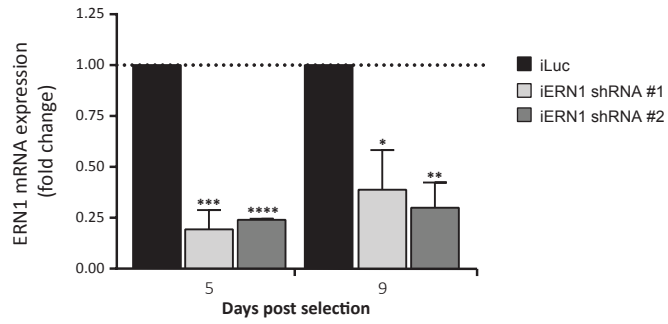
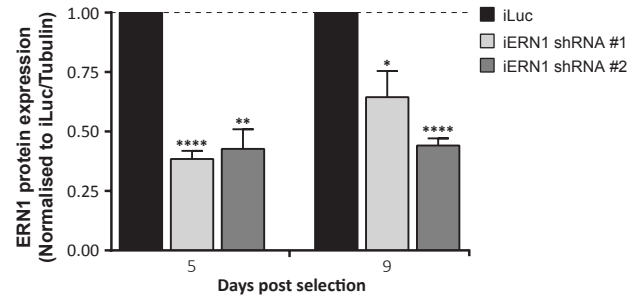
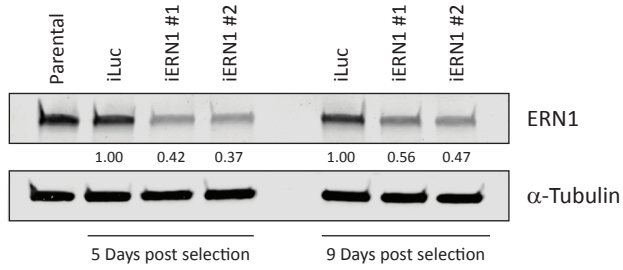
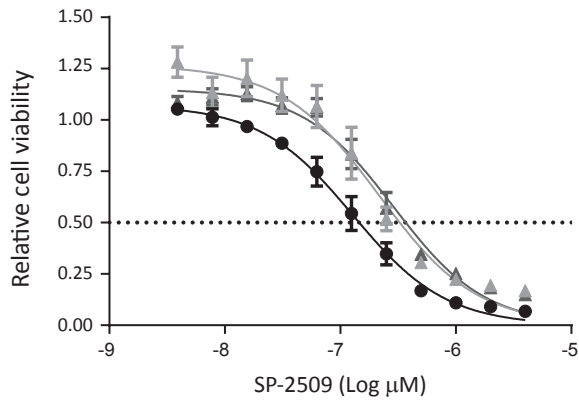
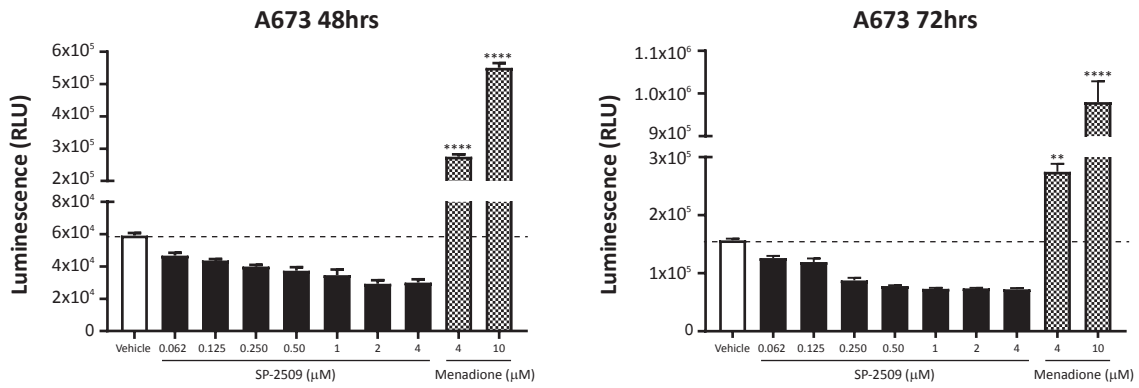


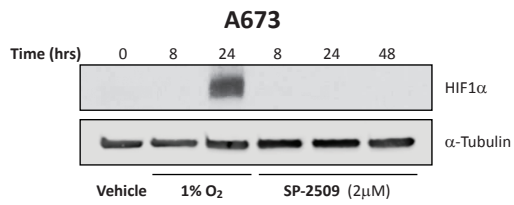
**C****D****E**

	SP-2509 72hr IC <sub>50</sub> ( $\mu$ M)	95% CI IC <sub>50</sub> ( $\mu$ M)
● iLuc	0.143 $\pm$ 0.032	0.108 - 0.150
▲ iERN1 shRNA #1	0.253 $\pm$ 0.030	0.135 - 0.310
▲ iERN1 shRNA #2	<b>0.295 <math>\pm</math> 0.046</b>	<b>0.208 - 0.394</b>

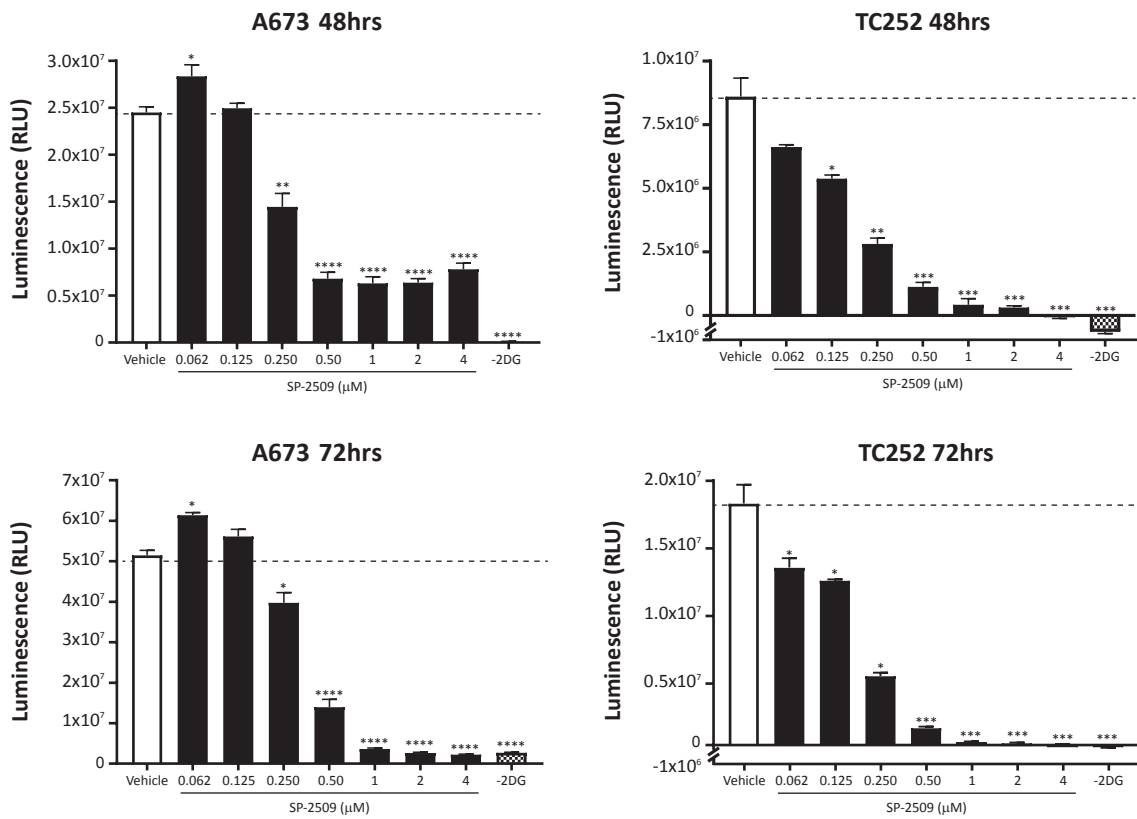
**F**

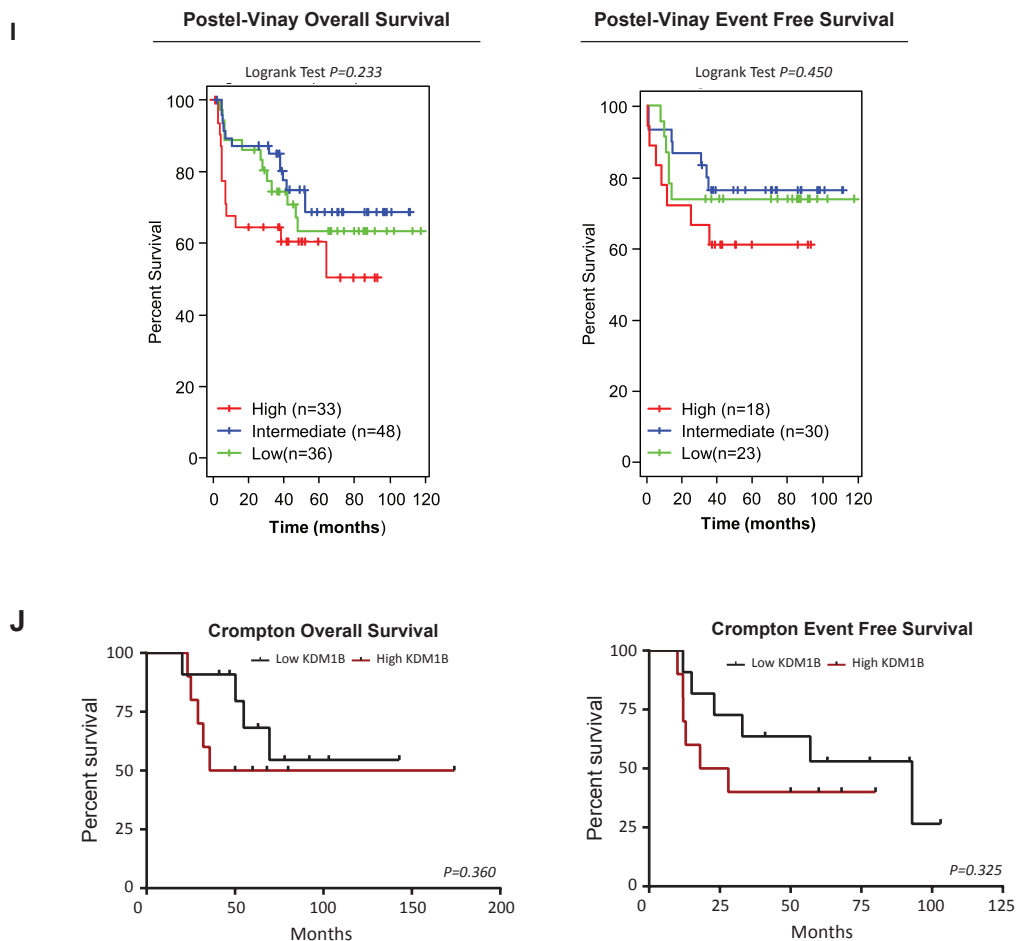


**G**



**H**





**Supplementary Figure S6:** Knockdown of *ERN1* significantly impairs the cytotoxic effects of SP-2509, with SP-2509 significantly reducing glucose uptake.

(A) Western blot analysis of ER-stress response genes in A673, TC252, ES-2 and EWS-502 cells treated with Thapsigargin (50nM) or SP-2509 (2 $\mu$ M) for the indicated times. (B) Basal rlog transformed counts (rld) of UPR genes obtained from RNA seq analysis of hypersensitive (A673, TC252, TC32) versus sensitive (EWS-502, ES-2, TC71) SP-2509 cell lines. Relative (C) mRNA and (D) protein expression levels of *ERN1* (*IRE1 $\alpha$* ) in A673 cells stably transduced with *iERN1* (#1 and #2) or *iLuc* control shRNA constructs following puromycin selection. Quantification of protein levels (densitometry) is also depicted. Protein normalized to  $\alpha$ -tubulin (loading control) and vehicle (DMSO) control. (E) Cell viability analysis (Cell Titer Glo) of A673 cells transduced with the indicated shRNA constructs and treated with SP-2509 (0-4 $\mu$ M) for 72hrs. (F) ROS accumulation in A673 cells treated with SP-2509 (0-4 $\mu$ M) or Menadione (ROS-generating compound) for 48 and 72hrs. (G) *HIF1 $\alpha$*  protein expression in A673 cells treated with SP-2509 (2 $\mu$ M) or grown under hypoxic conditions (1%  $O_2$ ) for the indicated times. (H) Glucose uptake in A673 and TC252 cells treated with SP-2509 (0-4 $\mu$ M) for the indicated times. Negative control: Omission of 2-deoxyglucose (-2DG). Data represents mean  $\pm$  SEM expression from three independent experiments. Asterisks denote statistical significance (\* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001, \*\*\*\* $P$ <0.0001). DPS: Days post selection. (I) Event free and overall survival of Ewing sarcoma patients stratified by high (median +0.5xMAD), intermediate and low (median -0.5xMAD) *KDM1B* expression. Expression data obtained from Postel-Vinay et al., 2012. Log-rank (Mantel Cox Test) used to determine survival significance. (J) Event free and overall survival of Ewing sarcoma patients stratified by high and low *KDM1B* expression. Expression data obtained from Crompton et al., 2014.