Analysis of human soft palate morphogenesis supports regional regulation of palatal fusion

SUPPLEMENTARY FIGURES

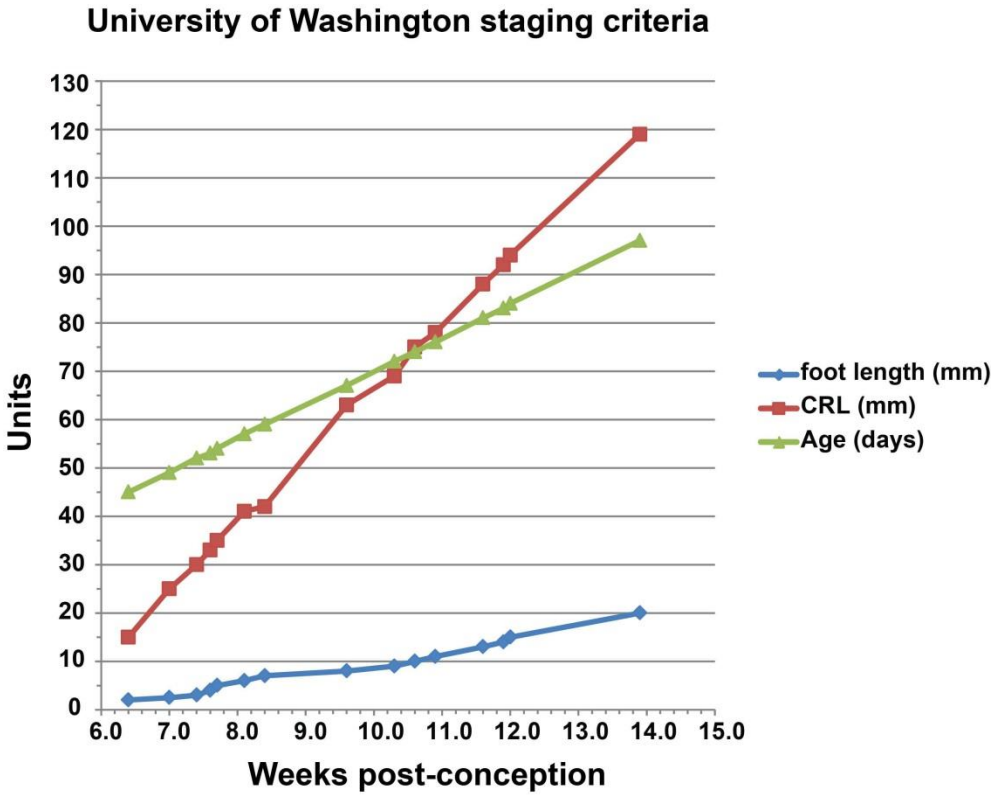

Figure S1. Staging criteria used by U of Washington included crown rump length (CRL) and foot length. Embryos are 6-8 weeks; fetuses are from 8 weeks onward. The increase in size in crown rump length is dramatic during the later embryonic and early fetal periods. Our sample covers the 8-10 week period.

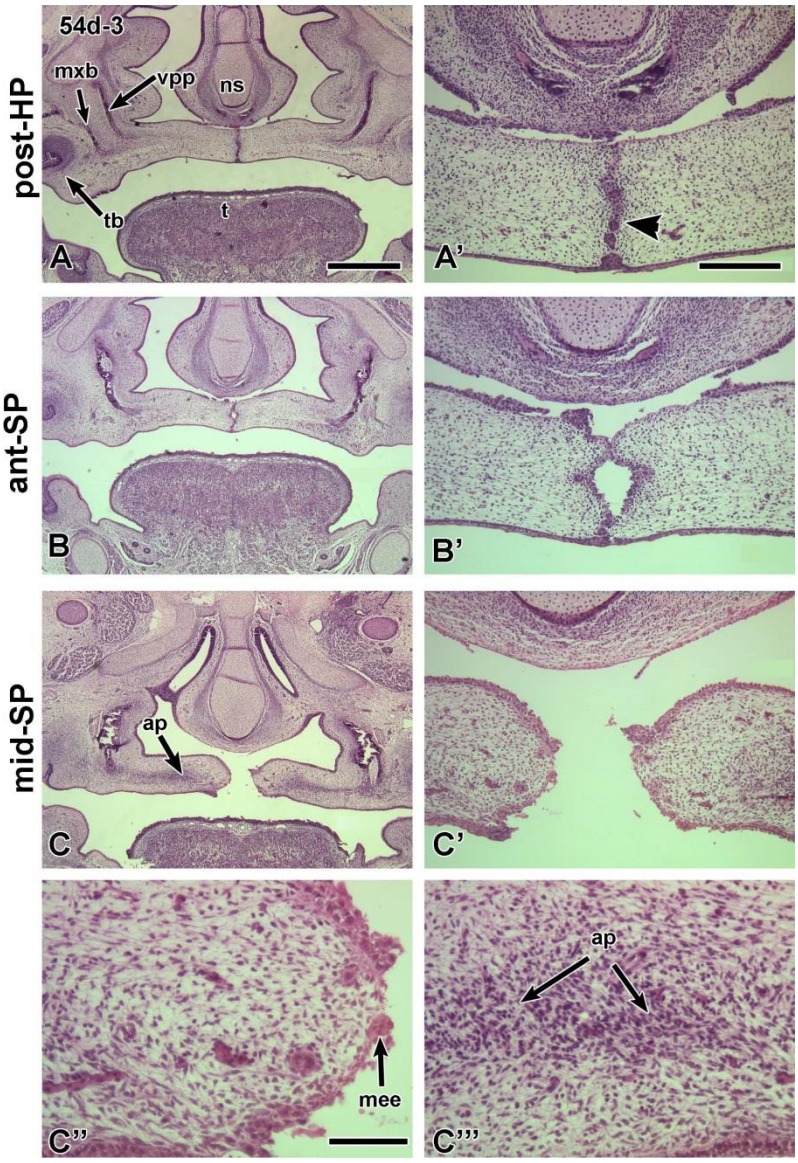

Figure S2. A 54d specimen stained with H and E with a partially fused anterior soft palate. A,A') The hard palate seam is starting to break up into islands of epithelium (arrowhead). B,B') a seam is forming in the boundary between the hard and soft palate. The dehiscence in the midline may be transient. C-C''') The palatal shelf medial edge epithelium is multilayered in some areas and a single layer in others. The aponeurosis is condensing prior to fusion.

Key: ap – aponeurosis, mee – medial edge epithelium, mxb – maxillary bone, ns – nasal septum, tb – tooth bud, vpp – vertical plate of the palatine bone. Scale bars – 1 mm for low power views. Bar in A' = 200 μm and applies to B', C'. Bar in C''= 100 μm and applies to C'''.

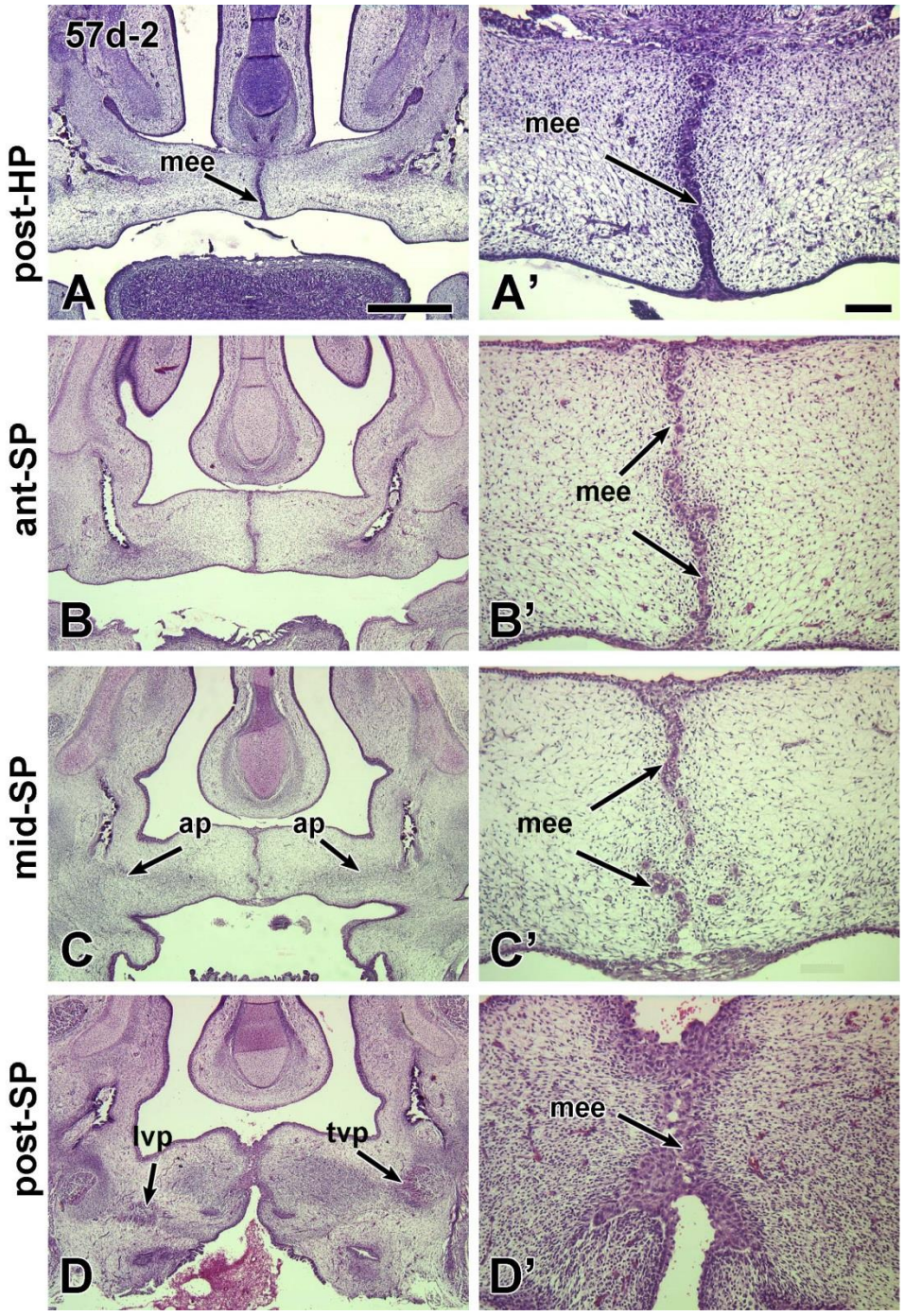

Figure S3. A 57d specimen stained with H and E demonstrating a seam throughout the soft palate. A,A') The hard palate seam is starting to break up into islands of epithelium (arrowhead). B,B') The medial edge epithelium is beginning to degrade in the anterior soft palate.C,C') The mid-soft palate contains the aponeurosis and remnants of the medial edge epithelium. D,D') the palatal shelves in the most posterior aspect of the soft palate are contacting and have a thick, disorganized medial edge epithelial seam. The levator veli palatini is in the right palatal shelf. Key: ap – aponeurosis, lvp – levator veli palatini, mee – medial edge epithelium, tvp – tensor veli palatini. Scale bars = 1 mm for low power views and 100 μm for the high power views.

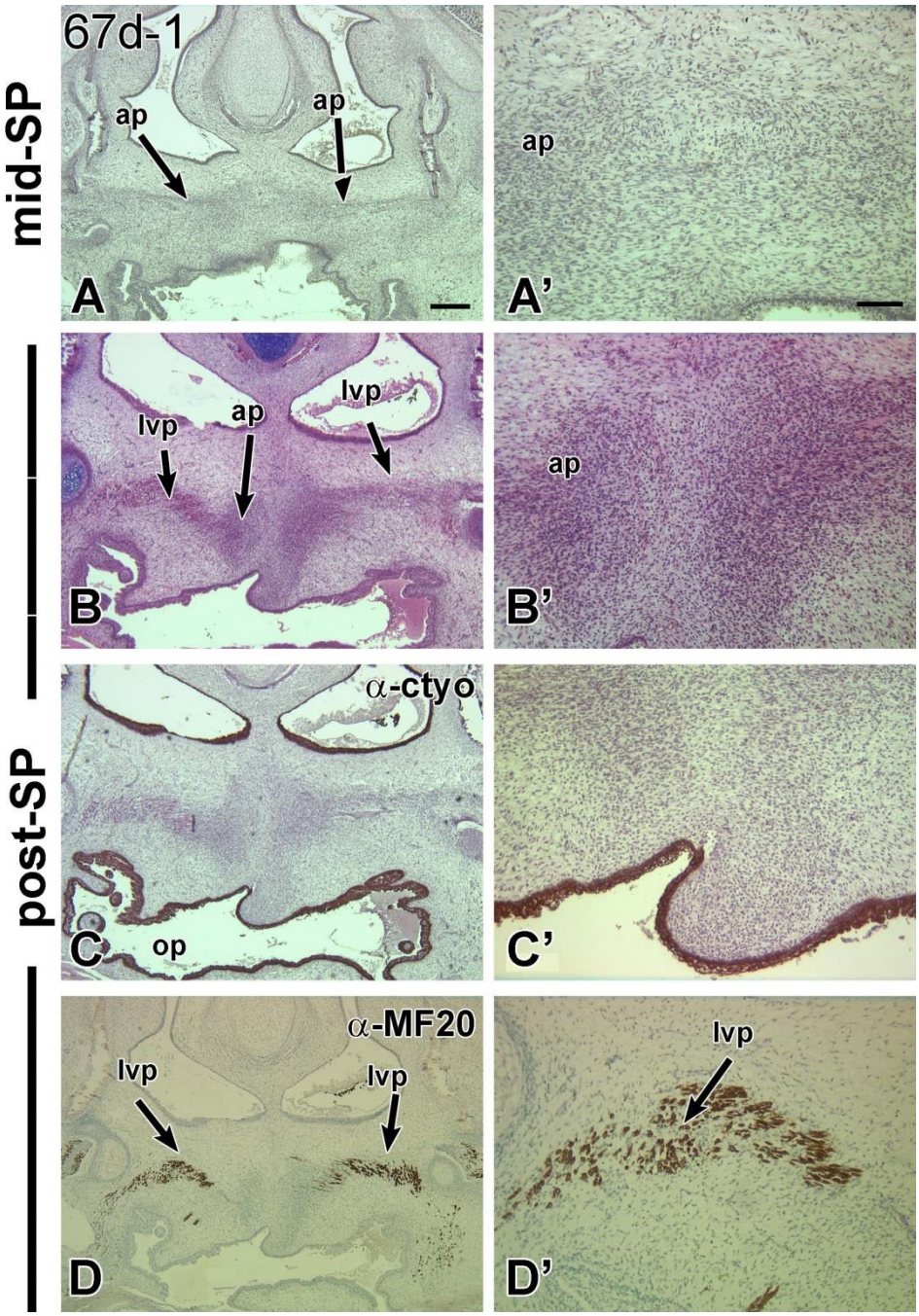

Figure S4. A 67d specimen sectioned through the soft palate. These are near adjacent sections stained with hematoxylin-Eosin, anti-cytokeratin or anti muscle (MF-20) antibody. A,A') staining of the narrow band that is the aponeurosis demarcates the mid-soft palate. B, B') staining of the levator veli palatini indicates that section is in the posterior soft palate. Note that even without specific antibody staining it is possible to distinguish muscle cells from aponeurosis fibroblasts. C.C') Positive cytokeratin staining in the endoderm of the oropharynx. There are no epithelial remnants in the soft palate. D,D') Staining for myoblasts in the levator veli palatini is present, however the aponeurosis is not stained. Key: ap – aponeurosis, lvp – levator veli palatini op – oropharynx. Scale bars Low power views = 500 µm, high power views = 100 µm.

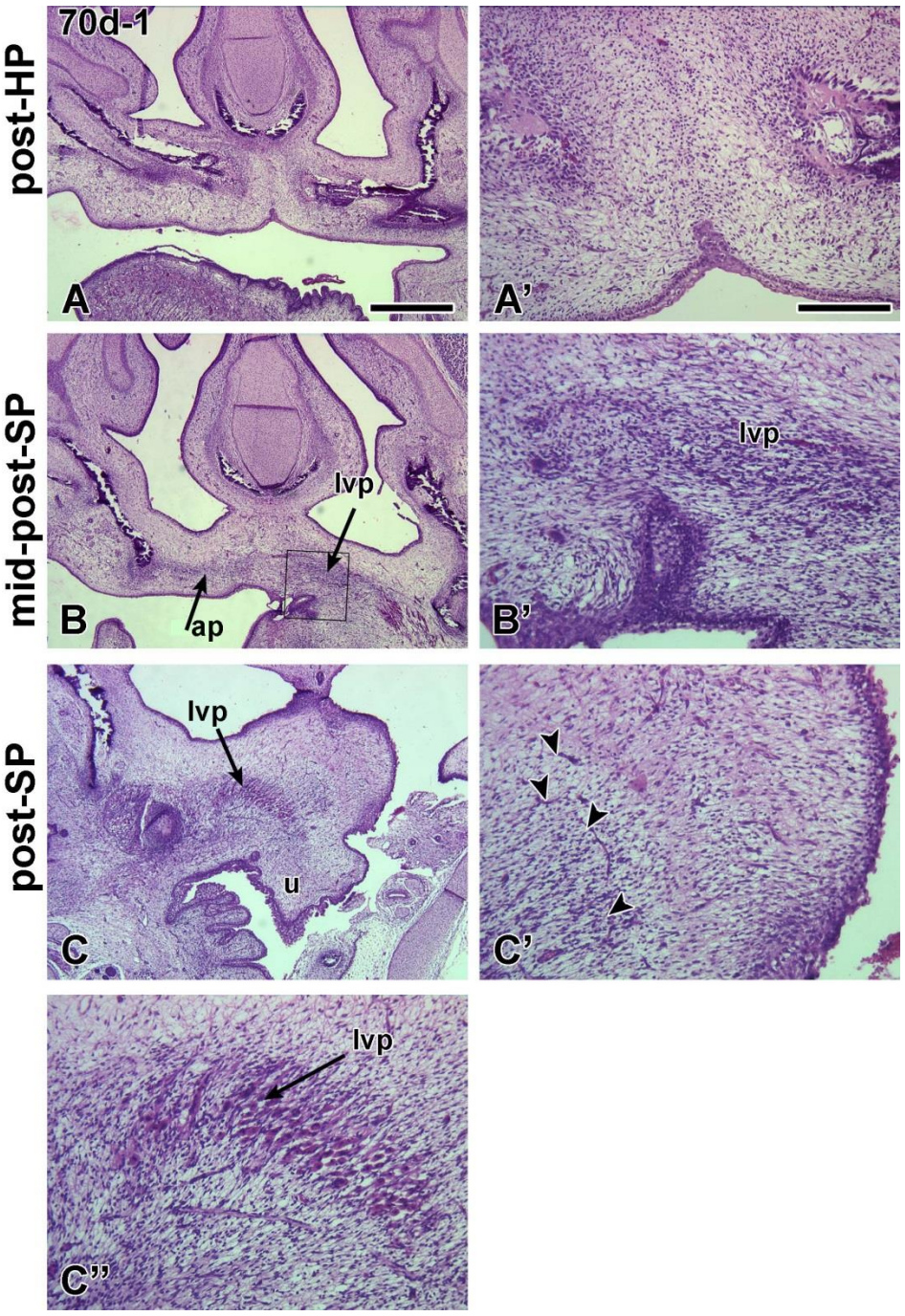

Figure S5. A 70d specimen with a fully fused hard and soft palate. This embryo was tilted to the left when sectioned so the left side is cut at a more posterior plane than the right side. A, A') very little medial seam remains in this region of the hard palate. B,B') The section is through the mid-soft palate an aponeurosis on the right side and the posterior soft palate in the left side where the musculature is forming. C, C'') A more posterior section, the right side contains the levator veli palatini. The uvula is forming inferiorly. C') Small blood vessels are very abundant (arrowheads). The mucosal epithelium is thin and disorganized. Key: lvp – levator veli palatini, u – uvula. Scale bar = 1 mm for low power views and 200 µm for high power views.