

Supplementary Information

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Supplementary Figure Legends

Supplementary Figure 1

Alignment of TEX11 protein sequences. Multiple sequence alignment was performed using CLUSTAL 2.0.9. Red font marks residues affected by the five *TEX11* missense mutations identified in infertile men.

Supplementary Figure 2

Analysis of chromosomal synapsis in spermatocytes from 25-day-old wild type and *Tex11* KI(WT);-Y mice. Surface spread nuclei from spermatocytes were immunostained with anti-SYCP1 and anti-SYCP2 antibodies. Representative images of spermatocytes at the pachytene stage are shown. XY chromosomes are indicated. This supplementary figure is related to Figure 3B.

Supplementary Figure 3

Number of MLH1 foci in spermatocytes from 3-month-old males. Representative images are shown for the following *Tex11* genotypes: A) -/Y, 17 MLH1 foci; B) KI;-/Y, 21 MLH1 foci; C) +/Y, 22 MLH1 foci; D) KI;+/Y, 26 MLH1 foci. This supplementary figure is related to Figure 4A.

Supplementary Figure 4

Analysis of chromosomal synapsis in spermatocytes from 3-month-old male mice. Surface spread nuclei from spermatocytes were immunostained with anti-SYCP1 and anti-SYCP2 antibodies. XY chromosomes are indicated (A-C). Representative images are shown for the following *Tex11* KI/KO (KI;-/Y) genotypes: A) wt; B) W118R; C) Q173R; D) KIV749A. The spermatocyte (D) displayed extensive chromosomal asynapsis. This supplementary figure is related to Figure 6E.

Supplementary Figure 5

Testicular maturation arrest in an infertile male patient with V748A mutation in *TEX11* (WHT2499). Testicular tissue was obtained by biopsy, sectioned, and stained with hematoxylin/eosin. The biopsy sample shows absence of post-meiotic germ cells such as round spermatids in the seminiferous tubule, whereas meiotic stage spermatocytes such as at the pachytene stage (arrows) are present. The boxed tubule in panel A is enlarged in panel B. Scale bar, 50 μ m.

Supplementary Table 1 Common sequence variants in human *TEX11* gene.

Position	Nucleotide change	Resultant change	Infertile males		Normal males
			AZ 246*	Fertile 93*	NIH diversity 82*
Exon 6	344A→G	Missense mutation, K115R; SNP: rs6525433	14 [#]	5	20
Exon 16	1306G→A	Missense mutation, E436K; SNP: rs4844247	11	4	10
Exon 26	2274A→T	Silent mutation	1		1
Exon 28	2496T→G	Missense mutation, D832E			2
Exon 28	2496T→C	Silent mutation; SNP: rs16991177	13	4	9
Exon 30	123T→C 3'UTR	Alteration in the first AATAAA signal	2	1	0
Intron 4	+35G→A	Intronic alteration; SNP: rs5937008	118	34	29
Intron 4	-22T→C	Intronic alteration	7	1	9
Intron 4	-24C→T	Intronic alteration	1	1	3
Intron 7	-26T→C	Intronic alteration; SNP: rs17301944	25	9	2
Intron 8	+11T→A	Intronic alteration	1	1	1
Intron 11	-27T→C	Intronic SNP: rs6653304	12	5	2
Intron 16	+48A→T	Intronic SNP: rs5980996	38	23	13
Intron 18	-25A→G	Intronic SNP: rs1325094	40	24	14
Intron 18	-23T→C	Intronic SNP: rs1325094	88	39	21
Intron 21	-10T→A**	Intronic alteration	2		
Intron 24	+89T→C	Intronic alteration			2
Intron 28	+58T→C	Intronic SNP	8	3	12
Intron 28	-3, -4 TC→AT	Intronic SNP: rs1536250, rs1536251	15	4	2

Notes: *Number of individuals screened; AZ, azoospermic males. **+1 refers to the first base of a given intron, and –1 the last base.

[#]Number of males with the given variant.

Supplementary Figure 1 Alignment of TEX11 protein sequences

<u>Species</u>		<u>Accession No.</u>
Human		NM_031276
Mouse		NM_031384
Macaq	Macaque	AB074448
Dog		XM_844423
Horse		XM_001490344
Cow		XM_593293
Rat		XM_576992
Oposs	Monodelphis domestica	XP_001368341
Chick	Gallus gallus	XM_420206
Danio	Danio rerio	XM_001922537
Tetra	Tetraodon nigroviridis	CAG09799
Branch	Branchiostoma floridae	XM_002210899
Coral	Acropora millepora	EZ003728
Nemat	Nematostella vectensis	XM_001630824

Multiple sequence alignment was performed using CLUSTAL 2.0.9.

Human	-----	MDNDDFFSMDFKEVVENLVTNDNS	24
Mouse	-----	MDRITDFYFLDFRESVKTLIITGNS	25
Macaq	-----	-----	1
Dog	-----	MTSLWEEVPSARRPALWIEFVERRLWLSWSCGEIVENLIVKDNS	44
Horse	-----	-----	MKIIENLIVKGNS
Cow	-----	-----	MSLLSFVISETIENLIATDIS
Rat	-----	-----	MIITGNS
Oposs	-----	-----	1
Chick	-----	-----	1
Danio	-----	-----	MEQFAVEVKDLSEKLLHRQSH
Tetra	-----	-----	1
Branch	MHVFLVFSTDLLRLLFKFNFKNLYLFFCRLKALEKETLPKCQATMIKALEDKDEDKVA	60	
Coral	-----	-----	1
Nemat	-----	-----	1

Human	PNIPEAIDRLFSDIANINRESMAEITDIQIEEMAVNLWNWALTIGGGWLVNEEQKIRLHY	84
Mouse	WRLQEMIDRFFTNISNFNRESLTEIQNIQIEEIAVNLWNWAVTKRVELSVRKNAAKLCY	85
Macaque	-----MIFFPWT-----LKIEEMAVNLWNWALTIGGGWHVNEERKIKLHY	40
Dog	PSIPEAINRLFTDIANINRESIAEIQDAQVEEMAVNLWNWAVTKRVDLVINEEQKAKLWY	104
Horse	PSIHEAIDRLFMDIANINRESMAEMQDAQVEEMAVNLWNWAVTRRVGLVISSEEQKAKLQH	73
Cow	PTTPAAIDRLFMDIGNINRESMAEIQDSQIEEMAVNLWNWIITNNIGLVINEEQKAKVRH	81
Rat	WRLHEMIDKFFMNISNFNRKSLTEVQNIQIEEIAVNLWNWATAKRLELSVRKSQAACLHY	67
Opossum	-----	1
Chick	-----	1
Danio	SDLDEVIDSLFKEILTLD--EAAKLQDSOLEEIAIQLWNWAVTKRVGTSITEEQKAKVRH	79
Tetra	-----	1
Branch	QRAETLSELLDKVSGLHGDSKNRTSSGELQNCAVNLWNIAVAKRAGGLSTGLNARLRH	120
Coral	-----	1
Nemat	-----	1

W117R

V142I

Human	VACKLLSMCEASFQSIQRLIMMNMRIGKEWLDAGNFLIADECFCQAAVASLEQLYVVKL	144
Mouse	IACKLVYMHGISVSSEEAIQRQILMNIKTGKEWLYTGNAQIADEFFQAAAMTDLERLYVRL	145
Macaque	VACKLLSMCEASFQSIQRLIMMNMRIGKEWLDAGNFLIADECFCQAAVASLEQLYIKL	100
Dog	VACKLVCVMCGSAASEEAIIRQIQLMNMKTGKGWWDVGNAVIADEFQAAAMAGLEQLYVVKL	164
Horse	VACKLVCMCEDSAVSEEAIQKQIQLMNMKTGKGWLDVGNAVTADEFQVAIAGLEQLYVRL	133
Cow	VACKLIRMCEHPDASEEAIHRQIQLMNMKTGKGWVN VGNVLADDFFQSAITSLEKLYSKI	141
Rat	IACKLVCMHGVSVSSEEAIQRQIQLMNMKTGKQWLDTGNAQIADEFFQAAAMANLEKLYSRL	127
Opossum	-----	1
Chick	-----MQCGVARRETSMRSAVPARSPPEQAAARMRPAVLSLSSGRAVLRDGLGHELLRELL	54
Danio	VACRLLYSCGPENPSESIVRKQIILMANKTGRWTLDCKNSKSADAFLSMAVNSLETLYSRL	139
Tetra	-----FSSYLQSLETLYSKL	15
Branch	LACQLAFKVSPLEGSEVVLKRRVMMASKTGRAWLDGGNPSMADNSLSSLASECLEKLTCSV	180
Coral	-----	1
Nemat	-----	1

Q172R

Human	IQRS----SPEADLTMEKITVESDHFRVLSYQAESAVAQGDFQRASMCVLQCKDMLMRLP	200
Mouse	MQSC----YTEANVCVYKMIVEKGIFHVLSYQAESAVAQGDFKKASLCVLCKDMLMRLP	201
Macaque	IQRS----SPEADLTMEKITVERDHFRVLSYQAESAVAQGDFQRASMCVLQCKDMLMRLP	156
Dog	MQRS----STEIHMALHKIAMERDLFKVLSYQAEAAVAQGDFHRAYTCTLRCKDMLMRFP	220
Horse	MQRS----STEAHVAMQKIAVERDLFKVLSYQAESAVAQGDFQRASACVLCKDVLMRVP	189
Cow	TQRS----PTEEHVMVQKNTVERDLLKVLSYQAEAAVAGGNFQKASRCILRCKDMLVRLP	197
Rat	MQSC----HTETNLSLYKNTVERLHCYVNWEIETAVAQGDFKKASICVLCKDMLIRIP	183
Opossum	-----	1
Chick	REEA----PPRIAALTEELFQAAAGALCEADGSAAEAVAQGDFQKAMQCVRCKDMLVRLP	110
Danio	TSHG----DGE-DMNTPKGDIKEKDLLRILSYQAESAVSQEQHQVAVSCIQRCKEILLRLP	194
Tetra	TSRT----DGSYDSTLSKEDVEKDLLRVLSFQAESALCQGNNAEALTCIQRCKDMLRLP	71
Branch	LSAAAEGKLSEDDKERQKTEIEKDMFKVFCYQAESAVAQEHDVAMRLAQRCKEMLARLP	240
Coral	-----	1
Nemat	-----AMSLGNNNSALECIEKAKAFLVKLP	25

T244I

Human	QMTSSLHHLCYNFGVETQKNNKYEESSFWLSQS ^Y DIGKMDKK-S	T GPEMLAKVLRLLATN	259
Mouse	NMTKYLHVLCYNLGIEASKRNYKESSFWLGSQ ^Y EIGKMDRR-S	V EPQMLAKTLRLLATI	260
Macaque	QMTSSLHHLCYNFGVG ^T QKNNKYEESSFWLSQS ^Y DIGKMDKK-S	T RPEMLAKVLRLLATN	215
Dog	KMTGYLHILCYNFGVETHQK ^N KYEESSFWLSQS ^Y DIGKMDKN-S	V GPEMLAKVLRLLAT	279
Horse	KKTRYLHILCYNFGVETYQK ^N KYEESSFWLSQS ^Y DIGKMEKN-S	I GPEMLAKVLRLLAT	248
Cow	QMVCYLHTVCYNGLQTYREN ^K YEE ^E SCFWLSQSFDIGKT ^D G-N	S VEPEMLAKVLRLLAT	256
Rat	EMTKYLHVLCYNFGE ^S SKQ ^N KYRESSFWLSQS ^Y DIGKMGKD-S	V EP ^E MLAKGLRLLATI	242
Opossum	-----	-----	1
Chick	RETRYLAILCYNFGVETYDCK ^K YEQSSFWLSQS ^Y DIGKMDMKYS	I GKEMQAKVLRLLAT	170
Danio	KETGYLSLICYNFGVDTYSQGKHEESTFWLSQS ^Y DIGKMNMKYS	P GSEMQAKVLRLLATV	254
Tetra	KDTAYL ^S LMCYNFGVDTYNMRKFEDSAIWLSQS ^Y DIGKINVKYA	P GSEIQAKILRLLATV	131
Branch	KETTFLAMLCYNFGVETYQK ^Q KYEEAVAWLR-----	-----	271
Coral	-----	-----	1
Nemat	KENATLSRLCYNFGVTTYNRREYEDCIEWLRESLELGKGKAP--	V GHKKQATT ^L RLIANA	83
Human	YLDWDDTKYYDKALNAVNL ^A NKEHLSSPGLFLKM ^A KILLKGETSNEE--	LLEAVMEILHLD	317
Mouse	YLNCGGEAYYT ^A KAFIAILIANKEHLHPAGLFLKM ^A RILMGN ^A SCNEE--	LLEAAKEILYLA	318
Macaque	YLDWDNTKYYDKALNAVNL ^A NKEHLN ^A STGLFLKM ^A KILLKGETSNEE--	LLEAVMEILHLD	273
Dog	YVDWDDQEYYDKALSAINLANKEHLNP ^A SGLFFKM ^A KILLKSERANEE--	LLEAVMEILHLD	337
Horse	YLDWDDREYYDKALSAINLANKEHSHPAGLFLKM ^A KILLKGEIA ^N EE--	LLEAVMETILHLD	306
Cow	YLDWDSSEYRD ^A KALNIINLANKEHLD ^A PVGLFLKV ^A KILLKGEIGNEE--	LFQNVMEILHLG	314
Rat	YLNCDDEAYYNKALIAVIVANKEHLD ^A PAGLFLKM ^A RILIKGKAFNEE--	LLEAAKEVLYLA	300
Opossum	-----	-----	1
Chick	YFEWDCSLYLDKALKAINLANEENLHPAGFFLKV ^A KILLKSGASDEG--	ISSAVA ^E FQHHE	228
Danio	YLEWDHQ ^A YLEKALQAVSLANKEHMQLSGLYLKIRILVKGCSPDEA--	VKSGLSELLDCE	312
Tetra	YLEWDCQRFQE ^A KALNAVNL ^A NECMSTSGLYLKIRILQTCGASDDD--	IRAGLNEILEAE	189
Branch	-----LTANLIFTGLANSEHSHPAGLHLKV ^A HILLVSDTVEDNRLVSAVT ^D CLHHPE	-----	322
Coral	-----	-----	1
Nemat	YLKWDS-----LANAEHPHPAGH ^A YLKIELLLLSDNTPMARLQSAFEALSLPD	-----	131
Human	MPLDFCLNI ^A KL ^A MDHERESVG ^A FHFLTI ^A IHERFKSSENIGKV ^A LILHTDMLLQRKEELLAK	377	
Mouse	MPLEFYLSIIQFLIDNKRESVGFRFLRIISDNFKSPEDRK ^A R ^A KILLFYIDTLLQKDQDMIAE	378	
Macaque	MPLDFCLNI ^A KL ^A MDHEGESVG ^A FHFLTI ^A IHERFKSSENIGKV ^A LLYIDMLLQRKEELLAK	333	
Dog	MSLD ^A FCLNVAK ^A LLMDHERESVG ^A FYFMKIICEHF ^A KSSENIGK ^A LLYIDMLLQRKEELLAK	397	
Horse	ASLDVCLNI ^A KL ^A MDHDRESVG ^A FYFLKIICEHF ^A KSSENIGK ^A LLHIDMLLERKEELLAK	366	
Cow	MTMN ^A FCLNIVK ^A LLMDHERDSVG ^A FYFLKIICEHFQ ^A SSEDTSKAQ ^A LLHIDMLLQRKEDLF ^A K	374	
Rat	MPL ^A FEFCLSI ^A IQFLIDNKRD ^A SVGFCFLKIIADHF ^A KLPEHRKR ^A ILLFYIDMLLQVDQDVIAE	360	
Opossum	-----MCL-----LARD ^A SVGFDFLKT ^A CDRFESSADVG ^A KGFLLV ^A K ^A LLQRKEDQLAK	48	
Chick	MSLD ^A FCLN ^A TA ^A KL ^A LEHGRESVG ^A FD ^A FLK ^A VAERFEASP ^A PDFG ^A KV ^A T ^A LLYIEFL ^A LQN ^A KRELLAK	288	
Danio	VPLEVCL ^A STV ^A K ^A LLVEENREALAFD ^A FLK ^A RVCQHFE ^A SSPEL ^A GSALLM ^A HIELL ^A LLQRDKELLAK	372	
Tetra	AALEEC ^A CL ^A STVN ^A LLMS ^A EDREV ^A LAF ^A FLK ^A RVCQHFEASP ^A DLGT ^A ALVLHAELL ^A LLQRGKELLAK	249	
Branch	LTVELALNTIK ^A LLIEHKRAQLALDG ^A V ^A KQLTQQFQASPELG ^A KV ^A YLL ^A LR ^A LLDNRQMQAAK	382	
Coral	-----	-----	1
Nemat	LTAQIGLSLAQLATRYKRTDLALNC ^A QTLATRFKN ^A SPDLAKIQLQHV ^A ELL ^A QNNQDQEAK	191	

Human	EKIEEIFLAHQTGRQLTAESMNWLHNILWRQAASSFEVQNYTDALQWYYYSLRFYST-DE	436
Mouse	EKIKDVLKGYQTRSRLSRDLVNLHNLWGKASRSVKVQKYADALHWYSYSLKLYEY-DK	437
Macaque	EKIEEILAHQTGRQLTAELKNWLHNILWRKATSSFEVQNYADALQWYYYSLRFYSP-DE	392
Dog	EKIDEIIIGQRTGRQLTTELVVCVCLHNILWKKAARSFEVQNYADALNWYYSLRFYAT-DR	456
Horse	EKIEEIVGHQTRGQLTTELVGCLHDILWKKAARSFEVQNYPDALQWYCYSRLYAS-DQ	425
Cow	EKVEEIIIGHQTRGPKLASELIGYFHNIIWQAAKSFEVHNFFDALHWYCYSRLFYAA-DQ	433
Rat	EKIKEILIDHQTRSRLTRALVNWLHNILWGKATRCVKVQNYADALRWYSYSLKLYEC-DQ	419
Opossum	KKVEDILTAHHAGKELPPQIISWLHTILWDRAAQNFQEAQNYFEALQWYNYSLFYPS-GQ	107
Chick	QKVEEIIIGHYTGKQLLPETLNRLHIILWDTAAKHYEAKSYSEALHWYNYSVSFYTP-GQ	347
Danio	QKIEDAITGHYTGKQLASQTLSSLHLLWDRASKNFEAKNFSEALQWYNYSLSFYKA-GE	431
Tetra	QKIEDIITDHYTGKQLSPQALTCLHVMLWDKACKYFEARNYPEALQWYNYSLSFFKA-GQ	308
Branch	ALVEDCITGHNTTQPLDTTRKFHLLLWEQAAQAYEANEYEEALRWYDYSRNLFSSNDR	442
Coral	-----	1
Nemat	DLVEDCITGHNTGQQLSPDICMFHLLLWEKAAN-----	225

Human	MDLDFTKLQRNMACCYLNLQQLDKAKEAVAEAERHDPRNVFTQFYIFKIAVIEGNSERAL	496
Mouse	ADLDLILKRNMVSCYLSLKQLDKAKEAIAEVEQKDPTHVFTRYYIFKIAIMEGDAFRAL	497
Macaque	MDLDFAKLQRNMACCYLNLQQLDKAKEAVAEAERHDPRNVFTQFYIFKIAVIEGNSERAL	452
Dog	TDLDLAKLRRNMASCYLHLKQLDKAKEAVIEAEQRDPNTNIFTQFYVFKIAVLEGNSRAL	516
Horse	MDLDLAKLQRNMACACYLHLKQLDKAKVAVAEEAEQRDPNTNIFTQFYVFKIAVLEGNSDRAL	485
Cow	SDLDLAKLQRNMASCYLHLGQFDKAKDAVIKAAQRDPRNIFTQFYIFKISIVEGNSGRAL	493
Rat	ADMVLVKLKRNMVSCYLSLKQLDKAKEALAEAEQQDPTNIFTQYYIFKIAILDGEAYRAL	479
Opossum	MDADFAKLQRNRASCYIHLKOLEKANAAVKEAERFDAANIFTQFIIFKIAVLRDNTEAL	167
Chick	IDQNLAKLQRNMASCYLHLKQIDKAKEAVKEAERCIDLNSIFTQFSVYKIAVMENDTDKAV	407
Danio	LDPNLAKLQRNRSSCFLHLQQLDKAKEAVEEAARIDSANIFTQFNIYKIAILENNAEKAA	491
Tetra	LDPNLAKLQRNRVSCFLQLKOLEKAKDAVKEAQRSDPDSIFTHFSVYKVAVLENNVERAA	368
Branch	HDNNMAKLERNRSACYLHLKQYDKALEAARQAETCDPTSANTQYALFKIGLLOQNSDAAI	502
Coral	-----	1
Nemat	-----LYERNKCSCYMLQDLKNASEAAAQRIEPNSPIVHFFQFKIALMKGDDNKAM	279

Human	QAIITLENILTDEESEDNDLVAERGSPTMLLSLAAQFALENGQQIVAEKALEYLAQHSED	556
Mouse	QVVSALKKSLMDGESEDRGLIEAGVSTLTILSLSIDFALENGQQFVAERALEYLCQLSKD	557
Macaque	QAIITLENVLTDEESEDNDLVAERGSPTMLLSLAAQFALENGQQIVAEKALEYLAQHSED	512
Dog	QAITTLEKLLE-EDPKENELLTDRDSPVMLLSIAAHFALENGQQIVAGKALEYLAQYSED	575
Horse	QAITSLLEHLLTAAEPEENNLLTDRGSTVMLLSLAAQFALEHGQQIVAGKALEYLAQYSED	545
Cow	QAIIFSLEYLLTTEEQNENNVTKRTSGVMLFCLAQFALENGQQVVAVKALENLAKYSEE	553
Rat	QIVSALKISMEE-DSEDCGLIENGVSTLRLSLCVEFALENEQQFVAQKALEYLIQLSKD	538
Opossum	TAVDALEKSVGNSAAQEKEVMKEENSSTAFLTFAAQFALENGQQDVARRALEYLSQHSQD	227
Chick	EAIIEMGKLAEKQSQHEDKLIVDESTSTNLLSLAAHIALENDQQVVAVRALEHLSEHSQD	467
Danio	AALRGIGILAKAPVSEDRLVTENAAANLLSLAAQIALEHEQQETAIALETLCHEHSED	551
Tetra	EALDAIGLLCKAPVSEDRLVSEDAASHLLKLAQMALENEQQETAMKALESCEHSED	428
Branch	EAIEKMGTVDEKGEENS-----TIEGLVCLAAQLAFFESNRDVAIRALERLVQHSHN	554
Coral	-----	1
Nemat	EAIKQISQSQAN-DHDNNNDVTTDEDDAHGLICLAAQLSLEQNNRSVAVKALDGILETSAS	338

Human	QEQLTAVKCLLRFLLPKIAEMPES-----	EDKKKEMDRLL	592
Mouse	PKEVLGGLKCLMRIILPQAFHMPES-----	EYKKKEMGRLW	593
Macaque	QEQLTAIKCLLRVVLPKIAEMPES-----	EDKKKEMDRLL	548
Dog	PQQVLTALKCLFCLVPRVSQMPES-----	ENKKKEMDRLL	611
Horse	PQQVLTALKCLFRLVLPKVSQMPES-----	ENKKKEMDRLL	581
Cow	PPQVLTALKCLFRLCLPRISQMPES-----	ENKRQEMDRIL	589
Rat	PREVLEALKCLVRIILPQAFYLPES-----	EIKKKEMNRLL	574
Opossum	LQQVLTALKCLIRLTVPOQISQTTECEGNRSIDLLETLFASPFIILRVVPLKRRRVKFSSMS-----	287	
Chick	CQQVFAALKCLVRLTLSKVEKE-----	EKRDNDIKSML	500
Danio	VPQTLTALRCLVRLALSTLENISE-----	ENRNASLDILL	586
Tetra	AGLVLSALRCLVRLVLSATEQLTE-----	GMRARGLVQED	463
Branch	IQQVLTAIRSSNDIILS-----		571
Coral	-----		1
Nemat	NKQVITALRCLIRLKLTISETDVKG-----	DVNGIMPYLKKAL	376

Exon 22-encoded aa sequence

Human	TCLNRAFKVLSQPFGEELSLESRANEAQWFRKTAWNLAQCDKDPMVMMREFFILSYK--	650	
Mouse	NYLNTALLKFSEYFNEAPSTLDYMVNDAWFRTKIAWNLAQSEKDLEAMKNFFMVSYK--	651	
Macaque	TCLNRAFKVLSQPFGEEDLSLESRVNEAQWFRKIAWNLAQCDKDPMVIMREFFILSYK--	606	
Dog	TCLNTALQKLGSQSLNGEALNSDSRAKEAHWFRKIAWNLAQCGDKLVTMREFFILSYK--	669	
Horse	TCLNIALLKLAQFFDGKASTSDSRTNEAHWFRKIAWNLAQCDKDPMVTMREFFMLSYK--	639	
Cow	AYFNTALLNLTQLFEGETWTLASKINEAHWFRKTAWNLAQCEKDPLSMREFFVLSYK--	647	
Rat	SYLDTALLKFSQHFDDTSSTLDPMVNDANWFRTKIAWNLAQSEKDLEKMKNFFMISYE--	632	
Opossum	NKLEIAHQRLAEPVAKENLTLDVWTNEAHWFRKIAWNLAQCEKCPGTMRDFFLLSYE--	345	
Chick	TYLTLAHQRLAEPFTEENLTRDIRTSEAHWFRKVAWNLAQQLKDCPEKMRDFFVLSFK--	558	
Danio	SYLKTAQKLSQVCHIPGQRAEQRSEDANWFRTIAWNLAQCEHSPVRMKDFFLSFQ--	644	
Tetra	RYQR-ALPRLSLSNLLHSDKPS-----FCAPAWNSALYCEKCPDRMRDFFVLSYQAS	514	
Branch	-----YLETAWNGLKGEDTNMMHQFFNMCFK--	599	
Coral	-----		1
Nemat	ECVNKMAALGPLADVQAEATWFMKIGNTILOQKLAPWNMALDSSESSSDLREFFLICYK--	434	

Human	MSQFCPSDQVILIARKTCLLMAVADLEQGRKASTAFAEQTMFLSRALEEIQTCNDIHNFL	710	
Mouse	LSLFCPLDQGLLIAQKTCCLVAAAADVLDLDRGRKAPTICEQNMLLRTALEQIKKCKVWNLL	711	
Macaque	MSQFCPSDQVILIARKTCLLMAAAADVLEQGRKASTAFAEQTMFLSRALEEIQTCNDIQNFL	666	
Dog	LSQFCPSDQVILIAQKTCCLMAAAADVLEQGRKASTAFAEQTRLLNRALEQIHKCRHTWNLL	729	
Horse	LSQFCPSDQVILIAQKTCCLMAAAADVLEQGRKASTTFQQVMLLNRALEQIHKCRDIWNLL	699	
Cow	MSLFCPSDQVILIAQKTCCLMAAAADVLDLQGRRASTAFAEQNCFLNRALDLIRKCRDIWNVL	707	
Rat	LSLFCPSDQGLLIAQKTCCLVAAAADVLEQGRKATTTYEQNKLLRMALEQIQCCKNVWNLL	692	
Opossum	LSQLCPSKETVLTTSQKACLLMVAADLELGRSASEASQQTELLSRALEHIQGCKEISNVL	405	
Chick	LSQFCPSDKAVLIAQKTCCLMAAAIDLELGRQEVTPSEQEWFNQALQHLQACKEIWKVL	618	
Danio	LSQLCAPDRAVLMQKTCLLMSAAASLEICRSSDHTEQQTELLTQTLENIQLCKEIWTI	704	
Tetra	LSQLCPPDRGLLTGQKTCLLMAAAASLELCRKS-PLSDQTEELTRVLEQIQTWCWEWKTL	573	
Branch	FSSLCPMDMANLVRKKTCALMAAAACLQSARNVSDPGEKMEMLKTIVLHVDAACRETREI	659	
Coral	-----		1
Nemat	-----KEALSETLTHVQNCRDLCKRI	455	

V748A

Human	KQTGTFNSNDSC EK-----	LLLYEFEVRAKLN--	DPLLESFLES VWELPHLETKT FETIA	763
Mouse	KKTGDFSGDDCG V-----	LLLYEFEVKTKTN--	DPSLSRFVDS WKMPDLECRTLET MA	764
Macaq	KQTGTFNSNDSC EK-----	LLLYEFEVRAKLN--	DPLLESFLES VWELPHLETKT FETIA	719
Dog	KETGDFSSDP CET-----	LLLYEFEVKAKMN--	DPLLDSFLES VWELPHLESKT FETIA	782
Horse	KETGDFSN DPCT-----	LLLYEFEVKAKMN--	DPFLDSFLES VWELPHLESKT FETIA	752
Cow	KTTGDFSRDP CET-----	LLILYEFEVRAKLN--	DPLLDHFLEP VWELPTLESKA FETMA	760
Rat	KQTGDFSGDDCG V-----	MLLYEFEVKTKTN--	DPSLHSFLQS WKMPGLESRTLE IMA	745
Oposs	KLAGDGSKDPT EN-----	LLLYEIEARAKLH--	DAGLTSLL ESVWELPOLET KILETIA	458
Chick	KLTGDFSKDPT DT-----	LLLYEFEARSKLN--	DPTLNNFMES VWEQP-HEIKTLE IAVA	670
Danio	KTSGISSQDKT DS-----	LLLYEFEARAKLN--	DPKLET VLESVLELDNIET KLLEVIA	757
Tetra	KASGNLSVDPT DTL-----	LLLYEFEARAKLN--	DPKVETVLEA VLELQNVEPKV LETIA	627
Branch	CNSKITDTDCSG DST-----	PILLCLYEFEAK AKLG-----	EPDLTQLVDR VMAMPQADAKT LETMA	716
Coral	-----	-----	HLG-DNNLSECLDM VEAMPHTDPNTF ETLA	29
Nemat	NGTHSIQGNKDK KDDSIILLI LYEFEAKA KLGEGDVS LESCLHKI IALPFCEAKT FETVA	-----	-----	515
		:	:	*
Human	IIAMEKPAH-----	YPLIAALKAKKK ALLLYKKEEP IDISQ-----	-----	798
Mouse	LLAMD KPAY-----	YPTIAHKAMKK LLL MYRKQEP VDVLK-----	-----	799
Macaq	IIAMEKPAH-----	YPFI ALKAKKK ALLLYKKEE SIDV SQ-----	-----	754
Dog	SLAVE MPAH-----	YPSIA ALKALKE ALLLYKK ESIDV LK-----	-----	817
Horse	SLAME MPAC-----	YPSIA ALKAKR ALLLHK RKESID VLK-----	-----	787
Cow	ALAME MPAC-----	YPSIA ALKAK KALFFYKR KESID VV R-----	-----	795
Rat	SLAMD KPAH-----	YPTIA QALK KLLLIYRR KVPINV LR-----	-----	780
Oposs	SVAME HPAH-----	YPDIA KKALRK KALSLYLO GESIDV AK-----	-----	493
Chick	SLAME PPAR-----	YPVLCK KALKSALN LYRKET TTDAV K-----	-----	705
Danio	VLAME PPAH-----	YPVLCK KALKIAL SLHRKQ PQVDLM R-----	-----	792
Tetra	ALAME PPAH-----	FPR LCK KALRVAL ALHRKQ PQOADL TR-----	-----	662
Branch	AVCC-----	-----	-----	720
Coral	ALAVESP VY-----	HRDLSMR SLRIA IKKCLAM DVIDFT K-----	-----	64
Nemat	GITNNEP FFPVIV WIALFAGP PRGVSM LIQDL LEEGKL ALHKI IFTRNN YFALPV HTFDF	-----	-----	575
	:			
Human	-YSKCMHN LVNL SVPDG ASNV ELCP LEEV WGYF FEDAL SHISRT -KDYPE MEIEL WLW MVKSW	-----	-----	856
Mouse	-YSVCMHN LIKLL VADEV WNISLY PLKEV QSHFK NTLSII RQN-E GYPEEE IVWLM IKSW	-----	-----	857
Macaq	-YSKCMHN LINL SVPDG ASNV ELCP LEEV WGYF FEDAL SHISHT -KDYPE MEIEL WLW MVKSW	-----	-----	812
Dog	-YSKCMHN LINL LPDG VPSTE LCPL EEVW GYFED ALSL ISHT-K GYPETE IELWL MIKSW	-----	-----	875
Horse	-YSKCMHN LINL LPVER VLSAEL CPLEEV WGYF FEDAL HLISHT-E GYPETE IELWL MVKSW	-----	-----	845
Cow	-YSQCMH SLVN LLVPDG VPNTEL CPMEEI WGNF EDALI FISQT-E GYPE MEVL CLMI KSW	-----	-----	853
Rat	-YSVCMHN LIDL LVS DERR WMALY PLKEV WGHL KSALSL IRQT-K GYPEEE IVWLM IKAW	-----	-----	838
Oposs	-FSKCLH SFINL SLPDE ALSTD DVCS LQDI WSTF EVAL RVISH S-E EYPQ VEIL WLMT KAW	-----	-----	551
Chick	-FSKCLH SLINL SLPTG VTDL DACV LQE VWGYF FEDAL SVS ST-D SYPE MEIEL WLW MTRAW	-----	-----	763
Danio	-CSNCLH SLIQL SLP NGVSE QPCV LEE VWGY YYEE EALSL IATT-ED FPE LEIEL WL LTRAW	-----	-----	850
Tetra	-CSKCVH SLIKL SLPS GVSE VEAR LEE VWE YYEE EALSM IAASP DDFPE METL WL LTRAW	-----	-----	721
Branch	-----	-----	-----	720
Coral	-LSKAFH SLSL AELAL GRGSS RDA ESKEE AWTLY QEII EFVEST DKGS YPE MELI WL MTRAW	-----	-----	72
Nemat	MFSKVF HSLA ELAL GRGSS RDA ESKEE AWTLY QEII EFVEST DKGS YPE MELI WL MTRAW	-----	-----	635

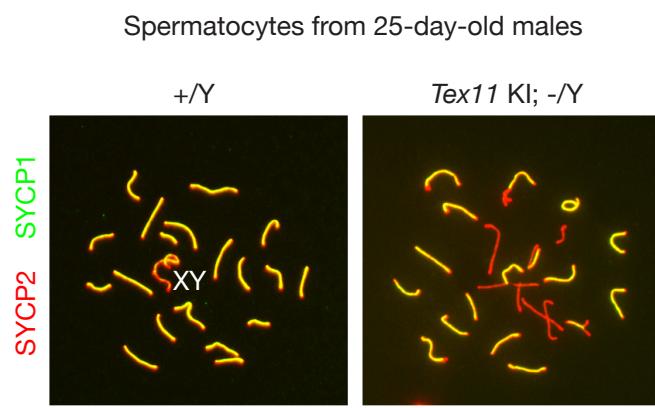
Human	NTGVLMFSRSKYASAEKWCGLALRFLNHLTSFKEYETQ-----	MNMLYSQ-----	902
Mouse	NIGILMSSKNKYISAERWAAMALDFLGHLSLTKTSYEAK-----	VNLLYAN-----	903
Macaque	NTGILMFSRSKYVSTEKWCGLALRFLDHLTSFKEYETQ-----	MNMLYSQ-----	858
Dog	NTGIFMYGRNKYVSAEKWCGLALRFLDRLGSLKRSYETQ-----	MNILYSE-----	921
Horse	NIGIFMYGSSKYVSAEKWCSLALRFLDHLGSLRRSYEIQ-----	MNVLYSQ-----	891
Cow	NIGIFLYSERMYVSTEKWCGLALRLLDYLGSLKSTYETQ-----	VNILYGE-----	899
Rat	NIGILMYSRNKYISAERWARMTLEFLDHGLGSLKTNYEAKHPFRLLPTSCLHAVSHSQKYL	898	
Opossum	NTGVSQYKEGMYATAEKWCGLGIRFLDHLGSLKKCYEGQ-----	MADLYGE-----	597
Chick	NTGIFQYTVGKYQEAEQWCGLGMRFLNHLGSLKRSYEGH-----	VSQMPES-----	809
Danio	NTGILLYSLAQYPEAERWCGLGMSFLRYLGSLQDSYQTQ-----	MAGLYSE-----	896
Tetra	NTGILLYSLAPYPAERWCGLAMSFVRHLGSLQQSYGTQ-----	VNS-----	763
Branch	-----	-----	720
Coral	-----	-----	72
Nemat	NCGINLFSSGRFDASEKWCATAMRLLQLRVGFKTNYESK-----	MTSVYSD-----	681

Human	LVEALSNNKGPFHEHGYWSKSD-----	925	
Mouse	LMEILDKKTD-----	LRSTEMTEQ-----	928
Macaque	LVEALSNNKGPFHEHGYWSKSD-----	-----	881
Dog	LVEALDRKKGSLFNEE-----	-----	937
Horse	LVEALDKNKGSLFNKECPLTEDRSQAPPCLGFFFFRLNLLRPHTCSSQDVVTSPSKSE	951	
Cow	LMEAEEKNRSAFNEE-----	-----	915
Rat	LQALLKGHKDDWTSPGWTHPTFTVGKVAGMAALQGQQLLSVKSPSSSSQPKPLLCRCLA	958	
Opossum	VLTKVERDKSLPPNEE-----	-----	613
Chick	-----	-----	809
Danio	VLDRLDKAKKNLIIIEE-----	-----	912
Tetra	-----	-----	763
Branch	-----	-----	720
Coral	-----	-----	72
Nemat	ILDRISRAAAQATVEE-----	-----	697

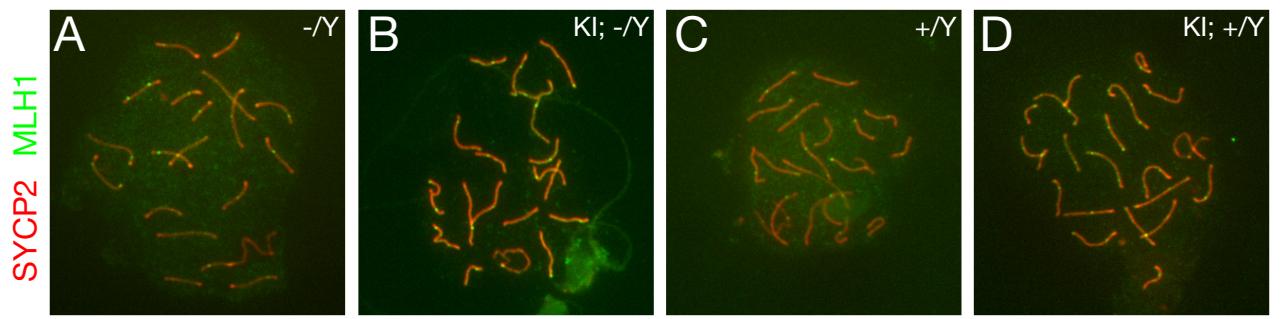
Human	-----	925	
Mouse	PP-----EDQGSVSSTNVAAQN-----HL-----	947	
Macaque	-----	-----	881
Dog	-----	-----	937
Horse	CRWQKRDRRTSRWFIQSNVKTSGYPMAPTGTALQIKSSWGFTAEEARLFSGKAHGIETLF	1011	
Cow	-----	-----	915
Rat	PPGANLSSEEENDQIQSCKPAVQGRNAQGHLITWAVSLTSQAVQLSRVCLSVSPRLFSALL	1018	
Opossum	-----	-----	613
Chick	-----	-----	809
Danio	-----	-----	912
Tetra	-----	-----	763
Branch	-----	-----	720
Coral	-----	-----	72

Human	-----	925
Mouse	-----	947
Macaq	-----	881
Dog	-----	937
Horse	AEGGTRCAGASSAISCK-----	1028
Cow	-----	915
Rat	LGVYENNNSDGFAPVLARERSMQEWRSHDE	1049
Oposs	-----	613
Chick	-----	809
Danio	-----	912
Tetra	-----	763
Branch	-----	720
Coral	-----	72
Nemat	-----	697

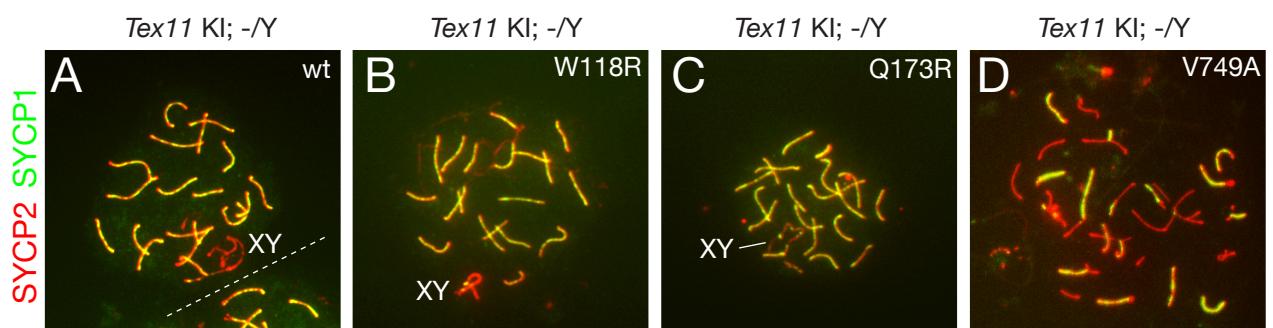
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

