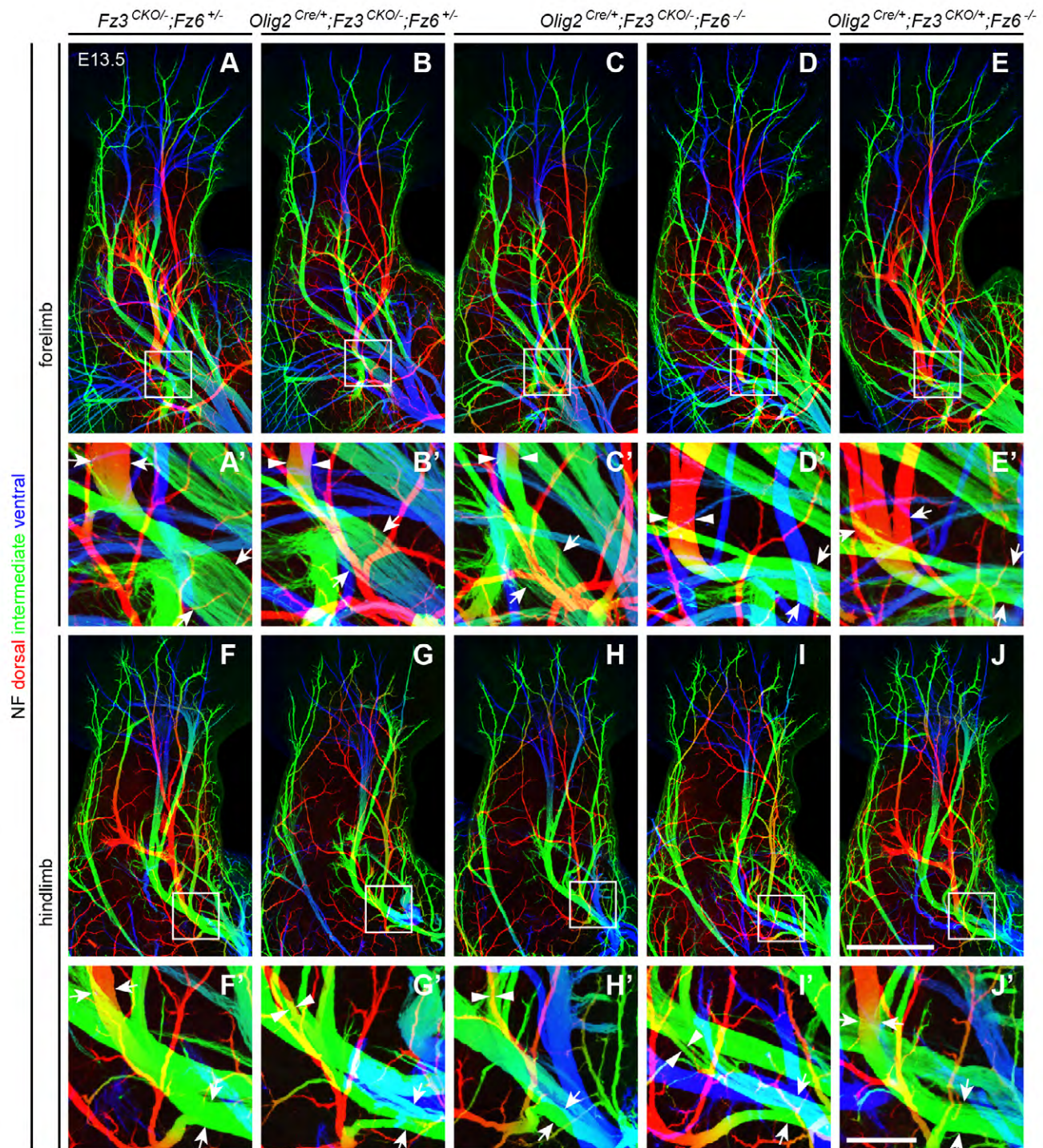


**Figure S1. Failure of  $Z/Fz3^C$  to rescue the palate closure defect in  $Fz1^{-/-};Fz2^{-/-}$  embryos.**

Coronal sections through the head at P0 show the tongue (bottom), the palate (middle), and the nasal cavities and olfactory turbinates (top). In  $Fz1^{-/-};Fz2^{-/-}$  and  $Fz1^{-/-};Fz2^{-/-};Z/Fz3^C$  embryos, the failure of palate closure allows the tongue to protrude upward into the nasal cavity. Scale bar, 1 mm.

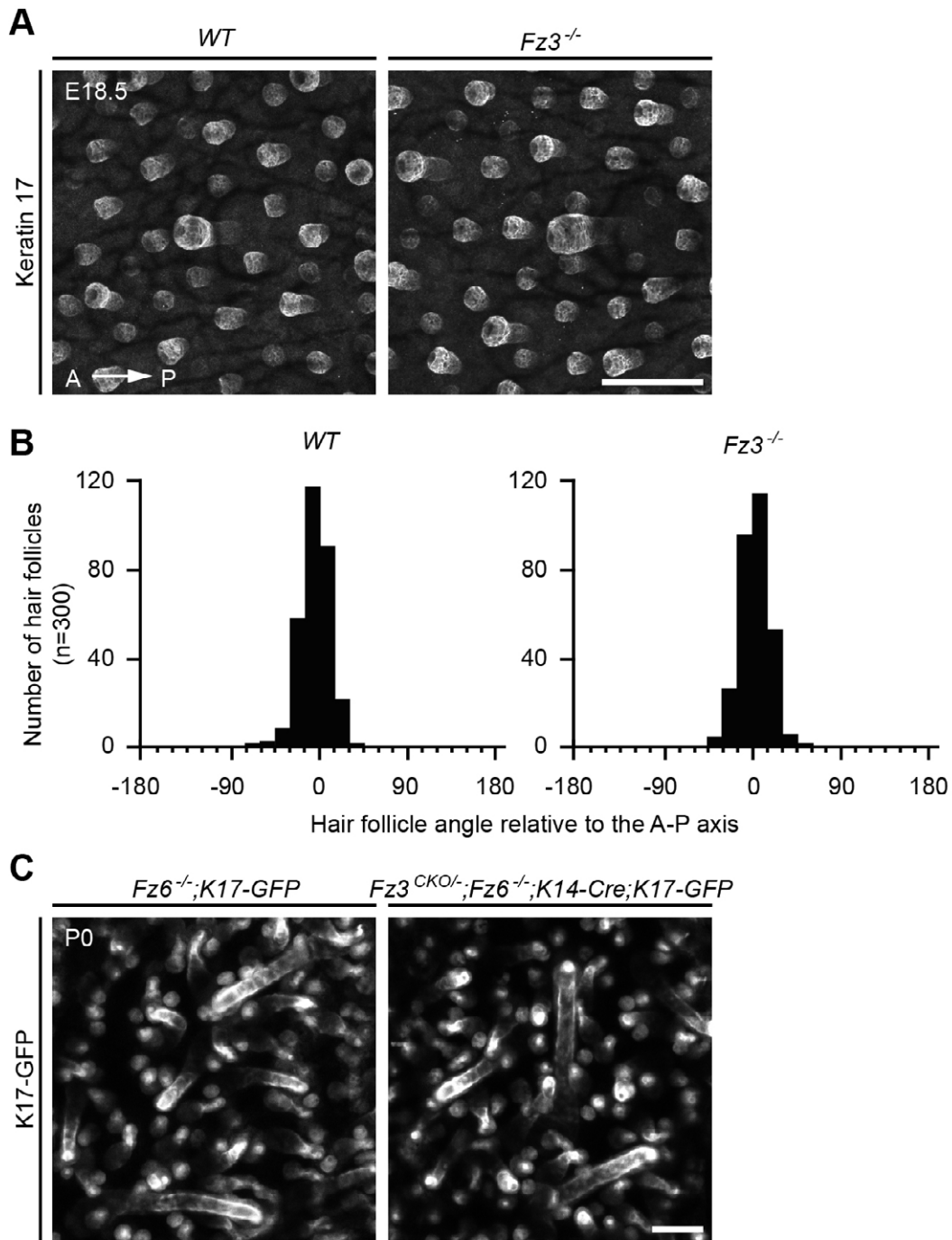


**Figure S2. *Fz6* does not play a redundant role with *Fz3* in motor axon innervation of the dorsal limb.**

(A-E') NF immunostaining of whole-mount E13.5 forelimbs. Boxed regions in (A-E) are magnified in (A'-E'). In *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/-</sup>;Fz6<sup>+/-</sup>* and *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/-</sup>;Fz6<sup>-/-</sup>* forelimbs, the dorsal nerve is thinned to similar extents (compare regions between arrows and arrowheads in B and B' vs. C-D'); limbs from two *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/-</sup>;Fz6<sup>-/-</sup>* embryos, 0803-6 and 0804-3, are shown). In *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/+</sup>;Fz6<sup>-/-</sup>* forelimbs (E and E') the dorsal nerve thickness is indistinguishable from the *WT* control (compare regions between arrows in A and A' vs. E and E'). Depth within the Z-stack along the dorsal to ventral axis is color-coded from red to blue. In this figure, arrows show normal nerve widths and arrowheads show reduced widths.

(F-J') NF immunostaining of whole-mount E13.5 hindlimbs. Boxed regions in (F-J) are magnified in (F'-J'). In *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/-</sup>;Fz6<sup>+/-</sup>* and *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/-</sup>;Fz6<sup>-/-</sup>* hindlimbs, the dorsal nerve is thinned to similar extents (compare regions between arrows and arrowheads in G and G' vs. H-I'). In *Olig2<sup>Cre/+</sup>;Fz3<sup>CKO/+</sup>;Fz6<sup>-/-</sup>* hindlimbs the dorsal nerve thickness is indistinguishable from the *WT* control (compare regions between arrows in F and F' vs. J and J'). Scale bars: (J), 500  $\mu$ m; (J'), 100  $\mu$ m.





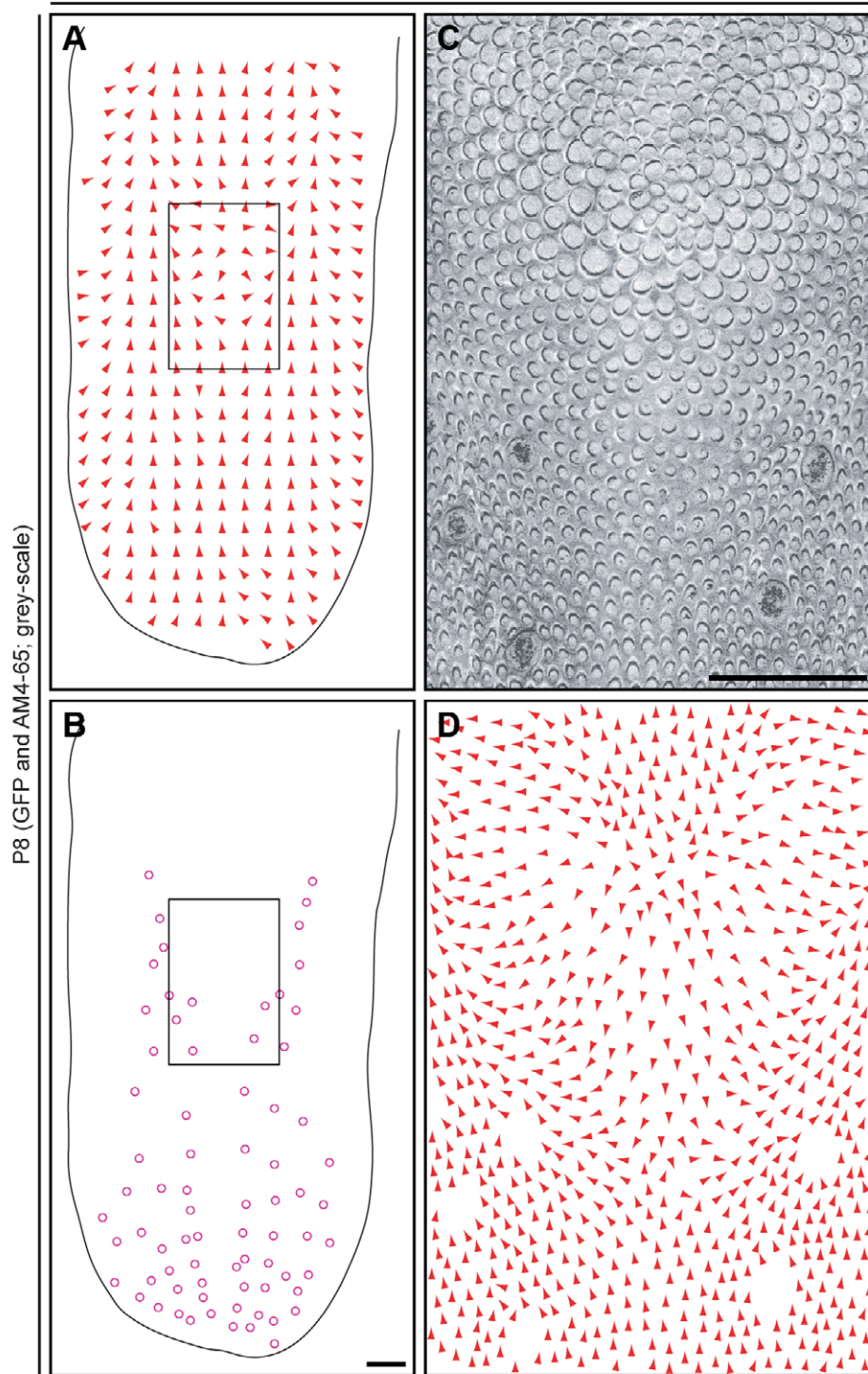
**Figure S3. Loss of *Fz3* has no effect on hair follicle orientation at E18.5.**

(A) Whole-mount back skin from *WT* and *Fz3<sup>-/-</sup>* E18.5 embryos immunostained for K17 to visualize hair follicles. A, anterior; P, posterior. Scale bars, 200  $\mu$ m.

(B) Quantification of hair follicle orientations relative to the A-P axis. Both distributions cluster tightly around zero degrees.

(C) At P0, the hair follicle orientation phenotype produced by combined loss of *Fz6* globally and *Fz3* in the epidermis is indistinguishable from the phenotype produced by global loss of *Fz6*. Whole-mount back skin from *Fz6<sup>-/-</sup>;K17-GFP* and *Fz3<sup>CKO/-</sup>;Fz6<sup>-/-</sup>;K14-Cre;K17-GFP* mice at P1 was imaged for GFP to visualize hair follicles. Scale bar, 100  $\mu$ m.

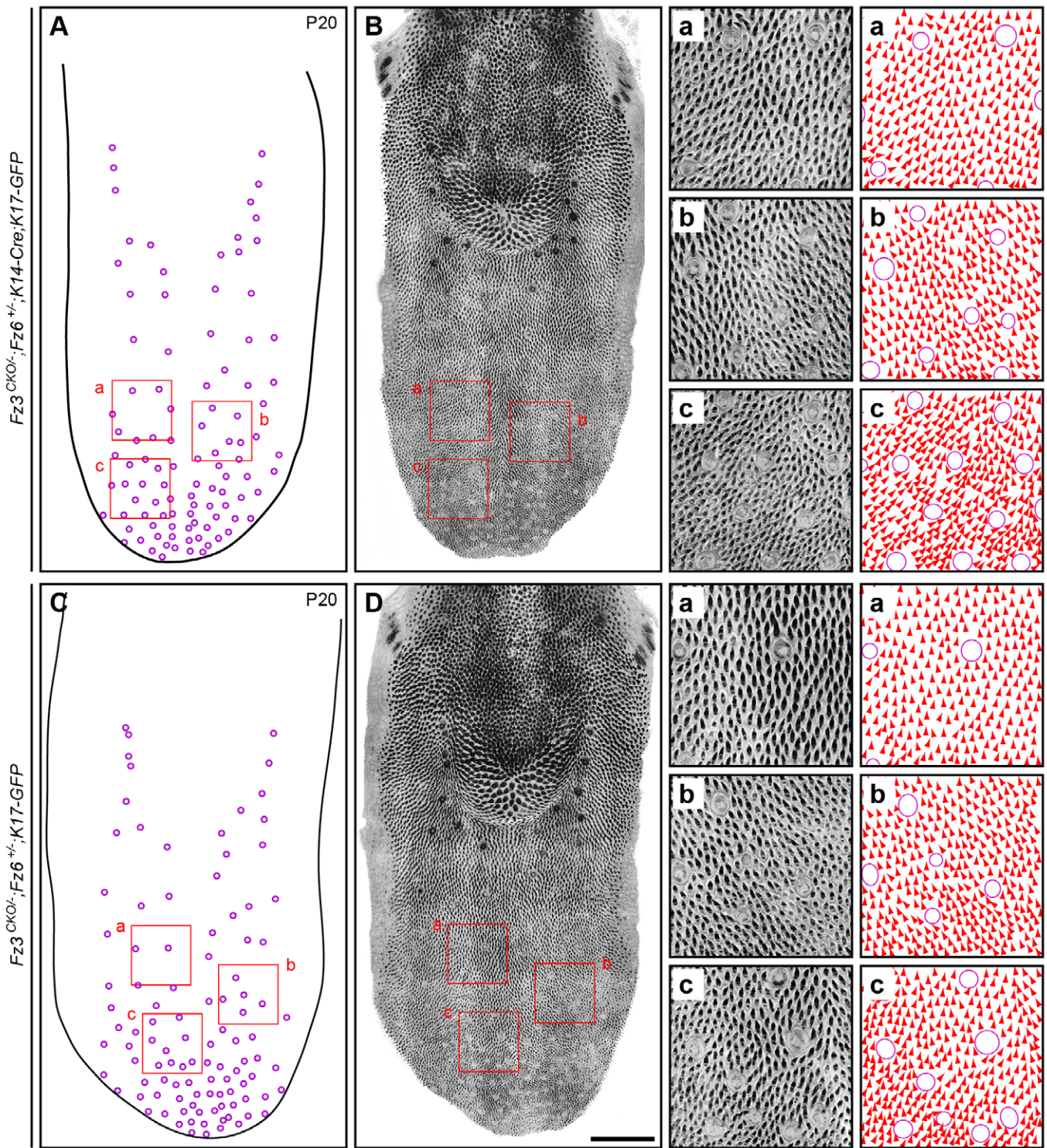
*Fz3<sup>CKO/+</sup>;Fz6<sup>-/-</sup>;K14-Cre;K17-GFP*



**Figure S4. Lingual papillae in and around the patterning center show contiguous variations in orientation.**

(A-D) the dorsal surface of a *Fz3<sup>CKO/+</sup>;Fz6<sup>-/-</sup>;K14-Cre;K17-GFP* tongue at P8 showing the global orientation of lingual papillae (A) and the locations of taste buds (B); both patterns match those of *WT* tongues. The tongue is outlined in black. Anterior is down. The grey scale image of epithelial GFP and AM4-65 dye fluorescence highlights the structure of the papillae. The region enclosed in the black rectangles in (A) and (B) is enlarged in (C) and (D). The orientation of each papilla in (C) is indicated with an arrowhead in (D). The “flower” pattern is present along the midline near the top of the images in (C) and (D), and the point of confluence of posterior and anterior facing papillae is present below the center of each image. Scale bars, 500  $\mu$ m.





**Figure S5. Patterning of lingual papillae is unperturbed in  $Fz3^{CKO/-};Fz6^{+/-};K14-Cre$  and  $Fz3^{CKO/-};Fz6^{+/-}$  tongues.**

(A-D) Dorsal view of a  $Fz3^{CKO/-};Fz6^{+/-};K14-Cre;K17-GFP$  tongue at P20 (A and B) and a  $Fz3^{CKO/-};Fz6^{+/-};K17-GFP$  tongue at P20 (C and D) showing the locations of taste buds (A and C) and epithelial morphology with AM4-65 and GFP fluorescence (B and D). In both cases the pattern matches that of *WT* tongues. Anterior is down. The “flower” pattern is present along the midline at ~70% of the distance from the tip to the base of the tongue. The grey scale images to the right (panels a-c) correspond to the locations of the red squares in the low magnification image at left. The orientation of each papilla in (a-c) was scored with an arrowhead. Scale bar, 1 mm.

```

Fz3 1 MAVSWIVFDLWLLTVFLGQIGGHSLSFCEPITLRMCQDLPYNTTFMPNLL 50
Fz6 1 MERS----PFLACILLPLVRGHSLSFTCEPITVPRCKMNTYNMTFFPNLM 46
* * * * * * . . * . *****.*****. * . ** ** ** .

Fz3 51 NHYDQQTAAALAMEPFHPMVNLDCSRDRFPFLCALYAPICMEYGRVTLPCR 100
Fz6 47 GHYDQGIAAVEMGHFLHLANLECSPNIEMLCQAFIPTCTEQIHVVLPKR 96
.**** ** . * * . ***.*** ** . * * * . * ****

Fz3 101 RLCQRAYSECSKLMEMFGVPWPEDECSRFPDCDEPYPRLVDLNLVGDPT 150
Fz6 97 KLCEKIVSDCKKLMDFGIRWPEELECNRLPHCDDTVPVTSHPHTELSGP 146
.**.. *.* ** . ** .***.*** ** * ** . *

Fz3 151 EGAPVAVQRDYGFWCPRELKIDPDLGYSFLHVRDCSPPCPNMYFRRELS 200
Fz6 147 QKKSQVPRDIGFWCPKHLRTSGDQGYRFLGIEQCAPPCCPNMYFKSDELD 196
. * ** ***** . * ** ** . * .*****. **

Fz3 201 FARYFIGLISIICLSATLFTFLTFLIDVTRFRYPERPIIFYAVCYMMVSL 250
Fz6 197 FAKSFIGIVSIFCLCATLFTFLTFLIDVRRFRYPERPIIYYSVCYSIVSL 246
** . ** . ** ** .***** ***** . * ** .**

Fz3 251 IFFIGFLEDRVACNASSPAQYKASTVTQGSHNACTMLFMVLYFFTMAG 300
Fz6 247 MYFVGFLLGNSTACNKADEKLELGDTVVLGSKNACSVFMFLYFFTMAG 296
..* .**** ** . ** ** .***.*** ** *****

Fz3 301 SVVWVILTITWFLAAVPKWGSEAIEKKALLFHASAWGIPGTLTIILLAMN 350
Fz6 297 TVVWVILTITWFLAAGRKWSCEAIEQKAVWFHAVAWGAPGLTVMLLAMN 346
.***** ** .***.*** ** ** ** ** ** ** .***.***

Fz3 351 KIEGDNISGVCFVGLYDVALRYFVLAPLCLYVVVGVSLLLAGIISLNRV 400
Fz6 347 KVEGDNISGVCFVGLYDLASRYFVLLPLCLCFVGLSLLLAGIISLNVH 396
* .***** ** ***** ** * ** .***** .

Fz3 401 RIEIPEKENQDKLVKFMIRIGVFSILYLVPLLVIIGCYFYEQAYRGIWE 450
Fz6 397 RQVIQHDGRNQEKLVKFMIRIGVFSGLYLVPLVTLGCVYVELVNRITWE 446
* * . **.* ***** ***** . .*** ** * **

Fz3 451 TTWIERCREYHIPCPYQVTQMSRPDLILFLMKYLMALIVGIPISIFWVGS 500
Fz6 447 MTWFSDHCHQYRIPCYPQANPKARPELALFMKYLMALIVGISAVFWVGS 496
** ..* .***.*** . **.* ** .***.*** ** .***.***

Fz3 501 KKTCEWASFFHGRKKEIVNESRQVLQEP-----DFAQSLLRDPNTP 543
Fz6 497 KKTCEWAGFFKRNKRDPISERRVLQESCEFFLKHNSKVKHKKKHGAP 546
*** ** ** . **.. .***.*** . . . *

Fz3 544 -----IIRKSRGTSTQGTSTHASSTQLAMVDDQRKAGSVHVKVSSYHG 587
Fz6 547 GPHRLKVISKSMGTSTGATTNHG-TSAMAIAHDHYLGQETSTEVHTSPEA 595
. * ** ***** * . * . . * . *

Fz3 588 SLHRSRDGRYTPCSYRMEERLPHGMSRLTDHS--RHSSSHRLNEQSR- 634
Fz6 596 SVKEGRADRANTPSAKDRDCGESAGPSSKLSGNRNGRESRAGGLKERSNG 645
*.. * * . * . * * . * * . * * . * * .

Fz3 635 -----HSSIRDLSNNP-----MTHITHGTS 654
Fz6 646 SEGAPSEGRVSPKSSVPETGLIDCSTSQAASSPEPTSLKGTSLPVHSAS 695
. * . * . * . *

Fz3 655 MNRVIEEDG TSA 666
Fz6 696 RARKEQGAGSHSDA 709
* . * .

```

**Figure S6. Alignment of mouse Fz3 and Fz6 amino acid sequences.**

The ligand-binding cysteine-rich domain is highlighted in red and the predicted transmembrane domain is highlighted in blue. Asterisks indicate amino acid identities and dots indicate similarities.

**Supplemental Table 1. Sources for protein sequences used to construct the dendrogram in Figure 7.**

*Caenorhabditis elegans* CFZ-2a: F27E11.3a  
*Caenorhabditis elegans* MOM-5: T23D8.1  
*Caenorhabditis elegans* MIG-1b: Y34D9B.1b

*Ciona intestinalis* Fz3/6: ENSCINP00000026933

*Drosophila melanogaster* Fz: FBpp0303135  
*Drosophila melanogaster* Fz2: FBpp0303228  
*Drosophila melanogaster* Fz3: FBpp0111841  
*Drosophila melanogaster* Fz4: FBpp0070977

*Gallus gallus* (chicken) Fz3: ENSGALP00000026786  
*Gallus gallus* (chicken) Fz6: ENSGALP00000025843

*Homo sapiens* Fz3: CCDS6069  
*Homo sapiens* Fz6: CCDS6298

*Mus musculus* Fz3: CCDS27212  
*Mus musculus* Fz6: CCDS27441

*Petromyzon marinus* (lamprey) Fz6: ENSPMAP00000000914

*Takifugu rubripes* Fz3: ENSTRUP00000011955  
*Takifugu rubripes* Fz6: ENSTRUP00000017077

*Xenopus tropicalis* Fz3: ENSXETP00000045728  
*Xenopus tropicalis* Fz6: ENSXETP00000008881

Sources:

CCDS: the Consensus CDS Database

ENS: Ensembl

FB: FlyBase

*C. elegans*: WormBase