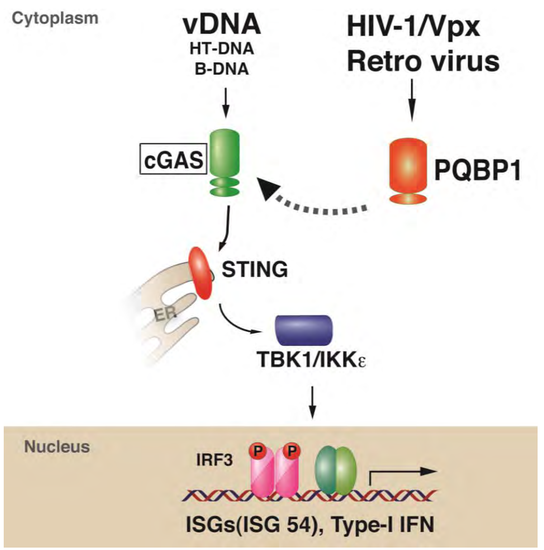
#352- Identification of Pattern Recognition Receptor for HIV-1 in Human Dendritic cells

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Abstract Dendritic cells (DCs) play a critical role in the immune response to viral infection through the facilitation of cell intrinsic antiviral activity and the activation of adaptive immunity. Productive infection of DCs by HIV-1 triggers an IRF3-dependent innate immune response, which has recently been shown to require the activity of cyclic GAMP synthase (cGAS)[7,11,12]. Here, we report the results of a targeted RNAi screen utilizing primary human monocyte-derived DCs (MDDCs) to identify cellular immune regulators that directly interface with HIV-1-encoded features to initiate this innate response. One of the strongest candidates that emerged from this screen was polyglutamine binding protein 1 **(**PQBP1). We found that PQBP1 is required for IRF3-dependent signalling in HIV-1-infected MDDCs and human cell lines. PQBP1 directly binds to reverse-transcribed HIV-1 DNA and initiates a cGAS and IRF3-dependent innate response in myeloid cells. Our results demonstrate that PQBP1 acts as a proximal sensor of a pathogen-associated molecular pattern (PAMP) encoded by HIV-1 DNA to activate cGAS/IRF3-dependent antiviral responses.



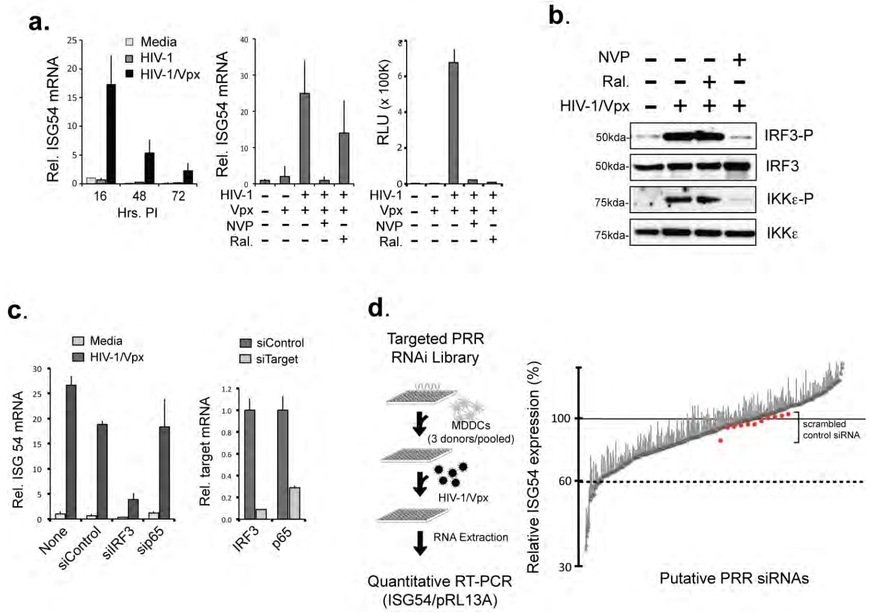


Figure 1: A targeted RNAi screen to identify innate immune sensors of HIV-1 infection. (a) *ISG54* mRNAs levels and viral reporter activities or (b) p-IRF3 and p-IKKε levels were measured from MDDCs infected with HIV-1/Vpx in the absence or presence of antiviral antiviral drugs, NVP or Ral. (c) MDDCs were transfected with siRNAs targeting IRF3 and NF-κB, and *ISG54* induction was measured. (d) A schematic depicting the screening process (left). The average and standard deviation of *ISG54* mRNA levels in MDDCs depleted of each putative sensor is shown (right). The scramble control activities are depicted in the large, red circles. The horizontal dotted line reflects the 3 standard deviation cut-off from the controls.

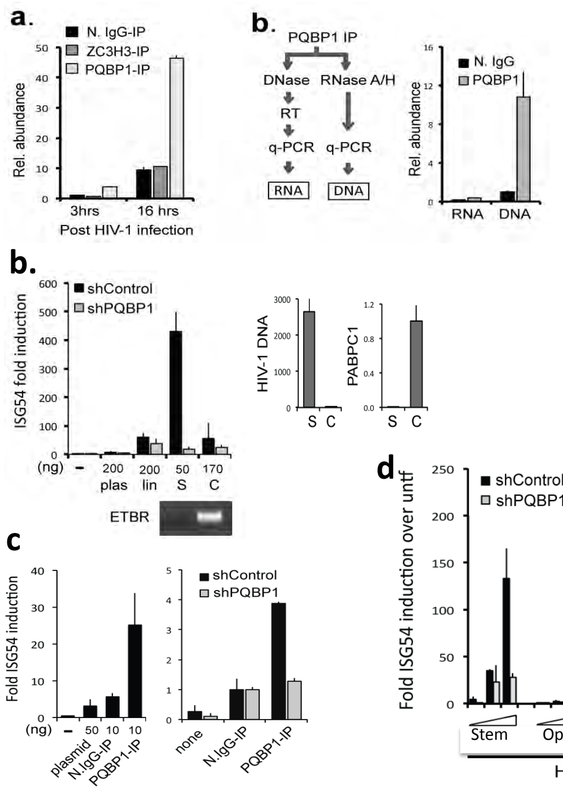


Figure 3: PQBP1 binds immunogenic HIV-1 DNA. (a) MDDCs were challenged with HIV-1/Vpx for 3 or 16 hr, followed by formaldehyde cross-linking. The abundance of HIV-1-encoded nucleic acids with the indicted Ips were evaluated using qRT-PCR. (b) Immunoprecipitants described in (*a*) were treated with either DNase or RNaseA/H and HIV-1 nucleic acid levels were measured. (c) Infected and cross-linked MDDCs lysates were separated into soluble non-chromatin (S) and chromatin (C) fractions. (c) DNA isolated from each fraction was electroporated into PMA-differentiated THP-1 cells harboring a indicated shRNA, and *ISG54* expression was measured (right). Rel. abundance of HIV-1 DNA vs cellular genomic DNA in each fraction is evaluated (bottom). DNA immuno-precipated with the PQbP1 protein were electroporated into the cells and assayed for *ISG54* induction. (d) PMA-THP cells transfected DNA oligoes , as indicated, and assayed for ISG54 induction.

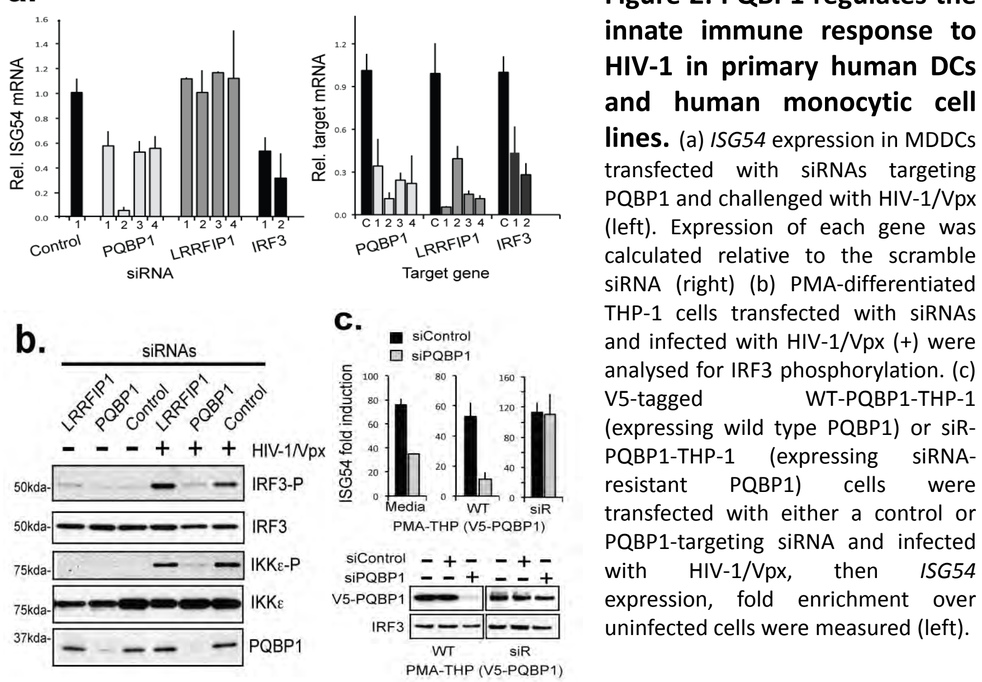


Figure 2: PQBP1 regulates the innate immune response to HIV-1 in primary human DCs and human monocytic cell lines. (a) *ISG54* expression in MDDCs transfected with siRNAs targeting PQBP1 and challenged with HIV-1/Vpx (left). Expression of each gene was calculated relative to the scramble siRNA (right) (b) PMA-differentiated THP-1 cells transfected with siRNAs and infected with HIV-1/Vpx (+) were analysed for IRF3 phosphorylation. (c) V5-tagged WT-PQBP1-THP-1 (expressing wild type PQBP1) or siR-PQBP1-THP-1 (expressing siRNA-resistant PQBP1) cells were transfected with either a control or PQBP1-targeting siRNA and infected with HIV-1/Vpx, then *ISG54* expression, fold enrichment over uninfected cells were measured (left).

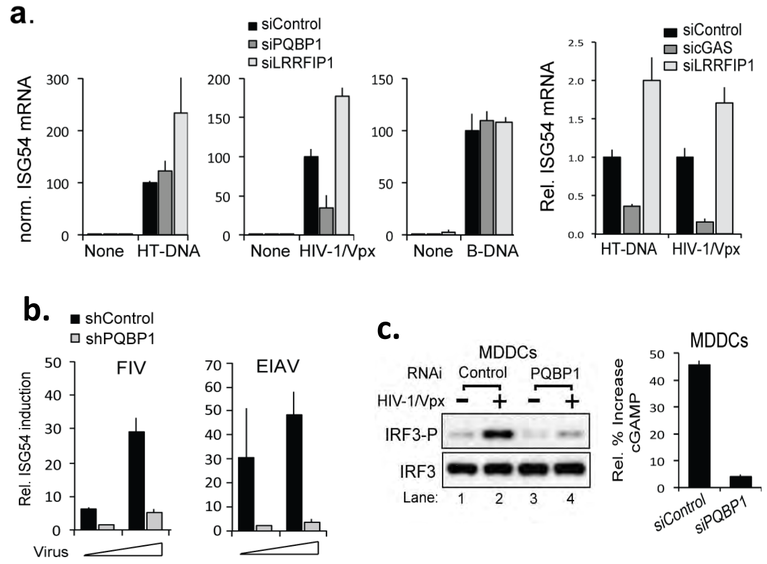


Figure 4: PQBP1 is a specific sensor of retroviral DNA. (a) MDDCs were transfected with indicated siRNAs and challenged with HIV-1/Vpx, B-DNA, or HT-DNA, and *ISG54* expression was measured. (b) PMA differentiated shControl or shPQBP1 THP-1 cells were infected with FIV or EIAV and *ISG54* mRNA induction was measured relative to untreated cells. (c) cGAMP levels in the lysates of MDDCs were assayed prior to and after HIV-1/Vpx infection. Cellular lysates were incubated with PFO-treated THP-1 cells, followed by western blot analysis to detect p-IRF3 (right) or directly quantified using LC-MS (left). The level of cGAMP was graphed as a % increase in HIV-1 infected cells over the untreated samples either in the presence or absence of PQBP1 protein.