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Supplemental Data

Biallelic *SUN5* Mutations Cause Autosomal-Recessive Acephalic Spermatozoa Syndrome

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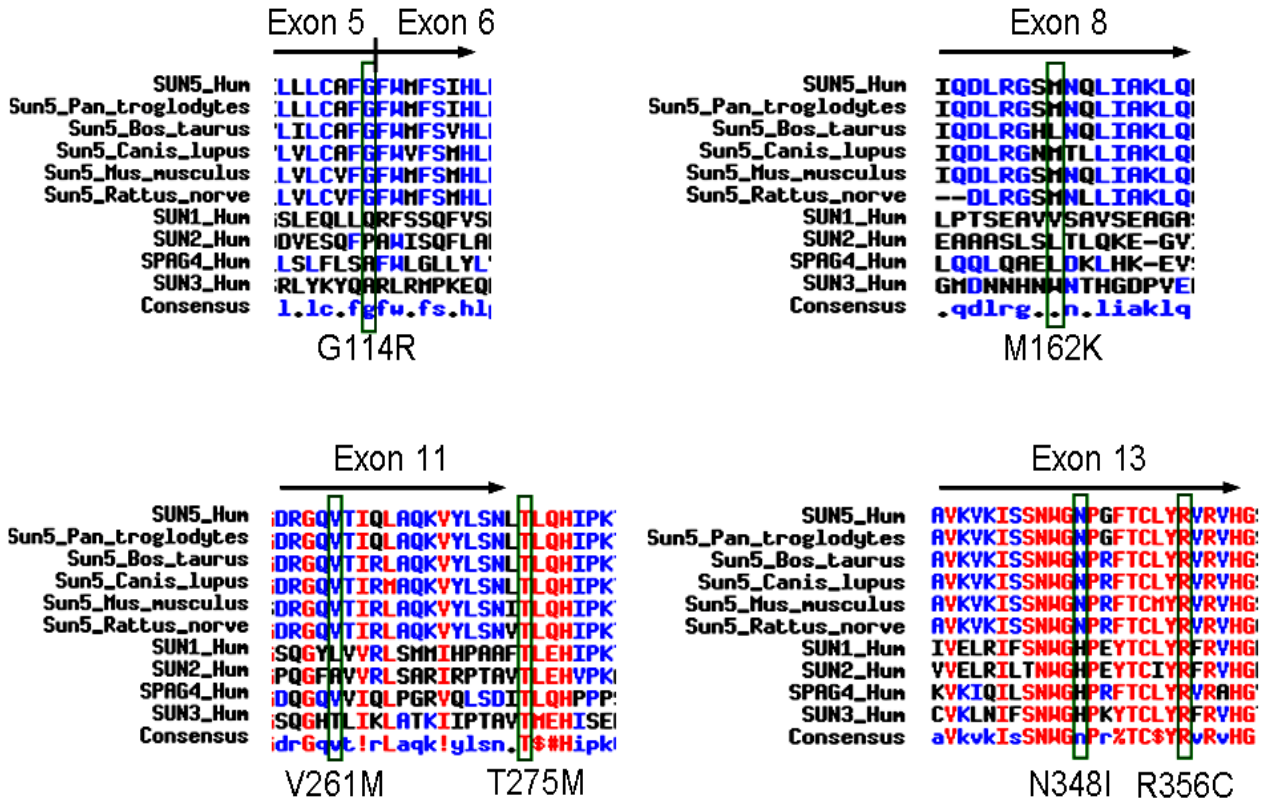

Figure S1

Conservation of SUN5 amino acid residues affected by missense mutations in the affected individuals with acephalic spermatozoa syndrome.

Partial amino acid sequence alignments across exons 5, 6, 8, 11 and 13 of selected SUN5 orthologs and paralogs are shown. The multiple sequence alignments have been performed using MultAlin. The amino acids in positions p.Gly114, p.Met162, p.Val261, p.Thr275, p.Asn348 and p.Arg356 are highlighted by a green rectangle.

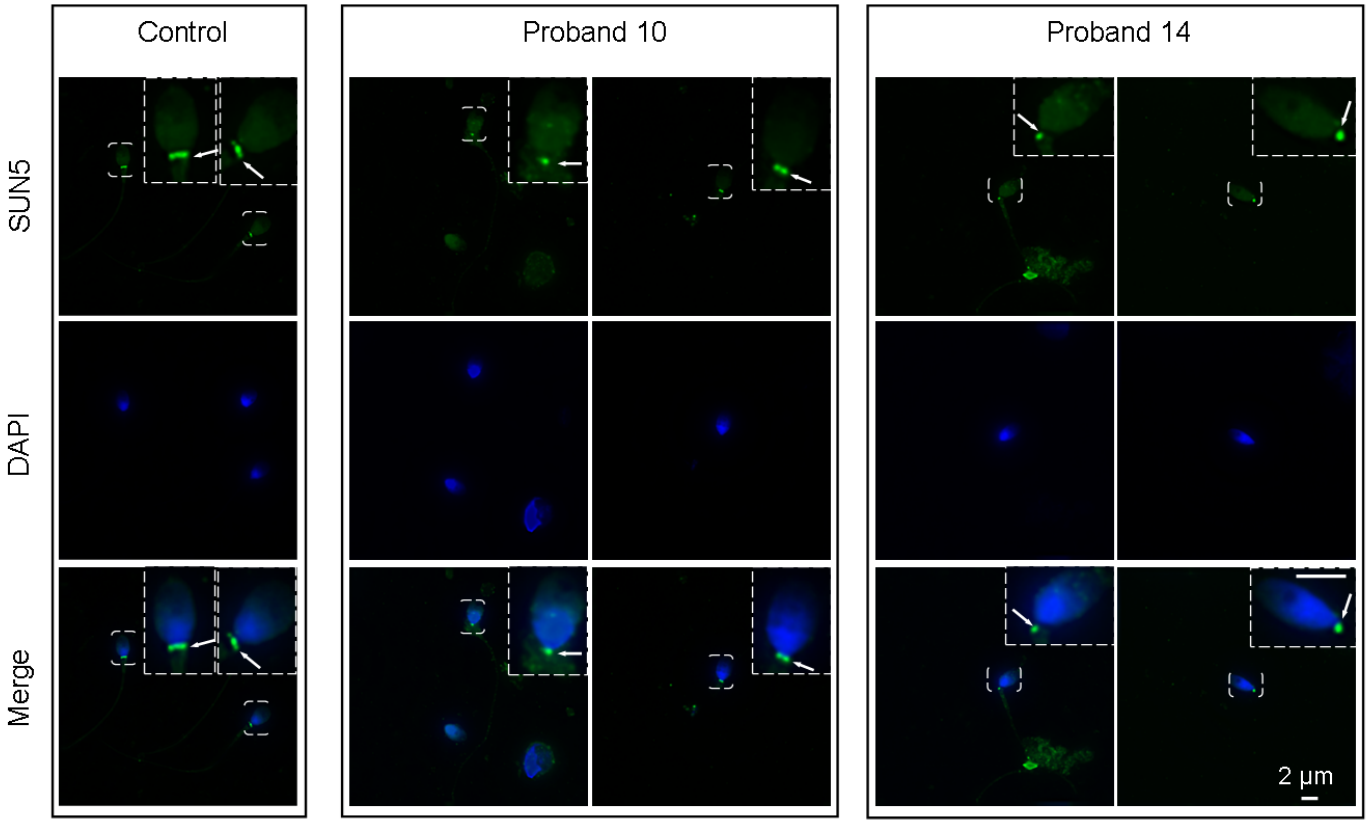

Figure S2

SUN5 immunofluorescence in sperm from controls and two affected individuals with acephalic spermatic syndrome without *SUN5* mutations.

SUN5 staining is confined to the head-tail junction region (exactly in the distal part of the inner nuclear membrane which is close to the distal end of the nucleus) in control sperm (highlighted by white arrow). This specific pattern of distribution is not changed in abnormal head-tail junction sperm (left panel) or in acephalic sperm (right panel) of two affected individuals (Proband 10 and Proband 14) with no *SUN5* mutations. DAPI indicates the nucleus in the sperm head. The scale is shown at the bottom right panel. (*Insets*) Digital enlargement of the respective sperm head-tail junction regions.

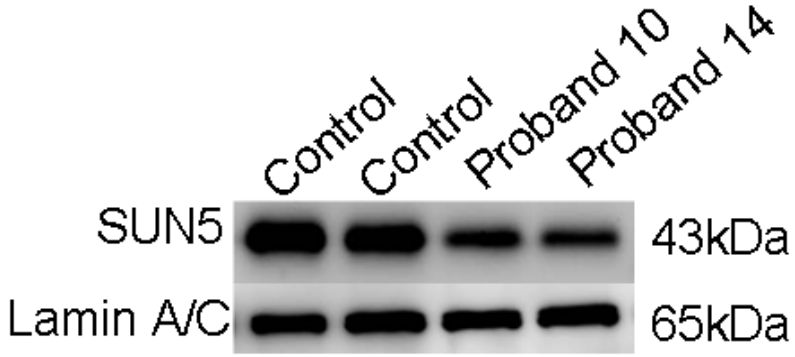

Figure S3

SUN5 levels in two affected individuals with acephalic spermatic syndrome without *SUN5* mutations.

Western blot showing SUN5 (Proteintech antibodies, 17495-1-AP) on ejaculated sperm from two controls and from two affected individuals (Proband 10 and Proband 14) with no *SUN5* variants identified.

Table S1. Semen parameters of affected individuals with acephalic spermatozoa syndrome grouped by *SUN5* genotype.

Mutation types	**M / M**		**– / M**				**– / –** [a]		**No MT**								
Patients	**F1:II-3**	**F2:II-4**	**F3:II-1**	**F4:II-1**	**F5:II-1**	**F6:II-2**	**F7:II-1**	**F8:II-2**	**P9**[b]	**P10**	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**	**P17**
volume (ml)	2.2[c]	3.3	3.9	1.9	3.0	1.2	1.8	2.8	3.3	2.7	3.6	3.1	1.2	1.1	2.8	2.1	3.8
Concentration [d]	5.3	3.2	1.5	10.8	3.7	7.6	6.5	4.9	2.2	3.3	2.3	3.4	6.8	9.2	3.8	5.5	2.5
Motility B+C (%) [e]	23.4	18.4	18.3	16.1	8.6	3.6	20.2	26.4	8.9	4.2	14.6	28.8	20.4	13.6	7.2	6.4	19.4
Percentages of different morphologic spermatozoa (%)																	
Normally formed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal head-tail junction	3.9	0.3	1.6	4.2	4.4	0.9	4.1	3.3	0.9	4.3	2.4	1.8	4.8	4.3	1.3	0.4	2.2
Decaudated	0.4	0.0	0.1	0.5	0.4	0.3	0.5	0.1	0.6	0.4	0.8	0.1	0.3	0.8	0.6	0.5	0.1
Acephalic	95.7	99.7	98.3	95.3	95.2	98.8	95.4	96.6	98.5	95.3	96.8	98.1	94.9	94.9	98.1	99.1	97.7

a. "– / –" represents a homozygous nonsense mutation or a splice site mutation that were predicted to cause no protein production. "– / M" represents a compound heterozygous mutation composed of a missense mutation and either a nonsense, or a frameshift, or a splice site mutation that were supposed to remain a mild function of SUN5 protein. "M / M" represents homozygous or heterozygous missense mutations that were supposed to remain some basic function of SUN5 protein. "No MT" represents no mutations identified. **b**. "P" represents proband. **c**. Values are means of semen parameters calculated from more than twice of ejaculated semen analyses. **d.** The unit of concentration is "$\times 10^6$ / ml". Sperm concentration was based on normally formed spermatozoa, abnormal head-tail junction spermatozoa, and decaudated spermatozoa. **e.** Mobility B+C (%) represents the total motility of normally formed spermatozoa, abnormal head-tail junction spermatozoa and acephalic spermatozoa, since no rapid progressive mobility sperm (grade A) was observed in all patients. **Note:** All groups showed oligozoospermia with less than 15×10^6/ml sperm （or 39×10^6 per ejaculate）(the normally formed spermatozoa, the abnormal head-tail junction spermatozoa and the decaudated heads observed in fresh semen were counted as sperm), and asthenospermia (the motility of the normally formed spermatozoa, the abnormal head-tail junction spermatozoa and the acephalic spermatozoa was less than 40%). No sperm showed rapid progressive motility (grade A).

Table S2. Statistical analysis of the semen parameters of the affected individuals according to *SUN5* genotype.

Mutation types	– / – (n = 2)	– / M (n = 4)	M / M (n = 2)	No MT (n = 9)	– / – vs – / M	– / – vs M / M	– / – vs No MT	– / M vs M / M	– / M vs No MT	M / M vs No MT
volume (ml)	2.3 ± 0.5 [a]	2.5 ± 0.6	2.8 ± 0.6	2.6 ± 0.3	0.81 [b]	0.61	0.63	0.78	0.85	0.87
Concentration [a]	5.7 ± 0.8	5.9 ± 2.1	4.3 ± 1.1	4.3 ± 0.8	0.93	0.39	0.30	0.52	0.52	0.96
Motility B+C (%)	23.3 ± 3.1	11.65 ± 3.4	20.9 ± 2.5	13.7 ± 2.7	0.08	0.61	0.11	0.10	0.65	0.26
Percentages of different morphologic spermatozoa (%)										
Normally formed	0	0	0	0	NA	NA	NA	NA	NA	NA
Abnormal head-tail junction	3.7 ± 0.4	2.8 ± 0.9	2.1 ± 1.8	2.5 ± 0.5	0.40	0.53	0.12	0.78	0.79	0.78
Decaudated	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	0.5 ± 0.1	0.96	0.76	0.49	0.68	0.23	0.22
Acephalic	96.0 ± 0.6	96.9 ± 1.0	97.7 ± 2.0	97.0 ± 0.6[b]	0.46	0.55	0.28	0.77	0.92	0.65

a. Values are expressed as Mean ± SEM. b. P values > 0.05 means the difference between two groups are not significant. NA: not applicable.

Table S3. Filtering of WES variants in two affected individuals F1:II-3 and F2:II-4

	P1	P2
Total variants	102874	111794
After excluding variants reported in dbSNP, 1000 genomes, EVS, Hapmap-CHB and ExAC (MAF > 1%).	1496	1465
Exonic non-synonymous or splice site variants, or coding indels	237	238
Homozygous or compound heterozygous (excluding X and Y chromosomes)	6	5
Homozygous	3	0
In homozygous region > 1Mb	2	-

Table S4 WES homozygous variants that survived filtering in affected individual F1:II-3

Genomic SNV	Gene	Change	phastCons score	ExAC allele frequency	ExAC homozygotes frequency	NNSplice	SIFT	Polyphen	Mutation Taster	OMIM	Homozygous interval
Chr9: 135523687 G>T	*DDX31*	c.1176-5C>A	0[a]	13/120940	0/120940	0.51 to 0.14[b]	-	-	-		0.18 Mb
Chr13: 52249312 G>A	*WDFY2*	c.G212A;p.Cys71Tyr	688	0	0	-	0.00 (D)[c]	**0.962 (D)**	1.000 (D)		3.97 Mb
Chr20: 31573615 G>A	*SUN5*	c.C824T;p.Thr275Met	501	4/121306	0/121306	-	0.00 (D)	**0.999 (D)**	1.000(D)		2.19 Mb

a. phastCons score reveals the conversation of the site. The scores were higher; the possibilities of being deleterious for the variants will be higher. **b**. NNSplice reveals the decrease of the acceptor site score from 0.51 down to 0.14. **c**. Scores were lower in SIFT or higher in either Polyphen or MutationTaster, the possibilities of being deleterious (D) for the mutations will be higher.

Table S5 WES heterozygous variants that survived filtering in the affected individual F2:II-4

Genomic SNV	Gene	Change	phastCons score	ExAC allele frequency	ExAC homozygotes frequency	SIFT	Polyphen	MutationTaster	OMIM
Chr2: 100938293	*LONRF*	c.G263T;p.Gly88Val	0	Not found	Not found	0.11 (T)	0.297 (B)	0.000 (N)[a]	
Chr2: 100938294	*LONRF*	c.G262T;p.Gly88Trp	0	Not found	Not found	0.01 (**D**)	0.957 (**D**)	0.000 (N)	
Chr11: 66307056	*ZDHHC*	c.C799T;p.Pro267Ser	469	Not found	Not found	0.33 (T)	0.994 (**D**)	1.000 (**D**)	
Chr11: 66307057	*ZDHHC*	c.G798T;p.Leu266Phe	469	2/111740	0/111740	0.20 (T)	0.923 (**D**)	1.000 (**D**)	
Chr17:76554252	*DNAH1*	c.G2116A;p.Glu706Lys	0	1/117758	0/117758	1.00 (T)	0.003 (B)	0.000 (N)	
Chr17: 76421553	*DNAH1*	c.A13015G;p.Met4339V	524	6/121214	0/121214	0.19 (T)	0.063 (B)	0.112 (N)	
Chr17: 76421543	*DNAH1*	c.A13025G:p.Lys4342Ar	524	5/121274	0/121274	0.23 (T)	0.191 (B)	0.804 (**D)**	
Chr20: 62191852	*HELZ2*	c.C7480G;p.Leu2494Val	505	16/120832	0/120832	0.34 (T)	0.063 (B)	0.001 (N)	
Chr20: 62196216	*HELZ2*	c.T3959C;p.Leu1320Pro	0	3/117818	0/117818	0.04 (**D**)	0.899 (**P**)	0.000 (N)	
Chr20: 31571674	*SUN5*	c.C1066T;p.Arg356Cys	440	6/121306	0/121306	0.00 (**D**)	0.999 (**D**)	1.000 (**D**)	
Chr20: 31583474	*SUN5*	c.T485A;p.Met162Lys	435	Not found	Not found	0.02 (**D**)	0.042 (B)	**0.778** (**N**)[b]	

a. The letter D in the brackets indicates deleterious, N neutral, T tolerated and B benign. **b.** The p.Met162Lys mutation is predicted to cause a loss of coil in SUN5.

Table S6. Genomic PCR primers used to amplify *SUN5* exons for Sanger sequencing.

Exon	F/R[a]	Primer Sequence (5' to 3')	PCR Size (bp)
1	F	TTGGCACTTTTCAGGGGAC	564
	R	TTGGAATTTCTGTCACCTGCA	
2/3	F	CAACCTGGGTCTTTTCCTTGA	684
	R	CCGATGGGTAGAACTCCAGA	
4	F	AACCAGTGCCCACCTGTAG	391
	R	GGATAGCTTTTCTGGTCTGGC	
5	F	TTGCCAAGTTCACAGGGTTG	489
	R	ACGGTTTGGGGAGAGCTTTA	
6	F	CGCCTTCTTCTCTTTGCCAG	483
	R	TGGATCTGGGTCAAGTCAGG	
7	F	TGTGGGAGAATAGATGGATGAAG	475
	R	TGCAATTAAGGATGCTGCCA	
8	F	ATGAATGGGTCCAGGGATGG	284
	R	AGATGTTTGGGGCAGAATGG	
9	F	GTTAGGGAGAGGACAGCGTC	299
	R	ATGAACTTCCCAGCGGCA	
10	F	ATAGTCAGGGCCCATCAGTG	481
	R	CAAAGCAAGCTGTCCCTCTG	
11	F	AGGGGATCAAAGGTGGGATG	376
	R	ACTTCCAGCCTTAACCAAGC	
12	F	TCTTCAGAGACCATGGAGCC	667
	R	AAGAAACACAGCAAGAGGGC	
13	F	TGTCTGTCCTTCTGGCCTC	437
	R	TCAGATGTGAAAGGCTAGCC	

a. F represents forward primers and R represents reverse primers.

Web Resources

MultAlin, http://multalin.toulouse.inra.fr/multalin/multalin.html;

ESEfinder,

http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home;

NNSplice, http://www.fruitfly.org/seq_tools/splice.html;

SIFT, http://sift.bii.a-star.edu.sg/;

Polyphen, http://genetics.bwh.harvard.edu/pph2/;

MutationTaster, http://www.mutationtaster.org/