APPENDIX

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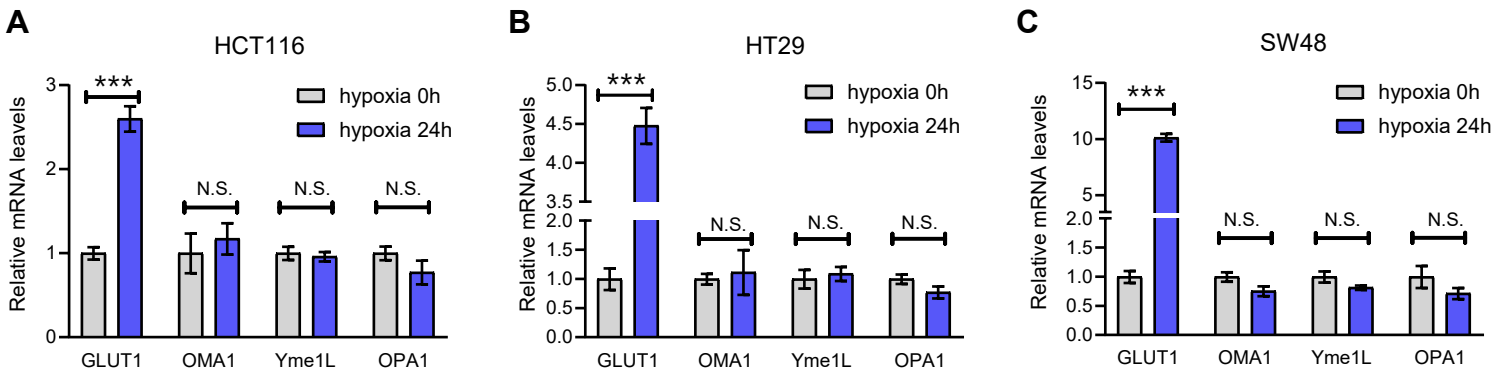
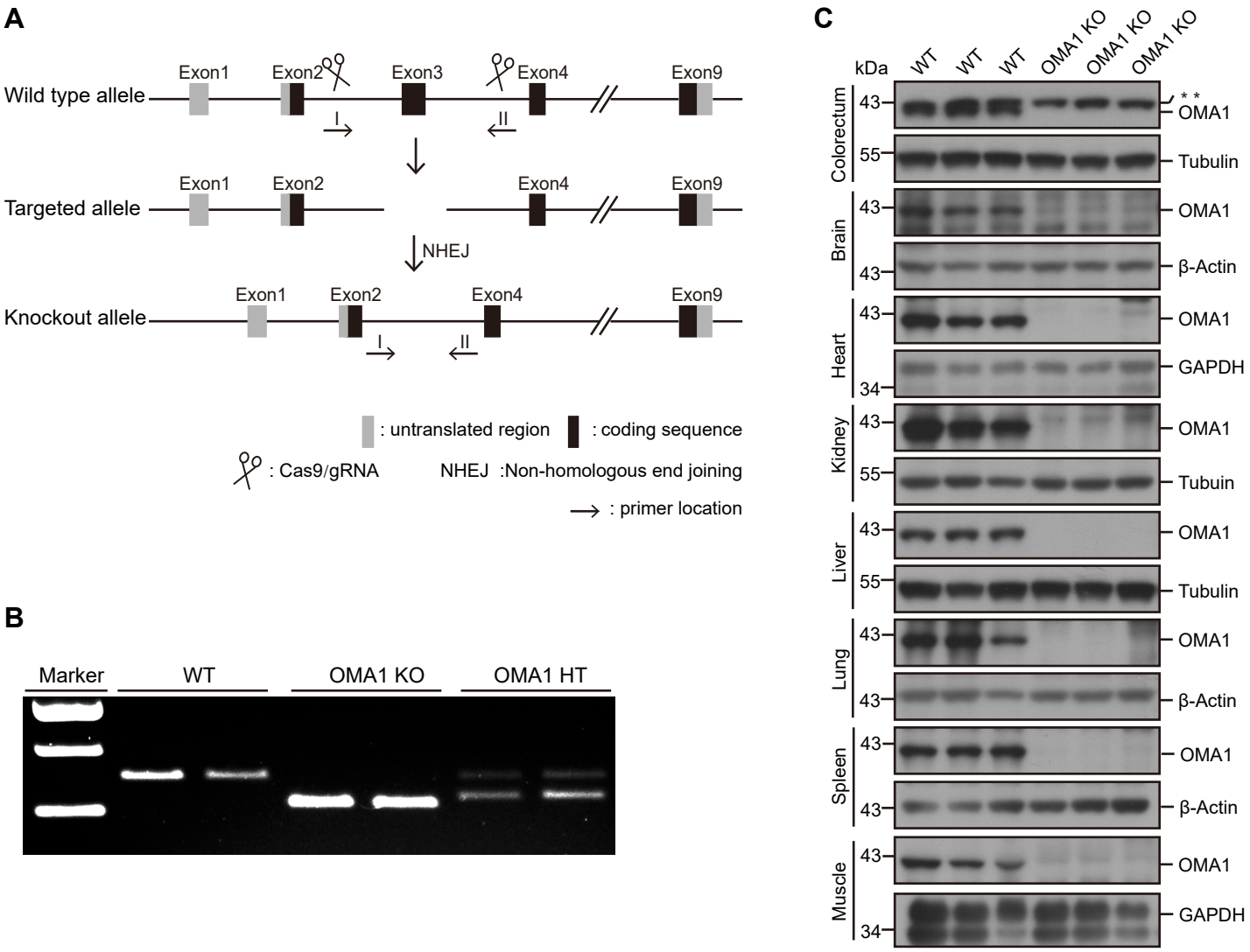


Appendix Figure S1. Hypoxia-activated OMA1 does not rely on the transcriptional upregulation.

A-C. Colorectal cancer cells HCT116 (A), HT29 (B), and SW48 (C) were cultured in normoxia or hypoxia (1% O_2) for 24 hours. Relative expressions of GLUT1, OMA1, Yme1L, and OPA1 mRNA in normoxia or hypoxia were analyzed by quantitative RT-PCR. GLUT1 was used as a positive control. Statistical significance was assessed by Student's t-test; error bars are presented as mean ± SD of three independent experiments; N.S., not significant; ***$p < 0.001$.

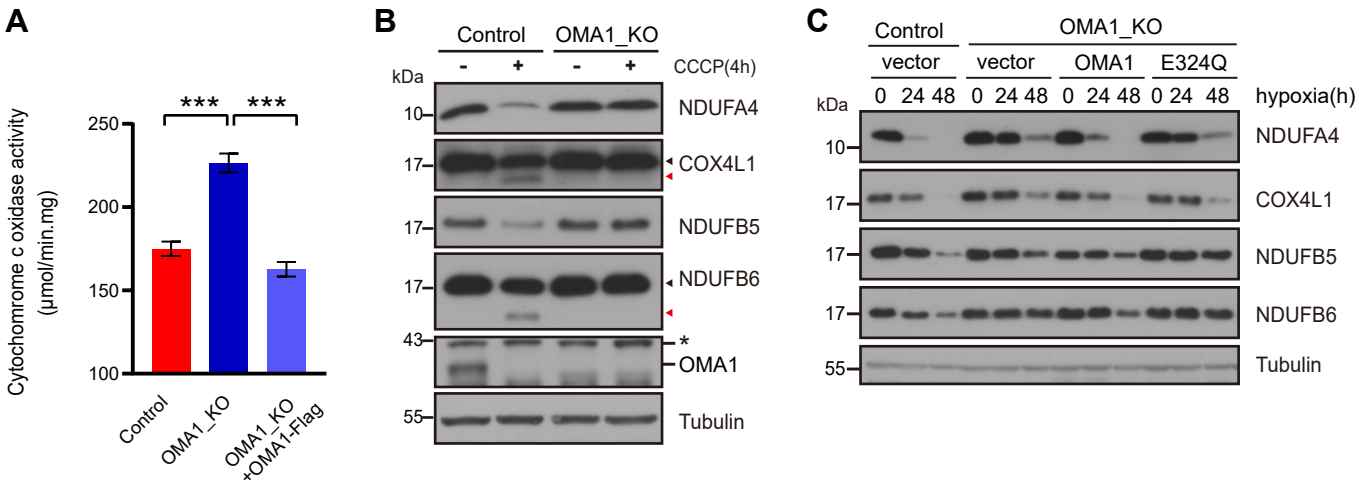


Appendix Figure S2. Generation of OMA1$^{-/-}$ mice via the CRISPR/CAS9 system.

A. Schematic representation of wild-type (WT) and knockout alleles, and the targeted locus. The exons are indicated with boxes, and the solid lines represent introns. To delete OMA1, the Cas9 (a nuclease guided by sgRNA) bound to the targeted genomic locus next to the PAM sequence, and generated a double-strand break. The break was then repaired by non-homologous end-joining (NHEJ), which leads to a 620 bp deletion mutations in the exon 3 of OMA1 gene.

B. The genotyping of OMA1 knockout mice was determined by PCR analysis. The PCR product for WT mice is 369 bp. The PCR product for OMA1 KO mice is 293 bp. The heterozygous (OMA1 HT) mice contain both products (representative data from three independent experiments).

C. Western blotting analysis of OMA1 expression in different organs from WT or OMA1 KO mice. Tubulin, β-Actin, or GAPDH was used as loading control in different organs. The asterisk indicates a nonspecific band.



Appendix Figure S3. OMA1 regulates mitochondrial cytochrome c oxidase activity and respiratory chain complexes components

A. Cytochrome c oxidase (COX) activities of control, OMA1 KO, or OMA1-Flag expressed OMA1 KO HCT116 cells were measured. Statistical significance was assessed by one-way ANOVA; error bars are presented as mean ± SD of three independent experiments; ***$p < 0.001$.

B. Control or OMA1 KO HCT116 cells were treated with vehicle (-) or 20 μm CCCP (+) for 4 hours. Cell lysates were then analyzed by Western blotting with antibodies against NDUFA4, COX4L1, NDUFB5, DNUFB6, or Tubulin. The black arrowhead indicated the full-length COX4L1 or NDUFB6, and the red arrowhead pointed to the cleaved band. The images are representative of three experiments.

C. WT, OMA1 KO and OMA1 KO HCT116 cells expressing WT-OMA1(OMA1-Flag) or proteolytic inactive OMA1(E324Q-Flag) were cultured in hypoxia (1% O_2) for 0, 24, or 48 hours, and the cell lysates were assessed by western blot with antibodies against NDUFA4, COX4L1, NDUFB5, and NDUFB6. Tubulin was used as a loading control (representative data from three independent experiments).

Appendix Table S1. Selected Tandem mass spectrometry (MS/MS) data from the eluted protein samples of the co-IP assay.

Gene Name	Description	MW (kDa)	Σ# Unique Peptides	Σ# PSMs
OMA1	Metalloendopeptidase OMA1, mitochondrial	60.08	6	25
MIC60/IMMT	Mitochondrial inner membrane protein	83.63	16	19
LPPRC	Leucine-rich PPR motif-containing protein	157.81	20	20
PGAM5	Serine/threonine-protein phosphatase PGAM5	31.98	9	13
AFG3L2	AFG3-like protein 2	88.53	8	8
YME1L1	ATP-dependent zinc metalloprotease YME1L1	86.40	3	3
MIC19/CHCHD3	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3	26.14	3	3
APOOL	MICOS complex subunit MIC27	29.14	2	2
PHB2	Prohibitin-2	33.28	2	3
PHB	Prohibitin	29.19	1	1
UQCRC2	Cytochrome b-c1 complex subunit 2, mitochondrial	48.41	4	4
NDUFS1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	79.42	4	4
AIFM1	Apoptosis-inducing factor 1	66.86	26	15

NDUFA4	Cytochrome c oxidase subunit NDUFA4	9.36	15	22
ATP5C1	ATP synthase subunit gamma, mitochondrial	32.98	3	4
ATP5B	ATP synthase subunit beta, mitochondrial	56.52	15	23
ATP5A1	ATP synthase subunit alpha	59.71	16	22
NDUFB5	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5	21.74	2	4
NDUFB6	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6	15.48	2	3
ATP5F1C	ATP synthase subunit gamma	32.98	3	4
COX2	Cytochrome c oxidase subunit 2	25.55	1	1
COX4L1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	19.56	1	3

Table S1. Selected Tandem mass spectrometry (MS/MS) data. OMA1-E324Q-Flag was transiently expressed in 293T cells, and co-immunoprecipitation (co-IP) assay was performed with anti-Flag M2 affinity gel. The eluted protein samples were analyzed by MS/MS and the indicated proteins from MS/MS data was displayed in this table. The indicated protein of molecular weight (MW), the number and of total peptide spectrum matching (PSMs), the number of total unique peptides (Coverage) identified by MS were shown. The “red” indicates these proteins were further confirmed to interact with OMA1 by co-IP assay.