



**Fig. S1. Migratory epithelial cells display spatially-dependent effects on speed within the bifurcation clefts.**

(A) Paired mean cell speeds ( $\mu\text{m}/\text{hour}$ ) were calculated from nuclei trajectories as track length divided by time for individual cells that were migrating between all three locations: a branch ( $8.77 \pm 1.26 \mu\text{m}/\text{hr}$ , pre-cleft), to a cleft ( $7.00 \pm 1.17 \mu\text{m}/\text{hr}$ ), and returned to the branch ( $8.89 \pm 2.67 \mu\text{m}/\text{hr}$ , post-cleft), or did two of the three locations (38 cells from 10 organoids,  $r = 7$ ). Quantification includes the data from Figure 1F. Paired analysis ANOVA reached significance (\*\*\*\* $p < 0.0001$ ). (B) Cellular persistence (path length divided by displacement length) was measured for branch ( $0.35 \pm 0.18$ ), cleft ( $0.28 \pm 0.17$ ), and stalk ( $0.22 \pm 0.13$ ) cells. Paired analysis ANOVA reached significance (\* $p < 0.05$ ; \*\*\*\* $p < 0.0001$ ). Mean  $\pm$  SD, 7 organoids,  $r = 4$ .