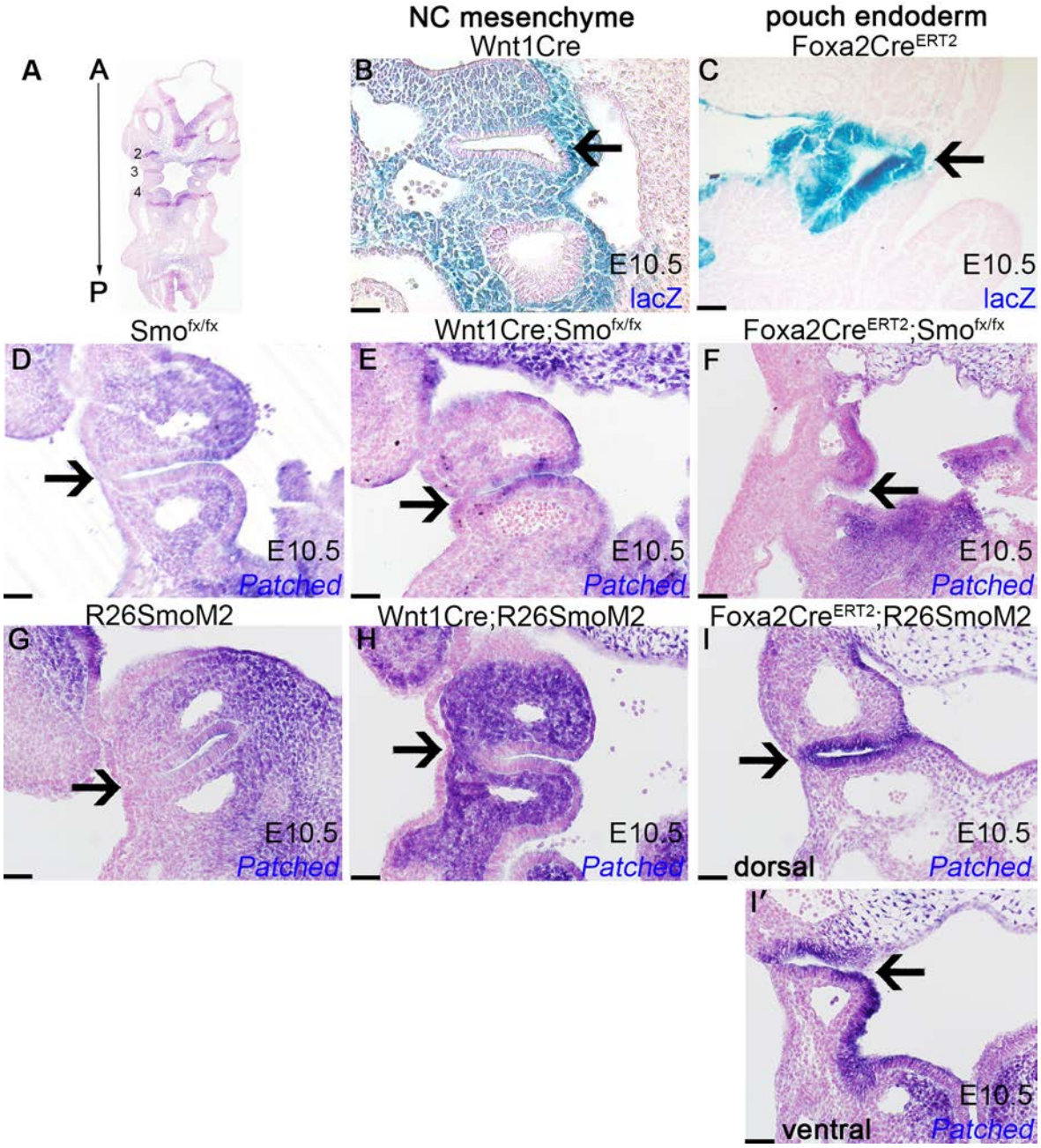

Figure S1. Validation of mouse models used in the study. Arrows in all panels indicate the 3rd pp. A: Coronal section showing anterior to posterior orientation (arrow) and locations of the 2nd 3rd and 4th pharyngeal pouches (numbers). B: Sagittal section showing activation of the R26R βgal reporter by

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Wnt1Cre. C: Transverse section showing activation of the R26R βgal reporter by *Foxa2Cre*^{ERT2} after injection of tamoxifen at E5.75. D-I: Coronal sections showing SHH signaling as assayed by *Ptch1* ISH at E10.5. Cre-negative littermate controls show that SHH signaling is not uniform throughout the endoderm (D, G). SHH signaling is absent in the mesenchyme when *Smo* is deleted by *Wnt1Cre* (E). SHH signaling is absent in the endoderm when *Smo* is deleted by *Foxa2Cre*^{ERT2} (F). SHH signaling is constitutively active in the mesenchyme when *SmoM2* is activated by *Wnt1Cre* (H). SHH signaling is constitutively active in the endoderm when *SmoM2* is activated by *Foxa2Cre*^{ERT2} (I). I' shows a more ventral section from the same embryo as in I. Scale bars: 50 μm (n = 3 for panels D - I).

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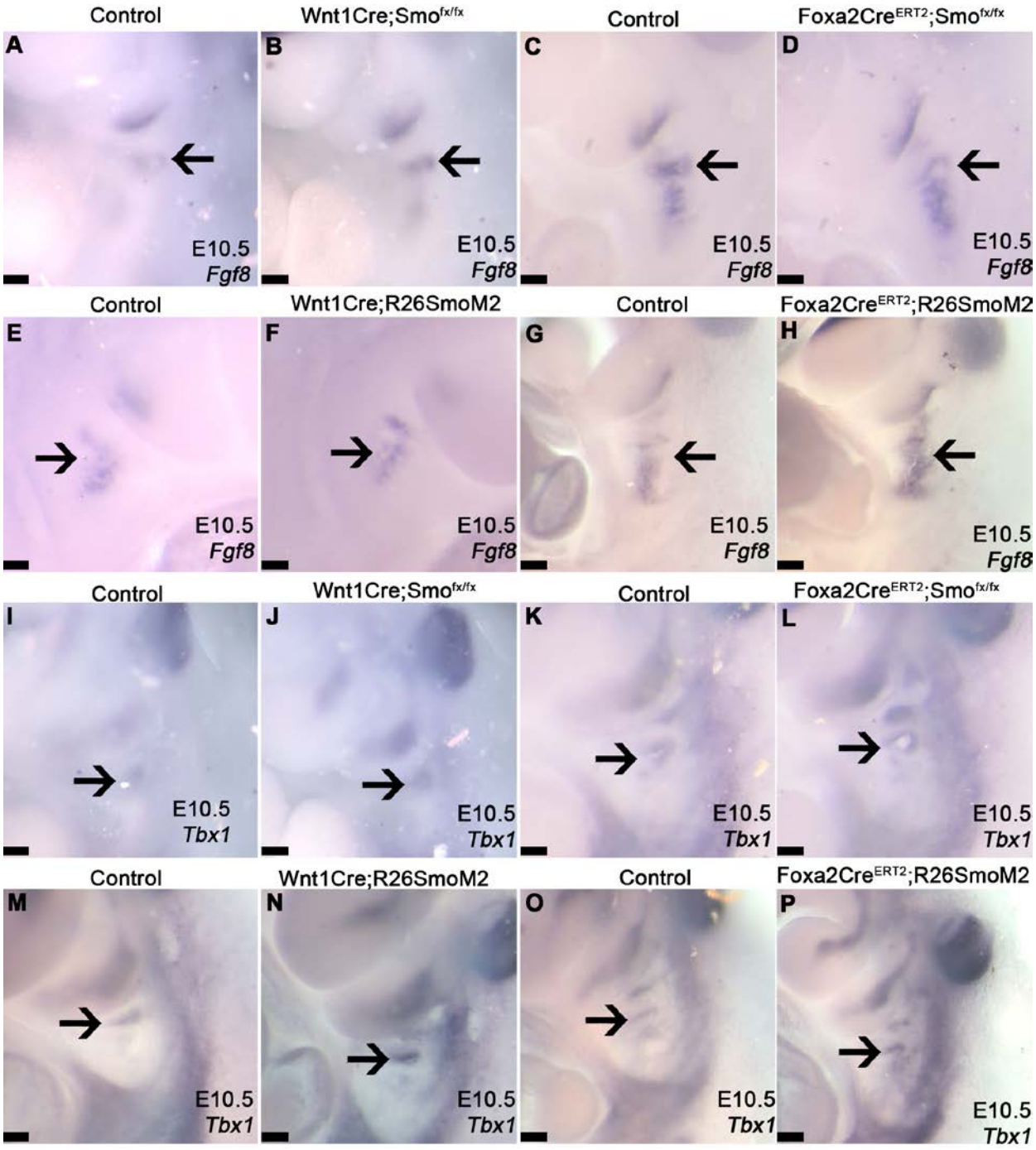

Figure S2. Markers of pharyngeal pouch patterning are unchanged at E10.5. A-H: Lateral views of whole-mount *Fgf8* ISH on mutants and littermate controls matched by somite stage at high magnification (A-D, 36 somites; E-F, 37 somites; G-H, 35 somites). Arrows indicate 3rd pharyngeal

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pouch. I-P: Lateral views of whole-mount *Tbx1* ISH on mutants and littermate controls matched by somite stage at high magnification (I-L, 36 somites; M-N, 37 somites; O-P, 35 somites). Arrows indicate 3rd pharyngeal pouch. Scale bar: 100 μm, ($n \geq 4$ for all panels except H, where n = 2). Controls A, C, and K are $Cre^{neg};Smo^{fx/fx}$. Control I is $Cre^{neg};Smo^{fx/+}$. Controls E, G, M, and O are $Cre^{neg};R26SmoM2/+$.

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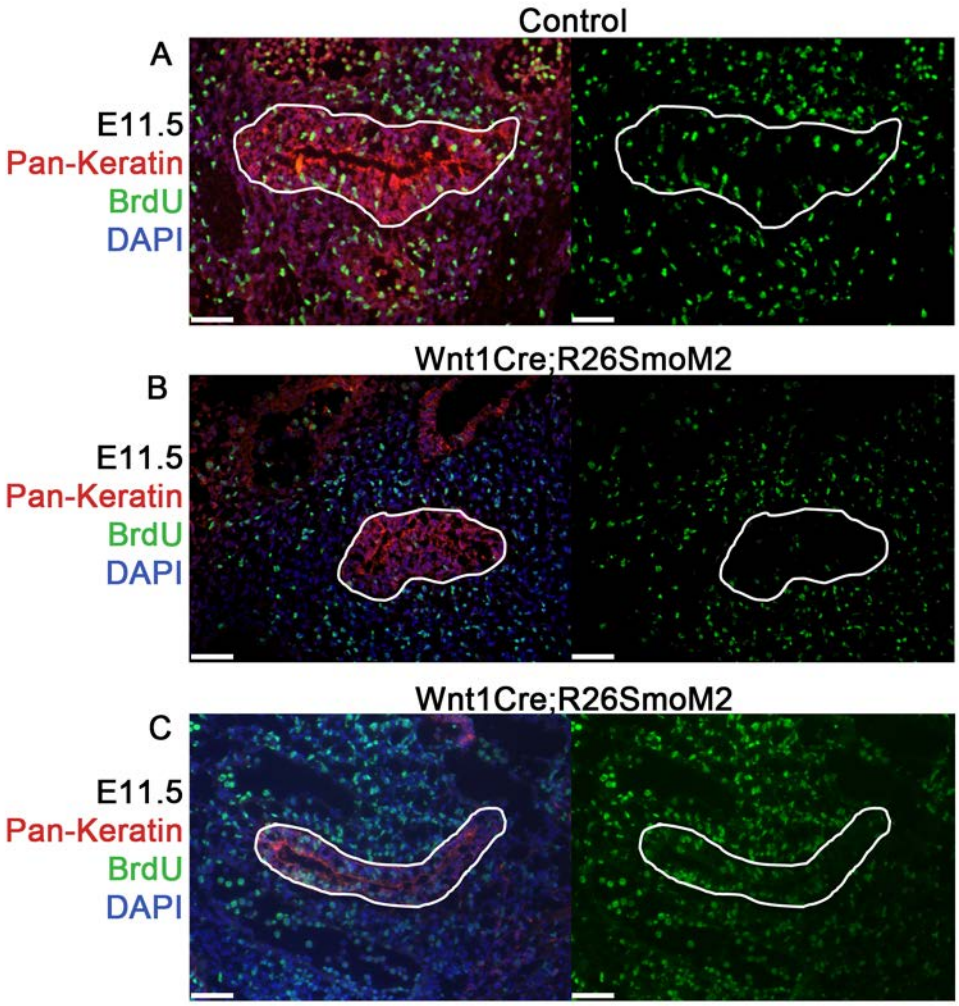

Figure S3. Proliferation in *Wnt1Cre;R26SmoM2* embryos at E11.5. A-C: Sagittal sections through E11.5 primordia stained with antibodies against BrdU (green) and Pan-Keratin (red), showing reduced proliferation in the mutants (n = 4). In all cases the primordium was clearly smaller than controls, but in some cases (B; n = 2) there were very few $BrdU^{+}$ cells in the primordium, while in others (C; n = 2) there appeared to be more, even though the epithelium clearly had not expanded. Primordia are outlined in white. Scale bar: 50 μm. (n = 4). Control A is Cre^{neg};*R26SmoM2/+*.

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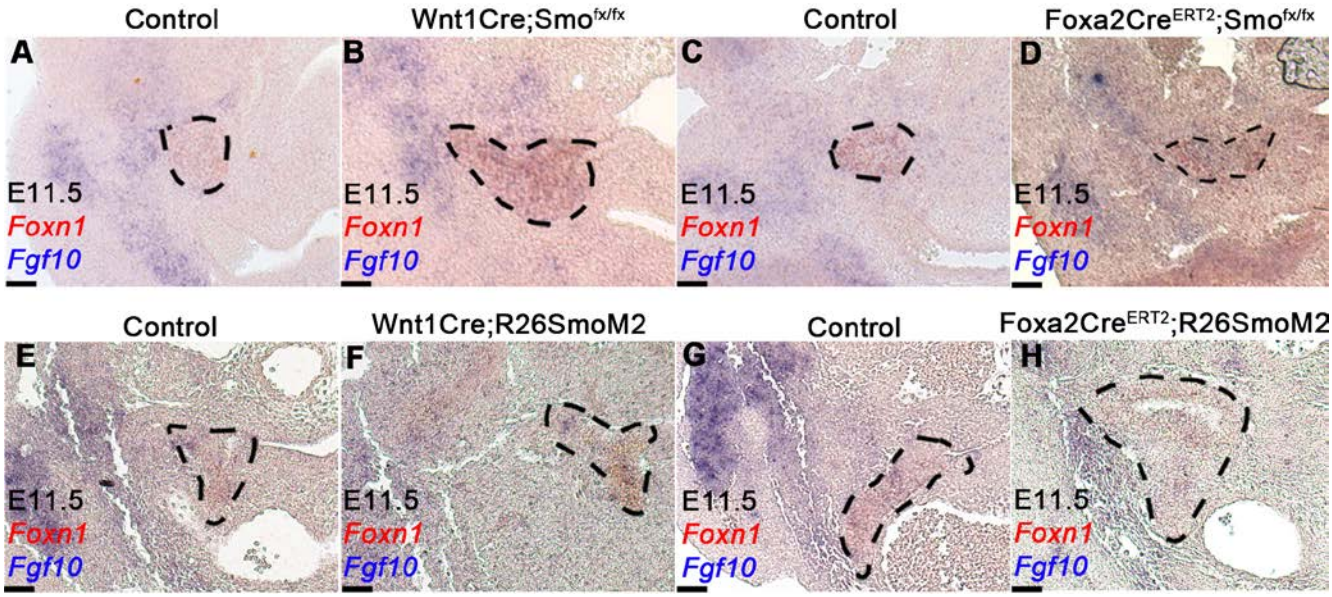

Figure S4. *Fgf10* expression in SHH signaling mutants. A-H: Transverse sections of dual-color *Foxn1* (red) and *Fgf10* (purple) ISH at E11.5. Pouch is outlined in black. Scale bars: 50 μm (n = 2 per panel, except B and H where n = 3). Controls A and C are $Cre^{neg};Smo^{fx/fx}$. Controls E and G are $Cre^{neg};R26SmoM2/+$.

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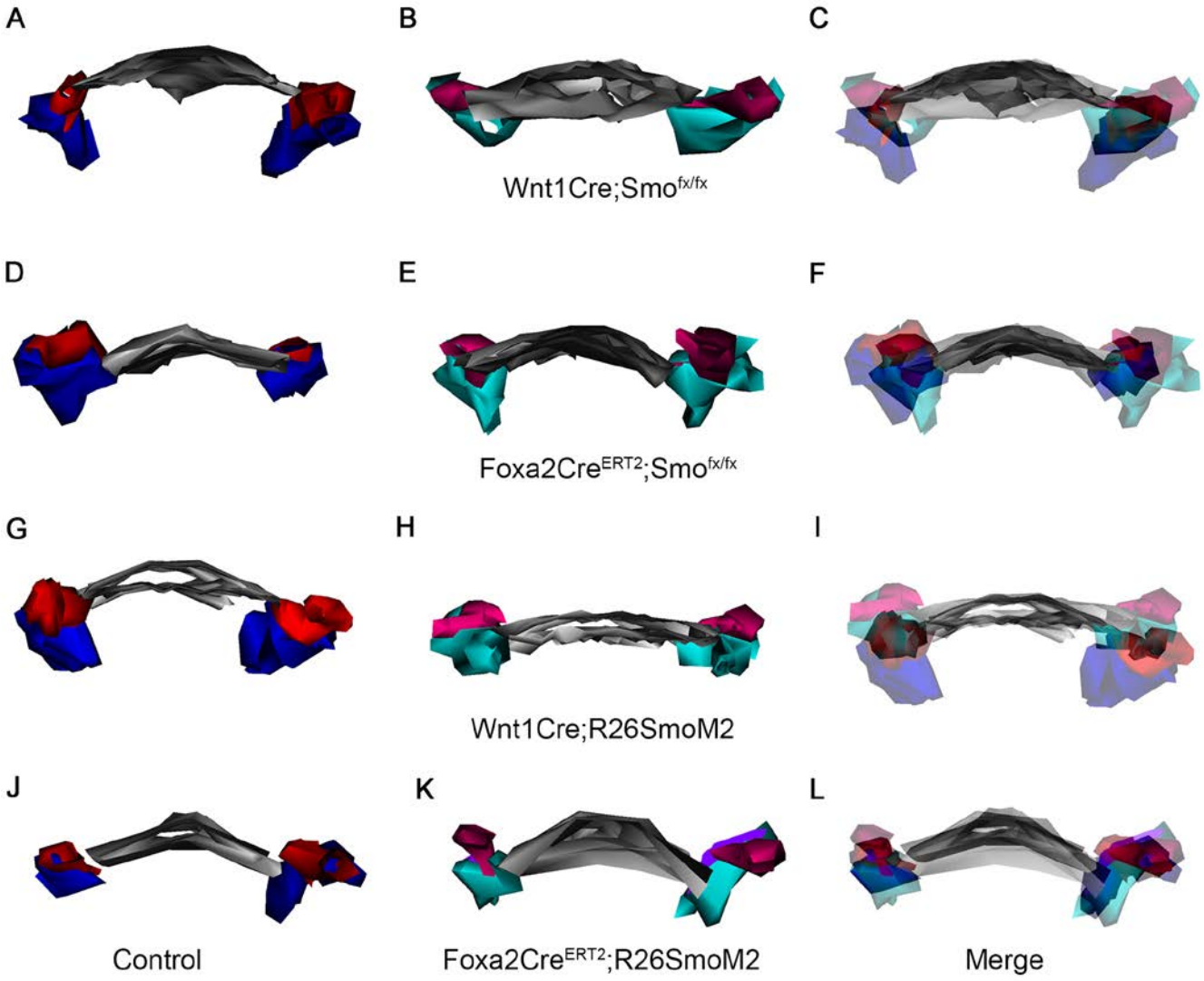

Figure S5. Pharynx shape and pouch location in SHH signaling mutants. A, D, G, J: Anterior views of 3-D reconstructions of wild type littermates at E11.5 showing the Gcm2 domain (red), the Foxn1 domain (blue), and the pharynx (gray). B, E, H, K: Anterior views of 3-D reconstructions of *Wnt1Cre;Smo*^{fx} (B), *Foxa2Cre*^{ERT2}*;Smo*^{fx} (E), *Wnt1Cre;R26SmoM2* (H), and *Foxa2Cre*^{ERT2}*;R26SmoM2* (K) embryos at E11.5 showing the Gcm2 domain (magenta), the Foxn1 domain (turquoise), and the pharynx (gray). C, F, I, L: Merged 3-D reconstructions for *Wnt1Cre;Smo*^{fx} (C), *Foxa2Cre*^{ERT2}*;Smo*^{fx}

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(F), *Wnt1Cre;R26SmoM2* (I), and *Foxa2Cre^{ERT2};R26SmoM2* (L). All reconstructions were generated from transverse sections of embryos stained by dual ISH for *Foxn1* and *Gcm2* (data not shown, n = 2 per genotype). Control A is *Cre^{neg};Smo$^{fx/fx}$*. Control D is *Cre^{neg};Smo$^{fx/+}$*. Controls G and J are *Cre^{neg};R26SmoM2/+*.

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