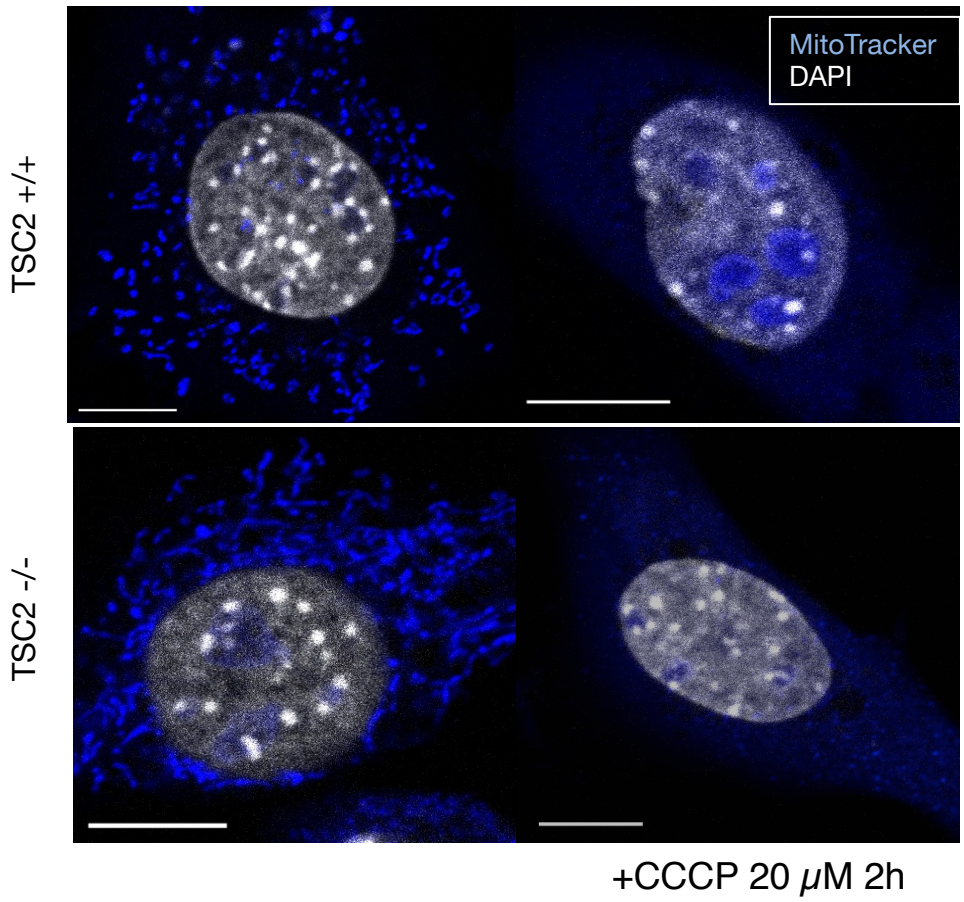
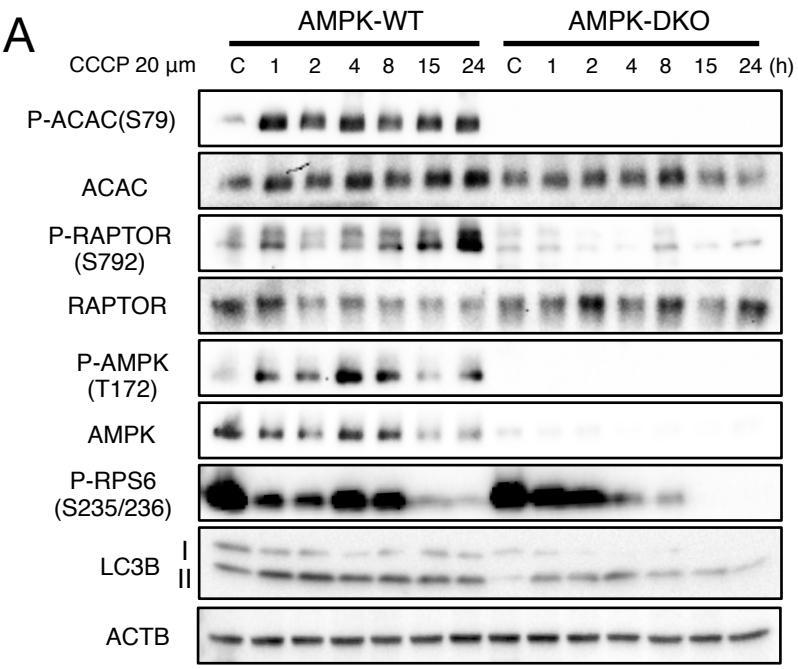
Figure S1

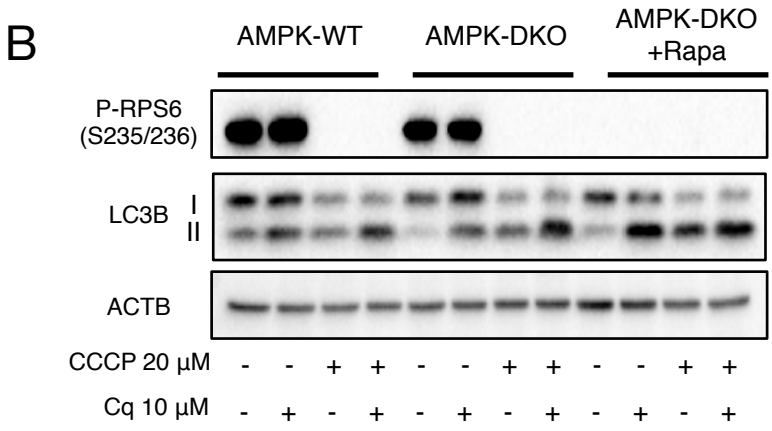
A



Supplemental Fig1
A)Loss of MitoTracker uptake after CCCP treatment reflects loss of mitochondrial membrane potential in both TSC2 -/- and control cells. Representative confocal microscope images from cells treated for 2h with 20 μM CCCP. Other times and doses display showed similar results, not shown. 10 μm bar

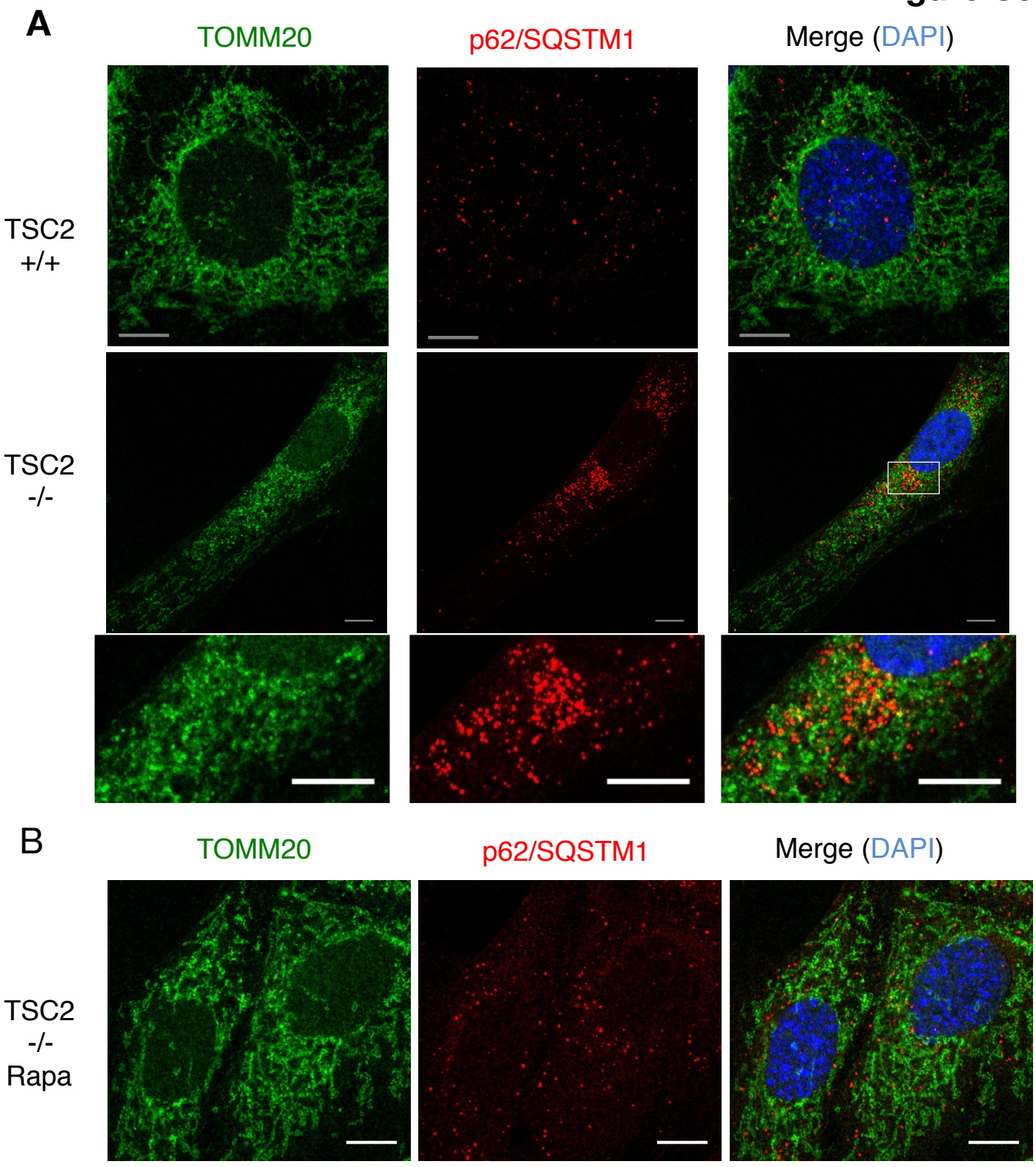
Figure S2





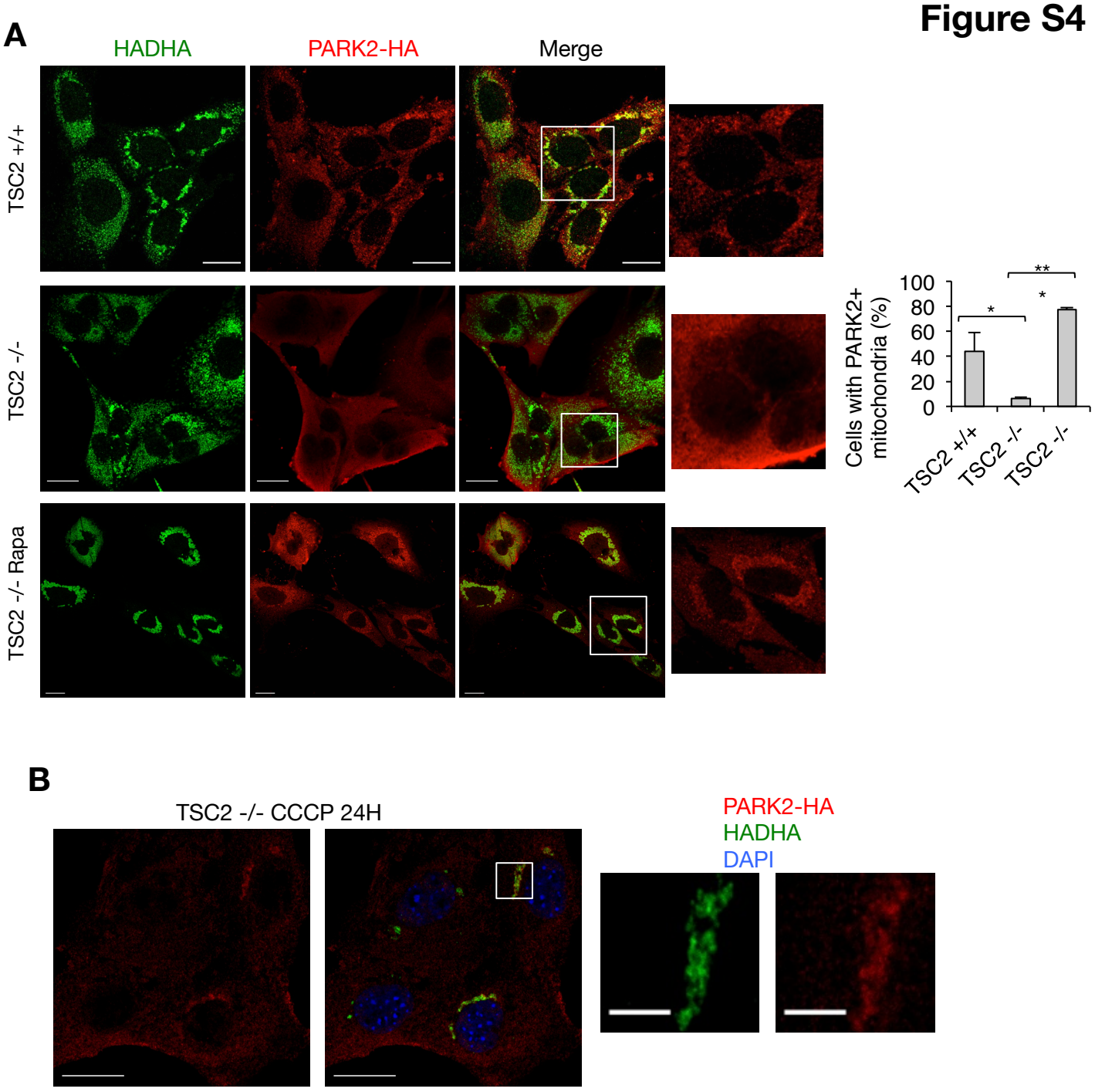
Supplementary Figure 2. CCCP-mediated mTORC1 downregulation and autophagy stimulation is AMPK independent. (A) AMPK-WT and AMPK-DKO cells were stimulated with CCCP for the indicated times. (B) Cells were stimulated with CCCP for 15h in the presence or absence of chloroquine (CQ) and/or rapamycin 20 nM (Rapa). Representative blots are shown.

Figure S3



Supplemental Fig3. p62/SQSTM1 positive mitochondria in TSC2 -/- cells. (A) Representative confocal microscopy images of TSC2 +/+ and TSC2 -/- MEFs. (B) TSC2 -/- were treated with 20 nM rapamycin for 15h.

Figure S4



Supplemental Fig4.
A) Impaired PARK2 translocation to mitochondria in valinomycin-treated treated TSC -/- cells. Cells were treated with valinomycin 1 µM for 3h. Quantification shows percentage of PARK2+ mitochondria, of 3 independent experiments. *P < 0.05, ***P < 0.001
B) PARK2 translocation to mitochondria in TSC2 -/- cells after prolonged CCCP treatment (20 µM CCCP, 24h). By the time that most mitochondria are cleared in control cells, mitochondrial PARK2 is still observed in TSC2 -/- cells. Bars represent 20 µM, or 5 µM in enlarged images

Table S1. List of antibodies

Antibody	Company	Ref	WB dilution	IF dilution
ACAC	Cell Signaling	#3662	1:1000	
P-ACAC	Cell Signaling	#3661	1:1000	
AMPK	Cell Signaling	#2352	1:1000	
P-AMPK	Cell Signaling	#2531	1:1000	
FOXO1	Cell Signaling	#2880		1:100
LC3B	Cell Signaling	#4108	1:1000	
RAPTOR	Cell Signaling	#4978	1:1000	
P-RAPTOR	Cell Signaling	#2083	1:1000	
RPS6	Cell Signaling	#2217	1:5000	
P-RPS6KB1	Cell Signaling	#9205	1:1000	
RPS6KB1	Cell Signaling	#9202	1:1000	
TSC2	Cell Signaling	#3612	1:1000	
TUBA1A	Cell Signaling	#2144	1:5000	
HADHA	Abcam	#ab54477	1:1000	1:200
TOMM20	Santa Cruz	#sc-11415		1:200
GFP	Santa Cruz	#sc-9996	1:1000	
SQSTM1	Progen	#GP62	1:2500	1:500
PINK1	Novus Biologicals	#BC100-494	1:1000	
P-RPS6	Thermo-Fisher	#MA5-15140	1:2500	
V5	Thermo-Fisher	#R960-25	1:2500	
HA	Roche	#12CA5		1:200
ACTB	Sigma	#A5316	1:5000	

Table S2. List of oligonucleotides

Gene	Forward	Reverse
Ppargc1a	AATCAGACCTGACACAACGC	GCATTCCTCAATTTCACCAA
Ppargc1b	CGCTCCAGGAGACTGAATCCAG	CTTGACTACTGTCTGTGAGGC
Actb	GACATGGAGAAGATCTGGCA	GGTCTCAAACATGATCTGGGT
Fis1	TGGTGTCTGTGGAGGATCTG	ATTGCGTGCTCTTGGACAC
Mfn1	ATGGCAGAAACGGTATCTCCA	CTCGGATGCTATTCGATCAAGTT
Mfn2	CGAGGCTCTGGATTCACTTC	CAACCAGCCAGCTTTATTCC
Opa1	ACCAGGAGAAGTAGACTGTGTCAA	TCTTCAAATAAACGCAGAGGTG
Drp1	GCTAGTCCACGTTTCACCAGA	TCCATGTGGCAGGGTCAT
Pink1	CTGAGATGCCTGAGTCGGTG	GTGCAGACGGTCTCTTGCT
Mycoplasma sp.	GGCGAATGGGTGAGTAACACG	CGGATAACGCTTGCGACCTATG