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Supplemental Data

GWAS Identifies 44 Independent Associated Genomic Loci for Self-Reported Adult Hearing Difficulty in UK Biobank

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Supplemental Data

Supplemental Data include six figures and three tables.

Figure S1a. Flow chart describing case-control assignment for the hearing difficulty (*HDiff*) phenotype.

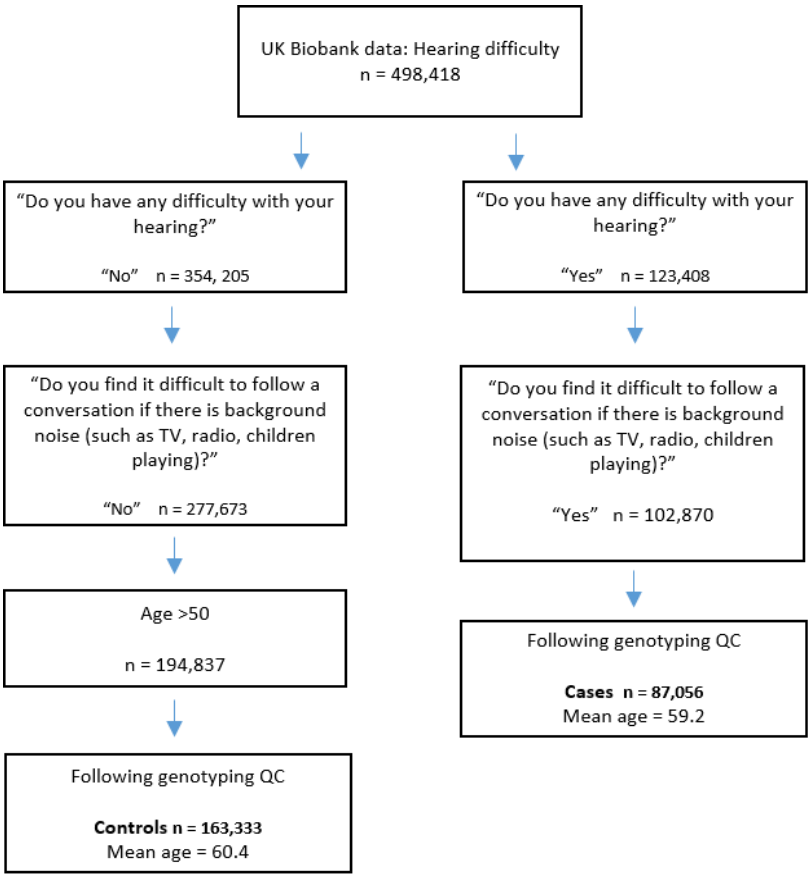

Figure S1a. Flow chart describing case-control assignment for the hearing difficulty (*HDiff*) phenotype. Participants answered questions as part of the UKBB questionnaire administered at UKBB assessment centres. Participants who answered 'Prefer not to answer', 'I am completely deaf' and 'Do not know' were removed from the analysis. Participants were removed from the control group if they answered "Yes" to "Do you use a hearing aid most of the time?" A lower age limit of 50 was implemented for controls to ensure age was consistent between the case and control groups due to the association of aging with the trait.

Figure S1b. Flow chart describing case-control assignment for the hearing aid use (*HAid*) phenotype

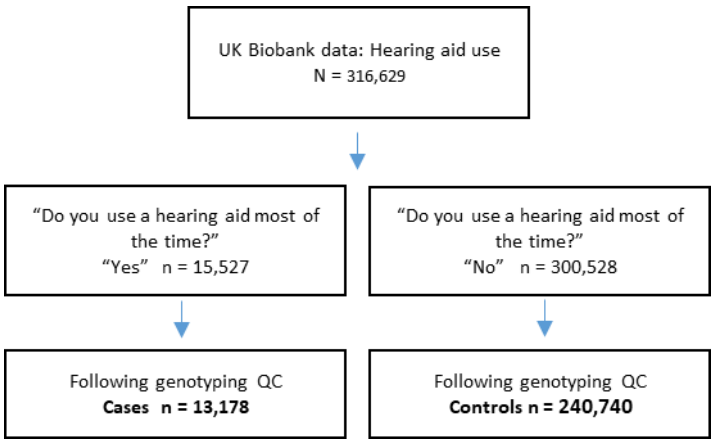

Figure S1b. Flow chart describing case-control assignment for the hearing aid use (*HAid*) phenotype. Participants answered questions as part of the UKBB questionnaire administered at the UKBB assessment centres. No information was collected regarding age at hearing aid prescription or cause of hearing loss.

Figure S2a. Q-Q plot of GWAS summary statistics, *HDiff* (left) and *HAid* (right).

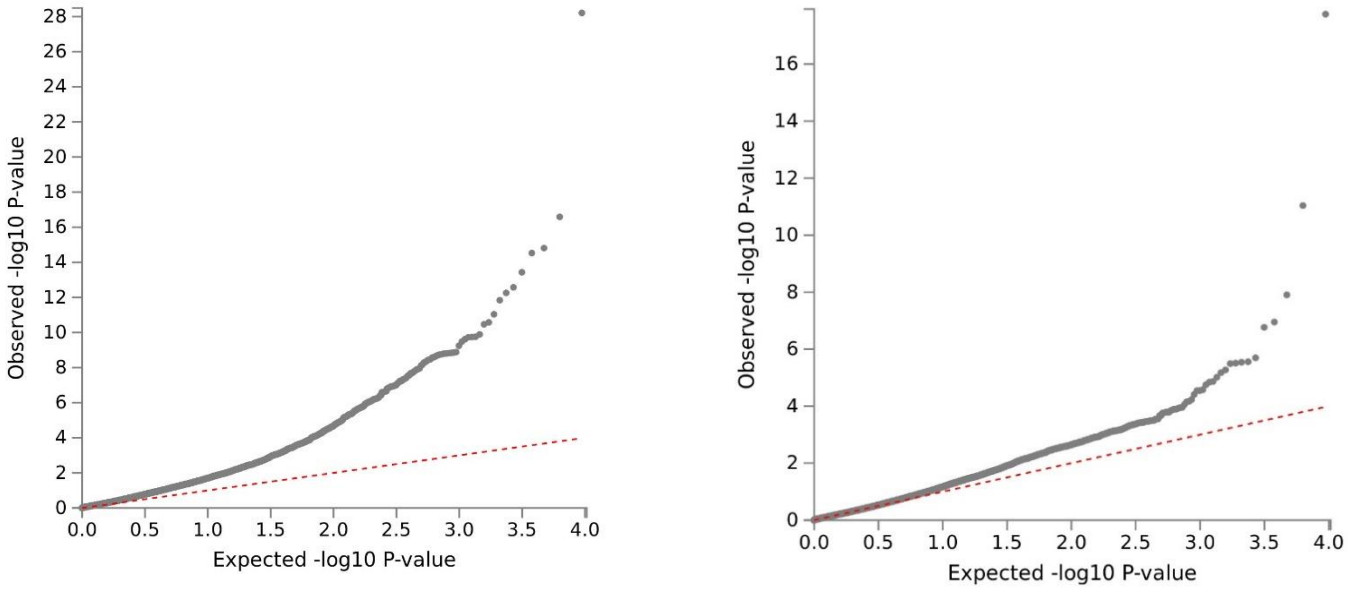

Figure S2a. Q-Q plot of GWAS summary statistics, *HDiff* (left) and *HAid* (right). The LD score regression intercepts for the two analyses were 1.032 for *HDiff* and 1.03 for *HAid*. The ratio (intercept-1)/(mean(χ^2)-1) for *HDiff* was 8% and represents the proportion of inflation in the χ^2 statistic that the intercept attributes to alternative explanations than polygenicity. The ratio for *HAid* was 5%.

Figure S2b. Q-Q plot of the gene-based test computed by MAGMA, *HDiff* (left) and *HAid* (right).

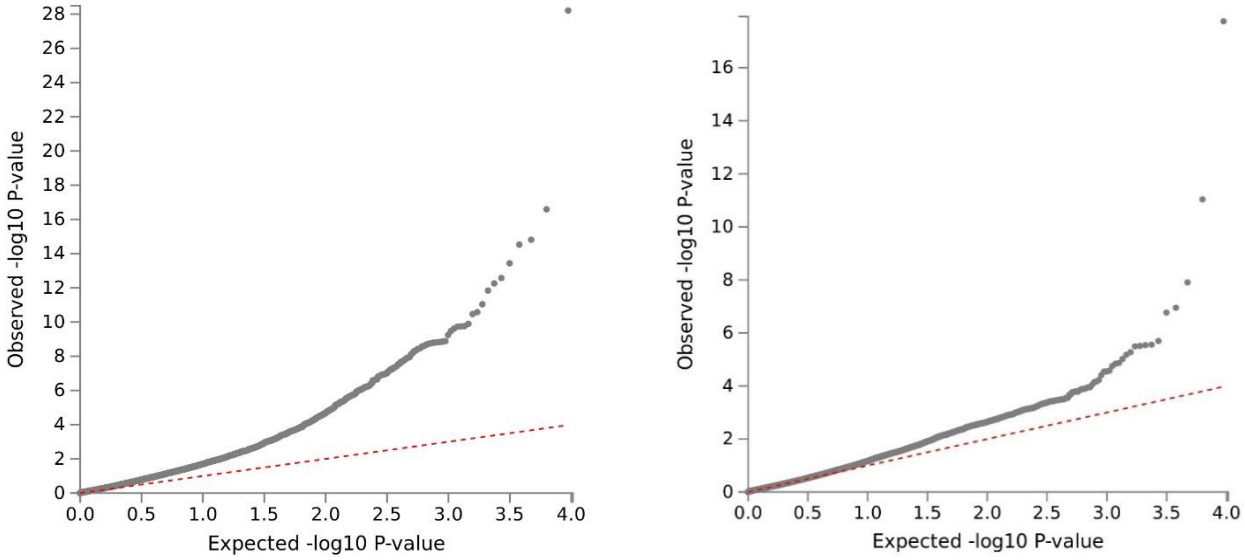

Figure S2b. Q-Q plot of the gene-based test computed by MAGMA, *HDiff* (left) and *HAid* (right).

Figure S3. Manhattan-Style plots of regional heritability across the genome for *HDiff* (a) and *HAid* (b)

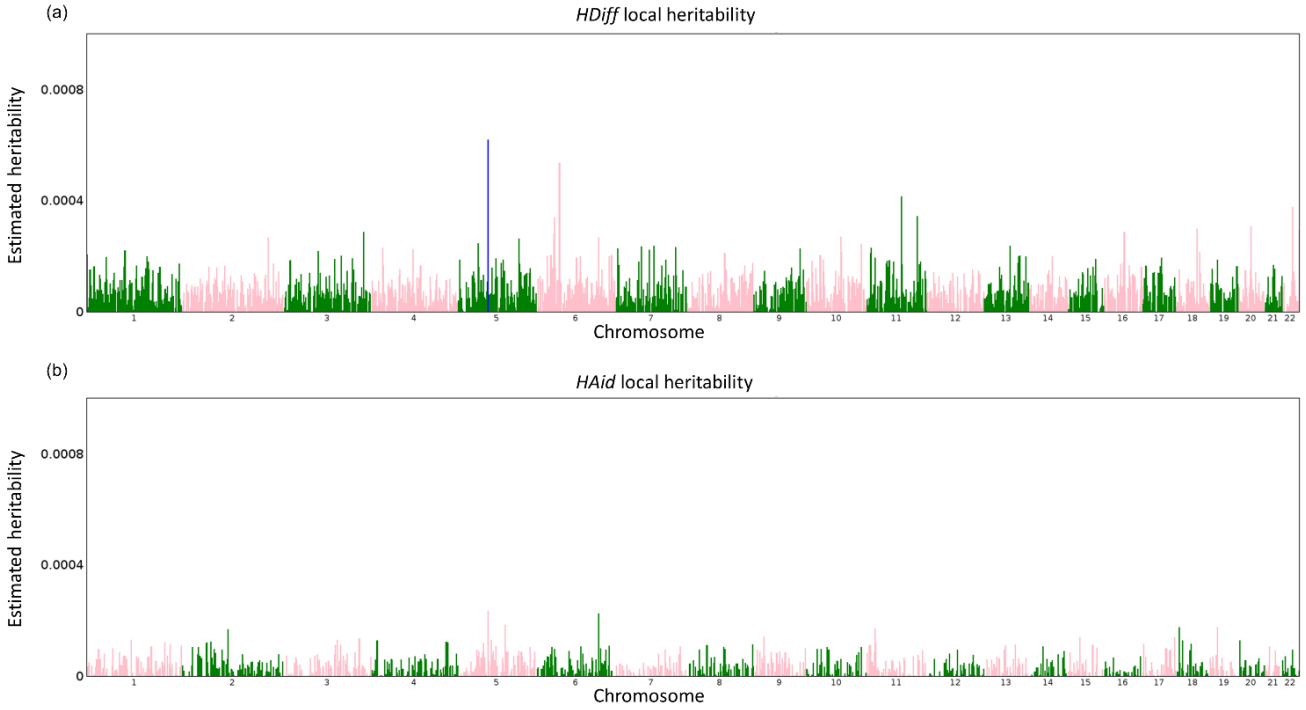

Figure S3. Manhattan-Style plots of Regional heritability across the genome for *HDiff* (a) and *HAid* (b). Estimated local SNP heritability for 1702 loci. Blue denotes an estimate of local_h2g = 0.0006, p<1.1579E-05on Chr5 for *HDiff* in the region between base positions 71240456- 73759326, here represented by 4404 SNPs. HESS total SNP heritability estimates were 0.1, SE=0.005 for *HDiff* and 0.011, SE=0.005 for *HAid*.

Figure S4. Locus plots displaying regions significantly associated in both *HDiff* and *HAid* analysis.

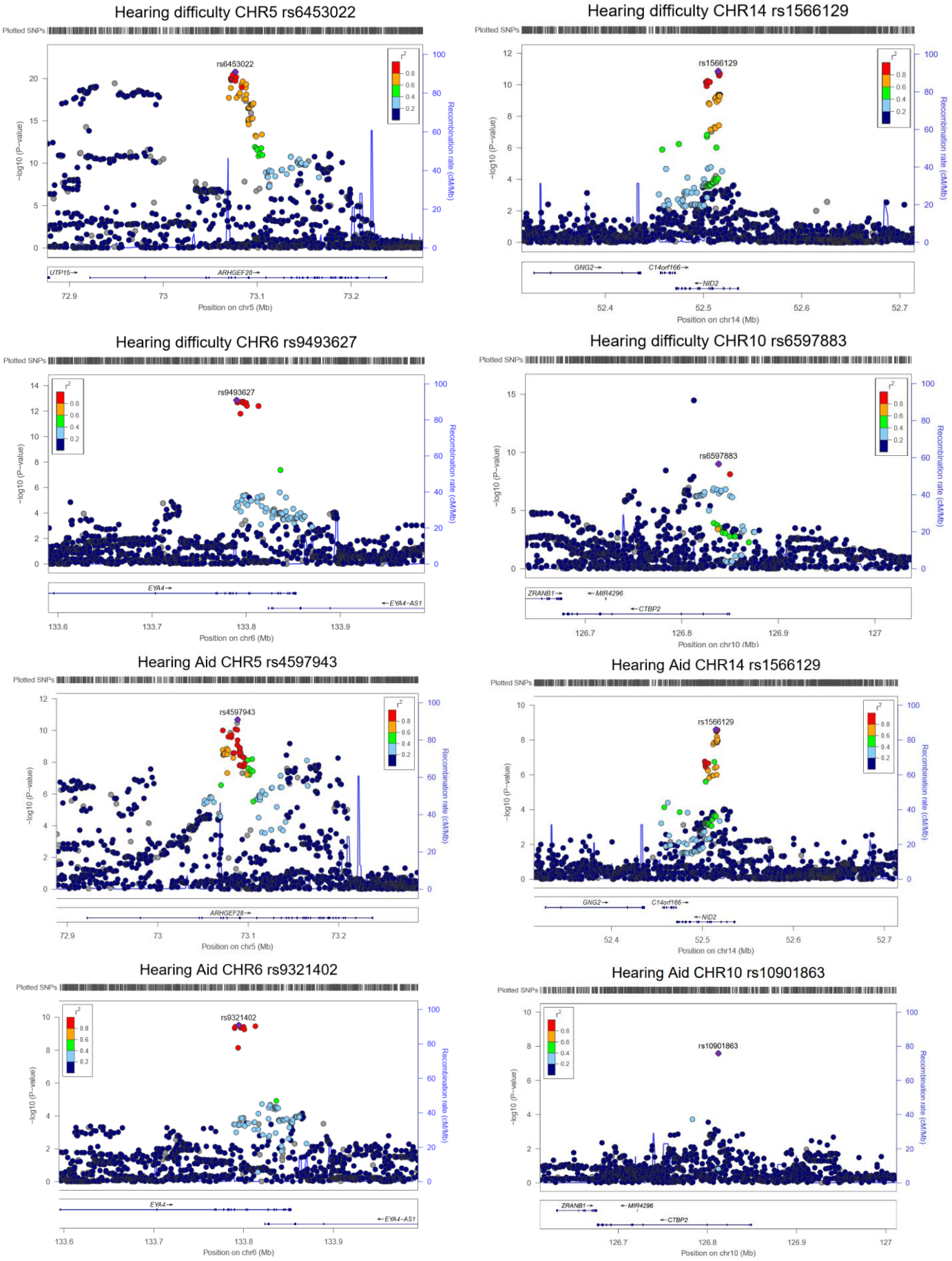

Figure S4. Locus plots displaying regions significantly associated in both *HDiff* and *HAid* analysis. Locus plots generated with *HDiff* summary statistics. Purple indicates lead independent SNP generated from GCTA-COJO conditional analysis. The colouring of remaining SNPs represents the correlation (r^2) to the lead SNP (purple).

Figure S5. MAGMA gene-property analysis for tissue specificity from average expression of 30 general tissue types from GTEx v6.

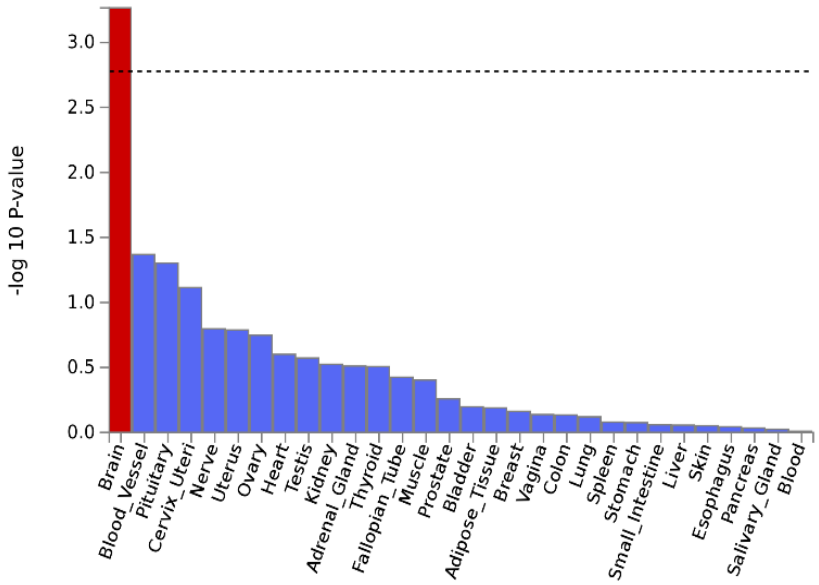

Figure S5. MAGMA gene-property analysis for tissue specificity from average expression of 30 general tissue types from GTEx v6. Relationships between tissue specific gene expression profiles (x-axis) and genetic association of genes (y-axis) is shown. Genetic associations of genes were performed in MAGMA and represent the aggregated effect of all SNPs in a gene. The dotted line indicates the Bonferroni-corrected level of 2.6E-06.

Figure S6. Immunofluorescence images of adult mouse cochlea Vibratome sections stained with proteins of interest (green), DAPI (blue) and Phalloidin (magenta)

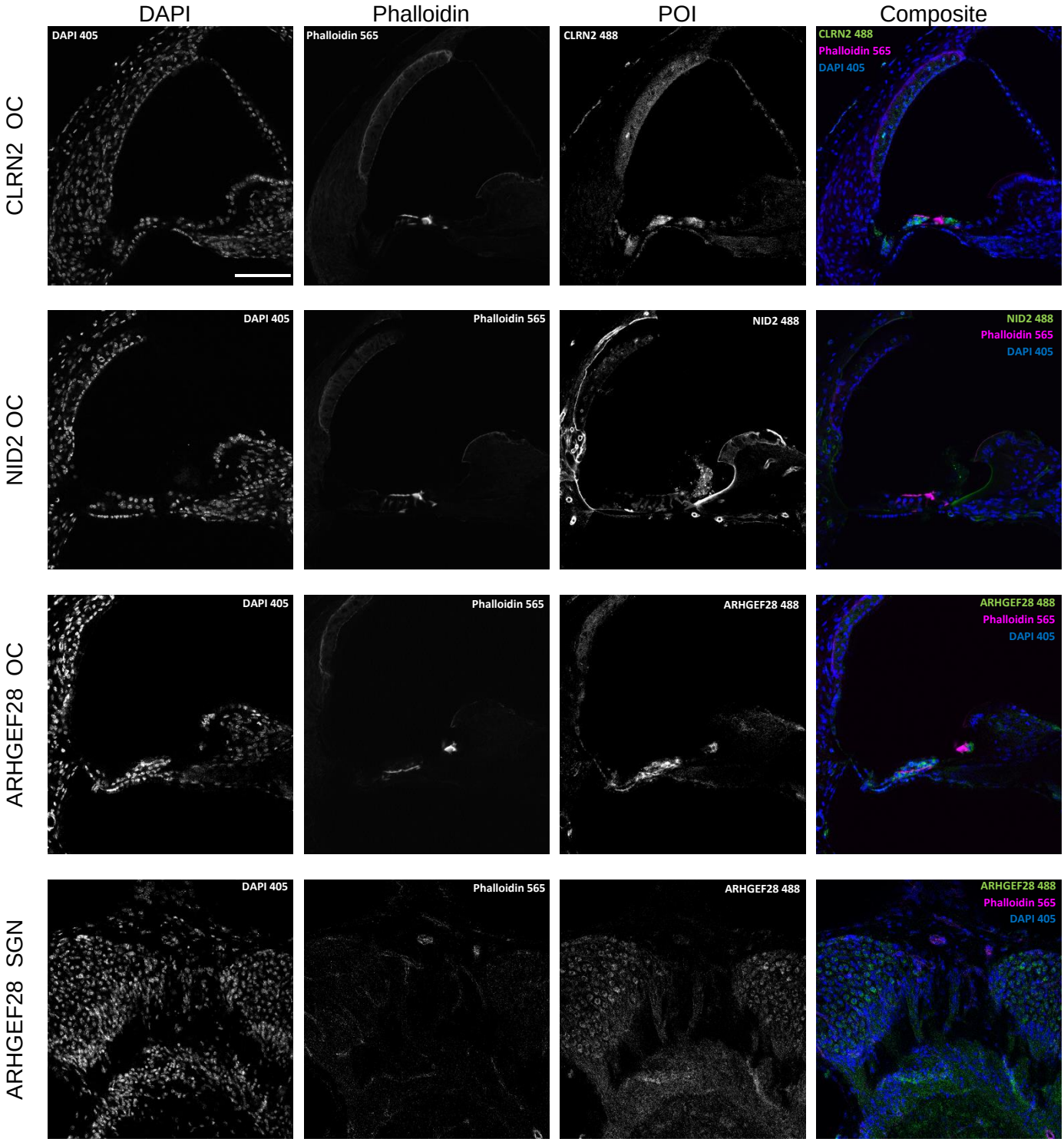

Figure S6. Immunofluorescence images of adult mouse cochlea Vibratome sections stained with proteins of interest (green), DAPI (blue) and Phalloidin (magenta)

Individual channels presented in greyscale, and combined for composite colour images as in Figure 4. Three panels display the organ of Corti (OC) regions (CLRN2 OC, NID2 OC, and ARHGEF28 OC) and one displays the Spiral Ganglion Neurons (SGN) region (ARHGEF28 SGN).

The scale bar in the top left image displays 100µm. This scale is consistent for all images in this figure.

Table S1. Summary statistics for *HDiff* phenotype from the replication meta-analysis of the white non-British UKBB sample, TwinsUK and ELSA

Marker Name	Allele1	Allele2	Weight	Zscore	P-value	Direction	Replication power p<0.05	Replication power p<0.0012
rs759016271	a	agtagtccacttttcttctttgcctg	29866	4.556	5.20E-06	+++	0.900	0.504
rs1566129	t	c	30894	3.534	0.00041	+++	0.661	0.195
rs36062310	a	g	30868	2.974	0.002938	+--	0.928	0.575
rs143282422	a	g	30274	2.652	0.007996	+-+	0.492	0.098
rs12225399	c	g	29802	2.624	0.008688	+++	0.610	0.160
rs6597883	t	c	30274	2.585	0.009748	++-	0.564	0.133
rs62033400	a	g	30851	2.513	0.01198	+++	0.600	0.153
rs7951935	t	g	29802	2.48	0.01312	++-	0.820	0.360
rs141403654	a	t	29802	-2.392	0.01674	---	0.477	0.091
rs35186928	a	g	29866	2.348	0.01885	+-+	0.785	0.314
rs17671352	t	c	30852	2.228	0.02587	++-	0.520	0.110
rs217289	a	g	29866	2.227	0.02597	+-+	0.524	0.112
rs62188635	t	c	30377	-2.189	0.02859	---	0.583	0.144
rs76837345	a	g	30318	-2.158	0.0309	---	0.499	0.101
rs55635402	a	g	29802	2.132	0.03301	+++	0.585	0.145
rs10824108	t	g	30274	1.982	0.04752	+-+	0.541	0.120
rs2236401	t	c	29866	1.873	0.06102	++-	0.561	0.131
rs5756795	t	c	30868	-1.696	0.08981	--+	0.679	0.209
rs7525101	t	c	30389	1.636	0.1018	+++	0.506	0.104
rs10475169	a	c	30356	-1.603	0.1088	-+-	0.516	0.108
rs4948502	t	c	30274	1.574	0.1155	+-+	0.553	0.127
rs9691831	a	g	30717	-1.512	0.1305	-+-	0.492	0.098
rs12938775	a	g	30852	-1.481	0.1385	-+-	0.509	0.105
rs4611552	t	c	30753	-1.434	0.1515	---	0.489	0.096
rs13093972	a	g	30098	-1.413	0.1577	--+	0.525	0.113
rs6453022	a	c	30356	1.327	0.1846	+--	0.912	0.532
rs835267	a	g	30274	1.17	0.242	++-	0.555	0.128
rs35414371	a	t	30856	1.163	0.245	+-+	0.657	0.192
rs9493627	a	g	29866	1.018	0.3086	+++	0.724	0.248
rs12027345	a	g	30389	-0.986	0.3244	--+	0.537	0.119

rs3890736	a	g	30318	-0.706	0.4805	+--	0.495	0.099
rs4947828	t	g	30717	-0.562	0.574	+--	0.571	0.137
rs13277721	a	g	30318	0.527	0.5983	+--	0.590	0.147
rs12552	a	g	30676	0.489	0.6249	+-+	0.480	0.093
rs6890164	a	g	30356	0.298	0.7658	+--	0.877	0.455
3:182069497_TA_T	t	ta	30098	0.298	0.766	++-	0.629	0.172
rs132929	a	g	30868	0.234	0.815	++-	0.731	0.256
rs10927035	t	c	30389	0.219	0.8268	+-+	0.476	0.091
rs34442808	t	ta	30356	0.218	0.8277	-++	0.560	0.131
rs9366417	a	g	29866	0.137	0.8911	+-+	0.490	0.097
rs62015206	t	c	30459	-0.071	0.9437	--+	0.522	0.111

Table S1. Summary statistics for *HDiff* phenotype from the replication meta-analysis of the white non-British UKBB sample, TwinsUK and ELSA. Marker Name, SNP ID; Allele1, the first allele for this marker in the first file where it occurs; Allele 2, the second allele for this marker in the first file where it occurs; Weight, the sum of the individual study weights (N) for this marker; Z-score, the combined z-statistic for the marker; P-value, meta-analysis p-value; Direction, direction of effect for each study ordered: white non-British UKBB sample, TwinsUK, ELSA; Replication power at $p<0.05$, estimated power to identify a replicated association at nominal significance; Replication power $p<0.0012$, estimated power to detect an association at significance threshold 0.05/41 = 0.0021, $p<0.0012$.

Table S2. Summary statistics for the *HAid* phenotype in the replication meta-analysis of white non-British UKBB sample, TwinsUK and ELSA

Marker Name	Allele1	Allele2	Weight	Zscore	P-value	Direction	Replication power p<0.05	Replication power p<0.00714
rs4597943	t	g	34475	2.833	0.004608	+++	0.695	0.413
rs7823971	a	c	34919	-1.886	0.05934	---	0.541	0.265
rs3915060	t	c	34359	-1.664	0.09605	--+	0.560	0.281
rs1566129	t	c	35139	0.784	0.4333	++-	0.602	0.318
rs10901863	t	c	32251	0.638	0.5234	+--	0.510	0.240
rs9321402	a	g	35101	0.58	0.5622	++-	0.649	0.364
rs9677089	a	c	34727	-0.537	0.5915	--+	0.653	0.368

Table S2. Summary statistics for the *HAid* phenotype in the replication meta-analysis of white non-British UKBB sample, TwinsUK and ELSA.
Marker Name, SNP ID; Allele1, the first allele for this marker in the first file where it occurs; Allele 2, the second allele for this marker in the first file where it occurs; Weight, the sum of the individual study weights (N) for this marker; Z-score, the combined z-statistic for the marker; P-value, meta-analysis p-value; Direction, direction of effect for each study ordered: white non-British UKBB sample, TwinsUK, ELSA; Replication power p<0.05, calculated power with replication sample parameters to achieve an association at nominal significance; Replication power p<0.00714, calculated power with replication sample parameters to achieve an association at the significance threshold of (0.05/7 = 0.00714) p<0.00714.

Table S3. Genetic correlation results with *HDiff* phenotype.

Trait	rg	se	p	Group
Wheeze or whistling in the chest in last year	0.3137	0.0306	1.35E-24	Breathing difficulty
Shortness of breath walking on level ground	0.3228	0.0429	5.60E-14	Breathing difficulty
Bring up phlegm/sputum/mucus on most days	0.342	0.0679	4.74E-07	Breathing difficulty
Long-standing illness_ disability or infirmity	0.3763	0.0305	5.48E-35	Health report / subjective wellbeing
Overall health rating	0.3156	0.0266	2.09E-32	Health report / subjective wellbeing
Health satisfaction	0.3405	0.0374	8.47E-20	Health report / subjective wellbeing
Other serious medical condition/disability diagnosed by doctor	0.3203	0.0409	4.80E-15	Health report / subjective wellbeing
Subjective well being	-0.3257	0.0421	1.06E-14	Health report / subjective wellbeing
Other eye problems	0.4311	0.0687	3.59E-10	Health report / subjective wellbeing
Had major operations	0.3196	0.06	9.84E-08	Health report / subjective wellbeing
Illnesses of siblings: None of the above (group 2)	-0.3436	0.0702	9.94E-07	Health report / subjective wellbeing
Former alcohol drinker	0.3136	0.0701	7.56E-06	Health report / subjective wellbeing
Tinnitus: Yes_ now most or all of the time	0.6	0.0562	1.40E-26	Hearing
Loud music exposure frequency	0.3224	0.0583	3.20E-08	Hearing
Frequency of tiredness / lethargy in last 2 weeks	0.4089	0.029	2.79E-45	Low mood /depression
Neuroticism score	0.315	0.0257	1.94E-34	Low mood /depression
Miserableness	0.3283	0.0273	2.69E-33	Low mood /depression
Seen doctor (GP) for nerves_ anxiety_ tension or depression	0.3447	0.0298	5.90E-31	Low mood /depression
Frequency of depressed mood in last 2 weeks	0.3361	0.0318	4.30E-26	Low mood /depression
Guilty feelings	0.3258	0.0309	4.98E-26	Low mood /depression
Loneliness_ isolation	0.3233	0.0333	2.99E-22	Low mood /depression
Frequency of unenthusiasm / disinterest in last 2 weeks	0.3239	0.0348	1.35E-20	Low mood /depression
Frequency of tenseness / restlessness in last 2 weeks	0.3046	0.0336	1.26E-19	Low mood /depression
Ever depressed for a whole week	0.371	0.041	1.45E-19	Low mood /depression
Illness_ injury_ bereavement_ stress in last 2 years: Financial difficulties	0.3098	0.0368	3.70E-17	Low mood /depression
Depressive symptoms	0.3314	0.0409	5.69E-16	Low mood /depression

Ever unenthusiastic/disinterested for a whole week	0.3445	0.0432	1.53E-15	Low mood /depression
Happiness	0.3148	0.0408	1.27E-14	Low mood /depression
Financial situation satisfaction	0.3437	0.0473	3.87E-13	Low mood /depression
Family relationship satisfaction	0.3118	0.0433	6.12E-13	Low mood /depression
Ever highly irritable/argumentative for 2 days	0.3192	0.0457	2.78E-12	Low mood /depression
Insomnia	0.3211	0.0484	3.31E-11	Low mood /depression
Illness_ injury_ bereavement_ stress in last 2 years: Serious illness_ injury or assault to yourself	0.3161	0.0485	7.36E-11	Low mood /depression
Neuroticism	0.3125	0.0724	1.60E-05	Low mood /depression
Chest pain or discomfort	0.3823	0.0341	3.60E-29	Pain
Pain type(s) experienced in last month: None of the above	-0.3225	0.0295	9.62E-28	Pain
Pain type(s) experienced in last month: Neck or shoulder pain	0.3686	0.0367	9.34E-24	Pain
Pain type(s) experienced in last month: Stomach or abdominal pain	0.3409	0.0418	3.73E-16	Pain
Pain type(s) experienced in last month: Hip pain	0.3079	0.0394	5.25E-15	Pain
Mouth/teeth dental problems: Toothache	0.428	0.0699	9.02E-10	Pain
Mouth/teeth dental problems: Painful gums	0.4514	0.0749	1.71E-09	Pain

Table S3. **Genetic correlation results with *HDiff* phenotype**. This table lists traits that had significant correlations with the *HDiff* phenotype and an rg < -0.3 or rg > 0.3. The traits are ordered by group, and by p-value order within each group.