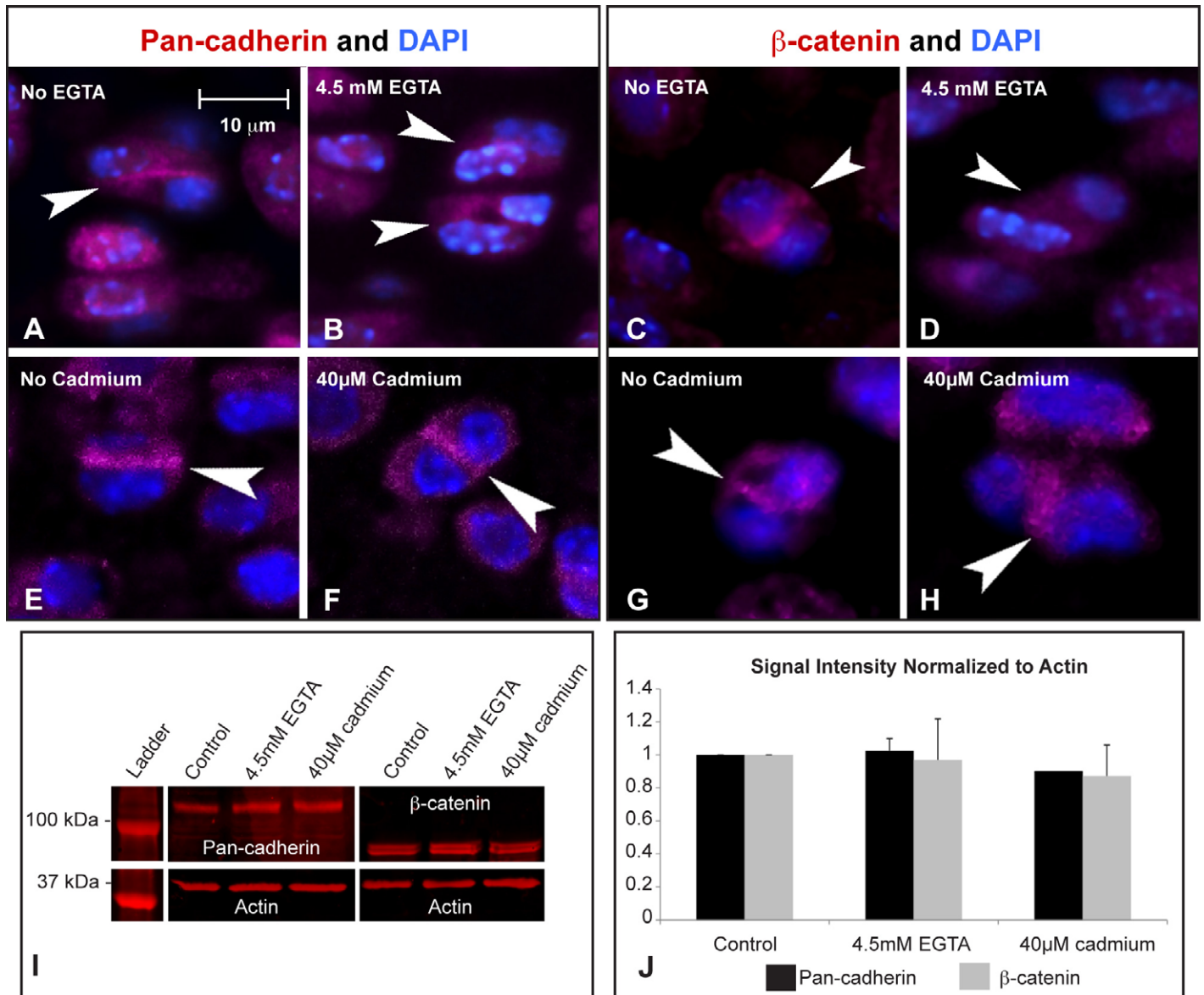


Supplementary Figure S1. Chondrocyte rearrangement is not accompanied by cell growth.

Measurements of both resting and proliferative chondrocytes along the longest (A) and shortest (B) dimensions of the cells as well as measurements of the perimeter (D) before and after division/rotation demonstrate that there is no significant change in the size or shape of the original undivided cell. The length-to-width ratio of the resting vs. proliferative zones (C) confirms the expected shapes of the chondrocytes – i.e. rounder for resting cells and discoid for proliferative. For all resting zone measurements $n=21$, and for all proliferative zone measurements $n=46$.



Supplementary Figure S2. EGTA treatment down-regulates cadherins and β -catenin in PSS explants.

Cranial base growth plates were cultured for 10 hours with no small molecule treatment (A, C, E, G), with 4.5 mM EGTA (B,D), or with 40mm cadmium chloride (F, H). Immunofluorescence was performed against cadherins (A-B, E-F) and β -catenin (C-D, G-H). Arrowheads indicate the daughter cell interface of recently divided proliferative chondrocytes. Localization of cadherins and β -catenin at the interface is detected in untreated explants but is lost in explants treated with EGTA. Cadherin localization is not disrupted by cadmium chloride treatment, and β -catenin is present at the interface in a more diffuse manner. Additionally, EGTA and cadmium treatment do not affect the overall protein levels of cadherins or β -catenin (I-J). Cranial base growth plates were harvested and cultured as explants in the specified conditions for 17 hours. Infrared fluorescent western blotting (I) for cadherins and β -catenin revealed that neither treatment resulted in a significant decrease in protein levels of the adhesion molecules after normalization to actin (J).



Supplementary Movie 1. Resting zone chondrocytes lack oriented rearrangement. This representative resting chondrocyte undergoes cytokinesis that is not aligned with divisions of surrounding cells and the daughter cells rotate slightly without orienting into a column. Many observed resting cells did not rearrange following cytokinesis. Each frame represents 30 minutes.



Supplementary Movie 2. Proliferative chondrocytes rotate around an interface to form clonal columns. The division plane of this representative proliferative chondrocyte bisects the long axis of the cell. The daughter cells remain closely associated as they rotate around their interface to align with the expanding column. Each frame represents 30 minutes.



Supplementary Movie 3. Chondrocyte shape does not determine the position of rotation termination. Chondrocytes at the border between the resting and proliferative zones are ellipsoidal and occasionally do not show planar alignment to nearby columns. This cell, one of two examples observed, has a division plane that bisects the long axis of the cell and rotates until stacked and aligned with nearby columns.



Supplementary Movie 4. Chelation of calcium inhibits daughter cell adhesion, causing premature rearrangement termination. Proliferative chondrocytes treated with EGTA divide normally. However, daughter cell rearrangement is incomplete and terminates before the cells are aligned with surrounding columns and the longitudinal axis of the growth plate. A neighboring cell divides near the end of this movie, suggesting overall tissue health.



Supplementary Movie 5. Competitive inhibition of calcium binding to daughter cell adhesion molecules causes premature rearrangement termination.

Cadmium chloride treatment does not affect proliferative chondrocyte division. However, daughter cell rearrangement is incomplete and terminates before the cells are aligned with surrounding columns and the longitudinal axis of the growth plate.