

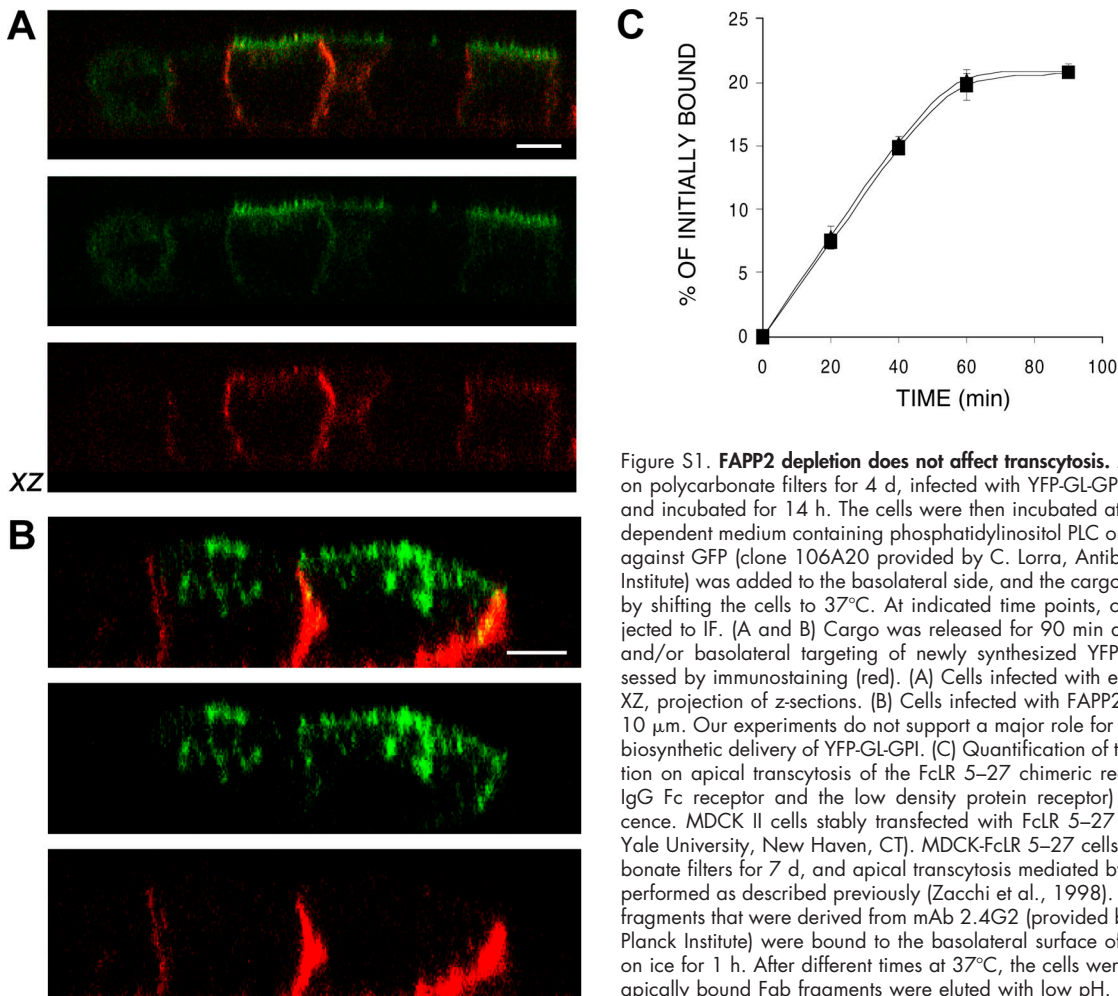
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#### Cloning of A-VSVG

A-VSVG-GFP is based on a previously described VSVG3 fluorescent protein (Toomre et al., 1999) and differs from it by having a mutated basolateral sorting signal ( $Y^{501}xxL^{504}-A^{501}xxA^{504}$ ). These mutations were made in order to minimize (in addition to steric hindrance by GFP that was attached adjacent to the tyrosine-based motif) potential interactions with components of the basolateral transport machinery. VSVG-tsO45 was PCR amplified with the following primers and cloned into pEGFP-C3 (CLONTECH Laboratories, Inc.): sense, 5'-TTCGAATTCGCCATGAAGTGCCTTTG-3'; and antisense, 5'-TTTGATCCCCACTAGTCTTCCAAGTCGGTTCATCTCTGCGTCTGTAGCAATCTGTC-3'.

#### Other oligonucleotide sequences

Oligonucleotides that were used to subclone into pShuttle are listed as follows: YFP-PH, sense (5'-GTCGACATGGTGAGCAAGGGCG-3') and antisense (5'-GCGGCCGCTCAAGTCCTGTATCAGT-3'); GFP-FAPP, sense (5'-GTCGACATGGTGAGCAAGGGCG-3') and antisense (5'-GCGGCCGCTCAGGAAGAGAAGGATGGAAG-3'); and GFP-FAPP2, sense (5'-GCGGCCGCTCAAGTCCTGTATCAGT-3') and antisense (5'-TCTAGATTATAAAGCGTCAGTATGGC-3'). The target sequence that was used for FAPP1 KD was 5'-GAACCTACTCAGATACAGA-3'. The target sequences that were used for FAPP2 KD were 5'-GGCTTGCTGACGATAGT-3' or 5'-GACTCTACTGTGACCTCT-3'.



**Figure S1. FAPP2 depletion does not affect transcytosis.** MDCK cells were grown on polycarbonate filters for 4 d, infected with YFP-GL-GPI-expressing adenovirus, and incubated for 14 h. The cells were then incubated at 20°C for 3 h in CO<sub>2</sub>-independent medium containing phosphatidylinositol PLC on the apical side. A mAb against GFP (clone 106A20 provided by C. Lorra, Antibody facility, Max Planck Institute) was added to the basolateral side, and the cargo was released from TGN by shifting the cells to 37°C. At indicated time points, cells were fixed and subjected to IF. (A and B) Cargo was released for 90 min at 37°C, and transcytosis and/or basolateral targeting of newly synthesized YFP-GL-GPI (green) was assessed by immunostaining (red). (A) Cells infected with empty adenovirus control. XZ, projection of z-sections. (B) Cells infected with FAPP2 KD adenovirus. Bars, 10 μm. Our experiments do not support a major role for transcytosis in the apical biosynthetic delivery of YFP-GL-GPI. (C) Quantification of the effect of FAPP2 depletion on apical transcytosis of the FcLR 5-27 chimeric receptor (composed of the IgG Fc receptor and the low density protein receptor) by electrochemiluminescence. MDCK II cells stably transfected with FcLR 5-27 (a gift from I. Mellman, Yale University, New Haven, CT). MDCK-FcLR 5-27 cells were grown on polycarbonate filters for 7 d, and apical transcytosis mediated by chimeric receptors was performed as described previously (Zacchi et al., 1998). In brief, biotinylated Fab fragments that were derived from mAb 2.4G2 (provided by C. Schnatwinkel, Max Planck Institute) were bound to the basolateral surface of transfected MDCK cells on ice for 1 h. After different times at 37°C, the cells were cooled on ice, and the apically bound Fab fragments were eluted with low pH. Intracellular and basolat-

eral Fab fragments, which were unaffected by the low pH treatment, were harvested by extracting the cells for 15 min at RT with lysis buffer. Biotinylated Fab fragments were quantified by an electrochemiluminescence assay on an imager. The detection of biotinylated Fab fragments that transcytosed to the apical cell surface (acid wash solution) and cell lysates was performed on streptavidin-coated standard Multi-Array 96 plates. Biotinylated Fab fragments were allowed to bind to the streptavidin-coated wells for 1 h at RT with agitation. The wells were washed twice with PBS/0.2% NP-40 and were incubated with 50 ng of the goat anti-rat IgG (Fab) 2 fragment. After another wash with 150 μl PBS/0.2% NP-40, 50 ng of the detection antibody Sulfo-TAG anti-goat IgG were added in 30 μl PBS/0.2% NP-40. Binding of the detection antibody was allowed to occur for 1 h at RT with agitation. The wells were washed again with 150 μl PBS/0.2% NP-40, 150 μl Read Buffer T was added, and the plate was read immediately. The output (ECL counts) was used to calculate the fraction of biotinylated Fab fragments at the cell surface. Transcytosis was determined from the amount of apically eluted biotinylated Fab as the percentage of the originally bound Fab fragments to the basolateral surface. Triangles, control; squares, FAPP2 KD cells. Data are means ± SEM from three independent experiments.

### Quantification of cell surface arrival of YFP-GL-GPI by electrochemiluminescence

Cells expressing YFP-GL-GPI were incubated at 20°C for 3 h in CO<sub>2</sub>-independent medium containing phosphatidylinositol PLC in the apical side. The cargo was released by shifting the cells to 37°C in the presence of cycloheximide for different times. YFP-GL-GPI that had arrived at the cell surface was then released by trypsin treatment for 15 min at 4°C. Intracellular YFP-GL-GPI, which was unaffected by the trypsin treatment, was harvested by extracting the cells for 15 min at RT with lysis buffer. YFP-GL-GPI was quantified by an electrochemiluminescence assay on an imager (SECTOR Imager 6000; Meso Scale Discovery). The imager's platform was based on Multi-Array technology (Meso Scale Discovery), which is a proprietary combination of patterned arrays and electrochemiluminescence detection enabling large numbers of measurements with exceptional sensitivity (10<sup>6</sup> molecules), wide dynamic range (six logs), and convenience. It uses custom multiwell plates with integrated electrodes to achieve rapid readouts that approach one plate per minute for 96-, 384-, or 1536-well plate formats. The measurement of YFP-GL-GPI was performed on standard Multi-Array 96 plates. The carbon surface in the wells was coated with 0.5 µl of a 20 ng/µl dilution of rabbit anti-GFP antibody KG41 (provided by P. Keller, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany) in 50 mM Tris, pH 7.5, 50 mM NaCl, and 0.015% Triton X-100. After the spots had completely dried, the wells were washed once with 150 µl PBS/0.2% NP-40. Then, 20 µl PBS, 0.06% NP-40, 0.006% SDS, 100 mM Hepes, pH 7.25, and 15 µl trypsin solution or cell lysate, respectively, were added. YFP-GL-GPI was allowed to bind to the immobilized capture antibody KG41 for 1 h at RT with agitation. After another wash with 150 µl PBS/0.2% NP-40, 20 ng of the detection antibody Sulfo-TAG-KG41 were added in 30 µl PBS/0.2% NP-40. Sulfo-TAG-KG41 was prepared by incubating antibody KG41 with Sulfo-TAG-NHS-Ester (Ruthenium [II] Tris-bipyridine and N-hydroxysuccinimide; Meso Scale Discovery) as suggested by the manufacturer. As KG41 is a polyclonal antibody recognizing multiple epitopes, it was possible to use the same antibody for both the capture and detection of YFP-GL-GPI. Identical results were obtained by a combination of rabbit and goat anti-GFP antibodies for capture and detection, respectively. Binding of the detection antibody was allowed to occur for 1 h at RT with agitation. The wells were washed again with 150 µl PBS/0.2% NP-40. 150 µl Read Buffer T (Meso Scale Discovery) was added, and the plate was read immediately. The output (ECL counts) was used to calculate the fraction of YFP-GL-GPI at the cell surface.

## References

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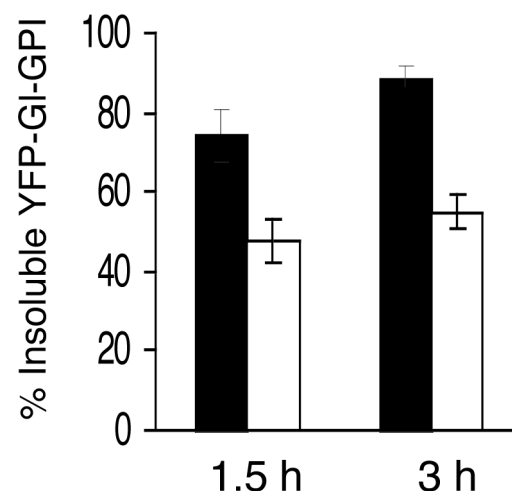


Figure S2. **FAPP2 depletion affects the detergent solubility of YFP-GL-GPI.** Control and FAPP2 KD MDCK cells were infected with YFP-GL-GPI-expressing adenovirus. 14 h later, the detergent solubility of YFP-GL-GPI was assessed as described previously (Schuck et al., 2003). In brief, cells were starved for 30 min in methionine-free medium, radiolabeled for 15 min with [<sup>35</sup>S]methionine, and chased for the indicated times. The cells were rinsed with ice-cold PBS containing calcium and magnesium, scraped into TNE, homogenized through a needle, and extracted on ice for 20 min in the presence of 1% (vol/vol) Triton X-100. Samples were adjusted to 40% OPTIPREP (Axis-Shield). Samples were overlaid with 30 and 5% OPTIPREP layers. Discontinuous gradients were centrifuged at 55,000 rpm for 2 h in an ultracentrifuge (model TLS55; Beckman Coulter). Two fractions were collected. YFP-GL-GPI was immunoprecipitated from these fractions with a polyclonal anti-GFP antibody and analyzed by 10% SDS-PAGE. YFP-GL-GPI signal was quantified using a PhosphorImager (model BAS-1800II; Fujifilm). Black bar, control cells; white bar, FAPP2 KD cells. The values plotted are means ± SEM of three independent experiments performed in duplicate.