Supplemental Material

TIN2 DYSKERATOSIS CONGENITAL MISSENSE MUTANTS ARE DEFECTIVE IN ASSOCIATION WITH TELOMERASE

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Running title: TIN2 DC mutants and telomerase association

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Supplementary Figure S1. TIN2 mutations in DC. *A*. Summary table of TIN2 mutations found in DC patients (1-3). *B*. Sequence conservation across mammalian species in the region of TIN2 frequently mutated in DC patients. The alignment was generated using Jalview (4) and conserved residues are shaded in blue. Arrows indicate residues targeted for mutations.

Supplementary Figure S2. Cell cycle and protein expression analysis of HTC75 cells expressing various TIN2 proteins. *A*. Cycling HTC75 cells expressing indicated TIN2 proteins were harvested, stained with PI, and analyzed by flow cytometry. *B*. Quantification of the relative protein level from Figure 3*A*. The results were normalized to the expression amount of TIN2-FLAG and Actin.

Supplementary Figure S3. DC mutations in TIN2 have minimal impact on its interaction with TPP1 and TRF1. Myc-tagged wild-type (WT) or mutant TIN2 were transiently co-expressed into 293T cells with FLAG-tagged TPP1 (*A)* or TRF1 (*B*). FLAG-tagged TPP1-ΔC22 served as a negative control. FLAG fusion proteins were immunoprecipitated with anti-FLAG beads, and the precipitated proteins were detected by western blotting (WB).

Supplementary Figure S4. TIN2 mutants that were expressed at endogenous levels did not alter TIN2-TPP1 interaction. *A*. Empty vector or TIN2 shRNA (shTIN2) was stably introduced into HT1080 cells. The shTIN2 cells were then used to establish stable cells that also expressed doxycycline (Dox) inducible RNAi-resistant SFB-tagged wild-type or mutant TIN2. Whole-cell extracts from these cells were blotted with the indicated antibodies. *B*. Anti-FLAG immunoprecipitates from cells shown in (*A*) were eluted with 3xFLAG peptides for quantitative immunoblotting. TIN2 protein mixtures were serially diluted to obtain a calibration curve in order to normalize the protein amount in each IP. The ratio of TPP1/SFB-TIN2 was also calculated for each IP.

Supplementary Figure S5. DC mutations in TIN2 led to reduced association with TERT. TAP-TERT and TPP1-FLAG were transiently co-transfected with GST alone, or GST-tagged wild-type or mutant TIN2 into 293T cells. *A*. Whole cell extracts (input) were analyzed by western blotting (WB). *B*. The GST fusion proteins were pulled down with glutathione beads and examined by western blotting. The mixture of precipitated proteins was serially diluted to obtain a calibration curve in order to normalize the protein amount in each pull-down assay. PAP, peroxidase-anti-peroxidase, was used to blot for protein A on the TAP-tag. *C*. Quantification of the relative amount of precipitated proteins in (*B*). The amount of TPP1-FLAG and TAP-TERT was normalized to GST-TIN2 level in the corresponding pull-down.

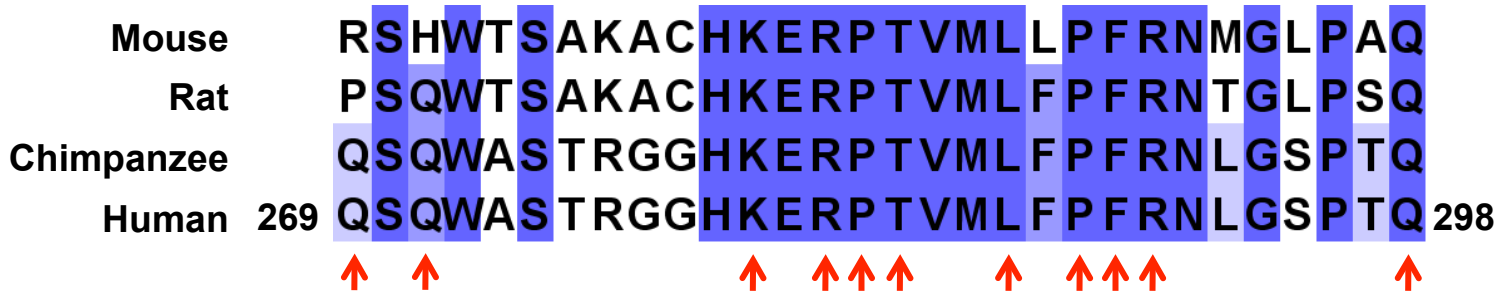
Supplementary Figure S6. TIN2 associates with less telomerase activity upon knockdown of TPP1. shRNAs against GFP or TPP1 were introduced into cells stably expressing Dox-inducible SFB-tagged TIN2. These cells were then treated with Dox, followed by immunoprecipitation with anti-FLAG antibodies. The immunoprecipitates were eluted with 3xFLAG peptides and used in western blotting (*A*) or Q-TRAP for telomerase activity (*B*) as described above. TIN2 protein mixtures were serially diluted to obtain a calibration curve in order to normalize the protein amount in each IP. The results were normalized based on the TIN2 protein amount calculated in (*A*). Error bars represent standard error from triplicates of each experiment. *p*-values were calculated using the Student *t*-test. * indicates $p<0.05$.

1. Walne, A. J., Vulliamy, T. J., Beswick, R., Kirwan, M., and Dokal, I. (2008) *Blood*, 3594-3600
2. Savage, S. A., Giri, N., Baerlocher, G. M., Orr, N., Lansdorp, P. M., and Alter, B. P. (2008) *Am J Hum Genet* **82**, 501-509
3. Sasa, G., Ribes-Zamora, A., Nelson, N., and Bertuch, A. (2011) *Clin Genet*
4. Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., and Barton, G. J. (2009) *Bioinformatics* **25**, 1189-1191

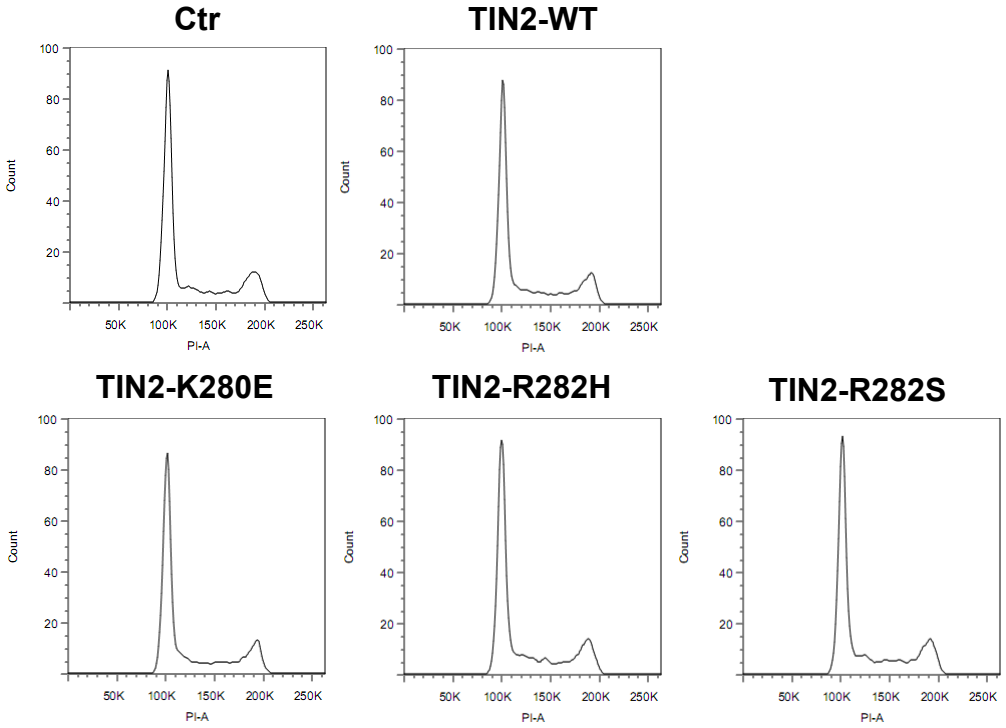
A

TIN2 mutations	Reported cases	TIN2 mutations	Reported cases
Q269X	1	P283H	1
Q271X	1	P283S	1
K280RfsX36	1	T284A	1
K280X	1	T284HfsX8	1
K280E	6	L287P	1
R282C	7	P289S	1
R282H	15	F290LfsX2	1
R282S	1	R291G	1
P283A	1	Q298RfsX19	1
Total	43		

B

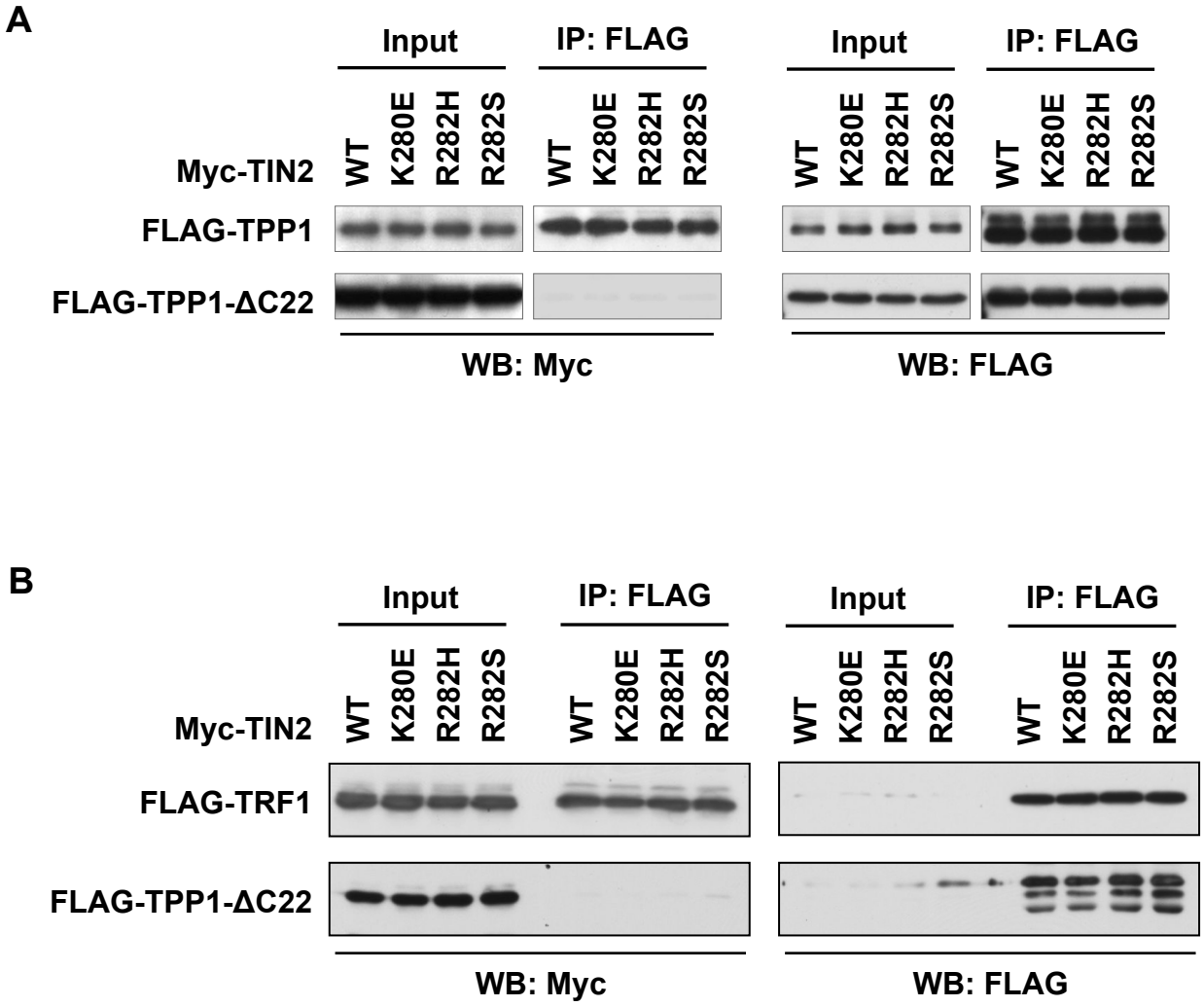


A

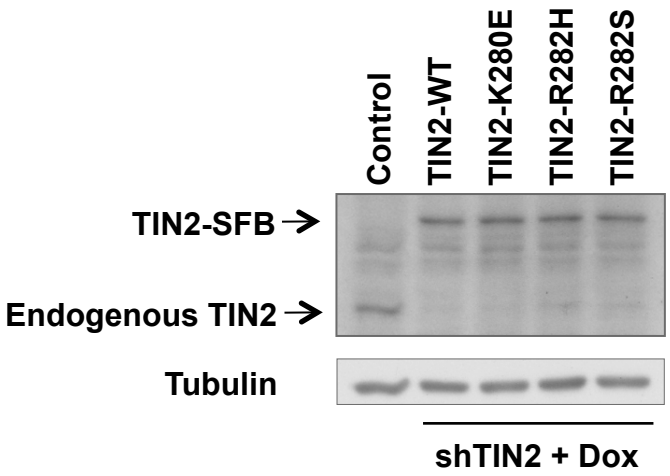


B

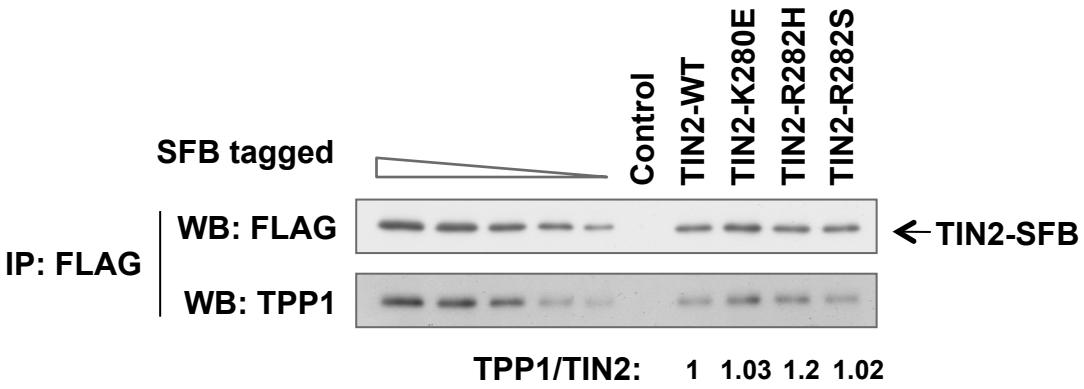
Relative Protein Levels (PD65)				
Cell line	TIN2-WT	TIN2-K280E	TIN2-R282H	TIN2-R282S
TRF1	1	1.05	1.05	1.19
TRF2	1	1.05	1.15	0.93
TPP1	1	1.09	1.06	1.08
RAP1	1	1.35	1.10	0.95

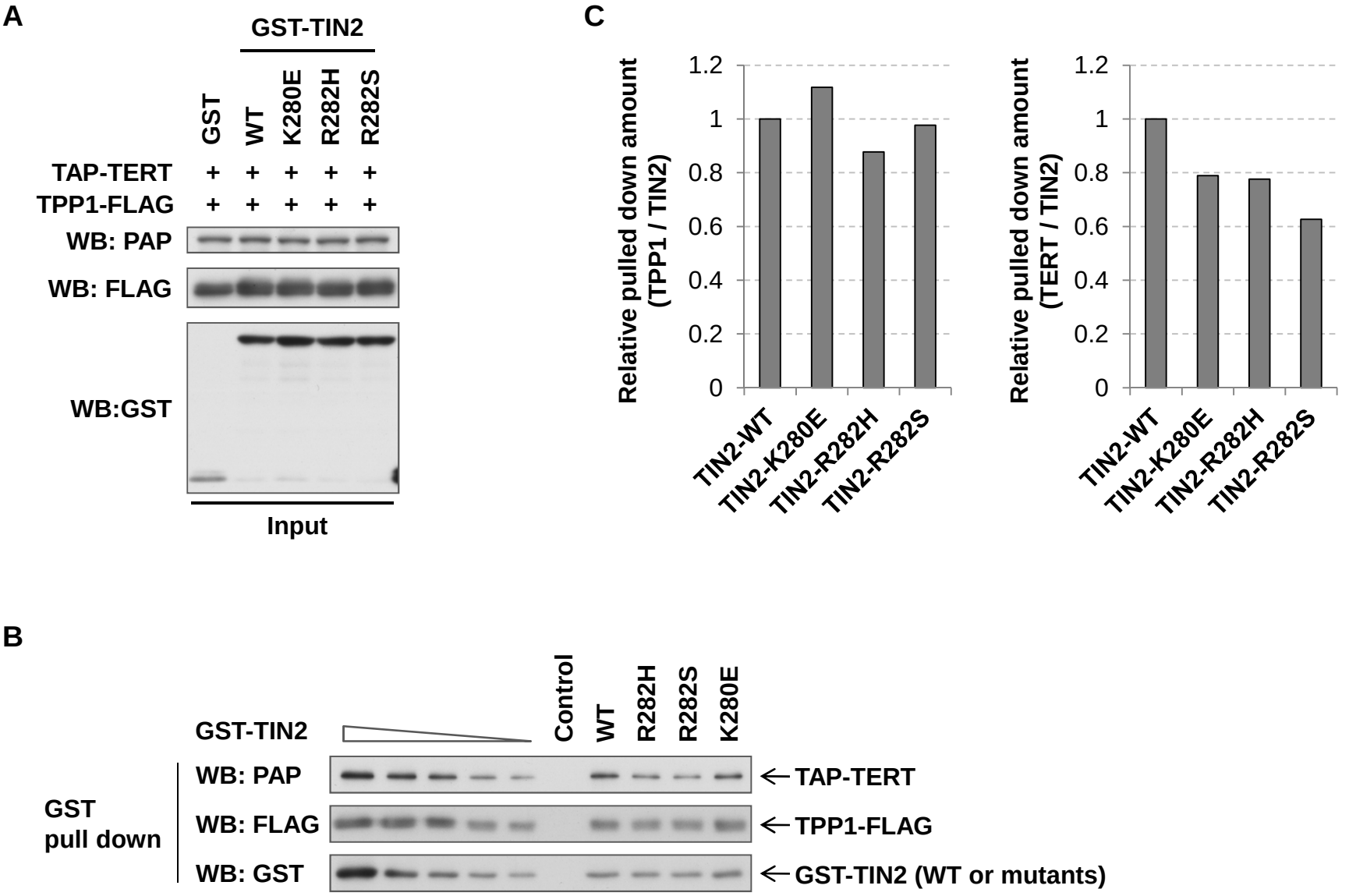

A
Input
IP: FLAG
Input
IP: FLAG
WT
K280E
R282H
R282S
Myc-TIN2
FLAG-TPP1
FLAG-TPP1-ΔC22
WB: Myc
WB: FLAG
B
Input
IP: FLAG
Input
IP: FLAG
Myc-TIN2
FLAG-TRF1
FLAG-TPP1-ΔC22
WB: Myc
WB: FLAG

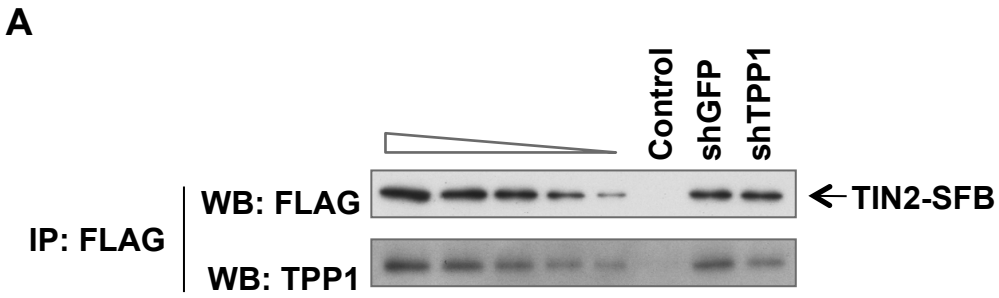
A

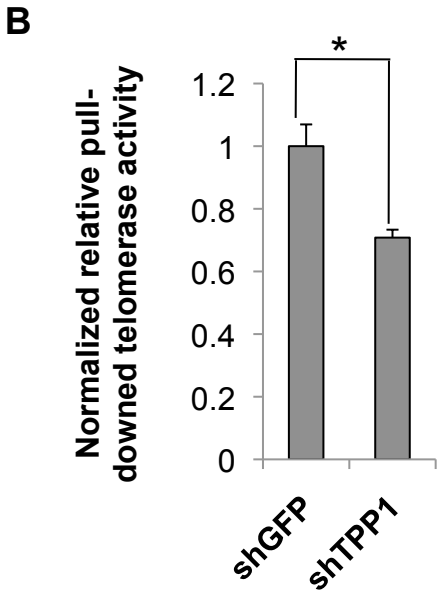

Control
TIN2-WT
TIN2-K280E
TIN2-R282H
TIN2-R282S
TIN2-SFB
Endogenous TIN2
Tubulin
shTIN2 + Dox

B


SFB tagged
Control
TIN2-WT
TIN2-K280E
TIN2-R282H
TIN2-R282S
IP: FLAG
WB: FLAG
WB: TPP1
TIN2-SFB
TPP1/TIN2:
1
1.03
1.2
1.02


A
GST-TIN2
GST
WT
K280E
R282H
R282S
TAP-TERT + + + + +
TPP1-FLAG + + + + +
WB: PAP
WB: FLAG
WB:GST
Input
C
Relative pulled down amount (TPP1 / TIN2)
1.2
1
0.8
0.6
0.4
0.2
0
TIN2-WT
TIN2-K280E
TIN2-R282H
TIN2-R282S
Relative pulled down amount (TERT / TIN2)
1.2
1
0.8
0.6
0.4
0.2
0
TIN2-WT
TIN2-K280E
TIN2-R282H
TIN2-R282S
B
GST-TIN2
Control
WT
R282H
R282S
K280E
GST pull down
WB: PAP
WB: FLAG
WB: GST
TAP-TERT
TPP1-FLAG
GST-TIN2 (WT or mutants)


A
Control
shGFP
shTPP1
IP: FLAG
WB: FLAG
WB: TPP1
TIN2-SFB


B
*
Normalized relative pull-downed telomerase activity
1.2
1
0.8
0.6
0.4
0.2
0
shGFP
shTPP1