SUPPLEMENTAL DATA

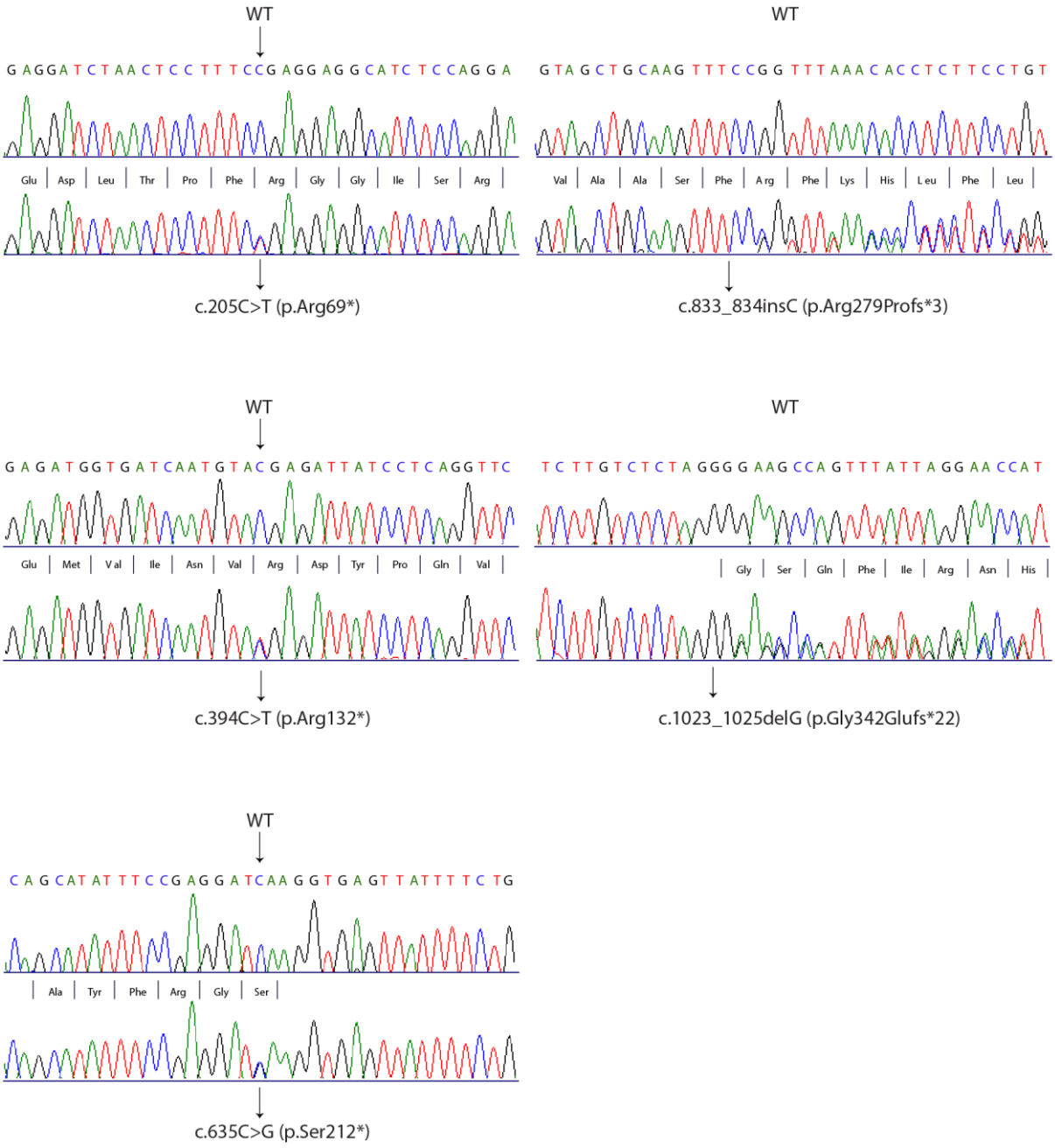

Figure S1 Results of sequencing analyses in *POGLUT1*: Mutations identified in individuals with DDD.

The mutations are given in comparison to the corresponding wild type sequences. Abbreviations: WT, wild type.

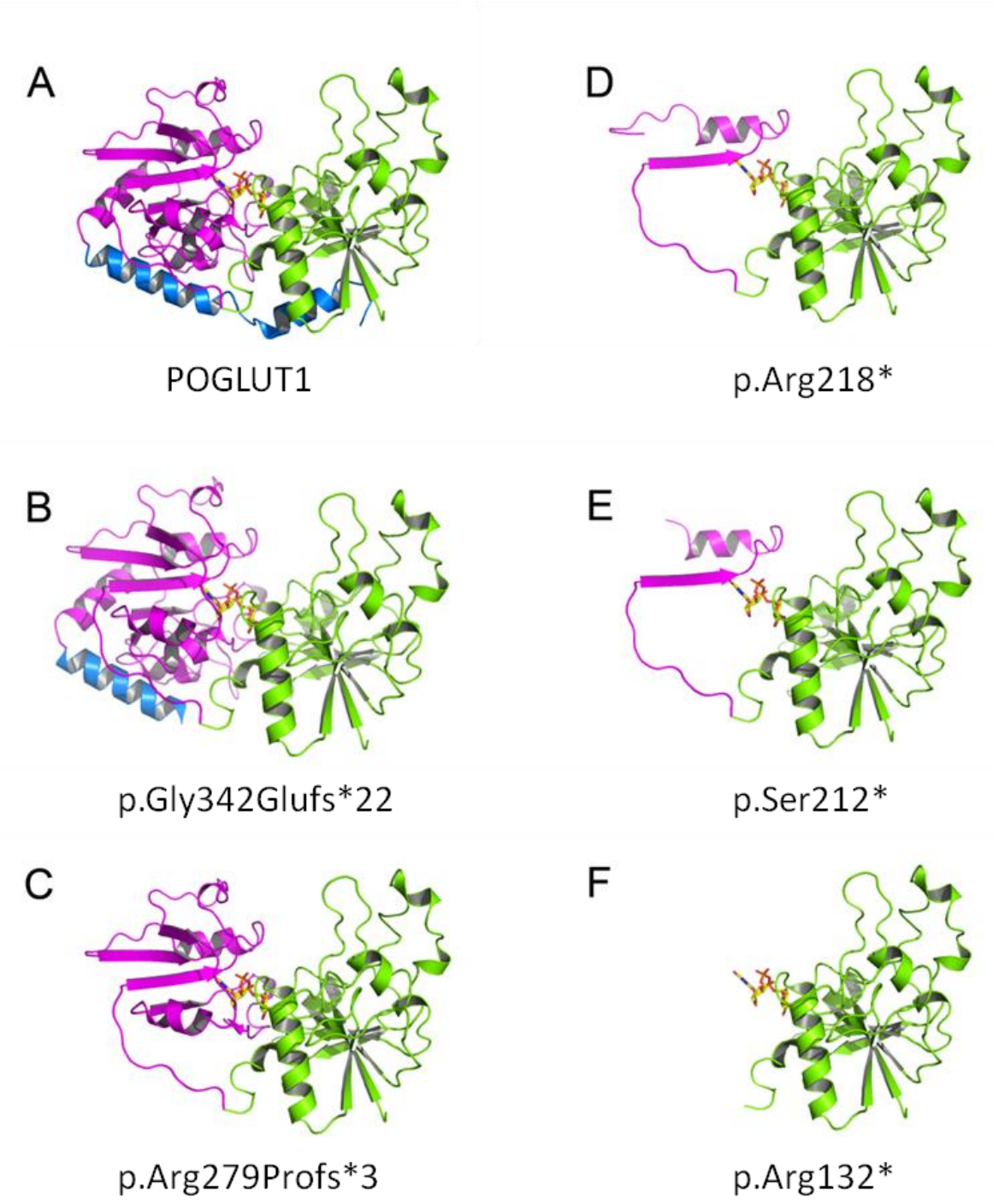

Figure S2 Three-dimensional structure of wild type POGLUT1 and five truncated forms caused by mutations resulting in a stop codon.

(A) Model of full length POGLUT1. The N-terminal domain (residues 1-180) is shown in green, the C-terminal domain (residues 181-349) is shown in magenta. The C-terminal helix (residues 350-384) connecting the two domains is shown in blue. UDP-glucose is shown in yellow.

(B) p.Gly342Glufs*22 results in the loss of the C-terminal helix, that extends into the N-terminal domain, most likely destabilizing the arrangement of the two domains and the substrate binding site.

(C) p.Arg279Profs*3 leads to the loss of the essential Arg279 as well as the C-terminal helix.
(D-F) Similarly, p.Arg218*, p.Ser212* and p.Arg132* result in a largely truncated form of POGLUT1 with partial or almost complete loss of the C-terminal domain.

A

GTCAGCAGCACAGTGGCCATGGAAAAAGAAAAACTC***TACAGCATATTTCCGAG***	Exon 6
GATCAAGGACAAGTCCAGAACGAGATCCTCTCATTCTTCTGTCTCGGAAAAAC	Exon 7
CCAAAACTTGTTGATGCAGAATACACCAAAAACCAGGCCTGGAAATCTATGAA	
AGATACCTTAGGAAAGCCAGCTGCTAAGGATGTCCATCTTGT**GGATCACTGCA**	Exon 8
AATACAAGTATCTGTTTAATTTTCGAGGCGTAGCTGCAAGTTTCCGGTTTAAA	Exon9
CACCTCTTCCTGTGTGGCTCACTTGTTTTCCATGTTGGTGATGAGTGGCTAGA	
ATTCTTCTATCCACAGCTGAAGCCATGGGTTCACTATATCCCAGTCAAAACAG	
ATCTCTCCAATGTCCA**AGAGCTGTTACAATTTGTAAAAGCAAATG**ATGATGTA	Exon 10
GCTCAAGAGATTGCTGAAAGGGGAAGCCAGTTTATTAGGAACCATTTGCAGAT	Exon 11
G***GATGACATCACCTGTTACTGG***GAGAACCTCTTGAGTGAATACTCTAAATTCC	
TGTCTTATAATGTAACGAGAAGGAAAGGTTATGATCAAATTATTCCCAAAATG	
TTGAAAACTGAACTATAG	

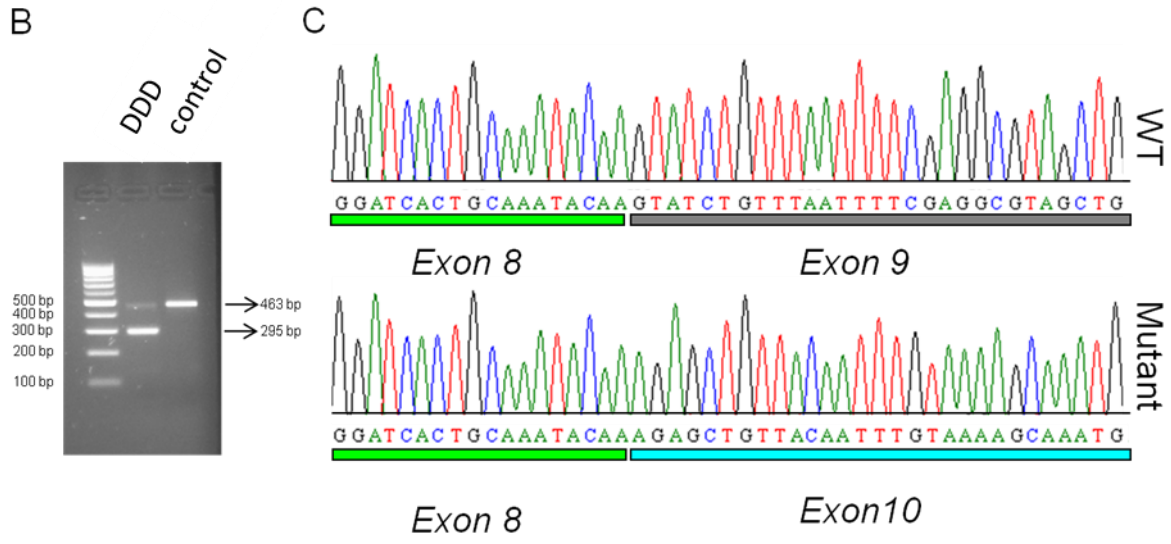

Figure S3 Analysis of the splice site mutation c.798-2A>C by total RNA isolation from blood.

Total RNA was isolated from blood collected in PAXgene Blood RNA Tubes by the use of the PAXgene Blood RNA Kit (Preanalytix, Hombrechtikon Switzerland). Total mRNA from the individual carrying the c.798-2A>C mutation and a control individual were reverse transcribed into cDNA with the use of the oligo(dT) primers and SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Darmstadt, Germany). Primers designed to bind regions in exons 6 and 11 were used for generating an amplicon of 463 bp by standard PCR. The PCR products were visualized by gel electrophoresis. The DNA bands were cut from the agarose gel and purified by Wizard SV Gel and PCR Clean-up System (Promega, Mannheim, Germany). The purified DNA was sequenced using the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA). (A) The coding sequence is given for

exons 6-11. Primers were designed to bind exon 6 and 11 (bold, italic sequences) to amplify a region of 463 bp. The bold underlined sequences in exons 8, 9 and 10 are observed in the electropherograms given in C. (B) Gel electrophoresis showing the wild type (463 bp) and mutated (295 bp) transcripts identified in the individual with DDD in comparison to the healthy control individual with only the wild type transcript. (C) The electropherograms showing the sequence of the wild type and mutant transcripts. In the mutated transcript exon 9 is abolished and exon 8 is followed directly by exon 10.

Abbreviations: WT, wild type

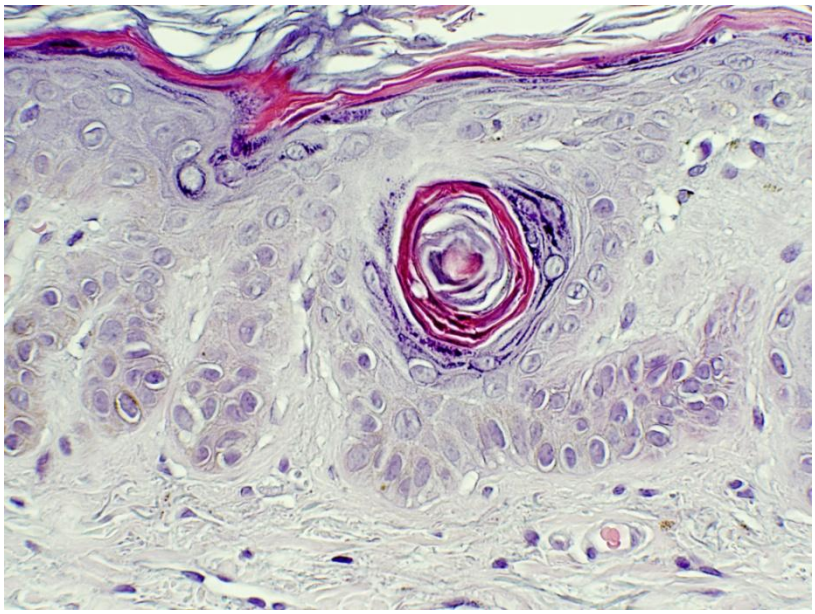

Figure S4 Typical histological findings of an individual affected by DDD with a *POGLUT1* mutation.

Typical histological findings seen in our affected individuals include digitiform reteacanthosis, pronounced hyperpigmentation at the tips of the rete ridges, small horn cysts, minor acantholysis and focal hypergranulosis.

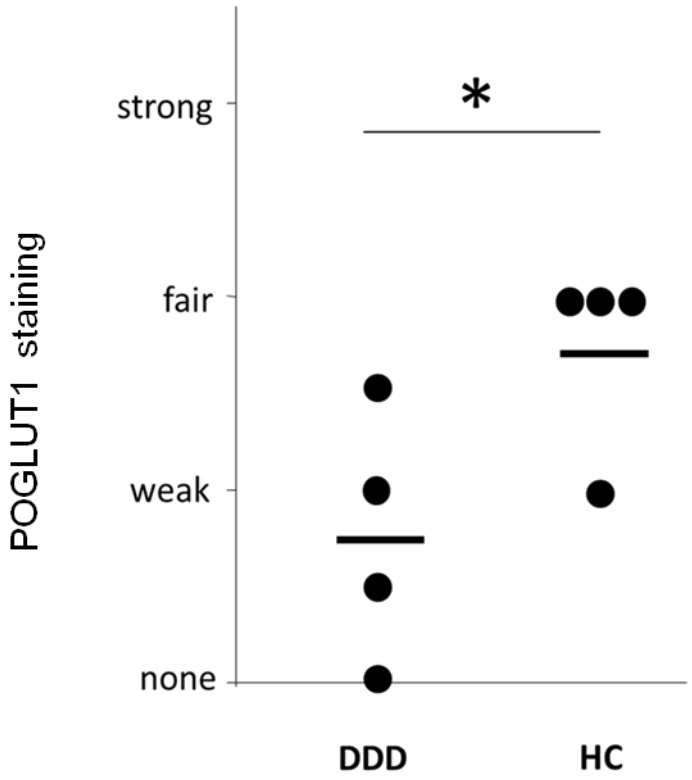

Figure S5 POGLUT1 in skin biopsies of healthy controls and individuals with DDD.

The mean staining intensity of POGLUT1 in the epidermis of individuals with DDD (n=4) and healthy controls (n=4) are given. The POGLUT1 staining was significantly weaker in lesional skin of individuals suffering from DDD (mean staining intensity, 1.75±0.25 SEM versus 0.75±0.32 SEM, $p<0.05$, Mann-Withney-U Test).

Abbreviations: HC, healthy control; SEM, standard error of the mean

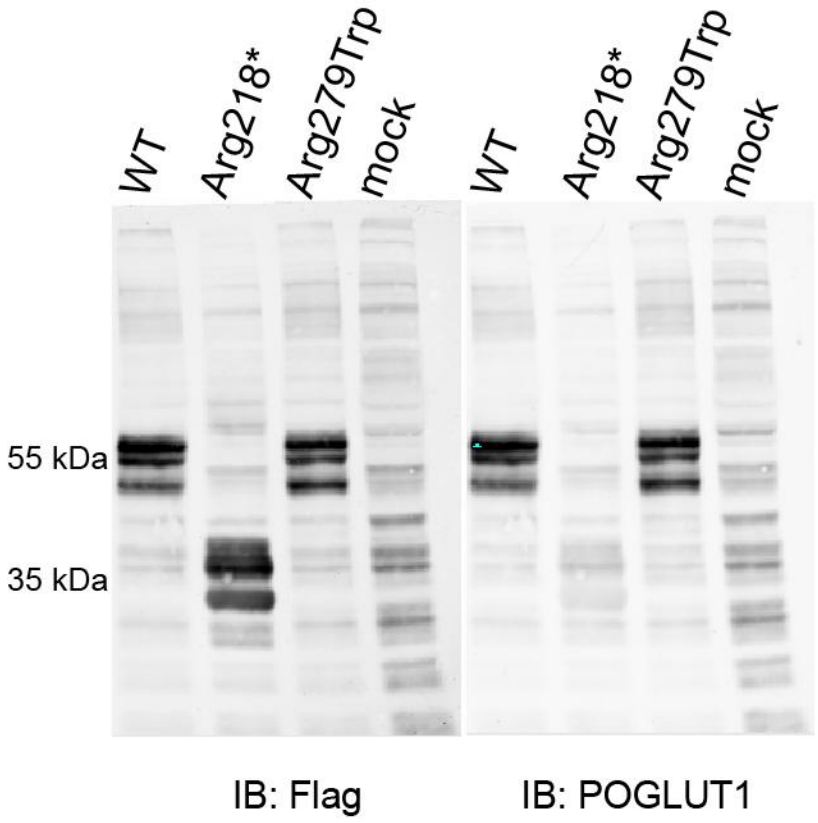

Figure S6 Immunoblot analysis of wild type and mutant POGLUT1.

POGLUT1, wild type and two of the identified mutants (p.Arg218* and p.Arg279Trp), fused to a C-terminal Strep/FLAG Tandem Affinity-tag (C-TAP-constructs) was expressed in HEK293T cells and analyzed by immunoblotting. Bands of the expected size were detected for the wild-type and the p.Arg279Trp variant, whereas the p.Arg218* truncation resulted in a protein of around 30 kDa. Two bands corresponding to higher molecular weights were observed which can be attributed to different posttranslational modifications of the protein.

Abbreviations: IB, immunoblot; WT, wild type; kDa, Kilodalton

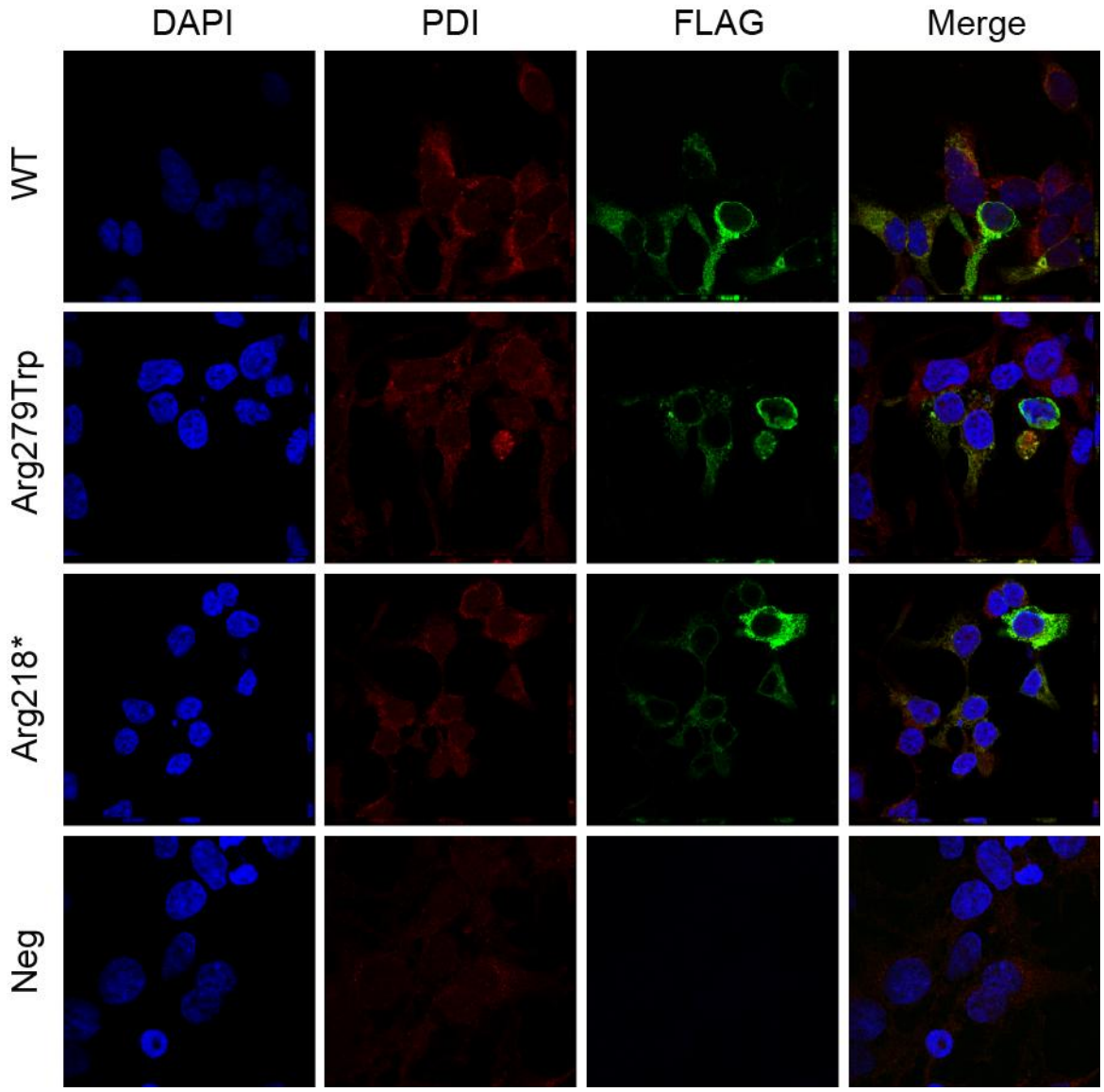

Figure S7 Immunofluroescence analysis of the C-TAP-POGLUT1 fusion proteins.

C-TAP-POGLUT1 variants were expressed in HEK293T cells and analyzed by immunofluorescence and confocal microscopy. No difference was observed in the subcellular localization of the wild type and mutant proteins.

Abbreviations: WT, wild type; neg, negative; DAPI, 4',6-diamidino-2-phenylindole; PDI, protein disulfide isomerase

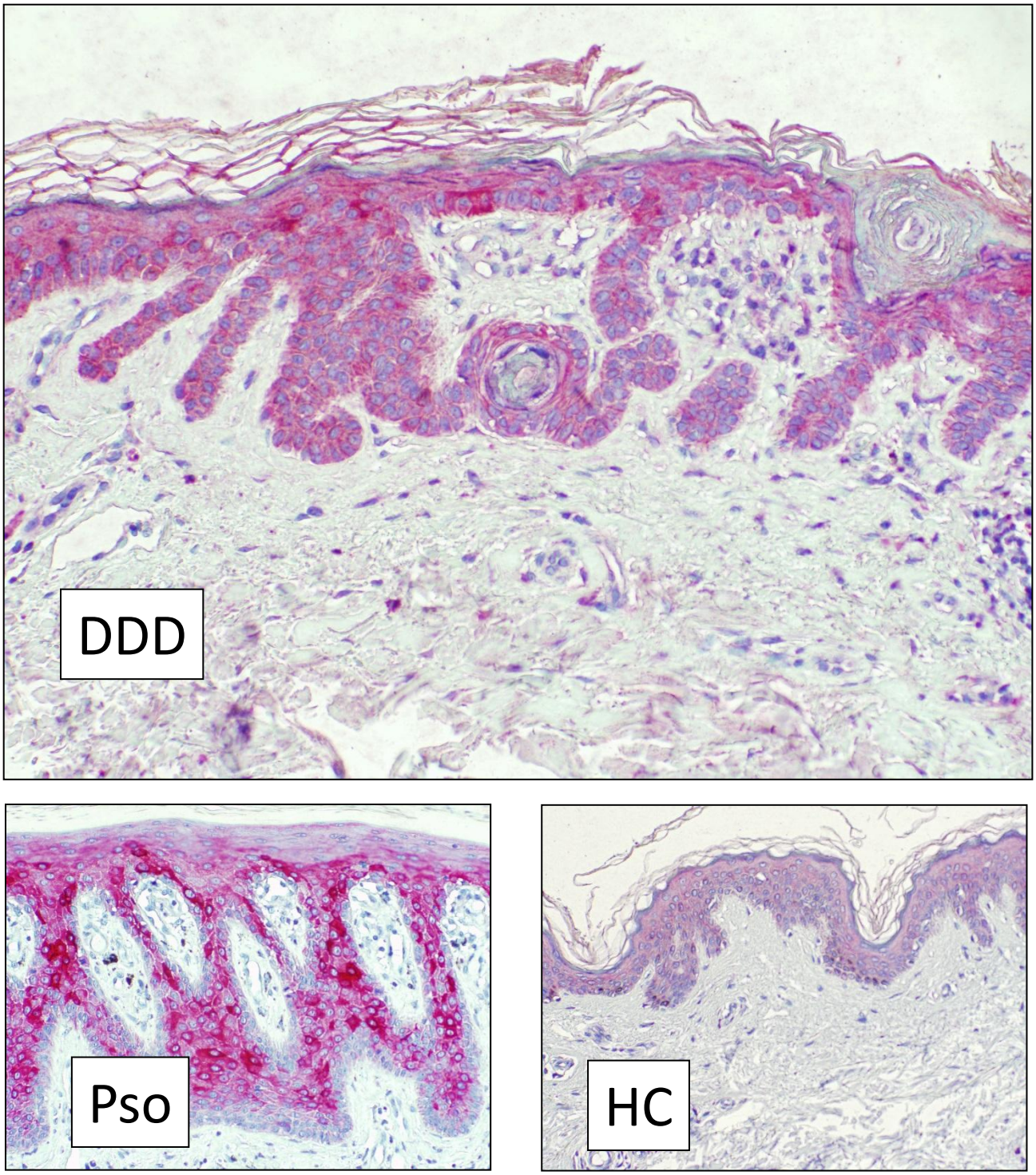

Figure S8 KRT5 in skin biopsies of individuals with DDD or psoriasis and healthy controls.

Immunohistological analyses of KRT5 in different skin conditions, using a monoclonal anti-KRT5 antibody (abin 614389) and REAL detection system with FAST-RED as chromogen. Depicted are representative findings in skin samples taken from individuals with DDD, psoriasis (Pso) and healthy controls (HC; n=4 resp.). Original magnification x200.

Table S1 Pipeline and filter parameters used for exome sequencing

Filter Parameter	Values
Reads variation allele frequency range	25-100%
Maximal number of allowed variations per gene	10
Gene burden fraction	60-100%
Maximal number seen in Epilepsy InhouseDB (n=511)	2
Maximal population variation frequency (dbSNP build 135; 1000genomes build 20110521; EVS build ESP6500)	0.5%
Minimal read coverage	15
Minimal variation quality	25
Maximal target distance	100
Transcript biotypes	protein_coding
Variation locations	COMPOSED;GENES;INTRON;CDS
Consequence types	Protein structure affected;Strong 5'SS/3'SS effects

For the data analysis, the Varbank pipeline v.2.1 and interface was used. Primary data were filtered according to signal purity by the Illumina Realtime Analysis (RTA) software v1.8. Subsequently, the reads were mapped to the human genome reference build hg19 using the BWA[1] alignment algorithm. GATK v.1.6[2] was used to mark duplicated reads, to do a local realignment around short insertions and deletions, to recalibrate the base quality scores and to call SNPs and short Indels.

Scripts developed in-house at the Cologne Center for Genomics were applied to detect protein changes, affected donor and acceptor splice sites, and overlaps with known variants. Acceptor and donor splice site mutations were analyzed with a Maximum Entropy model[3] and filtered for effect changes. In particular, we filtered for high-quality, rare autosomal variants. We also filtered against an inhouse-database containing variants from 511 exomes from individuals with epilepsy to exclude pipeline related artifacts (MAF<0.004). We then focused on genes with the highest burden of rare functional relevant variants.

Table S2 Primers used for sequencing of *POGLUT1* exons

Exon	Forward Primer (5‘-3‘)	Reverse Primer (5‘-3‘)	Amplicon (bp)
1	CAGCCTTCTCAGGGAAACTC	AATGGGTTACTCTCCAGGGTC	470
2	AATTCCTCCCTAGCGTTTCG	CTTGCCTTTCTGACCACTGC	339
3	GCTGCTCATTCTCACTCTCG	GGAGGGATCTGCTTGTTAGTC	384
4	GACTGATGATATGGAACACTGG	CCTCATCCTTACCATTATCCATC	365
5	GCTGACCTTTAATGTGATTCTG	AAATACTGACTGAGCACAGGG	1227
6	AATCTTCAGAAATGGTTAAAGCAC	CATCACATAATCAATGACCGAC	417
7	TTGTGCAGAGTCATTGCATTG	ACATTCACCTCACCACTGAC	319
8	TCTATTTAGAGCCACACTGAC	AGTGCATTTAGGCCCTGAGG	425
9	AACAACCTGTAGTCCTAGCC	TAGCCTACTTGGATAATAATGC	548
10	GGGTATCTTTAATCAGTGTGTGG	CCCAGGACAGGTTTAGTTCTG	400
11	TGTGGATATGATTGCCATAGG	TCAAGGTATAGGTGCCAAGC	409

Table S3 Primers used for cloning of POGLUT1

Plasmid name	Primer	Primer sequence (5’-3’)	Restriction enzyme
pAAV-CMV-N-TAP-POGLUT1	N-TAP	Fw: GCGGAATTCACCATGGATTATAAAGATGATGATG	EcoRI
		Rev:GCGGGATCCTGGTCCTGGTTTCTCGAACTGCGGGTG	BamHI
	POGLUT1	Fw:GCGGGATCCATGGAGTGGTGGGCTAGCTCGC	BamHI
		Rev:GCGGTCGACTCATAGTTCAGTTTTCAACATTTTGG (WT) Rev:GCGGTCGACTCATTCTGGACTTGTCCTTGATCC (R218*)	SalI
pAAV-CMV-POGLUT1-C-TAP	C-TAP	Fw:GCGGTCGACCCCGGACCCTGGAGCCACCCTCAGTTC	SalI
		Rev: GCGAAGCTTTCATTTATCATCATCATCTTTATAATC	HindIII
	POGLUT1	Fw:GCGGGATCCACCATGGAGTGGTGGGCTAGCTCGC	BamHI
		Rev:GCGGTCGACTAGTTCAGTTTTCAACATTTTGG (WT) Rev: GCGGTCGACTTCTGGACTTGTCCTTGATCC (R218*)	SalI

Table S4 Primers used for mutagenesis

Primer	Primer sequence (5'-3')
1	Fw: GGC GTA GCT GCA AGT TTC TGG TTT AAA CAC CTC TTC C
	Rev: GGA AGA GGT GTT TAA ACC AGA AAC TTG CAG CTA CGC C
2	Fw: GAG GCG TAG CTG CAA GTT TCT GGT TTA AAC ACC TCT TCC TG
	Rev: CAG GAA GAG GTG TTT AAA CCA GAA ACT TGC AGC TAC GCC TC
3	Fw: CGA GGC GTA GCT GCA AGT TTC TGG TTT AAA CAC CTC TTC CTG
	Rev: CAG GAA GAG GTG TTT AAA CCA GAA ACT TGC AGC TAC GCC TCG

Supplemental references

1. International Multiple Sclerosis Genetics Consortium (2009). The expanding genetic overlap between multiple sclerosis and type I diabetes. Genes Immun 10, 11-14.
2. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., et al. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20, 1297-1303.
3. Yeo, G., and Burge, C.B. (2004). Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. J Comput Biol 11, 377-394.