



Figure 6 figure supplement 1 Vpr inhibits NF- κ B p65 nuclear translocation and NF- κ B sensitive plasmid expression

(A) Induction of luciferase reporter in HEK293T cells transfected with CSLW, CMV-Luc, TK-Luc or M5P-Luc (10ng), and empty vector, or Vpr encoding vector (50 ng, 100 ng, 200 ng). Table shows the promoters driving the luciferase reporter in each plasmid. (B) Percentage of cells in Figure 6D with translocation coefficient greater than 0.5. (C) Single cell measurement of NF- κ B nuclear translocation in PMA differentiated THP-1 cells stimulated with LPS, or left unstimulated, and infected with HIV-1 GFP lacking Vpr or bearing Vpr (1 RT U/ml), or left uninfected (top panel). Percentage of cells with NF- κ B translocation coefficient greater than 0.5 plotted as a percentage (bottom panel). Data is analysed using two-way ANOVA: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$) compared to data from infection with HIV-1 lacking Vpr. (D) Quantification of GFP expression by densitometry for the immunoblot in Figure 6E. (E) Immunoblot detecting flag-Vpr, GFP or actin as a loading control from HEK293T cells transfected with empty vector, flag-tagged WT Vpr encoding vector or flag-tagged mutant Vpr encoding vector and CMV-GFP vector or left untransfected. Size markers are shown in kDa. Quantification of GFP expression by densitometry for the immunoblot is shown below. (F) Quantification of GFP expression by densitometry for the immunoblot in Figure 6G. (G) Immunoblot detecting GFP, or actin as a loading control, from HEK293T cells transfected with CMV-GFP, EF1 α -GFP or Ub-GFP plasmids (10 ng, 2 ng, 0.4 ng) and stimulated with TNF α (200 ng/ml) or left unstimulated. Size markers are shown in kDa. Quantification of GFP expression by densitometry for the immunoblot is shown below.