Supporting Information

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SI Methods

Viruses. Doubled deleted *vaccinia virus* expressing GFP (28) was propagated in Vero cells, while *Semliki Forest Virus* expressing GFP was grown and titered using baby hamster kidney cells. Viral titer were determined by standard plaque assay in Vero or U2OS cells.

***Primary ex-Vivo* Prostate Cancer Cell Cultures.** Radical prostatectomy specimens were obtained from the Jewish General Hospital (McGill University) from untreated, consenting patients diagnosed with prostate cancer, with Institutional Review Board approval. Both tumor areas and benign areas were sectioned under pathologic direction (Dr. T. Bismar) and confirmed by the adjacent surrounding histology. Specimens were washed immediately in cold, sterile PBS. After removing excess, damaged epithelium and stromal tissue, specimens were cut into small pieces and incubated for 10 min at 37 °C in 0.05% trypsin/0.53 mM EDTA (Wisent). Surface epithelium was mechanically separated to dissociate cells into a single cell suspension. Prostate cells were collected after centrifugation and resuspended in mammary epithelial growth medium (MEGM) (Clonetics). Cells were seeded on plastic dishes (Falcon) and media changed every 48 h to remove non-adherent cells (fibroblasts). The population isolated consisted on epithelial cells. After 1 week, MEGM medium was replaced with KSF medium supplemented with 5 mg/100 ml of bovine pituitary extract (BPE) (Gibco/BRL).

Titration of *VSV* from Whole Tissue Specimens. Primary tissue specimens were obtained from consenting patients who underwent tumor resection. All tissue specimens were processed within 48 h postsurgical excision. Samples were manually divided using a 15 mm scalpel blade into equal portions under sterile techniques. After the indicated treatment condition, samples were weighed and homogenized in 1 ml of PBS using a homogenizer (Kinematica AG-PCU-11). Serial dilutions of tissue preparations were prepared in serum free media and viral titers were quantified by standard plaque assay.

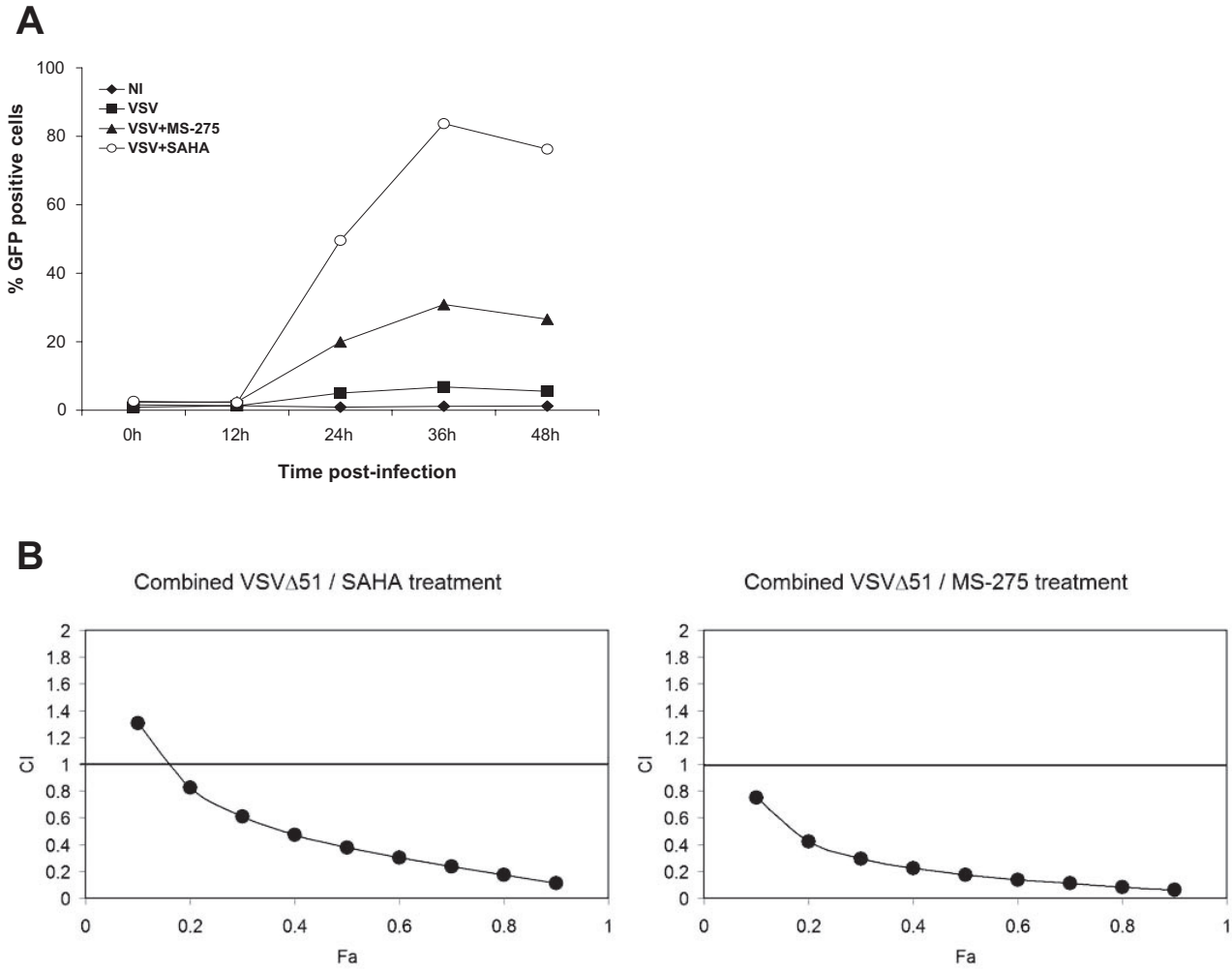
IFN ELISA. IFNα levels were measured using a Human IFN ELISA kit (PBL Biomedical). PC3 cells were treated or not with MS-275 (2 μM) or SAHA (5 μM) for 24 h and then infected with *VSV*-Δ51-GFP at 0.1 MOI. Culture medium (100 μl) was collected at different times postinfection and processed as per manufacturer's instructions. Samples were read on a DYNEX plate reader at primary wavelength of 450 nm (with a reference wavelength of 630 nm).

Western Blotting. Cells were lysed in standard Nonidet P-40 lysis buffer (50 mM Tris, pH7.4, 150 mM NaCl, 2 mM EDTA, 10% glycerol, 30 mM β-glycerophosphate, 1% Nonidet P-40 substitute (IGEPAL), 50 mM NaF, 0.1 M $NaVO_4$, 0.1 M PMSF, 0.001% protease inhibitor mixture (Sigma–Aldrich). Whole-cell extracts (50 μg) were run on a SDS-polyacrylamide gel and blotted with the following antibodies as indicated: IRF-7 (sc-9083; Santa Cruz), IRF-3 (sc-9082; Santa Cruz), ISG56 (a gift from Dr. Ganes Sen, Cleveland Clinic, Department of Molecular Genetics, Cleveland, OH), IKKε (BD Biosciences) (35), RIG-I (in-house) (35), *VSV* (Polyclonal antiserum to *VSV*) (3), cleaved caspase-3, cleaved caspase 9, caspase 8, acetylated histone 3 (Ac-H3), total H3 (all from Cell Signaling Inc.), and Actin (sc-8432; Santa Cruz).

Reverse Transcription and PCR. Total RNA from treated or control PC3 cells was isolated as per manufacturer's instruction (RNeasy; Qiagen). RNA (400 ng) was reverse transcribed with Oligo dT primers and SuperScript II reverse-transcriptase (Invitrogen). Five percent of RT reaction was used as template for PCR using Taq polymerase (Amersham, GE Healthcare), amplification was carried for 25–30 cycles. Primers used were as follows: IFN-α forward primer 5′-TTGCTCTCCTGTTGTGCTTC-3′ and reverse primer 5′-GGCCTTCAGGTAATGCAGAA-3′; IRF7 forward primer 5′-CTTCGTGATGCTGCGAGATA-3′ and reverse primer 5′-AAGCCCTTCTTGTCCCTCTC-3′; MxA 5′-CTGATCTCCAGGGTGATTAGCTCAT-3′ and reverse primer 5′-ATGGTTGTTTCCGAAGTGGACA-3′ and GAPDH forward primer 5′- AATCCCATCACCATCTTCCA-3′ and reverse primer 5′-TGAGTCCTTCCACGATACCA-3′.

IVIS Imaging. Mice were injected with D-luciferin (Molecular Imaging Products Company) (200 ml i.p. at 10 mg/ml in PBS) for Firefly luciferase imaging. Mice were anesthesized under 3% isofluorane (Baxter Corp.) and imaged with the In Vivo Imaging System 200 Series (Xenogen Corporation). Data acquistion and analysis was performed using Living Image v2.5 software. For each experiment, images were captured under identical exposure, aperture, and pixel binning settings, and bioluminescence is plotted on identical color scales.

Immunohistochemistry (IHC). Paraformaldehyde-fixed, 4 μm tissue sections were used for hematoxylin and eosin (H&E) staining or immunochemistry (IHC). IHC was performed using a Vecastain ABC kit for rabbit primary antibodies (Vector Labs), according to manufacturer's instructions. Primary antibodies used were rabbit polyclonal antibodies against *VSV* [gift from E. Brown (University of Ottawa, Ottawa, ON, Canada)], active caspase 3 ((BD PharMingen) and acetylated histone H3 (Upstate). For assessment of cell morphology, sections were stained with hematoxylin and eosin according to standard protocols. Whole tumor images were obtained with an Epson Perfection 2450 Photo Scanner while magnifications were captured using a Xeiss Axiophot HBO 50 microscope.



Drug : Virus (ratio)	CI Values at ED50	ED75	ED90
VSV:SAHA	**0.37835**	**0.20614**	**0.11331**
(31250 PFU:1uM)	+++	++++	++++
VSV:MS-275	**0.17471**	**0.09734**	**0.06088**
(25000 PFU:1uM)	++++	+++++	+++++

Range of CI	Symbol	Description
<0.1	+++++	Very strong synergism
0.1-0.3	++++	Strong synergism
0.3-0.7	+++	Synergism
0.7-0.85	++	Moderate synergism
0.85-0.90	+	Slight synergism
0.90-1.10	±	Nearly additive
1.10-1.20	–	Slight antagonism
1.20-1.45	– –	Moderate antagonism
1.45-3.3	– – –	Antagonism
3.3-10	– – – –	Strong antagonism
>10	– – – – –	Very strong antagonism

Fig. S1. *VSV*Δ51-GFP exhibits synergic *in vitro* cytotoxicity with two HDAC inhibitors. (*A*) Percentage of GFP-expressing cells by FACS analysis after infection with *VSV*-Δ51-GFP at 0.1 MOI. (*B*) PC3 cells were treated with serial dilutions of a fixed ratio combination mixture of *VSV* and either MS-275 or SAHA (5×10^5 PFU to 1 μM) in 96-well plates. Cytotoxicity was assessed using MTS reagent after 60 h. DMSO-treated cells were used as control. Combination indices (CI) were calculated according to the method of Chou and Talalay. Plots represent the algebraic estimate of the CI in function of the fraction of cells affected (Fa).

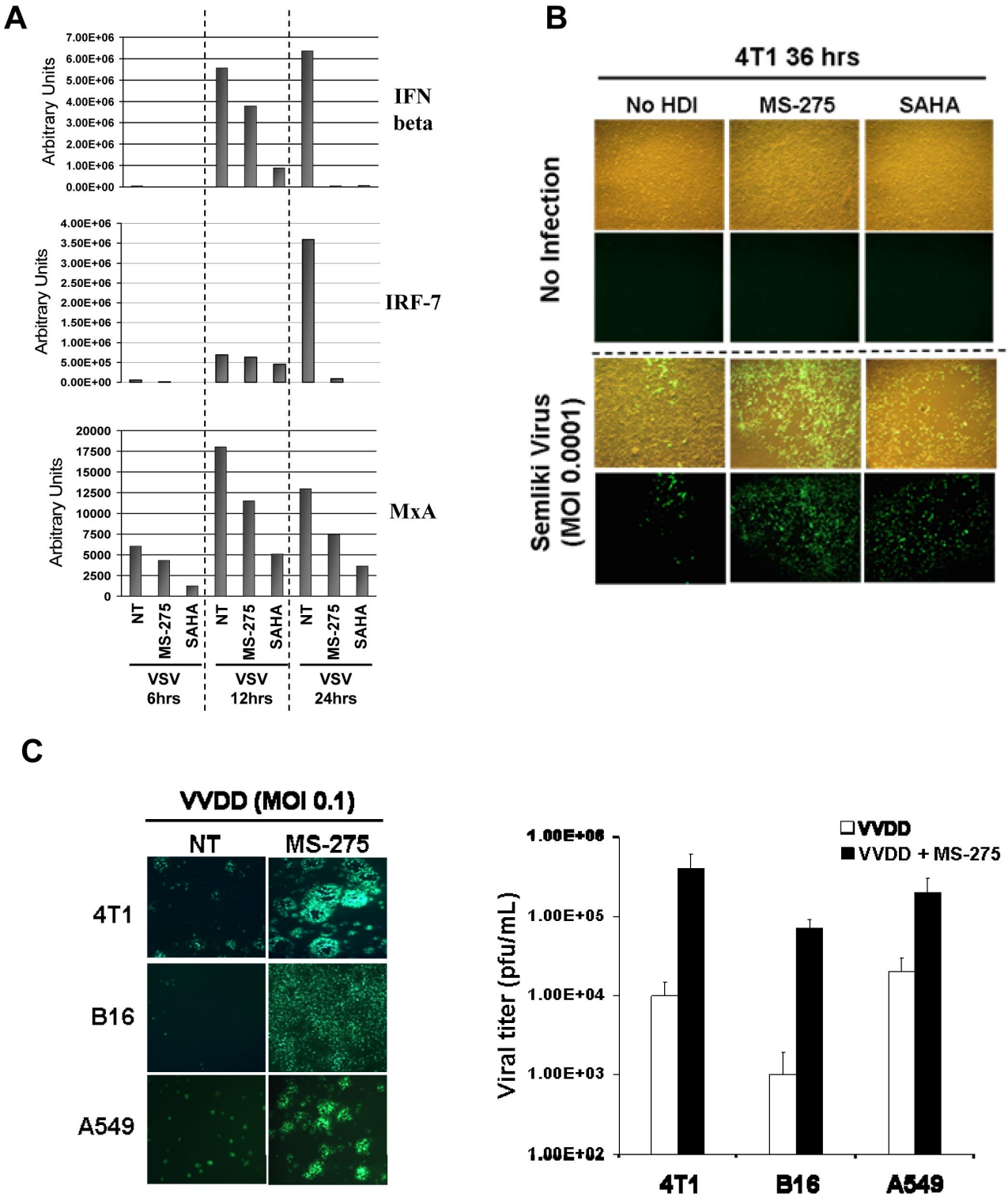


Fig. S2. (*A*) Quantification of the PCR fragments amplified by PCR in Fig. 3*B* was obtained using ImageQuant software after scanning the DNA gel in a Typhoon 9400 PhosphoImager (Amersham Pharmacia). All values were normalized to GAPDH. (*B*) and (*C*) HDIs enhance the oncolytic effects of *vaccinia* (VVDD) and *Semliki Forest* (SFV). (*B*) 4T1 cell were inoculated with SFV at MOI 10^{-4} together with either MS-275 (4 μM) or SAHA (4 μM). Micrographs were obtained at 36 h after virus inoculation. (*C*) Fluorescent micrographs obtained 72 h after the inoculation of 4T1, B16, and A549 cancer cells with VVDD-GFP at MOI 0.01. MS-275 (4 μM) was added at the time of viral inoculation and left in the media for 72 h, after which supernatants were collected and titered on U2OS cells.

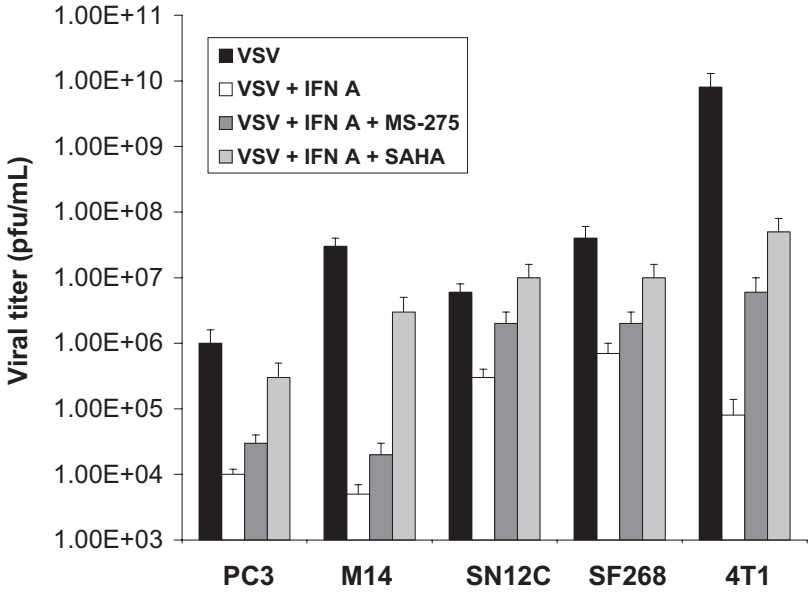


Fig. S3. HDIs can reverse the inhibitory effect of IFNα and restore *VSV* replication. Cell lines were treated with IFNα 24 h before *VSV* infection and viral titers in supernatants were determined 24 h later by standard plaque assay. Addition of HDIs simultaneously with IFNα reversed the effect of IFN and restored *VSV* replication.

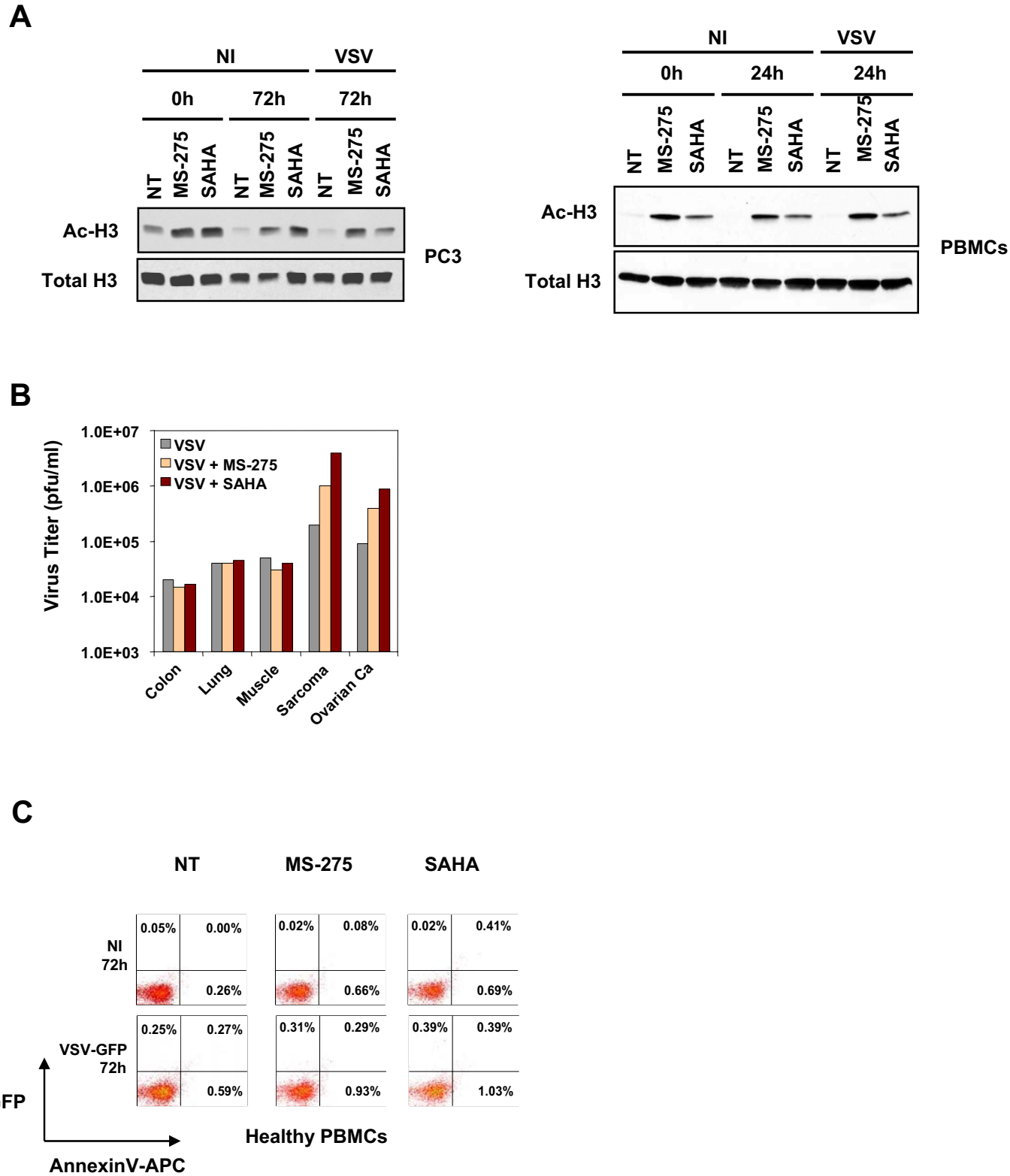


Fig. S4. (*A*) MS-275 or SAHA treatment inhibits histone deacetylases. PC3 cells or normal PBMCs were treated with MS-275 (2 μM) or SAHA (5 μM) for the specified times, in the presence or absence of *VSV*-Δ51-GFP infection. Following acid extraction of histones, hyperacetylation was assessed by immunoblot using an antibody against total or acetylated histone H3. (*B*) Viral titers determined by standard plaque assay after infection of tumor samples with *VSV*, as described for Fig. 4 (*C*) HDI treatment does not enhance *VSV*-Δ51-GFP replication and oncolysis in healthy PBMCs, as demonstrated by FACS analysis.

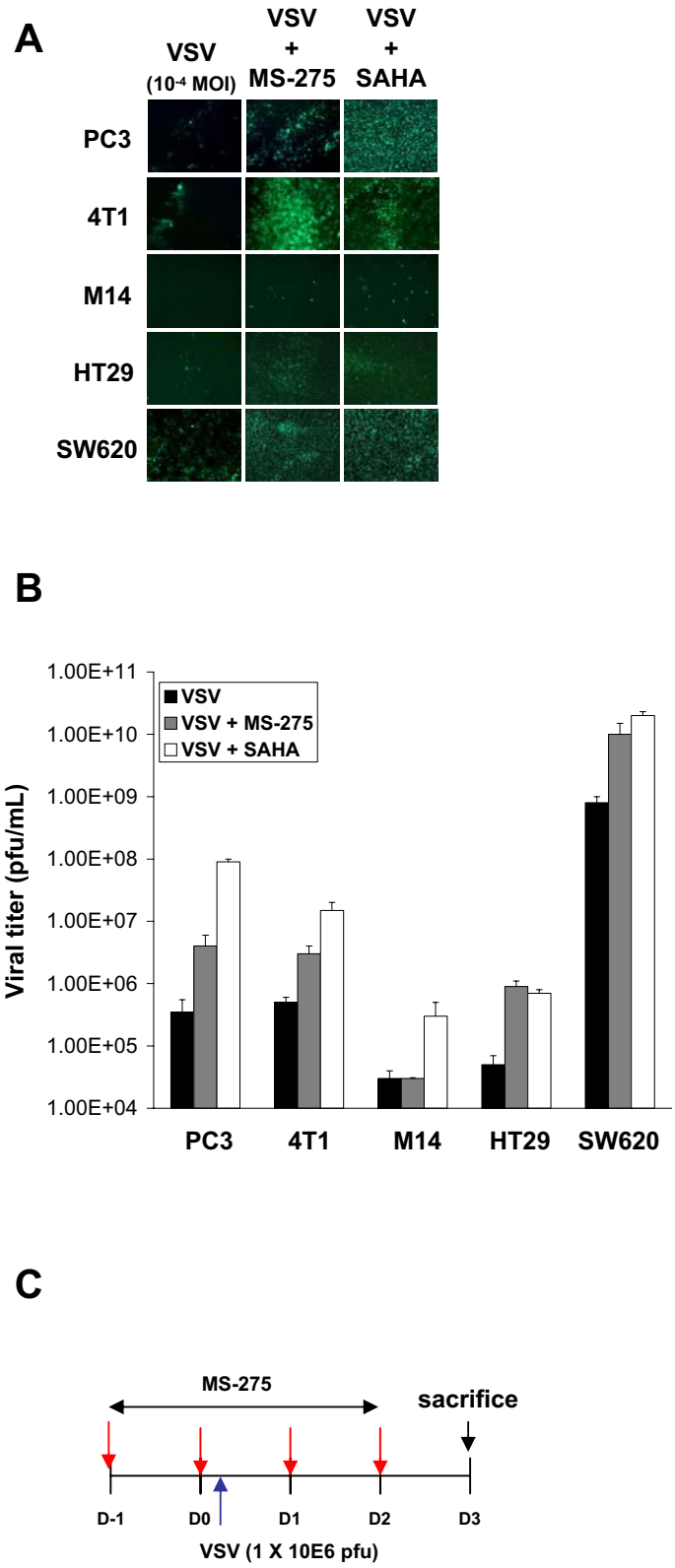


Fig. S5. Enhancement of *VSV* viral production by HDIs in partially resistant cell lines. (*A*) Various partially resistant cell lines were infected with *VSV*-Δ51-GFP (0.0001 MOI) with or without HDI pretreatment (MS-275 or SAHA at 4 μM). Viral replication was assessed 36h later by fluorescence microscopy for GFP expression. (*B*) Supernatants from the various treatment conditions were collected 24 h following viral infection and titered on Vero cells to quantify *VSV* yield. (*C*) Treatment schedule for tumor models shown in Fig. 5.

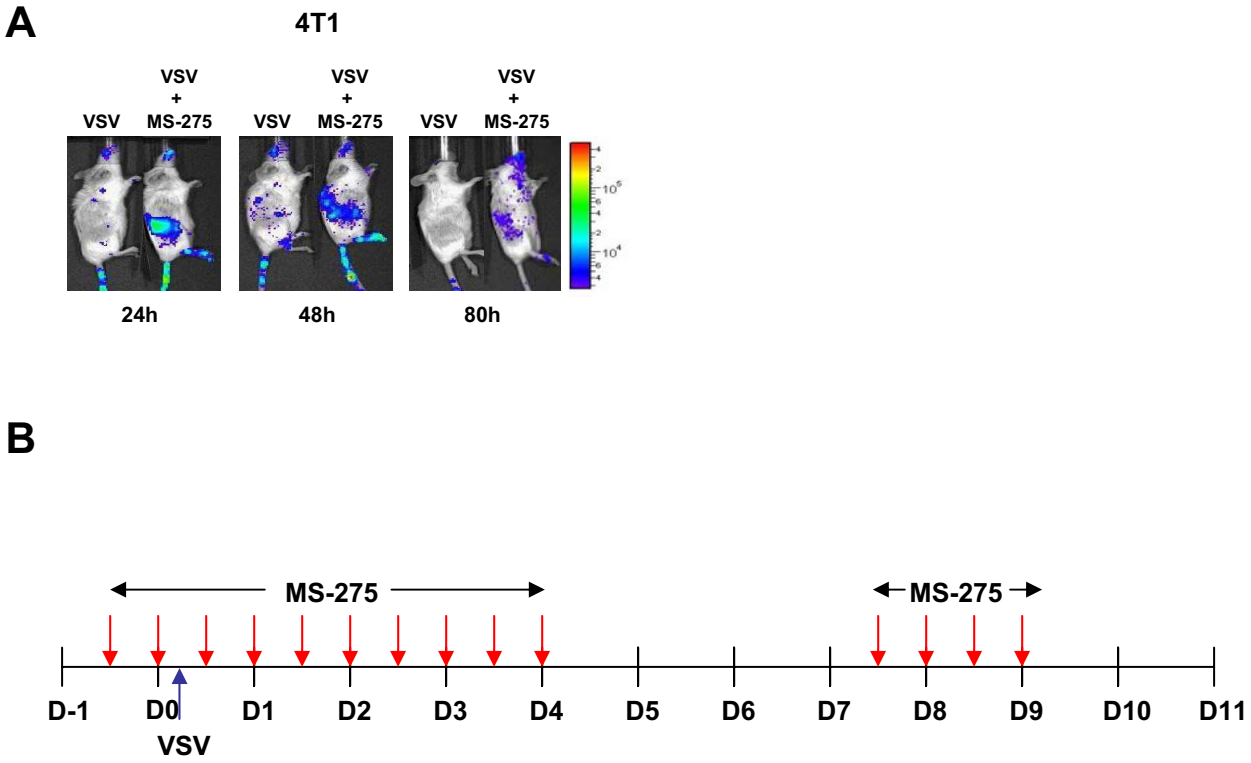


Fig. S6. (*A*) Viral replication in 4T1 immunocompetent model visualized by IVIS imaging. (*B*) Treatment schedule for SW620 tumor model (see Fig. 6).