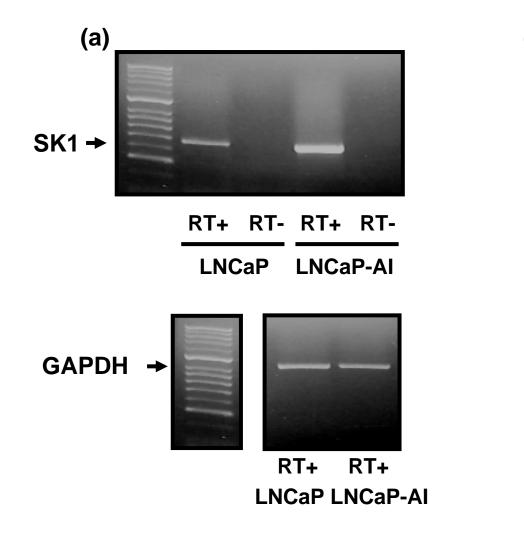
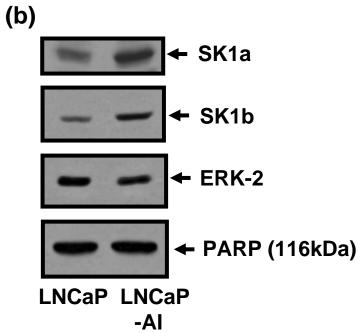
Supplementary Figures

- <u>Fig. 1</u> SK1a and SK1b expression in LNCaP and LNCaP-AI cells. (a) RT-PCR with gene specific primers comparing the mRNA expression of SK1 in LNCaP and LNCaP-AI cells. GAPDH expression was analysed using specific primers to ensure equal RNA sampling; (b) western blots comparing SK1a, SK1b and PARP protein expression in LNCaP and LNCaP-AI cells. The western blots were reprobed with anti-ERK-2 antibody to ensure comparable protein loading. Results are representative of 3 separate experiments.
- **Fig. 2 Densitometric quantification of SK1b immunoreactivity.** Immunoreactivity of western blots for SK1b (total cell lysate protein equalised/sample using the BCA protein assay) was quantified by densitometry and expressed as a percentage of the control (100%). Statistical analysis was performed using unpaired t-tests. *P* values were as follows: SKi (24h) *vs* control, <0.001; SKi (48h) *vs* control, <0.001; DMS *vs* control, <0.001; cycloheximide *vs* control, NS; cycloheximide/SKi *vs* SKi (24h), NS; MG132 (48h) *vs* control, NS; MG132/SKi *vs* SKi (48h), <0.01; fumonisin *vs* control, NS; fumonisin/SKi *vs* SKi (24h), <0.01; CA074Me *vs* control, <0.05; CA074Me/SKi *vs* SKi (48h), NS; MG132 (24h) *vs* control, NS; C2 ceramide *vs* control, <0.001; MG132/C2 ceramide *vs* C2 ceramide, <0.01; siRNA SK1 *vs* control, NS; siRNA SK1/SKi *vs* scrambled siRNA/SKi, <0.05; siRNA SK1/SKi *vs* siRNA SK1 alone < 0.05. Values of *n* were from 3-12 experiments. NS denotes not significant.
- <u>Fig. 3</u> SKi does not decrease SK1 and cyclin D1 mRNA expression. Bar chart showing that SKi $(10\mu M, 24 \text{ h})$ does not decrease SK1 and cyclin D1 mRNA expression in LNCaP cells as assessed using real time PCR. SKi $(10\mu M, 24 \text{ h})$ -treatment of LNCaP cells induces degradation of SK1b (Fig. 2a) and cyclin D1 (Fig. 7d), and we show here also induces degradation of SK1a in LNCaP cells.
- <u>Fig. 4</u> Lack of effect of the cathepsin B inhibitor, CA074Me on the SKi-induced degradation of SK1a and SK1b. Western blot showing the lack of effect of CA074Me ($10\mu M$, 48 h) on the SKi ($10\mu M$, 48 h)-induced degradation of SK1a and SK1b in LNCaP cells. The western blots were reprobed with anti-actin antibody to ensure comparable protein loading. Results are representative of 3 separate experiments.
- <u>Fig. 5</u> Lack of effect of dihydrosphingosine or C2-dihydroceramide on SK1a or SK1b expression. Western blot showing the lack of effect of dihydrosphingosine ($10\mu M$, 24 h) or C2-dihydroceramide ($50\mu M$, 24 h) on SK1 expression in LNCaP cells. The western blots were reprobed with anti-ERK-2 antibody to ensure comparable protein loading. Results are representative of 3 separate experiments.

Suppl Fig. 1



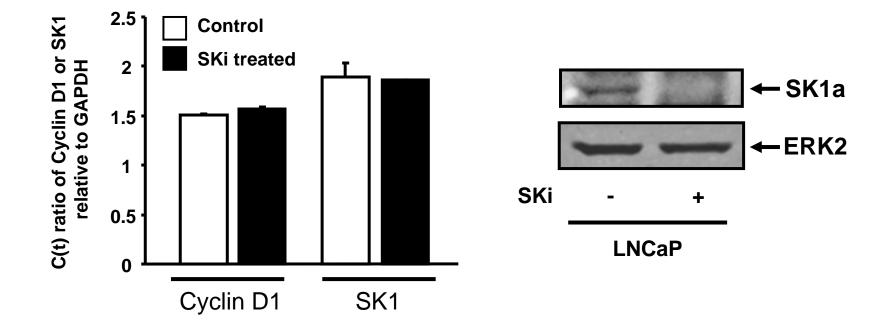


Suppl Fig. 2 180 160 140 % of control (100%) 120 100 80 60 40 20 MG132 MG1325K1 0 CAOTAMOSKI ZAM RE132/CER CHYLSKI FUNEKI SKI ABIN CHY DMS FUM 180 160 % of control (100%) 140 120 100 80 60 40 20 0

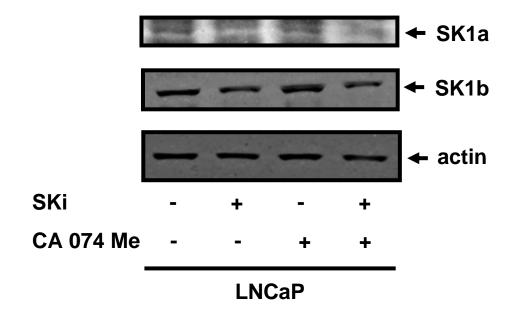
scr/SKI

sIRNA

sIRNA/SKI



Suppl Fig. 4



Suppl Fig.5

