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Figures and figure supplements

Cochlear progenitor number is controlled through mesenchymal FGF receptor signaling

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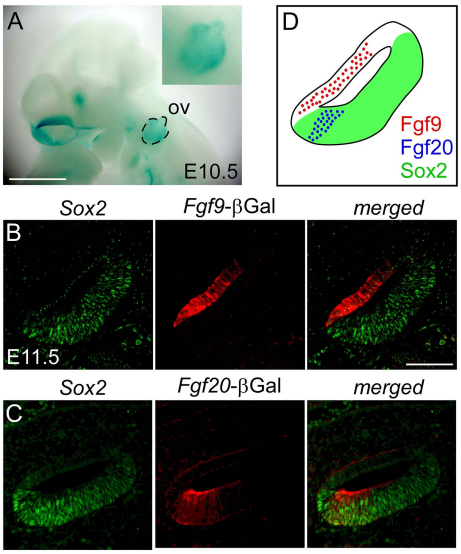

Figure 1. *Fgf9* and *Fgf20* are expressed in distinct regions of the otic vesicle. (**A**) βGal activity in an $Fgf9^{lacZ/+}$ embryo at E10.5 visualized with xGal staining. (**B**, **C**) βGal (red) and Sox2 (green) co-immunostaining showing that *Fgf9* (**B**) is expressed in Sox2- non-sensory epithelium and *Fgf20* (**C**) is expressed in $Sox2^{+}$ sensory epithelium at E11.5. (**D**) Schematic diagram of FGF9, FGF20, and Sox2 immunostaining showing that FGF9 and FGF20 are expressed in distinct domains in the otic vesicle. ov, otic vesicle, scale bars, 100 μm.

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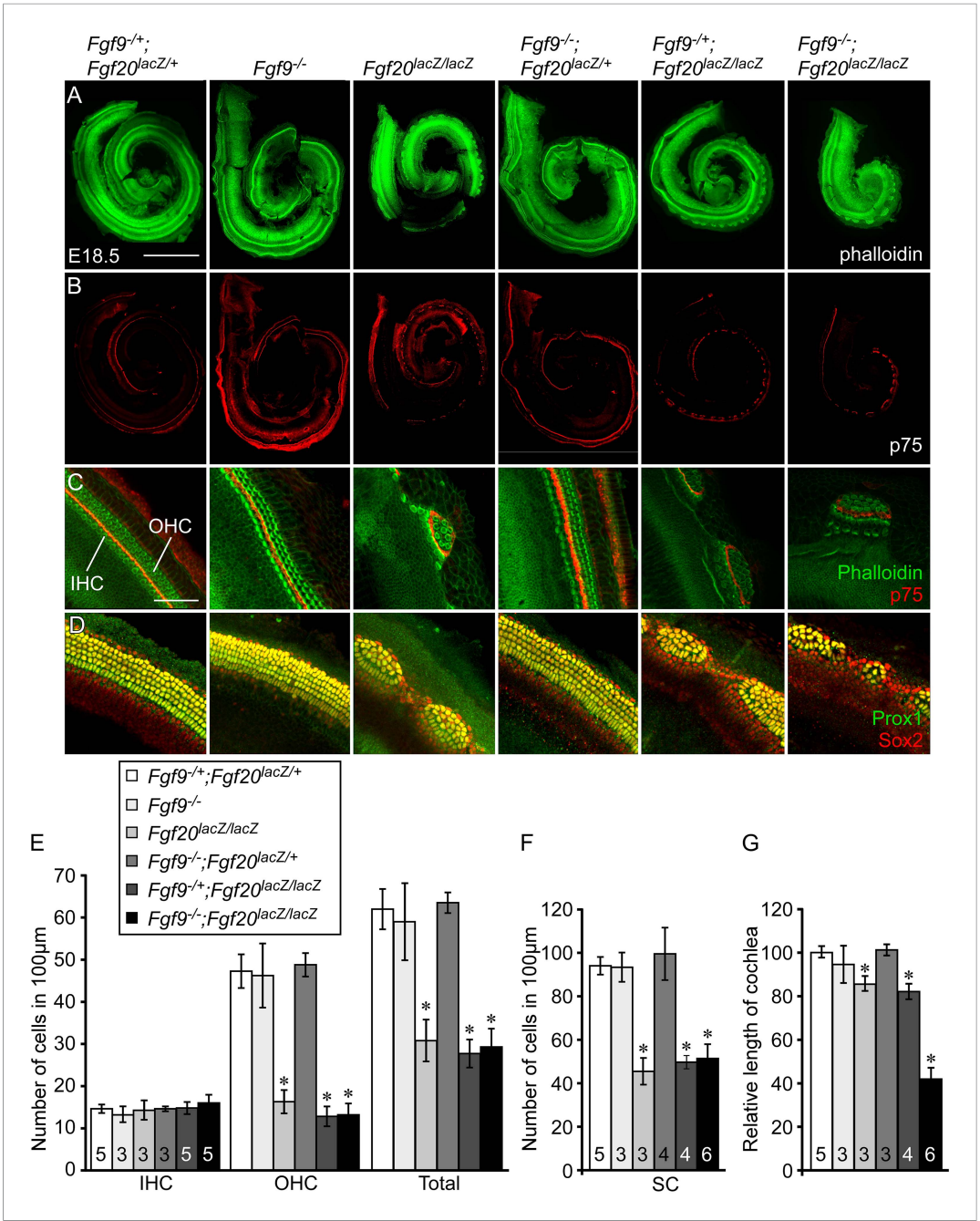

Figure 2. *Fgf9* and *Fgf20* regulate cochlear length. (**A**, **B**) Phalloidin (**A**) and p75 immunostaining (**B**) of E18.5 whole cochlea showing hair cells (HCs) (phalloidin) and pillar cells (p75) in the cochlear duct of *Fgf9*$^{-/+}$;*Fgf20*$^{lacZ/+}$, *Fgf9*$^{-/-}$, *Fgf20*$^{lacZ/lacZ}$, *Fgf9*$^{-/-}$;*Fgf20*$^{lacZ/+}$, *Fgf9*$^{-/+}$;*Fgf20*$^{lacZ/lacZ}$ and, *Fgf9*$^{-/-}$;*Fgf20*$^{lacZ/lacZ}$ embryos. (**C**) Phalloidin (green) and p75 immunostaining (red) showing the orientation of HCs, pillar cells, and gaps in the sensory epithelium. (**D**) Prox1 (green) and Sox2 (red) co-immunostaining showing supporting cells (SCs) (yellow, Prox1 and Sox2) and undifferentiated sensory progenitors (red, Sox2). (**E**–**G**) Measurement of HC number (**E**), SC number (**F**), and length of cochleae (**G**) of E18.5 embryos. Scale bars, **A**, 500 µm; **C**, 100 µm. For statistical analysis, all samples were compared with *Fgf9*$^{-/+}$;*Fgf20*$^{lacZ/+}$ double heterozygous controls. *p < 0.001. Sample numbers (n) are indicated in data bars.

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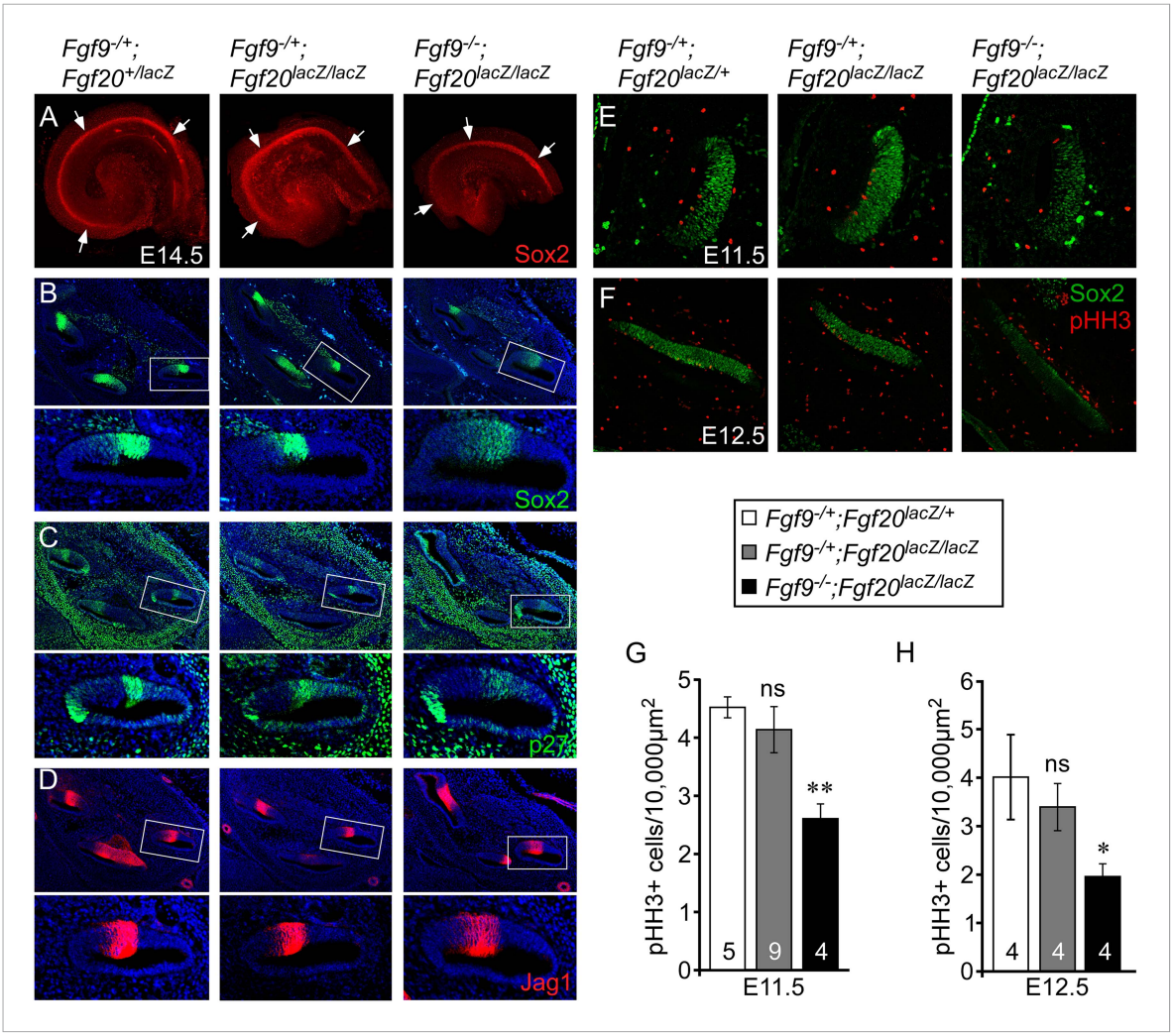

Figure 3. *Fgf9* and *Fgf20* are required for sensory progenitor proliferation. (**A**) Sox2 immunostaining of whole E14.5 cochlea to identify the progenitor domain (arrows). (**B**–**D**) Sox2 (**B**), p27 (**C**), and Jag1 (**D**) immunostaining of E14.5 $Fgf9^{-/+};Fgf20^{lacZ/+}$, $Fgf9^{-/+};Fgf20^{lacZ/lacZ}$, and $Fgf9^{-/-};Fgf20^{lacZ/lacZ}$ embryo sections. Boxed regions of the cochlear duct are magnified below each image and were chosen in regions where the sections perpendicularly transect the cochlear duct. (**E**, **F**) Sox2 and phospho-Histone H3 (pHH3) co-immunostaining of E11.5 (**E**) and E12.5 (**F**) $Fgf9^{-/+};Fgf20^{lacZ/+}$, $Fgf9^{-/+};Fgf20^{lacZ/lacZ}$ and, $Fgf9^{-/-};Fgf20^{lacZ/lacZ}$ embryo sections. (**G**, **H**) Measurement of $Sox2^{+}$ sensory progenitor proliferation at E11.5 (**G**) and E12.5 (**H**). All samples were compared with $Fgf9^{-/+};Fgf20^{lacZ/+}$ double heterozygous controls. *p < 0.01, **p < 0.001. Sample numbers (n) are indicated in data bars. See also ***Figure 3—figure supplements 1, 2***.

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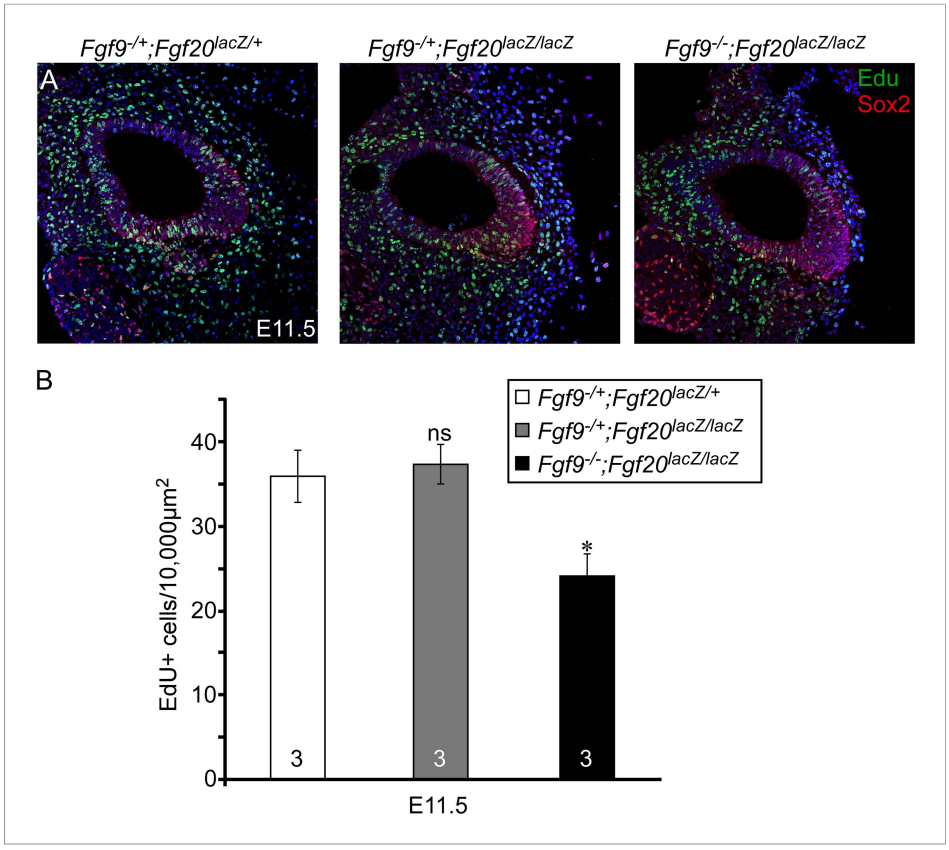

Figure 3—figure supplement 1. Proliferation of sensory progenitors. (**A**) Sox2 and EdU staining of E11.5 *Fgf9*$^{-/+}$;*Fgf20*$^{lacZ/+}$, *Fgf9*$^{-/+}$;*Fgf20*$^{lacZ/lacZ}$, and *Fgf9*$^{-/-}$;*Fgf20*$^{lacZ/lacZ}$ embryo sections. (**B**) Measurement of Sox2^{+} sensory progenitor proliferation at E11.5. All samples were compared with *Fgf9*$^{-/+}$;*Fgf20*$^{lacZ/+}$ double heterozygous controls. *p < 0.01. ns, not significant. Sample numbers (n) are indicated in data bars.

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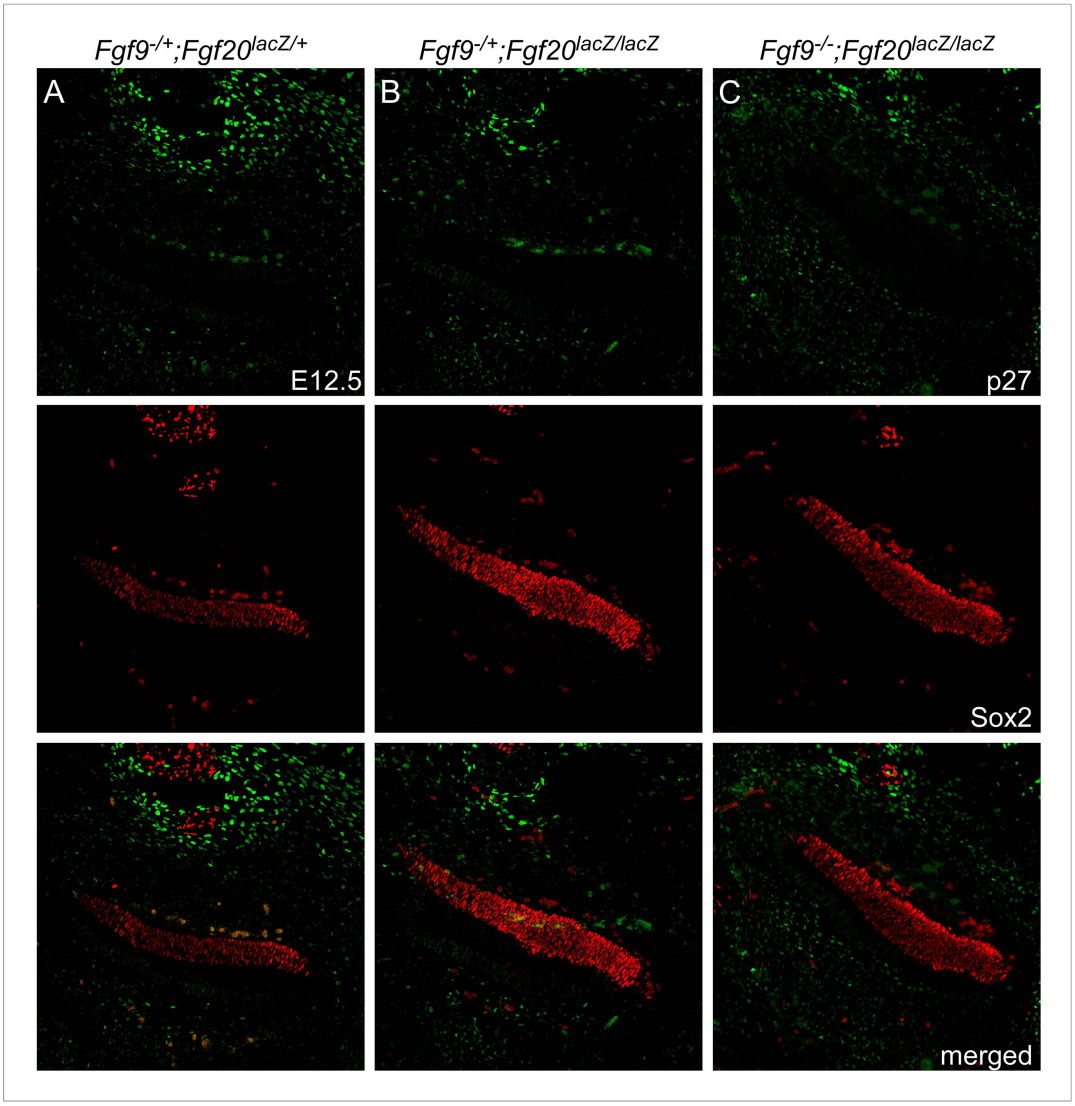

Figure 3—figure supplement 2. *Fgf9* and *Fgf20* loss do not cause premature cell cycle exit. (**A**–**C**) p27 (green) and Sox2 (red) co-immunostaining of E12.5 $Fgf9^{-/+};Fgf20^{lacZ/+}$ (**A**), $Fgf9^{-/+};Fgf20^{lacZ/lacZ}$ (**B**), and $Fgf9^{-/-};Fgf20^{lacZ/lacZ}$ (**C**) embryo sections.

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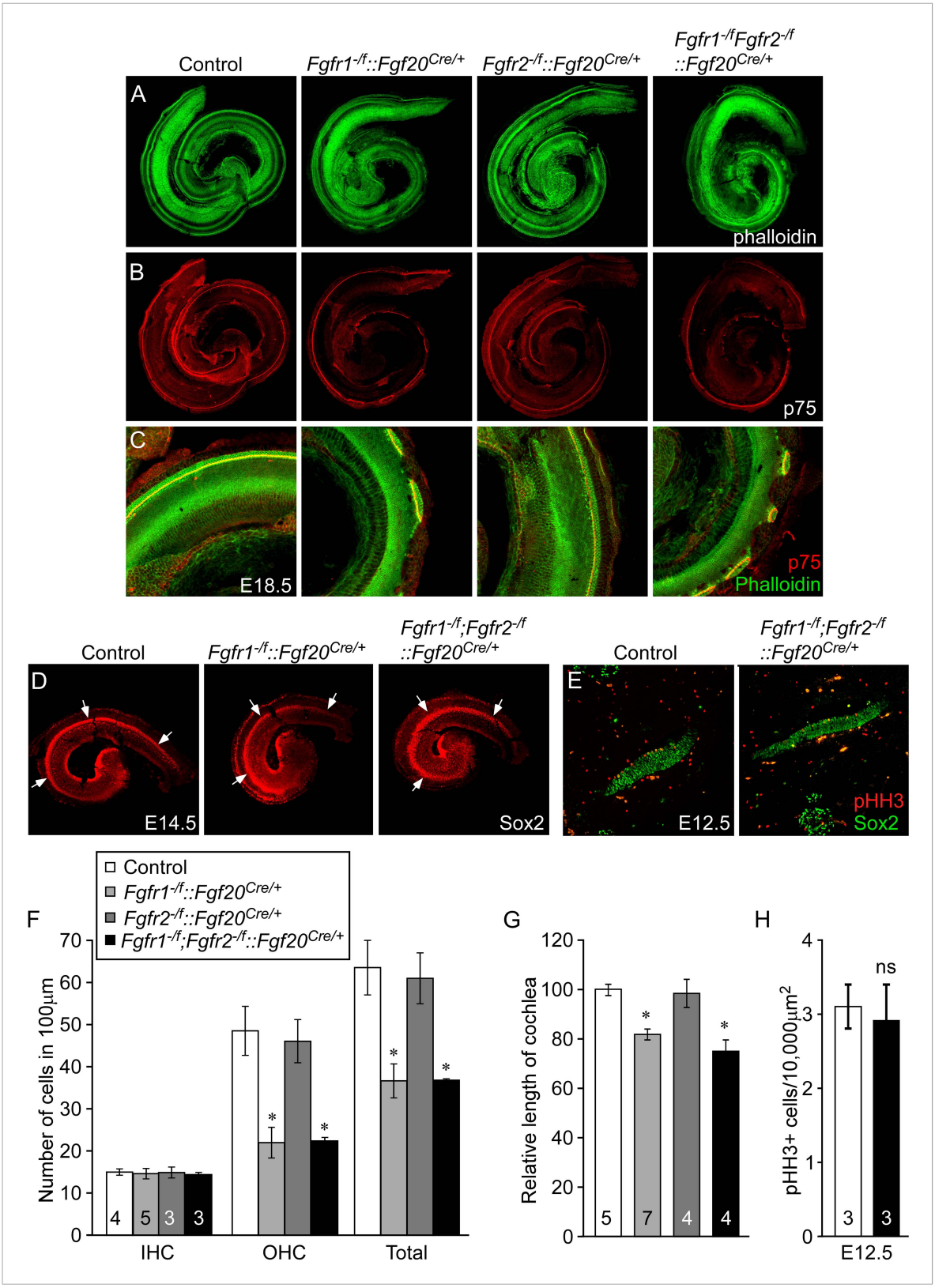

Figure 4. Cell-autonomous regulation of sensory progenitor differentiation requires epithelial *Fgfr1* but not *Fgfr2*. (**A**, **B**) Phalloidin (**A**) and p75 immunostaining (**B**) of E18.5 whole cochlea showing HCs (phalloidin) and pillar cells (p75) in the cochlear duct of control, *Fgfr1*$^{-/f}$::*Fgf20*$^{Cre/+}$ (*Fgfr1*$^{-/f}$;*Fgfr2*$^{+/f}$::*Fgf20*$^{Cre/+}$), *Fgfr2*$^{-/f}$::*Fgf20*$^{Cre/+}$ (*Fgfr1*$^{+/f}$;*Fgfr2*$^{-/f}$::*Fgf20*$^{Cre/+}$) and, *Fgfr1*$^{-/f}$;*Fgfr2*$^{-/f}$::*Fgf20*$^{Cre/+}$ embryos. (**C**) Phalloidin (green) and p75 immunostaining (red)

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Figure 4. Continued

showing the patterning of HCs and pillar cells in the cochlear duct. (**D**) Sox2 immunostaining of E14.5 whole cochlea to identify progenitor domains (arrows). (**E**) Sox2 and pHH3 co-immunostaining of E12.5 embryo sections. (**F**, **G**) Measurement of HC number (**F**) and length of cochleae (**G**) of E18.5 control embryos. (**H**) Measurement of Sox2^{+} sensory progenitor proliferation in E12.5 embryos. All samples were compared with controls. *p < 0.001; ns, not significant. Sample numbers (n) are indicated in data bars. See also ***Figure 4—figure supplements 1, 2***.

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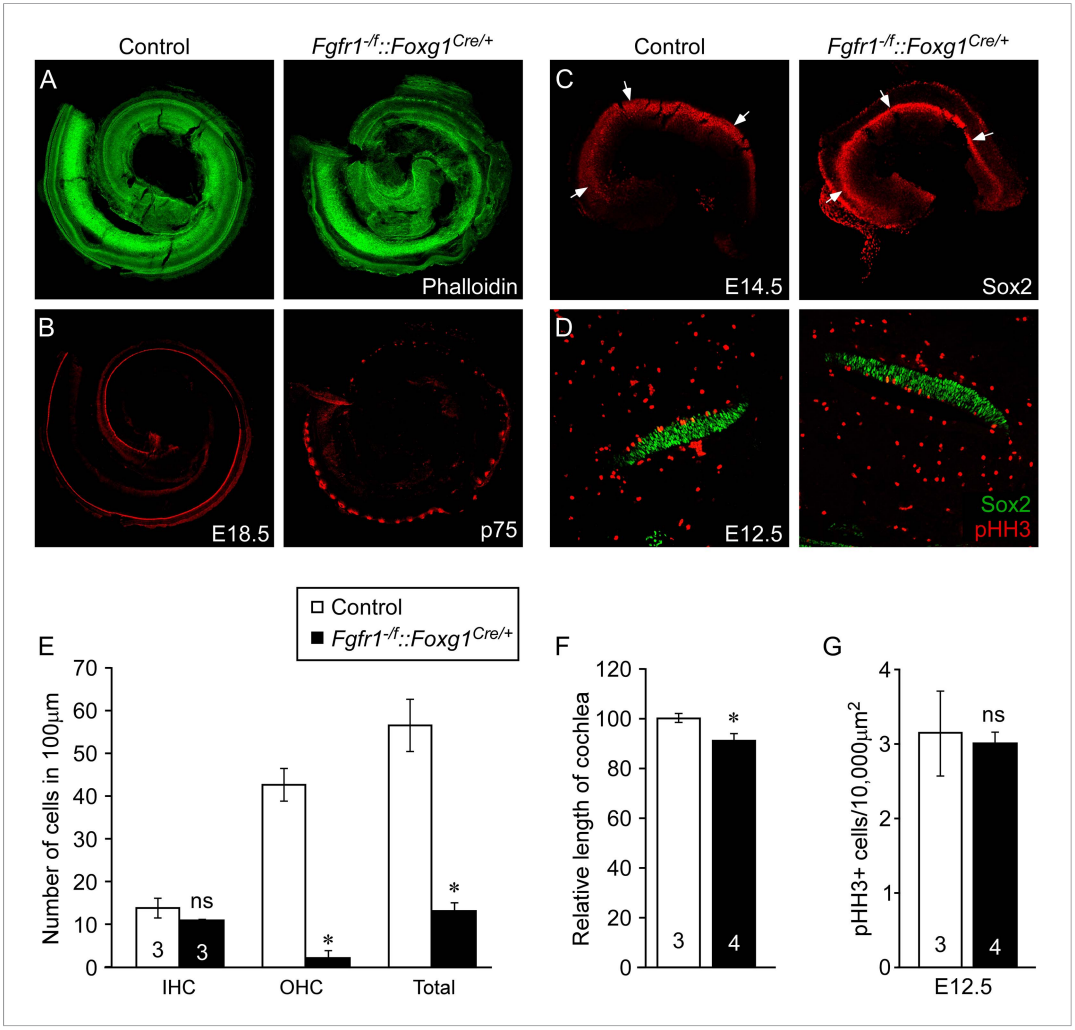

Figure 4—figure supplement 1. Epithelial *Fgfr1* is required for lateral compartment differentiation and HC and SC patterning. (**A**, **B**) Phalloidin (**A**) and p75 immunostaining (**B**) of E18.5 whole cochlea showing HCs (phalloidin) and pillar cells (p75) in the cochlear duct of control and *Fgfr1*$^{-/f}$::*Foxg1*$^{Cre/+}$ embryos. (**C**) Sox2 immunostaining of E14.5 whole cochlea to identify progenitor domains (arrows). (**D**) Sox2 and pHH3 co-immunostaining of E12.5 embryo sections. (**E**, **F**) Measurement of HC number (**E**) and length of cochleae (**F**) of E18.5 control and *Fgfr1*$^{-/f}$::*Foxg1*$^{Cre/+}$ embryos. (**G**) Measurement of Sox2^{+} sensory progenitor proliferation in E12.5 cochleae. *p < 0.001 in **E** and *p < 0.01 in **F**. Sample numbers (n) are indicated in data bars.

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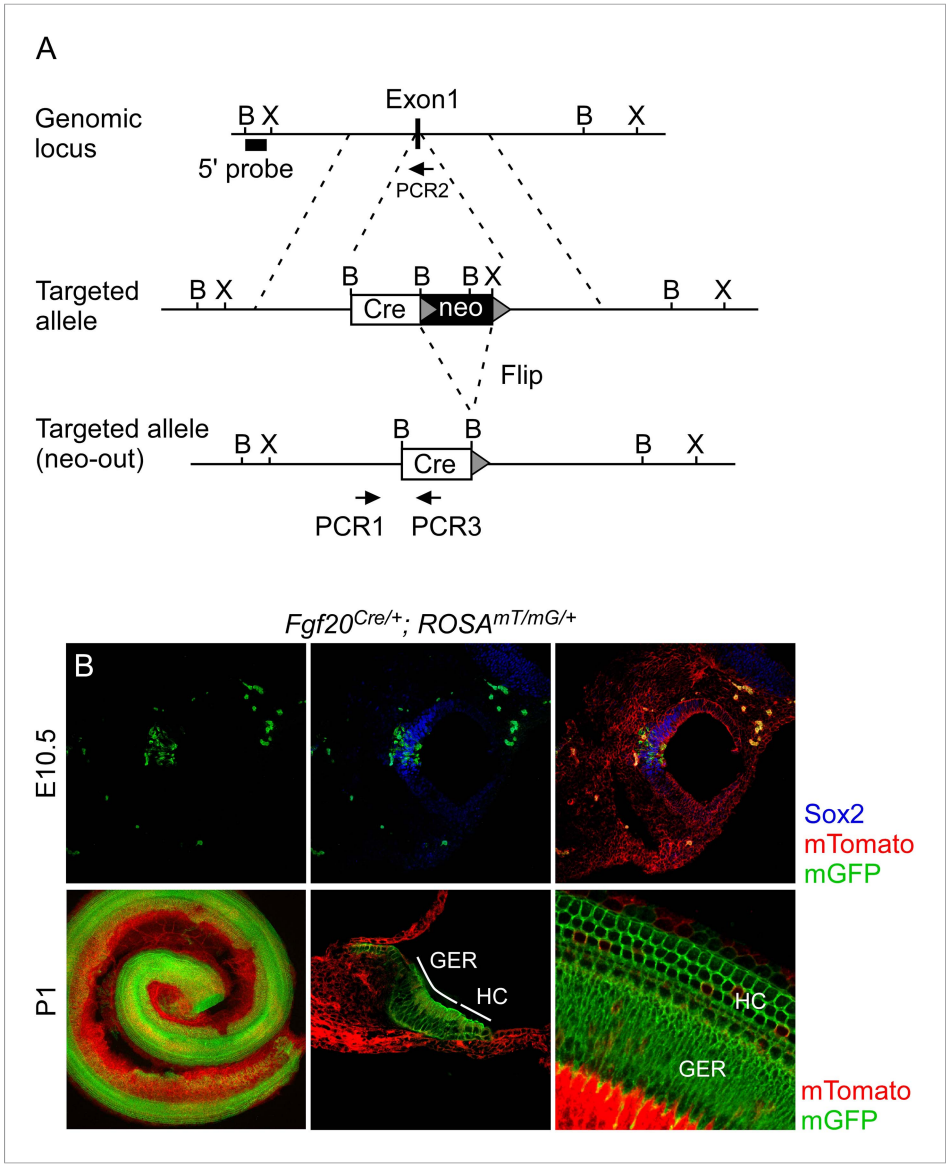

Figure 4—figure supplement 2. Generation of an *Fgf20*^{Cre} knockin mouse line. (**A**) Schematic diagram showing targeting of the *Fgf20* genomic locus. Homologous recombination in mouse ES cells was used to insert a GFP:Cre (Cre) gene and neo selection cassette (flanked by Flip recombination target sequences, grey triangles) into exon 1 of *Fgf20*. F1 mice were bred to mice that express Flip recombinase in the germline to excise the neo selection cassette. Arrows indicate PCR primers used for genotyping. B, BamH1; X, Xho1. (**B**) *Fgf20*$^{Cre/+}$; *ROSA26*$^{mT/mG/+}$ (*ROSA*$^{mT/mG/+}$) double transgenic mice showing the cumulative lineage of *Fgf20*^{Cre} expressing cells (green) in the Sox2^{+} prosensory domain (blue) at E10.5 and in HCs, SCs, and the greater epithelial ridge (GER) at P1. Recombination at the *ROSA26*$^{mT/mG}$ locus silences membrane localized Tomato (mT) and activates membrane localized GFP (mG).
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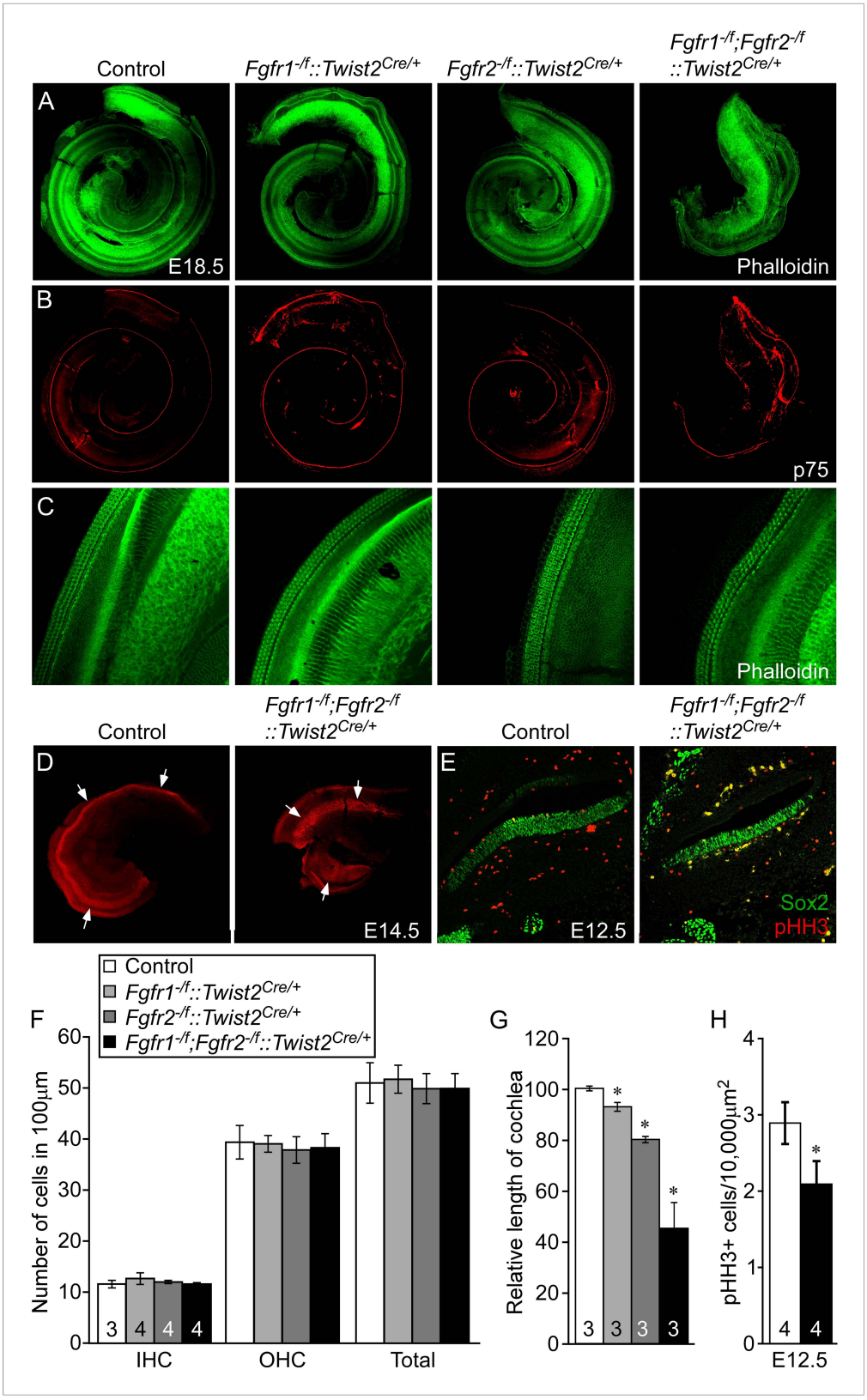

Figure 5. Mesenchymal *Fgfr1* and *Fgfr2* regulate the length of the cochlear duct and sensory progenitor proliferation. (**A**, **B**) Phalloidin (**A**) and p75 immunostaining (**B**) of E18.5 whole cochlea showing HCs (phalloidin) and pillar cells (p75) in the cochlear duct of control, *Fgfr1*$^{-/f}$*::Twist2*$^{Cre/+}$ (*Fgfr1*$^{-/f}$*;Fgfr2*$^{+/f}$*::Twist2*$^{Cre/+}$), *Fgfr2*$^{-/f}$*::Twist2*$^{Cre/+}$

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(*Fgfr1*$^{+/f}$;*Fgfr2*$^{-/f}$::*Twist2*$^{Cre/+}$), and *Fgfr1*$^{-/f}$;*Fgfr2*$^{-/f}$::*Twist2*$^{Cre/+}$ embryos. (**C**) Phalloidin (green) staining showing normal HC patterning in the cochlear sensory epithelium. (**D**) Sox2 immunostaining of E14.5 whole cochlea to identify progenitor domains (arrows). (**E**) Sox2 and pHH3 co-immunostaining of E12.5 cochlea sections. (**F**, **G**) Measurement of HC number (**F**) and length of cochleae (**G**) of E18.5 control, *Fgfr1*$^{-/f}$::*Twist2*$^{Cre/+}$ (*Fgfr1*$^{-/f}$;*Fgfr2*$^{+/f}$::*Twist2*$^{Cre/+}$), *Fgfr2*$^{-/f}$::*Twist2*$^{Cre/+}$ (*Fgfr1*$^{+/f}$;*Fgfr2*$^{-/f}$::*Twist2*$^{Cre/+}$), and *Fgfr1*$^{-/f}$;*Fgfr2*$^{-/f}$::*Twist2*$^{Cre/+}$ embryos. (**H**) Measurement of Sox2^{+} sensory progenitor (green) proliferation (red, pHH3) in E12.5 embryo sections. All samples were compared with controls. *$p < 0.001$. Sample numbers (n) are indicated in data bars. See also ***Figure 5—figure supplement 1***.

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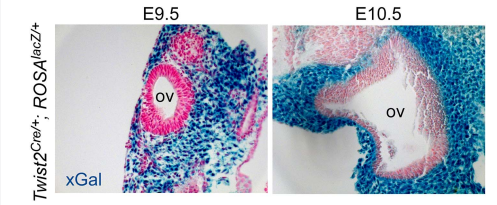

Figure 5—figure supplement 1. *Twist2*^{Cre} targeting of periotic mesenchyme. *Twist2*$^{Cre/+}$;*ROSA26*$^{lacZ/+}$ (*ROSA*-$^{lacZ/+}$) double transgenic mice showing βGal activity (xGal, blue) in periotic mesenchymal cells at E9.5 and E10.5. OV, otic vesicle.

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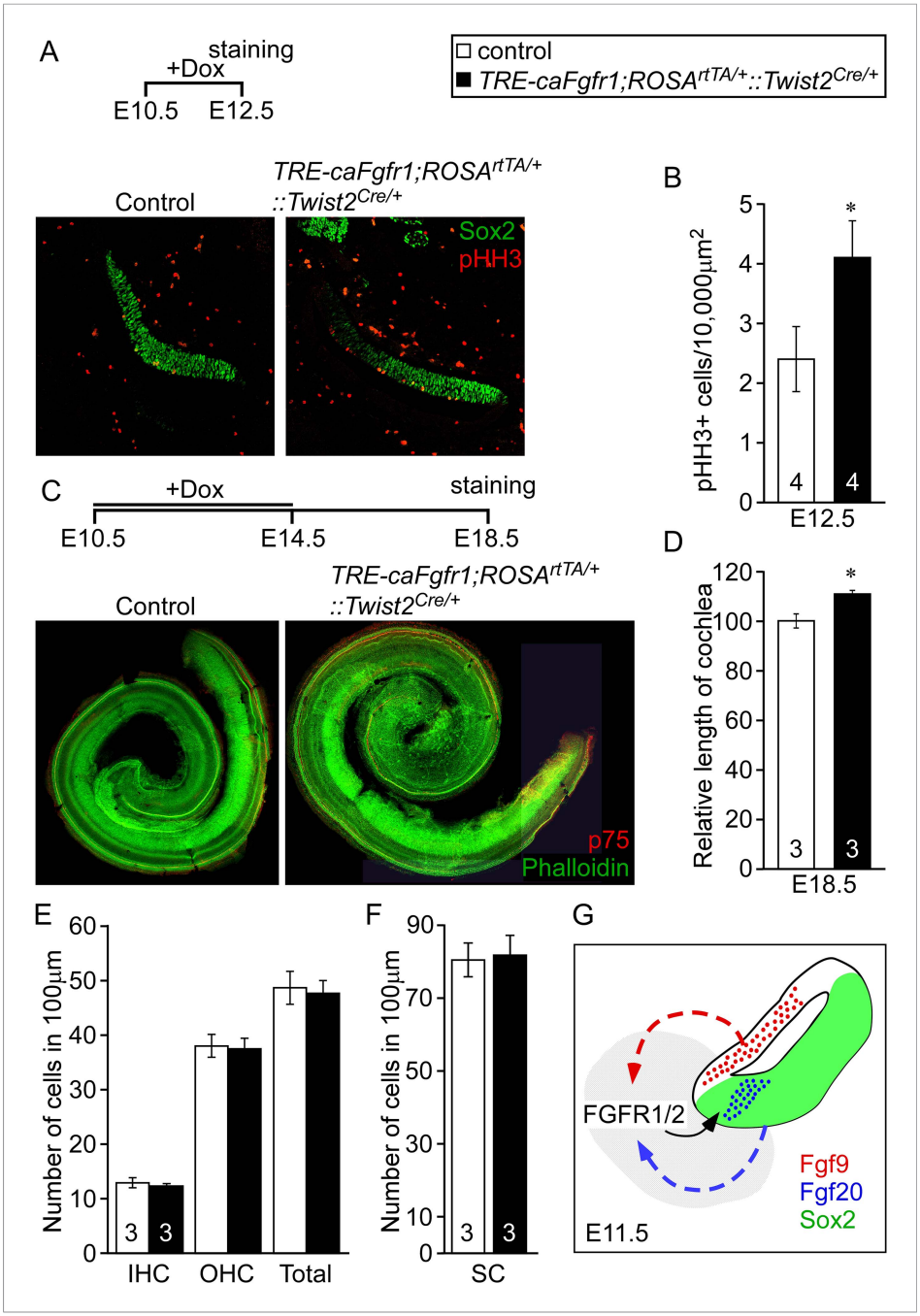

Figure 6. Ectopic activation of FGFR signaling in mesenchyme increases sensory progenitor proliferation and cochlear length. (**A**) Sox2 (green) and pHH3 (red) co-immunostaining of E12.5 control and *TRE-caFgfr1;ROSA*$^{rtTA/+}$*::Twist2*$^{Cre/+}$ embryo sections. (**B**) Measurement of Sox2^{+} sensory progenitor proliferation at E12.5. (**C**) Phalloidin (green) and p75 immunostaining (red) showing the patterning of HCs and pillar cells in the cochlear duct of control and *TRE-caFgfr1;ROSA*$^{rtTA/+}$*::Twist2*$^{Cre/+}$ embryos. Measurement of the length of the cochleae (**D**), HC number (**E**), and SC number (**F**) of E18.5 control and *TRE-caFgfr1;ROSA*$^{rtTA/+}$*::Twist2*$^{Cre/+}$ embryos. (**G**) Schematic diagram indicating the requirement for epithelial FGF9/20 signaling to mesenchymal FGFR1/2 to induce sensory progenitor proliferation. *$p < 0.01$ in **B** and *$p < 0.001$ in **D**. Sample numbers (n) are indicated in data bars. See also ***Figure 6—figure supplement 1***.

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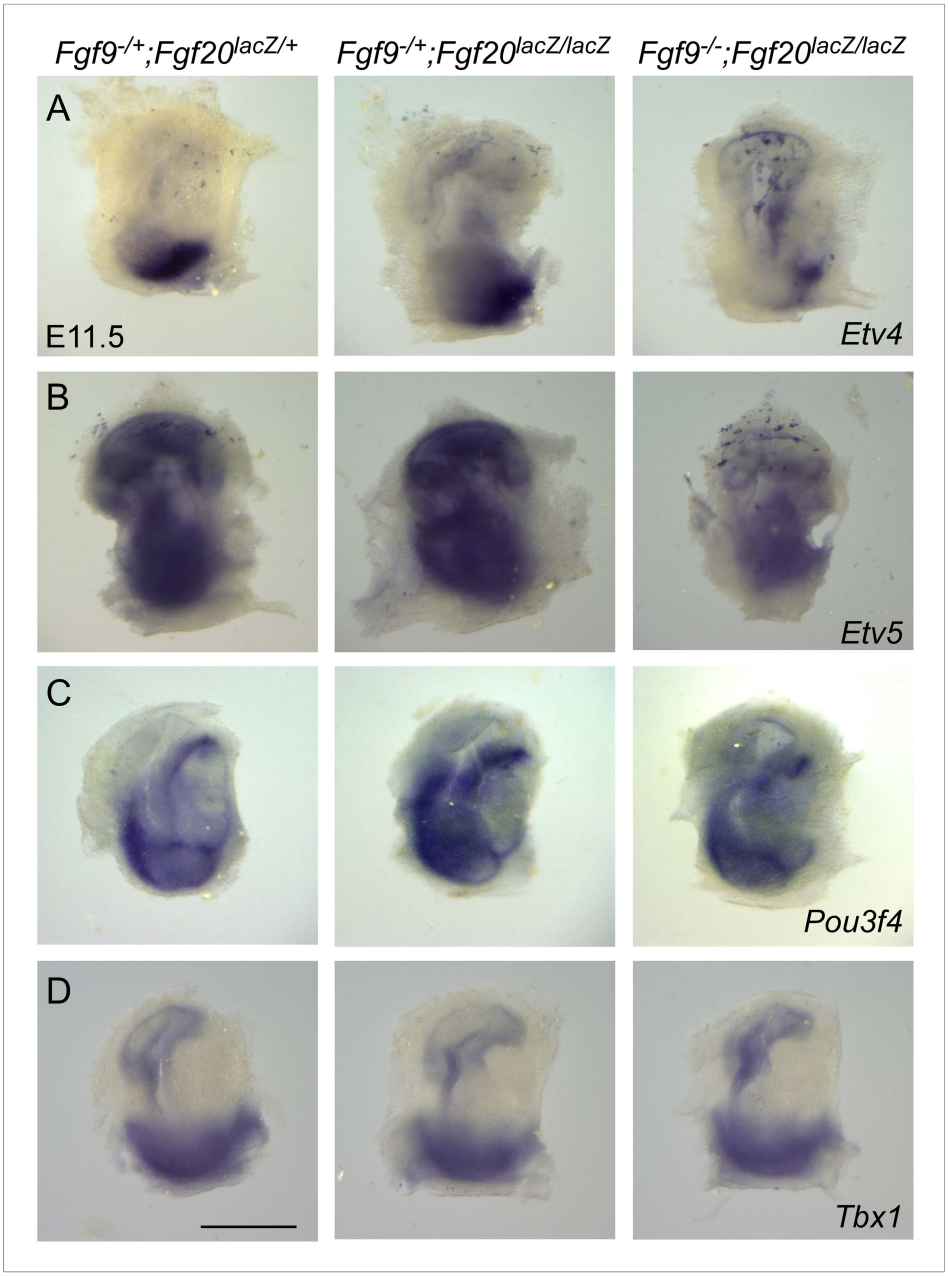

Figure 6—figure supplement 1. *Fgf9* and *Fgf20* regulate the expression of *Etv4* and *Etv5*, but not *Pou3f4* or *Tbx1*. (**A**–**D**) Wholemout mRNA in situ staining of *Etv4* (**A**), *Etv5* (**B**), *Pou3f4* (**C**), and *Tbx1* (**D**) genes on otic vesicles from E11.5 embryos. The genotypes of embryos analyzed are: $Fgf9^{-/+};Fgf20^{lacZ/+}$, $Fgf9^{-/+};Fgf20^{lacZ/lacZ}$ and, $Fgf9^{-/-};Fgf20^{lacZ/lacZ}$. Images are representative of at least three embryos for each probe and genotype. Scale bar, 500 μm.
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