

Supplemental Material

Tctex1d2 associates with short-rib polydactyly syndrome proteins and is required for ciliogenesis

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Supplemental Figures

Figure S1. Tctex1d2 shares amino acid similarity to Dynlt1 and is a member of the Tctex1d family of proteins.

Figure S2. Tctex1d2 cell cycle subcellular localization.

Figure S3. Depletion of Tctex1d2 or Wdr60 leads to ciliation defects.

Supplemental Tables

Table S1. Summary of LAP-Tctex1d2 interactors from non-ciliated and ciliated cells identified by mass spectrometry analysis.

A

DYNL1	-----MEDYQAAEETAFAVVD-----EVSNIIVKEAIESAIGNA	33
TCTEX1D2	MATSIGVSFSVSGDGVPEAEKNAGEPENTYILRPVQQRFRPSVVKDCIHAVLKEELANAE	60
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DYNL1	YQHSKVNQWTTNNVEQTLSQLTKLG-KPFKYIVTCVIMQKNGAGLHTASSCFWDSSTDGS	92
TCTEX1D2	YSPEEMPQLTKHLSSENIKDKLKEMGFDRYKMOVVQVIGEQRGEVFMASRCFWDADTDNY	120
	*. :: * *.::: * *.::: * *.::: * *.::: * *.::: *	
DYNL1	CTVRWENKTMKYCIVSAFGLSI- 113	
TCTEX1D2	THDVFMNDSLFCVVAAGCFYY 142	
	: *.::: *:::***	

B

DYNL1	-----MEEYHRRHCEV-----FNAEEAHNIVKECVDGLGGE-----D	34
TCTEX1D2	MATSIGVSFSVSGDGVPEAEKNAGEPENTYILRPVQQRFRPSVVKDCIHAVLKEELANAE	60
	: * ..::..* *: . .***::..** * :	
DYNL1	YNHNNINQWTASIVEQSLTHLVKLG-KAYKYIVTCAVVQKSAYGFHTASSCFWDDTSDGT	93
TCTEX1D2	YSPEEMPQLTKHLSSENIKDKLKEMGFDRYKMOVVQVIGEQRGEVFMASRCFWDADTDNY	120
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DYNL1	CTVRWENRTMNCIVNVFAIAIVL 116	
TCTEX1D2	THDVFMNDSLFCVVAAGCFYY- 142	
	: * : * * * .*	

C

TCTEX1D1	MMMSDNAKGRAAHSWKKRGSISLSNHEFWRKEIHGRIKDSMSTVSMEEPSQRDDISRL	60
TCTEX1D2	-----MATSIGVSFSVSGDGVPEAEKNAGEPEN	27
	: * *::*. . : *::.. . .	
TCTEX1D1	TVQMENTYQLGPPKHFPVTVNHLKDVVTSYLQVEEYEPQLCRMQTKTISEVIKAQVKD	120
TCTEX1D2	TYILRPVQQR----FRPSVVKDCIHAVLKEELANAEYSPEEMPQLTKHLSSENIKDKLKE	83
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TCTEX1D1	LMIPRYKLIVIVHIGQLNRQSILIGSRCLWDPKSDTFSSYVRNSSLFALANVYAVYLE	179
TCTEX1D2	MGFDRYKMOVVQVIGEQRGEVFMASRCFWDADTDNYTHDVFMNDSLFCVVAAGCFYY	142
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TCTEX1D3	MEKRGRGVKSSPIQTPNQTPQAPVTPRKERRPSMFEKEATQILRERLRESIHNQVYVE	60
TCTEX1D2	-----MATSIGVSFSVSGDGVPEAEKNAGEP-----ENTYILR	32
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TCTEX1D3	PPFDDSIADIGKEWKSALAKLKFANSYRMEPLKKFQAHSVETKVQQLTESLKDVKYDDK	120
TCTEX1D2	PVFQQ-----RFRPSVVKDCIHAVLKEELANAEYSPE	64
	* *::: *: * : : * * * . : * . :	
TCTEX1D3	VFSHLSLELADRILLAVKEFGYHYRYKFIKVLFIQKTQAINIASRWIWDIAWDSWVAAK	180
TCTEX1D2	EMPQLTKHLSSENIKDKLKEMGFDRYKMOVVQVIGEQRGEVFMASRCFWDADTDNYTHDV	124
	: : * : . * : * * : * * * : : : : : * : . : * * * * * * * . :	
TCTEX1D3	HEAESYVALVLVFALYYE 198	
TCTEX1D2	FMNDSLFCVVAAGCFYY 142	
	. : * . . : * . : *	

E

TCTEX1D4	MASRLPFGRQEENAKDSGRKPSFVRPRGCLPSIDEARPA GPAPASRRGSLGLAAS	60
TCTEX1D2	-----MATSIGVS	8
	. . . *	
TCTEX1D4	FSRRNSLVGPAGGPGQRPSLGFPVPLGSRVSFSGLPAPARWVAPSRYRTEFPVGERWEA	120
TCTEX1D2	FVSGDGVPEAEKNAGEP-----ENTYILRPVQQRFRP	41
	** : : . . * * : * * * . : : * : * * * : * : *	
TCTEX1D4	ARAQRALEAALAAGLHDACYSSDEAARLVRELCEQVHVRLRELSPPRYKLVCSSVLGPA	180
TCTEX1D2	SVKDCIHAVLKEELANAEYSPEEMPQLTKHLSSENIKDKLKEMGFDRYKMOVVQVIGEQR	101
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TCTEX1D4	GQGVHVVSRAWDVARDGLASVSYNTSLFAVATVHGLYCE 221	
TCTEX1D2	GEVGFMAASRCFWDADTDNYTHDVFMNDSLFCVVAAGCFYY 142	
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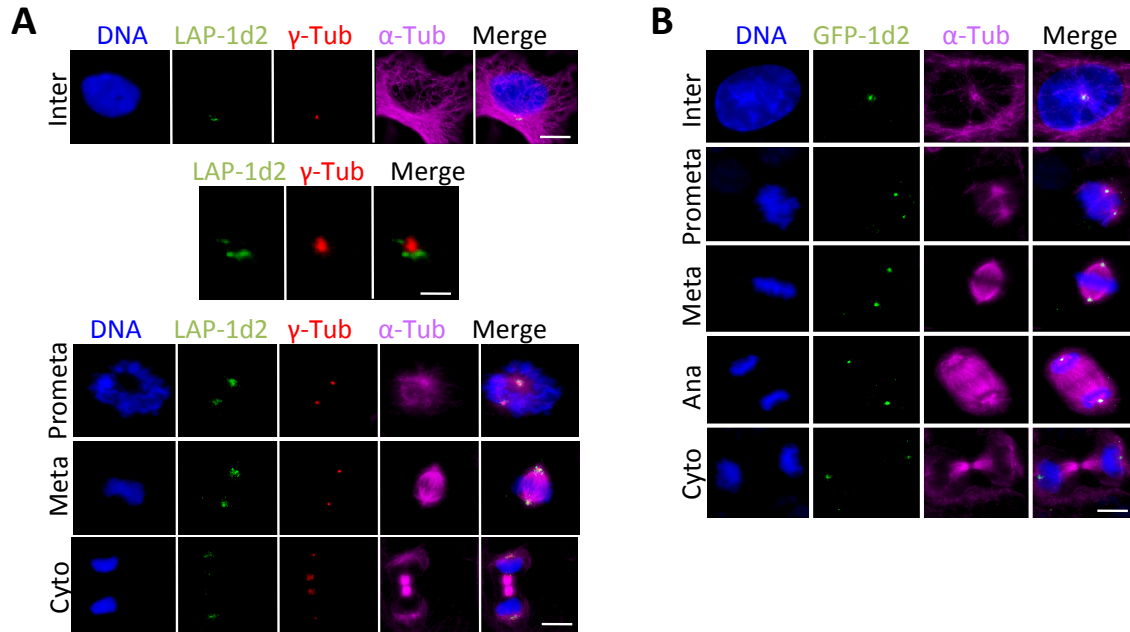


Figure S2. Tctex1d2 cell cycle subcellular localization. (A) Immunofluorescence microscopy of fixed HEK293-LAP-Tctex1d2 stable cells induced to express LAP-Tctex1d2 and stained with Hoechst 33342 DNA dye, and anti- α -tubulin and anti- γ -tubulin antibodies. Images show the cell cycle subcellular localization of LAP-Tctex1d2. Note that LAP-Tctex1d2 localizes to the microtubule organizing center (MTOC) in interphase and the spindle poles during mitosis and cytokinesis. Bar= 5 μ m. Panels in the second row show a zoom view of the MTOC region of interphase cells. Bar= 2 μ m. (B) Immunofluorescence microscopy of HeLa cells transiently transfected with pGLAP1-Tctex1d2. Cells were fixed and stained with Hoechst 33342 DNA dye and anti- α -tubulin and anti- γ -tubulin antibodies. Images show the cell cycle subcellular localization of LAP-Tctex1d2. Note that LAP-Tctex1d2 localizes to the microtubule organizing center (MTOC) in interphase and the spindle poles during mitosis and cytokinesis. Bar= 5 μ m.

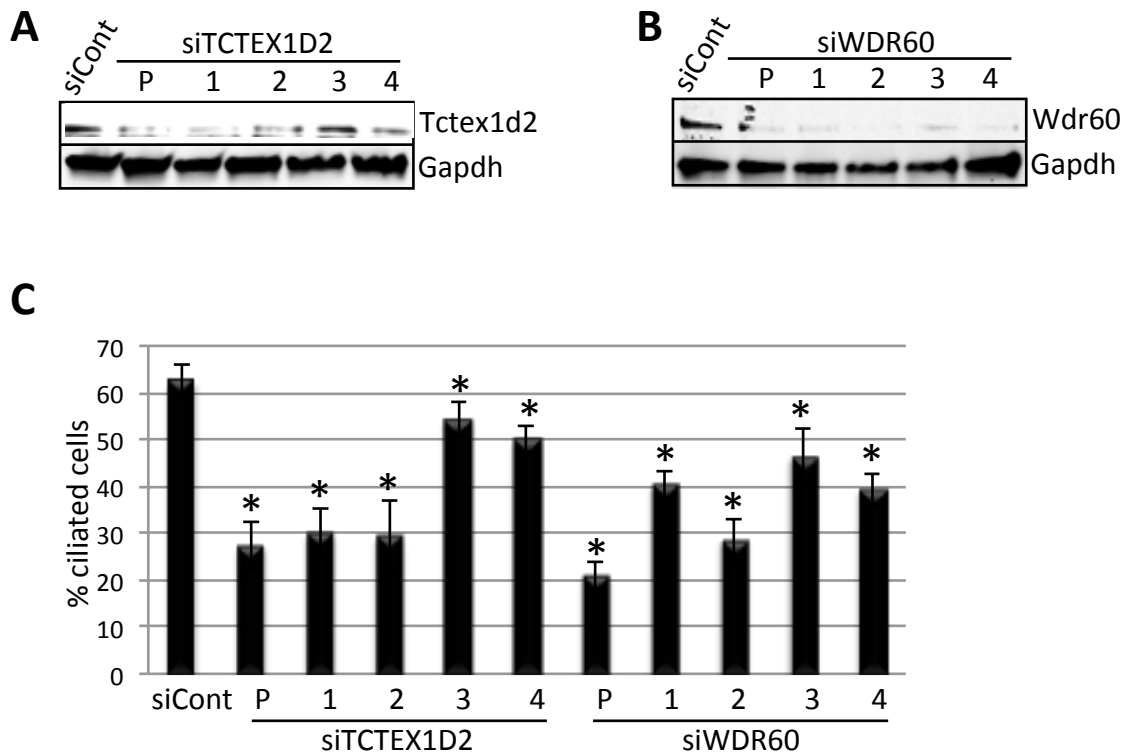


Figure S3. Depletion of Tctex1d2 or Wdr60 leads to ciliation defects. (A and B) siRNA knockdown of Tctex1d2 (A) or Wdr60 (B) protein levels. Immunoblot analysis showing that siRNA pool (P) and individual oligonucleotides (1-4) targeting *TCTEX1D2* (siTCTEX1D2) or *WDR60* (siWDR60) deplete Tctex1d2 or Wdr60 protein levels in hTERT-RPE cells compared to non-targeting control siRNA (siCont). (C) Quantitation of the percentage of ciliated cells siCont, siTCTEX1D2, and siWDR60-treated cells. Data represent the average \pm SD of 3 independent experiments, 100 cells counted for each, asterisks denote p values $< .05$.

Supplemental Tables

Table S1. Summary of LAP-Tctex1d2 interactors from non-ciliated and ciliated cells identified by mass spectrometry analysis. Table summarizes peptides identified in LAP-Tctex1d2 purifications from ciliated and non-ciliated cells by LC-MS/MS, including protein ID, number peptides identified, number of unique peptides, and percent protein coverage.