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Supplementary Information for

MICU1 imparts the mitochondrial uniporter with the ability to discriminate between Ca^{2+} and Mn^{2+}

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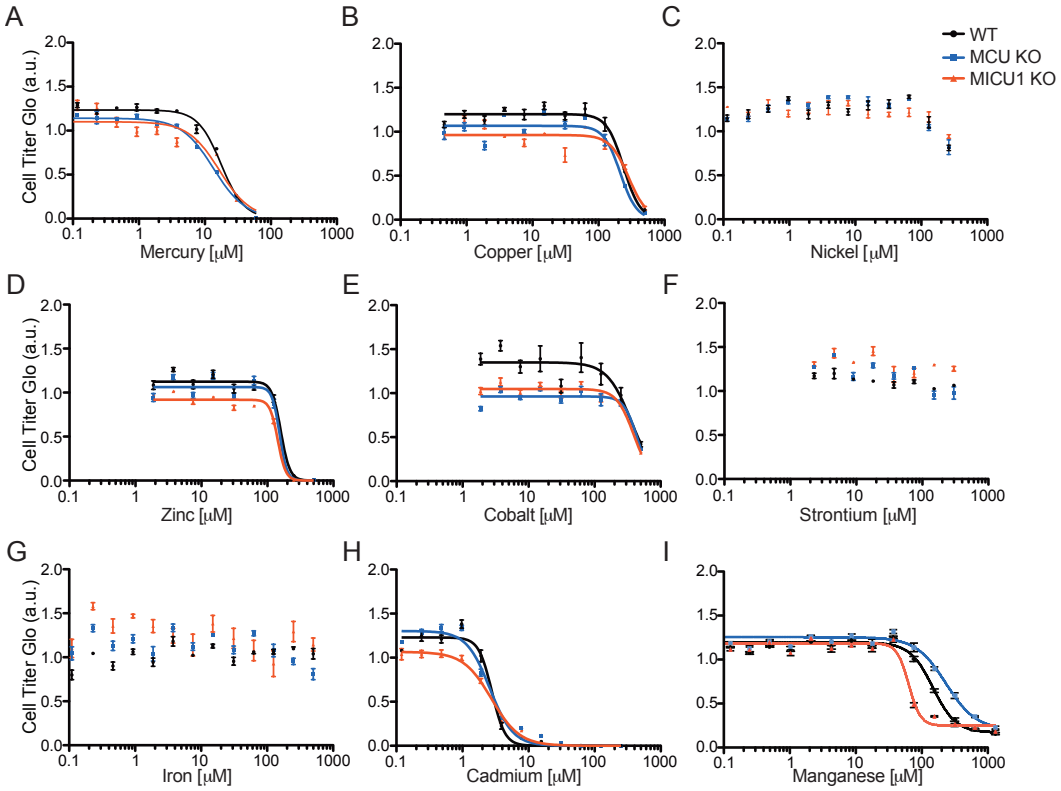

Fig. S1. Heavy metal screening of HEK-293T cells in glucose

(A-I). HEK-293T cells (WT, MCU KO, and MICU1 KO) were treated with a range of concentrations of metals in media containing high glucose. Cell Titer-Glo was used to measure cell viability. The y-axes are normalized to untreated controls (note that due to the log scale the untreated cells are not shown). Data shown represent the average ± SEM of six technical replicates. Note that (I) is plotted again in Figure 1A.

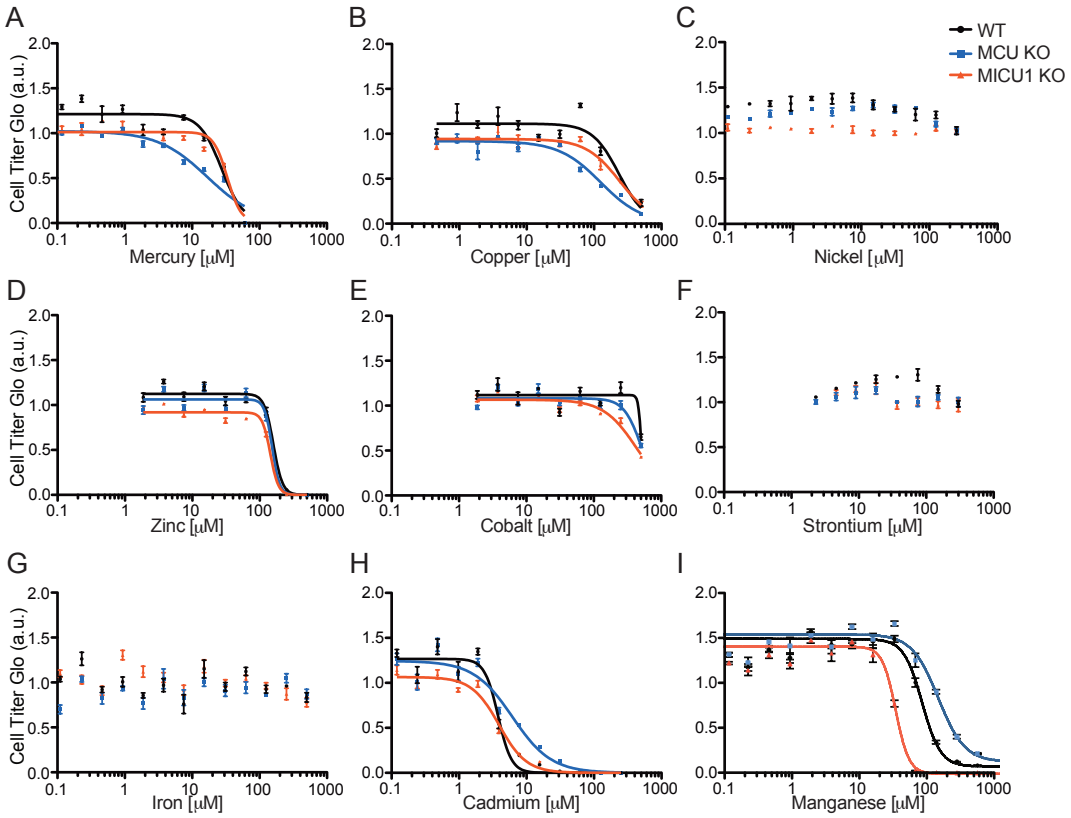

Fig. S2. Heavy metal screening of HEK-293T cells in galactose

(A-I) HEK-293T cells (WT, MCU KO, and MICU1 KO) were treated with a range of concentrations of metals in media lacking glucose but containing galactose. Cell Titer-Glo was used to measure cell viability. The y-axes are normalized to untreated controls (note that due to the log scale the untreated cells are not shown). Data shown represent the average ± SEM of six technical replicates. Note that (I) is plotted again in Figure 1B.

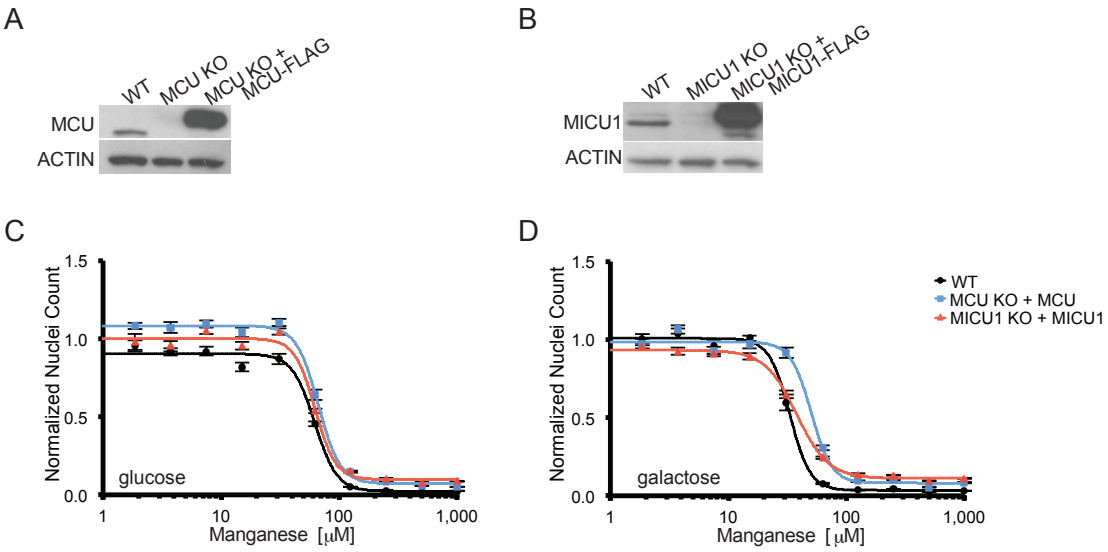

Fig. S3. Mn^{2+} toxicity differences between genotypes can be rescued by exogenous expression of the knocked out protein

(A) Immunoblot of whole cell lysates from WT, MCU KO and MCU KO with forced exogenous expression of MCU with a FLAG tag. (B) Immunoblot of whole cell lysates from WT, MICU1 KO and MICU1 KO with forced exogenous expression of MICU1 with a FLAG tag. (C and D) Nuclei counting was used to measure cell viability. HEK-293T cells (WT, MCU KO with forced exogenous MCU expression, and MICU1 KO with forced exogenous MICU1 expression) were treated with a range of concentrations of $MnCl_2$ in media with glucose (C) or lacking glucose but containing galactose (D). The y-axes are normalized to untreated controls (note that due to the log scale the untreated cells are not shown). Data shown represent the average ± SEM of six technical replicates.

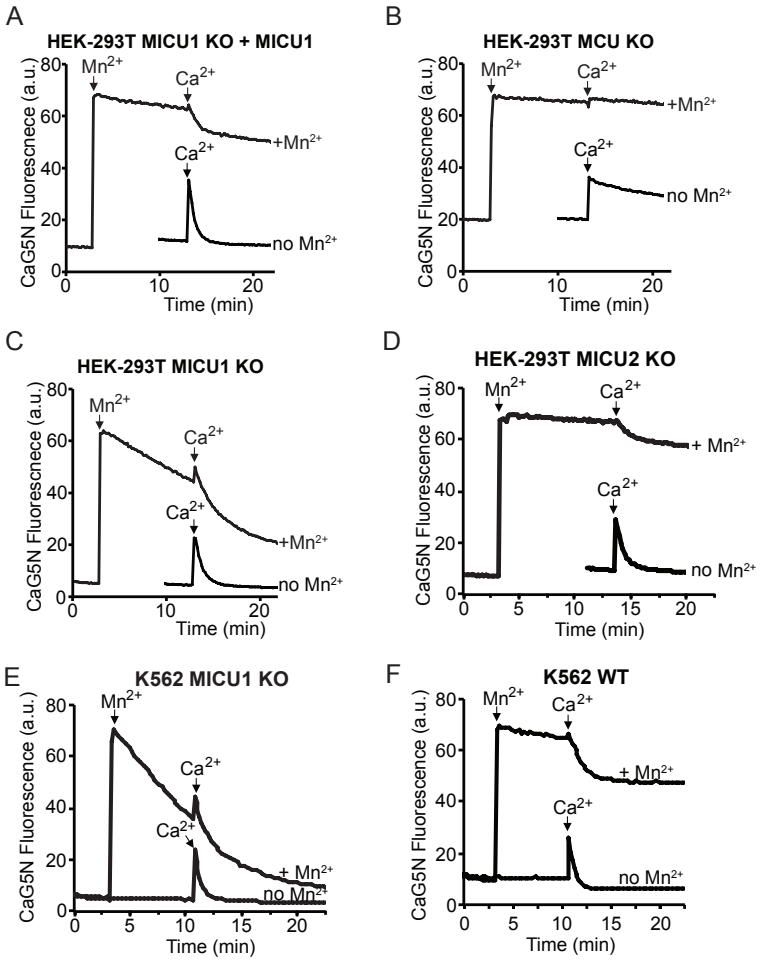

Fig. S4. Presence of Ca^{2+} promotes MCU-mediated Mn^{2+} uptake into mitochondria in HEK-293T or K562 cells even in the presence of MICU1

(A-D) MICU1 KO cells with exogenously expressed MICU1 (A), MCU KO cells (B), MICU1 KO cells (C), or MICU2 KO HEK-293T cells (D) were permeabilized and CaG5N was used to monitor extramitochondrial Mn^{2+} or Ca^{2+} levels. Ca^{2+} pulses indicated are 12 μM and Mn^{2+} pulses are 3 μM. Note that due to the higher affinity of CaG5N for Mn^{2+} most of the fluorescence signal comes from CaG5N-Mn^{2+} complex when both divalent cations are present. (E,F) MICU1 KO K562 cells (E) and WT K562 cells (F) were permeabilized and CaG5N was used to monitor extramitochondrial Mn^{2+} or Ca^{2+} levels. Ca^{2+} pulses are 12 μM and Mn^{2+} pulses are 3 μM. Representative traces of three biological replicates are shown for all experiments.

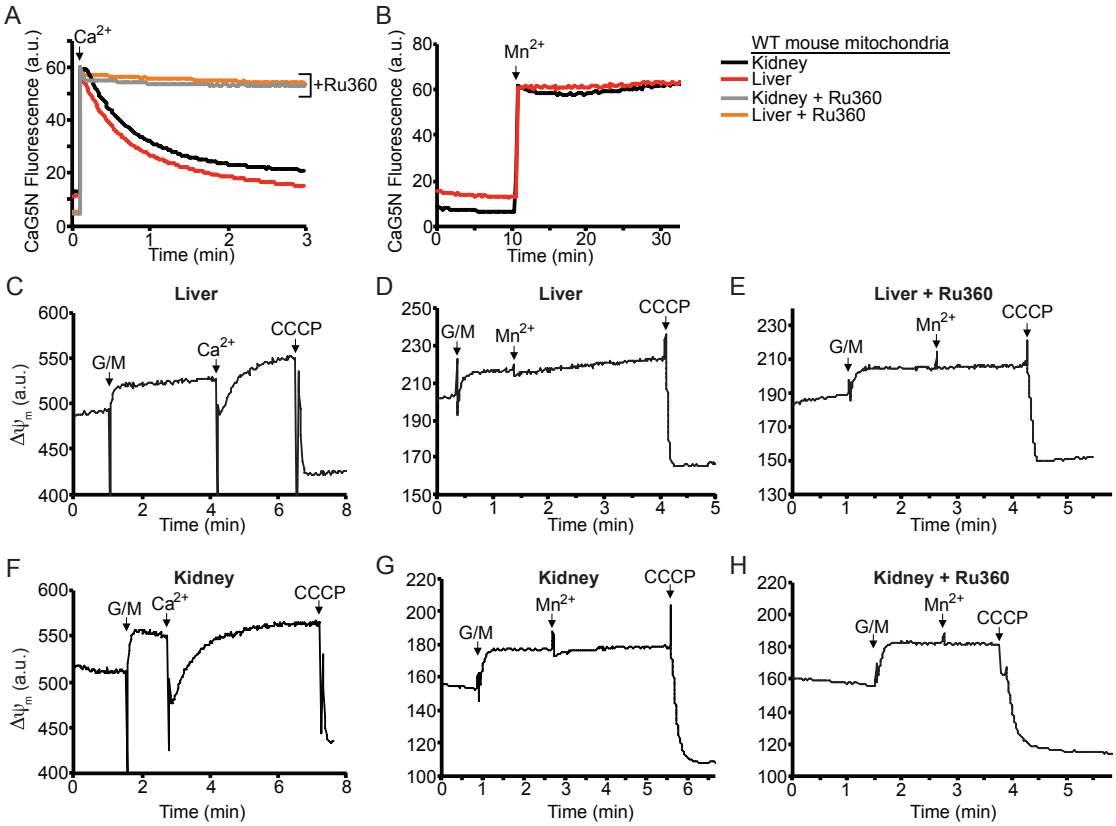

Fig. S5. WT Mouse kidney or liver mitochondria do not take up Mn^{2+}

(A and B) Isolated mouse liver or kidney mitochondria were assayed in the presence of the CaG5N fluorescent dye and given a pulse of either (A) 12 μM Ca^{2+} or 3 μM Mn^{2+}. CaG5N fluorescence is reporting on relative extramitochondrial [Mn^{2+} or Ca^{2+}]. Where indicated, 1 μM Ru360 was added to inhibit the uniporter and was present throughout the trace. (C-H) Mitochondrial membrane potential was monitored with TMRM and the signal is inverted (such that higher signal corresponds to increased membrane potential). G/M indicates the addition of 5 mM glutamate and malate to energize the mitochondria. Pulses of Ca^{2+} or Mn^{2+} indicated are 100 μM pulses and CCCP indicates the addition of 1 μM CCCP to uncouple mitochondria. Where indicated, 1 μM Ru360 was present throughout the trace. Traces shown are representative traces from three biological replicates for each.

Table S1. Values of LD_{50} for Mn^{2+} calculated from screens using nuclei counting

	Manganese LD50 (μM)	
HEK-293T cell line	**Glucose**	**Galactose**
WT	60 ± 2	33 ± 1
MCU KO	70 ± 2	66 ± 3
MICU1 KO	28 ± 1	10.7 ± 0.6
MCU KO + MCU	67 ± 1	50 ± 2
MICU1 KO + MICU1	61 ± 2	61 ± 1

LD_{50} values are listed in two different media conditions (containing glucose or galactose) for each HEK-293T cell genotype—WT, MCU KO, MICU1 KO, MCU KO with MCU-FLAG exogenously expressed (MCU KO + MCU) or MICU1 KO with MICU1-FLAG exogenously expressed (MICU1 KO + MICU1). The LD_{50} from a sigmoidal dose-response curve fit with variable slope along with the standard error of the fit are listed. All are significantly different than respective WT cells ($p < 0.05$). Graphical representation of the data is presented in Figure 1 and S3.