Expanded View Figures

Figure EV1. PIDD1 is recruited to the centriole distal appendage.

A Schematic outlining the competition growth assay used to measure fold change in the number of GFP+ cells treated with doxycycline compared to control, untreated GFP− cells.

B Graph showing the relative growth of doxycycline-treated PLK4^{Dox}; *TRIM37*$^{-/-}$ cells expressing an sgRNA targeting the indicated genes. Each dot displays measurements from a single experiment. Experiments were performed in polyclonal knockout cells. Data acquired across $n \geq 3$ biological replicates. Mean ± s.e.m.

C Representative images of PLK4^{Dox} RPE1 cells treated with and without doxycycline for two days and immunostained with the indicated antibodies. Scale bar = 5 μm.

D Representative images of wild-type and *PIDD1*$^{-/-}$ PLK4^{Dox} RPE1 cells treated with and without doxycycline for two days and immunostained with the indicated antibodies. Scale bar = 5 μm.

E Representative images of PLK4^{Dox} *PIDD1*-mNeonGreen DLD1 cells treated with and without doxycycline for 2 days and immunostained with the indicated antibodies. Scale bar = 5 μm.

F Representative images of *WT* or knockout PLK4^{Dox} cells immunostained with the indicated antibodies. Experiments were performed in PLK4^{Dox} monoclonal knockout cells. Scale bar = 5 μm.

G Quantification of the fraction of cells with the indicated protein localized at the centriole. Experiments were performed in PLK4^{Dox} monoclonal knockout cells. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

H Representative images of *WT* or knockout PLK4^{Dox} cells immunostained with the indicated antibodies. Experiments were performed in PLK4^{Dox} monoclonal knockout cells. Scale bar = 5 μm.

Data information: Asterisks indicate statistically significant differences between measurements (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, ****$P < 0.0001$). Statistics for all Figures were calculated using a two-tailed Student's *t*-test.
Source data are available online for this figure.

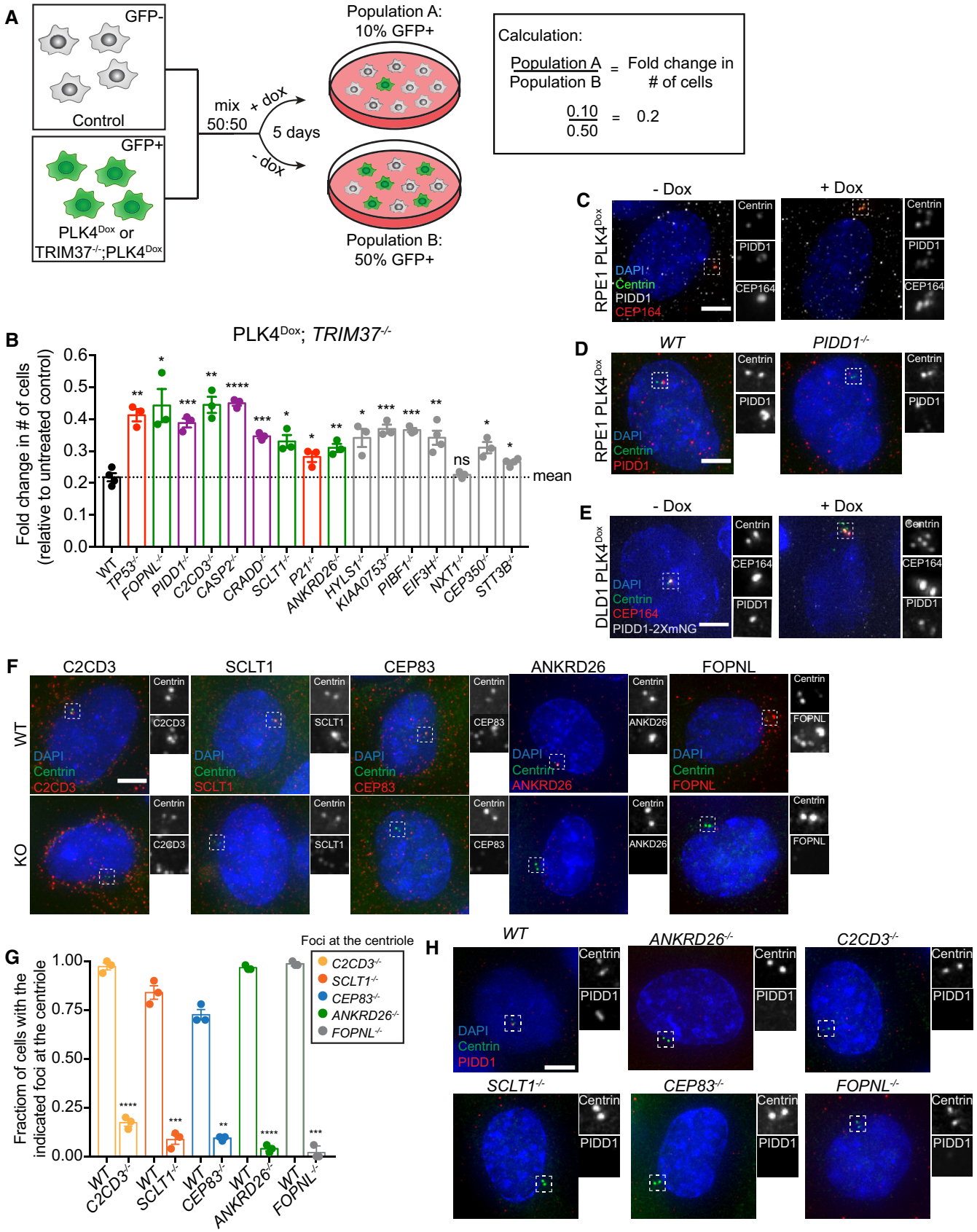

Figure EV1.

Figure EV2. ANKRD26 is not required for the assembly of functional centrosomes.

A Representative images of monoclonal PLK4^{Dox} knockout cells. Cells were stained with the indicated antibodies. Scale bar = 5 μm.

B Quantification of the fraction of cells with the indicated mitotic spindle orientation following treatment with the centrosome declustering agent griseofulvin. Experiments were performed in monoclonal PLK4^{Dox} knockout cells. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

C Representative images of PLK4Dox cells treated with and without doxycycline for two days then treated with griseofulvin for 24 h. Experiments were performed in monoclonal PLK4^{Dox} knockout cells. Cells were immunostained with indicated antibodies. Scale bar = 5 μm.

D Graph showing the relative growth of doxycycline-treated SAS6^{Dox} cells that were knocked out for the indicated genes. Experiments were performed in SAS6^{Dox} monoclonal knockout cells. Each dot displays measurements from a single experiment. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

E Quantification of centrosome number in SAS6^{Dox} cells expressing an sgRNA targeting the indicated genes. Experiments were performed in SAS6^{Dox} monoclonal knockout cells. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

F Quantification of the fold change in cycling cells with a DNA content > 4N following cytokinesis failure. Experiments were performed in PLK4^{Dox} monoclonal knockout cells. Each dot displays measurements from a single experiment. Data acquired across $n = 4$ biological replicates. Mean ± s.e.m.

G Schematic showing the treatment regime for the cytokinesis failure assay shown in (F). Cells were treated with cytochalasin B for 24 h to induce cytokinesis failure followed by treatment with EdU and DMN to mark S-phase cells and block progression through mitosis. The fraction of EdU+ cells with a DNA content > 4N was measured using flow cytometry.

Data information: Asterisks indicate statistically significant differences between measurements (*$P < 0.05$; ***$P < 0.001$, ****$P < 0.0001$). Statistics for all Figures were calculated using a two-tailed Student's *t*-test.
Source data are available online for this figure.

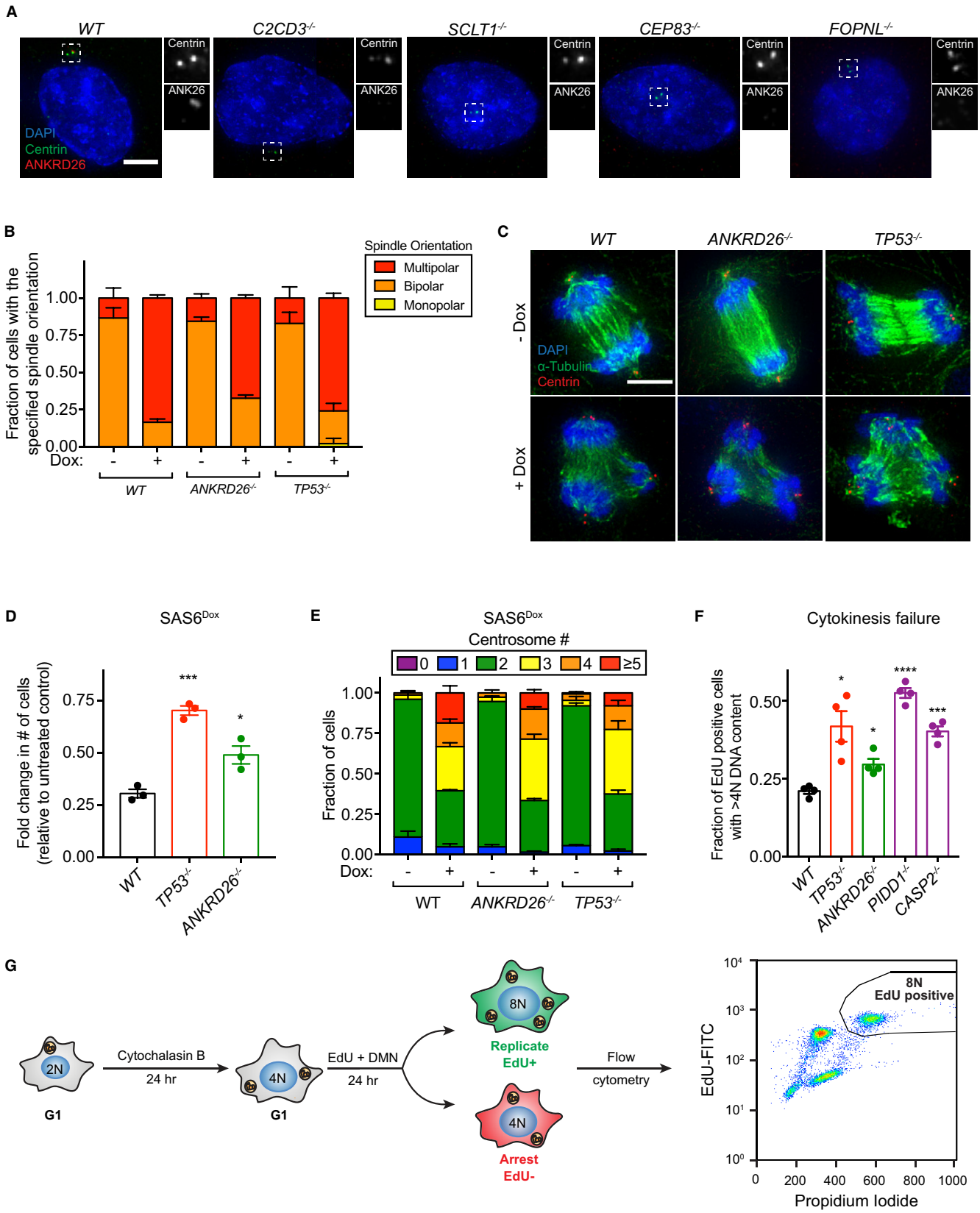

Figure EV2.

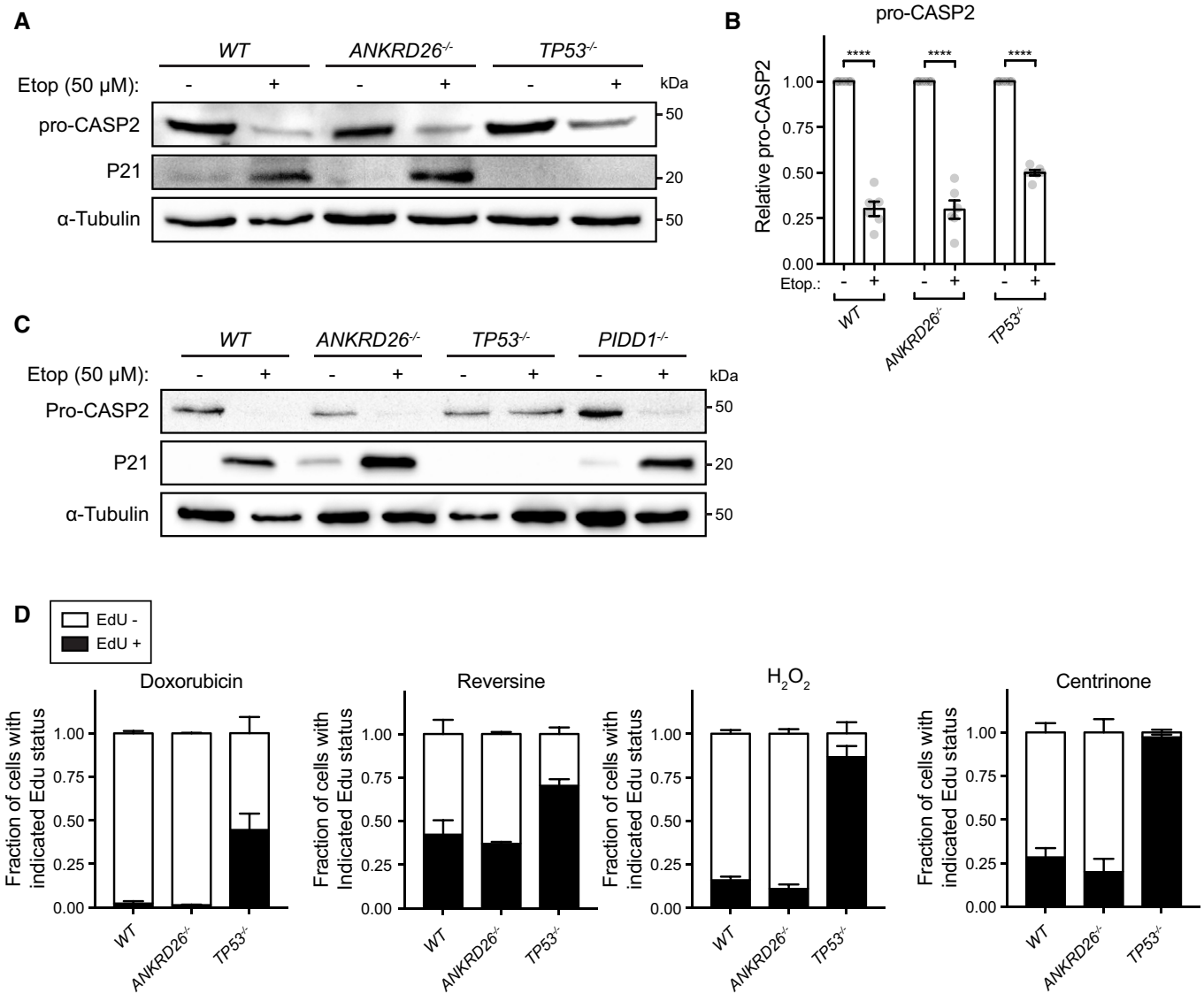

Figure EV3. ANKRD26 is not required to activate the PIDDosome in response to DNA damage.

A Western blot showing expression of pro-CASP2 and P21 following treatment with etoposide. Experiments were performed in monoclonal PLK4^{Dox} knockout cells.

B Quantification of pro-CASP2 levels following treatment with etoposide. Experiments were performed in monoclonal PLK4^{Dox} knockout cells. Each dot displays measurements from a single experiment. Data acquired across $n = 6$ biological replicates. Mean ± s.e.m.

C Western blot showing expression of pro-CASP2 and P21 following treatment with etoposide. Experiments were performed in PLK4^{Dox} cells knocked out for the indicated genes.

D Quantification of the fraction of proliferating cells following treatment with the indicated drugs/reagents. Experiments were performed in monoclonal PLK4^{Dox} knockout cells. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

Data information: Asterisks indicate statistically significant differences between measurements (****$P < 0.0001$). Statistics for all Figures were calculated using a two-tailed Student's *t*-test.
Source data are available online for this figure.

Figure EV4. An ANKRD26$^{\Delta M2}$ mutant fails to recruit PIDD1 to the distal appendage.

A Schematic representation of wild-type ANKRD26 and various mutants.

B Quantification of the fraction of cells with ANKRD26 localized to the mature mother centriole in monoclonal *ANKRD26*$^{-/-}$ PLK4^{Dox} cells expressing the indicated mCherry-ANKRD26 transgene. Each dot displays measurements from a single experiment. Data acquired across $n \geq 3$ biological replicates. Mean ± s.e.m.

C Quantification of the fraction of cells with PIDD1 localized to the mature mother centriole in monoclonal *ANKRD26*$^{-/-}$ PLK4^{Dox} cells expressing the indicated mCherry-ANKRD26 transgene. Each dot displays measurements from a single experiment. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

D Representative images of monoclonal *ANKRD26*$^{-/-}$ PLK4^{Dox} cells expressing the indicated mCherry-ANKRD26 transgene. Cells were immunostained with indicated antibodies. Scale bar = 5 μm.

E Schematic of the co-immunoprecipitation procedure performed in (F).

F HEK293FT cells were transfected with the indicated constructs. Cell lysates were split and subjected to co-immunoprecipitation with mCherry or GFP binder beads.

G Quantification of the fraction of cells with PIDD1 localized to the mature mother centriole in monoclonal *PIDD1*$^{-/-}$ PLK4^{Dox} cells expressing the indicated PIDD1 transgene. Each dot displays measurements from a single experiment. Data acquired across $n = 3$ biological replicates. Mean ± s.e.m.

H Representative images of monoclonal *PIDD1*$^{-/-}$ PLK4^{Dox} cells expressing the indicated PIDD1 transgene. Cells were immunostained with indicated antibodies. Scale bar = 5 μm.

Data information: Asterisks indicate statistically significant differences between measurements ($^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$). Statistics for all Figures were calculated using a two-tailed Student's *t*-test.

Source data are available online for this figure.

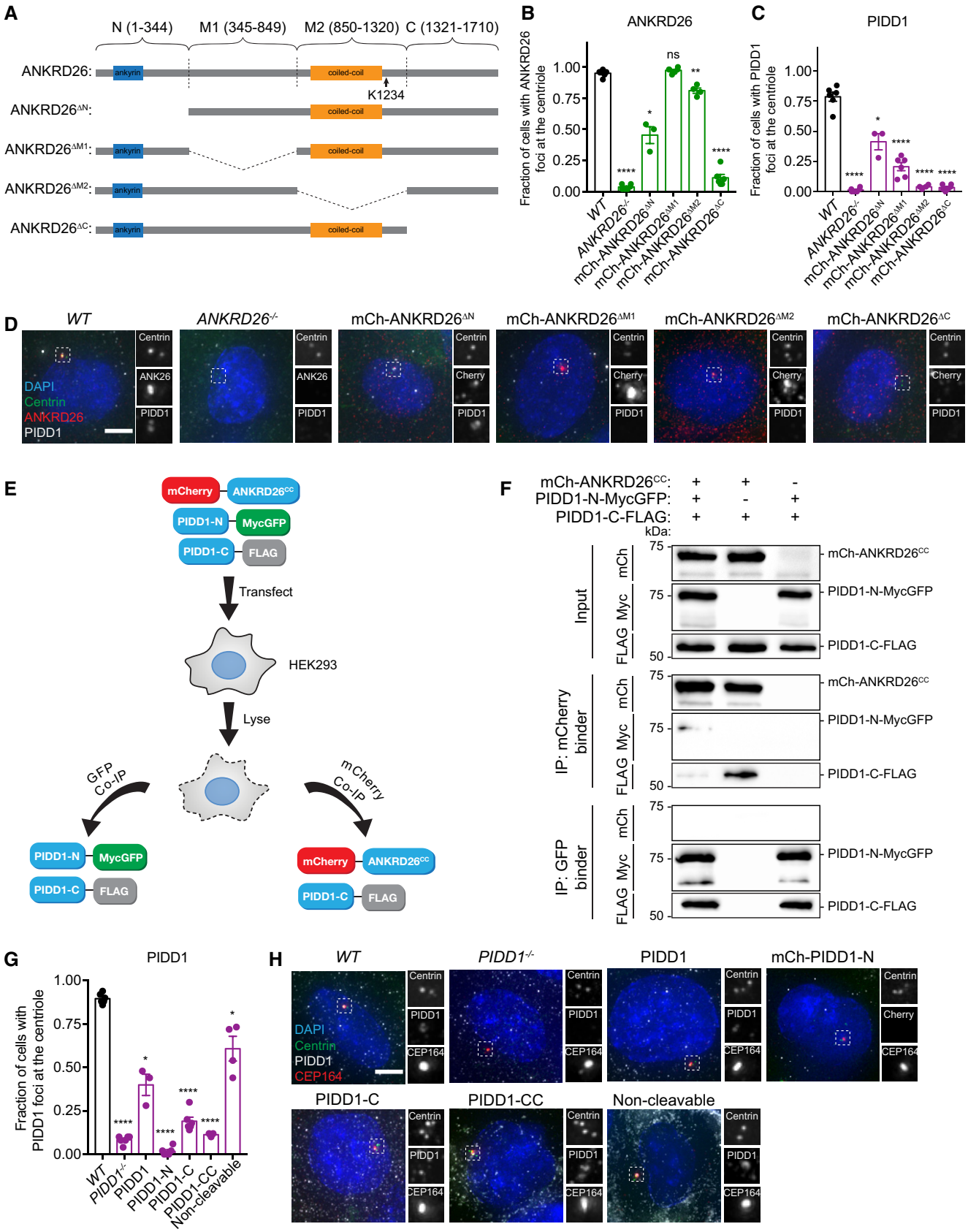
A
N (1-344)
M1 (345-849)
M2 (850-1320)
C (1321-1710)
ANKRD26:
ANKRD26ΔN:
ANKRD26ΔM1:
ANKRD26ΔM2:
ANKRD26ΔC:
ankyrin
coiled-coil
K1234
B
ANKRD26
Fraction of cells with ANKRD26 foci at the centriole
C
PIDD1
Fraction of cells with PIDD1 foci at the centriole
WT
ANKRD26-/-
mCh-ANKRD26ΔN
mCh-ANKRD26ΔM1
mCh-ANKRD26ΔM2
mCh-ANKRD26ΔC
D
DAPI
Centrin
ANKRD26
PIDD1
Cherry
E
mCherry
ANKRD26CC
PIDD1-N
MycGFP
PIDD1-C
FLAG
Transfect
HEK293
Lyse
GFP Co-IP
mCherry Co-IP
F
mCh-ANKRD26CC:
PIDD1-N-MycGFP:
PIDD1-C-FLAG:
kDa:
Input
IP: mCherry binder
IP: GFP binder
mCh
Myc
FLAG
G
PIDD1
Fraction of cells with PIDD1 foci at the centriole
PIDD1-N
PIDD1-C
PIDD1-CC
Non-cleavable
H
PIDD1-/-
mCh-PIDD1-N
CEP164

Figure EV4.

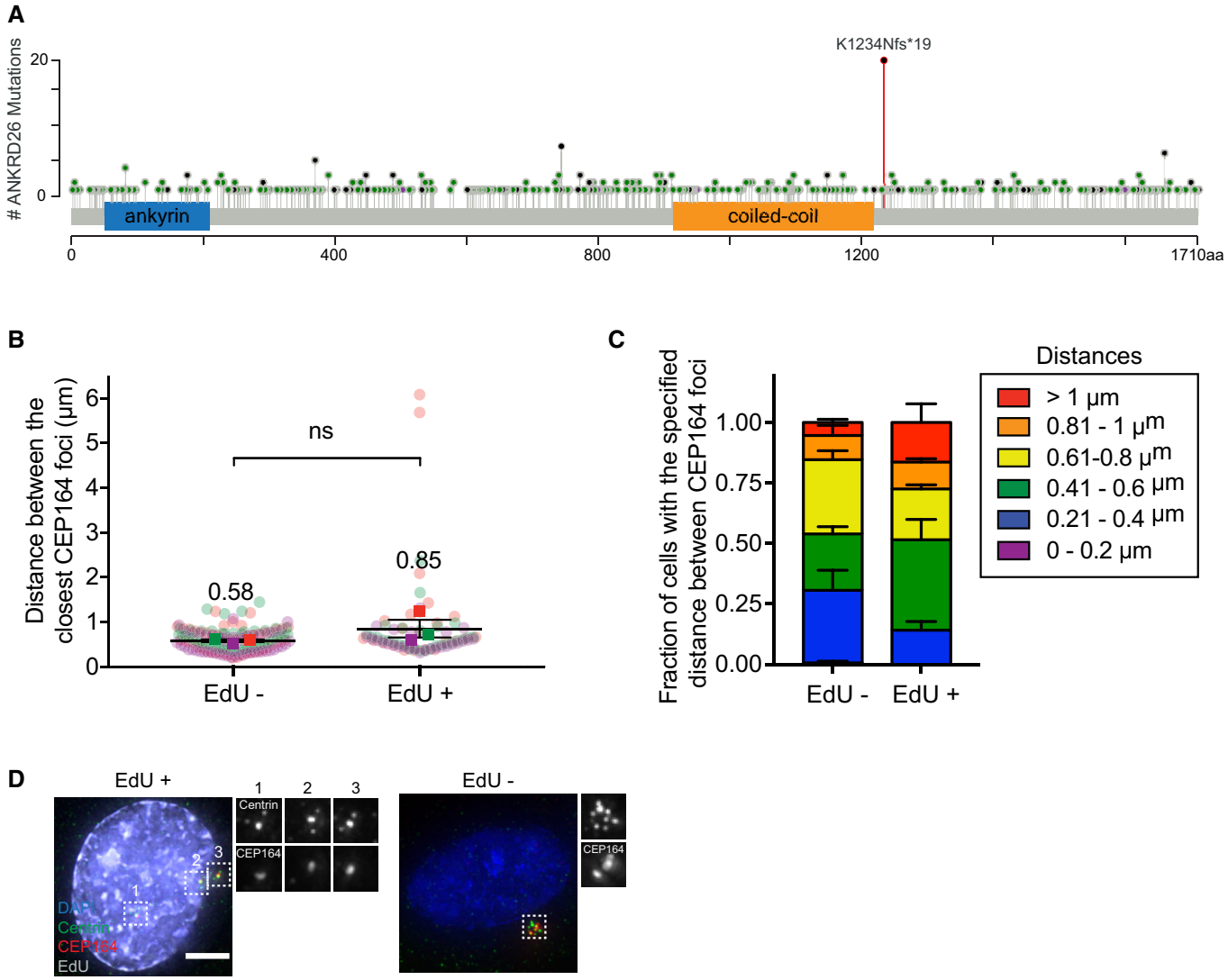

Figure EV5. A recurrent ANKRD26 mutation is observed in human tumors.

A Schematic showing the location of the 533 mutations in *ANKRD26* in human tumors. Black represents truncating mutations; green represents missense mutations; purple represents inframe mutations. The K1234Nfs*19 mutation is shown in red. Data from curated set of non-redundant studies in cBioPortal (Cerami *et al*, 2012).

B Plot showing the distance between the closest CEP164 rings in cycling (EdU+) and non-cycling (EdU− cells). Squares show the mean for each biological replicate; colored circles show individual data points from each of the replicates. Data acquired across $n = 3$, biological replicates, each with > 20 cells. *P* values, unpaired two-tailed *t*-test. Mean ± s.e.m.

C Stacked bar graphs of the same data shown in (B). Mean ± s.e.m.

D Representative images of PLK4^{Dox} RPE1 cells treated with and without doxycycline for two days and immunostained with the indicated antibodies. Scale bar = 5 µm.

Source data are available online for this figure.