Supplementary Figure Legends

Supplementary Figure 1. Gb3 levels are increased at the plasma membrane of α-gal A siRNA silenced cells. Surface staining of CD77/Gb3 in non-permeabilized MDCK control cells (left) and α-gal A siRNA silenced cells (right). Scale bar: 5 μm.

Supplementary Figure 2. Steady-state distribution of raft-associated and raft-independent basolateral cargoes is not perturbed by α-gal A siRNA silencing. Indirect immunofluorescence staining of the raft-independent cell adhesion protein E-cadherin (upper panels), the raft associated pump Na^+/K^+ ATPase (middle panels) and the raft associated scaffolding protein caveolin-1 (lower panels) in cells transfected with control siRNA (left) and α-gal A siRNA (right) for four days. Images taken at the level of the apical surface and at a medial plane are shown for each protein, and xz reconstructions are shown below. Scale bar: 5 μm

Supplementary Figure 3. Representative brightness vs. intensity histograms of GFP-GPI in untreated and antibody-clustered control and α-gal A silenced cells. Histograms (Brightness vs. Intensity) for control and α-gal A siRNA treated MDCK cells expressing GFP-GPI in the absence (-Ab) or presence (+Ab) of anti-GFP antibody. Individual points represent data from a single pixel within the region of interest (roi) shown adjacent to each plot. The y-axis values correspond to increasing brightness (i.e., a higher average oligomeric size of fluorescent particles in the pixel) while increasing x-axis values correspond to higher signal intensities within each pixel (i.e., higher levels of fluorescence intensity in that pixel). For example, a pixel containing large numbers of monomers would map to the intersection of a small y-axis value and large x-axis value. Conversely, a pixel containing a small number of larger oligomers would map to a larger y-axis value and a low value on the x-axis. Brightness values in these histograms are raw values of the sum of the molecular brightness and the detector noise and, in this plot, are not normalized to GFP. Subtraction of one from the B value gives ε (counts/dwell time/molecule). Multiplication of ε by the dwell time used for acquisition and divided by the average molecular brightness for GFP provides the normalized brightness as reported in Figure 5, which enables direct quantitation of oligomeric size (e.g., monomer, dimer, etc.). In the representative examples shown here, pixels bounded by the red rectangular box are mapped onto the adjacent roi image. Note that selected pixels map evenly across the entire imaged area of the cell (i.e., there are no brightness “hot-spots”). In contrast, numerous brighter pixels are evident in histograms of cells incubated with anti-GFP antibody, and these brighter pixels map to large clusters in the roi.

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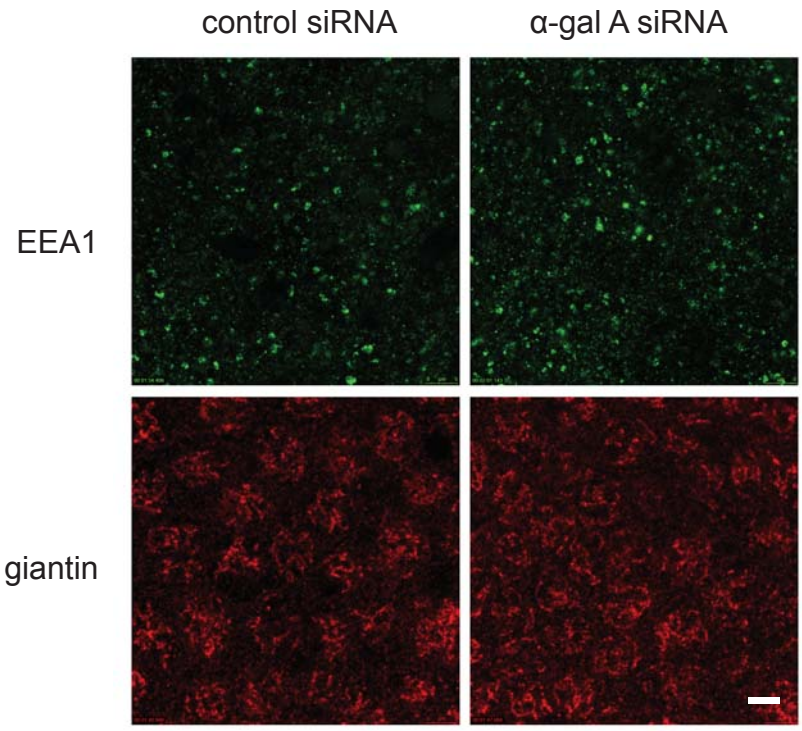
Supplementary Figure 1



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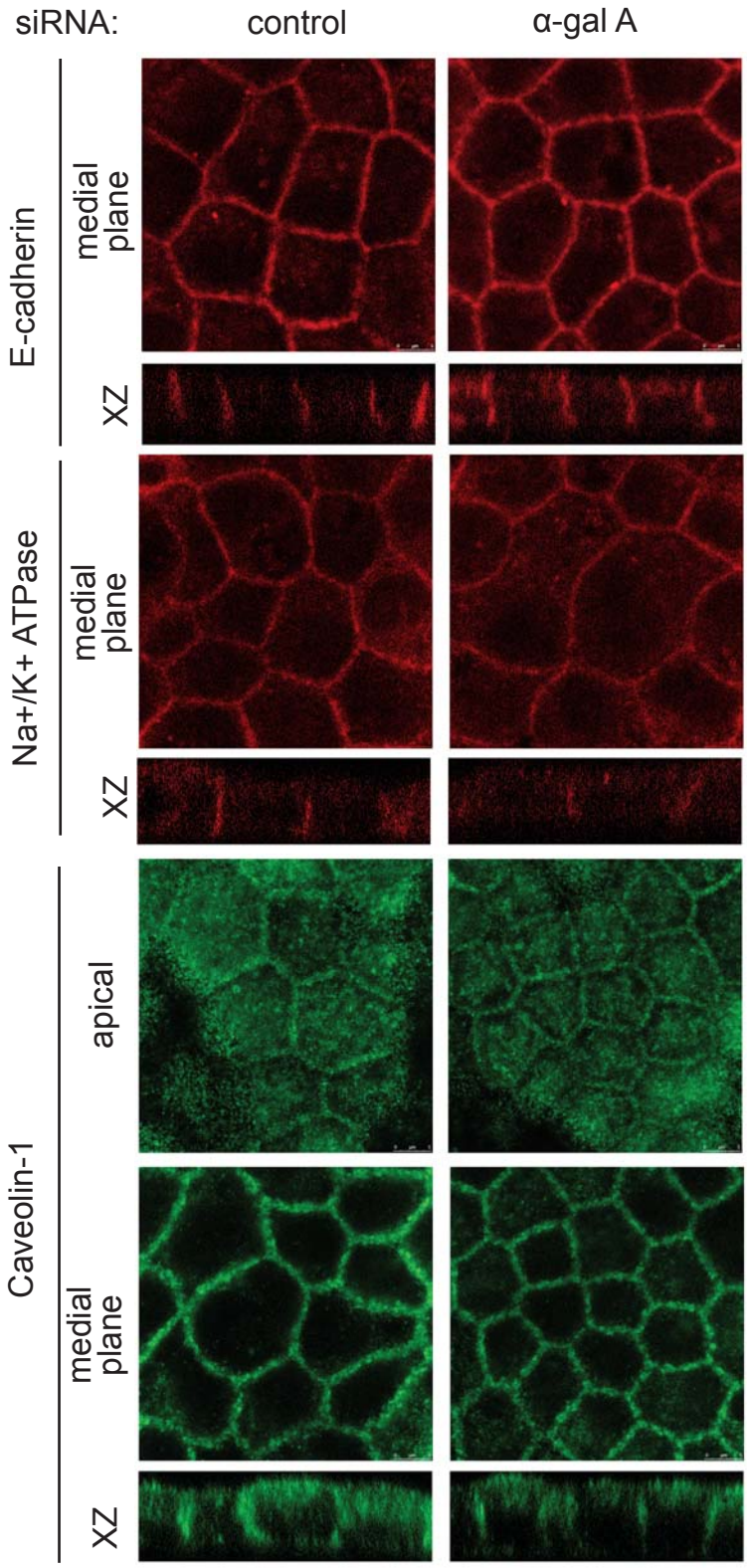
Supplementary Figure 2



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Supplementary Figure 3