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Supplemental Data

WDR11, a WD Protein that Interacts with Transcription Factor EMX1,Is Mutated in Idiopathic Hypogonadotropic Hypogonadism and Kallmann Syndrome

Hyung-Goo Kim, Jang-Won Ahn, Ingo Kurth, Reinhard Ullmann, Hyun-Taek Kim, Anita Kulharya, Kyung-Soo Ha, Yasuhide Itokawa, Irene Meliciani, Wolfgang Wenzel, Deresa Lee, Georg Rosenberger, Metin Ozata, David P. Bick, Richard J. Sherins, Takahiro Nagase, Mustafa Tekin, Soo-Hyun Kim, Cheol-Hee Kim, Hans-Hilger Ropers, James F. Gusella, Vera Kalscheuer, Cheol Yong Choi, Lawrence C. Layman

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Figure S1: Semi-quantitative RT-PCR analysis in rat tissues

Total RNA isolated from 11 rat IHH/KS-relevant tissues was used for RT-PCR using rat-specific *Wdr11* primers. 736 bp amplicons were detected in all tissues after 35 cycles; in particular, expression levels were higher in the ovary, testis, olfactory bulb and piri cortex. HPA: hypothalamus preoptic area; MBH: medial basal hypothalamus.

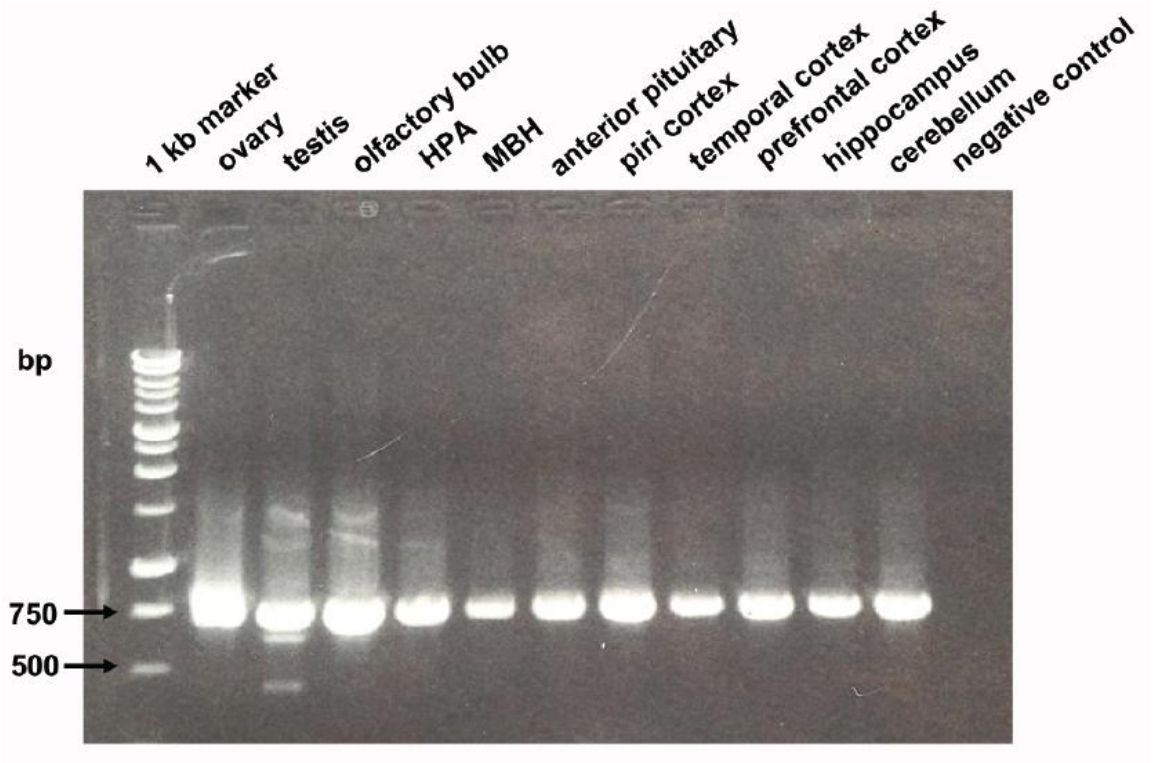


Figure S2: Evolutionary conservation of the residue Lys978

ClustalW multiple alignment of partial protein sequence of WDR11 orthologs. The position of residue K978 altered by one heterozygous nucleotide change of *WDR11* is marked by arrow and red letters in the corresponding segment of the multiple alignment. The amino acid residues that differ from the sequence of the human WDR11 protein are indicated blue. Lys978 residue is evolutionarily fully conserved in all thirteen available WDR11 orthologs.

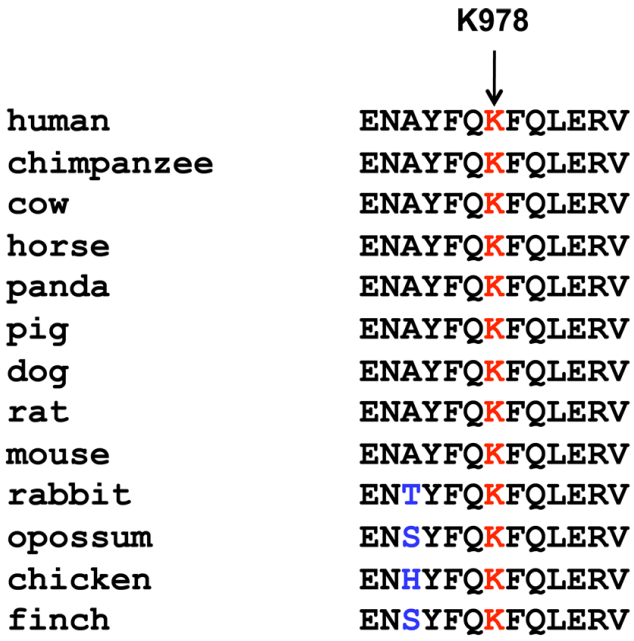
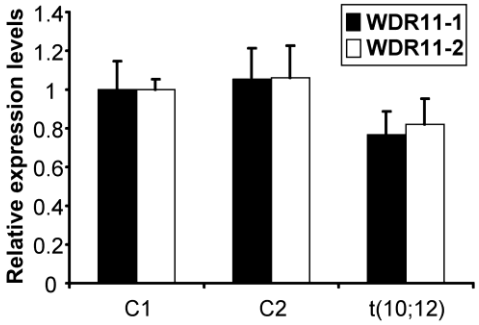


Figure S3: RT-qPCR and western blot analysis of t(10;12)

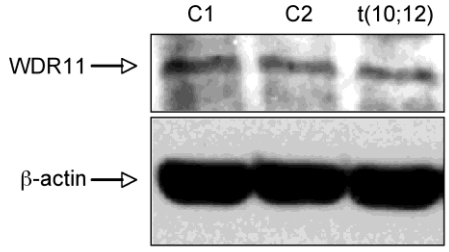
(A) *WDR11* mRNA levels in controls and the t(10;12) patient were measured by real time quantitative RT-PCR. Two different sets of primers (WDR11-1and -2) were used to validate *WDR11* mRNA expression, which was normalized against *GAPDH*. The level of *WDR11* mRNA in the t(10;12) patient was reduced by 20% compared to two male controls C1 and C2, suggesting the positional effect of the translocation breakpoint at 10q26.12. The data show the mean of three independent experiments ± SD.

(B) The anti-WDR11 antibody recognizes ~120kDa WDR11 protein in both male controls (C1 and C2) and t(10;12) lymphoblastoid cell extracts, showing about 10% reduced expression in t(10;12) (arrow). β-actin expression served as the internal loading control.

A



B



	C1	C2	t(10;12)
β-actin	179.8	161.3	164.7
WDR11	118.7	105.8	96.8
WDR11/B-actin	0.66	0.65	0.58
normalized to control	1	0.99	0.89

Figure S4: Overlay of 1PGU and WDR11

When we overlapped two replicas of the model of WDR11 (red) with the structure of 1PGU (blue) we found very good structural agreement, which led to the hypothesis that WDR11 may also dimerize by a similar mechanism involving two zinc coordination sites and might also be an actin binding protein. Because the two protein structures are not identical, deviations arising from their alignment make the position of the zinc ions (orange spheres) uncertain to a few angstroms. The sites of mutations in WDR11 are indicated with yellow spheres. To check our hypothesis we investigated whether WDR11 has a set of zinc-coordinating amino acids in the vicinity a putative zinc position, that we obtain, when we structurally align 1PGU with a dimer of WDR11. Because the two protein structures are not identical, deviations arising from their alignment make the position of the Zn ion uncertain to a few Angstrom. However, Fig. s7 clearly shows that also WDR11 has the required residues (ASP, GLU or HIS) for zinc binding in the vicinity of the putative zinc position. These residues are Asp 377, Glu 384, His 501, His 508, Glu 510. The two units, each of which has two beta propeller elements, are stabilized by two zinc ions as illustrated in Fig. S6 [1]. 1PGU contains two zinc binding sites in which a typical pattern of residues (green sticks) coordinates with the zinc ions (orange). Since these residues belong to different chains the zinc coordination acts as a tether stabilizing the dimer.

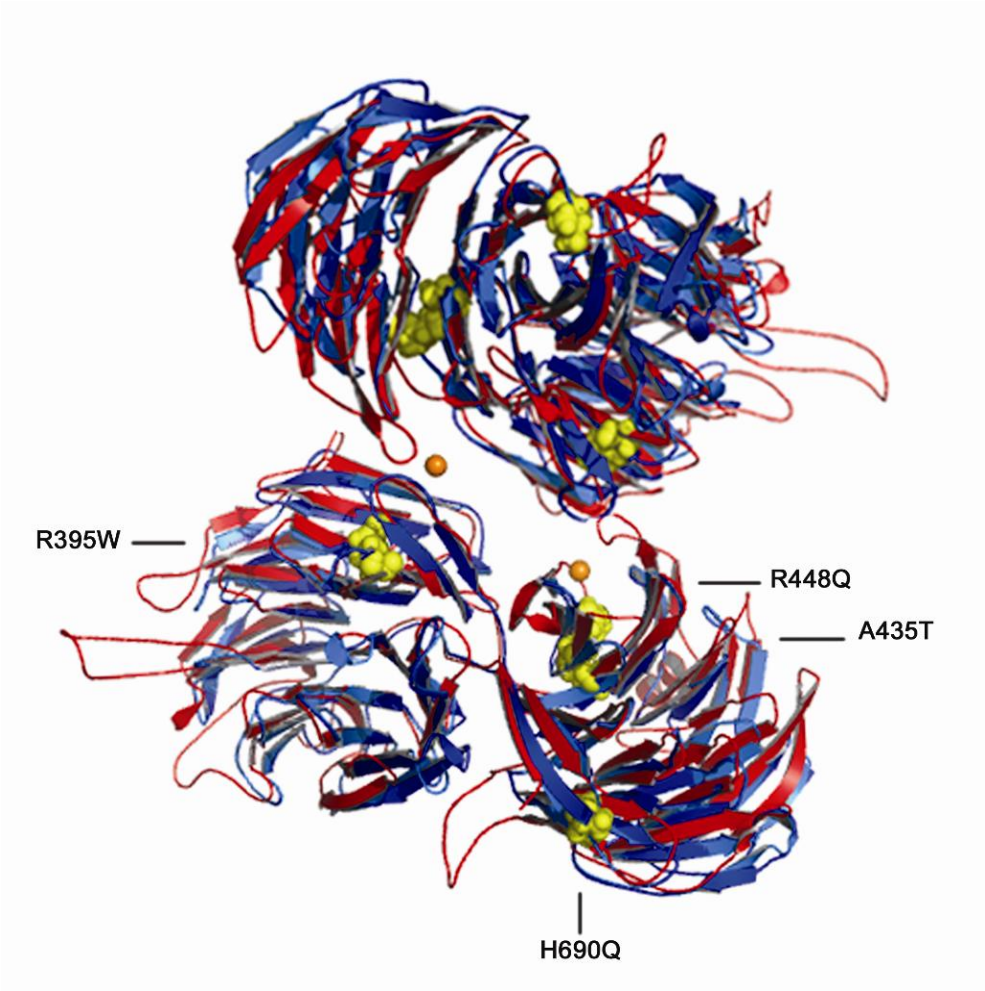

R395W
R448Q
A435T
H690Q

Figure S5: Protein-protein interaction region of WDR11

We used SPPIDER to detect protein-protein interaction regions (in bright cyan) in the model for WDR11 (non-interacting regions in dark blue), here shown in a view obtained by rotating panel A 90 degrees out of the plane on a horizontal axis. The Figure shows two prominent external interaction regions (labeled inner & outer surface), where WDR11 may interact with multiple other proteins, such as EMX1 or actin, as well as an intramolecular interaction region at the interface of the two propeller units. Four mutations (those that are visible are illustrated with orange sidechains and labeled) lie within protein-protein interaction regions of WDR11 and may thus directly influence binding to EMX1.

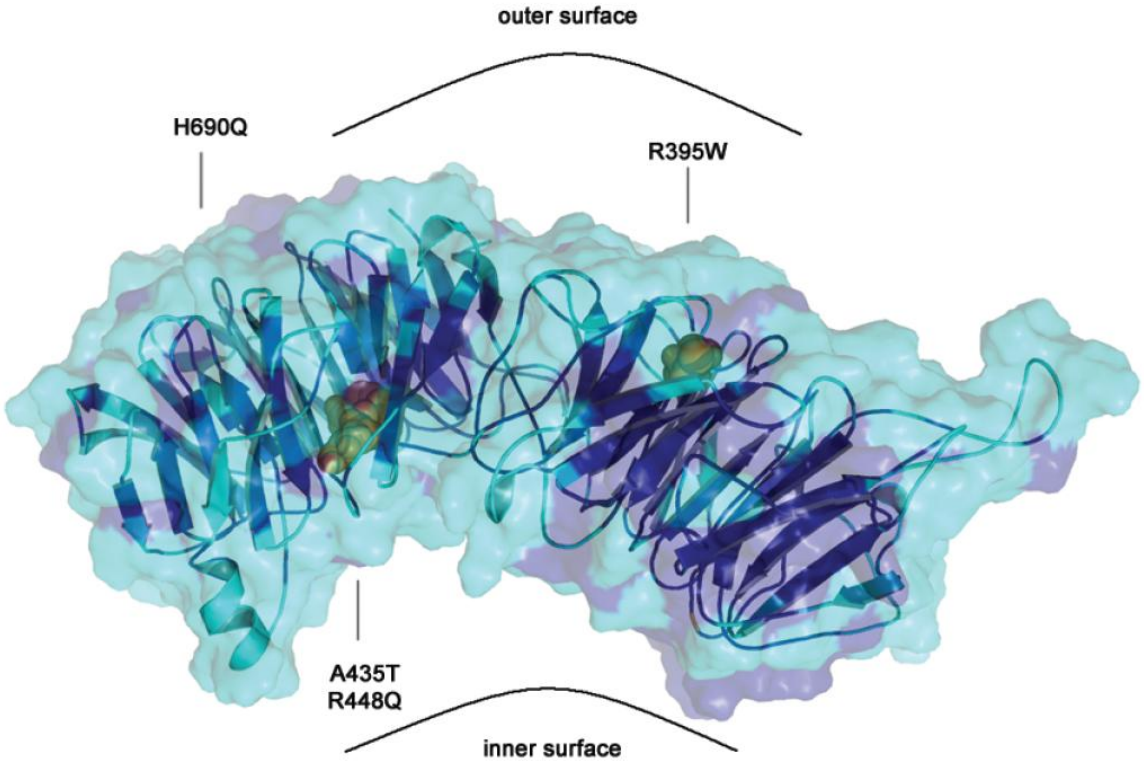


Figure S6: Two zinc ion binding sites of 1PGU

We have identified other beta-propeller proteins that are highly homologous to WDR11, in particular, actin-interacting protein 1 (pdb code 1PGU), which is comprised of a dimer of two double propeller units that are present in 1NR0 and WDR11. The two units, each of which has two β-propeller elements, are stabilized by two zinc ions (shown in orange) as illustrated here [1]. Zinc ions in proteins are coordinated by a highly conserved set of amino acids, most commonly His, Glu, Asp and Cys [2]. The experimental structure of 1PGU shows the interaction between zinc and amino acids Asp, His and Glu. Both Zn ions are stabilized by residues coming from different molecules in the dimer and they therefore act as tethers that hold the dimer together.

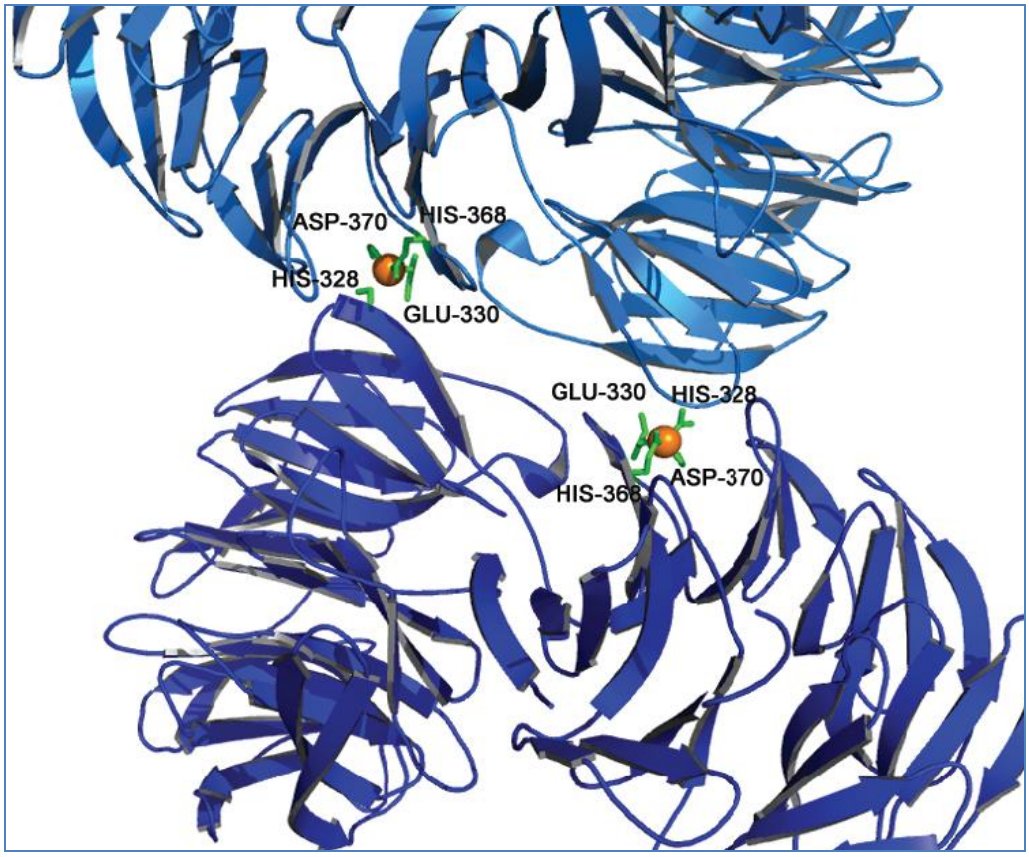


Figure S7: Glu, His and Asp residues in the model of WDR11 in the vicinity of the proposed zinc position based upon 1PGU

A single unit of the proposed dimer is shown. The presence of the required set of amino acids demonstrates that WDR11 might also bind zinc and form a dimer that is stabilized by the same mechanism as 1PGU. WDR11 has the required residues (Asp 377, Glu 384, His 501, His 508, Glu 510) for zinc binding in the vicinity of the putative zinc position.

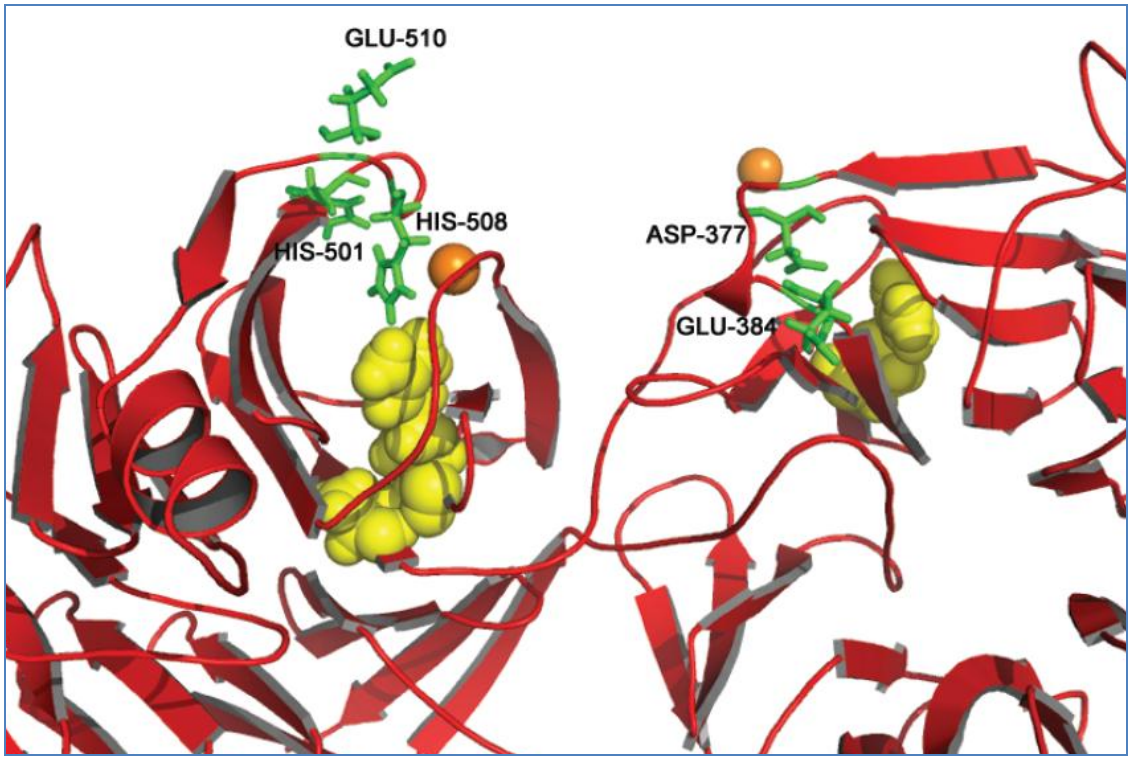


Table S1: SNPs of *WDR11* (NM_018117.11) found in IHH/KS patients and normal controls

Location	Nucleotide change	Amino-acid change	Frequency of second allele in patients	dbSNP or frequency of second allele in normal controls
exon 1	c.-6G>A	−	3/402	rs17100985 5/96 (1)[a]
exon 1	c.51G>T	Gly17Gly	3/402	rs35692153 4/96 (1)[a]
intron 2	IVS2-7G>C	−	91/402 (8)[a]	rs2241846 17/96 (5)[a]
exon 6	c.834G>A	Thr278Thr	164/402 (31)[a]	rs10886789 32/96 (7)[a]
exon 6	c.789C>G	Leu263Leu	1/402	0/96
exon 8	c.1066G>A	Val356Ile	0/402	rs34304988 3/936
exon 8	c.1113C>T	Ala371Ala	1/402	rs41287986 2/936
exon 10	c.1425G>A	Pro475Pro	0/402	6/1466
exon 11	c.1542C>T	His514His	5/402	rs12355108 2/96
intron 12	IVS12+25G>A	−	6/402 (1)[a]	rs7923412 2/96
intron 13	IVS13+11A>C	−	1/402	
exon 15	c.1899A>T	Ala633Ala	169/402 (35)[a]	rs7899928 40/96 (10)[a]
exon 18	c.2304A>G	Ala768Ala	76/402 (5)[a]	rs2289337 19/96 (2)[a]
intron 18	IVS18-9C>T		1/402	1/548
intron 21	IVS21+48A>C	−	1/402	0/96
intron 21	IVS21-5C>G	−	1/402	1/456
intron 23	IVS23-48C>T	−	2/402	1/456
intron 23	IVS23-30G>C	−	2/402	7/648 (2)[a]
exon 24	c.2932A>C	Lys978Gln	1/402	2/1174
exon 24	c.2958A>G	Leu986Leu	163/402 (29)[a]	rs1652727 216/648 (38)[a]
intron 26	IVS26+11C>T	−	7/402	3/456
intron 26	IVS26+39C>T	−	259/402 (72)[a]	rs1866516 279/456 (87)[a]
intron 26	IVS26-12C>T	−	1/402	no SNP data

exon 27	c.3318C>T	Ala1106Ala	1/402	1/96 rs34937000
exon 27	c.3363C>G	Val1121Val	73/402 (2)[a]	rs3740307 12/96 (3)[a]
exon 27	c.3393C>T	Leu1131Leu	1/402	rs12268298 4/96 (1)[a]

[a] Total number of homozygotes among the individuals screened

Table S2: WD domains in WDR11

WD domains in WDR11 predicted based upon the structural model (WD domains from 2 to 10) and confidently predicted from the server SMART (WD domains 1, 11, and 12)

Number	Name	Begin	End	Size (AA)	E-value	Blast Confirmation
1	WD	50	99	50	0.04	Begin/end 50-99
2	WD	126	164	39	__	__
3	WD	220	274	55	__	__
4	WD	277	324	48	__	__
5	WD	335	374	40	__	__
6	WD	428	467	40	__	__
7	WD	561	605	45	__	__
8	WD	609	646	38	__	__
9	WD	652	691	40	__	__
10	WD	708	736	29	__	__
11	WD	738	778	41	10.30	738-778
12	WD	781	822	42	13.80	781-822

Table S3: BAC clones used for FISH analysis of t(10;12)

FISH result of BACs from Chromosome 10q26 (coordinates are from NCBI Build 36/hg18 assembly of UCSC Genome Browser)

No.	BAC ID	Start (bp)	End (bp)	Proximal	Spanning	Distal
1	RP11-592D19	118,702,524	118,893,472	X		
2	RP11-714M16	121,088,268	121,281,904	X		
3	RP11-717L13	121,765,054	121,963,545	X		
4	RP11-254K03	121,923,506	122,098,857		X	
5	RP11-572P18	122,039,380	122,255,369			X
6	RP11-313F09	122,302,049	122,499,951			X
7	RP11-499E06	122,574,466	122,745,931			X
8	RP11-152I12	122,709,923	122,891,863			X
9	RP11-604B15	122,847,905	123,036,763			X
10	RP11-251P02	122,860,000	123,009,780			X
11	RP11-753P11	123,034,946	123,207,777			X
12	RP11-300A10	123,166,882	123,350,993			X
13	RP11-7N04	123,407,405	123,552,165			X
14	RP11-105F10	123,764,473	123,939,046			X
15	RP11-619E04	124,103,531	124,311,827			X
16	RP13-63P20	124,872,781	125,056,306			X
17	RP11-47H10	125,699,910	125,916,390			X
18	RP11-8D22	130,325,185	130,493,639			X
19	RP11-91E2	135,150,439	135,301,208			X

FISH result of BACs from Chromosome 12q12-12q13 (coordinates are from NCBI Build 36/hg18 assembly of UCSC Genome Browser)

No.	BAC ID	Start (bp)	End (bp)	Proximal	Spanning	Distal
1	RP11-88L2	42,459,839	42,629,204	X		
2	RP11-462E15	42,875,295	43,061,579	X		
3	RP11-424H10	43,693,902	43,853,320	X		
4	RP11-19E18	44,441,691	44,596,850	X		
5	RP11-659M15	44,897,959	45,111,797	X		
6	RP11-755G20	45,222,971	45,399,371	X		
7	RP11-618L22	45,523,763	45,703,378	X		
8	RP11-72L16	45,581,486	45,734,180	X		
9	RP11-23J18	45,755,428	45,925,226	X		
10	RP11-479I2	45,789,177	45,975,868	X		
11	RP11-677P12	45,813,066	45,983,115	X		
12	RP11-71G17	45,875,143	46,046,421	X		
13	RP11-464D5	45,880,805	46,073,952		X	
14	RP11-543D1	46,000,452	46,161,075			X
15	RP11-648F12	46,021,097	46,184,193			X
16	RP11-379M8	46,074,480	46,267,797			X
17	RP11-650J13	46,467,143	46,641,379			X
18	RP11-204C20	46,894,554	47,075,616			X
19	RP11-141C14	50,423,519	50,612,911			X
20	RP11-762I7	54,296,170	54,469,906			X

Table S4: **Primers used in this study**

Semi-quantitative RT-PCR in rat tissues (rat *WDR11*; XM_219377.5)	
Forward primer	5´-GACCTTGAAGTGAATCAAACAGTTGGCGTGATTGCAATTGAGCGAAC-3´
Reverse primer	5´-CACACGAGTGGACGCTTAGCTCCTTGTGTAGCAGACC-3´
Amplicon size	736 bp
Annealing temperature	68 ºC

WDR11 mutant constructs			
WDR11 mutants	Type	Outside primers	Mutant primers
m1	R395W	F01: 5´-GCTATCCACCCGCCAAATTA-3´	1183_mR1: 5´-TACTGTTCCATGAATTTCGATTACAAACTG-3´
		R01: 5´-CTGCTCACCATCTGAACCTC-3´	1183_mF1: 5´-CGAAATTCATGGAACAGTAGTTCTGGTGTG-3´
m2	A435T	F01: 5´-GCTATCCACCCGCCAAATTA-3´	1303_mR1: 5´-CAGCAATTGTACTTTGCCCAATCATGTTAT-3´
		R01: 5´-CTGCTCACCATCTGAACCTC-3´	1303_mF1: 5´-GGGCAAAGTACAATTGCTGGGGAAGAACAT-3´
m3	R448Q	F01: 5´-GCTATCCACCCGCCAAATTA-3´	1343_mR1: 5´-TGCACTTCCTGCAGAATTGAACCTCTGGGA-3´
		R01: 5´-CTGCTCACCATCTGAACCTC-3´	1343_mF1: 5´-CAATTCTGCAGGAAGTGCACCTCAAGTTCC-3´
m4	H690Q	F01: 5´-GCTATCCACCCGCCAAATTA-3´	2070_mR1: 5´-AACAGTGAGTTGATACACTTGGCCATCAAT-3´
		R01: 5´-CTGCTCACCATCTGAACCTC-3´	2070_mF1: 5´-AGTGTATCAACTCACTGTTGAAGGAAACTC-3´
m5	F1150L	F06: 5´-ATCGCTTGGAAAGGTGATAC-3´	3450_mR1: 5´-TGCTCTATCCAAGTATCTCATGCTGTGAAG-3´
		R02: 5´-CTTCAATGGGTTCTTCCTTG-3´	3450_mF1: 5´-GAGATACTTGGATAGAGCAGCCTTATTTGT-3´

Y2H and Binding Assay		
Construct	Top primer	Bottom primer
pENTR3C-WDR11 1-361 [N]	5´- GATGGATCCA TGTTGCCCTA CACAGTG -3´	5´ - GTAGTCGACC ATACTGAAGG GACGGAC -3´
pENTR3C-WDR11 362-830 [M]	5´- GATGGATCCG TGTGCTGTCC TGTCAAT -3´	5´ - GATGTCGACA AAGCACGCAG ACTTCAT -3´
pENTR3C-WDR11 831-1224 [C]	5´- GATGGATCCA GAATGGATGA ACAAGAG -3´	5´ - GATGTCGACT CACTCTTCAA TGGGTTC -3´
pENTR3C-WDR11 1-1224	5´- GATGGATCCA TGTTGCCCTA CACAGTG -3´	5´ - GATGTCGACT CACTCTTCAA TGGGTTC -3´
pENTR3C-Emx1 1-290	5´- GAT GAA TTC ATG TGC CTG GCT GGG TGC -3´	5´ - GAT CTC GAG CTA GTC ATT GGA GGT GAC -3´
pGBKT7-WDR11 1-830	5´- GATGGATCCA TGTTGCCCTA CACAGTG -3´	5´ - GATGTCGACA AAGCACGCAG ACTTCAT -3´

Mouse *in situ* hybridization analysis (NM_172255.3)		
WDR11 probe	Forward primer	Reverse primer
641-1035 of NM_172255.3	5´-GAATTCGGGAAGAAAGTGTACATCT CCAG-3´	5´-TCTAGATCATTTGATGTAGTACAGATGC-3´
2892-3492 of NM_172255.3	5´-GAATTCTAACCAAGGAAGGTGCTCC TAA-3´	5´-TCTAGATATCTCATGCTGTGAAGCGTCT-3´

Mutation Screening of *WDR11* (NM_018117.11)		
Exon number	Outer primer pair	Nested primer pair
1	F01: 5´-AGAGTGCGGAACCTAG-3´	F01: 5´-TAGGAAACTGAAGGCAAC-3´
	R01: 5´-GGCTATTATCACTTTTGTAACAA-3´	R01: 5´-TCATTGCGTGAGGGC-3´
2	F02: 5´-CAGATCTTTTATTTAAGTGGGATA-3´	F02: 5´-ATAAATACTGGCCTTTGGG-3´
	R02: 5´-ATGATCTAACAACTAGCATTTAATA-3´	R02: 5´-CCTGTATTGACATTAAGTCATATA-3´
3	F03: 5´-CCTTCAAAATGAAAACCAGTTT-3´	F03: 5´-TCTGTTTATTCTTGCTAAATGTTTA-3´
	R03: 5´-ATTTAGATCAGCAGAATAGG-3´	R03: 5´-CAATCAAGAGCTAACTTGGA-3´
4	F04: 5´-ATTAGTTTTTTCTCTGGAATATTGA-3´	F04: 5´-ACATTTGGGGCTGGTG-3´
	R04: 5´-GACTAGACATGCTTCTC-3´	R04: 5´-CTTCGCTCAGTCATTTTAAC-3´
5	F05: 5´-TAATGGACCACCTGTTTCT-3´	F05: 5´-TTAGAGAACATTCCCATTATGTT-3´
	R05: 5´-AAAGGCAATGTTAAATGCTGAA-3´	R05: 5´-TTAGGGCAGGGGACAA-3´
6	F06: 5´-GATGCCAACCCATGTT-3´	F06: 5´-ACTTCAGGGAATAGTTGTAC-3´
	R06: 5´-AAAAACAATCTAAAATAGTGTTGCT-3´	R06: 5´-CTAGTCTTTTGATAAATTGAAAAG-3´

7	F07: 5´-ATCCTGAGATATTTATTGAACTATT-3´	F07: 5´-AGTCACATGGGTAAAGACAA-3´
	R07: 5´-TCAAGAGATTAATCTTGGGC-3´	R07: 5´-GCTTATACTTTTTATGCCTCTTAT-3´
8	F08: 5´-TAATTGTAAAAAGTGAAGCCATG-3´	F08: 5´-TGACATTGCATTCATGAAGG-3´
	R08: 5´-ATAGGTGAAATAGCTCTAGC-3´	R08: 5´-GCAGATTTACATAATTCCCTAATA-3´
9	F09: 5´-GAGGGCTGGTTTTCTATAA-3´	F09: 5´-TGCTAGAGCTATTTCACC-3´
	R09: 5´-GGCACTTTTCTACCTAAGTA-3´	R09: 5´-CTCTGATTTATTTAGGAAAGAATG-3´
10	F10: 5´-AGTCATTGTGTCCTTAATAAC-3´	F10: 5´-CAGCCACTAAAGTACTACAT-3´
	R10: 5´-CAGTTGGAATAAAAGGACAG-3´	R10: 5´-ATGAGTATTTACAGTTTCACTTAG-3´
11	F11: 5´-ATTTTTTTTATAAGGAAGTGGACC-3´	F01: 5´-CAGAGAAATTCATTCTTCTTTGT-3´
	R11: 5´-TCTACTGCTTATGTTCTTTAC-3´	R01: 5´-ATCCTTGATTAACATAGCCATT-3´
12	F12: 5´-GTATGGTAATACAGTCTCAA-3´	F12: 5´-CAAATAAAAGGTCATGTTAAGATAA-3´
	R12: 5´-TAAACAATGATGAGGCCTTT-3´	R12: 5´-TTCTAGGGCAATTATTAGAGAATA-3´
13	F13: 5´-CCGAAAAAAACAGAAACAGATT-3´	F13: 5´-AAAAAACAGAAACAGATTAGAAGC-3´
	R13: 5´-GTTACTTCATCTTTTTAGAAGTAG-3´	R13: 5´-GAATTAAAAGTGATAGCAACTTAAT-3´
14	F14: 5´-GAGCTTTACCTTCTTATGAC-3´	F14: 5´-TTAATCATGGTGCTAAATGG-3´
	R14: 5´-CCAAGAAAATAAGAATTATCTCCA-3´	R14: 5´-CATTTATTTAGATCATCTTCTCTG-3´
15	F15: 5´-AGCCTTTCTCTAAGTAGGTA-3´	F15: 5´-TAAAATCTAAAGAACAAATGTGGA-3´
	R15: 5´-ATTATCTTTTTAGTAATCTGGGTAT-3´	R15: 5´-ATGTACTGAAAGATGAAACTAAGT-3´
16	F16: 5´-GCTGTTAAGGAGTGATTCAT-3´	F16: 5´-CATAATTAGCTGGGCAAATAT-3´
	R16: 5´-AGTATGGAACAAGTTTAATCG-3´	R16: 5´-TCGATAATATCTCCTTAAATAAAGT-3´
17	F17: 5´-GATAGGCAATGTAGCTCTT-3´	F17: 5´-TTTGTTAGTGTCTAAAGTCTGTTT-3´
	R17: 5´-GCAGGAGAATGGCATGA-3´	R17: 5´-CGGGAGTCAGAGCTT-3´
18	F18: 5´-GACCTTAAAGGGCAGG-3´	F18: 5´-TGTCAGATGTGGCCC-3´
	R18: 5´-GGTCTCAGAGACAGTG-3´	R18: 5´-AGTTGTTACCTTTAGAACACAAAT-3´
19	F19: 5´-TGGAAGATAGATTGCCAG-3´	F19: 5´-GCTGTTCAGTTCTAAACTTG-3´
	R19: 5´-ACTCAAGCTTCAAGTTAATATATTT-3´	R19: 5´-AAACTCAGATTCTAAGAAAATAAGA-3´
20	F20: 5´-AGACTCTCTCTGCTCTT-3´	F20: 5´-TAAGCGGTGACTACATTGAA-3´
	R20: 5´-TTCCACAAAGGCCCGA-3´	R20: 5´-GCAATTCTGTAGTGTCTCT-3´
21	F21: 5´-CTCAATAAGATAAAAGCAGATATTA-3´	F21: 5´-GGTCCTGAGTAGATTG-3´
	R21: 5´-CTCTGTCACTGTAACTAC-3´	R21: 5´-CTGAATAAGTACTTTTTCTGCAAA-3´
22	F22: 5´-AGAACACTCTCAGCCC-3´	F22: 5´-CTTCAACTTTTATAATTTCAGGGT-3´
	R22: 5´-CTAGATCTCACATTTAAAATGCA-3´	R22: 5´-CAATTTGATTTTTCCCTATTTGTT-3´
23	F23: 5´-CTTTCACCCTGTCAGG-3´	F23: 5´-CCAGTATCCCAGTGG-3´
	R23: 5´-CAAATGTACCGAGGGC-3´	R23: 5´-GGCTTATTTCTATTACTGAAC-3´
24	F24: 5´-GATAATATCTATCAACATTTTTGCT-3´	F24: 5´-TTCAAATTCTTATTAAATGTTTGGG-3´
	R24: 5´-TTTTTCTCATGAGGTTTATTGGTA-3´	R24: 5´-GTATTATTTTGAAAGAAAGCCTAAA-3´
25	F25: 5´-TAGTGTAGCACCTCTG-3´	F25: 5´-CTGTTTAATGTTTGTATTTGTAAAC-3´
	R25: 5´-ACAGTCTGGAGAATAAGTAG-3´	R25: 5´-ATAGTTCTCTCACAGGTTTTAT-3´
26	F26: 5´-TACCCTGGTTACTTGAG-3´	F26: 5´-GAGTAATAACAGTAAAACTCTTAAT-3´
	R26: 5´-GATCCATTTTTAAATAAGTAATGCA-3´	R26: 5´-ACTCATAAAGAATAGGAAAAGG-3´
27	F27: 5´-CAGAGTATGCAGCAGCT-3´	F27: 5´-GGCAGTGTGCTAGTCA-3´
	R27: 5´-AGTTCCATTCTCCTTTGG-3´	R27: 5´-TTGACAAACCTGAAATGTATACTT-3´
28	F28: 5´-CTGTAGATTTTTGTGTATTTAAACT-3´	F28: 5´-TGAGTATTCTTCCCTCCTT-3´
	R28: 5´-TGCAGCCACCTTCTTAT-3´	R28: 5´-TGATTCTTTGTCCTCTAATTTCT-3´
29	F29: 5´-AAAAGTCTCCTGTGTTCATC-3´	F29: 5´-ACACCAGGTCCCTTC-3´
	R29: 5´-GGCACACATAGTCTTAGAA-3´	R29: 5´-TCAAACAGCTCACAGG-3´

Confirmation of *WDR11* missense mutations			
Mutation	Location	Forward primer	Reverse primer
R395W	Exon 8	5´-CCATGCTTGACATTGCATTCATGAAGGAGTGATGC-3´	5´-CTTAAAATCTGCATTACGTATTAGGAATTCAAAGTATTAAGTCCAG-3´
A435T	Exon 10	5´-GATGCAATTTAAGTCCTATAGCCAGGTACAGAGTC-3´	5´-GCATTCCAATTCAATATAAGACAGTTGGAATAAAAGGACAG-3´
R448Q	Exon 10	5´-GTGTCCTTAATAACTACAGCCACTAAAGTACTACATTAAC-3´	5´-GAGTATTTACAGTTTCACTTAGTCTCTATCTCCTTTTAAAGC-3´
H690Q	Exon 16	5´-CATGTTTACCCATAATTAGCTGGGCAAATATTAGTTTCAGAC-3´	5´-GCTGGATAACTTTGGGAAAGTTACTTATTCTCCCACAG-3´
F1150L	Exon 28	5´-GCTTGTGTTTGGAGATGTGAGTATTCTTCCCTC-3´	5´-CTTCCTGCCTCCCTCCATCCTAGTC-3´

Long Range PCR for der(12) junction fragment	
Forward primer	Primer sequence
12q13.2-10kb-4208f	5´-CTCATATGTTTTCAAGAGGATCTTCTCAATATAACAATGTGTAGCTTAT-3´
12q13.2-10kb-4562f	5´-CTGCAACGATGAACAGATCAGAATAGGCGACATGC-3´
12q13.2-10kb-5338f	5´-GTTATATTGAAGCGTGAAATATTGATTACAATTTTGAAGCATAAAATATATCG-3´
12q13.2-10kb-5961f	5´-ATATTCTGTGCTTCAAAATAGTTGTAATCAATATTTGACACTTCAATATAAC-3´
12q13.2-10kb-7351f	5´-GTAATGACTTACTCACACCCCTGCCCCACTCTAAC-3´
Reverse primer	Primer sequence
10q26-10kb-3497r	5´-CAAAAGCTGGAGTGGCATTACTACTAAGTAACAGGTAGAACAAAAAC-3´
10q26-10kb-5710r	5´-CCCTGGTTTAATGTTTCTGCATTGCCTAAACAATGAGATTTAAAC-3´
10q26-10kb-5750r	5´-CTAGAGTTGTCTTCTGGATAGCCAGAACTGATCACC-3´
Long Range PCR for der(10) junction fragment	
Forward primer	Primer sequence
10q26-10kb-1851f	5´-CTGTAGGTCAAGCCTTTAATACCCTCCTATTAGAGGG-3´
10q26-10kb-2367f	5´-GCCAAAAAATGTTAGCCATTATTACAGAGTATTTCAGGAGTTCAG-3´
10q26-10kb-2801f	5´-ATCTACTTTATAAGGTCATGATGAACACTAGATTTAGCATGTGGTAAG-3´
10q26-10kb-2851f	5´-CCATGAAAATTTGCAGTATTATTATAGCAGTTGGAGGCCATG-3´
10q26-10kb-2914f	5´-GTATTGTGAAGAGGTCAAAAGCCAGGCTGCAGAG-3´

Reverse primer	Primer sequence
12q13.2-10kb-7630r	5´-CTGGACATTGGGTATCAAATTGCTGATACCCTGATCTG-3´
12q13.2-10kb-8180r	5´-CGATCTCAGCTCACTGCAACCTCTGTCTCC-3´
12q13.2-10kb-8836r	5´-GCTATGTAAGCAGTTATCAGCAATTTGACCCCAGTC-3´
12q13.2-10kb-9664r	5´-CTGTATACTTTCTGTCTTCATTTTATATCATCATTCCATGTTTTGAAAAAAGTT-3´
12q13.2-10kb-9890r	5´-GCTCAATTATAAAAGGAAAGTTGATTCTAGATAGGAGCAGGCCATTG-3´

Zebrafish *wdr11* gene (XM_682139.3) isolation by RT-PCR	
Forward primer	Reverse primer
5´-CGGGATCCGCCAAAACATTCCACCTTTTCAT-3´	5´-CACCATGGAGTCCATATCCTGAATGGGTTTG-3´

RT-qPCR of *WDR11* in balanced translocation patient	
Primer	Primer sequence
WDR11-RT-3117f	5´-GCAACCCACTGGATATATGCTATGACGTGCTCTG-3´
WDR11-RT-3213r	5´-GACCGTTTCACTTCCTGTAGATTAACCCTTTCTAGCTG-3´
WDR11-RT-3221f	5´-GATCATACAAGGAAATGTACAGACCAGCTACTGCTCTTG-3´
WDR11-RT-3370r	5´- GCCTGACGAGGTGACAGTAGTGACTAAACAGG-3´

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