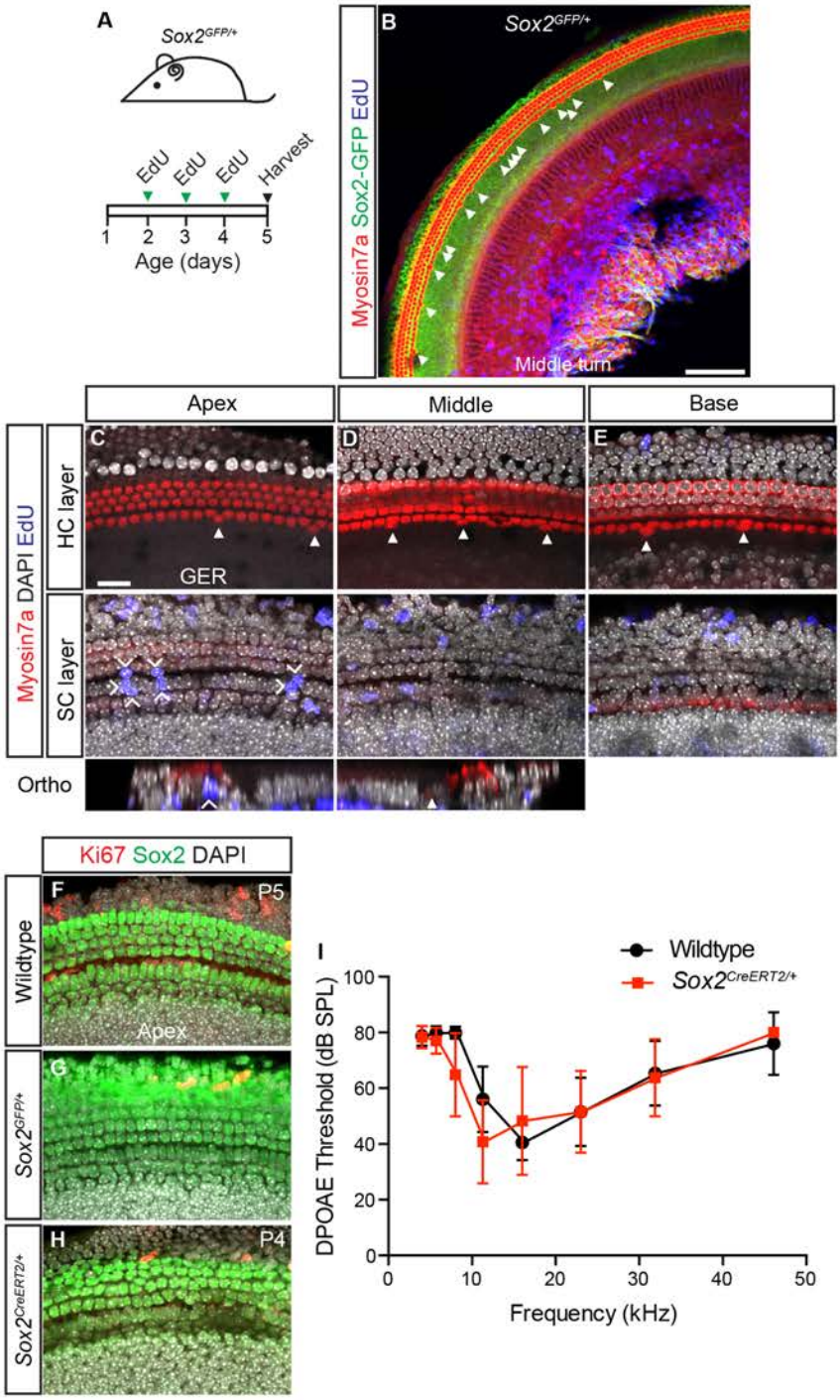
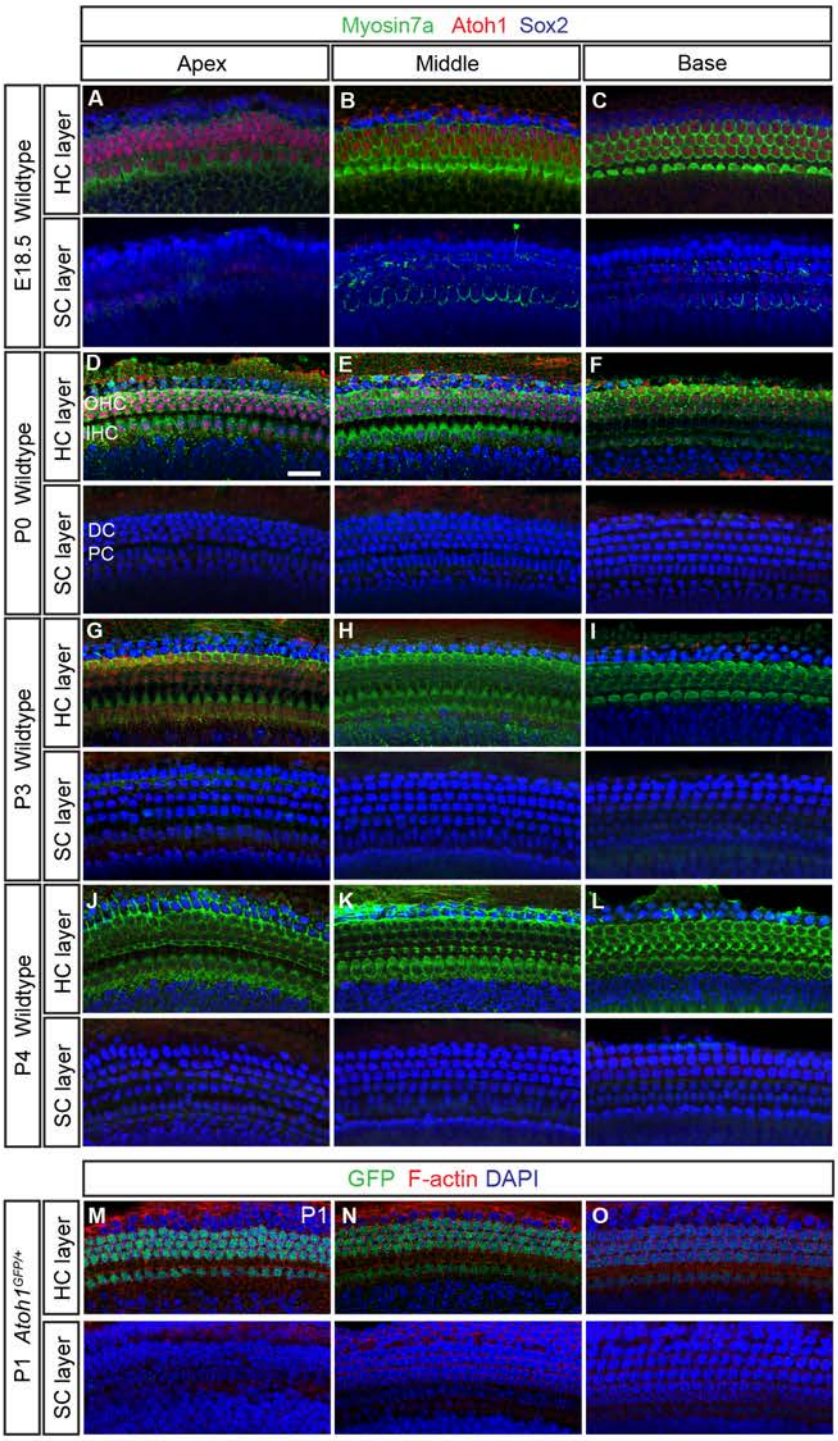
Figure S1



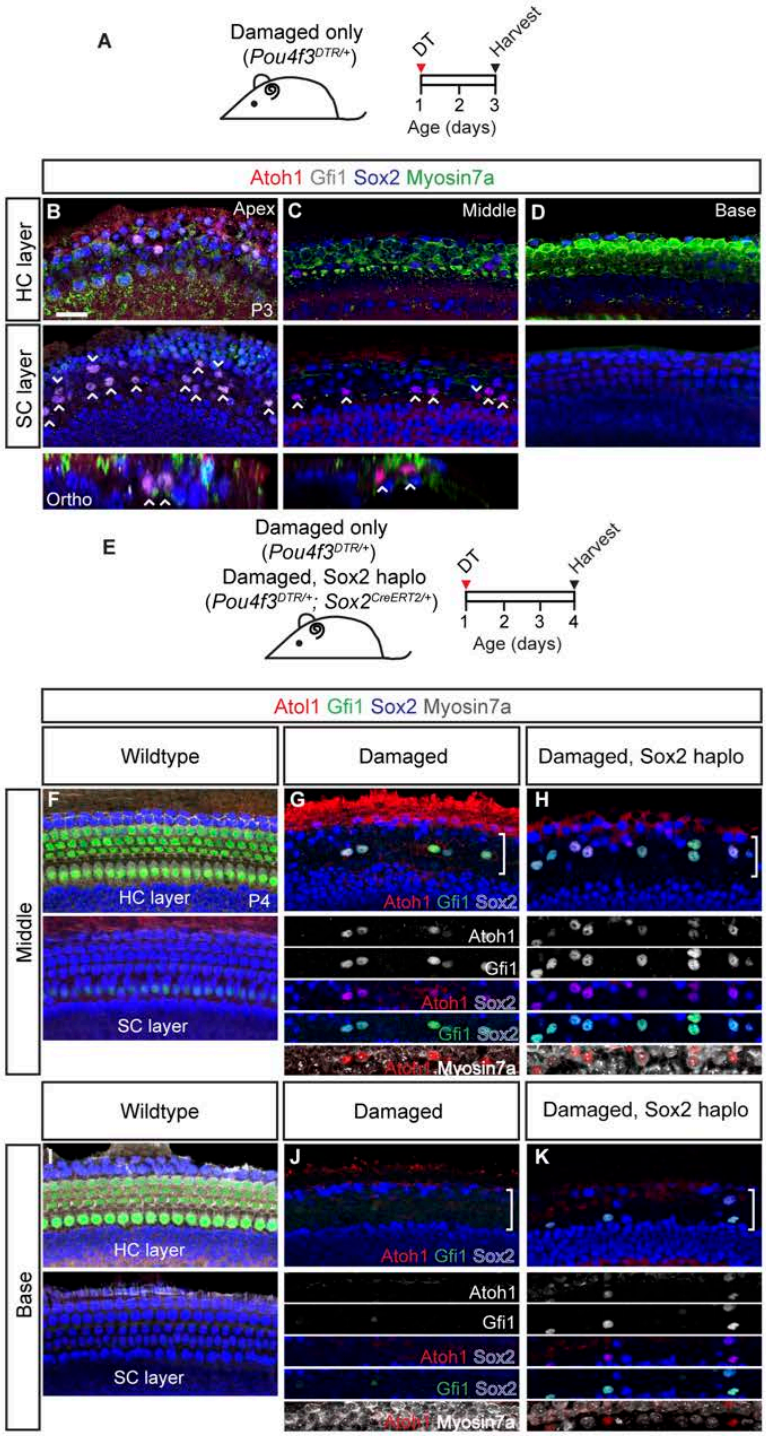
Supplementary Figure 1. Proliferation and ectopic hair cells in the postnatal *Sox2*$^{EGFP/+}$ cochlea. (**A**) Schematic of experimental paradigm. *Sox2*$^{EGFP/+}$ pups were injected EdU (P2-P4) and cochleae were examined at P5. (**B**) Low magnification image showing ectopic hair cells (arrowheads) adjacent to inner hair cells in the middle turn of the *Sox2*$^{EGFP/+}$ cochlea. (**C**-**E**) High magnification image of *Sox2*$^{EGFP/+}$ cochlea showing ectopic hair cells (arrowheads) in all three cochlear turns, and EdU-labeled supporting cells (chevron) in the pillar cell region in the apical turn. EdU-labeled cells in the middle and basal turns are outside the organ of Corti. (**F**-**H**) No Ki67-labeled hair cells or supporting cells were found in the wildtype, *Sox2*$^{EGFP/+}$ and *Sox2*$^{CreERT2/+}$ cochleae (P4-P5). (**I**) P30 *Sox2*$^{CreERT2/+}$ mice exhibit comparable DPOAE thresholds as wildtype littermates. GER=greater epithelial ridge. Data are shown as mean±S.D (two-tailed Student's t-test), n=3-8, scale bar in B= 100 μm, scale bar in C=20 μm.

Figure S2



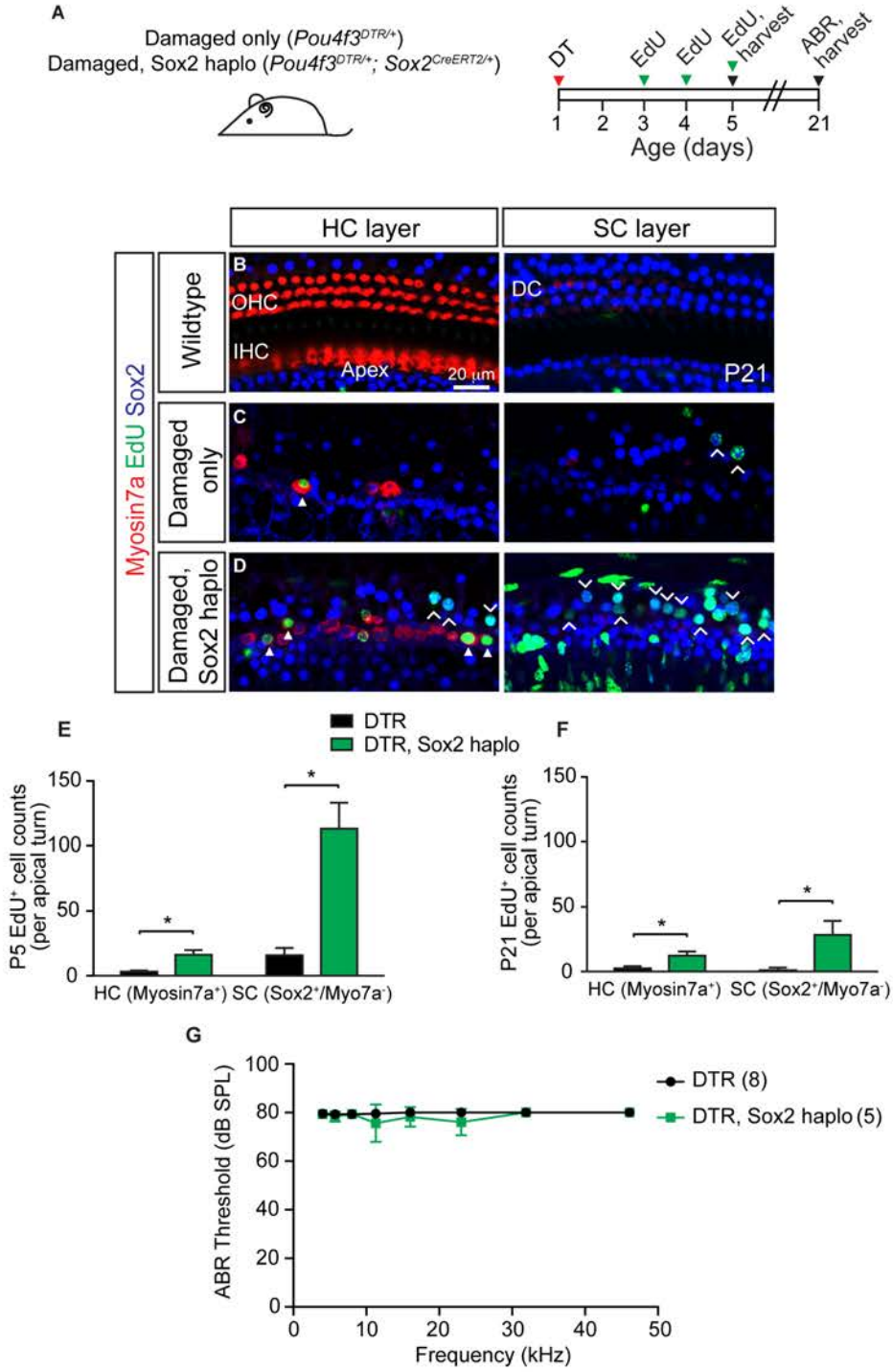
Supplementary Figure 2. Atoh1 expression pattern in the embryonic and neonatal cochlea. (**A**-**C**) Immunostaining of E18.5 wildtype cochlea showed Atoh1 expression in hair cells in an apex-to-base gradient, with weaker expression in basal hair cells. No Atoh1^{+} supporting cells were found. (**D**-**F**) At P0 Atoh1 remains expressed in hair cells in an apex-to-base gradient, with weaker expression in basal hair cells. No Atoh1^{+} supporting cells were detected. (**G**-**I**) At P3, Atoh1 expression was detected in the hair cells in the apical turn only. No Atoh1^{+} supporting cells were found. (**J**-**L**) At P4, no Atoh1 expression was detected in hair cells or supporting cells. (**M**-**O**) Atoh1-GFP expression in hair cells in an apex-to-base gradient in P1 *Atoh1*$^{GFP/+}$ mice. No Atoh1^{+} supporting cells were found at this age. OHC=outer hair cells, IHC=inner hair cells, PC=pillar cells, DC=Deiters' cells, n=3, scale bar=20 μm.

Figure S3



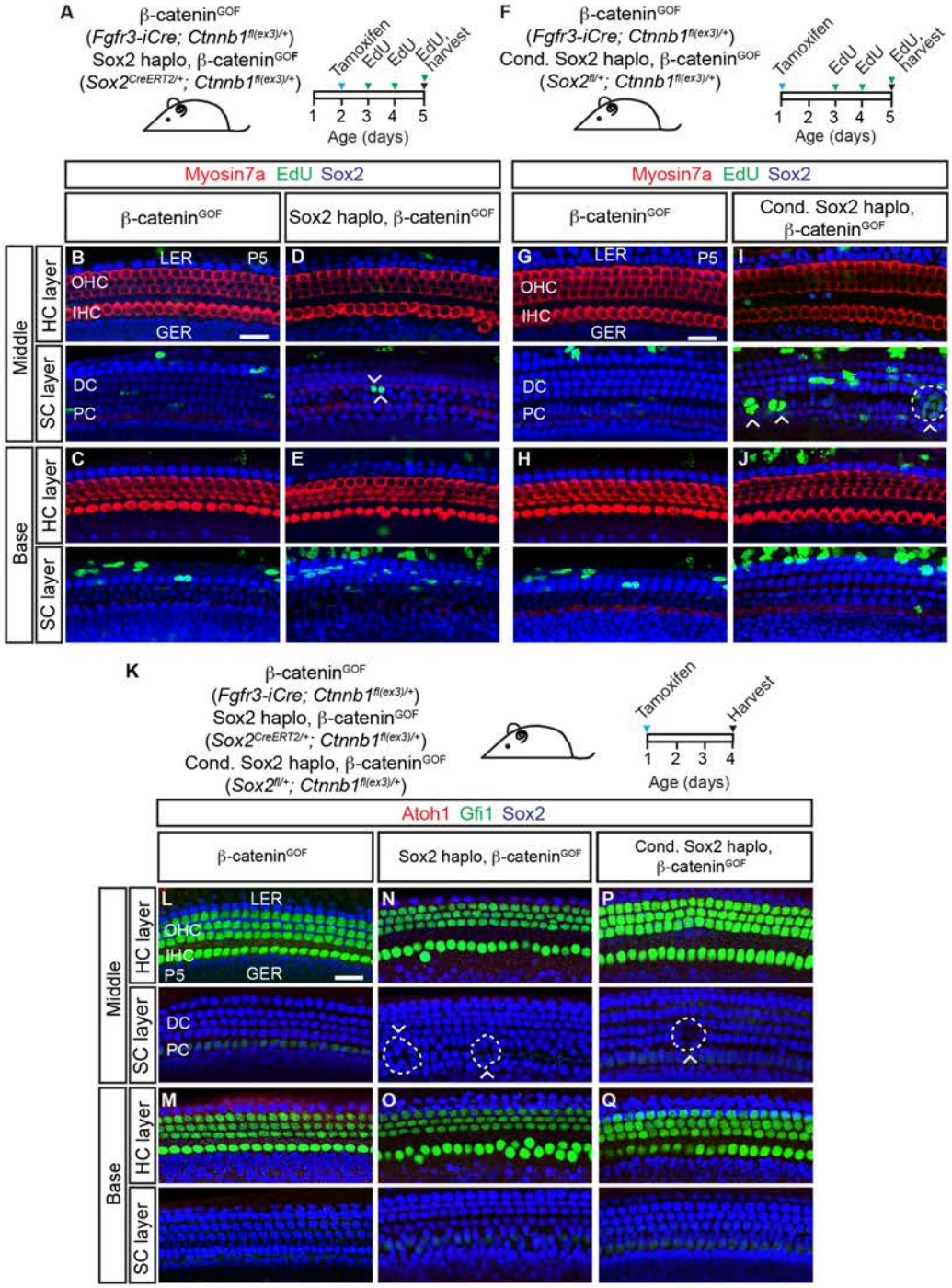
Supplementary Figure 3. Transitional cell formation after hair cell ablation. (**A**) Schematic of experimental paradigm. *Pou4f3*$^{DTR/+}$ mice were injected with diphtheria toxin (DT) at P1 and cochleae were harvested at P3. (**B**-**D**) After damage, Myosin7a^{+} hair cells no longer express Gfi1. In the apical and middle turns, many Atoh1^{+}, Gf1^{+}, Sox2^{+} transitional cells (chevron) were detected. No Atoh1^{+} or Gfi1^{+} transitional cells were detected in the basal turn. (**E**) *Pou4f3*$^{DTR/+}$ or *Pou4f3*$^{DTR/+}$*; Sox2*$^{CreERT2/+}$ mice were injected with DT at P1 and cochleae were examined at P4. (**F**, **I**) Hair cells (Myosin7a^{+} and Sox2^{-}) in middle and basal turns of wildtype cochlea expressed Gfi1 but not Atoh1. No Atoh1 of Gfi1 expression was detected in the supporting cells. (**G**, **J**) After damage, many Atoh1^{+}, Gfi1^{+}, Sox2^{+}, Myosin7a^{+} transitional cells were detected in the middle but not basal turn of the *Pou4f3*$^{DTR/+}$ cochlea. (**H**, **K**) In the *Pou4f3*$^{DTR/+}$*; Sox2*$^{CreERT2/+}$ (damaged, Sox2 haplo) cochlea, there were noticeably more Atoh1^{+}, Gfi1^{+}, Sox2^{+}, Myosin7a^{+} transitional cells in the middle and basal turns. n=3, scale bar=20 μm.

Figure S4



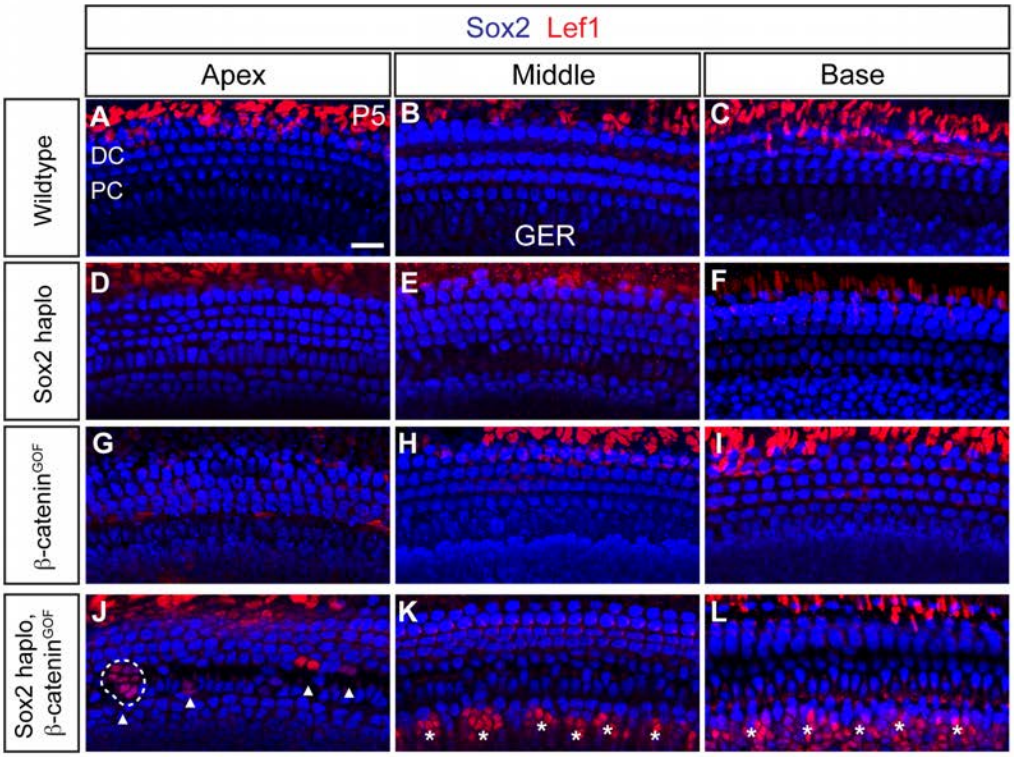
Supplementary Figure 4. Survival of regenerated hair cells and supporting cells. (**A**) Schematic of experimental timeline: hair cells were damaged at P1, EdU was injected at P3-P5 and cochleae were examined at P5 or P21. **(B)** In the wildtype P21 cochlea, there were no EdU-positive hair cells or supporting cells. (**C**) In the P21 damaged only cochlea, few hair cells remained alongside disorganized supporting cells. Few mitotically regenerated hair cells (arrowhead) and supporting cells (chevron) were observed in the apical turn. (**D**) In the apical turn of P21 damaged, Sox2 haplo cochlea, many mitotically regenerated hair cells and supporting cells were observed. (**E-F**) Quantifcation of EdU-positive hair cells and supporting cell in the apical turn of P5 and P21 cochleae. (**G**) Both P21 damaged only and damaged, Sox2 haplo animals displayed elevated ABR thresholds. OHC=outer hair cells, IHC=inner hair cells, DC=Deiters' cells, n=3-8, scale bar=20 μm. *p<0.05, Student's t-test.

Figure S5



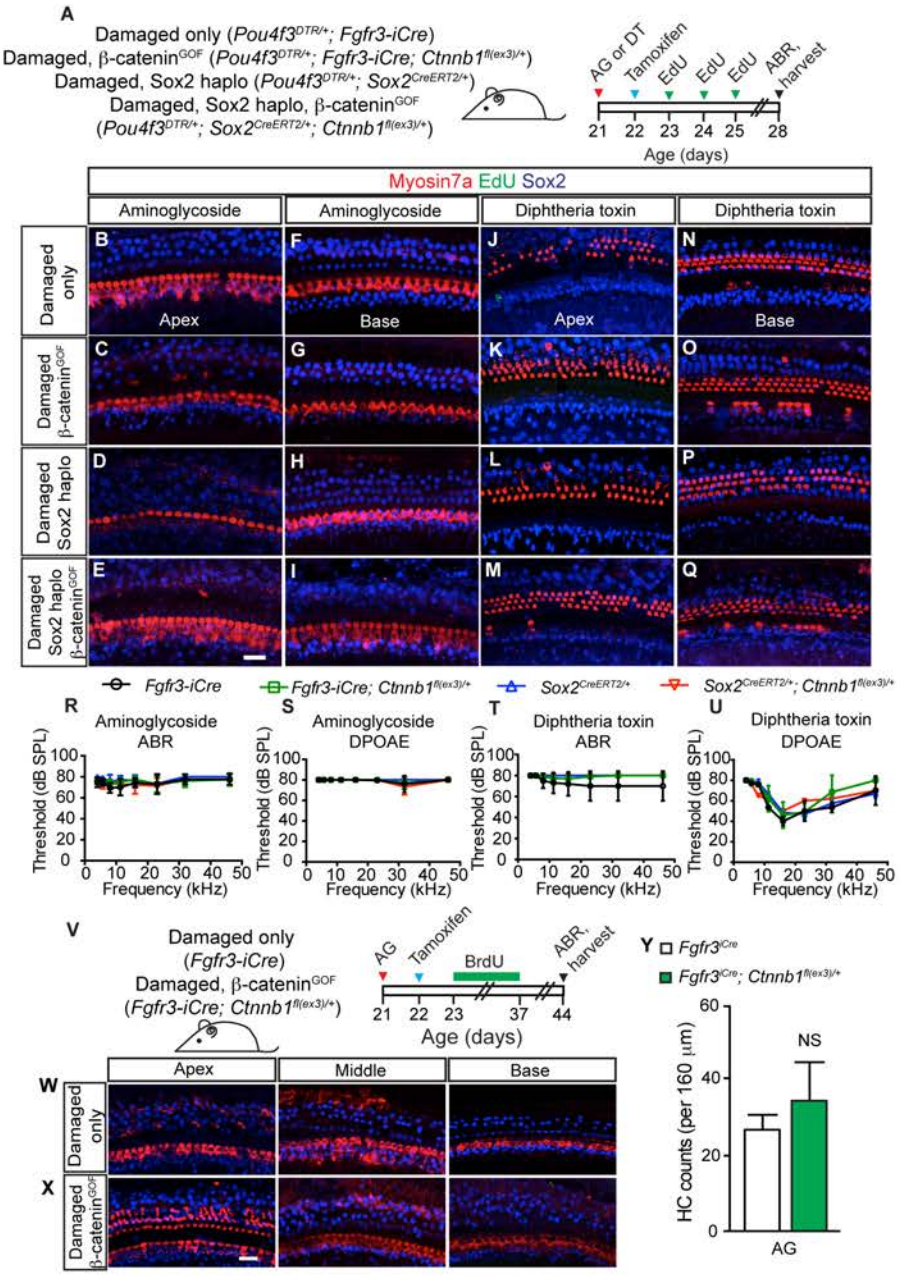
Supplementary Figure 5. Sox2 haploinsufficiency acts as a permissive signal for ß-catenin-induced proliferation in the undamaged cochlea. (**A**) Schematic of experimental paradigm. *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$ and *Sox2*$^{CreERT2/+}$*; Ctnnb1*$^{fl(ex3)/+}$ pups were injected with tamoxifen at P2, followed by EdU injection daily P3-P5, cochleae were examined at P5. (**B-C**) Middle and basal turns of *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$ cochleae revealed no EdU$^+$ hair cells or supporting cells. (**D-E**) A few EdU$^+$ supporting cells (chevron) were detected in the middle turn of the *Sox2*$^{CreERT2/+}$*; Ctnnb1*$^{fl(ex3)/+}$ cochlea. No EdU$^+$ supporting cells were found in the base. (**F**) *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$ and *Fgfr3-iCre; Sox2*$^{fl/+}$*; Ctnnb1*$^{fl(ex3)/+}$ pups were injected with tamoxifen at P1, followed by EdU injection P3-P5, cochleae were examined at P5. (**G-H**) No EdU$^+$ hair cells or supporting cells were detected in the middle and basal turns of *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$ cochleae. (**I-J**) EdU$^+$ supporting cells (chevron) were found in the middle turn of *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$*; Sox2*$^{fl/+}$ cochleae, some of which formed foci (dashed lines). No EdU$^+$ supporting cells were seen in the base. (**K**) *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$, *Sox2*$^{CreERT2/+}$*; Ctnnb1*$^{fl(ex3)/+}$ and *Fgfr3-iCre; Sox2*$^{fl/+}$*; Ctnnb1*$^{fl(ex3)/+}$ pups received tamoxifen at P1 and cochleae were harvested at P4. (**L-Q'**) Myosin7a$^+$ hair cells expressed Gfi1, but no Atoh1 or Gfi1 was detected in supporting cells in the *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$, *Sox2*$^{CreERT2/+}$*; Ctnnb1*$^{fl(ex3)/+}$ or, *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$*; Sox2*$^{fl/+}$ cochleae. (**N'**, **P'**) Foci of cells (dashed lines and chevron) were observed in the middle turn of *Sox2*$^{CreERT2/+}$*; Ctnnb1*$^{fl(ex3)/+}$ and *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$*; Sox2*$^{fl/+}$ cochleae, but not the basal turn. OHC=outer hair cells, IHC=inner hair cells, GER=greater epithelial ridge, LER=lesser epithelial ridge, PC=pillar cells, DC=Deiters' cells, n=3, scale bar=20 μm.

Figure S6



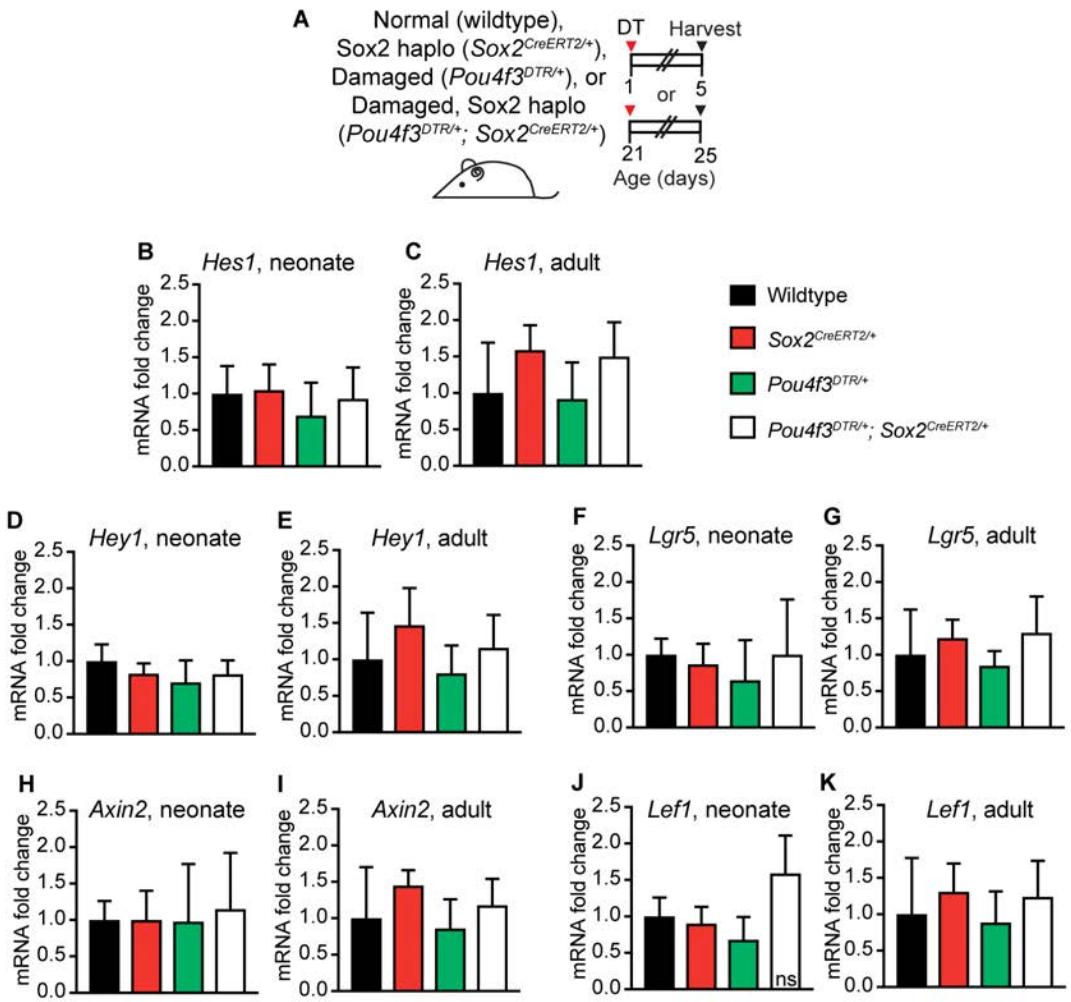
Supplementary Figure 6. Sox2 haploinsufficiency and ß-catenin stabilization induce Lef1 expression in the neonatal cochlea. (**A-I**) No foci or Lef1 expression was observed in the P5 wildtype, *Sox2*$^{CreERT2/+}$, or *Fgfr3-iCre; Ctnnb1*$^{fl(ex3)/+}$ cochleae. (**J-L**) Foci (dashed line) were observed in the pillar cell region in the apical turn of *Sox2*$^{CreERT2/+}$*; Ctnnb1*$^{fl(ex3)/+}$ cochlea. These foci and supporting cells in the organ of Corti (arrowheads) and greater epithelial ridge (GER) (asterisks) also expressed the Wnt target Lef1. PC=pillar cells, DC=Deiters' cells, n=3-4, scale bar=20 μm.

Figure S7



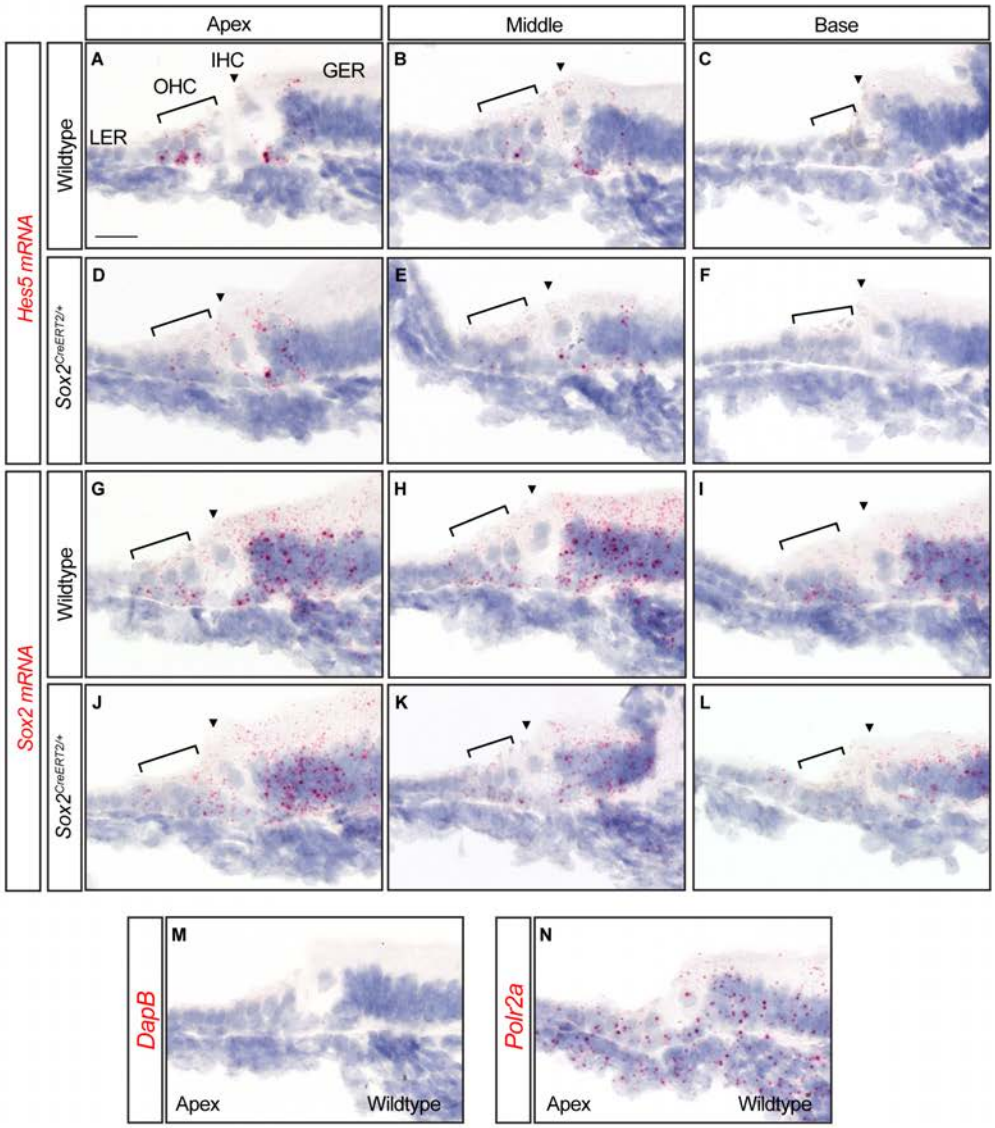
Supplementary Figure 7. Sox2 haploinsufficiency and ß-catenin stabilization in the damaged adult cochlea. (**A**) Schematic of transgenic mouse models and experimental timeline. Animals were damaged at P21, Cre was activated by tamoxifen at P22, EdU from P23-P25, and sacrificed post ABR at P28. (**B-I**) Aminoglycoside treatment caused outer hair cell loss in the apical and basal turns in all cochleae. No Myosin7a$^+$, Sox2$^+$ cells or EdU$^+$ hair cells or supporting cells were observed one week post treatment in all four groups. (**J-Q**) Severe inner hair cell loss and some outer hair cell loss was observed in the apex and base after diphtheria toxin treatment. No Myosin7a$^+$, Sox2$^+$ cells or EdU$^+$ hair cells or supporting cells were detected. A rare EdU$^+$ cell in the inner sulcus region is shown in (**J**). (**R-S**) Aminoglycoside treatment led to elevated thresholds in ABR and DPOAE without changes across genotypes one week post damage. (**T-U**) Diphtheria toxin treatment caused elevated ABR thresholds but not DPOAE thresholds. No changes in ABR or DPOAE thresholds were seen when measured one week after damage across genotypes tested. (**V**) Schematic of long term ß-catenin stabilization in *Fgfr3-iCre* and *Fgfr3-iCre; Ctnnb1$^{fl(ex3)/+}$* mice. Animals were damaged at P21, Cre was activated at P22 and BrdU was administered via drinking water from P23-P37, and sacrificed post ABR at P44. (**W-X**) No Myosin7a$^+$, Sox2$^+$ cells or BrdU$^+$ cells were observed 3 weeks after ß-catenin stabilization in the aminoglycoside-damaged cochlea. (**Y**) There was no significant difference in Myosin7a$^+$ hair cell counts along the length of the cochlea when comparing ß-catenin stabilization to control cochleae (two-tailed Student's t-test), n=3-5, scale bar=20 μm.

Figure S8



Supplementary Figure 8. Quantitative PCR of Notch and Wnt target genes in damaged and Sox2 haploinsufficient cochleae. (**A**) Neonatal (P1) and mature (P21) wildtype, *Sox2*$^{CreERT2/+}$, *Pou4f3*$^{DTR/+}$, and *Pou4f3*$^{DTR/+}$*; Sox2*$^{CreERT2/+}$ mice were treated with DT and cochleae were collected 4 days later. (**B-E**) There was no significant difference in the expression of the Notch target genes (*Hes1* and *Hey1*) in the neonate or adult cochleae. (**F-K**) Similarly, there was no significant difference in the expression of the Wnt target genes (*Lgr5*, *Axin2* or *Lef1*) in either the neonate or adult cochleae in all four genotypes examined. There was a downregulation of *Axin2*, *Hey1*, *Lef1* and *Lgr5* in the mature, wildtype cochlea relative to the neonate, wildtype cochlea. (one-way ANOVA, Holm-Sidak multiple comparisons, Student's t-test), n=4.

Figure S9



Supplementary Figure 9. *Hes5* and *Sox2 mRNA expression* in P5 wildtype and and *Sox2*$^{CreERT2/+}$ cochleae. (**A-C**) *In situ* hybridization using RNAscope shows *Hes5* expression in organ of Corti and greater epithelial ridge (GER) supporting cells in the wildtype cochlea. Expression was more robust in the apex and lower in the base. (**D**-**F**) *Hes5* expression also displayed an apical-basal gradient in supporting cells in the *Sox2*$^{CreERT2/+}$ cochlea, and is noticeably lower than wildtype cochlea. (**G-L**) *Sox2* expression was detected in organ of Corti and GER supporting cells in both wildtype and *Sox2*$^{CreERT2/+}$ cochleae, the latter of which appeared to display similar or slightly lower *Sox2* expression. (**M**) Negative control. (**N**) Positive control. Shown are representative images for 3 or more experiments. OHC=outer hair cells, IHC=inner hair cells, LER=lesser epithelial ridge. Scale bar=25 μm.

Supplementary table 1. Quantification of proliferative cells

P5	Apex		Middle		Base	
Genotypes	EdU^+ $Myo7a^+$	EdU^+ $Sox2^+$ $Myo7a^-$	EdU^+ $Myo7a^+$	EdU^+ $Sox2^+$ $Myo7a^-$	EdU^+ $Myo7a^+$	EdU^+ $Sox2^+$ $Myo7a^-$
Wildtype	0	0	0	0	0	0
Sox2-CreERT2	0	(10.2±4.8)	0	0	0	0
Sox2-EGFP	0	(22.8±13.8)	0	0	0	0
Fgfr3-iCreER; Ctnnb1-fl(ex3) (P2 tamox)	0	0	0	0	0	0
Sox2-CreERT2; Ctnnb1-fl(ex3) (P2 tamox)	0	3.5±0.1 (39.3±1.5)	0	0.3±0.1 (3.3±1.5)	0	0
Fgfr3-iCreER; Ctnnb1-fl(ex3) (P1 tamox)	0	0	0	0	0	0
Fgfr3-iCreER; Sox2-fl; Ctnnb1-fl(ex3) (P1 tamox)	0	2.5±0.7 (27.7±7.4)	0	4.0±1.2 (45.3±12.0)	0	0
Pou4f3-DTR	0.3±0.1 (3.2±0.8)	1.4±0.5 (15.8±5.6)	0	0	0	0
Pou4f3-DTR; Sox2-CreERT2	1.5±0.3 (16.2±3.6)	10.3±1.9 (113.2±20.1)	0	3.9±0.6 (43.2±6.3)	0	0
Pou4f3-DTR; Fgfr3-iCreER; Ctnnb1-fl(ex3) (P2 tamox)	0.8±0.2 (8.3±2.1)	5.2±1.4 (57.7±14.6)	0	0	0	0
Pou4f3-DTR; Sox2-CreERT2; Ctnnb1-fl(ex3) (P2 tamox)	1.4±0.2 (16.0±2.0)	16.0±2.8 (186.8±31.3)	0.2±0.1 (2.0±1.4)	3.1±0.4 (36.8±5.0)	0	0.1±0.2 (1.5±1.9)

-Shown are EdU^+ cell counts per 160 μm cochlear length from P5 mice. Also shown in parenthesis are counts per cochlear turn. Mean±S.D. n=3-6.

Supplementary Table 2. Quantification of transitional cells.

P4	Apex		Middle		Base	
Genotypes	Atoh1$^+$ Gfi1$^+$ Sox2$^+$ Myo7a$^+$	Atoh1$^+$ Gfi1$^+$ Sox2$^+$ Myo7a$^-$	Atoh1$^+$ Gfi1$^+$ Sox2$^+$ Myo7a$^+$	Atoh1$^+$ Gfi1$^+$ Sox2$^+$ Myo7a$^-$	Atoh1$^+$ Gfi1$^+$ Sox2$^+$ Myo7a$^+$	Atoh1$^+$ Gfi1$^+$ Sox2$^+$ Myo7a$^-$
Wildtype	0	0	0	0	0	0
Sox2-CreERT2	0	0	0	0	0	0
Fgfr3-iCreER; Ctnnb1-fl(ex3) (P1 tamox)	0	0	0	0	0	0
Sox2-CreERT2; Ctnnb1-fl(ex3) (P1 tamox)	0	0	0	0	0	0
Fgfr3-iCreER; Sox2-fl; Ctnnb1-fl(ex3) (P1 tamox)	0	0	0	0	0	0
Pou4f3-DTR	9.7±0.6	0	5.0±1.0	0	0.3±0.6	0
Pou4f3-DTR; Sox2-CreERT2	12.7±1.2	0	8.3±1.5	1.3±0.6	3.7±0.6	0.3±0.6
Pou4f3-DTR; Fgfr3-iCreER; Ctnnb1-fl(ex3) (P2 tamox)	4.3±1.5	6.0±1.7	2.7±1.2	4.3±1.5	0	0
Pou4f3-DTR; Sox2-CreERT2; Ctnnb1-fl(ex3) (P2 tamox)	21.3±3.2	3.0±1.0	12.7±0.6	4.0±1.0	4.3±2.1	4.3±0.6

-Shown are Atoh1$^+$, Sox2$^+$ transitional cell counts per 160 μm length from P4 cochleae. Mean±S.D. n=3-4.

Supplementary table 3. Quantification of Myosin7a^{+} hair cells

	Genotypes	Apex	Middle	Base
P5	Wildtype	84.8±4.4$^{\#}$	87.0±3.7$^{\#}$	76.8±3.4$^{\#}$
	Pou4f3-DTR	47.3±4.6	65.7±4.6	55.8±3.9
	Pou4f3-DTR; Sox2-CreERT2	57.8±5.3**	72.8±2.6**	58.2±3.4
P7	Wildtype	86.3±6.2$^{\#}$	88.3±2.9$^{\#}$	83.5±3.1$^{\#}$
	Pou4f3-DTR	47.0±4.6	57.3±2.5	36.0±6.6
	Pou4f3-DTR; Sox2-CreERT2	41.0±5.0	66.0±4.4**	47.0±3.6**
P10	Wildtype	87.7±2.3$^{\#}$	84.3±1.2$^{\#}$	79.0±6.1$^{\#}$
	Pou4f3-DTR	14.7±0.6	36.7±6.4	12.7±1.5
	Pou4f3-DTR; Sox2-CreERT2	41.0±4.0**	53.3±3.2**	26.3±2.5**
P21	Wildtype	79.3±4.2$^{\#}$	85.3±3.8$^{\#}$	77.3±3.2$^{\#}$
	Pou4f3-DTR	5.3±0.5	0	0
	Pou4f3-DTR; Sox2-CreERT2	17.5±3.9**	0	0

-Shown are Myosin7a^{+} hair cell counts per 160 µm cochlear length from P5, P7, P10, P21 mice. Mean±S.D. n=3-6. ** denotes p<0.01 between *Pou4f3*$^{DTR/+}$ and *Pou4f3*$^{DTR/+}$*; Sox2*$^{CreERT2/+}$ animals. $^{\#}$ denotes p<0.001 between wildtype and the other two groups (two-way ANOVA and Tukey post hoc test).