

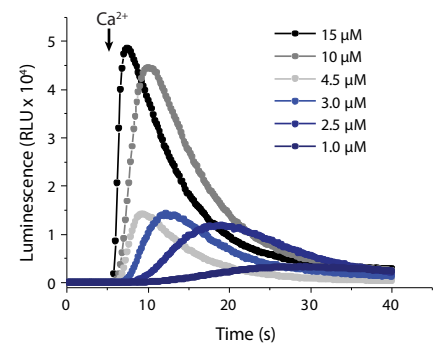
Supplemental Information

Systematic Identification of MCU Modulators

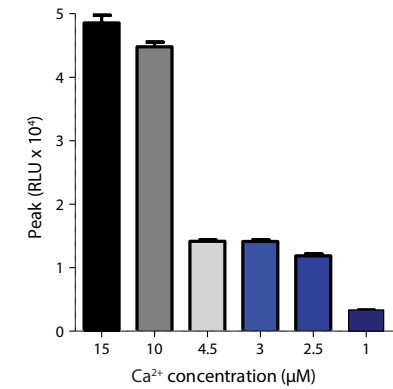
by Orthogonal Interspecies Chemical Screening

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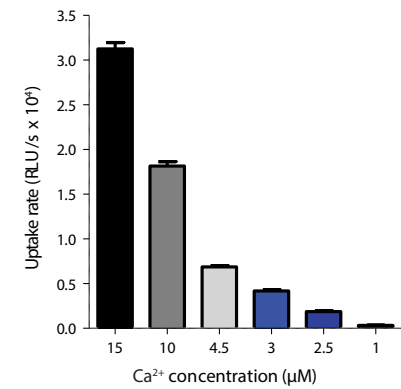
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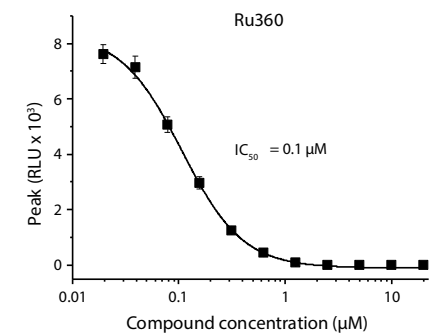
B



C



D



E

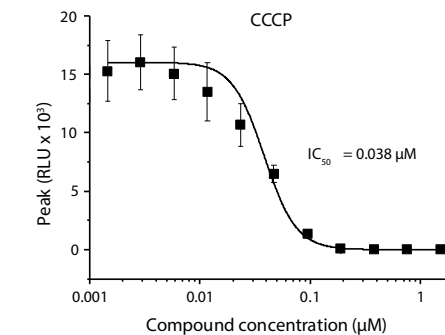


Figure S1

Figure S1, Related to Figure 3. Optimization of the permeabilized HeLa cell-based assay for drug screening

(A) Representative traces of AEQ-based light kinetics in mitochondria of permeabilized HeLa cells in response to different concentrations of Ca^{2+} .

(B) Quantification of maximal luminescence (peak) for traces in (A). Mean \pm SEM; n = 6.

(C) Quantification of rate of light emission (uptake rate) for traces in (A). Mean \pm SEM; n = 6.

(D) Ru360 dose-response curve derived from AEQ-based light kinetics in mitochondria of permeabilized HeLa cells. Data are fitted with a logistic function (continuous line) to determine the half maximal inhibitory concentration (IC_{50}). Mean \pm SEM; n = 3.

(E) Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) dose-response curve derived from AEQ-based light kinetics in mitochondria of permeabilized HeLa cells. Mean \pm SEM; n = 3. RLU, relative luminescence units.

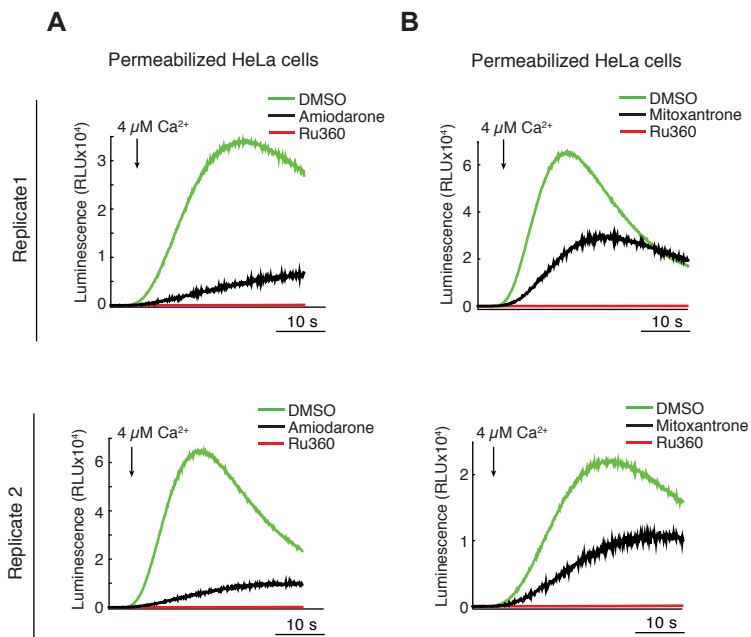


Figure S2

Figure S2, Related to Figure 3. Hits from the HeLa-permeabilized cell-based drug screen

(A) Biological duplicates of Ca^{2+} -dependent, AEQ-based light kinetics in mitochondria of permeabilized HeLa cells treated with 10 μM amiodarone. Averaged light kinetics are shown for DMSO (0.1%; n=8; negative control) and Ru360 (10 μM ; n=8; positive control). Data represent mean \pm SEM.

(B) Biological duplicates of Ca^{2+} -dependent, AEQ-based light kinetics in mitochondria of permeabilized HeLa cells treated with 10 μM mitoxantrone. Averaged light kinetics are shown for DMSO (0.1%; n=8; negative control) and Ru360 (10 μM ; n=8; positive control). Data represent mean \pm SEM.

RLU, relative luminescence units.

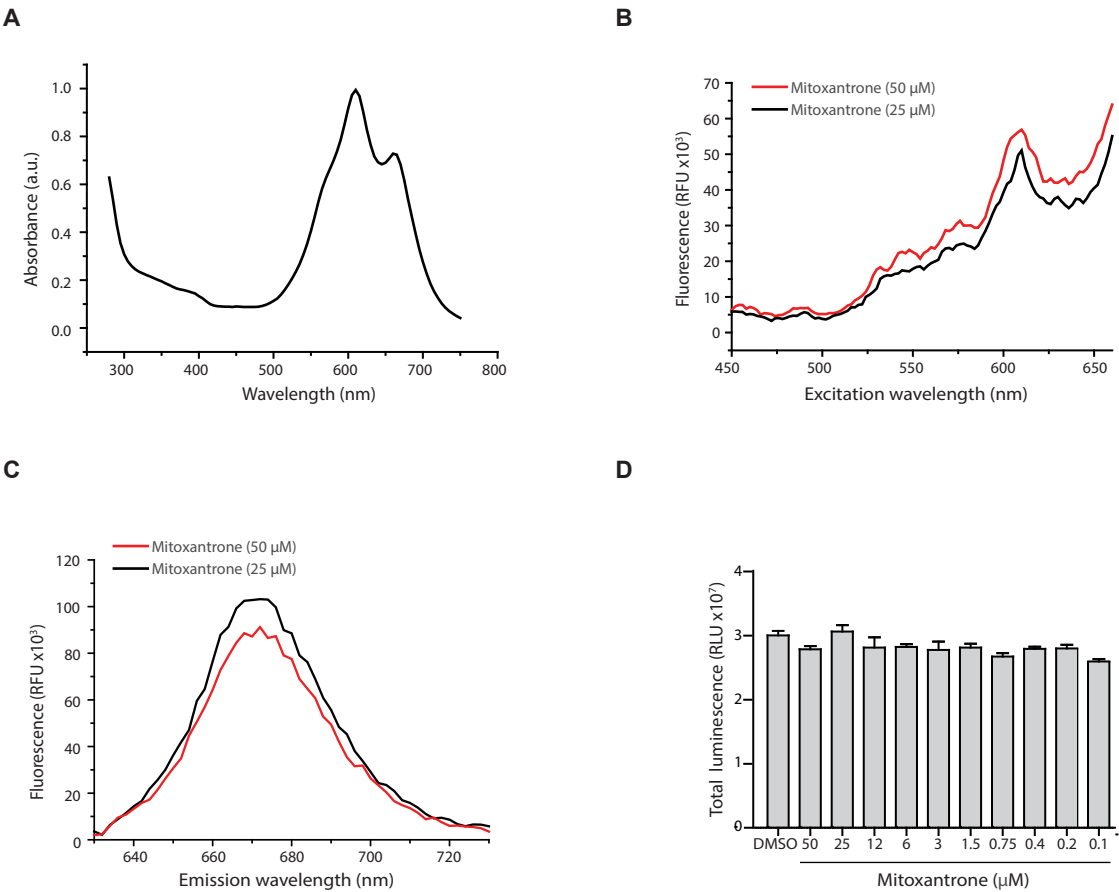


Figure S3

Figure S3, Related to Figure 3. Optical properties of mitoxantrone

(A) Absorbance spectrum of mitoxantrone (25 μ M) in PBS (pH 7.2).

(B) Fluorescence excitation (Em 690 nm) spectrum of mitoxantrone in PBS (pH 7.2).

(C) Fluorescence emission (Ex 605 nm) spectrum of mitoxantrone in PBS (pH 7.2).

(D) Effect of different concentrations of mitoxantrone on the total luminescence signal (RLU, relative luminescence units) from yeast mitochondria expressing mt-AEQ, reconstituted with native coelenterazine and lysed with Triton X-100 (2%) in the presence of 50 mM CaCl_2 .

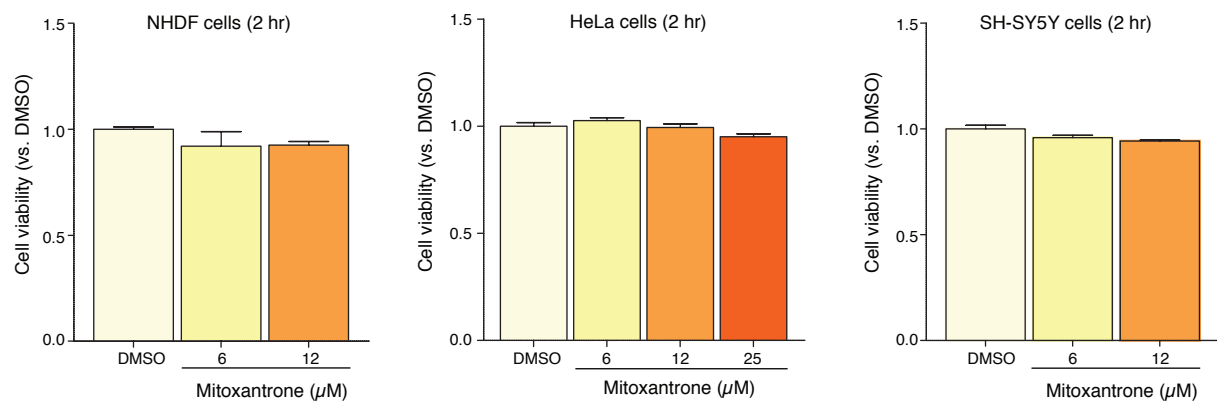
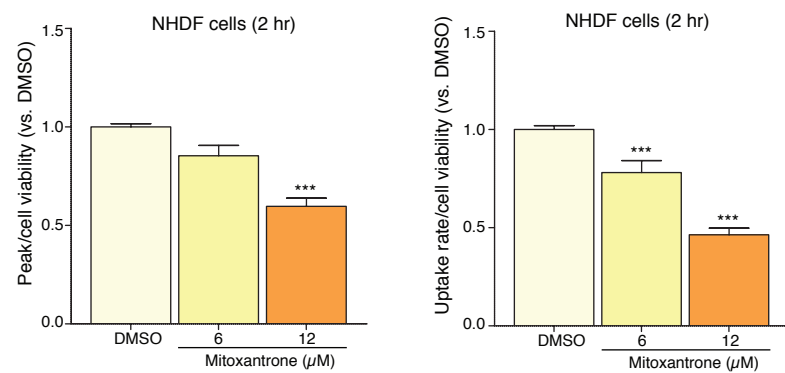
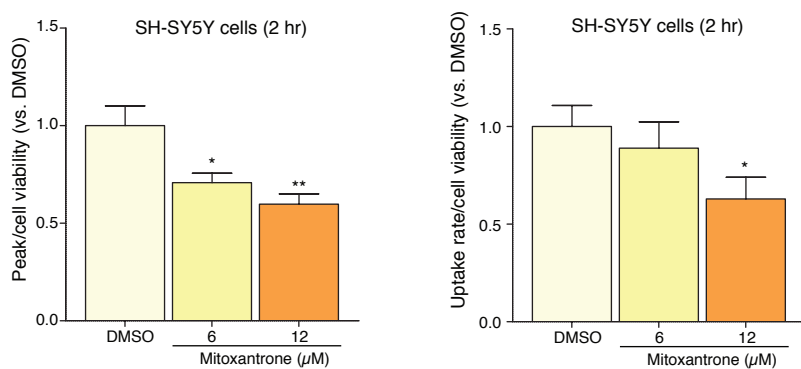
A**B****C****Figure S4**

Figure S4, Related to Figure 5. Effect of mitoxantrone on the viability of HeLa, NHDF, and SH-SY5Y cells

(A) Cell viability in NHDF human neonatal fibroblasts, HeLa and SH-SY5Y cells treated with mitoxantrone for 2 hours. Data are normalized to DMSO (0.2%). Mean \pm SEM; n = 4.

(B) Ca^{2+} -dependent, AEQ-based light kinetics in mitochondria of intact NHDF cells treated with different concentrations of mitoxantrone for 2 hours. Data are normalized to the number of viable cells. Mean \pm SEM; ***, $P < 0.001$; one-Way ANOVA; n = 12.

(C) Ca^{2+} -dependent, AEQ-based light kinetics in mitochondria of intact SH-SY5Y cells treated with different concentrations of mitoxantrone for 2 hours. Data are normalized to the number of viable cells. Mean \pm SEM; *, $P < 0.05$; **, $P < 0.01$; n = 12.

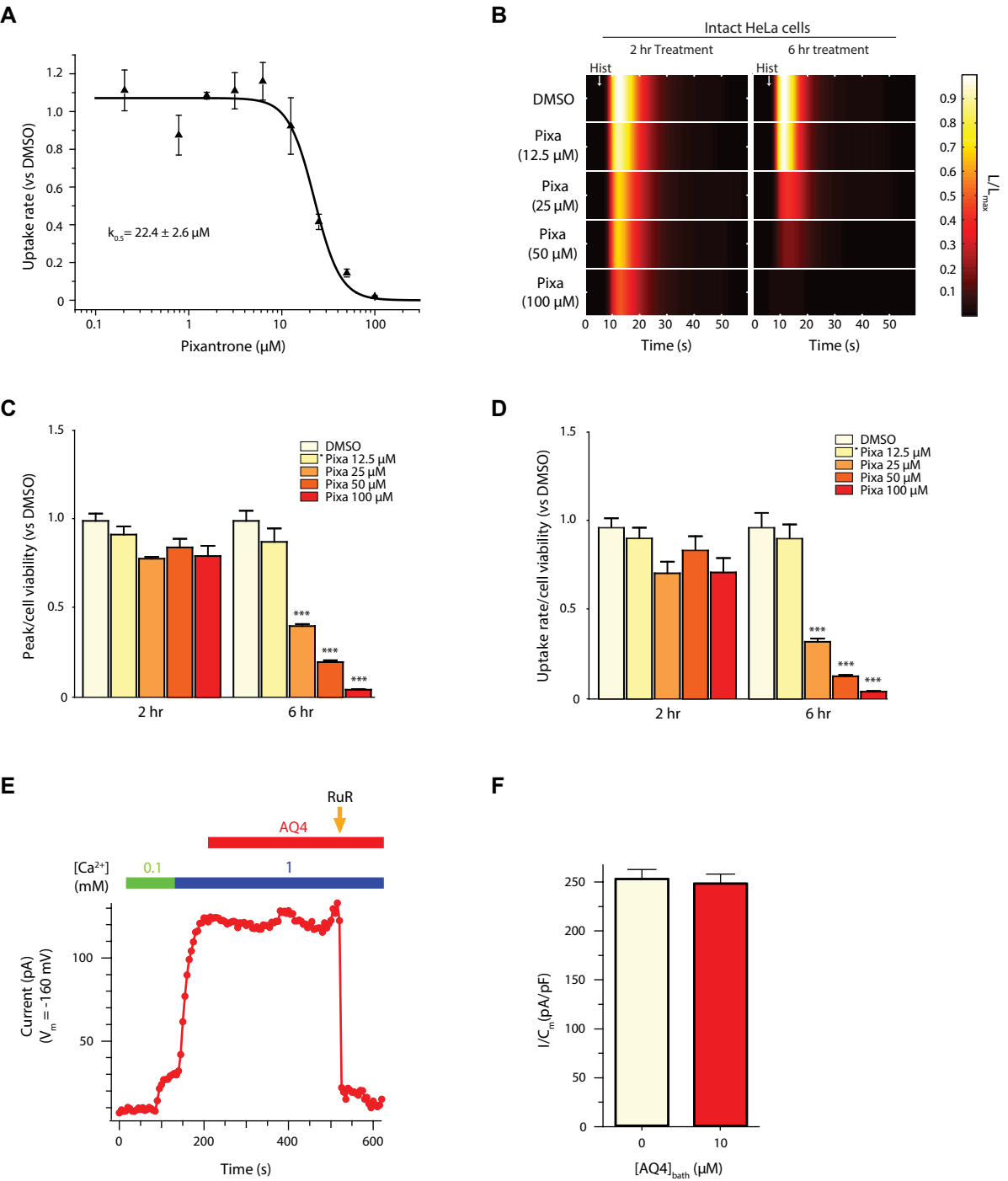


Figure S5

Figure S5, Related to Figure 6. Effect of pixantrone and AQ4 on mitochondrial calcium uptake

(A) Dose–response curve derived from AEQ-based light kinetics in mitochondria of permeabilized HeLa cells treated with pixantrone in response to 4 μM Ca^{2+} . Uptake rate values relative to DMSO (0.2%) are fitted with a Hill equation (continuous lines) to extract Michaelis constant ($k_{0.5}$). Mean \pm SEM; $n = 4$.

(B) Representative AEQ-based light kinetics in mitochondria of intact HeLa cells treated with either vehicle (DMSO, 0.2%) or different concentrations of pixantrone (Pixa) for 2 or 6 hr. Intracellular Ca^{2+} signaling was stimulated with 100 μM histamine (Hist). Data represent the ratio of luminescence (L) over maximal peak luminescence (L_{max}).

(C) Quantification of peak luminescence normalized to the number of viable cells. Mean \pm SEM; ***, $P < 0.001$; one-Way ANOVA; $n = 12$.

(D) Quantification of rate of light emission (uptake rate) normalized to the number of viable cells. Mean \pm SEM; ***, $P < 0.001$; one-Way ANOVA; $n = 12$.

(E) Representative time course of MCU current densities during exposure to 10 μM AQ4 in the bath solution. Each point represents the amplitude of MCU current at - 160 mV, sampled every 5 s. Bars (Top) indicate in bath $[\text{Ca}^{2+}]$ and period of drug exposure. Ruthenium Red (RuR, 200 nM) is added at end of each experiment.

(F) Quantification of MCU current densities (pA/pF) in 1 mM Ca^{2+} before and after AQ4 treatment (10 μM). Mean \pm SEM; $n = 3$.

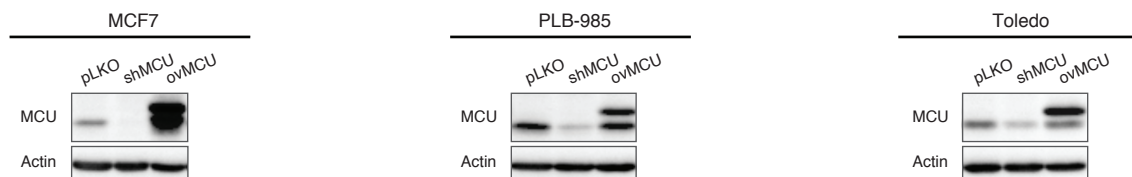
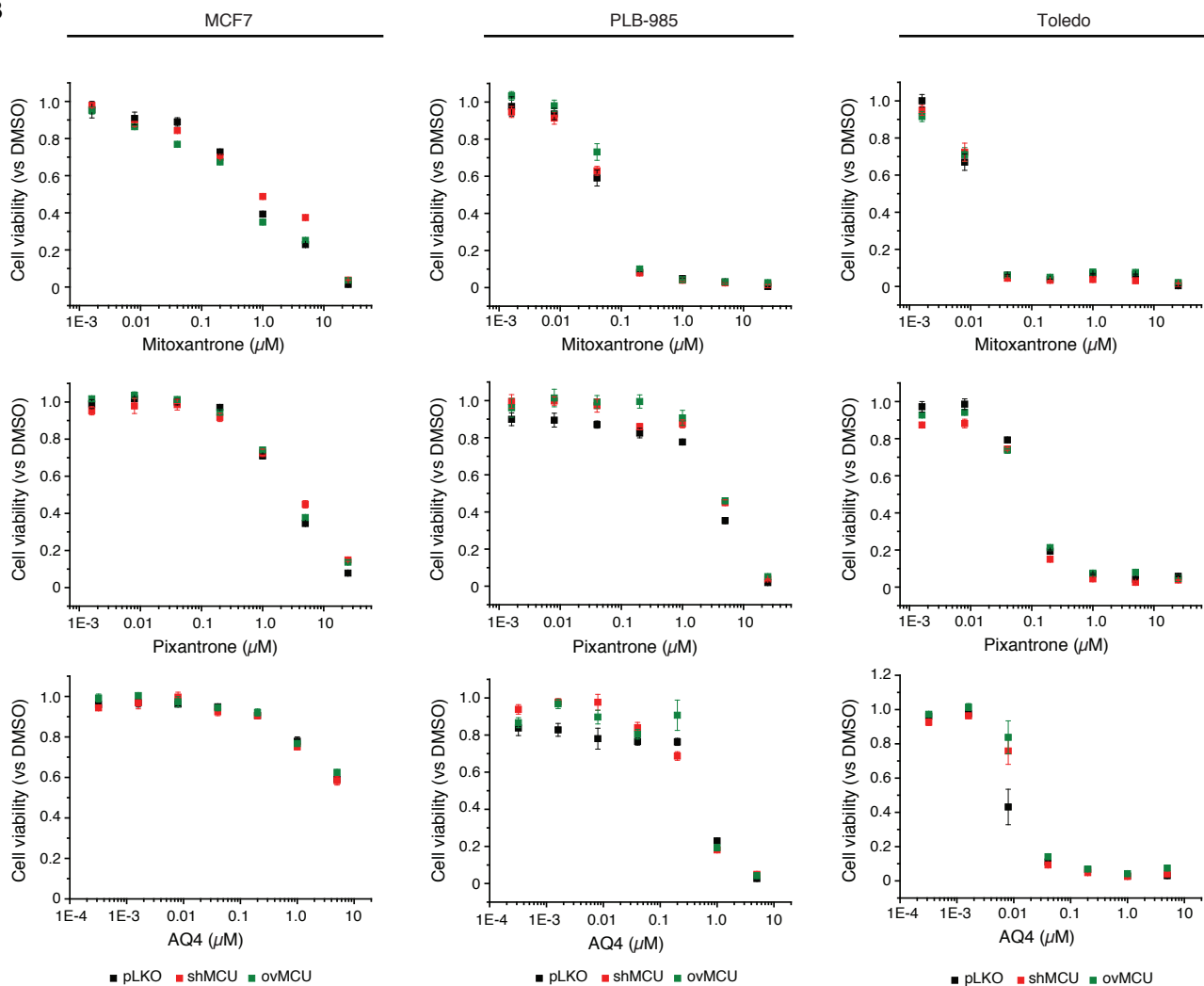
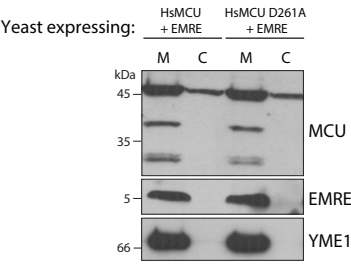
A**B****Figure S6**

Figure S6, Related to Figure 6. Effect of mitoxantrone and its analogs on the viability of cancer cell lines expressing different levels of MCU

(A) Immunoblot analysis of whole-cell lysate from human breast adenocarcinoma (MCF-7), human peripheral blood acute myeloid leukemia (PLB-985) and non-Hodgkin's B cell lymphoma (Toledo) cells expressing empty vector (pLKO), shRNA against MCU (shMCU; TRCN0000133861, 5'-GCAAGGAGTTTCTTTCTCTTT-3') or V5-tagged wild type human MCU (ovMCU).

(B) Analysis of cell viability in response to 48 h drug treatment. Mean \pm SEM; n = 4.

A



B

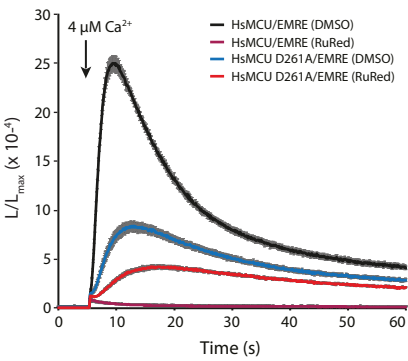


Figure S7

Figure S7, Related to Figure 7. Functional reconstitution of yeast mitochondria with wild type human EMRE and either wild type or mutated human MCU (D261A)

(A) Immunoblot analysis of mitochondrial (M) and cytosolic (C) fractions isolated from yeast cells co-expressing wild type human EMRE and either wild type or mutated human MCU (D261A). YME1 (mitochondrial i-AAA protease).

(B) Normalized AEQ-based light kinetics in yeast mitochondria after treatment with 0.2% DMSO or 5 μ M RuRed. Mean \pm SEM; n = 4.