Supplemental Materials

Molecular Biology of the Cell

Sankaran et al.

SUPPLEMENTAL FIGURE LEGEND

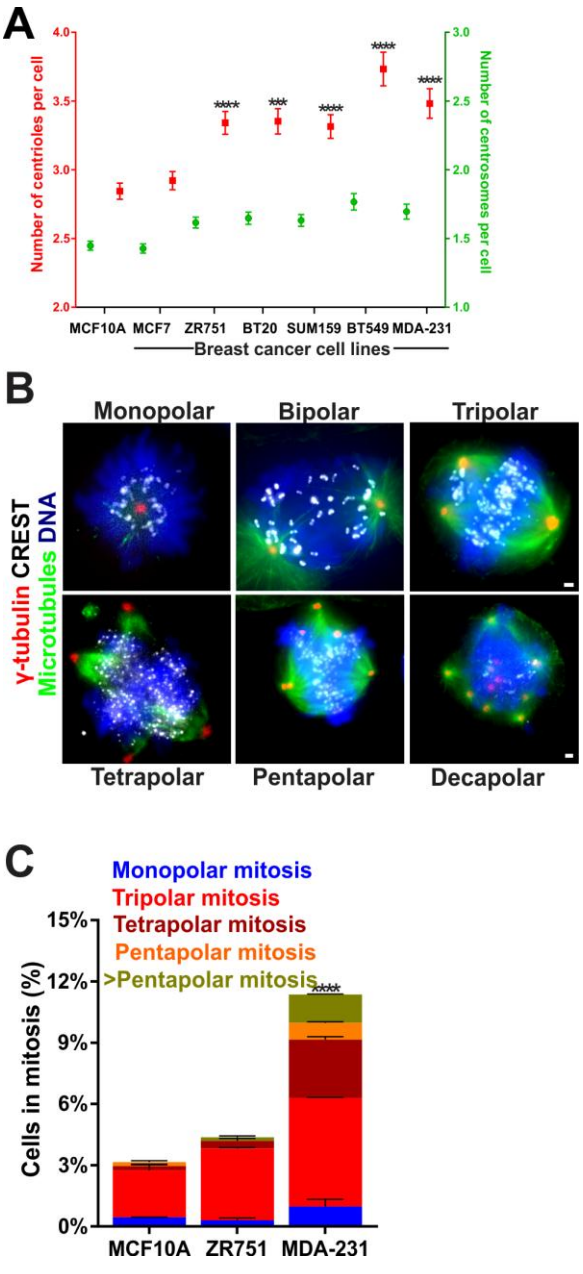


Figure S1: Aggressive breast cancer cells exhibit increased CA and chromosome mis-segregation. (A) Number of the total centrioles (red) and centrosomes (green) in all cell populations of breast cancer cell lines. (B) Monopolar, bipolar and multipolar mitoses in MDA-231 cells. Cells stained for PCM (γ-tubulin; red), microtubules (α-tubulin; green), DNA (Hoechst 33342; blue) and kinetochores (CREST; grayscale). (C) Percentage of mitotic cells exhibiting monopolar, bipolar and multipolar mitosis. Mean±SEM. (A,C) Statistical tests compare to MCF10A cells. Fischer's exact test and Mann-Whitney U test. *p<0.05, **p<0.01,***p<0.005 and ****p<0.0005. Scale bars, 1 μm.

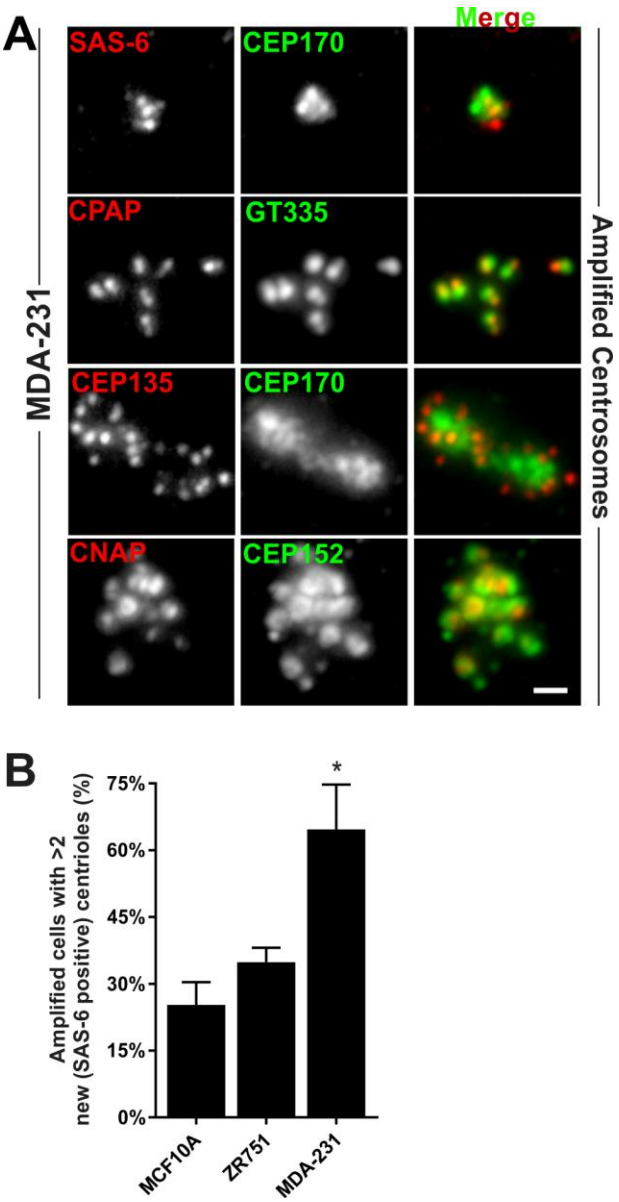


Figure S2: Centriole overduplication in breast cancer cells with amplified centrosomes. (A) Both non-amplified and amplified centrioles contain a full complement of representative centriole proteins. Panels from top to bottom are centrioles stained for SAS-6, CEP170; CPAP, glutamylated tubulin (GT335); CEP135, CEP170; CNAP and CEP152. (B) Percentage of cells with amplified centrioles that show new centriole overduplication (based on SAS-6 foci). Mean±SEM. Statistical tests compare to MCF10A cells. Student's t-test *p<0.05, **p<0.01, ***p<0.005 and ****p<0.0005. Scale bar, 1 μm.

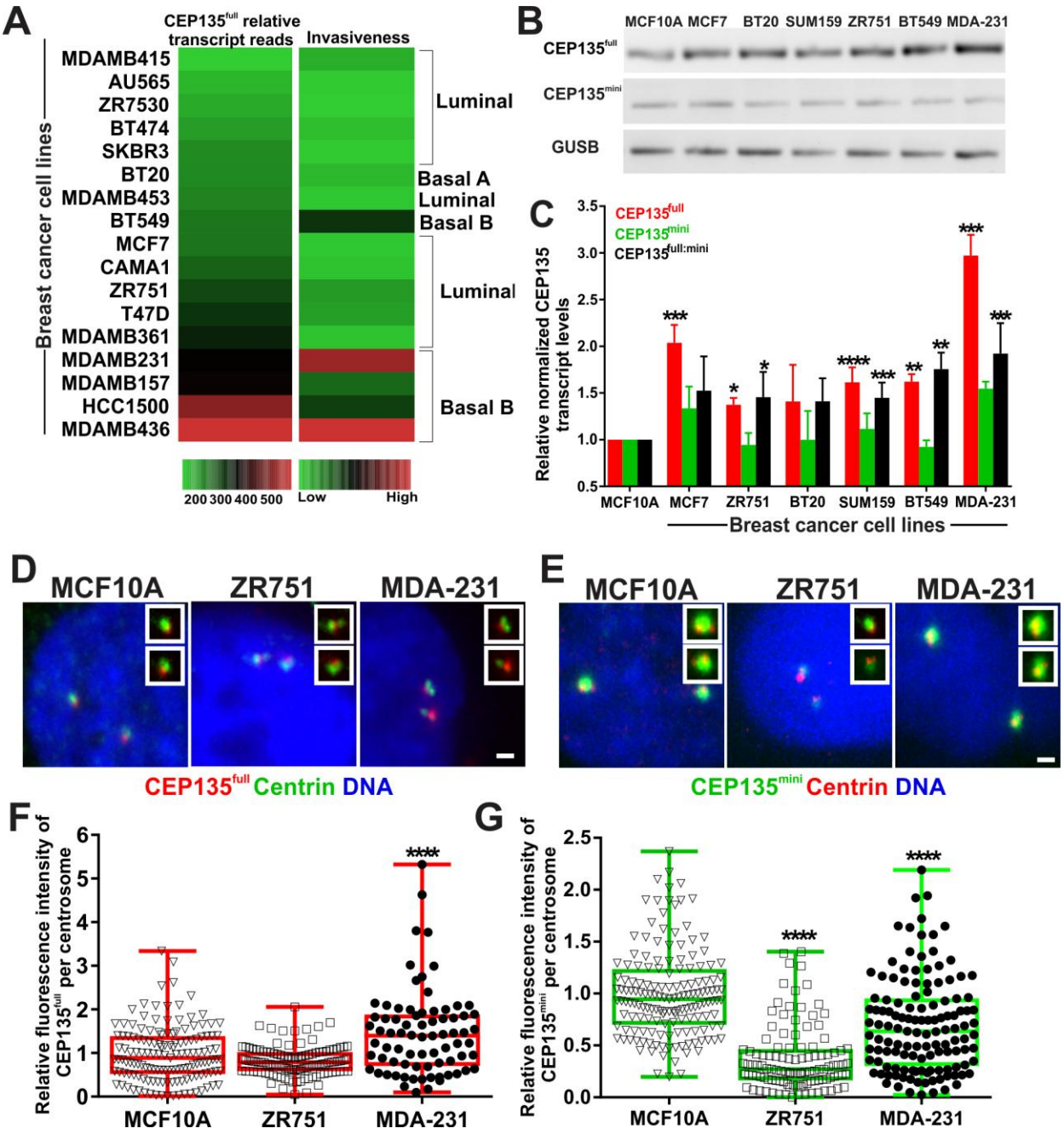


Figure S3: CEP135 transcript and protein levels are altered in breast cancer cells. (A) Comparison of CEP135^{full} transcript reads and invasiveness in various breast cancer cell lines (Adapted from (Barretina et al., 2012; Neve et al., 2006)). (B) CEP135^{full}, CEP135^{mini} and GUSB (control) RT-PCR in breast cancer cells. (C) CEP135^{full} (red) and CEP135^{mini} (green) transcript levels normalized to GUSB levels and represented relative to their corresponding transcript levels in MCF10A cells. The CEP135$^{full:mini}$ transcript ratio (black) in breast cancer cells is also represented relative to MCF10A cells. (D) CEP135^{full} (red) and centrin (green) at

centrosomes of G2 phase MCF10A, ZR751 and MDA-231 cells. Corresponding images in Figure 3E. (E) CEP135^{mini} (green) and centrin (red) at centrosomes of G2 phase MCF10A, ZR751 and MDA-231 cells. Corresponding images in Figure 3F. (F) Quantification of CEP135^{full} protein fluorescence intensity per G2 phase centrosome relative to MCF10A cells. (G) Quantification of CEP135^{mini} protein fluorescence intensity per G2 phase centrosome relative to MCF10A cells. (C,F,G) Statistical tests compare to MCF10A cells. Mean±SEM. Student's t-test and Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.005 and ****p<0.0005. Scale bars, 1 µm.

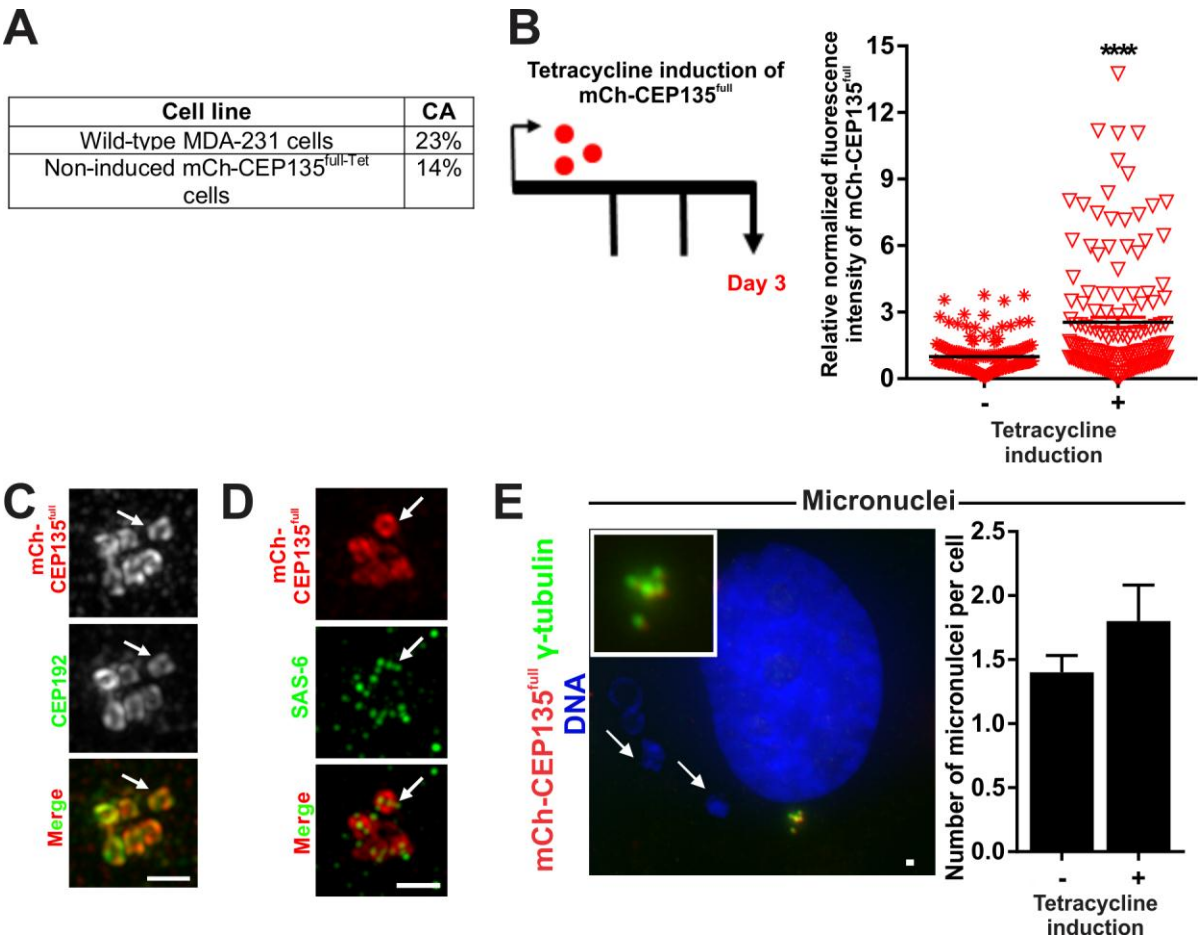


Figure S4: Elevated CEP135^{full} expression increases centriole number, CA and chromosome mis-segregation in breast cancer cells. (A) CA frequency in control MDA-231 and non-induced mCh-CEP135$^{Full-Tet}$ MDA-231 cell lines. (B) Left panel, schematic of tetracycline-induced mCh-CEP135$^{Full-Tet}$ MDA-231 cells. Right panel, relative normalized fluorescence intensity of mCh-CEP135^{full} in non-induced and induced mCh-CEP135$^{full-Tet}$ MDA-231 cells. (C) mCh-CEP135^{full} (red) localization relative to CEP192 (green). Arrows denote centrioles with mCh-

CEP135^{full} at the cylinder walls. (D) mCh-CEP135^{full} (red) localization relative to SAS-6 (green). Arrows denote SAS-6 foci at the core cylinder. (E) Left panel, micronuclei in mCh-CEP135$^{Full\text{-}Tet}$ MDA-231 cells. Arrows denote micronuclei. Right panel, micronuclei per cell in non-induced and induced mCherry-CEP135$^{full\text{-}Tet}$ MDA-231 cells. Mean±SEM. Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.005 and ****p<0.0005. Scale bars, 1 μm.

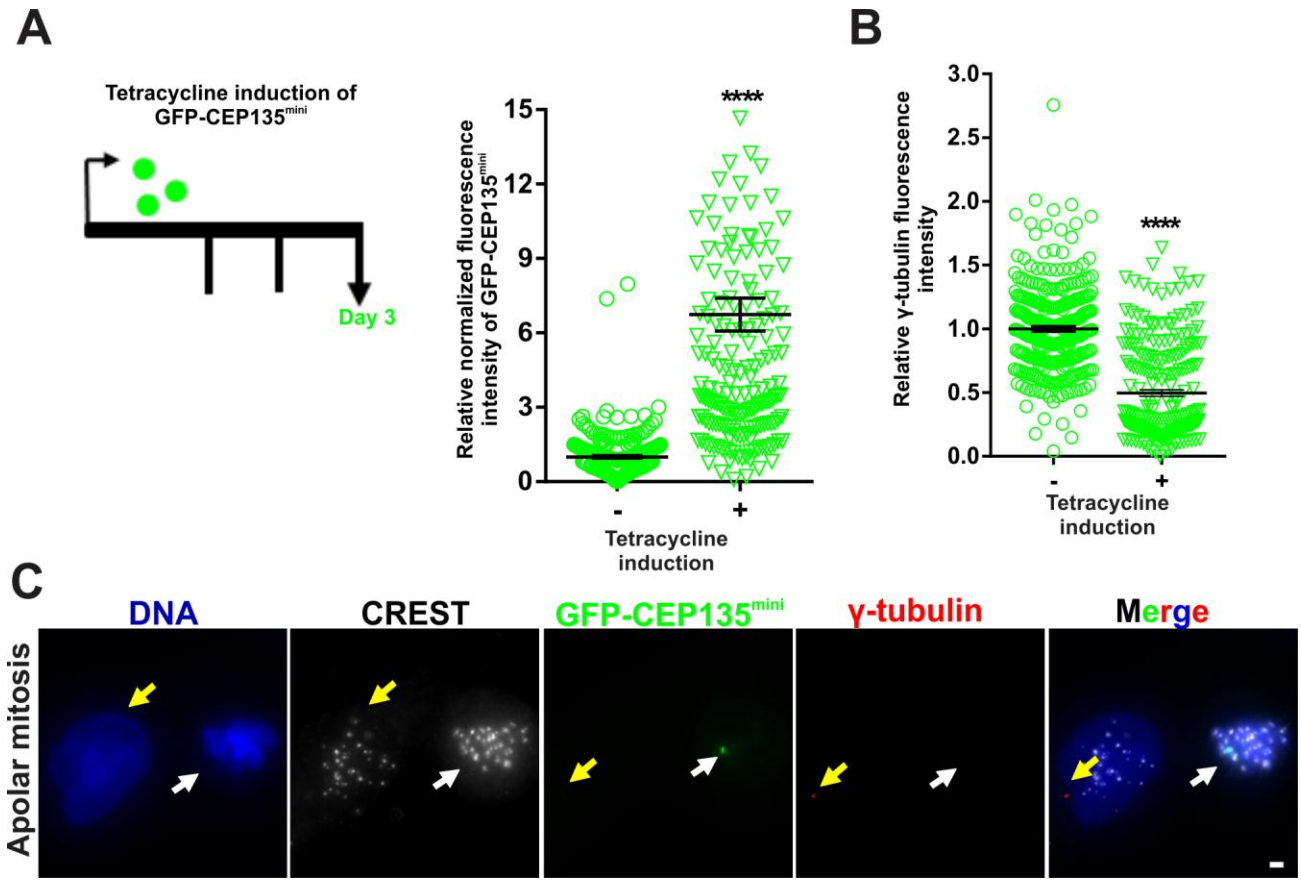
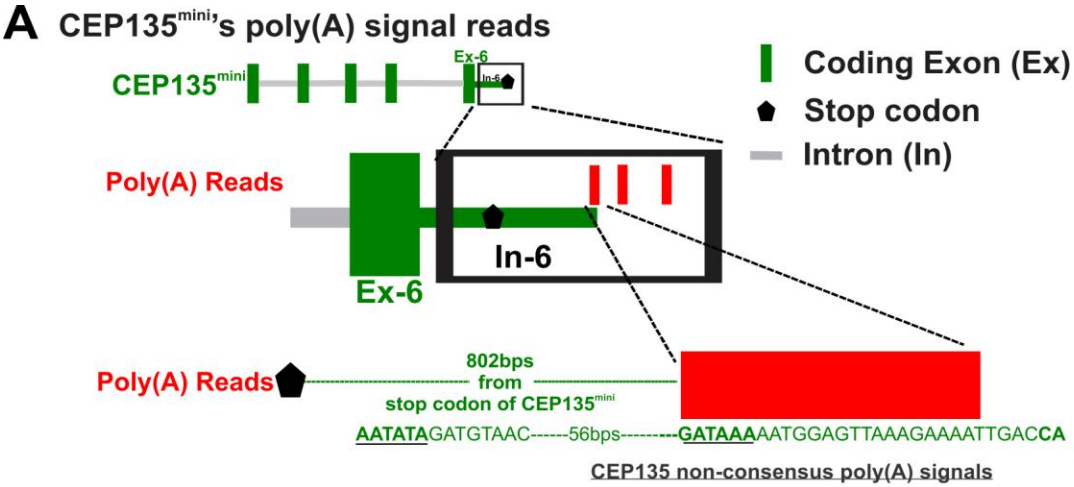
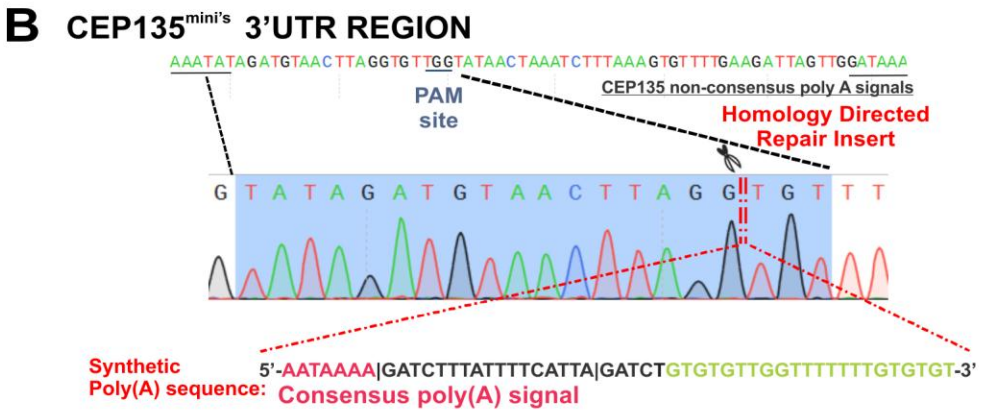
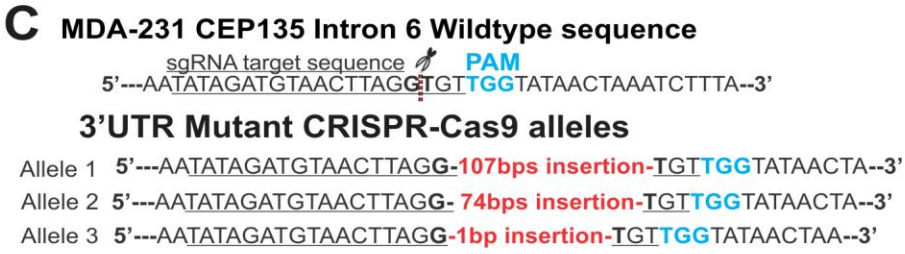


Figure S5: Elevated CEP135^{mini} expression is sufficient to decrease CA and alters chromosome segregation in breast cancer cells. (A) Left panel, schematic of GFP-CEP135$^{mini\text{-}Tet}$ MDA-231 cell induction. Right panel, quantification of the normalized fluorescence intensity of GFP-CEP135$^{mini\text{-}Tet}$ relative to non-induced cells. (B) Quantification of the relative fluorescence intensity of γ-tubulin in non-induced and induced GFP-CEP135$^{mini\text{-}Tet}$ MDA-231 cells. (C) Apolar, mitotic (white arrow) and neighboring interphase (yellow arrow) GFP-CEP135$^{mini\text{-}Tet}$ MDA-231 cells. Cells were stained for PCM (γ-tubulin; red), DNA (Hoechst 33342; blue) and kinetochores (CREST; grayscale). Mean±SEM. Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.005 and ****p<0.0005. Scale bars, 1 μm.







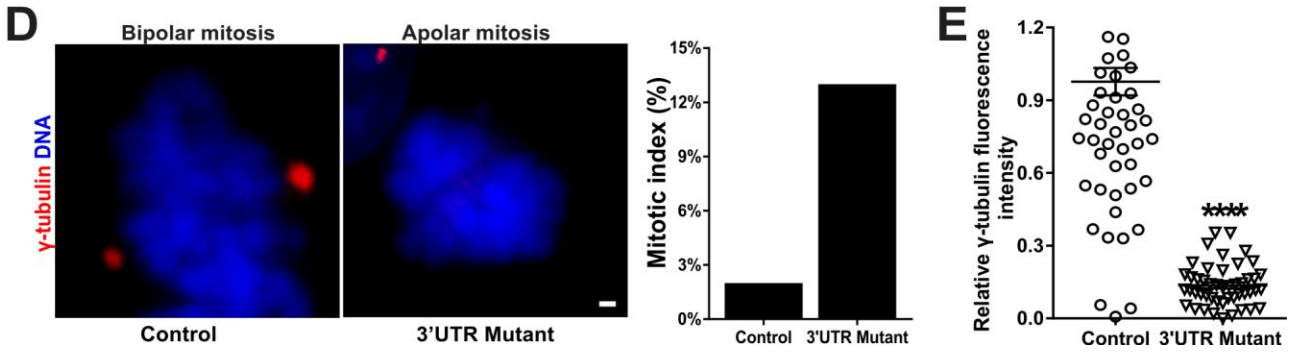


Figure S6: CEP135^{mini} is an alternatively polyadenylated isoform, and mutations near the CEP135^{mini} poly(A) signal reduce the CEP135$^{full:mini}$ ratio and centrosome number in breast cancer cells. (A) Top panel, schematic

of the CEP135^{mini} gene. Green bars denote coding exons (Ex) of CEP135^{mini}. Gray lines denote introns (In). Black pentagons denote translation stop codons. Middle panel, poly(A) signal reads (red) in intron 6 of the CEP135 locus from the 3'READS+ dataset (Zheng et al., 2016). Bottom panel, magnified view of the first poly(A) signal read (red) that is 802bps downstream of the CEP135^{mini} stop codon (pentagon). Underlined sequences represent CEP135^{mini}'s predicted polyadenylation signals and cleavage sites. (B) Top panel, the predicted poly(A) signals AAUAUA and GAUAAA in CEP135^{mini}'s 3'UTR region. Middle panel, 20 nucleotide Cas9 target region in CEP135's intron 6 utilized for the design of gRNA along with the PAM site (blue). Bottom panel, Synthetic poly(A) signal attempted for homology directed repair (HDR) knock-in into CEP135 intron 6. (C) Sequence of CEP135^{mini} 3'UTR CRISPR-induced mutant alleles. (D) Left panel, bipolar mitosis in a control MDA-231 cell and apolar mitosis in a CEP135^{mini} 3'UTR Mutant MDA-231 cell. Cells were stained for PCM (γ-tubulin, red) and chromosomes (Hoechst 33342, blue). Right panel, percentage of cells in mitosis in control and 3'UTR Mutant MDA-231 cells. (E) Relative γ-tubulin fluorescence intensity in control and 3'UTR Mutant MDA-231 cells. Mean±SEM. Mann-Whitney U test. *$p<0.05$, **$p<0.01$, ***$p<0.005$ and ****$p<0.0005$. Scale bars, 1 μm.