

P030 The ubiquitin ligase Nedd4.1 rescues HIV-1 budding defects in an L domain dependent manner

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The p6 region of HIV-1 Gag contains PTAP and LYPX_nL late domain motifs that facilitate the recruitment of vacuolar protein sorting (VPS) machinery via direct interaction with Tsg101 and Alix, respectively. Other retroviruses, including HTLV-1, MLV and RSV, exploit the VPS pathway by utilizing a PPXY late domain motif known to bind members of the Nedd4 HECT E3 Ubiquitin Ligase family. While HIV-1 does not contain a PPXY motif, we show that overexpression of the HECT E3 Ubiquitin Ligase Nedd4.1 efficiently rescued the release of an HIV-1 mutant lacking the PTAP motif (PTAP-). Rescue of HIV-1 PTAP- release by Nedd4.1 overexpression was unaffected by siRNA depletion of Tsg101. However, a mutation in Nedd4.1 known to abrogate ubiquitin ligase activity severely inhibited the ability of Nedd4.1 to rescue the HIV-1 PTAP- defect. This mutation had little effect on the ability of Nedd4.1 to co-immunoprecipitate HIV-1 Gag. Mutation of both the PTAP and LYPX_nL motifs within p6 reduced the ability of Nedd4.1 to rescue HIV-1 release. Concurrently, deletion of the entire p6 region resulted in a reduction in the amount of Gag co-immunoprecipitated by Nedd4.1. Overexpression of Nedd4.1 with wild type HIV-1 or a mutant containing only the PTAP motif stimulated virion release independently of Nedd4.1 ligase activity. Together, these data suggest that overexpression of the HECT E3 Ubiquitin Ligase Nedd4.1 stimulates HIV-1 release via two separate and novel L domain dependent mechanisms.