WDR62 is associated with the spindle pole and mutated in human microcephaly

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Supplementary Information

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Supplementary Table 1

Analysis of parallel sequencing data to find pathogenic mutations.

Mapped sequence data was filtered to remove non-coding, synonymous variants and those variants known to be non-pathogenic polymorphisms. This left a single candidate gene, *WDR62*, common to both mapping families.

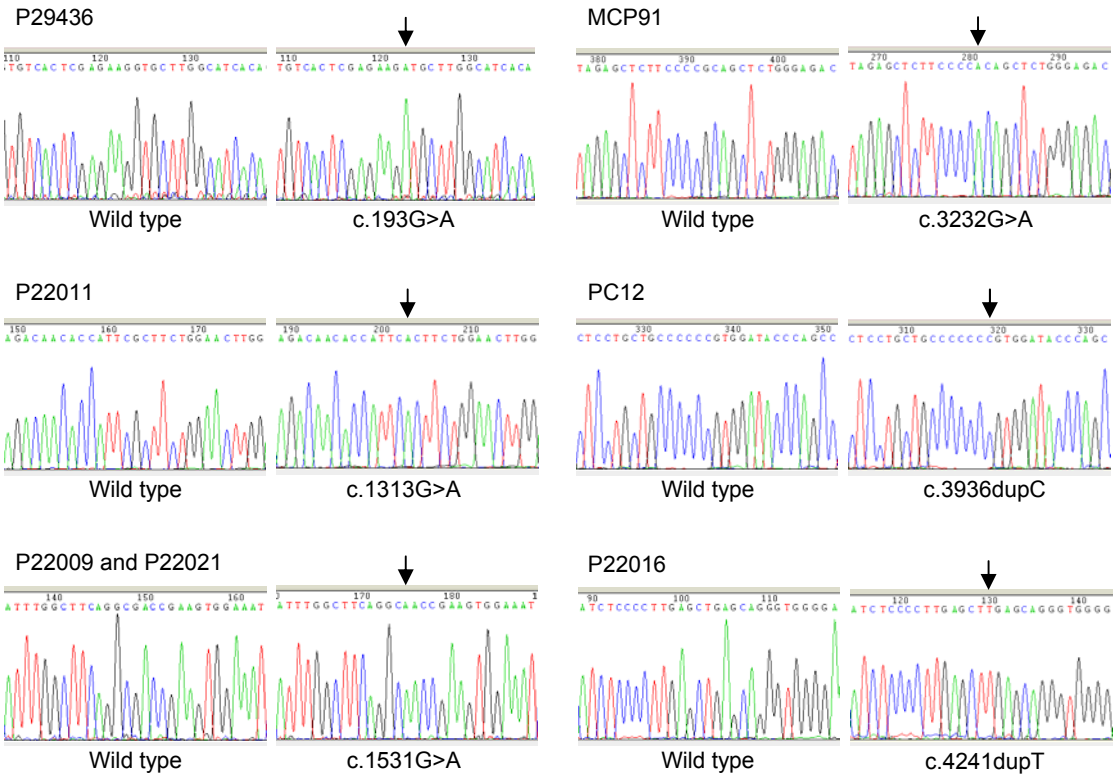
	P22011	**P22016**
Targeted region (no. of bases)	2,752,492	2,752,492
% coverage of reference	97.27	97.51
No. of unique matching reads	78,894	167,506
Range of depth of coverage of variants (no. of reads)	3-222	3-199
% of variants with depth of coverage of at least 10 reads	84.1	92.6
% of variants with depth of coverage of at least 20 reads	58.1	75.8
No. of coding variants	63	64
No. of coding variants not in UCSC Genome Browser SNP 130	10	13
No. of hand-curated non-synonymous coding variants not in UCSC Genome Browser SNP130	2	1
Gene(s) bearing non-synonymous coding variants	**WDR62** HKR1	**WDR62**

Supplementary Figure 1

Sequence chromatograms showing the six homozygous mutations identified in the seven pedigrees.

Black arrows indicate position of mutation. The chromatograms are in pairs with the relevant Pedigree number indicated above. For each pair a homozygous wild type sequence is shown on the left and to its right the homozygous mutation.

(For the PC12 chromatograms there is a known SNP, rs2074435, 5' to the polyC run, where the common allele is C**A**G coding for glutamine, and the rarer allele C**T**G coding for leucine. Both the control shown and the PC12 patient are homozygous for the rarer T allele.)



Supplementary Figure 2

Evolutionary analysis of the four *WDR62* missense mutations found in the study. Each mutation is given at the top of each table. In each table, species is stated on the left. In the middle the 20 amino acid sequence either side of the mutation are given. The mutation is in the centre position in bold by itself above the wild type amino acid it replaces. To the right are given the reference protein sequences used in this analysis. Where no sequence is give we derived the peptide sequence from available nucleotides form the relevant species followed by translation and curation. Stars indicate that all amphibians and Drosophila had the same amino acid sequence. MAPKBP1 is the only homologue of WDR62 in mammals and so is also shown to allow assessment of the degree of evolutionary conservation. Grey shading of amino acids indicates deviations from the human canonical sequence. For the p.Ala1078Thr mutation more species are given, as we cannot determine if this is an atavistic mutation or a harmless (but very rare) polymorphism.

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p.Val65Met

mutation	**M**	
human	TASEETVQNRVSLEK**V**LGITAQNSSGLTCDP	NP_001077430
chimpanzee	TASEETVQNRVSLEK**V**LGITAQNSSGLTCDP	XP_001162576
mouse	AAPEDTVQNRVTLEK**V**LGITAQNSSGLTCDP	NP_666298
rat	AAPEDTVQNRVTLEK**V**LGITAQNSSGLTCDP	XP_001075797
danio	GSHRRDIHNRVVLEK**V**LGITASSSSALTCDP	XP_002664595
human MAPKBP1	GNRREDLSSKVTLEK**V**LGITVSGGRGLACDP	NP_001122080
frog*	GTKKEDLSSKVTLEK**V**LGITVSAGRGLSCDP	NP_001086542
melanogaster*	KRNDEQLMDRIKLKK**V**LGLTVCSNAALDVSP	NP_608630

p.Arg438His

mutation	**H**	
human	LPSGSFLTCSSDNTI**R**FWNLDSSPDSHWQKN	NP_001077430
chimpanzee	LPSGSFLTCSSDNTI**R**FWNLDSSPDSHWQKN	XP_001162576
mouse	LPSGTFLTCSSDNTI**R**FWNLDSASDTRWQKN	NP_666298
rat	LPSGTFLTCSSDNTI**R**FWNMDSGSDTQWRKN	XP_001075797
danio	LSPGSFLTCSSDNTI**R**LWRSE-SPQSHSAAL	XP_002664595
human MAPKBP1	LPPSSFITCSSDNTI**R**LWNTESSGVHGSTLH	NP_001122080
frog*	LPPNSFITCSSDSTI**R**LWNMETSNIHGTALH	NP_001086542
melanogaster*	LPEECFVTCSSDDTI**R**VWGLDGCTNNDIYRR	NP_608630

p.Asp511Asn

mutation	**N**	
human	RVMQVSPDGQHLASG**D**RSGNLRIHELHFMDE	NP_001077430
chimpanzee	RVMQVSPDGQHLASG**D**RSGNLRIHELHFMDE	XP_001162576
mouse	RVMQVSPDGQHLASG**D**RSGNLRIHELHFMDE	NP_666298
rat	RVMQVSPDGQHLASG**D**RSGNLRIHELHFMDE	XP_001075797
danio	RVLGISPDGQHLAAG**D**RSGNLRIFGLQFMDE	XP_002664595
human MAPKBP1	RSVCVSPNGQHLASG**D**RMGTLRVHELQSLSE	NP_001122080
frog*	RSVCVSPNGQHLASG**D**RTGTLRVHELQSMTE	NP_001086542
melanogaster*	RCIKISPELQHLASG**D**RCGNIRVYSLVNLRL	NP_608630

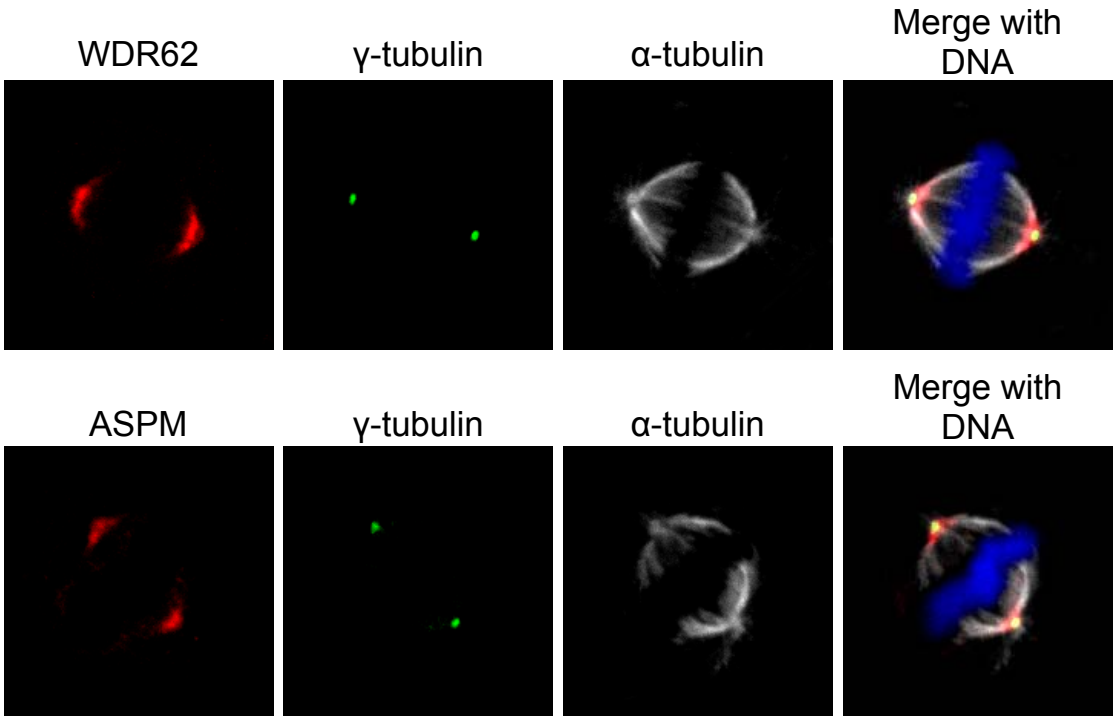
p.Ala1078Thr

mutation	**T**	
human	HFETLTESPCRELFP**A**ALGDVEASEAEDHFF	NP_001077430
chimpanzee	HFETLTESPCRELFP**A**ALGDVEASEAEDYFF	XP_001162576
orang-u-tan	HFETLTESPCRELFP**A**ALGDVEASEAEDYFF	
rhesus	HFETLTESPCRELFP**A**ALGDVEASEAEDYFF	
marmoset	HFETLTESPCRELFP**T**ALGDTEASEAEDYFF	
horse	HFETLTDAPPEELFH**G**SLRDVQASEAEDYFN	XP_001492683
pig	HFETLTDAPAEELFH**G**SLRDLKASEAKDDFF	NP_001121933
mouse	HFETLTDAPTEELFH**G**SLGDIKISETEDYFF	NP_666298
rat	HFETLTDAPAEELFP**G**SLGDIKISETDDYFF	XP_001075797
panda	HFETLTDAHPEELFH**G**ALRDLEASESEDFFN	EFB29891
cow	HFETLTDAQPEELFL**G**SLRDLKAPETEDDFF	XP_870429
rabbit	HFETLTDAHPEELFH**G**SLGDANVPEAEDVFL	XP_002722275
danio	HFDTLGVAT-NEKFN**T**DLSSLQPT-TESNFL	XP_002664595
human MAPKBP1	------------------------------	NP_001122080
frog*	------------------------------	NP_001086542
melanogaster*	------------------------------	NP_608630

Supplementary Figure 3

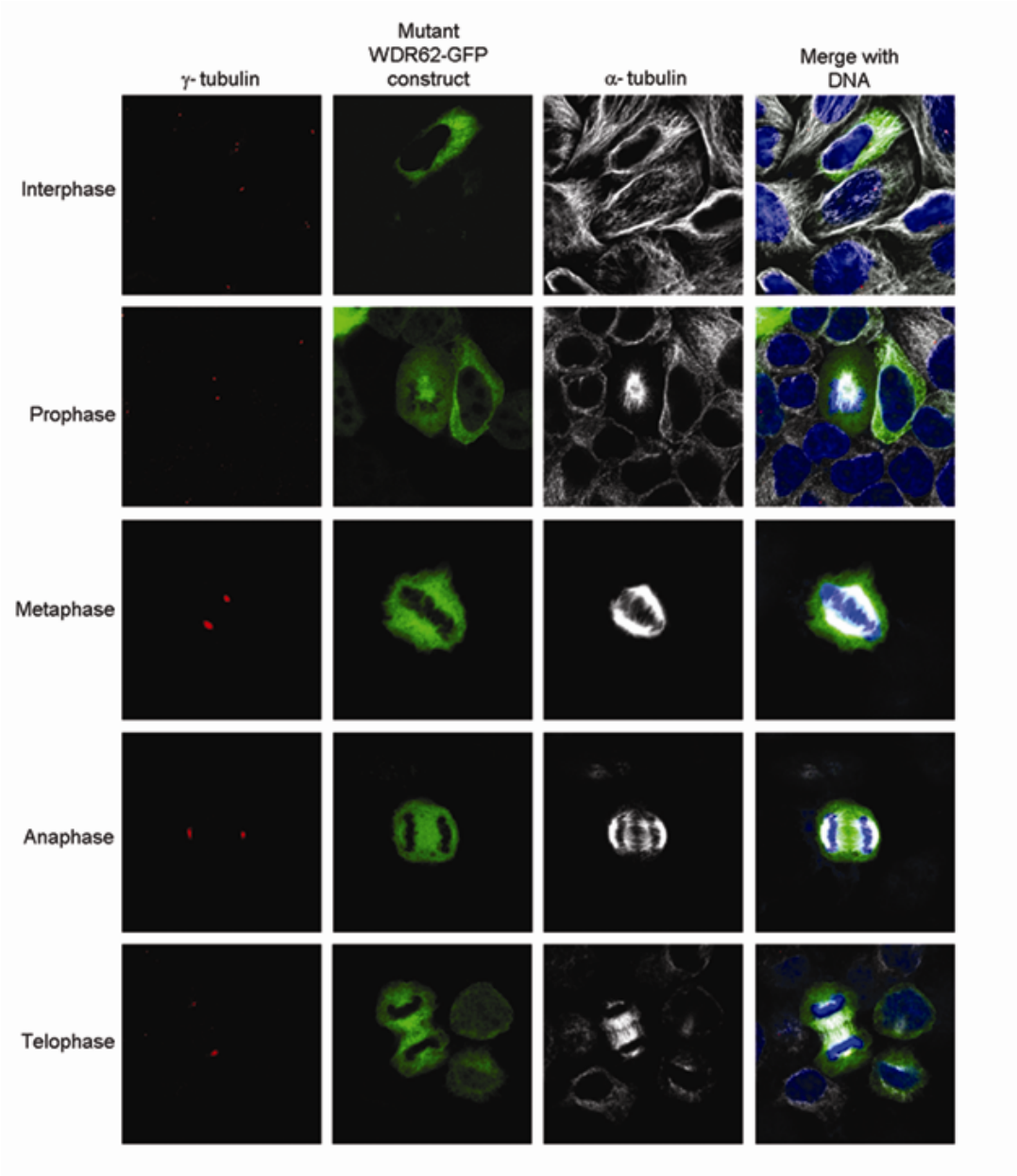
Comparison of endogenous WDR62 and ASPM staining in metaphase HeLa cells.

HeLa cells were stained with ASPM and WDR62 antibodies. Both WDR62 and ASPM antibodies are raised in rabbit so could not be used concurrently. The top panel shows WDR62 (red), and the lower panel ASPM (red). Cells were costained with γ-tubulin (green) to mark centrosomes, α-tubulin (white) to mark the mitotic spindle, with DNA (blue) stained with DAPI. ASPM and WDR62 have an apparently identical subcellular localization at the spindle poles in metaphase. A representative cell in metaphase was picked for each panel of four images, however >50 cells were assessed which gave identical results.



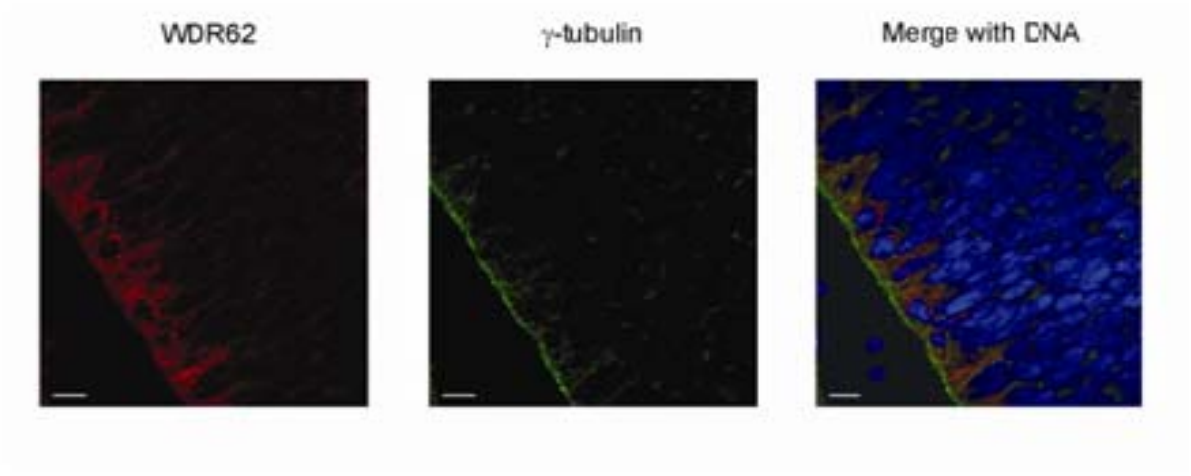
Supplementary Figure 4

Overexpression of WDR62-GFP c.4241dupT mutant construct in HeLa cells. The c.4241dupT/Leu1414LeufsX14 mutation was engineered into a WDR62-GFP construct and expressed in HeLa cells. The 4 by 5 panel shows that the mutant WDR62 does not accumulate at the spindle pole during mitosis (in 50 of 50 cells analyzed). This can be compared to wild type cells in Figure 4. Cells were stained with antibodies against γ-tubulin (red) marking the centrosome, α-tubulin (white) marking the microtubules of the mitotic spindle, with DNA (blue) stained with DAPI. WDR62-GFP protein (green) was directly visualized. Scale bar denotes 5μm.



Supplementary Figure 5

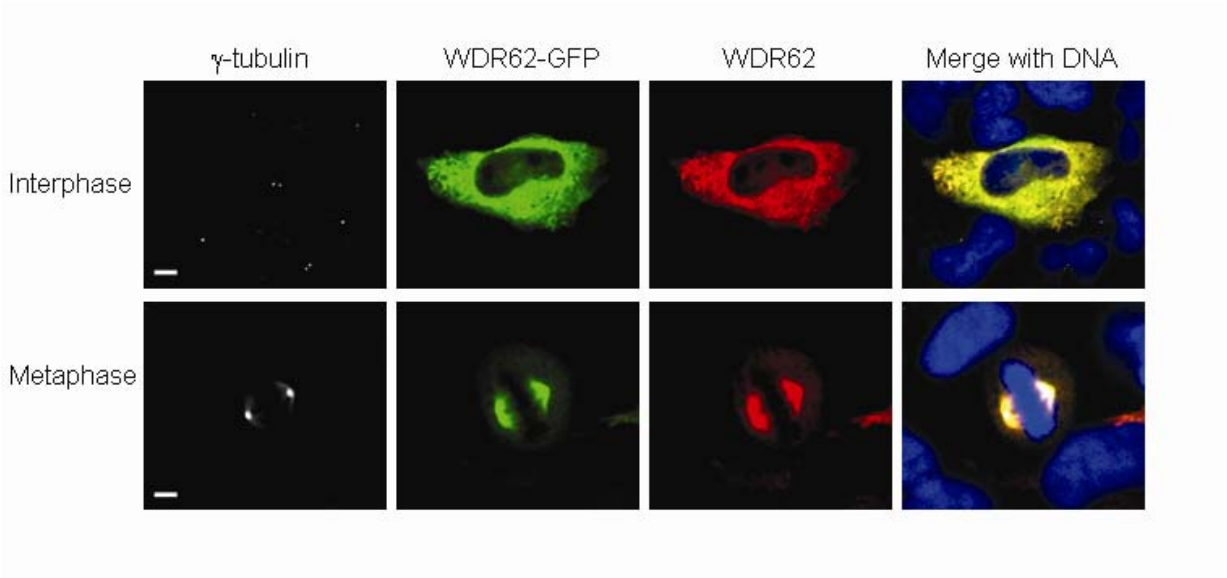
Details of Figure 5c showing human embryonic cerebral cortex neuroepithelium. Figure 5c is a close up of human CS22 neuroepithelium stained with WDR62 (red), γ-tubulin (green) and DAPI (blue). This Supplementary Figure shows the whole confocal microscopy image from which Figure 5 is taken. Here the WDR62 (red) and γ-tubulin (green) antibody staining are shown separately, as well as the composite on the right showing the nuclei stained with DAPI (blue). This image shows full depth of the human embryonic neuroepithelium, the region containing the apical neural precursor cells. WDR62 is localized only at the apical margin and only in some cells (presumably those undergoing mitosis). Scale bars denote 10μm.



Supplementary Figure 6

Immunohistochemistry to assess the specificity of the WDR62 antibody used in this study.

To assess antibody specificity HeLa cells transfected with wild type WDR62-GFP construct were costained with the WDR62 antibody used in this study and an anti-GFP antibody. Representative cells in interphase and metaphase are shown. WDR62 and GFP antibody staining patterns are identical indicating antibody specificity. Cells were stained with antibodies against γ-tubulin (white) to mark centrosomes, GFP (green), WDR62 (red), with DNA (blue) stained with DAPI.



Supplementary Figure 7

Western blot to assess the specificity of the WDR62 antibody used in this study. HeLa cells transfected with the wild type WDR62-GFP construct and untransfected controls were lysed and the cell lysates probed with anti-WDR62 (left blot) and anti-GFP (right blot) antibodies. The untransfected controls lane shows a single band (of the size expected for WDR62) when probed with the WDR62 but not the GFP antibody, corresponding to endogenous WDR62. The WDR62-GFP transfection lysates lane shows a double band when probed with WDR62, the smaller band corresponding to endogenous WDR62, plus a slightly larger band corresponding to the WDR62-GFP fusion protein. When probed with GFP it shows a single prominent band of the size expected for the WDR62-GFP fusion protein. These results demonstrate WDR62 antibody specificity.