Supplementary Information Material and Methods:

Immunofluorescence

Immunostaining for HIF-1α was performed on cells grown on glass cover-slips (density of 5,5 × 10^4 cells per well and in 6-well plates) and fixed with 3% paraformaldehyde, 2% sucrose, PBS1x solution for 10 min RT. Fixed cells were permeabilized with 0.5% Triton-X100, PBS1x solution for 5 min RT and blocked with 2% BSA PBS1x for 1 h at 37 °C. HIF-1α was detected using polyclonal antibody (NB100-479, Novus Biological) 1:400 and FITC-conjugated goat anti-mouse immunoglobulin (Sigma-Aldrich, USA) 1: 800 in 2% BSA PBS1x solution. Nuclear counterstaining with DAPI (1:5000 in PBS1x) was performed after removal of excess secondary antibody. Cells were mounted on microscope slides with Vectashield mounting medium for fluorescence (Vector Company, USA). Immunostaining was visualized with a SP8 High-Resolution STED (Stimulated Emission Depletion) Zeiss Fluorescence Microscope (Zeiss, Germany).

Superoxide Production

Mitochondrial Superoxide Indicator (Red) Assay Kit (Abcam, UK) was utilized as a sensitive detection reagent for mitochondrial superoxide in adherent cells. In all the assayed conditions, cells were plated in 96-well plates with a density of 8 x 10^3 cells per well. Fresh medium and cells without probe were used as controls. After treatments, all the conditions were washed with PBS1x / 0.2% BSA and maintained in Hank's balanced Salt Solution (Thermo Fisher Scientific, USA). According to manufacturer instructions, the absorbance was measured using a micro-plate reader TECAN NanoQuant Infinite M200 (Tecan, Swiss) with a specific Ex/Em 540/580 nm. H_2O_2 10 mM for 10 min was used as a positive control of mitochondrial superoxide production.

Isolation and Fractionation of Mitochondria

Isolated functional mitochondria and lysed mitochondria extracts will be obtained using the Thermo Fisher Mitochondria Isolation Kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. HeLa cells were plated in 100 mm dishes with a density of 2 x 10^6 cells per dish by duplicates. α-tubulin and TOMM20 were used as cytosolic and mitochondria control marker respectively.

Statistical analysis

All the experiments were performed at least 3 times, except the TEM assays which were repeated twice. The data are presented as the means ± standard errors of the means (SEMs) and calculated with GraphPad Prism 10 software. Differences between conditions were analyzed with ordinary one-way or two-way ANOVA with Bonferroni post hoc correction and considered statistically significant when the *p value* was < 0.05.

Supplementary Table S1: List of Primary and Secondary Antibodies used in this study

Name	Concentration	Reference	Secondary Ab
anti-HIF-1α	1:4000	NB100-479, Novus	Rabbit
anti-PARP-1 (C2-10)	1:1000	MA3-950, Thermo	Mouse
anti-PAR 10HA	1:2000	4335-MC-100, Biotech	Mouse
anti-SOD2 (A-2)	1:3000	sc-133134, SantaCruz	Mouse
anti- FIS1 (E3K9O)	1:1000	32525, Cell Signaling	Rabbit
anti-p-DRP1(S616) (D9A1)	1:1000	4494S, Cell Signaling	Rabbit
anti-PARKIN	1:2000	2132S, Cell Signaling	Rabbit
anti p-PINK1 (Ser628)	1:1000	PA5-105356, Invitrogen	Rabbit
anti-PINK1 (D8G3)	1:1000	6946S, Cell Signaling	Rabbit
anti-TOMM20	1:2000	11802-1-AP, Proteintech	Rabbit
anti-HK2 (EPR20839)	1:1000	ab209847, AbCam	Mouse

anti-CA9 (EPR23055)	1:1000	ab243660, AbCam	Rabbit
anti-LC3I/II	1:750	PD014, MBL	Rabbit
anti-ULK1 (H-240)	1:1000	sc-33182, Santa Cruz	Rabbit
anti-pULK1(S467)	1:1000	sc-8418, Santa Cruz	Rabbit
anti-p-AMPk(Thr172) (40H9)	1:1000	2535, Cell Signaling	Rabbit
anti- AMPKα (D5A2)	1:2500	5831, Cell Signaling	Rabbit
anti-p-$p70^{S6k}$ (T389)	1:1000	9205S, Cell Signaling	Rabbit
anti-$p70S6^{k}$ (H-9)	1:1000	sc-8418, Santa Cruz	Mouse
anti-GAPDH (14C10)	1:5000	2118, Cell Signaling	Rabbit
anti-βactin (AbGEa)	1:10000	MA1-744, Thermo	Mouse
anti-αtubulin	1:10000	236-10501, Thermo	Mouse

Supplementary Table S2: human siRNA sequences for human cancer cells

Scrambler SCR (h) Fw 5'-CUUUGGGUGAUCUACGUUA-3'	Rv 5'-UAACGUAGAUCACCCAAAG-3'
siPARP1 (h) Fw 5'-GAAGAUGGUGGACCCGGAG-3'	Rv 5'-CUCCGGGUCCACCAUCUUC-3'
siATG5 (h) Fw: 5'-CCCUCUAUCAGGAUGAGAUTT-3'	Rv: 5'-AUCUCAUCCUGA UAGAGGGTT-3'
siATG7 (h) Fw: 5'-ACUAAAAGGGGCAAACUGCAG-3'	Rv: 5'-GCAGUUUGCCCCUUUUAGUAG-3'

Supplementary Table S3: List of RT-qPCR primers used in the study (Forward/Reverse 5'-3')

Bnip3	5'- GTCTGGACGGAGTAGC -3'	5'- GGCCGACTTGACCAAT -3'
Beclin1	5'- ACCGTGTCACCATCCAGGA -3'	5'- GAAGCTGTTGGC ACTTTCTGT -3'
Fis1	5'- GTCCAAGAGCACGCAGTTTG -3'	5'- ATGCCTTTACGGATGTCATCATT -3'
Mff	5'- ACTGAAGGCATTAGTCAGCGA -3'	5'- TCCTGCTACAACAATCCTCTCC -3'
Opa1	5'- TGCCTGACATTGTGTGGGAAA -3'	5'- TTCCGGAGAACCTGAGGTAA -3'
Mfn1	5'- AGTTGGAGCGGAGACTTAGC -3'	5'- ATCGCCTTCTTAGCCAGCAC – 3'
Mfn2	5'- AATCTGAGGCGACTGGTGAC -3'	5'- GGACATTGCGCTTCACCTTC -3'
Hk2	5'- TCACGGAGCTCAACCATGAC -3'	5'- CCCAAAGCACACGGAAGTTG -3'
Pdk1	5'- CTGTGATACGGATCAGAAACCG -3'	5'- TCCACCAAACAATAAAGAGTGCT -3'
Pfkfb3	5'- ATCTACCTGAACGTGGAGTCCGTCTG -3'	5'- TCAGTGTTTCCTGGAGGAGTCAGC-3'
Pfkfb4	5'- TTAATTTTGGAGAACAGAATGGC -3'	5'- CGTAGCCTCATCACTGTCGC -3'
βactin	5'- CGACAGGATGCAGAAGGAGAT -3'	5'- CAAGAAAGGGTGTAACGCAACTA -3'
36B4	5'- CAGATTGGCTACCCAACTGTT -3'	5'- GGCCAGGACTCGTTTGTACC - 3'