SUPPLEMENTAL FIGURES

Figure 1. Human parainfluenza virus type 3 (HPIV3) infectivity in RWPE-1 (RWPE) and PC-3 cells. a. HPIV3 infection measured by plaque assay at 40h post-infection. b. MTT cell viability assay of cells infected with HPIV3 for 48h. MTT assay values are mean ± standard deviation of 6 wells and triplicate experiments. Uninfected (-) cells indicate 100% cell viability. Plaque assay values expressed as pfu/ml represent mean ± standard deviation for three independent determinations. Standard deviations are shown by the error bars.
RESULT: HPIV3 does not possess oncolytic activity.

Figure 2. Prostate tumor growth in the dorsal flank of nude mice following RSV administration. Effect of intra-tumorally administered RSV on tumor growth in mice harboring the tumor xenograft in the dorsal flank. Tumors were allowed to develop first. At day 15 RSV injection was initiated. Injection was given every 2 days for the period shown in the plot. Each treatment group consisted of five animals (n=5) and the data represent the mean and standard deviation of each group. The complete experiment has been repeated twice with similar results.
RESULT: Regression of prostate tumor growth in the dorsal flank of mice following RSV injection.

Figure 3. Measurement of TNF-α (TNF) concentration in culture supernatants of RSV infected PC-3 cells. Medium supernatants collected from PC-3 cells infected with RSV (0h-12h) were subjected to ELISA analysis with human-TNF specific ELISA kit (BD Biosciences). Amount of TNF deduced from the ELISA assay was expressed as pg/ml and each value represents the mean ± standard deviation for three determinations.
RESULT: RSV infection of PC-3 cells results in secretion of TNF-α.

Figure 4. NF-κB inhibitory property of SN50 peptide. a. NF-κB specific EMSA of nuclear extracts prepared from mock infected and RSV infected (at 12h post-infection) PC-3 cells in the presence of either SN50 peptide or control SN50 peptide. The same amount of nuclear extracts used for the EMSA assay was blotted with HDAC-2 antibody. RWPE (b) or PC-3 (c) cells incubated with either cell permeable NF-κB inhibitory peptide SN50 (30 μM) (Calbiochem) or cell permeable control SN50M peptide (50 μM) (Calbiochem) were treated with TNF-α (TNF) (30 ng/ml) for 3h. Nuclear extracts prepared from these cells were subjected to Western blot analysis with antibody against NF-κB p65 subunit (Santa Cruz Biotechnology). Loading efficiency is shown by the uniform presence of a non-specific (NS) band in all lanes.
RESULT: NF-κB inhibitory SN50 peptide is active, since it inhibited nuclear translocation of NF-κB following treatment of cells with TNF-α. In contrast, the control SN50M peptide did not inhibit NF-κB nuclear translocation. Similarly, SN50 also appreciably reduced the levels of activated NF-κB in RSV infected PC-3 cells.

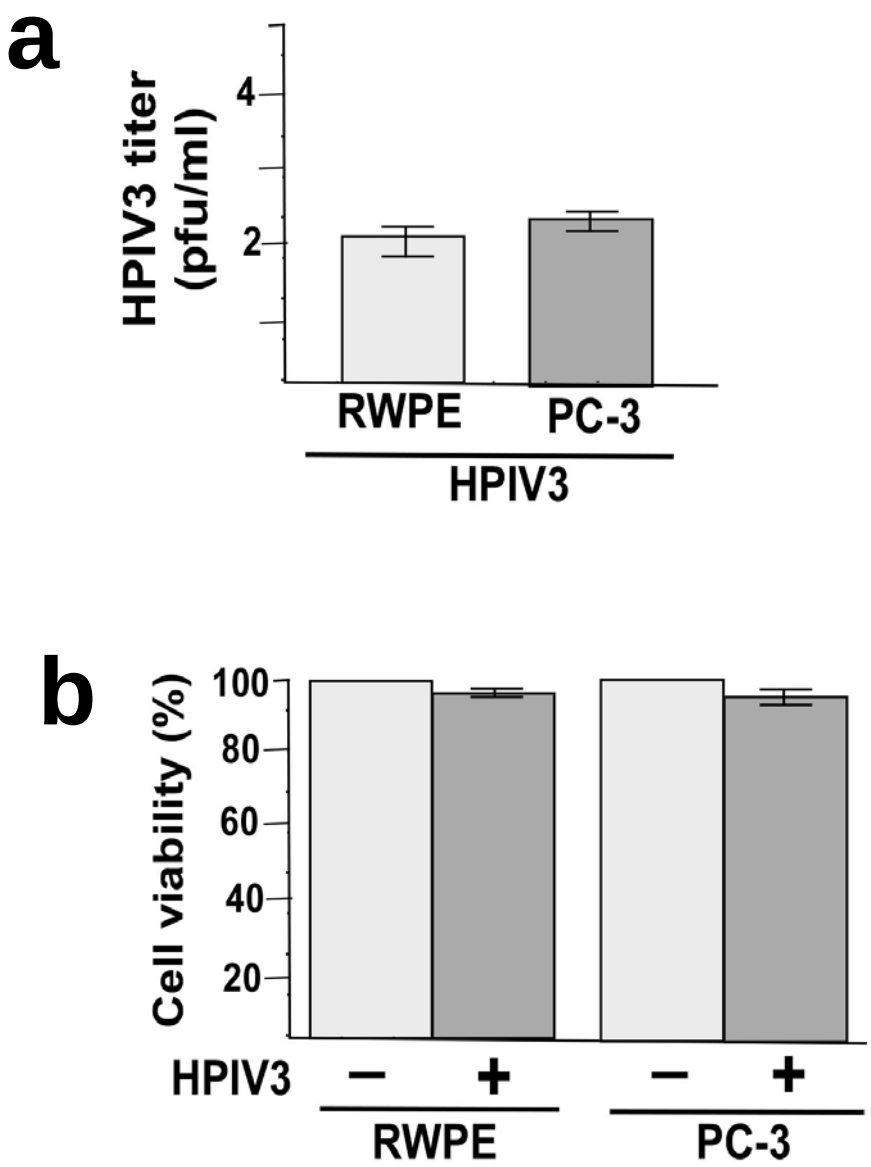
Figure 5. Role of apoptosis in RSV mediated inhibition of NF-κB activity in PC-3 cells. NF-κB specific EMSA of nuclear extracts prepared from mock infected and RSV infected (at 12h post-infection) PC-3 cells in the presence of either control inhibitor or general caspase inhibitor zVAD.
RESULT: Apoptosis of PC-3 cells is not a pre-requisite for inhibition of NF-κB activity in RSV infected PC-3 cells.

Figure 6. Akt activity in RSV infected cells. Cell lysates obtained from PC-3 cells infected with RSV for 0h-16h were subjected to Western blot analysis with phosphorylated-Akt (Phospho-Akt), Akt protein and β-actin (loading control) antibodies. Lysates obtained from RSV infected (12h infection) cells treated with DMSO (control) or wortmannin (WRT) (200 nM) were also subjected to Western blot analysis. The

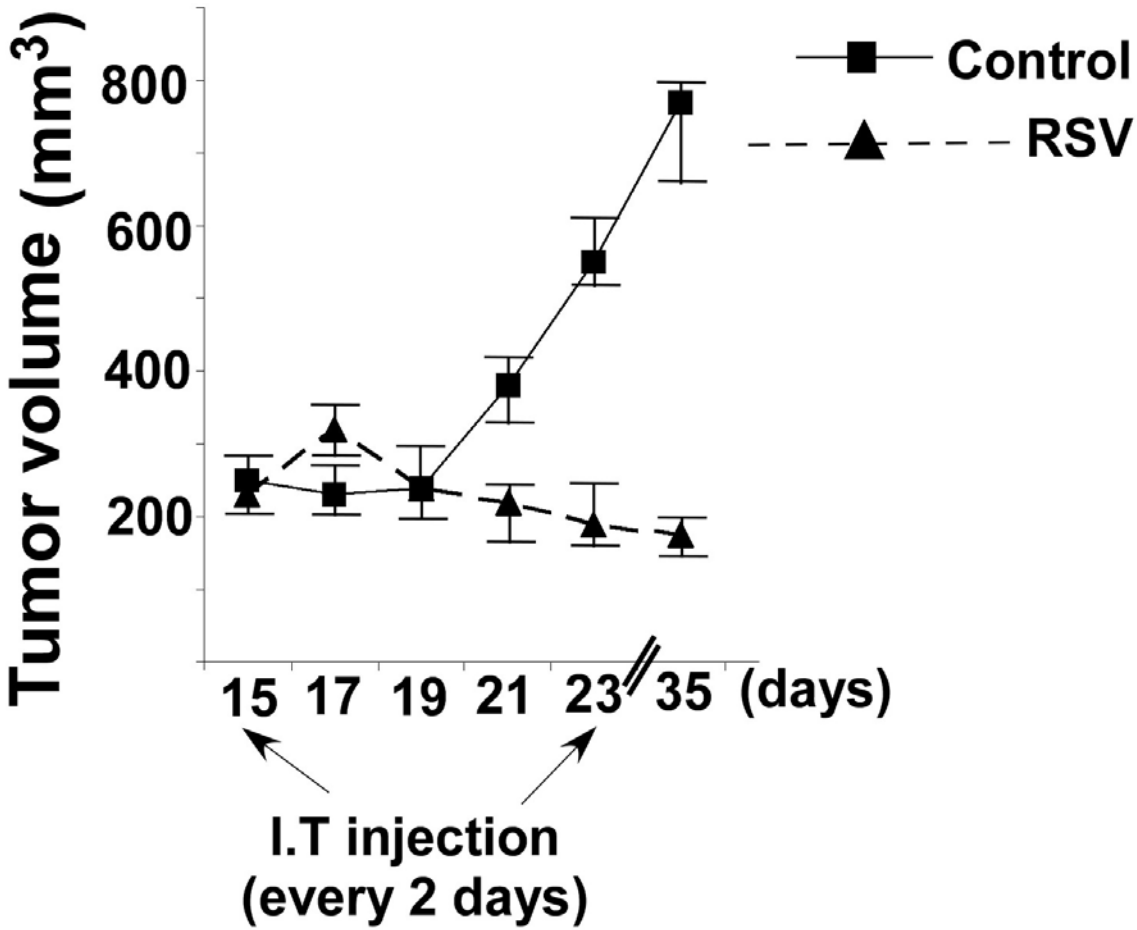
protein bands from these blots were quantified and plotted to demonstrate activation of Akt. After normalizing with β-actin expression, Akt activation was measured by calculating the ratio of phosphorylated-Akt / total Akt protein. The result represents mean ± standard deviation for three independent experiments.
RESULT: Akt is marginally activated in PC-3 cells infected with RSV.

Figure 7. Role of Akt in anti-apoptotic function of RSV infected RWPE and PC-3 cells. a. RWPE cells infected in the presence of DMSO (control) (-Wortmannin) or wortmannin (+Wortmannin) (200 nM) for 0h, 24h and 36h were subjected to apoptosis assay. b. PC-3 cells infected in the presence of either DMSO (control) or wortmannin (200 nM) for 0h and 12h were subjected to apoptosis assay. % apoptotic cells were calculated based on the total number of cells present during each experimental set. Apoptotic values represent mean ± SD for three determinations.
RESULT: Akt does not play an important role in anti-apoptotic activity since inhibition of Akt activity by wortmannin did not alter apoptosis status of RSV infected RWPE and PC-3 cells.

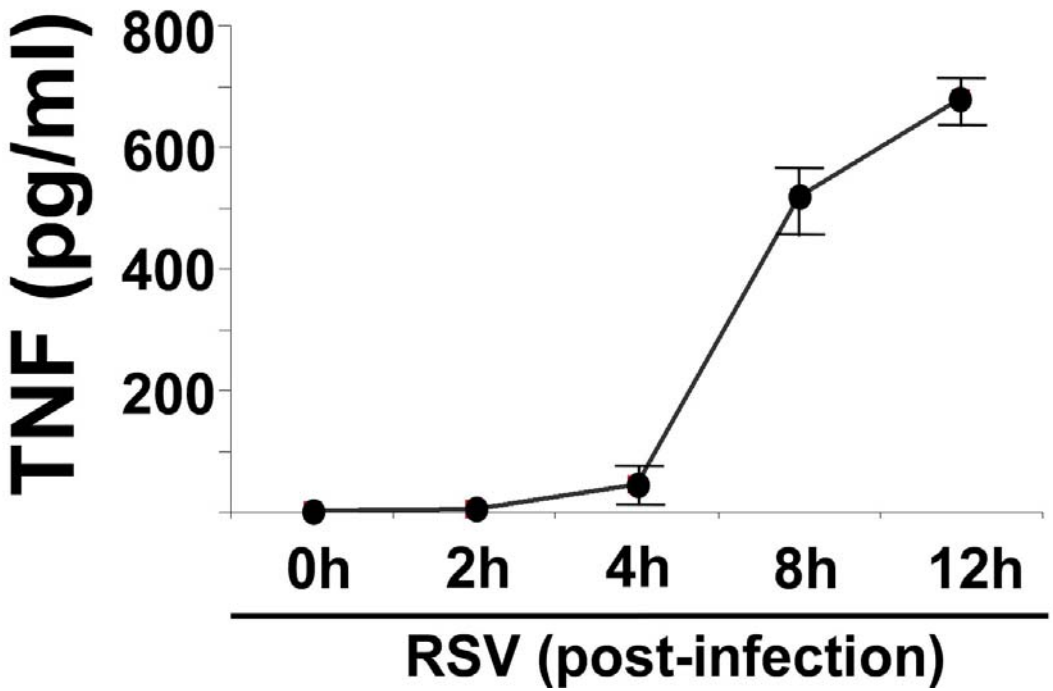
Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3



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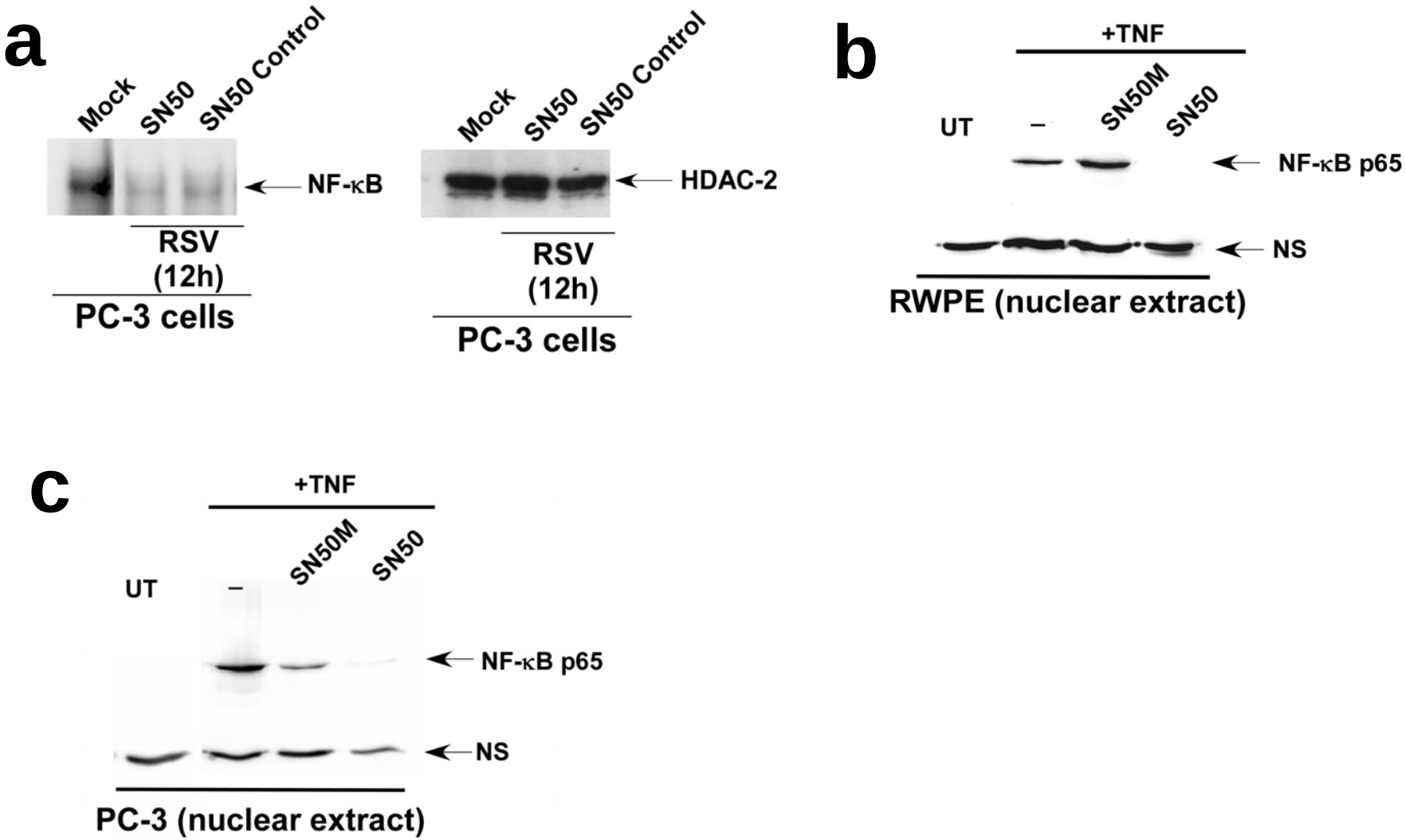
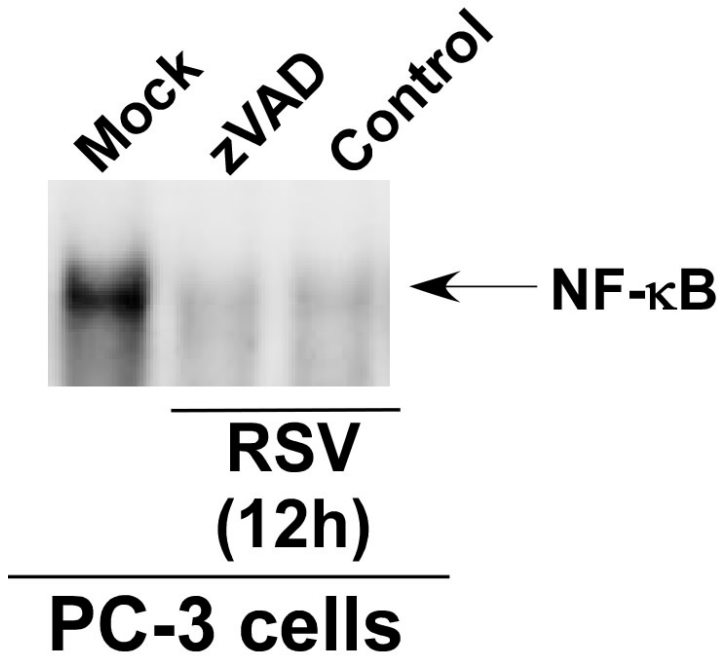


Supplementary Fig. 5



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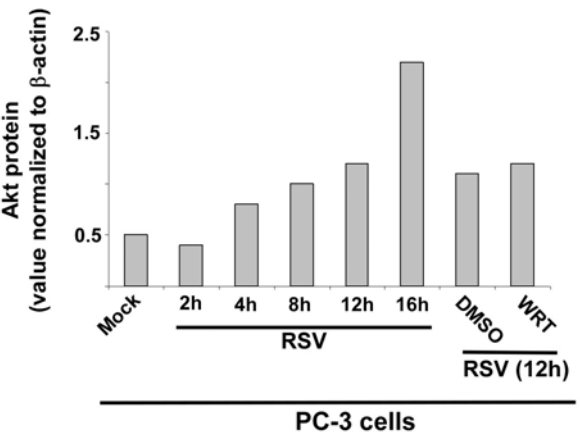

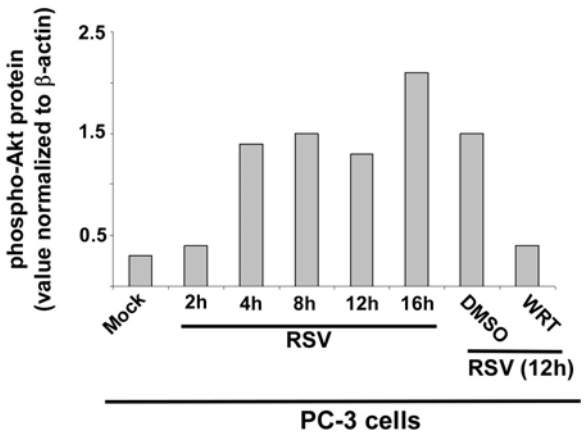

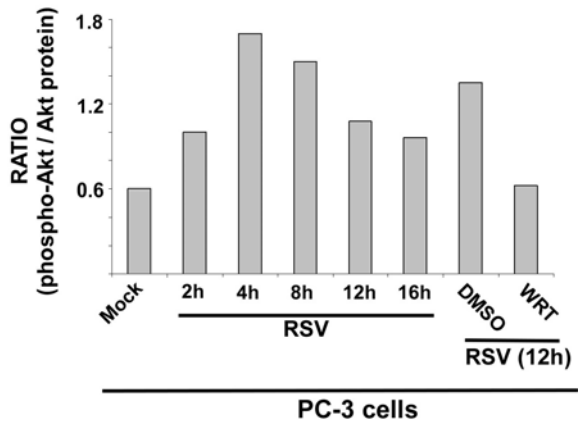

Supplementary Fig. 7