

Expanded View Figures

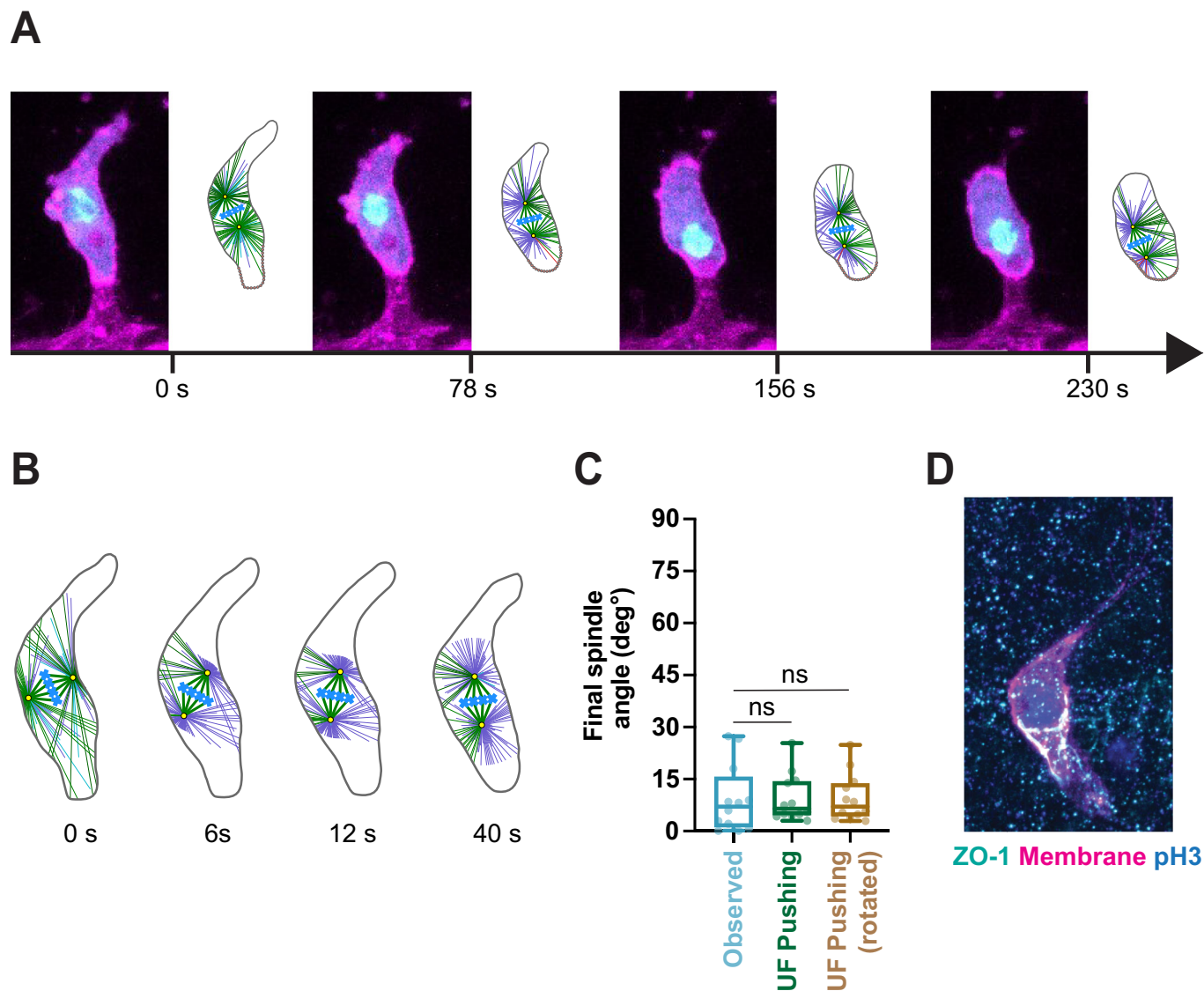
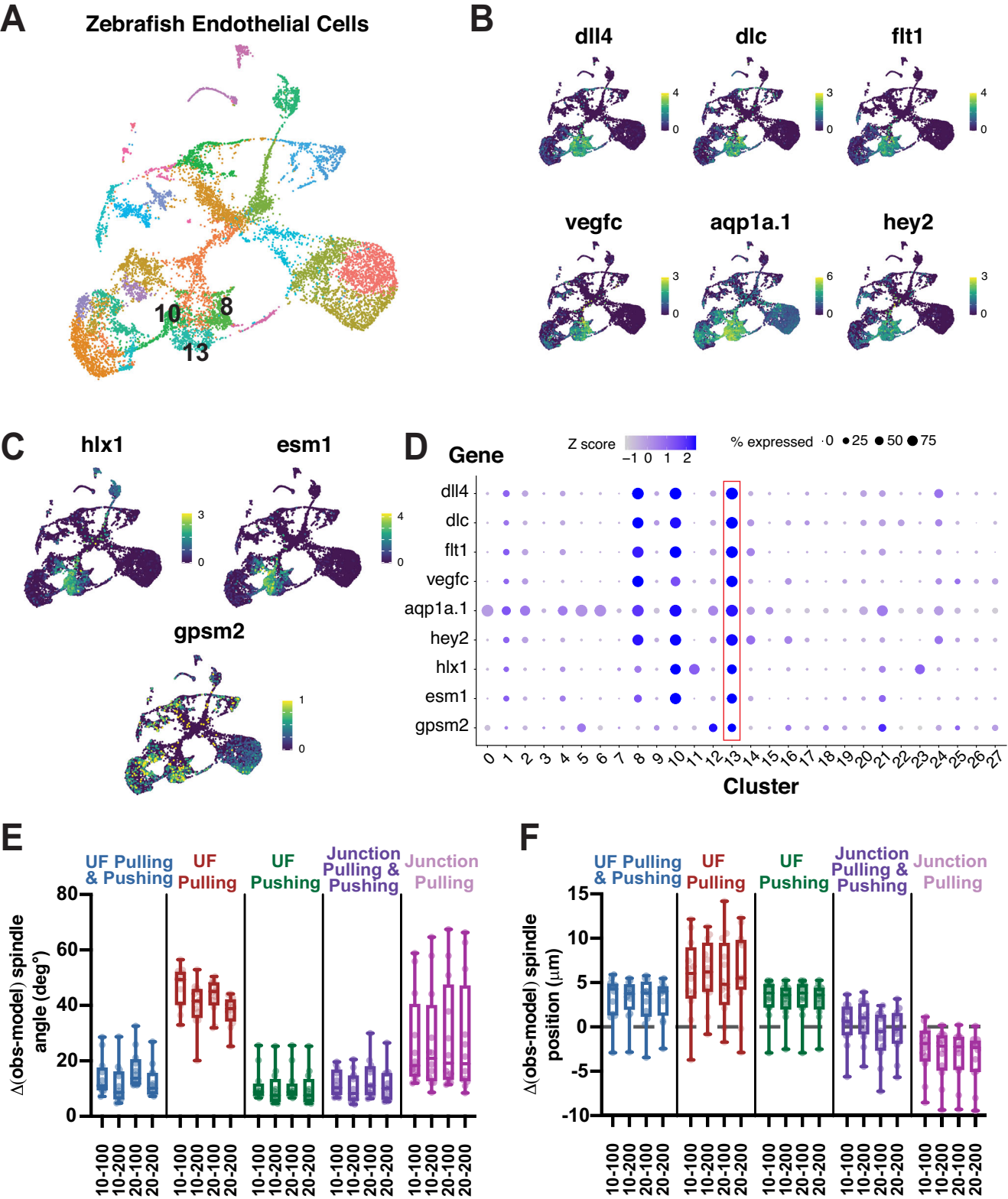


Figure EV1. Pushing forces alone can reproduce correct alignment for initially misoriented spindles.

(A) Microscopy images alongside representative simulation snapshots at corresponding time points, illustrating how the model reproduces observed cell shape changes during division. (B) Representative simulation snapshots of a cell with the spindle initially misoriented by 90°, shown at successive time points. The spindle aligns correctly along the cell's long axis despite initial misorientation. (C) Final spindle angle comparison between experimental data (cyan) and two simulated conditions: uniform pushing (green) and uniform pushing with initially misoriented spindles (brown). Boxplots show the 25th, 50th, and 75th percentiles, with whiskers spanning the full data range ($n = 12$ observed cells). The center line represents the median. Each dot represents the mean of 40 simulation runs for a single cell. Pushing forces alone can recapitulate experimentally observed spindle orientation regardless of initial angle. Statistical significance was assessed using the paired Wilcoxon signed-rank test. (D) Cell-cell junctions at the base of a branching endothelial cell, which may serve as polarity cues biasing the spindle downward. Lyn-mCherry (magenta) expressing zebrafish tip endothelial cell with mosaic Tubulin-GFP (cyan) undergoing an asymmetric cell division. Cell-cell junctions are marked by ZO-1 (white).



◀ **Figure EV2. Junctional pulling and pushing robustly predict spindle angle and position across parameter settings.**

(A–D) Single-cell RNA-seq data analysis shows *gpsm2* (LGN ortholog) expression in ISV. (A) UMAP plot of 10874 cells with 28 distinct clusters. (B, C) UMAP feature plots with expression of selected arterial markers (*dll4*, *dlc*, *flt1*, *vegfc*, *aqp1a.1*, and *hey2*) (B) and ISV markers (*hlx1* and *esm1*) (C). Clusters 8, 10, and 13 show enriched expression of arterial markers. ISV markers *hlx1* and *esm1* are predominantly expressed in clusters 10 and 13. *gpsm2* expression, though low, is visibly enriched in the region corresponding to clusters 10 and 13 (colorbar upper limit set to 1 for visibility). (D) Dot plot showing expression of selected genes across all clusters. Red box highlights a cluster expressing arterial markers, ISV markers and *gpsm2*. Comparison of observed and predicted final spindle angle (E) and position (F) under different simulated conditions across multiple parameter pairs ($N_{MT} - N_{FG}$). For spindle angle (left), values represent the absolute difference between observed and model predictions. For spindle position (right), values represent the signed difference between observed and model predictions. Boxplots show the 25th, 50th, and 75th percentiles with whiskers spanning the full range ($n = 12$ observed cells). The center line represents the median. Each dot corresponds to the average of 40 simulation runs for a single cell. Conditions are color-coded as follows: blue, combined pulling and pushing with uniformly distributed FGs; red, pulling only with uniformly distributed FGs; green, pushing only without FGs; purple, combined pulling and pushing with FGs localized to cell–cell junctions; pink, pulling only with FGs localized to cell–cell junctions.