Pharmacological inhibition of Polo-like kinase 1 (PLK1) by BI-2536 decreases the viability and survival of hamartin and tuberin deficient cells via induction of apoptosis and attenuation of autophagy

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Supplementary Materials and Methods

Immunohistochemistry. This study was approved by the Institutional Review Board of Drexel University College of Medicine. Five µm-thick sections of formalin-fixed paraffin-embedded LAM patient-derived lung specimens (provided by Dr. Elizabeth P. Henske) were deparaffinized with xylenes (Fisher Scientific), and rehydrated in a series of graded ethanol. For antigen retrieval, sections were boiled in 10 mM sodium citrate-trisodium salt pH 6.0 for 10 min. Endogenous peroxidase activity was blocked with 3% H₂O₂ in PBS for 30 min. Non-specific background was reduced by blocking with 2% FBS in PBS for 30 min, followed by streptavidin and biotin blocking according to the manufacturer's instructions (Vector Laboratories). The sections were stained with primary antibodies (anti-pS235/236-S6, Cell Signaling 2212, 1:50 in PBS; and anti-PLK1, Invitrogen 37-7100, 1:50 in PBS) in a humidified chamber overnight at 4°C. Samples were incubated with Zymax goat anti-rabbit and anti-mouse biotinylated IgG secondary antibodies (1:1,000, Invitrogen), labeled with HRP-conjugated streptavidin (Invitrogen), and the chromogenic reaction was developed using Liquid DAB Substrate (Biogenex). Sections were counterstained with Harris modified hematoxylin (Fisher Scientific).
**Figure S1.** PLK1 protein levels correlate with phosphorylation of ribosomal protein S6 in LAM patient-derived lung specimens. Representative micrographs of two specimens (A003 and A005) stained for pS235/236-S6 (left) and PLK1 (right). Scale bar 100 µm.
Figure S2. PLK1 protein levels are rapamycin-sensitive. (A) Isogenic hamartin deficient 208-P2 and hamartin re-expressing 208-T3 MEF, and (B) isogenic tuberin deficient ELT3-V3 and tuberin re-expressing ELT3-T3 cells were treated with 20 nM rapamycin for 24 hours, or vehicle control, lysed, and the lysates were immunoblotted with the indicated antibodies. The PLK1 / α-tubulin ratios (normalized to the vehicle control-treated samples from each cell type) are indicated bellow the PLK1 immunoblots.
**Figure S3.** BI-2536 decreases the viability of hamartin deficient cells. Hamartin deficient 208-P2 and hamartin re-expressing 208-T3 MEF were treated with increasing concentrations of BI-2536 for 48 and 96 hours (left and right panel, respectively), and viability was measured by MTT conversion. $n = 8$, *, ** and *** indicate $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
**Figure S4.** Pharmacological inhibition of PLK1 decreases the clonogenic survival of hamartin deficient cells. Hamartin deficient 208-P2 and hamartin re-expressing 208-T3 MEF were treated with increasing concentrations of the PLK1 inhibitors BI-2536 (A) or Compound 1 (B) for three days, and clonogenic survival was measured seven days after replating 300 viable cells in 100 mm dishes. $n = 3$, *, ** and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
Figure S5. BI-2536 induces apoptosis and attenuates autophagy in hamartin deficient cells.

Hamartin deficient 208-P2 and hamartin re-expressing 208-T3 MEF (A), and Tsc1−/− and Tsc1+/− MEF (B) were treated with vehicle control (DMSO), BI-2536, or rapamycin at the indicated concentrations for 1-3 days, and lysates were immunoblotted with the indicated antibodies.