Nelms et al., https://doi.org/10.1083/jcb.201609035

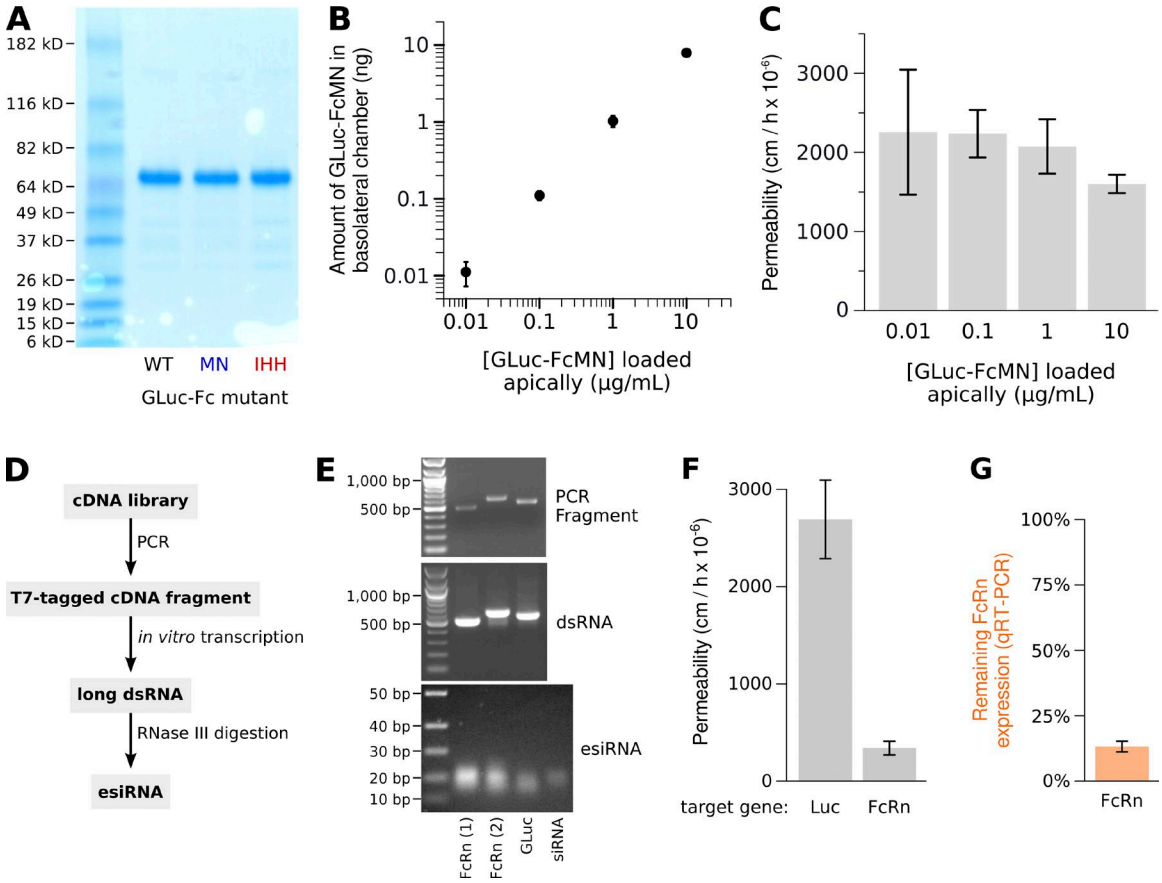


Figure S1. **Development of model for FcRn transcytosis and esiRNA gene KD.** (A) SDS-PAGE of the purified recombinant Gluc-Fc fusion protein, including wild type (WT), high-affinity binding (MN), and nonbinding (IHH) Fc mutants. (B and C) Dose dependency of Gluc-FcMN transport (mean ± SD; $n = 3$ filters × 2 technical replicates). (D and E) Schematic for preparation of esiRNAs and validation of intermediate and final products. (F and G) Validation of esiRNA KD for FcRn. MDCK cells transfected with esiRNA against FcRn exhibit strongly inhibited transcytosis of Gluc-FcMN compared with MDCK cells transfected with control nontargeting esiRNA against luciferase ($P < 0.05$ by a *t* test; $n = 3$ filters; F), and reduced FcRn expression as measured by quantitative RT-PCR (mean ± SD; $P < 0.05$ by a *t* test; $n = 3$ filters × 2 technical replicates; G). The degree of inhibition for Gluc-FcMN transport (80%; F) correlates closely to the degree of gene suppression (mean ± SD; G). Quantitative PCR primers used for expression measurements are for FcRn 5′-AGGAGGAGCTCTGTTGT GGA-3′ and 5′-CAAATCAGCATCCTGGGCCT-3′.

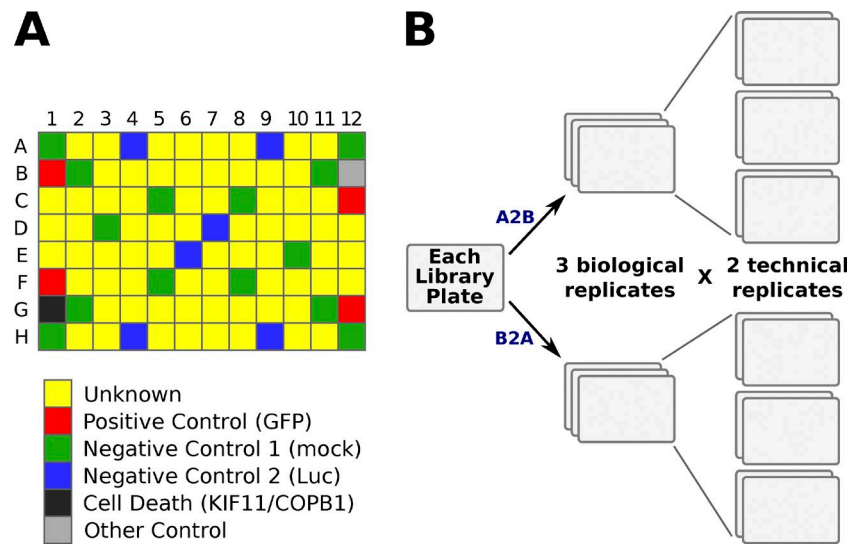


Figure S2. **Plate layout and replication design.** (A) Plate layout showing the position of all controls. (B) Diagram of replication schema.

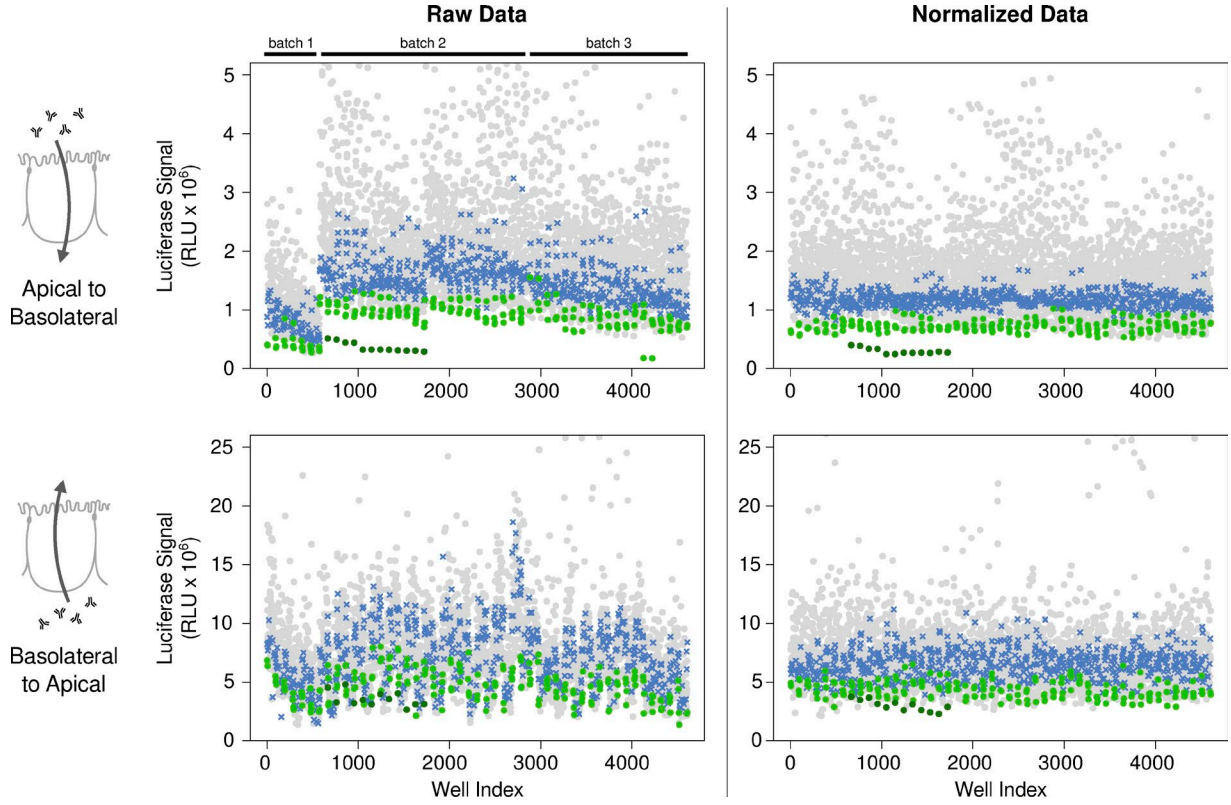


Figure S3. **Plot of normalized (right) and unnormalized (left) luciferase measurements from the screen.** Each dot corresponds to a single luciferase measurement; there are six measurements for each esiRNA (three biological × two technical replicates). Green dots, positive control 1 (esiRNA against FcRn-GFP, weak KD); dark green dots, positive control 2 (esiRNA against FcRn-GFP, strong KD); blue crosses, negative control wells (mock transfection); gray dots, other esiRNAs.

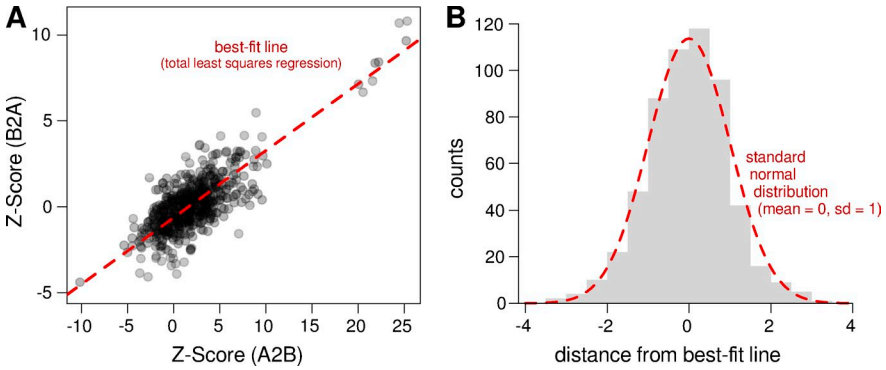


Figure S4. **Comparison of apical to basolateral (A2B) and basolateral to apical (B2A) transport.** (A) Z-scores for A2B and B2A transport for all esiRNAs; dotted red line, the best fit line from a total least squares regression. (B) Histogram of the distance from the best fit line. If both directions of transport are fully explained by the best fit line, this histogram is expected to fit a standard normal distribution (mean = 0, SD = 1). The standard normal distribution, plotted as a dotted red line, fits the observed data.

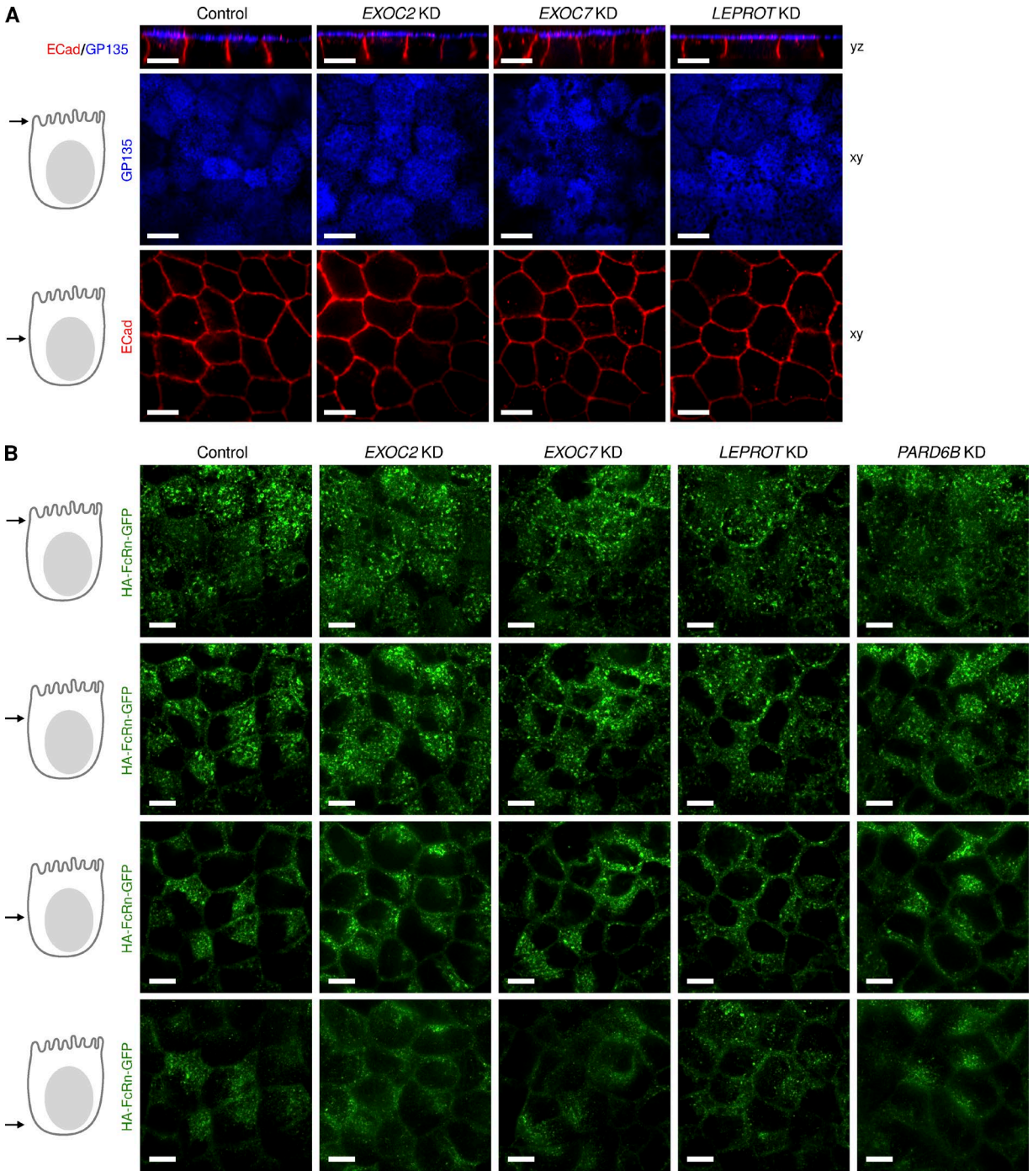


Figure S5. **Assessment of membrane polarity and endosome morphology.** (A) Immunolocalization of the apical membrane marker GP135 (blue) and lateral membrane marker E-cadherin (red) after esiRNA depletion of *EXOC2*, *EXOC7*, and *LEPROT*. Control indicates treatment with nontargeting control esiRNA against Gaussia luciferase. (B) Localization of HA-tagged FcRn-EGFP after esiRNA depletion of the indicated genes by direct confocal microscopy. Cartoon on left indicates the focal plane for each row of images. Bars, 10 µm.

**Provided online are four Excel tables. Table S1 shows the relative permeabilities for all genes used in the pilot screen. Table S2 shows the final list of genes curated for the screen, the list of genes curated in the high-throughput and literature gene sets, and the list of genes curated based on their containing a domain linked to trafficking. Table S3 shows the list of genes and sequences used to prepare esiRNA for the full and pilot screens and the second esiRNAs prepared for the validation studies. Table S4 shows the entire list of ranked Z-scores for each gene studied.**