Meitinger et al., http://www.jcb.org/cgi/content/full/jcb.201604081/DC1

A

	clone	Gene	gRNA	library #
screen 1	1	TP53BP1	GTATACCTGCTTGTCCTGTT	HGLibA_58342
		SF3A2	CCTCATTGTTGTGAAGTGTC	HGLibB_43642
	2	PCNT	GGCTGTCGATGCGTCTGTCC	HGLibA_42929
		TP53BP1	GTATACCTGCTTGTCCTGTT	HGLibA_58342
	3	USP28	TGAGCGTTTAGTTTCTGCAG	HGLibB_53255
		KLK13	ACTCCTCATCTGAGCGAAGT	HGLibB_25207
		C9Orf64	GTATATTCCGAACCTGATCC	HGLibB_06744
	4	SPARCL1	TTTGTTGGAGGACAAGTCAC	HGLibB_46364
		TRIM37	CTCCCCAAAGTGCACACTGA	HGLibB_51413
	5	ADAM21	TCCTGAACGATTTCTCTCAA	HGLibB_00752
		TP53BP1	CTGCTCAATGACCTGACTGA	HGLibB_50991
	6	ZNF823	TGAACTTCACACAAGAGGAG	HGLibB_56755
		USP28	CTGCTGCAGCTCCGCAGTCA	HGLibA_60609
		hsa-mir-7159	CTTGGCATTTCTATGTTAGT	HGLibA_28919
screen 2	1	TP53BP1	GTATACCTGCTTGTCCTGTT	HGLibA_58342
		CTPS1	TTGACTCACCACGGGATGAC,	HGLibA_11667
	2	TP53BP1	CTGCTCAATGACCTGACTGA	HGLibB_50991
		SEZ6L2	ATTGTGGATGGTGCCACCAC	HGLibB_43632
		-	CTGCTCACTGACGTGACGGAC	-
		-	ACTTCCTGAGAACTGCGTGC	-
	3	TP53BP1	GTATACCTGCTTGTCCTGTT	HGLibA_58342
		NRG2	AGCCACGCAGATGCCCGTGA	HGLibA_39966
		NonTargetingControlGuideForHuman_0874	TCATGCTTGCTTGGGCAAAA	HGLibA_65257
	4	USP28	TGAGCGTTTAGTTTCTGCAG	HGLibB_53255
		-	GCCCGTCCAGCCTAGCACT	-
		DBT	CACTTCCTGAAAACAACTGC	HGLibB_12392
	5	TP53BP1	GTATACCTGCTTGTCCTGTT	HGLibA_58342
		SYCE2	TCGCAGTTCTCTTCCCACCG	HGLibA_55207
	6	USP28	TGAGCGTTTAGTTTCTGCAG	HGLibB_53255
		-	TGAACTTCACACAATAGGA	-
		-	ACTGCCTGTCGACGTCGAC	-
	7	TP53BP1	GTATACCTGCTTGTCCTGTT	HGLibA_58342
		BRD1	GTTAAATAGGATTGCGAATC	HGLibB_04767
	8	USP28	TGAGCGTTTAGTTTCTGCAG	HGLibB_53255
		DHRS13	AGTGGCAAAGGCCCGCACCG	HGLibA_13126
		TOMM40L	AGTGAAGCTCGTTGTCAACA	HGLibA_58257
	9	TRIM37	CTCCCCAAAGTGCACACTGA	HGLibB_51413

B

#	gRNA library	Gene	counts	
			centrinone	untreated
1	HGLibA_08821	CDKN1A	2008039	13723
2	HGLibB_08815	CDKN1A	1590038	10720
3	HGLibB_50984	TP53	1221915	7843
4	HGLibB_50985	TP53	1184166	7895
5	HGLibA_26076	hsa-mir-4731	870348	5710
6	HGLibB_20190	GRAPL	815396	5439
7	HGLibB_06744	C9orf64	635230	4259
8	HGLibA_58337	TP53	566927	3635
9	HGLibA_58335	TP53	511028	3652
10	HGLibA_58342	TP53BP1	463115	3428
11	HGLibB_50991	TP53BP1	407157	2969
12	HGLibB_49833	TMEM119	367835	2511
13	HGLibA_43470	PFN4	363562	2384
14	HGLibB_25207	KLK13	339303	2308
15	HGLibA_63972	ZNF76	332635	2309
16	HGLibB_00752	ADAM21	301287	2406
17	HGLibB_47214	ST6GALNAC3	300129	2280
18	HGLibB_53255	USP28	287950	1795
19	HGLibB_35409	PCDHA8	272185	1985
20	HGLibA_08820	CDKN1A	269767	1889

#	gRNA library #	Gene	counts	
			centrinone	untreated
21	HGLibB_46364	SPARCL1	244621	1673
22	HGLibA_10012	CLECL1	234986	1525
23	HGLibB_54049	WDR5	219262	1513
24	HGLibB_24626	KIAA0895L	213848	1458
25	HGLibB_49756	TMED6	203719	1788
26	HGLibA_28356	hsa-mir-6781	195381	1359
27	HGLibA_52561	SLC5A5	185426	1233
28	HGLibB_08779	CDK5RAP2	182520	1434
29	HGLibA_19433	GMPS	168051	1178
30	HGLibB_51413	TRIM37	159383	1487
31	HGLibA_47816	RBM28	159187	1182
32	HGLibA_49688	RTN2	150774	1017
33	HGLibA_39516	NOA1	145033	983
34	HGLibB_33013	NUP50	144201	1016
35	HGLibB_56755	ZNF823	133056	1083
36	HGLibA_60609	USP28	107705	817
37	HGLibB_20860	HAUS6	88840	908
38	HGLibA_64356	ZWILCH	81402	602
39	HGLibB_50986	TP53	79573	657

Figure S1. **Results from the CRISPR/Cas9 screen.** (A) Table shows the gRNAs identified in each of the 15 type 1 colonies obtained in both screens. For this analysis, gRNAs were amplified from each colony, cloned, and identified by Sanger sequencing. All type 1 colonies contained a gRNA targeting *TP53BP1* (blue), *USP28* (green), or *TRIM37* (red). (B) For the first screen, a second approach was also taken in which all 32 colonies (6 type 1 and 26 type 2) were pooled. The gRNAs were then amplified and subjected to Illumina sequencing. The top hits from this analysis (according to the gRNA counts) included the genes encoding the expected type 2 hits p53 (*TP53*; orange) and p21 (*CDKN1A*; purple) as well as the type 1 hits *TP53BP1*, *USP28*, and *TRIM37*.

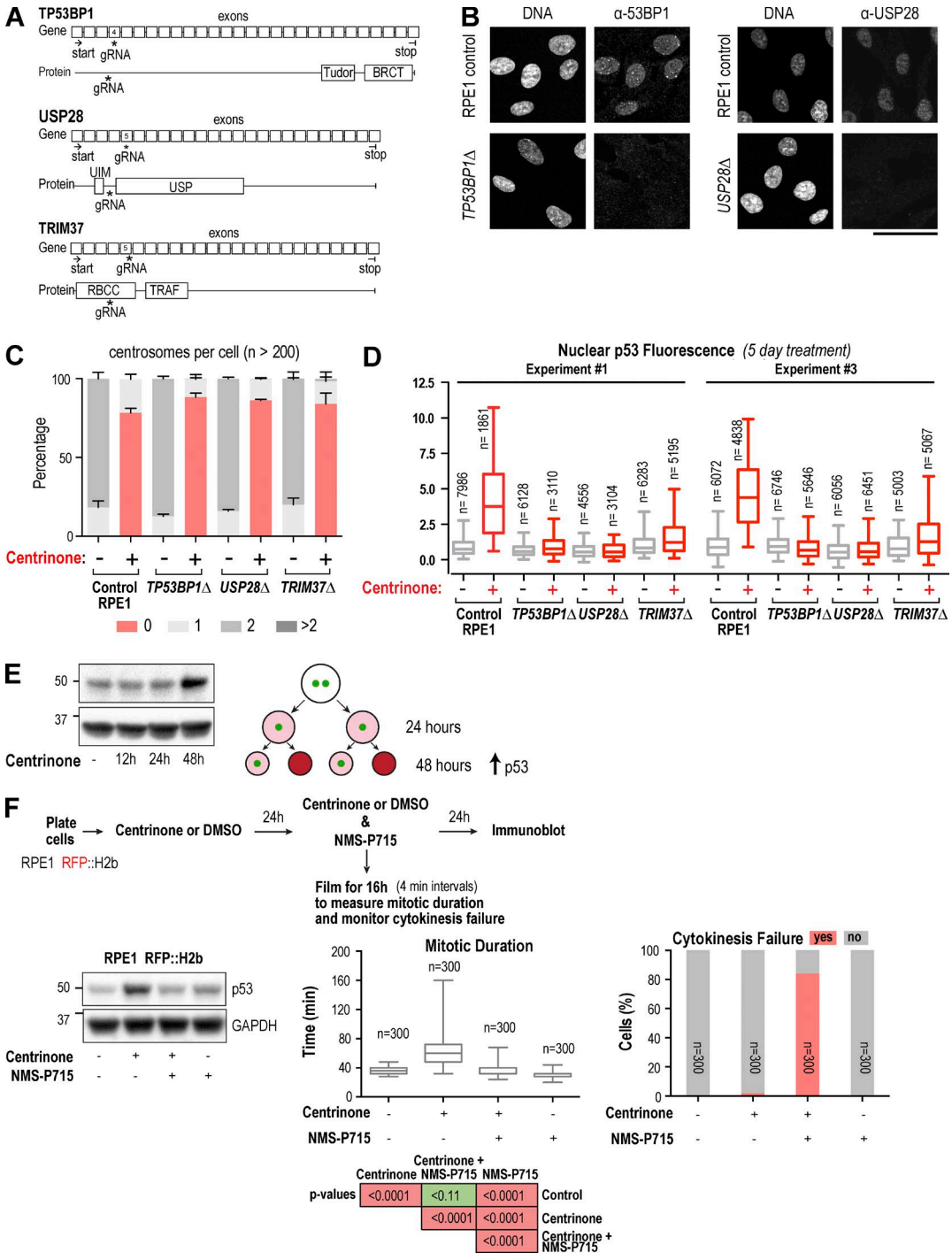
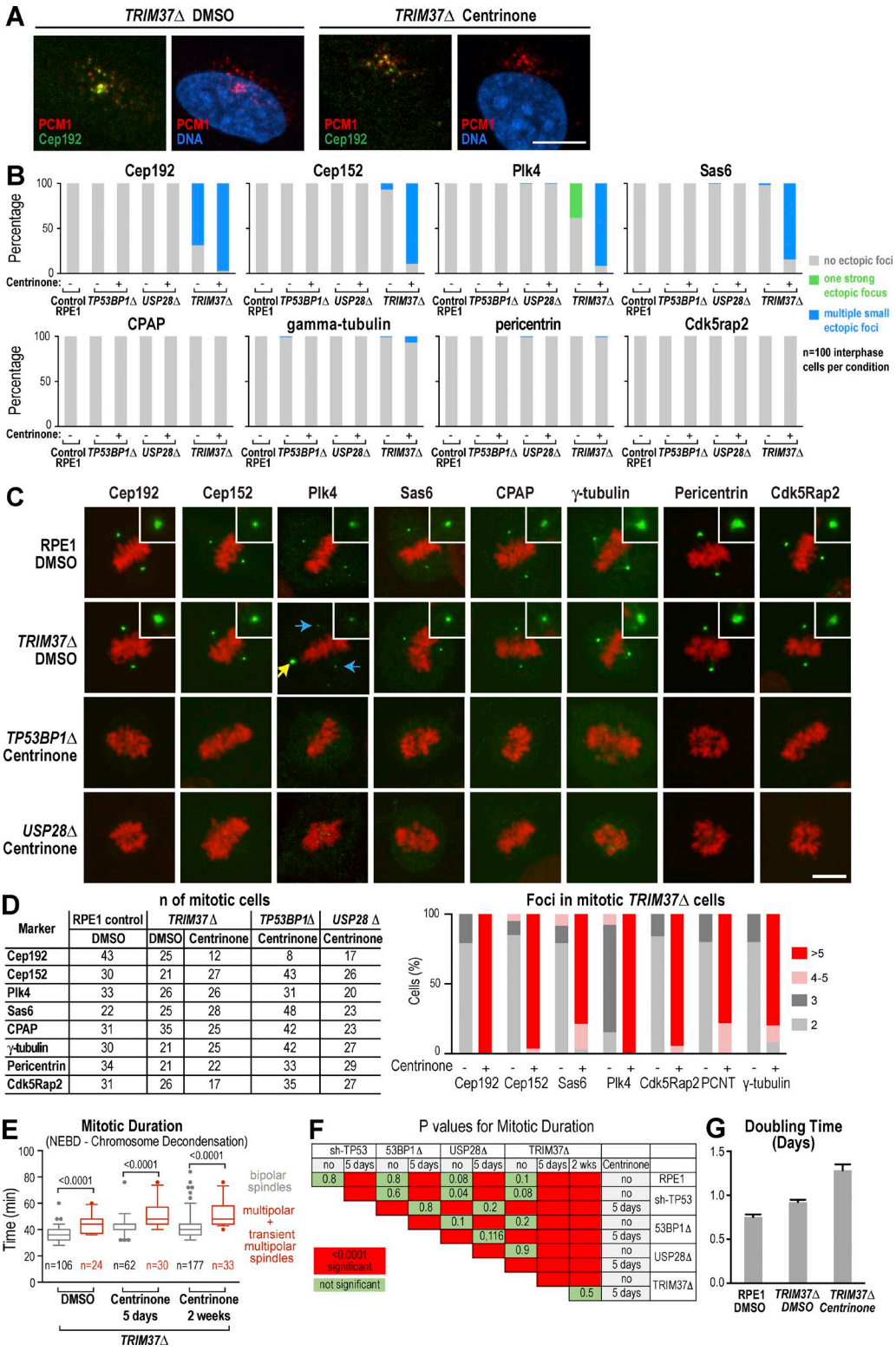
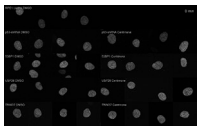


Figure S2. **Generation and characterization of single *TP53BP1*, *USP28*, and *TRIM37* knockout mutants.** (A) Locations of the gRNAs (asterisks) used to generate the single deletion mutants in the *TP53BP1*, *USP28*, and *TRIM37* genes, along with the locations in the corresponding proteins. (B) Immunofluorescence images acquired using spinning disk confocal optics of control (top row) or knockout (bottom row) RPE1 cells stained for DNA and 53BP1 (left) or DNA and USP28 (right). Bar, 50 µm. (C) Graph shows the centrosome number distribution in control or knockout RPE1 cell lines after 5-d treatment with DMSO or centrinone. Centrosomes were identified as foci costaining for γ-tubulin and Cep192. Data from three experiments were analyzed. Error bars are SD. (D) Control or knockout RPE1 cell lines were treated with centrinone or DMSO (vehicle control) for 5 d and fixed and stained for DNA and with antibodies to Cep192 and p53. The graph shows mean nuclear p53 fluorescence for two independent experiments (experiments 1 and 3; data for experiment 2 are plotted in Fig. 2 E). Graph shows 5–95% box-and-whiskers plots. (E) Immunoblot shows p53 levels after 12, 24, and 48 h of centrinone treatment. Schematic summarizes the result relative to the kinetics of centrosome depletion (Wong et al., 2015). (F) Schematic outlines the experiment performed to test whether reducing mitotic duration using the Mps1 inhibitor NMS-P715 can suppress centrinone-induced p53 elevation. Cells were pretreated for 24 h with centrinone, at which point the majority of cells have one centrosome and do not yet have elevated p53 (see schematic and blot in E). The cells were then incubated with centrinone plus the specific Mps1 inhibitor NMS-P715 for an additional 24 h to determine whether it was possible to block the p53 elevation that normally occurs as the first wave of cells go from having one to zero centrosomes. In addition to monitoring the experiment by Western blotting, cells (which expressed RFP::H2B) were monitored by live cell filming. Shown are an immunoblot for p53 levels (left), a graph plotting mitotic duration, shown as 5–95% box-and-whiskers plots (middle), and a graph plotting the percentage of cells that failed chromosome segregation and cytokinesis (right).



Marker	RPE1 control	TRIM37Δ		TP53BP1Δ	USP28 Δ
	DMSO	DMSO	Centrinone	Centrinone	Centrinone
Cep192	43	25	12	8	17
Cep152	30	21	27	43	26
Plk4	33	26	26	31	20
Sas6	22	25	28	48	23
CPAP	31	35	25	42	23
γ-tubulin	30	21	25	42	27
Pericentrin	34	21	22	33	29
Cdk5Rap2	31	26	17	35	27

Figure S3. **Analysis of foci containing centrosomal markers in *TP53BP1Δ*, *USP28Δ*, and *TRIM37Δ*.** (A) Immunostaining shows colocalization between PCM-1 and CEP192 in *TRIM37Δ* cells treated for 5 d with DMSO or centrinone. (B) Graphs plot the percentage of interphase cells with foci containing the indicated centrosomal markers. (C) Immunofluorescence images of mitotic control RPE1, *TRIM37Δ*, *TP53BP1Δ*, and *USP28Δ* cells stained for DNA (red) and with antibodies to the indicated centrosomal proteins (green) after 5-d treatment with DMSO or centrinone (data for *TRIM37Δ* is reproduced from Fig. 4 D to facilitate comparison). Images are representative, and each marker was equivalently scaled. Yellow arrow points to bright ectopic Plk4 focus; blue arrows point to centrioles. (D) Table (left) shows number of mitotic cells analyzed for the analysis shown in Figs. 4 D and S3 C and for the quantification (right) of foci of centrosomal proteins of DMSO- and centrinone-treated mitotic *TRIM37Δ* cells. (E) Graph plots mitotic duration, shown as 5–95% box-and-whiskers plots, of bipolar and multipolar mitoses for DMSO and centrinone-treated *TRIM37Δ* cells. (F) P-values for the mitotic duration experiment shown in Fig. 5 C. (G) Doubling times in days for DMSO-treated control RPE1 and *TRIM37Δ* mutant cells, as well as *TRIM37Δ* mutant cells treated for >2 wk with centrinone (n = 3–4 triplicate measurements and SEMs are plotted). Bars, 10 µm.



Video 1. **Mitosis in control DMSO-treated RPE1 and DMSO- and centrinone-treated p53 knockdown (*sh-TP53*), *TP53BP1Δ*, *USP28Δ*, and *TRIM37Δ* cells.** Cells were treated for 5 d with DMSO or centrinone before collection of a 5 × 2-µm confocal *z*-series every 4 min for 8 h. Four representative examples are shown for each condition. Images are maximum-intensity projections. Images were acquired on a CV7000 spinning disk confocal system (Yokogawa Electric Corporation) equipped with a 40× (0.95 NA) U-PlanApo objective and 2,560 × 2,160-pixel sCMOS camera (Andor Technology). Playback rate is 1,168× real time (5 frames/s).

Table S1. **Cell lines**

Cell line	Background	Description
MF101	hTERT RPE-1 (ATCC)	H2B-mRFP
MF102	hTERT RPE-1 (ATCC)	H2B-mRFP; sh-p53
MF103	hTERT RPE-1 (ATCC)	*TP53BP1Δ*
MF104	hTERT RPE-1 (ATCC)	*USP28Δ*
MF105	hTERT RPE-1 (ATCC)	*TRIM37Δ*
MF106	hTERT RPE-1 (ATCC)	*TP53BP1Δ*; H2B-mRFP
MF107	hTERT RPE-1 (ATCC)	*USP28Δ*; H2B-mRFP
MF108	hTERT RPE-1 (ATCC)	*TRIM37Δ*; H2B-mRFP

Reference

Wong, Y.L., J.V. Anzola, R.L. Davis, M. Yoon, A. Motamedi, A. Kroll, C.P. Seo, J.E. Hsia, S.K. Kim, J.W. Mitchell, et al. 2015. Cell biology. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. *Science.* 348:1155–1160. http://dx.doi.org/10.1126/science.aaa5111