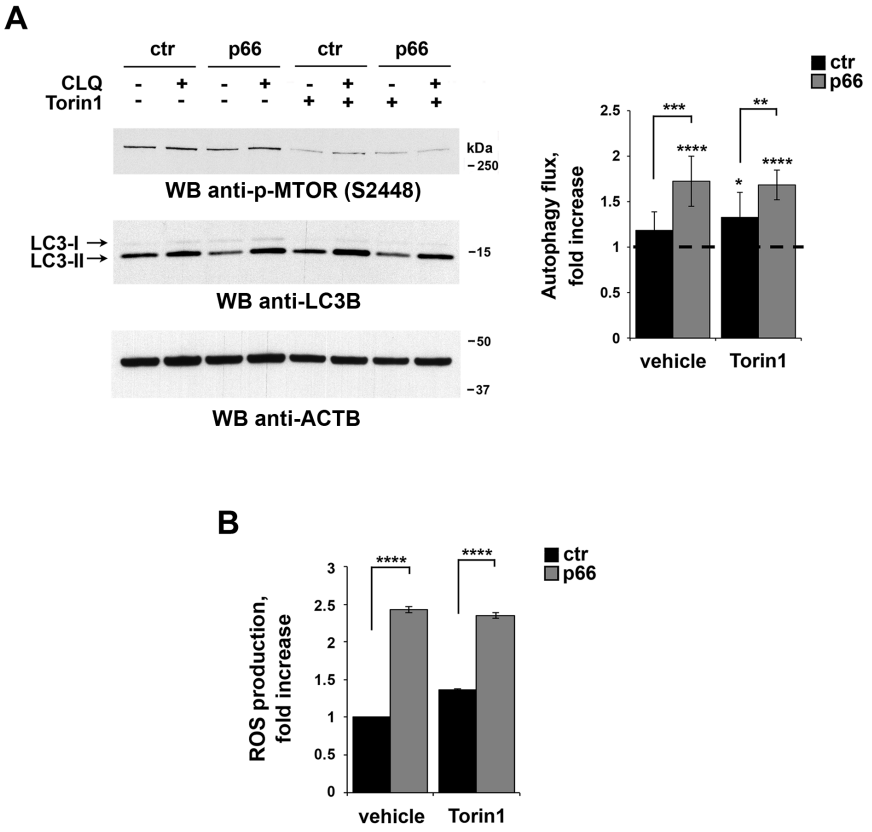

A
ctr
p66
ctr
p66
CLQ
Torin1
kDa
250
WB anti-p-MTOR (S2448)
LC3-I
LC3-II
15
WB anti-LC3B
50
37
WB anti-ACTB
ctr
p66
Autophagy flux, fold increase
2.5
2
1.5
1
0.5
0
***
****
**
*
****
vehicle
Torin1
B
ctr
p66
ROS production, fold increase
3
2.5
2
1.5
1
0.5
0
****
****
vehicle
Torin1

Figure S1

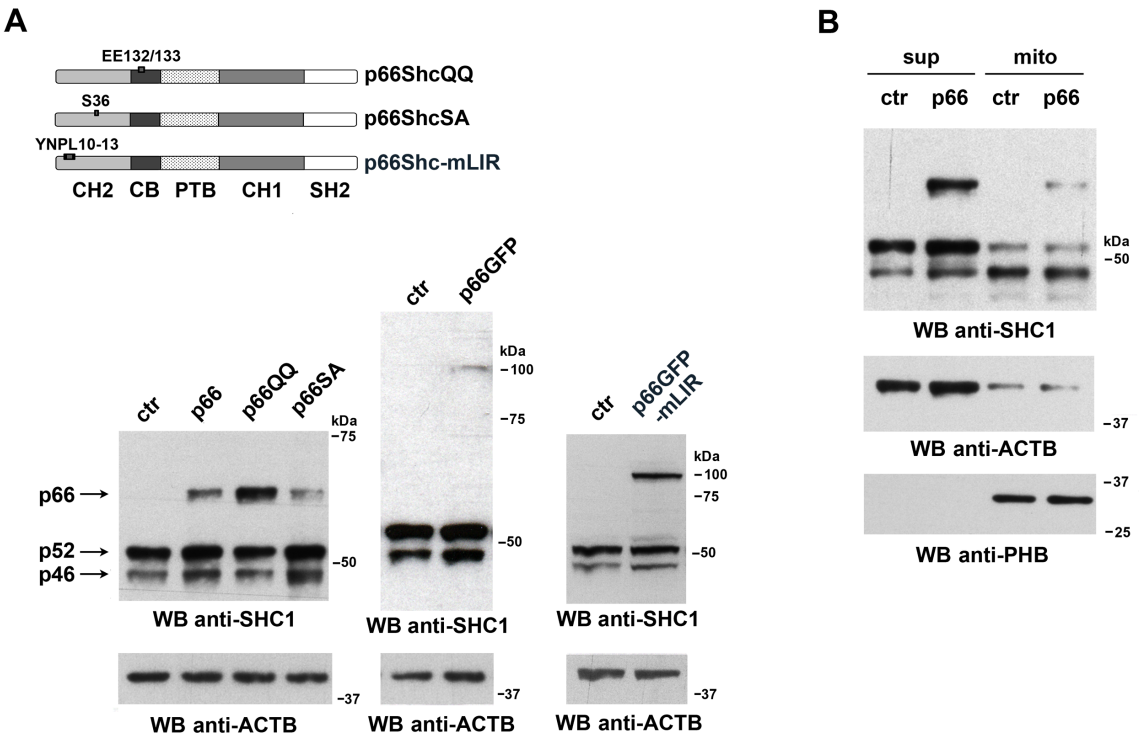

A
EE132/133
p66ShcQQ
S36
p66ShcSA
YNPL10-13
p66Shc-mLIR
CH2 CB PTB CH1 SH2
ctr
p66
p66QQ
p66SA
kDa
75
p66
p52
p46
50
WB anti-SHC1
37
WB anti-ACTB
ctr
p66GFP
kDa
100
75
50
WB anti-SHC1
37
WB anti-ACTB
ctr
p66GFP
mLIR
kDa
100
75
50
WB anti-SHC1
37
WB anti-ACTB
B
sup
mito
ctr
p66
ctr
p66
kDa
50
WB anti-SHC1
37
WB anti-ACTB
37
25
WB anti-PHB

Figure S2

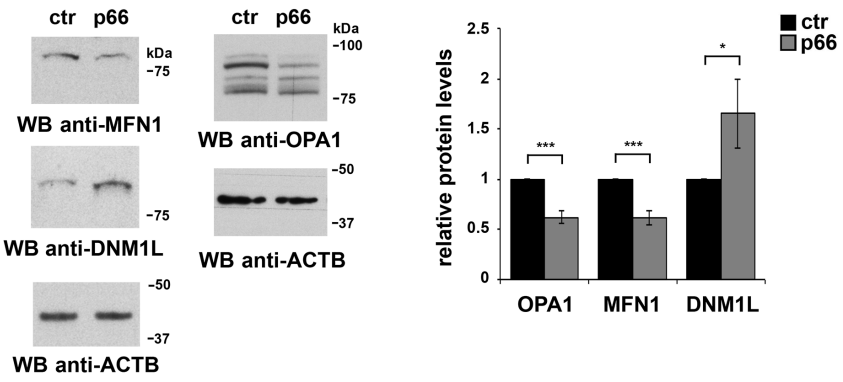

ctr
p66
kDa
75
WB anti-MFN1
75
WB anti-DNM1L
50
37
WB anti-ACTB
ctr
p66
kDa
100
75
WB anti-OPA1
50
37
WB anti-ACTB
relative protein levels
2.5
2
1.5
1
0.5
0
***
***
*
OPA1
MFN1
DNM1L
ctr
p66

Figure S3

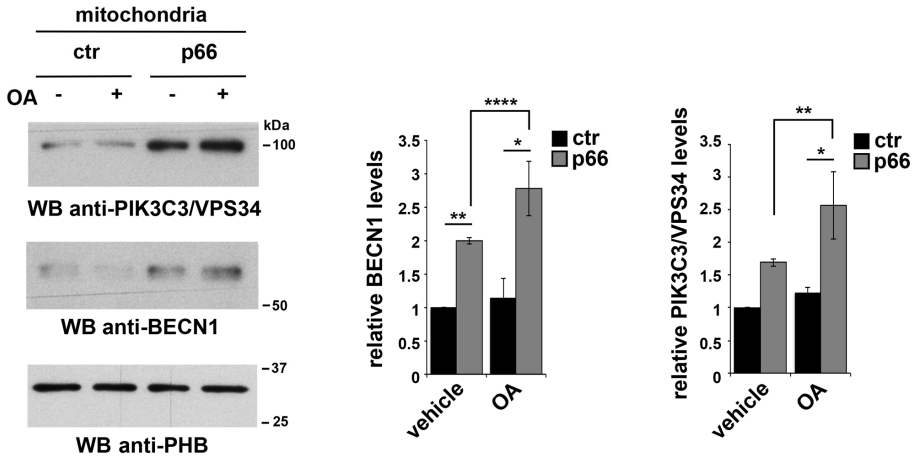

mitochondria
ctr
p66
OA - + - +
kDa
100
WB anti-PIK3C3/VPS34
50
WB anti-BECN1
37
25
WB anti-PHB
relative BECN1 levels
ctr
p66
vehicle
OA
relative PIK3C3/VPS34 levels

Figure S4

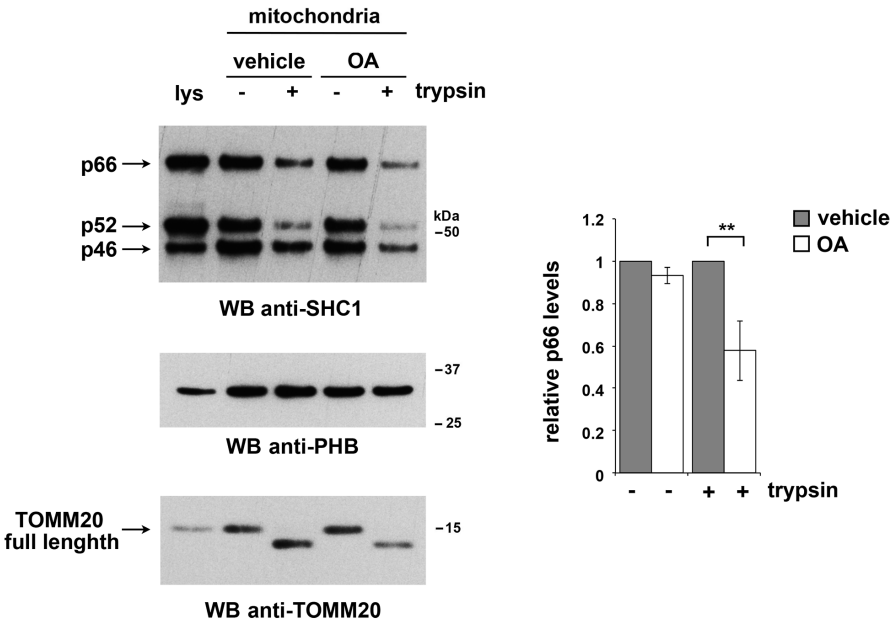

mitochondria
vehicle
OA
lys
-
+
-
+
trypsin
p66
p52
p46
kDa
50
WB anti-SHC1
37
25
WB anti-PHB
TOMM20
full lenghth
15
WB anti-TOMM20
relative p66 levels
1.2
1
0.8
0.6
0.4
0.2
0
**
vehicle
OA
- - + + trypsin

Figure S5

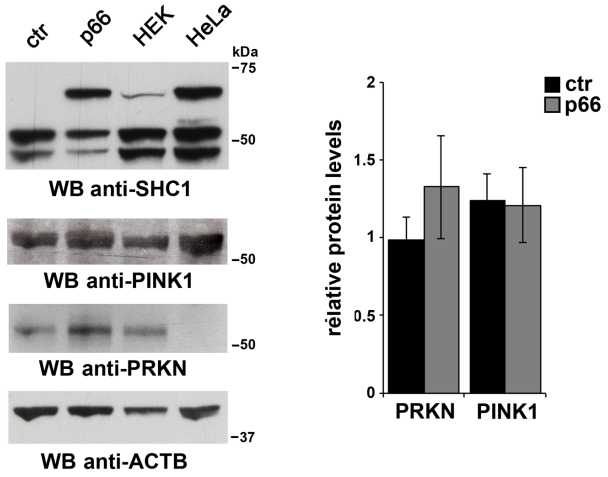
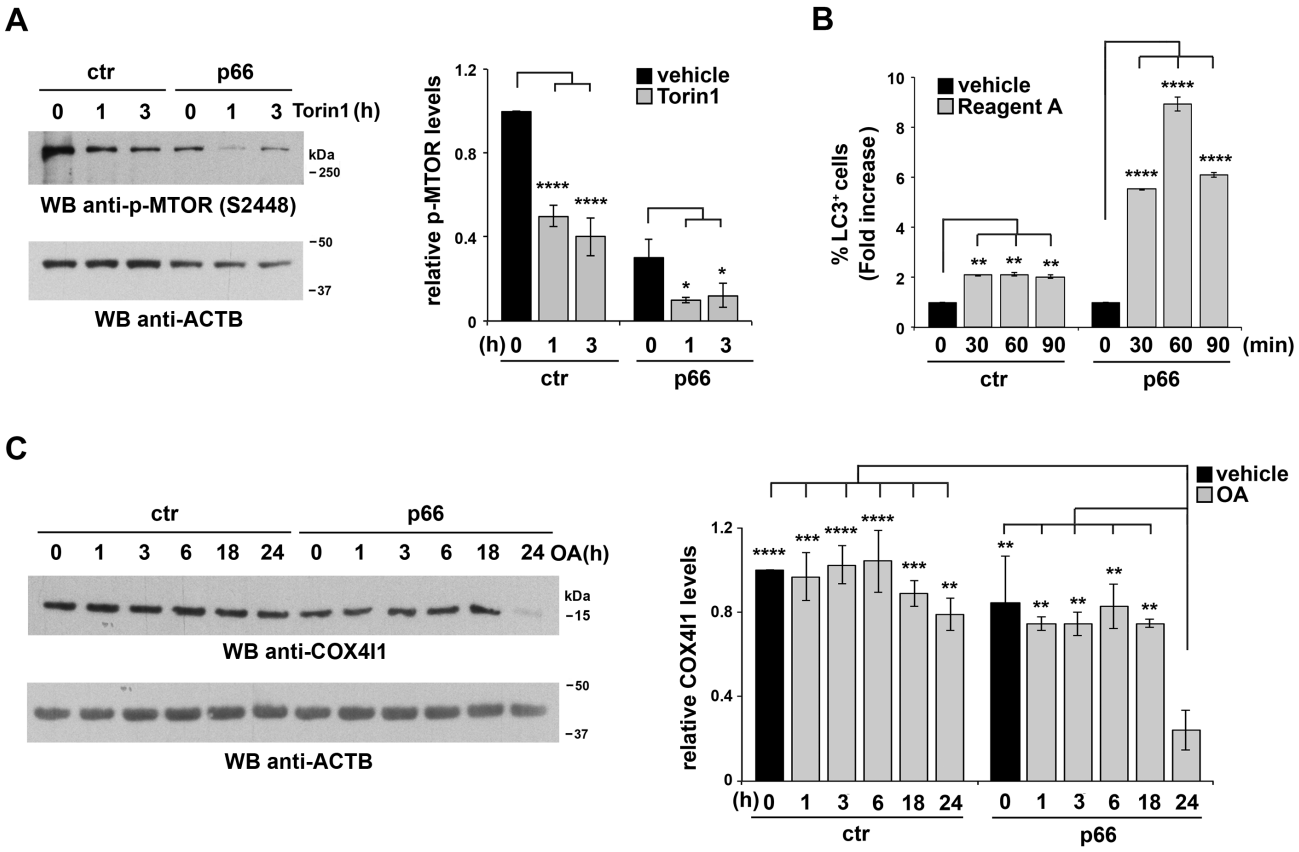

ctr
p66
HEK
HeLa
kDa
75
50
WB anti-SHC1
50
WB anti-PINK1
50
WB anti-PRKN
37
WB anti-ACTB
relative protein levels
2
1.5
1
0.5
0
ctr
p66
PRKN
PINK1

Figure S6



D

% cell viability

Treatment	ctr	p66	p66SA	p66QQ	p66GFP	p66GFP -mLIR
untreated	92.4±1.1	93±0.8	89±2.3	86±2.9	90±1.8	88±2.2
Reagent A	90±2.2	92±1.7	91±1.5	87±2.8	94±0.8	91±2.2
OA	90.3±2	88±3.1	87±1.7	86±3.4	93±0.8	89±1.8
Torin1	91±1.2	93±0.8				

Figure S7

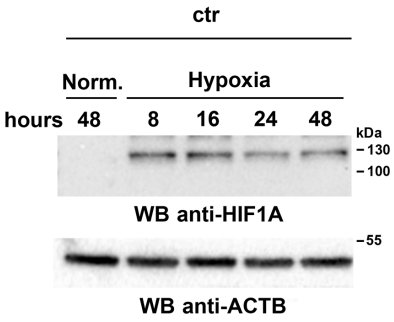

ctr
Norm.
Hypoxia
hours
48
8
16
24
48
kDa
130
100
WB anti-HIF1A
55
WB anti-ACTB

Figure S8

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. p66SHC promotes MTOR-independent autophagy in B cells. **(A)** Immunoblot analysis of the autophagy marker LC3B in lysates of ctr and p66 cells untreated or treated with a downstream inhibitor of the autophagy pathway, chloroquine (CLQ), or an upstream activator of autophagy, Torin1, alone or in combination. ACTB was used as a loading control. The histogram shows the quantification of autophagy flux as the difference in LC3-II/ACTB between CLQ-treated and untreated cells (mean fold ± SD accumulation of LC3B-II in samples treated with CLQ compared to the vehicle control; vehicle control value=1, dashed line) (n≥3). (**B**) Flow cytometric analysis of ctr and p66 cells loaded with the ROS-sensitive probe CM-H_2DCFDA untreated or treated with an upstream activator of autophagy, Torin1. The histogram shows the relative ROS production using as reference control (empty vector) ctr cells set as 1 (n=3). ****P≤0.0001; ***P≤0.001; **P≤0.01; *P≤0.05 (one-way ANOVA).

Figure S2. p66SHC has both a cytosolic and a mitochondrial intracellular localization. **(A)** Immunoblot analysis of p66SHC in lysates of the ctr, p66, p66QQ and p66SA MEC transfectants, and of the MEC transfectants expressing GFP-tagged wild-type p66SHC (p66GFP) or the GFP-tagged p66SHC LIR mutant (p66GFP-mLIR). ACTB was used as a loading control. Schematic presentation of the domain structure of p66SHC highlighting the localization of the amino acid residues substituted in the mutants, which are schematized at the top of the panel. **(B)** Immunoblot analysis of p66SHC in lysates from post-mitochondrial supernatants and mitochondrial fractions of ctr and p66 cells. PHB was used as a purity control of the post-mitochondrial supernatants and mitochondrial fractions. ACTB was used as a loading control. The immunoblots shown are representative of n≥3 independent experiments.

Figure S3. p66SHC-dependent B cell mitophagy is coupled to mitochondrial dynamics. Immunoblot analysis of OPA1, MFN1 and DNM1L in lysates of ctr and p66 cells. ACTB was used as a loading control. The histogram shows the quantification of OPA1, MFN1 and DNM1L in multiple experiments (n≥3). The data are expressed as mean±SD. ***P≤0.001; *P≤0.05 (Student's t-test).

Figure S4. Immunoblot analysis of BECN1 and PIK3C3/VPS34 in lysates from purified mitochondria of the ctr and p66 MEC transfectants, untreated or treated for 15 min with OA (n≥3). PHB was used to assess the purity of mitochondrial fractions. The histograms in the right part of the panel show the quantification of BECN1 and PIK3C3/VPS34 in multiple experiments (n≥3). The data are expressed as mean±SD. ****P≤0.0001 **P≤0.01; *P≤0.05 (one-way ANOVA).

Figure S5. Immunoblot analysis of p66SHC in lysates from purified mitochondria of the ctr and p66 MEC transfectants, untreated or treated for 4 h with OA (n≥3). Purified mitochondria were left untreated or digested with trypsin (n≥3). TOMM20 was used as a control of OMM degradation by trypsin and PHB as control of a trypsin-resistant IMM protein. The histogram shows the quantification of p66SHC in multiple experiments (n≥3). **P≤0.01 (Student's t-test).

Figure S6. Immunoblot analysis of p66SHC, PINK1 and PRKN in lysates of ctr, p66, HEK (positive control) and HeLa (negative control) cells. ACTB was used as a loading control. The histogram shows the quantification of PINK1 and PRKN in multiple experiments (n≥3).

Figure S7. Time course analysis of ctr and p66 cell treatment with Torin1 (**A**), the lysosome inhibitor "Autophagy Reagent A" (CLQ) (**B**), or OA (**C**). Samples treated with Torin1 and OA were analyzed by immunoblot using antibodies to p-MTOR and COX4I1, respectively. ACTB was used as loading control. (**D**) Viability of ctr MEC cells or the MEC transfectants expressing wild-type p66SHC (p66), the p66SHC-QQ (p66QQ) mutant, the p66SHC-SA (p66SA), or GFP-tagged wild-type p66SHC (p66GFP) or the GFP-tagged p66SHC LIR mutant (p66GFP-mLIR) at the longest time point for each treatment, measured using trypan blue exclusion. The data are expressed as mean±SD. ****P≤0.0001; ***P≤0.001; **P≤0.01; *P≤0.05 (one-way ANOVA).

Figure S8. Time course analysis of HIF1A induction in ctr MEC cells either in normoxic or hypoxic conditions. Samples were analyzed by immunoblot using antibodies to HIF1A. ACTB was used as a loading control.