Supplementary Figures

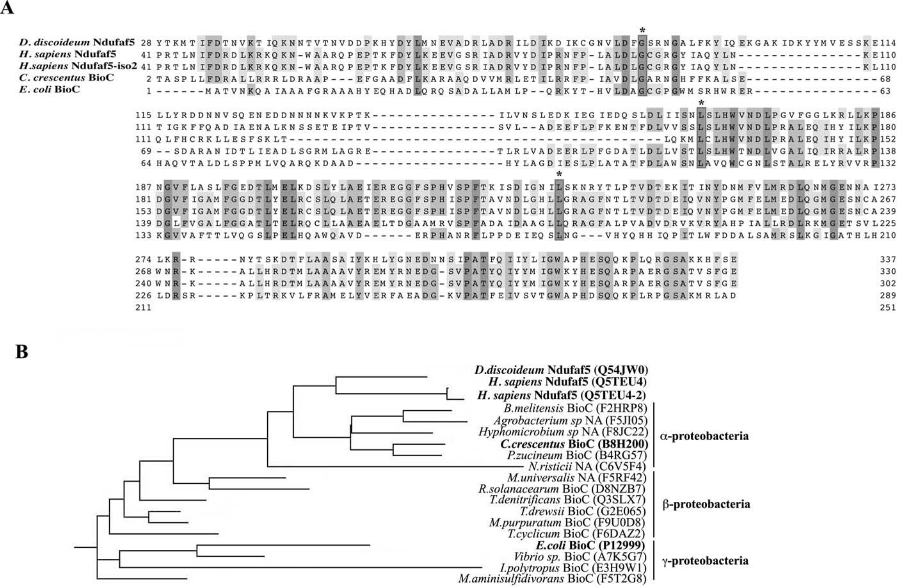
FIGURE S1. Bioinformatic analyses of Ndufaf5 and BioC. (A) Alignment of Ndufaf5 and BioC proteins suggests that they might be truly orthologs. Figure shows both Ndufaf5 isoforms from human, *Dictyostelium* Ndufaf5 and BioC proteins from *E.coli* and *C.crescentus*. Grey intensity represents the identity percentage. Asterisks represent conserved amino acids cited in the text (G86, L165 and L235). **(B)** Phylogenetic tree of Ndufaf5-BioC family. Ndufaf5 protein from human, *D.discoideum* and a representative group of orthologs in α, β and γ-protebacteria (19 proteins), obtained previously using Blast, were used. Branch lengths represent % of evolution. The number between brackets are Uniprot entries. NA, Not Annotated.

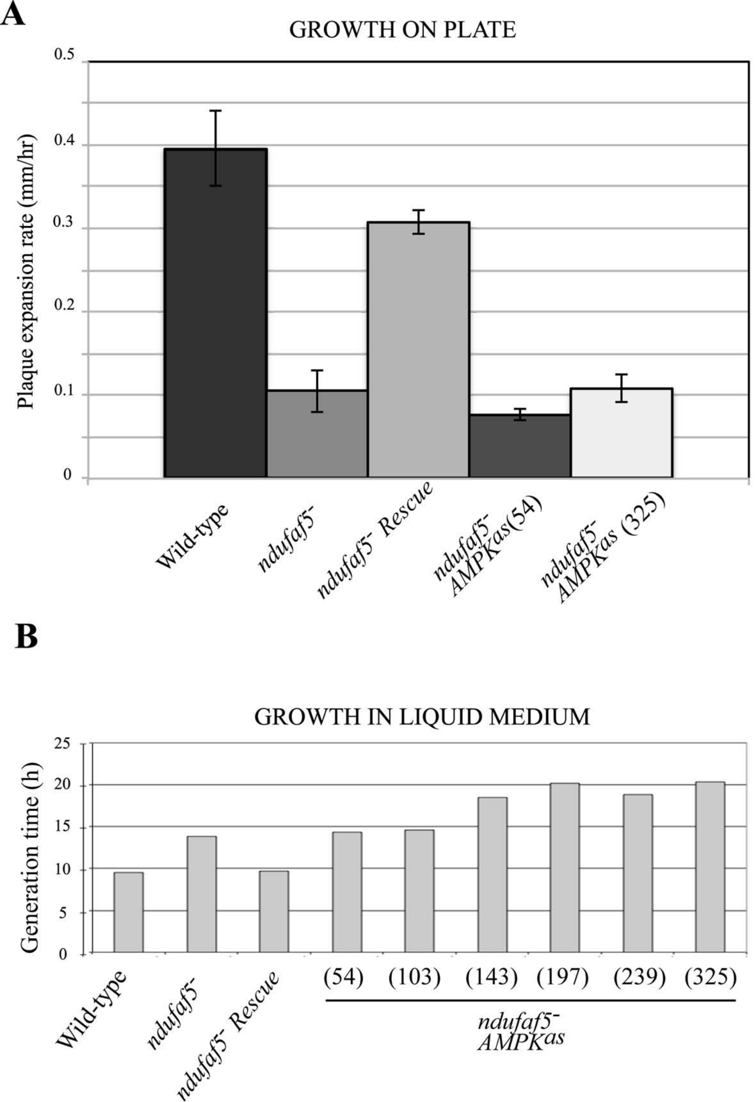
FIGURE S2. *ndufaf5*$^-$ growth rates are complemented in *ndufaf5*$^-$ Rescue strain but not by AMPK inhibition. **(A)** Growth rates in association with bacteria (SM plates) were obtained from at least 3 independent experiments. Numbers between brackets indicate the copy number of the AMPK^{as} construct. **(B)** A representative experiment of growth in HL5 liquid medium is shown. Numbers between brackets indicate the copy number of the AMPK^{as} construct.

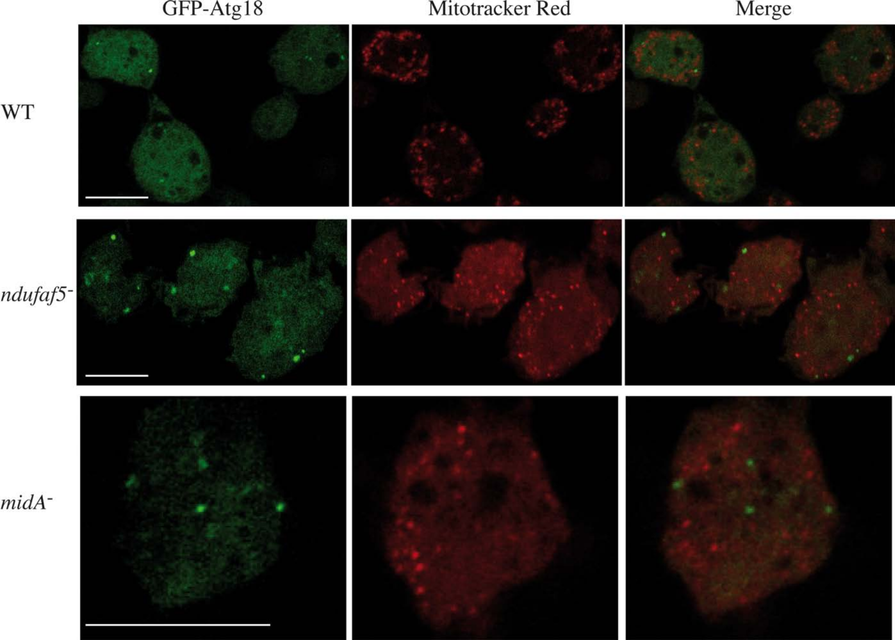
FIGURE S3.

Confocal analyses of GFP-Atg18 and mitochondria I. Wild-type, *ndufaf5*$^-$ and *midA*$^-$ growing cells transfected with the autophagic marker GFP-Atg18 were stained with the mitochondrial marker Mitotracker Red, fixed and visualized by confocal microscopy. No colocalization was observed. Bar, 10 μm.

FIGURE S4. Confocal analyses of GFP-Atg18 and mitochondria II. Wild-type, *ndufaf5*$^-$ and *midA*$^-$ growing cells transfected with the autophagic marker GFP-Atg18 were fixed, stained with DAPI and visualized by confocal microscopy. Nuclei and mitochondria were stained by DAPI (Red color was assigned to the fluorescence emission of DAPI). The signal was not found to colocalize with GFP-Atg18 puncta. Bar, 10 μm.


A
D. discoideum Ndufaf5
H. sapiens Ndufaf5
H.sapiens Ndufaf5-iso2
C. crescentus BioC
E. coli BioC
*
28 YTKMTIFDTNVKTIQKNNTVTNVDDPKHYDYLMNEVADRLADRILDIKDIKCGNVLDFGSRNGALFKYIQEKGAKIDKYYMVESSKE 114
41 PRTLNIFDRDLKRKQKN-WAARQPEPTKFDYLKEEVGSRIADRVYDIPRNFP-LALDLGCGRGYIAQYLN---------------KE 110
41 PRTLNIFDRDLKRKQKN-WAARQPEPTKFDYLKEEVGSRIADRVYDIPRNFP-LALDLGCGRGYIAQYLN---------------KL 110
2 TASPLLFDRALLRRRLDRAAP---EFGAADFLKARAAQDVVMRLETILRRFP-IAVDLGARNGHFFKALSE---------------- 68
1 ------MATVNKQAIAAAFGRAAAHYEQHADLQRQSADALLAMLP--QRKYT-HVLDAGCGPGWMSRHWRER--------------- 63
*
115 LLYRDDNNVSQENEDDNNNNKVKPTK---------------ILVNSLEDKIEGIEDQSLDLIISNLSLHWVNDLPGVFGGLKRLLKP 186
111 TIGKFFQADIAENALKNSSETEIPTV---------------SVL--ADEEFLPFKENTFDLVVSSLSLHWVNDLPRALEQIHYILKP 180
111 QLFHCRKLLESFSKLT---LQKMLCLHWVNDLPRALEQIHYILKP 152
69 --SDARANIDTLIEADLSGRMLAGRE---------------TLRLVADEERLPFGDATLDLLVSTLSLHWTNDLVGALIQIRRALRP 138
64 HAQVTALDLSPPMLVQARQKDAAD------------------HYLAGDIESLPLATATFDLAWSNLAVQWCGNLSTALRELYRVVRP 132
*
187 NGVFLASLFGEDTLMELKDSLYLAEIEREGGFSPHVSPFTKISDIGNILSKNRYTLPTVDTEKITINYDNMFVLMRDLQNMGENNAI 273
181 DGVFIGAMFGGDTLYELRCSLQLAETEREGGFSPHISPFTAVNDLGHLLGRAGFNTLTVDTDEIQVNYPGMFELMEDLQGMGESNCA 267
153 DGVFIGAMFGGDTLYELRCSLQLAETEREGGFSPHISPFTAVNDLGHLLGRAGFNTLTVDTDEIQVNYPGMFELMEDLQGMGESNCA 239
139 DGLFVGALFGGATLTELRQCLLAAEAELTDGAAMRVSPFADAIDAAGLLQRAGFALPVADVDRVKVRYAHPIALLRDLRKMGETSVL 225
133 KGVVAFTTLVQGSLPELHQAWQAVD------ERPHANRFLPPDEIEQSLNG---VHYQHHIQPITLWFDDALSAMRSLKGIGATHLH 210
274 LKR-R------NYTSKDTFLAASAIYKHLYGNEDNNSIPATFQIIYLIGWAPHESQQKPLQRGSAKKHFSE 337
268 WNR-K------ALLHRDTMLAAAAVYREMYRNEDG-SVPATYQIYYMIGWKYHESQARPAERGSATVSFGE 330
240 WNR-K------ALLHRDTMLAAAAVYREMYRNEDG-SVPATYQIYYMIGWKYHESQARPAERGSATVSFGE 302
226 LDRSR------KPLTRKVLFRAMELYVERFAEADG-KVPATFEIVSVTGWAPHDSQQKPLRPGSAKMRLAD 289
211 251
B
D.discoideum Ndufaf5 (Q54JW0)
H. sapiens Ndufaf5 (Q5TEU4)
H. sapiens Ndufaf5 (Q5TEU4-2)
B.melitensis BioC (F2HRP8)
Agrobacterium sp NA (F5JI05)
Hyphomicrobium sp NA (F8JC22)
C.crescentus BioC (B8H200)
P.zucineum BioC (B4RG57)
N.risticii NA (C6V5F4)
M.universalis NA (F5RF42)
R.solanacearum BioC (D8NZB7)
T.denitrificans BioC (Q3SLX7)
T.drewsii BioC (G2E065)
M.purpuratum BioC (F9U0D8)
T.cyclicum BioC (F6DAZ2)
E.coli BioC (P12999)
Vibrio sp. BioC (A7K5G7)
I.polytropus BioC (E3H9W1)
M.aminisulfidivorans BioC (F5T2G8)
α-proteobacteria
β-proteobacteria
γ-proteobacteria


A
GROWTH ON PLATE
Plaque expansion rate (mm/hr)
0.5
0.4
0.3
0.2
0.1
0
Wild-type
ndufaf5⁻
ndufaf5⁻ Rescue
ndufaf5⁻ AMPKas(54)
ndufaf5⁻ AMPKas (325)
B
GROWTH IN LIQUID MEDIUM
Generation time (h)
25
20
15
10
5
0
Wild-type
ndufaf5⁻
ndufaf5⁻ Rescue
(54)
(103)
(143)
(197)
(239)
(325)
ndufaf5⁻ AMPKas


GFP-Atg18
Mitotracker Red
Merge
WT
ndufaf5^{-}
midA^{-}

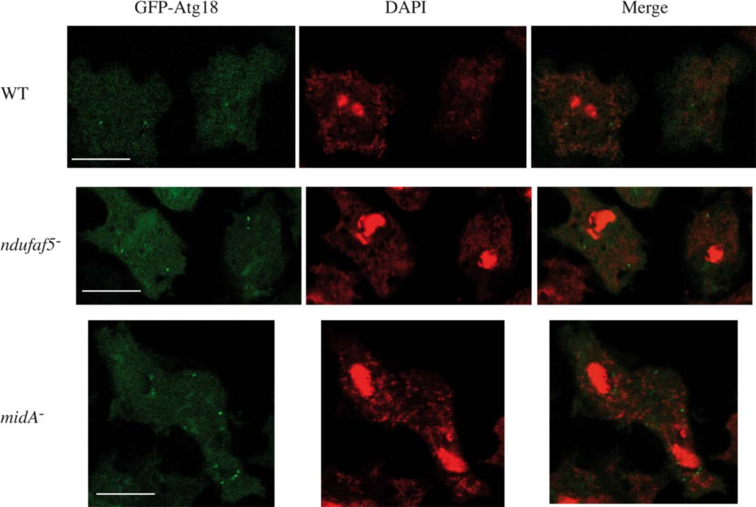

GFP-Atg18
DAPI
Merge
WT
ndufaf5⁻
midA⁻

TABLE S1. Oligonucleotides used for KO verification and site- directed mutagenesis

EXPERIMENT	OLIGO FORWARD	OLIGO REVERSE
GENOMIC KO VERIFICATION	1. AACTTGAGAATCCAATAGTTGC	2. CAAATAATAATTAACCAACCCAAG
RNA KO VERIFICATION	3.TAGGATCCATGTTAAGAACAACATTTAGAAAAGG	4. CCCAATGTAATGAGAAATTACTTATAATTAAATC
CONTROL (DDB_G0268840)	5.CGGGATCCGCATGTAACATCCAAAGATTTTAG	6.CGGGATCCCTATATTATTATTTTTTAATAAATCAAATGG
DIRECTED MUTAGENESIS M1	7.GGTAATGTTTTAGATTTTGTTAGTAGAAATGGAGC	8.GCTCCATTTCTACTAACAAAATCTAAAACATTACC
DIRECTED MUTAGENESIS M2	9. GATTTAATTATAAGTAATTTCTCATTACATTGGG	10. CCCAATGTAATGAGAAATTACTTATAATTAAATC
DIRECTED MUTAGENESIS M3	11. GATATTGGTAACATTCCATCAAAGAATAGATAC	12. GTATCTATTCTTTGATGGAATGTTACCAATATC

Organism	E-value	Eukaryote	Bacteria	GenBank accession
Polysphondylium	7e-102	Metazoa		EFA86651.1
Drosophila	3e-67	Metazoa		NP_610922.1
Laccaria	6e-66	Fungi		XP_001877001.1
Danio rerio	3e-65	Metazoa		NP_001076363.1
Xenopus	2e-64	Metazoa		NP_001016398.1|
Homo sapiens	6e-62	Metazoa		NP_077025.2
Mus musculus	2e-60	Metazoa		NP_081369.2
Anopheles	4e-60	Metazoa		XP_309862.3
Aspergillus	8e-60	Fungi		XP_662522.1
Neurospora	4e-57	Fungi		XP_956725.2
Pichia	9e-54	Fungi		XP_002492537.1
Yarrowia	5e-51	Fungi		XP_501354.1
Oriza sativa	2e-50	Plantae		EEC69002.1
Vitis vinifera	4e-50	Plantae		XP_002279511.1
Arabidopsis	1e-46	Plantae		XP_002890535.1
Rhodospirillum	5e-46		alphaproteobacteria	YP_425827.1
Magnetospirilum	1e-45		alphaproteobacteria	YP_420968.1
Rhodomicrobium	1e-45		alphaproteobacteria	YP_004013051.1
Ochrobactrum	4e-45		alphaproteobacteria	YP_001369577.1
Brucella	6e-44		alphaproteobacteria	YP_001628309.1
Agrobacterium	3e-41		alphaproteobacteria	NP_357097.2
Caenorhabditis	7e-41	Metazoa		NP_496949.1
Rhodoseudomonas	1e-40		alphaproteobacteria	YP_530416.1
Rickettsi	4e-19		alphaproteobacteria	YP_001492229.1

TABLE S2. Phylogenetic distribution of Ndufaf5 homologous proteins. *Dictyostelium* Ndufaf5 protein was subjected to Blast and representative homologous showing significant E-values and covering most of the sequence were selected from prokaryotes, metazoan, plants and fungi kingdoms.