Supporting Information

Cell-based high-throughput screening assay identifies 2', 2'-difluoro-2'-deoxycytidine Gemcitabine as potential anti-poliovirus agent

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# Equal contribution

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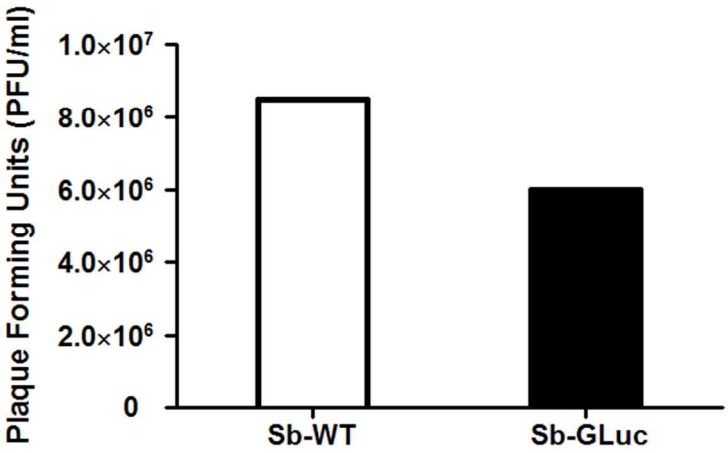
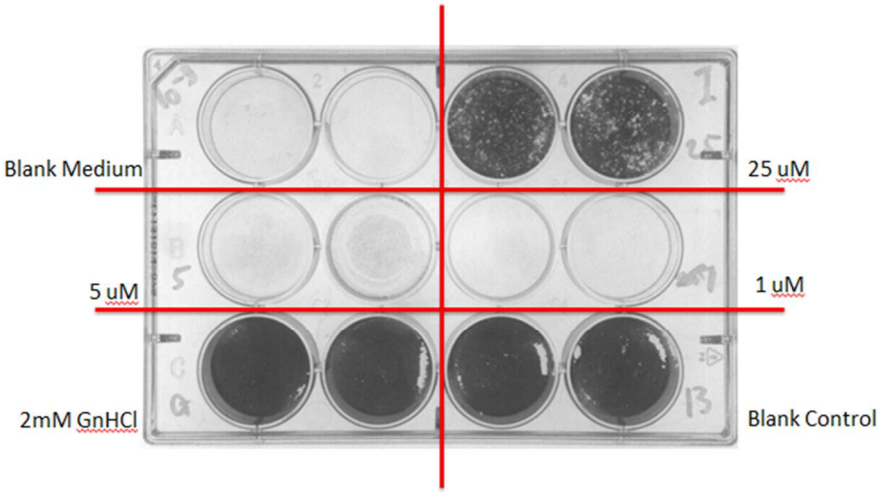


Figure S1. Growth pattern of Sb-GLuc and Sb-WT

HeLa cells were infected by Luciferase construct (Sb-GLuc) or wild type Sabin strain (Sb-WT).

Plaque assays were performed after 3-day incubation.

a.



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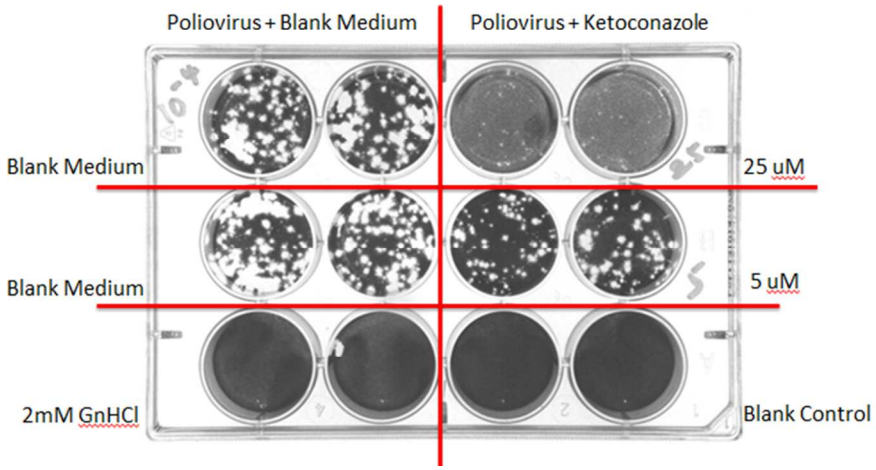


Figure S2. Itraconazole and Ketoconazole confer partial protective effects on HeLa cells against WT-PV infection.

a) 10^6 HeLa cells were infected by 2×10^5 PFU WT-PV (M.O.I. = 0.2) and treated with 0, 5 or 25μM of Itraconazole.

b) 10^6 HeLa cells were infected by 2×10^4 PFU WT-PV (M.O.I. = 0.02) and treated with 0, 5 or 25μM of Ketoconazole.

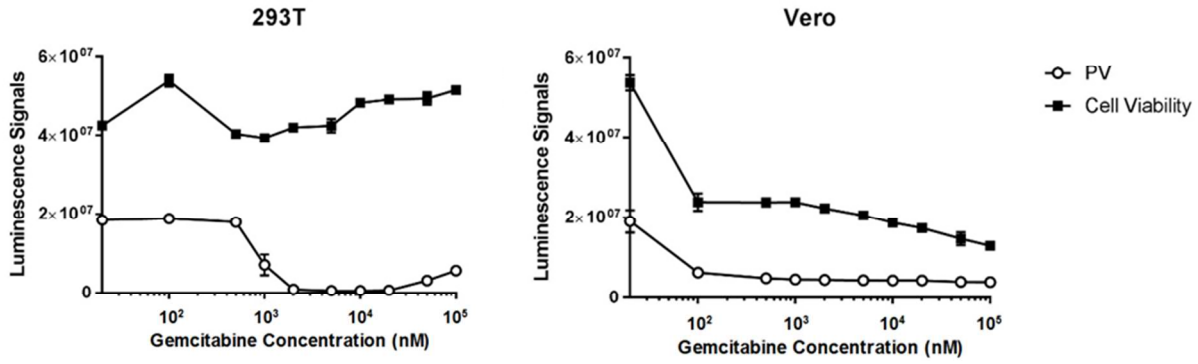
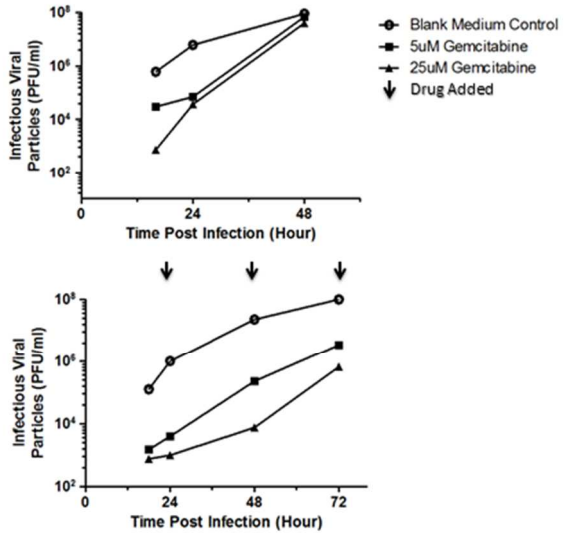


Figure S3. Inhibition of PV replication and cell viability on 293T and Vero cells. 293T cells and Vero cells were treated in the same condition as HeLa cell. Gemcitabine inhibited PV viral replication without cytotoxic effect in 293T cells. Vero cells are sensitive to Gemcitabine treatment and the inhibitory effect on PV replication is likely due to the cytotoxic effect in the cells.

a.



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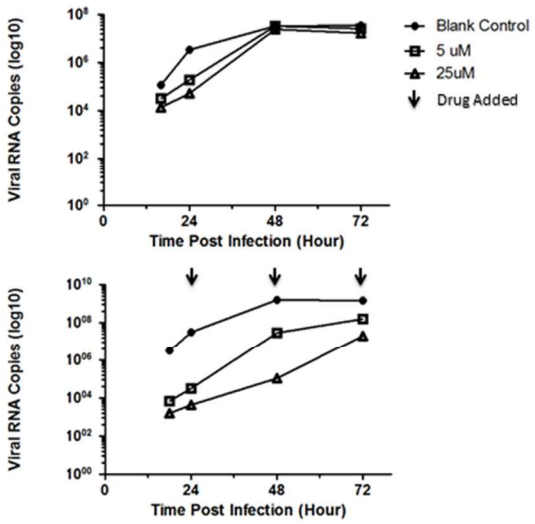


Figure S4. Daily dosage of Gemcitabine confers inhibitory effect against PV

a) PFUs of WT-PV treated by Gemcitabine. WT-PV 10^6 HeLa cells were infected by 2×10^4 PFU WT-PV (M.O.I. = 0.02) and treated with 0, 5 or 25μM of Gemcitabine. The drug was delivered either at the beginning of the incubation (upper) or every 24-hour (down). The arrows indicate the administration of additional dosages. Virus were harvested after 16, 24, 48 and 72-hour incubation, and the PFU were determined by plaque assays.

b) Viral RNA copy numbers of WT-PV treated by Gemcitabin. WT-PV 10^6 HeLa cells were infected by 2×10^4 PFU WT-PV (M.O.I. = 0.02) and treated with 0, 5 or 25μM of Gemcitabine. The drug was delivered either at the beginning of the incubation (upper) or every 24-hour (down). The arrows indicate the administration of additional dosages. Virus were harvested after 16, 24, 48 and 72-hour incubation, and the viral RNA copy numbers were determined by aRT-PCR

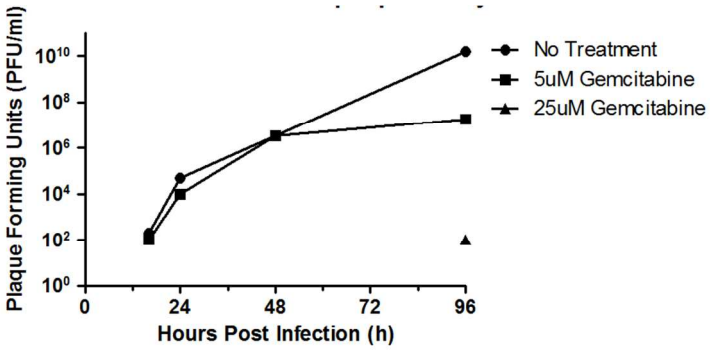


Figure S5. 25μM Gemcitabine completely inhibits WT-PV replication in HeLa cells

4×10^5 HeLa cells were infected by 100 PFU WT-PV, and 0, 5 or 25μM of Gemcitabine was added every 24 hours during the incubation. Virus was harvested after 16, 24, 48 and 72-hour incubation, and the PFU were determined by plaque assays.

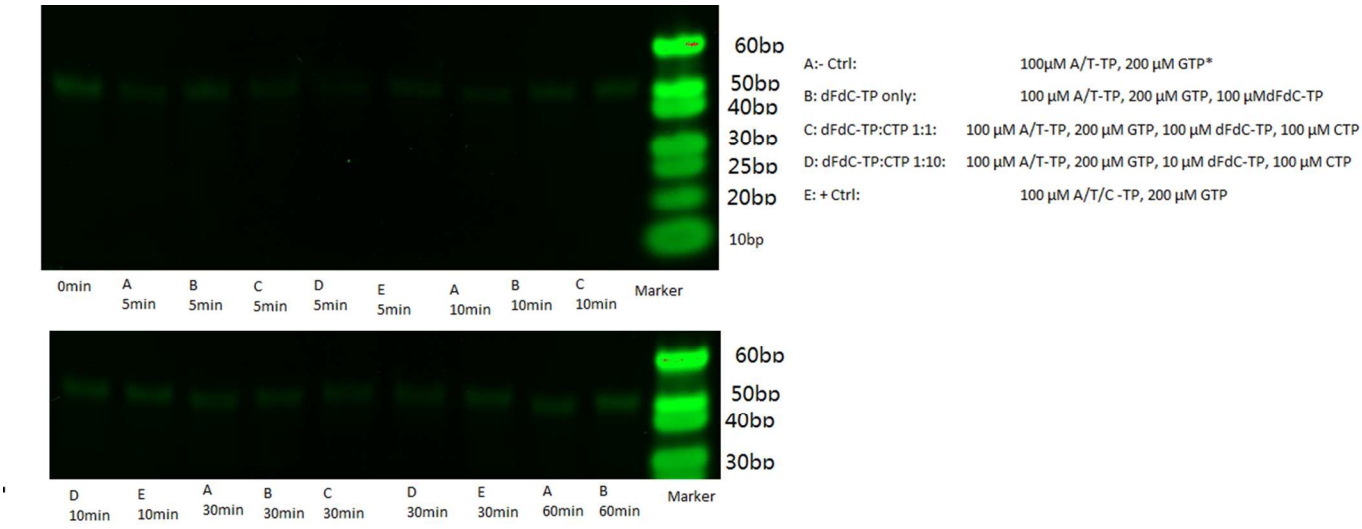
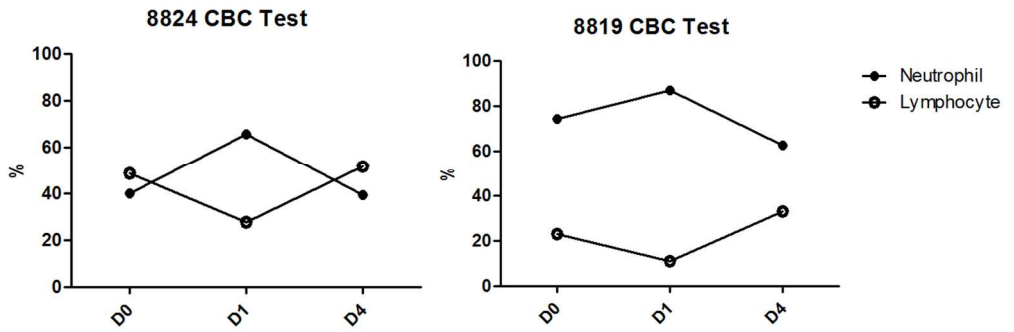


Figure S6. In vitro DNA elongation using human DNA polymerase α. The assay was performed following the method described in Clarke, et al. *BMC* 2015. Recombined human DNA polymerase α (ProFoldin) were incubated with different concentration of dFdC-TP and the products were collected at 5, 10, 30min.

a.



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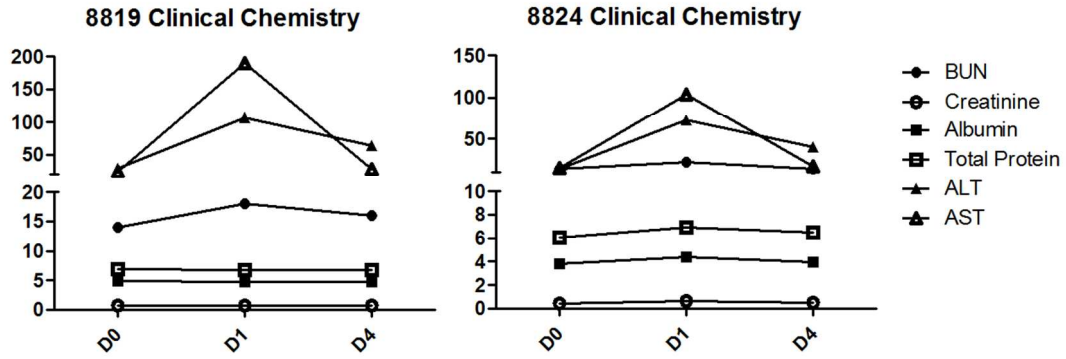

Figure S7, Gemcitabine treatment confers subtle and reversible changes in immune cell counts or liver/kidney functions. a) Clinical lab and b) Biochemistry test results of healthy rhesus macaques that received 24mg/kg Gemcitabine treatment. Blood were obtained before Gemcitabine administration, 1-day and 4-day post Gemcitabine administration.

Table S1. Other forms of nucleoside analogs do not remarkably inhibit PV replication

Index	Ec	Vc	Compound Name	Mechanism of Action
01A03	62.71%	47.54%	Azaguanine-8	Purine antimetabolite
01C17	0.00%	93.78%	Idoxuridine	deoxyuridine analog
01O08	0.00%	102.77%	Didanosine	Adenosine analog
02C16	0.00%	99.37%	Lamivudine	Cytidine analog
02N12	43.97%	92.40%	Cytarabine	Cytidine analog
03F06	0.00%	97.53%	Abacavir Sulfate	Guanosine analog
03N22	36.25%	91.76%	Stavudine	Thymidine analog
04C14	0.00%	86.80%	Trifluridine	deoxyuridine analog
04F15	24.46%	85.79%	Fludarabine	Purine analog
04G07	60.28%	94.58%	Ribavirin	Guanosine analog