SUPPLEMENARY MATERIAL

Creatine kinase-1 regulates the mitochondrial permeability transition pore in a process that provides evidence for alternative forms of the complex

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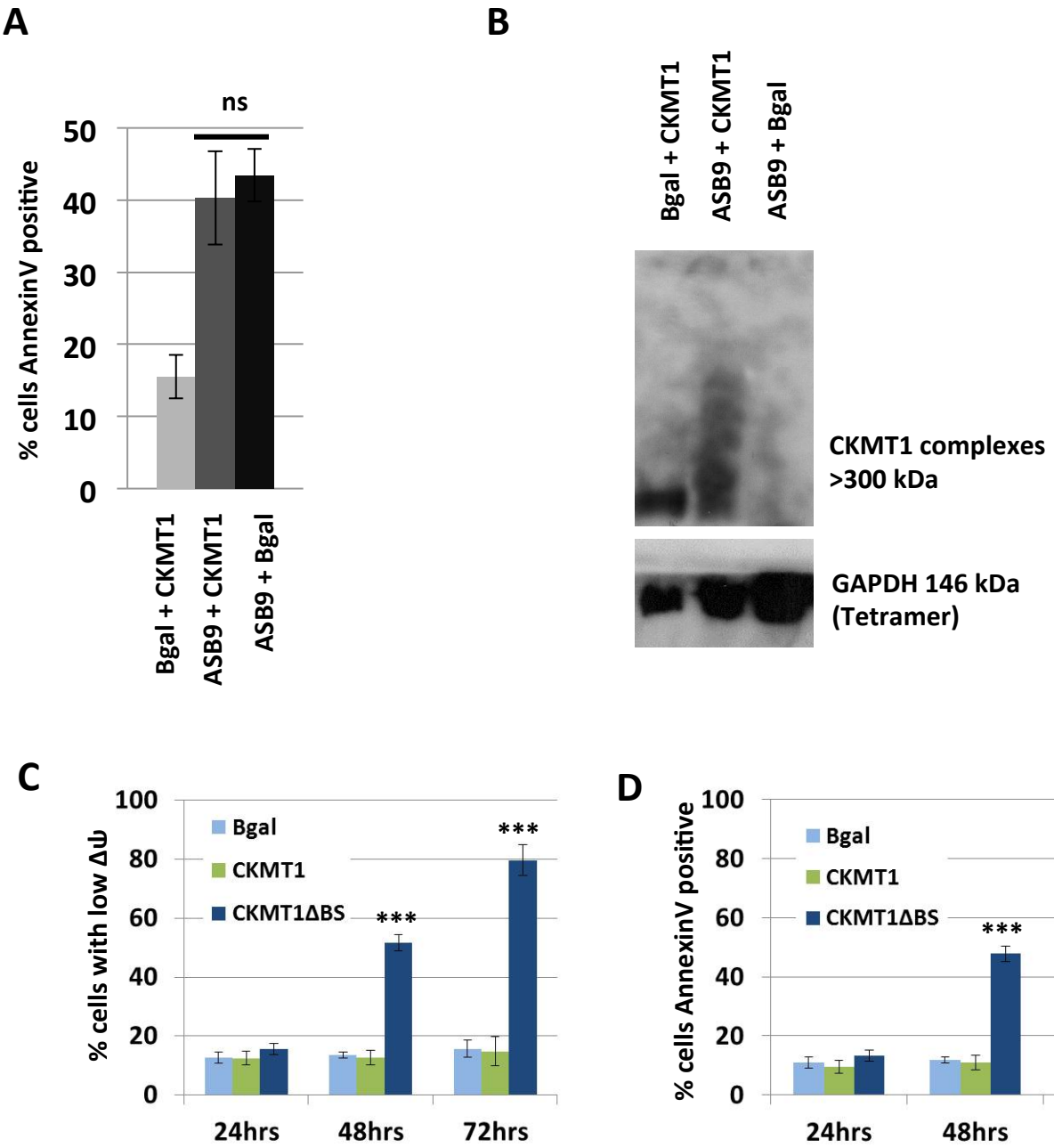
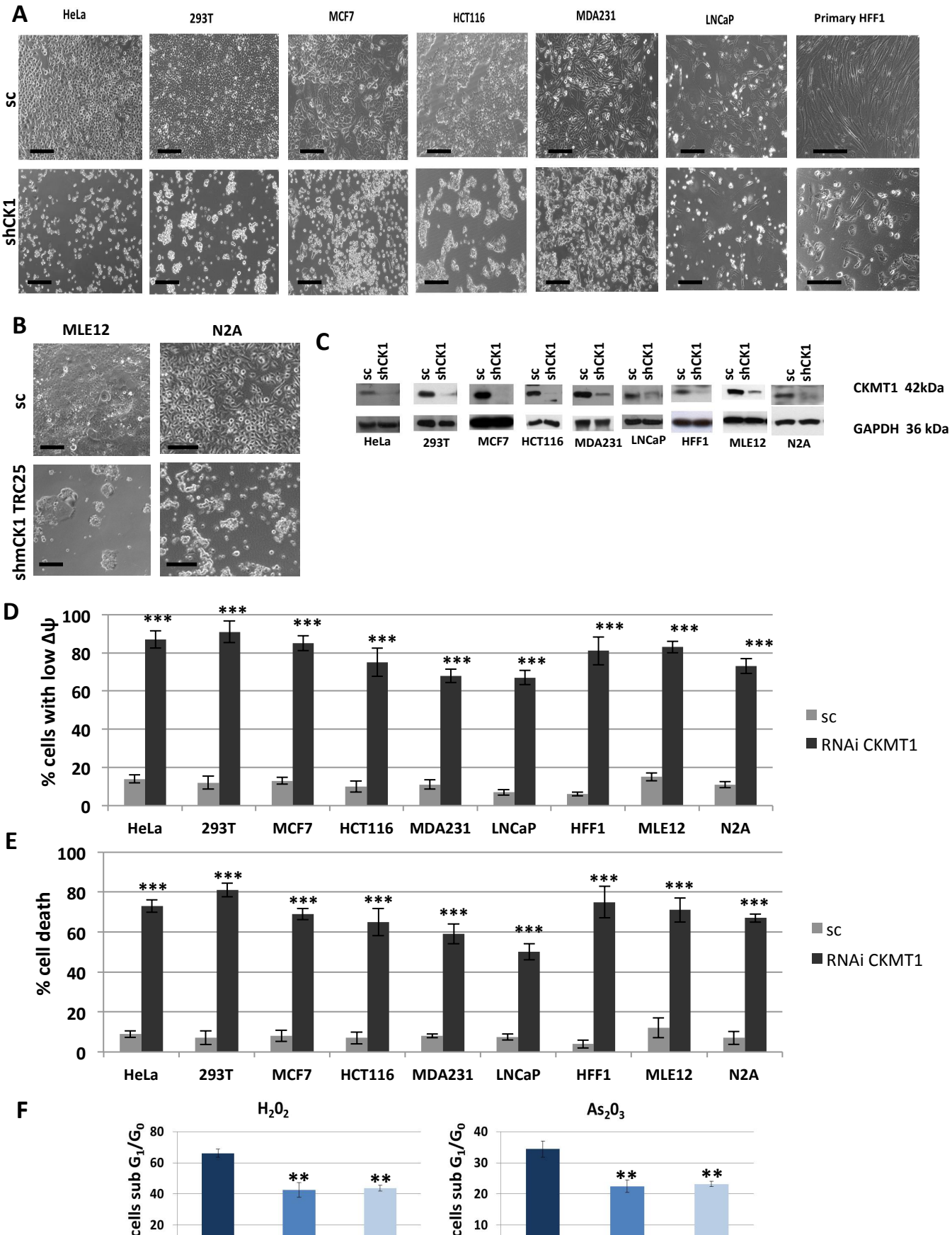
Supplementary figure S1.



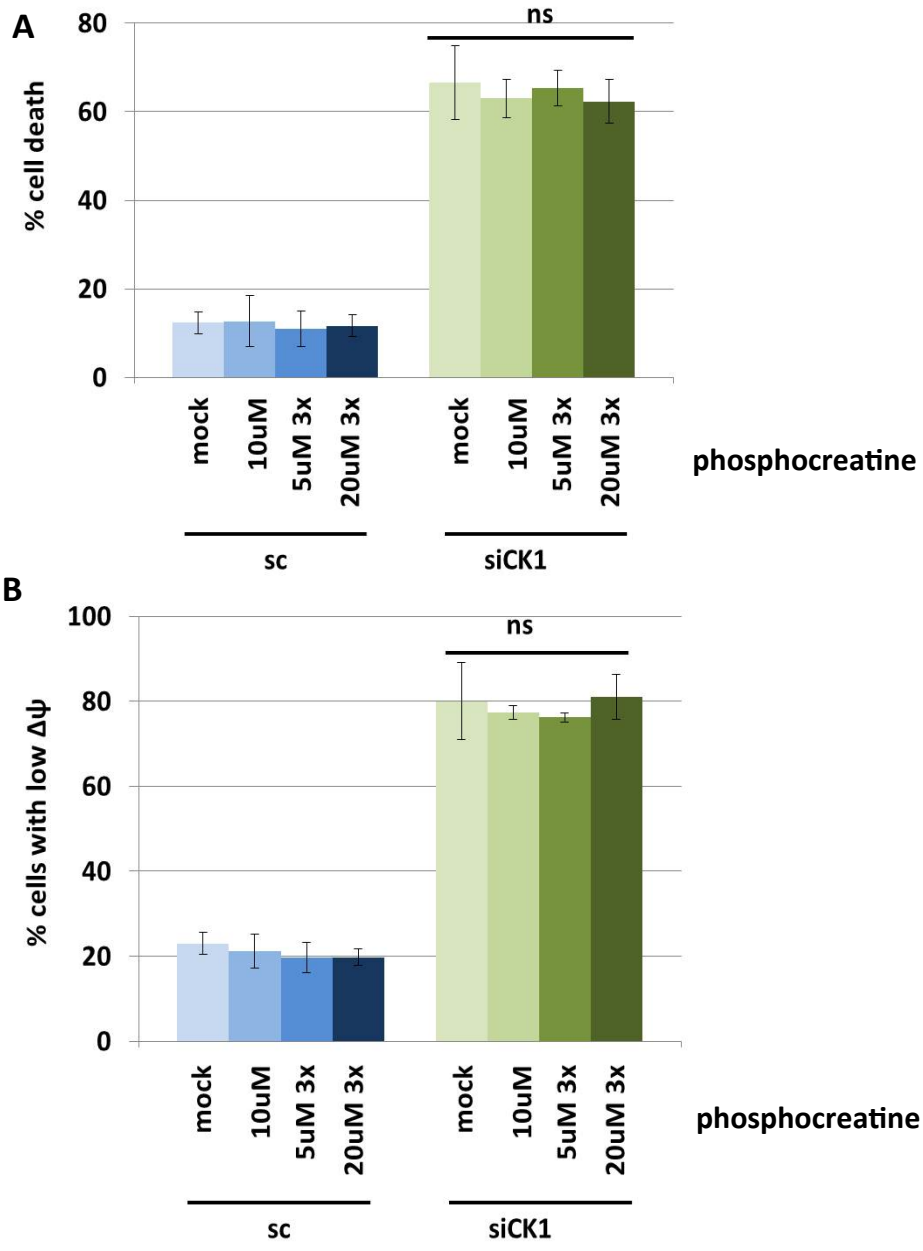
Supplementary figure S1. Co-overexpression of WT CKMT1 does not inhibit ASB9-induced apoptosis and overexpression of the CKMT1ΔBS deletion mutant induces apoptosis. (A) HeLa were cells transfected with the indicated combinations of beta-galactosidase (Bgal), CKMT1 and ASB9 plasmids and apoptosis assessed 72hrs post-transfection. (B) Native blue gel electrophoresis of protein lysates recovered from (A) show ubiquitination of co-overexpressed CKMT1. Overexpression of the deletion mutant CKMT1ΔBS (CKMT1 with amino acids 135 to 145 deleted whose interaction with ASB9 is abrogated) results in significant mitochondrial membrane potential dissipation (C) and apoptosis induction (D); ***p<0.001 student's t-test.

Supplementary figure S2.



Supplementary figure S2. Knock-down of CKMT1 results in the dissipation of the mitochondrial membrane potential and apoptotic cell death in transformed human and mouse cell lines as well as human primary cells and overexpression of CKMT1 inhibits apoptosis independently of its enzymatic activity. Cells were infected with viral particles encoding for shRNA constructs specifically targeting the human/ mouse CKMT1 coding sequence (shCK1/shmCK1 TRC25) or a scrambled sequence (sc); compare supplementary table S1. 4-7 days post transfection phase contrast microscopy pictures of representative fields were acquired. Bars correspond to 100 μm (A-B). The CKMT1 expression level was assessed by Western blotting (C) and the mitochondrial membrane potential (D) as well as apoptosis (E) were measured using $DiOC_6$ and annexin V staining and subsequent FACS analysis. Knock-down of CKMT1 results in highly significant mitochondrial depolarization and apoptosis in HeLa, 293T, MCF7, HCT116, MDA231 and LNCaP cells, as well as in primary human foreskin fibroblasts (HFF1) and the murine cell lines MLE12 and N2A. (F) Plasmids coding for luciferase (Luc), CKMT1 and the enzymatically inactive CKMT1mut E266→L266 were transfected into HeLa cells. 24h post transfection apoptosis was induced by 500μM hydrogen peroxide or 10μM arsenic trioxide for 24h and cell death assessed by propidium iodide staining and subsequent FACS analysis. ***$p<0.001$, **$p<0.01$ (student's t-test).

Supplementary figure S3.



Supplementary figure S3. Phosphocreatine supplementation does not rescue from mitochondrial depolarization upon depletion of endogenous CKMT1. HeLa cells were transfected with siRNA constructs targeting the coding sequence of CKMT1 (siCK1) or a scrambled sequence (sc). Cells were supplemented twice daily with the indicated doses of phosphocreatine right after transfection for the next 92 hours. (**A**) Cell death was assessed by PI exclusion staining and subsequent FACS analysis. (**B**) The mitochondrial membrane potential was assessed by $DiOC_6$ staining and subsequent FACS analysis every 24 hours. Representative data acquired 96 hours post transfection is shown. Ns, non-significant.

Table S1

name	official name	specificity	target sequence
shCK1	TRCN0000006058	homo sapiens	GTGATCCAAGAGCGACACAAT
siCK1	n.a	homo sapiens	TGAAGCACACCACGGATCT
shmCK1 TRC27	TRCN0000024927	mus musculus	CGGCAACATGAAGAGAGTGTT
shmCK1 TRC25	TRCN0000024925	mus musculus	GCCACTGCTGAGCAAAGATAA

Table S1. List of all siRNAs used in this study to target human or murine CKMT1