Supplemental Information

Engraftment of Human Stem Cell-Derived

Otic Progenitors in the Damaged Cochlea

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Non-amikacin treated group

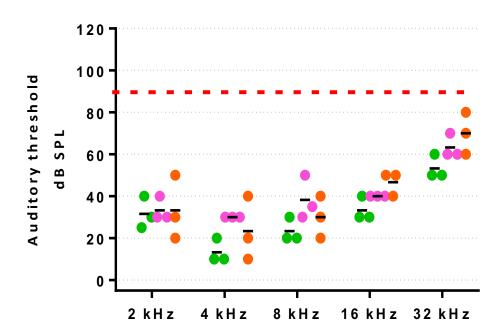


Figure S1. Delivery of hOPCs in Cochlea without Auditory Dysfunction

ABRs were registered for each animal before engraftment, 4 days and 14 days post-engraftment in amikacin-treated animals. The auditory thresholds of each animal were compared before and 14 days after engraftment and no shifts in thresholds were observed at the frequencies tested.

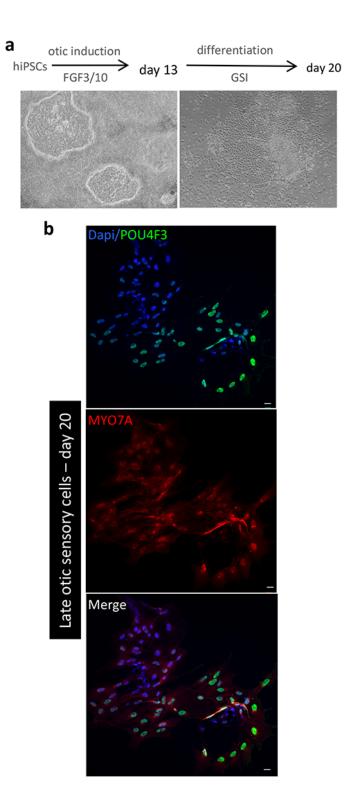


Figure S2. Expression of Late Otic Sensory Cell Markers after Prolonged Differentiation *in Vitro*

(a) Otic/placodal progenitors from day 13 were maintained for one more week in culture medium supplemented with GSI. (b) After one week *in vitro* (i.e. day 20), a subset of differentiated cells displayed co-expression of two known late otic sensory (MYO7A and POU4F3) markers. Scale bar = $20 \mu m$

Table S1. List of human qPCR primers

Gene Name	Forward Primer	Rerverse Primer	
DLX5	GCTAGCTCCTACCACCAGTAC	GGTTTGCCATTCACCATTCTCA	
GAPDH	ACACCATGGGGAAGGTGAAG	GTGACCAGGCGCCCAATA	
GATA3	CACGGTGCAGAGGTACCC	AGGGTAGGGATCCATGAAGCA	
PAX2	CGGCTGTGTCAGCAAAATCC	GCTTGGAGCCACCGATCA	
PAX8	GCCCAGTGTCAGCTCCATTA	GCTGTCCATAGGGAGGTTGAA	
BMP4	CCACAGCACTGGTCTTGAGTA	GGTCCCTGGGATGTTCTCC	

Table S2. List of antibodies

	Specie	Provider	Reference	Dilution
PAX2	rabbit	Eurogentec	PRB-276P	1:100
IBA1	rabbit	Wako	019-19741	1:200
MYO7A	mouse	DHSB	138-1	1:200
MYO7A	rabbit	Proteus	PTS-25-6790-C050	1:200
POU4F3	mouse	Abnova	H-5459-M01(DB9310)	1:100
SOX2	goat	Santa-Cruz	SC-17320	1:200

Video S1. A Grafted Mature Guinea-pig Cochlea that was Rendered Transparent with

Bone Clarity. Human stem cell derived otic progenitors (shown in red) appear distributed along the basal-to-apical longitudinal axis of the damaged cochlea. The ability to see human otic progenitor cell behavior is crucial for stimulating research into the development of a cell-based therapy to cure deafness.