## NFIB Regulates Embryonic Development of Submandibular Glands

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Appendix



**Appendix Figure 1.** Mucin presence and distribution is altered in  $Nfib^{-/-}$  mice at E16.5 and E18.5. Slides were prepared as described in the Materials and Methods. Slides were stained with a mucicarmine stain (Newcomer Supply, Middleton, WI, USA). The red arrow indicates mucin expression in the cytoplasm.

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**Appendix Figure 2.** Hematoxylin and eosin staining of E16.5 mouse tissue sections. Formalin-fixed E16.5 and E18.5 SMGs were dissected out, embedded in paraffin, and stained with hematoxylin and eosin. No sublingual gland is evident in either the wild-type or  $Nfib^{-/-}$  mice at age E16.5.



**Appendix Figure 3.** Ductal and terminal end bud lumen formation in E18.5 mouse tissue sections. Salivary glands were dissected, embedded in paraffin, and stained with ZO-1 (green) and E-cadherin (red) antibodies and propidium iodide (blue), as described in the Materials and Methods. Red arrows indicate ductal lumen formation and yellow arrows indicate terminal end bud lumen formation.



**Appendix Figure 4.** Negative control staining of E16.5 and E18.5 mouse tissue sections. Sections were incubated with Alexa Fluor 488–conjugated goat anti-rabbit (1:500 dilution in 5% goat serum; Sigma, St. Louis, MO, USA) and Alexa Fluor 633–conjugated goat anti-mouse (1:500 dilution in 5% goat serum; Sigma), as described in the Materials and Methods.