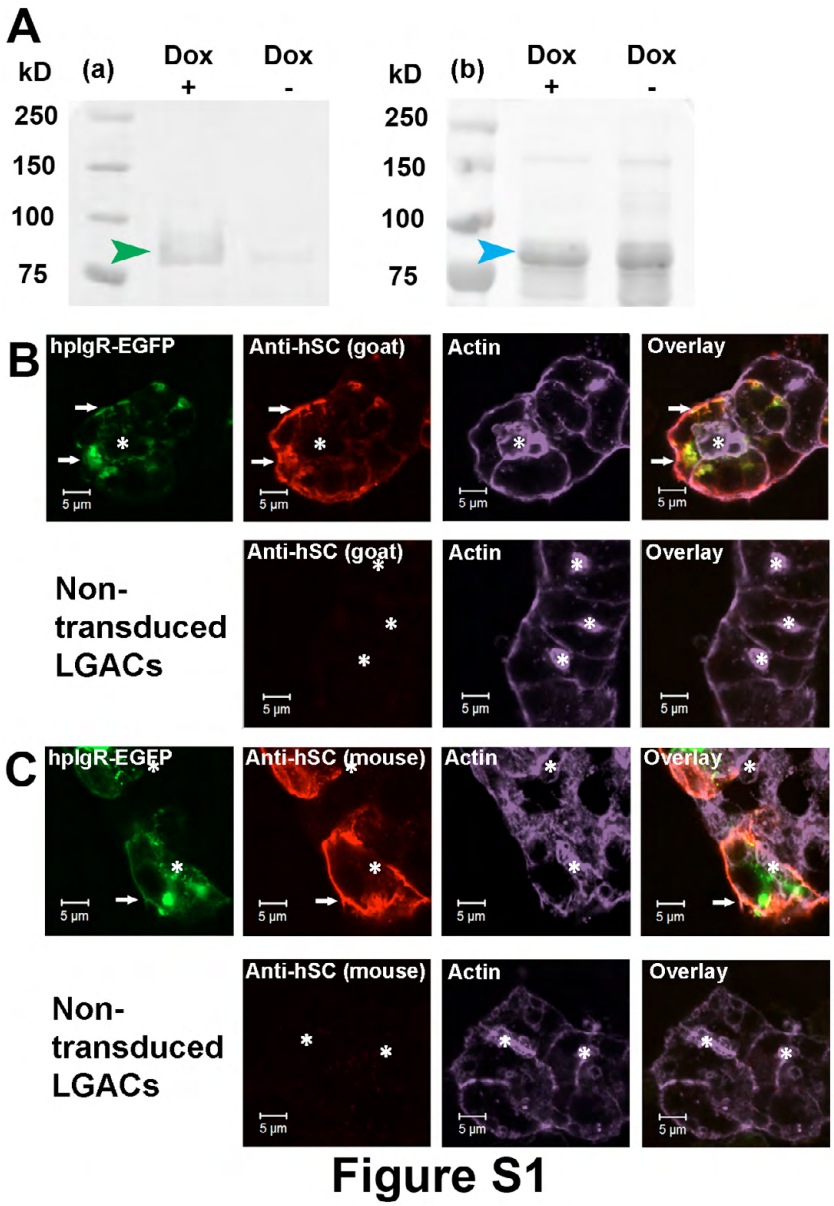


Figure S1

Fig. S1. Goat and mouse anti-hSC antibodies distinguish hSC from endogenous rabbit SC. A) LGACs were co-transduced with Ad hpIgR-EGFP and Adeno-X Tet-On®, with (+) or without (-) 0.1 µg/mL doxycycline. Supernatant from LGACs cultured overnight after transduction was concentrated with a Vivaspin-500 concentrator, and analyzed by western blotting, using primary goat anti-hSC antibody (a) or sheep anti-rabbit SC serum (b), and secondary IRDye®800-conjugated donkey anti-goat or Alexa Fluor®-680-conjugated donkey anti-sheep antibodies, respectively.. B) Live LGACs expressing hpIgR-EGFP or non-transduced LGACs were fixed, permeabilized and labeled with primary goat anti-hSC antibody, secondary Alexa Fluor®-568-conjugated donkey anti-goat antibody and Alexa Fluor®-647-conjugated Phalloidin. C) Live LGACs expressing hpIgR-EGFP or non-transduced LGACs were incubated with mouse anti-hSC antibody at 4°C for 1 hour. After rinsing, cells were fixed, permeabilized and labeled with secondary Alexa-Fluor-568-conjugated goat anti-mouse antibody and Alexa Fluor®-647-conjugated phalloidin. White arrows, colocalization; *, lumena; scale bar: 5 µm.

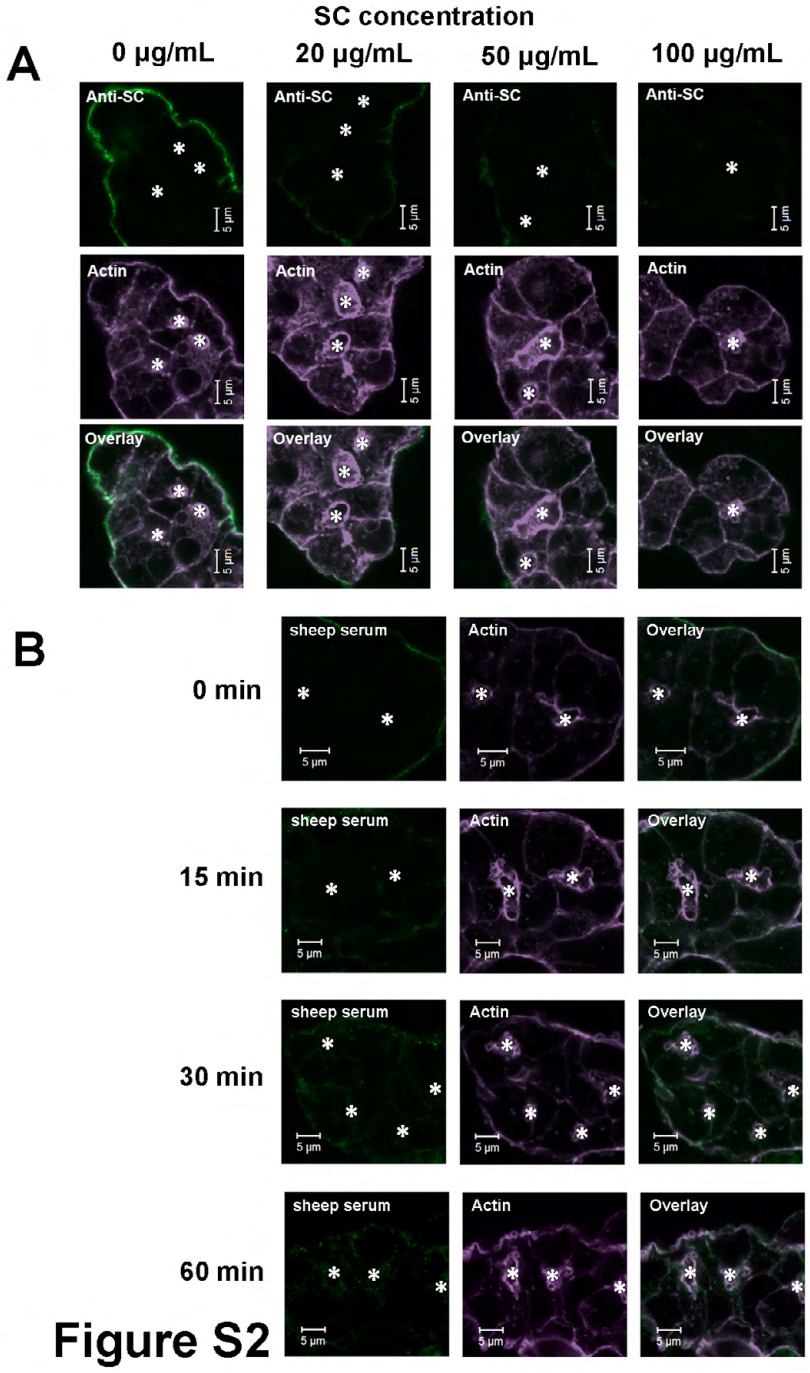


Fig. S2. Sheep anti-SC serum specifically recognizes pIgR on the basolateral membrane of LGACs. A) non-transduced LGACs were incubated with sheep anti-SC serum and purified rabbit SC at scaling up concentrations for 1 hour at 4°C. After extensive rinses, LGACs were fixed, permeabilized, and labeled with secondary Alexa-Fluor-488-conjugated donkey anti-sheep antibody and Alexa-Fluor-647-conjugated phalloidin. B) non-transduced LGACs were incubated with normal sheep serum for 1 hour at 4°C, rinsed and incubated at 37°C. Cells were fixed at time points indicated, permeabilized, and labeled with secondary Alexa-Fluor-488-conjugated donkey anti-sheep and Alexa Fluor®-647-conjugated phalloidin. *, lumena; scale bar: 5 µm.

Fig. S3. Characterization of the regulated secretory pathway in LGACs. A) LGACs were fixed, permeabilized and labeled with primary rabbit anti-Rab3D serum, primary mouse anti-γ-adaptin antibody, secondary Alexa Fluor®-488-conjugated donkey anti-rabbit and Alexa Fluor®-568-conjugated goat anti-mouse antibodies, as well as Alexa Fluor®-647-conjugated phalloidin. Actin stained with Alexa Fluor®-647-conjugated phalloidin is displayed in purple in the overlay image. High magnification image displays a region of 10 x 10 µm

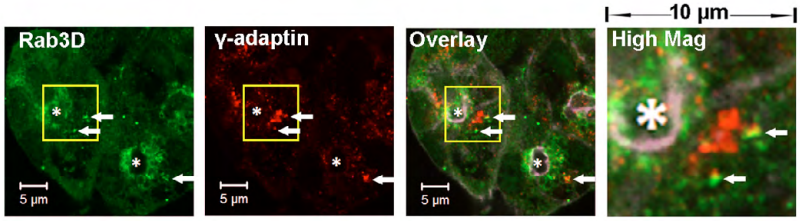


Figure S3

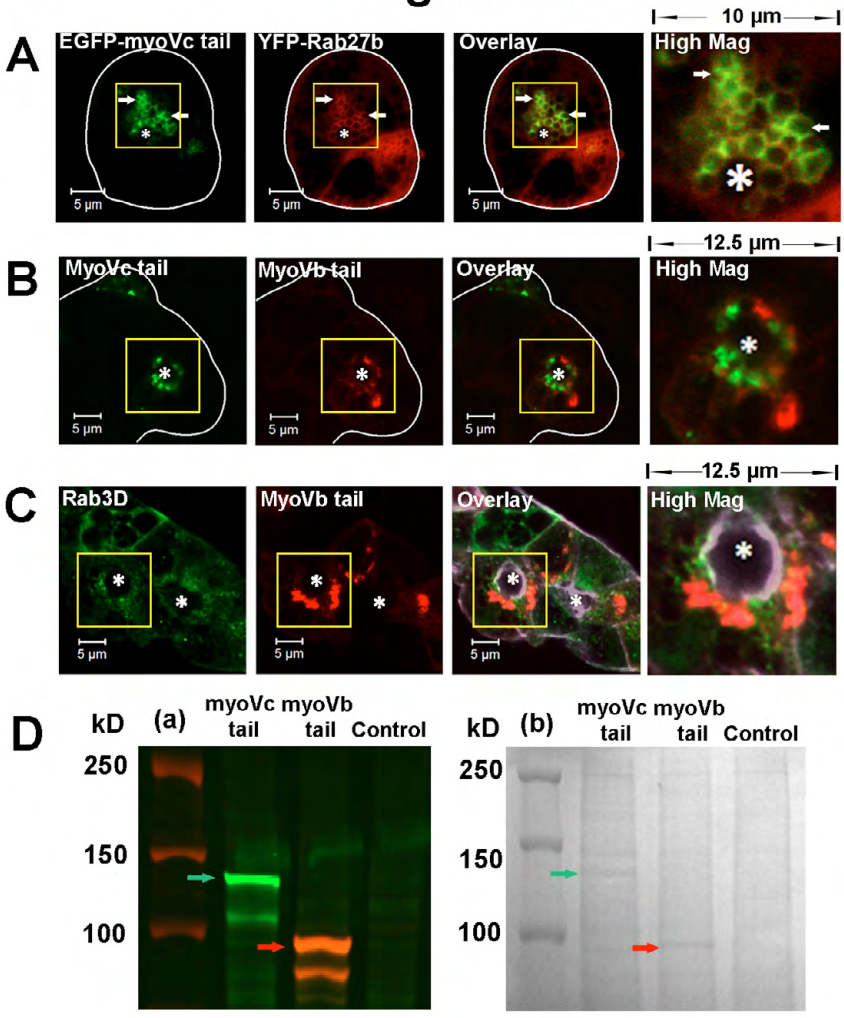
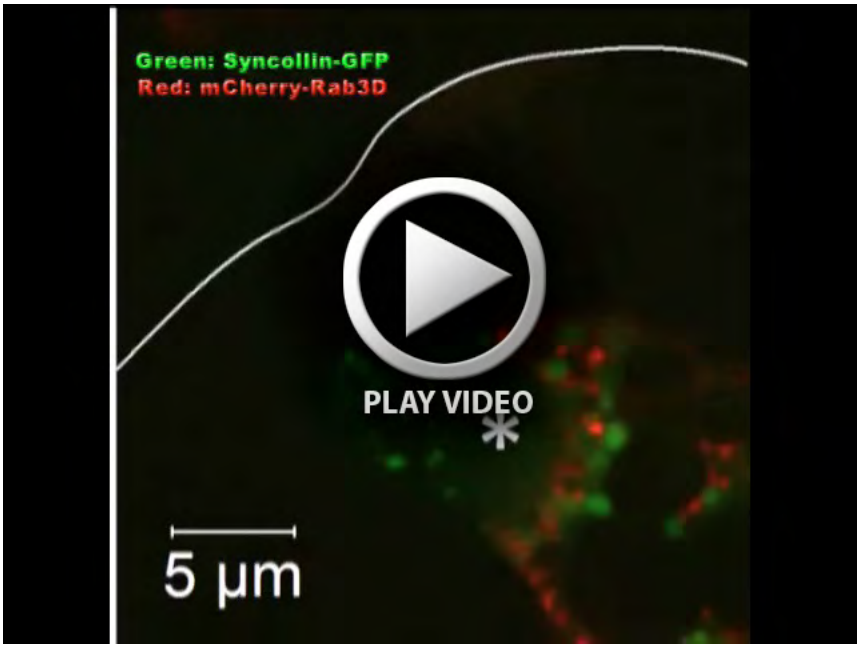
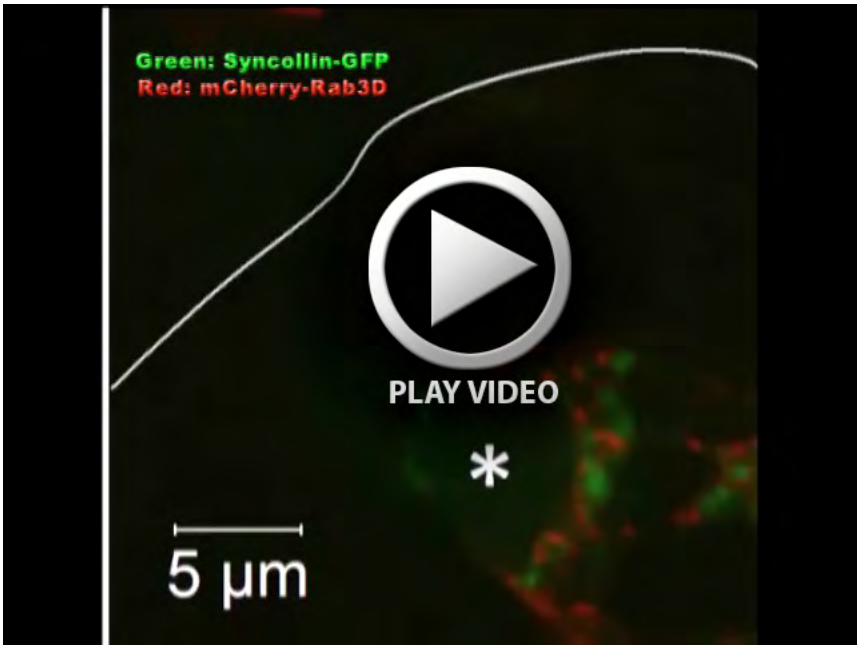


Figure S4

Fig. S4. Characterization of over-expressed mCherry-myosin Vb tail and EGFP-myosin Vc tail in LGACs. A) Live LGACs co-expressing EGFP-myosin Vc tail and YFP-Rab27b were observed by confocal fluorescence microscopy. Cellular outlines were obtained by comparing the respective image with the differential interference contrast (DIC) image. B) Live LGACs co-expressing hpIgR-EGFP and mCherry-myosin Vb tail were observed by confocal fluorescence microscopy. Cellular outlines were obtained by comparing the respective image with the differential interference contrast (DIC) image. C) LGACs expressing mCherry-myosin Vb tail were fixed, permeabilized and labeled with primary rabbit anti-Rab3D serum, secondary Alexa Fluor®-488-conjugated donkey anti-rabbit antibody and Alexa Fluor®-647-conjugated phalloidin. Actin stained with Alexa Fluor®-647-conjugated phalloidin is displayed in purple in the overlay image. High magnification image displays a region of 10 x 10 µm (A) or 12.5 x 12.5 µm (B, C) from the yellow boxed image to the left. *, lumena; scale bar: 5 µm. D) The LGACs expressing EGFP-myosin Vc tail, mCherry-myosin Vb tail, or LifeAct–TagRFP (control) were lyzed with 4x SDS-PAGE loading buffer and resolved by 7.5% SDS-PAGE gels, which were subsequently used for further western blotting (a) or commassie blue staining (b). Primary rabbit anti-myosin Vc tail and goat anti-myosin Vb antibodies, as well as secondary IRDye®800-conjutated goat anti-rabbit and IRDye®700-conjugated donkey anti-goat antibodies were used for western blotting. Green arrow, EGFP-myosin Vc tail; red arrow, mCherry-myosin Vb tail.



Movie 1. Syncollin-GFP-enriched vesicles and mCherry-Rab3D-enriched vesicles in resting LGACs. LGACs grown on a 35-mm glass-bottomed plate were co-transduced with Ad constructs on the second day of culture for co-expression of syncollin-GFP and mCherry-Rab3D. After 0.1 µg/mL doxycycline induction overnight, LGACs were mounted in a 37°C incubation chamber and imaged. ~7.5-minute of real time is represented in this movie. *, lumena; scale bar: 5 µm.



Movie 2. Syncollin-GFP-enriched vesicles and mCherry-Rab3D-enriched vesicles in LGACs stimulated with CCh. LGACs grown on a 35-mm glass-bottomed plate were co-transduced with Ad constructs on the second day of culture for co-expression of syncollin-GFP and mCherry-Rab3D. After 0.1 µg/mL doxycycline induction overnight, LGACs were mounted in a 37°C incubation chamber and imaged immediately after application of 100 µM CCh. ~7.5-minute of real time is represented in this movie. *, lumena; scale bar: 5 µm.

Table S1. DNA and protein sequences

DNA sequence of the coding region of Ad pIgR-EGFP construct:

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3090bp

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1026 AA

DNA sequence of the coding region of Ad mCherry-Rab3D construct:

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1416 bp

Sequence of the translated mCherry-Rab3D

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467AA

Theoretical pI/Mw: 5.13 / 51706.88