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Supplementary Information:

Low dose laulimalide represents a novel molecular probe for investigating microtubule organization

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Figure S1. Docetaxel treatment does not prevent entry into mitosis. (A) Time-lapse images from G2/M phase, using mCherry tubulin and GFP-histone-H2B HeLa cells treated with 30 nM taxotere, synchronized at G2/M using a double thymidine block as described in the text. As with laulimalide, cells enter prophase and repartition interphase tubulin into an aberrant multipolar structure with circularly-distributed chromosomal arrays. Times in hours:minutes:seconds indicate the period of incubation in docetaxel. Tubulin is shown in red and histone H2B in green. Scale bar represents 13 µm. B) Electron micrographs of prometaphase HeLa cells treated with 30 nM laulimalide (left) and 30 nM docetaxel (right) for 2.5 hours, showing further evidence of circular chromosomal arrays. Scale bar represents 2 µm.
Figure S2. Aster formation occurs near or at nuclear envelope breakdown under laulimalide treatment. (A) Electron micrograph of an early prometaphase HeLa cell treated with 30 nM laulimalide for 2 hours. (B) and (C) are zoomed in images of the boxed areas in (A), showing evidence of nuclear envelope breakdown and adjacent microtubule aster formation. Asters are indicated with the filled black arrows and the nuclear envelope with the open black arrows. Scale bars are 2 µm (A) and 100 nm (B and C).
Figure S3. Docetaxel and laulimalide treatment of asynchronous HeLa cells result in acentrosomal pole formation. (A) Cells treated with 1 nM laulimalide (top) and 1 nM docetaxel (bottom) showing the formation of centrosomal and acentrosomal poles and the emergence of a circular arrangement of chromosomes around the poles. (B) Cells treated with DMSO (top) and 30 nM laulimalide (bottom) for 2.5 hours show only two poles containing γ-tubulin. Centrosomes (red) were detected using a γ-tubulin antibody, tubulin (green) with a β-tubulin antibody and DNA (blue) with DAPI. Scale bar represents 5µm.
**Figure S4.** Docetaxel treatment results in a decrease in spindle pole distances. Pole-to-pole distances were determined by measuring the spacing between centrosomes of 22-25 HeLa cells arrested at metaphase with MG132, and treated with DMSO as a control, 30 nM laulimalide or 30 nM docetaxel for approximately 2 hours. For docetaxel-treated cells, distances were derived from cells displaying the dominant multipolar phenotype, selecting only those cells with an obvious bipolar orientation (i.e., avoiding cells with misaligned centrosomal poles).
Figure S5. Revised model of one laulimalide binding site on the MT lattice, showing its location between two parallel protofilaments of the MT lattice. Laulimalide (cyan spheres) is located between adjacent β-tubulin monomers in the interprotofilament space. The molecular footprint measured using mass shift methods is shown in red. The location of doublecortin as a example of interprotofilament-binding protein is shown in yellow, and tubulin protofilaments in green. Figure based on pdb entry 2XRP, using Pymol. Doublecortin is shown for reference only, as it appears in the 2XRP structure. The authors are not inferring a relationship to the laulimalide binding site.