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Supplemental Data

ELOVL5 Mutations Cause Spinocerebellar Ataxia 38

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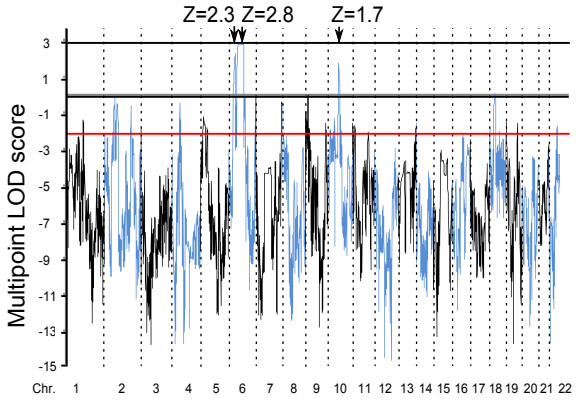
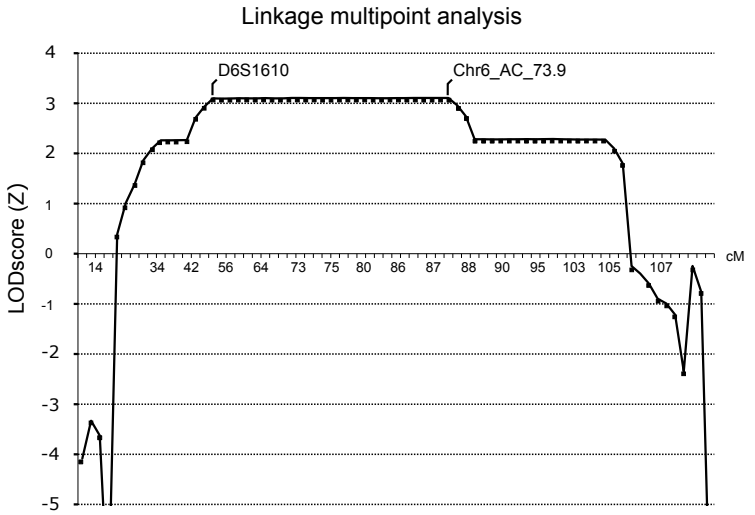
Figure S1

A



B



C

Haplotype analysis

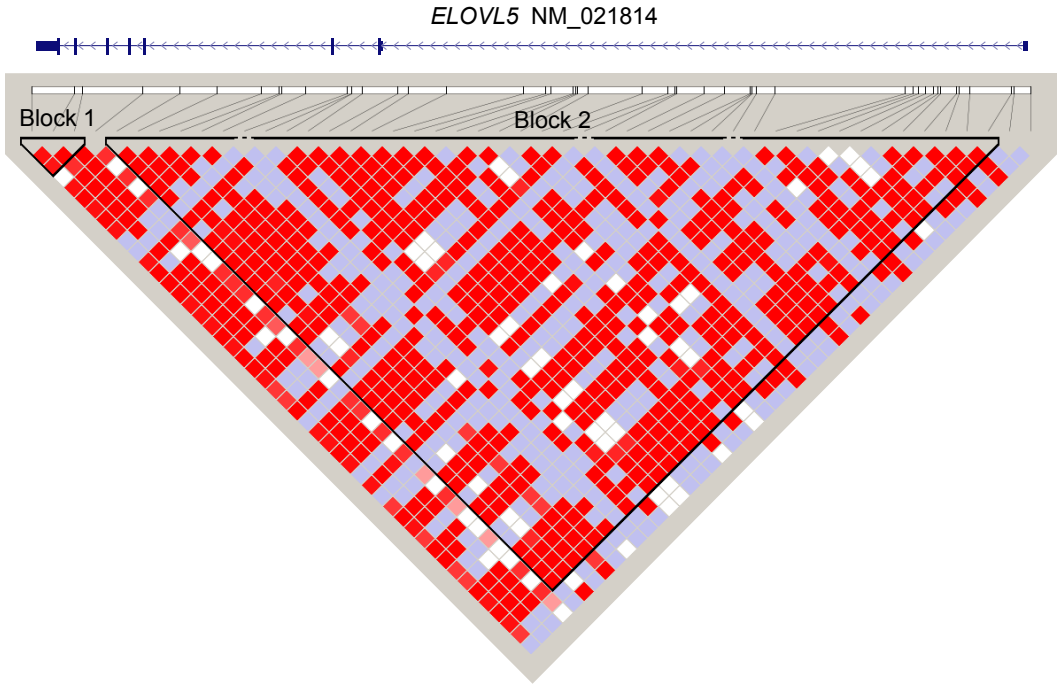
	1/III-6	3/III-4	2/IV-8	
D6S282	2	2	2	
D6S452	4	4	1	
D6S272	3	3	2	
SNP Haplotype	4	4	4	*ELOVL5*
D6S1573	3	3	1	
D6S257	2	2	2	
Chr6_TG69	3	3	1	

Figure S1. Genetic linkage and haplotyope analysis of SCA38 families.

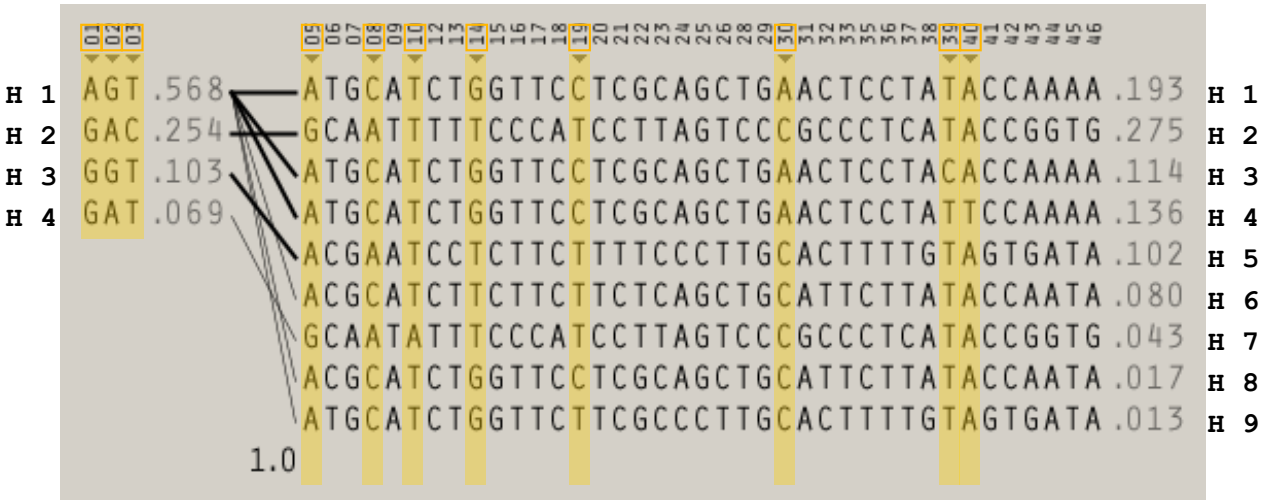
Panel A displays multipoint linkage analysis between markers D6S289 and D6S434 in family SCA38-01-BS. Genotypes were assigned using the Bead Studio genotyping module software (Illumina) and multipoint linkage analysis was performed using Merlin ver 1.0 under an autosomal dominant model with a gene frequency of 0.0001, equal allele frequencies for all markers and a penetrance set at 0.8. Panel B. Two point (not shown) and multipoint genetic linkage analyses were performed with MLINK and GeneHunter software in family SCA38-01-BS. Calculations were performed using three liability classes (95% at a ge >70 yrs.; 85% at age >50 years; 80% at age >40 yrs.) and disease frequency of 0.0001.A significant multipoint LOD score (Zmax = +3.08) was found between markers D6S1610 and Chr6_AC_73.9. cM: centi Morgan. Panel C shows the haplotype of ELOVL5 surrounding genomic region. Families 1 and 3 share the same STR haplotype, and all three share a common SNP haplotype.

Figure S2

A



B



C

	BLOCK 1			BLOCK 2		
	01 02 03	H	F	05 08 10 14 19 30 39 40	H	F
1/III-6	A G T	1	.568	A C T G C A C A	3	.114
	A G T	1	.568	A C T G C A T T	4	.136
3/III-4	A G T	1	.568	A C T G C A T T	4	.136
	A G T	1	.568	A C T T T C T A	6	.080
2/IV-8	A G T	1	.568	A C T G C A T A	1	.568
	A G T	1	.568	A C T G C A T T	4	.136

Figure S2 SNP genotype analysis.

SNP genotype data referred to Tuscan population in Italy (TSI) were downloaded from the HapMap database (Rel 27,Phase II+III, NCBI B36 assembly). Linkage disequilibrium (LD) profile (A) and haplotypes (B) of the ELOVL5 locus (chr6:53,240,155-53,321,901) wass assessed using HalpoView (ver.4.1). The eleven TagSNPs identified using HaploView default analysis (panel B, highlighted in yellow) were genotyped by direct sequencing or by restriction fragment length analysis. Inferred haplotypes for one subject of each family are reported in panel C. Haplotype 4 (H4) of the block 2 segregates with the c.689G>T mutation.

Figure S3

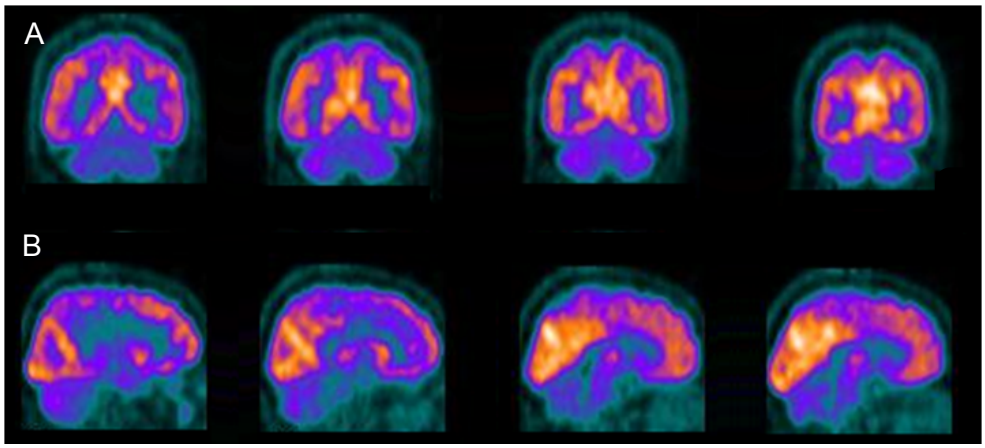


Figure S3. Brain FDG-PET of a SCA38 affected subject.
Brain Fluorodeoxy glucose (FDG)- Positron Emission Tomography (PET) study in the affected subject III-10 of family SCA38-BS-01 demonstrates a significant hypometabolism in the cerebellum . Coronal (A) and sagittal (B) sections are reported.

Figure S4

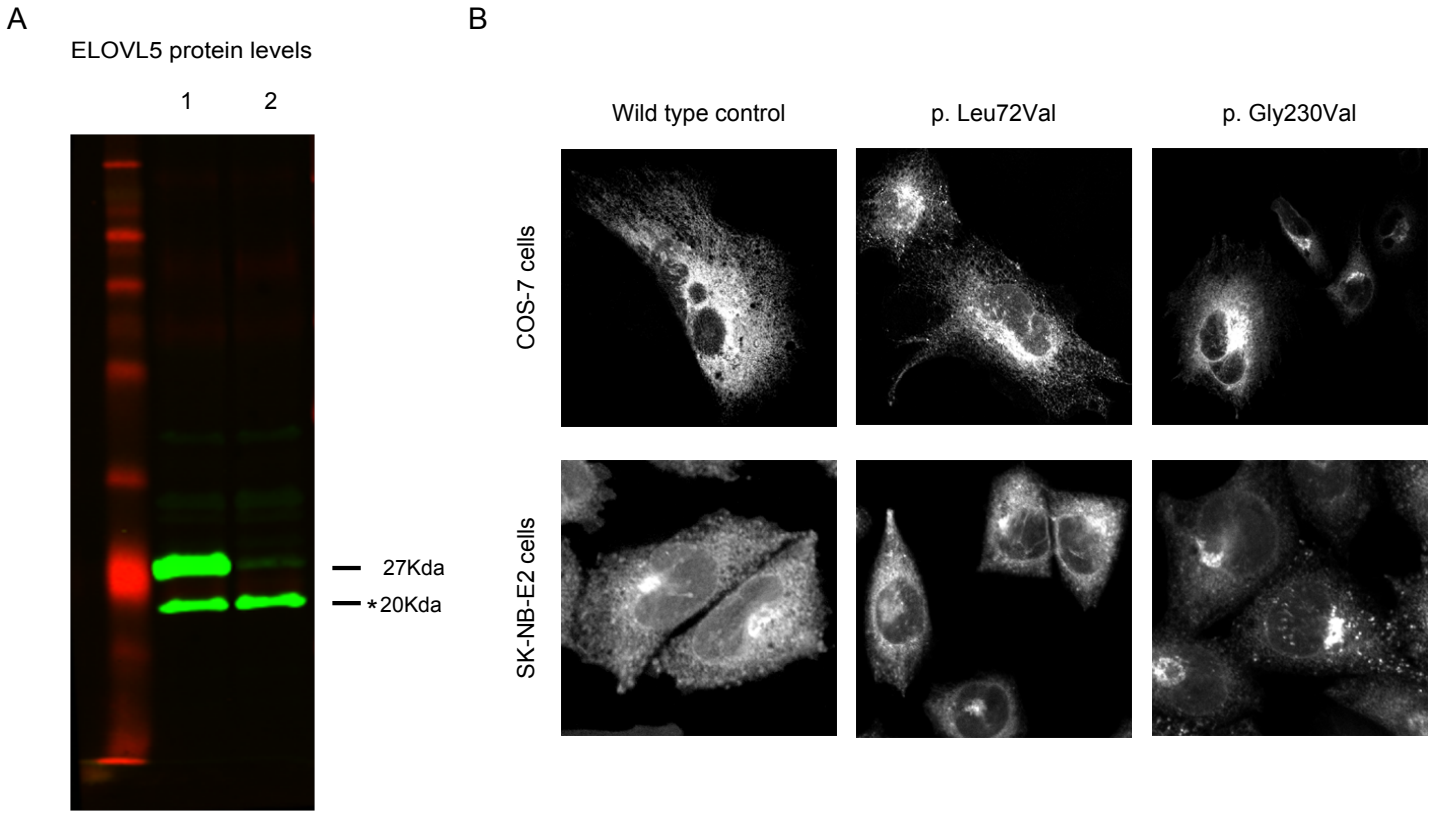


Figure S4. Elovl5 protein levels in wild-type and knockout mice and subcellular localization of the altered ELOVL5 in cells.
Panel A shows a Western blot from liver homogenates of wild-type and *Elovl5*-/- stained with the Antibody #C15621 (Assay bYoTech) * indicates an uncharacterized protein signal. Lanes 1 and 2 are wild-type and knockout mice respectively. Panel B shows cells (COS-7 and SK-N-BE2) transfected with the wild type and mutated *ELOVL5* cDNAs (p. Leu72Val or p. Gly 230 Val).

Figure S5

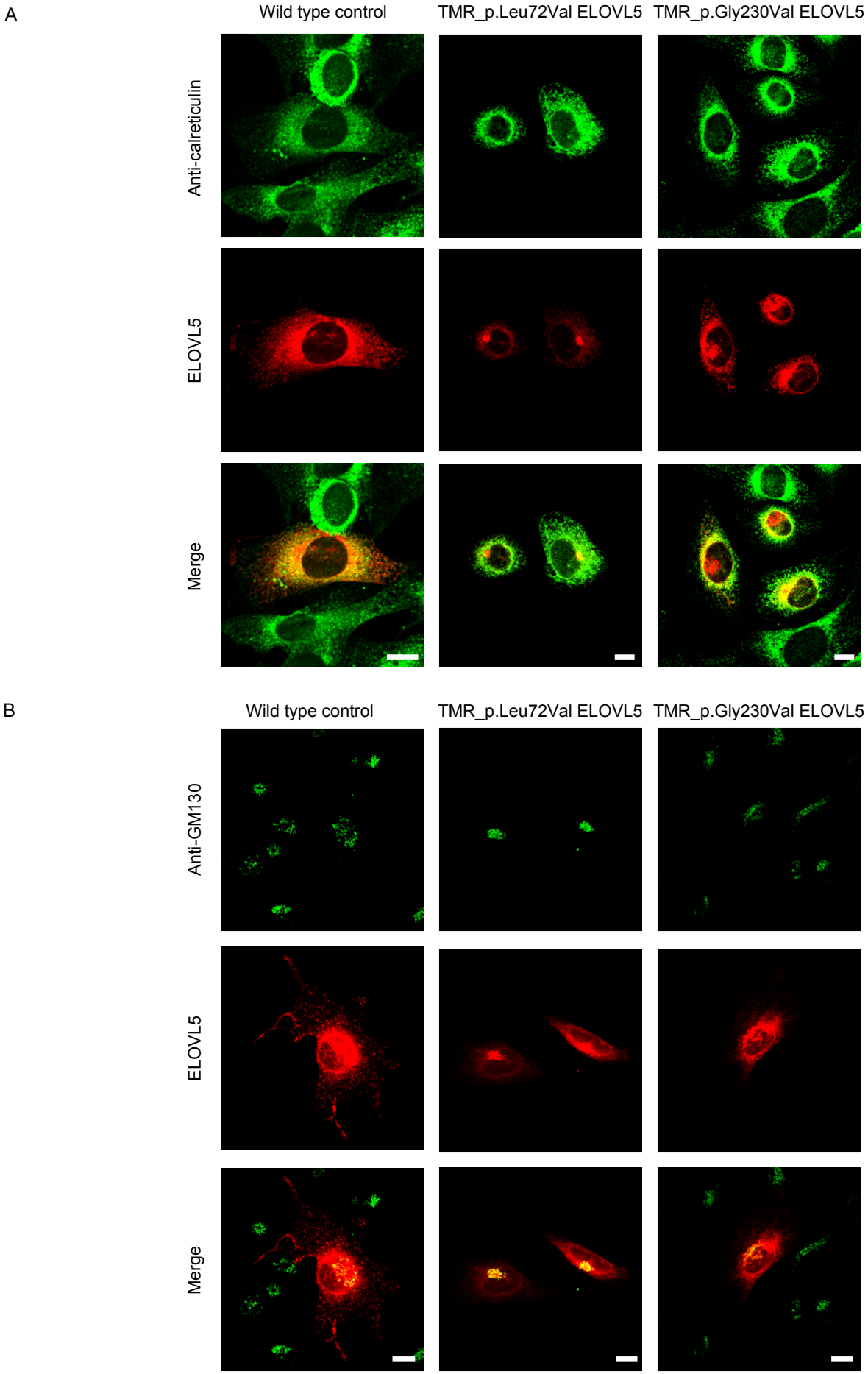


Figure S5 Subcellular localization of altered ELOVL5 in NIH/3T3 cells.
Panel A and B show NIH/3T3 cells transfected with the wild-type and mutated *ELOVL5* cDNAs (p.Leu72Val and p.Gly230Val). For details see legend of Figure 3.

Figure S6

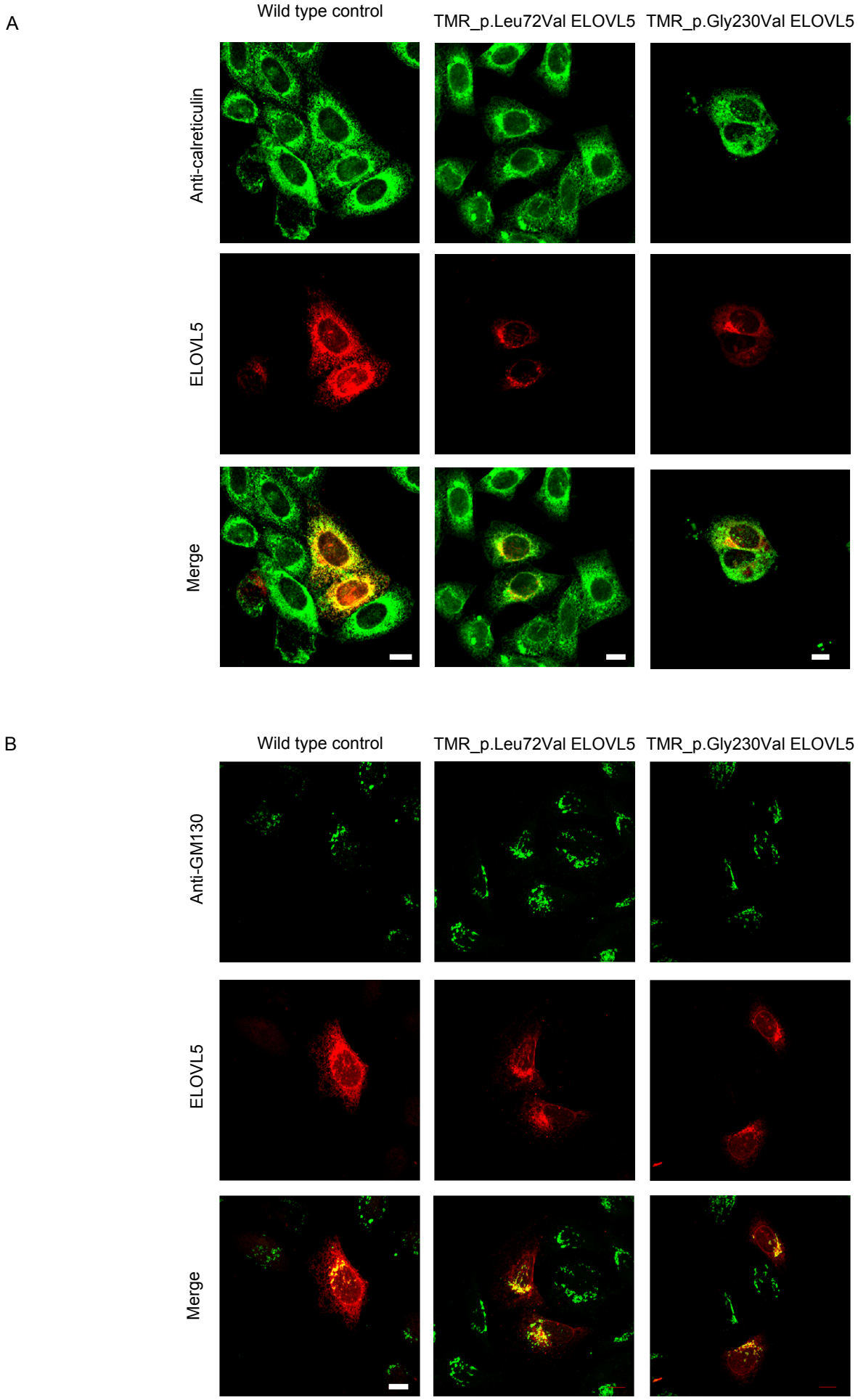


Figure S6 Subcellular localization of altered ELOVL5 in HeLa cells.
Panel A and B show HeLa cells transfected with the wild-type and mutated *ELOVL5* cDNAs (p.Leu72Val and p.Gly230Val). For details see legend of Figure 3.

Figure S7

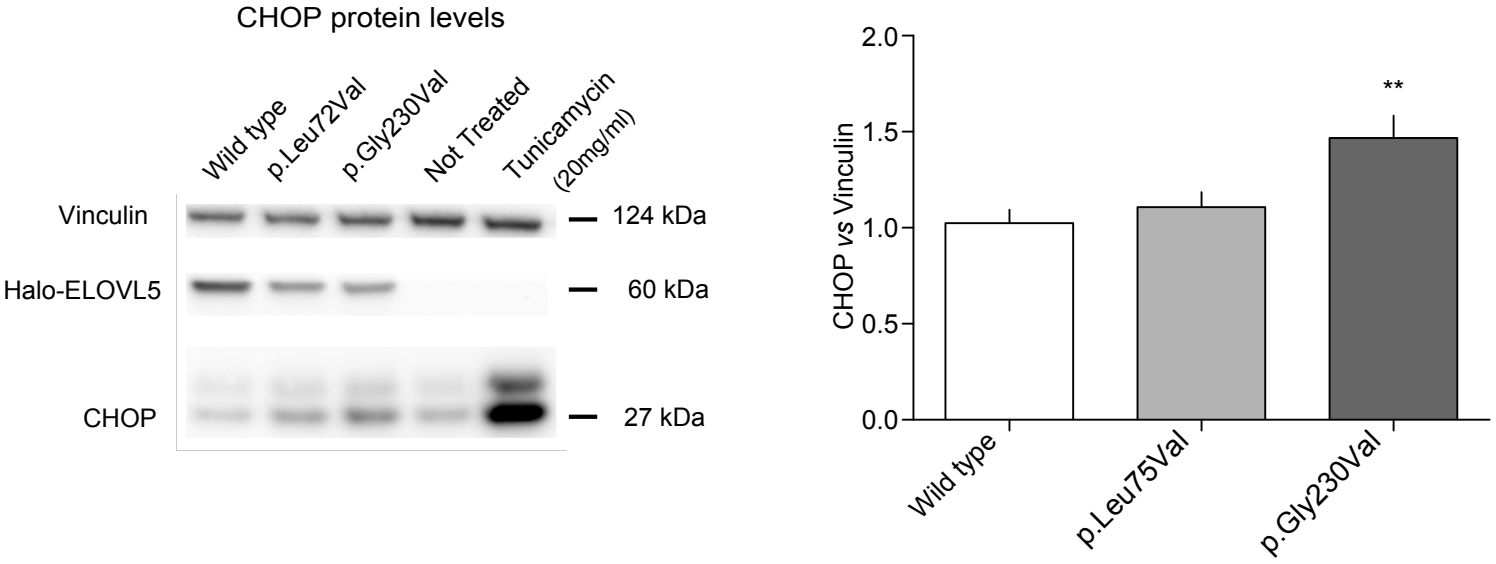


Figure S7. CHOP is enhanced in COS-7 p.Gly230Val transfected cells.

Cell lysates were collected after 48 hours of transfection with the wild-type or mutated *ELOVL5* cDNAs (p.Leu72Val and p.Gly230Val). Western blots were performed using a pre-cast Nu-PAGE 4-12% Bis-Tris gel (Life Technologies) and decorated with CHOP (D46F1, Cell Signaling), Vinculin (AB6039, Millipore), HaloTag (G9281, Promega) using chemioluminescence. The panel shows a statistically significant increase of CHOP protein levels in cells overexpressing p.Gly230Val ELOVL5 but not in those with the wild-type protein (mean ± S.E.M.; **p=0.002, one-tailed unpaired t-test). Using the p.Leu72Val ELOVL5 the increase was not significant.

Western blots from left to right: total protein lysate from COS-7 transfected cells with wild-type, p.Leu72Val and p.Gly230Val ELOVL5; untransfected cells; Tunicamycin (T7765, Aurogene) treated cells (20 mg/ml for 4 hrs at 37°C)(positive UPR control). CHOP band intensities were acquired using a Chemidoc apparatus (Biorad), and analyzed using Image Lab 3.0 (Biorad). CHOP was normalized against Vinculin (see histograms).

Table S1. Microsatellite markers used in linkage analysis

Microsatellite marker	Primer
Chr6_TG69_F	5’-gtctcacccacgttgttgtaaatg
Chr6_TG69_R	5’-acatcgaagagatatcagcactcct
Chr6_TG73_F	5’-cacaagaatggctcactacagcttg
Chr6_TG73_R	5’-ggctcagcatggtggctcat
Chr6_AC73_9_F	5’-aagacattgtcaatccttttaagcaa
Chr6_AC73_9_R	5’-ttgagacttaacgaggcctaaggag
Chr6_AC76_F	5’-aaggcatgcatagtttgattacttca
Chr6_AC76_R	5’-ctgagatgggatgttgtaattcctg
Chr6_AC97_F	5’-tagaggcaaagaacctttgggattc
Chr6_AC97_R	5’-tactctcaacaggcagcatgaaatc
Chr6_GT97.4_F	5’-tttagctgtggagagttgccttttg
Chr6_GT97.4_R	5’-ggagtgattctctgcagaatgagga
Chr6_GT99_F	5’-atccctttctgatgtttagcttgg
Chr6_GT99_R	5’-tctgatggagaataaatgaggacca
Chr6_TG100_F	5’-gggttaccgggtgtaagtttaacag
Chr6_TG100_R	5’-tcaaaaagtgagagtgtggtcattg

Table S2. Exon primers for the *ELOVL5* gene

Exon	**Forward primer**	**Reverse primer**	**T_a**
2	5’-aaggaactgaacttaaccttgtctg	5’-tgccactgataatggtgaaatctat	54°C
3	5’-gaacatgaataccaggtgacttta	5’-cctcatttaggaatacaatttagga	54°C
4	5’-tctgcaactaataaagttcaatgagga	5’-agtgaggttatgggccattttgtatt	56°C
5	5’-cttggggcactcgggcttct	5’-aaaaagggttttgtgtgcataggta	56°C
6	5’-gttggaagtcatctctctttctgattc	5’-agttcaaaacaagaaaattccctaatc	54°C
7	5’-gtatgtgttgtgttcatgaagtgactg	5’-gctccacatgcccattaagtaaata	56°C
8	5’-ctcaggagtttctccttgataagattt	5’-actattgtaggccagactagttacagc	54°C

Note: PCR conditions to amplify *ELOVL5* exons were: 10 µMol primers, 60 ng of genomic DNA, and KAPA-fast 2G kit (Kapa Biosystems, Inc., MA, USA) in a 25 µl final volume under standard amplification conditions. PCR products were purified using Agencourt AMPure XP - PCR Purification (Beckman Coulter, Miami, FL, USA) and sequenced with the Big Dye v3.1 kit (Applied Biosystems, Foster City, CA, USA). Products were purified using Agencourt CleanSEQ - Dye Terminator Removal (Beckman Coulter) and run on a ABI-3730 platform, using POP7 polymer (Applied Biosystem). Electropherograms were analyzed with the SeqScape version 2.6 software (Applied Biosystems).

Table S3. *ELOVL5* mutation screening

Variant	Number of cases	Reported in databases	Effect	Note
c.324+4C>T	1	No	Possible donor splice site change (0.62 to 0.37, Aplice site predictor)	cDNA analysis in lymphoblastoid cells showed a normal splicing between exon 3 and 5
p.Tyr233Cys	2	Exome variant server: rs: 41273880	Predicted as disease causing (0.98 mutation taster)	Present in 10/8590 (EA); 2/4404 (AA)

Table S4. Pathogenicity prediction by bioinformatics analysis

Amino acid change	Provean	Mutation Assessor	PolyPhen2	SNP&Go	PhD-SNP	PANTHER
	<2.5 (deleterious)	>0.85 (low) >1.94 (medium)	>0.8 damaging	>0.5 (disease)	>0.5 (disease)	>0.5 (disease)
p.Leu72Val	-0.86	**1.76**	0.156	**0.653**	**0.623**	**0.847**
p.Gly230Val	**-4.1**	**2.24**	**0.99**	**0.761**	**0.815**	**0.761**

Note: Figures in bold indicate values above the pathogenicity threshold

Table S5. Tag SNPs in the *ELOVL5* genomic region

tagSNP #	tagSNP rs	Block #	Forward primer	Reverse primer	Ta °C
2	rs9463895	1	5’-gtgtcagatgcccctctagc	5’-aacagagggatttgggaagg	56-63
3	rs2235722	1	5’-cctccctacatttgaagagtgc	5’-aacagagggatttgggaagg	56-63
5	rs2235723	2	5’-ctaatagcactgcgaaaattgg	5’-gcacctcaaacagcagttcc	56-63
8	rs2294867	2	5’-gtggtcacacacctgtaatgg	5’-aaaaaccccgcttcttcc	56-63
10	rs13206121	2	5’-gactggtgagatctgcatttagg	5’-caaactgcttaccaccagagg	56-63
14	rs3736732	2	5’-aaggaactgaacttaaccttgtctg	5’-ataatggtgaaatctat	56-63
19	rs209485	2	5’-cctctccctaggactgtttgc	5’-cccttatgtagtgccctttcc	56-63
30	rs209500	2	5’-tgaccacgtttatcctgttcc	5’-taggtgaggaacagtttatgtcc	56-63
39	rs209512	2	5’-ccattaagggccactgtgc	5’-cctgagcttaagcagtctgg	59-66
40	rs12207094	2	5’-ccattaagggccactgtgc	5’-cctgagcttaagcagtctgg	59-66

PCR conditions were: restriction fragment length analysis for rs2073040 (SNP #1) was performed using BglII (New England Biolabs) according to the manufacturer’s instructions.

Table S6. Real time PCR assays

Gene	Exons	Forward primer	Reverse primer	UPL
ELOVL5	2-3	5'-cccttccatgcgtccata	5'-gattgtcagcacaaactgaagc	#31
ELOVL6	4-5	5'- caaagcacccgaactaggag	5'- ggtgataccagtgcaggaaga	#38

Note: VIC-labeled pre-designed TaqMan gene expression assay for TATA-Binding protein (*TBP*) (Hs00427620_m1, Applied Biosystems) was used as normalizer. Reactions were carried out on an ABI 7500 Fast real-time PCR machine using the ABI TaqMan Universal PCR master mix according to the manufacturer (Life Technologies). Assays efficiencies were in a range 90-100%. Samples were run in triplicate, and the mean Ct value was used for calculations using the ΔΔCt method.[32]

Table S7. Primers for site-directed plasmid mutagenesis

Amino acid change	Forward primer	Reverse primer	T_a
p.Leu72Val	5'-gtgtataaccttggagtcacactgctgtctc	5'-gagacagcagtgtgactccaaggttatacac	58°C
p.Gly230Val	5'-gcacattccctcttgtttggttgtatttcca	5'-tggaaatacaaccaaacaagagggaatgtgc	56°C