SUPPLEMENTARY FIGURE LEGENDS

Figure S1

Detection of endogenous TcFEB in primary hepatocytes. Protein extracts from 3 controls and 3 TcFEB-/- primary hepatocyte cell lines derived from an albumin-CRE driven conditional TcFEB KO mouse, were probed with TFEB (MyBiosource) and tubulin antibodies. A cross-reacting band corresponding to the TcFEB protein is clearly detected in control samples only. The very faint band detected in TcFEB-/- cells is likely the result of incomplete penetrance of CRE-mediated cleavage.

Figure S2

Amino acids induce TFEB molecular weight shift. Immunoblotting of protein extracts isolated from HEK-293T cells transfected either TFEB-3xFLAG or with an empty vector were nutrient starved for 50 min, or starved and then stimulated for 10 mins with amino acids (A.A.). Antibody used were p-T389-S6K, S6K and FLAG.

Figure S3

Detection of p-S142-TFEB using a p-S142-TFEB antibody. Immunoblotting of protein extracts isolated from HeLa cells transfected with an empty vector, with TFEB-3XFLAG or with TFEB-^{S142A}-3XFLAG were probed with p-S142-TFEB or Flag antibodies.

Figure S4

Analysis of the effects of serine-to-alanine, or threonine-to-alanine, mutations on TFEB subcellular localization. FLAG immunostaining (red) of HeLa cells that express TFEB-3xFLAG carrying different serine-to-alanine mutations. Nuclei were stained with dapi (blue). Scale bars represent 10μm.

Figure S5

Torin 1 treatment induces lysosomal clustering of TFEB-GFP. HeLa cells that stably express TFEB-GFP were treated with vehicle (top) or Torin 1 (bottom), imaged by the OPERA system (Perkin Elmer). Yellow arrows indicate lysosomal clusters of TFEB-GFP in vehicle-treated cells. Scale bars represent 10μm.

Figure S6

Torin 1 causes nuclear and lysosomal accumulation of TFEB in Rag^{CA}-expressing HEK-293T cells. Cells were co-transfected with TFEB-GFP and either Rap2A or Rag^{CA}, and either left untreated or treated with Torin 1 for 1h.

Figure S7

Quantitative polymerase chain reaction (qPCR) of TFEB target genes in primary hepatocytes from control (flox/flox) and Tcfeb-/- (flox/flox; alb-Cre) mice. The level of target genes in Tcfeb-/- samples is expressed as fold change over control samples. Values represent means±SD of three independent hepatocyte preparations (3 mice/genotype). Student t test (two tailed) * = P value ≤ 0.05.

Figure S8

Nuclear translocation of TFEB-GFP in TSC+/+ and TSC2-/- MEFs induced by either amino acid starvation, chloroquine or Torin 1. 2x10^6 TSC2+/+ and TSC2-/- MEFs were transfected with 1µg of a TFEB-GFP plasmid by nucleofection, and plated at a density of 300,000 cells/dish in 35mm, glass-bottom Mattek dishes. The next day, MEFs were either switched to fresh culture media, or to fresh media containing Torin 1 or Cholroquine, or transferred to RPMI without amino acids supplemented with 10% dialyzed FBS. After 1h, MEFs were fixed in 4% PFA, stained with DAPI and imaged by spinning disk confocal.

Scale bars represent 10µm.

Figure S9

Nuclear translocation of endogenous TFEB in TSC2+/+ and TSC2-/- MEFs induced by either amino acid starvation, chloroquine or Torin 1. MEFs were plated at a density of 300,000 cells in 35mm dishes containing 2 glass coverslips each. The next day, MEFs were either switched to fresh culture media, or to fresh media containing Torin 1 or Cholroquine, or transferred to RPMI without amino acids supplemented with 10% dialyzed FBS. After 1h, coverslips were fixed in 4% PFA, stained with antibodies against endogenous TFEB and RagC and with DAPI, and imaged by spinning disk confocal.

Scale bars represent 10µm.

SUPPLEMENTARY MOVIE LEGENDS

Movie S1

Spinning disk confocal movie of a MEF co-transfected with TFEB-GFP (green) and mRFP-Rab7 (red), showing TFEB localization to Rab7-positive lysosomes. Playback speed = 64x.

Movie S2

Spinning disk confocal movie of a MEF co-transfected with TFEB-GFP (green) and mRFP-Rab7 (red) and treated with Torin 1 at the start of the imaging period. The movie shows how the progressive appearance of TFEB-GFP signal on several lysosomes parallels its massive accumulation in the nucleus. Playback speed = 900x.

Movie S3

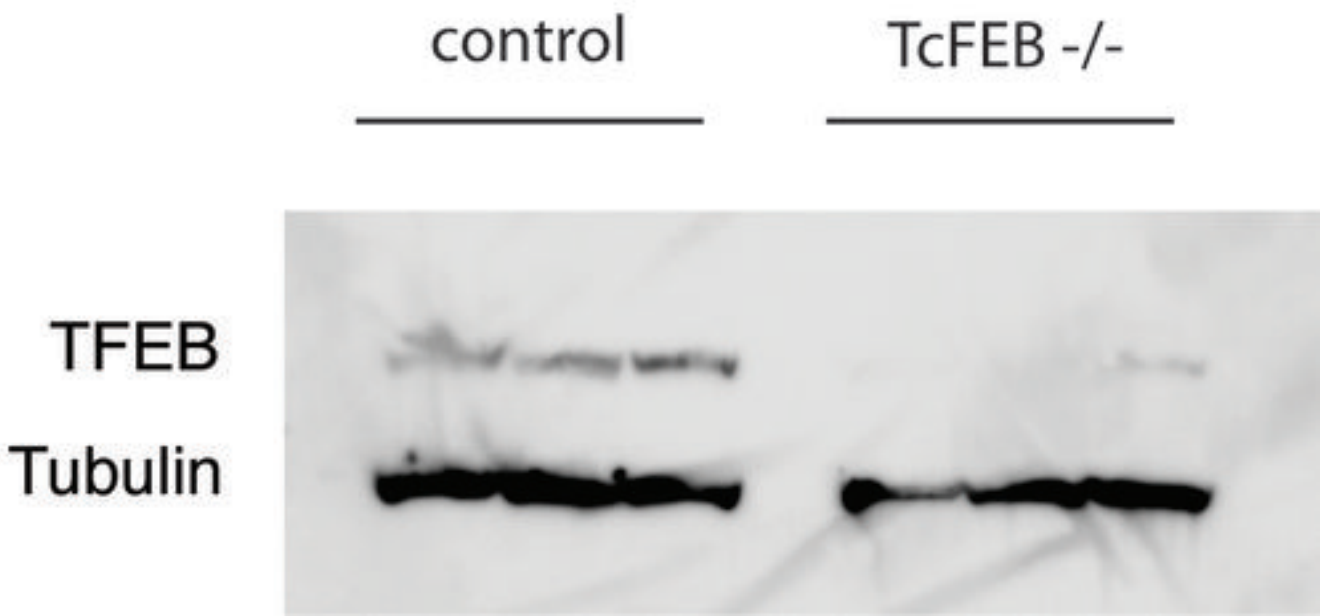
Spinning disk confocal movie of a TFEB-GFP expressing HeLa cell treated with Torin 1 at the start of the imaging period. The movie shows how the progressive appearance of TFEB-GFP signal on several lysosomes parallels its massive accumulation in the nucleus. Playback speed = 600x.

Movie S4

FRAP experiment on TFEB-GFP positive lysosomes from a control MEF (*left*) and a MEF treated with Torin 1 (*right*). Photobleaching was achieved with

high power 488nm laser light. The movie shows the differences in the speed and amount of fluorescence recovery between the two conditions. Playback speed = 96x.

Figure S1



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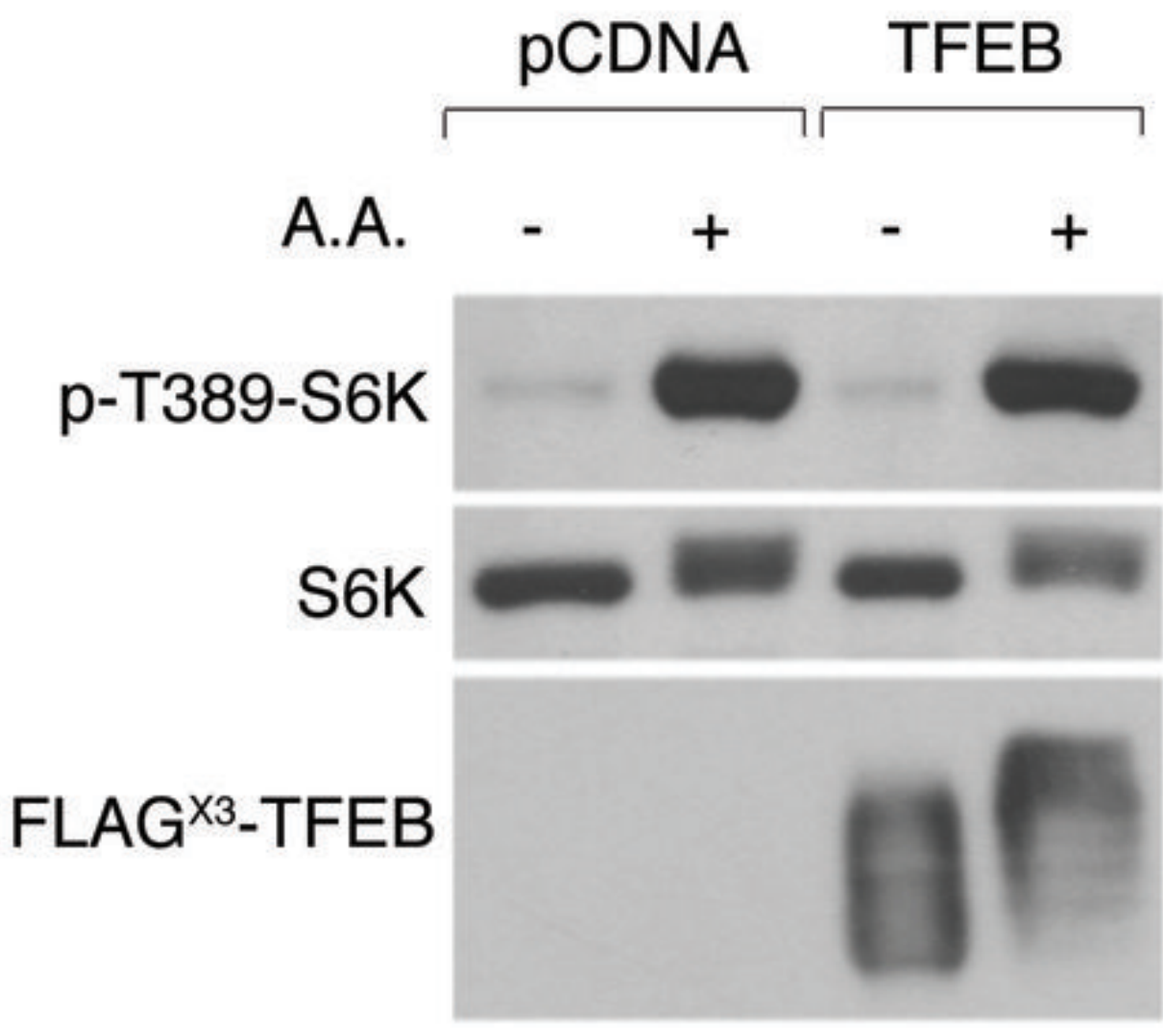

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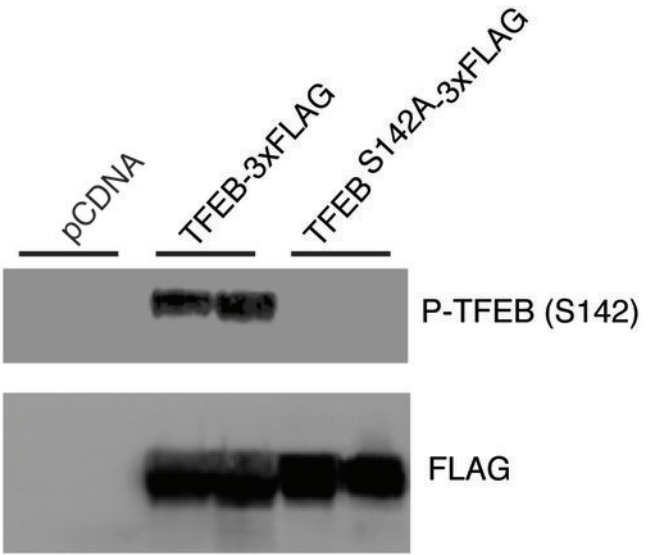

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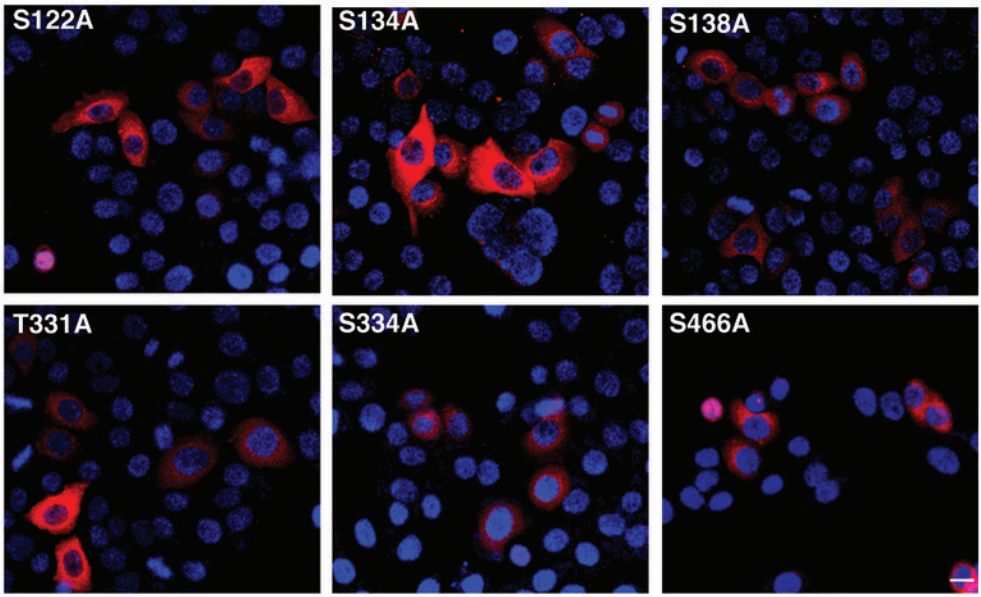

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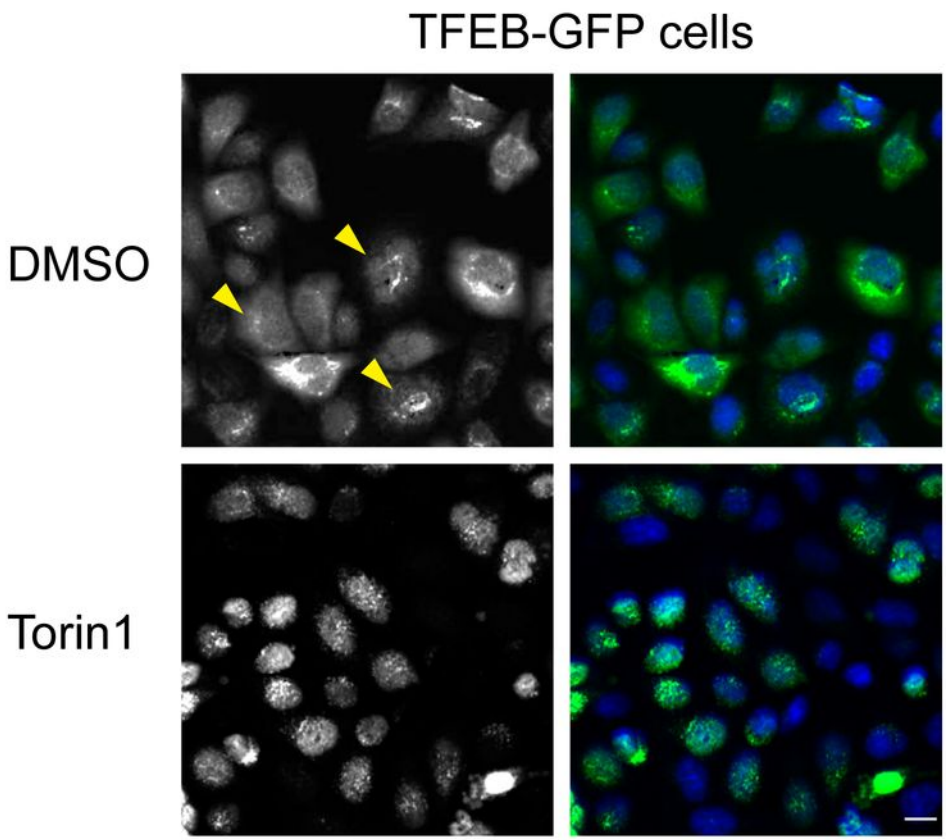

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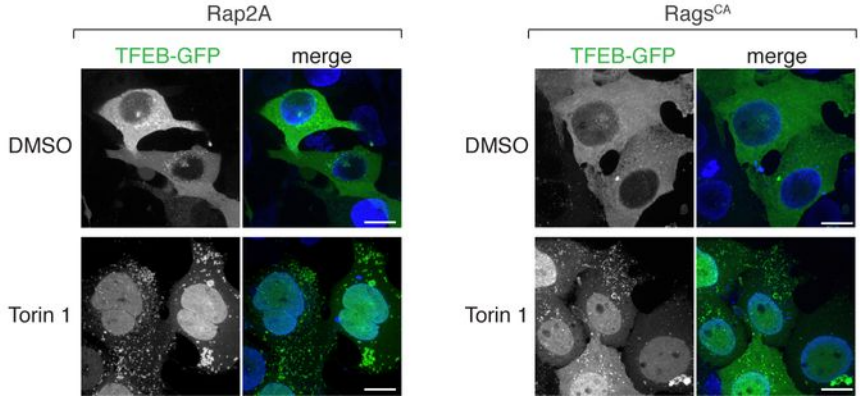
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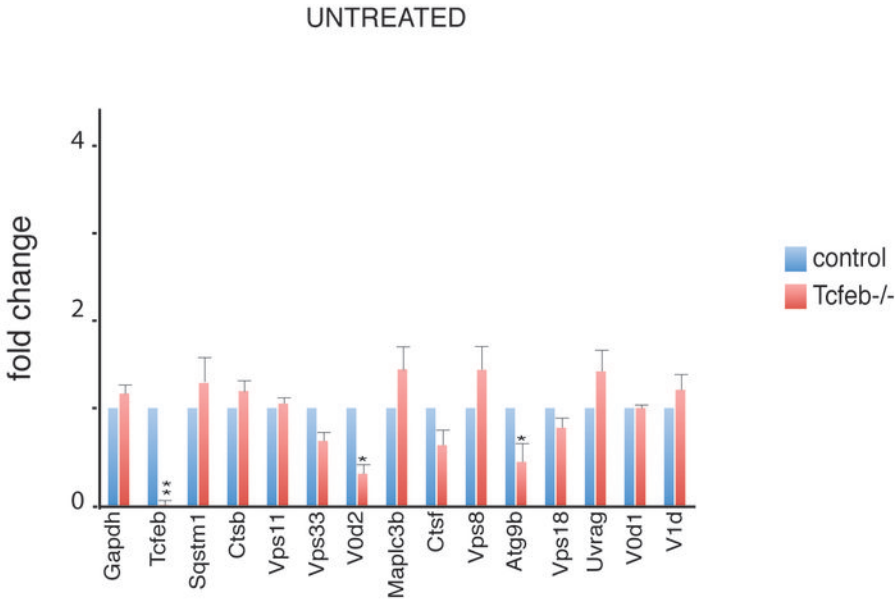
Rags^CA
TFEB-GFP
merge
DMSO
Torin 1

Figure S7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V1d
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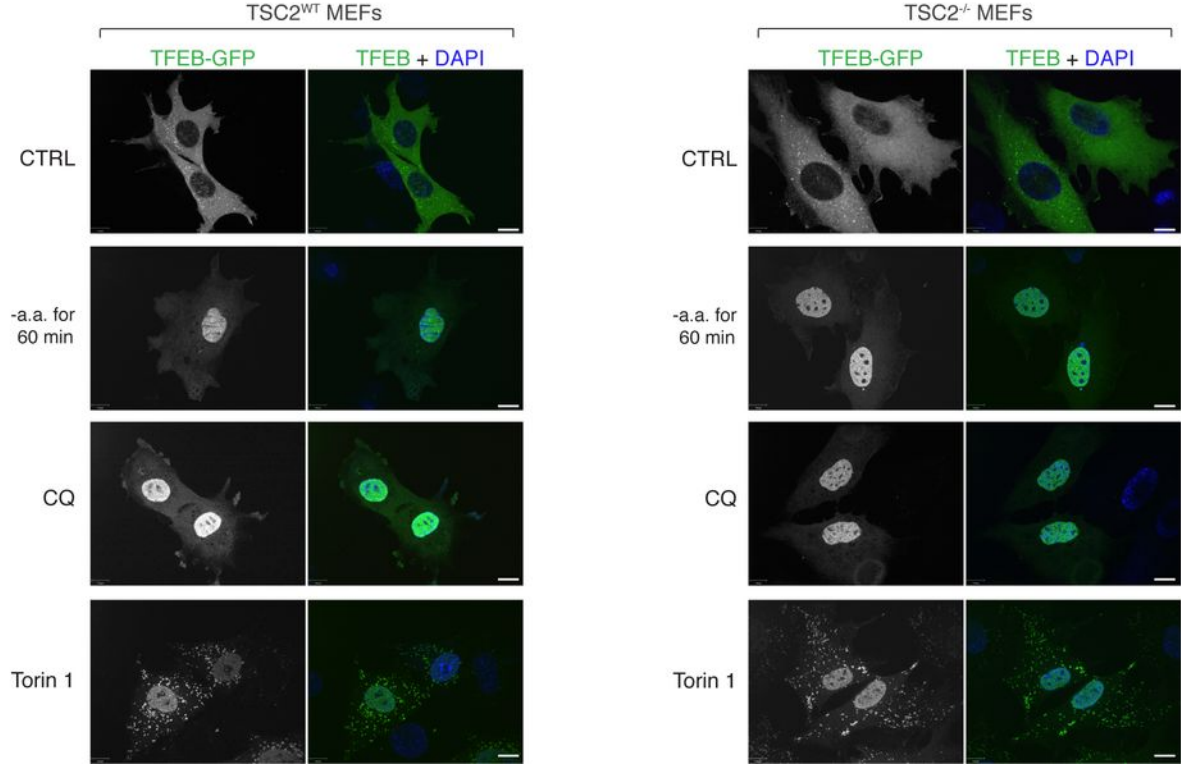
Figure S8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TFEB-GFP
TFEB + DAPI
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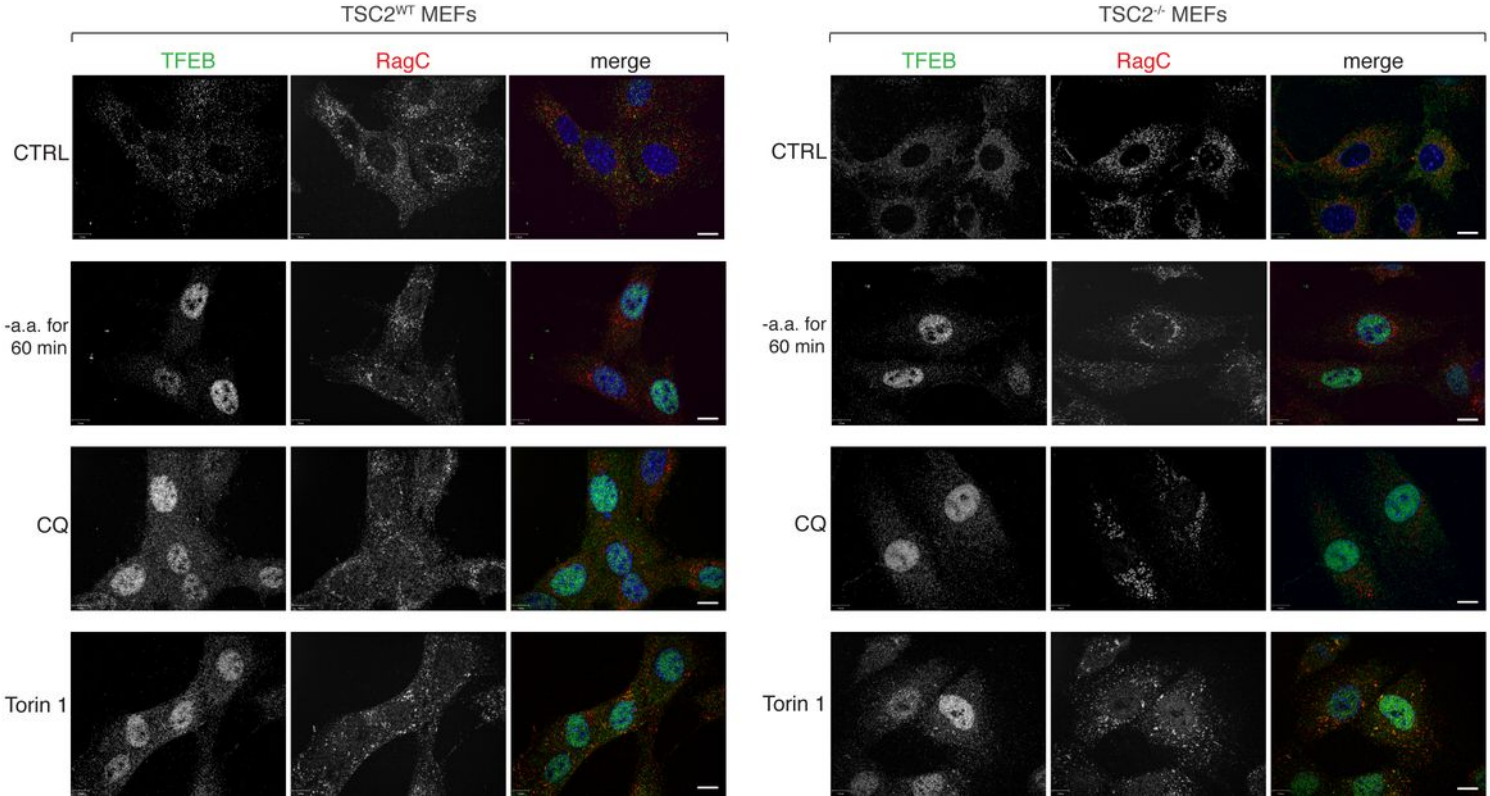
-a.a. for 60 min
CQ
Torin 1
TSC2-/- MEFs
TFEB-GFP
TFEB + DAPI
CTRL
-a.a. for 60 min
CQ
Torin 1

Figure S9


TSC2WT MEFs
TFEB
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CQ
Torin 1
TSC2-/- MEFs
TFEB
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CTRL
-a.a. for 60 min
CQ
Torin 1