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Characterisation of Lentiviral Accessory Protein Vpx as an NF-{kappa-x}B antagonist

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Purpose of study: To investigate a new role of lentiviral accessory protein Vpx in antagonism of the NF-{kappa-x}B and IRF3 inflammatory pathways in DNA sensing. This study was supported by the Pathological Society Undergraduate Bursary.

Methods: Cell-line luciferase reporter assays were carried out in 293T cells reconstituted for DNA sensing molecules and transfected with Vpx or Vpx mutants. Immunoprecipitation experiments in 293T cells were carried out with over-expressed Vpx and NF-{kappa-x}B signalling pathway members. Cell-line reporter assays were also carried out in DCAF1 knockdown cells, achieved by transient knock-down with siRNAs.

Summary of results: Cell-line reporter assays showed Vpx directly antagonises NF-{kappa-x}B, but not IRF3, activation downstream of DNA-sensing. NF-{kappa-x}B antagonism was preserved in Vpx binding mutants described in the literature. Immunoprecipitation experiments showed that Vpx interacts with several NF-{kappa-x}B signalling molecules and likely targets key NF-{kappa-x}B subunit p65 for degradation. Transient knockdown experiments indicated Vpx antagonism of NF-{kappa-x}B is independent of host co-factor DCAF1.

Conclusions: Our experiments suggest a novel phenotype of Vpx to antagonise NF{kappa-x}B signalling, distinct from previously described roles of the protein. Notably, Vpx antagonism of NF-{kappa-x}B is independent of host co-factor DCAF1, representing an entirely novel mechanism of Vpx action. We believe this phenotype may have implications for evasion of DNA-sensing and host immune responses {1ITALIC}in vivo{0ITALIC} which warrant further investigation in the context of infection.