

OHC-TRECK: A Novel System Using a Mouse Model for Investigation of the Molecular Mechanisms Associated with Outer Hair Cell Death in the Inner Ear

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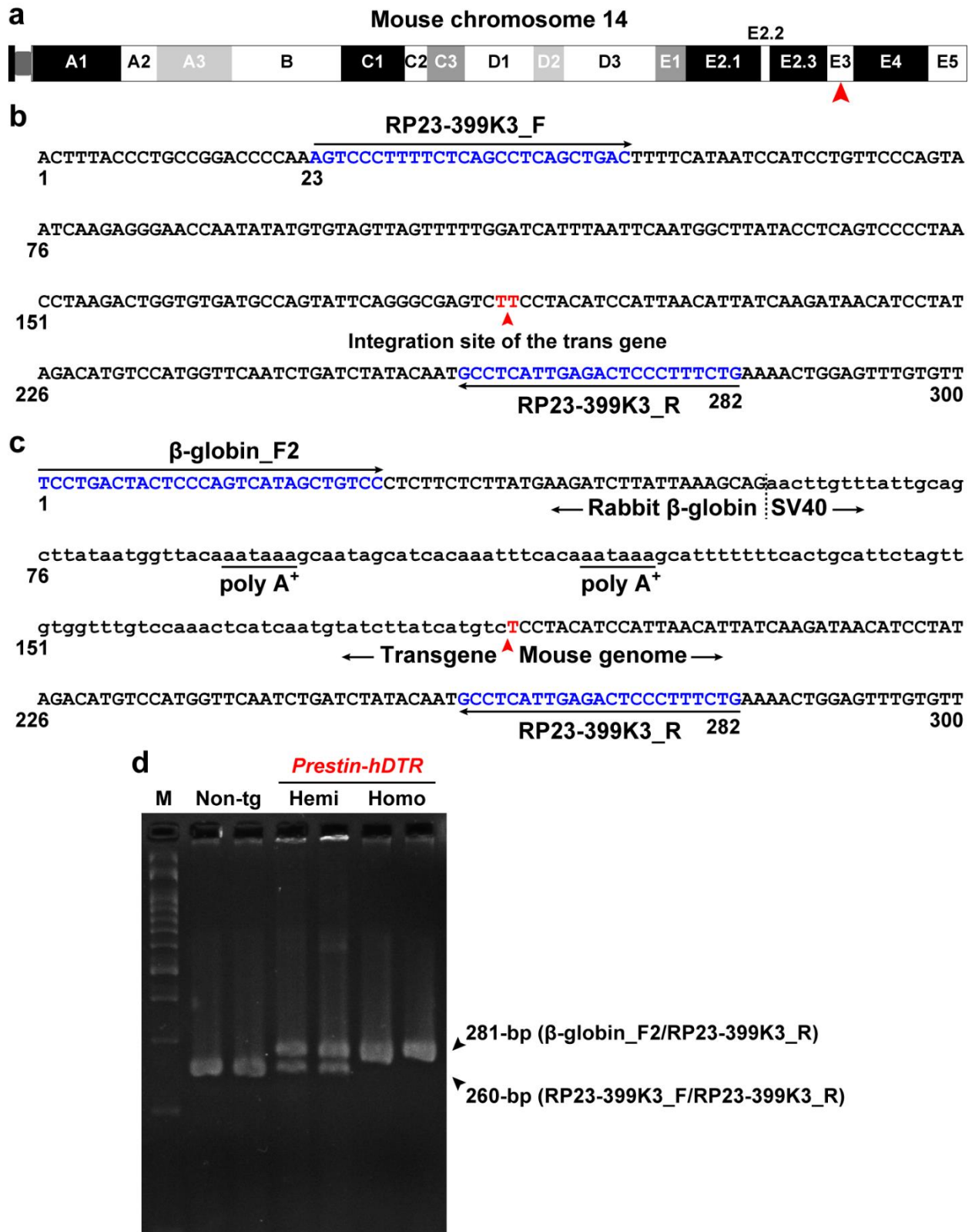
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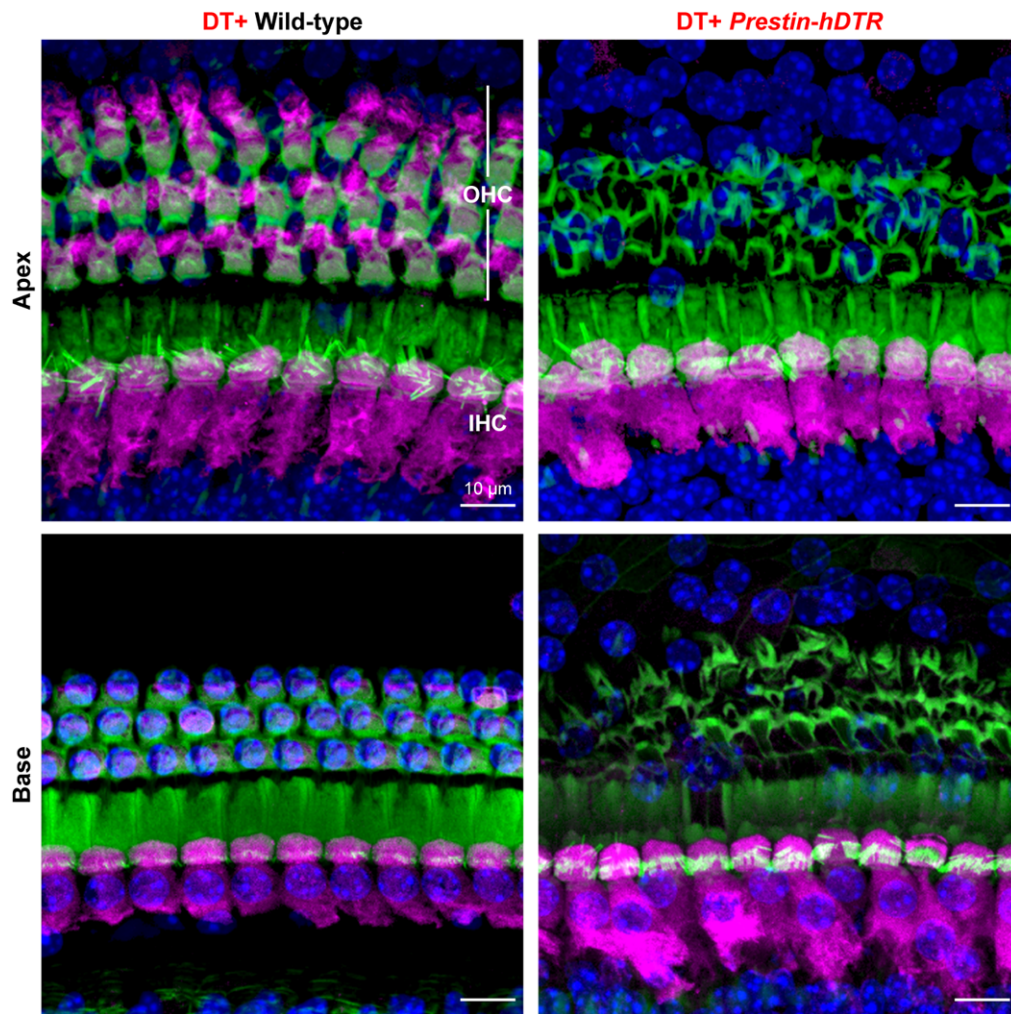
Supplementary Methods

Detection of the integration site of the transgene. The integration site of the transgene was detected by ligation-mediated PCR¹. Genomic DNA was isolated from the livers of wild-type and *Prestin-hDTR* mice using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). DNA was digested using *Dra*I and *Pvu*II at 37°C for 90 min, ligated to the linkers (L25: 5'-GCG GTG ACC CGG GAG ATC TGA ATT C-3' and L11: 5'-GAA TTC AGA TC-3') at 16°C overnight, and incubated at 95°C for 5 min. The sample was amplified by PCR using a KOD FX Neo instrument (TOYOBO, Osaka, Japan), the β -globin_F1 primer (CCC CTG CTG TCC ATT CCT TAT TCC ATA G) designed for the transgene construct and L25 linker primers (1/10 volume of the β -globin_F1 primer) with the following conditions: 40 cycles of 98°C for 10 sec and 68°C for 2 min. The PCR product was diluted 1:100 with distilled water and amplified by PCR using a KOD FX Neo instrument, the β -globin_F2 primer (TCC TGA CTA CTC CCA GTC ATA GCT GTC C) and L25 linker primers with the same conditions as those used for the first PCR. The second PCR product was purified using the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA, USA) and sequenced. The sequence data were aligned with the mouse genome sequence using BLAST. The integration site was confirmed by PCR using Takara Ex Taq (Takara Bio Inc., Kyoto, Japan) and the β -globin_F2, RP23-399K3_F (AGT CCC TTT TCT CAG CCT CAG CTG AC), and RP23-399K3_R (CAG AAA GGG AGT CTC AAT GAG GC) primers.

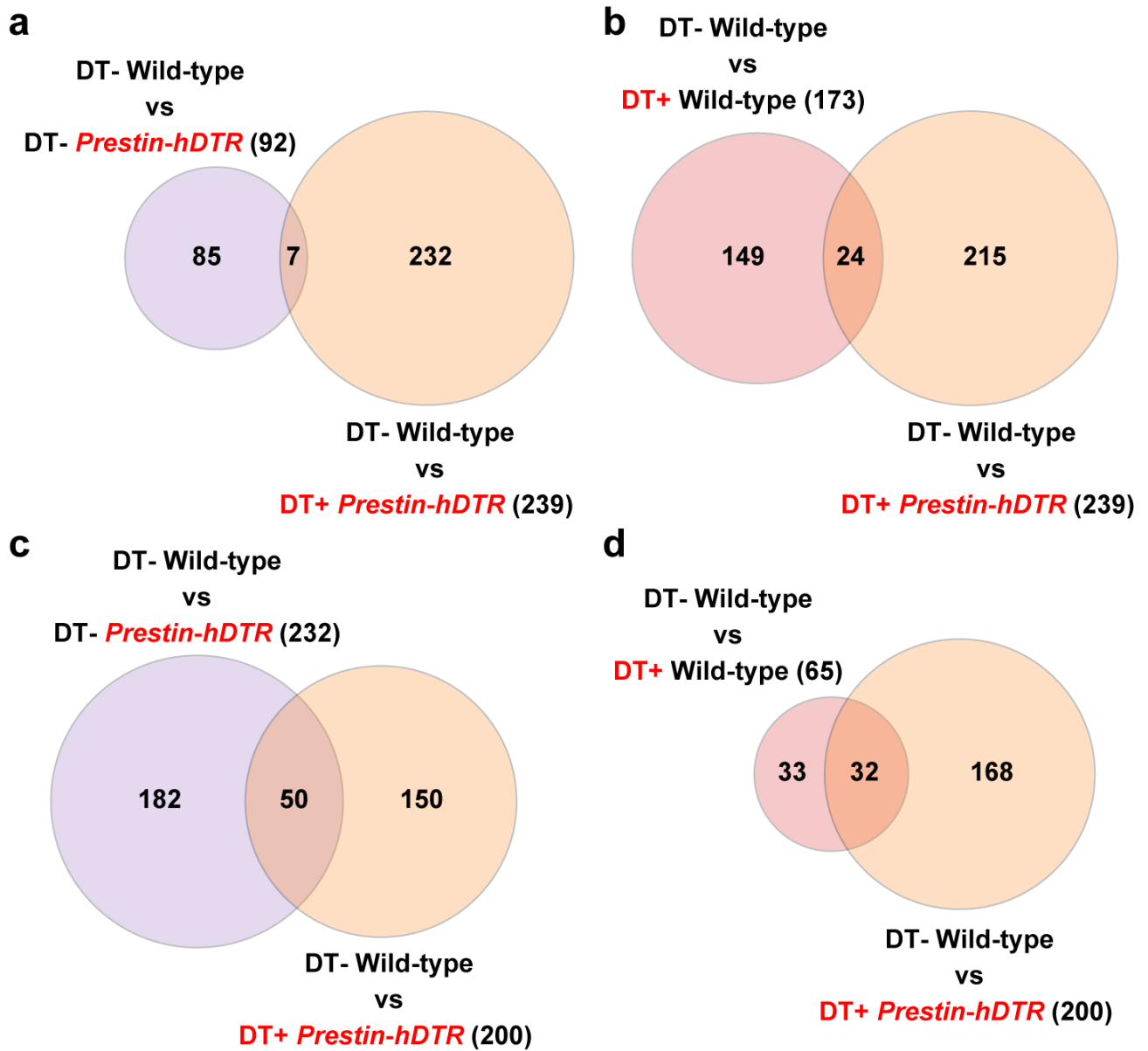
Supplementary Figures



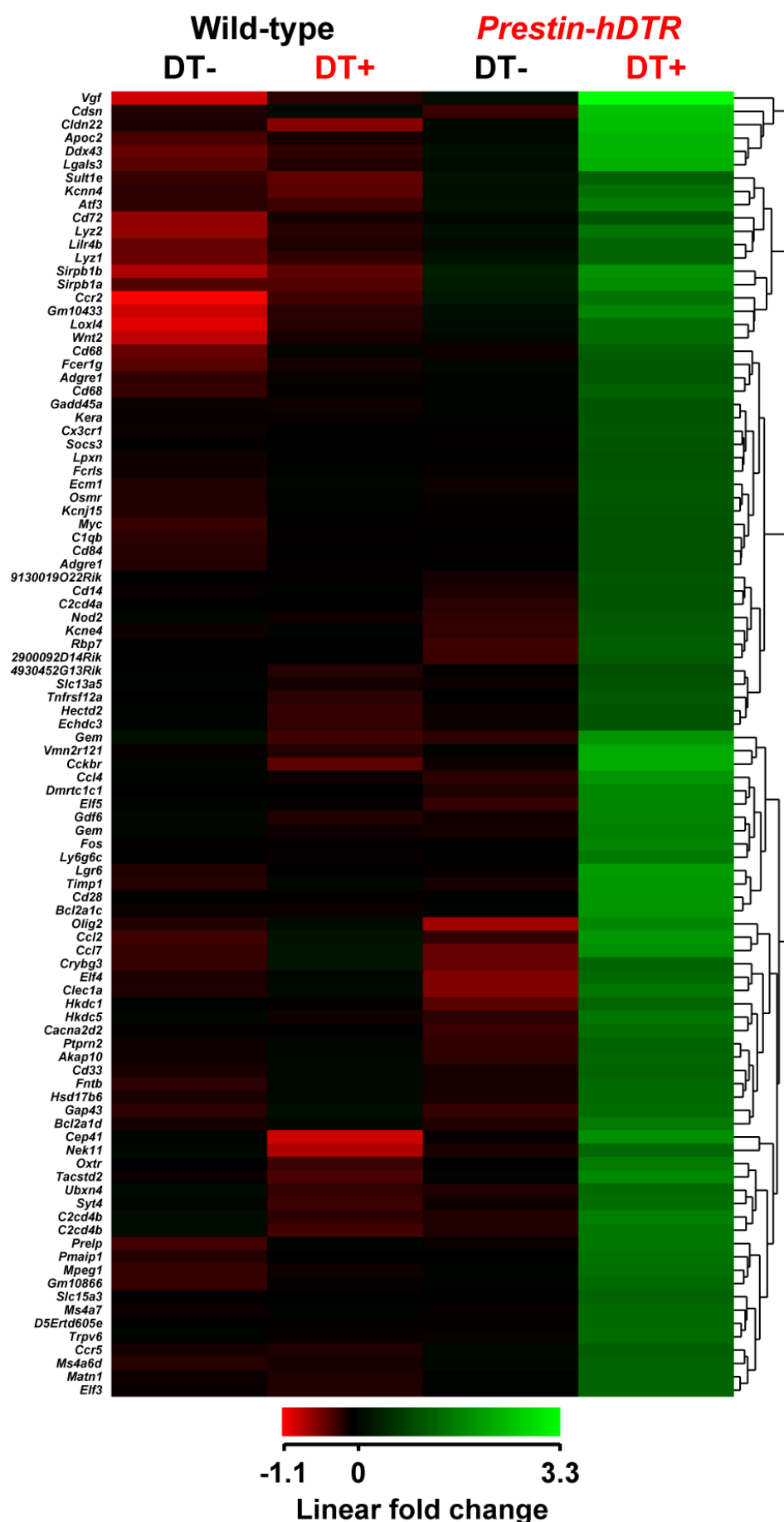
Supplementary Fig. S1. Detection of a chromosomal integration site of the transgene in *Prestin-hDTR* mice. **(a)** Diagram of mouse chromosome 14 and an integration site (red arrowhead) of the transgene. The illustration of the cytogenetic map was modified from Ensembl². **(b and c)** Genome sequences in the vicinity of the integration site of the transgene in non-transgenic (non-tg) **(b)** and *Prestin-hDTR* (tg) **(c)** mice. Although the genome sequence in the vicinity of the integration site is not annotated in public genome databases, this sequence was included within a BAC clone (RP23-399K3) (GenBank: AC154574.2), which is mapped on the E3 region of mouse chromosome 14³. Nucleotide sequences of the integration site and primers used for genotyping of non-tg and tg mice are highlighted in red and blue, respectively. **(d)** Genotyping of non-tg, hemizygous tg (Hemi), and homozygous tg (Homo) mice. PCR using the RP23-399K3_F/RP23-399K3_R primer set amplified a band at 259 bp in non-tg and Hemi mice, whereas PCR using the β -globin_F2/RP23-399K3_R primer set amplified a band at 281 bp in Hemi and Homo mice. M: marker (100-bp ladder).



Supplementary Fig. S2. Confocal images of the hair cells from the apical and basal areas of the cochleae of DT-treated (DT+) wild-type and *Prestin-hDTR* mice. The cochleae of *Prestin-hDTR* mice were stained with anti-myosin VI (MYO6) antibody (magenta), phalloidin (green), and DAPI (blue).



Supplementary Fig. S3. Effects of transgene (**a**, **c**) and DT administration (**b**, **d**) on gene expression in mice, as detected by microarray analysis. Venn diagrams illustrate differentially up- (**a**, **b**) and downregulated (**c**, **d**) genes (fold change $\geq \pm 2$) in cochlear RNA of untreated (DT-) wild-type vs DT- *Prestin-hDTR* and DT- wild-type vs DT-treated (DT+) *Prestin-hDTR* mice (**a**, **c**) and of DT- wild-type vs DT+ wild-type and DT- wild-type and DT+ *Prestin-hDTR* mice (**b**, **d**).



Supplementary Fig. S4. Heat map showing expression profiles of 98 common probes (94 genes) upregulated in *Prestin-hDTR* mice. The 98 common probes were isolated by comparison of differentially expressed genes in the cochlear RNA of untreated (DT-) wild-type vs DT-treated (DT+) *Prestin-hDTR*, DT- *Prestin-hDTR* vs DT+ *Prestin-hDTR*, and DT+ wild-type vs DT+ *Prestin-hDTR* mice (**Fig. 7b**).

Supplementary Tables

Supplementary Table S1. List of genes of upregulated genes with ≥ 2 -fold change in the cochlea RNA of DT+ *Prestin-hDTR* mice.

Gene Symbol	Description [RefSeq]	Entrez Gene ID	Probe Name	Fold change
				DT+/DT-
<i>Vgf</i>	Mus musculus VGF nerve growth factor inducible (Vgf), mRNA [NM_001039385]	381677	A_55_P2085060	18.38
<i>Ddx43</i>	Mus musculus DEAD (Asp-Glu-Ala-Asp) box polypeptide 43 (Ddx43), mRNA [NM_001191044]	100048658	A_55_P2005060	6.79
<i>Apoc2</i>	Mus musculus apolipoprotein C-II (Apoc2), transcript variant 1, mRNA [NM_001277944]	11813	A_51_P334979	6.31
<i>Cdsn</i>	Mus musculus corneodesmosin (Cdsn), mRNA [NM_001008424]	386463	A_55_P2190994	6.26
<i>Sirpb1b</i>	Mus musculus signal-regulatory protein beta 1B (Sirpb1b), mRNA [NM_001173460]	668101	A_55_P2033041	6.23
<i>Lgals3</i>	Mus musculus lectin, galactose binding, soluble 3 (Lgals3), transcript variant 1, mRNA [NM_001145953]	16854	A_55_P2171116	6.22
<i>Gm10433</i>	Mus musculus predicted gene 10433 (Gm10433), long non-coding RNA [NR_045282]	100038378	A_55_P2062558	6.10
<i>Ccr2</i>	Mus musculus chemokine (C-C motif) receptor 2 (Ccr2), mRNA [NM_009915]	12772	A_51_P245989	6.06
<i>Cldn22</i>	Mus musculus claudin 22 (Cldn22), mRNA [NM_029383]	75677	A_55_P2112459	6.02
<i>Loxl4</i>	Mus musculus lysyl oxidase-like 4 (Loxl4), transcript variant 1, mRNA [NM_001164311]	67573	A_55_P2044143	5.24
<i>Wnt2</i>	Mus musculus wingless-type MMTV integration site family, member 2 (Wnt2), mRNA [NM_023653]	22413	A_55_P2065059	4.78
<i>Ccl2</i>	Mus musculus chemokine (C-C motif) ligand 2 (Ccl2), mRNA [NM_011333]	20296	A_51_P286737	4.70
<i>Vmn2r121</i>	Mus musculus vomeronasal 2, receptor 121 (Vmn2r121), mRNA [NM_001100616]	100038941	A_55_P2090152	4.69
<i>Sirpb1a</i>	Mus musculus signal-regulatory protein beta 1A (Sirpb1a), transcript variant 3, mRNA [NM_001002898]	320832	A_55_P1955308	4.60
<i>Cckbr</i>	Mus musculus cholecystokinin B receptor (Cckbr), mRNA [NM_007627]	12426	A_52_P596375	4.48
<i>Lgr6</i>	leucine-rich repeat-containing G protein-coupled receptor 6 [Source:MGI Symbol;Acc:MGI:2441805] [ENSMUST00000044828]	329252	A_52_P75777	4.44
<i>Timp1</i>	Mus musculus tissue inhibitor of metalloproteinase 1 (Timp1), transcript variant 1, mRNA [NM_001044384]	21857	A_55_P1985850	4.32
<i>Lyz2</i>	Mus musculus lysozyme 2 (Lyz2), mRNA [NM_017372]	17105	A_51_P321150	4.31
<i>Ccl7</i>	Mus musculus chemokine (C-C motif) ligand 7 (Ccl7), mRNA [NM_013654]	20306	A_51_P436652	4.25
<i>Cd28</i>	Mus musculus CD28 antigen (Cd28), mRNA [NM_007642]	12487	A_55_P1979491	3.90

<i>Bcl2a1c</i>	Mus musculus B cell leukemia/lymphoma 2 related protein A1c (Bcl2a1c), mRNA [NM_007535]	12046	A_52_P299771	3.88
<i>Olig2</i>	Mus musculus oligodendrocyte transcription factor 2 (Olig2), mRNA [NM_016967]	50913	A_52_P223626	3.72
<i>Ccl4</i>	Mus musculus chemokine (C-C motif) ligand 4 (Ccl4), mRNA [NM_013652]	20303	A_51_P509573	3.64
<i>Prelp</i>	Mus musculus proline arginine-rich end leucine-rich repeat [NM_054077]	116847	A_55_P2144736	3.59
<i>Cep41</i>	Mus musculus centrosomal protein 41 (Cep41), transcript variant 1, mRNA [NM_031998]	83922	A_52_P122673	3.55
<i>Atf3</i>	Mus musculus activating transcription factor 3 (Atf3), mRNA [NM_007498]	11910	A_52_P452689	3.52
<i>Tacstd2</i>	Mus musculus tumor-associated calcium signal transducer 2 (Tacstd2), mRNA [NM_020047]	56753	A_51_P257938	3.44
<i>Dmrtc1c1</i>	Mus musculus DMRT-like family C1c1 (Dmrtc1c1), mRNA [NM_001142691]	71083	A_55_P2180292	3.38
<i>Gem</i>	Mus musculus GTP binding protein (gene overexpressed in skeletal muscle) (Gem), mRNA [NM_010276]	14579	A_55_P2179027	3.36
<i>Cd72</i>	Mus musculus CD72 antigen (Cd72), transcript variant 1, mRNA [NM_001110320]	12517	A_55_P2180415	3.36
<i>Lilr4b</i>	Mus musculus leukocyte immunoglobulin-like receptor, subfamily B, member 4B (Lilr4b), transcript variant 2, mRNA [NM_001291892]	14727	A_55_P2063736	3.35
<i>Lyz1</i>	Mus musculus lysozyme 1 (Lyz1), mRNA [NM_013590]	17110	A_55_P2181738	3.34
<i>Fos</i>	Mus musculus FBJ osteosarcoma oncogene (Fos), mRNA [NM_010234]	14281	A_52_P262219	3.32
<i>Elf5</i>	Mus musculus E74-like factor 5 (Elf5), transcript variant 1, mRNA [NM_010125]	13711	A_51_P455866	3.24
<i>Mpeg1</i>	Mus musculus macrophage expressed gene 1 (Mpeg1), mRNA [NM_010821]	17476	A_51_P390538	3.22
<i>Bcl2a1d</i>	Mus musculus B cell leukemia/lymphoma 2 related protein A1d (Bcl2a1d), mRNA [NM_007536]	12047	A_55_P1978424	3.21
<i>Pmaip1</i>	Mus musculus phorbol-12-myristate-13-acetate-induced protein 1 (Pmaip1), mRNA [NM_021451]	58801	A_51_P477121	3.16
<i>Clec1a</i>	Mus musculus C-type lectin domain family 1, member a (Clec1a), transcript variant 1, mRNA [NM_175526]	243653	A_52_P624434	3.16
<i>Gdf6</i>	Mus musculus growth differentiation factor 6 (Gdf6), mRNA [NM_013526]	242316	A_52_P28960	3.16
<i>Cd68</i>	CD68 antigen [Source:MGI Symbol;Acc:MGI:88342] [ENSMUST00000108654]	12514	A_55_P2067583	3.11
<i>Kcnn4</i>	Mus musculus potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4 (Kcnn4), transcript variant 1, mRNA [NM_008433]	16534	A_51_P389636	3.11
<i>Slc15a3</i>	Mus musculus solute carrier family 15, member 3 (Slc15a3), mRNA [NM_023044]	65221	A_52_P467389	3.04
<i>Gap43</i>	Mus musculus growth associated protein 43 (Gap43), mRNA [NM_008083]	14432	A_55_P2096867	3.02

<i>Gem</i>	Mus musculus GTP binding protein (gene overexpressed in skeletal muscle) (Gem), mRNA [NM_010276]	14579	A_55_P2111790	3.01
<i>Oxtr</i>	Mus musculus oxytocin receptor (Oxtr), mRNA [NM_001081147]	18430	A_55_P2013203	2.99
<i>Ly6g6c</i>	Mus musculus lymphocyte antigen 6 complex, locus G6C (Ly6g6c), mRNA [NM_023463]	68468	A_55_P2141395	2.96
<i>Fntb</i>	Mus musculus farnesyltransferase, CAAX box, beta (Fntb), mRNA [NM_145927]	110606	A_55_P2076852	2.96
<i>Crybg3</i>	PREDICTED: Mus musculus beta-gamma crystallin domain containing 3 (Crybg3), transcript variant X1, mRNA [XM_006522095]	224273	A_55_P2052709	2.91
<i>Fcer1g</i>	Mus musculus Fc receptor, IgE, high affinity I, gamma polypeptide (Fcer1g), mRNA [NM_010185]	14127	A_51_P405476	2.89
<i>C2cd4b</i>	Mus musculus C2 calcium-dependent domain containing 4B (C2cd4b), mRNA [NM_001081314]	75697	A_51_P415395	2.86
<i>Hdhd5</i>	Mus musculus cat eye syndrome chromosome region, candidate 5 (Cecr5), mRNA [NM_144815]	214932	A_52_P565279	2.80
<i>Hsd17b6</i>	Mus musculus hydroxysteroid (17-beta) dehydrogenase 6 (Hsd17b6), mRNA [NM_013786]	27400	A_52_P590154	2.79
<i>Elf4</i>	Mus musculus E74-like factor 4 (ets domain transcription factor) (Elf4), mRNA [NM_019680]	56501	A_51_P448856	2.78
<i>Cd68</i>	Mus musculus CD68 antigen (Cd68), transcript variant 1, mRNA [NM_001291058]	12514	A_55_P1961943	2.77
<i>Sult1e1</i>	Mus musculus sulfotransferase family 1E, member 1 (Sult1e1), mRNA [NM_023135]	20860	A_51_P493649	2.77
<i>Cacna2d2</i>	Mus musculus calcium channel, voltage-dependent, alpha 2/delta subunit 2 (Cacna2d2), transcript variant 1, mRNA [NM_001174047]	56808	A_55_P2122879	2.75
<i>Ms4a6d</i>	Mus musculus membrane-spanning 4-domains, subfamily A, member 6D (Ms4a6d), mRNA [NM_026835]	68774	A_51_P149714	2.71
<i>C2cd4b</i>	Mus musculus C2 calcium-dependent domain containing 4B (C2cd4b), mRNA [NM_001081314]	75697	A_55_P2054261	2.68
<i>Ms4a7</i>	Mus musculus membrane-spanning 4-domains, subfamily A, member 7 (Ms4a7), transcript variant 3, mRNA [NM_001276398]	109225	A_55_P1958165	2.66
<i>Akap10</i>	Mus musculus A kinase (PRKA) anchor protein 10 (Akap10), mRNA [NM_019921]	56697	A_52_P75866	2.63
<i>Cd33</i>	Mus musculus CD33 antigen (Cd33), transcript variant 1, mRNA [NM_001111058]	12489	A_66_P109986	2.60
<i>Trpv6</i>	Mus musculus transient receptor potential cation channel, subfamily V, member 6 (Trpv6), mRNA [NM_022413]	64177	A_55_P2111444	2.59
<i>Syt4</i>	Mus musculus synaptotagmin IV (Syt4), mRNA [NM_009308]	20983	A_52_P628915	2.58
<i>D5Ertd605e</i>	Mus musculus DNA segment, Chr 5, ERATO Doi 605, expressed (D5Ertd605e), long non-coding RNA [NR_033625]	100039805	A_55_P2035326	2.56

<i>Ptpn2</i>	Mus musculus protein tyrosine phosphatase, receptor type, N polypeptide 2 (Ptpn2), mRNA [NM_011215]	19276	A_55_P1977369	2.55
<i>Adgre1</i>	Mus musculus adhesion G protein-coupled receptor E1 (Adgre1), mRNA [NM_010130]	13733	A_55_P2018934	2.55
<i>Matn1</i>	Mus musculus matrilin 1, cartilage matrix protein (Matn1), mRNA [NM_010769]	17180	A_55_P2171221	2.53
<i>Elf3</i>	Mus musculus E74-like factor 3 (Elf3), transcript variant 2, mRNA [NM_007921]	13710	A_51_P480241	2.49
<i>Hkdc1</i>	Mus musculus hexokinase domain containing 1 (Hkdc1), mRNA [NM_145419]	216019	A_51_P322972	2.47
<i>Ccr5</i>	Mus musculus chemokine (C-C motif) receptor 5 (Ccr5), mRNA [NM_009917]	12774	A_52_P578732	2.46
<i>Myc</i>	Mus musculus myelocytomatosis oncogene (Myc), transcript variant 1, mRNA [NM_010849]	17869	A_52_P108346	2.45
<i>Ecm1</i>	Mus musculus extracellular matrix protein 1 (Ecm1), transcript variant 1, mRNA [NM_007899]	13601	A_66_P104815	2.45
<i>Gm10866</i>	PREDICTED: Mus musculus predicted gene 10866 (Gm10866), misc_RNA [XR_141279]	100038696	A_55_P2157537	2.42
<i>Kcnj15</i>	Mus musculus potassium inwardly-rectifying channel, subfamily J, member 15 (Kcnj15), transcript variant 2, mRNA [NM_019664]	16516	A_55_P2164090	2.40
<i>Osmr</i>	Mus musculus oncostatin M receptor (Osmr), transcript variant 1, mRNA [NM_011019]	18414	A_51_P319460	2.39
<i>Ubxn4</i>	Mus musculus UBX domain protein 4 (Ubxn4), mRNA [NM_026390]	67812	A_52_P401475	2.36
<i>C1qb</i>	Mus musculus complement component 1, q subcomponent, beta polypeptide (C1qb), mRNA [NM_009777]	12260	A_51_P351860	2.35
<i>Kcne4</i>	Mus musculus potassium voltage-gated channel, Isk-related subfamily, gene 4 (Kcne4), mRNA [NM_021342]	57814	A_51_P508838	2.34
<i>Rbp7</i>	Mus musculus retinol binding protein 7, cellular (Rbp7), mRNA [NM_022020]	63954	A_52_P343627	2.34
<i>Cd84</i>	Mus musculus CD84 antigen (Cd84), transcript variant 1, mRNA [NM_013489]	12523	A_55_P2054445	2.33
<i>Nek11</i>	Mus musculus NIMA (never in mitosis gene a)-related expressed kinase 11 (Nek11), mRNA [NM_172461]	208583	A_51_P262325	2.31
<i>Adgre1</i>	Mus musculus adhesion G protein-coupled receptor E1 (Adgre1), mRNA [NM_010130]	13733	A_55_P2144386	2.29
<i>Cx3cr1</i>	Mus musculus chemokine (C-X3-C motif) receptor 1 (Cx3cr1), mRNA [NM_009987]	13051	A_52_P99810	2.28
<i>Fcrls</i>	Mus musculus Fc receptor-like S, scavenger receptor (Fcrls), mRNA [NM_030707]	80891	A_52_P231075	2.25
<i>2900092D14Rik</i>	Mus musculus RIKEN cDNA 2900092D14 gene (2900092D14Rik), long non-coding RNA [NR_027891]	73100	A_55_P2319478	2.25
<i>Tnfrsf12a</i>	Mus musculus tumor necrosis factor receptor superfamily, member 12a (Tnfrsf12a), transcript variant 1, mRNA [NM_013749]	27279	A_51_P131408	2.23

<i>Gadd45a</i>	Mus musculus growth arrest and DNA-damage-inducible 45 alpha (Gadd45a), mRNA [NM_007836]	13197	A_51_P296608	2.23
<i>Kera</i>	Mus musculus keratocan (Kera), mRNA [NM_008438]	16545	A_51_P123655	2.21
<i>9130019O22Rik</i>	Mus musculus RIKEN cDNA 9130019O22 gene (9130019O22Rik), mRNA [NM_030226]	78921	A_55_P2044827	2.17
<i>Lpxn</i>	Mus musculus leupaxin (Lpxn), mRNA [NM_134152]	107321	A_52_P577748	2.17
<i>Socs3</i>	Mus musculus suppressor of cytokine signaling 3 (Socs3), mRNA [NM_007707]	12702	A_51_P474459	2.16
<i>Cd14</i>	Mus musculus CD14 antigen (Cd14), mRNA [NM_009841]	12475	A_51_P172853	2.16
<i>Nod2</i>	Mus musculus nucleotide-binding oligomerization domain containing 2 (Nod2), mRNA [NM_145857]	257632	A_55_P2359797	2.16
<i>C2cd4a</i>	Mus musculus C2 calcium-dependent domain containing 4A (C2cd4a), mRNA [NM_001163143]	244911	A_55_P2147897	2.12
<i>Hectd2</i>	Mus musculus HECT domain containing 2 (Hectd2), transcript variant 2, mRNA [NM_172637]	226098	A_52_P502141	2.07
<i>Slc13a5</i>	Mus musculus solute carrier family 13 (sodium-dependent citrate transporter), member 5 (Slc13a5), mRNA [NM_001004148]	237831	A_55_P1963508	2.04
<i>Echdc3</i>	enoyl Coenzyme A hydratase domain containing 3 [Source:MGI Symbol;Acc:MGI:1915106] [ENSMUST00000114941]	67856	A_55_P2131190	2.04
<i>4930452G13Rik</i>	Mus musculus RIKEN cDNA 4930452G13 gene (4930452G13Rik), long non-coding RNA [NR_045060]	73989	A_55_P2204168	2.01

Supplementary Table S2. Gene ontological categories of upregulated genes with statistical significance ($P < 0.05$) in the cochlea RNA of DT+ *Prestin-hDTR* mice.

GO accession no.	GO term	P-value	Gene Symbol
GO:0032760	Positive regulation of tumor necrosis factor production	1.62E-04	<i>Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Fcer1g, Nod2</i>
GO:1903557	Positive regulation of tumor necrosis factor superfamily cytokine production	1.62E-04	<i>Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Fcer1g, Nod2</i>
GO:0097529	Myeloid leukocyte migration	5.63E-04	<i>Ccl2, Ccl4, Ccl7, Ccr2, Cx3cr1, Fcer1g, Lgals3</i>
GO:0006950	Response to stress	9.70E-04	<i>Atf3, Bcl2a1c, Bcl2a1d, C1qb, Cckbr, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd84, Ecm1, Elf3, Elf4, Fcer1g, Fntb, Fos, Gadd45a, Lgals3, Lgr6, Lyz1, Lyz2, Myc, Nek11, Nod2, Oxtr, Pmaip1, Ubxn4, Vgf</i>
GO:0070098	Chemokine-mediated signaling pathway	9.70E-04	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cx3cr1</i>
GO:0048522 GO:0051242	Positive regulation of cellular process	1.49E-03	<i>Apoc2, Atf3, Bcl2a1c, Bcl2a1d, Cckbr, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd28, Cd84, Cx3cr1, Ecm1, Elf3, Elf4, Elf5, Fcer1g, Fntb, Fos, Gadd45a, Gdf6, Kcnn4, Lgals3, Lgr6, Myc, Nod2, Olig2, Osmr, Oxtr, Pmaip1, Sirpb1a, Socs3, Sult1e1, Syt4, Tacstd2, Timp1, Tnfrsf12a, Wnt2</i>
GO:0030595	Leukocyte chemotaxis	1.49E-03	<i>Ccl2, Ccl4, Ccl7, Ccr2, Cx3cr1, Fcer1g, Lgals3</i>
GO:1903555	Regulation of tumor necrosis factor superfamily cytokine production	1.52E-03	<i>Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Fcer1g, Nod2</i>
GO:0006952 0002217 0042829	Defense response	1.52E-03	<i>C1qb, Cckbr, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd84, Ecm1, Elf3, Elf4, Fcer1g, Lgals3, Lyz1, Lyz2, Nod2</i>
GO:0032680	Regulation of tumor necrosis factor production	1.52E-03	<i>Cd14, Ccr2, Ccl2, Fcer1g, Ccl4, Ccr5, Nod2</i>
GO:0048518 0043119	Positive regulation of biological process	1.63E-03	<i>Apoc2, Atf3, Bcl2a1c, Bcl2a1d, Cacna2d2, C1qb, Cckbr, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd28, Cd84, Cx3cr1, Elf3, Elf4, Elf5, Ecm1, Fcer1g, Fntb, Fos, Gadd45a, Gdf6, Kcnn4, Lgals3, Lgr6, Myc, Nod2, Olig2, Osmr, Oxtr, Pmaip1, Sirpb1a, Socs3, Sult1e1, Syt4, Tacstd2, Timp1, Tnfrsf12a, Wnt2</i>
GO:0006955	Immune response	2.75E-03	<i>Cd14, Ccr2, Ccl2, C1qb, Fcer1g, Ccl7, Elf4, Ccl4, Ccr5, Cx3cr1, Cd28, Adgre1, Cd84, Adgre1, Lgals3, Nod2</i>

GO:0034097	Response to cytokine	6.07E-03	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cx3cr1, Fntb, Myc, Osmr, Socs3, Timp1</i>
GO:0002548	Monocyte chemotaxis	6.36E-03	<i>Ccl4, Ccl7, Ccr2, Lgals3</i>
GO:1990266	Neutrophil migration	6.36E-03	<i>Ccl2, Ccl4, Ccl7, Fcer1g, Lgals3</i>
GO:0030593	Neutrophil chemotaxis	6.36E-03	<i>Ccl2, Ccl4, Ccl7, Fcer1g, Lgals3</i>
GO:0009897	External side of plasma membrane	6.36E-03	<i>Adgre1, Ccr2, Ccr5, Cd14, Cd33, Cd28, Fcer1g, Lgals3, Osmr</i>
GO:0050776	Regulation of immune response	6.40E-03	<i>Bcl2a1d, C1qb, Ccr2, Cd14, Cd28, Cd84, Ecm1, Fcer1g, Kcnn4, Lgals3, Lpxn, Nod2</i>
GO:0050900	Leukocyte migration	6.49E-03	<i>Ccl2, Ccl7, Ccl4, Ccr2, Cx3cr1, Fcer1g, Lgals3</i>
GO:0002684	Positive regulation of immune system process	6.49E-03	<i>Bcl2a1d, C1qb, Ccl2, Ccl4, Ccr2, Cd14, Cd28, Cd84, Fcer1g, Fos, Kcnn4, Lgals3, Nod2</i>
GO:0060326	Cell chemotaxis	6.66E-03	<i>Ccl2, Ccl4, Ccl7, Ccr2, Cx3cr1, Fcer1g, Lgals3</i>
GO:0071674	Mononuclear cell migration	7.50E-03	<i>Ccl4, Ccl7, Ccr2, Lgals3</i>
GO:0071621	Granulocyte chemotaxis	7.50E-03	<i>Ccl2, Ccl4, Ccl7, Fcer1g, Lgals3</i>
GO:0006954	Inflammatory response	7.50E-03	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Ecm1, Elf3, Fcer1g, Nod2</i>
GO:0008284	Positive regulation of cell proliferation	7.60E-03	<i>Atf3, Ccl2, Cckbr, Ccr2, Cd28, Cx3cr1, Ecm1, Fntb, Lgals3, Myc, Nod2, Osmr, Timp1, Wnt2</i>
GO:0097530	Granulocyte migration	7.60E-03	<i>Ccl2, Ccl4, Ccl7, Fcer1g, Lgals3</i>
GO:0051240	Positive regulation of multicellular organismal process	9.11E-03	<i>Cacna2d2, Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Cd28, Cd84, Cx3cr1, Ecm1, Fcer1g, Fos, Gdf6, Lgals3, Nod2, Olig2, Oxtr, Tnfrsf12a, Wnt2</i>
GO:0007166	Cell surface receptor signaling pathway	9.11E-03	<i>Adgre1, Bcl2a1c, Bcl2a1d, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd28, Cx3cr1, Fcer1g, Fos, Gdf6, Lgr6, Myc, Osmr, Socs3, Tnfrsf12a, Wnt2</i>
GO:0048247	Lymphocyte chemotaxis	1.19E-02	<i>Ccl2, Ccl4, Ccl7, Ccr2</i>
GO:0045087 0002226	Innate immune response	1.19E-02	<i>C1qb, Ccl2, Ccl4, Ccl7, Cd14, Cd84, Elf4, Fcer1g, Lgals3, Nod2</i>
GO:0090264	Regulation of immune complex clearance by monocytes and macrophages	1.19E-02	<i>Ccl2, Ccr2</i>
GO:0090265	Positive regulation of immune complex clearance by monocytes and macrophages	1.19E-02	<i>Ccl2, Ccr2</i>
GO:0001819	Positive regulation of cytokine production	1.19E-02	<i>Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Cd28, Cd84, Fcer1g, Nod2</i>
GO:0032701	Negative regulation of interleukin-18 production	1.19E-02	<i>Cd84, Nod2</i>

GO:0048584	Positive regulation of response to stimulus	1.19E-02	<i>Bcl2a1d, C1qb, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd28, Cd84, Ecm1, Fcer1g, Gadd45a, Gdf6, Kcnn4, Lgr6, Nod2, Pmaip1, Tnfrsf12a, Wnt2</i>
GO:0032879	Regulation of localization	1.42E-02	<i>Apoc2, Cacna2d2, Cckbr, Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Cd84, Cx3cr1, Ecm1, Fcer1g, Kcne4, Kcnj15, Kcnn4, Lgals3, Lgr6, Myc, Oxtr, Pmaip1, Sirpb1a, Syt4, Tacstd2</i>
GO:0030100	Regulation of endocytosis	1.42E-02	<i>Apoc2, Ccl2, Cd14, Fcer1g, Lgals3, Sirpb1a, Syt4</i>
GO:1905517	Macrophage migration	1.48E-02	<i>Ccl2, Cx3cr1, Lgals3</i>
GO:0048246	Macrophage chemotaxis	1.48E-02	<i>Ccl2, Cx3cr1, Lgals3</i>
GO:0032653	Regulation of interleukin-10 production	1.60E-02	<i>Cd28, Cd84, Fcer1g, Nod2</i>
GO:0045765	Regulation of angiogenesis	1.60E-02	<i>Ccl2, Ccr2, Ccr5, Cx3cr1, Ecm1, Lgals3, Tnfrsf12a</i>
GO:0098552	Side of membrane	1.64E-02	<i>Adgre1, Ccr2, Ccr5, Cd14, Cd28, Cd33, Fcer1g, Gem, Lgals3, Osmr</i>
GO:0002682	Regulation of immune system process	1.64E-02	<i>Bcl2a1d, C1qb, Ccl2, Ccl4, Ccr2, Cd14, Cd28, Cd84, Ecm1, Fcer1g, Fos, Kcnn4, Lgals3, Lpxn, Nod2</i>
GO:0010033	Response to organic substance	1.79E-02	<i>Atf3, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd68, Cx3cr1, Fcer1g, Fntb, Fos, Gdf6, Myc, Nod2, Osmr, Oxtr, Ptprn2, Socs3, Timp1, Ubxn4, Vgf</i>
GO:1901342	Regulation of vasculature development	2.00E-02	<i>Ccl2, Ccr2, Ccr5, Cx3cr1, Ecm1, Lgals3, Tnfrsf12a</i>
GO:0072676	Lymphocyte migration	2.06E-02	<i>Ccl2, Ccl4, Ccl7, Ccr2</i>
GO:0019221	Cytokine-mediated signaling pathway	2.08E-02	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cx3cr1, Osmr, Socs3</i>
GO:0005886 GO:0005904	Plasma membrane	2.18E-02	<i>Adgre1, Akap10, C2cd4a, C2cd4b, Cacna2d2, Cckbr, Ccr2, Ccr5, Cd14, Cd28, Cd33, Cd68, Cd72, Cd84, Cldn22, Cx3cr1, Fcer1g, Gap43, Gem, Kcne4, Kcnj15, Kcnn4, Lgals3, Lgr6, Lpxn, Ly6g6c, Nod2, Osmr, Oxtr, Sirpb1a, Slc13a5, Syt4, Tacstd2, Tnfrsf12a, Trpv6, Wnt2</i>
GO:0051094	Positive regulation of developmental process	2.25E-02	<i>Cacna2d2, Ccr2, Ccr5, Cd28, Cx3cr1, Ecm1, Fos, Gdf6, Lgals3, Olig2, Oxtr, Socs3, Sult1e1, Tacstd2, Tnfrsf12a, Wnt2</i>
GO:0051049	Regulation of transport	2.27E-02	<i>Apoc2, Cacna2d2, Cckbr, Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Cd84, Fcer1g, Kcne4, Kcnj15, Kcnn4, Lgals3, Oxtr, Pmaip1, Sirpb1a, Syt4</i>

GO:0032103	Positive regulation of response to external stimulus	2.27E-02	<i>Ccl2, Ccl4, Ccr2, Ccr5, Cd28, Fcer1g, Nod2</i>
GO:0060627	Regulation of vesicle-mediated transport	2.65E-02	<i>Apoc2, Ccl2, Ccr2, Cd14, Cd84, Fcer1g, Lgals3, Sirpb1a, Syt4</i>
GO:0006935	Chemotaxis	2.65E-02	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cx3cr1, Fcer1g, Gap43, Lgals3</i>
GO:0042330	Taxis	2.69E-02	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cx3cr1, Fcer1g, Gap43, Lgals3</i>
GO:0071677	Positive regulation of mononuclear cell migration	2.75E-02	<i>Ccl2, Ccr2, Lgals3</i>
GO:0071944	Cell periphery	3.11E-02	<i>Adgre1, Akap10, C2cd4a, C2cd4b, Cacna2d2, Cckbr, Ccr2, Ccr5, Cd14, Cd28, Cd33, Cd68, Cd72, Cd84, Cldn22, Cx3cr1, Fcer1g, Gap43, Gem, Kcne4, Kcnj15, Kcnn4, Lgals3, Lgr6, Lpxn, Ly6g6c, Nod2, Osmr, Oxtr, Sirpb1a, Slc13a5, Syt4, Tacstd2, Tnfrsf12a, Trpv6, Wnt2</i>
GO:1903306	Negative regulation of regulated secretory pathway	3.11E-02	<i>Ccr2, Cd84, Syt4</i>
GO:0002699	Positive regulation of immune effector process	3.26E-02	<i>Ccl2, Ccr2, Cd28, Cd84, Fcer1g, Nod2</i>
GO:0050727	Regulation of inflammatory response	3.26E-02	<i>Ccr2, Ccr5, Cd28, Cx3cr1, Fcer1g, Socs3, Nod2</i>
GO:0048583	Regulation of response to stimulus	3.47E-02	<i>Atf3, Bcl2a1d, C1qb, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd28, Cd84, Cx3cr1, Ecm1, Fcer1g, Gadd45a, Gdf6, Kcnn4, Lgals3, Lgr6, Lpxn, Nod2, Pmaip1, Socs3, Syt4, Timp1, Tnfrsf12a, Vgf, Wnt2</i>
GO:0051050	Positive regulation of transport	3.53E-02	<i>Cckbr, Ccl2, Ccl4, Ccr5, Cd14, Fcer1g, Kcnn4, Lgals3, Oxtr, Pmaip1, Sirpb1a, Syt4</i>
GO:0045920	Negative regulation of exocytosis	3.79E-02	<i>Ccr2, Cd84, Syt4</i>
GO:0031727	CCR2 chemokine receptor binding	3.79E-02	<i>Ccl2, Ccl7</i>
GO:0080134	Regulation of response to stress	3.81E-02	<i>Ccl2, Ccr2, Ccr5, Cd14, Cd28, Cx3cr1, Fcer1g, Gadd45a, Gdf6, Nod2, Pmaip1, Socs3, Tnfrsf12a</i>
GO:1903532	Positive regulation of secretion by cell	4.09E-02	<i>Cckbr, Ccr5, Cd14, Fcer1g, Kcnn4, Lgals3, Oxtr, Syt4</i>
GO:0001836	Release of cytochrome c from mitochondria	4.14E-02	<i>Bcl2a1c, Bcl2a1d, Pmaip1</i>
GO:0046903	secretion	4.37E-02	<i>Cckbr, Fcer1g, Kcnn4, Nod2, Oxtr, Ptprn2, Syt4, Trpv6, Vgf</i>
GO:0001637	G-protein coupled chemoattractant receptor activity	4.37E-02	<i>Ccr2, Ccr5, Cx3cr1</i>
GO:0019956	Chemokine binding	4.37E-02	<i>Ccr2, Ccr5, Cx3cr1</i>
GO:0051239	Regulation of multicellular organismal process	4.37E-02	<i>Apoc2, Cacna2d2, Cckbr, Ccl2, Ccl4, Ccr2, Ccr5, Cd14, Cd28, Cd84, Cx3cr1, Ecm1, Fcer1g, Fos, Gdf6, Lgals3, Matn1, Nod2, Olig2, Oxtr, Syt4, Tacstd2, Tnfrsf12a, Wnt2</i>

GO:0004950	Chemokine receptor activity	4.37E-02	<i>Ccr2, Ccr5, Cx3cr1</i>
GO:0071345	Cellular response to cytokine stimulus	4.37E-02	<i>Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cx3cr1, Myc, Osmr, Socs3</i>
GO:0042127	Regulation of cell proliferation	4.37E-02	<i>Atf3, Cckbr, Ccl2, Ccr2, Ccr5, Cd28, Cx3cr1, Ecm1, Fntb, Lgals3, Myc, Nod2, Osmr, Pmaip1, Tacstd2, Timp1, Wnt2</i>
GO:0002376	Immune system process	4.37E-02	<i>Adgre1, Bcl2a1d, C1qb, Ccl2, Ccl4, Ccl7, Ccr2, Ccr5, Cd14, Cd28, Cd84, Cx3cr1, Elf4, Fcer1g, Kcnn4, Lgals3, Nod2, Pmaip1</i>
GO:0042803	Protein homodimerization activity	4.49E-02	<i>Apoc2, Atf3, Bcl2a1c, C1qb, Cd28, Cdsn, Fcer1g, Gadd45a, Gdf6, Olig2, Syt4</i>
GO:0002828	Regulation of type 2 immune response	4.64E-02	<i>Ccr2, Ecm1, Nod2</i>
GO:0002253	Activation of immune response	4.71E-02	<i>Bcl2a1d, C1qb, Cd14, Cd28, Fcer1g, Nod2</i>

Supplementary Table S3. List of genes of downregulated genes with ≤ -2 -fold change in the cochlea RNA of DT+ *Prestin-hDTR* mice. *The GeneID was changed to 108168154.

Gene Symbol	Description [RefSeq]	Entrez Gene ID	Probe Name	Fold change
				DT+/DT-
<i>Strc</i>	Mus musculus stereocilin (Strc), mRNA [NM_080459]	140476	A_55_P2004001	-8.52
<i>Chrna10</i>	Mus musculus cholinergic receptor, nicotinic, alpha polypeptide 10 (Chrna10), mRNA [NM_001081424]	504186	A_55_P1991244	-7.60
<i>Gm46479</i>	PREDICTED: Mus musculus uncharacterized LOC108168195 (LOC108168195), ncRNA [XR_001781413]	108168195	A_55_P1958544	-7.25
<i>Chrna9</i>	Mus musculus cholinergic receptor, nicotinic, alpha polypeptide 9 (Chrna9), mRNA [NM_001081104]	231252	A_51_P381069	-6.79
<i>Gm46479</i>	PREDICTED: Mus musculus uncharacterized LOC108168195 (LOC108168195), ncRNA [XR_001781413]	108168195	A_55_P2051166	-5.92
<i>Ocm</i>	Mus musculus oncomodulin (Ocm), mRNA [NM_033039]	18261	A_55_P2094916	-5.74
<i>Gm3161</i>	predicted gene 3161	100041143*	A_55_P2019838	-5.51
<i>Myo15</i>	Mus musculus myosin XV (Myo15), transcript variant 1, mRNA [NM_010862]	17910	A_55_P1969977	-5.48
<i>Ocm</i>	Mus musculus oncomodulin (Ocm), mRNA [NM_033039]	18261	A_55_P2073825	-5.15
<i>Gfi1</i>	Mus musculus growth factor independent 1 (Gfi1), transcript variant 1, mRNA [NM_010278]	14581	A_55_P1977008	-4.20
<i>Gm3161</i>	predicted gene 3161	100041143*	A_55_P1957867	-4.17
<i>Ppp1r17</i>	Mus musculus protein phosphatase 1, regulatory subunit 17 (Ppp1r17), mRNA [NM_011153]	19051	A_52_P355124	-3.90
<i>Lhx3</i>	Mus musculus LIM homeobox protein 3 (Lhx3), transcript variant 1, mRNA [NM_001039653]	16871	A_55_P2031272	-3.88
<i>Barhl1</i>	Mus musculus BarH-like 1 (Drosophila) (Barhl1), transcript variant 1, mRNA [NM_019446]	54422	A_52_P65084	-3.86
<i>Mkrn2os</i>	Mus musculus makorin, ring finger protein 2, opposite strand (Mkrn2os), mRNA [NM_001101431]	70291	A_52_P512427	-3.24
<i>Vwa3a</i>	Mus musculus von Willebrand factor A domain containing 3A (Vwa3a), mRNA [NM_177697]	233813	A_66_P139647	-3.23
<i>Pjvk</i>	Mus musculus pejvakin (Pjvk), mRNA, mRNA [NM_001080711]	381375	A_55_P2181381	-3.04
<i>Pou4f3</i>	Mus musculus POU domain, class 4, transcription factor 3 (Pou4f3), mRNA [NM_138945]	18998	A_55_P1997220	-2.93
<i>Tomt</i>	Mus musculus transmembrane O-methyltransferase (Tomt), transcript variant 2, mRNA [NM_001282088]	791260	A_55_P2121106	-2.92

<i>Tcap</i>	Mus musculus titin-cap (Tcap), mRNA [NM_011540]	21393	A_52_P249733	-2.86
<i>Tmc1</i>	Mus musculus transmembrane channel-like gene family 1 (Tmc1), mRNA [NM_028953]	13409	A_55_P2147026	-2.83
<i>Tctex1d1</i>	Mus musculus Tctex1 domain containing 1 (Tctex1d1), transcript variant 1, mRNA [NM_026100]	67344	A_55_P2051270	-2.82
<i>Mei1</i>	Mus musculus meiotic double-stranded break formation protein 1 (Mei1), transcript variant 2, mRNA [NM_001310435]	74369	A_51_P406835	-2.73
<i>Grxcr2</i>	Mus musculus glutaredoxin, cysteine rich 2 (Grxcr2), mRNA [NM_001033426]	332309	A_66_P106204	-2.72
<i>Elovl2</i>	Mus musculus elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2 (Elovl2), transcript variant 2, mRNA [NM_001311121]	54326	A_55_P2062737	-2.71
<i>Sec14l4</i>	Mus musculus SEC14-like lipid binding 4 (Sec14l4), mRNA [NM_146013]	103655	A_51_P194853	-2.67
<i>Slc8a2</i>	Mus musculus solute carrier family 8 (sodium/calcium exchanger), member 2 (Slc8a2), transcript variant 1, mRNA [NM_148946]	110891	A_55_P2095563	-2.62
<i>Ackr4</i>	Mus musculus atypical chemokine receptor 4 (Ackr4), mRNA [NM_145700]	252837	A_52_P153291	-2.53
<i>Palm3</i>	Mus musculus paralemmin 3 (Palm3), mRNA [NM_028877]	74337	A_55_P2127934	-2.51
<i>Bpifa1</i>	Mus musculus BPI fold containing family A, member 1 (Bpifa1), mRNA [NM_011126]	18843	A_51_P202331	-2.46
<i>Zp1</i>	Mus musculus zona pellucida glycoprotein 1 (Zp1), mRNA [NM_009580]	22786	A_55_P2123626	-2.42
<i>9530056K15Rik</i>	9530056K15Rik RIKEN cDNA 9530056K15 gene	320650	A_55_P2360271	-2.41
<i>Acbd7</i>	Mus musculus acyl-Coenzyme A binding domain containing 7 (Acbd7), mRNA [NM_030063]	78245	A_55_P2053324	-2.34
<i>Klhl14</i>	Mus musculus kelch-like 14 (Klhl14), mRNA [NM_001081403]	225266	A_55_P2048085	-2.26
<i>Ackr4</i>	AY072938 chemokine receptor CCX CKR (exp=-1; wgp=0; cg=0), complete [TC1596586]	252837	A_55_P2050226	-2.21
<i>Col8a2</i>	Mus musculus collagen, type VIII, alpha 2 (Col8a2), mRNA [NM_199473]	329941	A_55_P1960631	-2.21
<i>Lmod3</i>	Mus musculus leiomodrin 3 (fetal) (Lmod3), mRNA [NM_001081157]	320502	A_51_P427964	-2.21
<i>Gm1043</i>	predicted gene 1043 [Source:MGI Symbol; Acc:MGI:2685889] [ENSMUST00000213782]	381634	A_66_P139530	-2.16
<i>Clrn2</i>	Mus musculus clarin 2 (Clrn2), mRNA [NM_001163317]	624224	A_55_P2057806	-2.10
<i>Pcp4</i>	Mus musculus Purkinje cell protein 4 (Pcp4), mRNA [NM_008791]	18546	A_51_P253984	-2.09

Supplementary Table S4. Gene ontological categories of downregulated genes with statistical significance ($P < 0.05$) in the cochlea RNA of DT+ *Prestin-hDTR* mice.

GO accession no.	GO term	P-value	Gene Symbol
GO:0007605	Sensory perception of sound	8.11E-11	<i>Barhl1, Chrna9, Chrna10, Grxcr2, Myo15, Pjvk, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0050954	Sensory perception of mechanical stimulus	2.26E-10	<i>Barhl1, Chrna9, Chrna10, Grxcr2, Myo15, Pjvk, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0048839	Inner ear development	1.74E-08	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap, Tmc1, Tomt</i>
GO:0043583	Ear development	4.74E-08	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap, Tmc1, Tomt</i>
GO:0042472	Inner ear morphogenesis	3.06E-07	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap</i>
GO:0042471	Ear morphogenesis	1.01E-06	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap</i>
GO:0007423	Sensory organ development	3.48E-06	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0042491	Inner ear auditory receptor cell differentiation	3.48E-06	<i>Chrna9, Chrna10, Col8a2, Gfi1, Myo15, Pou4f3, Strc, Tcap, Tmc1, Tomt</i>
GO:0090596	Sensory organ morphogenesis	4.45E-06	<i>Chrna9, Chrna10, Col8a2, Gfi1, Myo15, Pou4f3, Strc, Tcap, Tmc1, Tomt</i>
GO:0035315	Hair cell differentiation	4.91E-06	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0042491	Inner ear auditory receptor cell differentiation	3.48E-06	<i>Chrna9, Chrna10, Strc, Tmc1</i>
GO:0050910 0009592 0055128	Detection of mechanical stimulus involved in sensory perception of sound	4.91E-06	<i>Chrna9, Chrna10, Strc, Tmc1</i>
GO:0060563	Neuroepithelial cell differentiation	1.50E-05	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0050982	Detection of mechanical stimulus	1.52E-05	<i>Chrna9, Chrna10, Strc, Tcap, Tmc1</i>
GO:0009612	Response to mechanical stimulus	2.81E-05	<i>Chrna9, Chrna10, Gfi1, Strc, Tcap, Tmc1</i>
GO:0060113	Inner ear receptor cell differentiation	3.05E-05	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0042490	Mechanoreceptor differentiation	4.44E-05	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0048562	Embryonic organ morphogenesis	1.52E-04	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap</i>
GO:0050974	Detection of mechanical stimulus involved in sensory perception of sound	1.58E-04	<i>Chrna9, Chrna10, Strc, Tmc1</i>
GO:0002065	Columnar/cuboidal epithelial cell differentiation	1.95E-04	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0009581	Detection of external stimulus	3.86E-04	<i>Chrna9, Chrna10, Strc, Tcap, Tmc1</i>
GO:0009582	Detection of abiotic stimulus	3.86E-04	<i>Chrna9, Chrna10, Strc, Tcap, Tmc1</i>

GO:0048568	Embryonic organ development	0.0015099	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap</i>
GO:0009913 0043355	Epidermal cell differentiation	0.0015099	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0060117	Auditory receptor cell development	0.0020491	<i>Strc, Tmc1, Tomt</i>
GO:0098858	Actin-based cell projection	0.0027265	<i>Bpifa1, Grxcr2, Myo15, Strc, Tmc1</i>
GO:0009605	Response to external stimulus	0.0027265	<i>Ackr4, Bpifa1, Chrna9, Chrna10, Gfi1, Lhx3, Palm3, Pou4f3, Strc, Tcap, Tmc1</i>
GO:0048598 0048828	Embryonic morphogenesis	0.0086246	<i>Chrna9, Chrna10, Gfi1, Myo15, Pou4f3, Strc, Tcap</i>
GO:0030240	Skeletal muscle thin filament assembly	0.0103704	<i>Lmod3, Tcap</i>
GO:0032420	Stereocilium	0.0107737	<i>Myo15, Strc, Tmc1</i>
GO:0008544	epidermis development	0.012116	<i>Gfi1, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0003008	System process	0.012116	<i>Barhl1, Chrna9, Chrna10, Dfnb59, Grxcr2, Lmod3, Myo15, Pou4f3, Slc8a2, Strc, Tcap, Tmc1, Tomt</i>
GO:0009887	Animal organ morphogenesis	0.0151258	<i>Chrna9, Chrna10, Col8a2, Gfi1, Myo15, Pou4f3, Strc, Tcap</i>
GO:0002066	Columnar/cuboidal epithelial cell development	0.0155943	<i>Strc, Tmc1, Tomt</i>
GO:0060119	Inner ear receptor cell development	0.0155943	<i>Strc, Tmc1, Tomt</i>
GO:0032421	Stereocilium bundle	0.0155943	<i>Myo15, Strc, Tmc1</i>
GO:0014866	Skeletal myofibril assembly	0.0155943	<i>Lmod3, Tcap</i>
GO:0048513	Animal organ development	0.0325121	<i>Barhl1, Chrna9, Chrna10, Col8a2, Gfi1, Lhx3, Lmod3, Myo15, Pou4f3, Strc, Tcap, Tmc1, Tomt</i>
GO:0032426	Stereocilium tip	0.0334263	<i>Strc, Tmc1</i>
GO:0050877	Nervous system process	0.0373343	<i>Barhl1, Chrna9, Chrna10, Dfnb59, Grxcr2, Myo15, Pou4f3, Slc8a2, Strc, Tmc1, Tomt</i>
GO:0007600	Sensory perception	0.0376413	<i>Barhl1, Chrna9, Chrna10, Dfnb59, Grxcr2, Myo15, Pou4f3, Strc, Tmc1, Tomt</i>
GO:0048699	Generation of neurons	0.0475618	<i>Barhl1, Gfi1, Lhx3, Ocm, Pcp4, Pou4f3, Strc, Tmc1, Tomt</i>

Supplementary Table S5. Primers used for qRT-PCR analysis in this study.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
<i>Apoc2</i>	GCATGGATGAGAAACTCAGGG	AAAATGCCTGCGTAAAGTGCTC	4
<i>Barhl1</i>	CAAAGTGAAGGAGGAGGGCG	GTGTCGGTGAGGTTGAGCGA	5
<i>Ccl2</i>	GAGGAAGGCCAGCCCAGCAC	TGGGGCGTTAACTGCATCTGGC	6
<i>Ccl4</i>	GCCCTCTCTCTCTCTTGCT	CTGGTCTCATAGTAATCCATC	7
<i>Ccl7</i>	TGTCCCTGGGAAGCTGTTAT	CTTTGGAGTTGGGGTTTTCA	This study
<i>Cd28</i>	GCCTTACCTAGACAACGAGA	CCAAACAGCTTAGGAGATGAC	This study
<i>Cdsn</i>	CTGATGGCCGGTCTTATTCT	GCTGTTGGAGCCAGTCTTTC	8
<i>Chrna9</i>	GTCCCTCTGATAGGAAAATACTAC	CTAAGGCAGCTCTCACCCAC	This study
<i>Chrna10</i>	GTTTCGTGACCTGTTTGCCAACT	GGTACAAGGTCAGCACTTGGTTC	This study
<i>Cldn22</i>	GGGCTTAGTCTTCCGAACG	CTTCCAGTGCGGCAAGTAGT	This study
<i>Gapdh</i>	TCGGTGTGAACGGATTTG	CGTGAGTGGAGTCATACTGGA	This study
<i>Gfi1</i>	GAGCAACACAAGGCAGTG	TCTTGCCACAGATCTTACAGTC	9
<i>Grxcr2</i>	CCAGCCTCTGTTCAATGACTAC	CCTCATGAAGTCCCTTTGTCC	This study
<i>Lgals3</i>	CAACGCAAACAGGATTGTTC	CGTGTTACACACAATGACTCTCC	10
<i>Lhx3</i>	CCCCACCCATGAGGGTGCT	GAGCCAGGGGAAGCAGAGGC	11
<i>Loxl4</i>	CCGCTGCAAGTATGATGG	GTTCTGAGTCGCTGTTC	12
<i>Mpeg1</i>	GTGAAACAAAAGCCAGACAGAGCCT	TCATGGCGCAGATGGTTTTGGC	13
<i>Myo15</i>	ACCAACAGATGCTCACTGTCTCTG	ATAGAAGGCAGGTTGCGTATGGAG	This study
<i>Ocm</i>	AGACAACG CCAGAGTGGATAC	TCAAGGACTTGGTCTCTGACTC	This study
<i>Pjvk</i>	TTCATCTACCTGGACGGTGCCTT	TCCAGGTAAAGCTGGGAACGAGA	This study
<i>Pou4f3</i>	ATGCGCCGAGTTTGTCTCC	GGGCTTGAACGGATGATTCT	14
<i>Sirpb1b</i>	GTCACCTCTGCTGATTCCGG	GTCACCTGTCTGCTGAGGGAC	This study
<i>Slc26a5</i>	TGTTCTCTCGGCTTGGACTAC	GTCAATGTACACATCCGTGTCAG	This study
<i>Sox2</i>	ACTTTTGTCCGAGACCGAGAA	CGCGGCCGGTATTTATAATC	15
<i>Strc</i>	CCTGACGCTCATGCTTCGTTC	CAGCCAGGACACTGTCGTTG	This study
<i>Tmc1</i>	CATCTGCAGCCAACCTTGGTGTGT	AGAGGTAGCCGGAATTCAGCCAT	16
<i>Tomt</i>	ACTTCGGATTGAGGAGCGGGCTTT	ACCAGCCGCATCAGAATCTGACCT	This study
<i>Vgf</i>	TCGACCATCGCTCATACTCC	CGTGAAGGTTTTTCATGACCAAC	This study
<i>Vglut3</i>	TGGTGCTTGCTGTAGGATTTA	CCACGCCATTTGAGATCC	17
<i>Wnt2</i>	CACCCTGGACAGAGATCACA	ACAACGCCAGCTGAAGAGAT	18

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