nature cell biology

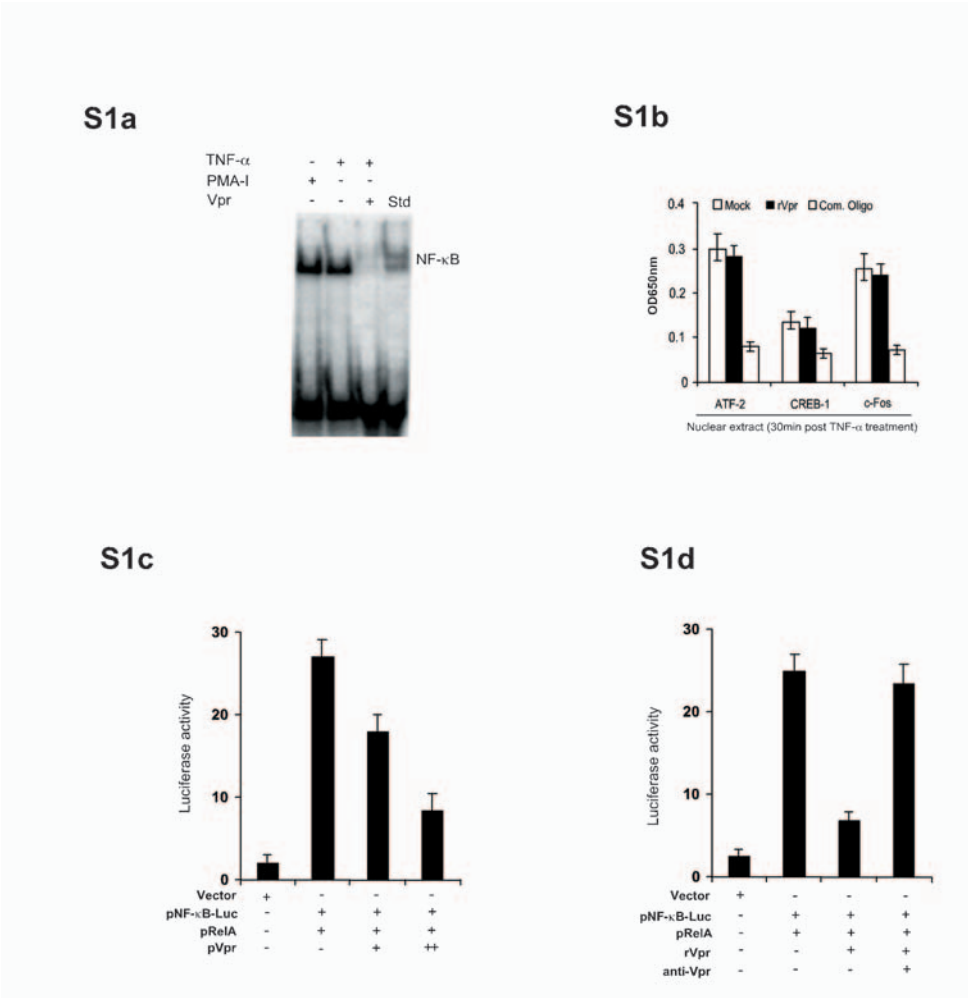

Figure S1 Vpr specifically inhibits NF-κB transcriptional activation. (**a**) DNA binding effects of NF-κB in cells treated with rVpr. Cell nuclear extracts were prepared from the rVpr treated cells incubated with or without TNF-α (5ng/ml) for 3 hrs and incubated with a oligonucleotide radiolabelled probe corresponding to the consenus-binding site for NF-κB as described in Methods. Their respective DNA binding activity in the presence and absence of Vpr was determined. PMA-I stimulated cells were also loaded as an additional control for NF-κB activity. (**b**) HeLa cells were treated with 5ng/ml TNF-α as indicated time period in the presence or absence of rVpr (10pg). Cells were collected as indicated time period and nuclear proteins were extracted for ELISA-based transcription factor assay. Nuclear extracts (10μg) were incubated in 96-well plates lined with oligonucleotide specific for ATF-2, CREB-1 and c-Fos. Absorbance (O.D) was measured at 650nm. The assay was performed in triplicate. Values and bars represents mean (n=3) and SD. (**c**-**d**) Vpr mediated repression of RelA. (**c**) CV-1 cells were transfected with the NF-κB dependent reporter plasmid (2μg), with pRelA plasmid (1μg), and with pCMVLacZ (1μg) with or without Vpr plasmid at different concentrations (2μg and 4μg). (**d**) In control antibody experiments, rVpr (10pg) was also added and cotreated with an anti-Vpr antibody and Luciferase activity was measured thereafter using a reporter assay system, and chemiluminescent activity was measured as described in Methods.

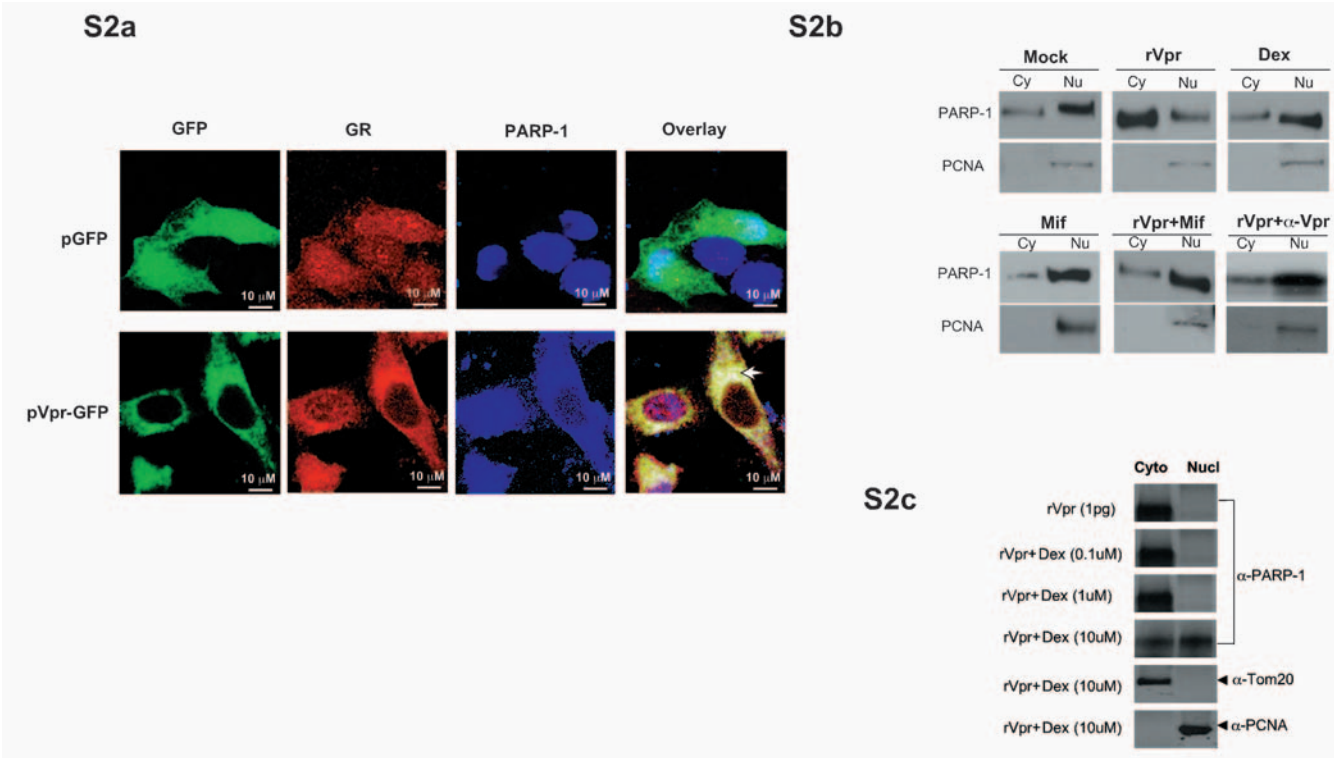

Figure S2 Dexamethasone can antagonize Vpr's effect on PARP-1 localization. (**a**) Vpr, GR and PARP-1 colocalize to cytoplasmic and peri-nuclear regions. Immunofluorescence analysis of HeLa cells that were transiently transfected with GFP vector or pVpr-GFP (2μg). At 36h post-transfection, transfected cells were fixed and immunostained with anti-GR and anti-PARP-1 antibody as described in Methods. Arrow indicates the colocalization of all three proteins. (**b**) Dexamethasone is insufficient to regulate the localization of PARP-1. HeLa cells were treated with Mock, rVpr (10pg), Dex (1μM), Mifpristone (1μM), rVpr (10pg)+Mifpristone (1μM), or Vpr (10pg)+anti-Vpr(1:200) and nuclear/cytoplasmic contents were measured for PARP-1. (**c**) High concentration of Dex is sufficient to reverse Vpr's effects on PARP-1 nuclear localization. Total nuclear and cytosol extracts were prepared from rVpr (10pg) or rVpr+Dex treated cells at indicate concentrations post 30 hours treatment. Nuclear/Cytoplasmic extracts were blotted for PARP-1 and Tom20/PCNA as a fractionation control.

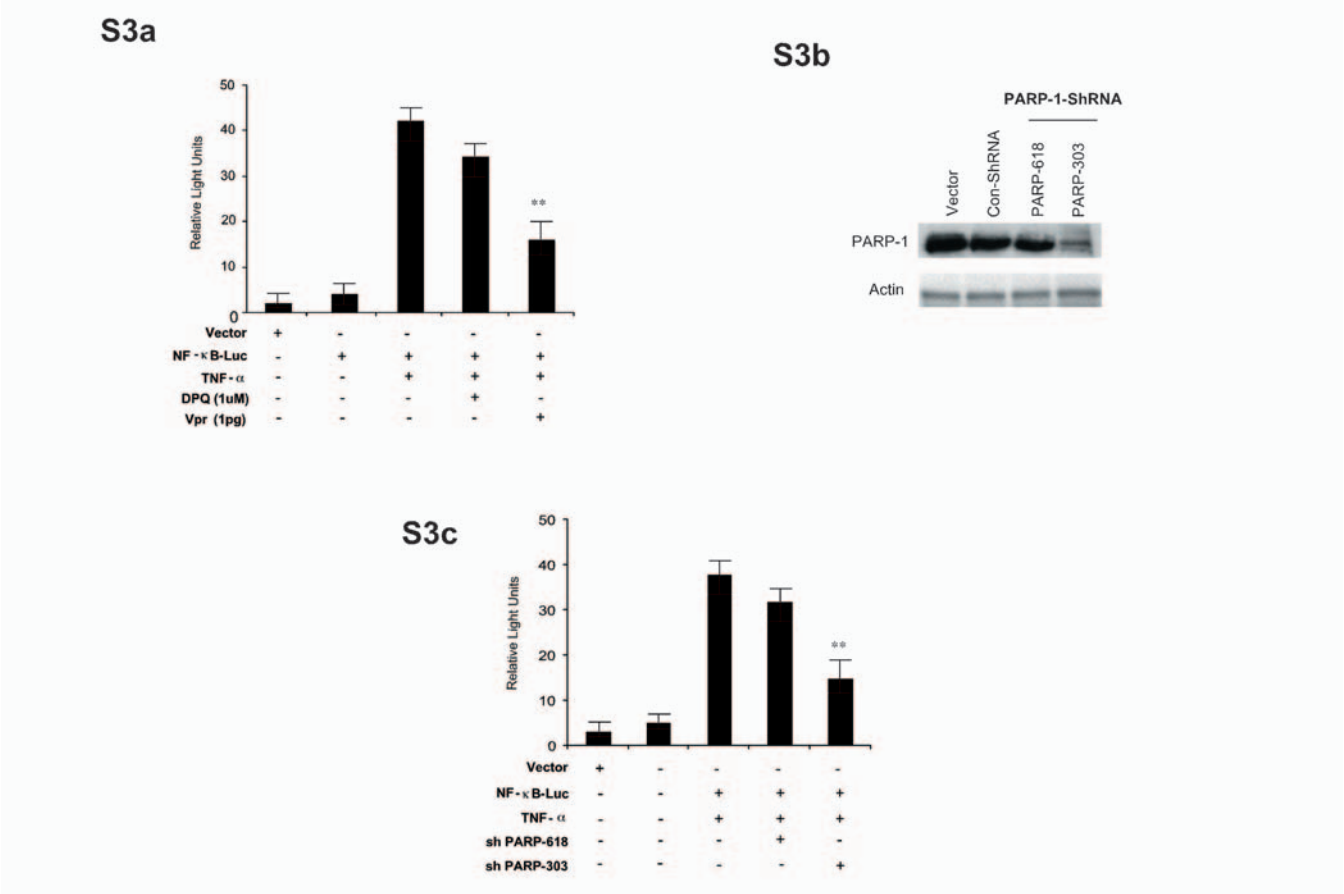

Figure S3 PARP-1 but not its enzymatic activity is required for NF-κB transcription. (**a**) Vpr-mediated NF-κB suppression is not associated with the enzymatic functions of PARP-1. HeLa cells were transfected with the NF-κB dependent reporter plasmid (2μg), with pCMVLacZ (1μg) and treated with or without rVpr (10pg) or DPQ (1μM) and luciferase was measured as described in Methods. An asterisk (**) over a bar indicates a statistically significant lower ratio of the experimental compared with the control ($p< 0.005$). (**b**) shRNA mediated depletion of PARP-1. HeLa were transfected with 5μg of siRNA control vector or PARP-1 shRNA expression constructs as indicated clones. Cell lysates were extracted 48hrs post-transfection and immunoblotting was performed by using an anti-PARP-1 antibody and an actin antibody as a control for equal loading. (**c**) Luciferase assay measuring NF-κB activation with shRNA directed against PARP-1 from (**a**) is shown. An asterisk (**) over a bar indicates a statistically significant lower ration of the experimental compared with the control ($p<0.05$).