Supplemental Material

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

Ankur A. Gholkar, Silvia Senese, Yu-Chen Lo, Joseph Capri, William J. Deardorff, Harish Dharmarajan, Ely Contreras, Emmanuelle Hodara, Julian P. Whitelegge, Peter K. Jackson and Jorge Z. Torres

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.

Supplemental Figures

A

```
DYNLT1          -----------------MEDYQAAEETAFVVD----------EVSNIVKEAIESAIGGNA 33
TCTEX1D2        MATSIGVSFSVGDGVPEAEKNAGEPENTYILRPVFQQRFRPSVVKDCIHAVLKEELANAE 60
                                  *.  .  *.::::            *.: :: .::. :..

DYNLT1          YQHSKVNQWTTNVVEQTLSQLTKLG-KPFKYIVTCVIMQKNGAGLHTASSCFWDSSTDGS 92
TCTEX1D2        YSPEEMPQLTKHLSENIKDKLKEMGFDRYKMVVQVVIGEQRGEGVFMASRCFWDADTDNY 120
                *. .:: * *.:: *:  .:*.::* . :* :*  ** ::.* *:. ** ****:.**.

DYNLT1          CTVRWENKTMYCIVSAFGLSI- 113
TCTEX1D2        THDVFMNDSLFCVVAAFGCFYY 142
                    : *.:::*:*:***
```

B

```
DYNLT3          --------------MEEYHRHCDEVG--------FNAEEAHNIVKECVDGVLGGE----D 34
TCTEX1D2        MATSIGVSFSVGDGVPEAEKNAGEPENTYILRPVFQQRFRPSVVKDCIHAVLKEELANAE 60
                               : * .::..*          *: .   .:**:*:..**  *    :

DYNLT3          YNHNNINQWTASIVEQSLTHLVKLG-KAYKYIVTCAVVQKSAYGFHTASSCFWDTTSDGT 93
TCTEX1D2        YSPEEMPQLTKHLSENIKDKLKEMGFDRYKMVVQVVIGEQRGEGVFMASRCFWDADTDNY 120
                *. ::: * *  : *:   :* ::* . ** :*  .: :: . *.. ** ****: :*.

DYNLT3          CTVRWENRTMNCIVNVFAIAIVL 116
TCTEX1D2        THDVFMNDSLFCVVAAFGCFYY- 142
                    : * :: *:* .*.
```

C

```
TCTEX1D1        MMMSDNAKGRAAHSWKKRGSISSLSNHEFWRKEIHGRIKDSMSTVSYMEEPSQRDDISRL 60
TCTEX1D2        --------------------------------MATSIGVSFSVGDGVPEAEKNAGEPEN 27
                                                :   *  *:*. . : *..:. . ..

TCTEX1D1        TVQMENTYQLGPPKHFPVVTVNHILKDVVTSYLQVEEYEPELCRQMTKTISEVIKAQVKD 120
TCTEX1D2        TYILRPVFQQR----FRPSVVKDCIHAVLKEELANAEYSPEEMPQLTKHLSENIKDKLKE 83
                *  :. .:*      *   .*:. :: *:.. *   **.**   *:** :** ** ::*:

TCTEX1D1        LMIPRYKLIVIVHIGQLNRQSILIGSRCLWDPKSDTFSSYVFRNSSLFALANVYAVYLE 179
TCTEX1D2        MGFDRYKMVVQVVIGEQRGEGVFMASRCFWDADTDNYTHDVFMNDSLFCVVAAFGCFYY 142
                : : ***::* * **: . :.:::.***:**..:*.::  ** *.***.:. .:. :
```

D

```
TCTEX1D3        MEKRGRGVKSSPIQTPNQTPQQAPVTPRKERRPSMFEKEAYTQILRERLRESIHNVQYVE 60
TCTEX1D2        --------MATSIGVSFSVGDGVPEAEKNAGEP--------------------ENTYILR 32
                         ::.* .. .. : .* : ::  .*                   .*.  :.

TCTEX1D3        PPFDDSIADIGKEWKSALAKLKFANSYRMEPLKKFQAHSVETKVQQILTESLKDVKYDDK 120
TCTEX1D2        PVFQQ----------------------------RFRPSVVKDCIHAVLKEELANAEYSPE 64
                * *::                             :*:.  *:  :: :*.*.* :.:*. :

TCTEX1D3        VFSHLSLELADRILLAVKEFGYHRYKFIIKVLFIQKTGQAINIASRWIWDIAWDSWVAAK 180
TCTEX1D2        EMPQLTKHLSENIKDKLKEMGFDRYKMVVQVVIGEQRGEGVFMASRCFWDADTDNYTHDV 124
                 :.:*: .*::.*   :**:*:.***::::*:: :: *:.: :*** :**   *.:.

TCTEX1D3        HEAESYVALVLVFALYYE 198
TCTEX1D2        FMNDSLFCVVAAFGCFYY 142
                .  :* ..:* .*. :*
```

E

```
TCTEX1D4        MASRPLPPGRQEEENAKDSGRKPSPVRPRGCLPSIDEARPAGPGPAPASRRGSMLGLAAS 60
TCTEX1D2        ----------------------------------------------------MATSIGVS 8
                                                                     .:..*

TCTEX1D4        FSRRNSLVGPGAGPGGQRPSLGPVPPLGSRVSFSGLPLAPARWVAPSYRTEPVPGERWEA 120
TCTEX1D2        FSVGDGVPEAEKNAGEP---------------------------ENTYILRPVFQQRFRP 41
                **  :.:  .  ..*                             :*  .**  :*:..

TCTEX1D4        ARAQRALEAALAAGLHDACYSSDEAARLVRELCEQVHVRLRELSPPRYKLVCSVVLGPRA 180
TCTEX1D2        SVVKDCIHAVLKEELANAEYSPEEMPQLTKHLSENIKDKLKEMGFDRYKMVVQVVIGEQR 101
                : .: .:.*.*   * :* **.:* .:*.:.*.*::: :*:*:.  ***:* .**:* :

TCTEX1D4        GQGVHVVSRALWDVARDGLASVSYTNTSLFAVATVHGLYCE 221
TCTEX1D2        GEGVFMASRCFWDADTDNYTHDVFMNDSLFCVVAAFGCFYY 142
                *:**.:.**.:**.  *. :   : * ***.*.:..* :
```

Figure S1. Tctex1d2 shares amino acid similarity to Dynlt1 and is a member of the Tctex1d family of proteins. (**A-E)** Amino acid alignment of Tctex1d2 with Dynlt1 (Tctex1) and Tctex1d family members. Amino acid sequences used for alignments were derived from NCBI as follows Dynlt1 (NP_006510.1), Dynlt3 (NP_006511.1), Tctex1d1 (NP_689878.2), Tctex1d2 (Q8WW35.2), Tctex1d3 (NP_777570.1), and Tctex1d4 (NP_001013654.1). “*****” identical match, ":” conserved substitution, "." semi-conserved substitution.

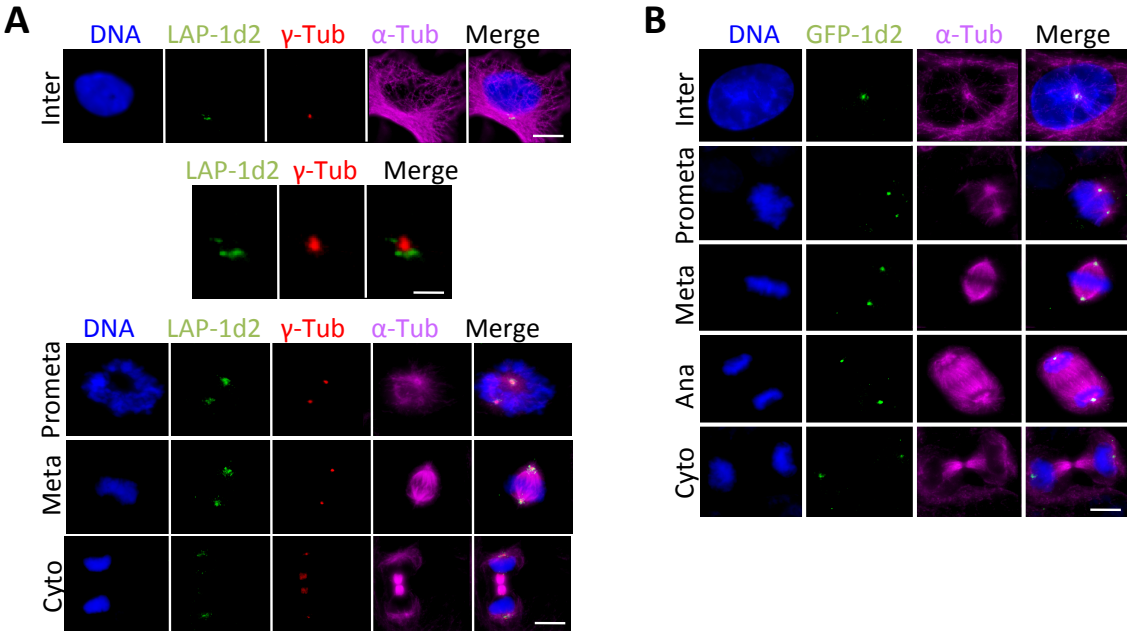

Figure S2. Tctex1d2 cell cycle subcellular localization. (**A**) Immunofluorescence microscopy of fixed HEK293-LAP-Tctex1d2 stable cells induced to express LAP-Tctex1d2 and stained with Hoechst 33342 DNA dye, and anti-α-tubulin and anti-γ-tubulin antibodies. Images show the cell cycle subcellular localization 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= 5μm. Panels in the second row show a zoom view of the MTOC region of interphase cells. Bar= 2μm. (**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= 5μm.

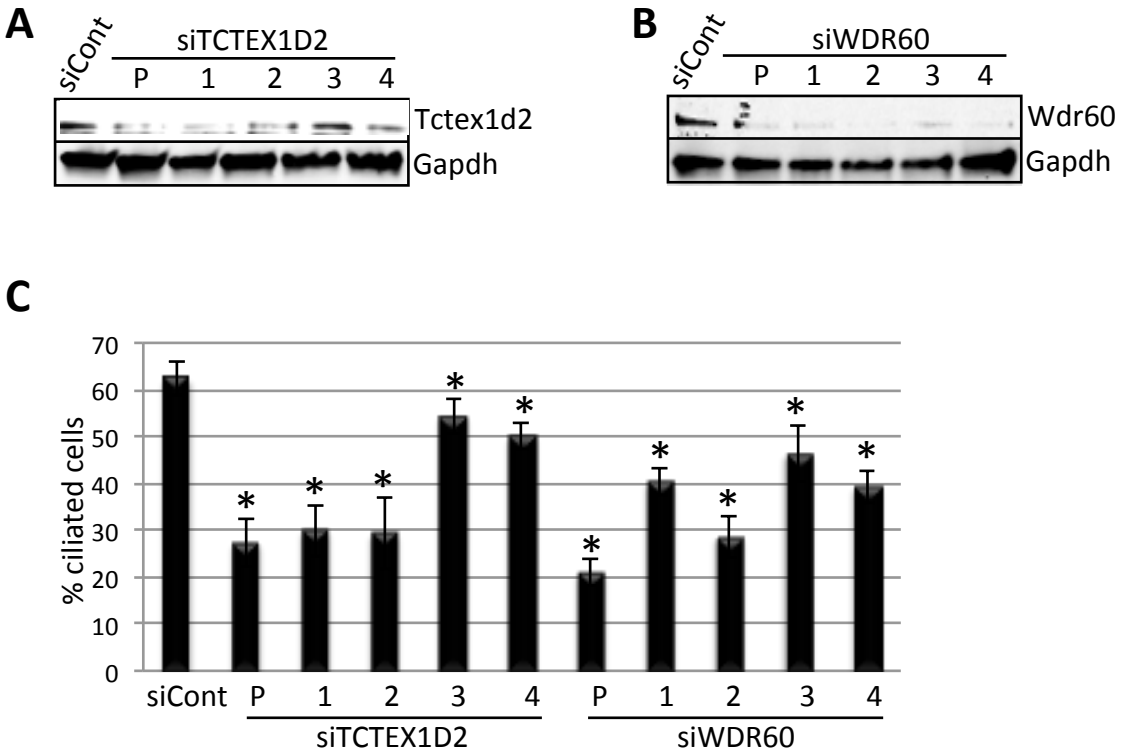

Figure S3. Depletion of Tctex1d2 or Wdr60 leads to ciliation defects. (**A** and **B**) siRNA knockdown of Tctex1d2 (**A**) or Wdr60 (**B**) protein levels. Immunoblot analysis showing that siRNA pool (P) and individual oligonucleotides (1-4) targeting *TCTEX1D2* (siTCTEX1D2) or *WDR60* (siWDR60) deplete 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 ± 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.