Figure S1

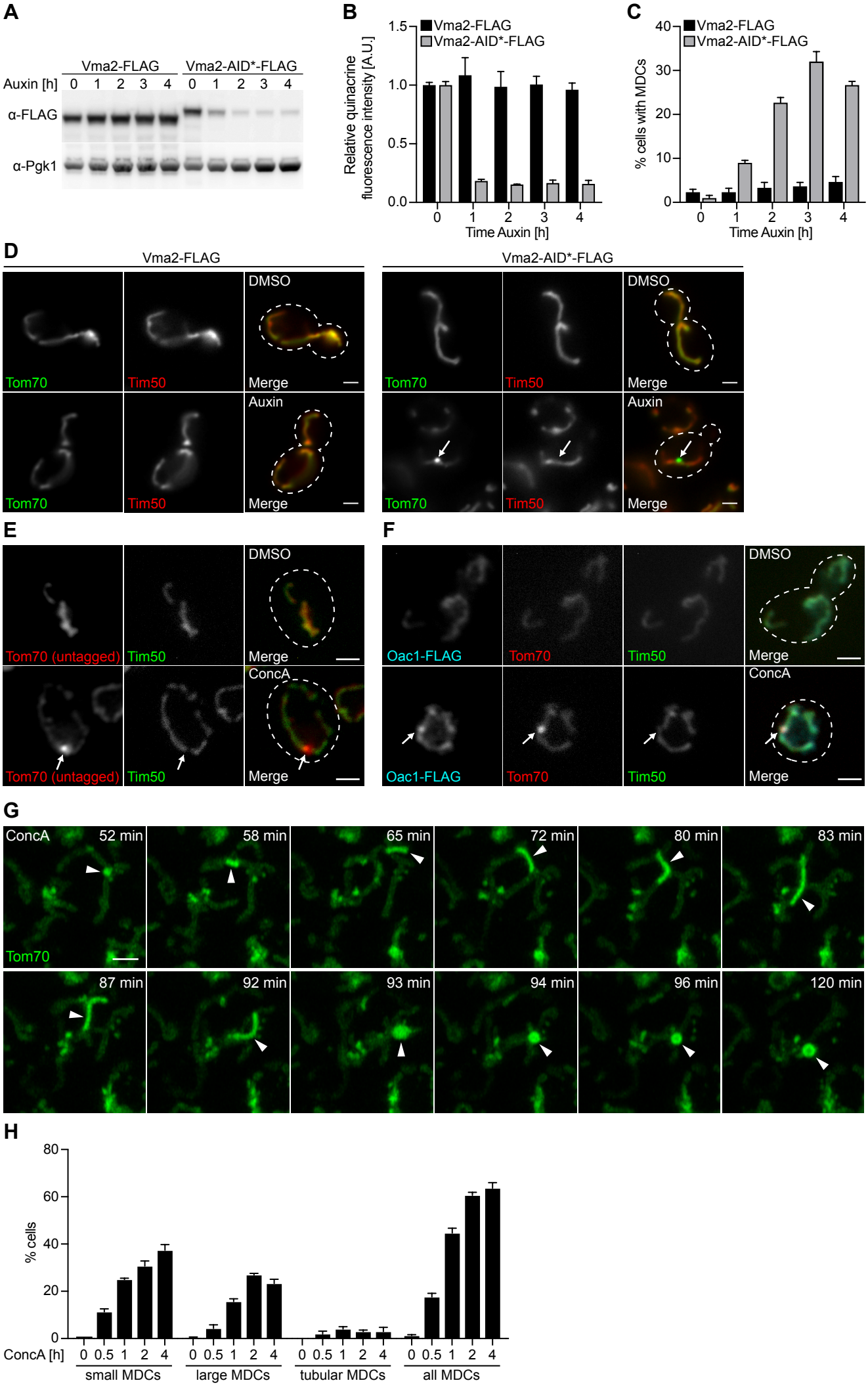

A
Vma2-FLAG
Vma2-AID*-FLAG
Auxin [h] 0 1 2 3 4 0 1 2 3 4
α-FLAG
α-Pgk1
B
Vma2-FLAG
Vma2-AID*-FLAG
Relative quinacrine fluorescence intensity [A.U.]
Time Auxin [h]
C
Vma2-FLAG
Vma2-AID*-FLAG
% cells with MDCs
Time Auxin [h]
D
Vma2-FLAG
Vma2-AID*-FLAG
DMSO
Auxin
Tom70
Tim50
Merge
E
DMSO
ConcA
Tom70 (untagged)
Tim50
Merge
F
DMSO
ConcA
Oac1-FLAG
Tom70
Tim50
Merge
G
ConcA
Tom70
52 min
58 min
65 min
72 min
80 min
83 min
87 min
92 min
93 min
94 min
96 min
120 min
H
% cells
ConcA [h] 0 0.5 1 2 4
small MDCs
large MDCs
tubular MDCs
all MDCs

Figure S1. V-ATPase Inhibition Induces MDC Formation, Related to Figure 1.

(A) Western blot showing time course of auxin-induced Vma2-AID*-FLAG depletion.

(B) Time course of auxin-induced inhibition of vacuole acidification due to Vma2-AID*-FLAG depletion assayed by staining with pH-dependent dye quinacrine. Error bars show mean ± standard error of *N* = three replicates with *n* = 30 cells per replicate.

(C) Quantification of Vma2-AID*-FLAG-depletion induced MDC formation over time. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

(D) Widefield images of auxin-induced MDC formation in the indicated yeast strains expressing Tom70-GFP and Tim50-mCherry. White arrow marks MDC. Scale bar = 2 μm.

(E) Widefield image of concanamycin A (ConcA) treated yeast cells expressing Tim50-GFP fixed and stained for endogenous, untagged Tom70. White arrow marks MDC. Scale bar = 2 μm.

(F) Widefield image of ConcA treated yeast cells expressing Oac1-FLAG, Tom70-mCherry and Tim50-GFP fixed and stained for FLAG. White arrow marks MDC. Scale bar = 2 μm.

(G) Time-lapse images of ConcA-induced MDC formation in yeast cells expressing Tom70-GFP. Images were acquired over 120 minutes (min). Arrowhead marks MDC. Scale bar = 2 μm.

(H) Time-course analysis of MDC morphology in ConcA treated cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

Figure S2

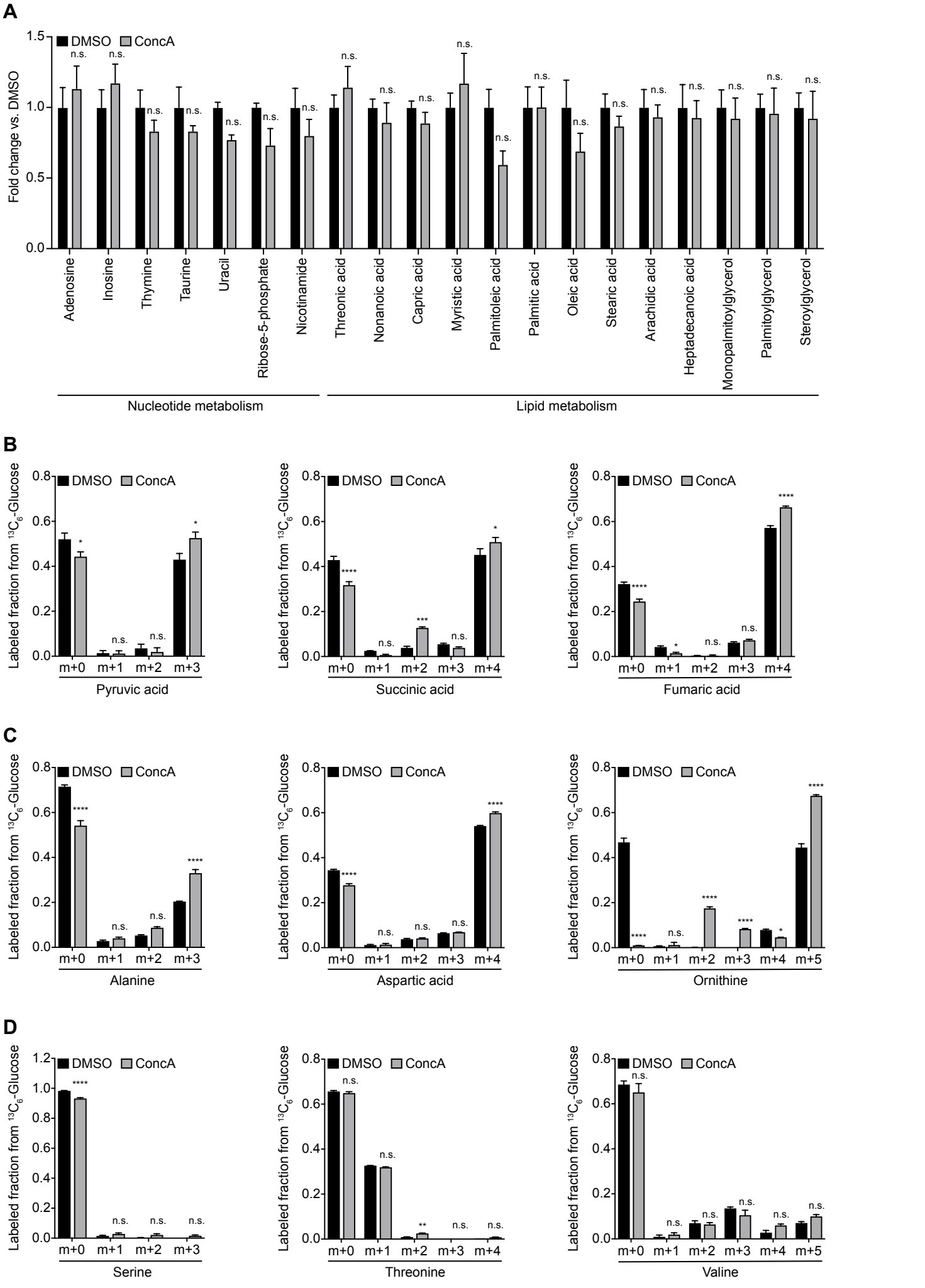

A
DMSO
ConcA
Fold change vs. DMSO
Adenosine
Inosine
Thymine
Taurine
Uracil
Ribose-5-phosphate
Nicotinamide
Threonic acid
Nonanoic acid
Capric acid
Myristic acid
Palmitoleic acid
Palmitic acid
Oleic acid
Stearic acid
Arachidic acid
Heptadecanoic acid
Monopalmitoylglycerol
Palmitoylglycerol
Steroylglycerol
Nucleotide metabolism
Lipid metabolism
B
Labeled fraction from $^{13}C_6$-Glucose
Pyruvic acid
Succinic acid
Fumaric acid
C
Alanine
Aspartic acid
Ornithine
D
Serine
Threonine
Valine
m+0
m+1
m+2
m+3
m+4
m+5
n.s.

Figure S2. Metabolic remodeling in ConcA treated cells, Related to Figure 2.

(A) Analysis of whole cell metabolite levels in *wild-type* cells treated with concanamycin A (ConcA) for three hours. Error bars show mean ± standard error of *N* = four replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, two-way ANOVA with Holm-Šídák test.

(B) Analysis of ^{13}C-labeling of pyruvic acid, succinic acid, and fumaric acid from glucose carbons in *wild-type* cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. Error bars show mean fraction labeled ± standard error of *N* = four replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, *p < 0.0333, ***p < 0.0002, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

(C) Analysis of ^{13}C-labeling of alanine, aspartic acid, and ornithine from glucose carbons in *wild-type* cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. Error bars show mean fraction labeled ± standard error of *N* = four replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, *p < 0.0333, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

(D) Analysis of ^{13}C-labeling of serine, threonine, and valine from glucose carbons in *wild-type* cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. Error bars show mean fraction labeled ± standard error of *N* = four replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, **p < 0.0022, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

Figure S3

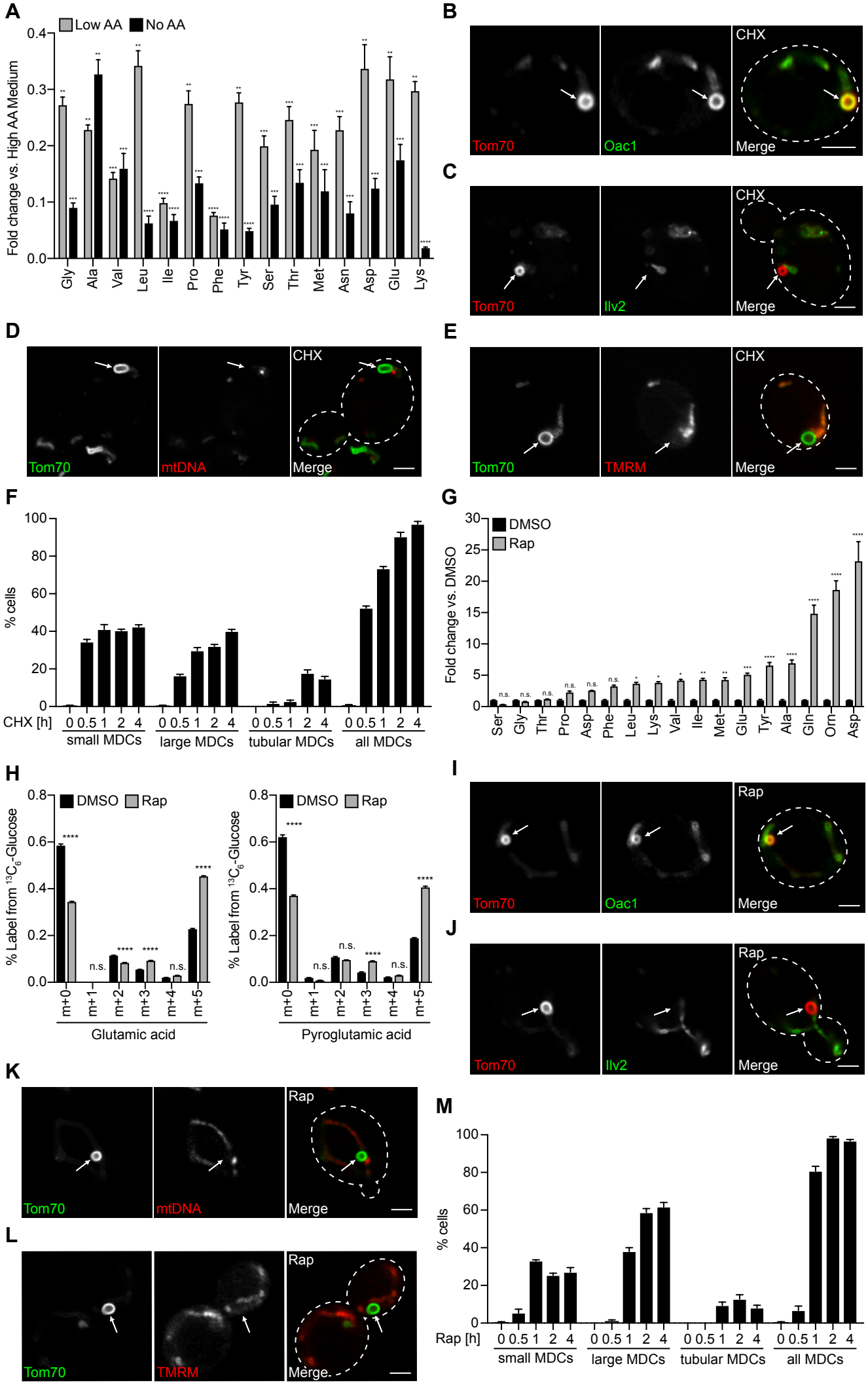

A
Low AA
No AA
Fold change vs. High AA Medium
Gly
Ala
Val
Leu
Ile
Pro
Phe
Tyr
Ser
Thr
Met
Asn
Asp
Glu
Lys
B
Tom70
Oac1
CHX
Merge
C
Tom70
Ilv2
CHX
Merge
D
Tom70
mtDNA
CHX
Merge
E
Tom70
TMRM
CHX
Merge
F
% cells
CHX [h]
0 0.5 1 2 4
small MDCs
large MDCs
tubular MDCs
all MDCs
G
DMSO
Rap
Fold change vs. DMSO
Ser
Gly
Thr
Pro
Asp
Phe
Leu
Lys
Val
Ile
Met
Glu
Tyr
Ala
Gln
Orn
Asp
H
DMSO
Rap
% Label from $^{13}C_6$-Glucose
m+0
m+1
m+2
m+3
m+4
m+5
Glutamic acid
Pyroglutamic acid
I
Tom70
Oac1
Rap
Merge
J
Tom70
Ilv2
Rap
Merge
K
Tom70
mtDNA
Rap
Merge
L
Tom70
TMRM
Rap
Merge
M
% cells
Rap [h]
0 0.5 1 2 4
small MDCs
large MDCs
tubular MDCs
all MDCs

Figure S3. Characteristics of CHX- and Rap-induced MDCs, Related to Figure 3.

(A) Relative amino acid levels in yeast cells grown in media containing low or no amino acids (AA) compared to high AA medium assayed by GC-MS. Error bars show mean ± standard error of *N* = three replicates. Statistical comparison shows difference to high AA media. **p < 0.0022, ***p < 0.0002, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

(B) Super-resolution image of cycloheximide (CHX) treated yeast cells expressing Oac1-GFP and Tom70-mCherry. White arrow marks MDC. Scale bar = 2 μm.

(C) Super-resolution image of CHX treated yeast cells expressing Ilv2-GFP and Tom70-mCherry. White arrow marks MDC. Scale bar = 2 μm.

(D) Super-resolution images of CHX treated yeast cells expressing Tom70-GFP, stained with DAPI to label mitochondrial DNA. White arrow marks MDC. Scale bar = 2 μm. % of MDCs with DAPI = 0 ± 0.33, N = 3 replicates, 50 MDCs (n) analyzed per replicate (mean ± standard error).

(E) Super-resolution images of CHX treated yeast cells expressing Tom70-GFP stained with fluorescent membrane potential indicator TMRM. White arrow marks MDC. Scale bar = 2 μm. % of MDCs with TMRM = 0 ± 0.02, N = 3 replicates, 30 MDCs (n) analyzed per replicate (mean ± standard error).

(F) Time-course analysis of MDC morphology in CHX treated cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

(G) Relative amino acid levels in yeast cells grown in high amino acid medium and treated with rapamycin (Rap) assayed by GC-MS. Error bars show mean ± standard error of *N* = three replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, *p < 0.0333, **p < 0.0022, ***p < 0.0002, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

(H) Analysis of ^{13}C-labeling of glutamic acid and pyroglutamic acid from glucose carbons in *wild-type* cells treated for three hours with Rap in the presence of $^{13}C_6$-glucose. Error bars show mean fraction labeled ± standard error of *N* = four replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

(I) Super-resolution image of Rap treated yeast cells expressing Oac1-GFP and Tom70-mCherry. White arrow marks MDC. Scale bar = 2 μm.
(J) Super-resolution image of Rap treated yeast cells expressing Ilv2-GFP and Tom70-mCherry. White arrow marks MDC. Scale bar = 2 μm.
(K) Super-resolution images of Rap treated yeast cells expressing Tom70-GFP, stained with DAPI to label mitochondrial DNA. White arrow marks MDC. Scale bar = 2 μm. % of MDCs with DAPI = 0 ± 0, N = 3 replicates, 50 MDCs (n) analyzed per replicate (mean ± standard error).
(L) Super-resolution images of Rap treated yeast cells expressing Tom70-GFP stained with fluorescent membrane potential indicator TMRM. White arrow marks MDC. Scale bar = 2 μm. % of MDCs with TMRM = 0.01 ± 0.33, N = 3 replicates, 50 MDCs (n) analyzed per replicate (mean ± standard error).
(M) Time-course analysis of MDC morphology in Rap treated cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

Figure S4

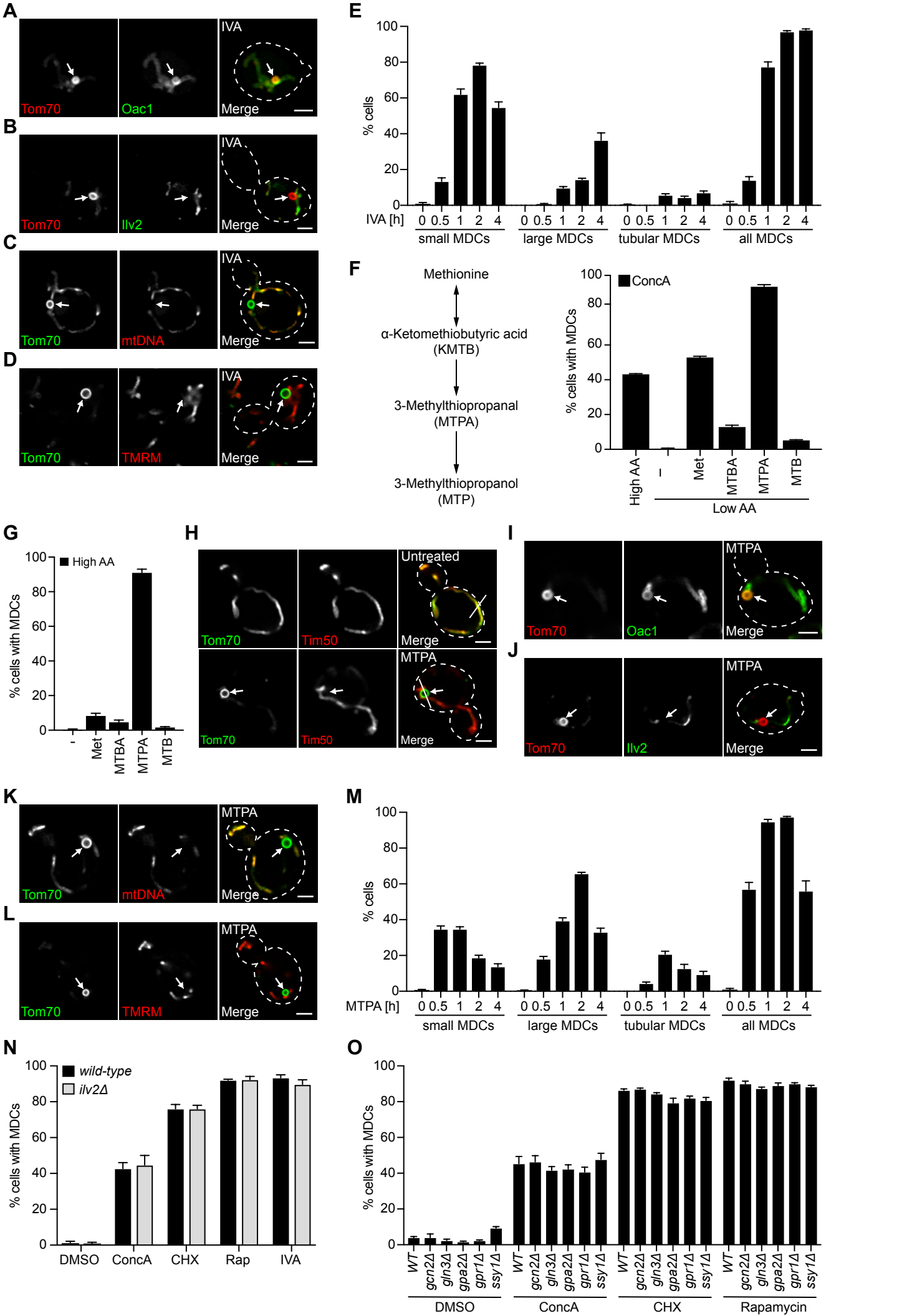

A
Tom70
Oac1
IVA
Merge
B
Tom70
Ilv2
IVA
Merge
C
Tom70
mtDNA
IVA
Merge
D
Tom70
TMRM
IVA
Merge
E
% cells
IVA [h]
0 0.5 1 2 4
small MDCs
large MDCs
tubular MDCs
all MDCs
F
Methionine
α-Ketomethiobutyric acid (KMTB)
3-Methylthiopropanal (MTPA)
3-Methylthiopropanol (MTP)
ConcA
% cells with MDCs
High AA
-
Met
MTBA
MTPA
MTB
Low AA
G
High AA
% cells with MDCs
H
Tom70
Tim50
Untreated
MTPA
Merge
I
Tom70
Oac1
MTPA
Merge
J
Tom70
Ilv2
MTPA
Merge
K
Tom70
mtDNA
MTPA
Merge
L
Tom70
TMRM
MTPA
Merge
M
% cells
MTPA [h]
small MDCs
large MDCs
tubular MDCs
all MDCs
N
wild-type
ilv2Δ
% cells with MDCs
DMSO
ConcA
CHX
Rap
IVA
O
% cells with MDCs
WT
gcn2Δ
gln3Δ
gpa2Δ
gpr1Δ
ssy1Δ
DMSO
ConcA
CHX
Rapamycin

Figure S4. Leucine and Methionine Derivatives Activate MDC Formation, Related to Figure 4.

(A) Super-resolution images isovaleraldehyde (IVA)-induced MDC formation in yeast cells expressing Tom70-mCherry and Oac1-GFP. White arrow marks MDC. Scale bar = 2 μm.

(B) Super-resolution images IVA-induced MDC formation in yeast cells expressing Tom70-mCherry and Ilv2-GFP. White arrow marks MDC. Scale bar = 2 μm.

(C) Super-resolution images IVA-induced MDC formation in yeast cells expressing Tom70-GFP, stained with DAPI to label mitochondrial DNA. White arrow marks MDC. Scale bar = 2 μm.

(D) Super-resolution images IVA-induced MDC formation in yeast cells expressing Tom70-GFP stained with fluorescent membrane potential indicator TMRM. White arrow marks MDC. Scale bar = 2 μm.

(E) Time-course analysis of MDC morphology in IVA treated cells. Error bars show mean ± standard error of N = three replicates with n = 100 cells per replicate.

(F) Quantification of concanamycin A (ConcA)-induced MDC formation in low amino acid medium supplemented with methionine (Met) or its catabolites α-ketomethiobutyric acid (KMTB), 3-methylthiopropanal (MTPA) or 3-methylthiopropanol (MTP). Error bars show mean ± standard error of N = three replicates with n = 100 cells per replicate.

(G) Quantification of MDC formation in high amino acid medium supplemented with Met, KMTB, MTPA or MTP in the absence of drug treatment. Error bars show mean ± standard error of N = three replicates with n = 100 cells per replicate.

(H) Super-resolution images of MTPA-induced MDC formation in yeast cells expressing Tom70-GFP and Tim50-mCherry. White arrow marks MDC. Scale bar = 2 μm.

(I) Super-resolution images of MTPA treated yeast cells expressing Tom70-mCherry and Oac1-GFP. White arrow marks MDC. Scale bar = 2 μm.

(J) Super-resolution images of MTPA-induced MDC formation in yeast cells expressing Tom70-mCherry and Ilv2-GFP. White arrow marks MDC. Scale bar = 2 μm.

(K) Super-resolution images of MTPA-induced MDC formation in yeast cells expressing Tom70-GFP, stained with DAPI to label mitochondrial DNA. White arrow marks MDC. Scale bar = 2 μm.

(L) Super-resolution images of MTPA-induced MDC formation in yeast cells expressing Tom70-GFP stained with fluorescent membrane potential indicator TMRM. White arrow marks MDC. Scale bar = 2 μm.
(M) Time-course analysis of MDC morphology in MTPA treated cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.
(N) Quantification of ConcA-, cycloheximide (CHX)-, rapamycin (Rap)-, and IVA-induced MDC formation in *wild-type* and *ilv2*Δ cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.
(O) Quantification of ConcA-, CHX-, and Rap-induced MDC formation in *wild-type*, *gcn2*Δ, *gln3*Δ, *ilv2*Δ, *gpa2*Δ, *gpr1*Δ, and *ssy1*Δ cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

Figure S5

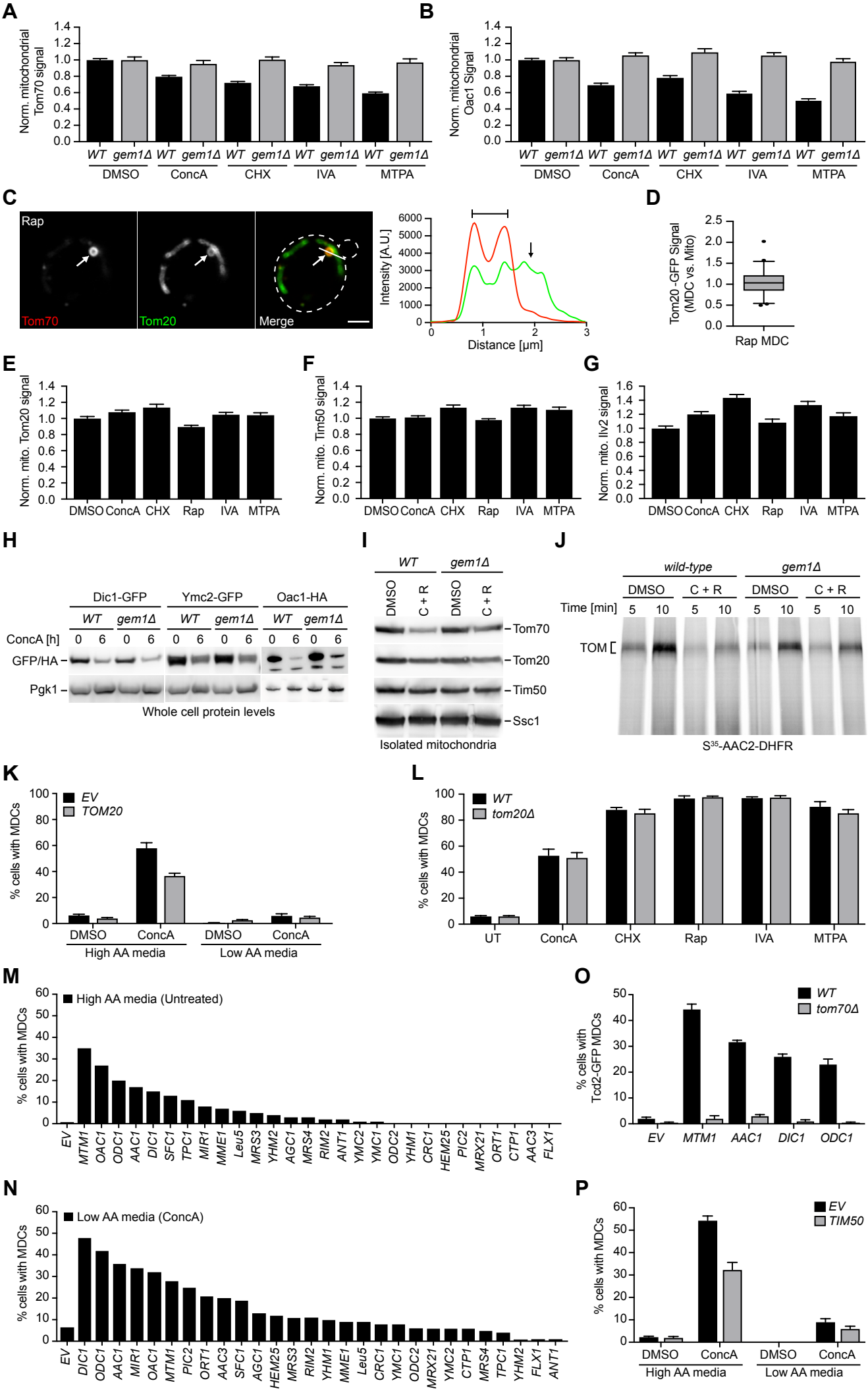

A
Norm. mitochondrial Tom70 signal
WT gem1Δ WT gem1Δ WT gem1Δ WT gem1Δ WT gem1Δ
DMSO ConcA CHX IVA MTPA
B
Norm. mitochondrial Oac1 Signal
C
Rap
Tom70
Tom20
Merge
Intensity [A.U.]
Distance [µm]
D
Tom20 -GFP Signal (MDC vs. Mito)
Rap MDC
E
Norm. mito. Tom20 signal
DMSO ConcA CHX Rap IVA MTPA
F
Norm. mito. Tim50 signal
G
Norm. mito. Ilv2 signal
H
Dic1-GFP
Ymc2-GFP
Oac1-HA
ConcA [h] 0 6 0 6 0 6 0 6 0 6 0 6
GFP/HA
Pgk1
Whole cell protein levels
I
WT
gem1Δ
DMSO
C + R
Tom70
Tom20
Tim50
Ssc1
Isolated mitochondria
J
wild-type
gem1Δ
Time [min] 5 10 5 10 5 10 5 10
TOM
S35-AAC2-DHFR
K
EV
TOM20
% cells with MDCs
High AA media
Low AA media
L
WT
tom20Δ
UT ConcA CHX Rap IVA MTPA
M
High AA media (Untreated)
EV MTM1 OAC1 ODC1 AAC1 DIC1 SFC1 TPC1 MIR1 MME1 Leu5 MRS3 YHM2 AGC1 MRS4 RIM2 ANT1 YMC2 YMC1 ODC2 YHM1 CRC1 HEM25 PIC2 MRX21 ORT1 CTP1 AAC3 FLX1
N
Low AA media (ConcA)
EV DIC1 ODC1 AAC1 MIR1 OAC1 MTM1 PIC2 ORT1 AAC3 SFC1 AGC1 HEM25 MRS3 RIM2 YHM1 MME1 Leu5 CRC1 YMC1 ODC2 MRX21 YMC2 CTP1 MRS4 TPC1 YHM2 FLX1 ANT1
O
% cells with Tcd2-GFP MDCs
EV MTM1 AAC1 DIC1 ODC1
P
EV
TIM50
DMSO ConcA DMSO ConcA
High AA media
Low AA media

Figure S5. MDCs Selectively Remove Tom70 and SLC25A Carrier Proteins From Mitochondria, Related to Figure 5.

(A) Normalized mitochondrial Tom70-GFP fluorescence in *wild-type (WT)* and *gem1*Δ cells treated with concanamycin A (ConcA), cycloheximide (CHX), isovaleraldehyde (IVA), or 3-methylthiopropanal (MTPA) compared to DMSO. Error bars show mean ± standard error of *N* = three replicates with *n* = 15 cells per replicate. This graph shows additional data generated in the experiments used for the related panel in Figure 5E. The DMSO control is duplicated from Figure 5E for comparison purposes.

(B) Normalized mitochondrial Oac1-GFP fluorescence in *WT* and *gem1*Δ cells treated with ConcA, CHX, IVA, or 3- MTPA compared to DMSO. Error bars show mean ± standard error of *N* = three replicates with *n* = 15 cells per replicate. This graph shows additional data generated in the experiments used for the related panel in Figure 5F. The DMSO control is duplicated from Figure 5F for comparison purposes.

(C) Super-resolution images (left) and line-scan analysis (right) of rapamycin (Rap)-induced MDC formation in yeast cells expressing Tom70-mCherry and Tom20-GFP. White arrow marks MDC. White line marks fluorescence intensity profile position. Black arrow marks mitochondrial tubule. Bracket marks MDC. Scale bar = 2μm.

(D) Box plot showing the normalized fluorescence intensity of Tom20-GFP in Rap-induced MDCs compared to the adjacent mitochondrial tubule. Error bars show median ± 95 % confidence interval of *n* = 45 cells from *N* = three per replicates with *n* = 15 cells per replicate.

(E) Normalized mitochondrial Tom20-GFP fluorescence in cells treated with ConcA, CHX, Rap, IVA, or MTPA compared to DMSO. Error bars show mean ± standard error of *N* = three replicates with *n* = 15 cells per replicate.

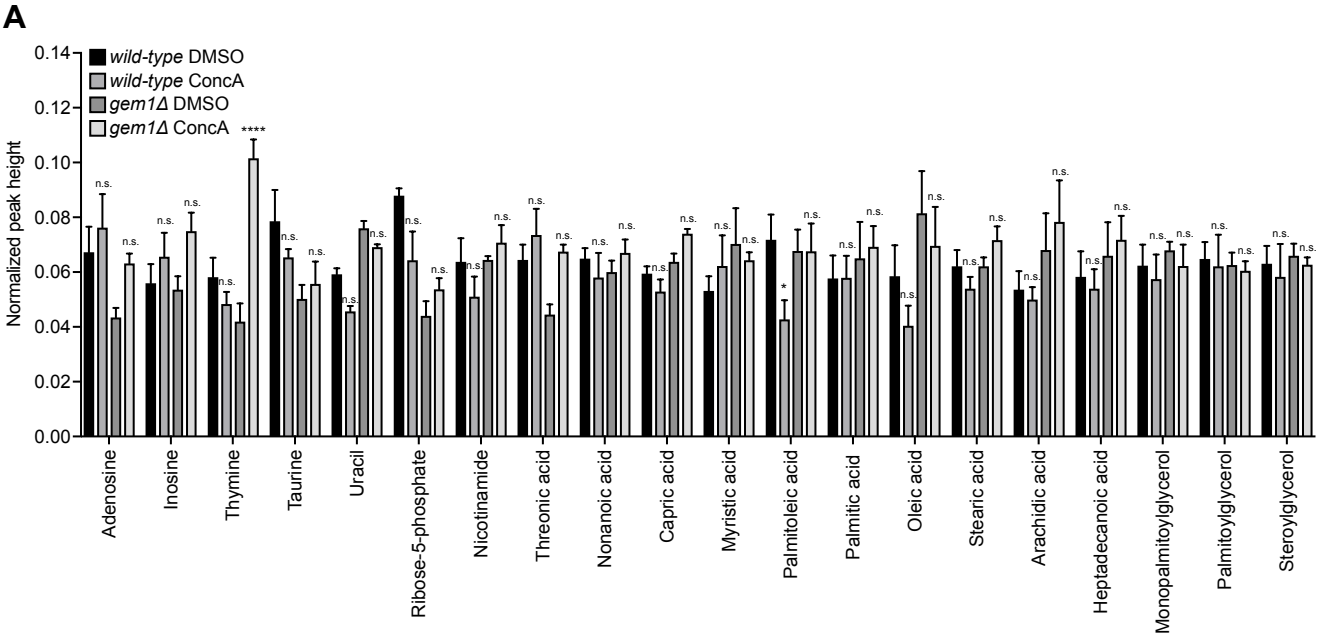
(F) Normalized mitochondrial Tim50-GFP fluorescence in cells treated with ConcA, CHX, Rap, IVA, or MTPA compared to DMSO. Error bars show mean ± standard error of *N* = three replicates with *n* = 15 cells per replicate.

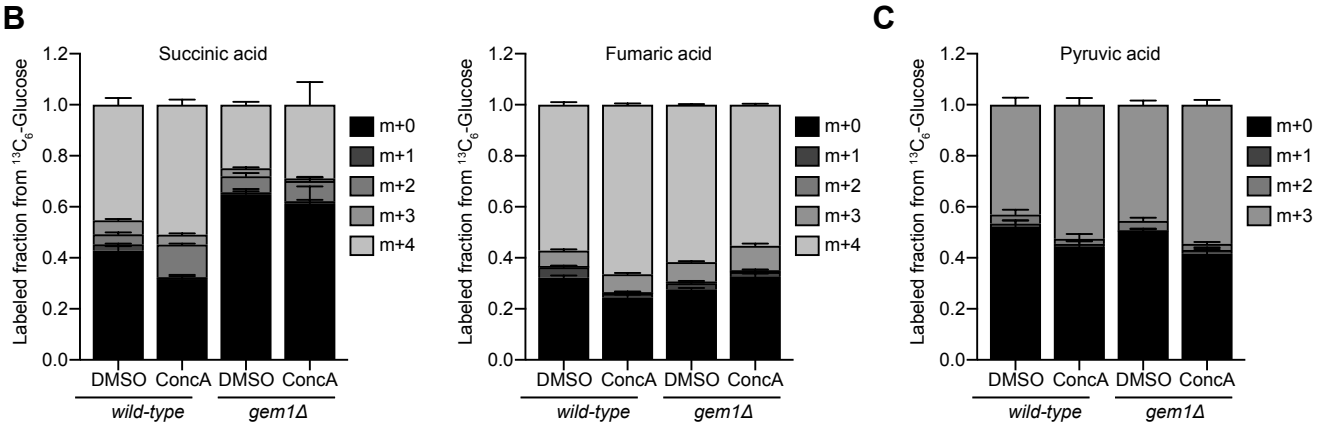
(G) Normalized mitochondrial Ilv2-GFP fluorescence in cells treated with ConcA, CHX, Rap, IVA, or MTPA compared to DMSO. Error bars show mean ± standard error of *N* = three replicates with *n* = 15 cells per replicate.

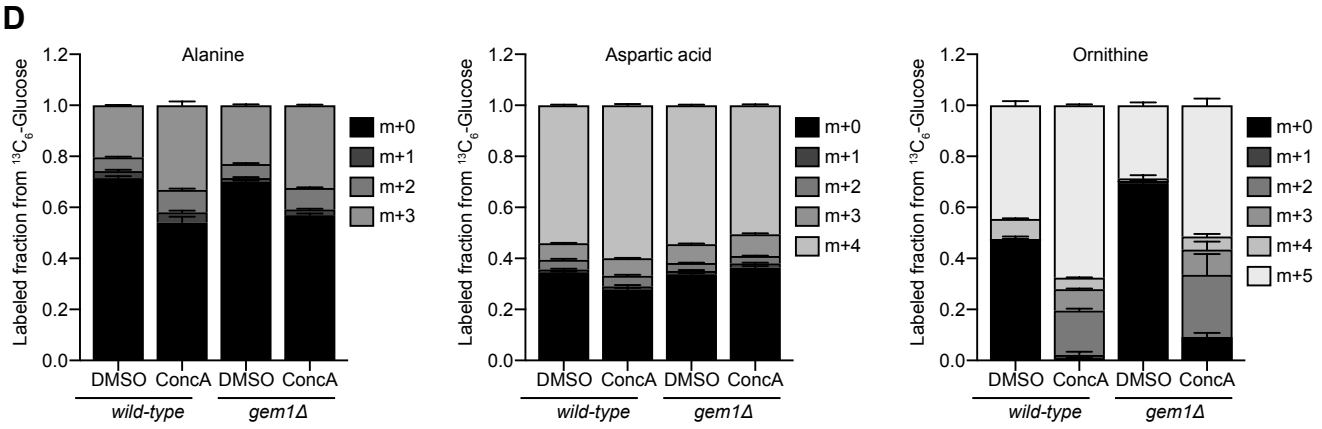
(H) Whole cell protein steady state levels in *WT* and *gem1*Δ cells treated with ConcA.

(I) Protein steady state levels in mitochondria isolated from *WT* and *gem1*Δ cells treated with a combination of ConcA and Rap (C + R) for six hours.
(J) Binding of ^{35}S-AAC2-DHFR to the translocase of the outer membrane (TOM) complex of mitochondria isolated from *WT* and *gem1*Δ cells treated with C + R.
(K) Quantification of ConcA-induced MDC formation in *TOM20* overexpressing or empty vector (*EV)* control cells in high and low amino acid (AA) media. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.
(L) Quantification of ConcA, CHX, Rap, IVA, and MTPA-induced MDC formation in *WT* and *tom20*Δ cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.
(M) Quantification of MDC formation in cells expressing the indicated mitochondrial carrier in high AA media in absence of drug treatment.
(N) Quantification of MDC formation in cells expressing the indicated mitochondrial carrier in low AA media in presence of ConcA.
(O) Quantification of MDC formation in *WT* and *tom70*Δ cells expressing the indicated carrier protein or *EV* control grown in high AA media in absence of drug treatment. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.
(P) Quantification of ConcA-induced MDC formation in *TIM50* overexpressing or *EV* control cells in high and low AA media. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

Figure S6


A
wild-type DMSO
wild-type ConcA
gem1Δ DMSO
gem1Δ ConcA
Normalized peak height
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
****
*
n.s.
Adenosine
Inosine
Thymine
Taurine
Uracil
Ribose-5-phosphate
Nicotinamide
Threonic acid
Nonanoic acid
Capric acid
Myristic acid
Palmitoleic acid
Palmitic acid
Oleic acid
Stearic acid
Arachidic acid
Heptadecanoic acid
Monopalmitoylglycerol
Palmitoylglycerol
Steroylglycerol


B
Succinic acid
Fumaric acid
C
Pyruvic acid
Labeled fraction from $^{13}C_6$-Glucose
m+0
m+1
m+2
m+3
m+4
DMSO ConcA DMSO ConcA
wild-type
gem1Δ


D
Alanine
Aspartic acid
Ornithine
Labeled fraction from $^{13}C_6$-Glucose
m+0
m+1
m+2
m+3
m+4
m+5
DMSO ConcA DMSO ConcA
wild-type
gem1Δ

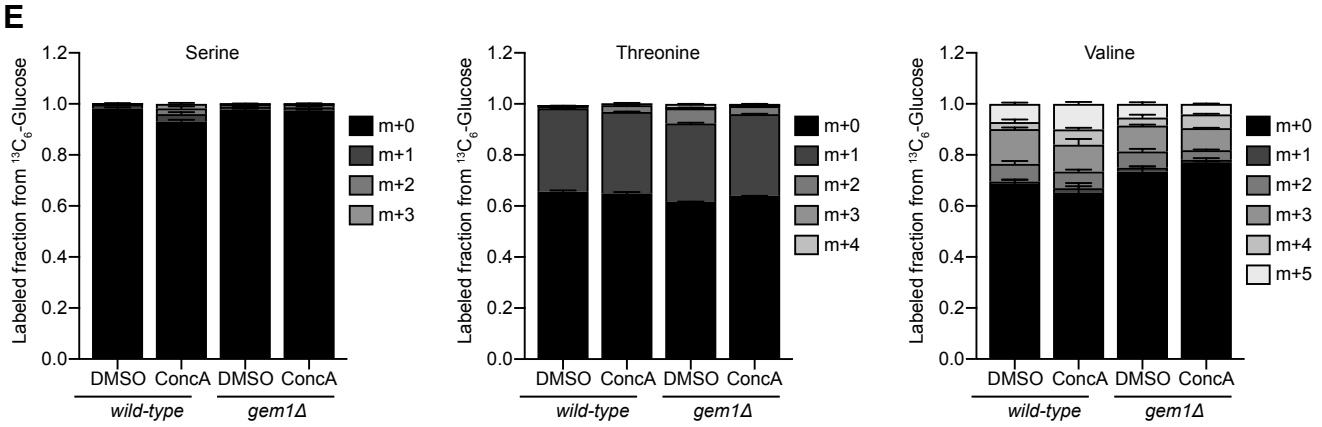

E
Serine
Threonine
Valine
Labeled fraction from $^{13}C_6$-Glucose
m+0
m+1
m+2
m+3
m+4
m+5
DMSO ConcA DMSO ConcA
wild-type
gem1Δ

Figure S6. MDC Formation Promotes Cellular Metabolic Remodeling in V-ATPase Inhibited Cells, Related to Figure 6.

(A) Normalized metabolite levels in *wild-type (WT)* and *gem1*Δ cells treated with concanamycin A (ConcA) for three hours. This graph shows additional data generated in the experiments used for Supplemental Figure S2A. *WT* control graphs show the same data as in Supplemental Figure S2A but normalized to the sum of all corresponding metabolite intensity levels (peak heights) to allow for better comparison to *gem1*Δ cells. Error bars show mean ± standard error of *N* = four replicates. Statistical comparison shows difference to the corresponding DMSO control. n.s., not significant, *p < 0.0333, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.

(B) Analysis of ^{13}C-labeling of succinic acid and fumaric acid from glucose carbons in *wild-type* and *gem1*Δ cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. This graph shows additional data generated in the experiments used for Supplemental Figure SB. *WT* control graphs were duplicated for comparison. Error bars show mean fraction labeled ± standard error of *N* = four replicates.

(C) Analysis of ^{13}C-labeling of pyruvic acid from glucose carbons in *wild-type* and *gem1*Δ cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. This graph shows additional data generated in the experiments used for Supplemental Figure SB. *WT* control graphs were duplicated for comparison. Error bars show mean fraction labeled ± standard error of *N* = four replicates.

(D) Analysis of ^{13}C-labeling of alanine, aspartic acid, and ornithine from glucose carbons in *wild-type* and *gem1*Δ cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. This graph shows additional data generated in the experiments used for Supplemental Figure S2C. *WT* control graphs were duplicated for comparison. Error bars show mean fraction labeled ± standard error of *N* = four replicates.

(E) Analysis of ^{13}C-labeling of serine, threonine, and valine from glucose carbons in *wild-type* and *gem1*Δ cells treated for three hours with ConcA in the presence of $^{13}C_6$-glucose. This graph shows additional data generated in the experiments used for Supplemental Figure S2D. *WT* control graphs were duplicated for comparison. Error bars show mean fraction labeled ± standard error of *N* = four replicates.

Figure S7

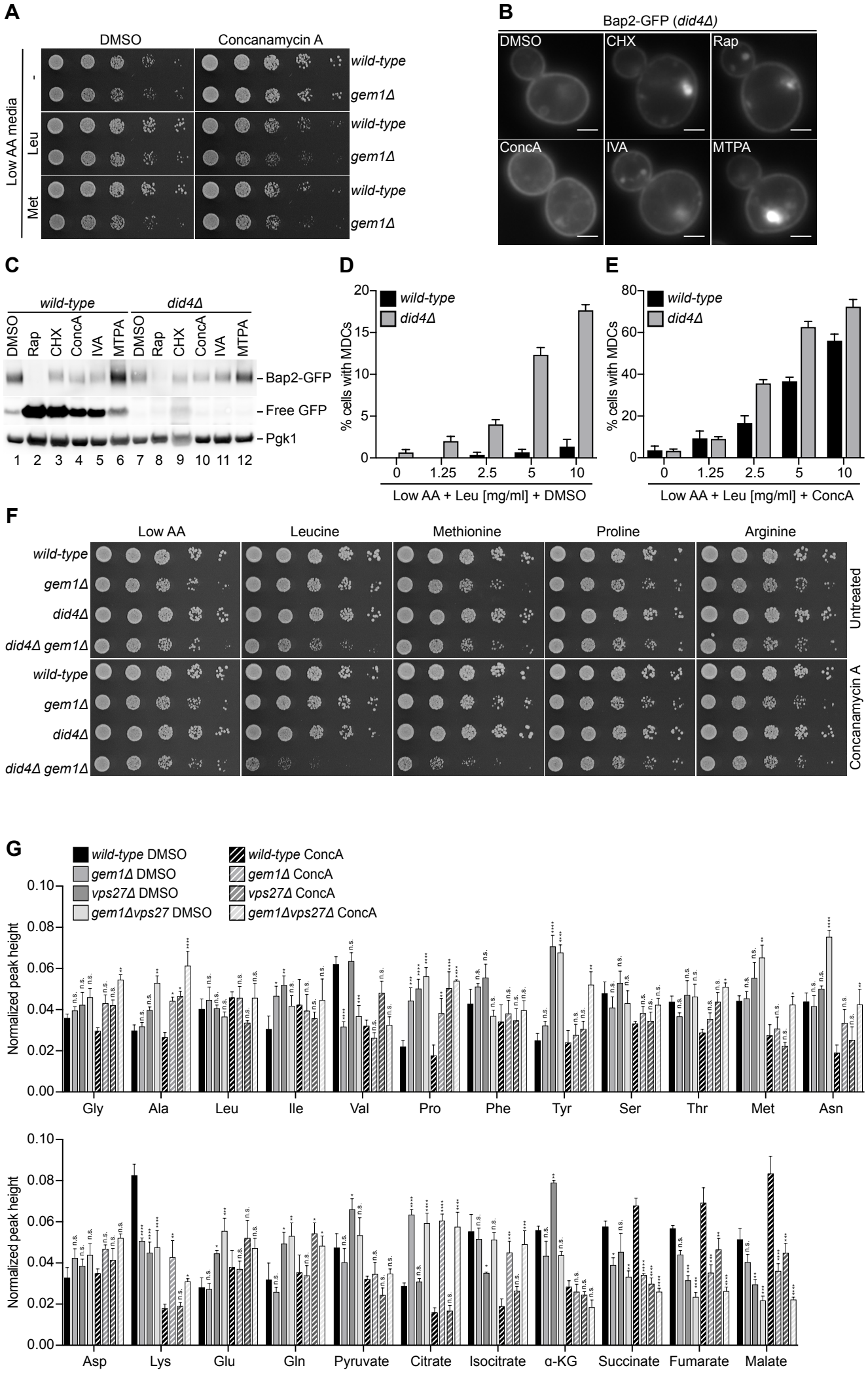

A
DMSO
Concanamycin A
Low AA media
-
Leu
Met
wild-type
gem1Δ
wild-type
gem1Δ
wild-type
gem1Δ
B
Bap2-GFP (did4Δ)
DMSO
CHX
Rap
ConcA
IVA
MTPA
C
wild-type
did4Δ
DMSO
Rap
CHX
ConcA
IVA
MTPA
DMSO
Rap
CHX
ConcA
IVA
MTPA
Bap2-GFP
Free GFP
Pgk1
1 2 3 4 5 6 7 8 9 10 11 12
D
wild-type
did4Δ
% cells with MDCs
0
5
10
15
20
0
1.25
2.5
5
10
Low AA + Leu [mg/ml] + DMSO
E
wild-type
did4Δ
% cells with MDCs
0
20
40
60
80
0
1.25
2.5
5
10
Low AA + Leu [mg/ml] + ConcA
F
Low AA
Leucine
Methionine
Proline
Arginine
wild-type
gem1Δ
did4Δ
did4Δ gem1Δ
wild-type
gem1Δ
did4Δ
did4Δ gem1Δ
Untreated
Concanamycin A
G
wild-type DMSO
gem1Δ DMSO
vps27Δ DMSO
gem1Δvps27 DMSO
wild-type ConcA
gem1Δ ConcA
vps27Δ ConcA
gem1Δvps27Δ ConcA
Normalized peak height
0.00
0.02
0.04
0.06
0.08
0.10
Gly
Ala
Leu
Ile
Val
Pro
Phe
Tyr
Ser
Thr
Met
Asn
Asp
Lys
Glu
Gln
Pyruvate
Citrate
Isocitrate
α-KG
Succinate
Fumarate
Malate

Figure S7. MDCs Cooperate with MVBs to Protect Cells from Amino Acid Toxicity, Related to Figure 7.

(A) Growth of *wild*-type and *gem1*Δ cells in presence and absence of concanamycin A (ConcA) on low amino acid media supplemented with 10mg/ml leucine or methionine.

(B) Widefield images showing stabilization of Bap2-GFP on the plasma membrane in rapamycin (Rap), cycloheximide (CHX), ConcA, isovaleraldehyde (IVA) or 3-methylthiopropanal (MTPA) treated *did4*Δ cells. Bright structures resemble typical class E compartments found in ESCRT mutants. Scale bar = 2 μm.

(C) Western blot analysis of Bap2-GFP clipping upon treatment with Rap, CHX, ConcA, IVA and MTPA in *wild-type* and *did4*Δ cells.

(D) Quantification of MDC formation in low amino acid media supplemented with the indicated amount of leucine in *wild-type (WT)* and *did4*Δ cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

(E) Quantification of ConcA-induced MDC formation in low amino acid media supplemented with the indicated amount of leucine in *wild-type (WT)* and *did4*Δ cells. Error bars show mean ± standard error of *N* = three replicates with *n* = 100 cells per replicate.

(F) Growth of *wild-type*, *gem1*Δ*, did4*Δ and *gem1*Δ *did4*Δ strains in presence and absence of ConcA on low amino acid media supplemented with 10mg/ml leucine, methionine, proline or arginine.

(G) Normalized whole cell amino acid and TCA cycle metabolite abundance in *wild-type*, *gem1*Δ*, vps27*Δ and *gem1*Δ *vps27*Δ strains grown in low amino acid medium, shifted to low amino acid medium supplemented with 10mg/ml leucine and treated with DMSO or ConcA. Error bars show mean fraction labeled ± standard error of *N* = three replicates. Statistical comparison shows difference to the corresponding *wild-type* control. n.s., not significant, *p < 0.0333, **p < 0.0022, ***p < 0.0002, ****p < 0.0001, two-way ANOVA with Holm-Šídák test.