

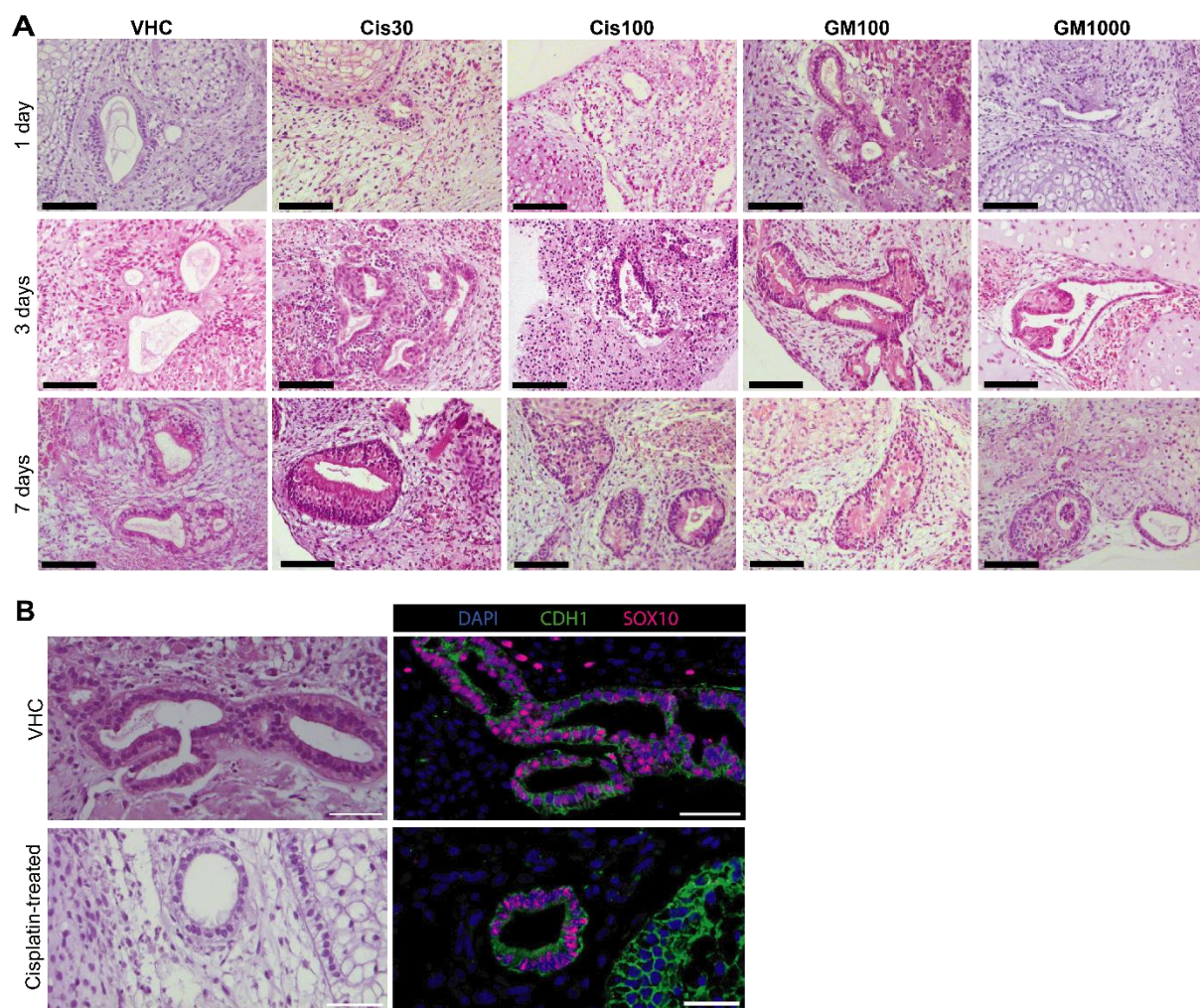


Fig. S1. Overview of representative images for all time points and treatment conditions. Images were selected to best represent each condition and/or timepoint and enable direct comparison across the dataset. A) H&E imaging of VHC versus cisplatin and gentamicin exposed samples. Cisplatin concentrations were 30 and 100 μM , and gentamicin concentrations were 100 and 1000 μM . Scale bars = 100 μm . B) Panels showing H&E and IF staining of VHC vs. cisplatin-treated samples at 1 day post-treatment, containing epithelium (CDH1, green), supporting cells (SOX10, magenta), and cell nuclei (DAPI, dark blue). Scale bars = 50 μm .

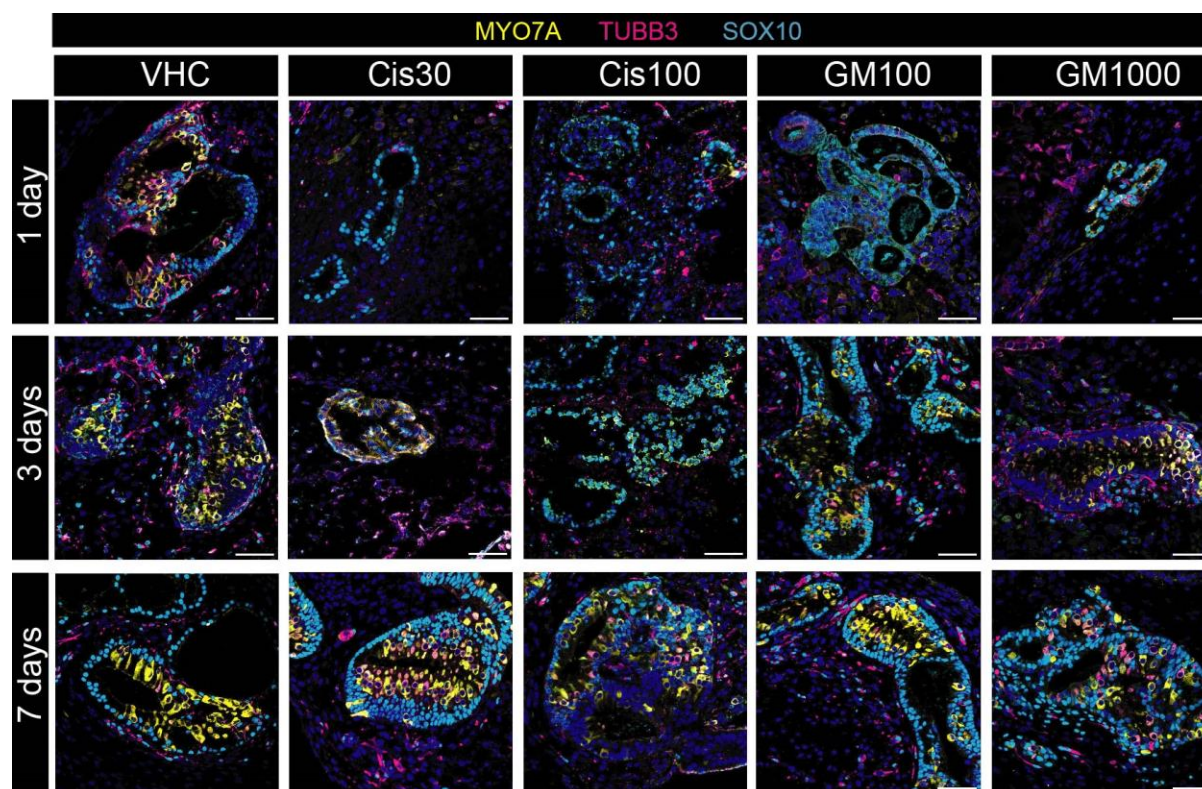


Fig. S2. Otic vesicles containing hair cells, neurons, and otic epithelium analysis over time. Panels showing IF staining of otic vesicles of VHC vs. ototoxic compound-treated samples at 1 day, 3 days, and 7 days post-treatment, containing hair cells (MYO7A, yellow), neurons (TUBB3, magenta), otic epithelium (SOX10, cyan), and cell nuclei (DAPI, dark blue) for treated and untreated samples over time. The 1 day VHC, Cis100 and GM1000 images are also shown in Fig. 3; the 3 days GM1000 image and 7 days VHC, Cis100 and GM1000 images are also shown in Fig. 4. Scale bar = 50 μ m.

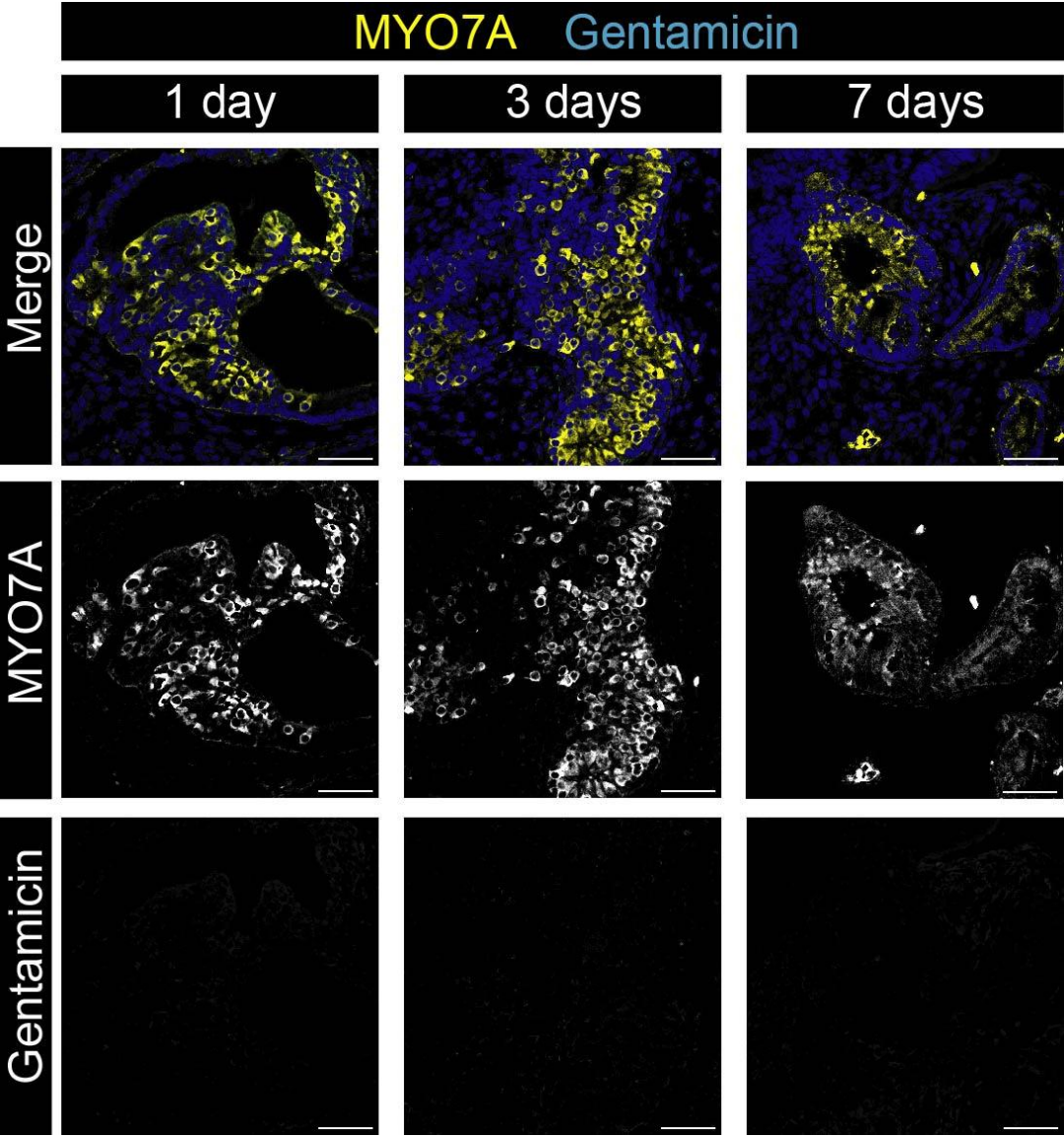


Fig. S3. Gentamicin absence in VHC samples. Panels showing IF staining of VHC samples at 1 day, 3 days and 7 days post-treatment, containing hair cells (MYO7A, yellow), cell nuclei (DAPI; dark blue), and gentamicin (cyan). Scale bars are 50 μ m.

Table S1. Morphological criteria observed in H&E-stained sections of inner ear organoids. The table outlines structural features used to interpret hair cells, neurons, otic vesicles, and related components, as well as indicators of damage. H&E staining provides histological context (e.g., vesicle formation, epithelial organization, signs of degeneration) but does not confer lineage specificity. Definitive identification of otic structures is established through immuno-fluorescence markers (CDH1, SOX10, or MYO7A, TUBB3). The presence vs. absence was semi-quantitatively scored based on a three-level scoring.

Structure / Cell Type	H&E morphological features	Interpretation / Limitation	Semi-quantitative scoring
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Hair cells (type I & type II)	Tall, columnar cells at the luminal side of a vesicle. Aligned atop supporting cells.	Suggestive of sensory hair cells, but confirmed with MYO7A co-expression in later analysis	0 = Absence 1 = Few 2 = Presence
Neurons	Small, round to oval cell bodies with dense, basophilic nuclei and little cytoplasm. Arranged in clusters or as peripheral processes extending towards the vesicle. Axon-like protrusions are commonly observed to run beneath the basal layer of epithelial vesicle, and connecting to the hair cells.	Suggestive of neurons, but confirmed with TUBB3 co-expression in later analysis.	0 = Absence 1 = Partial 2 = Presence
Otic vesicle	Organized epithelium surrounding a central lumen, often with a basal layer of supporting cells and hair cells atop of those cells.	Morphology indicates vesicular epithelium. CDH1 / SOX10 co-expression confirms otic origin.	Not applicable for scoring
Vesicle lumen	Clear, acellular central space of an otic vesicle, bordered by epithelium.	Indicates epithelial vesicle formation, but by itself does not specify otic lineage.	0 = Absence / collapse 1 = Partial 2 = Presence / Intact
Stratification (supporting cells + hair cells)	Two- or multi-layered appearance: basal nuclei of presumable supporting cells with a more apical row of presumable hair cell cells facing the lumen.	Suggestive of sensory epithelium, but in later analysis supported by marker staining.	0 = Monolayer 1 = Partial 2 = Multilayered
Basal membrane	Thin eosinophilic line at the base of the epithelium separating epithelial cells from the surrounding mesenchyme.	Indicates epithelial polarity and organization. Not otic-specific.	0 = Disrupted 1 = Partial 2 = Intact
Signs of damage (e.g., pyknotic nuclei, cell body damage)	Condensed, hyperchromatic nuclei. Fragmented or irregular nuclear morphology within epithelial or neuronal layers. Cytoplasmic shrinkage or disruption of cell bodies, with the presence of cell debris.	Hallmark of cell death / damage: not lineage-specific.	Not applicable for scoring

Table S2. List of antibodies used in this study.

Primary antibodies						
Marker	Host	Clonality	Company	Cat. No.	Dilution	RRID
MYO7A	Mouse	Monoclonal	DSHB	138-1-s	1:50	AB_2282417
MYO7A	Rabbit	Polyclonal	Proteus	25-6790	1:500	AB_10015251
SOX10	Goat	Polyclonal	Thermo Fisher Scientific	PA5- 47001	1:100	AB_2608449
Cleaved Caspase-3	Rabbit	Polyclonal	Cell Signaling Technology	9961S	1:400	AB_2341188
Tubulin beta 3 Class III (TUBB3)	Mouse	Monoclonal	R&D Systems	MAB1195	1:100	AB_357520
Cisplatin- DNA-adduct	Rat	Monoclonal	Merck Millipore	MABE416	1:100	AB_3678988
Gentamicin	Mouse	Monoclonal	Biosynth	10-1541	1:100	AB_3683114
Ki-67	Rabbit	Polyclonal	Abcam	Ab15580	1:100	AB_443209
CDH1 (E- cadherin)	Mouse	Polyclonal	BD Biosciences	610182	1:20	AB_397581
Phospho- Histone H2A.X	Rabbit	Monoclonal	Cell Signaling Technology	9718	1:500	AB_2118009

(γH2AX)						
Peripherin (PRPH)	Chicken	Polyclonal	Invitrogen	PA1-10012	1:5000	AB_1086584
Ki-67	Rat	Monoclonal	Invitrogen	14-5698-82	1:200	AB_10854564
SOX2	Mouse	Monoclonal	BD Biosciences	561469	1:100	AB_10694256
Oncomodulin (OCM)	Rabbit	Polyclonal	Novus Biologicals	NBP2-14568	1:200	AB_3261947
Secondary antibodies						
Host	Antibody Against		Alexa Fluor	Fisher Scientific Catalog No.	RRID	
Donkey	Mouse IgG (H+L)		488	A21202	AB_141607	
Donkey	Rat IgG (H+L)		488	A21208	AB_2535794	
Goat	Chicken IgY		488	A11039	AB_2534096	
Donkey	Rabbit IgG (H+L)		594	A21207	AB_141637	
Donkey	Mouse IgG (H+L)		594	A21203	AB_2535789	
Donkey	Goat IgG (H+L)		680	A21084	AB_141494	
Donkey	Rabbit IgG (H+L)		680	A10043	AB_2534018	
Donkey	Mouse IgG (H+L)		680	A10038	AB_11180593	

Table S3. List of primers used in this study.

Marker		Sequence (5' to 3')
<i>H2AX</i>	Forward	GAT CGC CGA TTT CGG TCT GG
	Reverse	GTG CTT GGA TTG CCG AGT TG
<i>SOD1</i>	Forward	AGG CAT GTT GGA GAC TTG GG
	Reverse	TGC TTT TTC ATG GAC CAC CAG
<i>RPS18 (housekeeping)</i>	Forward	ATA CAG CCA GGT CCT AGC CA
	Reverse	AAG TGA CGC AGC CCT CTA TG