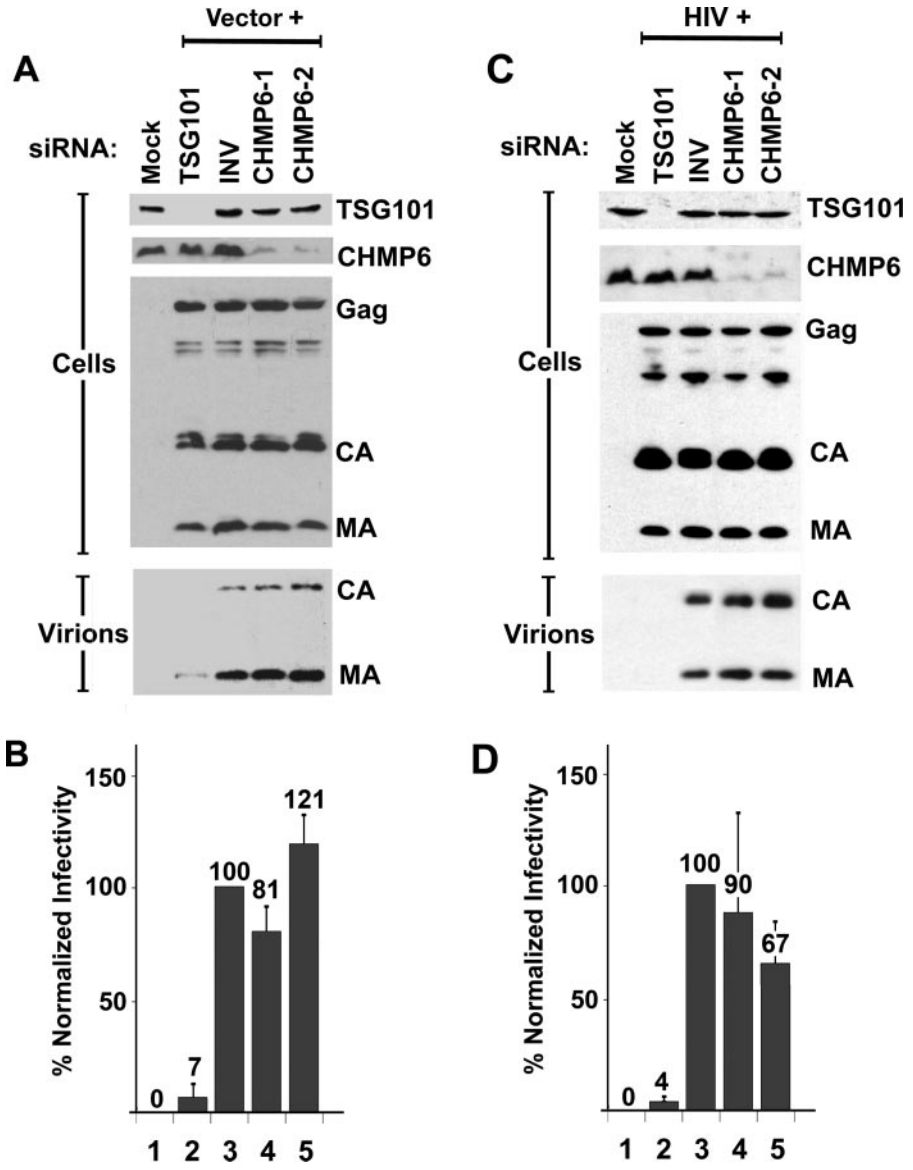

FIG. 8. HIV release and infectivity are minimally affected by CHMP6 depletion. (A) Western blot analysis of 293T cell extracts showing the efficiency of siRNA depletion of TSG101 (positive control; lane TSG101 in the top gel) or CHMP6 (lanes CHMP6-1 and -2 in the second gel from the top) and the effects on Gag protein expression (third gel from the top) and virus-like particle release (virions; bottom gel) from a cotransfected HIV-1 vector system. CHMP6 was depleted using two different siRNAs (denoted CHMP6-1 and CHMP6-2). Lanes Mock and INV are negative controls showing cells that were mock transfected without the HIV-1 vector or cotransfected with an inverted siRNA against TSG101 (INV), respectively. Cellular Gag, CA, and MA levels were monitored to ensure equal transfection and protein expression levels. The extracellular CA and MA levels reflect the relative efficiencies of virus-like particle release. The TSG101 signal intensity for TSG101-depleted cells was 2% of that of the control, and the CHMP6 signal intensities for CHMP-6-depleted cells were 12% (CHMP6-1) and 3% (CHMP6-2) relative to the control. Quantification of at least three repetitions of this experiment showed the following levels of MA and CA release: inverted siRNA, 100% (defined as the control); CHMP6-1, 133% ± 14%; and CHMP6-2, 105% ± 35%. The data were collected 48 h after the second siRNA transfection. (B) HIV-1 viral-vector titers produced by cells depleted of CHMP6 or TSG101 (positive control). Vector transduction levels were normalized to the negative control (INV) and averaged from four independent experiments. The data were collected 48 h after the second siRNA transfection. (C) Western blot analysis showing the efficiency and effects of siRNA-mediated silencing of the CHMP6 protein. The experiment was similar to that shown in panel A except that wild-type HIV-1_{NL4-3} virus was used. Quantification of at least three repetitions of this experiment showed the following levels of MA and CA release: inverted siRNA, 100% (defined as the control); CHMP6-1, 109% ± 22%; and CHMP6-2, 94% ± 38%. The data were collected 26 h after the second siRNA transfection. (D) HIV-1 titers produced by cells depleted of CHMP6 or TSG101 (positive control). Viral transduction levels were normalized to the negative control (INV) and averaged from eight independent experiments. The data were collected 26 h after the second siRNA transfection. The error bars represent standard deviations.

stimulation for 2 h, >90% of the EGFR was internalized and degraded (column 2). Importantly, EGFR degradation was significantly reduced by treatment with siRNAs against TSG101 (column 4), EAP20 (column 5), and CHMP6 (column 6), but not by treatment with a control siRNA (column 3). Depletion of the three ESCRT components did not affect receptor internalization but rather induced intracellular accumulation of internalized receptors, consistent with a defect in