were refined using REFMAC5[63] with TLS refinement using TLSMD[64] and TLSANL[65] in the CCP4 suite[66]. Structures were analyzed with PROCHECK[67] and figures were generated using PYMOL[68].

Infectivity Assays

293T cells were seeded in 6-well plates at $8x10^5$ cells per well and co-transfected with a pEGFP-VPS4A expression vector and an HIV-1 vector system (packaging and transfer plasmids kindly provided by D. Trono, and envelope plasmid kindly provided by J. Burns). Briefly, 12 μl Fugene 6 Transfection Reagent (Roche Diagnostics) was combined with 85.14 μl Optimem (no additives) and incubated at room temperature for 5 minutes. 1 μg of pCMVDR8.2, 1 μg of pWPTS-nlsLacZ, 0.36 μg phCMV-VSVG, and 0.5 μg of wild type or mutant pEGFP-VPS4A was combined with the transfection mixture (final volume of 100 μl) and incubated at room temperature for 20 minutes. Virions were harvested ~48 h post-transfection and used to transduce the reporter HeLa-M cell line as described previously[38] or used in Western blotting experiments to analyze virion release.

Virion Release Assays

Virion-containing supernatants and proteins from transfected cells were prepared for Western blotting as described[69; 70]. Primary antibodies used were: rabbit anti-HIV-1 MA (1:1000, D. Trono), rabbit anti-HIV-1 CA UT415 (1:1000, Covance), rabbit anti -VPS4A UT289 (1:000, Covance), and mouse anti-γ-Tubulin GTU-88 (1:5000, Abcam). Secondary antibodies used were: goat anti-rabbit ALEXA680nm (1:20,000,