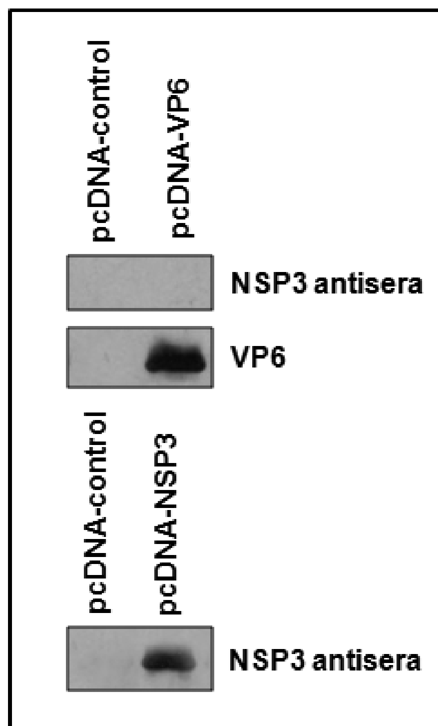
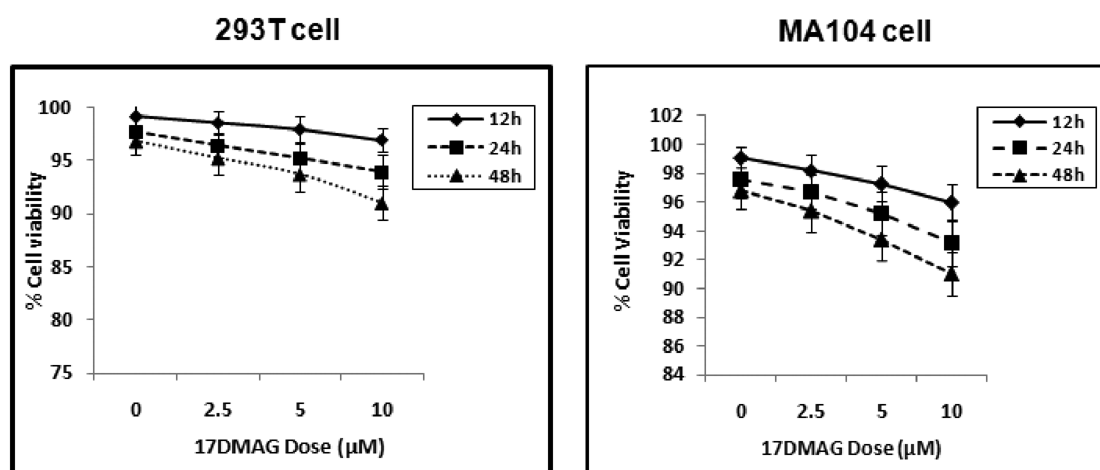



# Supplementary 1

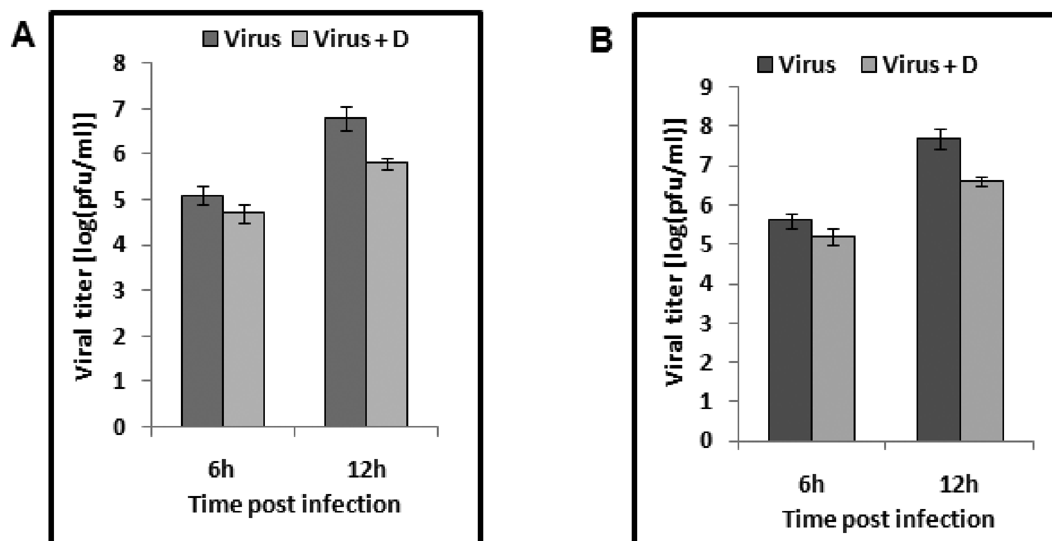
To exclude the possible cross-contamination of NSP3 antisera with VP6 anibody, VP6 (in pcDNA-VP6) expressed in 293T cells was subjected to western immunoblotting with NSP3 antisera (upper panel). The blot shows no reactivity of NSP3 antisera with VP6 . Expression of both VP6 (from pcDNA-VP6) and NSP3 (from pcDNA-NSP3) were confirmed by immunoblotting with respective antibodies (middle and bottom panel) against pcDNA vector control.




Supplementary 2

Viability of 293T and MA104 cells treated with 17DMAG. Cytotoxicity of 17 DMAG was determined by MTS reduction assay with cell titer 96 ® aqueous One Solution Cell Proliferation assay kit (Promega) measuring metabolic activity of cells following treatment for 12, 24, and 48h. 293T and MA104 cells cultured in serum free media were supplemented with increasing concentrations of the inhibitor ( 0-10μM) At each end point, cells were treated with MTS solution in serum free medium for 4 h at 37ºC in a humified 5% $CO_2$ atmosphere. The quantity of the soluble formazan which was produced when MTS got bioreduced by NADPH or NADH produced by dehydrogenase enzymes in metabolically active cells was measured spectrophotometrically at 490 nm . The absorbance was directly proportional to the number of living cells in culture. The percent viability was calculated considering 100% viability for untreated control cells at 0 h post drug treatment. At 24 and 48h, percent viability was 96.5-95.2%; 95.2-93.7%; and 93.9-91% for 293T cells and 96.7-95.4%; 95.1-93.4% and 93.2-90.7% for MA104 cells in presence of 2.5μM-5μM-10μM of 17DMAG respectively.

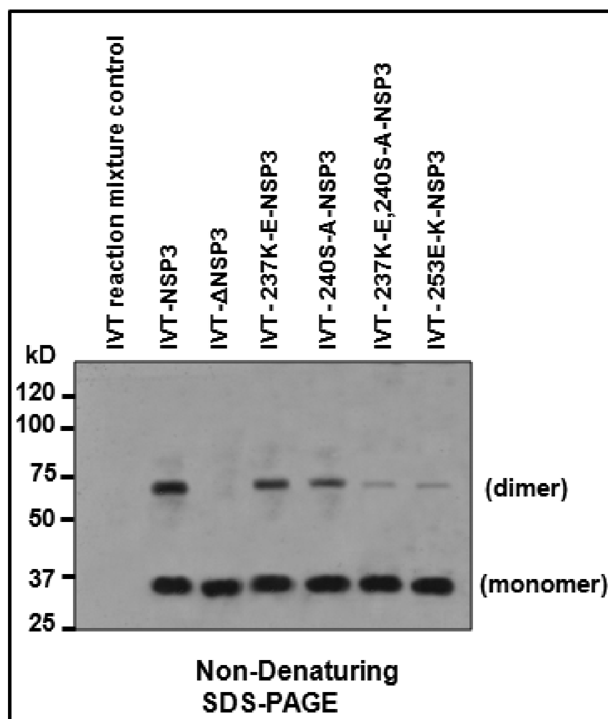
## Supplementary 3



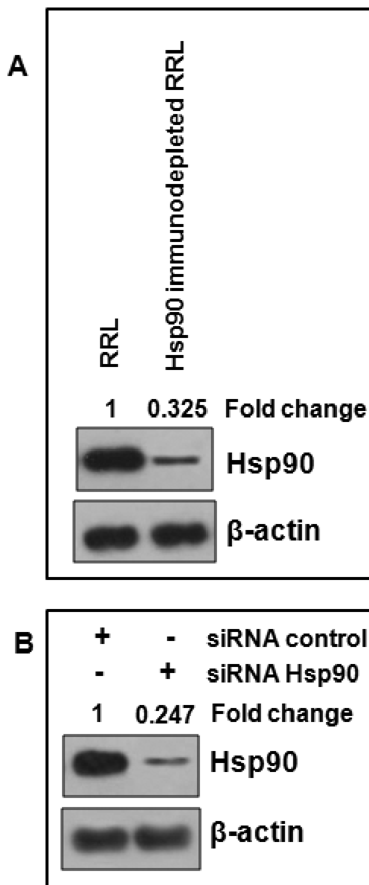

Viral Titer in presence or absence of 17DMAG (D)

Virus growth assays were done by infecting the MA104 cells with rotavirus SA11 (moi of 1, Fig 3A) and (moi of 3, Fig 3B) in the presence or absence of Hsp90 inhibitor 17DMAG (5μM). After 6 and 12 h post treatment virus titers, as determined by plaque assay, showed ~0.35- 0.4 and ~0.9- 1 log inhibition of growth rates respectively compared to only virus infection. The data represent the means of three experiments (n=3, $P<0.05$).

Supplementary 4

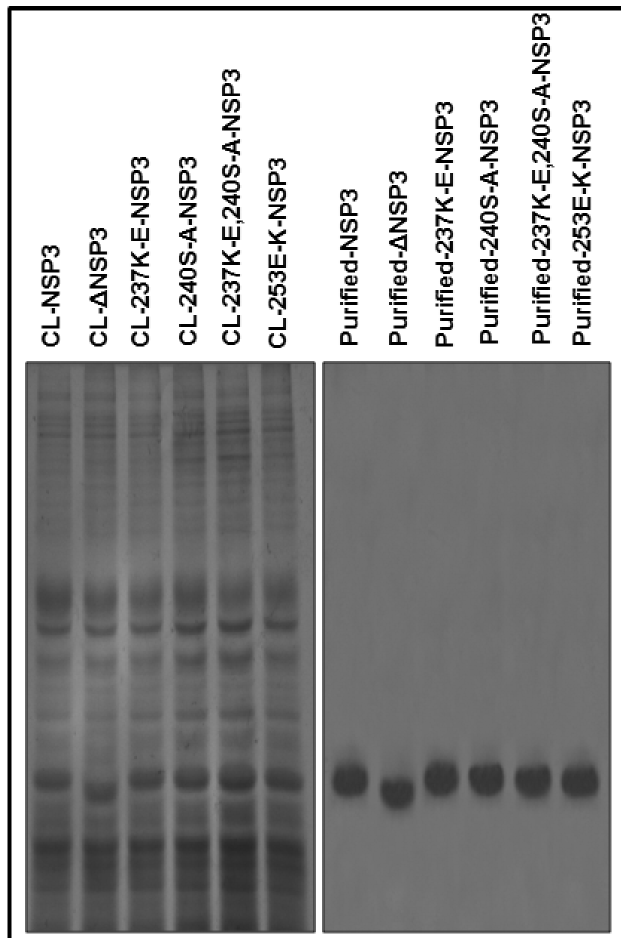



Full length NSP3 (pcD-NSP3), the aa 225-258 deletion (Δ-NSP3) and various point mutants of the same region were subjected to *in vitro* coupled transcription-translation (IVT) in presence of Transcend™ biotinylated-Lysyl-tRNA for 90 min and the products were seperated in nondenaturing SDS-PAGE at $4^{0}$C followed by immunoblotting using streptavidin-HRP. Apart from deletion mutant, both dimer and monomers of NSP3 are observed for wild type and different point mutants, however point mutants which poorly interact with Hsp90 show dimeric form relatively less abundant from wild type NSP3 whereas deletion mutant has only monomeric form.




Supplementary 5

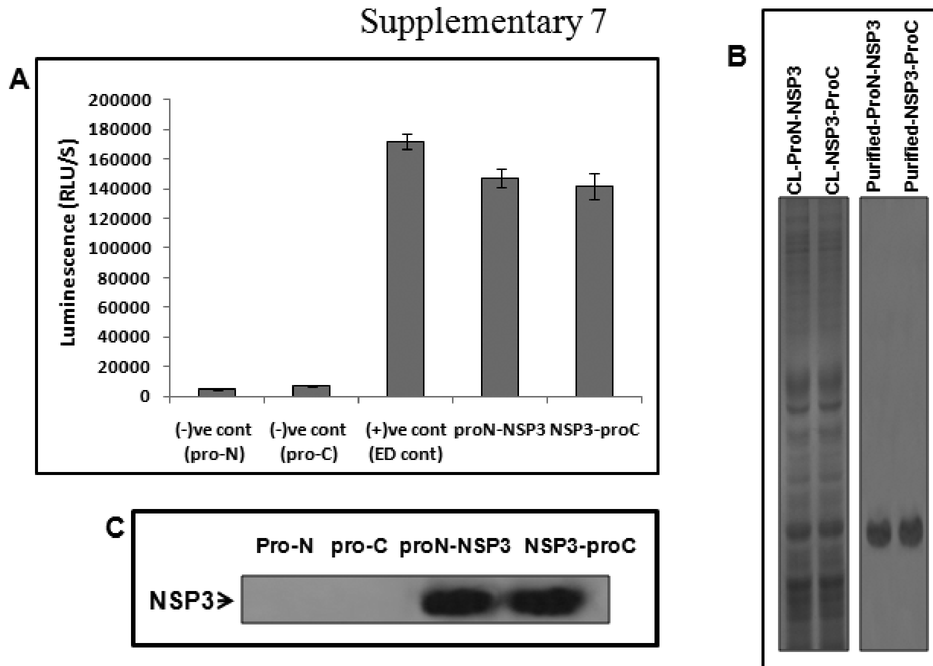
(A) 500 µl of rabbit reticulocyte lysate was incubated overnight with 10 µg of Hsp90 monoclonal antibody at $4^0$c. The immunocomplex was mixed with protein A sepharose for 4 h and removed through centrifugation. The process was repeated twice to make clarified lysate immunodepleted from Hsp90 and was verified through western blotting against Hsp90. >60% depletion in Hsp90 level was observed compared to control. (B) Downregulation of Hsp90 expression in the presence of Hsp90 siRNA (Ambion ID: s6995), (0.5 nM X 2: 48 h) as analyzed by western blotting. 293T cells that were transfected with HSP90 siRNA or matched negative control siRNA were lysed and Hsp90 protein expression was measured by western blotting. >3 fold (> 70%) knockdown in Hsp90 protein level was observed in cells treated with the gene specific siRNA compared to cells treated with negative control siRNA (B). In both experiments β- actin expression was measured as endogenous control to check equal protein loading.




Supplementary 6

Expression of full length as well as deletion and different point mutants of NSP3 in 293T cells and their purification. The cDNA encoding SA11 NSP3 and its deletion mutant (aa 225-258) was cloned in pcDNA 6b. Different point mutants were produced from full length NSP3 clone. Plasmids were introduced in 293T cells. Proteins expressed under the control of CMV promoter were resolved by SDS-PAGE and stained with Coomassie blue (left panel). Recombinant proteins were purified on Ni-NTA magnetic agarose deads under native conditions and were resolved by SDS-PAGE and stained with Coomassie blue (right panel).

# Supplementary 7




Expression of proN-NSP3 and NSP3-proC fusion protein. Plasmid pProN-NSP3 and pNSP3-ProC fusion contracts expressed under CMV promoter were monitored by measuring luminescence of prolabel tag using Prolabel Chemiluminiscent Detection kit (Clontech) against proN and proC vector control. The Prolabel-NSP3 fusion expression was compared to the ED positive control (Kit) [panel A]. The Prolabel-NSP3 fusion proteins were purified with anti-NSP3 antibody-sepharose bead. Briefly, 500 µl of cell lysates of 293T cells expressing Prolabel-NSP3 protein was diluted 1:1 with binding buffer (10 mM HEPES-NaOH, pH 7, 0.1 M NaCl, 2mM PMSF) and was allowed to bind with 1ml of 50% anti-NSP3-sepharose resin slurry overnight by continuous mixing. After washing 3 times with pre-elution buffer [0.1X TBS (2mM Tris-HCl, pH 7.5, 15 mM NaCl)], the bound proteins were eluted with 0.1 M glycine, pH 3 at room temperature and collected into tubes preloaded with equal volume of cold 1M Tris-HCl, pH 6 to neutralize the eluate. Purification of the protein was validated through SDS-PAGE (panel B) and expression of purified Prolabel-NSP3 protein was verified by western blotting with NSP3 specific antibody ( panel C).