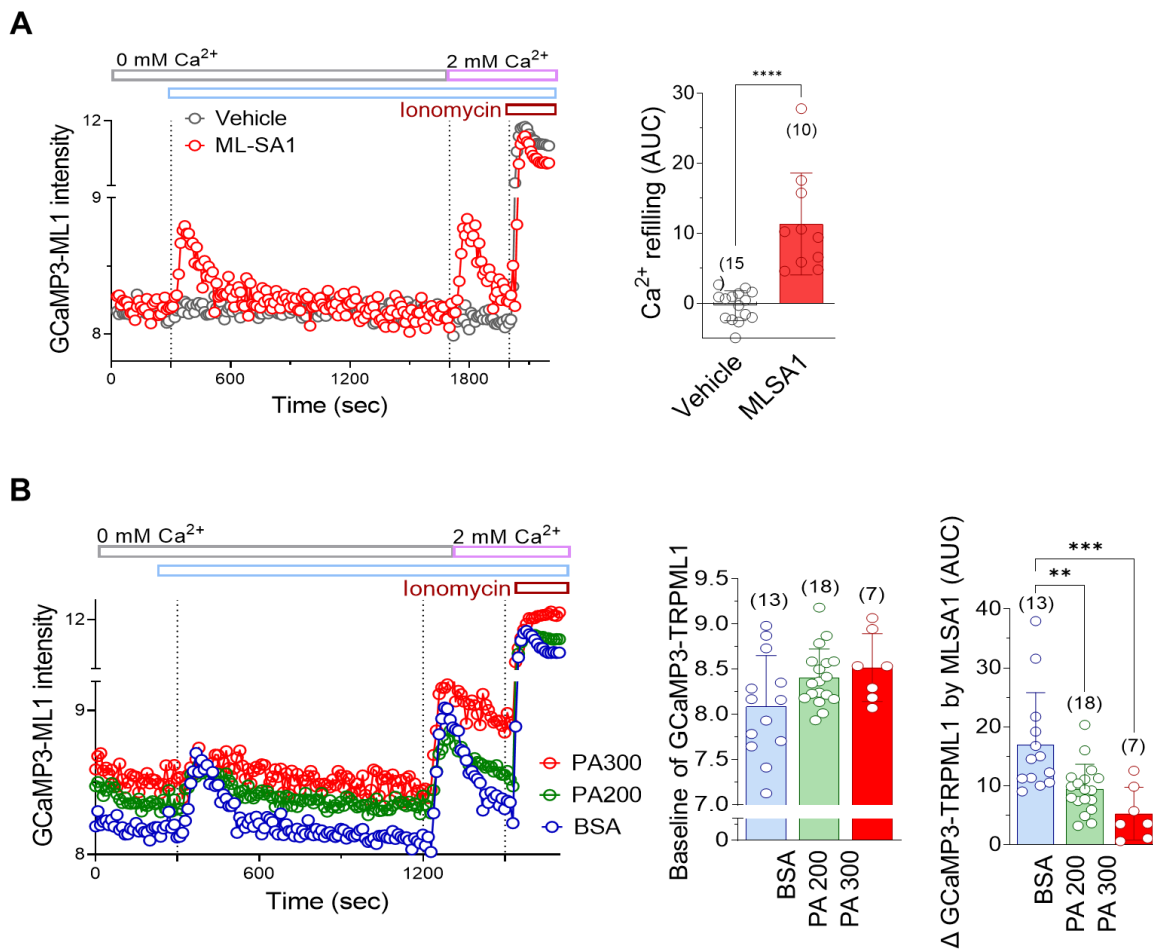
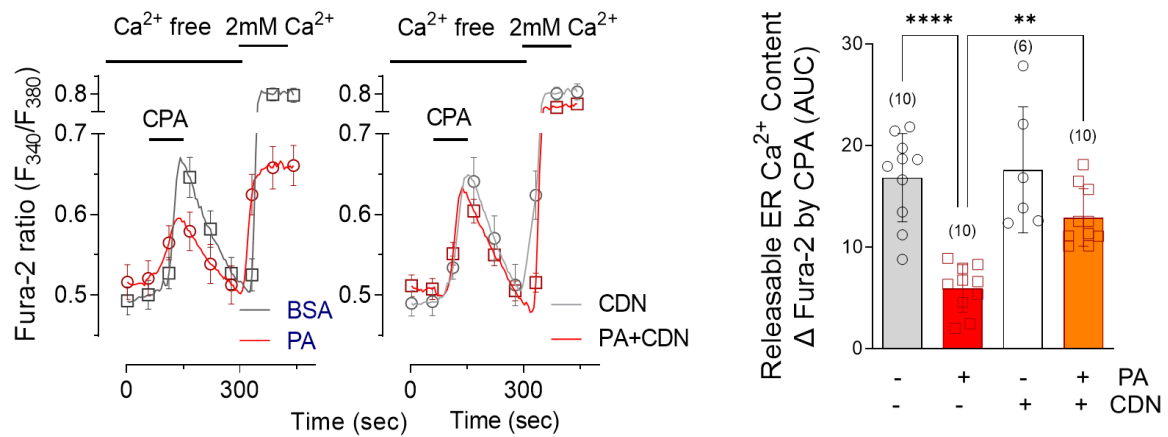


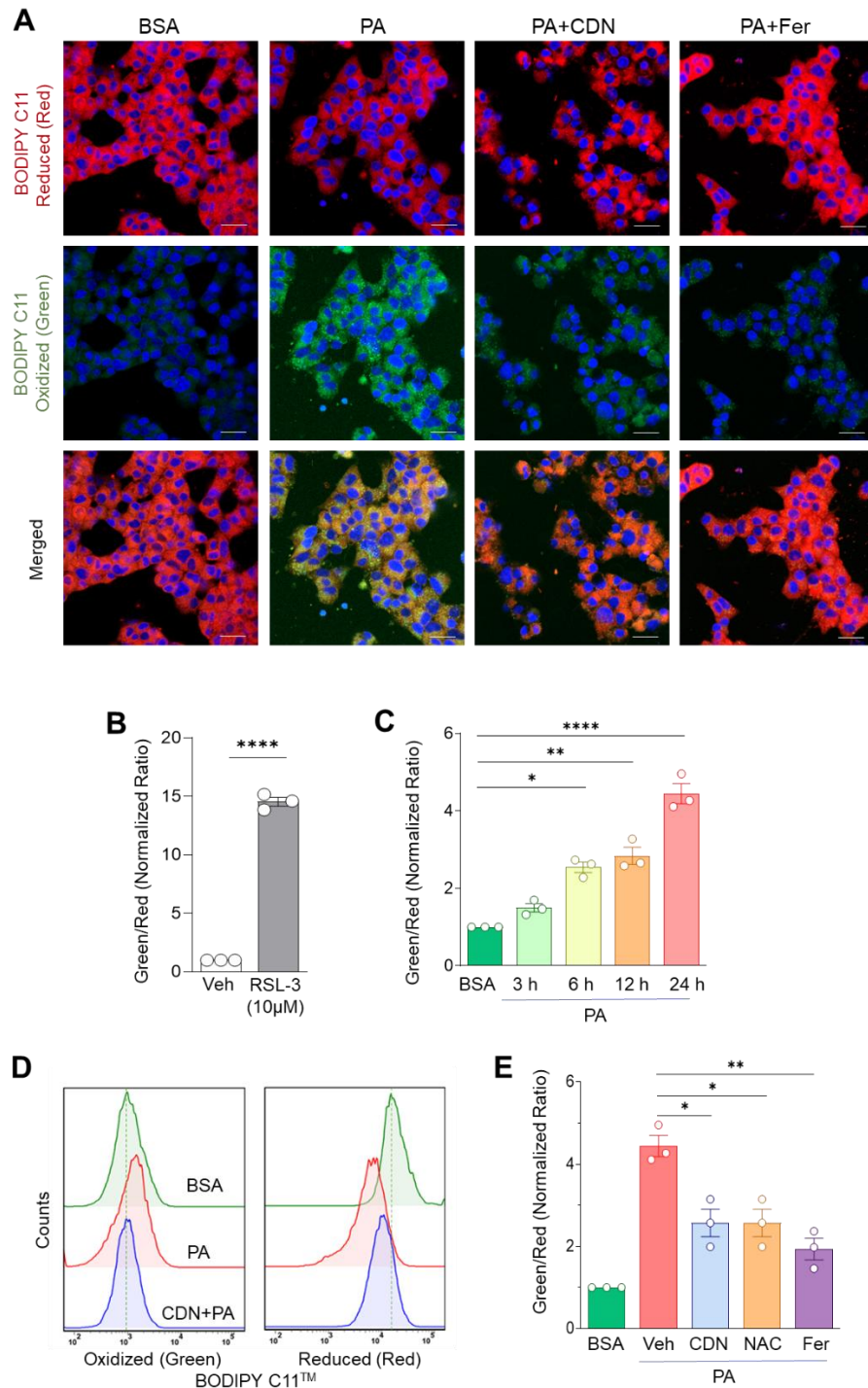
Supplementary Figure S1. Overexpression of TFEB alleviated palmitate-induced autophagy impairment and cytotoxicity. (A & B) MIN6 cells were infected with adenoviruses encoding either wild type TFEB (Ad/CMV/V5-DEST-TFEB-HA) or an empty vector control (Ad/CMV/V5-DEST). Expression of TFEB was confirmed by Western blot using anti-TFEB (A), anti-HA (B), and anti-V5 (B). (C-G) Overexpression of TFEB upregulated lysosomal protein LAMP1 (E), restored p62 clearance (F), and prevented Caspase-3 activation (G) in palmitate-treated MIN6 cells. Data are presented as means \pm standard errors from three independent experiments. Statistical significance was determined using unpaired two-tailed Student's t-test (D) or one-way ANOVA with post-hoc Tukey multiple comparison test (E-G). * $p < 0.05$; ** $p < 0.01$.



Supplementary Figure S2. Perilyosomal Ca^{2+} measurement using high-throughput fluorescence reader with GCaMP3-TRPML1-expressing cells. (A) Perilyosomal Ca^{2+} rise upon extracellular Ca^{2+} addition, mediated by ML-SA1-triggered SOCE. **(B)** Elevated the baseline level of perilyosomal Ca^{2+} , but abolished ML-SA1 response by palmitate (PA) in multi-well plates seeded with GCaMP3-TRPML1-expressing HEK cells. Fluorescence intensity from each well were measured by using FlexStation. (n) is the number of analyzed wells from at least 3 independent experiments. Statistical significance was determined using unpaired two-tailed Student's t-test (A) or one-way ANOVA with post-hoc Tukey multiple comparison test (B). ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

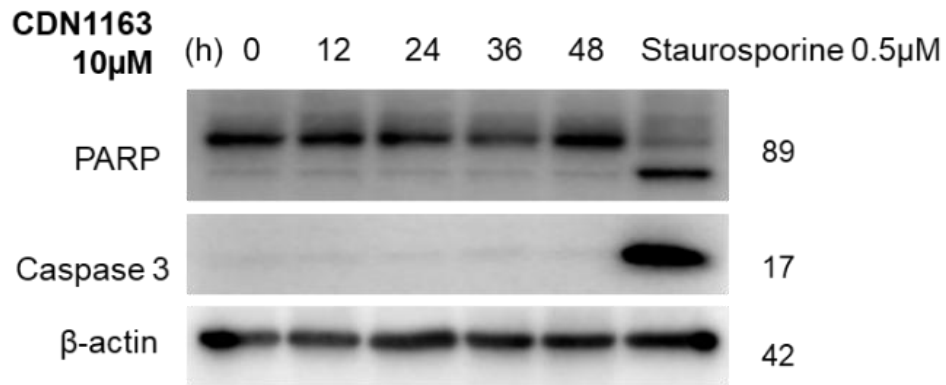


Supplementary Figure S3. SERCA activator recovers ER Ca²⁺ depletion by palmitate. ER Ca²⁺ release due to cyclopiazonic acid (CPA) was reduced in palmitate-treated MIN6 cells, which was normalized by pretreatment with CDN1163, a SERCA activator. Data are presented as means \pm standard deviations and (n) is the number of analyzed cells from more than 3 independent experiments. Statistical significance was determined using one-way ANOVA with post-hoc Tukey multiple comparison test. ** $p < 0.01$; **** $p < 0.0001$.



Supplementary Figure S4. SERCA activator and antioxidant mitigate palmitate-induced ferroptotic lipid peroxidation. (A) Lipid peroxidation associated with ferroptosis in MIN6 cells was estimated by confocal imaging using BODIPY C11; increased green and decreased red fluorescence indicating ferroptosis. **(B-E)** BODIPY

C11 fluorescence shifts were analyzed by flow cytometry. RSL-3, a ferroptosis inducer through glutathione peroxidase inhibition, increased the green-to-red fluorescence ratio (B). Palmitate also elevated this ratio in a time-dependent manner, suggesting ferroptotic lipid peroxidation. CDN1163, a small-molecule SERCA activator (D & E), and the antioxidant N-acetyl cysteine (NAC; E) reduced palmitate-induced ferroptosis. Additionally, ferrostatin (Fer), a ferroptosis inhibitor, abolished palmitate-induced peroxidation, confirming that the lipid peroxidation was ferroptotic. Data are expressed as means \pm standard errors from three independent experiments. Statistical significance was determined using unpaired two-tailed Student's t-test (B) or one-way ANOVA with post-hoc Tukey multiple comparison test (C, E). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.



Supplementary Figure S5. CDN1163, a SERCA activator, does not induce cytotoxicity. Cytotoxicity was assessed by detecting PARP cleavage and activated Caspase-3 via Western blot analysis. Incubating MIN6 cells with CDN1163 (10 μ M) for 48 h showed no signs of cytotoxicity, whereas treatment with staurosporine (0.5 μ M, 8 h) resulted in PARP cleavage and activated Caspase-3.

Supplementary Table 1. Reagents and Tools

Reagents/Resources	Reference/Source	Identifier/Catalogue Number
Antibodies		
Rabbit anti-SERCA2	Cell Signaling T.	Cat. 9580, RRID:AB_10827913
Rabbit anti-p62	Cell Signaling T.	Cat.8025, RRID: AB_10562134
Rabbit anti-LC3	Cell Signaling T.	Cat.12741, RRID: AB_2278129
Rabbit anti-p-p70S6K	Cell Signaling T.	Cat.9234, RRID: AB_330944
Rabbit anti-t-p70S6K	Cell Signaling T.	Cat.9202, RRID: AB_330930
Rabbit anti-p-ACC	Cell Signaling T.	Cat.3661, RRID: AB_330337
Rabbit anti-t-ACC	Cell Signaling T.	Cat.3662, RRID: AB_2223182
Rabbit anti-p-AMPK	Cell Signaling T.	Cat.2535, RRID: AB_330330
Rabbit anti-t-AMPK	Cell Signaling T.	Cat.2532, RRID: AB_10622186
Rabbit anti Cleaved Caspase 3	Cell Signaling T.	Cat.9661, RRID: AB_2341188
Rabbit anti-TFEB	Cell Signaling T.	Cat.37785, RRID: AB_2799103
Rabbit anti-LaminA/C	Cell Signaling T.	Cat.2032, RRID: AB_2136278
Mouse anti- β actin	Proteintech	Cat. 20536-1-AP, RRID: AB_10700003
Mouse anti-alpha tubulin	DSHB	Cat# 12G10, RRID: AB_1157911
Rabbit anti-LAMP2	Cell Signaling T.	Cat. 49067, RRID: AB_2798843
Mouse anti-LAMP1	Santa Cruz Bio.	Cat. 20011, RRID: AB_626853
Rabbit anti-TFEB	Proteintech	Cat. 13372-1, RRID: AB_2199611
Mouse anti-V5-Tag	Invitrogen	Cat. R960-25, RRID: AB_2556564
Rabbit anti-HA-Tag	Cell Signaling T.	Cat. 3724, RRID:AB_1549585
Rabbit anti-PARP	Cell Signaling T.	Cat. 9542, RRID:AB_2160739
Chemicals, Reagents		
CDN1163	Sigma Aldrich	Cat. SML1682
Torin-1	Sigma Aldrich	Cat. 475991
CM-H2DCFDA	Invitrogen	Cat. C6827
mitoSOX	Invitrogen	Cat. M36008
GPN	Santa Cruz Biotechnology	Cat. G6149
MLSA1	Sigma Aldrich	Cat. SML0627
Ionomycin	Alomone labs	Cat. I-700
Menadione	Sigma Aldrich	Cat. M5625

H ₂ O ₂	Sigma Aldrich	Cat. H1009
MitoTEMPO	Sigma Aldrich	Cat. SML0737
BAPTA-AM	Sigma Aldrich	Cat. A1076
W7	Sigma Aldrich	Cat. A4844
KN62	Sigma Aldrich	Cat. K1125
Verapamil	Sigma Aldrich	Cat. V4629
Nimodipine	Sigma Aldrich	Cat. N149
Diazoxide	Sigma Aldrich	Cat. D9035
Fura-2 AM	Invitrogen	Cat. IN-F1201
JC-1	Invitrogen	Cat. T3168
Cyclopiazonic acid (CPA)	Sigma Aldrich	Cat. C1530
Oligomycin	Sigma Aldrich	Cat. 75351
FCCP	Sigma Aldrich	Cat. C2920
Rotenone	Sigma Aldrich	Cat. R8875
Anitmycin A	Sigma Aldrich	Cat. A8674
Paraformaldehyde	Sigma Aldrich	Cat. P6148
Sodium Palmitate	Sigma Aldrich	Cat. P9767
Bovine serum albumin	Sigma Aldrich	Cat. A6003
DAPI Fluoromount-G	Roche	Cat. 10236276001
Ribo EX	GeneAll	Cat. 301-001
Beta-mercaptoethanol	Sigma Aldrich	Cat. M6250
RIPA buffer	SupremeTech	Cat. ST1090-RI
Phosphatase inhibitor (PhosSTOP)	Roche	Cat. 4906845001
Protease inhibitor	Roche	Cat. 5892791001
X-tremeGENE HP DNA Transfection Reagent	Roche	Cat. 6366236001
OptiMEM I	Thermo Fisher Scientific	Cat. 31985-062
BODIPY TM 581/591 C11	Thermo Fisher Scientific	Cat.D3861
Critical commercial assays		
MTT	Sigma Aldrich	Cat. M2128
Cell Death Detection ELISA kit	Roche	Cat. 11774425001
Adeno-X TM Rapid Titer kit	Takara	Cat. 632250
Experimental models		
MIN6	AddexBio	RRID:CVCL_0431
C57BL/6N	DBL	
Software and algorithms		
Prism 9.1	GraphPad	GP9-1988787-RLRM-

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Others

siATP2a2	Dharmacon	Cat. M040968-02-0050
Human SERCA2a (pcDNA3.1+)	Addgene	Cat. 75187
Human SERCA2b (pcDNA3.1+)	Addgene	Cat. 75188
pCAG G-CEPIA1er	Addgene	Cat. 105012
2mt-CEPIA plasmid	Addgene	Cat. 58218
GCaMP3-TRPML1	from Prof. Xu	
LC3-GFP-RFP	Addgene	Cat. 21074
TFEB-pEGFP	Addgene	Cat. 38119
pcDNA3-Lyso-TORCAR	Addgene	Cat. 64929
