Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Yatsenko AN, Georgiadis AP, Röpke A, et al. X-linked *TEX11* mutations, meiotic arrest, and azoospermia in infertile men. N Engl J Med 2015;372:2097-107. DOI: 10.1056/NEJMoa1406192

Supplementary Materials

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Methods

Immunohistochemistry

The normal mouse and monkey tissue were obtained from the biobank of CeRA. All tissue sections became available from previous experimental studies on animals approved by LANUV NRW. Mouse, primate, and human testicular tissue sections were stained with primary antibody against TEX11 (goat polyclonal anti-TEX11, ab99461, dilution of 1:100; Abcam, Cambridge, MA) and visualized using 3, 3´-diaminobenzidine (D4168, Sigma-Aldrich, St. Louis, MO) and hematoxylin as counterstain. Paraffin wax was removed from testicular tissue sections using ProTaqs Clear solvent (Quartett Immunodiagnostika and Biotechnologie, Berlin, Germany), and sections were subsequently rehydrated in a series of decreasing concentrations of ethanol. After rinsing with distilled water and Tris-buffered saline (TBS), sections were placed into citrate buffer (pH 6) and heated by microwave for 12 min for antigen retrieval. After cooling to room temperature (RT), sections were washed in TBS, and non-specific peroxidases were blocked with 3% H_2O_2 for 15 min at RT. To block non-specific binding sites, sections were incubated with 10% chicken serum in TBS containing 5% bovine serum albumin for 20 min at RT. Primary antibodies against TEX11 (goat polyclonal anti-TEX11, ab99461, dilution of 1:100; Abcam) were applied, and sections were incubated overnight in a humid chamber at 4°C. After three washes in TBS, primary antibody was detected using the secondary antibody (chicken-anti-goat-HRP, sc-2984, dilution 1:250; Santa Cruz Biotechnology, Dallas, TX). Staining was visualized using 3, 3´-diaminobenzidine (D4168, Sigma-Aldrich) and hematoxylin as counterstain.

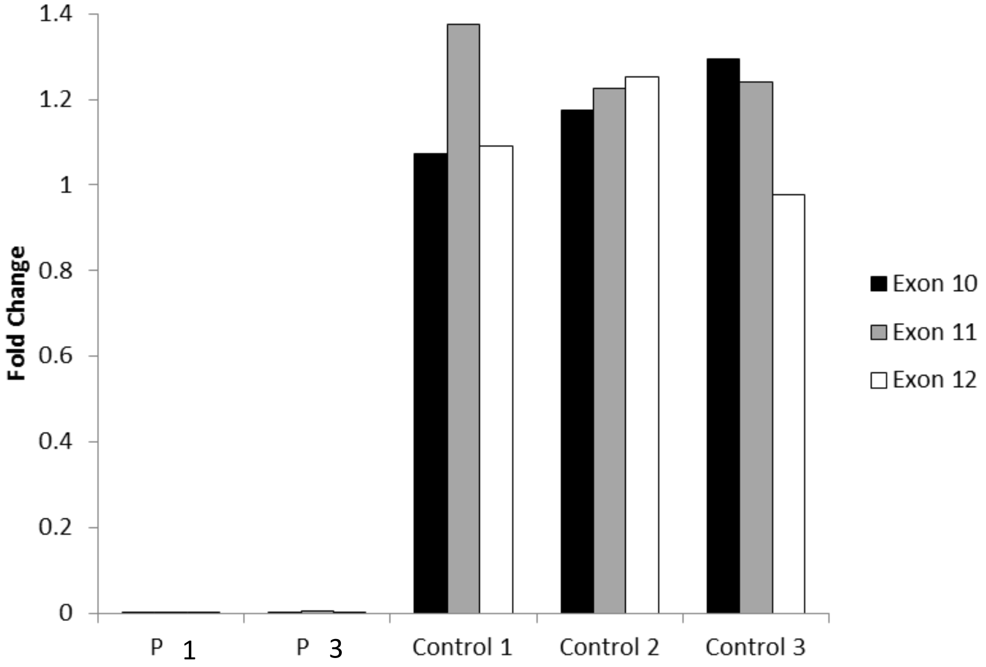

Figure S1. qPCR analysis of exons 10-12 of *TEX11*. Samples fold-change calculated by delta Ct with beta-actin serving as an internal control. Patients 1 and 3 showed no amplification of *TEX11* exons 10-12 products. Normal samples 1, 2, and 3 served as controls and display a fold-change of 1.19 +/- 0.12, consistent with one copy of *TEX11*.

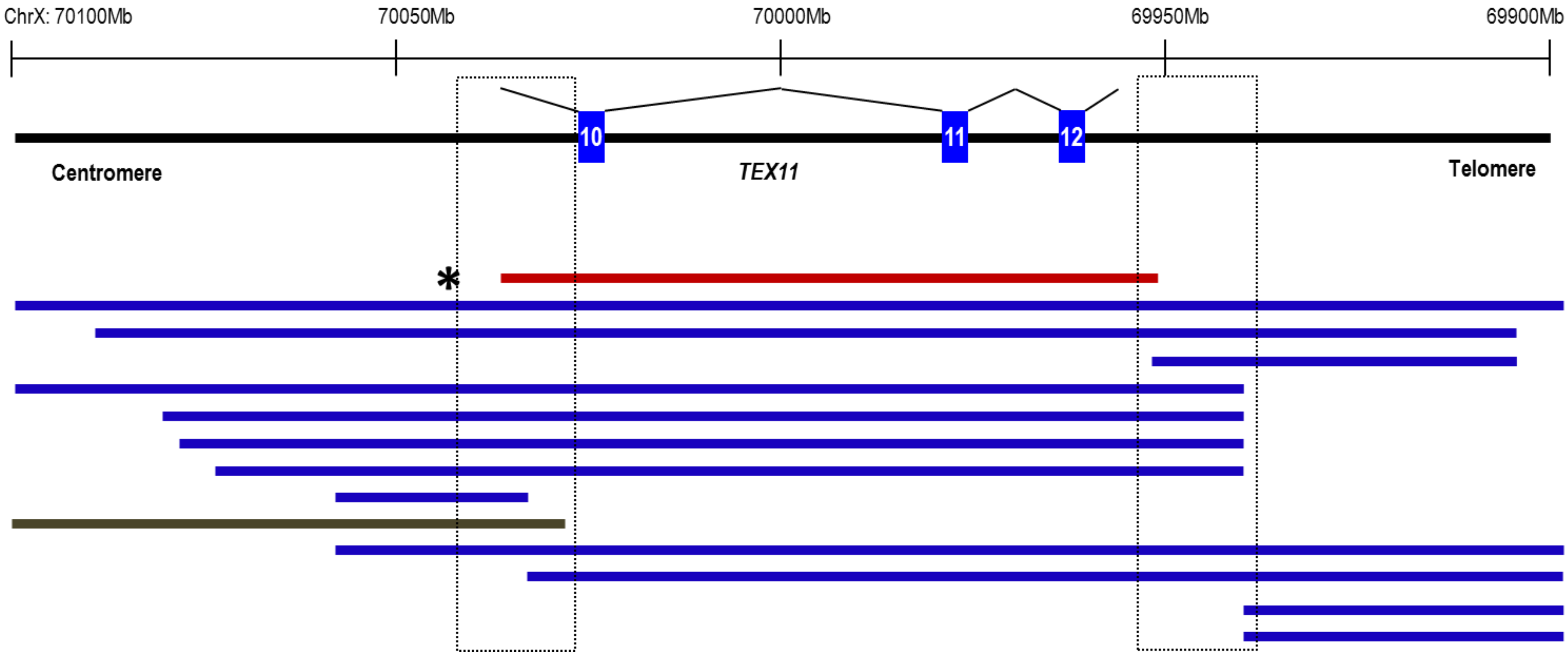

Figure S2. Reported structural variations in a general population from the Database of Genomic Variants (DGV) around exons 10-12 of *TEX11*. The asterisked 99 Kb deletion (red line) represents the CNV found in Patients 1 and 3. The duplications (blue lines) and complex CNVs (brown line) are reported structural variations in the DGV. Dashed rectangles indicate that the area around breakpoints of the deletion found in Patients 1 and 3. Note that breakpoints of CNVs are clustered around the Patients 1 and 3 deletion breakpoints, indicating DNA intervals prone to breakage.

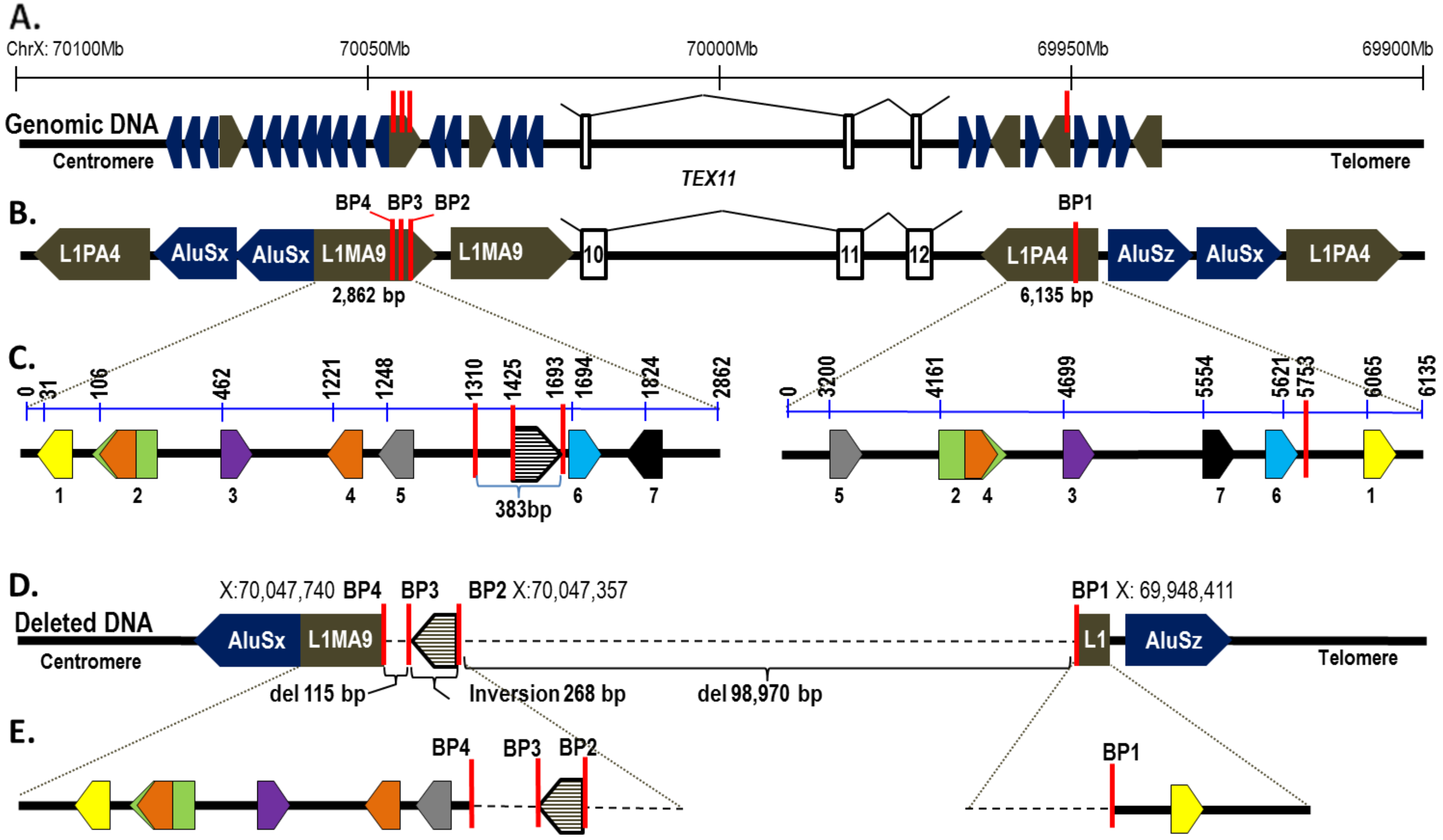

Figure S3. Breakpoint analysis for the deletion of exons 10-12 in patients 1 and 3. A. Reference DNA depicting LINE (brown arrows) and Alu (blue arrows) repeats flanking four breakpoint regions (orange vertical lines) around exons 10-12 (black outlined open rectangles). **B.** Magnified view of significant LINE and Alu repeats around breakpoints. **C.** Genomic structure of L1MA9 and L1PA4 repeats with shared microhomology around breakpoints BP1-4. Colored arrows correspond to identical microhomology regions in opposing LINE repeat. Blue line and numbers indicate position of microhomology region in LINE repeat. **D.** *TEX11* (black solid line) deletion of 115 bp (dotted lines), a short inversion of 268 bp in intron 9 (striped arrow), and a 98,970 pb deletion of exons 10-12 (dotted lines). **E.** A magnified view of breakpoint regions inside of LINE repeats.

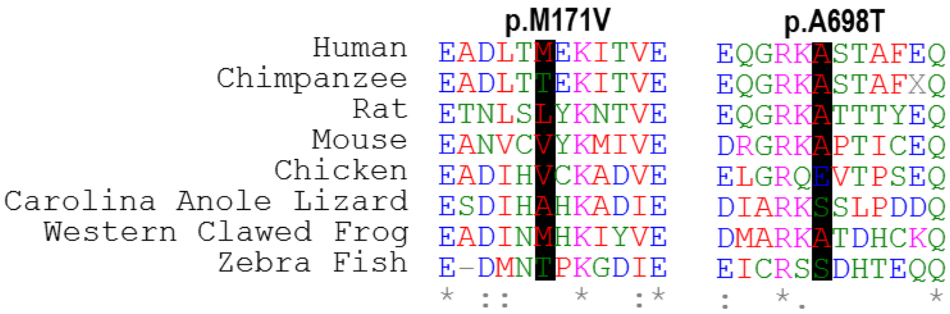

Figure S4. Multiple protein alignment of 2 missense mutations in the TEX11 amino acid sequence. The methionine amino acid at position 171 was changed to valine, and is not conserved, but it is adjacent to a conserved region. The protein sequence is highly conserved from amino acid position 189 through the entire SPO22 domain. The conserved alanine-698, was mutated to threonine and falls into another conserved region.

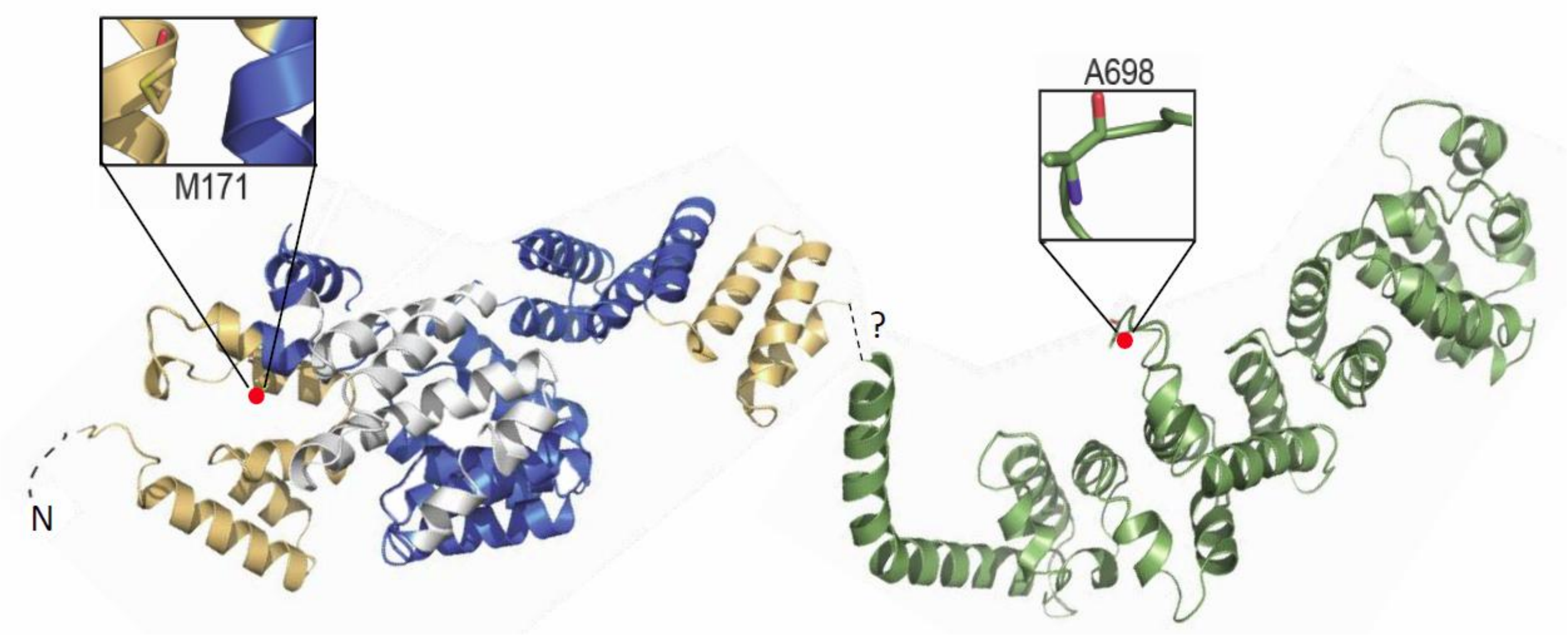

Figure S5. Tertiary TEX11 protein structure prediction. Diagram of the top tertiary structure model from Phyre2[15]. The N-terminal two-thirds of TEX11, modeled with template 2XPI, is shown in blue and gold: the SPO22 domain shown in blue; the segment of SPO22 that is deleted in p.218del79aa in white; TPR1 and TPR2 in gold. The C-terminal region of TEX11, modeled with template structure 4I2W, is shown in green. Predicted locations of amino acid changes in the genetic study are shown as red dots. Each inset shows the amino acid of interest as sticks on the cartoon representation of the backbone in an orientation that best shows their modeled positions. The N-terminal unmodeled region and the linker between the two structural models are shown as dotted lines. The orientation of the two domains relative to one another has not been modeled, as indicated by a question mark.

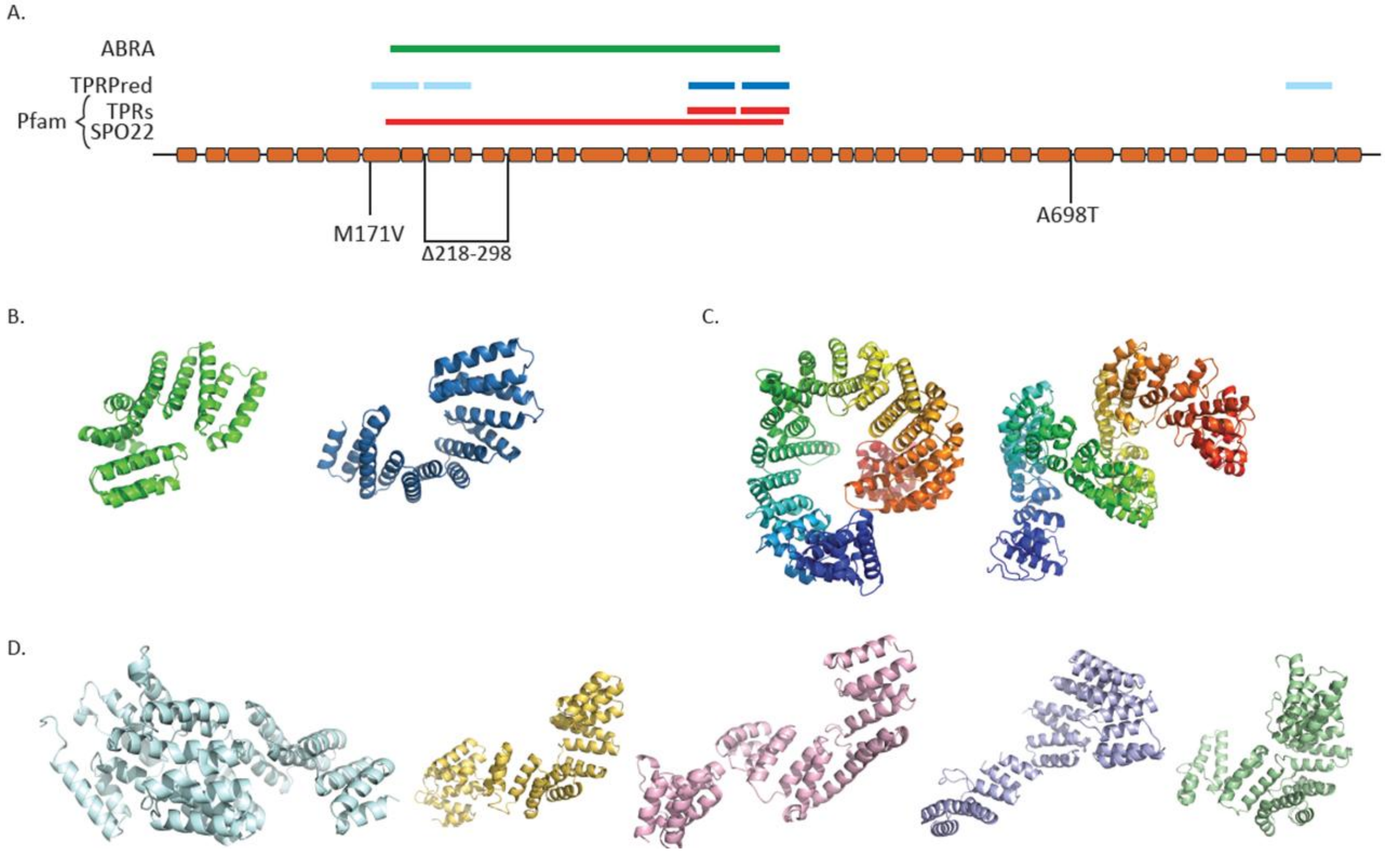

Figure S6. **Three dimensional structural protein modeling of TEX11 reveals many potential arrangements of its helical motifs. A.** Linear diagram of the secondary structure prediction of TEX11. Each orange region represents a predicted α-helix. Locations of the missense mutations and deletion analyzed in this study are shown. Red bars represent elements detected by Pfam [18]. Blue bars represent TPRs predicted by TPRPred[19], with p-values < 10^{-4} represented by dark blue bars, and p-value = 10^{-4} in light blue bars. The green bar represents the TPRs predicted by ABRA. **B**. Diagrams of the two most probable models of the Spo22 domain of TEX11 modeled by I-Tasser[17,20]. **C.** Diagrams of the two highest ranked tertiary structure models from I-Tasser of TEX11[17,20]. **D.** Diagrams of the five top ranked tertiary structure models from Phyre2 of the N-terminal two-thirds of TEX11[15].

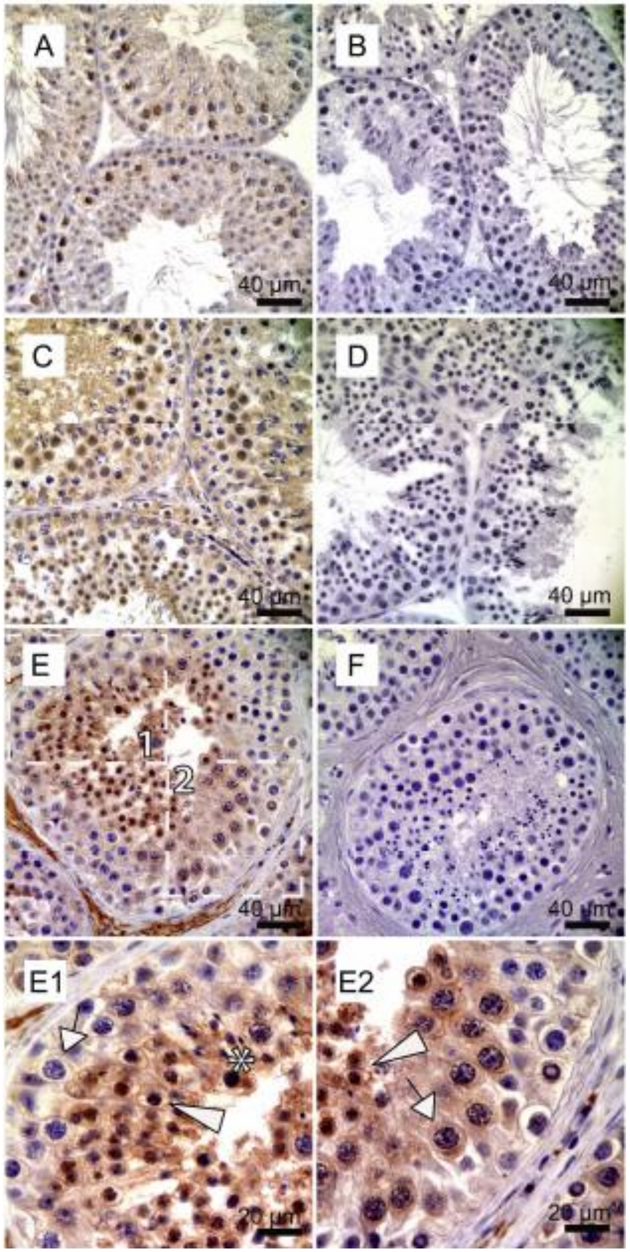

Figure S7. **Immunohistochemical detection of TEX11 in normal testicular tissue sections of mouse (A-B), macaque (*Macaca fascilaris*) (C-D), and human (E-F).** Higher magnifications of dashed subframes of Fig. E are shown in E1 and E2. Positive signal for TEX11 is visible as brown precipitate. Hematoxylin was used as counterstain. Exchange of the TEX11 antibody with a corresponding IgG antibody was used as a control for method specificity (B, D, F). In the mouse testis the positive signal is most prominent in spermatocytes (A). In primates (C) and humans (E, E1, E2), the positive staining was detected at later stages of germ cell development in comparison to the mouse. TEX11 is absent in early spermatocytes, designated by arrow (E1), and expression becomes visible in late spermatocytes, designated by arrow (E2). The staining is also present in round (designated by arrowhead) and elongating spermatids (designated by asterisk). Scale bars represent the indicated distances.

Table S1. Genomic DNA primers for 30 coding exons of *TEX11* isoform 1 (NM_001003811.1)* and 2 (NM_031276.2).

Primers name	Forward	Reverse	Length (bp)
Ex 2, isoform 2	CCCTTTCATGGGCTTTTAACTTTAC	CCACCGCGCCCAGCAATATTTAG	274
Ex 3, isoform 1	TGTTCATGTCATATCTTTGGTGCTT	GACAAGAGTTGGCTTGGCACAGT	527
Ex 3, isoform 1 seq	TCTTTCTTTCTTTCCTTAG	-	-
Ex 4	TCCTTCATGCTGTATTCATTTGG	CGAGCACATATGTTCCCATTGGT	422
Ex 5	TGGGTAAGTTTTTGCGGGGAGAGG	TGAGGGAGGCCATTTGCTTGAGA	411
Ex 6	CAACAAAACTGGCTTCCTTCAATT	GGAACACTGAAAAGGAACACCAT	319
Ex 7+8	TGCTAACTGTTGCTTTTTGTTTTCTG	TCACCCTTAGCCACCCCATAGA	632
Ex 9	ACTCTGTGTCACCAAAGATTACCACTT	GCTTTTTCAAATGCCCTTTCTGG	460
Ex 10	TCTCCTAGGCTGGAGTGATGTGG	AAGTCAGAGAAGGTTTAAACTGCCAAA	301
Ex 11	TTGTAGTGCAAAACAGAAAAGTTGGA	GATGTTAAATGTTGTGCCCAATAGC	323
Ex 12	GACTGTGGTCGCTTAAGGAAATGC	AACATAGGAATTTGCGGGGACAC	431
Ex 13	TGGGAAATATCCTCAGGGATAGAACA	TGGGTGACAATATTTTCCCTTTCC	406
Ex 14	TGACACTCTCCAGCACTGGATGTTA	ATATTGCCAAGGTTGGTCTCAAGC	401
Ex 15	TCTGGCTTATTTTGCTCACACTG	GGAAGAAATCCATTGACTTGAGG	351
Ex 16	AGGAGAATCAGGCAGCAGTACC	CTTGCACAATGCTCATTAACACTC	415
Ex 17	TTGACCAAGACTGATAATAAAATGCA	TTCTCTCTTTCTTTGGGAAGTTTCA	502
Ex 18	TTGCATTAGCATTCTAGAAGAGCAC	ATAACCTTCCACGGTTTCTTTTTGC	442
Ex 19	TGGTGTTCAAATCCTCCTTGAAA	GGCATGAAAATATGAGGCAAAAC	423
Ex 20	GGGGCTAATGCAAATGTGTCCTG	CACCCTCCTCTGCTTCGCTATTT	408
Ex 21	CTTCTCCCTCCCAGAGTAATCAA	AAGACTGGCCCCAAAACCTGTAT	288
Ex 22	TGAGGGCACTGGGAATGAGTAAC	TCTCTGCTTGTGAATGAAGAAACCA	412
Ex 23	ACTTTTGGTATGCACCCCTACAC	CCACAGCTACAAATGAAAGATCCA	328
Ex 24	AAGATTGGAATTGACGAGGGTCTC	GCCCCACATTCTTATGTTATACCC	455
Ex 25	GTGCTTTTCCAGCTGAATTTTCTCAT	GAAGAGGCCACACCAAAAGTAACC	424

Ex 26	AAGGAAATGTGCAAATAGAGGCTAA	AAGCTCCTTTCAAACTGTGTTACC	301
Ex 27	TCTGTCTACAGCAGTGAAGATGAC	TGGCATTAGAGAAGGAGAAACAAAG	439
Ex 28	CACCATGCTACTCTGCCCAGAAA	TTCACAAGAATGCCCCCAGATCAT	438
Ex 29	GGCCAGATAAAACCGAGGGAAG	TTCAGAAACCCAGGAAAATTCTGTA	420
Ex 30	GTGCTCTCCTCCTGCACTCTCC	CAAGGAGGATGGGGTTGAGTCCT	416
Ex 31	GGGGATTCCTTTTCCTCAAGAGA	TTCAGCATGCTTTTATTTTAGAAAGTT	410

**Exons are labeled according to isoform 1.*

All forward and reverse primers were used for both PCR and Sanger Sequencing, excluding exon 3 isoform 1. For this exon, forward and reverse primers were used for PCR, the reverse primer was used for sequencing in the reverse direction, and the exon 3 isoform 1 seq primer was used for sequencing in the forward direction.

Table S2. Genomic DNA primers for quantitative PCR of *TEX11* exons 10-12 isoform 1 (NM_001003811.1).

Primer name	Forward	Reverse	Length (bp)
Ex 10	ACTTCAAGTCTTCATCATCTCTGT	ATTCCCGAGTTTACATTCAGTTTT	123
Ex 11	AGCTATGATATTGGGAAGATGGA	ATGGGGAGTACGCTATTATAATTTT	137
Ex 12	ACAGGCTAAAGTTCTACGGCTA	AGCCCCATACCTTGTTTGCTAG	110
ACTB	CGTACCACTGGCATCGTGAT	GCTCGGTGAGGATCTTCATGA	148

Table S3. Total copy number variation (CNV) report for 15 azoospermic men analyzed by array comparative genomic hybridization (aCGH). CNV is Copy number variant, Chr denotes chromosome, kb is kilo bases or thousand bases, cytoband is chromosome band, DGV is the database of genomic variants.

CNV	Chr	Start	End	Size, kb	Genome coordinates	Cytoband	#Probes	Copy number	Number of genes	Number of patients	DGV
1	1	12,846,934	12,870,823	23,889	chr1:12846934-12870823	p36.21	4	loss	0	2	present
2	1	144,986,366	145,064,610	78,244	chr1:144986366-145064610	q21.1	19	loss	1	2	present
3	1	149,041,933	149,230,698	188,765	chr1:149041933-149230698	q21.2	23	loss	1	8	present
4	1	151,333,124	151,405,013	71,889	chr1:151333124-151405013	q21.3	16	gain	3	1	present
5	1	1,521,481	1,540,027	18,546	chr1:1521481-1540027	p36.33	6	loss	0	2	present
6	1	152,553,255	152,581,944	28,689	chr1:152553255-152581944	q21.3	7	gain	1	13	present
7	1	1,651,368	1,664,221	12,853	chr1:1651368-1664221	p36.33	3	loss	1	1	present
8	1	169,227,144	169,230,491	3,347	chr1:169227144-169230491	q24.2	1	loss	1	4	present
9	1	16,927,124	17,253,438	326,314	chr1:16927124-17253438	p36.13	16	gain	2	1	present
10	1	17,091,225	17,253,438	162,213	chr1:17091225-17253438	p36.13	9	gain	0	1	not present
11	1	191,826,625	191,864,379	37,754	chr1:191826625-191864379	q31.2	6	loss	0	1	present
12	1	196,742,735	196,756,290	13,555	chr1:196742735-196756290	q31.3	3	loss	1	4	present
13	1	206,318,836	206,331,282	12,446	chr1:206318836-206331282	q32.1	4	loss	1	1	not present
14	1	248,339,940	248,672,268	332,328	chr1:248339940-248672268	q44	49	gain	0	10	present

15	1	59,997,718	60,052,475	54,757	chr1:59997718-60052475	p32.1	12	loss	1	3	present
16	1	64,841,584	64,848,598	7,014	chr1:64841584-64848598	p31.3	3	loss	0	1	present
17	1	72,768,855	72,795,480	26,625	chr1:72768855-72795480	p31.1	5	loss	0	9	present
18	2	126,996,699	127,050,268	53,569	chr2:126996699-127050268	q14.3	4	loss	0	1	present
19	2	132,078,028	132,197,678	119,650	chr2:132078028-132197678	q21.1	16	gain	0	3	present
20	2	180,069,605	180,070,733	1,128	chr2:180069605-180070733	q31.2	3	gain	0	7	present
21	2	18,189,720	18,251,750	62,030	chr2:18189720-18251750	p24.2	10	loss	0	1	present
22	2	186,043,764	186,094,608	50,844	chr2:186043764-186094608	q32.1	4	loss	0	1	present
23	2	202,410,290	202,420,696	10,406	chr2:202410290-202420696	q33.1	3	loss	0	1	not present
24	2	242,753,511	242,762,406	8,895	chr2:242753511-242762406	q37.3	3	gain	1	4	present
25	2	242,856,588	243,038,331	181,743	chr2:242856588-243038331	q37.3	40	loss	0	1	present
26	2	34,697,718	34,730,142	32,424	chr2:34697718-34730142	p22.3	7	loss	0	7	present
27	2	34,702,930	34,730,142	27,212	chr2:34702930-34730142	p22.3	6	loss	0	2	present
28	2	37,958,541	38,002,391	43,850	chr2:37958541-38002391	p22.2	11	loss	0	3	present
29	2	89,601,864	90,265,119	663,255	chr2:89601864-90265119	p11.2	35	loss	0	2	present
30	3	162,514,534	162,611,115	96,581	chr3:162514534-162611115	q26.1	9	loss	0	12	present
31	3	195,354,124	195,454,513	100,389	chr3:195354124-195454513	q29	12	gain	2	4	present

32	3	195,419,168	195,444,214	25,046	chr3:195419168-195444214	q29	10	loss	2	2	present
33	3	37,980,926	37,986,165	5,239	chr3:37980926-37986165	p22.2	3	loss	1	3	present
34	3	46,796,031	46,848,728	52,697	chr3:46796031-46848728	p21.31	6	loss	0	2	present
35	3	56,608,466	56,619,604	11,138	chr3:56608466-56619604	p14.3	3	loss	0	1	present
36	3	75,767,533	75,793,273	25,740	chr3:75767533-75793273	p12.3	5	gain	0	1	present
37	3	89,394,229	89,416,802	22,573	chr3:89394229-89416802	p11.1	5	loss	1	2	present
38	4	131,949,297	132,352,577	403,280	chr4:131949297-132352577	q28.3	32	gain	0	1	present
39	4	186,401,069	186,419,493	18,424	chr4:186401069-186419493	q35.1	4	loss	0	1	present
40	4	190,678,649	190,721,966	43,317	chr4:190678649-190721966	q35.2	7	gain	0	1	present
41	4	34,787,128	34,814,779	27,651	chr4:34787128-34814779	p15.1	3	loss	0	1	present
42	4	39,963,379	40,088,977	125,598	chr4:39963379-40088977	p14	26	gain	2	1	not present
43	4	65,418,993	65,486,011	67,018	chr4:65418993-65486011	q13.1	10	gain	0	1	present
44	4	69,387,056	69,483,277	96,221	chr4:69387056-69483277	q13.2	12	loss	1	13	present
45	4	78,115,834	78,211,152	95,318	chr4:78115834-78211152	q21.1	19	loss	0	1	present
46	5	104,274,226	104,292,080	17,854	chr5:104274226-104292080	q21.2	3	loss	0	2	present
47	5	140,223,256	140,236,399	13,143	chr5:140223256-140236399	q31.3	4	loss	11	2	present
48	5	150,204,997	150,221,011	16,014	chr5:150204997-150221011	q33.1	4	loss	0	4	present

49	5	155,471,484	155,488,272	16,788	chr5:155471484-155488272	q33.2	4	gain	1	1	present
50	5	17,662,300	17,697,930	35,630	chr5:17662300-17697930	p15.1	4	loss	0	1	present
51	5	180,410,254	180,429,788	19,534	chr5:180410254-180429788	q35.3	5	gain	1	3	present
52	5	21,427,452	21,524,011	96,559	chr5:21427452-21524011	p14.3	6	loss	0	1	present
53	5	32,107,383	32,167,638	60,255	chr5:32107383-32167638	p13.3	13	gain	2	2	present
54	5	685,256	766,095	80,839	chr5:685256-766095	p15.33	4	gain	1	2	present
55	5	759,169	857,264	98,095	chr5:759169-857264	p15.33	6	gain	0	2	present
56	6	109,446,615	109,546,279	99,664	chr6:109446615-109546279	q21	19	loss	0	1	not present
57	6	111,223,803	111,414,820	191,017	chr6:111223803-111414820	q21	37	gain	2	1	present
58	6	124,436,517	124,468,315	31,798	chr6:124436517-124468315	q22.31	8	gain	1	4	present
59	6	165,725,547	165,737,665	12,118	chr6:165725547-165737665	q27	3	gain	0	1	present
60	6	259,881	291,594	31,713	chr6:259881-291594	p25.3	7	gain	0	5	present
61	6	28,520,009	29,615,842	1,095,833	chr6:28520009-29615842	p22.1	195	gain	7	1	not present
62	6	29,854,870	29,896,710	41,840	chr6:29854870-29896710	p22.1	8	loss	0	2	present
63	6	299,363	378,956	79,593	chr6:299363-378956	p25.3	18	loss	0	2	present
64	6	31,349,905	31,450,159	100,254	chr6:31349905-31450159	p21.33	16	loss	2	2	present
65	6	31,785,578	31,794,898	9,320	chr6:31785578-31794898	p21.33	4	loss	3	1	present

66	6	32,427,088	32,546,882	119,794	chr6:32427088-32546882	p21.32	17	loss	2	15	present
67	6	32,601,344	32,654,142	52,798	chr6:32601344-32654142	p21.32	11	loss	2	8	present
68	6	56,917,608	56,953,602	35,994	chr6:56917608-56953602	p12.1	8	loss	0	1	present
69	6	78,979,172	79,023,328	44,156	chr6:78979172-79023328	q14.1	4	gain	0	8	present
70	7	109,443,100	109,453,033	9,933	chr7:109443100-109453033	q31.1	4	loss	0	1	present
71	7	119,577,193	119,629,793	52,600	chr7:119577193-119629793	q31.31	10	loss	0	1	present
72	7	133,788,914	133,797,387	8,473	chr7:133788914-133797387	q33	3	gain	0	7	present
73	7	141,750,430	141,792,094	41,664	chr7:141750430-141792094	q34	9	loss	1	2	present
74	7	143,951,931	143,953,472	1,541	chr7:143951931-143953472	q35	3	loss	0	8	present
75	7	152,456,633	152,459,393	2,760	chr7:152456633-152459393	q36.1	3	gain	0	1	present
76	7	20,662,985	20,758,193	95,208	chr7:20662985-20758193	p21.1	20	gain	1	1	not present
77	7	53,461,023	53,583,184	122,161	chr7:53461023-53583184	p12.1	13	loss	0	1	present
78	7	64,691,376	65,308,719	617,343	chr7:64691376-65308719	q11.21	63	gain	1	1	present
79	7	76,433,522	76,527,501	93,979	chr7:76433522-76527501	q11.23	7	gain	0	1	present
80	7	85,162,484	85,198,014	35,530	chr7:85162484-85198014	q21.11	3	loss	0	1	present
81	8	137,693,236	137,850,185	156,949	chr8:137693236-137850185	q24.23	13	loss	0	2	present
82	8	144,990,160	145,021,210	31,050	chr8:144990160-145021210	q24.3	12	gain	2	5	present

83	8	15,421,661	15,433,690	12,029	chr8:15421661-15433690	p22	4	loss	1	1	present
84	8	39,234,992	39,386,158	151,166	chr8:39234992-39386158	p11.22	28	loss	0	12	present
85	8	4,329,470	4,357,521	28,051	chr8:4329470-4357521	p23.2	6	loss	1	2	present
86	8	7,169,490	7,786,708	617,218	chr8:7169490-7786708	p23.1	10	gain	10	2	present
87	8	7,239,491	7,786,708	547,217	chr8:7239491-7786708	p23.1	9	loss	10	1	present
88	9	119,533,116	119,562,311	29,195	chr9:119533116-119562311	q33.1	8	loss	1	1	present
89	9	139,407,170	139,419,558	12,388	chr9:139407170-139419558	q34.3	4	gain	1	1	present
90	9	43,505,843	43,836,428	330,585	chr9:43505843-43836428	p12	6	gain	0	2	present
91	10	101,507,355	101,514,326	6,971	chr10:101507355-101514326	q24.2	3	loss	1	1	not present
92	10	132,360,482	132,370,482	10,000	chr10:132360482-132370482	q26.3	3	gain	0	5	present
93	10	135,236,007	135,378,761	142,754	chr10:135236007-135378761	q26.3	25	gain	3	1	present
94	10	46,968,072	47,695,889	727,817	chr10:46968072-47695889	q11.22	54	gain	3	1	present
95	10	47,007,565	47,148,546	140,981	chr10:47007565-47148546	q11.22	25	gain	1	1	present
96	10	56,448,627	56,468,820	20,193	chr10:56448627-56468820	q21.1	5	gain	1	1	present
97	10	59,850,715	59,878,505	27,790	chr10:59850715-59878505	q21.1	6	gain	0	1	present
98	11	18,949,929	18,960,666	10,737	chr11:18949929-18960666	p15.1	3	loss	1	1	present
99	11	25,610,678	25,622,569	11,891	chr11:25610678-25622569	p14.3	3	loss	0	2	present

100	11	55,361,638	55,450,788	89,150	chr11:55361638-55450788	q11	17	loss	0	13	present
101	11	55,372,753	55,430,765	58,012	chr11:55372753-55430765	q11	11	loss	0	2	present
102	11	5,785,900	5,805,665	19,765	chr11:5785900-5805665	p15.4	4	loss	0	6	present
103	11	7,815,017	7,826,975	11,958	chr11:7815017-7826975	p15.4	6	loss	0	2	present
104	12	10,579,158	10,593,748	14,590	chr12:10579158-10593748	p13.2	4	loss	1	1	present
105	12	11,221,333	11,249,210	27,877	chr12:11221333-11249210	p13.2	5	loss	1	13	present
106	12	33,960,021	34,756,209	796,188	chr12:33960021-34756209	p11.1	43	gain	1	1	present
107	12	9,637,323	9,698,517	61,194	chr12:9637323-9698517	p13.31	8	gain	0	15	present
108	13	19,858,137	19,892,981	34,844	chr13:19858137-19892981	q12.11	8	gain	0	1	present
109	13	57,760,478	57,783,723	23,245	chr13:57760478-57783723	q21.1	5	loss	0	1	present
110	13	63,410,535	63,441,072	30,537	chr13:63410535-63441072	q21.31	3	loss	0	1	present
111	13	69,240,911	69,263,772	22,861	chr13:69240911-69263772	q21.33	6	loss	0	2	present
112	13	84,115,188	84,152,921	37,733	chr13:84115188-84152921	q31.1	4	loss	0	2	present
113	14	106,531,557	106,559,103	27,546	chr14:106531557-106559103	q32.33	7	loss	1	4	present
114	14	106,535,379	106,653,147	117,768	chr14:106535379-106653147	q32.33	11	gain	1	1	present
115	14	106,785,927	106,810,907	24,980	chr14:106785927-106810907	q32.33	3	gain	1	3	present
116	14	106,873,961	106,927,426	53,465	chr14:106873961-106927426	q32.33	12	loss	1	2	present

117	14	106,875,948	106,931,193	55,245	chr14:106875948-106931193	q32.33	12	loss	1	1	present
118	14	107,148,739	107,180,095	31,356	chr14:107148739-107180095	q32.33	7	gain	1	3	present
119	14	19,376,762	20,420,849	1,044,087	chr14:19376762-20420849	q11.2	35	loss	1	10	present
120	14	45,189,654	45,222,609	32,955	chr14:45189654-45222609	q21.2	3	loss	0	1	present
121	14	74,001,651	74,012,568	10,917	chr14:74001651-74012568	q24.3	3	loss	1	5	present
122	14	80,106,284	80,113,460	7,176	chr14:80106284-80113460	q31.1	3	loss	1	2	present
123	14	95,103,155	95,177,742	74,587	chr14:95103155-95177742	q32.13	11	gain	0	1	present
124	15	102,394,651	102,480,888	86,237	chr15:102394651-102480888	q26.3	6	loss	0	3	present
125	15	20,190,548	22,332,237	2,141,689	chr15:20190548-22332237	q11.1	70	loss	1	14	present
126	15	20,849,110	22,173,977	1,324,867	chr15:20849110-22173977	q11.2	48	gain	1	1	present
127	15	22,168,276	22,578,630	410,354	chr15:22168276-22578630	q11.2	37	gain	0	1	present
128	15	24,406,896	24,587,080	180,184	chr15:24406896-24587080	q11.2	6	loss	1	1	present
129	15	34,727,647	34,795,913	68,266	chr15:34727647-34795913	q14	12	loss	0	5	present
130	16	15,048,676	15,120,666	71,990	chr16:15048676-15120666	p13.11	11	loss	1	1	present
131	16	21,475,039	21,593,631	118,592	chr16:21475039-21593631	p12.2	6	loss	0	2	present
132	16	32,471,625	33,651,765	1,180,140	chr16:32471625-33651765	p11.2	31	gain	0	10	present
133	16	34,472,017	34,744,872	272,855	chr16:34472017-34744872	p11.2	22	gain	0	3	present

134	16	55,799,203	55,822,392	23,189	chr16:55799203-55822392	q12.2	6	loss	0	4	present
135	16	55,842,389	55,848,225	5,836	chr16:55842389-55848225	q12.2	3	gain	1	2	present
136	16	70,155,006	70,193,942	38,936	chr16:70155006-70193942	q22.1	5	loss	0	2	present
137	16	72,095,998	72,107,365	11,367	chr16:72095998-72107365	q22.2	3	gain	1	1	present
138	16	74,394,080	74,407,341	13,261	chr16:74394080-74407341	q23.1	3	loss	0	2	present
139	16	75,538,398	75,572,540	34,142	chr16:75538398-75572540	q23.1	8	gain	2	1	present
140	16	78,372,097	78,381,281	9,184	chr16:78372097-78381281	q23.1	3	loss	1	4	present
141	16	83,200,264	83,207,885	7,621	chr16:83200264-83207885	q23.3	3	loss	1	1	not present
142	16	88,747,384	88,763,519	16,135	chr16:88747384-88763519	q24.3	5	loss	1	1	present
143	17	10,410,275	10,434,595	24,320	chr17:10410275-10434595	p13.1	7	gain	2	1	not present
144	17	34,437,475	34,475,514	38,039	chr17:34437475-34475514	q12	7	gain	0	1	present
145	17	44,165,726	44,210,822	45,096	chr17:44165726-44210822	q21.31	17	loss	1	10	present
146	17	78,093,616	78,109,158	15,542	chr17:78093616-78109158	q25.3	5	gain	2	1	present
147	19	20,607,860	20,701,620	93,760	chr19:20607860-20701620	p12	8	loss	0	2	present
148	19	20,830,072	20,996,917	166,845	chr19:20830072-20996917	p12	31	gain	0	1	present
149	19	35,847,189	35,861,544	14,355	chr19:35847189-35861544	q13.12	4	loss	1	2	present
150	19	50,426,600	50,472,122	45,522	chr19:50426600-50472122	q13.33	11	gain	4	1	present

151	19	52,136,261	52,148,589	12,328	chr19:52136261-52148589	q13.41	3	loss	0	2	present
152	19	53,522,243	53,550,020	27,777	chr19:53522243-53550020	q13.41	6	gain	0	1	present
153	19	53,927,854	54,005,410	77,556	chr19:53927854-54005410	q13.42	14	gain	0	1	present
154	19	54,730,830	54,742,404	11,574	chr19:54730830-54742404	q13.42	3	loss	0	2	present
155	19	6,377,061	6,386,806	9,745	chr19:6377061-6386806	p13.3	3	gain	1	1	not present
156	20	1,551,034	1,580,958	29,924	chr20:1551034-1580958	p13	7	gain	1	14	present
157	20	60,924,636	60,935,883	11,247	chr20:60924636-60935883	q13.33	3	gain	1	2	present
158	21	10,861,051	11,056,805	195,754	chr21:10861051-11056805	p11.2	12	gain	1	1	present
159	22	16,133,474	17,340,318	1,206,844	chr22:16133474-17340318	q11.1	36	loss	3	1	present
160	22	24,390,255	24,395,353	5,098	chr22:24390255-24395353	q11.23	1	loss	0	11	present
161	22	25,664,618	25,919,542	254,924	chr22:25664618-25919542	q11.23	54	gain	0	1	present
162	22	30,332,258	30,360,514	28,256	chr22:30332258-30360514	q12.2	7	loss	1	1	not present
163	22	35,829,283	35,860,068	30,785	chr22:35829283-35860068	q12.3	5	gain	0	5	not present
164	22	39,359,112	39,385,485	26,373	chr22:39359112-39385485	q13.1	6	loss	2	2	present
165	22	42,896,699	42,949,585	52,886	chr22:42896699-42949585	q13.2	8	gain	1	4	present
166	X	103,258,679	103,303,380	44,701	chrX:103258679-103303380	q22.2	11	gain	1	2	present
167	X	119,037,491	119,055,778	18,287	chrX:119037491-119055778	q24	5	gain	1	1	present

168	X	154,788	169,113	14,325	chrX:154788-169113	p22.33	5	gain	0	1	present
169	X	154,946,353	155,226,048	279,695	chrX:154946353-155226048	q28	39	gain	2	1	present
170	X	159,430	216,099	56,669	chrX:159430-216099	p22.33	21	gain	0	2	present
171	X	184,528	187,823	3,295	chrX:184528-187823	p22.33	3	gain	0	1	not present
172	X	52,858,608	52,905,175	46,567	chrX:52858608-52905175	p11.22	9	gain	1	1	not present
173	X	56,093,719	56,098,999	5,280	chrX:56093719-56098999	p11.21	3	loss	0	1	not present
174	X	61,091	170,147	109,056	chrX:61091-170147	p22.33	16	gain	0	3	present
175	X	62,405,221	62,501,768	96,547	chrX:62405221-62501768	q11.1	4	gain	0	1	present
176	X	69,956,055	70,045,530	89,475	chrX:69956055-70045530	q13.1	14	loss	1	1	not present
177	X	70,397	169,113	98,716	chrX:70397-169113	p22.33	13	gain	0	13	present
178	Y	11,091	11,528	437	chrY:11091-11528	p11.32	1	gain	0	3	not present
179	Y	11,529	120,147	108,618	chrY:11529-120147	p11.32	15	gain	0	1	not present
180	Y	20,397	163,421	143,024	chrY:20397-163421	p11.32	30	gain	0	6	not present
181	Y	9,641,061	9,650,194	9,133	chrY:9641061-9650194	p11.2	3	loss	0	1	present

Table S4. qPCR analysis of exons 10-12 of *TEX11* isoform 1 (NM_001003811.1).

	CONTROL	Ct- TEX11 ex10				Avg. Ct	St. Dev.	Ct- bACT				Avg. Ct	St. Dev.	DCt	DDCt	Fold change
Exon 10		well 1	well 2	well 3	well 4			well 1	well 2	well 3	well 4					
	Agilent Control	29.76	29.43	28.93	29.86	29.49	0.42	28.05	28.00	27.87	28.11	28.01	0.10	1.49	0.00	1.00
	UNKNOWN	**Ct- TEX11 ex10**				**Avg. Ct**	**St. Dev.**	**Ct- bACT**				**Avg. Ct**	**St. Dev.**	**DCt**	**DDCt**	**Fold change**
		well 1	well 2	well 3	well 4			well 1	well 2	well 3	well 4					
	P1 (p.218del79aa)	40	40	40	40	40.00	0.00	28.65	28.37	*	28.24	28.42	0.21	11.58	10.09	0.00
	P3 (p.218del79aa)	40	40	40	40	40.00	0.00	28.96	28.02	28.99	28.93	28.72	0.47	11.28	9.79	0.00
	Control 1	29.78	29.77	29.69	29.69	29.73	0.05	28.14	28.21	28.61	28.42	28.34	0.21	1.39	-0.10	1.07
	Control 2	29.84	29.44	29.14	29.40	29.46	0.29	28.10	28.27	28.35	28.08	28.20	0.13	1.26	-0.23	1.18
	Control 3	29.65	29.77	29.31	28.90	29.41	0.39	28.28	28.23	28.36	28.29	28.29	0.05	1.12	-0.37	1.30
Exon 11	**CONTROL**	**Ct- TEX11 ex11**				**Avg. Ct**	**St. Dev.**	**Ct- bACT**				**Avg. Ct**	**St. Dev.**	**DCt**	**DDCt**	**Fold change**
		well 1	well 2	well 3	well 4			well 1	well 2	well 3	well 4					
	Agilent Control	32.10	32.60	31.46	31.13	31.82	0.66	28.05	28.00	27.87	28.11	28.01	0.10	3.82	0.00	1.00
	UNKNOWN	**Ct- TEX11 ex11**				**Avg. Ct**	**St. Dev.**	**Ct- bACT**				**Avg. Ct**	**St. Dev.**	**DCt**	**DDCt**	**Fold change**
		well 1	well 2	well 3	well 4			well 1	well 2	well 3	well 4					
	P1 (p.218del79aa)	40	40	40	40	40.00	0.00	28.65	28.37	*	28.24	28.42	0.21	11.58	7.76	0.00
	P3 (p.218del79aa)	40	40	40	40	40.00	0.00	28.96	28.02	28.99	28.93	28.72	0.47	11.28	7.46	0.01
	Control 1	31.74	31.51	31.86	31.71	31.70	0.15	28.14	28.21	28.61	28.42	28.34	0.21	3.36	-0.46	1.37
	Control 2	31.82	31.73	31.74	31.63	31.73	0.08	28.10	28.27	28.35	28.08	28.20	0.13	3.53	-0.29	1.23
	Control 3	31.74	32.12	31.57	31.76	31.80	0.23	28.28	28.23	28.36	28.29	28.29	0.05	3.51	-0.31	1.24
Exon 12	**CONTROL**	**Ct- TEX11 ex12**				**Avg. Ct**	**St. Dev.**	**Ct- bACT**				**Avg. Ct**	**St. Dev.**	**DCt**	**DDCt**	**Fold change**
		well 1	well 2	well 3	well 4			well 1	well 2	well 3	well 4					
	Agilent Control	24.57	24.68	24.65	24.83	24.68	0.11	28.05	28.00	27.87	28.11	28.01	0.10	-3.32	0.00	1.00
	UNKNOWN	**Ct- TEX11 ex12**				**Avg. Ct**	**St. Dev.**	**Ct- bACT**				**Avg. Ct**	**St. Dev.**	**DCt**	**DDCt**	**Fold change**
		well 1	well 2	well 3	well 4			well 1	well 2	well 3	well 4					
	P1 (p.218del79aa)	35.19	37.12	35.91	36.20	36.10	0.80	28.65	28.37	*	28.24	28.42	0.21	7.68	11.01	0.00
	P3 (p.218del79aa)	35.48	37.06	40.00	34.86	36.85	2.30	28.96	28.02	28.99	28.93	28.72	0.47	8.13	11.45	0.00
	Control 1	24.77	24.84	24.95	25.01	24.89	0.11	28.14	28.21	28.61	28.42	28.34	0.21	-3.45	-0.13	1.09
	Control 2	24.52	24.49	24.54	24.66	24.55	0.08	28.10	28.27	28.35	28.08	28.20	0.13	-3.65	-0.33	1.25
	Control 3	24.92	24.90	25.03	25.14	25.00	0.11	28.28	28.23	28.36	28.29	28.29	0.05	-3.29	0.03	0.98

Table S5. Polymorphic variants found in AZ patients and controls.

DNA change	Exon	RNA/Protein	Patients (%)	Controls (%)	dbSNP	ESP6500 Minor Allele frequency (EU ethnic /AA/Overall) (%)
c.389A>G	ex5	p.K130R	4.50%	3.1%	rs6525433	0.9367/36.2712/13.7676
c.1351G>A	ex15	p.E451K	4.50%	3.1%	rs4844247	3.909/10.6649/6.3618
c.2541T>C	ex27	p.D847D	5.60%	5.20%	rs16991177	4.2658/10.3259/6.466
c.2566-3T>C	ex28	intron 27	3.26%	6.77%	rs1536250	9.6611/29.9087/17.0122
c.2566-4A>T	ex28	intron 27	3.26%	6.77%	rs1536251	9.2301/29.8305/16.7093