onto the Vpx_{sm} (blue ribbon)-DCAF1-CtD (white surface) structure. SAMHD1-CtD is shown as red ribbon.

Extended Data

Extended Data Table 1. Data collection and refinement statistics

*Values in parentheses are for highest-resolution shell.

Extended Data Table 2: Intermolecular interactions

*HB – hydrogen bond, HI – hydrophobic interaction, SB – salt bridge # Bi-functional residues are highlighted in red

Extended Data Table 3: Vpx and Vpr mutations

Extended Data Figure 1. **Experimental electron density**. Experimental electron density observed after solvent flattening for DCAF1, Vpx and SAMHD1 is shown as light blue wireframe, contoured at 1 σ. The backbone Cα traces of the final refined protein structure are shown in green ribbon representation.

Extended Data Figure 2. M**odel of the hijacked $CRL4^{DCAF1}$ ubiquitin ligase complex.** Vpx_{sm} (blue) bound to the substrate specificity module DCAF1-CtD (grey) is shown in cartoon representation as in **Figure 1c**. SAMHD1-CtD is shown as red spheres. The DDB1 adaptor is shown as green cartoon. The inset to the right shows the superposition of the DDB2 helical hairpin (H-box, orange), which inserts into the binding groove created by DDB1 β-propellers 1 and 3 (BPA, BPC), and the N-terminus of DCAF1-CtD

presented in this study. The CUL4A scaffold is represented as orange semi-transparent surface, the ROC1 RING module as purple spheres. Due to conformational freedom of the DDB1-CUL4A connection, the two most extreme conformations of CUL4A with respect to DDB1 available in the Protein Data Bank were modelled. See Methods for modelling procedures and PDB codes. The model clearly shows that in both extreme CUL4 conformations, the ROC1 RING finger (purple spheres) is well positioned to reach the SAMHD1 protein, which would be attached at the N-terminal end of the SAMHD1-CtD. The SAMHD1 globular fold is most probably mobile with respect to the fixed position of SAMHD1-CtD due to the flexibility of the sequence stretch between SAMHD1 residues 583 (the last ordered residue of PDB code 3U1N [5]) and 606 (the first ordered residue of SAMHD1-CtD presented here).

Extended Data Figure 3. Analogous mechanism of restriction factor counteraction in SIV/HIV-2 and HIV-1 Vpx and Vpr. SAMHD1 provides a potent post-entry block against immunodeficiency viruses in non-cycling cells. Its dNTP-triphosphohydrolase activity lowers the cellular dNTP pool preventing viral reverse transcription. HIV-2/SIVs use their Vpx and Vpr accessory proteins to modify the host cell's CUL4A-DDB1-DCAF1 ubiquitin ligase specificity towards SAMHD1, resulting in its proteasomal degradation and ultimately raising dNTP levels making the cells permissive to viral replication. Sequence similarity and comparative functional analysis suggest that the ancestral HIV-1 accessory protein Vpr employs a similar mechanism to exploit the CUL4A-DDB1-DCAF1 system in order to induce proteasomal degradation of a yet undiscovered cellular factor whose absence causes cell cycle arrest in the G2 phase, promoting viral replication and pathogenesis *in vivo*.