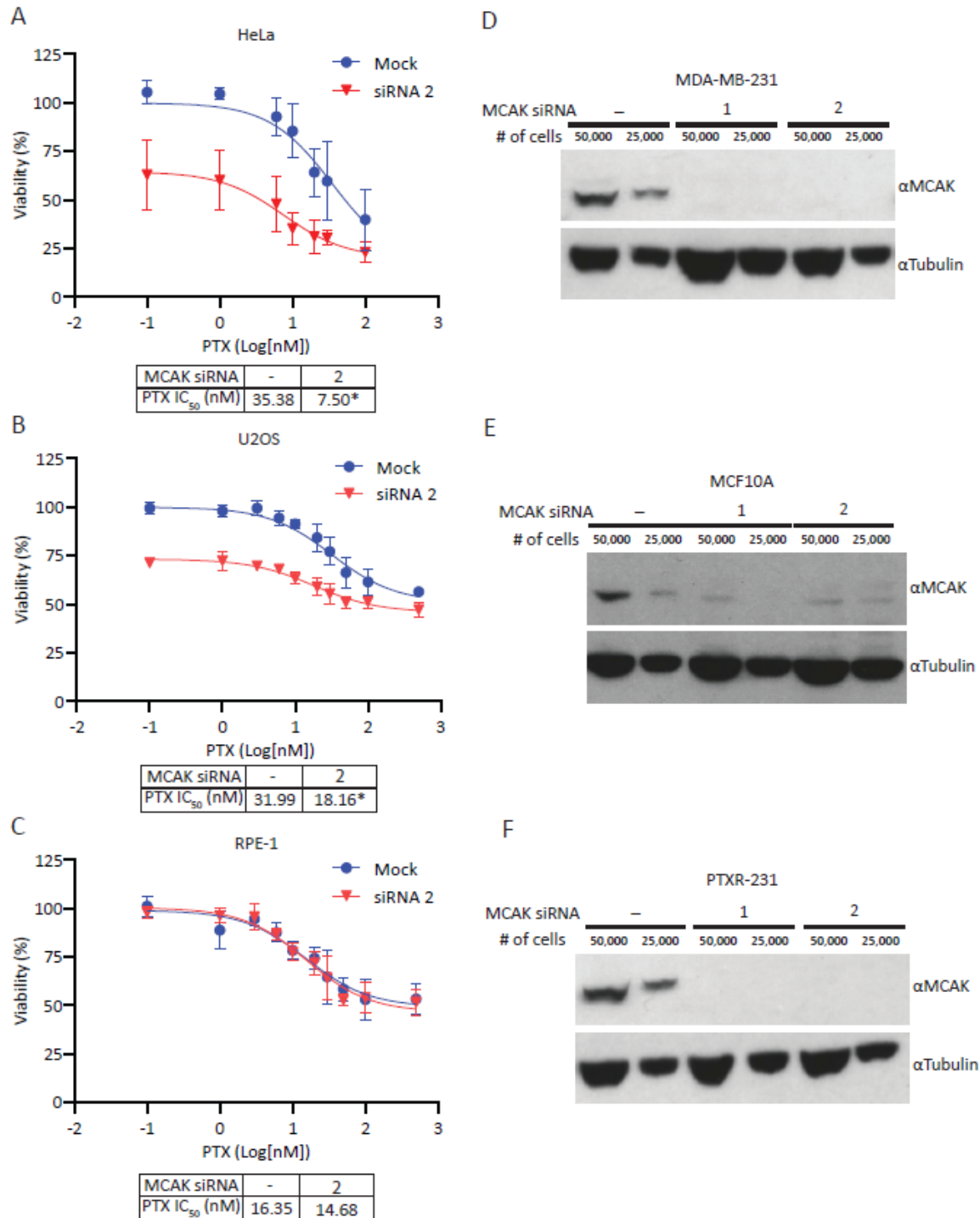
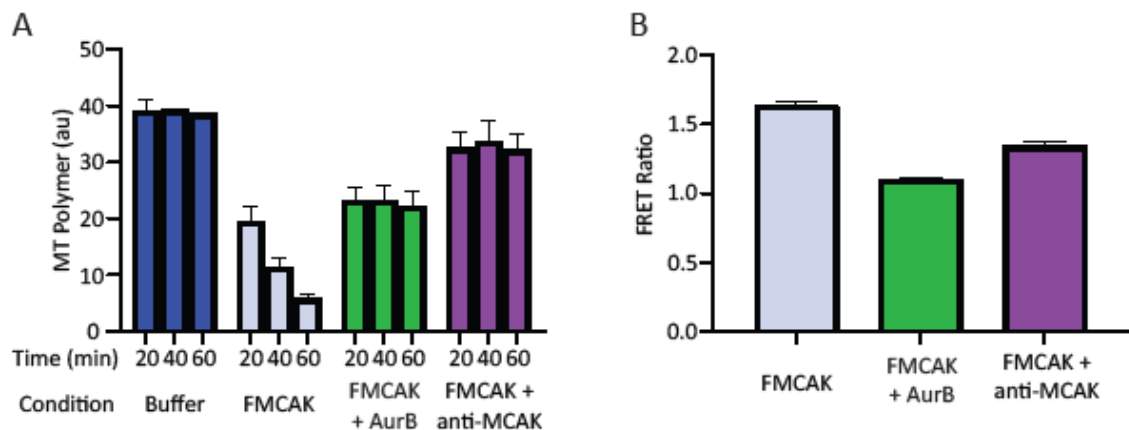


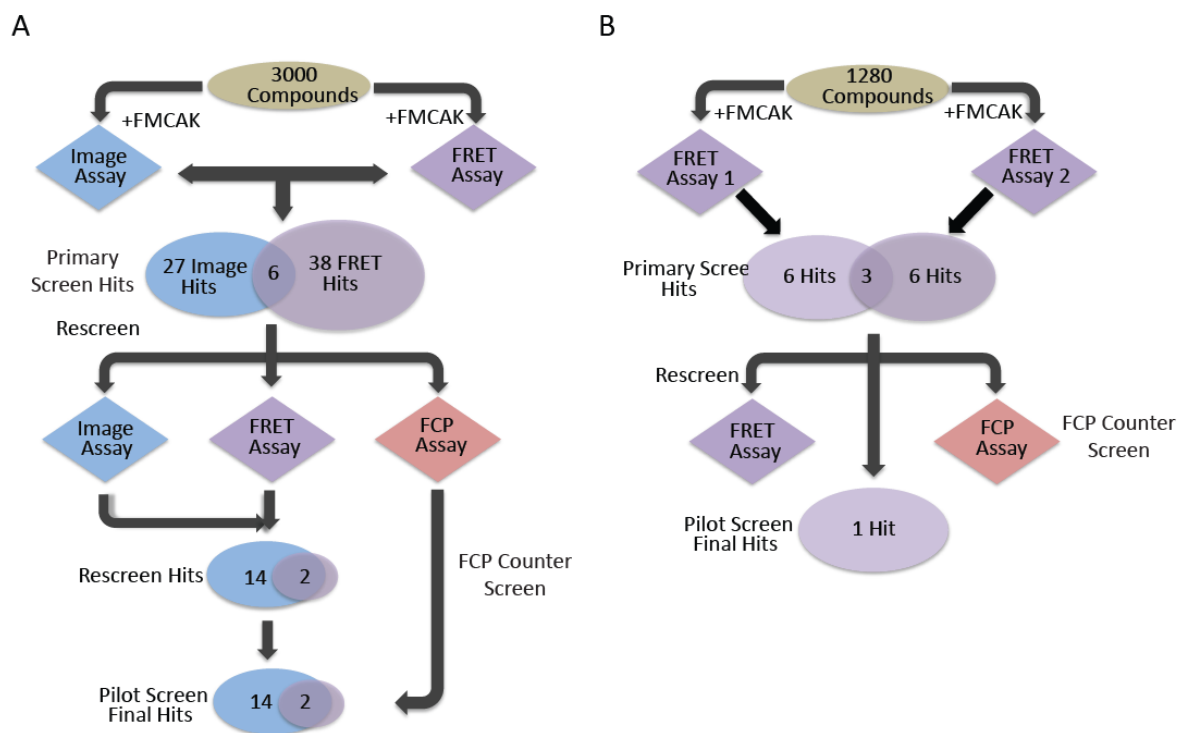
**Figure S1.** MCAK overexpression correlates with poor prognoses. **(A)** MCAK/KIF2C expression data from TCGA BC dataset from breast cancer tumors of different receptor status (Normal N = 112, LumA = 516, LumB = 261, HER2 = 80, Basal = 167) plotted as box-and-whisker plots where the box represents the upper and lower quartiles, and the whiskers represent the upper and lower ranges. ANOVA with Tukey's post hoc test for significance was used to compare groups. \*\*\*\* p < 0.0001. LumA=Luminal A; LumB=Luminal B; HER2=HER2-enriched. **(B)** MCAK/KIF2C expression data from GSE25066 dataset from breast tumors in which patients had either a pathological Complete Response (pCR; N = 196) or had Residual Disease (RD; N = 312). MCAK/KIF2C levels are plotted for each condition as a box-and-whisker plot. The Mann-Whitney test for significance was used to compare groups. \*\*\*\* p<0.0001.



**Figure S2.** MCAK loss sensitizes cancer cells to taxanes. **(A-C)** Dose response curves and associated IC<sub>50</sub> values of paclitaxel in HeLa **(A)**, U2OS **(B)**, and RPE-1 **(C)** cells from n = 6, 5, and 4 independent experiments respectively in which duplicate values were averaged and normalized versus control DMSO treatment. The log(inhibitor) vs response (three parameters) analysis in prism was used to generate curves and compare IC<sub>50</sub> values. \* p<0.05. **(D-F)** Western blot of control Neg2 (-) or MCAK knockdown cells with two independent siRNAs (1 or 2) in either MDA-MB-231 **(D)**, MCF10A **(E)** or PTXR-231 **(F)** cells. Blots were probed with anti-MCAK (top) or anti-alpha-tubulin (bottom).

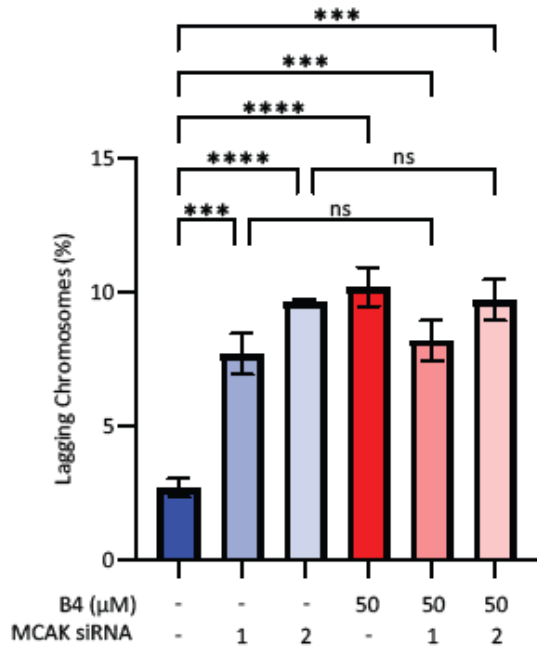


**Figure S3.** Controls for screening assays. **(A)** FIJI quantification of MT polymer amounts under the indicated conditions. Bars represent mean  $\pm$  SD of 10 replicates in a single 96-well plate and represents two independent experiments. **(B)** The FRET ratio (mean  $\pm$  SD, N=10) was calculated for each condition as the ratio of the 525 to 475 nm emissions using a filter-based assay in an Appliskan plate reader. Representative of two independent experiments.

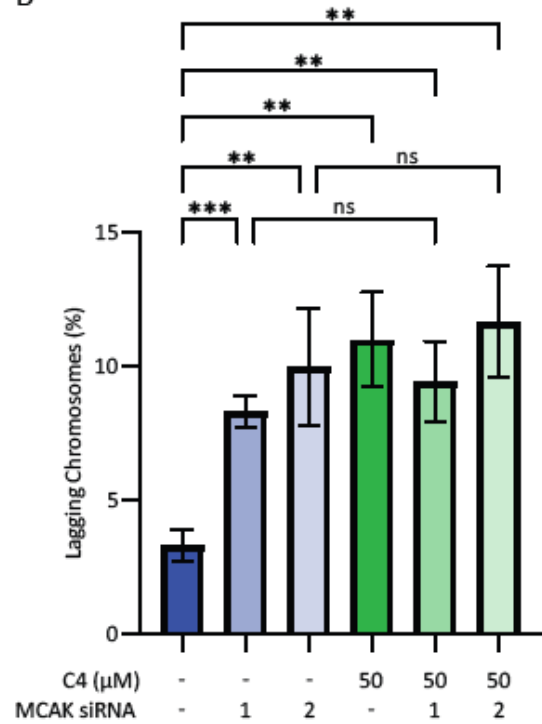


**Figure S4.** Summary of screens used to identify MCAK inhibitors. **(A)** Flowchart schematic of 3000-compound screen using both a MT depolymerization assay and a FRET-based assay. **(B)** Flowchart schematic of 1280-compound screen done in duplicate with the optimized FRET-based assay.

A

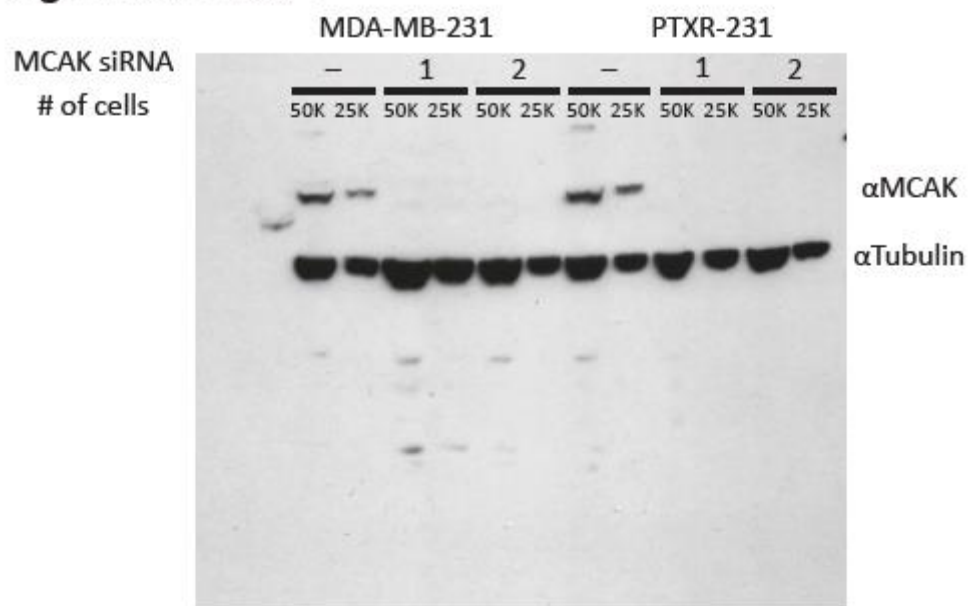


B

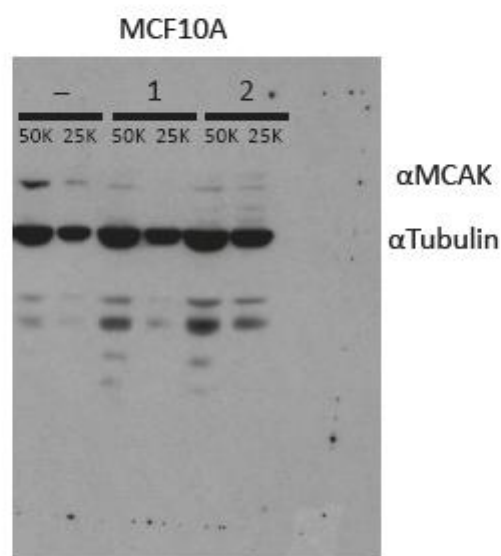


**Figure S5.** MCAK aneuploidy with knockdown in HeLa cells. **(A, B)** Percentage of HeLa cells in anaphase with lagging chromosomes. Cells were treated with control or MCAK siRNAs and DMSO, 50 μM B4 **(A)**, or 50 μM C4 **(B)**. 100 anaphase cells were counted per condition for each of three independent experiments and categorized as containing or not containing a lagging chromosome. Conditions were compared with a Student's *t*-test. ns = not significant, \*\*  $p<0.01$ , \*\*\*  $p<0.001$ , \*\*\*\*  $p<0.0001$ .

**Figure S2 D and F**



**Figure S2 E**



**Figure S5** Whole of western blot figures