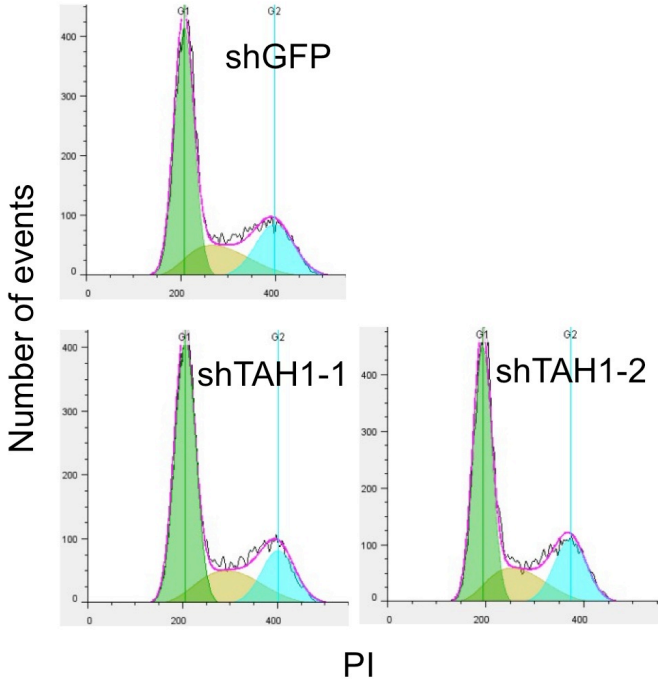
Fig. S1. TAH1 knockdown does not affect cell cycle progression. (**A**) FACS analysis of cell cycle profiles of control (GFP knockdown) and TAH1 knockdown cells. (**B**) Quantification of data from (A).

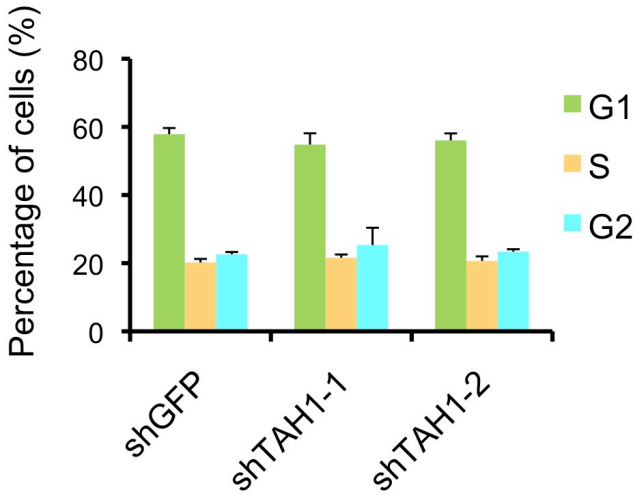
Fig. S2. SMC5 knockdown reduces c-circle information. (**A**) Knockdown efficiency of SMC5 siRNA (si-SMC5) was assessed by quantitative real-time PCR. (**B**) Varying amount of genomic DNA from control and SMC5 knockdown U2OS cells was used for CC assays. HTC75 cells were used as negative controls. (**C**) Quantification of data from (B).

Fig. S3. TRF2 knockdown leads to increased telomere-dysfunction-induced foci (TIF) in ALT cells. (**A**) Knockdown efficiency of TRF2 shRNA (shTRF2) was assessed by western blotting. shRNA against GFP was used as a negative control. (**B**) Representative images of TIF analysis in control and TRF2 knockdown U2OS cells using anti-53BP1 antibodies (red) and TeloC-FITC probes (green). Arrows indicate superimposable foci. (**C**) 53BP1 and telomere co-staining foci (TIFs) were scored for individual cells in each cell line and graphed. Blue lines mark the average number of TIFs per cell. Error bars indicate standard errors (n=3). ***, P<0.001. (**D**) Percentages of TIF-positive cells were calculated based on data from (B) and (C). Cells with ≥3 co-localization foci were scored as TIF positive. Error bars indicate standard errors (n=3). **, P<0.01.

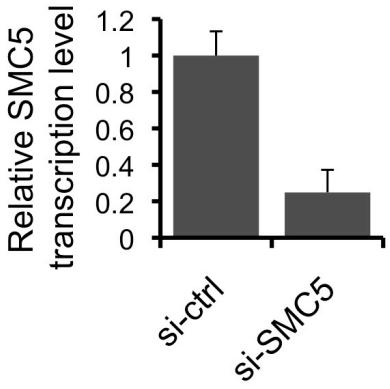
A


shGFP
shTAH1-1
shTAH1-2
Number of events
PI

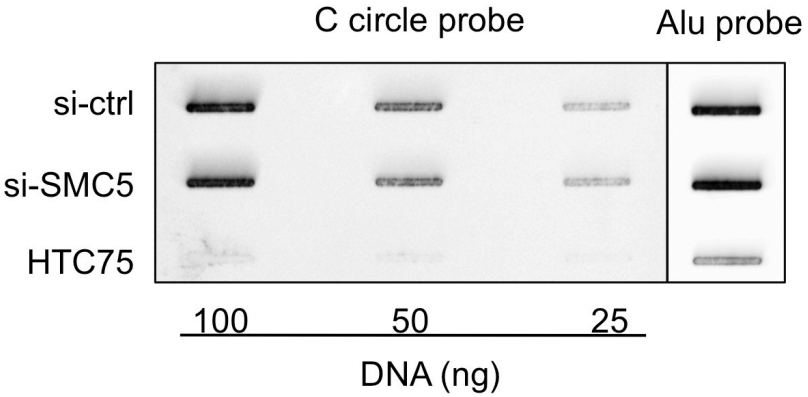
B


Percentage of cells (%)
80
60
40
20
0
G1
S
G2
shGFP
shTAH1-1
shTAH1-2

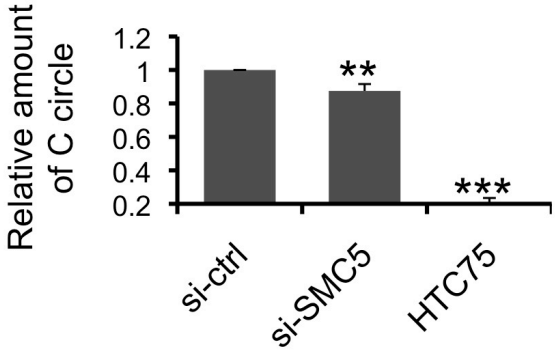
A

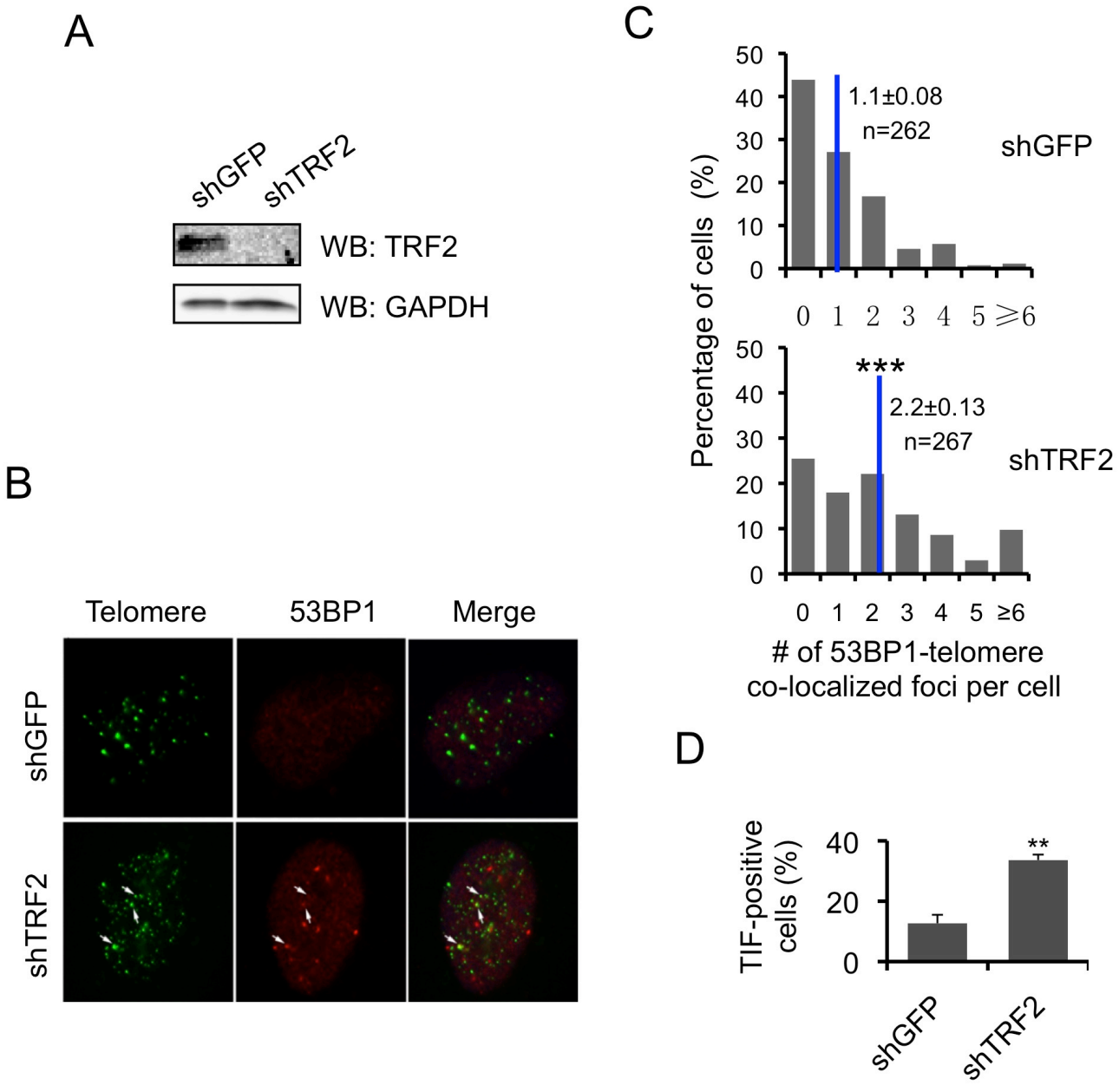

Relative SMC5 transcription level
1.2
1
0.8
0.6
0.4
0.2
0
si-ctrl
si-SMC5

B


C circle probe
Alu probe
si-ctrl
si-SMC5
HTC75
100
50
25
DNA (ng)

C


Relative amount of C circle
1.2
1
0.8
0.6
0.4
0.2
**
***
si-ctrl
si-SMC5
HTC75


A
shGFP
shTRF2
WB: TRF2
WB: GAPDH
B
Telomere
53BP1
Merge
shGFP
shTRF2
C
Percentage of cells (%)
1.1±0.08
n=262
shGFP
***
2.2±0.13
n=267
shTRF2
# of 53BP1-telomere
co-localized foci per cell
D
TIF-positive
cells (%)
**
shGFP
shTRF2