SUPPLEMENTARY MATERIAL

FIGURE S1

A Mapping data from the human deafness family. Genomewide SNP mapping in the deafness family revealed candidate regions with homozygosity by descent on chromosomes 6, 7 and 11. *AHI1* is localized about 5 Mb centromeric of the chromosome 6 candidate locus. **B** Western blot of fibroblast lysates that were probed with polyclonal rabbit IgG antibodies against AHI1. AHI1 isoforms (red arrows) are expressed in fibroblasts from a homozygous mutation carrier, II:4, and in fibroblasts from a healthy control proband (ctrl.). PA5-30901 (left half of the panel) recognizes a region betweeen AHI1 residues 383-596, PA31846 (right half of the panel) recognizes a region between residues 1-231. As internal control, the monoclonal mouse anti ß-actin (Sigma, Cat.-Nr. A2228) antibody recognizing human β-actin was used.

FIGURE S2

A Detail of the family 1 pedigree showing SNP genotyping data of the siblings from genome-wide linkage analysis. Because *AHI1* locates within an HBD region (between SNPs rs2327578 and rs724875) shared by the three deaf individuals (II:3, II:5, II:6) and the healthy person II:4, the p.Arg1066* mutation can be considered truly homozygous in all these individuals. **B** Overview of the family including the parents, showing heterozygous haplotypes in the parents.

FIGURE S3

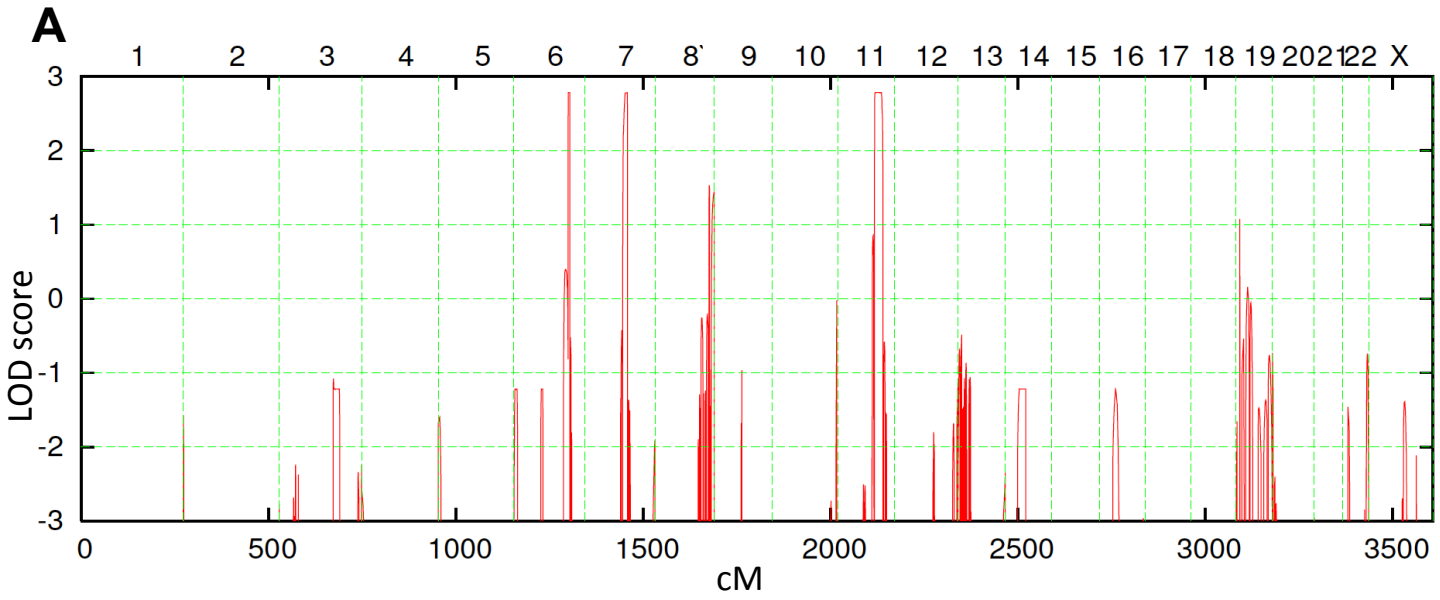
A Evolutionary conservation of the p.Ser671 residue. **B** WD40 repeats of AHI1 as predicted by ScanProsite. Note that ScanProsite predicts only four of the seven WD40 repeats. **C** ScanProsite predicts loss of the WD40 repeat affected by the p.Ser671Leu mutation.

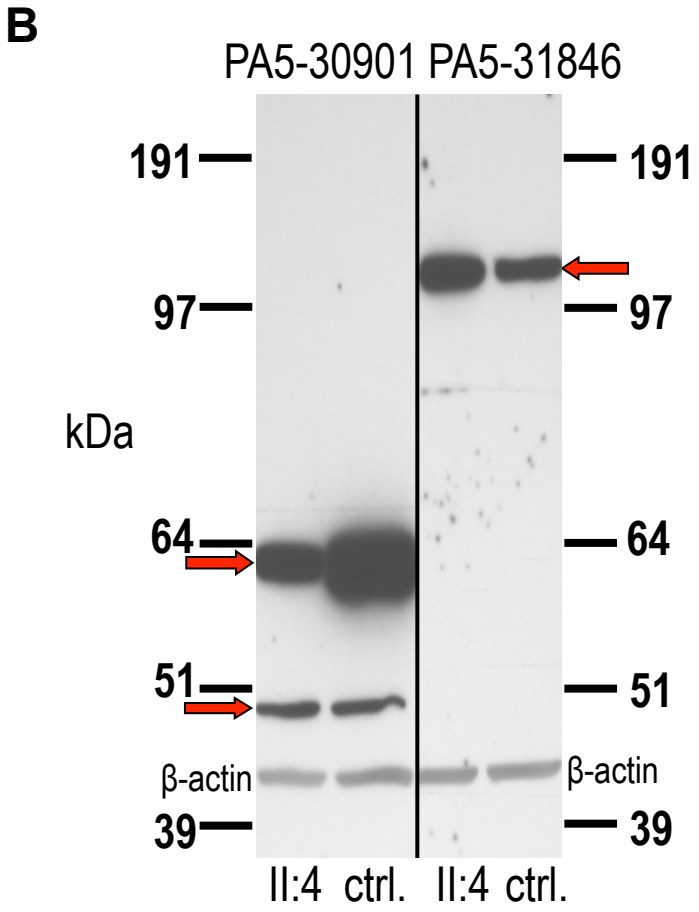
TABLE S1

Secondary structure prediction of AHI1 by the WDSP algorithm. Each WD40 repeat starts from Loop_da, followed by Strand a, Loop_ab, Strand_b, Loop_bc, Strand_c, Loop_cd, and Strand d. Thus, KHLFSL is the Strand-d of WD7. The red residues are involved in hydrogen bond networks.

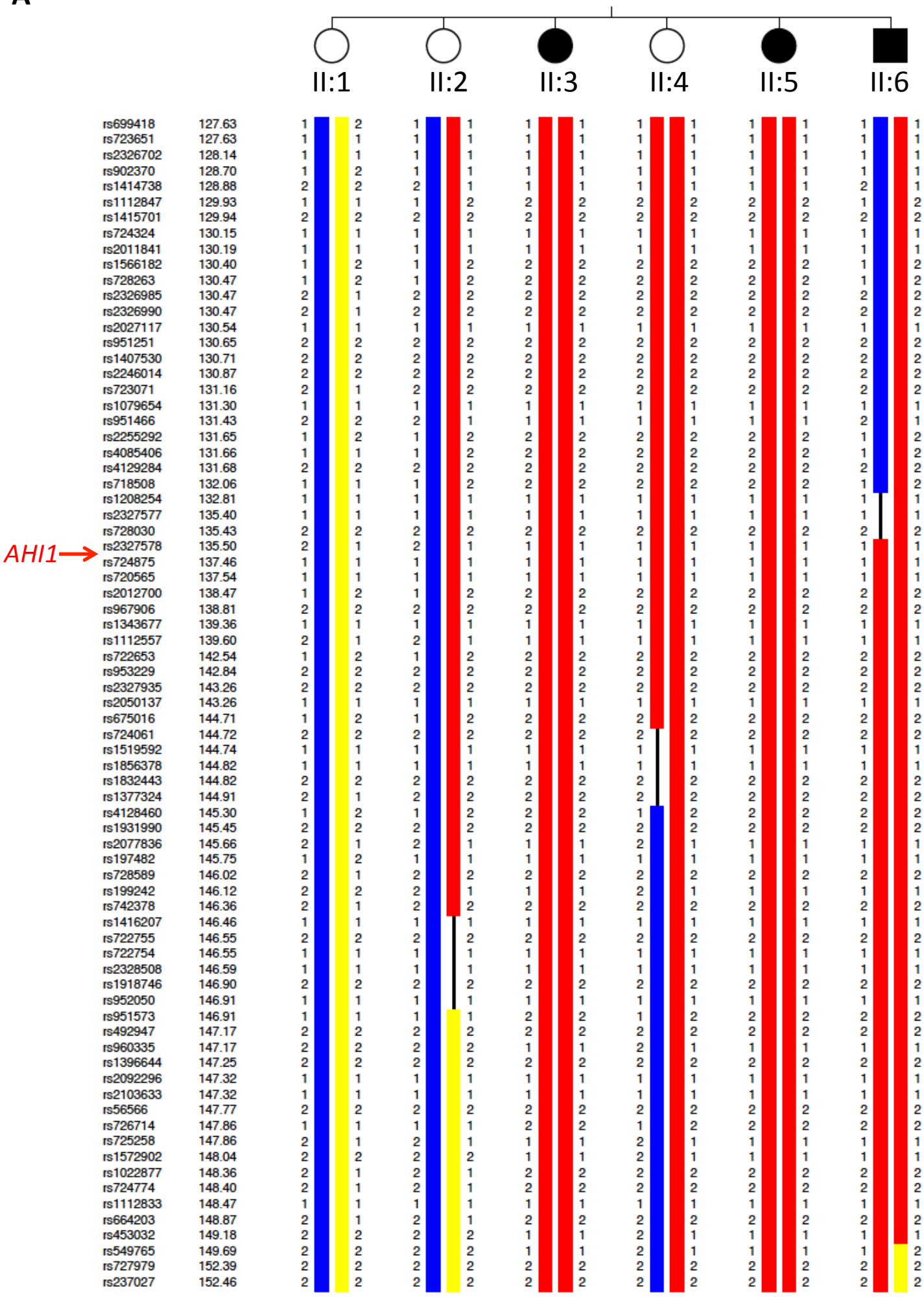
TABLE S2

Secondary structure prediction of TLE1 by the WDSP algorithm.

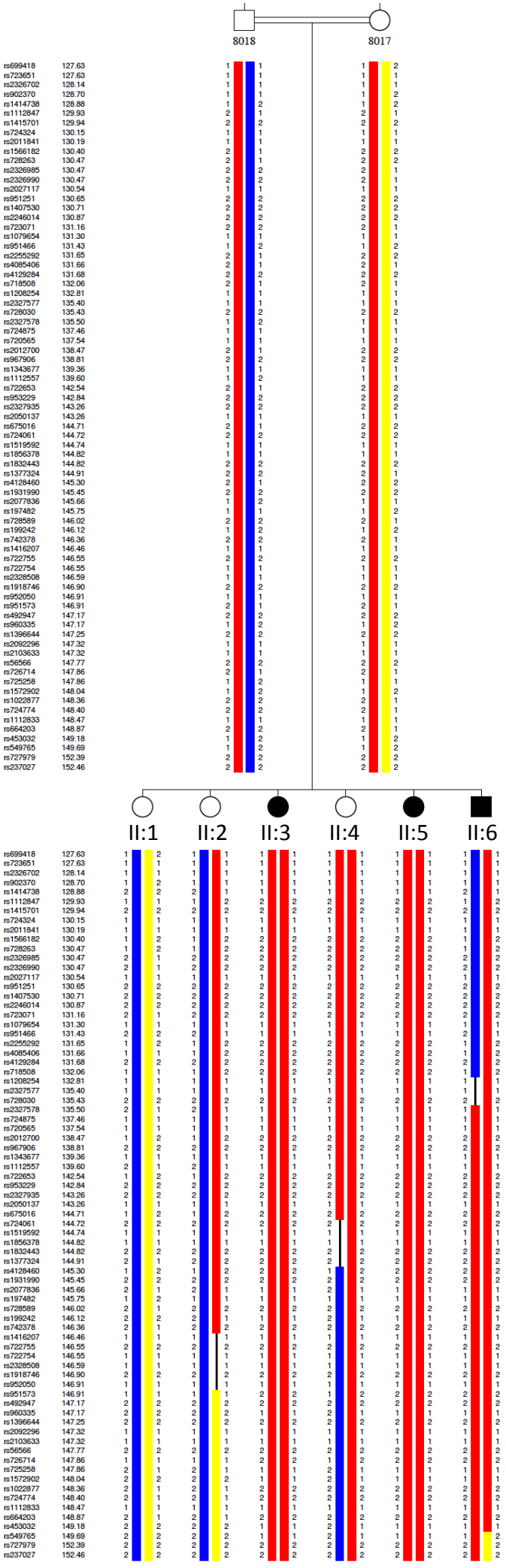

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
LOD score
3
2
1
0
-1
-2
-3
0 500 1000 1500 2000 2500 3000 3500
cM


B
PA5-30901 PA5-31846
191
97
64
51
kDa
β-actin
39
191
97
64
51
β-actin
39
II:4 ctrl. II:4 ctrl.

A



B



A

p.Ser761Leu

```
LCFDTEGHHMYSGDCTGVIVVWN
LCFDTEGHHMYSGDCTGVIVVWN
ICFDDEGHHMYSGDCIGVIVVWD
LCFDPEGSRMFSADNSGLIIVWG
LCFDIEGLQMYSGDS--------
:*** ** :*:*.*
```

Human
Rhesus monkey
Mouse
Zebrafish
African clawed frog

B

C

Table S1. Secondary structure prediction of AHI1 by the WDSP algorithm. Each WD40 repeat starts from Loop_da, followed by Strand a, Loop_ab, Strand_b, Loop_bc, Strand_c, Loop_cd, and Strand d. Thus, KHLFSL is the Strand-d of WD7. The red residues are involved in hydrogen bond networks.

Repeats	Score	Start	End	Strand_d	Loop_da	Strand_a	Loop_ab	Strand_b	Loop_bc	Strand_c	Loop_cd	H_bonds
WD1	76	600	644	KHLFSL	NAGERG	CFCLDF	SHNGR	ILAAAC	ASRDGY	PIILYE	IPSG	NA
WD2	143	645	688	RFMREL	CGHLNI	IYDLSW	SKDDH	YILTSS	SDG	TARIWK	NEINNT	Tetrad
WD3	124	689	734	NTFRVL	PHPSF	VYTAKF	HPAVRE	LVVTGC	YDS	MIRIWK	VEMREDSA	Tetrad
WD4	117	735	788	ILVRQF	DVH737KSF	INSLCF	DTEGH	HMYS761GD	CTG	VIVVW771N	TYVKINDLEHSVHHWT	Triad
WD5	48	789	833	INKEIK	ETEFKGIP	ISYLEI	HPNGK	RLLIHT	KDS	TLRIMD	LRILV	NA
WD6	82	834	877	ARKFVG	AANYREK	IHSTLT	PCGT	FLFAGS	EDG	IVYVWN	PETGEQ	NA
WD7	75	878	919	VAMYSD	LPFKSP	IRDISY	HPFEN	MVAFCA	FGQNEPI	LLYIYD		NA

Table S2. Secondary structure prediction of TLE1 by the WDSP algorithm.

Repeats	Score	Start	End	Strand_d	Loop_da	Strand_a	Loop_ab	Strand_b	Loop_bc	Strand_c	Loop_cd	H_bonds
WD1	107	476	512	RQINTL	NHGEVS	VCAVTI	SNPTR	HVYTGG	KG	CVKVWD	ISHPGNK	Triad
WD2	94	519	559	SPVSQL	DCLNRDNYH	IRSCKL	LPDGC	TLIVGG	EAS	TLSIWD	LAAPTP	NA
WD3	90	565	603	RIKAEL	TSSAPA	CYALAI	SPDSK	VCFSCC	SDG	NIAVWD	LHNQ	NA
WD4	134	607	645	TLVRQF	QGHTDG	ASCIDI	SNDGT	KLWTGG	LDN	TVRSWD	LREG	Tetrad
WD5	71	649	686	RQLQQH	DFTSQ	IFSLGY	CPTGE	WLAVGM	ESS	NVEVLH	VNK	NA
WD6	113	689	727	PDKYQL	HLHESC	VL702SSLKF	AYCGK	WFVS715TG	KDN	LLNAWR	TPYGA	Tetrad
WD7	80	732	768	SIFQSK	ESSS	VLSCDI	SVDDK	YIVTGS	GDK	KATVYE		NA