Supplemental Inventory

Supplemental Figures

Supplemental Tables

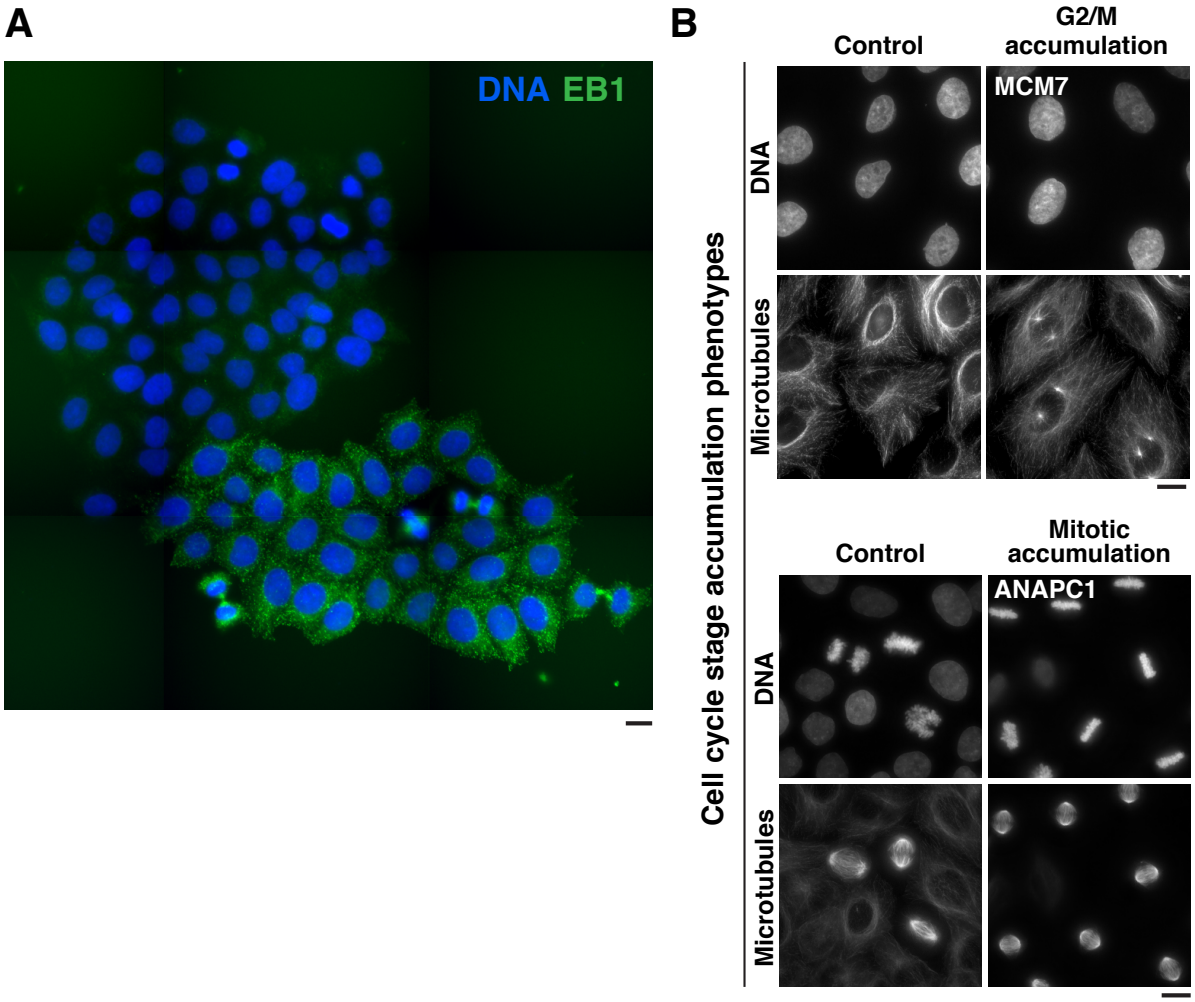

Figure S1. Features and phenotypes of the inducible knockout collection. Related to Figure 1. A) Immunofluorescence image of *MAPRE1* knockout cells stained for DNA (blue) and EB1 (green), indicating clonal outgrowth of knockout and wild type cells. The image was assembled by stitching of multiple adjacent panels using the DeltaVision software. B) Representative immunofluorescence images of the mitotic and interphase accumulation defects used to categorize the collection. Scale bars, 15 µm.

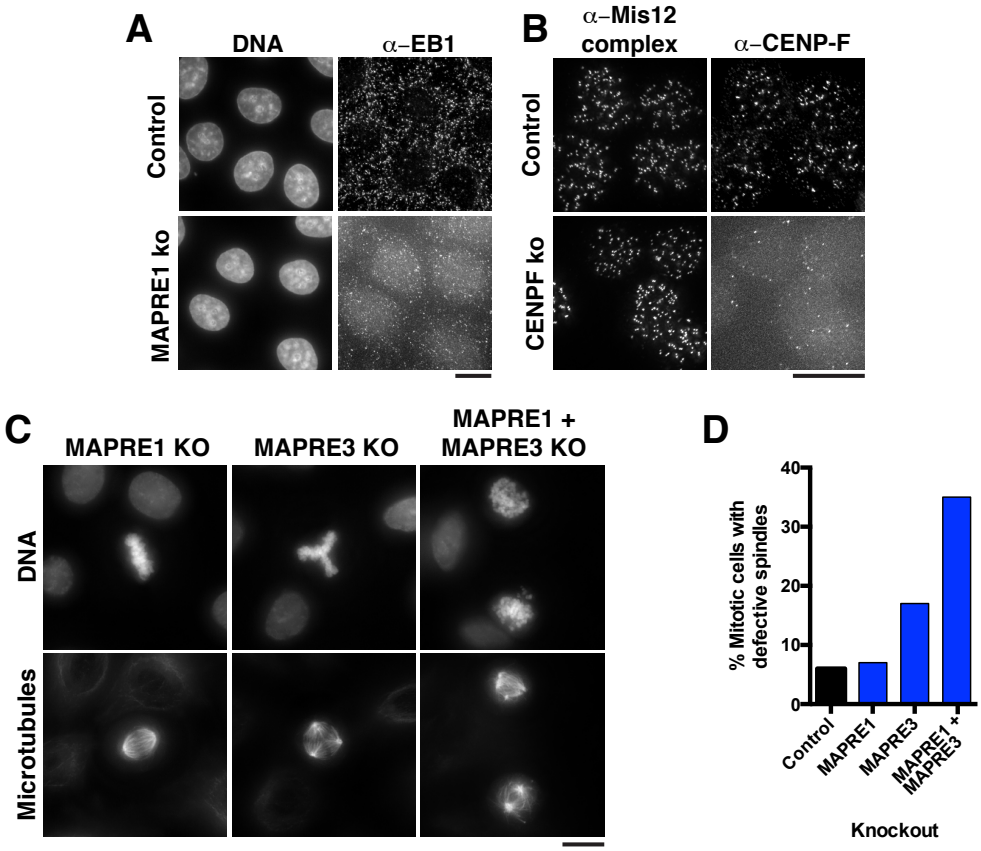

Figure S2. Long-term depletion to disrupt stable processes using the inducible knockouts. Related to Figure 3. A and B) Replicates of the immunofluorescence images shown in Fig. 3C, in which the panel testing for the knockout has been scaled to be as bright as possible to show the absence of the corresponding protein. C) Representative immunofluorescence images showing the increased severity of mitotic phenotypes observed upon double knockout of *MAPRE1* (EB1) and *MAPRE3* (EB3). D) Quantification of the increased frequency of mitotic phenotypes observed upon double knockout of *MAPRE1* (EB1) and *MAPRE3* (EB3), n = 100 cells.

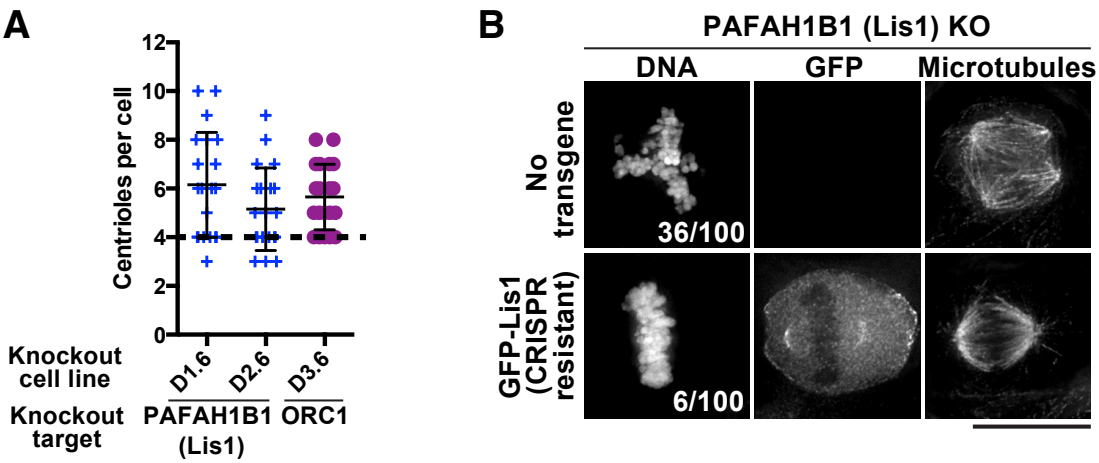

Figure S3. Origins of multipolarity. **Related to Figure 4.** A. Quantification of centriole numbers in cells in which *PAFAH1B1* and *ORC1* are knocked out using distinct guides. B) Representative immunofluorescence images showing mitotic spindle structure in the absence (top) or presence (bottom) of a stable GFP-Lis1 transgene in which the CRISPR targeting sequence has been mutated. Numbers represent proportion of mitotic cells exhibiting defects.

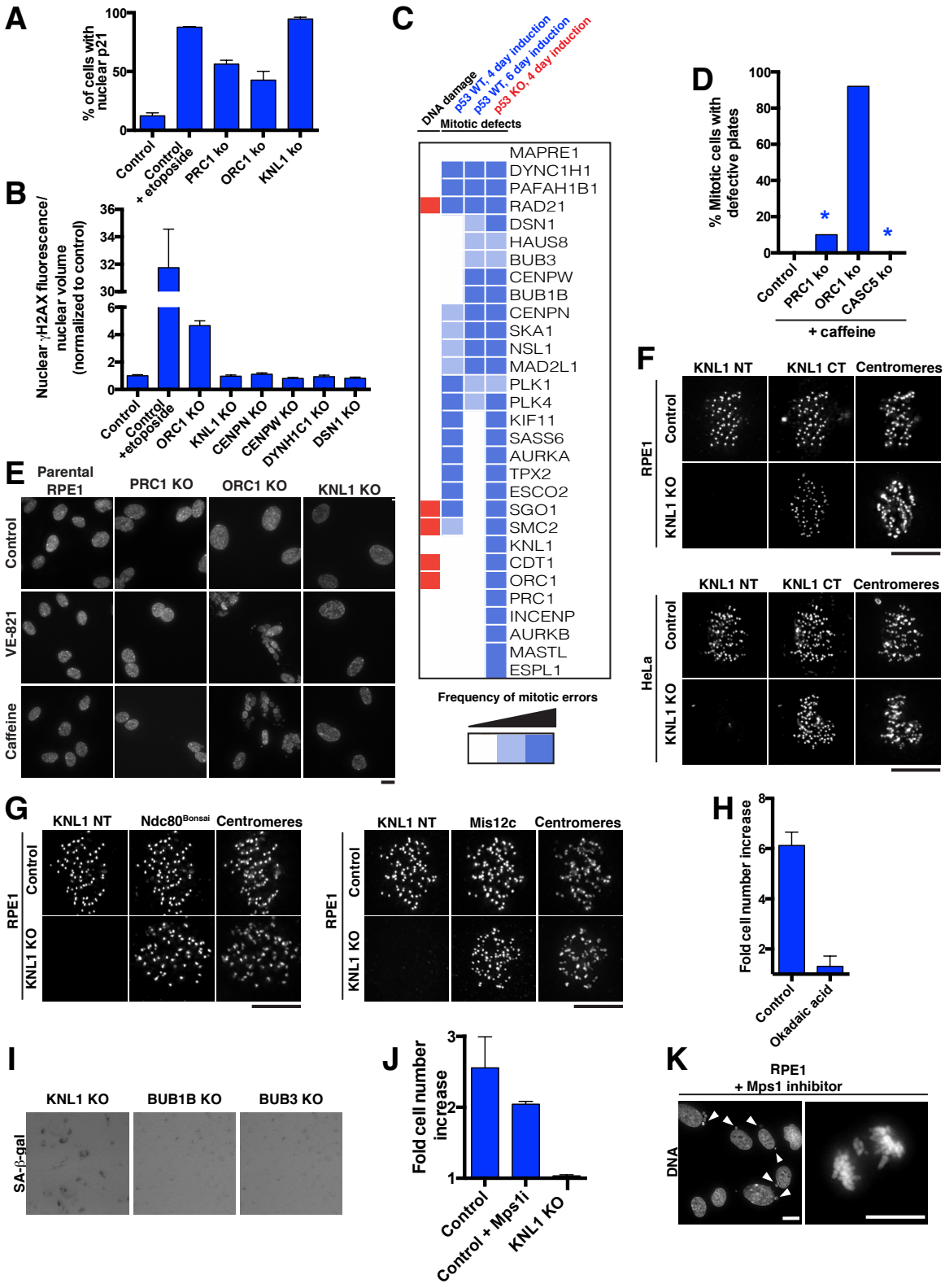

Figure S4. Mechanisms of activation of p53-dependent arrests in the cell cycle knockouts. Related to Figure 7. A) Quantification of the proportion of cells in which p21 is nuclear

following four days of Cas9 induction in the indicated knockouts. Error bars represent standard deviation. B) Quantification of nuclear γH2AX fluorescence in the indicated knockouts after four days of Cas9 induction. Error bars represent s.e.m, n = 50 cells. C) Assessment of increased DNA damage based on gamma-H2AX staining in the RPE1 cell cycle knockouts. Red bars indicate increased gamma-H2AX staining. The heat map demonstrating the phenotypic categorization of the indicated knockouts in presence or absence of *TP53* knockout after 4 or 6 days is reproduced from Figure 6E for comparison. D) Quantification of the percentage of mitotic cells with defective plates in the indicated knockouts following four days of Cas9 induction and 2 days of caffeine treatment, n = 50 cells unless designated by an asterisk (*), which indicates that insufficient mitotic cells were present to achieve this n. In these cases, n > 10 cells. E) Representative immunofluorescence images showing interphase nuclei in the indicated knockouts following four days of Cas9 induction and 2 days of treatment with the indicated small molecules. F) Representative immunofluorescence images of *KNL1* knockout HeLa and RPE1 stained for N- and C-terminal epitopes of KNL1. Centromeres are marked with anti-centromere antibody. G) Representative immunofluorescence images of *KNL1* knockout RPE1 cells stained for the associated kinetochore complexes, the Mis12 complex and the Ndc80 complex. Centromeres are marked with anti-centromere antibody. H) Quantification of fold increase in cell number over 2.5 days in control RPE1 cells that have been treated with Okadaic acid for four days. Error bars represent standard deviation. I) Representative brightfield images of the indicated knockout cells stained for SA-β-galactosidase after four days of Cas9 induction. J) Quantification of fold increase in cell number over 24 hours in control RPE1 cells that have been treated with Mps1 inhibitor for four days and RPE1 *KNL1* knockout cells in which Cas9 has been induced for 4 days. Error bars represent standard deviation. K) Representative DNA images of parental RPE1 cells that have been treated with the Mps1 inhibitor AZ-3146 for four days. Arrowheads indicate micronuclei. Scale bars, 15 μm.

Supplemental Tables

Supplemental tables provided as attached spreadsheets
Please note that Tables S1 and S4 contain multiple tabs

Table S1. Knockout cell lines used in this study. Related to STAR Methods.

Table S2. Functional categorization of targets used in this study based on previous published reports. Related to Figure 1. The number 1 in a column designates that functions related to the indicated category have been reported for the indicated gene product.

Table S3. Disease mutations reported for genes targeted in this study. **Related to Figure 1.**

Table S4. Phenotypic categorization of HeLa inducible CRISPR/Cas9 knockouts. Related to Figure 2. Tab 1 reports the phenotypes observed in every cell line. Tab 2 reports the phenotypes observed in one cell line for each target for which the most potent phenotype was observed. If no phenotype was observed with any sgRNA, the selection of the cell line for inclusion in Tab 1 was arbitrary. Frequently observed phenotypes are designated with the number 2, and infrequently observed phenotypes are designated with the number 1, as outlined in Figure 2.

Table S5. Phenotypic categorization of RPE1, U2OS and DLD1 inducible CRISPR/Cas9 knockouts. Related to Figure 5. Frequently observed phenotypes are designated with the number 2, and infrequently observed phenotypes are designated with the number 1, as outlined in Figure 2.

Table S6. Antibodies used in this study. Related to STAR Methods.

Antigen	**Species**	**Source**	**Fixation for immunofluorescence**
Tubulin (DM1a) conjugated to FITC	Mouse	Sigma	10 min in 3.8% formaldehyde in PBS
Tubulin (DM1a) unconjugated	Mouse	Sigma	10 min in 3.8% formaldehyde in PBS
Centrin2	Rabbit	Cheeseman lab (Backer et al., 2012)	4-5 min in ice cold methanol
EB1	Mouse	BD Biosciences 610535	5 min in ice cold methanol
EB1	Rabbit	Santa Cruz sc-15347	Western only
Mis12 complex	Rabbit	Cheeseman lab	5 minutes pre-extraction in PBS + 0.5 % Triton-X, then

			10 minutes in 3.8% formaldehyde in PBS (hereafter, “pre-extraction”)
CENP-F	Mouse	Abcam ab90	Pre-extraction
P21	Rabbit	Abcam ab109520	10 min in 3.8% formaldehyde in PBS
H2AX phospho-S139	Mouse	Millipore clone JBW309	Pre-extraction
YAP	Mouse	Santa Cruz clone 63.7	10 min in 3.8% formaldehyde in PBS
KNL1 N terminus	Mouse	Yanagida lab (Kiyomitsu et al., 2007)	Pre-extraction
KNL1 central region	Rabbit	(Cheeseman et al., 2008)	Pre-extraction
Ndc80-Bonsai	Rabbit	Cheeseman lab (Schmidt et al., 2012)	Pre-extraction
Anti-centromere antibodies	Human	Antibodies, Inc.	Pre-extraction