Supplementary Materials for

Spatially resolved cell polarity proteomics of a human epiblast model


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The PDF file includes:

- Figs. S1 to S8
- Legends for tables S1 to S4
- Legends for movies S1 to S4
- References

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/17/eabd8407/DC1)

- Tables S1 to S4
- Movies S1 to S4
Figure S1. APEX2-based proximity biotinylation shows high spatial specificity in the hPSC-cyst epiblast model, Related to Figure 1

(A) Western blot analyses of d3 hPSC-cysts carrying indicated DOX-inducible APEX constructs, blotted using antibodies for indicated proteins that were used to generate APEX fusion constructs (anti-PODXL, -EZRIN, -ATP1B1, -SDC1) as well as with anti-FLAG.

(B) d3 APEX2 hPSC-cysts generated from distinct APEX2 lines (Fig. 1A) were stained for pluripotency markers (OCT4 and NANOG) and F-actin (Phalloidin). Cysts generated from five APEX2 hESC lines maintain pluripotency. Nuclei = Hoechst (blue). Scale bars = 20µm.

(C) APEX2 labeling analysis in Figure 1E including negative controls (BP only and H2O2 only), stained with apical markers (pERM, PODXL-APEX2, EZRIN-APEX2, APEX2-NES) and basolateral membrane markers (ECAD, APEX2-ATP1B1, SDC1-APEX2). Omitting
either BP or H$_2$O$_2$ abolished the biotinylated signal. Note that BP and H$_2$O$_2$ samples are identical to Fig. 1E. Nuclei = Hoechst (blue). Scale bars = 20µm.

(D) Whole cell lysate streptavidin blot analysis including H$_2$O$_2$ only negative controls. Total protein staining is shown adjacent to the streptavidin blot. Note that the endogenous biotinylated proteins were present in all conditions (indicated by red arrowheads) as previously described (95).
Figure S2. Cutoff analysis and ratiometric scatter plots of additional biological replicates, Related to Figure 2
(A,B) Histograms and ROC curves of apical replicate 2 (A) and basolateral replicate 2 (B), illustrating how the TMT ratio cutoffs were determined for each replicate.

(C,D) Histograms and ROC curves of apical replicate 3 (C) and basolateral replicate 3 (D).

(E) Correlation of other biological replicates.

(F) Venn diagrams showing protein overlap between three apical replicates (left) and three basolateral replicates (right). The datasets after Filter 3 were presented for each apical/basolateral replicate.

(G) Venn diagram showing overlap between the final apical and basolateral territory proteomes. There are 611 proteins in total from these two proteomes. 30 proteins are shared by both proteomes and are considered non-polar membrane proximal proteins.

(H) Scatter plots showing enrichment of proteins after Filters 1 and 2. All proteins detected in apical replicate 2 (PODXL-APEX2, upper left), basolateral replicate 2 (APEX2-ATP1B1, upper right), apical replicate 3 (EZRIN-APEX2, lower left) and basolateral replicate 3 (SDC1-APEX2, lower right) are shown. Each protein is colored based on the UniProt annotation. Selected highly enriched proteins are labeled. Dotted lines indicate cut-offs used in Filter 1 and Filter 2.

(I) Data for additional replicates shown in Fig. 2I. Ratiometric scatter plots based on normalized TMT ratios of apical #2 (top, x-axis – 127N/131) and #3 (bottom, x-axis – 127C/131) and basolateral #2 (top, y-axis – 128C/131) and #3 (bottom, y-axis – 129N/131) of 4454 proteins revealing a clear enrichment of proteins from apical (red), basolateral (blue) and non-polar (green) lists in distinct domains of the scatter plot. Vertical and horizontal dotted lines indicate Filter 1 cut-offs for apical and basolateral, respectively. A cohort of well-established apical and basolateral proteins as are labeled. Some proteins identified through Filter 4 are indicated in gray fonts.
Gene Ontology - Molecular Function

Gene Ontology - Biological Process

Gene Ontology - Cellular Component
Figure S3. Additional GO analysis data, Related to Figure 3

(A-C) Top 10 enriched Cellular Compartment (A), Biological Process (B) and Molecular Function (C) GO terms of further narrowed apical territory proteome (250 proteins) and basolateral territory proteome (252 proteins). Note the 250 proteins are shared by all three apical replicates shown (Fig. S2F).

(D-F) Top 10 enriched Cellular Compartment (D), Biological Process (E) and Molecular Function (F) GO terms of 139 proteins unique to PODXL-APEX2 Replicate #1 and Replicate #2 after Filter 3 (green) and 182 proteins unique to the EZRIN-APEX2 sample (orange). See Fig. S2F for intersection.

(G) Intersection of protein lists from proteomes of the hPSC-cyst apical membrane territory (from this study), with published MDCK proteomes from the Pals1 territory (apical to the tight junction) and Par3 territory (at the tight junction) (17).
Figure S4. Validation of novel polarized proteins in hPSC-cysts

(A) Novel proteins selected for validation.

(B) Analysis of seven novel apical territory proteins using IF (LAMTOR1, SNX27 and STX7) or stable transgene expression (BASP1-HA, ECE1-HA, AP1G1-mCherry and EGFP-RAB35) in d2 hPSC-cysts, showing direct colocalization (BASP1 and ECE1) with known apical markers (PODXL or pERM), or sub-apical localization (LAMTOR1, SNX27, AP1G1 and STX7).

(C) Analysis of six novel basolateral territory proteins using IF (EPHB4, SPINT1, NEO1, MPZL1) or stable transgene expression (BMPR1A-Myc, ACVR1B-Myc) in d2 hPSC-cysts. Staining for apical markers (PODXL or WGA - membrane) and E-CADHERIN (basolateral) reveals colocalization at E-CAD+ basolateral membranes.

(D) Analysis of three unpolarized proteins. IF of d2 hPSC-cyst stained for SNAP23 (top), SLC1A5 (middle), LCK (bottom), E-CADHERIN and WGA.

Note: Transgene expression of BASP1-HA or EGFP-RAB35 results in defective hPSC-cyst morphogenesis in some cysts; only intact hPSC-cysts were selected for co-localization analyses in Fig. S4B. Defects are shown in Fig. 4C.

Nuclei = Hoechst (blue) for all images. Scale bars = 10µm.
Figure S5. Design and validation of hESC CRISPR/Cas9 knockout lines, Related to Figure 4

(A–E) Sequence of original (WT) and mutant (KO) hESC cell lines of targeted genes (EZR – A, RAB35 – B, SNX27 – C, BASP1 – D, AP1G1 - E). Western blot analyses (right) are shown for all targeted lines except for RAB35 (antibody not available). PAM sequence (green), target sequence (red), insertion (blue), deletion (red dashed lines) are as indicated.

(F) D2 RAB35-KO hPSC-cyst with inverted polarity (seen in <5% of cysts, 5 out of 154).

(G) D2 RAB35 overexpressing hPSC-cyst with multiple small PODXL* lumens.

(H) D2 SNX27-KO hPSC-cyst with a small and unexpanded PODXL*, pERM* lumen. Nuclei = Hoechst (blue) for all IF images. Scale bars = 10µm.
Figure S6. Additional characterization data of AP1G1-KO hPSC monolayer, Related to Figure 5

(A) Top, bright field images of dense monolayers from control, AP1G1-KO and AP1G1-KO rescue cells. Scale bars, 50µm.
Bottom, 3D reconstruction of control, AP1G1-KO and AP1G1-KO rescue monolayers, stained with indicated markers. XZ planes are shown to reveal cellular organization along the apicobasal axis. Note that the gray channel in AP1G1-KO rescue samples (identical images shown in Fig. 5F) indicates sub-apically enriched AP1G1-mCherry.

(B) D2 control and AP1G1-KO hPSC-cyst expressing CDC42-EGFP, stained for pERM and E-CAD.

(C,D) D2 (C) and D5 (D) control and AP1G1-KO hPSC-cyst stained for indicated markers. Nuclei = Hoechst (blue) for all images. Scale bars, 10µm in (B,C), 20µm in (D).
Figure S7. Additional images and characterizations of time-lapse imaging, Related to Figure 6C

(A) Time-lapse snapshots of control (top, Movie 3) and AP1G1-KO (bottom, Movie 4) hPSC-cysts at 00:00, 11:00 and 19:00. For images in Fig. 6C, control and KO samples are selected from 3µm at 00:00, and 9 µm at 11:00 and 19:00.

(B) Optical section of mTnG hPSC aggregates with apicosomes (only membrane-tdTomato is shown). Organized mT* intracellular structures (red) colocalize with pERM* apicosomes (green).

Scale bar = 100µm in (A) and 10µm in (B).
Figure S8. Effects of PODXL overexpression in hPSC-cysts lacking AP1G1
(A) Confocal images of control and AP1G1-KO hPSC aggregates expressing the PODXL-mCherry construct prior to triggering cyst formation (d0, left). Defective apicosome formation in AP1G1-KO cells is partially rescued by expression of PODXL-mCherry. Middle panel: percent of cells with apicosomes in the presence or absence of added PODXL-mCherry. Right panel: apicosome size quantitation at 24hr (small (≤15 µm²) vs. large (>15 µm²)) at 24hr. Data represent mean ± STDV shown, two-way ANOVA with Tukey’s multiple comparison tests, n > 150 per condition. χ² test was used for the apicosome size statistical analysis (n = 176 for control, 154 for control expressing PODXL-mCherry, and 156 for AP1G1-KO expressing PODXL-mCherry).

(B) Confocal images of d2 control and AP1G1-KO hPSC-cysts (No OE, left) as well as control and AP1G1-KO hPSC-cysts expressing PODXL-mCherry (right), stained with indicated markers. Note partially expanded lumen in AP1G1-KO cysts over-expressing PODXL-mCherry.

(C,D) NHERF1 localization in control and AP1G1-KO d2 hPSC-cysts. Increase in gain (overexposure in (C)) reveals that NHERF1 is localized to ectopic PODXL vesicles in KO cysts, in addition to the EZRIN+/PODXL+ apical lumen.

(E) Western blot showing NHERF1 expression in the presence and absence of AP1G1.

(F) Expression of NHERF1 in freshly plated hPSC aggregates (d0), co-stained with indicated markers. NHERF1 is localized to the apicosome as well as PODXL+ apicosome precursor vesicles.

Scale bar = 10µm in all images.
Table S1. hPSC-cyst cell polarity proteome dataset and lists of proteins after Filter 1 and Filter 2
(A) hPSC-cyst 10-plex MS dataset.
(B) UniProt human protein database used in this study.
(C) List of 495 mitochondrial matrix proteins.
(D-I) List of proteins after Filter 1: D – PODXL-APEX2 #1 list; E – PODXL-APEX2 #2; F – EZR-APEX2; G – APEX2-ATP1B1 #1; H – APEX2-ATP1B1 #2; I – SDC1-APEX2.
(J-O) List of proteins after Filter 2: J – PODXL-APEX2 #1 list; K – PODXL-APEX2 #2; L – EZR-APEX2; M – APEX2-ATP1B1 #1; N – APEX2-ATP1B1 #2; O – SDC1-APEX2.

Table S2. Apical, basolateral and non-polar proteomes in hPSC-cyst
(A) List of 139 proteins unique to PODXL-APEX2 Replicate #1 and Replicate #2.
(B) Interaction analyses for APEX2-tagged proteins. Known binding partners were identified using BioGrid. Proteins without an intracellular domain or abundant RNA expression in hPSC, as well as proteins with known nuclear, ER, Golgi and/or mitochondrial localization were omitted from the analysis.
(C-E) List of proteins after Filter 3: C – apical (389); D – basolateral (252); E – non-polar (30).
(F) List of previously unidentified apical and basolateral membrane enriched proteins based on UniProt and GO annotations.
(G) UniProt- and GO-based functional annotation of proteins found in apical (389) and basolateral (252) membrane territory lists.
(H) List of 182 proteins found in EZRIN-APEX2 but not in PODXL-APEX2 Replicate #1 and Replicate #2 after Filter 3.

Table S3. Lists of apical, basolateral and non-polar proteins after Filter 4
(A-C) List of proteins after Filter 4: A – apical; B – basolateral; C – non-polar.

Table S4. Lists of reagents used in this study
(A-D) Lists of antibodies (A), primers (B), obtained plasmid (C) and newly generated plasmid (D) used in this study

Movie 1. 3D reconstruction of control d3 hPSC-cyst stained for DNA (blue, top left) F-ACTIN (green, top right), PODXL (orange, bottom left), revealing a hollow central lumenal cavity in control samples. Bottom right panel shows overlay of F-ACTIN and PODXL.

Movie 2. 3D reconstruction of AP1G1-KO d3 hPSC-cyst stained for DNA (blue, top left) F-ACTIN (green, top right), PODXL (orange, bottom left), revealing a thin disk-shaped lumen and scattered cytosolic PODXL vesicles. Bottom right panel shows overlay of F-ACTIN and PODXL.

Movie 3. High-resolution time-lapse imaging of control mTnG hPSC-aggregates in Fig. 6D. Images are taken at 20-min intervals. Six optical sections were captured in the Z-axis with 3µm steps, starting in close proximity to the coverslips (0µm) to 15µm, to accommodate the increase in cyst height over time. A hollow central lumenal cavity is seen by 16-hr. Scale bar = 100µm.

Movie 4. High-resolution time-lapse imaging of AP1G1-KO mTnG hPSC-aggregates in Fig. 6D. Images are taken at 20-min intervals. Six optical sections were captured in the Z-axis with 3µm steps, starting in close proximity to the coverslips (0µm) to 15µm, to accommodate the increase in cyst height over time. While radial organization is seen, a hollow lumen is not formed. Scale bar = 100µm.
REFERENCES AND NOTES


