

Supplementary Materials for

MCART1/SLC25A51 is required for mitochondrial NAD transport

Nora Kory, Jelmi uit de Bos, Sanne van der Rijt, Nevena Jankovic, Miriam Göra, Nicholas Arp, Izabella A. Pena, Gyan Prakash, Sze Ham Chan, Tenzin Kunchok, Caroline A. Lewis, David M. Sabatini*

*Corresponding author. Email: sabatini@wi.mit.edu

Published 9 September 2020, *Sci. Adv.* **6**, eabe5310 (2020)
DOI: [10.1126/sciadv.abe5310](https://doi.org/10.1126/sciadv.abe5310)

This PDF file includes:

Figs. S1 to S4

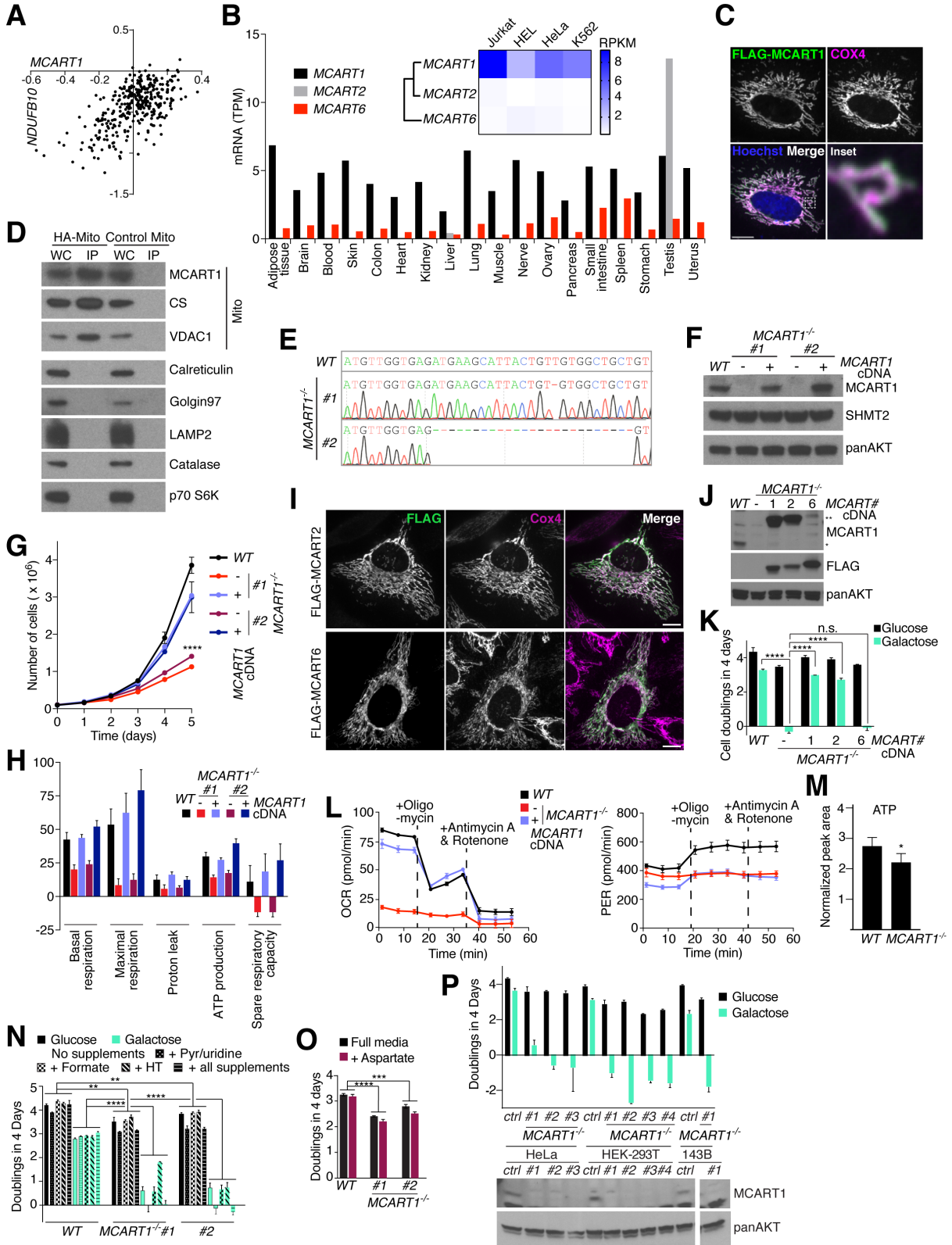
Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/sciadv.abe5310/DC1)

Data tables S1 and S2

Supplementary Figures

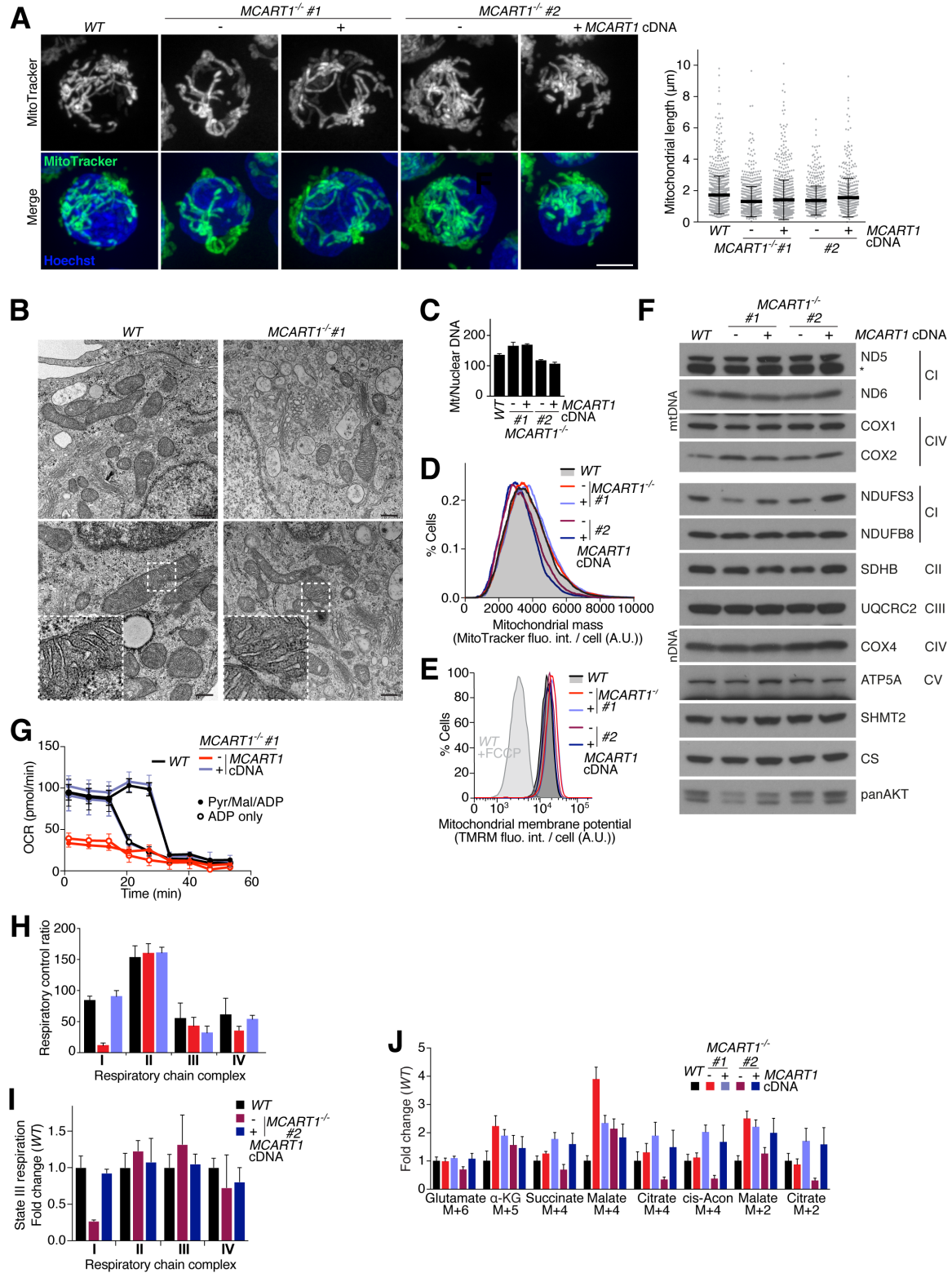
Figure S1



(A) *MCART1* is co-essential with *NDUFB10*. Gene essentiality scores for *MCART1* and *NDUFB10* from a panel of 341 cancer cell lines (8) are plotted. **(B)** mRNA levels of human *MCART* homologs in commonly used cell lines and normal tissues. RPKM (Reads Per Kilobase Million) levels were extracted from the Cancer Cell Line Encyclopedia (56) and TPM (Transcripts Per Kilobase Million) levels were extracted from GTEx Portal V7. **(C)** *MCART1* localizes to mitochondria. HeLa cells expressing FLAG-*MCART1* were processed for immunofluorescence detection and spinning disk-confocal microscopy. FLAG epitope – green; inner mitochondrial membrane (COX4) – magenta; overlap of channels – white. Scale bar, 10 μ m. **(D)** Endogenous *MCART1* co-purifies with mitochondria. HA-immunoprecipitates and cell lysates from wild-type cells expressing an HA-mito tag or a control MYC-mito tag were analyzed by immunoblotting for the following proteins: CS – citrate synthase; VDAC1 – voltage-dependent anion channel; CALR – calreticulin; GOLGA1 – Golgin subfamily A member; LAMP2 – lysosome-associated membrane glycoprotein; CAT – catalase; RPS6KB1 – Ribosomal protein S6 kinase beta-1. **(E)** Next generation sequencing confirms homozygous 1 or 25 bp frame-shift deletions in the *MCART1* open reading frame in two single-cell derived clones. **(F)** Immunoblot of *MCART1* in *MCART1*-null clones, or null clones re-expressing the *MCART1* cDNA. SHMT2 – serine hydroxymethyl transferase **(G)** *MCART1*-null cells have a proliferation defect in full RPMI media (mean \pm SD; $n = 3$). The asterisks denote statistically significant differences between knockout and wild-type clones or knockout clones re-expressing the *MCART1* cDNA, respectively. **(H)** Basal and maximal respiration, proton leak, ATP production and spare respiratory capacity are decreased in *MCART1*-null cells as measured by Seahorse extracellular flux analysis (mean \pm SD; $n \geq 13$ technical replicates). Graphs were generated from data in Fig. 11 **(I)** Human *MCART* homologs localize to mitochondria. HeLa cells expressing FLAG-*MCART* cDNA constructs were processed for immunofluorescence detection and spinning disk-confocal microscopy. FLAG epitope – green; inner mitochondrial membrane (COX4) – magenta; overlap of channels – white. Scale bar, 10 μ m. **(J)** Immunoblot of *MCART1*-null cells expressing FLAG-*MCART* cDNA constructs. *indicates endogenous *MCART1*. ** indicates FLAG-*MCART1*. **(K)** Human *MCART2* but not *MCART6* rescues the mitochondrial respiration defect of cells lacking *MCART1*. Asterisks denote statistically significant differences in proliferation in galactose between the cells expressing the empty vector and the solute carrier homologs, respectively (mean \pm SD; $n = 3$). **(L)** Oxygen consumption rate (OCR) and proton efflux rate (PER) were measured by Seahorse extracellular flux analysis with sequential treatment of oligomycin and antimycin A/rotenone (mean \pm SD; $n = 5$ technical replicates). **(M)** Cellular ATP concentrations in wild-type and *MCART1*-null cells. Data from two independent experiments were combined (mean \pm SD; $n = 6$). **(N)** Supplementation of RPMI media of cells with metabolites known to alleviate mitochondrial dysfunction does not rescue the proliferation defect of *MCART1*-null cells (mean \pm SD; $n = 3$). Pyr – pyruvate; HT – hypoxanthine-thymidine. **(O)** Supplementation of RPMI media of cells stably expressing the plasma membrane aspartate transporter SLC1A3 with 10 mM aspartate does not rescue the proliferation defect of *MCART1*-null cells (mean \pm SD; $n = 3$). **(P)** HeLa, HEK-293T and 143B cells lacking *MCART1* are unable to proliferation using galactose as the carbon-source. Multiple knockout clones for HeLa and HEK-293T, one clone for 143B cells is shown. In the bottom panel, loss of *MCART1* in knockout clones was confirmed by western blot.

** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

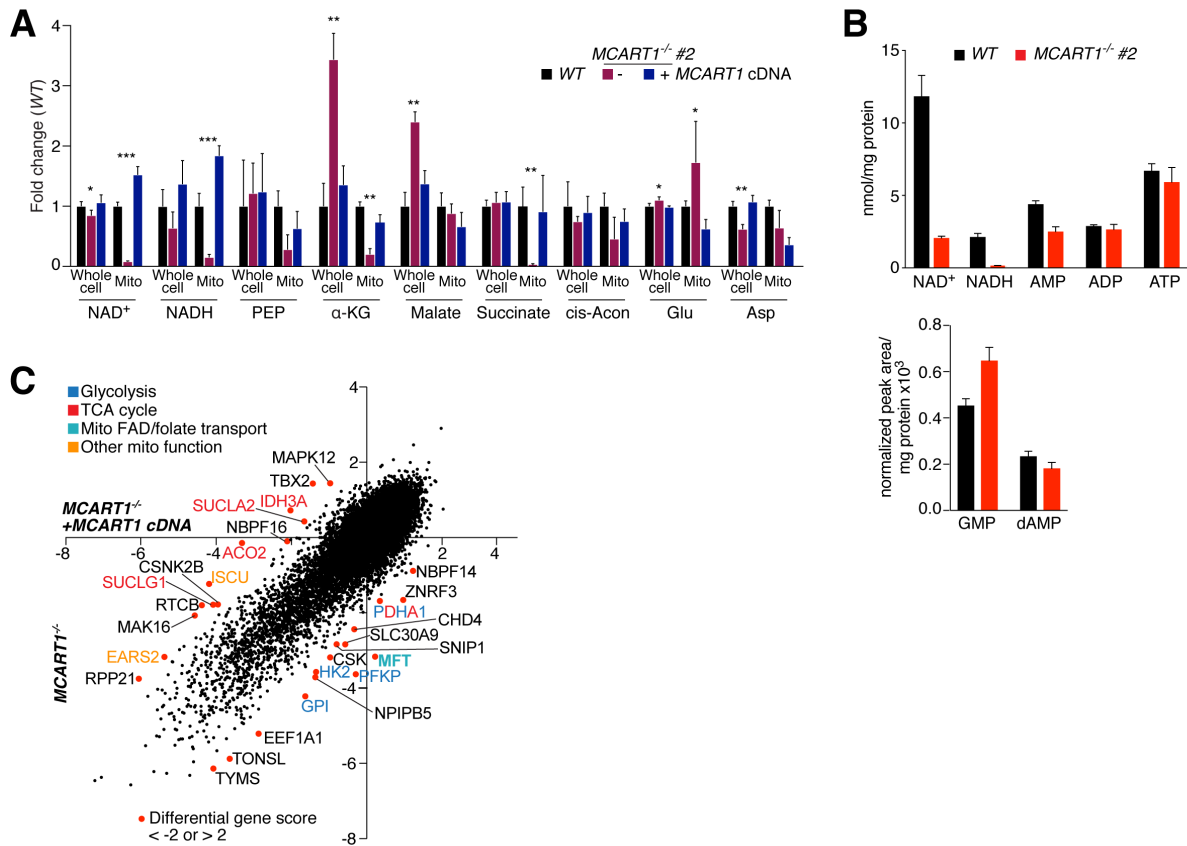
Figure S2



(A) Loss of MCART1 does not affect mitochondrial morphology and length. Max intensity z-projections of confocal images of mitochondria visualized by MitoTracker Green

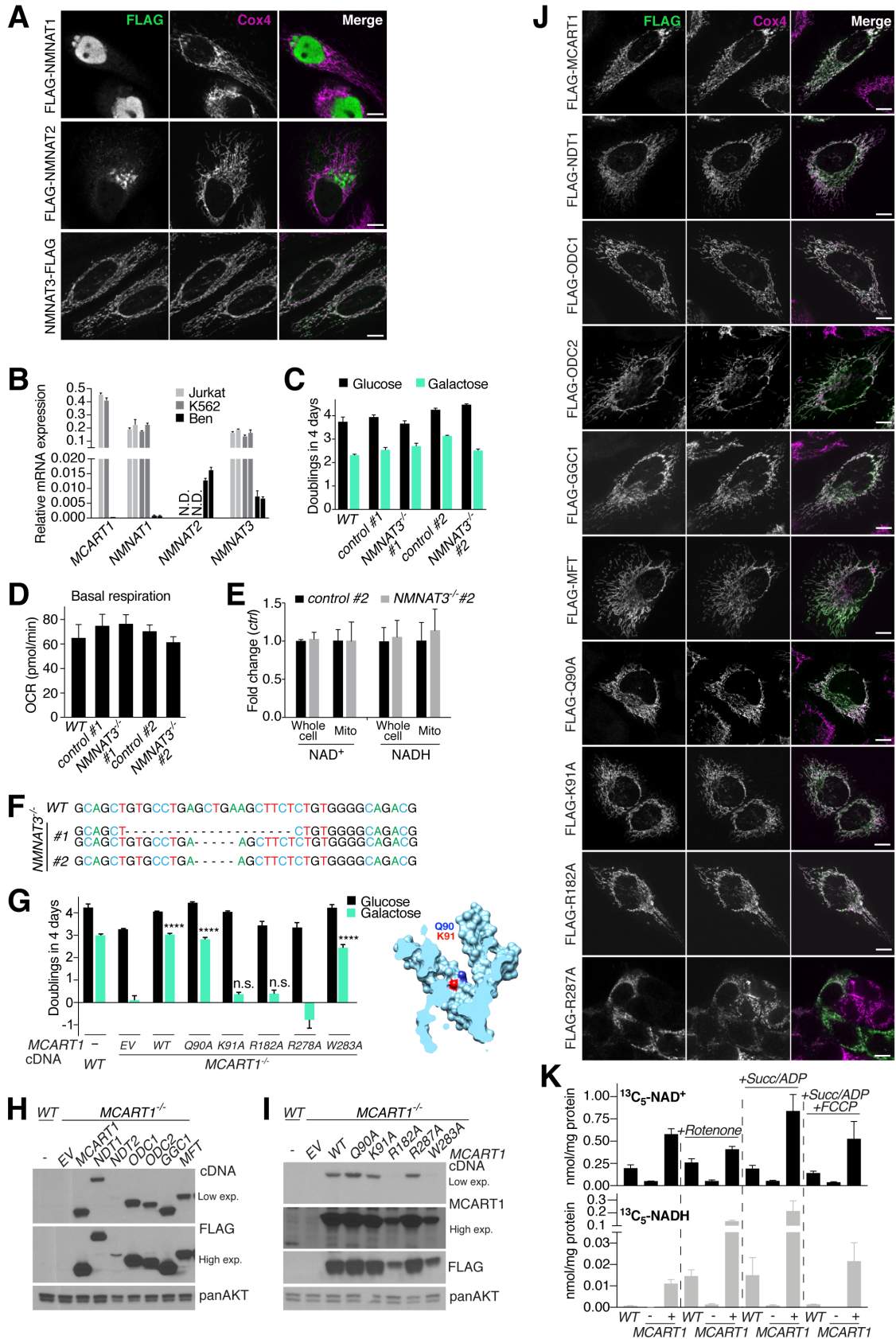
(green) were used to measure mitochondrial length of indicated Jurkat cells. Nuclei were stained with Hoechst DNA stain (blue) (mean \pm SD; $n > 500$; **** $P < 0.0001$). N.s. – not significant. Scale bar, 5 μ m. **(B)** Electron microscopy reveals mostly normal cristae morphology in *MCART1*-null mitochondria. Two 3x magnified inset are shown. Scale bar, 200 nm. **(C)** Loss of *MCART1* does not affect mtDNA content (mean \pm SD; $n = 3$ technical replicates). Mitochondrial (mt) DNA was quantified by qPCR and normalized to genomic DNA. **(D)** Loss of *MCART1* does not affect mitochondrial mass per cell as determined by flow cytometry analysis of indicated Jurkat cells stained with MitoTracker Green. The histograms were normalized and smoothed (A.U. – arbitrary units). **(E)** Loss of *MCART1* does not affect relative mitochondrial membrane potential as assessed by flow cytometry analysis of Jurkat cells stained with tetramethylrhodamine, methyl ester, and perchlorate (TMRM). Indicated cells were treated with 10 μ M FCCP. The histograms were normalized and smoothed. **(F)** Loss of *MCART1* only marginally affects protein levels of mitochondrially encoded (upper panel) and nuclear encoded (lower panel) mitochondrial respiratory chain complex subunits. Lysates prepared from indicated cells were equalized for total protein amounts and analyzed by immunoblotting for indicated proteins. CI – complex I; CII – complex II; CIII – complex III; CIV – complex IV; mtDNA – mitochondrially encoded; nDNA – nuclear encoded; CS – citrate synthase. **(G)** *MCART1*-null cells are unable to oxidize exogenous substrate via respiratory complex I. Oxygen consumption rate (OCR) measured by Seahorse extracellular flux analysis of indicated cells permeabilized and supplemented with ADP and complex I substrates or ADP only (mean \pm SD; $n = 3$ technical replicates). Mal – malate; perm – permeabilizer; pyr – pyruvate; rot – rotenone. **(H)** Respiratory control ratio determined by Seahorse extracellular flux analysis of indicated cells permeabilized and supplemented with ADP and different respiratory complex substrates (mean \pm SD; $n = 3$ technical replicates). **(I)** Complex I-dependent state 3 respiration is diminished in *MCART1*-null clone #2 as determined by Seahorse extracellular flux analysis (mean \pm SD; $n = 3$ technical replicates). **(J)** TCA cycle intermediates are still produced in *MCART1*-null cells at the whole cell level. Jurkat cells were incubated in RPMI media containing 2 mM $^{13}\text{C}_5$, $^{15}\text{N}_2$ -glutamine as the sole glutamine source for 2 hours before metabolites were extracted.

Figure S3



(A) NAD⁺ and NADH are depleted in mitochondria of *MCART1*-null clone #2 and TCA cycle intermediates are reduced. Whole cell and mitochondrial metabolite levels in indicated cells were measured by LC-MS using the Mito-IP method (mean ± SD; $n = 4$). Asterisks denote statistically significant differences of *MCART1*-null samples with both wild-type cells and cells re-expressing the *MCART1* cDNA ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). PEP – phosphoenolpyruvate; α-KG – α-Ketoglutarate; cis-Acon – cis-aconitate; Glu – glutamate; Asp – aspartate. **(B)** Absolute levels of metabolites in mitochondria isolated by differential centrifugation. Metabolites were measured by LC-MS, quantified based on standard curves and normalized to mitochondrial protein content. Normalized peak areas are shown for GMP and dAMP (mean ± SD; $n = 3$ technical replicates). **(C)** Gene scores from *MCART1*-reexpressing control cells were plotted against those from *MCART1*-null cells. Genes with a differential score of <-2 or >2 are annotated as hits. PDHA1 is labeled in red and blue as it connects glycolysis with the TCA cycle. mito – mitochondrial; TCA – tricarboxylic acid; FAD – flavin adenine dinucleotide.

Figure S4



(A) FLAG-tagged nicotinamide mononucleotide adenylyltransferase (NMNAT) isoforms localize to the nucleus, the Golgi and mitochondria, respectively. HeLa cells expressing FLAG-NMNAT cDNA constructs were processed for immunofluorescence detection and spinning disk-confocal microscopy. FLAG epitope – green; inner mitochondrial membrane (COX4) – magenta; overlap of channels – white. Scale bar, 10 μ m. **(B)** Jurkat and K562 cells express *MCART1*, *NMNAT1*, and *NMNAT3*. mRNA levels were quantified by qPCR relative to *β -ACTIN*. Two primer pairs each were used for *NMNAT1*, 2 and 3. N.D. – not detected. (Mean \pm SD; $n = 3$ technical replicates). **(C)** Human *NMNAT3*-null cells have no mitochondrial respiration defect. Single-cell-derived *NMNAT3* knockout or control Jurkat cells were cultured in media containing glucose or galactose as the carbon source. (Mean \pm SD; $n = 3$). N.s. – not significant. **(D)** Human *NMNAT3*-null cells have no mitochondrial respiration defect. A mitochondrial stress test was performed by Seahorse extracellular flux analysis on single-cell-derived *NMNAT3* knockout or control Jurkat cells. (Mean \pm SD; $n > 10$ technical replicates). **(E)** Mitochondrial NAD levels do not depend on *NMNAT3*. (Mean \pm SD; $n = 4$). N.s. – not significant. **(F)** Next generation sequencing confirms 20 bp and 5 bp or homozygous 5 bp frame-shift deletions in the *NMNAT3* open reading frame in two single-cell derived clones. **(G)** Mutation of lysine 91 or arginine 278 to alanine abolishes the ability of *MCART1* to rescue growth on galactose. *MCART1*-null cells infected with wild-type *MCART1* cDNA serve as control cells. (mean \pm SD; $n = 3$; **** $P < 0.0001$). EV – empty vector. Positions of glutamine 90 and lysine 91 in the predicted structure of *MCART1* are shown on the right. **(H)** Immunoblot of *MCART1*-null cells expressing FLAG-*MCART1*, MFT or yeast transporter cDNA constructs. Lysates prepared from indicated cells were equalized for total protein amounts and analyzed by immunoblotting for the FLAG-epitope or the levels of the indicated proteins. **(I)** Immunoblot of *MCART1*-null cells expressing wild-type or mutant FLAG-*MCART1* cDNA constructs. Lysates prepared from indicated cells were equalized for total protein amounts and analyzed by immunoblotting for the FLAG-epitope or the levels of the indicated proteins. **(J)** FLAG-tagged yeast NAD⁺ transporter NDT1, the 2-oxodicarboxylate transporters ODC1 and ODC2, the GTP/GDP transporter GGC1, human MFT and different *MCART1* point mutants localize to mitochondria. NDT2 expression was not detectable. HeLa cells expressing FLAG-tagged cDNA constructs were processed for immunofluorescence detection and spinning disk-confocal microscopy. FLAG epitope – green; inner mitochondrial membrane (COX4) – magenta; overlap of channels – white. Scale bar, 10 μ m. **(K)** Transport of NAD⁺ into mitochondria in depends on *MCART1*. Mitochondria purified from wild-type, *MCART1*-null or *MCART1*-null cells overexpressing the *MCART1* cDNA were incubated with 50 μ M stably isotope labeled ¹³C₅-NAD⁺ for 10 min, where indicated in the presence of 5 μ M rotenone, succinate and ADP or succinate, ADP and 5 μ M FCCP. Levels of taken up ¹³C₅-NAD⁺ and generated ¹³C₅-NADH were quantified by LC-MS (mean \pm SD; $n = 3$ uptake reactions).

Supplementary Data

Data Table S1 (separate file)

Metabolites abundances in *MCART1*-null mitochondrial fractions without or with *MCART1* cDNA (Fig, 3A)

Data Table S2 (separate file)

Guide counts and gene scores from *MCART1* synthetic lethality screen