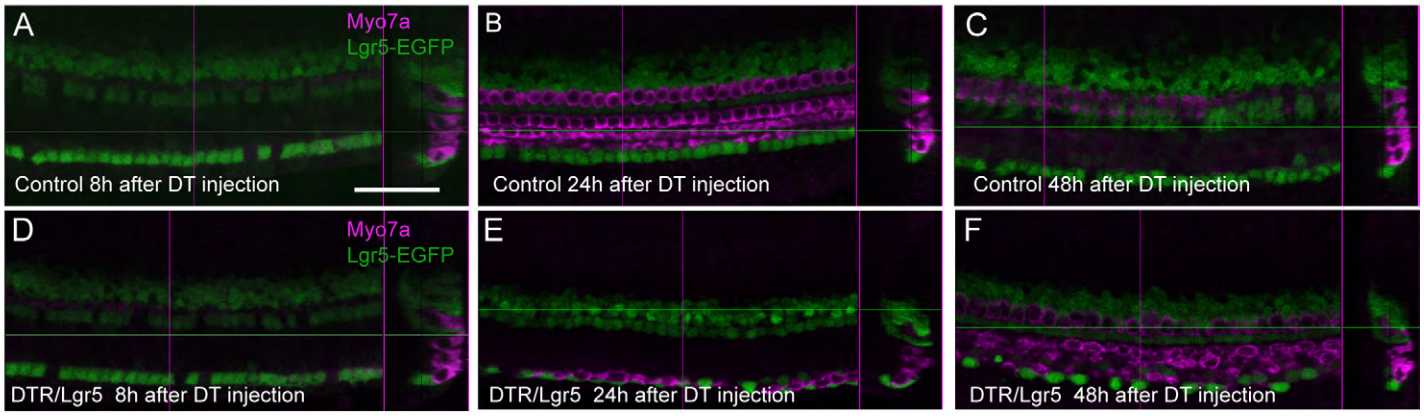

Fig. S1. Expression of Lgr5 after HC ablation in the neonatal mouse cochlea. Representative confocal images of Lgr5-EGFP expression (green) in the apical turn of control (Pou4f3$^{+/+}$; Lgr5$^{CreER/+}$) (A-C) and Pou4f3$^{DTR/+}$; Lgr5$^{CreER/+}$ cochleae (D-F) 8, 24, and 48 hours after DT injection at P1. HCs are labeled by myo7a (magenta). Scale Bar: 50µm.

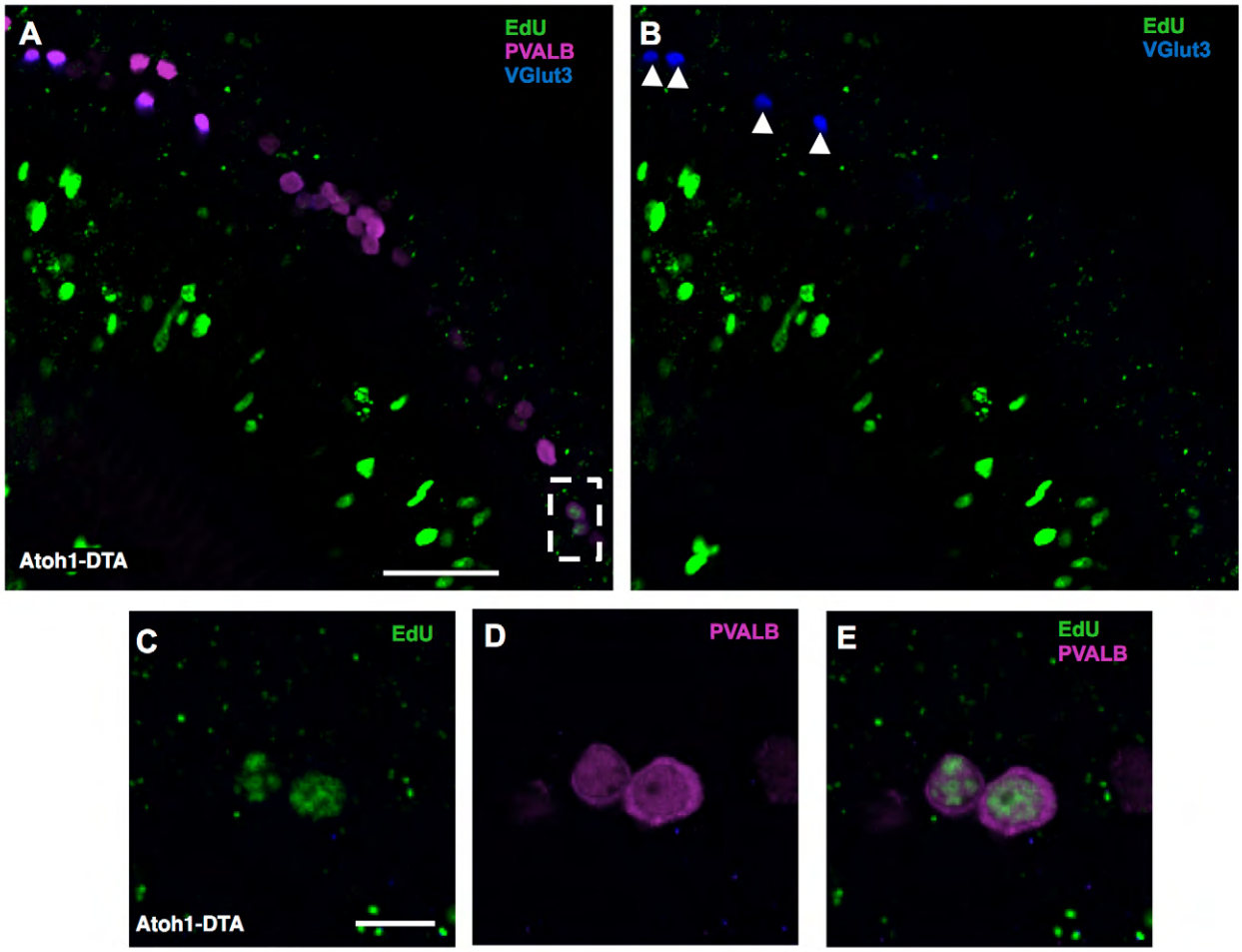

Fig. S2. Regenerated HCs do not express the inner HC marker VGlut3. Representative confocal images of EdU+ (green) HCs in the apical turn of Atoh1DTA mice at P10 after EdU injection at P4. EdU+ HCs were co-labeled with the non-selective HC marker parvalbumin (PVALB, magenta), but did not express the inner HC marker, VGlut3 (blue). C-D High magnification of square in A. Arrowheads in B label VGlut3+ cells. Scale bars: in A-B=50µm, in C-E=10µm.

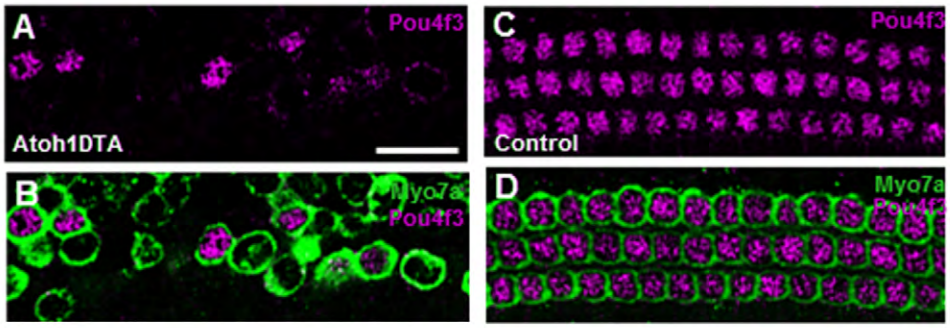

Fig. S3. Lack of Pou4f3 expression in regenerated HCs. Representative confocal images of myo7a+ (green) cells that express the HC survival factor, Pou4f3 (magenta), at P6 in the apical turn of Atoh1DTA (A-B) and control mice (lacking either the Cre or DTA allele) (C-D). Scale Bar=50µm.

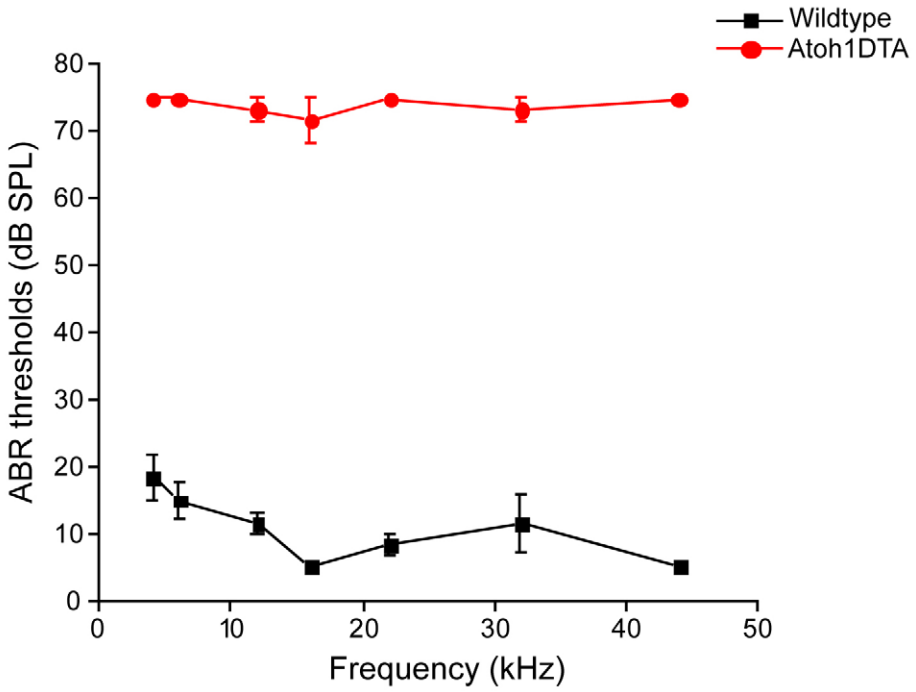

Fig. S4. Hearing loss after hair cell ablation. (A) After HCs were ablated at P0-1, P30 Atoh1DTA mice displayed elevated thresholds for auditory brainstem responses (ABR) in comparison to wild-type littermates (*n*=3 for each group).

Table S1. Genotyping primers

ROSA26^{DTA} genotyping	
Forward primer	5’ TGACGATGATTGGAAAGGGT 3’
Reverse primer	5’ TGAGCACTACACGCGAAGCA 3’
Hes5-nlsLacZ genotyping	
Forward primer	5’ CCGAAATCCCGAATCTCTATC 3’
Reverse primer	5’ ATCACACTCGGGTGATTACGA 3’
Pou4f3 DTR genotyping	
Pou4f3 1566 primer	5' CCGACGGCAGCAGCTTCATGG 3’
Pou4f3 1518 primer	5' GTCAAAAAATGTGCCTTAGAG 3’
Pou4f3 1567 primer	5' CACTTGGAGCGCGGAGAGCTA 3’

The primers used for genotyping ROSA26^{DTA} mice, Hes5-nlsLacZ mice, and Pou4f3$^{DTR/+}$ mice are listed. Genotyping for all other mouse lines has been previously described (see Methods).

Table S2. Summary of fate mapping and mitotic labeling experiments

A. Fate mapping of SCs using Pou4f3$^{DTR/+}$; Lgr5$^{CreER/+}$; ROSA26$^{CAG\text{-}tdTomato/+}$ mice

Cell ablation method	Age analyzed	n value
DTR: DT inj. at P1	P7	4

Myo7a+/tdTomato+ cells (whole turn)

	Apical	Middle	Base
CTL	12.0 ± 1.5	2.0 ± 0.7	0.5 ± 0.3
DTR	99.0 ± 4.6***	22.8 ± 6.5***	2.3 ± 0.9*

Myo7a+/Sox2+ cells (whole turn)

	Apical	Middle	Base
CTL	--	--	--
DTR	34.3 ± 3.8	8.0 ± 2.3	--

Myo7a+/Sox2+/tdTomato+ cells (whole turn)

	Apical	Middle	Base
CTL	--	--	--
DTR	18.3 ± 3.0	3.3 ± 1.3	--

B. Fate mapping of SCs using Atoh1DTA; Hes5-nlsLacZ$^{+/-}$ mice

Cell ablation method	Age analyzed	n value
DTA: Tam inj. at P0/P1	P2	3

Myo7a+/LacZ+ cells (whole turn)

	Apical	Middle	Base
CTL	--	--	--
Atoh1DTA	58.3 ± 30.2**	9.3 ± 7.9	1.3 ± 1.3

C. Mitotic labeling using Pou4f3$^{DTR/+}$ mice

Cell ablation method	EdU injection	Age analyzed	n value
DTR: DT inj. at P1	P3, P4, & P5	P7	4-6

Sox2+/EdU+/myo7a-negative cells (225 μm region counted per turn)

	Apical	Middle	Base
CTL	--	--	--
DTR	13.2 ± 3.7	5.8 ± 1.6	3.5 ± 1.5

Myo7a+/EdU+ cells (whole turn)

	Apical	Middle	Base
CTL	--	--	--
DTR	11.0 ± 1.8	--	--

Myo7a+/Sox2+/EdU+ cells (whole turn)

	Apical	Middle	Base
CTL	--	--	--
DTR	4.7 ± 1.3	--	--

D. Mitotic labeling using Atoh1DTA mice

Cell ablation method	EdU injection	Age analyzed	n value
DTA: Tam inj. at P0/P1	P2- P5	24h after EdU inj.	3

Sox2+/EdU+ cells (whole turn)

CTL	P2	P3	P4	P5
Apical	--	--	--	--
Middle	--	--	--	--
Base	--	--	--	--

Atoh1DTA	P2	P3	P4	P5
Apical	2.7 ± 1.8	1.7 ± 1.2	3.0 ± 2.1	2.7 ± 1.5
Middle	--	3.0 ± 2.1	2.0 ± 2.0	1.7 ± 1.7
Base	--	--	1.3 ± 1.3	5.3 ± 1.5

Myo7a+/EdU+ cells (whole turn)

CTL	P2	P3	P4	P5
Apical	--	--	--	--
Middle	--	--	--	--
Base	--	--	--	--

Atoh1DTA	P2	P3	P4	P5
Apical	2.0 ± 2.0	3.3 ± 0.9	3.7 ± 2.3	1.0 ± 0.6
Middle	--	--	--	--
Base	--	--	--	--

Myo7a+/ Sox2+/EdU+ cells (whole turn)

CTL	P2	P3	P4	P5
Apical	--	--	--	--
Middle	--	--	--	--
Base	--	--	--	--

Atoh1DTA	P2	P3	P4	P5
Apical	1.3 ± 1.3	1.7 ± 0.3	2.0 ± 1.2	1.0 ± 0.6
Middle	--	--	--	--
Base	--	--	--	--

E. Mitotic labeling and fate mapping in Pou4f3$^{DTR/+}$; Lgr5$^{CreER/+}$; ROSA26$^{CAG\text{-}tdTomato/+}$ mice

Cell ablation method	EdU injection	Age analyzed	n value
DTR: DT inj. at P1	P3, P4, & P5	P7	3-6

Myo7a+/tdTomato+/EdU+ cells (whole turn)

	Apical	Middle	Base
CTL	--	--	--
DTR	6.7 ± 0.6	--	--

Myo7a+/tdTomato+ cells (EdU-negative) (whole turn)

	Apical	Middle	Base
CTL	9.8 ± 2.1	1.3 ± 0.5	0.3 ± 0.5
DTR	106.3 ± 14.8***	25.7 ± 5.5**	2.7 ± 1.5*

F. Supporting cell counts in Pou4f3$^{DTR/+}$; Lgr5$^{CreER/+}$; ROSA26$^{CAG\text{-}tdTomato/+}$ mice

Cell ablation method	Age analyzed	n value
DTR: DT inj. at P1	P7	3-5

tdTomato+ organ of Corti cells (Myo7a+ and Sox2+/myo7a-negative cells lateral to GER) (225 μm region counted per turn)

	Apical	Middle	Base
CTL	70.3 ± 1.5	64.7 ± 1.5	60.6 ± 1.6
DTR	64.9 ± 0.9*	44.7 ± 1.4**	40.7 ± 0.4**

tdTomato+ organ of Corti supporting cells (Sox2+/myo7a-negative cells lateral to GER using IHC as reference) (225 μm region counted per turn)

	Apical	Middle	Base
CTL	68.0 ± 1.2	63.3 ± 1.9	60.6 ± 1.6
DTR	48.5 ± 0.4**	41.2 ± 0.7**	40.3 ± 0.3**

tdTomato+ GER cells (225 μm region counted per turn)

	Apical	Middle	Base
CTL	100.7 ± 5.6	84.7 ± 6.1	70.4 ± 9.0
DTR	111.6 ± 11.2	85.0 ± 1.5	75.6 ± 4.5

***p<0.001, **p<0.01, *p<0.05

The mouse model used, method of cell ablation, EdU injection paradigm, age analyzed, and *n* value for all experiments are listed. Raw counts of double or triple labeled cells in each turn of the cochlea are presented as mean ± s.e.m.