Figure S1. Misu localizes to the spindle and the nucleoli. (A) Expression of endogenous Misu (green) is weak at the spindle in T47D cells. (B) Overexpressed Misu (green) localized to mitotic microtubules. (C and D) EF2 antibody detects Misu (green) at the spindle in mitosis (asterisks) and the nucleoli in interphase (arrows). Cells are counterstained with DAPI (blue). Bars, 10 µm.
Figure S2. Depletion of Misu expression using RNAi in different breast cancer cell lines results in defective mitosis. (A–C) Misu (green) is absent from nucleoli and spindle fibers when cells are transduced with Misu RNAi but not when an Scr RNAi construct is used. Bar, 10 µm. (D–F) Western blots showing knockdown of Misu in response to Misu RNAi in HCC1954 cells (D), Misu siRNA in HCC1954 cells (E), and Misu siRNA in Cal51 cells (F). (G–J, top) Misu RNAi depletes Misu in BT20 (G), Cal51 (H), MT3 (I), and PMC42 (J) and causes mitotic defects (bottom). Spindles are visualized using α-tubulin antibody (red), and DNA is stained with DAPI (blue). Bars, 5 µm. (K–N) Quantification of mitotic defects from G–J. Data in K–N are representative of two experiments.
Figure S3. Depletion of Misu does not result in DNA double-strand breaks and specificity of 18S rRNA probe. (A and B) Scr and Misu RNAi cells have similar levels of phosphorylated histone γH2A.X (red). DNA is counterstained with DAPI (blue). (C) Quantification of levels of phosphorylated histone γH2A.X. (D and E) Staining of 18S rRNA (green) using an Alexa Fluor 488–labeled DNA probe (D) is lost when incubated with the corresponding sense oligo before staining (E). Spindles are visualized using α-tubulin antibody (red). (F) Colocalization of 18S rRNA (green) and Misu (red) at nucleoli and the spindle. Error bars indicate mean ± SD. Bars, 10 µm.
Figure S4. Localization of 18S rRNA to the spindle is independent of RNA, Misu localizes to the spindle in the absence of active translation, and localization of NuSAP to the spindle varies with cell cycle and fixation method. (A and B) Localization of 18S rRNA (green) to the spindle is not affected by depletion of Misu using RNAi. DNA is counterstained with DAPI (blue). (C) Quantification for A and B. (D and E) Localization of Misu (green) to the spindle (α-tubulin; D, red) is not affected by treatment with puromycin (E). DNA is counterstained with DAPI. (F–I) NuSAP staining in metaphase (F and H) and anaphase (G and I) varies depending on fixation methods. (F) In some cells, NuSAP labels both the central (arrows) and distal spindle (arrowheads). (G) When fixed with methanol, NuSAP stains both the distal (arrow) and central spindle (arrowhead). (H) In the majority of cells, NuSAP labels the distal spindle with higher intensity (arrowheads) and spindle poles (arrows). (I) When fixed with paraformaldehyde, NuSAP staining can only be found at the central spindle (arrowheads). Error bars indicate mean ± SD. Bars, 10 µm.
Figure S5. **Depletion of Misu or RNA in HCC1954 cells has no effect on the localization of aurora A, TPX2, or TACC3 during mitosis.** (A–F) Spindle assembly factors aurora A (A and B, red), TPX2 (C and D, red), and TACC3 (E and F, red) localize to the spindle in the presence of Misu (Scr RNAi; A, C, and E, green) and when Misu is depleted by RNAi (Misu RNAi; B, D, and F). (G) Quantification for A–F. (H–P) Spindle assembly factors aurora A (H and I, red), TPX2 (J and K, red), TACC3 (L and M, red), and NuSAP (N and O, green) localize to the spindle when cells are treated with RNase (+RNase; I, K, M, and O). DNA is counterstained with DAPI (blue). Error bars indicate mean ± SD. Bars, 10 µm.