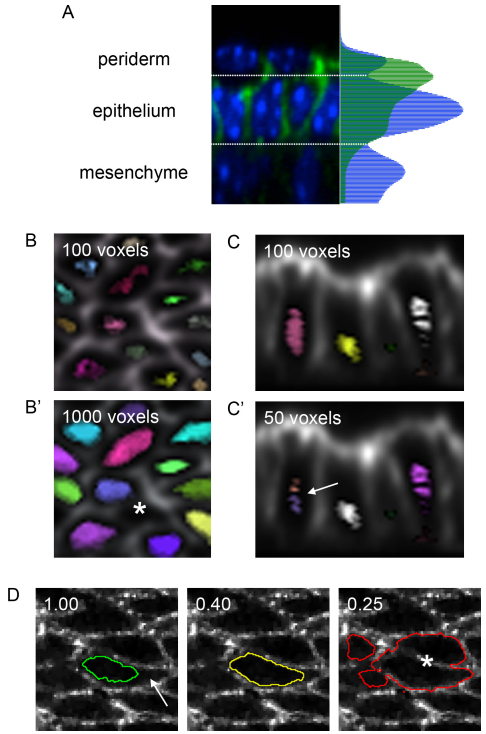

Figure S1. Image analysis.
(A) Z reconstruction from a confocal stack, with DAPI labelled nuclei (blue) and beta-catenin immunofluorescence (green) localised to cell membranes (apical up). Periderm, epithelium and mesenchyme nuclei were aligned to generate maxima in the average blue channel intensity across a slice (plotted at right). Minima in this channel were used to define crop lines defining the epithelial layer (dotted white lines). A maximum in the average beta-catenin intensity corresponding to the apical epithelial junctions was used to identify the upper crop line, or position it where the peridermal DAPI staining was weak. (B,C) Cells were identified using minima in beta-catenin staining of at least 100 voxels in size (see Methods for details). Beta-catenin staining in greyscale with local intensity minima above the respective volume threshold in random bright colours. The size threshold for the minima affected the performance of the method as follows. If the threshold is set too high, small cells where the size of the cell minimum falls below threshold size, are missed (e.g. asterisk in B' using 1000 voxel threshold). If the threshold is set too low cells can be double counted because they contain two local minima due to variation in staining intensity within a cell (e.g. arrow in C', threshold 50 voxels). Undercounting is obvious in XY projection (B, B'), over-counting more so in XZ projection because of intensity variation in the Z-axis (C, C'). (D) Segmentation parameters for Active Contour Level set segmentation were chosen to best extract cell shape without the volume bleeding into adjacent cells. This is illustrated for the *Curvature* parameter. A single slice through the outline of a cell volume is shown segmented with three different curvature values (beta-catenin staining of cell outlines in greyscale), the larger the curvature value, the less the object can deform from spherical. For large parameter values (e.g. 1.00, in green) the extremities of cells are not captured (arrow), because the contour stops enlarging when it hits the narrow sides of the cell. For small values (e.g. 0.25, in red), the volume bleeds across boundaries into adjacent cells (asterisk). The optimal segmentation parameters thus identified were *Advection* = 20, *Propagation* = 1, *Curvature* = 0.4, *Grayscale tolerance* = 10 and *Convergence* = 0.0075.

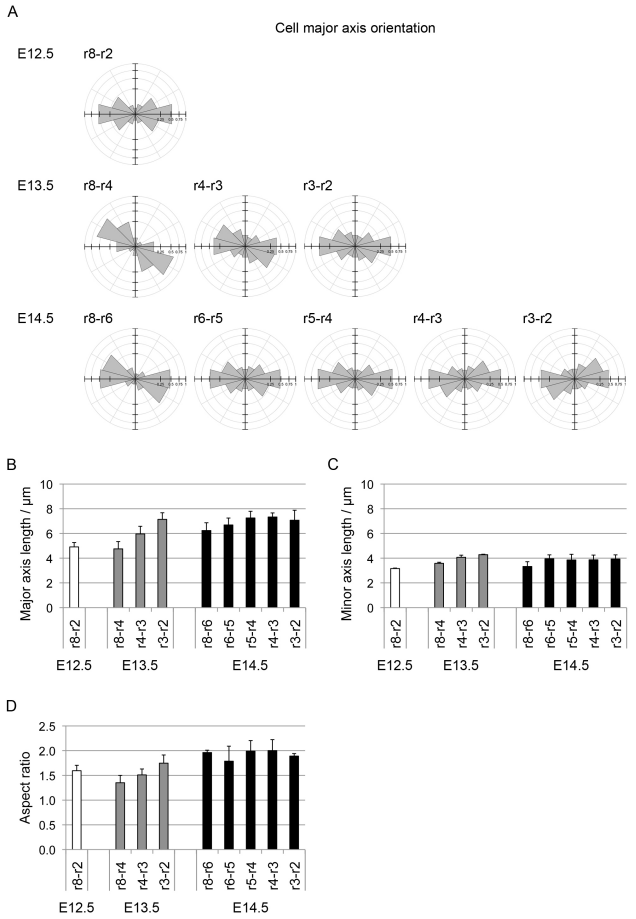

Figure S2. Cell orientations show as anteroposterior bias throughout the inter-rugal epithelium.
(A). Radial plots of cell principal axis orientations at E12.5, E13.5 and E14.5 for each inter-rugal interval. Orientations of cells measured from segmented objects as the orientation of the best-fit ellipse in the XY plane at the level of the centroid. All intervals show a strong bias in their orientations along the AP axis, although for E13.5 r8-r4 intervals the orientation is about 30º off the AP axis so that the XY bounding box slightly underestimates the cell's aspect ratio only for these cells. Scale shows proportion of cell divisions in given direction. Anterior right, medial up. For each interval orientations are pooled from three palates.
(B-D). Histograms showing (B) major axis length, (C) minor axis length and (D) aspect ratio (major/minor) of ellipses fitted to segmented cell volumes at the Z-level of their centroids. Data are from three specimens per state. Error bars = 1 s.d.

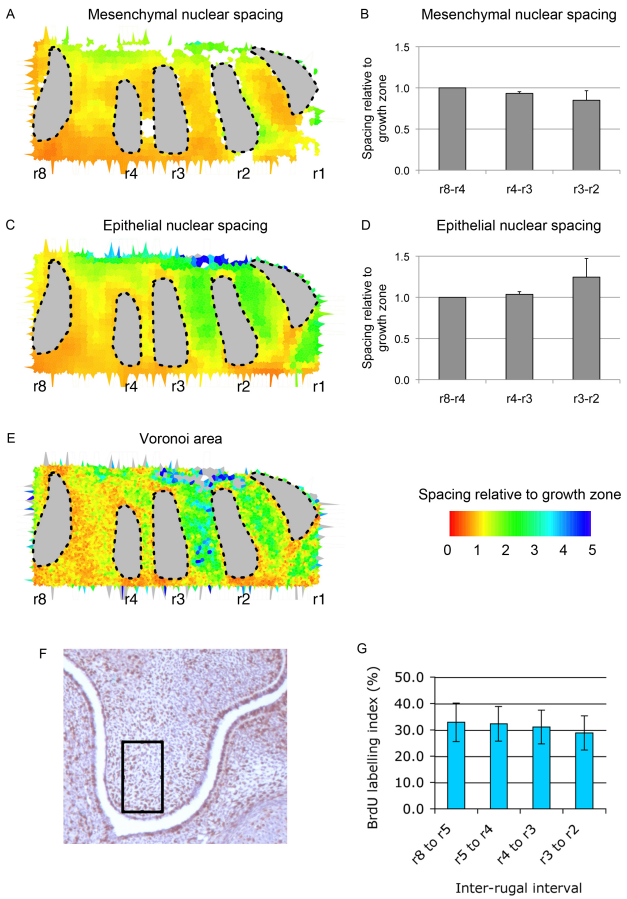

Figure S3. Patterns of cell spacing and proliferation in the mesenchyme differ from the epithelium. (A) Heat map of the local nuclear spacing (inverse DAPI staining intensity) in E13.5 mesenchyme shows near-uniformity along the palate. Rugae greyed out to exclude dipping epithelium and local subrugal condensations. Regional averages for three specimens are quantified in (B), error bars = 1 standard deviation. (C) Heart map of the local nuclear spacing in epithelium shows different pattern from underlying mesenchyme, with increasing nuclear spacing from posterior to anterior. Regional averages for three specimens are quantified in (D), error bars = 1 standard deviation. (E) Voronoi area heat map based on cell segmentation validates DAPI-based nuclear spacing map (C). (F,G) Proliferation measured by BrdU incorporation in palatal shelf mesenchyme. (F) Single 7 µm section from E13.5 head showing BrdU incorporating nuclei (brown) in palatal shelf (nuclei counterstained with heamotoxylin). Black box shows sample area (lingual left dorsal up). Histogram showing average mesenchymal BrdU index averaged across every other section from mid ruga to mid ruga. Error bars = 1 standard deviation. Mesenchymal BrdU shows no elevation in Growth Zone as seen in epithelium. Different patterns of spacing and proliferation in epithelium and mesenchyme suggest that epithelial cell behaviours are not just a passive response to the mesenchyme.