SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Mouse genes targeted in the siRNA library.

The gene symbol, protein name, and gene ID are shown for genes targeted in the siRNA library.

Figure S2. Depletion of SIK3 affects the duration of mitosis but not the DNA damage checkpoint.

(A) Depletion of SIK3 slows down mitosis. HeLa cells expressing histone H2B-GFP were transfected with control siRNA or siSIK3. After 24 h, individual cells were tracked for 24 h. Each horizontal bar represents one cell (*n*=50). Key: grey=interphase; black=mitosis (from DNA condensation to anaphase or cell death); truncated bars=cell death.

(B) Depletion of SIK3 does not abrogate the G_2 DNA damage checkpoint. HeLa cells expressing histone H2B-GFP were transfected with control siRNA or siSIK3. After 24 h, the cells were irradiated with 15 Gy of IR. After 16 h, the cells were either untreated or incubated with the CHK1 inhibitor UCN-01. Individual cells were tracked with live-cell imaging for 900 min. Each horizontal bar represents one cell (*n*=50). Key: grey=interphase; black=mitosis (from DNA condensation to anaphase or cell death); truncated bars=cell death.

Figure S3. Depletion of SIK3 increases the cytotoxicity of antimitotic drugs in HCT116.

HCT116 cells expressing histone H2B-GFP were transfected with control siRNA or siSIK3. After 24 h, the cells were treated with 10 ng/ml of nocodazole or 20 ng/ml of Taxol. After 2 h, individual cells were tracked using time-lapse microscopy for 24 h. Each horizontal bar represents one cell (n=50). Key: grey=interphase; black=mitosis (from DNA condensation to anaphase or cell death); truncated bars=cell death. The duration of mitosis was quantified (average ±95% CI). Transfection of siSIK3 significantly increased the duration of mitosis after nocodazole or Taxol challenge ($p < 0.001$; unpaired t-test).

SUPPLEMENTAL VIDEO LEGENDS

Video S1. Depletion of SIK3 delays mitotic exit.

HeLa cells expressing histone H2B-GFP were transfected with siSIK3. After 24 h, the cells were subjected to live-cell imaging. Channels for bright field (left) and histone H2B-GFP (right) are shown. The representative video was captured at 5 min/frame.

Video S2. Depletion of SIK3 enhances microtubule inhibitor-mediated mitotic arrest and cell death.

HeLa cells expressing histone H2B-GFP were transfected with siSIK3. After 24 h, the cells were treated with 6.25 ng/ml of nocodazole. After 2 h, individual cells were subjected to live-cell imaging. Channels for bright field (left) and histone H2B-GFP (right) are shown. The representative video was captured at 5 min/frame.

Video S3. Depletion of SIK3 enhances AURKB inhibitor-mediated mitotic slippage.

HeLa cells expressing histone H2B-GFP were transfected with siSIK3. After 24 h, the cells were treated with 12.5 nM of Barasertib. After 2 h, individual cells were subjected to live-cell imaging. Channels for bright field (left) and histone H2B-GFP (right) are shown. The representative video was captured at 5 min/frame.

Video S4. Depletion of SIK3 enhances PLK1 inhibitor-mediated metaphase arrest and cell death.

HeLa cells expressing histone H2B-GFP were transfected with siSIK3. After 24 h, the cells were treated with 1.25 nM of BI-2536. After 2 h, individual cells were subjected to

live-cell imaging. Channels for bright field (left) and histone H2B-GFP (right) are shown. The representative video was captured at 5 min/frame.

Video S5. Depletion of SIK3 enhances Eg5 inhibitor-mediated early mitotic arrest and cell death.

HeLa cells expressing histone H2B-GFP were transfected with siSIK3. After 24 h, the cells were treated with 1.25 nM of SB743921. After 2 h, individual cells were subjected to live-cell imaging. Channels for bright field (left) and histone H2B-GFP (right) are shown. The representative video was captured at 5 min/frame.