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Supplemental information

A pathogenic variant in the uncharacterized *RNF212B* gene results in severe aneuploidy male infertility and repeated IVF failure

Moran Gershoni, Tslil Braun, Ron Hauser, Shimi Barda, Ofer Lehavi, Mira Malcov, Tsvia Frumkin, Yael Kalma, Shmuel Pietrokovski, Eli Arama, and Sandra E. Kleiman

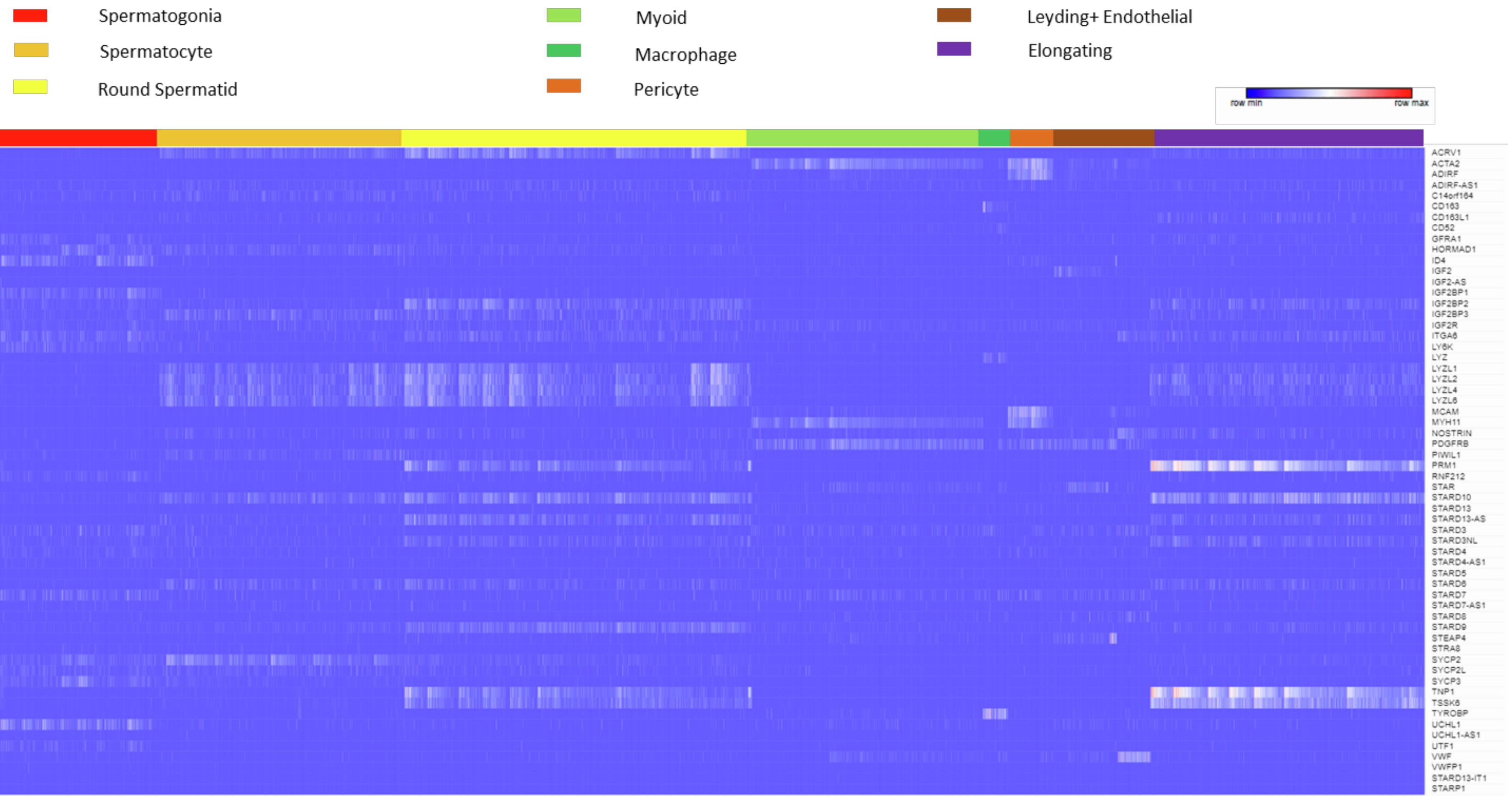


Figure S1. Hierarchical clustering of testis single-cell RNA-seq obtained from[1] using spermatogenic cell markers (X axis, as detailed in the Materials and Methods section).

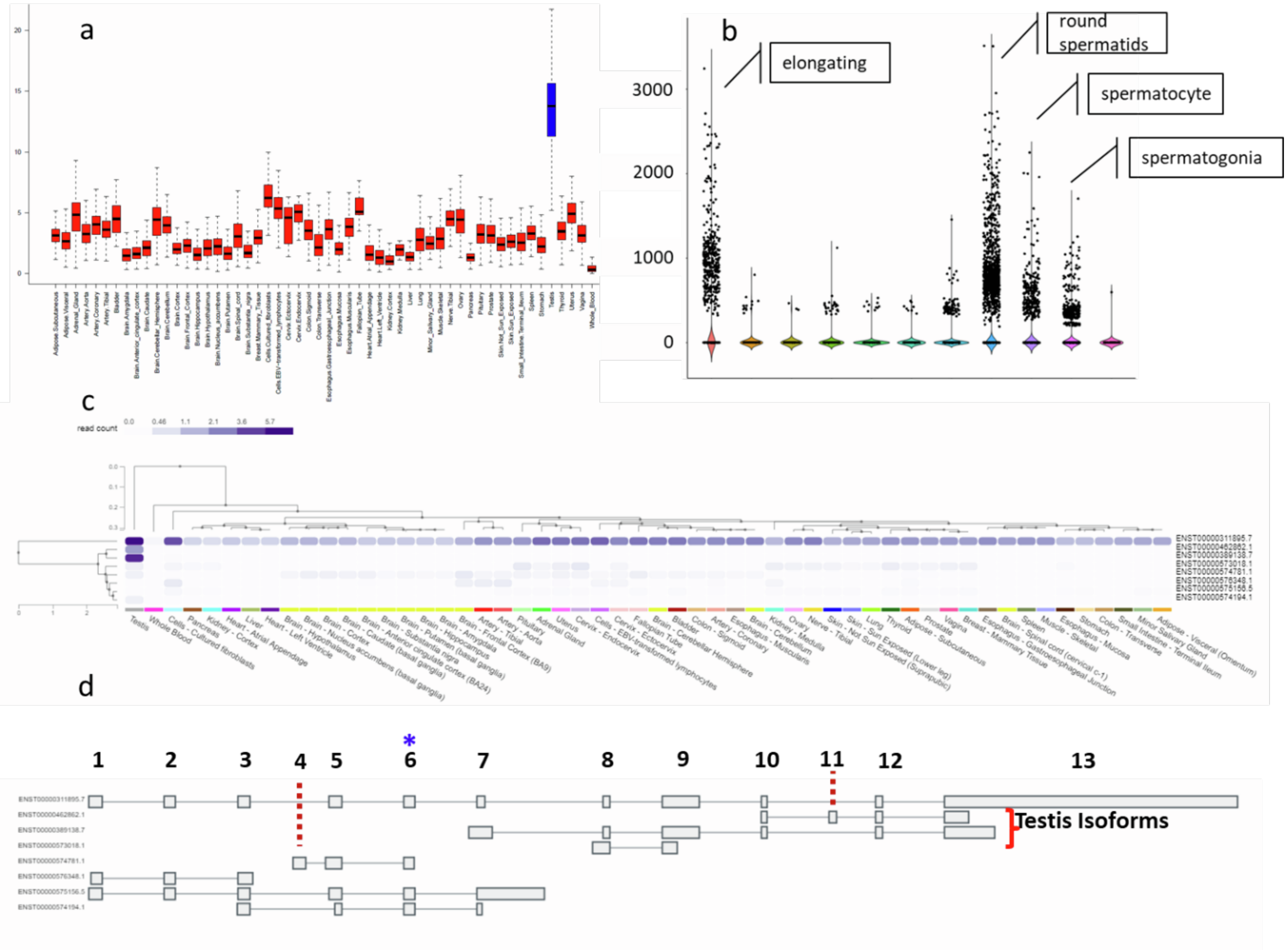


Figure S2. ERCC4 expression analysis. a) Expression pattern of ERCC4 across 53 human tissues obtained from GTEx[20], blue bar denotes testis tissue. b) Testis single-cell RNA-seq analysis of ERCC4 shows increased expression levels in round spermatids and elongating cells. c) Expression of ERCC4 transcript isoforms shows two ERCC4 testis-specific transcripts. d) schematic structure of ERCC4 as inferred from the GTEx data shows that

ERCC4 testis-specific isoforms do not harbor the exon with the candidate variant. The Blue asterisk denotes the variant location. Dashed lines point to the exon number.

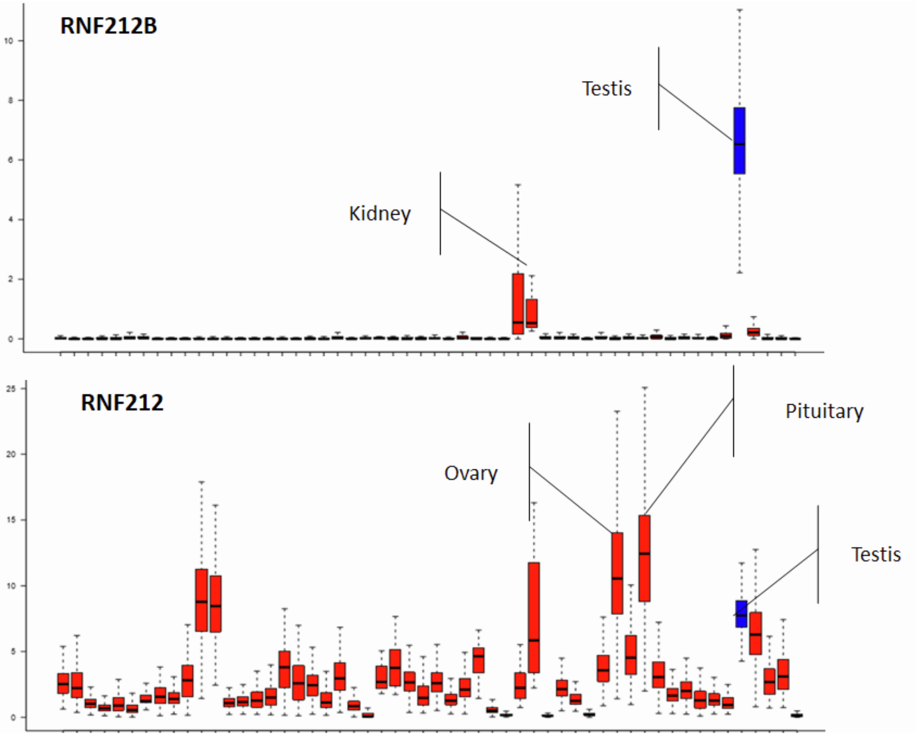
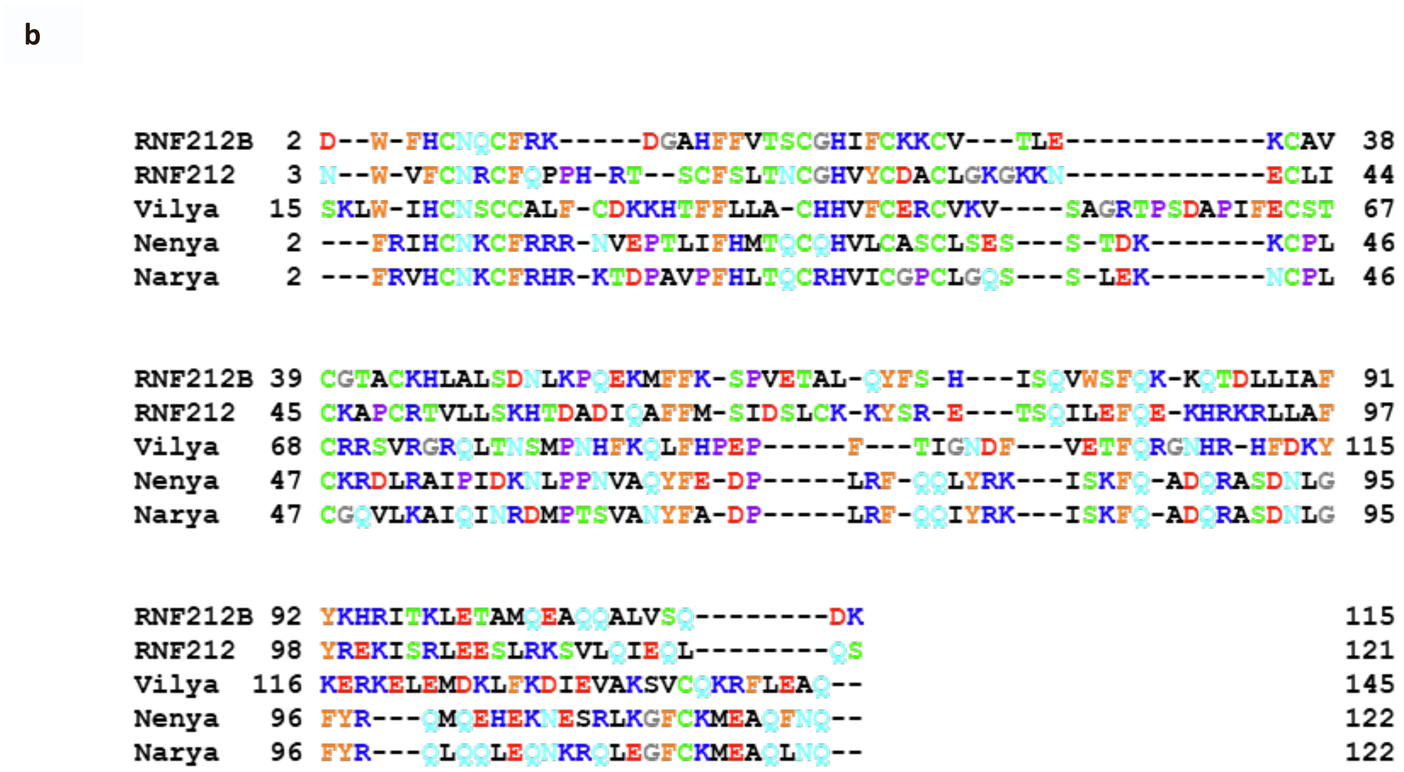


Figure S3. RNF212B bulk RNA-seq expression analysis. Expression of RNF212B and RNF212 in Transcript per million (TPM) values (Y-axis) across 53 human tissues (X-axis). Blue bars denote testis tissue. RNF212B shows almost testis-exclusive expression (with some expression in two kidney tissues), contrary to RNF212, which shows high expression in Ovary and several somatic tissues.


a
RNF212_calmi
100
RNF212_ambra
72
RNF212_carca
50
RNF212_chipl
29
RNF212_scyca
RNF212_manja
RNF212_ailme
96
RNF212_oryaf
41
39
RNF212_balmu
47
RNF212_cersi
58
29
RNF212_taloc
RNF212_molmo
84
53
RNF212_musmu
100
72
RNF212_human
100
RNF212-ie_human
RNF212_vomur
61
63
RNF212_tacac
100
RNF212_ornan
RNF212_promu
100
RNF212_pogvi
40
RNF212_gopev
57
96
RNF212_allmi
40
RNF212_melun
96
RNF212_galga
53
RNF212_micun
68
RNF212_xentr
RNF212_latch
RNF212_polse
100
29
RNF212_erpca
100
RNF212_lepoc
RNF212_angan
44
100
RNF212_salsa
92
RNF212_xipco
62
RNF212_cheun
64
RNF212_astca
48
96
RNF212_takru
RNF212B_erpca
92
RNF212B_angan
100
RNF212B_saltu
86
RNF212B_xipco
92
RNF212B_astca
40
RNF212B_cheun
96
RNF212B_takru
RNF212B_calmi
100
98
RNF212B_scyca
99
RNF212B_carca
76
RNF212B_hetze
79
RNF212B_pripe
RNF212B_latch
RNF212B_tacac
97
38
RNF212B_ornan
60
RNF212B_vomur
77
RNF212B_oryaf
80
RNF212B_human
RNF212B_musmu
54
15
RNF212B_taloc
31
46
23
RNF212B_cersi
31
RNF212B_ursar
39
RNF212B_manja
41
40
57
RNF212B_molmo
15
RNF212B_phyca
100
RNF212B_balmu
RNF212B_pogvi
100
37
RNF212B_promu
87
RNF212B_allmi
55
RNF212B_gopev
71
100
RNF212B_melun
RNF212B_melga
RNF212B_xentr
68
RNF212B_geosa
RNF212l_petma
100
RNF212l_enttr
98
RNF212l_letre
100
RNF212l_letca
41
RNF212l_sacko
71
RNF212l_ptyfl
RNF212l_brafl
100
RNF212l_brabe
93
RNF212l_brala
RNF212l_astru
86
RNF212l_acapl
99
41
RNF212l_patmi
RNF212l_strpu
100
RNF212l_lytva
Jawed vertebrates RNF212
Jawed vertebrates RNF212B
Mammals
Reptiles
Birds
Amphibians
Lobe-finned fish (coelacanth)
Ray-finned fish
Cartilaginous fish
Lampreys
Lancelets
Hemichordates
Echinoderms



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RNF212B  2 D--W-FHCNQCFRK-----DGAHFFVTSCGHIFCKKCV---TLE------------KCAV  38
RNF212   3 N--W-VFCNRCFQPPH-RT--SCFSLTNCGHVYCDACLGKGKKN------------ECLI  44
Vilya   15 SKLW-IHCNSCCALF-CDKKHTFFLLA-CHHVFCERCVKV----SAGRTPSDAPIFECST  67
Nenya    2 ---FRIHCNKCFRRR-NVEPTLIFHMTQCQHVLCASCLSES---S-TDK-------KCPL  46
Narya    2 ---FRVHCNKCFRHR-KTDPAVPFHLTQCRHVICGPCLGQS---S-LEK-------NCPL  46


RNF212B 39 CGTACKHLALSDNLKPQEKMFFK-SPVETAL-QYFS-H---ISQVWSFQK-KQTDLLIAF  91
RNF212  45 CKAPCRTVLLSKHTDADIQAFFM-SIDSLCK-KYSR-E---TSQILEFQE-KHRKRLLAF  97
Vilya   68 CRRSVRGRQLTNSMPNHFKQLFHPEP-----F---TIGNDF---VETFQRGNHR-HFDKY 115
Nenya   47 CKRDLRAIPIDKNLPPNVAQYFE-DP-----LRF-QQLYRK---ISKFQ-ADQRASDNLG  95
Narya   47 CGQVLKAIQINRDMPTSVANYFA-DP-----LRF-QQIYRK---ISKFQ-ADQRASDNLG  95


RNF212B 92 YKHRITKLETAMQEAQQALVSQ--------DK                            115
RNF212  98 YREKISRLEESLRKSVLQIEQL--------QS                            121
Vilya  116 KERKELEMDKLFKDIEVAKSVCQKRFLEAQ--                            145
Nenya   96 FYR---QMQEHEKNESRLKGFCKMEAQFNQ--                            122
Narya   96 FYR---QLQQLEQNKRQLEGFCKMEAQLNQ--                            122
```

Figure S4. RNF212-like proteins from vertebrates, hemichordates, and echinoderms. a) Dendrogram calculated from the RING zinc-finger sequence domain of representative proteins from species of various taxa, as denoted in the figure inset. Bootstrap values out of 100 are marked for each junction. The jawed vertebrate regions are marked and labeled. The Echinoderm cluster was used as an outgroup to root the tree. Note the congruence of the topology throughout the tree with established taxonomy of the different well-supported clusters. Sequence sources and descriptions are in Table S5. b) RING zinc-finger domains of Human RNF212 and RNF212B, and Drosophila melanogaster Vilya, Nenya, and Narya proteins. This domain is common in all RNF212-like protein family members.

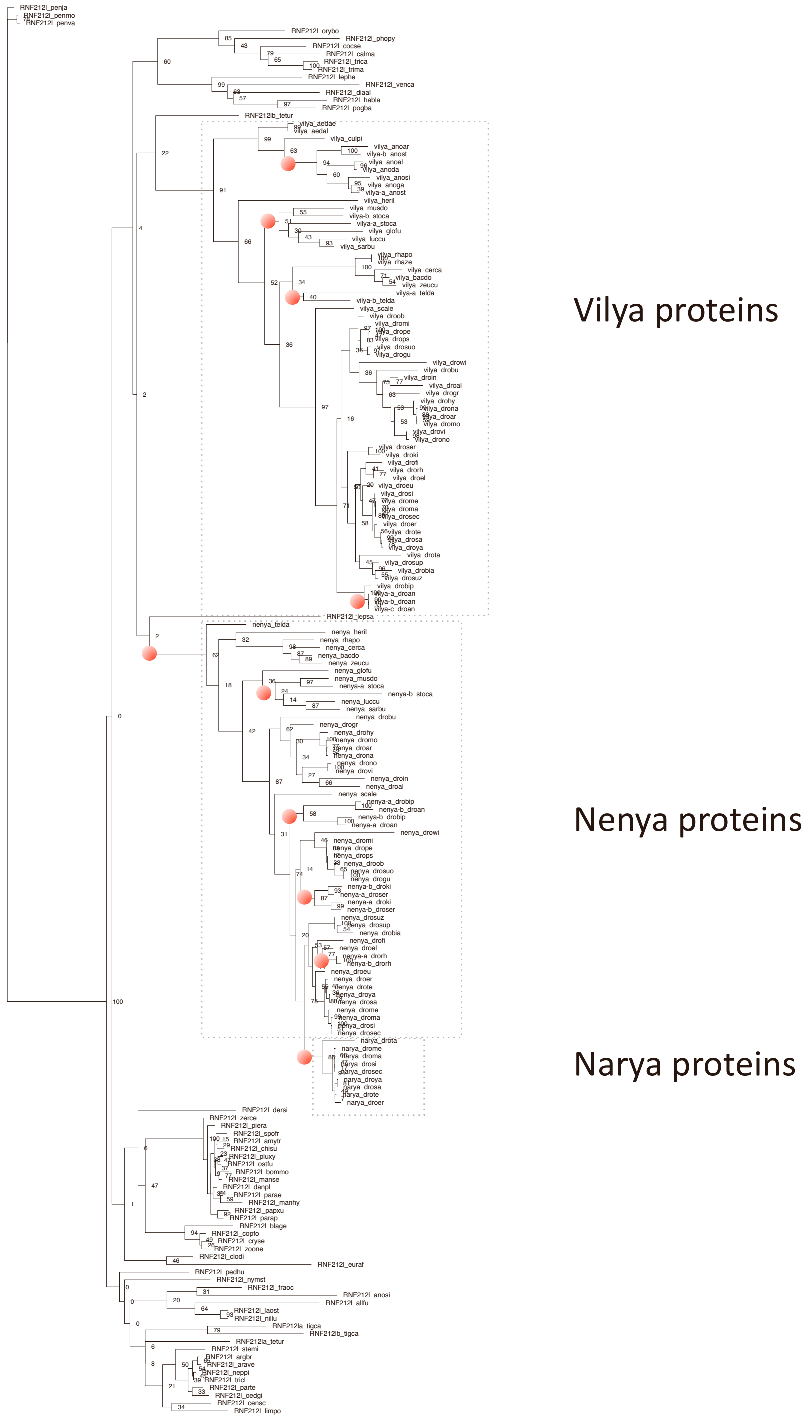
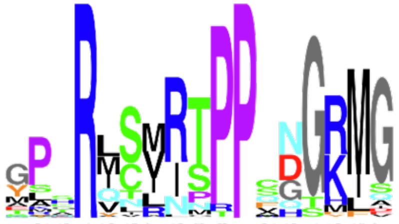

RNF212l_penja
RNF212l_penmo
RNF212l_penva
RNF212l_orybo
RNF212l_phopy
RNF212l_cocse
RNF212l_calma
RNF212l_trica
RNF212l_trima
RNF212l_lephe
RNF212l_venca
RNF212l_diaal
RNF212l_habla
RNF212l_pogba
RNF212lb_tetur
vilya_aedae
vilya_aedal
vilya_culpi
vilya_anoar
vilya-b_anost
vilya_anoal
vilya_anoda
vilya_anosi
vilya_anoga
vilya-a_anost
vilya_heril
vilya_musdo
vilya-b_stoca
vilya-a_stoca
vilya_glofu
vilya_luccu
vilya_sarbu
vilya_rhapo
vilya_rhaze
vilya_cerca
vilya_bacdo
vilya_zeucu
vilya-a_telda
vilya-b_telda
vilya_scale
vilya_droob
vilya_dromi
vilya_drope
vilya_drops
vilya_drosuo
vilya_drogu
vilya_drowi
vilya_drobu
vilya_droin
vilya_droal
vilya_drogr
vilya_drohy
vilya_drona
vilya_droar
vilya_dromo
vilya_drovi
vilya_drono
vilya_droser
vilya_droki
vilya_drofi
vilya_drorh
vilya_droel
vilya_droeu
vilya_drosi
vilya_drome
vilya_droma
vilya_drosec
vilya_droer
vilya_drote
vilya_drosa
vilya_droya
vilya_drota
vilya_drosup
vilya_drobia
vilya_drosuz
vilya_drobip
vilya-a_droan
vilya-b_droan
vilya-c_droan
RNF212l_lepsa
nenya_telda
nenya_heril
nenya_rhapo
nenya_cerca
nenya_bacdo
nenya_zeucu
nenya_glofu
nenya_musdo
nenya-a_stoca
nenya-b_stoca
nenya_luccu
nenya_sarbu
nenya_drobu
nenya_drogr
nenya_drohy
nenya_dromo
nenya_droar
nenya_drona
nenya_drono
nenya_drovi
nenya_droin
nenya_droal
nenya_scale
nenya-a_drobip
nenya-b_droan
nenya-b_drobip
nenya-a_droan
nenya_drowi
nenya_dromi
nenya_drope
nenya_drops
nenya_droob
nenya_drosuo
nenya_drogu
nenya-b_droki
nenya-a_droser
nenya-a_droki
nenya-b_droser
nenya_drosuz
nenya_drosup
nenya_drobia
nenya_drofi
nenya_droel
nenya-a_drorh
nenya-b_drorh
nenya_droeu
nenya_droer
nenya_drote
nenya_droya
nenya_drosa
nenya_drome
nenya_droma
nenya_drosi
nenya_drosec
narya_drota
narya_drome
narya_droma
narya_drosi
narya_drosec
narya_droya
narya_drosa
narya_drote
narya_droer
RNF212l_dersi
RNF212l_zerce
RNF212l_piera
RNF212l_spofr
RNF212l_amytr
RNF212l_chisu
RNF212l_pluxy
RNF212l_ostfu
RNF212l_bommo
RNF212l_manse
RNF212l_danpl
RNF212l_parae
RNF212l_manhy
RNF212l_papxu
RNF212l_parap
RNF212l_blage
RNF212l_copfo
RNF212l_cryse
RNF212l_zoone
RNF212l_clodi
RNF212l_euraf
RNF212l_pedhu
RNF212l_nymst
RNF212l_fraoc
RNF212l_anosi
RNF212l_allfu
RNF212l_laost
RNF212l_nillu
RNF212la_tigca
RNF212lb_tigca
RNF212la_tetur
RNF212l_stemi
RNF212l_argbr
RNF212l_arave
RNF212l_neppi
RNF212l_tricl
RNF212l_parte
RNF212l_oedgi
RNF212l_censc
RNF212l_limpo
Vilya proteins
Nenya proteins
Narya proteins

Figure S5. Arthropods' RNF212-like proteins dendrogram. Regions of Vilya, Nenya, and Narya proteins are marked and labeled. Red spheres mark likely positions of gene duplications. Bootstrap values out of 100 are marked for each junction. Sequence sources and descriptions are in Supplementary Table 5.


Mammals
Reptiles
Birds
Amphibians
Lobe-finned fish (coelacanth)
Ray-finned fish
Cartilaginous fish
Lampreys
Lancelets
Tunicates
Hemichordates
Echinoderms
Brachiopods
Mollusks
Arthropods
Nematodes
Segmented worms
Cnidaria
Ctenophores
Sponges
Choanoflagellates
Fungi
Protists
Jawed vertebrates RNF212B
Jawed vertebrates RNF212
Narya
Nenya
Vilya

Figure S6. Sequence relation between RNF212-like proteins (appendix) . Dendrogram calculated from the RING zinc-finger sequence domain (figure S4b of diverse proteins, colored by phylogenetic groups as shown in the inset). Bootstrap values out of 100 are marked for each junction. The jawed vertebrate regions, and arthropod Vilya, Nenya, and Narya-like proteins are marked and labeled. Sequence sources and descriptions are in Supplementary Table 5.



Human	176	GPARISMISPPQDGRMG
Lamprey	223	GSSRVSMRNPPAEGRMG
Lancelet	210	GPARITMRTPPIDGKMG
Tunicate	212	EKPRMSLRSPPVGGKIQ
Acorn worm	220	GPGRITMRTPPVDGKMG
Sea-urchin	217	GPARYCIRTPPSGGRMG
Spider	191	LPQRMCVITPPMNGKLG
Octopus	234	MSSRVSIRTPPSQGRID
Leech	233	GPSRLSMRTPPINGRIA
Brachiopod	196	MASRLSVRTPPVHGRLG
Coral	202	GPTRLSVRTPPCNGFMG
Hydra	255	SNGRISLRTPPVNGKLG
Sponge	356	APGRLSIRTPPINGKMG

Figure S7. Protein sequence motif present in RNF212 proteins of jawed vertebrates and other animals. Top, sequence logo of the motif. Bottom, motif sequences and protein start coordinates from representative species: Human (accession NP_001353848.1), lamprey (*Petromyzon marinus*, XP_032830699.1), lancelet (*Branchiostoma floridae*, XP_035678696.1), tunicate (*Ciona intestinalis*, XP_002131044.1), acorn worm (*Saccoglossus kowalevskii*, XP_006816460.1), sea-urchin (*Strongylocentrotus purpuratus*, XP_030831903.1), spider (*Nephila pilipes*, GFS75922.1, octopus (*Octopus sinensis*, XP_029633755.1), leech (*Hirudo medicinalis*, MPNW01000810.1), brachiopod (*Lingula anatina*, XP_013394848.1), coral (*Pocillopora damicornis*, XP_027037663.1), hydra (*Hydra vulgaris*, XP_012561882.1), sponge (*Amphimedon queenslandica*, XP_019860695.1).

Supplementary Table 1. PCR primers, annealing temperature, product size, and fragments size after restriction treatment for detection of candidate mutations

GENE	Variant	Objective	Primers (5’ -> 3’)	PCR		Restriction ^		
				Annealing (^{o}c)	Product size (bp)	Enzyme	Normal allele (bp)	Mutant allele (bp)
RNF212B	Exon8:c.C448T:p.R 150X	PCR, sequencing and RFLP	F- GTTGGAGAGCAATGGGTTTT R- GGCCAATTTGTTGTACATGG	60	399	Taq I	208+235	399
		Rt-PCR	F- CTTCACCGGCTTCAACTCAT R- GCAGGACTGGTAGCTGGAAA	64	cDNA:182 gDNA:1730			
ERCC4	exon6:c.A1062C:p. K354N	PCR and sequencing	F- GGAAGACAGGATGACAGCCA R- ACACACTTTTCACATGGCCA	60	341			
		RFLP	mF- TTCCAGATGCCAAAATGAGTAA**G**A	60	170	MBO II	34+36+100	136+34
VCX3B	exon3:c.C385G:p.Q 129E	PCR and sequencing	F- AAAGGGGCTGCGACAAAGAT R- ATAGGGGAGTACTTGGCCGT	62	605+485			

^After restriction, PCR products were analyzed on agarose gel (3 %).

Both enzymes were from NEB (USA).

F, forward primer; R-reverse primer; mF mismatch forward primer; bp, base pairs.

Supplementary Table 2. CNVs analysis around RNF212B R150X PV

	Location at chromosome 14												
Men tested	21769717	22110223	22507947	22687399	22781471	23026660	23069623	23295947	**23731887**	23904379	24042993	24339720	24397229
									RNF212B-mut				
P-1	**363/363**	**270/270**	**286/286**	**313/313**	**426/426**	**327/327**	**275/275**	**225/225**		**266/266**	**251/251**	**323/323**	**233/233**
P-2	**363/363**	**270/270**	**286/286**	**313/313**	**426/426**	**327/327**	**275/275**	**225/225**		**266/266**	**251/251**	**323/323**	**233/233**
Fertile brother	**363**/366	**270**/270	**286**/292	**313**/313	**426**/428	**327**/333	269/**275**	**225**/229		**266**/266	**251**/251	**323**/323	**233**/243
PS-1	**363/366**	**270/276**	**280/284**	**313/326**	**428/428**	**327//327**	**275/275**	**225/225**		**266/278**	**251/251**	**323/323**	**241/249**

Supplementary Table 3. Likely functional candidate mutations in patients P-1 and P-2

Chr[1]	**Start**[2]	**Ref**[3]	**Alt**[4]	**GENE**[5]	**FUNC**[6]	**SIFT**[7]	**Mut. Taster**[8]	**gnomAD exome**[9]	**gnomAD genome**[10]	**Testis r-score**[11]
14	**23731887**	**C**	**T**	**RNF212B**	**stop**	**.**	**A**	**0.0002**	**.**	**0.99**
16	**14026102**	**A**	**C**	**ERCC4**	**NS**	**D**	**D**	**.**	**.**	**0.75**
20	47614885	G	A	ARFGEF2	NS	D	D	0.001	0.0003	0.21
2	1.49E+08	G	A	MBD5	NS	T	D	0.0023	0.0017	0.11
2	1.61E+08	C	T	ITGB6	NS	D	D	0.0081	0.0035	0.09
20	33511162	G	A	ACSS2	NS	T	D	0.0006	0.0002	0
20	44470575	G	A	ACOT8	NS	D	D	0.005	0.0044	0
20	40049450	T	A	CHD6	NS	D	N	0.0052	0.0029	-0.009
1	1.86E+08	G	A	HMCN1	NS	T	D	0.0009	0.0002	-0.02
1	1.86E+08	C	T	HMCN1	NS	D	D	0.0009	0.0001	-0.01
6	90121647	-	TCC	RRAGD	INS					-0.02
20	25060143	G	C	VSX1	NS	T	D	0.0034	0.0029	-0.02
2	1.61E+08	A	G	LY75	NS	D	N	3.25E-05	.	-0.04
2	1.67E+08	C	T	SCN7A	NS	D	N	4.08E-05	6.46E-05	-0.04
X	1.14E+08	G	A	LRCH2	NS	D	D	0.0003	0.0001	-0.06

1-2.Chromosomal position.

3. Reference allele (hg19).

4. Alternative allele.

5. HGNC gene symbol.

6. Transcriptional consequence. NS=non synonymous; INS=insertion; stop= stop-gain

7-8. functional impact prediction by SIFT[7] and Mutation tester[8] .D= deleterious; A= affect; T= tolerant; N= neutral

9-10. minor allele frequencies obtained from the gnomAD[45]

11. The Pearson correlation co-efficiency score for testis-specific expression (see material and method).

Supplementary Table 4. FISH findings of blastomeres after fertilization with P-1 sperm cells

Embryo number	Stage	Blastomeres tested	Fish findings
1	I	2	t21, m22
2	I	1	m13, m18, m22
3	I	2	m13, m22
4	I-II	2	m16, m18, m22, n13
5	I	1	t13
6	I arrest	2	m16, m18 ,n22
7	II-III arrest	1	haploid cell

A sampling of embryos was performed on day 3. The assessment was for chromosomes 13, 16, 18, 21 and 22

Stage I: <10% fragmentation and similar cell sizes. Stage II: 10-20% fragmentation, and cells were not all of the similar sizes.

t: trisomy

m: monosomy

n: nullisomy