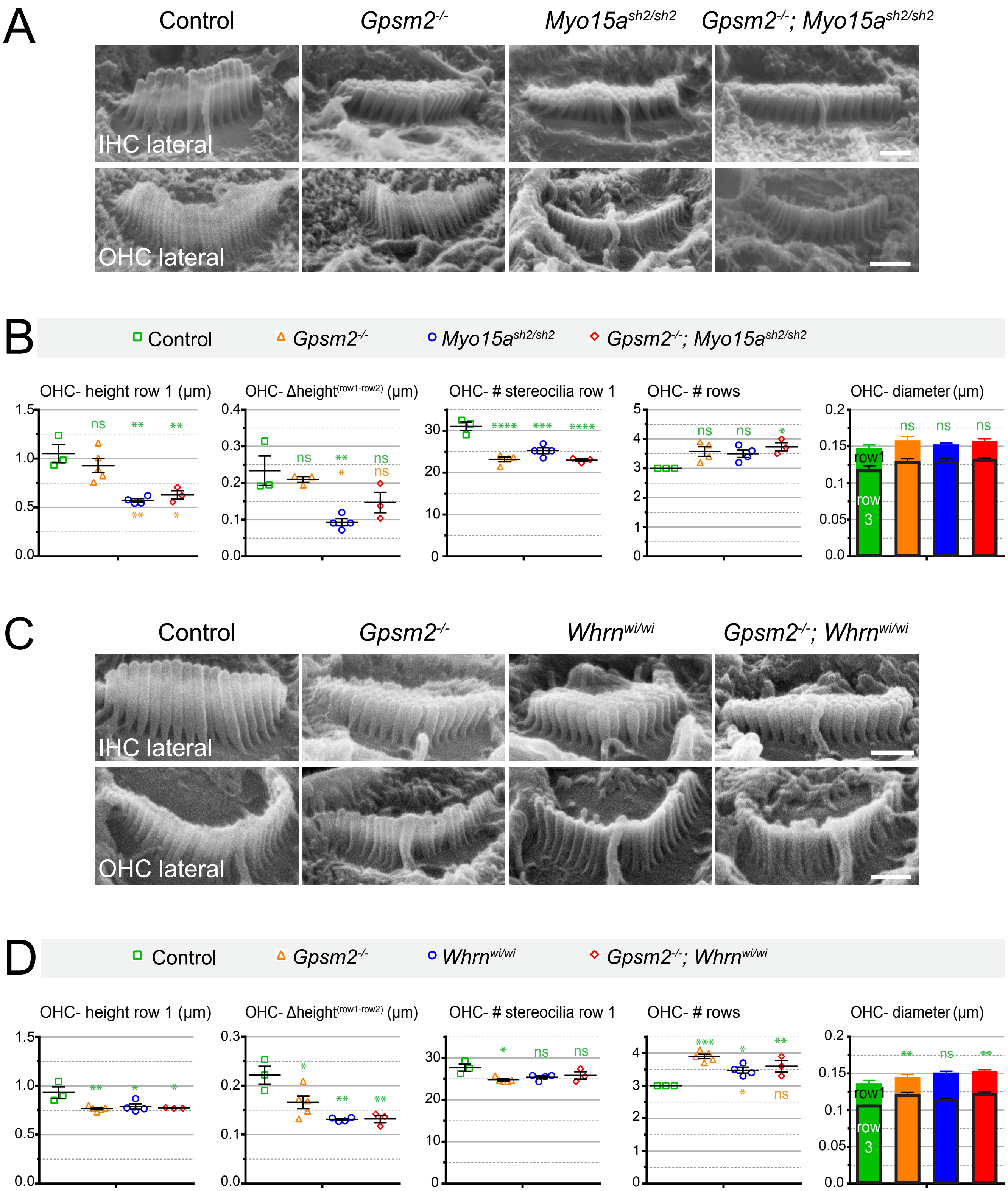


A
Control
Gpsm2-/-
Myo15ash2/sh2
Gpsm2-/-; Myo15ash2/sh2
IHC lateral
OHC lateral
B
Control
Gpsm2-/-
Myo15ash2/sh2
Gpsm2-/-; Myo15ash2/sh2
OHC- height row 1 (µm)
OHC- Δheight(row1-row2) (µm)
OHC- # stereocilia row 1
OHC- # rows
OHC- diameter (µm)
row1
row 3
C
Control
Gpsm2-/-
Whrnwi/wi
Gpsm2-/-; Whrnwi/wi
IHC lateral
OHC lateral
D
Control
Gpsm2-/-
Whrnwi/wi
Gpsm2-/-; Whrnwi/wi
OHC- height row 1 (µm)
OHC- Δheight(row1-row2) (µm)
OHC- # stereocilia row 1
OHC- # rows
OHC- diameter (µm)
row1
row 3

Figure S1. Similar hair bundle defects in *Gpsm2$^{-/-}$*, *Myo15a$^{sh2/sh2}$* *and (Gpsm2$^{-/-}$*; *Myo15a$^{sh2/sh2}$)* double-mutant and in *Gpsm2$^{-/-}$*, *Whrn$^{wi/wi}$* and (*Gpsm2$^{-/-}$*; *Whrn$^{wi/wi}$)* double mutants at P6/P7 – related to Figure 1. **(A)** Lateral views of IHC and OHC hair bundles from *Gpsm2$^{-/-}$*, *Myo15a$^{sh2/sh2}$, and Gpsm2$^{-/-}$*; *Myo15a$^{sh2/sh2}$* double mutant and unaffected littermate control using SEM. **(B)** Quantification of OHC stereocilia height in row 1, height differential between row 1 and row 2, number of stereocilia in row 1, total number of rows and stereocilia diameter in row 1 (solid-colored bars) and row 3 (black-edged bars) for the genotypes indicated. **(C)** Lateral views of IHC and OHC hair bundles from *Gpsm2$^{-/-}$*, *Whrn$^{wi/wi}$, and Gpsm2$^{-/-}$*; *Whrn$^{wi/wi}$* double mutant and unaffected littermate control using SEM. **(D)** Same set of OHC quantification as in (B) for the genotypes indicated. See Figure 1 for IHC quantifications and medial views of hair bundles in IHCs and OHCs, legend to Figure 1 for details on quantifications, and Tables S1, S2 for data values. Scale bars are 1µm.

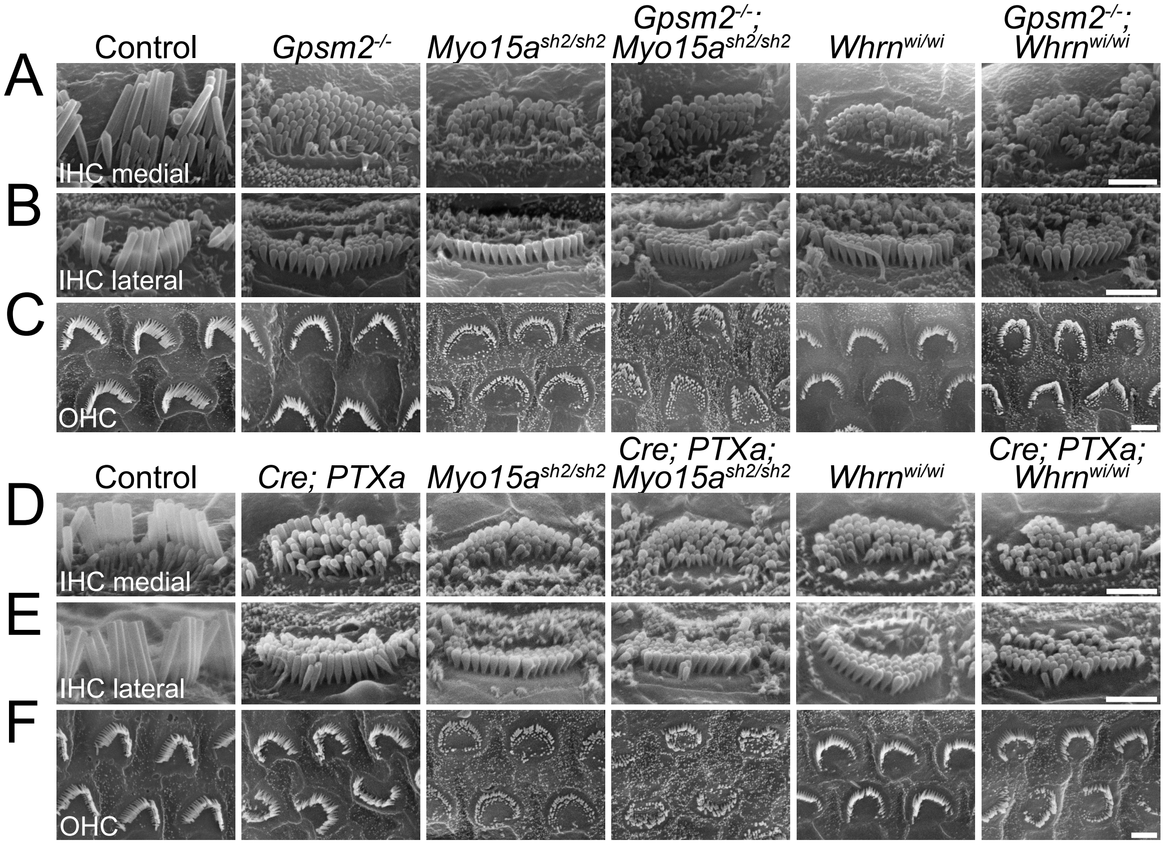

Control
Gpsm2-/-
Myo15ash2/sh2
Gpsm2-/-; Myo15ash2/sh2
Whrnwi/wi
Gpsm2-/-; Whrnwi/wi
A
B
C
IHC medial
IHC lateral
OHC
Control
Cre; PTXa
Myo15ash2/sh2
Cre; PTXa; Myo15ash2/sh2
Whrnwi/wi
Cre; PTXa; Whrnwi/wi
D
E
F
IHC medial
IHC lateral
OHC

Figure S2. Similar mature hair bundle defects in *Gpsm2$^{-/-}$*, *PTXa, Myo15a$^{sh2/sh2}$* , *Whrn$^{wi/wi}$* single mutants and (*Gpsm2$^{-/-}$; Myo15a$^{sh2/sh2}$*), (*Gpsm2$^{-/-}$; Whrn$^{wi/wi}$*), *(PTXa; Myo15a$^{sh2/sh2}$)*, *(PTXa; Whrn$^{wi/wi}$*) double mutants – related to Figure 1. (A-C) *Gpsm2^{-}* was crossed to either *Myo15a^{sh2}* or *Whrn^{wi},* and P16 IHCs were imaged with SEM from a medial (top) or lateral (middle) perspective, and OHCs were imaged from a top perspective (bottom) in the genotypes indicated. **(D-F)** *Atoh1-Cre; PTXa* was crossed to either *Myo15a^{sh2}* or *Whrn^{wi},* and P21 IHCs and OHCs were imaged as in (A-C). (B) and (D) represent the middle cochlear turn, and (A), (C), (E) and (F) represent the apical turn. *PTXa* OHCs were misoriented as reported previously (9). Scale bars are 2μm.

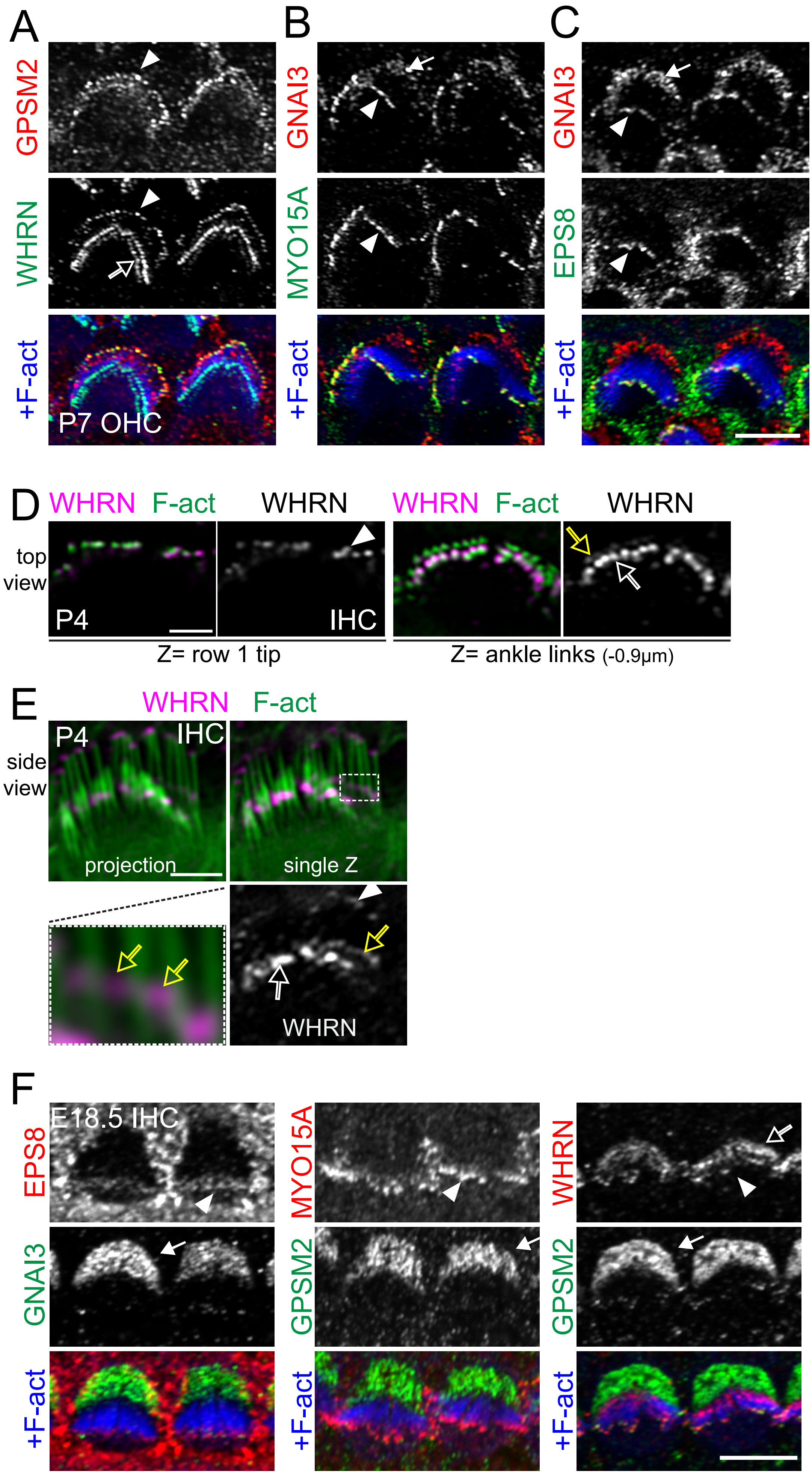
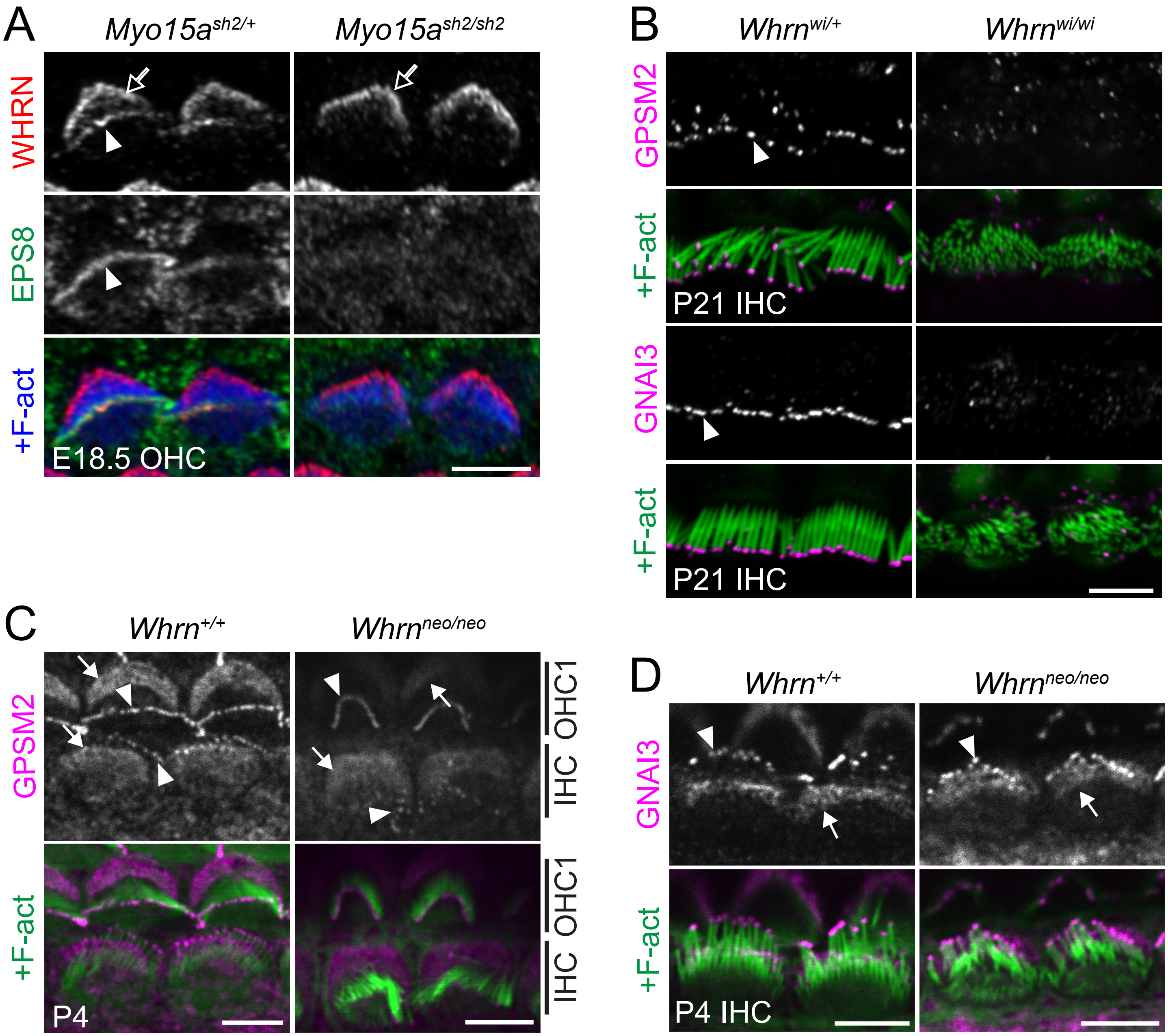
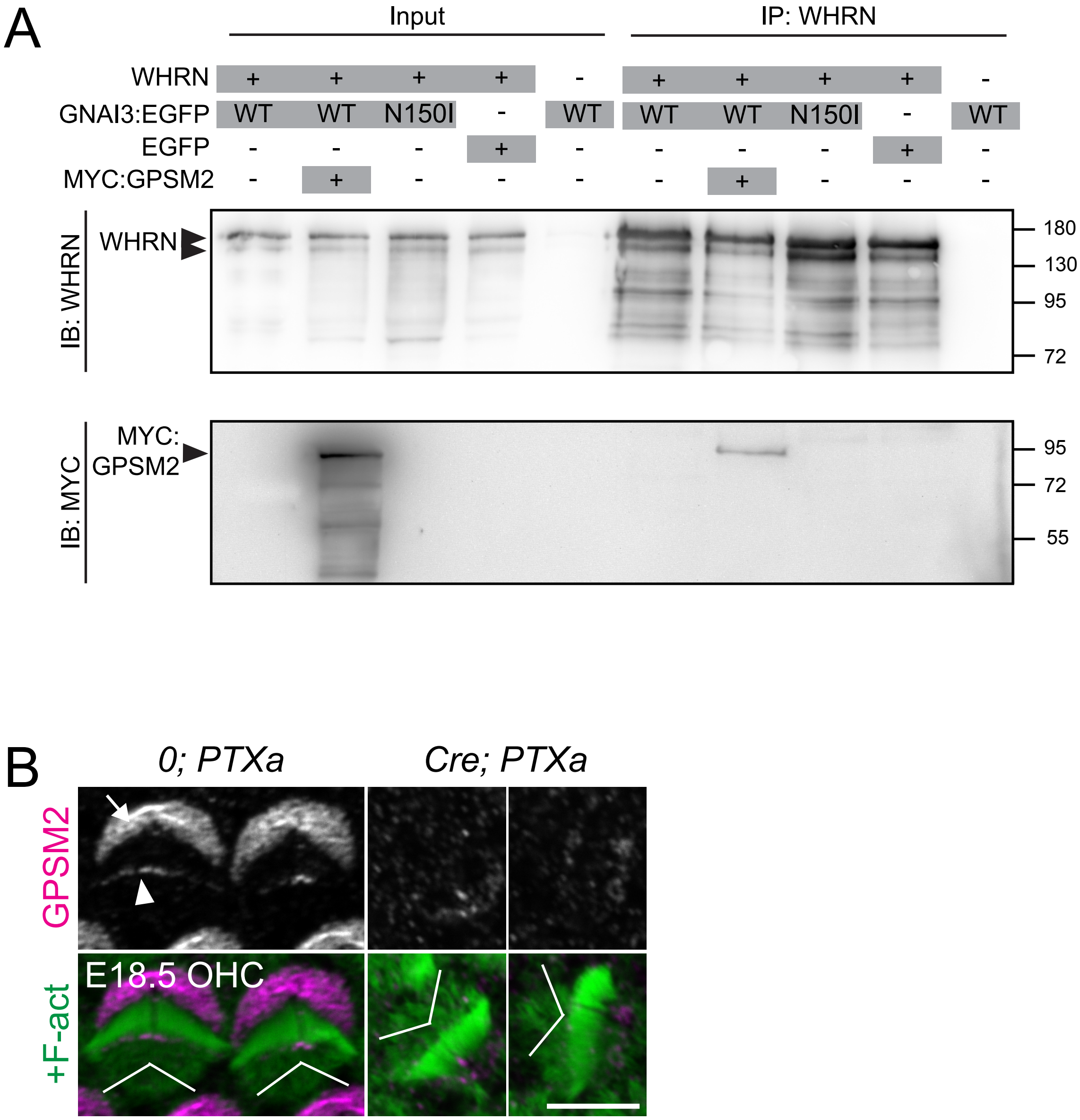

A
GPSM2
WHRN
+F-act
P7 OHC
B
GNAI3
MYO15A
+F-act
C
GNAI3
EPS8
+F-act
D
WHRN F-act
WHRN
top view
P4
IHC
Z= row 1 tip
WHRN F-act
WHRN
Z= ankle links (-0.9µm)
E
WHRN F-act
P4
IHC
side view
projection
single Z
WHRN
F
E18.5 IHC
EPS8
GNAI3
+F-act
MYO15A
GPSM2
+F-act
WHRN
GPSM2
+F-act

Figure S3. Distinct stereocilia distribution for WHRN-GPSM2-GNAI3 and MYO15A-EPS8 in postnatal and embryonic hair cells – related to Figure 2. (A-C) Coimmunostainings for GPSM2+WHRN (A), GNAI3+MYO15A (B) and GNAI3+EPS8 (C) in P7 OHCs one turn above the cochlear base. All proteins colocalized at row 1 tips (solid arrowheads; see also Figure 2A-C for P0 and P7 IHCs). In addition, GPSM2 and GNAI3 were detected at the bare zone (solid arrow), and WHRN localized to the base of stereocilia (hollow arrows). **(D-E)** WHRN immunostaining in P4 IHCs. Uncompressed mounting revealed WHRN localization at row 1 tips (D, left panels) and at ankle links level (D, right panels) at different positions of an Airyscan z-series of an IHC with a straight bundle. In a z-series where the bundle is tilted sideways laterally (E), WHRN was not detected at stereocilia tips in row 2 and was limited to row 1 tips (arrowhead) and ankle link regions. Yellow and white hollow arrows indicate signal tentatively associated with links between row 1 stereocilia (lower WHRN amounts; see magnified inset) and links between row 1 and row 2 stereocilia (higher WHRN amounts), respectively. **(F)** E18.5 IHCs coimmunostainings at the cochlear base for the proteins indicated. GPSM2 and GNAI3 protein levels are lower in IHCs than OHCs at E18.5 (see Figure 2F), and costainings in IHCs yield results similar to those seen at E16.5, with tips (arrowheads) showing abundant MYO15A-EPS8 and barely detectable WHRN-GPSM2-GNAI3 signals. Solid arrows indicate the bare zone, solid arrowheads indicate row 1 stereocilia tips, and hollow arrows indicate stereocilia base. (A-E) were acquired in Airy mode (A-C, single z; D-E, z-series). Scale bar is 5µm (A-C, F), 2µm (D-E).



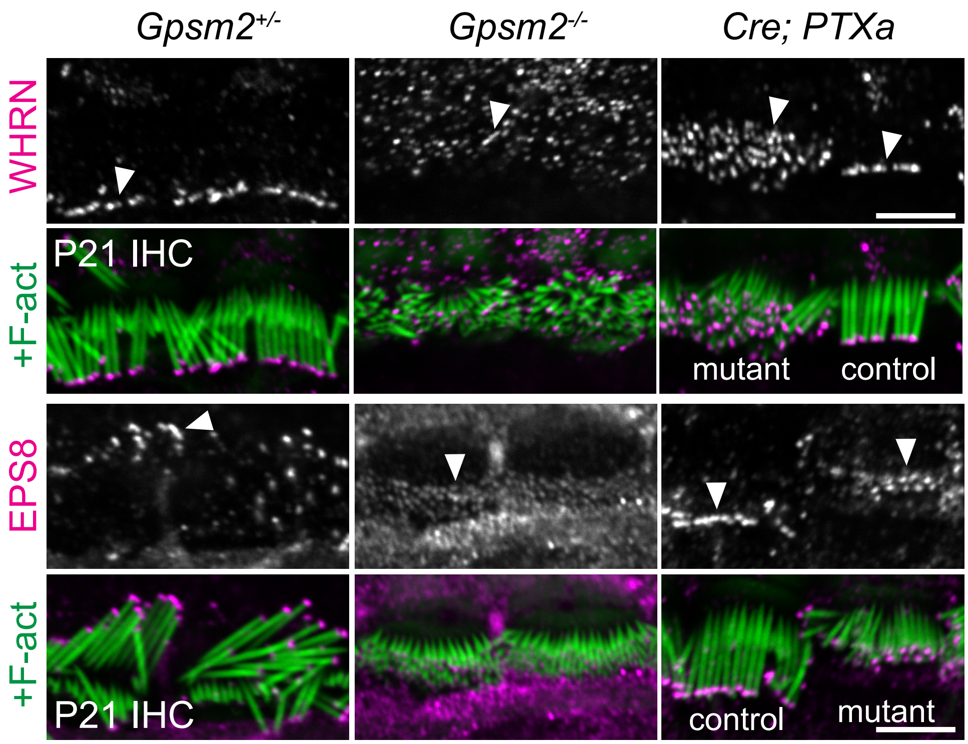
A
$Myo15a^{sh2/+}$
$Myo15a^{sh2/sh2}$
WHRN
EPS8
+F-act
E18.5 OHC
B
$Whrn^{wi/+}$
$Whrn^{wi/wi}$
GPSM2
+F-act
P21 IHC
GNAI3
+F-act
P21 IHC
C
$Whrn^{+/+}$
$Whrn^{neo/neo}$
GPSM2
+F-act
P4
OHC1
IHC
OHC1
IHC
D
$Whrn^{+/+}$
$Whrn^{neo/neo}$
GNAI3
+F-act
P4 IHC

Figure S4. MYO15A and WHRN are required for GPSM2-GNAI3 enrichment at row 1 stereocilia tips – related to Figure 3. (A) Coimmunostaining for WHRN and EPS8 in *Myo15a$^{sh2/sh2}$* mutants and a littermate control in E18.5 OHCs at the cochlear base**.** Like GPSM2 and GNAI3 (Figure 3A), WHRN and EPS8 enrichment at tips required MYO15A. **(B)** Immunostaining for GPSM2 and GNAI3 in P21 *Whrn$^{wi/wi}$.* As in E18.5 OHCs and P4 IHCs and OHCs (Figure 3), GPSM2 and GNAI3 required WHRN to localize to stereocilia tips in mature IHCs*.* **(C-D)** Immunostaining for GPSM2 (C) and GNAI3 (D) in P4 *Whrn$^{neo/neo}$* mutants specifically lacking WHRN long isoform (31). GPSM2 and GNAI3 were enriched normally at stereocilia tips, suggesting that WHRN short isoform is sufficient for their trafficking there. Solid arrows indicate the bare zone, hollow arrows indicate the stereocilia base and solid arrowheads indicate row 1 stereocilia tips. Scale bars are 5μm.



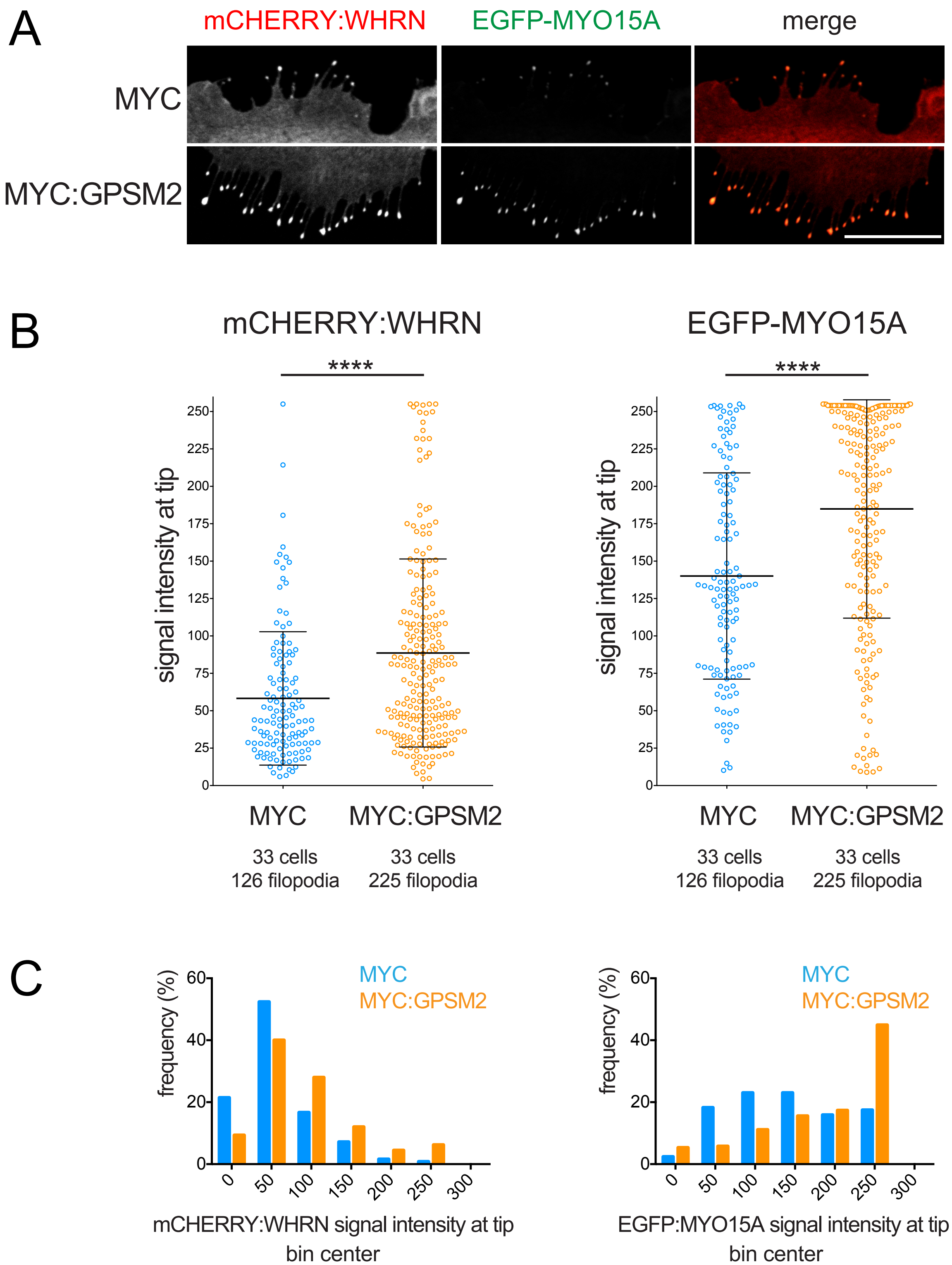
A
Input
IP: WHRN
WHRN
GNAI3:EGFP
EGFP
MYC:GPSM2
WT
N150I
IB: WHRN
WHRN
180
130
95
72
IB: MYC
MYC:
GPSM2
95
72
55
B
0; PTXa
Cre; PTXa
GPSM2
+F-act
E18.5 OHC

Figure S5. GNAI3 directly binds to WHRN and GNAI signaling is required to enrich GPSM2 at stereocilia tips – related to Figure 4. (A) Control blots related to Figure 4C where input (lanes 1-5) and immunoprecipitated (IP) protein extracts (lanes 6-10) were incubated with WHRN or MYC antibodies as indicated. **(B)** GPSM2 immunostaining in E18.5 *Atoh1-Cre; PTXa* OHCs. Normal GPSM2 enrichment at tips (arrowhead) required GNAI function. *PTXa* OHCs were misoriented as reported previously (9). Solid arrows indicate the bare zone and solid arrowheads indicate row 1 stereocilia tips. Scale bar is 5µm.



Gpsm2+/-
Gpsm2-/-
Cre; PTXa
WHRN
+F-act
P21 IHC
mutant
control
EPS8
+F-act
P21 IHC
control
mutant

Figure S6. GPSM2 and GNAI are required to enrich high amounts of WHRN-EPS8 at row 1 tips in mature IHCs – related to Figure 5. WHRN and EPS8 immunostainings in P21 IHCs. Like MYO15A (Figure 5E), WHRN and EPS8 were enriched in about equal amounts lower than seen in control row 1 in all stereocilia rows in *Gpsm2*$^{-/-}$ and *Atoh1-Cre; PTXa* mutants (arrowheads). Internal control IHCs with an apparently normal phenotype are indicated for *Atoh1-Cre; PTXa* (see Results and Methods). Solid arrowheads indicate row 1 stereocilia tips. Scale bars are 5µm.



A
mCHERRY:WHRN
EGFP-MYO15A
merge
MYC
MYC:GPSM2
B
mCHERRY:WHRN
EGFP-MYO15A
****
****
signal intensity at tip
250
225
200
175
150
125
100
75
50
25
0
MYC
MYC:GPSM2
33 cells
126 filopodia
33 cells
225 filopodia
C
frequency (%)
60
40
20
0
MYC
MYC:GPSM2
0
50
100
150
200
250
300
mCHERRY:WHRN signal intensity at tip
bin center
EGFP:MYO15A signal intensity at tip
bin center

Figure S7. GPSM2 increases MYO15A-WHRN accumulation at filopodia tips – related to Figure 5. COS-7 cells were transfected with pEGFP-C2-MYO15A and pCS2-mCHERRY:WHRN along with either MYC alone or MYC:GPSM2. After 36 hours, cells were passaged, seeded at low confluence and fluorescent signals were measured at filopodia tips after fixation. **(A)** Representative images of COS-7 cells with mCHERRY:WHRN and EGFP:MYO15A colocalizing at filopodia tips. **(B)** Plot of signal intensity at tips (33 cells per condition in 3 independent experiments; MYC: n=126 filopodia; MYC:GPSM2: n=225 filopodia. Bars represent SD). GPSM2 tended to increase the accumulation of both WHRN and MYO15A at filopodia tips. Note that GPSM2 also increased the total number of filopodia observed. Presence of GPSM2 in cotransfection was not tested. Filopodia were selected and signal at tips quantified according to published methods (49). Intensity range: 0-255 (RGB); Mann-Whitney U test, $p<0.0001$****. **(C)** Frequency distribution of the data in (B). GPSM2 increased the proportion of filopodia with high MYO15A-WHRN accumulation at the expense of filopodia with low accumulation. Bin=50. Scale bar is 5μm (A).

		P7, 0.5T			
		Control	*Gpsm2* $^{-/-}$	*Myo15a* $^{sh2/sh2}$	*Gpsm2* $^{-/-}$; *Myo15a* $^{sh2/sh2}$
height row 1 (µm) IHC	a1	1.896 ± 0.165 (n= 10 cells)	0.7 ± 0.065 (n= 11 cells)	0.869 ± 0.078 (n= 10 cells)	0.753 ± 0.04 (n= 10 cells)
	a2	1.891 ± 0.121 (n= 10 cells)	0.783 ± 0.099 (n= 10 cells)	0.897 ± 0.095 (n= 10 cells)	0.901 ± 0.063 (n= 10 cells)
	a3	1.514 ± 0.125 (n= 10 cells)	0.675 ± 0.065 (n= 10 cells)	0.909 ± 0.044 (n= 10 cells)	0.814 ± 0.057 (n= 10 cells)
	a4		0.89 ± 0.078 (n= 10 cells)	0.886 ± 0.047 (n= 10 cells)	
	a5		0.677 ± 0.05 (n= 10 cells)		
	avg ± SEM	1.767 ± 0.127	0.745 ± 0.041	0.89 ± 0.008	0.823 ± 0.043
diameter row 1 (µm) IHC	a1	0.286 ± 0.018 (n= 10 cells)	0.279 ± 0.01 (n= 11 cells)	0.286 ± 0.014 (n= 10 cells)	0.275 ± 0.018 (n= 10 cells)
	a2	0.291 ± 0.015 (n= 10 cells)	0.299 ± 0.017 (n= 10 cells)	0.267 ± 0.016 (n= 10 cells)	0.293 ± 0.012 (n= 10 cells)
	a3	0.298 ± 0.014 (n= 10 cells)	0.275 ± 0.021 (n= 10 cells)	0.291 ± 0.017 (n= 10 cells)	0.259 ± 0.012 (n= 10 cells)
	a4		0.292 ± 0.02 (n= 10 cells)	0.277 ± 0.021 (n= 10 cells)	
	a5		0.294 ± 0.017 (n= 10 cells)		
	avg ± SEM	0.292 ± 0.003	0.288 ± 0.005	0.28 ± 0.005	0.276 ± 0.01
diameter row 3 (µm) IHC	a1	0.123 ± 0.008 (n= 10 cells)	0.244 ± 0.019 (n= 10 cells)	0.254 ± 0.02 (n= 10 cells)	0.222 ± 0.016 (n= 10 cells)
	a2	0.126 ± 0.011 (n= 10 cells)	0.255 ± 0.019 (n= 10 cells)	0.224 ± 0.016 (n= 10 cells)	0.261 ± 0.014 (n= 10 cells)
	a3	0.141 ± 0.012 (n= 10 cells)	0.235 ± 0.019 (n= 10 cells)	0.244 ± 0.02 (n= 10 cells)	0.238 ± 0.017 (n= 10 cells)
	a4		0.25 ± 0.012 (n= 10 cells)	0.221 ± 0.021 (n= 10 cells)	
	a5		0.251 ± 0.013 (n= 10 cells)		
	avg ± SEM	0.13 ± 0.006	0.247 ± 0.003	0.236 ± 0.008	0.24 ± 0.011
# stereocilia row 1 IHC	a1	16 ± 0.816 (n= 10 cells)	18 ± 0.632 (n= 11 cells)	17.6 ± 0.516 (n= 10 cells)	17.7 ± 1.252 (n= 10 cells)
	a2	16.1 ± 0.876 (n= 10 cells)	17.8 ± 0.789 (n= 10 cells)	17.1 ± 0.994 (n= 10 cells)	17.143 ± 1.351 (n= 14 cells)
	a3	16.667 ± 0.985 (n= 12 cells)	18.083 ± 1.443 (n= 12 cells)	17.9 ± 1.101 (n= 10 cells)	17.6 ± 1.713 (n= 10 cells)
	a4		18.7 ± 1.059 (n= 10 cells)	18.1 ± 0.876 (n= 10 cells)	
	a5		17.4 ± 1.075 (n= 10 cells)		
	avg ± SEM	16.256 ± 0.208	17.997 ± 0.212	17.675 ± 0.217	17.481 ± 0.171
# rows IHC	a1	3.8 ± 0.422 (n= 10 cells)	5.1 ± 0.568 (n= 10 cells)	4.4 ± 0.516 (n= 10 cells)	4.3 ± 0.483 (n= 10 cells)
	a2	3.2 ± 0.422 (n= 10 cells)	4.5 ± 0.527 (n= 10 cells)	4.6 ± 0.516 (n= 10 cells)	4.5 ± 0.527 (n= 10 cells)
	a3	3.5 ± 0.527 (n= 10 cells)	5.1 ± 0.316 (n= 10 cells)	4.6 ± 0.516 (n= 10 cells)	4.7 ± 0.483 (n= 10 cells)
	a4		4.6 ± 0.516 (n= 10 cells)	4.3 ± 0.483 (n= 10 cells)	
	a5		5 ± 0 (n= 10 cells)		
	avg ± SEM	3.5 ± 0.173	4.86 ± 0.129	4.475 ± 0.075	4.5 ± 0.115
Δheight (row 1-row 2) (µm) IHC	a1	1.003 ± 0.124 (n= 10 cells)	0.166 ± 0.024 (n= 10 cells)	0.112 ± 0.019 (n= 10 cells)	0.156 ± 0.039 (n= 10 cells)
	a2	0.82 ± 0.105 (n= 10 cells)	0.091 ± 0.024 (n= 10 cells)	0.135 ± 0.021 (n= 10 cells)	0.114 ± 0.015 (n= 10 cells)
	a3	0.763 ± 0.082 (n= 10 cells)	0.174 ± 0.056 (n= 10 cells)	0.143 ± 0.017 (n= 10 cells)	0.155 ± 0.026 (n= 10 cells)
	a4		0.142 ± 0.022 (n= 10 cells)	0.116 ± 0.029 (n= 10 cells)	
	a5		0.125 ± 0.034 (n= 10 cells)		
	avg ± SEM	0.862 ± 0.072	0.14 ± 0.015	0.127 ± 0.007	0.142 ± 0.014
height row 1 (µm) OHC	a1	1.235 ± 0.052 (n= 10 cells)	0.826 ± 0.081 (n= 10 cells)	0.543 ± 0.069 (n= 10 cells)	0.557 ± 0.028 (n= 10 cells)
	a2	0.987 ± 0.207 (n= 10 cells)	0.891 ± 0.098 (n= 10 cells)	0.579 ± 0.077 (n= 10 cells)	0.707 ± 0.043 (n= 10 cells)
	a3	0.933 ± 0.111 (n= 10 cells)	0.758 ± 0.084 (n= 10 cells)	0.546 ± 0.062 (n= 10 cells)	0.623 ± 0.056 (n= 10 cells)
	a4		1.008 ± 0.067 (n= 10 cells)	0.622 ± 0.047 (n= 10 cells)	
	a5		1.16 ± 0.153 (n= 10 cells)		
	avg ± SEM	1.052 ± 0.093	0.929 ± 0.071	0.573 ± 0.018	0.629 ± 0.043
diameter row 1 (µm) OHC	a1	0.156 ± 0.009 (n= 10 cells)	0.162 ± 0.011 (n= 10 cells)	0.155 ± 0.022 (n= 10 cells)	0.156 ± 0.011 (n= 10 cells)
	a2	0.142 ± 0.01 (n= 10 cells)	0.164 ± 0.007 (n= 10 cells)	0.153 ± 0.012 (n= 10 cells)	0.163 ± 0.009 (n= 10 cells)
	a3	0.146 ± 0.01 (n= 10 cells)	0.141 ± 0.01 (n= 10 cells)	0.148 ± 0.011 (n= 10 cells)	0.152 ± 0.013 (n= 10 cells)
	a4		0.155 ± 0.014 (n= 10 cells)	0.155 ± 0.01 (n= 10 cells)	
	a5		0.17 ± 0.008 (n= 10 cells)		
	avg ± SEM	0.148 ± 0.004	0.158 ± 0.005	0.153 ± 0.002	0.157 ± 0.003
diameter row 3 (µm) OHC	a1	0.127 ± 0.01 (n= 10 cells)	0.125 ± 0.012 (n= 10 cells)	0.136 ± 0.01 (n= 10 cells)	0.13 ± 0.007 (n= 10 cells)
	a2	0.11 ± 0.013 (n= 10 cells)	0.134 ± 0.017 (n= 10 cells)	0.118 ± 0.007 (n= 10 cells)	0.133 ± 0.016 (n= 10 cells)
	a3	0.119 ± 0.007 (n= 10 cells)	0.123 ± 0.01 (n= 10 cells)	0.128 ± 0.006 (n= 10 cells)	0.135 ± 0.014 (n= 10 cells)
	a4		0.137 ± 0.008 (n= 10 cells)	0.136 ± 0.015 (n= 10 cells)	
	a5				
	avg ± SEM	0.119 ± 0.005	0.13 ± 0.003	0.13 ± 0.004	0.133 ± 0.001
# stereocilia row 1 OHC	a1	29 ± 1.944 (n= 10 cells)	23.7 ± 2.627 (n= 10 cells)	23.5 ± 1.179 (n= 10 cells)	23.4 ± 2.366 (n= 10 cells)
	a2	32.5 ± 1.434 (n= 10 cells)	23.4 ± 2.413 (n= 10 cells)	26.9 ± 3.929 (n= 10 cells)	23.1 ± 2.183 (n= 10 cells)
	a3	31.6 ± 2.066 (n= 10 cells)	21.4 ± 1.897 (n= 10 cells)	25.2 ± 2.201 (n= 10 cells)	22.3 ± 1.703 (n= 10 cells)
	a4		24.1 ± 1.792 (n= 10 cells)	25.2 ± 2.044 (n= 10 cells)	
	a5				
	avg ± SEM	31.033 ± 1.049	23.15 ± 0.601	25.2 ± 0.694	22.933 ± 0.328
# rows OHC	a1	3 ± 0 (n= 10 cells)	3.2 ± 0.422 (n= 10 cells)	3.2 ± 0.422 (n= 10 cells)	3.5 ± 0.527 (n= 10 cells)
	a2	3 ± 0 (n= 10 cells)	3.8 ± 0.422 (n= 10 cells)	3.4 ± 0.516 (n= 10 cells)	3.7 ± 0.483 (n= 10 cells)
	a3	3 ± 0 (n= 10 cells)	3.4 ± 0.516 (n= 10 cells)	3.6 ± 0.516 (n= 10 cells)	4 ± 0.471 (n= 10 cells)
	a4		3.9 ± 0.316 (n= 10 cells)	3.8 ± 0.422 (n= 10 cells)	
	a5				
	avg ± SEM	3 ± 0	3.575 ± 0.165	3.5 ± 0.129	3.733 ± 0.145
Δheight (row 1-row 2) (µm) OHC	a1	0.314 ± 0.031 (n= 10 cells)	0.217 ± 0.029 (n= 10 cells)	0.12 ± 0.014 (n= 10 cells)	0.138 ± 0.057 (n= 10 cells)
	a2	0.192 ± 0.045 (n= 10 cells)	0.218 ± 0.057 (n= 10 cells)	0.072 ± 0.016 (n= 10 cells)	0.104 ± 0.015 (n= 10 cells)
	a3	0.195 ± 0.033 (n= 10 cells)	0.194 ± 0.048 (n= 10 cells)	0.091 ± 0.012 (n= 10 cells)	0.199 ± 0.015 (n= 10 cells)
	a4			0.091 ± 0.015 (n= 10 cells)	
	a5				
	avg ± SEM	0.234 ± 0.04	0.21 ± 0.008	0.094 ± 0.01	0.147 ± 0.028

Table S1. Data from *Gpsm2* $^{-}$ X *Myo15a* ^{sh2} mice used to graph stereocilia quantifications, Related to Figures 1, S1.

			P6, 0.5T			
			Control	*Gpsm2* $^{-/-}$	*Whrn* $^{wi/wi}$	*Gpsm2* $^{-/-}$; *Whrn* $^{wi/wi}$
height row 1 (µm)	IHC	a1	1.587 ± 0.064 (n= 10 cells)	0.764 ± 0.065 (n= 10 cells)	0.752 ± 0.101 (n= 10 cells)	0.804 ± 0.045 (n= 10 cells)
		a2	1.566 ± 0.108 (n= 10 cells)	0.821 ± 0.049 (n= 10 cells)	0.81 ± 0.061 (n= 10 cells)	0.809 ± 0.042 (n= 10 cells)
		a3	1.77 ± 0.149 (n= 10 cells)	0.861 ± 0.054 (n= 10 cells)	0.855 ± 0.037 (n= 10 cells)	0.836 ± 0.027 (n= 10 cells)
		a4		0.731 ± 0.038 (n= 10 cells)	0.76 ± 0.061 (n= 10 cells)	
		a5		0.841 ± 0.07 (n= 10 cells)		
		avg ± SEM	1.767 ± 0.127	0.745 ± 0.041	0.89 ± 0.008	0.823 ± 0.043
diameter row 1 (µm)	IHC	a1	0.247 ± 0.01 (n= 10 cells)	0.27 ± 0.015 (n= 10 cells)	0.268 ± 0.009 (n= 10 cells)	0.264 ± 0.02 (n= 10 cells)
		a2	0.258 ± 0.015 (n= 10 cells)	0.271 ± 0.012 (n= 10 cells)	0.273 ± 0.014 (n= 10 cells)	0.276 ± 0.018 (n= 10 cells)
		a3	0.255 ± 0.013 (n= 10 cells)	0.282 ± 0.021 (n= 10 cells)	0.269 ± 0.017 (n= 10 cells)	0.293 ± 0.017 (n= 10 cells)
		a4		0.257 ± 0.014 (n= 10 cells)	0.281 ± 0.024 (n= 10 cells)	
		a5		0.276 ± 0.023 (n= 10 cells)		
		avg ± SEM	0.292 ± 0.003	0.288 ± 0.005	0.28 ± 0.005	0.276 ± 0.01
diameter row 3 (µm)	IHC	a1	0.141 ± 0.023 (n= 10 cells)	0.218 ± 0.014 (n= 10 cells)	0.223 ± 0.014 (n= 10 cells)	0.211 ± 0.015 (n= 10 cells)
		a2	0.148 ± 0.009 (n= 10 cells)	0.216 ± 0.013 (n= 10 cells)	0.195 ± 0.01 (n= 10 cells)	0.206 ± 0.015 (n= 10 cells)
		a3	0.137 ± 0.012 (n= 10 cells)	0.223 ± 0.01 (n= 10 cells)	0.212 ± 0.013 (n= 10 cells)	0.261 ± 0.016 (n= 10 cells)
		a4		0.221 ± 0.015 (n= 10 cells)	0.217 ± 0.012 (n= 10 cells)	
		a5		0.219 ± 0.014 (n= 10 cells)		
		avg ± SEM	0.13 ± 0.006	0.247 ± 0.003	0.236 ± 0.008	0.24 ± 0.011
# stereocilia row 1	IHC	a1	17.2 ± 0.919 (n= 10 cells)	19.7 ± 1.494 (n= 10 cells)	18.4 ± 1.174 (n= 10 cells)	19.1 ± 1.287 (n= 10 cells)
		a2	17.4 ± 0.966 (n= 10 cells)	20 ± 1.414 (n= 10 cells)	19.5 ± 0.972 (n= 10 cells)	19.4 ± 0.699 (n= 10 cells)
		a3	17.1 ± 1.101 (n= 10 cells)	19 ± 1.333 (n= 10 cells)	19.4 ± 1.647 (n= 10 cells)	16.9 ± 1.37 (n= 10 cells)
		a4		19.4 ± 0.966 (n= 10 cells)	19.1 ± 1.969 (n= 10 cells)	
		a5		19.5 ± 1.509 (n= 10 cells)		
		avg ± SEM	16.256 ± 0.208	17.997 ± 0.212	17.675 ± 0.217	17.481 ± 0.171
# rows	IHC	a1	4 ± 0 (n= 10 cells)	4.8 ± 0.422 (n= 10 cells)	4.3 ± 0.483 (n= 10 cells)	4.3 ± 0.483 (n= 10 cells)
		a2	3.9 ± 0.316 (n= 10 cells)	4.5 ± 0.527 (n= 10 cells)	4.7 ± 0.483 (n= 10 cells)	4.5 ± 0.527 (n= 10 cells)
		a3	3.8 ± 0.422 (n= 10 cells)	4.8 ± 0.422 (n= 10 cells)	4.7 ± 0.483 (n= 10 cells)	4.3 ± 0.483 (n= 10 cells)
		a4		4.7 ± 0.483 (n= 10 cells)	4.5 ± 0.527 (n= 10 cells)	
		a5		4.6 ± 0.516 (n= 10 cells)		
		avg ± SEM	3.5 ± 0.173	4.86 ± 0.129	4.475 ± 0.075	4.5 ± 0.115
Δheight (row 1-row 2) (µm)	IHC	a1	0.578 ± 0.113 (n= 10 cells)	0.061 ± 0.015 (n= 10 cells)	0.106 ± 0.052 (n= 10 cells)	0.105 ± 0.015 (n= 10 cells)
		a2	0.653 ± 0.081 (n= 10 cells)	0.062 ± 0.014 (n= 10 cells)	0.096 ± 0.016 (n= 10 cells)	0.117 ± 0.021 (n= 10 cells)
		a3	0.499 ± 0.07 (n= 10 cells)	0.108 ± 0.015 (n= 10 cells)	0.118 ± 0.015 (n= 10 cells)	0.123 ± 0.018 (n= 10 cells)
		a4		0.111 ± 0.019 (n= 10 cells)	0.067 ± 0.013 (n= 10 cells)	
		a5		0.133 ± 0.027 (n= 10 cells)		
		avg ± SEM	0.862 ± 0.072	0.14 ± 0.015	0.127 ± 0.007	0.142 ± 0.014
height row 1 (µm)	OHC	a1	0.852 ± 0.115 (n= 10 cells)	0.801 ± 0.085 (n= 10 cells)	0.774 ± 0.064 (n= 10 cells)	0.771 ± 0.071 (n= 10 cells)
		a2	0.898 ± 0.104 (n= 10 cells)	0.733 ± 0.065 (n= 10 cells)	0.742 ± 0.041 (n= 10 cells)	0.769 ± 0.087 (n= 10 cells)
		a3	1.045 ± 0.11 (n= 10 cells)	0.774 ± 0.071 (n= 10 cells)	0.869 ± 0.064 (n= 10 cells)	0.777 ± 0.079 (n= 10 cells)
		a4		0.758 ± 0.047 (n= 10 cells)	0.764 ± 0.065 (n= 10 cells)	
		a5		0.768 ± 0.112 (n= 10 cells)		
		avg ± SEM	1.052 ± 0.093	0.929 ± 0.071	0.573 ± 0.018	0.629 ± 0.043
diameter row 1 (µm)	OHC	a1	0.135 ± 0.007 (n= 10 cells)	0.143 ± 0.009 (n= 10 cells)	0.148 ± 0.013 (n= 10 cells)	0.154 ± 0.017 (n= 10 cells)
		a2	0.131 ± 0.012 (n= 10 cells)	0.152 ± 0.013 (n= 10 cells)	0.156 ± 0.009 (n= 10 cells)	0.151 ± 0.007 (n= 10 cells)
		a3	0.144 ± 0.005 (n= 10 cells)	0.147 ± 0.009 (n= 10 cells)	0.151 ± 0.013 (n= 10 cells)	0.156 ± 0.007 (n= 10 cells)
		a4		0.134 ± 0.008 (n= 10 cells)	0.151 ± 0.016 (n= 10 cells)	
		a5		0.151 ± 0.013 (n= 10 cells)		
		avg ± SEM	0.148 ± 0.004	0.158 ± 0.005	0.153 ± 0.002	0.157 ± 0.003
diameter row 3 (µm)	OHC	a1	0.106 ± 0.016 (n= 10 cells)	0.131 ± 0.015 (n= 10 cells)	0.108 ± 0.013 (n= 10 cells)	0.125 ± 0.013 (n= 10 cells)
		a2	0.099 ± 0.008 (n= 10 cells)	0.118 ± 0.01 (n= 10 cells)	0.112 ± 0.008 (n= 10 cells)	0.121 ± 0.014 (n= 10 cells)
		a3	0.107 ± 0.01 (n= 10 cells)	0.125 ± 0.011 (n= 10 cells)	0.12 ± 0.013 (n= 10 cells)	0.125 ± 0.01 (n= 10 cells)
		a4		0.117 ± 0.012 (n= 10 cells)	0.112 ± 0.009 (n= 10 cells)	
		a5		0.114 ± 0.007 (n= 10 cells)		
		avg ± SEM	0.119 ± 0.005	0.13 ± 0.003	0.13 ± 0.004	0.133 ± 0.001
# stereocilia row 1	OHC	a1	27.5 ± 1.581 (n= 10 cells)	24.3 ± 1.494 (n= 10 cells)	24.3 ± 1.494 (n= 10 cells)	24.4 ± 1.35 (n= 10 cells)
		a2	29.2 ± 2.394 (n= 10 cells)	25.7 ± 2.003 (n= 10 cells)	25.8 ± 2.394 (n= 10 cells)	25.6 ± 2.119 (n= 10 cells)
		a3	26.2 ± 1.476 (n= 10 cells)	24.4 ± 1.35 (n= 10 cells)	25.8 ± 1.814 (n= 10 cells)	27.4 ± 1.897 (n= 10 cells)
		a4		24.6 ± 1.265 (n= 10 cells)	25.5 ± 1.08 (n= 10 cells)	
		a5		24.6 ± 1.713 (n= 10 cells)		
		avg ± SEM	31.033 ± 1.049	23.15 ± 0.601	25.2 ± 0.694	22.933 ± 0.328
# rows	OHC	a1	3 ± 0 (n= 10 cells)	4 ± 0 (n= 10 cells)	3.4 ± 0.516 (n= 10 cells)	3.9 ± 0.316 (n= 10 cells)
		a2	3 ± 0 (n= 10 cells)	3.8 ± 0.422 (n= 10 cells)	3.3 ± 0.483 (n= 10 cells)	3.6 ± 0.516 (n= 10 cells)
		a3	3 ± 0 (n= 10 cells)	3.7 ± 0.483 (n= 10 cells)	3.5 ± 0.527 (n= 10 cells)	3.3 ± 0.483 (n= 10 cells)
		a4		3.9 ± 0.316 (n= 10 cells)	3.7 ± 0.483 (n= 10 cells)	
		a5		4.1 ± 0.316 (n= 10 cells)		
		avg ± SEM	3 ± 0	3.575 ± 0.165	3.5 ± 0.129	3.733 ± 0.145
Δheight (row 1-row 2) (µm)	OHC	a1	0.222 ± 0.029 (n= 10 cells)	0.132 ± 0.023 (n= 10 cells)	0.128 ± 0.023 (n= 10 cells)	0.142 ± 0.034 (n= 10 cells)
		a2	0.19 ± 0.025 (n= 10 cells)	0.148 ± 0.031 (n= 10 cells)	0.127 ± 0.021 (n= 10 cells)	0.137 ± 0.023 (n= 10 cells)
		a3	0.253 ± 0.038 (n= 10 cells)	0.169 ± 0.013 (n= 10 cells)	0.135 ± 0.019 (n= 10 cells)	0.117 ± 0.013 (n= 10 cells)
		a4		0.172 ± 0.042 (n= 10 cells)	0.134 ± 0.017 (n= 10 cells)	
		a5		0.209 ± 0.029 (n= 10 cells)		
		avg ± SEM	0.234 ± 0.04	0.21 ± 0.008	0.094 ± 0.01	0.147 ± 0.028

Table S2. Data from Gpsm2^{-} X Whrn^{wi} mice used to graph stereocilia quantifications, Related to Figures 1, S1.