Jen et al., Supplementary Figures:

Supplementary Figure 1: Gene ontology analysis of 672 non-hair cell genes that are up-regulated in hair cells in *Gfi1* knockout mice

Supplementary Figure 2: Known motif analysis of 732 *Gfi1* binding loci in hair cells.

Supplementary Figure 3: DNA sequences corresponding to the Rasd2 peaks in Figure 5 that contain binding sites for ATOH1 but not GFI1 transcription factors.

Supplementary Figure 4: ChIP-QPCR analysis of ATOH1 and GFI1 binding to the same distal regulatory elements in the *Anxa4* and *Atoh1* genes, both of which are direct targets of ATOH1. Cochlear epithelium from neonatal *Atoh1-GFP* mice were processed for ChIP using antibodies for GFP (to pull down ATOH1-bound DNA) and GFI1, with IgG as a negative control. QPCR amplification of the DNA was carried out with previously published primers to regions of the *Atoh1* and *Anxa4* loci [38]

Supplementary Figure 5: Full images of the blots of the co-immunoprecipitation experiments in Figure 6D. The labels are the same as those in Figure 6D

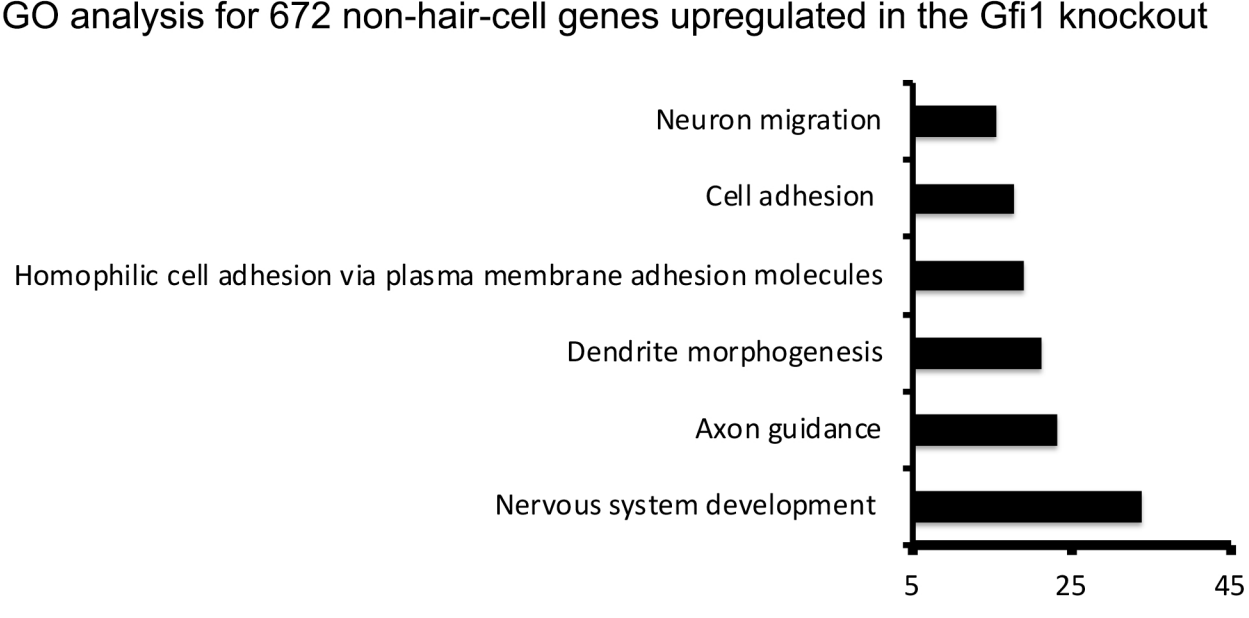
Jen et al., Supplementary Tables:

Supplementary Table S1: Sheet 1 – list of 1378 hair cell enriched genes (Figure 1A). Sheet 2: 90 hair cell-enriched genes up-regulated in the GFI1 knockout cochlea and 473 hair cell-enriched genes down-regulated in the Gfi1 knockout cochlea (Figure 1B)

Supplementary Table S2: Sheet 1 - List of 732 GFI1 binding loci with their closest mapped gene name. Sheet 2 - list of 3792 ATOH1 binding loci with their closest mapped gene name

Supplementary Table S3: Sheet 1 - List of hair cell loci with GFI1 binding sites that are either up-regulated (8 genes) or down-regulated (51 genes) in the Gfi1 knockout. Sheet 2 - List of hair cell loci with GFI1 and ATOH1 binding sites that are either up-regulated (4 genes) or down-regulated (40 genes) in the *Gfi1* knockout. Sheet 3 - List of non-hair cell gene loci with GFI1 binding sites that are upregulated (39 genes) in the *Gfi1* knockout

Supplementary Table S4: Sheet 1 - List of 350 loci uniquely binding GFI1 in hair cells. Sheet 2 - List of 386 loci binding GFI1 and ATOH1 in hair cells. Sheet3 - List of 3402 loci uniquely binding ATOH1 in hair cells.

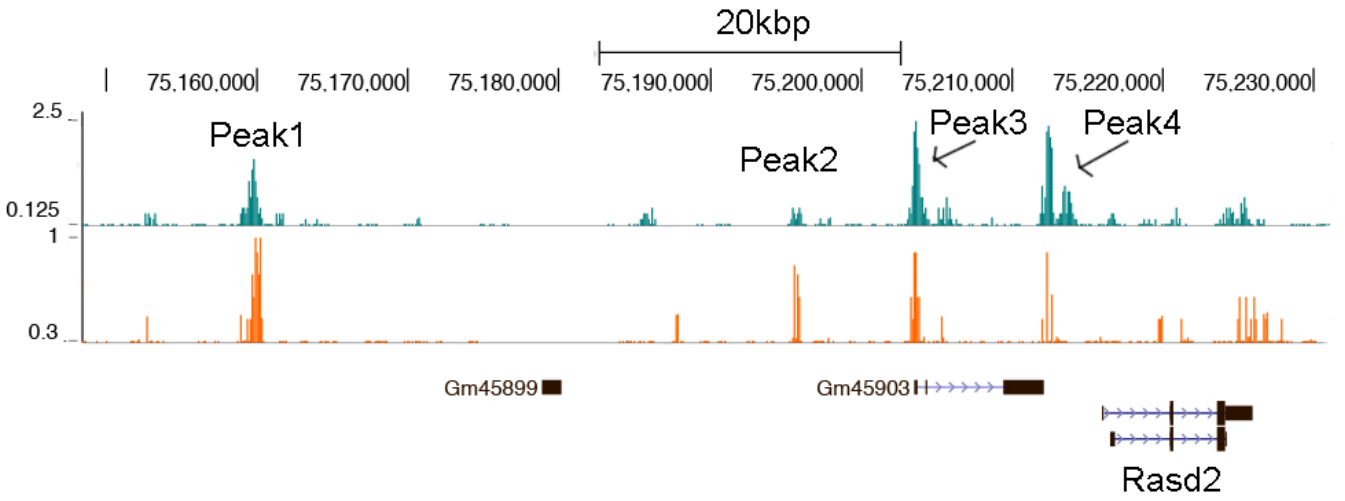


Supplementary Figure 1

Known motif enrichment analysis for Gfi1 binding loci

bHLH transcription factors	Motif	P-value
NeuroG2		1e-12
NeuroD1		1e-11
Atoh1		1e-11
Ascl1		1e-10
BHLH15		1e-10

Supplementary Figure 2



Peak 1(chr8:75157573-75159079)

CAAATCAGTTGCCCATCTCACTAAGTGCCTGTGCCTCTCCCTGGGTTGCT
GCTGAGCTGAGCATGGCAGGTAGAGTGTATGGCTATATGGGCTAGGGAAC
TCCAGGGGAACTCCGTGGGGACATTCCCGAAGCTCCCTGGAAGCTGCTAA
TTGAATCCTATTCTCTGCTCCCTGGGAGGCTGACAGCTGAAAGGAGCTCA
GCACAGAGATAATGAACTTGAGACCTCTGAAGTTGCTGCTCTCTTTGCAT
ACAAAGATCCTGGCCTCTATCCTGCACTTACTAAAAACTCCTGAGAGCTG
GGGCCAGGCAGCTTTGCCCAAAGGCGATCTGCCTGCTCTGTTCTAGGTCC
AGAAGGGCAATGATCTTGGGGAACTGTGCTCATCAATGCATGTCTCCTAG
GAGCTGCCCCTGCTGGCACCCAGCACCCACTCAGCATCCCACTCGTACAC
GGTGACGTGCTGGGTGGAGGGGGCAGAGAGGAGATGTCAACATGACTGAC
ATGCCAAATGCCATGTGAGCCTTGGAGTGCCACTCATCTGGGGGACTGAA
GAGCAAGCTGGAGCTACTATCCTGCCAGAGGGGACAGGTACAGCAGCTTC
TGTCCTGAAAGATCCTTGAACTCCCTGCCTCAATTTCTAAAGAGAAAGTG
AGGCTCAGAAAGACAAGATCAGTGTGCAGTGCCCAGGTTGGGGACACATA
TCAACTCTACAATTCATTGTTATGTGATTGCGTGAAGATAGGCCTATAAT
CCCAGTACAGGAGTGGAGGCAAAAGAATAAGGAACAGAATGTCATCCTTC
ACAGCATAGCAAGCTCCTAGCCAGCCTGGGCAACAGGTAAACTGTCCAAA
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ACAGAATCAATCAGTCAAGTCTTATCATGCCTCTGGATAATAGACTCCTC
ATATACATGAGTAAATGGGCTCAGAGTGATTAAGGAAGATATCTTAGGTT
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CCTATATTAGTTGAGTCCAGCTACCTAGGGCCCCAGGCCCTCTGCGTCTC
CCTCGAGTCTAAGACTATGGGTGGGCTGTTATGTCTGCTTGGCATTCAGA
TGGGTTCTGATCCCCTGGCTCCTGTCACATGAACTTTACCCACTGAGCCA
TCTCACCAGCGTCTCATGGCAGAATTTCATGGTGGGTTGTTATTGTTGGA
CAGAAACTTGATTTTTAGGCTGTGTGTCTCAGGAACTCATCGCCCTTTTG
GAACTTGTGCTCTCTGGTTCCCATGGAGATGAGGAGAGCTGGTCCTTGCC
CAGCATGATACTAGGTTGGTTTGTTCTATTTGTAATCACAGAAGACTGTG
AGGTAAGGCCCTGGCCCTTGGGGACCTGGGGATGGGACCCTTAGCATGCT
CTTGGTGCACTCCAGCCTGAGATATCCCCATCAGAGAAGGGTGGGAGGGA
ATAAGTT

Motif ID	Alt ID	Sequence Name	Strand	Start	End	p-value	q-value	Matched Sequence
MA0461.1	Atoh1	mm10_dna	+	1005	1012	4.87e-05	0.141	CAGCTGGT

Peak 3 (chr8:75202167-75203672)

ACATGCAGTCAGATTCTTTTACTCGGAAGTGGGTTGCCTTCTAAAGGTCA
TCTCATGAAAGAAGTTGCTGGATCAGTGCAATGCCACGTCAACTTGATTG
CTGAGTCGACACACAGAACCTGGCAGGATAGCACATCGGAGGAGGTGGTT
ATTCACACTTGCTAGGTGACAGGCCACTTGTCTTTTATGGGTTCAATAAA
GATTGCTTTCAAGGTCTAAGAACACCATCATAGGACGATGGGCTGGCTGG
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CCAAGTTCTGAAATTACAGATGTGTGGCACTATGCTTAAGCTATGTGCTG
CTGGGGATGGCACTCAGCATGCTAGGCAGGCACTACCAACTGAGCCATGC
CACCAAACCTTGTTTGTTTTTTCTTTCATTTGGGACCAGAGTTCACCTTG
TTATAGGTCTGGGCTATACAATAGCCATAGAGACAGATTTTGGTTGGTGT
GGGAGTGAGTACTGTCCTCCTGAGGTAGGGTGACAGAGTAGAGAGTGGAG
GATGGATACTGTGTCAGGGACCAATAGTCTTCATATAGACCTGTAGAAGC
TGGCTCCTACTTGGGAAATGTAAGCCTGACCAGTTATAAGGCTTGCCCCT
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GGGGAGATAGCATCTGCAAGACAGGATAGCTCTTCTGGGAGATGCTTGGC
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TGCTGCAGAGGAGACAGTAGAAGACCTGGTGGCTCTGAATCCCAGAACCT
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GATCCTCCAAGTAACATGCCTGAAAAACACAGATGGCATCTGAGGACATG
TGCCACCTCCTCAACTGCTCACACTCATATTTTCAGCATTGTCACCCTGA
TCCTCAAATGTGGGGACATGGTCATGAGGTGGCCCAGAGGAGGGTTTCCT
GCCTTGAGGACTGAGCAAGGAGAATGACGCCATTCCATTTCCTCCTGTGG
CTACGA

Motif ID	Alt ID	Sequence Name	Strand	Start	End	p-value	q-value	Matched Sequence
MA0461.1	Atoh1	mm10_dna	+	1330	1337	2.2e-05	0.0634	CAGATGGC
MA0461.1	Atoh1	mm10_dna	-	755	762	4.87e-05	0.0703	CAGATGGT
MA0461.2	Atoh1	mm10_dna	-	755	764	5.71e-05	0.169	AACAGATGGT

Peak 2 (chr8:75194608-75195109)

TGTTTTGTGCTCTATGTCTCTGCTGTCACAATGCACAAAGGCATGTTGGA
CAATAGCTCTCCAGGGGATATAGCTCATCTCAGCCCCTCTATCAGGCAGG
CACAGATCTGTGAGCTCAGCCCTCCATCCTGGCTGCCTTCCACTACCCCA
CCTCTCCACCCTCCCCTGCCCCCCCCCCCCCCCAGGCTCCTCTCTTCTG
AGGATGACTCACACCCTGCTGACTCCACTCAGCCCATCAACTGCTCTTCT
CTGCAACTGGAGGAGCTGCTGTCTCGCTTATTTATTGAAACTGGCAAACA
CAGGCTCTGAGTTAATAGGTACAAGAGGGTAGCCAGAGGAAAACCCCCTT
CCCACCTCCTTCCCCAGCCTGGCTCTGGGAGAGGATGATCCTTTGCATAT
GCTAATATGAAGCTGTCTAGACGCTTTGCTTTCCAGGTGCTGTGCACAAA
TGGTAGCCCTTAGAGCTAACAAACACCTGACACAGGTGACTTATTTATAA

Motif ID	Alt ID	Sequence Name	Strand	Start	End	p-value	q-value	Matched Sequence
MA1467.1	ATOH1(var.2)	mm10_dna	+	264	273	5.81e-05	0.0539	AGCTGCTGTC

Peak 4 (chr8:75211114-75212130)

TATATCACTGGGAAGCCTGCTCTTTTCTCAAGGGAAATGGAGGAACAGTA
GATTGGGGGGGGGGGGAAGAGGAGTGATGCGGAGGGGGGTAGAGAGGTTT
GGTGGGAGGTGAGACTGTATTTGGAATGTATTGTATGAGAGAAGAATAAA
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GAGGGAGCCTTAGGGAGTACAAAGGAGGAGGAGGCTTCAACACGTGGTCC
ATCCGGAGAGACCTGAGTGTAGGGAAGGGGACGAACTTGGGTACATGTTG
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TGTGGATCTGGATCTGGTCACTGGGGTGTGACCCACTCATCGAGGAGGAC
TTGGCTGAAAGCCCAAGGATTGGGTTCCTGTGCCAGCTCAGAGCTGGCCA
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CTCCCAGCTGCAGCCTTCCAGCTGAGGCACCCTGCTCTGGCTGCCTCCTG
CTGCCAAGCTCACAGGCTCTGTGCGTCAGGCCCAGTGCAAACTCTCCGGG
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CTACATTGAAGCCTAGACTTAGGCCTTAAACCCCGGCCCAAGGGGACCGG
CAAACCACCTCTTCCTGGCCCCTCCCCCATCCCGATGCATCTTCTCTCAT
ATAGTATTCCTGGAGGATGTCAAGATACTTTAATGTTTGATGAGCAACCC
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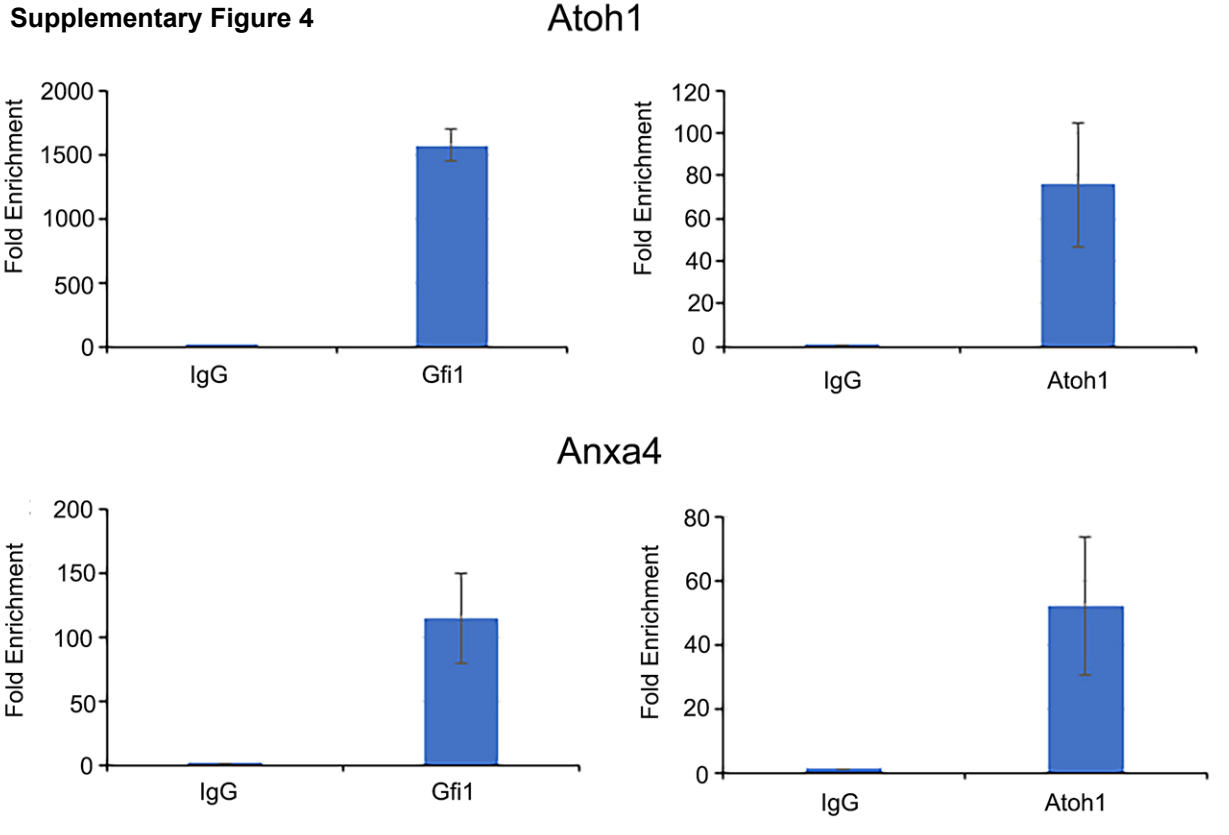
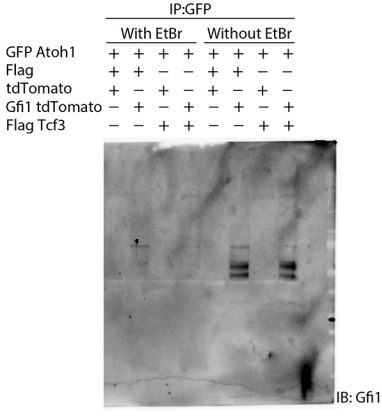
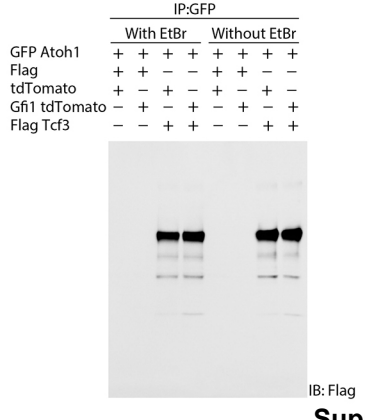
GGACTTGGCAGTATTGA

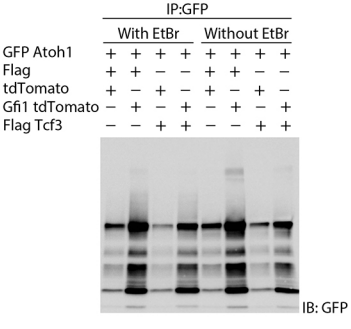
Supplementary Figure 3

Supplementary Figure 4









Supplementary Figure 5