

(Supplemental material)

Table S1. Target information of shRNA sequence.

ID	Sequence	Start	GC (%)
JAG1-RNAi(1)	GAATGGAGTACATCGTATA	4049	36.84
JAG1-RNAi(2)	GTTCAACCTGACAGTATTA	847	36.84
JAG1-RNAi(3)	GAGCACATTTGCAGTGAAT	3340	42.11
JAG1-RNAi(4)	AGGATAACTGTGCGAACAT	3275	42.11
JAG2-RNAi(1)	CTCACACAAATTCACCAAA	3993	36.84
JAG2-RNAi(2)	TCTACCAGTGCAAGAACTT	3845	42.11
JAG2-RNAi(3)	CTGCTACGACCTGGTCAAT	2466	52.63
JAG2-RNAi(4)	ACTGCCATATCAACGTCAA	1772	42.11
DLL1-RNAi(1)	ACGTCATATCCGAGGAGAA	2439	47.37
DLL1-RNAi(2)	CGGGCTGTTCAACTTCAAA	2397	47.37
DLL1-RNAi(3)	CCGACAAGAATGGCTTCAA	2196	47.37
DLL1-RNAi(4)	TGTTCTAATGGTGCCAAGT	1556	42.11

Control: NC

Sequence: TTCTCCGAACGTGTCACGT

Table S2. shRNA sequence.

ID	5'	Stem	Loop	Stem	3'
Jag-1-shRNA1-a	Ccgg	ccGAATGGAGTACATCGT ATA	CTCGAG	TATACGATGTACTCCA TTCGG	TTTTTg
Jag-1-shRNA1-b	aattcaaaaa	ccGAATGGAGTACATCGT ATA	CTCGAG	TATACGATGTACTCCA TTCGG	
Jag-1-shRNA2-a	Ccgg	ccGTTCAACCTGACAGTA TTA	CTCGAG	TAATACTGTCAGGTTG AACGG	TTTTTg
Jag-1-shRNA2-b	aattcaaaaa	ccGTTCAACCTGACAGTA TTA	CTCGAG	TAATACTGTCAGGTTG AACGG	
Jag-1-shRNA3-a	Ccgg	cgGAGCACATTTGCAGTG AAT	CTCGAG	ATTCAGTCAAATGTG CTCCG	TTTTTg
Jag-1-shRNA3-b	aattcaaaaa	cgGAGCACATTTGCAGTG AAT	CTCGAG	ATTCAGTCAAATGTG CTCCG	
Jag-1-shRNA4-a	Ccgg	ccAGGATAACTGTGCGA ACAT	CTCGAG	ATGTTGCGACAGTTAT CCTGG	TTTTTg
Jag-1-shRNA4-b	aattcaaaaa	ccAGGATAACTGTGCGA ACAT	CTCGAG	ATGTTGCGACAGTTAT CCTGG	
Jag-2-shRNA1-a	Ccgg	ctCTCACACAAATTCACC AAA	CTCGAG	TTTGGTGAATTTGTGT GAGAG	TTTTTg
Jag-2-shRNA1-b	aattcaaaaa	ctCTCACACAAATTCACC AAA	CTCGAG	TTTGGTGAATTTGTGT GAGAG	
Jag-2-shRNA2-a	Ccgg	gcTCTACCAGTGCAAGA ACTT	CTCGAG	AAGTTCTTGCACTGGT AGAGC	TTTTTg

Jag-2-shRNA2-b	aattcaaaaa	gcTCTACCAGTGCAAGA ACTT	CTCGAG	AAGTTCTTGCACTGGT AGAGC	
Jag-2-shRNA3-a	Ccgg	cgCTGCTACGACCTGGTC AAT	CTCGAG	ATTGACCAGGTCGTA GCAGCG	TTTTTg
Jag-2-shRNA3-b	aattcaaaaa	cgCTGCTACGACCTGGTC AAT	CTCGAG	ATTGACCAGGTCGTA GCAGCG	
Jag-2-shRNA4-a	Ccgg	caACTGCCATATCAACGT CAA	CTCGAG	TTGACGTTGATATGGC AGTTG	TTTTTg
Jag-2-shRNA4-b	aattcaaaaa	caACTGCCATATCAACGT CAA	CTCGAG	TTGACGTTGATATGGC AGTTG	
Dll-1-shRNA1-a	Ccgg	gtACGTCATATCCGAGGA GAA	CTCGAG	TTCTCCTCGGATATGA CGTAC	TTTTTg
Dll-1-shRNA1-b	aattcaaaaa	gtACGTCATATCCGAGGA GAA	CTCGAG	TTCTCCTCGGATATGA CGTAC	
Dll-1-shRNA2-a	Ccgg	ctCGGGCTGTTCAACTTC AAA	CTCGAG	TTTGAAGTTGAACAGC CCGAG	TTTTTg
Dll-1-shRNA2-b	aattcaaaaa	ctCGGGCTGTTCAACTTC AAA	CTCGAG	TTTGAAGTTGAACAGC CCGAG	
Dll-1-shRNA3-a	Ccgg	cgCCGACAAGAATGGCT TCAA	CTCGAG	TTGAAGCCATTCTTGT CGGCG	TTTTTg
Dll-1-shRNA3-b	aattcaaaaa	cgCCGACAAGAATGGCT TCAA	CTCGAG	TTGAAGCCATTCTTGT CGGCG	
Dll-1-shRNA4-a	Ccgg	ccTGTCTAATGGTGCCA AGT	CTCGAG	ACTTGGCACCATTAG AACAGG	TTTTTg
Dll-1-shRNA4-b	aattcaaaaa	ccTGTCTAATGGTGCCA AGT	CTCGAG	ACTTGGCACCATTAG AACAGG	

Figure S1

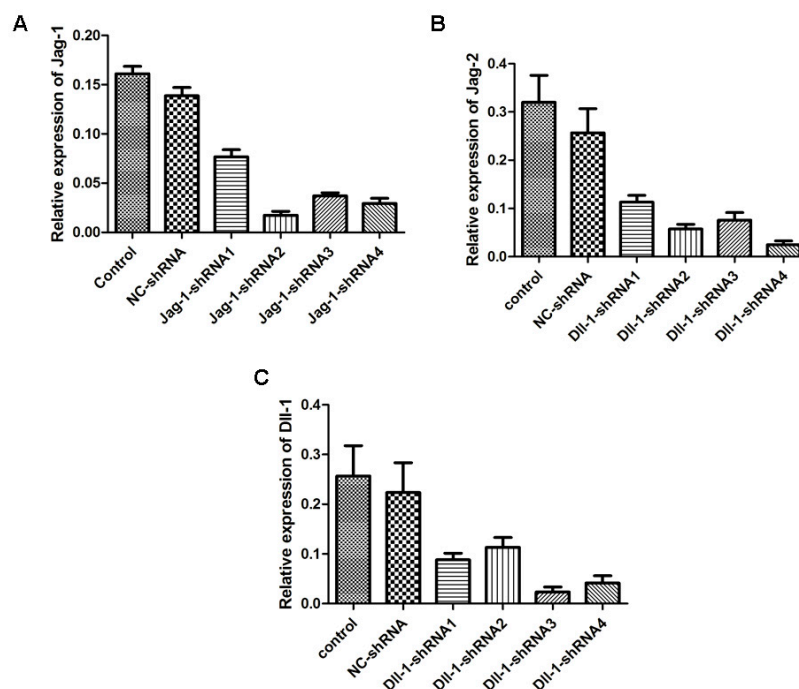


Figure S1. Efficiency of shRNA vectors. (A) JAG-1-shRNA: Cells on day 12 of otic progenitor differentiation were transfected with JAG-1-shRNA (1–4) and NC-shRNA. (B) JAG-2-shRNA: Cells on day 5 of hair cell differentiation were transfected with JAG-2-shRNA (1–4) and NC-shRNA. (C) DLL-1-shRNA: Cells on day 5 of hair cell differentiation were transfected with DLL-1-shRNA (1–4) and NC-shRNA. After 48 h of transfection, the total RNA was extracted and real-time PCR were performed to test the effects of various shRNAs on the expression of JAG-1, JAG-2, and DLL-1 respectively. Control: no transfection. The statistical results were significant ($p < 0.05$).

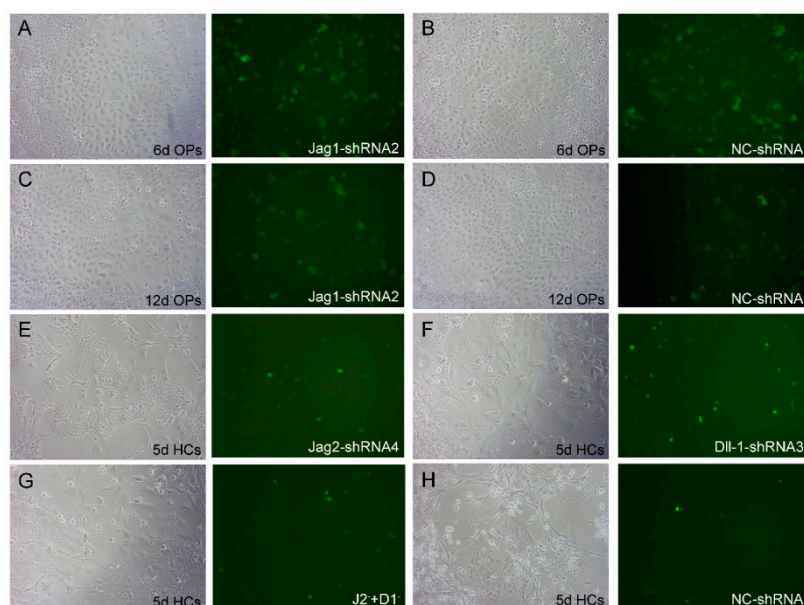
Figure S2

Figure S2. Infection efficiency of lentiviruses harboring shRNA. (A) Cell morphology and GFP expression in cells infected with lentivirus harboring JAG-1-shRNA2 on day 6 of otic progenitor differentiation. (B) Cell morphology and GFP expression in cells infected with lentivirus harboring NC-shRNA on day 6 of otic progenitor differentiation. (C) Cell morphology and GFP expression in cells infected with lentivirus harboring JAG-1-shRNA2 on day 12 of otic progenitor differentiation. (D) Cell morphology and GFP expression in cells infected with lentivirus harboring NC-shRNA on day 12 of otic progenitor differentiation. (E) Cell morphology and GFP expression in cells infected with lentivirus harboring JAG-2-shRNA4 on day 5 of hair cell differentiation. (F) Cell morphology and GFP expression in cells infected with lentivirus harboring DLL-1-shRNA3 on day 5 of hair cell differentiation. (G) Cell morphology and GFP expression in cells infected with lentiviruses harboring JAG-2-shRNA4 and DLL-1-shRNA3 on day 5 of hair cell differentiation. (H) Cell morphology and GFP expression in cells infected with lentivirus harboring NC-shRNA on day 5 of hair cell differentiation.

Figure S3

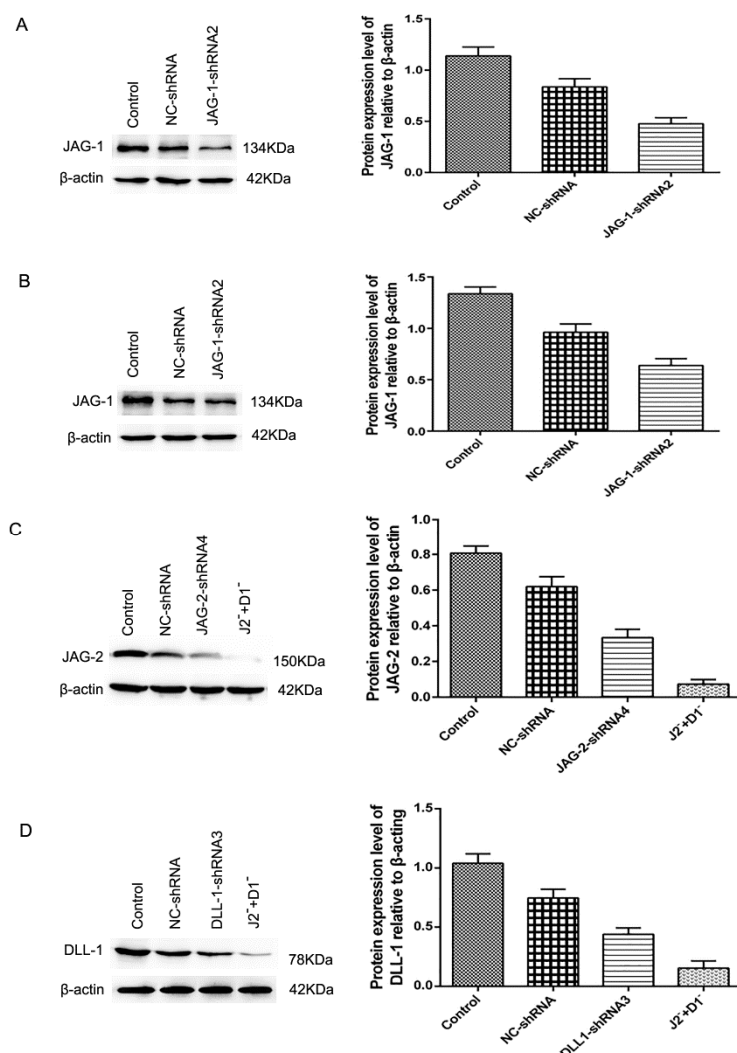


Figure S3. Identification of cells stably infected by the lentivirus. (A) and (B) Western blot analysis of cells infected with lentivirus harboring JAG-1-shRNA2 on day 6 and 12 of otic progenitor differentiation using an antibody specific for JAG-1. (C) and (D) Western blot analysis of cells infected with lentivirus harboring JAG-2-shRNA4 and DLL-1-shRNA3 at day 5 of hair cell differentiation using JAG-2 and DLL-1 specific antibodies.

Uninfected cells (Control) and cells infected with lentivirus harboring NC-shRNA were used as controls. The statistical results were significant ($p < 0.05$)