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Supplemental Data

Mutations in *SNRPE,* which Encodes a Core Protein of the Spliceosome, Cause Autosomal-Dominant Hypotrichosis Simplex

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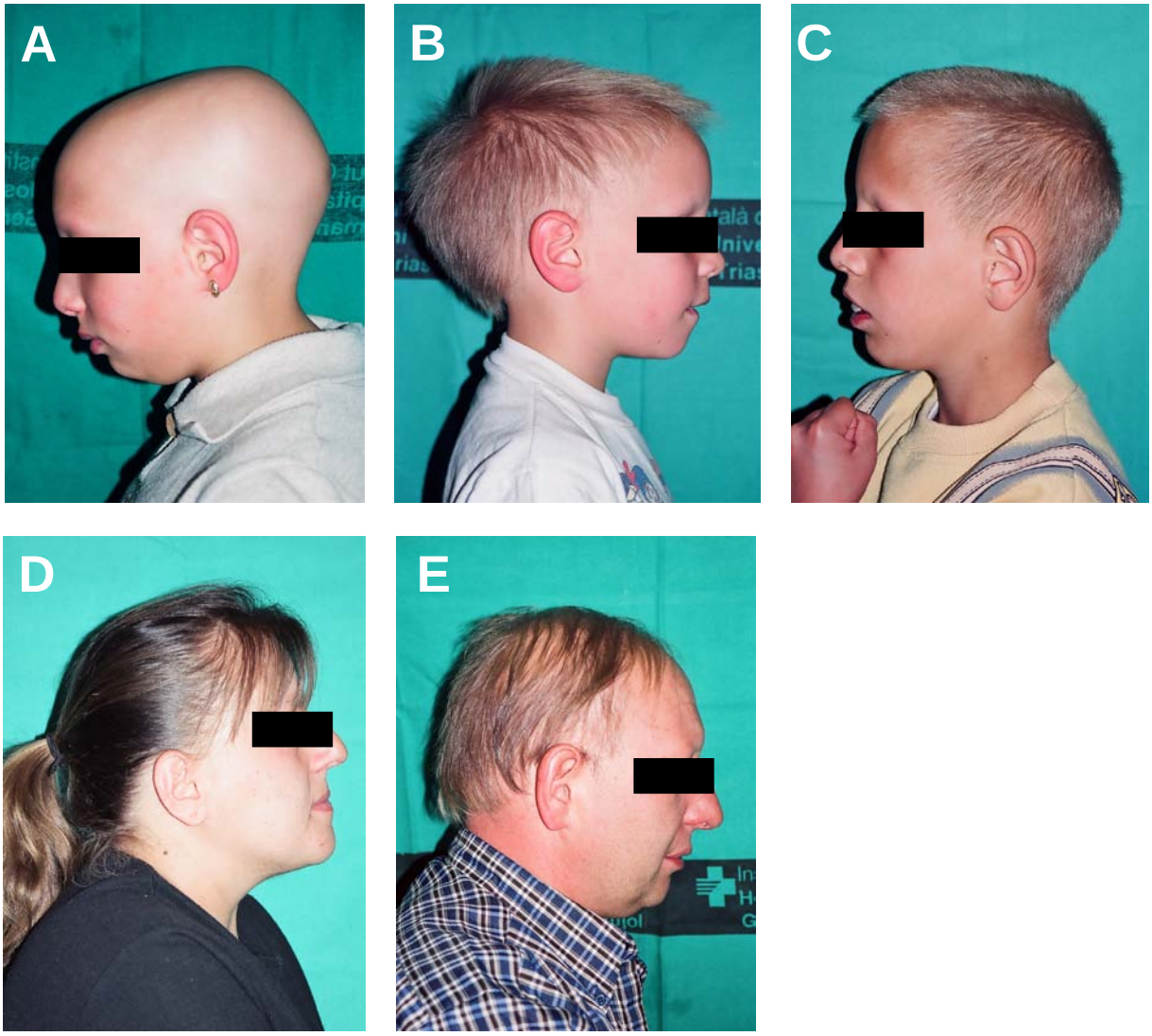

Figure S1. Affected individuals from the Spanish hypotrichosis simplex family, lateral view.
(A) Index case (III:3) of the Spanish family. The picture was taken when she was 10 years old. She shows complete alopecia of the scalp and is devoid of eyebrows and eyelashes.
(B) Cousin of the index person at the age of 8 years. He has almost normal scalp hair, but sparse eyebrows and eyelashes.
(C) Cousin of the index person at the age of 10 years. His hair at the scalp is almost normal but he has no eyebrows and no eyelashes.
(D) Mother of the Spanish family. Her scalp hair is almost normal, but the eyebrows are sparse. Eyelashes are not visible.
(E) Father of the Spanish family. His scalp hair is sparse. Eyelashes and eyebrows are not present. Also, he does not have axillary hair.

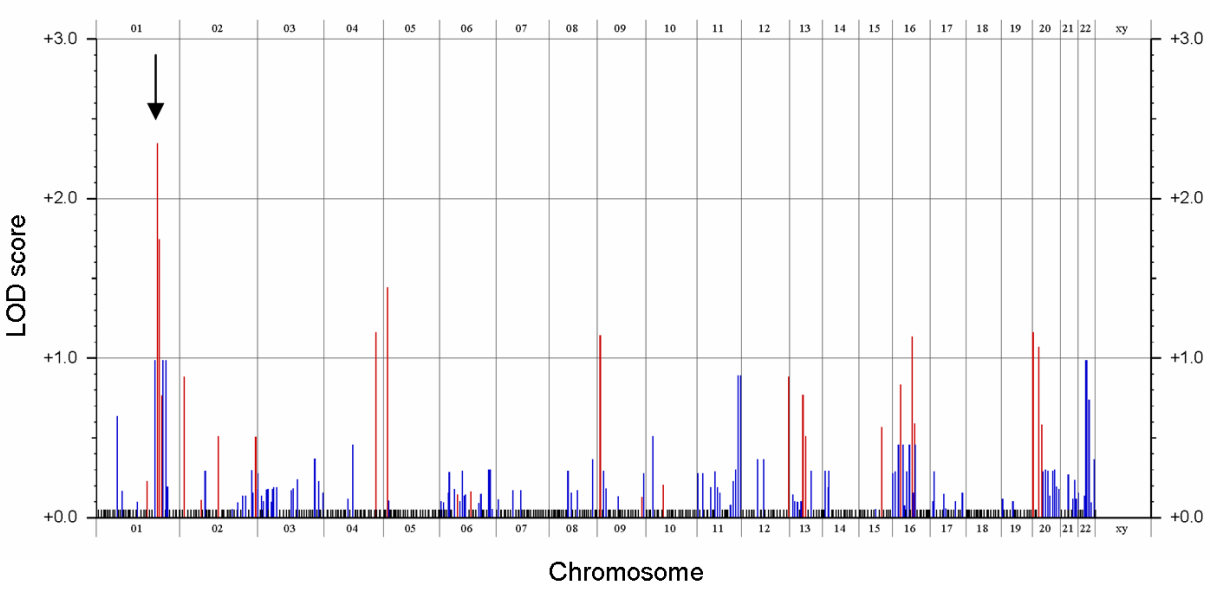

Figure S2. Multipoint LOD-score analysis of the Spanish family.
The analysis was performed using the software LINKAGE version 5.21. LOD-scores are plotted against all SNP markers distributed across the genome. We observed a LOD-score of 2.35 at chromosome 1 which is indicated by an arrow.

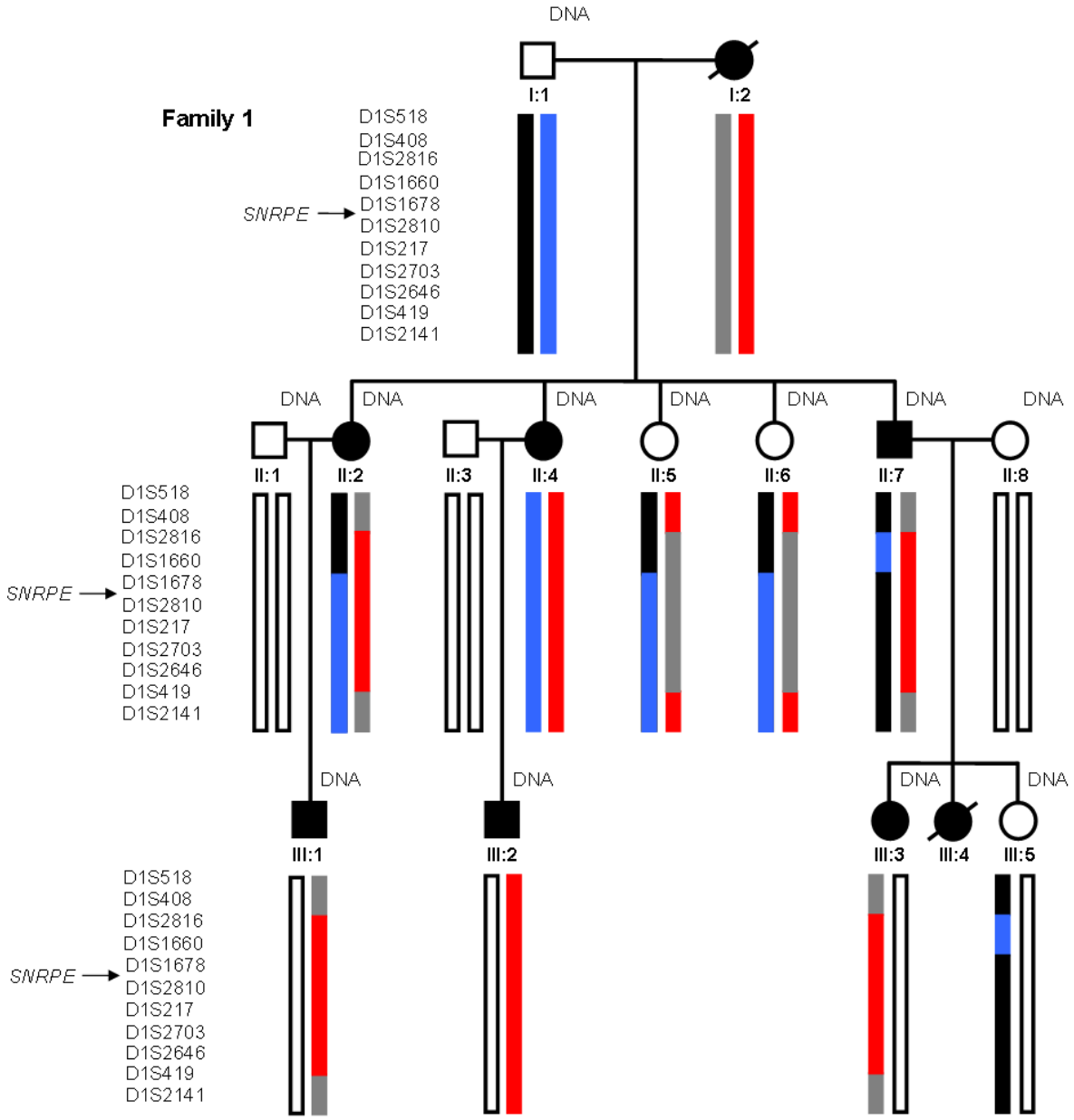

Figure S3. Pedigree of the Spanish hypotrichosis family.
Marker haplotypes on chromosome 1 that are linked to the disease are indicated by red bars. Affected family members are shown in black, circles and squares denote females and males respectively. Microsatellite markers are given on the left, and the location of *SNRPE* is indicated by an arrow.

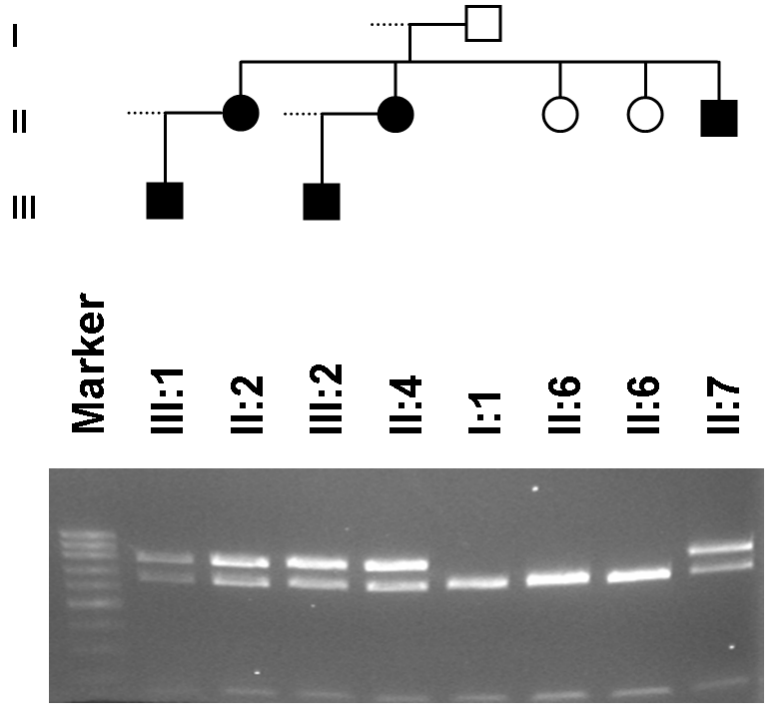

Figure S4. *NcoI* digestion of *SNRPE* PCR products of family 1.
The c.1A>G mutation identified in family 1 from Spain eliminates an *NcoI* restriction site (CC/ATGG). Therefore, an *NcoI* digestion of PCR products was performed with individuals from family 1 to verify the presence of the c.1A>G mutation in the affected individuals.

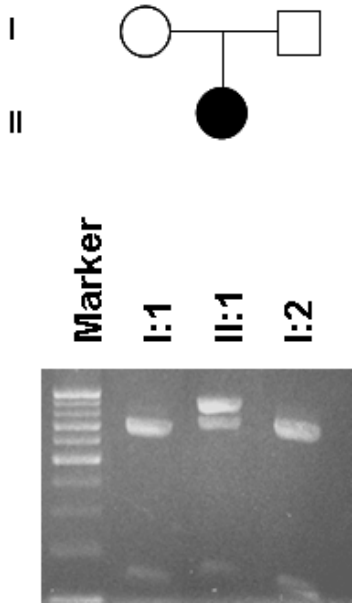

Figure S5. *NcoI* digestion of *SNRPE* PCR products of family 2.
Segregation of the c.1A>G mutation was tested by *NcoI* digestion in family 2 from Great Britain. The mutation was identified in the affected girl, but not in her parents, indicating a *de novo* mutation.

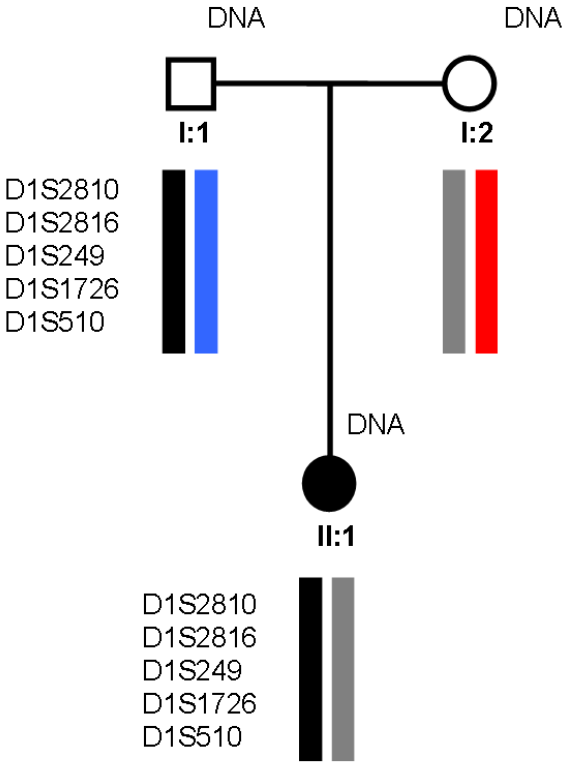

Figure S6. Confirmation of paternity in the British family.
We confirmed paternity in the British family by analysing 5 microsatellite markers around *SNRPE* (D1S2810, D1S2816, D1S249, D1S1726 and D1S510). The affected family member is shown in black, circles and squares denote females and males respectively. Microsatellite markers are given on the left.

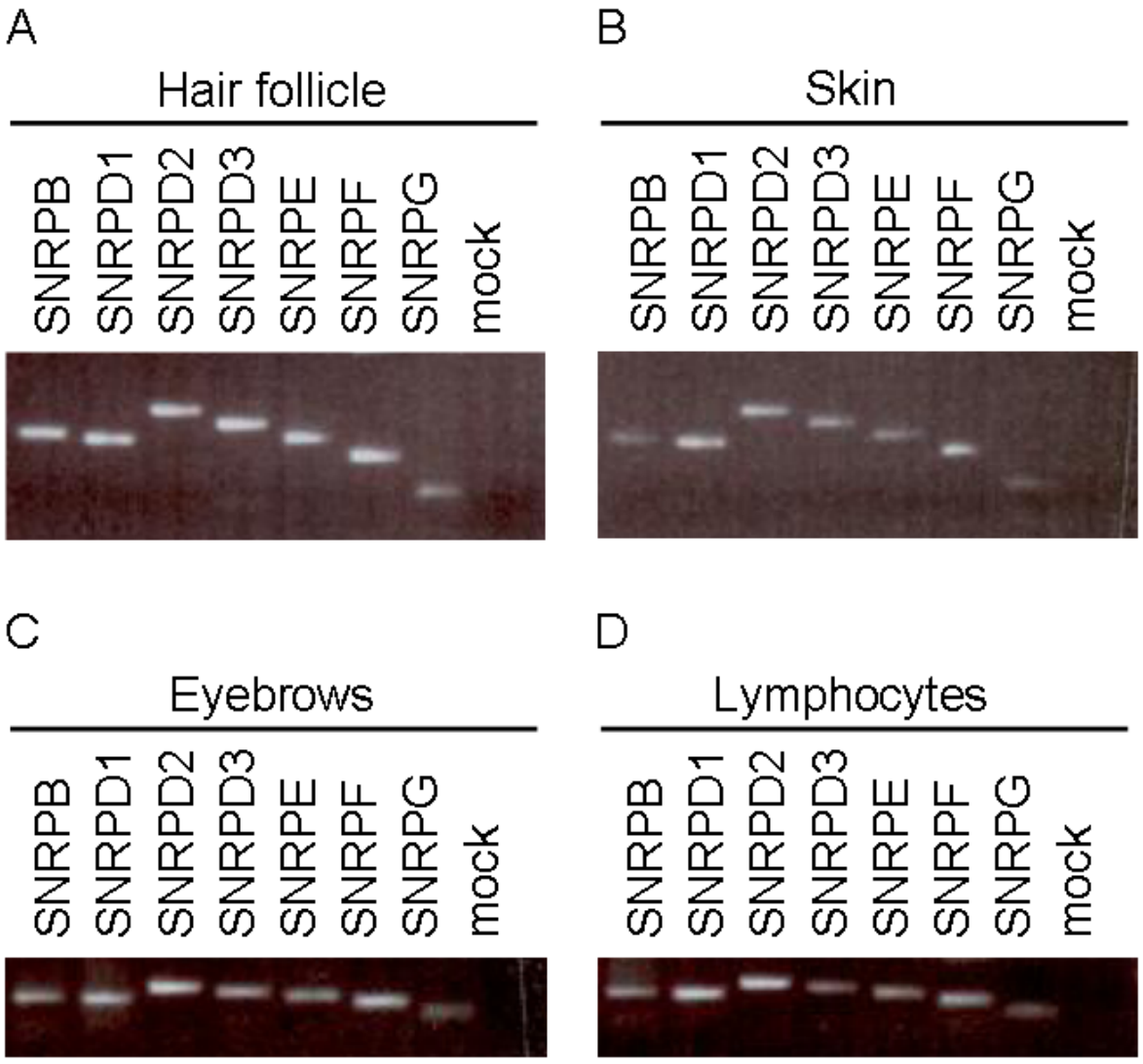

Figure S7. Expression analysis of genes encoding different SM core proteins. We isolated RNA from plucked human hair follicle cells of the scalp and eyebrows, skin biopsies and lymphocytes of control individuals by use of the RNeasy Micro Kit (Qiagen, Hilden, Germany). This was followed by a reverse transcriptase PCR with random hexamers. The obtained cDNA was used as template for end-point PCR using the gene specific primers given in Table S3. Shown is the expression of the genes *SNRPB*, *SNRPD1*, *SNRPD2*, *SNRPD3*, *SNRPE*, *SNRPF* and *SNRPG* in (A) hair follicle cells, (B) skin, (C) evebrow cells, and (D) immortalized lymphocytes of healthy controls. The seven proteins encoded by these genes constitute the Sm core of U1, U2, U4, and U5 snRNPs.

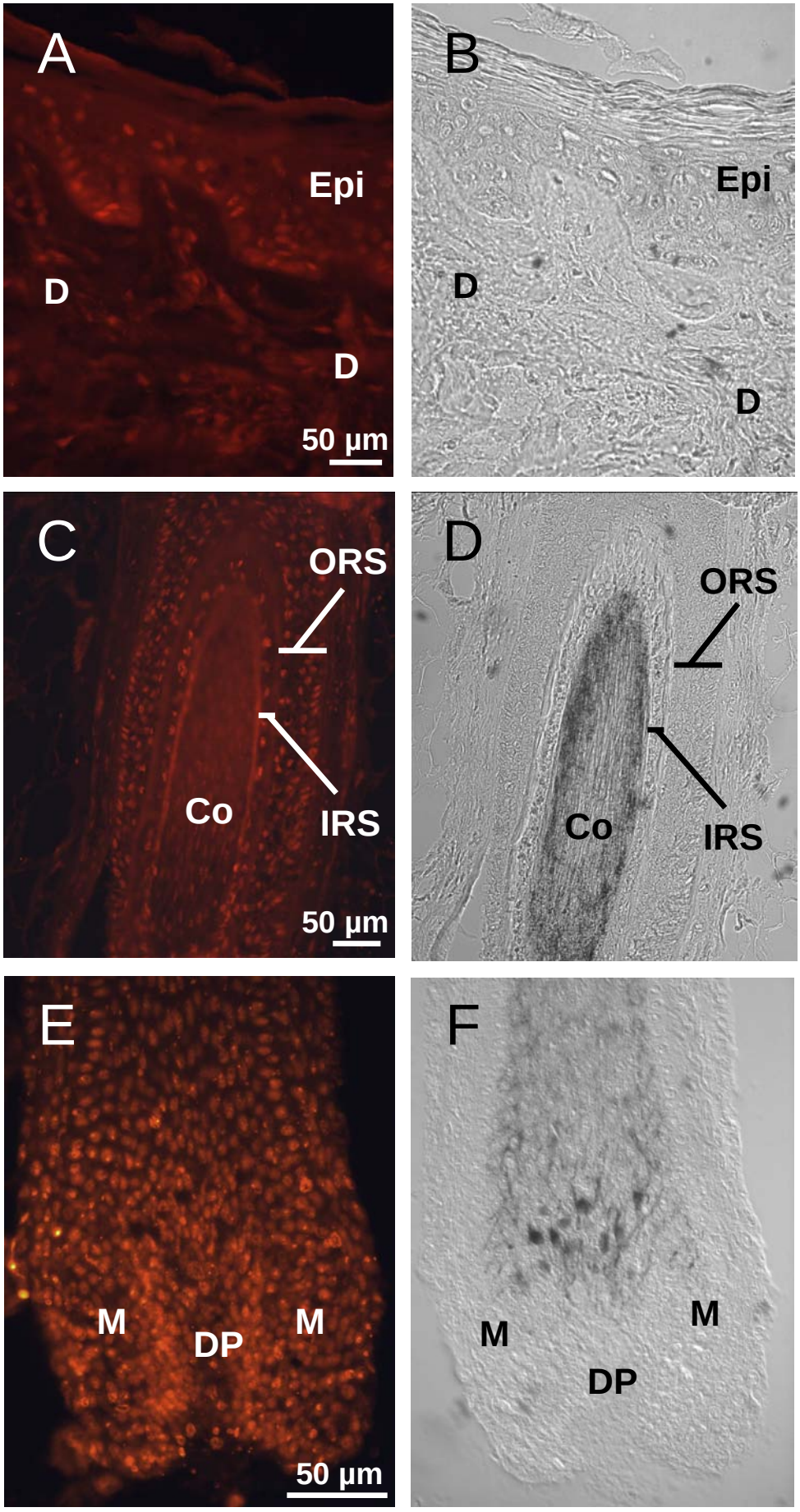

Figure S8. SNRPE immunoreactivity in human scalp skin.

SNRPE immunoreactivity was investigated in biopsies of human scalp skin that had been fixed over night in 4 % formaldehyde in Bouin solution and embedded in paraffin. Rabbit polyclonal antibody against SNRPE (20407-1-AP, ProteinTech, Chicago, USA) was used at a dilution of 1:50. Cy3-conjugated sheep anti rabbit IgG F(ab)2 from Sigma (cat. No. C-2306) was used at a dilution of 1:800. Reference size bar is given in A, C, and E. A, C, and E show SNRPE immunoreactivity. B, D, and F show the same sections as in A, C, and E, respectively, using differential interference microscopy (DIC) (Noma, Nomarski optics). Epi, epidermis; D, dermis; Co, hair shaft cortex; IRS, inner root sheath; ORS, outer root sheath; M, matrix; DP, dermal papilla.

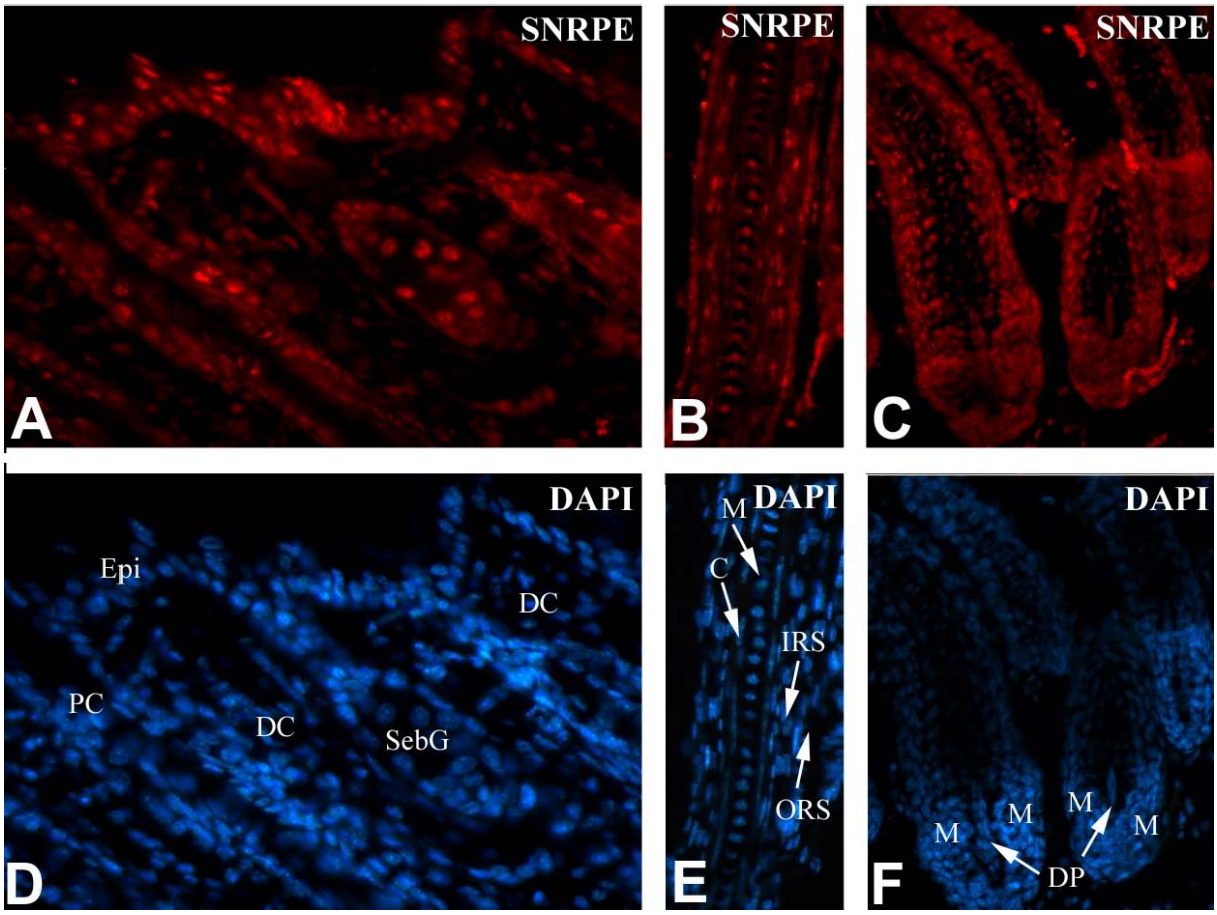

Figure S9. Immunohistochemical analyis of SNRPE in murine skin.
SNRPE immunoreactivity was investigated in dorsal skin biopsies from 9 day old C57BL/6J mice that had been fixed over night in 4 % formaldehyde in PBS and embedded in paraffin. Rabbit polyclonal antibody against SNRPE (20407-1-AP, ProteinTech, Chicago, USA) was used at a dilution of 1:50. Cy3-conjugated sheep anti rabbit IgG F(ab)2 from Sigma (cat. No. C-2306) was used at a dilution of 1:800. A, B, and C SNRPE immunoreactivity, D, E, and F DAPI nuclear stain.
DC, dermal cells; PC, pilary canal; Epi, epidermis; SebG, sebaceous gland; M in E, medulla; C, cortex of hair shaft; IRS, inner root sheath; ORS, outer root sheath; M in F, matrix; DP, dermal papilla.

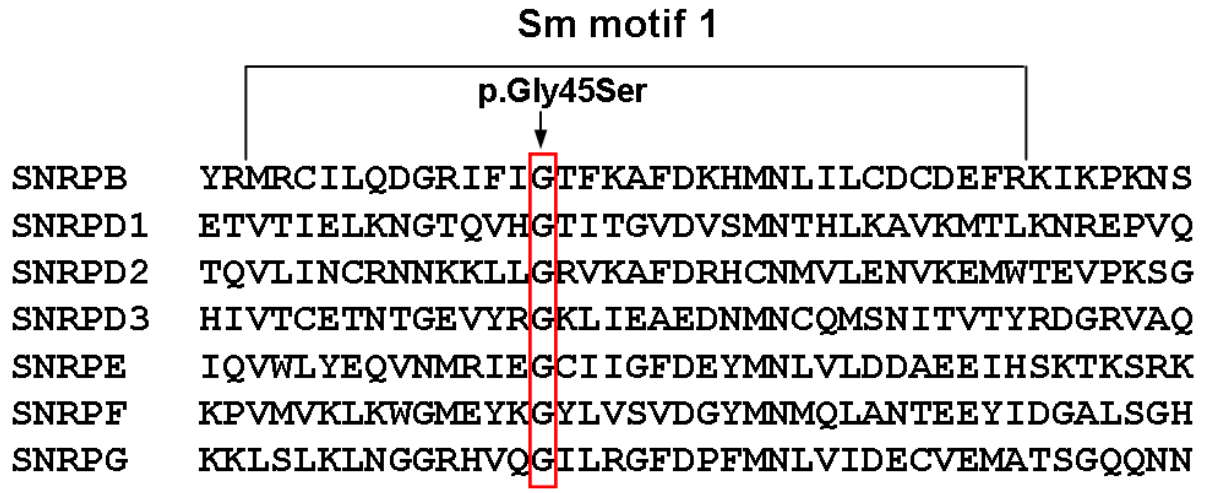

Figure S10. Bioinformatic analysis of different SNRP proteins.
The analysis revealed, that the Gly45 in the Sm motif 1 residue (boxed) is conserved in all SNRPs, pointing to its functional importance.

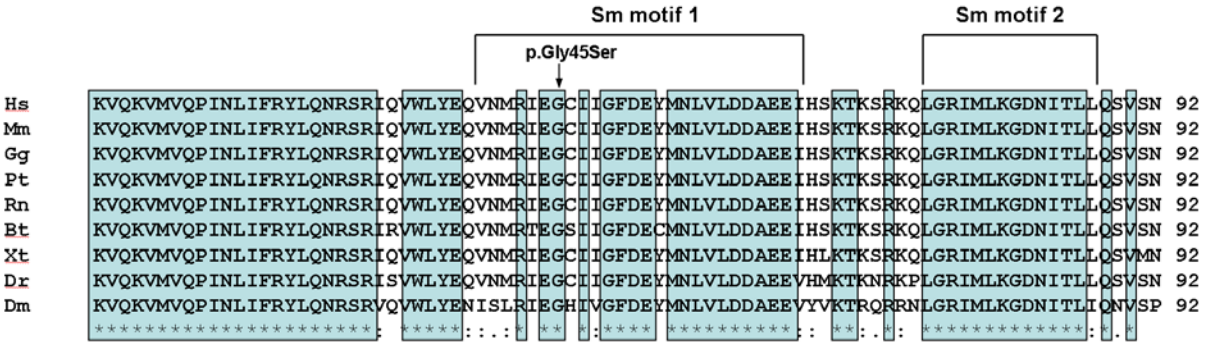

Figure S11. Partial amino acid sequence of the human SNRPE protein in comparison with orthologs from other species.
There is a high homology of Sm motif 1 and Sm motif 2. The Gly45 residue in Sm motif 1 is conserved from human to drosophila. Species abbreviations are as follows: Hs, *Homo sapiens*; Mm, *Mus musculus*; Gg, *Gallus gallus*; Pt, *Pan troglodytes*; Rn, *Rattus norvegicus;* Bt, *Bos taurus*, Xt *Xenopus tropicalis*. Dr, *Danio rerio*, Dm, *Drosophila melanogaster.*

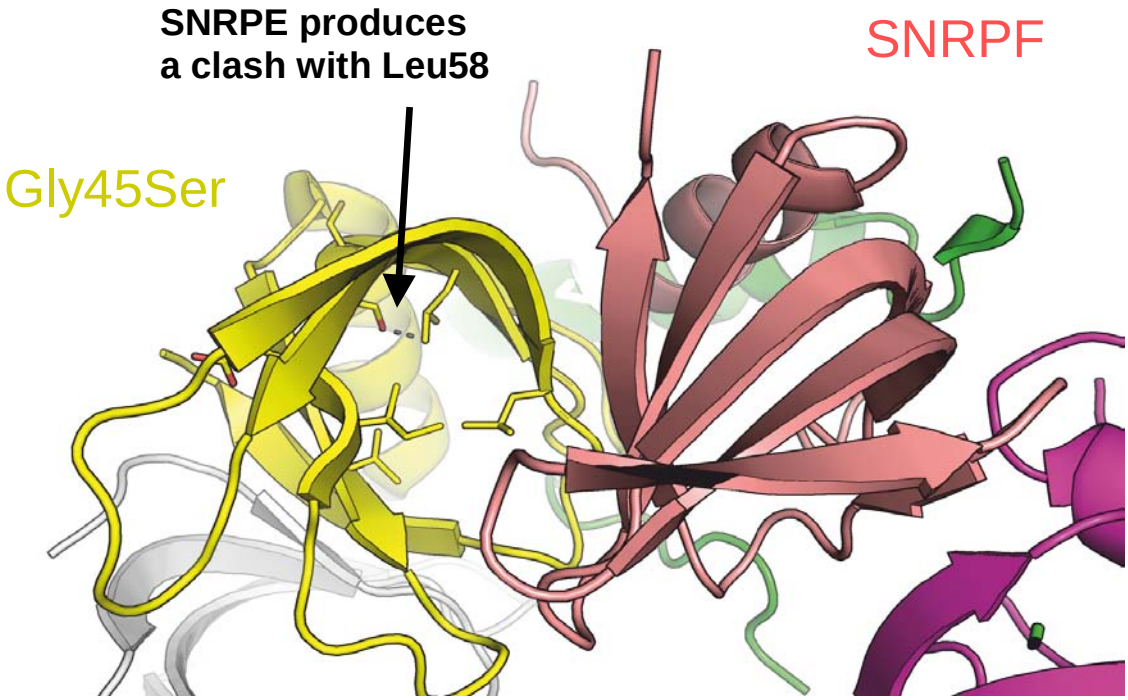

Figure S12. Protein modelling of mutant SNRPE p.Gly45Ser.
The substitution disrupts the hydrophobic core of SNRPE and produces a clash with Leu58. However, it does not disrupt core formation with the other SNRPs, like SNRPF.

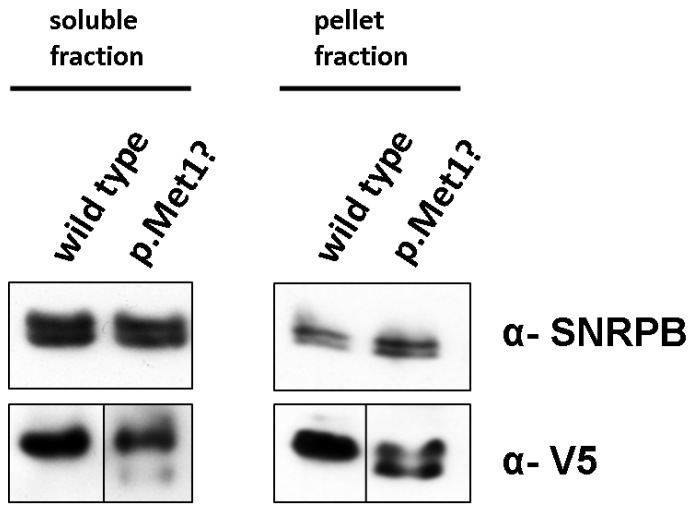

Figure S13. Comparison of the presence of full-length and truncated SNRPE in cell pellet versus soluble fraction.
Western Blot analyses were performed with cell pellets and soluble fraction of SNRPE wild type and p.Met1?. Note the higher presence of the truncated form in the pellet fraction.

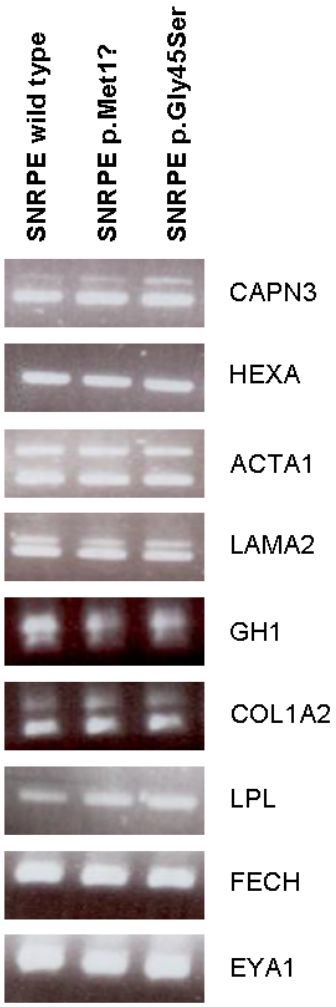

Figure S14. Splicing analyses of cells expressing *SNRPE* wild type or mutant constructs. We co-transfected HEK293T cells with SNRPE wild type/p.Met1?/p.Gly45Ser and different minigenes (Fu at al., 2011; *CAPN3*, *HEXA*, *ACTA1*, *LAMA*2, *GH1*, *COL1A2*, *LPL*, *FECH* and *EYA1*). Cells were harvested 30 hours post transfection and RNA was isolated with the RNeasy Micro Kit (Qiagen, Hilden, Germany). cDNA synthesis was performed with 1µg RNA using the Protoscript First Stand cDNA Synthesis Kit (NEB, Ipswich, MA) and random hexamers. After that, we performed non-quantitative end-point PCR with T7 and BGH primers. We observed no different splicing patterns between cells transfected with SNRPE wild type, p.Met1? or p.Gly45Ser.

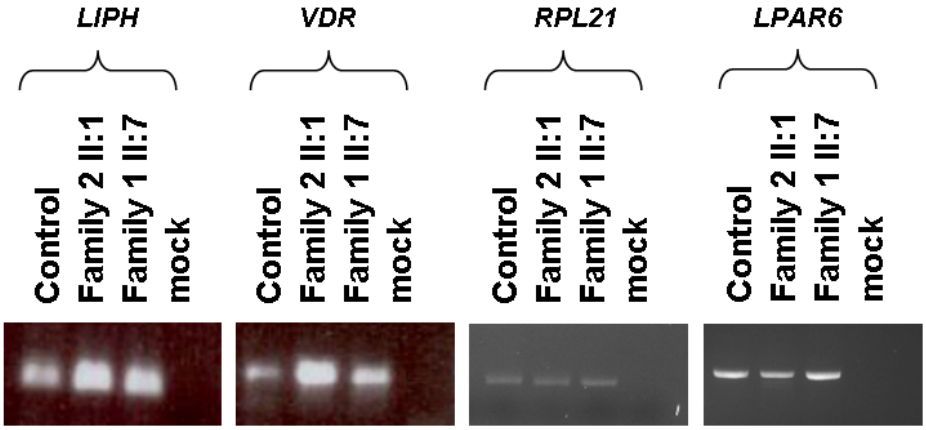

Figure S15. Analysis of mRNA splicing of genes, that are involved in monogenic isolated alopecias in humans.
We isolated RNA from immortalized lymphocytes of a control and individual II:7 from family 1 and also individual II:1 from family 2 by use of the RNeasy Micro Kit (Qiagen, Hilden, Germany). Both patients carry the *SNRPE* mutation c.1A>G (p.Met1?) in a heterozygous state. We performed cDNA sythesis with random hexamers and after that we used these cDNAs as template for non-quantitative end-point PCRs with primers for genes (*LIPH, VDR, RPL21* and *LPAR6;* primer sequences are available upon request), that have been shown to be involved in monogenic isolated alopecias in humans. We could not detect different splicing products in the affected individuals from family 1 and 2 and the control, indicating that the *SNRPE* mutation does not lead to aberrant splicing of the above mentioned genes in patients.

Table S1. Excluded candidate loci in family 1 originating from Spain.

Excluded locus	**Respective gene**
6p21	Corneodesmosin (*CDSN*)
8p21	Hairless (*HR*)
12q13	Vitamin D receptor (*VDR*)
16q22	Cadherin 3 (*CDH3*)
12q13	Keratin gene cluster
17q11	Winged-helix transcription factor nude (*WHN*)
17q21	Keratin gene cluster

Prior to a genome-wide linkage analysis, we excluded a number of candidate loci that were known to be involved in hair loss by that time.

Table S2. Exclusion of positional candidate genes in chromosomal region 1q31.3-q41 by direct sequencing.

Symbol	Full name
ZBTB41	zinc finger and BTB domain containing 41
LHX9	LIM homeobox 9
NEK7	NIMA-related kinase 7
ATP6V1G3	ATPase, H+ transporting, lysosomal, V1 subunit
PTPRC	protein tyrosine phosphatase, receptor type, C
NR5A2	nuclear receptor subfamily 5, group A, member 2
ZNF281	zinc finger protein 281
DDX59	DEAD (Asp-Glu-Ala-Asp) box polypeptide 59
GPR25	G protein-coupled receptor 25
TMEM9	transmembrane protein 9
PKP1	plakophilin 1 isoform 1b
LAD1	ladinin 1
CSRP1	cysteine and glycine-rich protein 1 isoform 1
BC030568	Homo sapiens ribosomal protein S10 pseudogene 7, mRNA
LMOD1	leiomodin 1 (smooth muscle)
TIMM17A	translocase of inner mitochondrial membrane 17
RNPEP	arginyl aminopeptidase (aminopeptidase B)
ELF3	E74-like factor 3 (ets domain transcription)
GPR37L1	G-protein coupled receptor 37 like 1
ARL8A	ADP-ribosylation factor-like 8A
PTPN7	protein tyrosine phosphatase, non-receptor type
RABIF	RAB-interacting factor
ADIPOR1	adiponectin receptor 1
PPFIA4	protein tyrosine phosphatase, receptor type, f
MYOG	myogenin
ADORA1	adenosine A1 receptor
BTG2	B-cell translocation gene 2
FMOD	fibromodulin precursor
PRELP	proline arginine-rich end leucine-rich repeat
OPTC	opticin precursor
ATP2B4	plasma membrane calcium ATPase 4 isoform 4a
LAX1	lymphocyte transmembrane adaptor 1 isoform a
SOX13	SRY-box 13
PPP1R15B	protein phosphatase 1, regulatory subunit 15B

CNTN2	contactin 2 precursor
TMEM81	transmembrane protein 81
RBBP5	retinoblastoma binding protein 5
DSTYK	receptor interacting protein kinase 5 isoform 1
TMCC2	transmembrane and coiled-coil domain family 2
LEMD1	LEM domain-containing protein 1 (LEMP-1) (Cancer/testis antigen 50) (CT50)
PCTK3	PCTAIRE protein kinase 3 isoform a
ELK4	ELK4 protein isoform a
SLC45A3	prostein
AVPR1B	arginine vasopressin receptor 1B
IKBKE	IKK-related kinase epsilon
DYRK3	dual-specificity tyrosine-(Y)-phosphorylation
MAPKAPK2	mitogen-activated protein kinase-activated
IL10	interleukin 10 precursor
IL19	interleukin 19 isoform 1 precursor
IL20	interleukin 20 precursor
IL24	interleukin 24 isoform 1 precursor
C1orf116	specifically androgen-regulated protein isoform
LAMB3	laminin, beta 3 precursor
IRF6	interferon regulatory factor 6
TRAF5	TNF receptor-associated factor 5
SLC30A1	solute carrier family 30 (zinc transporter)
NEK2	NIMA-related kinase 2
LPGAT1	lysophosphatidylglycerol acyltransferase 1
PPP2R5A	protein phosphatase 2, regulatory subunit B
ATF3	activating transcription factor 3 isoform 1

Given is the approved symbol in the left column and the full name in the right column.

Table S3. Primers for amplification, sequencing, expression analysis and cloning of *SNRPE* and expression analysis of the other Sm proteins (SNRPB, SNRPD1, SNRPD2, SNRPD3, SNRPF, SNRPG).

Primer denomination	Sequence
Primer for sequencing of the coding region of *SNRPE*	
SNRPE_1F	5'-ACGTGACTTCATGGCTAGAGG-3'
SNRPE_2R	5'-GAACACAGCGATCGCTCAGGT-3'
SNRPE_3F	5'-TGCAGGAGATAAGCCCTTGGT-3'
SNRPE_3R	5'-AAGGCTAAACAGCAGCACCTG-3'
SNRPE_3Fseq	5'-TCGGCCTCCGAAAGTGCTGG-3'
SNRPE_4F	5'-GGTGGGGCTTGAGAAGAGTG-3'
SNRPE_4R	5'-GGAGAAGCCAAAATGGAGTGT-3'
SNRPE_5F	5'-CCAGTGAACACCAGTGTTCC-3'
SNRPE_5R	5'-GCAACGAATGGTGTTACTGTG-3'
SNRPE_5Fseq	5'-AAATATTGTTCAAAAACTGG-3'
Primer for expression analysis of *SNRPE* and genes encoding other Sm proteins	
SNRPE_1F2	5'-GTGGCCAGGGTCAGAAAGTGC-3'
SNRPE_5R2	5'-TTTGTAGCAGAGTAATATTATCTCC-3'
SNRPB_ExprF	5'-TCAATGACAGTAGAGGGACC-3'
SNRPB_ExprR	5'-ATACTGGCTGTGGCAGCAGC-3'
SNRPD1_ExprF	5'-GAAGAACGGAACACAGGTCC-3'
SNRPD1_ExprR	5'-CTCTTCCTGCAACAGCTTCC-3'
SNRPD2_ExprF	5'-ATGAGCCTCCTCAACAAGCC-3'
SNRPD2_ExprR	5'-TTCCGCAGGACCACGATGAC-3'
SNRPD3_ExprF	5'-TGACATGTGAGACGAACACC-3'
SNRPD3_ExprR	5'-TCCACGTCCTCTTCCTCTTG-3'
SNRPF_ExprF	5'-AGTAGCCTGCAACATTCGGC-3'
SNRPF_ExprR	5'-CCCAGATGTCCAGACAAAGC-3'
SNRPG_ExprF	5'-AAGCTCACCCTCCCGAGTTG-3'
SNRPG_ExprR	5'-CACATTCATCTATCACAAGG-3'
Primer for cloning SNRPE wild type and mutant constructs	
Primer_WT/p.Gly45Ser_F	5'-CGAAGCTTCCGCCGCCATGGCGTACCGTGGCCAGGGTCA-3'
Primer_p.Met1?_F	5'-CGAAGCTTCCGCCGCCGTGGCGTACCGTGGCCAGGGTCA-3'

Site-directed mutagenesis_p.Gly45Ser_F	5'-GAATATGCGGATAGAA**A**GCTGTATCATTGGTTTTGATGAG-3'
Site-directed mutagenesis_p.Gly45Ser_R	5'-AACCAATGATACAGC**T**TTCTATCCGCATATTCACTTGCT-3'
Primer_R	5'-GCCTCGAGTTGGAGACACTTTGTAGCAGAGTAATATTA-3'

The primer SNRPE_3Fseq and SNRPE_5Fseq were only used for sequencing reaction. For cloning of the *SNRPE* mutation c.1A>G (p.Met1?) and the wild type sequence, the R primer was combined with one of the two given F primers. To clone the c.133G>A mutation (p.Gly45Ser), we used the wild type construct as starting material and performed a site-directed mutagenesis with the respective mutagenesis primers. All constructs were cloned by use of the enzymes *HindIII* and *XhoI* (recognition sequences are underlined).