

# **CCDC11 Acts as a Scaffold to Assemble the ESCRT Membrane-Scission Machinery at Viral Budding Sites for HIV-1 Release: Identifying a Novel Therapeutic Strategy for Antiviral Therapy**

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We previously reported that Coiled-Coil Domain Containing 11 (CCDC11) is critical for the production of Human Immunodeficiency Virus (HIV) from Human Embryonic Kidney (HEK) 293T cells, likely through recruitment of Endosomal Sorting Complex Required for Transport (ESCRT) machinery. To further extend the role of CCDC11 in this process, we attempted to knockout CCDC11 in human cervical cancer HeLa cells, which is a widely used model for HIV research, using the CRISPR-Cas9 technology. We identified three CCDC11-deficient HeLa cell lines with distinct mutations after sequencing, and a significant reduction of CCDC11 protein levels was verified by western blotting and immunofluorescence microscopy. As expected, HIV-1 release into culture media from the CCDC11-deficient cells was dramatically decreased. Consistent with our hypothesis that CCDC11 is required for viral budding, CCDC11 partially colocalized with Virus-Like Particles (VLPs), produced by ectopic expression of the HIV-1 Gag structural protein, at the plasma membrane of HeLa cells. In addition, we found that CCDC11 colocalizes with the ESCRT-I component TSG101 and the ESCRT-III components CHMP2A and CHMP4B, but not with ALIX, CHMP6, or CHMP8. In support of these findings, we found that CCDC11 physically interacts with CHMP2A and CHMP4B. Collectively, our data confirms our previous results that CCDC11 plays a key role in HIV production and provides the first evidence that CCDC11 promotes HIV budding through direct recruitment of CHMP2A and CHMP4B to viral budding sites at the plasma membrane. Protein-protein interactions between CCDC11 and CHMP2A and CHMP4B can be exploited as a novel therapeutic strategy for antiviral therapy.