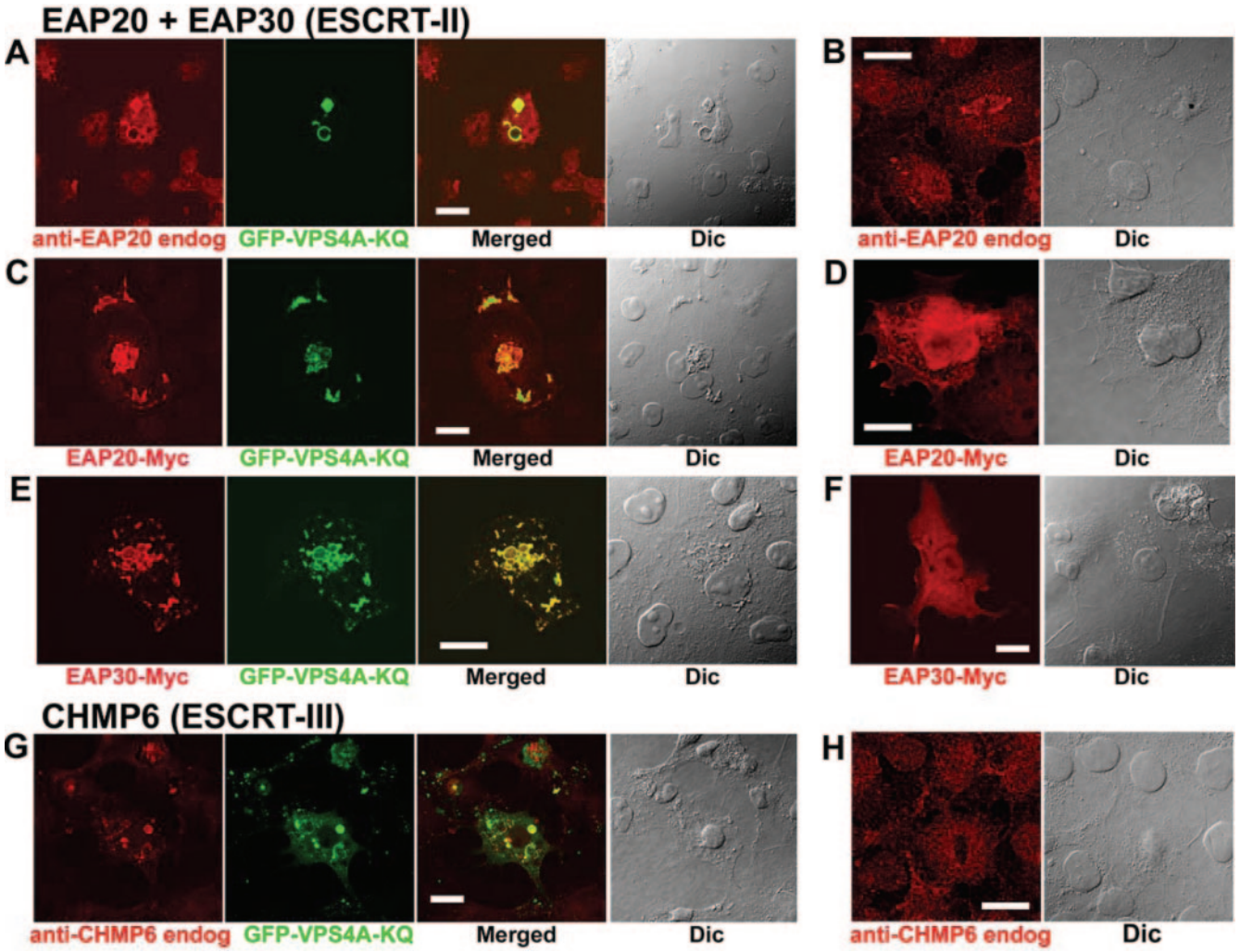

FIG. 6. Localization of ESCRT-II and ESCRT-III proteins by immunofluorescence. COS7 cells expressing fluorescent, exogenous, dominant-negative GFP-VPS4A$_{K173Q}$ (denoted GFP-VPS4A-KQ) were cotransfected and/or costained to detect the following proteins: (A) endogenous (endog) EAP20 protein (antibody UT461), (C) exogenous EAP20-Myc (anti-Myc antibody), (E) exogenous EAP30-myc (anti-Myc), and (G) endogenous CHMP6 (UT463). Column 1 (from left), ESCRT proteins alone (red); column 2, GFP-VPS4A-KQ proteins alone (green); column 3, overlaid (merged) images (colocalization on class E compartments is in yellow); column 4, differential interference contrast (Dic) images. Panels B, D, F, and H show the protein distributions in the absence of dominant-negative VPS4. Scale bars, 20 μm. Note that our two EAP20 antibodies also stained COS7 and human osteosarcoma cell centrosomes and spindles (Fig. 6B), and one of our two CHMP6 antibodies, UT 464, exhibited a punctate nuclear staining pattern in addition to staining class E membranes (not shown).

depleted cells was unexpected, and the implications of this observation were examined further by testing the requirement for CHMP6/ESCRT-III in virus release. CHMP6 was selected for study because it binds EAP20, forms the only known connection between ESCRT-II and ESCRT-III, is the only myristoylated CHMP protein (3, 88), and appears to initiate ESCRT-III assembly in yeast (3).

The effects of CHMP6 depletion on the release and infectivity of HIV-1 vectors and virus are shown in Fig. 8. As expected, virus release and infectivity were again substantially reduced upon siRNA depletion of TSG101 (Fig. 8A to D, lanes "TSG101" and bars 2), whereas treatment with an inverted control siRNA had no significant effect on virus release (defined as 100% infectivity; lanes "INV" and bars 3). Two different siRNAs were used to knock down CHMP6 (designated CHMP6-1 and CHMP6-2), and both siRNAs decreased CHMP6 levels very significantly (Fig. 8A and C, "Cells," second row) without affecting Gag protein expression or processing (third row). As with EAP20 depletion, CHMP6 depletion again failed to reduce HIV-1 release or infectivity substantially (Fig. 8A and 8C, "Virions," and B and D). Although there was some variability in multiple repetitions of this experiment, the average viral titers nevertheless fell within 40% of control levels following treatment with both CHMP6-1 and CHMP6-2 (Fig. 8B and D). At a later time point (48 h), viral infectivities were reduced four- to fivefold, possibly owing to secondary effects on general protein trafficking. We therefore conclude that normal cellular levels of CHMP6 are not required for efficient HIV-1 release or infectivity.

EAP20 and CHMP6 are required for efficient downregulation of the EGF receptor. The EGFR is normally present on the cell surface, where it can bind EGF and transmit growth