**Supplementary Information:**

**An Eg5 inhibitor LY2523355 shows potent schedule-dependent antitumor activity in xenograft tumor models by mitotic arrest and induction of apoptosis in cancer cells**

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Supplementary Figures and Legends 1-5

Supplementary Tables 1-2

***Supplementary Figure 1:*** A) Inhibition of microtubule-stimulated Eg5 ATPase activity by LY2523355; B) The microtubule-stimulated ATPase activity of recombinant Eg5, KHC, CENPE and MKLP1 motor domains in the presence of 200 M LY2523355; C) comparison of effects of LY2523355 with microtubule poisons vincristine and paclitaxel on microtubule polymerization in the presence (left panel) or absence (right panel) of GTP. The measures were repeated 3 times and were done as described previously (33).

***Supplementary Figure 2:*** A) HCT116 cells were treated with LY2523355 and analyzed by flow cytometry after propidium iodide staining at 18 h and 48 h; B) similar mitotic phenotype of normal, non-transformed human cells (HUVEC) and human cancer cells (HeLa) in the presences of 25 nM LY2523355 for 20 hours. Cells were stained for tubulin (green), pericentrin (red) and DNA (blue); C) mitotic phenotype of monopolar spindle of HeLa cells treated with 25nM LY2523355 for 20 hours. Cells were stained for Eg5 (green), pericentrin (red) and DNA (blue). Please note that Eg5 is normally localized to the mitotic spindle in mitotic cells and that LY2523355 inhibited Eg5 localization to the mitotic spindle, indicating that Eg5 function is required for its localization to the mitotic spindle; D) Real-time quantitative growth measurement of HeLa cells in the presence of various concentrations of LY2523355. Cell growth was measured continuously in an Incucyte instrument. The lower panel of micrographs show the inhibition of HeLa cell growth in various concentrations of LY2523355; E) Apoptotic index of HCT116 cells treated with LY2523355 at various time points. Apoptotic markers were measured and quantified at various times after LY2523355 addition by Acumen.

***Supplementary Figure 3***: A) Immunofluorescent microscopy of mitotic cells. Phospho-histone H2AX colocalized with condensed DNA in cells arrested at mitosis by LY253355; B) Knock-down of SAC protein Mad2 with a specific siRNA was confirmed by immunoblotting. C) Mitotic index of HeLa cells determined by Acumen based on histone H3 phosphorylation 24 h after treatment with LY2523355 in the presence or absence of Mad2 siRNA; D) Caspase 3/7 activity measured 48 h after treatment with LY2523355 in the presence or absence of Mad2 siRNA.

***Supplementary Figure 4***: A) the antitumor activity of LY2523355 in representative sensitive (LXFE-397) and a resistant (LXFL-529) PDX lung cancer models. The studies with PDX tumor models were carried out as previously described (35) with 10 mice per treatment group at the doses and schedules indicated; B) anticancer activity of LY2523355 in sensitive PDX tumors is associated with mitotic arrest and cell death. LY2523355 was administered by IV bolus on Days 0, 4, and 8 at 30 mg/kg dose. Tumor tissues were harvested 24 h after each dose and examined by immunohistochemistry for the mitotic marker, phosphorylated histone H3; a) In vehicle control tumors, only a few scattered phospho-histone H3-immuno-positive mitotic cells are present. The two xenograft tumors pictured on the top are resistant to treatment with LY2523355 (left, large cell lung carcinoma; right, ovarian carcinoma). The two xenograft tumors on the bottom are sensitive to treatment with LY2523355 (left, lung squamous cell carcinoma; right, breast carcinoma) (bar equals 100 µm); b) In LY2523355-treated tumors, phospho-histone H3-positive cells are greatly increased in the two sensitive xenograft tumors in the bottom row (inset shows example of monopolar morphology of a cell blocked in mitosis), while changes in the number of phospho-histone H3-positive mitotic cells do not occur or are small in the resistant xenograft tumors in the top row (bar equals 100 µm); c) Higher magnification of a xenograft tumor that is sensitive to treatment (lung squamous cell carcinoma) showing abundant necrotic cell debris (arrows) scattered among the numerous phospho-histone H3-positive cells; C) quantification of phospho-histone H3-positive cancer cells in the PDX tumors shown above in panel B. Note the increase in phospho-histone H3 cells with LY2523355 treatment in the two sensitive tumor models, lung squamous cell carcinoma denoted LXFE397 and breast carcinoma denoted MAXF1384 but not in the other tumor types; D) quantification of phospho-histone H3-positive proliferating epidermal cells (per one millimeter length of epidermis) from mouse ears collected from the tumor-bearing mice. Note that while phospho-histone 3 positive cells only increase in the tumor tissues of the LY2523355-sensitive tumor types, immunopositive cells increase in the skin of all treated animals, indicating the response in the skin is related to LY2523355 exposure. An example of the immunohistochemical label in the ear skin of vehicle and LY2523355 treated mice is shown in the main paper in Figure 5C.

***Supplementary Figure 5***: A) Pictures show mitotic arrest (phospho-histone H3, pHH3) and apoptosis (TUNEL) of p388 cells by LY2523355 48 h after continuous infusion at 0.416 mg/kg/h; B) Percentage of p388 cancer cells positive for histone H3 phosphorylation (pHH3) 72 h after continuous infusion: group 1, vehicle control; group 2 at 0.052 mg/kg/h; group 3 at 0.104 mg/kg/h; group 4 at 0.208 mg/kg/h; and group 5 at 0.416 mg/kg/h.