

A
E15.5 Mouse Kidney

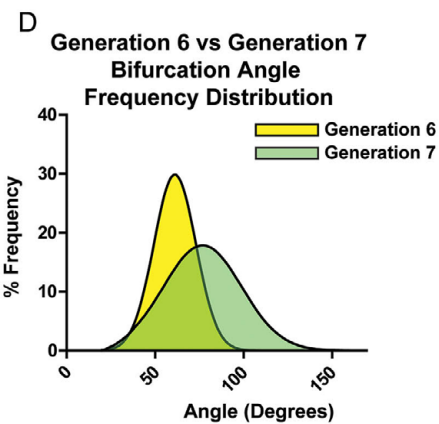
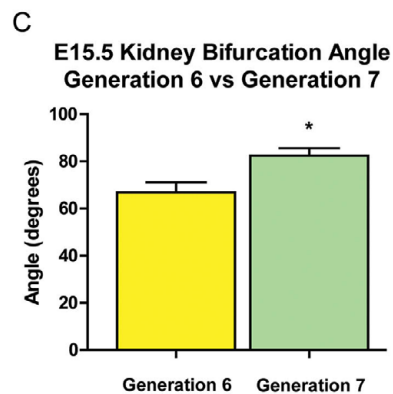
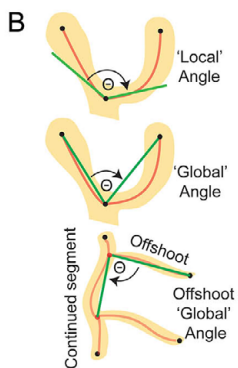
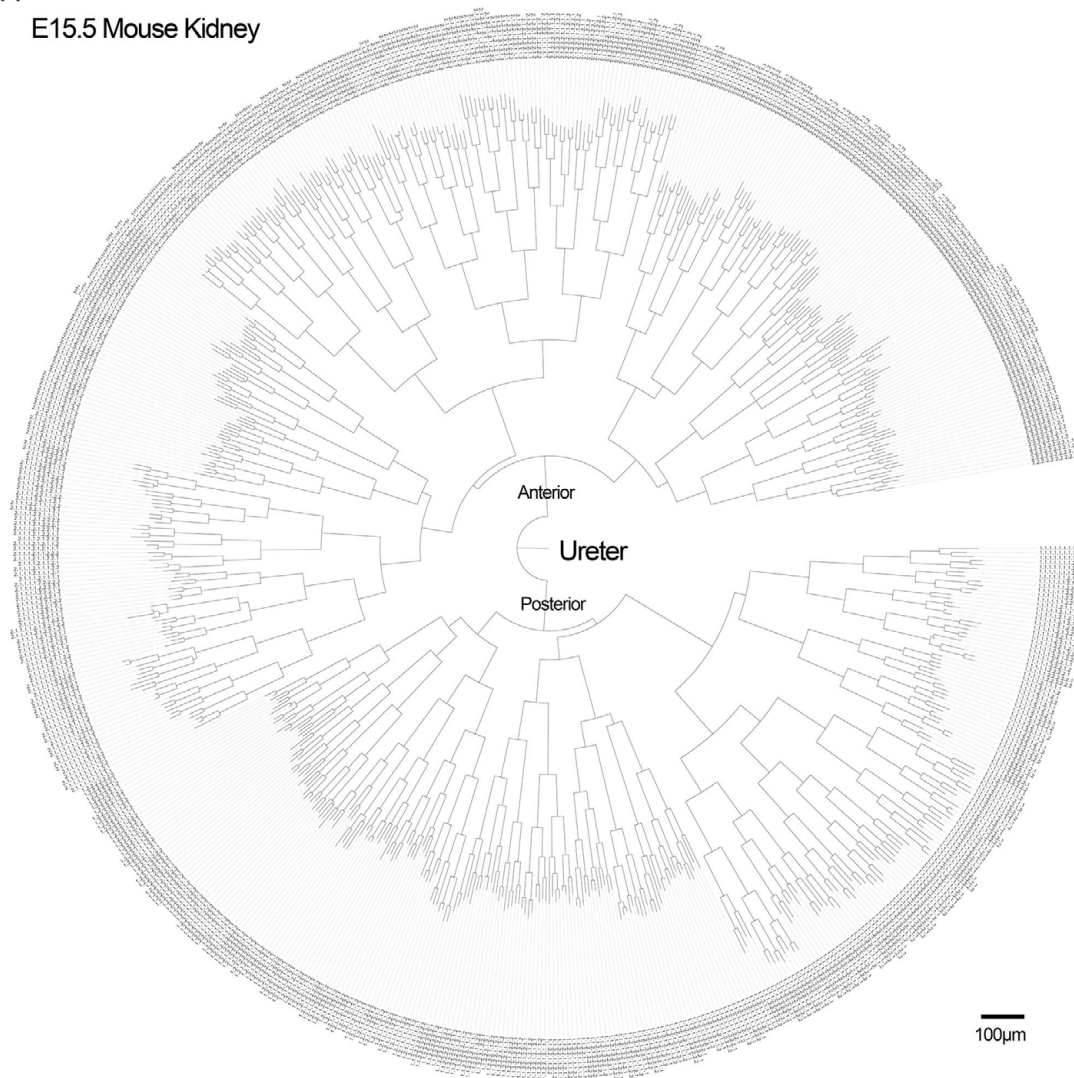


Fig. S1. Tree Surveyor enables mapping of branches and analysis of branch angles. (A) E15.5 C57Bl/6 mouse ureteric tree, circular phylogram rendered using JSTree (<http://lh3lh3.users.sourceforge.net/jstree.shtml>). Cladogram tree indicates the bifurcating nature and subtree clustering of the developing kidney; horizontal distances are not to scale but vertical distances are. (B) Angles between segments are measured in two ways: first, by calculating the ‘local’ angle between two rays (green) that begin at the branch node, and are projected to the spline’s (red) first derivative (which is notionally the first point at which a spline bends). A ‘global’ angle is measured between rays linearly projected from the branch node directly to the next branch nodes (or terminal nodes). ‘Global’ angles for offshoots on continuing segments are calculated differently, using the next offshoot node on the continuing segment (or terminal node if no further offshoots are present). (C,D) Measurement of global angles in E15.5 kidneys indicates that there is a significant shift in global bifurcation angle between generation 6 and generation 7 (C) with the a wider global bifurcation angle spread in generation 7 (D). Error bars represent s.e.m.

E14.5 Embryonic C57BL/6 Mouse Lung
Circular Phylogram

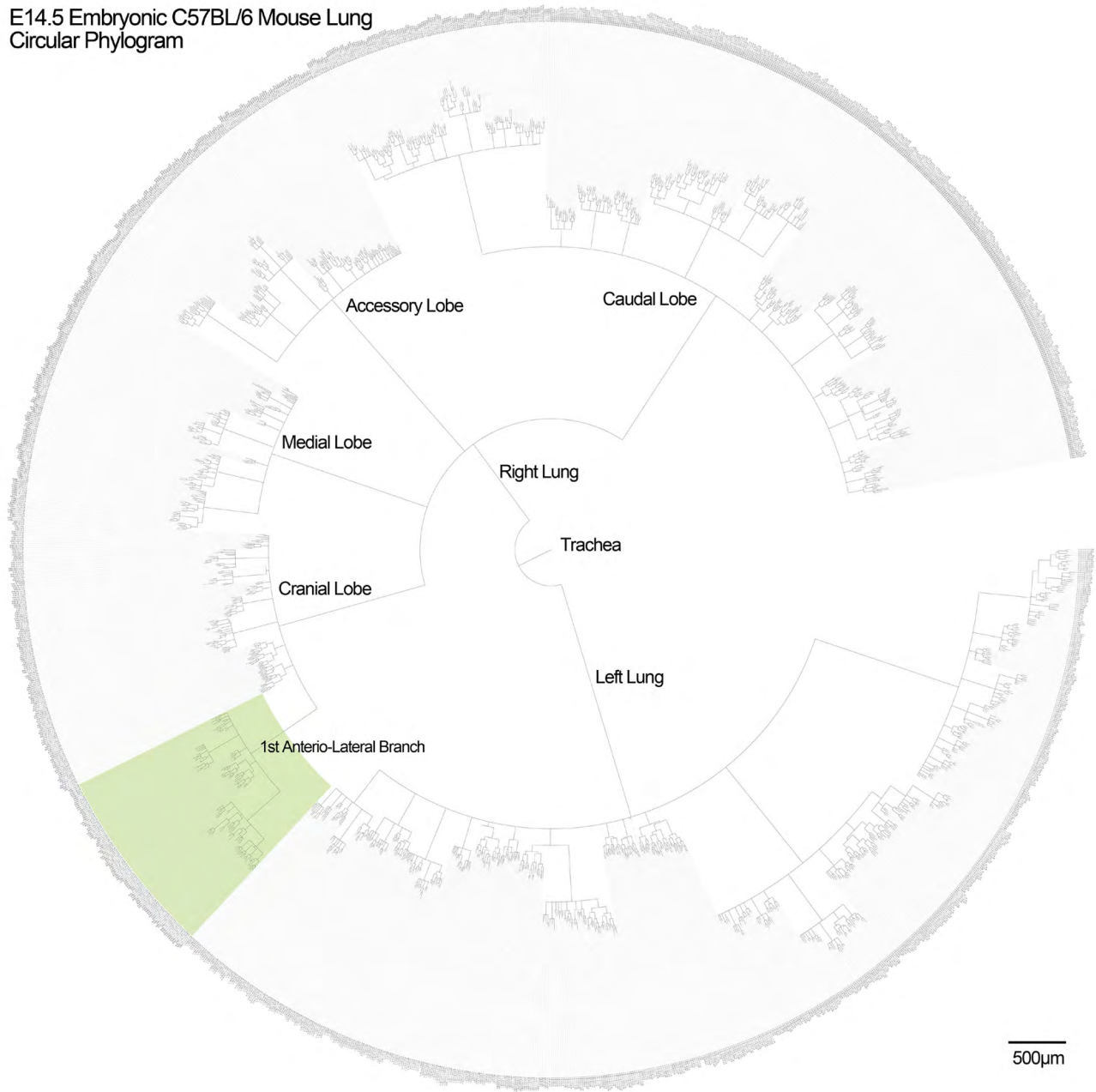


Fig. S2. A simplified view of complex domain branching networks. Tree Surveyor was used to analyse an E14.5 C57Bl/6 mouse lung and generate a Newick file. This was used to render a circular phylogram with JSTree (<http://lh3lh3.users.sourceforge.net/jstree.shtml>). The branching group analysed in Fig. 3 and Fig. S4 is highlighted in green.

E15 C57Bl/6J - Right Cranial Lobe - Antero-Lateral Branch 1

Metzger et al.
R.Cr.AL1 Lineage
CD1 Strain

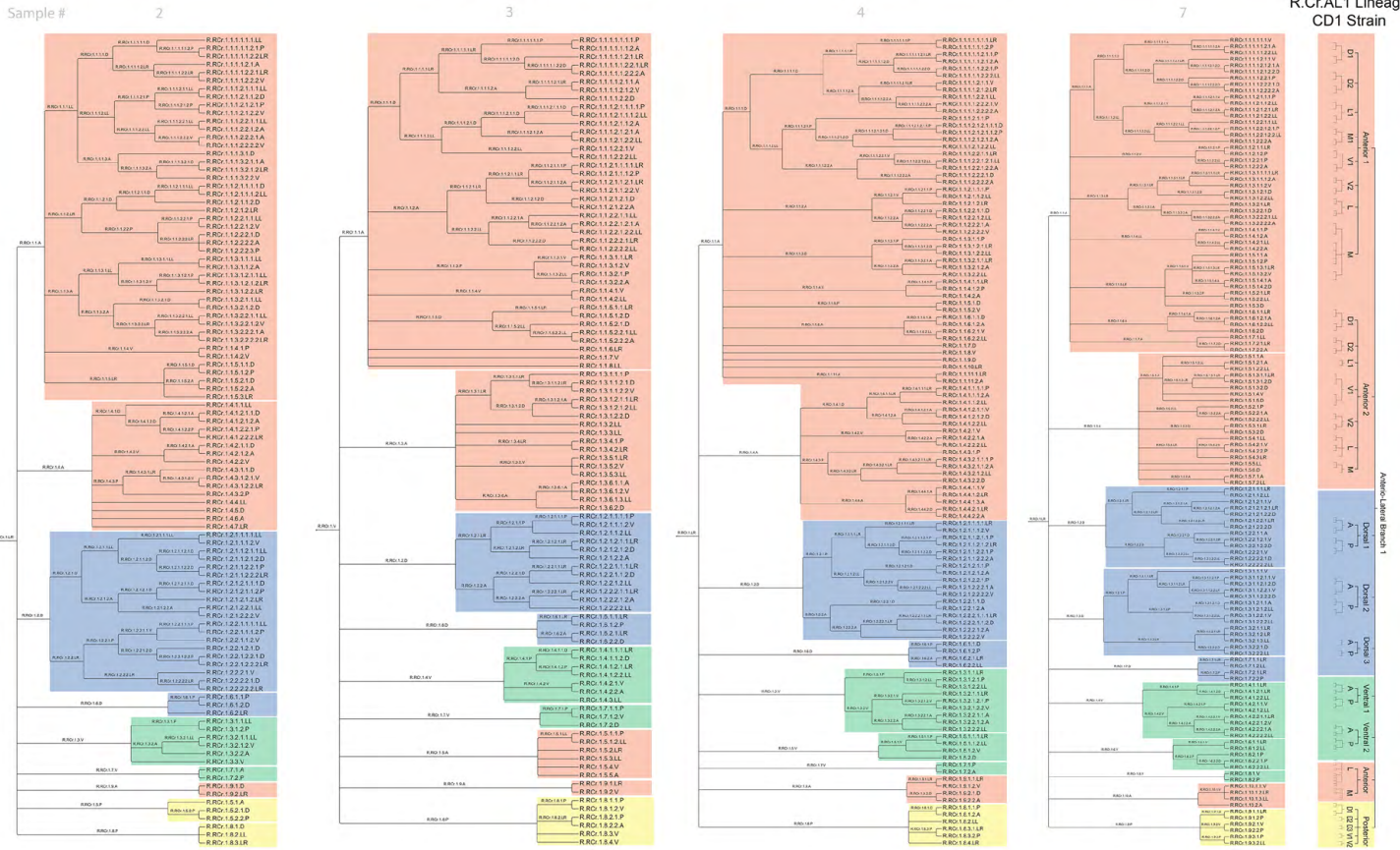


Fig. S3. Tree Surveyor analyses domain branching in lungs and highlights similarities and differences from known branching patterns. Cladogram of right cranial lobe, antero-lateral (AL) branch 1, from four E14.5 embryonic mouse lungs. The pattern and direction exhibited by the R.Cr.AL1 group is remarkably consistent, and compares favourably with the same segment mapped by Metzger and colleagues (Metzger et al., 2008). RCr prefix indicates that the segments are from the right cranial lobe, and suffixes A, P, D, V, LL, LR represent anterior, posterior, dorsal, ventral, lateral left and lateral right, respectively. Medial (M) notation was used by Metzger and colleagues but not used here as the axes set by TS are respective to the axis, which is manually aligned to the data.

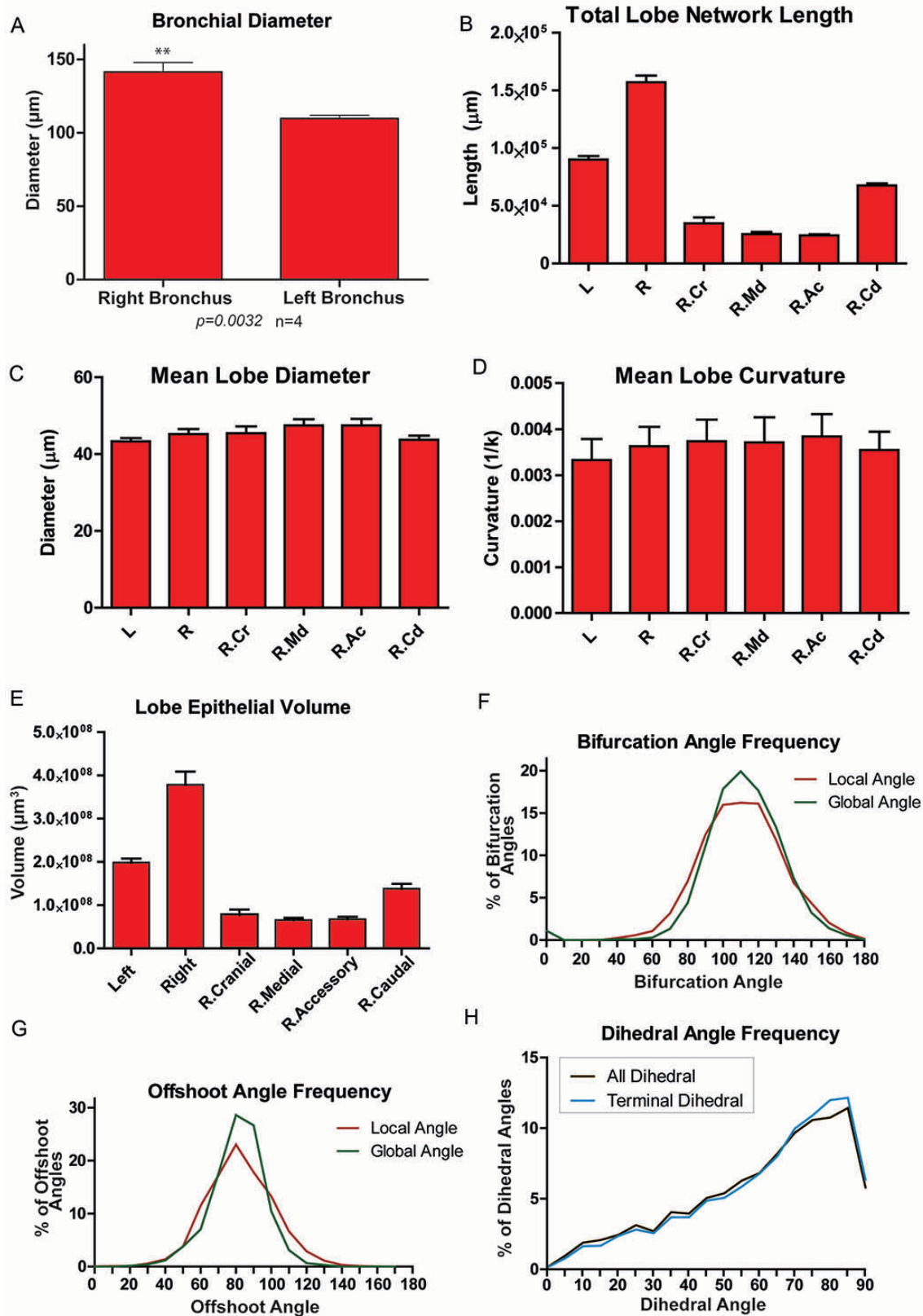


Fig. S4. Embryonic lung lobe and branching angle assessment with Tree Surveyor. (A) At E14.5 the right bronchus has significantly greater diameter than the left bronchus ($P=0.0032$, $n=4$ lungs). (B-E) Analysis of individual lobes from E14.5 embryonic mice indicates that the right lung has a greater volume than the left. When broken down into individual lobes, the left bronchial lobe has the greatest volume and length, followed by the right caudal lobe, while the cranial, medial and accessory lobes are similar in length and volume (B,C). Mean diameter and curvature is consistent across all lobes (D,E). (F) Bifurcation angle frequency distribution is similar between local and global angles, with means of 113.2° and 116.6° , respectively. (G) Using both local and global angle measurements, the angle that offshoots branch off of domain branches, such as the bronchus, has a mean of 82.3° and 81.0° . (H) The dihedral angle frequency distribution for all dihedral angles throughout the lungs mirrors that of the terminal (alveolar) axial dihedral rotations. Error bars represent s.e.m.