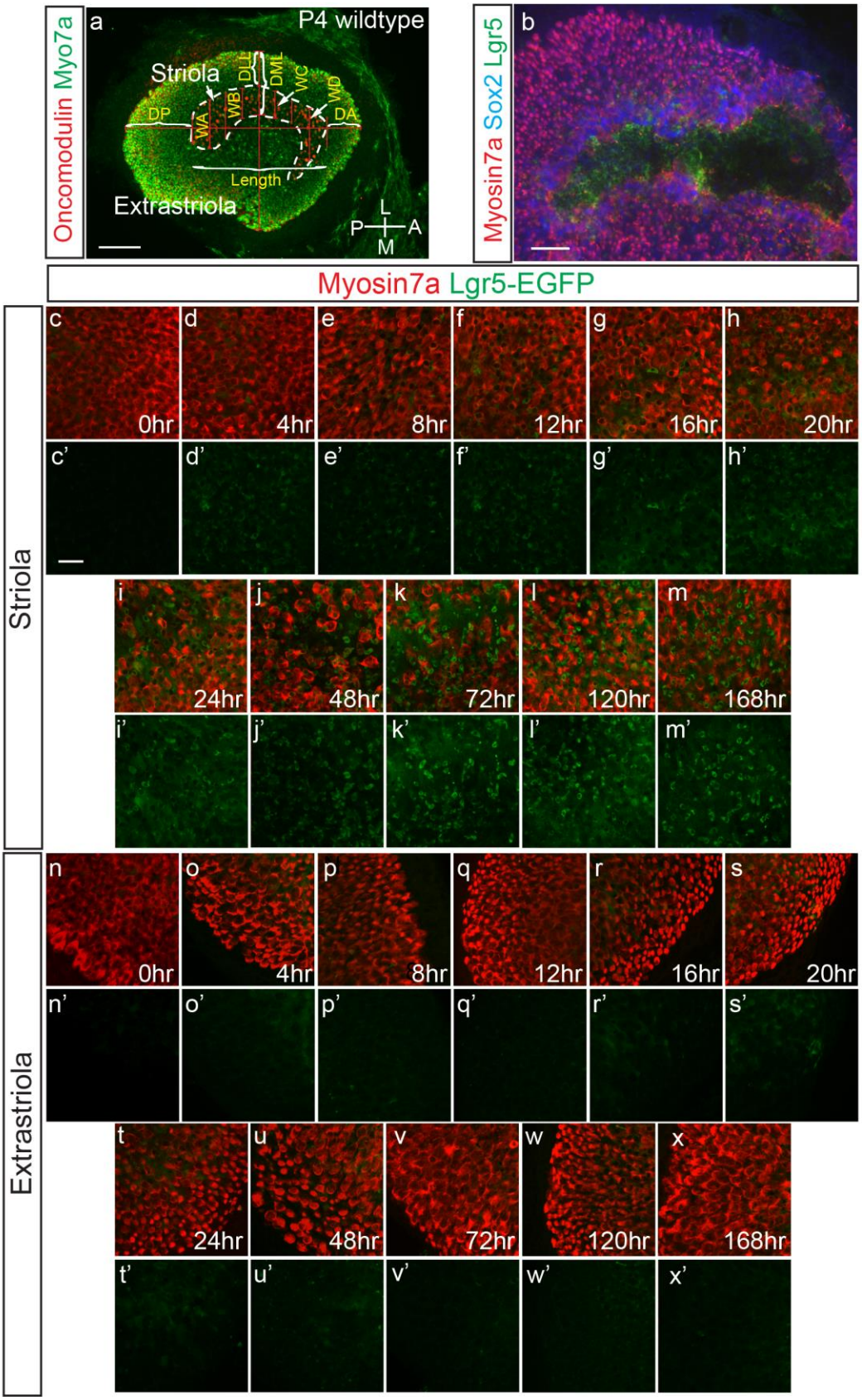
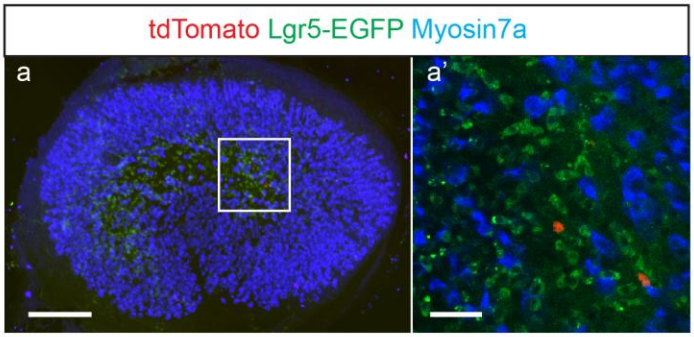
Supplementary figure 1



Supplementary Figure 1. Time-course of *Lgr5* expression after damage (related to Figure 1).

(**a**) Whole mount utricle from P4 wildtype mice immunostained for oncomodulin to define the striolar region, as outlined by white dashed lines. The length of the striola represents the longest distance in the anterior-posterior axis. We divided the striolar region along this axis into four regions whose midway widths were individually assessed (WA, WB, WC, WD). DP and DA represent the distances between oncomodulin+ domain and the posterior and anterior edges of the sensory epithelium, respectively. DLL and DML represent the distances between the lateral and medial boundaries of the oncomodulin+ domain, respectively, to the lateral edge of the sensory epithelium. (**b**) Mechanically damaged utricles cultured for 4 days without neomycin. (**c-x**) Utricles from P3 *Lgr5*$^{EGFP\text{-}CreERT2/+}$ mice were cultured with neomycin (1.0 mM) and tissues were fixed and immunostained for Myosin7a at defined time-points (0-168 hr). Green signals represent endogenous EGFP activity and all images were taken at identical microscope settings. Representative confocal images of the striolar (**c-m**) and extrastriolar regions (**n-x**). Scale bars, (**a**) 100 μm; (**b**) 50 μm; (**c-s**) 20 μm.

Supplementary figure 2



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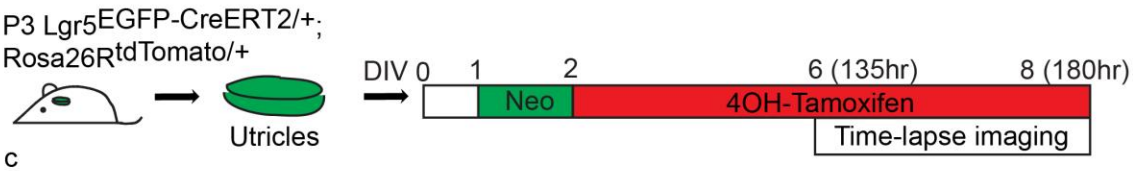

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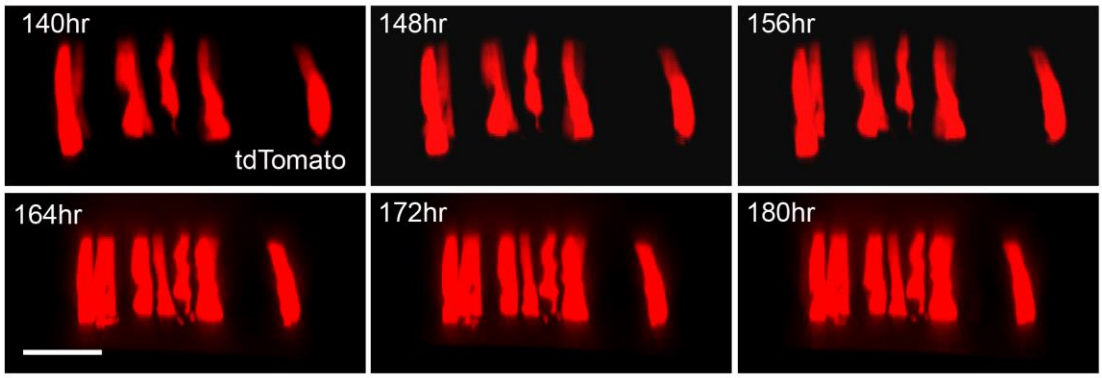
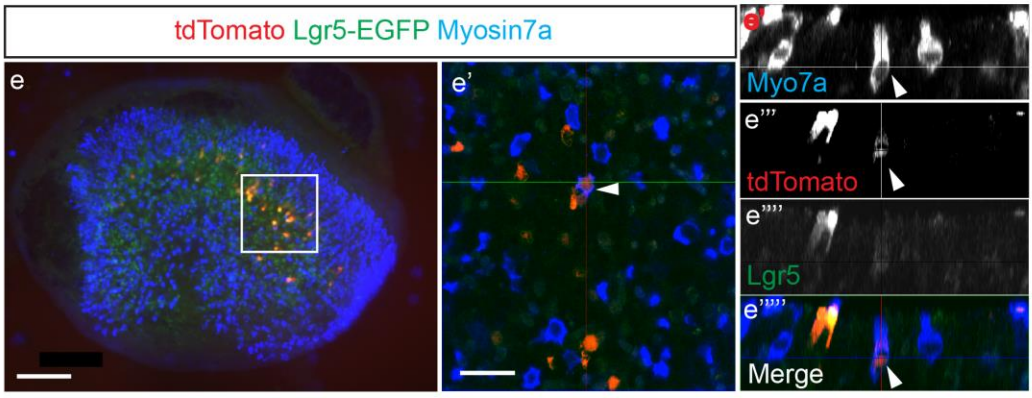


d

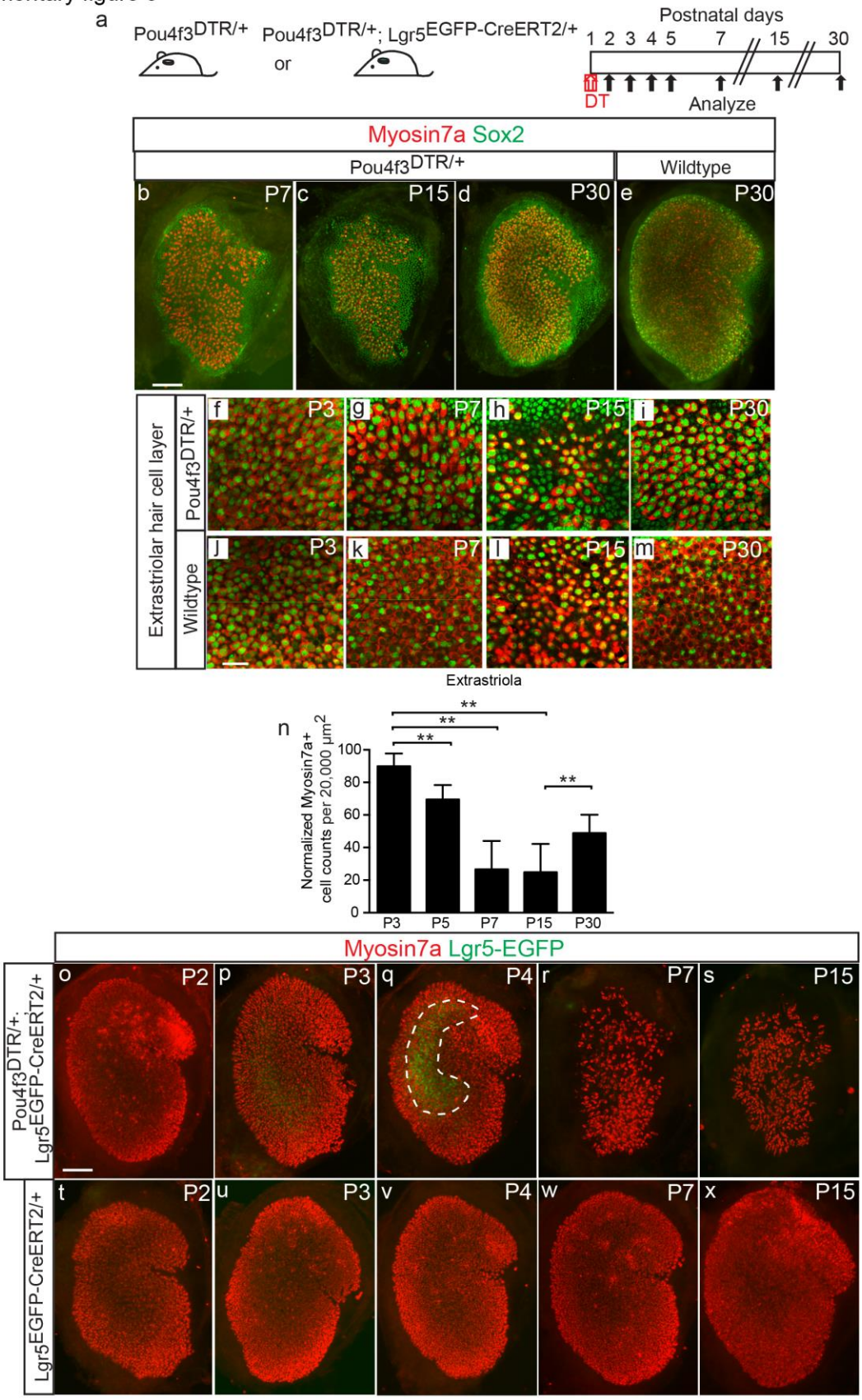
Utricle number	Tracked Cells	Tracked SC	Initial HC	Final SC	SC to HC	% SC to HC
1	8	8	0	7	1	---
2	12	11	1	11	0	---
3	11	11	0	11	0	---
4	3	3	0	2	1	---
Total	34	33	1	31	2	6%



Supplementary Figure 2. Time-lapse and quantification of traced cells from facultative Lgr5+ cells (related to Figure 2).

(**a**) Utricles from P3 *Lgr5*$^{EGFP\text{-}CreERT2/+}$*; Rosa26R*$^{tdTomato/+}$ mice were treated with neomycin and then 48hr 4OH-tamoxifen to fate-map Lgr5+ cells. Few tdTomato+ cells were noted at this time point, and no tdTomato+, Myo7a+ cells were found. (**a'**) High magnification image taken of the striolar region. (**b**) Schematic for time-lapse imaging of traced Lgr5+ cells after neomycin-mediated hair cell ablation *in vitro*. tdTomato+ cells within EGFP+ regions were identified and z-stack images were taken of these cells every hour for a total of 45 hr. (**c**) 3D reconstruction of z-stack images captured at defined time-points, showing 8 tall and slender tdTomato+ cells, which were characterized as supporting cell-like cells (SC) throughout the period analyzed. (**d**) From *Lgr5*$^{EGFP\text{-}CreERT2/+}$*; Rosa26R*$^{tdTomato/+}$ mice, 34 cells from 4 utricles were imaged and tracked for 45-97 hr (average=83.2 hr). At the start of the time-lapse imaging, thirty-three cells were tall and slender and classified as supporting cells. One cell was flask-shaped and classified as a hair cell (HC). Among the 33 supporting cells, 2 acquired a hair cell shape over time. No cell division was noted. Utricle #3 is shown in Figure S2B and #4 in Figure 2I. (**e**) Representative images of utricles immunostained for Myosin7a after 45 hr of time-lapse imaging and 8 days of culture. Overall, we detected 4 tdTomato+/Myo7a+ cells from 48 tdTomato+ cells (8.3±9.5% from 3 organs that were analyzed via time-lapse imaging). (**e'**) High magnification image of the striolar region. Arrowhead points to a tdTomato+, Myosin7a+ cell. (**e''-e''''**) represent orthogonal view of (**e'**). Scale bars, (**a, e**) 100 μm; (**c**) 10 μm; (**a', e'**) 20 μm.

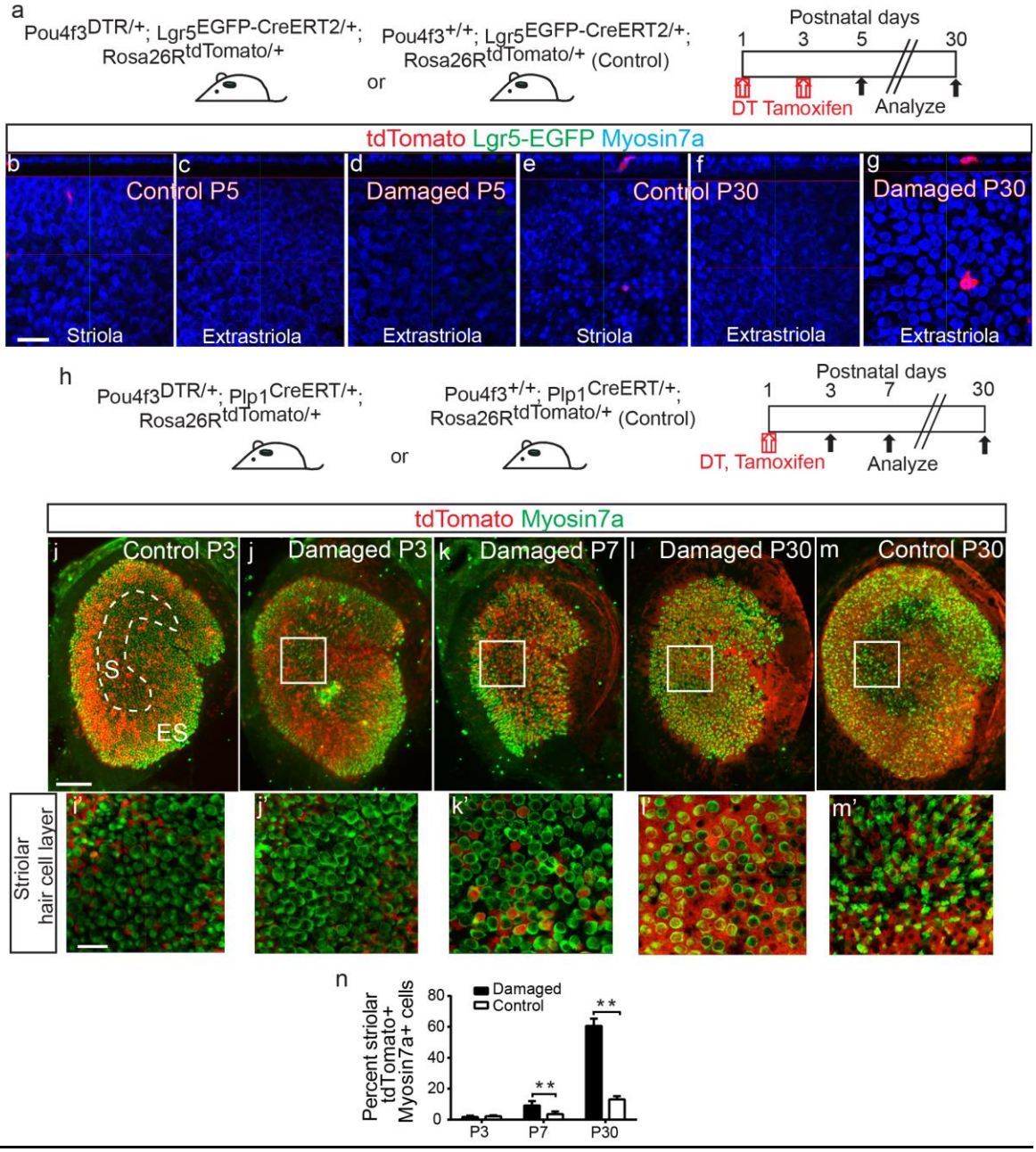
Supplementary figure 3



Supplementary Figure 3. Hair cell ablation in *Pou4f3-DTR* mice (related to Figure 4).

(**a**) Schematic of hair cell ablation and detection of Lgr5+ cells. (**b**-**m**) Representative images of utricles from wildtype and *Pou4f3*$^{DTR/+}$ mice injected with diphtheria toxin (DT) at P1. All tissues were immunostained for Myosin7a and Sox2. There was a progressive loss of hair cells in both the striolar and extrastriolar domains from P3-15 (Figure 4). A partial repopulation of hair cells was noted at P30. (**n**) Quantification of Myosin7a+ cells per 20,000 μm^2 in the extrastriola (normalized to control). (**o**-**q**) DT-mediated hair cell loss led to robust Lgr5-EGFP expression in striolar Sox2+ supporting cells at P3-4 (also see Figure 4). (**r**-**s**) EGFP expression was markedly less at P7 and undetectable at P15. (**t**-**x**) No detectable Lgr5-EGFP signals in age-matched non-damaged controls. n=4-12 for **n** (4 for P3, 8 for P5, and 12 for P7, P15 and P30). Data shown as mean±S.D. **p<0.01, Student's *t*-tests. Scale bars, (**b-e** and **o-x**) 100 μm; (**f-m**) 20 μm.

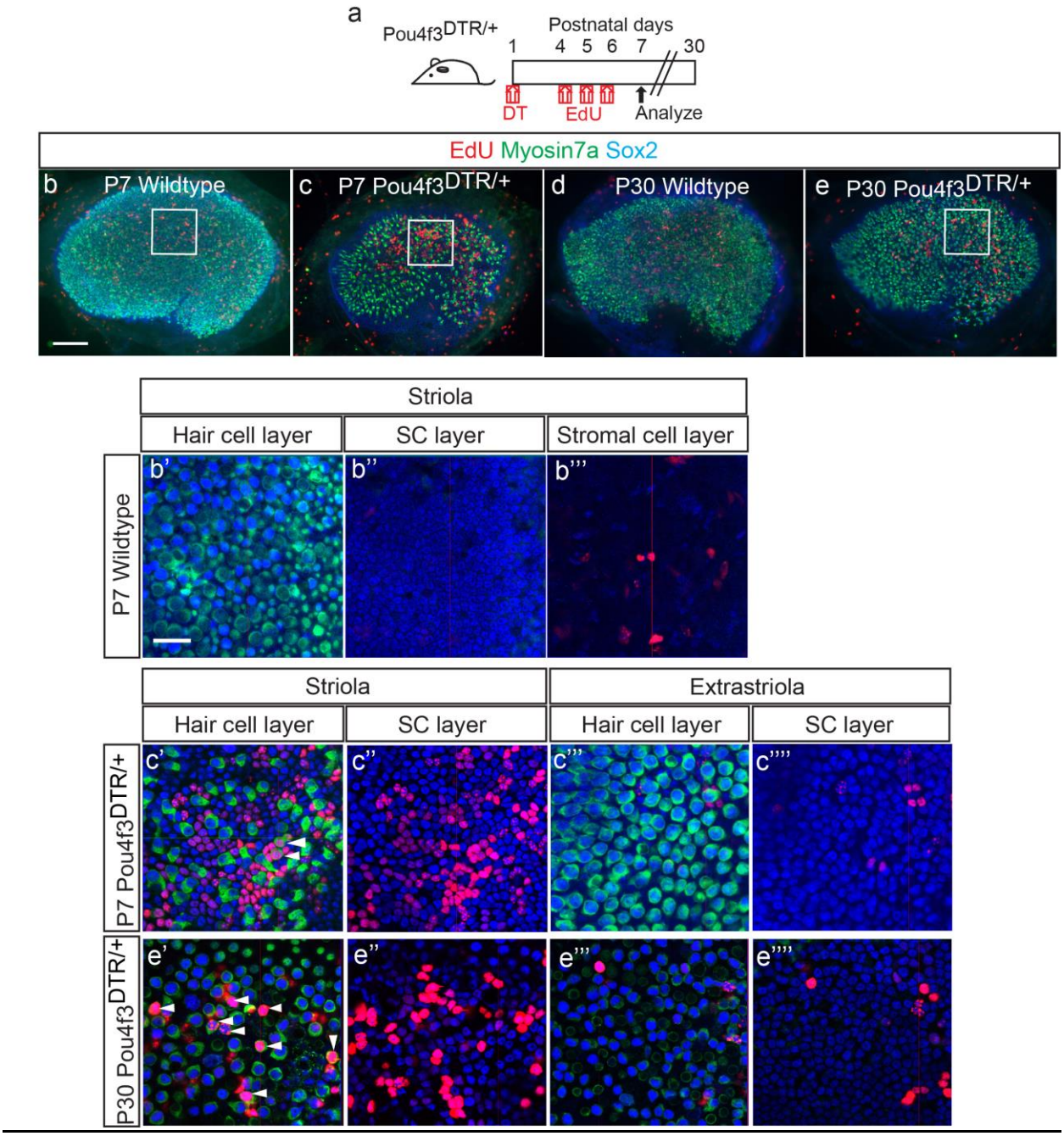
Supplementary figure 4



Supplementary Figure 4. *In vivo* lineage tracing after hair cell ablation (related to Figure 5 and Figure 6)

(**a**) Schematic of *in vivo* hair cell ablation and lineage tracing of Lgr5+ cells. Animals lacking the *Pou4f3* allele were used as parallel undamaged controls. In undamaged controls, almost no traced cells were found at P5 and P30 (Figure 5). (**b-c**) High magnification images of striolar and extrastriolar domains of P5 control organs. After hair cell ablation, Lgr5-EGFP and tdTomato labeling were readily detected in the striolar region (Figure 5). (**d**) Representative image showing the absence of Lgr5-EGFP or tdTomato labeling in the extrastriolar region after damage. (**e-f**) Striolar and extrastriolar domains from P30 controls. (**g**) Extrastriolar region showing rare tdTomato+ cells in the damaged P30 organs. (**h**) Schematic of fate-mapping Plp1+ supporting cells. (**i-m**) Tamoxifen led to tdTomato labeling in both striolar (S) and extrastriolar regions (ES) (see Figure 6 for the extrastriola). DT led to hair cell loss at P7 followed by regeneration at P30. Most hair cells were tdTomato+ in the P30 damaged utricles; whereas only a subset of hair cells were tdTomato+ in the P30 undamaged controls. In striolar region, rare Myosin7a+ cells were tdTomato-labeled in P3 control tissues (**i'**). The damaged organs showed progressively more traced hair cells from P7 to P30 (**k'-l'**). At P30, more tdTomato-labeled hair cells were noted in the damaged organs relative to controls (**l'-m'**). (**n**) Quantification shows that more Myosin7a+ hair cells from the striola of the damaged organs were traced relative to age-matched controls. n=4-8 in **n**. Data shown as mean±SD. **p<0.01, Student's *t*-tests. Scale bars, (**i-m**) 100 μm; (**b-g** and **i'-m'**) 20 μm.

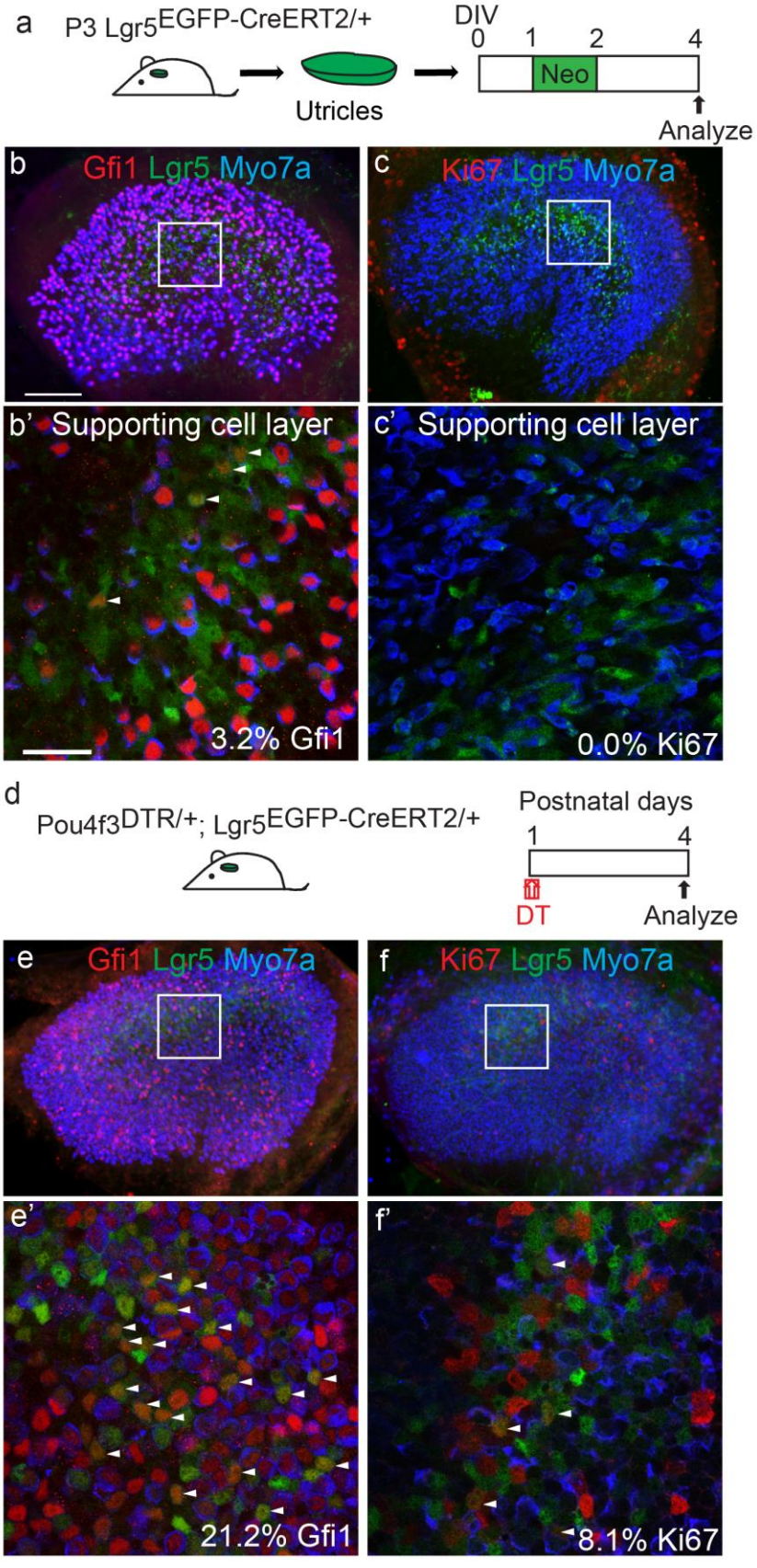
Supplementary figure 5



Supplementary Figure 5. Mitotic tracing after hair cell ablation (related to Figure 6)

(**a**) Schematic of *in vivo* hair cell ablation and pulse-chase experiments using *Pou4f3-DTR* mice. Wildtype animals receiving DT and EdU served as age-matched controls. (**b-b’’’**) Undamaged utricles contained few EdU-labeled cells at P7, most of which resided in the stromal layer underneath the sensory epithelium. (**c**) After damage, EdU robustly labeled in the striolar region of the sensory epithelium at P7. (**c’-c’’**) High magnification confocal images show that EdU labeled few hair cells (arrowheads) and many supporting cells in the striolar region at P7 (taken from inset in **c**). (**c’’’-c’’’’**) No EdU+ hair cells and rare EdU-labeled supporting cells (SC) were found in the extrastriolar region at P7. (**d**) Representative micrograph of undamaged P30 utricle, showing few EdU-labeled cells. (**e**) At P30, many EdU-labeled cells occupied the central sensory epithelium. (**e’-e’’**) Many EdU-labeled hair cells (arrowheads) and supporting cells were detected in the striolar region of damaged, P30 utricles (taken from inset in **e**). (**e’’’-e’’’’**) Rare EdU-labeled hair cells and supporting cells were found in the extrastriolar region. Scale bars: (**b-e**) 100 μm; (**b’-e’’’’**) 20 μm.

Supplementary figure 6



Supplementary Figure 6: Characterization of damaged-recruited Lgr5+ cells *in vivo* and *in vitro* (related to Figures 1, 2, and 6)

(**a**) Neomycin was used to induce hair cells loss in utricles from P3 *Lgr5*$^{EGFP\text{-}CreERT2/+}$ mice *in vitro*. (**b-c**) Damage-recruited of Lgr5+ cells expressed Gfi1 (3.2±1.9%) and no Ki67+, Lgr5+ cells were detected. (**d**) Schematic of *in vivo* hair cell ablation and the use of *Lgr5-EGFP* reporter mice. (**e-f**) A subset of damage-recruited Lgr5+ cells expressed Gfi1 (21.2±5.7%) and Ki67 (8.1±3.5%). The majority of Gfi1+, Lgr5+ (Myosin7a-negative) cells resided in the supporting cell layer. Scale bars, (**b-c** and **e-f**) 100 μm; (**b'-c'** and **e'-f'**) 20 μm.

Supplementary Table 1. Hair cell counts and sensory epithelium dimensions of utricles from P3 wildtype and *Lgr5*$^{EGFP\text{-}CreERT2/+}$ mice (related to Figure 1)

Mice (P3)	Myosin7a+ sensory epithelium area (mm^2)	Striolar Myosin7a+ cells*	Extrastriolar Myosin7a+ cells*
Wildtype	0.2 ± 0.02	204.8 ± 15.6	235.3 ± 17.5
Lgr5$^{EGFP\text{-}CreERT2/+}$	0.2 ± 0.01 (p=0.59)	215.3 ± 3.51 (p=0.27)	234.3 ± 12.1 (p=0.93)

Each category is shown as average ± S.D. n=3-4. P values are determined by Student's *t*-tests and represent comparisons between organs from P3 wildtype and *Lgr5*$^{EGFP\text{-}CreERT2/+}$ mice.

*Cell counts per 20,000 μm^2.

Supplementary Table 2. Dimensions and locations of oncomodulin+ and Lgr5+ domains (related to Figures 1 and 4).

	Oncomodulin*	Lgr5-EGFP after neomycin *in vitro*#	Lgr5-EGFP after diphtheria toxin *in vivo*~
Total Myosin7a+ sensory epithelium area (mm^2)	0.20 ± 0.01	0.20 ± 0.01 (p=0.89)	0.19 ± 0.01 (p=0.20)
Area expressing marker of interest (mm^2)	0.04 ± 0.01	0.05 ± 0.01 (p=0.15)	0.05 ± 0.004 (p=0.21)
Length (anterior-posterior)	0.37 ± 0.03	0.40 ± 0.01 (p=0.18)	0.41 ± 0.03 (p=0.12)
Width A (medial-lateral)	0.10 ± 0.03	0.12 ± 0.03 (p=0.43)	0.14 ± 0.02 (p=0.13)
Width B (medial-lateral)	0.08 ± 0.01	0.10 ± 0.02 (p=0.14)	0.12 ± 0.02 (p=0.01)
Width C (medial-lateral)	0.07 ± 0.003	0.10 ± 0.01 (p=0.01)	0.10 ± 0.04 (p=0.26)
Width D (medial-lateral)	0.14 ± 0.01	0.16 ± 0.01 (p=0.04)	0.14 ± 0.03 (p=0.96)
Distance to sensory epithelium edge (posterior)	0.17 ± 0.02	0.19 ± 0.02 (p=0.47)	0.13 ± 0.02 (p=0.04)
Distance to sensory epithelium edge (anterior)	0.09 ± 0.01	0.08 ± 0.01 (p=0.40)	0.07 ± 0.01 (p=0.06)
Distance between lateral domain and lateral edge	0.09 ± 0.004	0.09 ± 0.01 (p=0.28)	0.06 ± 0.03 (p<0.01)
Distance between medial domain and lateral edge	0.18 ± 0.02	0.20 ± 0.01 (p=0.19)	0.17 ± 0.03 (p=0.60)

Each category is shown as average ± S.D. in mm or mm^2. n=3-5 organs. P values are determined by Student's *t*-tests and represent comparisons between Lgr5-EGFP+ and oncomodulin+ areas in corresponding categories. See supplementary Fig. 1a for illustration of measurements. Oncomodulin expression was undetectable in cultured, undamaged utricles and could not be used for comparison. After DT-mediated damage

in vivo, oncomodulin+ hair cells decreased and resided within Lgr5-EGFP+ region (Fig. 4o).

*Utricles from P4 wildtype mice.

#Utricles from P3 *Lgr5*$^{EGFP\text{-}CreERT2}$ mice, damaged by neomycin *in vitro* (48 hr post damage).

~Utricles from P4 *Pou4f3*$^{DTR/+}$*; Lgr5*$^{EGFP\text{-}CreERT2}$ mice which were injected with DT at P1.

Supplementary Table 3. Measurements of cells and areas after *in vivo* hair cell ablation (related to Figure 4).

Postnatal Age	P3	P5	P7[#]	P15[#]	P30
Control striolar Myosin7a+ cells*	204.8 ± 15.6	223.4 ± 26.7	250.2 ± 30.8	256.3 ± 31.1	235.7 ± 49.6
Pou4f3-DTR striolar Myosin7a+ cells*	179.7± 5.6 (p<0.05)	149.1 ± 15.6 (p<0.0001)	79.4 ± 52.6 (p<0.0001)	74.5 ± 52.5 (p<0.0001)	150.7 ± 23.5 (p<0.0001)
Control extrastriolar Myosin7a+ cells*	235.3 ± 17.5	285.4 ± 18.8	294.4 ± 37.1	301.3± 21.6	286.3 ± 54.9
Pou4f3-DTR extrastriolar Myosin7a+ cells*	211.7 ± 18.1 (p=0.079)	198.5 ± 25.1 (p<0.0001)	79.4 ± 52.6 (p<0.0001)	74.5 ± 52.5 (p<0.0001)	139.8 ± 32.3 (p<0.0001)
Control Myosin7a+ sensory epithelium area (mm2)	0.18 ± 0.02	0.18 ± 0.01	0.21 ± 0.02	0.22 ± 0.01	0.22 ± 0.01
Pou4f3-DTR Myosin7a+ sensory epithelium area (mm2)	0.17 ± 0.01 (p=0.41)	0.15 ± 0.01 (p<0.0001)	0.12 ± 0.03 (p<0.0001)	0.10 ± 0.04 (p<0.0001)	0.14 ± 0.01 (p<0.0001)

Each category is shown as average ± S.D. n=4-6 at P3, 8 at P5, 12 at P7, P15 and P30. P values are determined by Student's *t*-tests and represent comparisons between damaged and control tissues at each age.

*Cell counts per 20,000 μm^2.

#Striolar and extrastriolar areas in damaged organs are analyzed together at P7 and P15.

Supplementary Table 4. Quantification of Lgr5- and Plp1-traced cells (related to Figures 5 and 6).

Genotypes	Area	Postnatal Age	P3	P5	P7	P30
Lgr5$^{EGFP\text{-}CreERT2/+}$; *Rosa26R*$^{tdTomato/+}$	Striola	**Control** tdTomato+ Myosin7a+ cells*^	N/A	0.5 ± 0.6	N/A	0.9 ± 0.5
		Pou4f3-DTR tdTomato+ Myosin7a+ cells*^	N/A	0.4 ± 0.5 (p=0.80)	N/A	13.5 ± 4.4 (p<0.0001)
		Control total Myosin7a+ cells*^	N/A	239.0 ± 21.6	N/A	233.3 ± 14.8
		Pou4f3-DTR total Myosin7a+ cells*^	N/A	170.0 ± 9.2 (p<0.005)	N/A	125.8 ± 23.4 (p<0.0001)
		Control total tdTomato+ cells*^	N/A	0.8 ± 1.0	N/A	1.2 ± 0.7
		Pou4f3-DTR total tdTomato+ cells*^	N/A	10.6 ± 4.4 (p<0.01)	N/A	22.7 ± 7.6 (p<0.0001)
Plp1$^{CreERT/+}$; *Rosa26R*$^{tdTomato/+}$	Striola	**Control** tdTomato+ Myosin7a+ cells*#	4.5 ± 1.3	N/A	9.3 ± 4.3	36.7 ± 7.4
		Pou4f3-DTR tdTomato+ Myosin7a+ cells*#	3.5 ± 1.8 (p=0.30)	N/A	10.1 ± 4.9 (p=0.76)	79.5 ± 12.7 (p<0.005)
		Control total Myosin7a+ cells*#	209.8 ± 10.4	N/A	258.0 ± 25.7	278.2 ± 32.4
		Pou4f3-DTR total Myosin7a+ cells*#	198.9 ± 9.4 (p=0.13)	N/A	95.9 ± 38.1 (p<0.0001)	131.0 ± 17.0 (p<0.0001)
	Extrastriola	**Control** tdTomato+ Myosin7a+ cells*#	8.9 ± 1.9	N/A	38.8 ± 2.6	72.6 ± 20.1
		Pou4f3-DTR tdTomato+ Myosin7a+ cells*#	3.4 ± 2.2 (p<0.005)	N/A	24.6 ± 11.6 (p<0.05)	105.8 ± 9.9 (p<0.01)
		Control total Myosin7a+ cells*#	259.1 ± 20.5	N/A	310.1 ± 22.9	315.7 ± 29.1
		Pou4f3-DTR total Myosin7a+ cells*#	221.5 ± 18.6 (p<0.05)	N/A	95.9 ± 38.1 (p<0.0001)	130.4 ± 10.4 (p<0.0001)

Each category is shown as average ± S.D. n=4-5 at P5, 26-28 at P30 in *Lgr5*$^{EGFP\text{-}CreERT2/+}$; *Rosa26R*$^{tdTomato/+}$ group. n=4-8 in *Plp1*$^{CreERT/+}$; *Rosa26R*$^{tdTomato/+}$ group. P values are determined by Student's *t*-tests and represent comparisons between damaged and control tissues at each age.

*Cell counts per 20,000 μm^2.

^Control mice were *Lgr5*$^{EGFP\text{-}CreERT2/+}$; *Rosa26R*$^{tdTomato/+}$, damaged were *Pou4f3*$^{DTR/+}$; *Lgr5*$^{EGFP\text{-}CreERT2/+}$; *Rosa26R*$^{tdTomato/+}$.

#Control mice were *Plp1*$^{CreERT/+}$; *Rosa26R*$^{tdTomato/+}$, damaged were *Pou4f3*$^{DTR/+}$; *Plp1*$^{CreERT/+}$; *Rosa26R*$^{tdTomato/+}$.

Supplementary Table 5. Quantification of EdU-labeled cells (related to Figure 7).

Genotypes				Striola		Extrastriola	
Pou4f3$^{DTR/+}$	*Lgr5*$^{EGFP\text{-}CreERT2/+}$	*Catnb*$^{flox\ (exon3)/+}$	Expected effects	EdU+/ Myosin7a+ hair cells	EdU+ supporting cells	EdU+/ Myosin7a+ hair cells	EdU+ supporting cells
-	+	-	No damage, no stable ß-catenin	0	0.7 ± 0.4	0.1 ± 0.2	0
	-	+					
-	+	+	No damage, stable ß-catenin	0	1.9 ± 1.8	0	0.7 ± 1.0
+	+/-	-	Damage, no stable ß-catenin	14.0 ± 13.6	95.7 ± 21.7	2.8 ± 3.6	60.6 ± 42.7
	-	+/-					
+	+	+	Damage, stable ß-catenin	29.6 ± 4.6 (p<0.001)*	113.2 ± 16.4 (p<0.05)*	3.1 ± 2.4 (p=0.79)*	38.3 ± 18.0 (p=0.09)*

Shown are cell counts per 20,000 µm^2 regions. Each category represents average ± S.D. n=18 for organs with damage and no ß-catenin stabilization, 5-8 for all other groups. *P values are determined by Student's *t*-tests and represent comparisons between damaged tissues with stabilized ß-catenin and damaged tissues without stabilized ß-catenin.

Supplementary Table 6. Quantification of hair cell and proliferation markers in Lgr5 lineage cells *in vivo* and *in vitro* (related to Figures 1, 2, and 6).

		P4	P5	P7	P30
*In vivo**	Percent Lgr5+ cells expressing Myosin7a	2.5±1.1% (n=4)	N/A	N/A	N/A
	Percent Lgr5+ cells expressing Gfi1	21.2±5.7% (n=4)	N/A	N/A	N/A
	Percent Lgr5 traced cells expressing Myosin7a	N/A	3.1±4.5% (n=5)	N/A	60.9±9.1% (n=28)
	Percent Lgr5+ cells expressing Ki67	8.1±3.5% (n=4)	N/A	N/A	N/A
	Percent Lgr5 traced cells labeled with EdU	N/A	N/A	53.0±14.5% (n=8)	48.5±16.3% (n=4)

		4 DIV	7 DIV	14 DIV
In vitro#	Percent Lgr5+ cells expressing Myosin7a	2.5±1.3% (n=4)	N/A	N/A
	Percent Lgr5+ cells expressing Gfi1	3.2±1.9% (n=4)	N/A	N/A
	Percent Lgr5 traced cells expressing Myosin7a	0.0% (n=4)	12.8±2.5% (n=3)	20.4±4.5% (n=3)
	Percent Lgr5+ cells expressing Ki67	0.0% (n=3)	0.0% (n=5)	N/A
	Percent Lgr5 traced cells labeled with EdU	N/A	0.0% (n=5)	N/A

Percentages are calculated from cell counts made from 20,000 μm^2 regions. N's represent the number of organs examined in each category/timepoint.

**Pou4f3*$^{DTR/+}$; *Lgr5*$^{EGFP\text{-}CreERT2/+}$ or *Pou4f3*$^{DTR/+}$; *Lgr5*$^{EGFP\text{-}CreERT2/+}$; *Rosa26R*$^{tdTomato/+}$ animals are used.

#*Lgr5*$^{EGFP\text{-}CreERT2/+}$ or *Lgr5*$^{EGFP\text{-}CreERT2/+}$; *Rosa26R*$^{tdTomato/+}$ animals are used.

Supplementary Table 7. Primers for genotyping and quantitative PCR.

Lgr5-EGFP-CreERT2	Forward	5’-CTGCTCTCTGCTCCCAGTCT-3’
	WT Reverse	5’-ATACCCCATCCCTTTTGAGC-3’
	Mutant Reverse	5’-GAACTTCAGGGTCAGCTTGC-3’
Rosa26R-tdTomato	WT Forward	5’- AAGGGAGCTGCAGTGGAGTA-3’
	WT Reverse	5’-CCGAAAATCTGTGGGAAGTC-3’
	Mutant Forward	5’-GGCATTAAAGCAGCGTATCC-3’
	Mutant Reverse	5’-CTGTTCCTGTACGGCATGG-3’
Pou4f3-DTR	Forward	5’-GTCAAAAAATGTGCCTTAGAG -3’
	Mutant Reverse	5’-CCGACGGCAGCAGCTTCATGGTC-3’
	WT Forward	5’-CACTTGGAGCGCGGAGAGCTAG-3’
Plp1-CreERT	WT Forward	5’- CTAGGCCACAGAATTGAAAGATCT-3’
	WT Reverse	5’-GTAGGTGGAAATTCTAGCATCATCC-3’
	Mutant Forward	5’-GCGGTCTGGCAGTAAAAACTATC-3’
	Mutant Reverse	5’-GTGAAACAGCAT TGCTGTCACTT-3’
Catnb-flox (exon3)	Mutant Forward	5’-AACTGGCTTTTGGTGTCGGG-3’
	Mutant Reverse	5’-TCGGTGGCTTGCTGATTATTTC-3’
Sox2	Forward	5’-ATGAACGGCTGGAGCAACGGCA-3’
	Reverse	5’-TCACATGTGCGACAGGGGCAGT-3’
Lgr5	Forward	5’-TCTTCACCTCCTACCTGGACCT-3’
	Reverse	5’-GGCGTAGTCTGCTATGTGGTGT-3’
Pou4f3	Forward	5'-ACCCAA ATTCTCCAGCCTACAC-3'
	Reverse	5'-GGCGAGATGTGCTCAAGTAAGT-3'
GAPDH	Forward	5'-TGTGTCCGTCGTGGATCTGA-3'
	Reverse	5'-CCTGCTTCACCACCTTCTTGAT-3'