Supplementary Materials:

Supplementary Materials and Methods

Cell culture

MDA-MB-231, Cal51 and DLD1 cell lines were grown in DMEM supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, and 50 μg/mL penicillin/streptomycin at 37°C and 5% CO_2. RPE1 cells were grown in DMEM/F12 supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, and 50 μg/mL penicillin/streptomycin at 37°C and 5% CO_2. MCF10A cells were grown in DMEM/F12 supplemented with 5% (vol/vol) horse serum, 20 ng/mL hEGF, 0.5 mg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 μg/mL insulin, and 50 μg/mL penicillin/streptomycin at 37°C and 5% CO_2. Paclitaxel (LC Laboratories) was diluted in DMSO and used at the indicated final concentrations. CW-069 (MedChem Express, 50 μM), reversine (Sigma-Aldrich, 1 μM), AZ3146 (Selleck Chemicals,1 μM), AZ82 (Cayman Chemical, 1.25 μM), and GSK923295 (AdooQ Bioscience, 50 nM) used in cell culture experiments were dissolved in DMSO. In the inducible Mad1 and Plk4 experiments, 2 μg/mL tet or doxycycline (dox) was added for 24 hours or 48 hours prior to the addition of paclitaxel, respectively.

Orthotopic experiments

All animal studies were performed in compliance with all relevant ethical regulations for animal testing and research. The study was approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. $5x10^6$ cells were injected orthotopically into the inguinal mammary fat pads of 5-week-old female athymic nude mice. Three mice were injected with parental MDA-MB-231 cells and three mice were injected with MDA-MB-231 cells that inducibly express Mad1-YFP on both sides (2 tumors per mouse). All mice were

started on dox chow (Teklad TD.120769, containing 625 mg/kg dox) the day before cell injection. Tumor size was measured every 2-3 days using calipers. Tumor volumes were calculated using the formula $V = width^2 \times length/2$. Mice were treated with sterile saline or 30 mg/kg paclitaxel iv every other day for 5 days once tumors reached a minimum volume of 75 mm^3.

Cell viability assays

Metabolic cell viability was determined using an MTT assay (VWR) . Briefly, cells were counted and plated in 6 well plates. On the day(s) of measurement, cells were incubated with 1 mg/mL MTT reagent (3-(4,5 dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide) for 3 hours. Viable cells contain NADH oxidoreductase enzymes which convert the MTT substrate to formazan. Formazan was liberated from cells by adding 800 µL of DMSO and 100 µL Sorenson's glycine buffer (0.1M glycine, 0.1M NaCl, pH:10.5 with 0.1M NaOH) to each well, followed by a 10 min incubation at 37°C. 3 x100 µL of solution was transferred to 3 wells in 96 well plates for triplicate measurements of each condition. Formazan, an indirect measure of metabolic cell viability, was colorimetrically detected by measuring absorbance on a plate reader at 540 nm.

Colony formation assays were performed by plating MDA-MB-231 or Cal51 cells at a density of 400 or 600 cells/well, respectively, in 6 well plates. Cells were allowed to attach overnight, and drugs were added the following day. After 14 days, cells were stained with crystal violet and the number of colonies were counted.

For cell counting assays to quantify live and dead cells, 25,000 cells were plated in 6 cm dishes. Cells were allowed to attach overnight, and drugs were added the following day. On days

selected for timepoint analysis, media and PBS rinse were collected in addition to trypsinized cells. Cells were pelleted, resuspended, and diluted in trypan blue before counting on a hemocytometer. Live and dead cells were counted to determine the percent dead cells.

Immunofluorescence in cells and primary patient cohort

Cellular immunofluorescence was performed as in (*37*). Staining was performed with antibodies to α-tubulin (YL 1/2; Bio-Rad, MCA77G), pericentrin (Abcam, ab4448) diluted 1:1000 or centrin (Millipore Sigma, 04-1624) diluted 1:5000 for one hour at room temperature. For patient immunohistochemistry, 5 μm sections of formalin-fixed, paraffin-embedded tissue sections were subjected to antigen retrieval in citrate buffer, serum-blocked overnight, and stained with rabbit anti-NuMA antibody (a kind gift from Duane Compton), mouse anti-γ-tubulin (Sigma, T6557), and pan-cytokeratin (to mark epithelial cells; Novus Biologicals, NBP2-33200AF647) antibodies diluted 1:100 overnight at 4°C. Alexa Fluor-conjugated secondary antibodies (Invitrogen) were used at 1:200 for one hour at room temperature. DNA was stained using DAPI. Ki67 visual estimate performed by a fellowship-trained subspecialist in surgical pathology of the breast (S.M.M), according to standard clinical practice.

Imaging response criteria

Baseline imaging was performed by ultrasound as part of a complete diagnostic imaging evaluation including mammography. All imaging was performed as closely as possible to the beginning of treatment. Tumors were again evaluated by ultrasound within two weeks of completion of paclitaxel chemotherapy, and prior to any additional chemotherapy or surgery. A single board-certified breast imaging radiologist with seven years of experience (A.M.F,) retrospectively reviewed the reports and saved images from the prospectively performed ultrasound exams. Imaging response was evaluated using RECIST 1.1 criteria (*26*) and the single

longest dimension measured on the baseline and post-paclitaxel ultrasounds. Three patients were excluded from this analysis due to the inability to accurately determine tumor measurements (1), deviation from treatment protocol (1), or withdrawal of patient consent (1).

Paclitaxel measurements

Cells in 6 cm dishes were treated with the indicated concentrations of paclitaxel in 3 mL total volume once they reached 90-100% confluence. After 20 hours, media was collected, and cells were pelleted and resuspended in 1 mL ddH_2O. Both cell and media samples were stored at -20°C until the day of analysis. Cell resuspensions were thawed and sonicated immediately before sample preparation for LC-MS/MS. For solid phase extraction, cell and media samples were applied to an Ostro plate (Waters,186005518). Prior to extraction, deuterium-labeled internal standard paclitaxel-d5 (Toronto Research Chemicals; P132502) was added via diluent to facilitate quantification of sample recovery. Samples were eluted using acetonitrile +1% formic acid with positive pressure (30-50 psi) of nitrogen into a collection plate. LC-MS/MS analysis was performed using a LC-UV-MS/MS system (QTrap 5500; ABSciex) equipped with a Waters Acquity UPLC System binary pump). A Phenomenex Kinetex C18 column (30 mm × 2.1 mm × 2.6 μm) was used with a gradient of acetonitrile/water/formic acid of 65:35:0.1 (v/v/v) to 100:0:0.1 at 0.35 mL/min flow rate. To monitor and quantify the concentrations of paclitaxel and paclitaxel-d5, a multiple-reaction monitoring (MRM) method was developed with signature ion fragments for each molecule. Calibration curves were obtained using paclitaxel at 0.2, 1, 5, 10, 50, 100, 250, 500, and 1000 ng/mL. Quantification was carried out based on peak area of the MRM transition and the linear calibration curve for each compound.

Patient tissue core biopsies were analyzed as in (*10*). Briefly, core biopsies were thawed on ice and core length, diameter, and weight were recorded. Intratumoral concentrations were

calculated using core tissue volume (cc; Table 1). Calculations based on core tissue weight (g) gave similar results (Table S5). In this case, the final molar concentration (μM) calculations assume that tissue core density is equivalent to that of water (1 g/cm^3 or 1 g/mL). Patient biopsies were homogenized in EDTA drug-free human plasma + 10% acetonitrile and 10 μg/mL docetaxel (Sigma-Aldrich; 01885-5MG) was added as internal standard prior to pre-extraction to facilitate quantification of sample recovery. Homogenized samples were then applied to C18 Bond Elut solid-phase extraction columns (Agilent) and eluted with acetonitrile by gravity or low vacuum. Solvents were then evaporated to dryness using nitrogen gas and samples were reconstituted in 100 μL acetonitrile and 100 μL of 35 mM acetic acid. Analysis was performed by monitoring the signal of a 50 μL injection at 235 nm during an isocratic elution with 56% 35 mM acetic acid, 44% acetonitrile on an analytical HPLC instrument (Shimadzu LC-20AD with SPD-20A UV Detector and a Waters Nova-Pak C18 4-μm 4.6 × 150 mm column). Calibration curves were obtained using paclitaxel at 10, 25, 50, 100, 250, 500, and 750 ng/mL. Quantification was carried out based on peak area and the linear calibration curve for each compound.

Microscopy

Images were acquired on a Nikon Ti-E inverted microscope driven by Nikon Elements software with focus-drift compensation. Images are maximum projections from 0.2 μm z-stacks collected with a 60×/1.4 or 100×/1.4 numerical aperture (NA) objective after deconvolution using the AQI 3D Deconvolution module in Elements. For MCF10A and Cal51 (transgene) timelapse analysis, cells were placed under 10% CO_2 flow at ~30 mL/min in a heated chamber at 37°C. Images were acquired at 3-min and 4-min intervals, for phase-contrast and fluorescent timelapse, respectively, using a 20×, 0.1 NA objective and focus drift compensation. For MDA-

MB-231 and Cal51 (endogenously tagged) 24-hour timelapse analysis, cells were imaged using a Tokai Hit stage top incubator with 5% CO2. Five 2 μm z-planes were acquired every 3 or 7 min, respectively using a 40×/0.75 NA objective and focus drift compensation. Maximum projections of in-focus planes or the maximally focused single z plane were assembled in Elements, exported as jpg files, and converted to .avi files in Fiji/ImageJ. For long term microscopy experiments to determine cell fate, cells were imaged using a Tokai Hit and 5% CO_2. Images were acquired every 4-5 minutes for 64-72 hours with three to five 2 μm z-planes. Cells were treated with drugs approximately 1 hour before observation unless otherwise noted. For Fig. 3A-E, cells were treated with paclitaxel 20 hours before observation. The ratio of time spent multipolar vs bipolar after anaphase onset in Fig. 3E was quantified by multiplying the number of frames a cell spent multipolar after anaphase onset by the frame rate (4 minutes) and dividing by the number of frames a cell spent bipolar times the frame rate (4 minutes).

Metastatic cohort immunofluorescence and scoring

For patient immunohistochemistry, 5 μm sections of formalin-fixed, paraffin-embedded tissue sections were subjected to antigen retrieval in citrate buffer, serum-blocked for 30 minutes, and stained with anti-rabbit β-tubulin III (Abcam, ab18207), anti-rabbit P glycoprotein 1 (P-gp1; Abcam, ab129450), Ki67 (Abcam, ab15580), or anti-mouse pan-cytokeratin (Abcam, ab7753). Primary antibodies were diluted 1:1000 for pan-cytokeratin and 1:5000 for the others. They all were incubated for 1 hour at room temperature. Mouse and rabbit HRP-conjugated secondary antibodies (Abcam) were used at 1:500 for 30 minutes at room temperature followed by TSA Plus fluorophore dyes diluted 1:50 (Akoya Biosciences). DNA was stained using DAPI. Ki-67 stain was quantified by dividing the number of Ki-67 positive cells by the total number of cells. The rest of the protein markers were calculated with H-Score using the following formula:

3x(percentage of strongly stained cells) + 2x(percentage of moderately stained cells) + 1x(percentage of weakly stained cells) + 0x(percentage of unstained cells), resulting in a range of 0 to 300.

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) was performed using standard techniques, as reported elsewhere (*13*). For each case, a set of 2 tissue slides were used to stain for probes to centromeres 4, 10, and 17 and 3, 7, and 9, respectively. The number of probed chromosomes were counted in 20 cells per section. The average percent of non-modal chromosomes was calculated as a measurement of chromosomal instability as in (*13*).

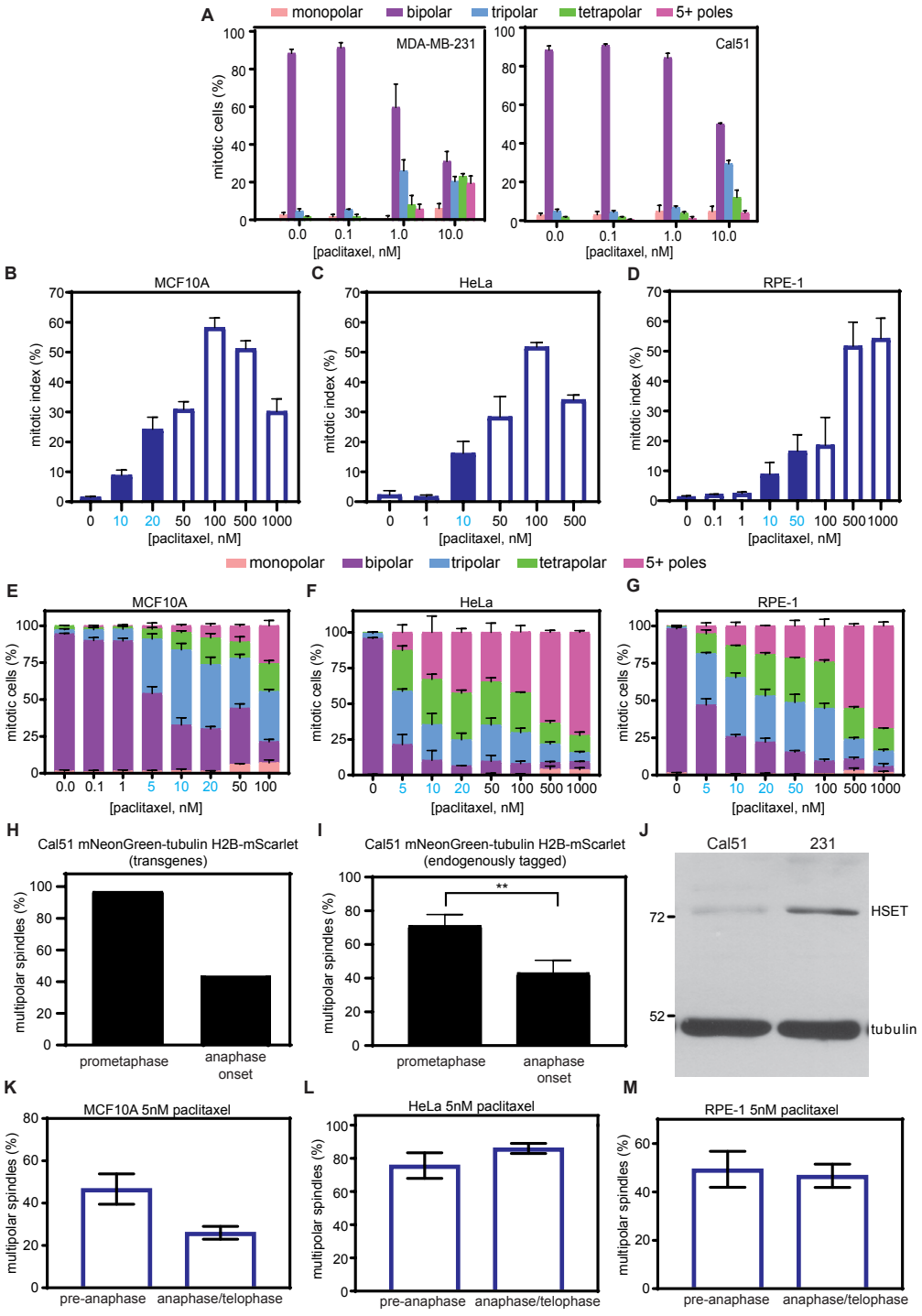

Fig. S1. Clinically relevant intracellular concentrations of paclitaxel induce multipolar spindles without mitotic arrest in multiple cell types. A) Mean mitotic spindle pole number +/- SEM in breast cancer cell lines in response to increasing concentrations of paclitaxel. n≥100

cells in each of 3 replicates. **B-D**) Mitotic index quantification in increasing paclitaxel concentrations in MCF10A (**B**), HeLa (**C**), and RPE-1 (**D**) cells. Clinically relevant doses of paclitaxel (as measured in table S2) are indicated by blue font and solid blue bars. n≥2. **E-G**) Spindle polarity in increasing paclitaxel concentrations in MCF10A (**E**), HeLa (**F**), and RPE-1 (**G**) cells. Paclitaxel doses in blue font represent clinically relevant concentrations. n=3. **H-I**) Timelapse analysis of Cal51 cells. Multipolarity before and at anaphase onset in Cal51 cells with fluorescent chromosomes and microtubules due to (**H**) viral-mediated expression of mNeonGreen-tubulin and histone H2B-mScarlet or (**I**) CRISPR/Cas9-mediated labeling of endogenous histone H2B and α-tubulin. Paclitaxel was added either 20 hours before imaging (**H**) or when initiating imaging (I). n ≥25 cells each from one (**H**) or three (**I**) biological replicates. **I**) Data represent mean +/- SEM. **J**) Immunoblot showing HSET expression in Cal51 and MDA-MB-231 cells. **K-M**) MCF10A, HeLa or RPE-1 cells after treatment with 5 nM paclitaxel. Quantification of multipolar spindles +/- SEM in pre-anaphase cells (n≥100 cells from each of 3 replicates) and in anaphase and telophase cells (n≥50 cells from each of 3 independent replicates) in response to paclitaxel in MCF10A (**K**), HeLa (**L**), and RPE-1 (**M**) cells. ***P*<0.001.

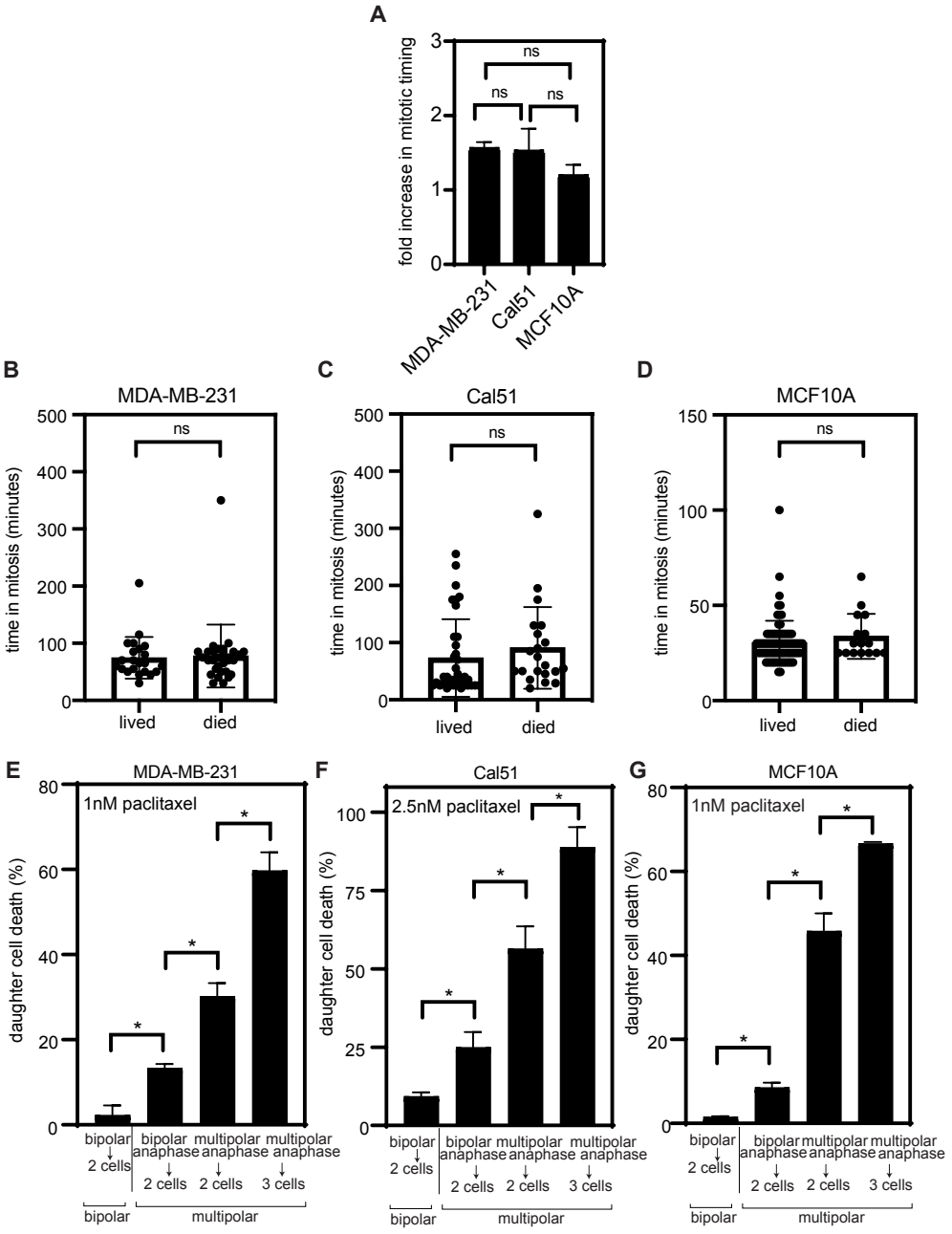

Fig. S2. Persistent multipolarity, rather than mitotic delay, causes paclitaxel-induced cell death. A-G) Results of long term timelapse microscopy of control and paclitaxel treated cells expressing fluorescent histone H2B and α-tubulin. **A**) Mean fold increase in mitotic duration +/- SD, relative to DMSO treated control cells, after treatment with 1 nM paclitaxel in MDA-MB-231 and MCF10A cells and 2.5 nM paclitaxel in Cal51 cells. n≥25 cells per replicate from two

independent replicates. **B-D**) Mean time in mitosis +/- SD, measured as time from NEB to anaphase onset, for divisions in paclitaxel that resulted in viable daughter cells (left) and divisions in which at least one daughter cell died (right) in MDA-MB-231 (**B**), Cal51 (**C**), and MCF10A (**D**) cells. n≥25 cells per replicate from two independent replicates. **E-G**) Quantitation of daughter cell death (mean +/- SEM) in MDA-MB-231 (**E**), Cal51 (**F)**, and MCF10A (**G**) cells after specified types of divisions in paclitaxel. n≥50 daughter cells per replicate from two independent replicates. **P*<0.05. ns=not significant.

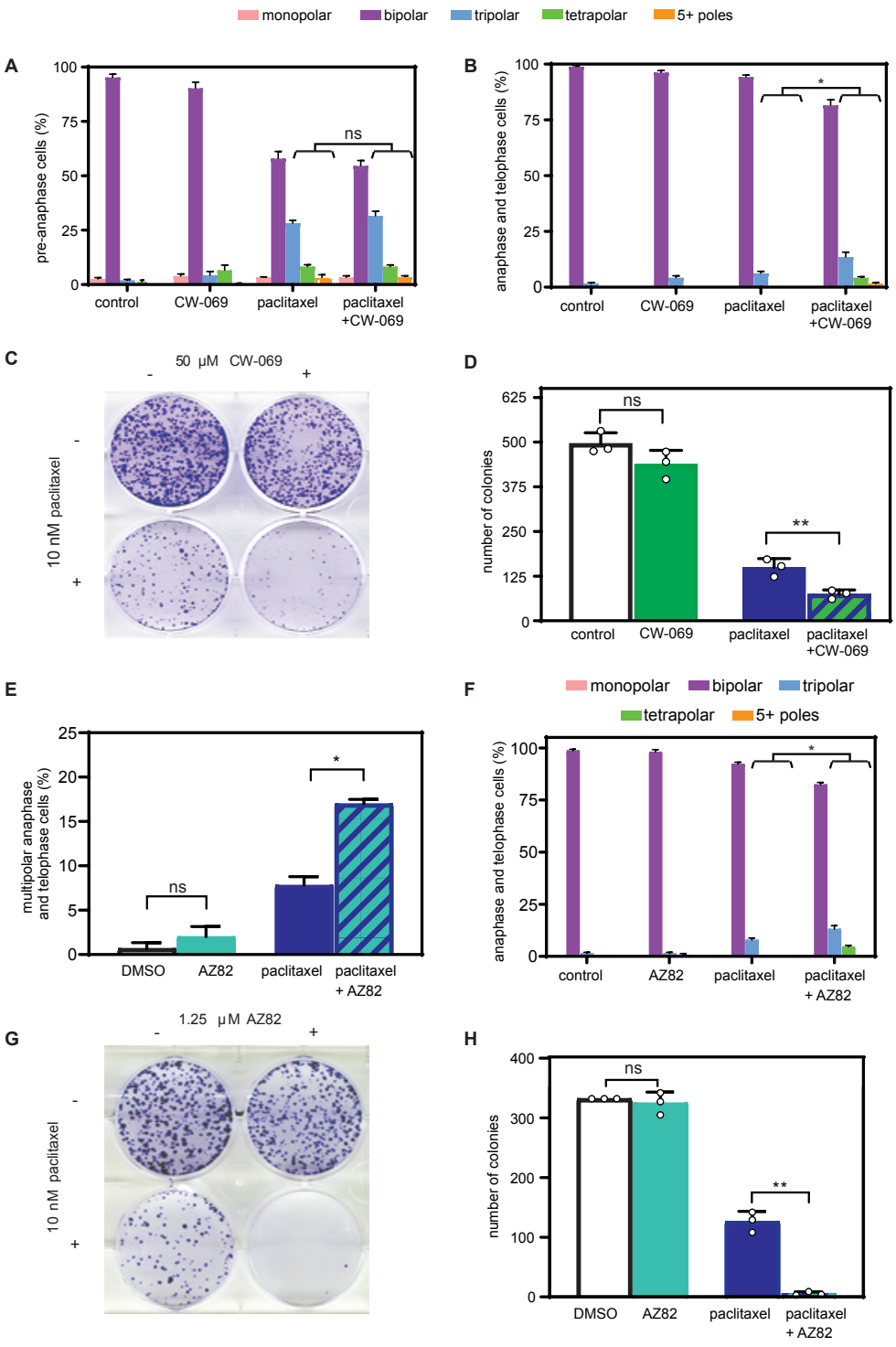

Fig. S3. HSET inhibition impairs multipolar spindle focusing and increases paclitaxel cytotoxicity. A-B) CW-069 treatment in Cal51 cells. Quantification of spindle polarity in (**A**) pre-anaphase and (**B**) anaphase and telophase cells from fixed analysis. Data represent the mean

+/- SEM from three replicates. n≥100 (**A**) or 50 (**B**) cells per replicate. **C**) Representative colony formation assay in the presence of paclitaxel, CW-069, or paclitaxel and CW-069. **D**) Mean number of colonies formed +/- SD in the presence of DMSO control, paclitaxel, CW-069, or both. n=3. **E-F**) Quantification of spindle polarity in anaphase and telophase cells from fixed analysis. Data represent the mean +/- SEM from three replicates of ≥50 cells per replicate. **G**) Representative colony formation assay in the presence of paclitaxel, 1.25 μM AZ82, or both. **H**) Mean number of colonies formed +/- SD in the presence of DMSO control, paclitaxel, AZ82, or both. n=3. *P<0.05. **P<0.001. ns=not significant.

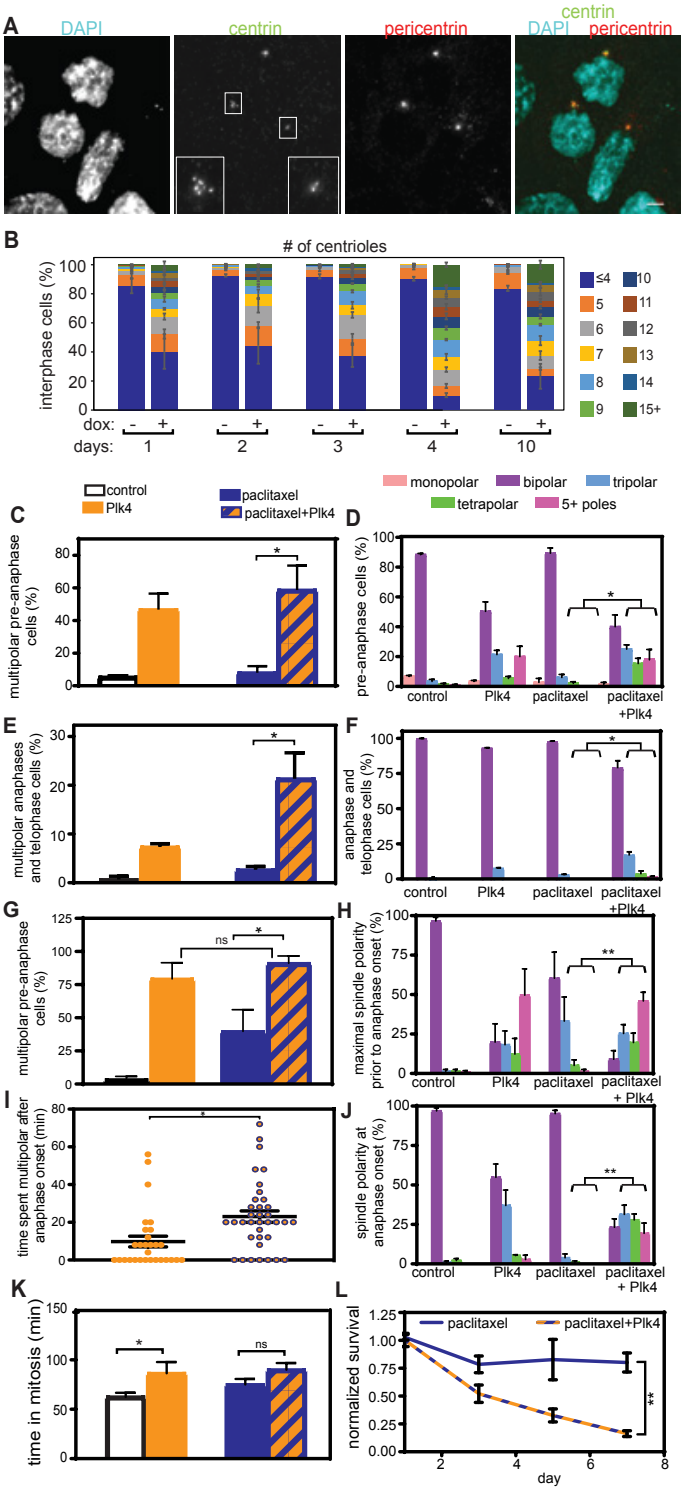

Fig. S4. Plk4 overexpression causes centriole amplification and increases paclitaxel-induced multipolar divisions and cytotoxicity in MCF10A cells. A-B) Tet-inducible Plk4 expression in MCF10A cells. **A**) Representative images of centrioles (centrin) and centrosomes (pericentrin) in

interphase. Insets show enlargements of normal centrosome with 2 centrioles (right) and centrosome with centriole amplification (left). Scale bar = 5 μm. **B**) Quantification +/- SEM of centriole number after inducible expression of Plk4. n=3 independent experiments of ≥100 cells each. **C-F**) Plk4 overexpression increases the incidence of multipolar spindles and multipolar divisions in a subclinical dose of paclitaxel (1 nM). **C**) Spindle polarity (mean +/- SEM) in pre-anaphase cells assessed by fixed analysis. n≥100 pre-anaphase cells in each of three replicates. **D**) Breakdown of spindle polarity in **C**. **E**) Spindle polarity (mean +/- SEM) in anaphase and telophase cells quantified by fixed analysis. n≥50 anaphase and telophase cells from each of three independent replicates. **F**) Breakdown of spindle polarity in **E**. **G-K**) Quantitation of timelapse analysis of Plk4-inducible MCF10A cells expressing histone H2B-mNeonGreen and mScarlet-tubulin treated with vehicle, doxycycline to induce Plk4, 1 nM paclitaxel, or doxycycline and paclitaxel. Data represent mean +/- SEM. n=2-4 independent movies. Quantification of pre-anaphase cell multipolarity (**G**) and the maximal number of poles in pre-anaphase multipolar spindles (**H**). **I**) Time spent multipolar after anaphase onset. Each dot represents an individual cell. **J**) Breakdown of spindle polarity of cells at anaphase onset. **K**) Time in mitosis (mean +/- SEM), from NEB to daughter cell sitting. **L**) Normalized MTT growth curves (mean +/- SEM) in 1 nM paclitaxel with and without Plk4 overexpression. Each curve is normalized to the -paclitaxel (DMSO treated) control. n=3. *P<0.05. **P<0.001. ns=not significant.

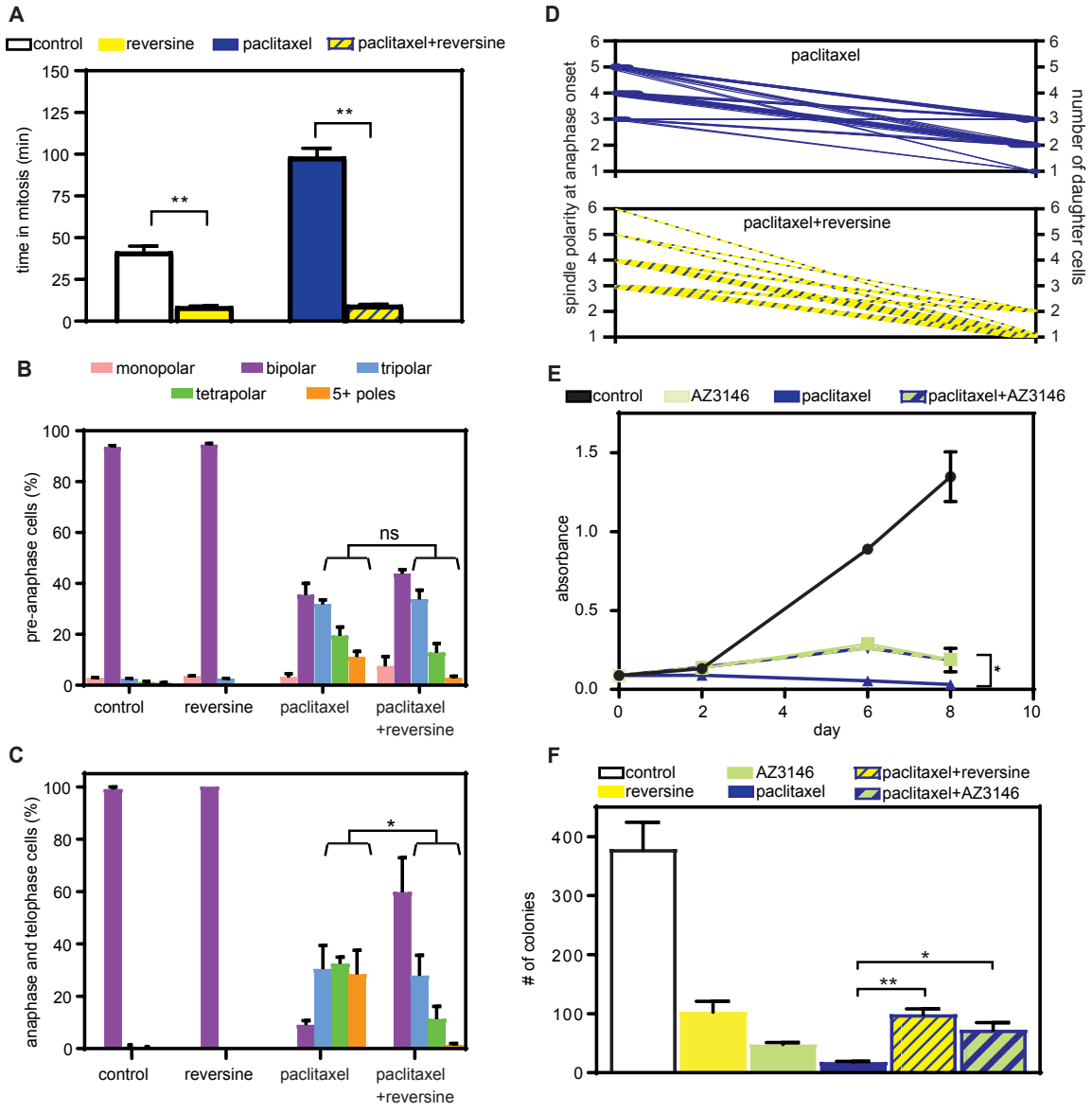

Fig. S5. Mps1 inhibition reduces sensitivity to paclitaxel in MDA-MB-231 cells. A) Time in mitosis, quantified by 24-hour fluorescent timelapse microscopy at 3-minute intervals as the time from NEB to anaphase onset. Data represent mean +/- SEM of two movies. n=81, 89, 89, 76 cells, respectively, for each condition. **B-C**) Spindle polarity (mean +/- SEM) in (**B**) pre-anaphase and (**C**) anaphase and telophase cells assessed by fixed analysis. n≥100 cells in each of three (B) or four (C) independent replicates. **D**) Multipolar spindle focusing after anaphase onset

in reversine treated cells. Each cell is represented by a line connecting the number of spindle poles at anaphase onset (left) and the number of daughter cells formed (right). **E-F**) The effect of the Mps1 inhibitor, AZ3146, on cell survival in paclitaxel. **E**) MTT metabolic viability assay (mean +/- SEM). n=2. **F**) Colony formation assay following treatment with reversine or AZ3146 in a clinically relevant dose of paclitaxel (10 nM) in MDA-MB-231 cells. n≥2. *P<0.05. **P<0.001. ns=not significant.

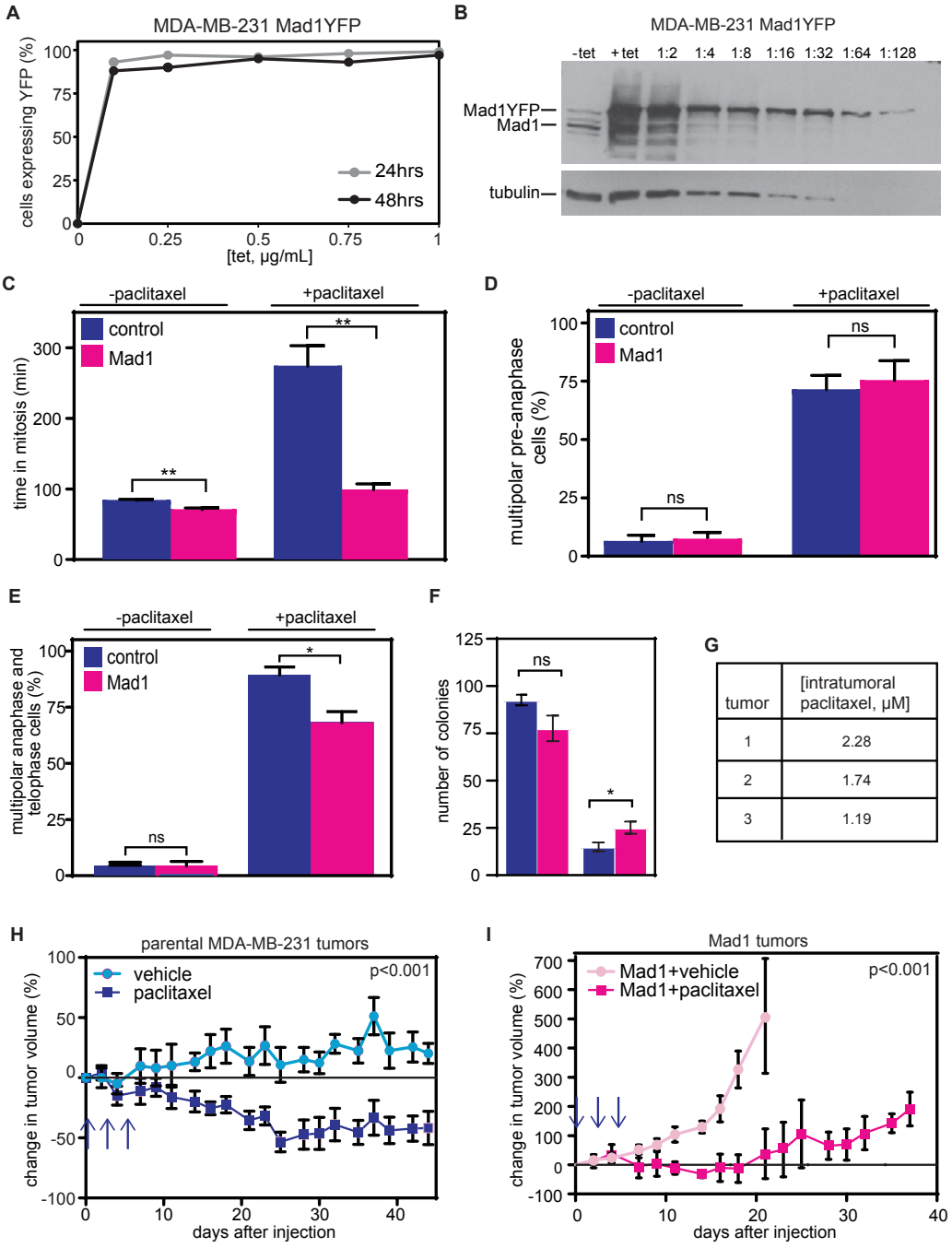

Fig. S6. Mad1 upregulation in MDA-MB-231 cells decreases multipolar divisions and response to paclitaxel. A-B) Uniform, tet-inducible expression of Mad1 in MDA-MB-231 cells. **A**) Percent of MDA-MB-231 cells expressing Mad1-YFP 24 and 48 hours after addition of the indicated doses of tet. **B**) Immunoblot showing Mad1 expression 24 hours after addition of 2

μg/mL tet. **C-F**) Effects of Mad1 upregulation in MDA-MB-231 cells treated with 10 nM paclitaxel. **C**) Time in mitosis, assessed as the time from cell rounding to the time the first daughter cell sat down. Data represent the mean +/- SEM of two brightfield movies. n=103, 100, 67 and 87 cells for control, Mad1, paclitaxel, and paclitaxel+Mad1, respectively. **D**) Spindle polarity (mean +/- SEM) in pre-anaphase cells quantified by fixed analysis. n≥100 cells per replicate in each of 3 biological replicates. **E**) Spindle polarity (mean +/- SEM) in anaphase and telophase cells quantified by fixed analysis. n≥50 cells per replicate in each of 3 biological replicates. **F**) Colony formation assay. Data represent mean +/- SEM. n=3. **G**) HPLC measurements of orthotopic intratumoral drug levels 20 hours after iv injection with 30 mg/kg paclitaxel. Each tumor contains a clinically relevant concentration. **H-I**) Percent change in tumor volume after vehicle or paclitaxel treatment in (**H**) parental or (**I**) Mad1 upregulated tumors. Arrows indicate days of paclitaxel treatments. n=6 tumors per treatment condition. **P*<0.05. ***P*<0.001. ns=not significant.

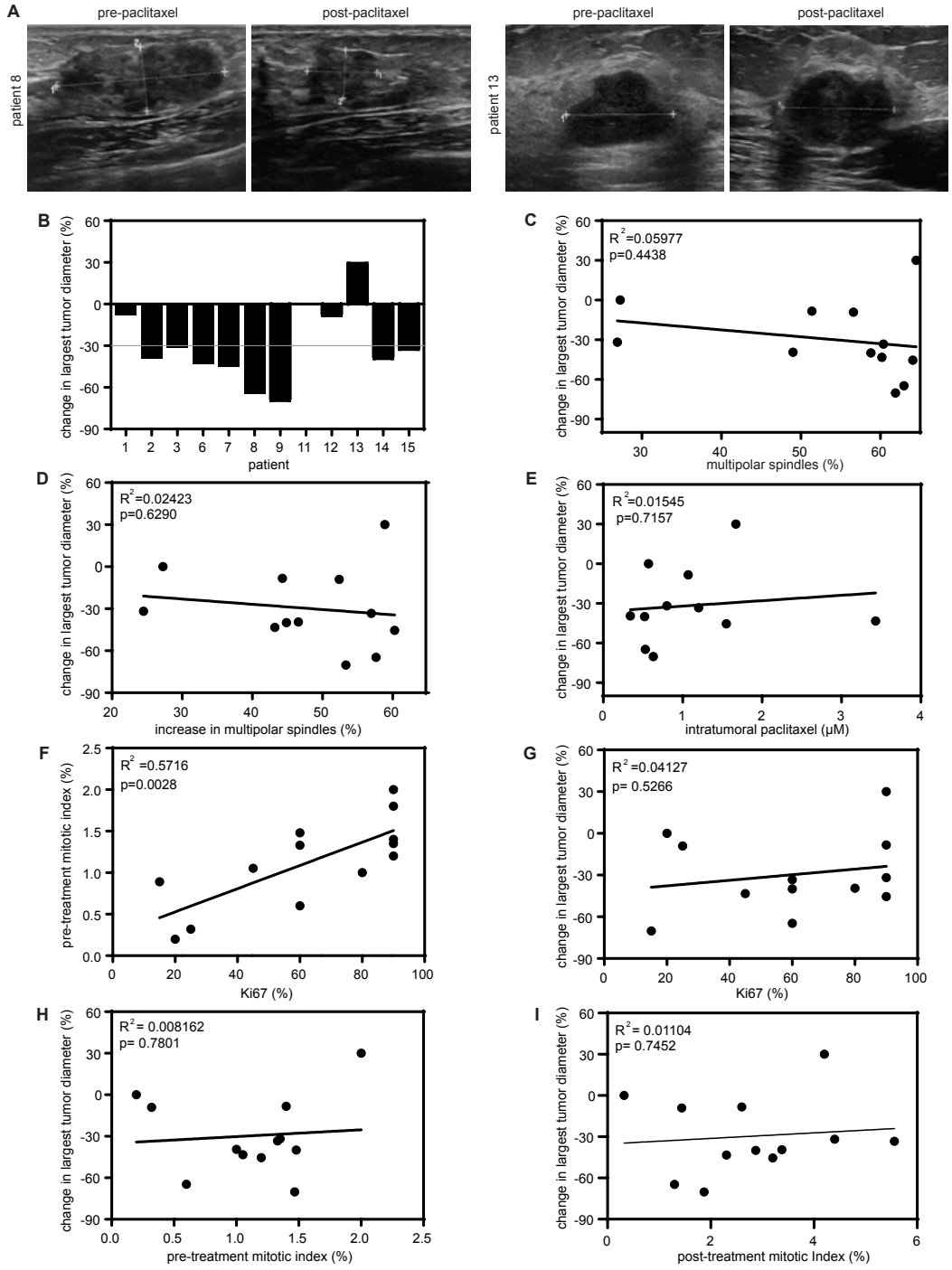

Fig. S7. Patient response cannot be predicted by current measures. A) Representative breast ultrasound images of a paclitaxel responder (left) and non-responder (right). **B**) Waterfall plot showing the percent change in largest tumor diameter following paclitaxel treatment. Response to paclitaxel is defined as having a decrease of ≥30% in the largest tumor diameter (grey line),

according to RECIST 1.1 criteria (*26)*. Three patients were excluded from response analysis due to the inability to accurately determine tumor measurements (patient 5), deviation from treatment protocol (patient 4), or withdrawal of patient consent (patient 10). **C-E**) Tumor response does not correlate with percent multipolar spindles in patients 20 hours after treatment with paclitaxel (**C**), the increase in multipolar spindles between pre-treatment and 20 hours post-paclitaxel treatment (**D**), or the intratumoral concentration of paclitaxel (**E**). Note that the vast majority of mitotic cells detected in patient tumors have not yet entered anaphase, precluding analysis of the extent to which specific tumors focus multipolar spindles as they proceed through mitosis. **F-G**) Ki-67, a measure of tumor proliferation, correlates with mitotic index (**F**), but not tumor response (**G**). **H-I**) Tumor response does not correlate with mitotic index before (**H**) or after (**I**) 20 hours of paclitaxel.

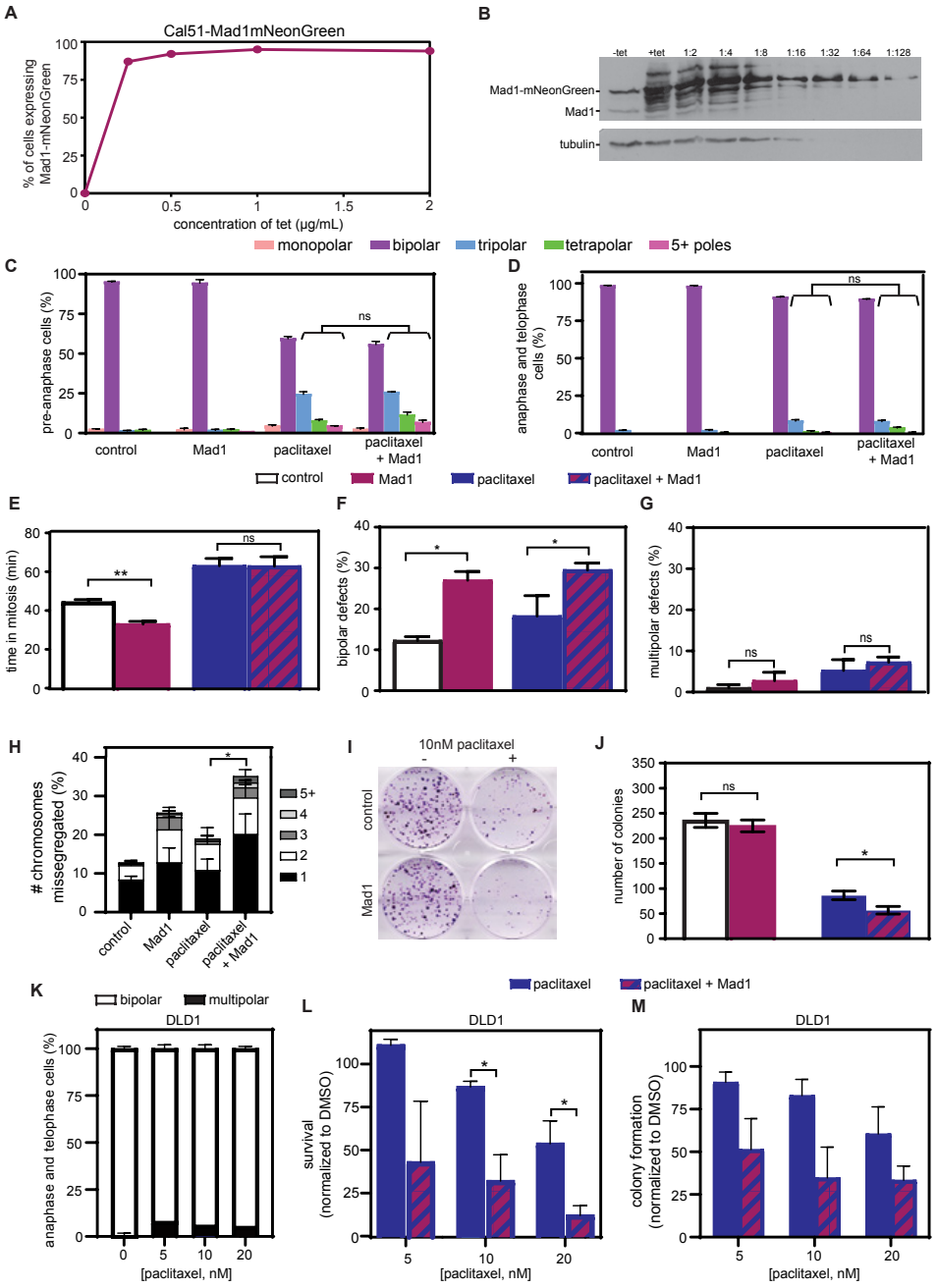

Fig. S8. Inducible expression of Mad1 increases CIN on bipolar spindles and sensitivity to paclitaxel in Cal51 and DLD1 cells. A) Quantification of the percent of Cal51 cells expressing Mad1-mNeonGreen 24 hours after addition of the indicated concentrations of tet. **B**) Immunoblot showing level of Mad1 expression in Cal51 cells 24 hours after addition of 2 µg/mL tet. Image is representative of 3 blots. **C-J**) Effects of Mad1 upregulation in Cal51 cells treated with 10 nM

paclitaxel. **C**) Spindle polarity (mean +/- SEM) in pre-anaphase cells (n≥100 cells in each of 3 independent replicates) or **D)** anaphase and telophase cells (n≥50 cells in each of 3 independent replicates) assessed by fixed analysis. **E**) Mitotic timing, assessed by brightfield timelapse microscopy, as the time from NEB to anaphase onset. Values indicate mean +/- SEM from ≥40 cells. **F-H**) Quantification of the percent of cells with mitotic errors with **F**) bipolar or **G**) multipolar mitotic spindles. Data represent mean +/- SEM from ≥ 50 anaphase and telophase cells. n≥4. **H**) Quantification of the number of aberrant chromosomes per cell. **I**) Representative image of colony formation assay. **J**) Quantification of I. Data represent mean +/- SEM. n=5 independent experiments. **K-M**) Mad1 upregulation in DLD-1 cells treated with the indicated concentrations of paclitaxel. **K**) Quantification of spindle polarity in anaphase and telophase cells. n=50 cells in each of 3 independent replicates **L**) Cell survival as assessed by cell counts (mean +/- SEM) after 10 days of treatment with low nanomolar doses of paclitaxel. n≥2. **M**) Clonogenic survival (mean +/- SEM) relative to DMSO treated controls. n=2. **P*<0.05. ***P*<0.001. ns=not significant.

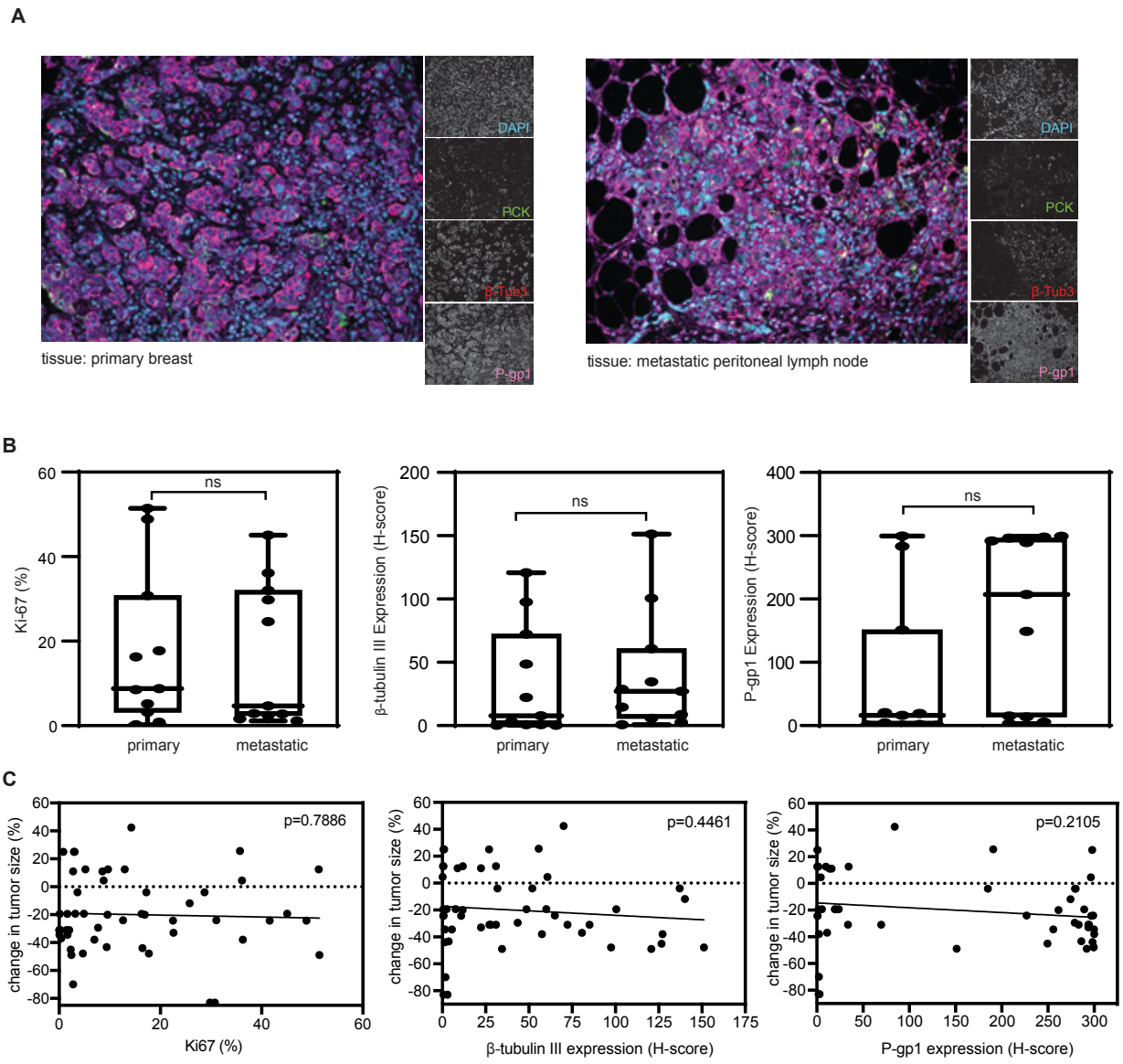

Fig. S9. Previously suggested biomarkers do not correlate with taxane response in metastatic breast cancer. A) Potential biomarkers were measured by quantitative immunofluorescence in matched primary (left) and metastatic (right) tumor tissues. PCK=pan-cytokeratin. β-Tub3=β-tubulin III. P-gp1=phospho-glycoprotein 1. **B**) Expression of Ki67, β-Tub3 and P-gp1 does not significantly differ between primary and metastatic breast tumors. **C**) Expression of Ki67, β-Tub3 and P-gp1 does not correlate with response to taxane in metastatic breast cancer.

Table S1. 80 mg/m^2 paclitaxel trial patient characteristics.

Patient	Subtype	Stage	Age	Race	Ethnicity	Ki67
1	TNBC	T1N0M0	38	White	Non-Hispanic or Latino	90%
2	TNBC	T2N2M0	62	White	Non-Hispanic or Latino	80%
3	TNBC	T2N2M0	59	White	Non-Hispanic or Latino	90%
4	TNBC	T2N2M0	58	White	Non-Hispanic or Latino	90%
5	ER+/PR+/HER2-	T3N1M0	57	White	Non-Hispanic or Latino	25%
6	TNBC	T2N2M0	50	White	Non-Hispanic or Latino	45%
7	ER+/PR+/HER2-	T2N1M0	46	White	Non-Hispanic or Latino	90%
8	TNBC	T2N2M0	43	White	Non-Hispanic or Latino	60%
9	ER+/PR+/HER2-	T2N1M0	37	White	Non-Hispanic or Latino	15%
10	consent withdrawn					
11	ER+/PR+/HER2-	T3N1M0	52	White	Non-Hispanic or Latino	20%
12	ER+/PR+/HER2-	T2N0M0	55	Black	Non-Hispanic or Latino	25%
13	TNBC	T2N0M0	49	White	Non-Hispanic or Latino	90%
14	ER-/PR+/HER2-	T1N0M0	44	White	Non-Hispanic or Latino	60%
15	TNBC	T2N0M0	37	White	Non-Hispanic or Latino	60%

TNBC: Triple negative breast cancer. ER: Estrogen Receptor. PR: Progesterone Receptor. HER2: human epidermal growth factor receptor 2. TNM: primary tumor (T), regional lymph nodes (N), distant metastases (M). T1 indicates a tumor ≤ 2 cm, T2 indicates a tumor >2 cm but no more than 5 cm, and T3 indicates a tumor >5 cm in greatest dimension. Node (N) indicates the regional lymph node status according to the American Joint Committee on Cancer (AJCC). All patients in this study were M0, meaning there was no evidence of metastatic spread. Patient 9

was diagnosed with invasive lobular carcinoma. All other patients were diagnosed with invasive ductal carcinoma.

Table S2. Intracellular paclitaxel measurements.

	[Intracellular paclitaxel, μM] ± SEM			
treatment (nM)	MDA-MB-231	MCF10A	HeLa	RPE
DMSO	ND	ND	ND	ND
0.01	-	ND	-	-
1	-	0.22 ± 0.06	-	-
5	6.8 ± 2.61	1.24 ± 0.11	0.94 ± 0.07	0.84 ± 0.05
10	8.0 ± 2.01	2.56 ± 0.43	1.86 ± 0.14	1.79 ± 0.09
20	17.7 ± 5.49	4.72 ± 0.70	2.79 ± 0.14	2.84 ± 0.43
50	39.9 ± 13.75	11.19 ± 1.90	9.51 ± 0.21	7.17 ± 0.99
100	55.2 ± 12.01	22.48 ± 4.06	18.21 ± 2.70	10.99 ± 1.04
500	78.7 ± 16.14	-	43.01 ± 1.62	25.82 ± 0.85
1000	104.4 ± 12.20	-	47.39 ± 2.41	32.15 ± 5.94

Intracellular paclitaxel levels were measured by LC-MS/MS 20 hours after addition of paclitaxel to cell culture media. Values represent mean ± SEM. n=3. ND indicates not detected. (-) indicates not tested.

Table S3. CENP-E inhibition is synergistic with clinically relevant doses of paclitaxel.

Compound 1	Compound 2	Fraction Affected	parameters			Combination Index (CI)
[paclitaxel, nM]	[GSK923295, nM]		m	Dm	r	
1	0	0.18	0.74103 +/- 0.13876	3.53579	0.95122	NA
5	0	0.54				NA
10	0	0.788				NA
20	0	0.868				NA
500	0	0.961				NA
0	25	0.075	3.12554 +/- 0.32587	59.4719	0.9893	NA
0	50	0.286				NA
0	75	0.663				NA
0	100	0.864				NA
1	50	0.355	NA	NA	NA	1.65082
5	50	0.812				0.72281
10	50	0.832				0.83041
20	50	0.872				0.87968
500	50	0.963				2.03580

Chou-Talalay non-constant ratio synergy testing of paclitaxel and the CENP-E inhibitor GSK923295 in Cal51 cells. Combination Index (CI)=1 indicates an additive response, CI>1 an antagonistic one, and CI<1 is synergistic. m = kinetic order of single drug curves, Dm = IC_{50}, r = linear correlation coefficient for median affect plot, NA=not applicable.

Table S4. Metastatic patient characteristics and taxane response.

Patient Characteristics (n=37)	
Age (Mean +/- SD)	59.7 +/- 12.8
Hormone receptor positive (%)	54 (20/37)
HER2 positive (%)	22 (8/37)
TNBC (%)	24 (9/37)
Stable disease (%)	51 (19/37)
Partial response (%)	41 (15/37)
Progressive disease (%)	8 (3/37)
Mean reduced tumor size (%)	-34
Range of reduced tumor size (%)	-4 to -83
Mean duration of taxane therapy (days)	177.9
Range of taxane therapy (days)	43 to 883

TNBC: Triple negative breast cancer. Hormone receptor positive (ER: Estrogen Receptor or PR: Progesterone Receptor positive). HER2: human epidermal growth factor receptor 2. Stable disease, partial response, and progressive disease were evaluated by RECIST 1.1 guidelines (*26*).

Table S5: Paclitaxel concentration measurements in patient tumors by tumor weight.

patient	[plasma paclitaxel, μM]	[intratumoral paclitaxel, μM]	degree of concentration
1	0.023	1.17	51x
2	0.040	0.39	10x
3	0.030	0.56	19x
4	0.027	0.72	27x
5	0.031	ND	-
6	0.020	3.61	181x
7	0.013	2.24	172x
8	0.011	0.53	48x
9	0.020	0.60	30x
11	0.038	0.88	23x
12	0.040	ND	-
13	0.094	1.98	21x
14	0.048	0.66	14x
15	0.035	2.16	62x

Intratumoral and plasma paclitaxel levels were measured by HPLC analysis 20 hours after the first dose of paclitaxel. Paclitaxel was quantified per tumor weight assuming a tumor density of 1 g/cm^3 or 1 g/mL. Paclitaxel was not determined (ND) in the tumor biopsies for subjects 5 and 12 due to insufficient tumor tissue collected by biopsy. Patient 10 withdrew consent.

Movie S1. Normal mitosis in Cal51 cell. Timelapse analysis of control (DMSO treated) Cal51 cell with endogenously tagged histone H2B-mScarlet and mNeonGreen-tubulin showing typical bipolar spindle elongation during anaphase.

Movie S2. Abbreviated multipolar anaphase in paclitaxel. Timelapse analysis of Ca51 cell undergoing a short, multipolar anaphase elongation in 10 nM paclitaxel.

Movie S3. Rapid focusing of a paclitaxel-induced multipolar spindle in anaphase. Timelapse analysis of Ca51 cell in 10 nM paclitaxel displaying rapid focusing of a multipolar spindle shortly after anaphase onset resulting in a pseudo bipolar division and two daughter cells.

Movie S4. Control mitosis in MCF10A cell. Timelapse analysis of control (DMSO treated) MCF10A cells expressing histone H2B-mNeonGreen and mScarlet-tubulin. A normal bipolar division produces two daughter cells that remain viable for the duration of the timelapse.

Movie S5. Chromosome missegregation on paclitaxel-induced multipolar spindle that focuses after anaphase onset. Timelapse analysis of Plk4 inducible MCF10A cell +doxycycline that, in the presence of 1 nM paclitaxel, establishes a transient multipolar spindle that is subsequently focused to produce two daughter cells with unequal genetic content, one of which subsequently dies.