Supplementary material

Inhibitors of mitochondrial Kv1.3 channels induce Bax/Bak-independent death of cancer cells

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Legends to supplementary figures:

Figure S1. Expression of Bax, Bcl-xL and caspase-3 does not differ between CTLL-2/pJK and CTLL-2/Kv1.3 cells.

(A) Expression of Bcl-xL, Caspase-3 and Bax does not differ between CTLL-2/pJK or CTLL-2/Kv1.3 as shown by Western blot using specific antibodies. While Kv1.3 is present only in CTLL-2/Kv1.3 cells, KCa3.1 is expressed in both lines. GAPDH was used as loading control. **(B)** CTLL-2/pJK and CTLL-2/Kv1.3 were incubated with MDRi (see Fig. 1) and 1 μM ShK. Apoptosis was analyzed by FACS using Annexin V and propidium iodide staining. Average % of cell death ± SD is shown (n=5). Shk was without significant effect.

Figure S2. Inhibition of mitoKv1.3 is necessary to induce tumor cell death.

(A) Calcium-dependent potassium channels are not involved in apoptotic signalling in human Jurkat leukemic T and CTLL-2 lymphocytes. Cell death was determined by FACS analysis (average ± SD, n=3, *$p<0.05$, **$p<0.01$) upon treatment of the indicated cells with KCa3.1 inhibitor TRAM-34 and KCa2.2 specific inhibitor UCL1684 at the indicated concentrations expressed in μM. C. control; S:

staurosporine. Psora-4 and MDRi were applied as in Figure 1. **(B)** Induction of oxidative stress induces cell death in both CTLL-2/pJK and CTLL-2/Kv1.3 cells, indicating that this pathway is independent or downstream and is not mediated by the presence of Kv1.3. Cell death was measured by FACS analysis, after treatment of CTLL-2 cell with different μM concentrations of Menadione (n=3; *p<0.05).

Figure S3. Identification of mitoKv1.3 in SAOS-2 and B16F10 cells.

(A) Membrane enriched fraction and purified mitochondria obtained from SAOS-2 cells as described in Material and Methods were analyzed by Western blot using two different antibodies against Kv1.3 (65 kDa). Mitochondrial purification was checked showing the enrichment of Bak (29 kDa) and the decrease of PMCA (135 kDa) and SERCA (110 kDa) at equal total protein concentration loaded (50 μg/lane). The same blots were used for Kv1.3 detection (2 blots), and after stripping, for the detection of Bak, SERCA and PMCA. **(B)** Mitochondria were Percoll-purified from cultured B16F10 cells, lysed and separated by 7.5% SDS-PAGE and western blotted with anti-Kv1.3 antibodies. **(C)** Cell death in B16F10 cells induced by Psora-4 or PAP-1 applied alone or in combination with MDRi (tamoxifen 5 μM) (n=4; ***p<0.001).

Figure S4. Kv1.3 inhibitors induce ROS production and mitochondrial membrane depolarization in human Jurkat leukemic T cells.

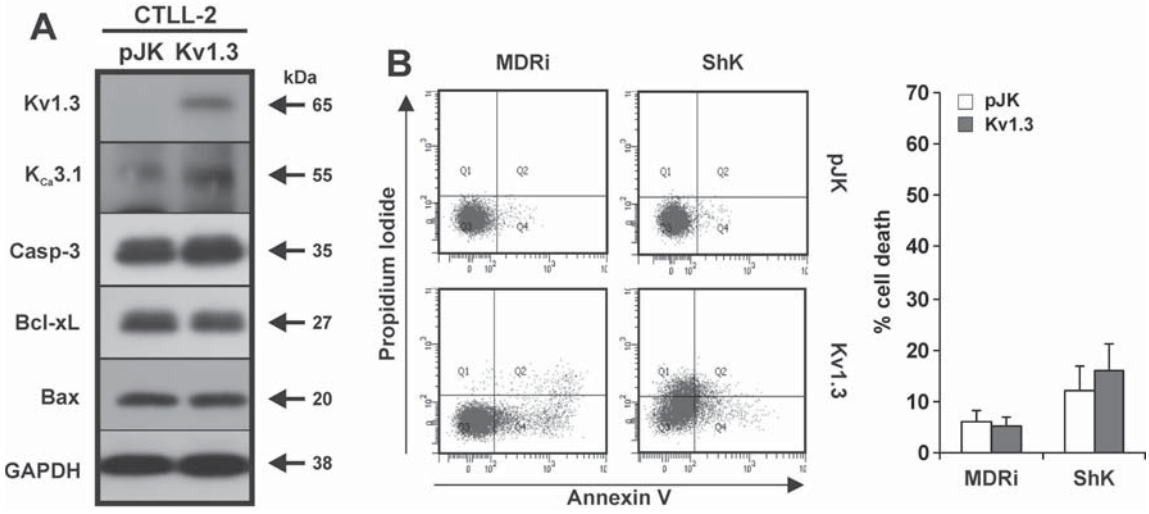
(A) Staining with the Mitosox probe reveals and increase in ROS production in human Jurkat leukemic T lymphocytes upon treatment with Kv1.3 inhibitors (MDRi: CSH 4 μM + Prob. 100 μM; 20 μM Psora, PAP; 1 μM Clofazimine, ShK). The Y scale is an arbitrary fluorescence units. (n=6; *p<0.05; **p<0.01). **(B)** The decrease of TMRM fluorescence upon treatment with 20 μM Psora, PAP or 1 μM Clofaziminine (with MDRi: CSH 4 μM + Prob. 100 μM) in Bax/Bak-deficient human Jurkat leukemic T cells (left panel) is prevented by 30 min pre-treatment with 4 μM CSA (right panel). No effects on mitochondrial membrane potential were observed in cells untreated (CTRL) or treated with 1 μM ShK (n=6; **p<0.01).

Figure S5. Identification of mitoKv1.3 and induction of autophagy in MEF DKO cells.

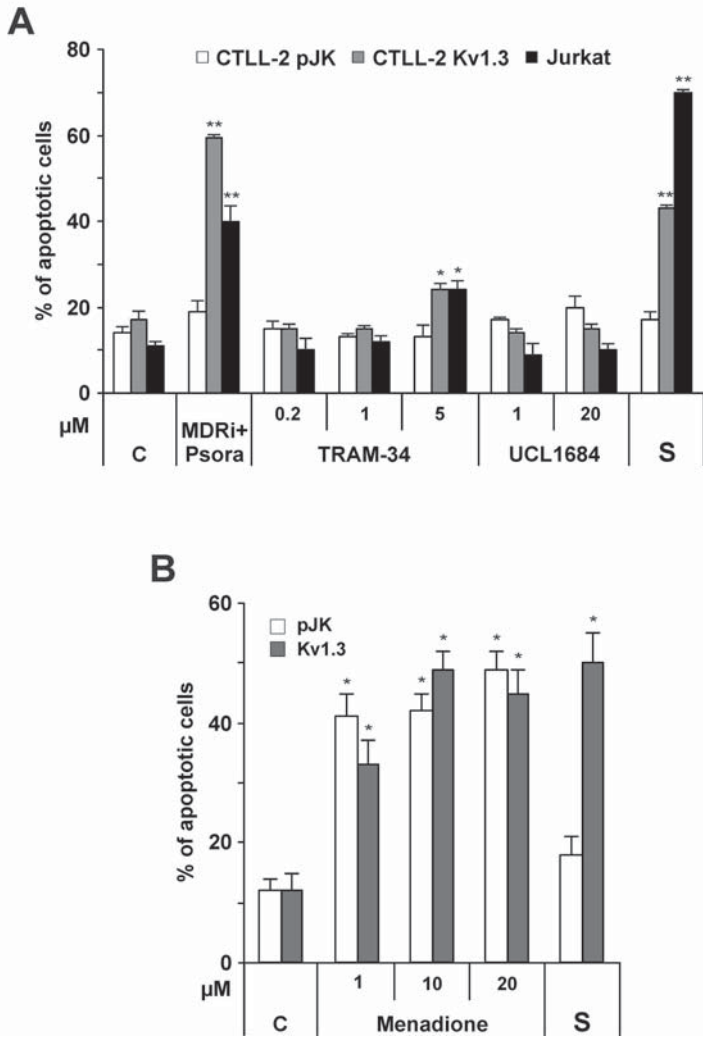
(A) Mitochondria were Percoll-purified from Bax/Bak-deficient MEFs, the mitochondria were lysed, separated on 7.5% SDS-PAGE, blotted and analysed with anti-Kv1.3 antibodies followed by

chemoluminescence visualization. The studies show expression of Kv1.3 in mitochondria from MEFs. Shown is a representative result of three similar experiments. **(B)** Induction of autophagy was observed following LC3 I to LC3 II conversion by Western blot with an anti-LC3 antibody. Total protein extracts were prepared from Bax/Bak-deficient MEFs, upon treatment with Kv1.3 inhibitors (50 μg/lane of protein; 1: untreated; 2: MDRi+ 20 μM Psora; 3: MDRi + 20 μM PAP; 4: MDRi + 1 μM Clofazimine; 5: 1 μM ShK; 6: 1 μM Staurosporine; MDRi: CSH 4 μM + Prob. 100 μM). This is a representative blot of three independent experiments. **(C)** Inhibition of autophagic pathway by addition of 3-methyl-adenine (3-MA; 1 mM) or Clorochine (Cloro; 15 μM) is not perturbing cell death observed by MTT assay after treatment of Bax/Bax-deficient MEFs with Kv1.3 inhibitors (cells were treated as reported in panel B; control: untreated cells; stauro: 1 μM staurosporine).

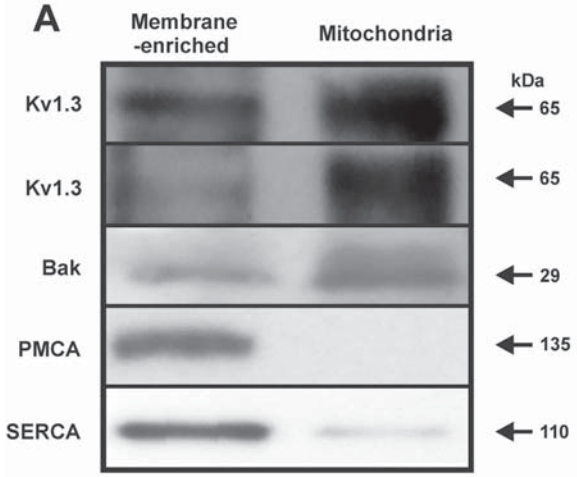
Supplementary Figure 1

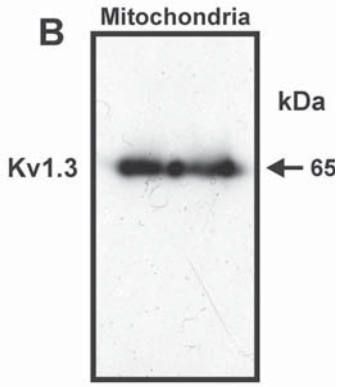


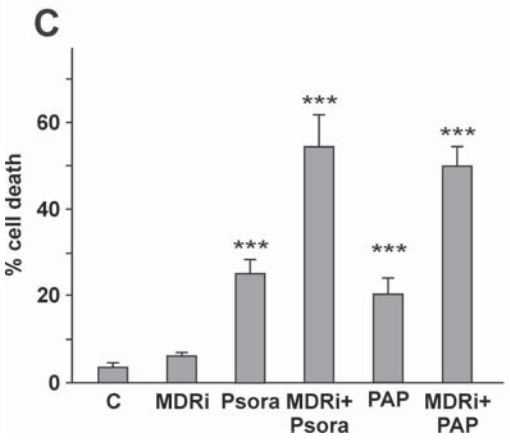
Supplementary Figure 2



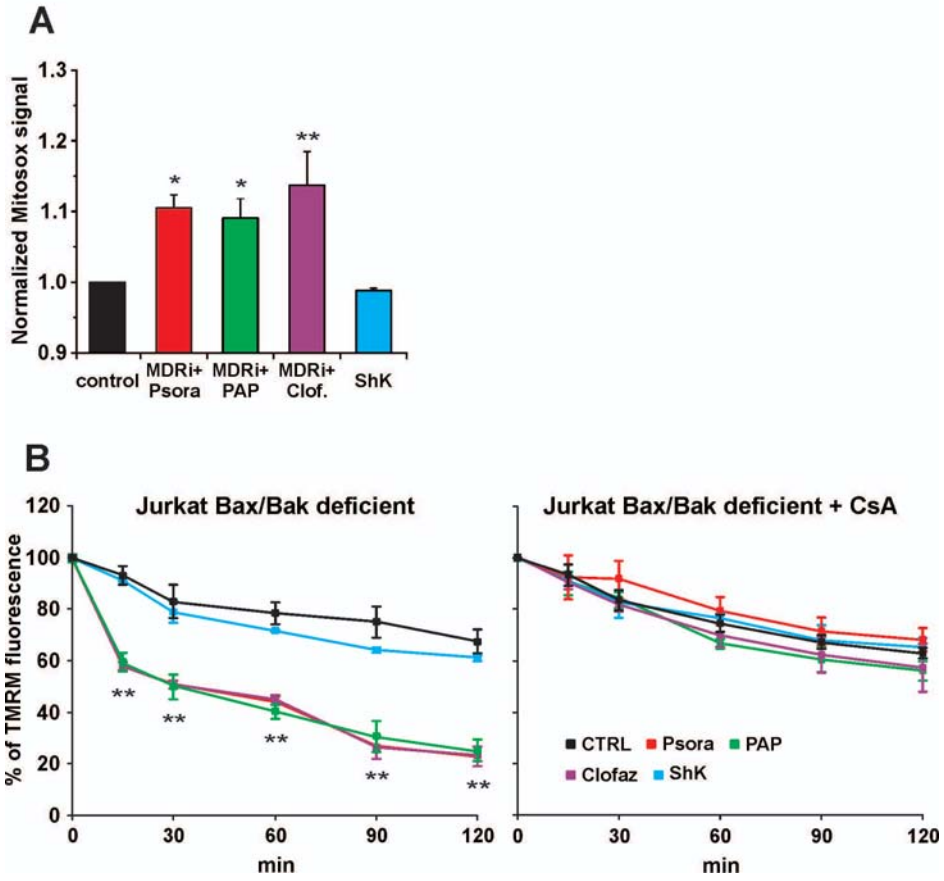
Supplementary Figure 3







Supplementary Figure 4



Supplementary Figure 5