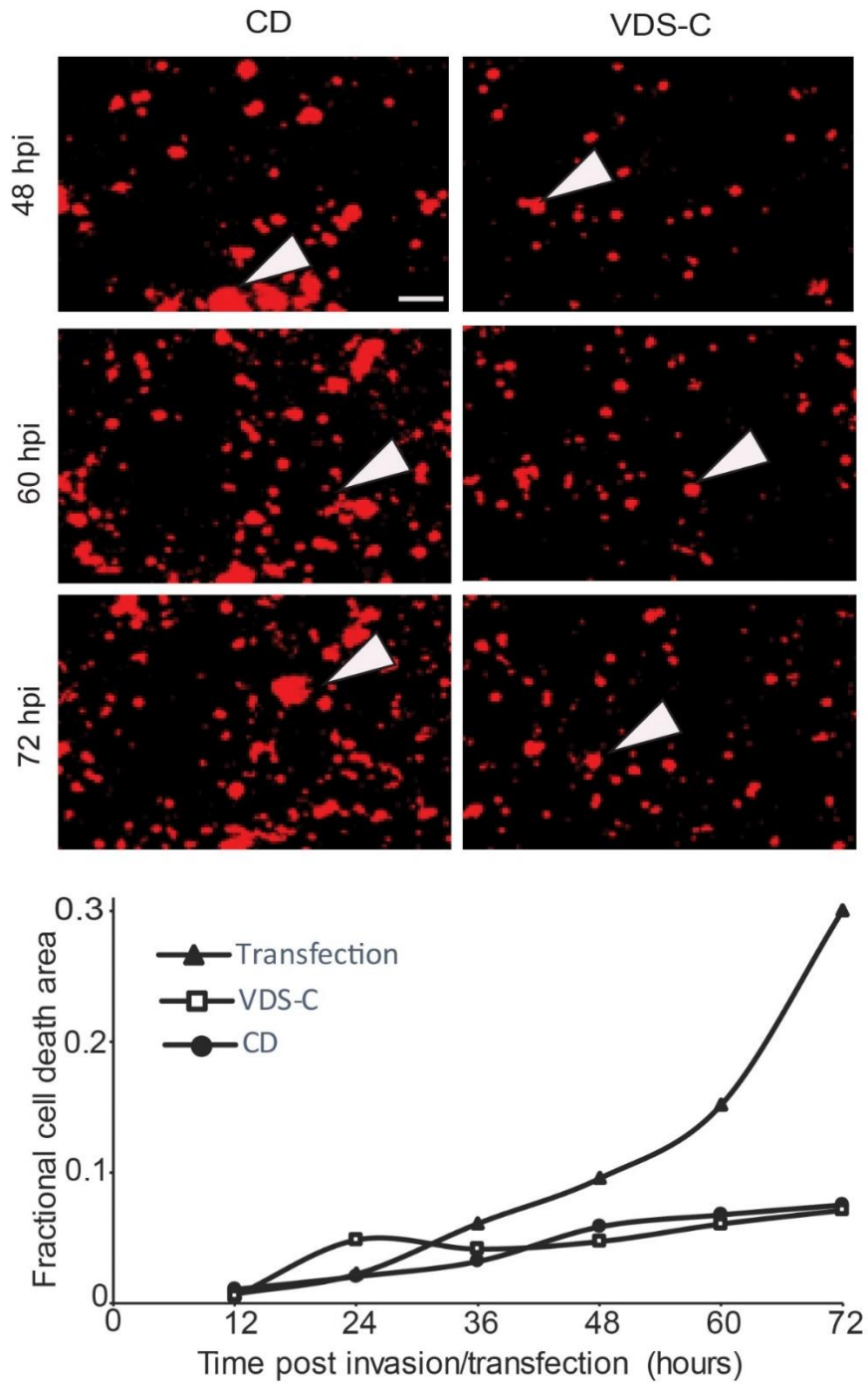
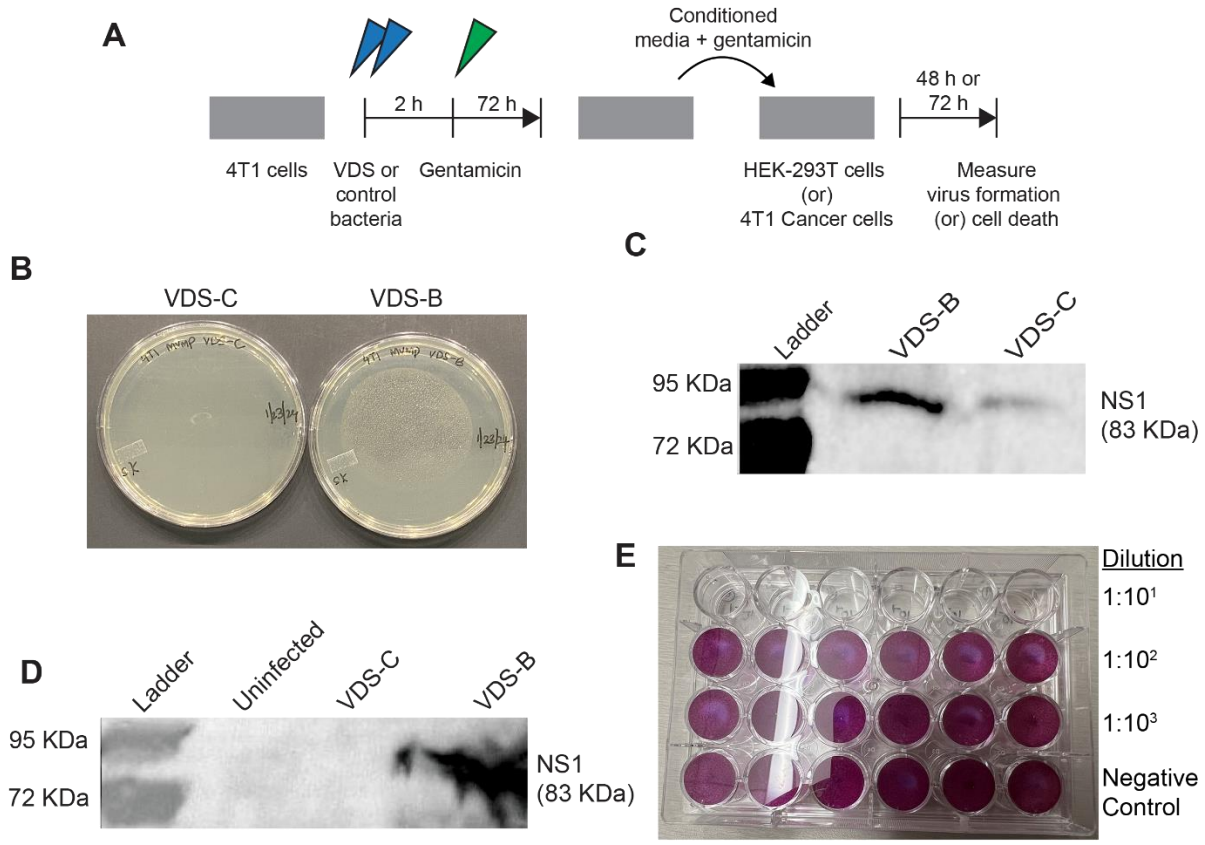
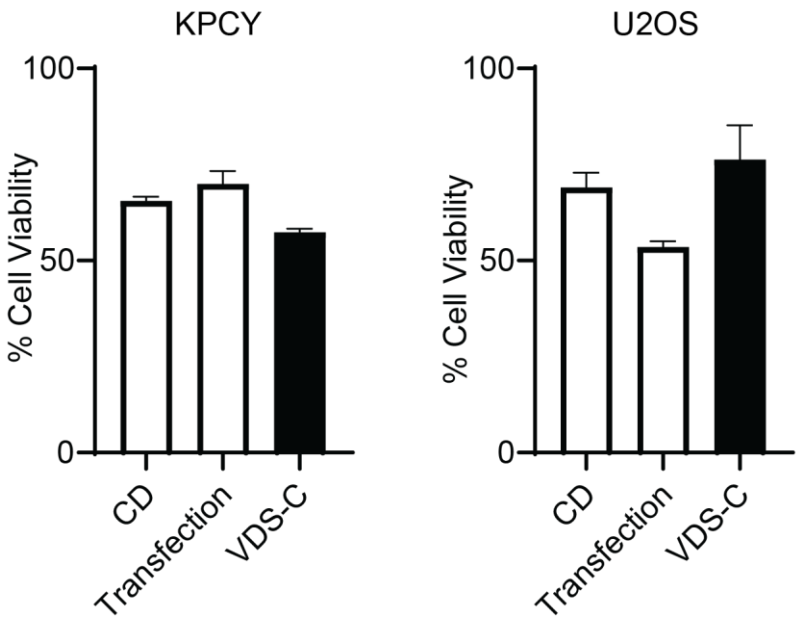
Supplemental Materials



Supplementary Figure 1: VDS-C did not cause cell death, related to Figure 6. The cancer cell death for VDS-C did not increase over time as was comparable to the cell death by the bacterial control. In comparison, cells death increased in cells transfected with the MVMp plasmid. Scale bar is 50 μm.



Supplementary Figure 2: Virus re-infection and induced cell death, related to Figure 7. Three experiments were performed to measure the infectivity of the virus particles produced after delivery by VDS. These experiments are replicates of the experiments in Figure 7B&C using alternative techniques. **A)** Each of the experiments used a similar procedure to generate viruses. Cancer cells (4T1) were co-cultured with VDS-B or VDS-C (*blue arrows*) for two hours to allow for cell invasion. Extracellular bacteria were cleared by addition (*green arrow*) of gentamicin (50 μg/ml). During the following 72 hours, the intracellular bacteria lysed, the virus plasmid was delivered, and virus particles were produced by infected cells. After this period, the culture media were collected and added to fresh media. Gentamycin (50 μg/mL) was added to prevent bacterial growth. **B)** One milliliter of conditioned media was plated on agar plates. None of the media from either bacterial strain (VDS-B or VDS-C) contained viable bacteria. **C)** After the conditioned medium from cells treated with VDS-B was diluted 40:60 with fresh media and added to HEK-293T cultures, the cells formed NS1 from MVM. Conditioned media from cells treated with VDS-C formed less NS1. **D)** In a replicate experiment, conditioned media from uninfected cells, cells administered VDS-B or VDS-C were diluted 40:60 with fresh media and applied to HEK-293T cultures. The cells that received media from VDS-B cultures produced NS1, which matches the results in Figure 7B. **E)** To measure the induction of cell death by produced virus, the conditioned media was diluted at three decreasing dilutions ($1:10^1$, $1:10^2$ and $1:10^3$) and applied to 4T1 cells. After 72 hours, the media was removed and the cells were stained with crystal violet. The produced viral particles killed all cells (*empty, clear wells*) that received ten-fold (1:10) diluted medium. Control cells and higher dilutions remained viable (*violet*). This result matches the experiment in Figure 7C.



Supplementary Figure 3: VDS-C did not induce cell death in either KPCY cell or U2OS cells, related to Figure 8. There was no significant difference in the cell viability of the cells infected by VDS-C compared to bacterial controls (strain CD) in KPCY cells or U2OS cells.

Supplemental Table S1. Primers for gene knockouts, related to Star methods.

Name	Primer Sequence	Template	Gene
SK 033	GTGACAGTTAAAAACGACAGCATACAGAGCACATTCC TCTTCCACGATTAgtcttgagcgattgtgtaggc	Pkd4	SbcB Forward
SK 034	TTACACAATCTCGGTTGCGTACTGCCACAGTGATTTCA GCAACCCCAGCTaattagccatggtccatatgaatatc	Pkd4	SbcB Reverse
SK 047	ATGCGCATCCTCCACACCTCTGACTGGCATCTGGGACA AAATTTCTACAGgtcttgagcgattgtgtaggc	Pkd4	SbcCD Forward
SK 048	TTACTCCACGGCAAACGCTTTGTCCAGTTTGCTATAAC CAAGCCCATTGAaattagccatggtccatatgaatatc	Pkd4	SbcCD Reverse
SK 029	ATGTCACTGACGCGCCTTTTAATCAAAGACTTCCGCAA CATAGAAAATGCgtcttgagcgattgtgtaggc	Pkd4	RecF Forward
SK 030	TTAATCCGTTATTTTACCCTTTTCCACGGTAAACATCTT CGAATTTTCATaattagccatggtccatatgaatatc	Pkd4	RecF Reverse
SK 031	GTCGCCGAGACTCTTGATCCTCTGCGCTTGCCCCTGAC AGGCGAGCGTTTgtcttgagcgattgtgtaggc	Pkd4	RecB Forward
SK 032	CGCCTCCTCACTCATTTCACCTGCAAACATATCATCCA GTTGGTTAATTAaattagccatggtccatatgaatatc	Pkd4	RecB Reverse
SK 043	GTGAAACAACAGAGACAACTTCGTCGGCGCGAGGCTG ATGAGACGGCGGAgtcttgagcgattgtgtaggc	Pkd4	RecJ Forward
SK 044	TCATAGCGGCCAAATATCATCGATAATAATCTGTAAA CTACGGTTGCCGCaattagccatggtccatatgaatatc	Pkd4	RecJ Reverse
SK 039	ATGGCTATCGACGAAAACAAACAGAAAGCGTTGGCGG CAGCACTGGGCCAgtcttgagcgattgtgtaggc	Pkd4	RecA Forward
SK 040	TTAAAAATCTTCGTTGGTTTCTGCAACGCCTTCGCTAT CGTCAACGGCGAaattagccatggtccatatgaatatc	Pkd4	RecA Reverse