FIG. 10. Colocalization of EGF and lysosomal fluid phase markers upon depletion of TSG101, EAP20, and CHMP6. (A) HeLa cells were treated with the designated siRNA, Alexa 488-dextran (green), and EGF-Alexa Fluor 555 streptavidin (red) as described in Materials and Methods. (B) Quantification of the fractions of cells showing some colocalization of Alexa 488-dextran and EGF-Alexa Fluor 555 streptavidin under the different conditions described for panel A. The quantification of EGF (red) coincident with lysosome dextran (green) was performed in single-plane overlay analyses using the Olympus Fluoview software. Cells were scored as positive if the overlap coefficient (R) was greater than 0.5. The error bars represent standard deviations. (C) Western blot analysis showing the efficiency of siRNA-mediated depletion of TSG101, EAP20, and CHMP6.

requirements for MHC-I downregulation are similar to the requirements for HIV-1 budding.

We examined the trafficking of EGF to the lysosome in cells depleted of TSG101/ESCRT-I, EAP20/ESCRT-II, or CHMP6/ESCRT-III. HeLa cells depleted of TSG101/ESCRT-I, EAP20/ESCRT-II, or CHMP6/ESCRT-III were incubated with the fluid phase marker Alexa 488-dextran for 18 h, followed by a 4-h chase in dextran-free medium, which allows the fluid phase marker to accumulate in lysosomes (12, 78). As shown above, the unstimulated EGFR is predominantly localized to the plasma membrane. Incubation of cells with EGF-Alexa Fluor 555 streptavidin induced the internalization of the EGFR, and after 30 min, fluorescent EGF colocalized with lysosomes in 41% of the cells examined (Fig. 10, "INV,")

(86/208 cells). In contrast, cells depleted of TSG101/ESCRT-I showed no colocalization of EGF with lysosomes ("TSG101") (0/29 cells tested). Cells depleted of either EAP20/ESCRT-II or CHMP6/ESCRT-III showed intermediate phenotypes, with 7 to 10% of the cells showing EGF staining at the lysosome. To control for possible differences between different siRNA oligonucleotides, we also tested one of the siRNA oligonucleotides used to deplete EAP20 in the study by Bowers et al. This oligonucleotide (EAP20-2) also efficiently depleted EAP20 levels (Fig. 10C) and again gave an intermediate EGF localization phenotype (Fig. 10A and B, "EAP20-2") (19/258 cells positive for EAP20-2 versus 10/140 cells for EAP20-1 and 22/213 cells for CHMP6-2).

As will be reported elsewhere, we have also now found that