Maldonado-Báez et al., http://www.jcb.org/cgi/content/full/jcb.201208172/DC1

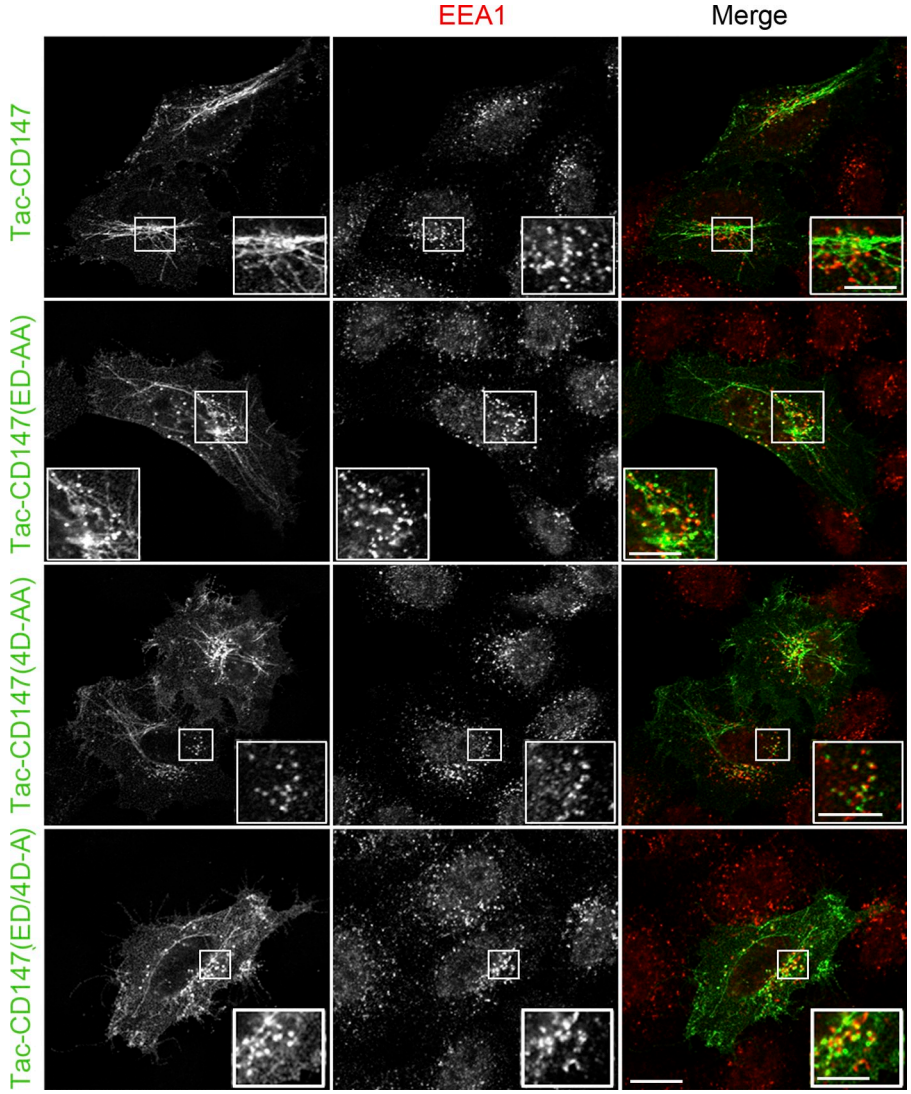


Figure S1. **Conserved acidic clusters in the carboxyl-terminal sequence of CD147 are important for sorting.** Site-directed mutagenesis analysis of two conserved acidic clusters in the cytoplasmic tail of CD147. The acidic clusters were independently mutated to Ala in the context of Tac-147-147 chimeric protein (green color in the merge image). The point mutants were expressed in HeLa cells, and their itineraries were followed after 1 h of internalization at 37°C. Cells were rinsed and fixed, and the cell surface antibody was then blocked with unlabeled goat anti–mouse IgG in the absence of saponin. EEA1 (red staining in the merge image) was labeled with a rabbit anti-EEA1 antibody. Alexa Fluor 488–conjugated goat anti–mouse antibody was used to detect internalized CIE cargo proteins, and Alexa Fluor 594–conjugated goat anti–rabbit antibody was used to detect EEA1. Insets show enlarged views of the boxed regions. Bars: (main panels) 10 µm; (insets) 5 µm.

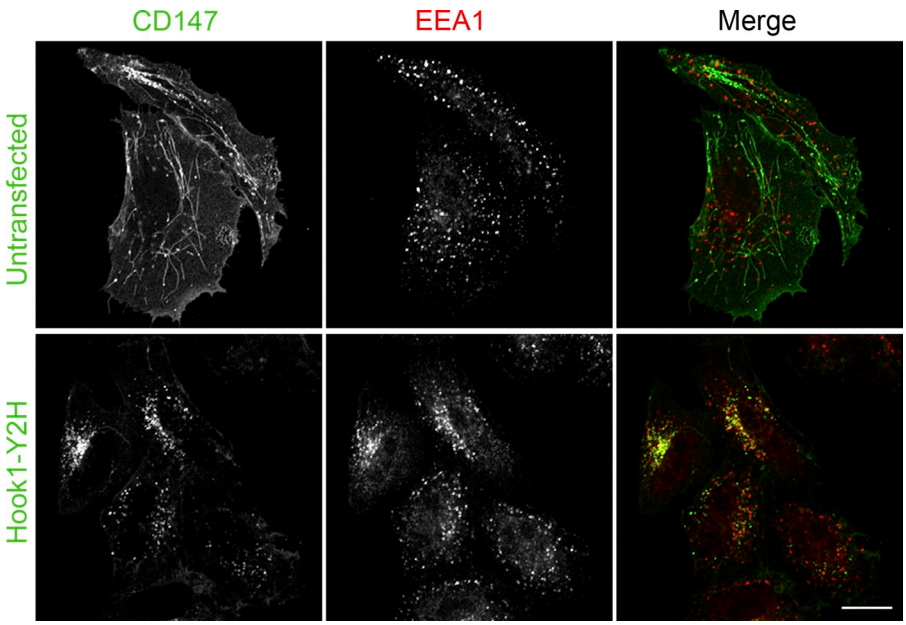
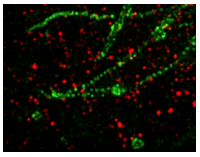
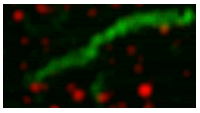


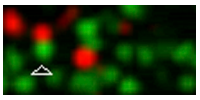
Figure S2. **Expression of Hook1-Y2H redirects CIE cargo to the EEA1-associated endosomal compartment.** HeLa cells overexpressing Hook1-Y2H were incubated with anti-CD147 antibody for 30 min at 37°C. After internalization, the cells were rinsed twice with PBS and fixed in PBS/2% formaldehyde for 10 min at room temperature. Surface anti-CD147 antibody was blocked with unlabeled goat anti–mouse IgG in the absence of saponin. EEA1 was localized using a rabbit anti-EEA1 antibody. Proteins were visualized using Alexa Fluor 488–conjugated goat anti–mouse and Alexa Fluor 594–conjugated goat anti–rabbit secondary antibodies to visualized CD147 and EEA1, respectively. Bar, 10 µm.



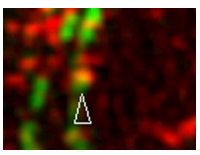
Video 1. **CD147 and Hook1 colocalize on tubular endosomes and endosomal structures.** After antibody internalization (30 min at 37°C), endogenous CD147 (green; Alexa Fluor 488) and endogenous Hook1 (red; Alexa Fluor 568) were visualized using super-resolution fluorescence microscopy in HeLa cells (SIM; DeltaVision OMX V4; Applied Precision). The video shows a 3D SIM image of internalized CD147 (green) with Hook1 (red) rotated from 0° to 14° (from Fig. 4 C).



Video 2. **Localization of Hook1 at the end of tubular endosome loaded with CD147.** Movie of a 3D SIM image (from Fig. 4 C, box 1) from 0° to 18° with 2° increments showing internalized endogenous CD147 (green; Alexa Fluor 488) with endogenous Hook1 (red; Alexa Fluor 568) on the end of a membrane tubule. Images were acquired using super-resolution fluorescence microscopy (SIM; DeltaVision OMX V4; Applied Precision).



Video 3. **CD147 and Hook1 colocalize on endosomes.** HeLa cells were allowed to internalize antibody-bound CD147 for 30 min at 37°C. After internalization, cells were processed for immunofluorescence and stained for Hook1 using a rabbit anti-Hook1 antibody. The video shows a SIM image of internalized CD147 (green; Alexa Fluor 488) with Hook1 (red; Alexa Fluor 568) rotated from 0° to 10° in 2° increments showing Hook1 with a CD147-containing endosome (from Fig. 4 C, box 2). Images were generated using super-resolution fluorescence microscopy (DeltaVision OMX V4; Applied Precision).



Video 4. **Endogenous CD98 and endogenous Hook1 colocalize in tubular endosomes.** HeLa cells were incubated with anti-CD98 antibody for 30 min at 37°C. Internalized CD98 was visualized using Alexa Fluor 488–conjugated secondary antibody, and Hook1 was labeled with rabbit anti-Hook1 and Alexa Fluor 568–conjugated secondary antibody. Super-resolution fluorescence microscopy was used to image the internalized CD98 (green) with Hook1 (red) in the middle of a membrane tubule (SIM image rotated from 0° to 12° in 2° increments; DeltaVision OMX V4; Applied Precision).