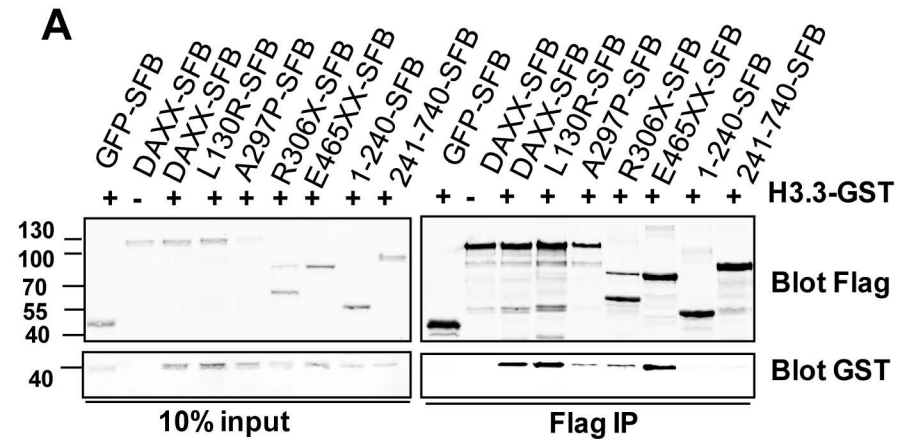
Supplementary Figure Legend

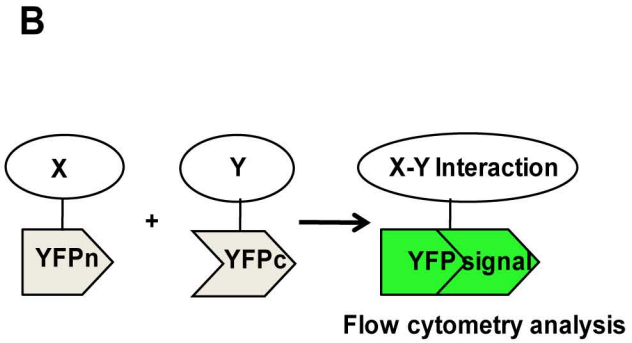
Figure S1. **(A)** 293T cells transiently expressing SFB-tagged wild-type and mutant DAXX or GST-tagged H3.3 were used for immunoprecipitations (IP) with anti-Flag antibodies. The immunoprecipitates were then blotted with the indicated antibodies. **(B)** BiFC principle. **(C)** Cells co-expressing YFPn-DKC1 and various YFPc-tagged proteins were analyzed by flow cytometry for fluorescence complementation. PDK1 and TCAB1 served as negative and positive controls respectively. **(D)** Cells co-expressing YFPn-DKC1 and various YFPc-tagged proteins were analyzed by western blotting. Arrowhead indicates YFPn-tagged DKC1. * indicates various YFPc-tagged proteins.

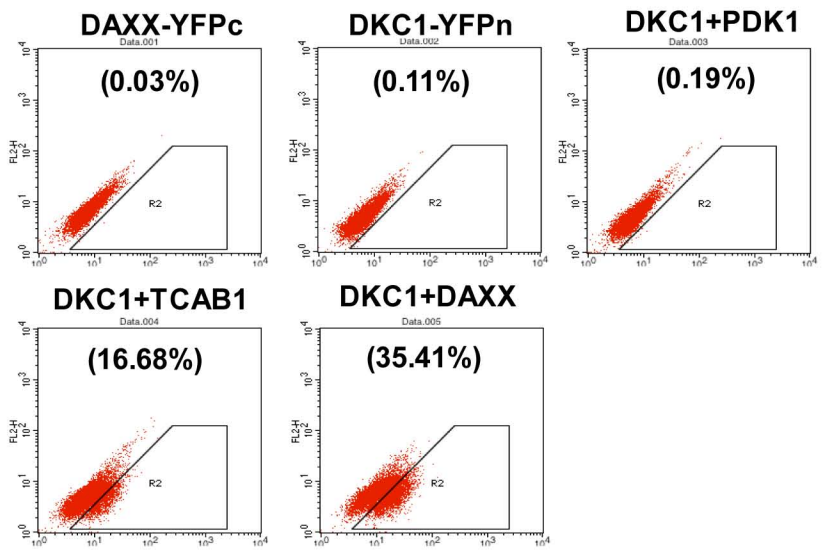
Figure S2. DAXX deletion mutations do not affect the overall telomerase activity. **(A)** 293T cells transiently expressing SFB-tagged wild-type and deletion mutants of DAXX were analyzed by Q-TRAP to assess total telomerase activity. Error bars represent standard error (n=3). **(B)** SFB-tagged wild-type and truncation mutants of DAXX were transiently expressed in 293T cells. DAXX proteins were immunoprecipitated and western blotted using anti-flag antibodies.

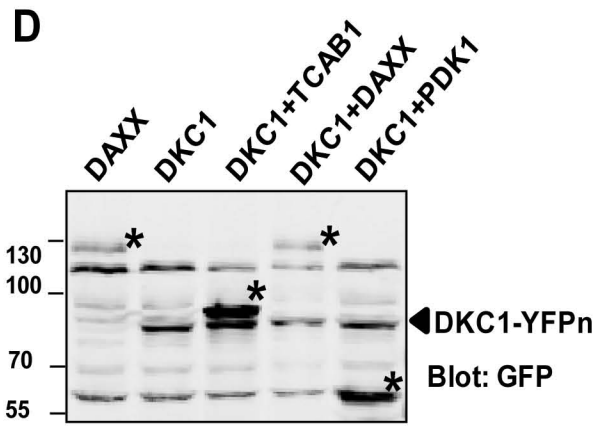
Figure S3. DAXX disease mutations do not affect the overall telomerase activity. **(A)** 293T cells transiently expressing SFB-tagged wild-type and disease mutants of DAXX were analyzed by Q-TRAP to assess total telomerase activity. Error bars represent standard error (n=3). **(B)** SFB-tagged wild-type and disease mutants of DAXX were transiently expressed in 293T cells. DAXX proteins were immunoprecipitated and then western blotted using anti-flag antibodies.

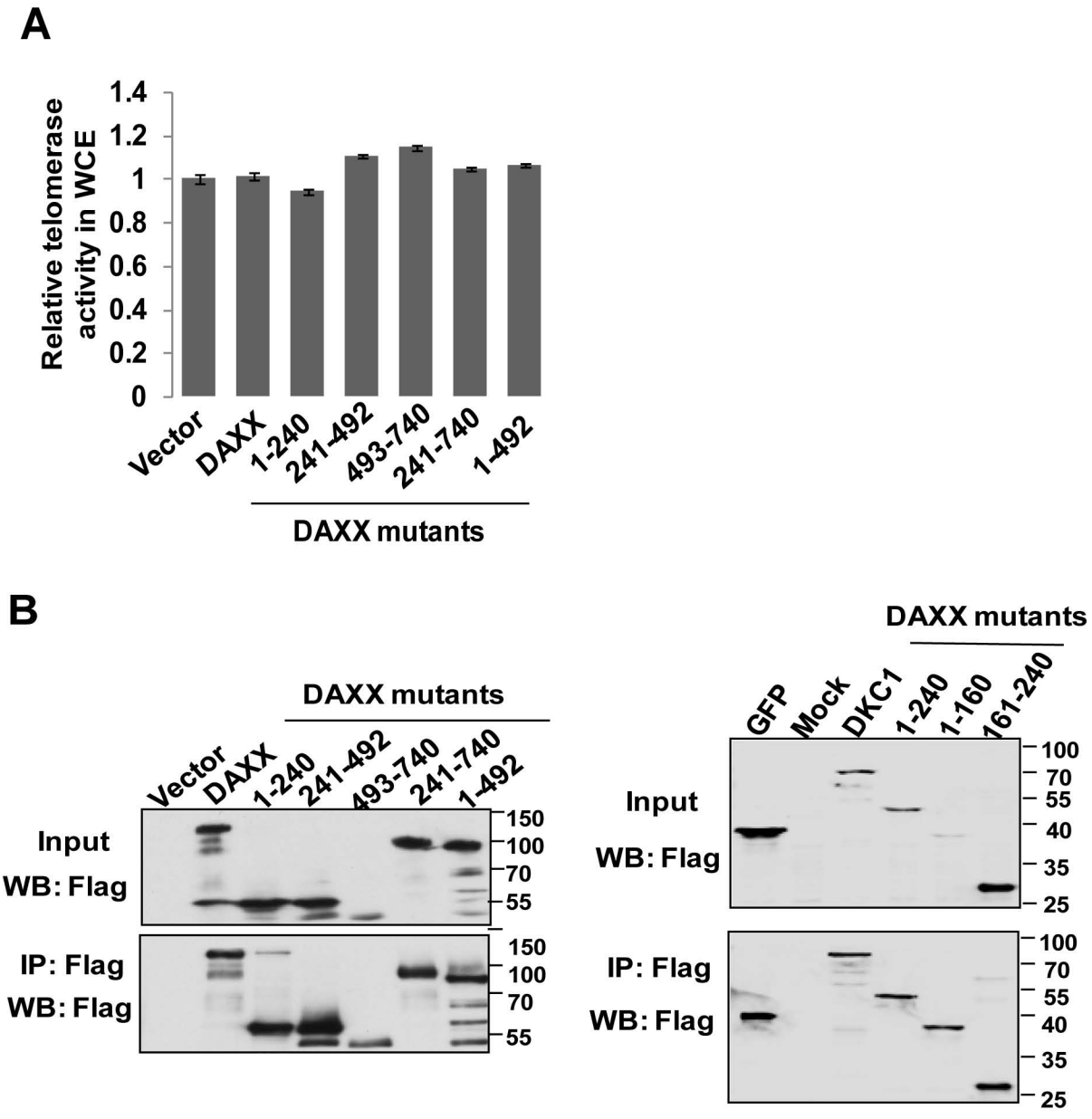
Figure S4. Endogenous DAXX expression would not change in DAXX mutants cells. Endogenous DAXX protein levels in cells used for experiments described for Fig. 1B **(A)**, Fig. 2C **(B)**, Fig. 2D **(C)**, and Fig. 2E **(D)** were examined by western blotting. GAPDH were used as loading control.

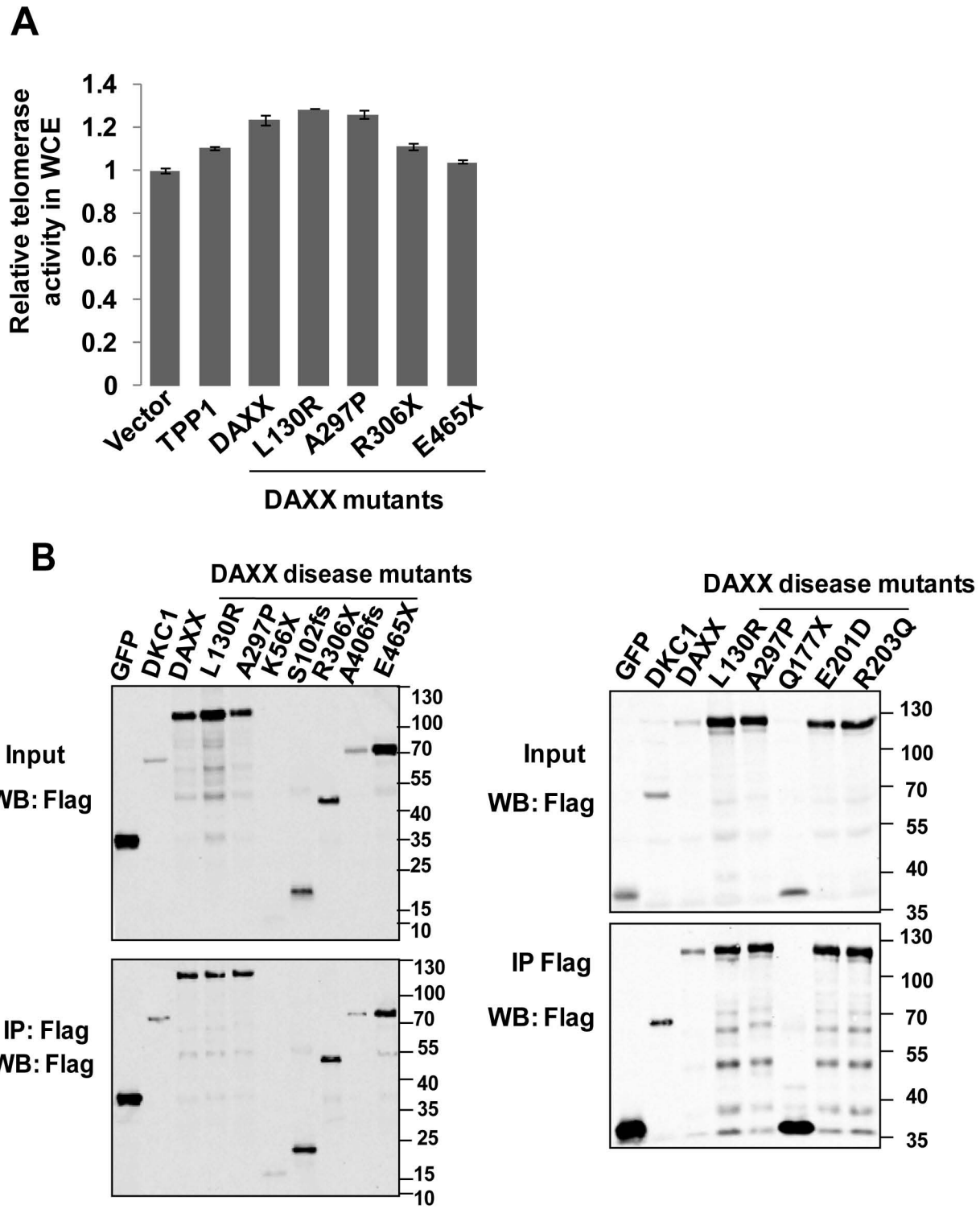

A
GFP-SFB
DAXX-SFB
DAXX-SFB
L130R-SFB
A297P-SFB
R306X-SFB
E465XX-SFB
1-240-SFB
241-740-SFB
GFP-SFB
DAXX-SFB
DAXX-SFB
L130R-SFB
A297P-SFB
R306X-SFB
E465XX-SFB
1-240-SFB
241-740-SFB
H3.3-GST
130
100
70
55
40
Blot Flag
40
Blot GST
10% input
Flag IP

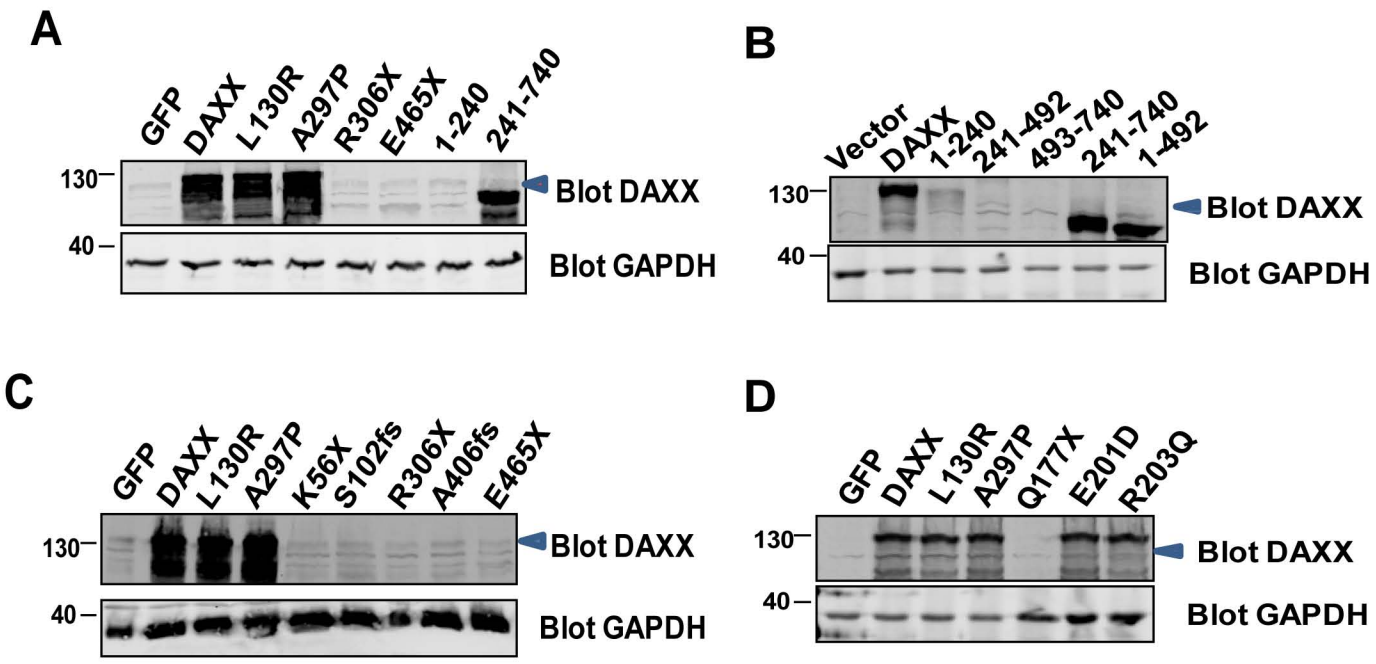

B
X
Y
X-Y Interaction
YFPn
+
YFPc
YFP signal
Flow cytometry analysis


C
DAXX-YFPc
(0.03%)
DKC1-YFPn
(0.11%)
DKC1+PDK1
(0.19%)
DKC1+TCAB1
(16.68%)
DKC1+DAXX
(35.41%)
R2


D
DAXX
DKC1
DKC1+TCAB1
DKC1+DAXX
DKC1+PDK1
130
100
70
55
DKC1-YFPn
Blot: GFP


A
Relative telomerase activity in WCE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Vector
DAXX
1-240
241-492
493-740
241-740
1-492
DAXX mutants
B
DAXX mutants
Vector
DAXX
1-240
241-492
493-740
241-740
1-492
Input
WB: Flag
IP: Flag
WB: Flag
150
100
70
55
DAXX mutants
GFP
Mock
DKC1
1-240
1-160
161-240
Input
WB: Flag
IP: Flag
WB: Flag
100
70
55
40
35
25


A
Relative telomerase activity in WCE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Vector
TPP1
DAXX
L130R
A297P
R306X
E465X
DAXX mutants
B
DAXX disease mutants
GFP
DKC1
DAXX
L130R
A297P
K56X
S102fs
R306X
A406fs
E465X
Input
WB: Flag
IP: Flag
WB: Flag
130
100
70
55
40
35
25
15
10
DAXX disease mutants
GFP
DKC1
DAXX
L130R
A297P
Q177X
E201D
R203Q
Input
WB: Flag
IP Flag
WB: Flag
130
100
70
55
40
35


A
GFP
DAXX
L130R
A297P
R306X
E465X
1-240
241-740
130
40
Blot DAXX
Blot GAPDH
B
Vector
DAXX
1-240
241-492
493-740
241-740
1-492
130
40
Blot DAXX
Blot GAPDH
C
GFP
DAXX
L130R
A297P
K56X
S102fs
R306X
A406fs
E465X
130
40
Blot DAXX
Blot GAPDH
D
GFP
DAXX
L130R
A297P
Q177X
E201D
R203Q
130
40
Blot DAXX
Blot GAPDH

Disease	nucleotide(cDNA)	protein	Mutation type	Function inactivation	Interaction by co-IP	Telomerase activity by IP-TRAP	Localization with Cajal body	Localization with telomere	Telomere length	Telomerase enzyme assay
–	Wildtype	DAXX	None	None	ATRX, H3.3, telomerase	Positive	Positive	Positive	Normal	
Colon Adenocarcinoma	9C>T	T3T	Nonsense	ATRX, H3.3, telomerase activity, Cajal body, telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Colon Adenocarcinoma	42C>T	D14D	Nonsense	ATRX, H3.3, telomerase activity, Cajal body, telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Lung Adenocarcinoma	93G>A	A31A	Nonsense	ATRX, H3.3, telomerase activity, Cajal body, telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	102delT	S33fs	Indel	ATRX,H3.3,telomerase activity,Cajal body, telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	166A>T	K56X	Nonsense	ATRX, H3.3, telomerase activity, Cajal body, telomere	N.D.	Negative	Negative	N.D.	N.D.	N.D.
PanNET	211delC	F70fs	Indel	ATRX, H3.3, telomerase activity,Cajal body, telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	306_309delGGCG	S102fs	Indel	ATRX, H3.3, telomerase activity, Cajal body, telomere	N.D.	Negative	N.D.	N.D.	N.D.	N.D.
PanNET	389T>G	L130R	Missense	ATRX	loss ATRX binding	Positive	Positive	Positive	Normal	Yes

Lung Adenocarcinoma	529C>T	Q177X	Nonsense	Cajal body, telomerase activity， telomere	N.D.	Reduced	N.D.	N.D.	N.D.	N.D.
Uterine Corpus Endometrioid Carcinoma	603G>T	E201D	Missense	H3.3, Cajal body, telomerase activity， telomere	N.D.	Reduced	N.D.	N.D.	N.D.	N.D.
Paediatric GBM	712C>T	R238X	Nonsense	H3.3, Cajal body, telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	889G>C	A297P	Missense	ATRX, H3.3, telomerase activity, Cajal body, telomere	Reduced ATRX, H3.3, telomerase binding	Reduced	Reduced	Reduced	Reduced	Yes
PanNET	c.916C>T	R306X	Nonsense	H3.3, Cajal body, telomere	Reduced H3.3 binding	Positive	Reduced	Reduced	Reduced	N.D.
PanNET	925_926insAGT(hom)	L309Q	Missense	H3.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	1219delG	A406fs	Indel	Cajal body, telomere	N.D.	Positive	Reduced	N.D.	N.D.	N.D.
PanNET	1342G>T(hom)	E448X	Nonsense	telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	1393G>T(hom)	E465X	Nonsense	telomere	Normal	Positive	Positive	Reduced	Reduced	Yes
PanNET	1403delA(hom)	L467fs	Indel	telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	1766delC	S588fs	Indel	telomere	N.D.	Positive	Positive	N.D.	N.D.	N.D.
Paediatric GBM	1885_1886insC	P628fs	Indel	telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	1976delA	K658fs	Indel	telomere	N.D.	Positive	Positive	N.D.	N.D.	N.D.
PanNET	1980delG(hom)	G660fs	Indel	telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PanNET	2042_2043insT	A680fs	Indel	telomere	N.D.	Positive	Positive	N.D.	N.D.	N.D.
PanNET	2120delC	L706fs	Indel	telomere	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Paediatric GBM： Paediatric Glioblastoma multiforme				**Red: experimemtal confirmed**						
PanNET： Pancreatic neuroendocrine tumors				**Blue: predicted**						
N.D.: not determined										

Table S1. A comprehensive list of DAXX mutations found in patients. Wildtype DAXX is shown as reference. DAXX disease mutants were examined for their ability to interact with other proteins, localization, and effect on telomeres. These data are summarized in the last 6 columns. Abbreviations: 1. GBM, Glioblastoma multiforme. PanNET, pancreatic neuroendocrine tumors. 2. Indel, insertion or deletion. 3. del, deletion. ins, insertion. Hom, homozygous. 4. fs, frame shift. X denotes mutation that results in stop codon. 5. Red: experimemtally confirmed. Blue: predicted. 6. N.D., not determined.