Expanded View Figures

Figure EV1. Phosphorylation of TFEB S401 induced by oxidative stress. Related to Fig 1.

A Table showing mass spectrometry analysis of the abundance ratios of TFEB phosphorylated peptides in S401 from $NaAsO_2$-treated cells versus control cells.

B Immunoblot analysis of protein lysates from U2OS cells expressing TFEB-WT-FLAG treated with 150 μM $NaAsO_2$ or 250 nM Torin-1 for 1 h. Two different rabbit polyclonal antibodies raised against a TFEB-S401 phospho-specific peptide were tested.

C Quantification of the nuclear localization of recombinant TFEB in ARPE-19 cells expressing TFEB-WT-FLAG or TFEB-S401A-FLAG and treated with 250 nM Torin-1 or 250 μM $NaAsO_2$ for 1 h or EBSS for 4 h. Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, (ns) not significant as compared to the same treatment in TFEB-WT-FLAG overexpressing cells, with > 300 cells counted per trial from three independent experiments.

D Immunoblot analysis of protein lysates from ARPE-19 cells treated with 250 μM $NaAsO_2$ for 1 h were subjected to subcellular fractionation. Immunoblots are representative of at least three independent experiments.

E Relative quantitative RT–PCR analysis of the mRNA expression of lysosome- (MCOLN1 and ATP6V1C1), autophagy- (UVRAG), mitochondria- (PGC-1α), cell cycle- (CDKN1a), and heme catabolism-related (HMOX1) genes in ARPE-19 cells overexpressing either TFEB-WT-FLAG, TFEB-S401A-FLAG, or TFEB-S401D-FLAG. Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, (ns) not significant and $*P < 0.01$ from three independent experiments.

Data information: $n = 3$ biological replicates (each dot represents a biological replicate).
Source data are available online for this figure.

A

Annotated Sequence	Modifications in TFEB	$NaAsO_2$ / Control
[L]. SHSLSFGGREDEGPPGYPEPL.[A]	1xPhospho [S401(100)]	6.23
[L]. SHSLSFGGREDEGPPGYPEPL.[A]	1xPhospho [S401(100)]	5.55
[L].SFGGREDEGPPGYPEPL.[A]	1xPhospho [S401(100)]	5.80
[L].DFSHSLSF.[G]	1xPhospho [S401(100)]	6.30

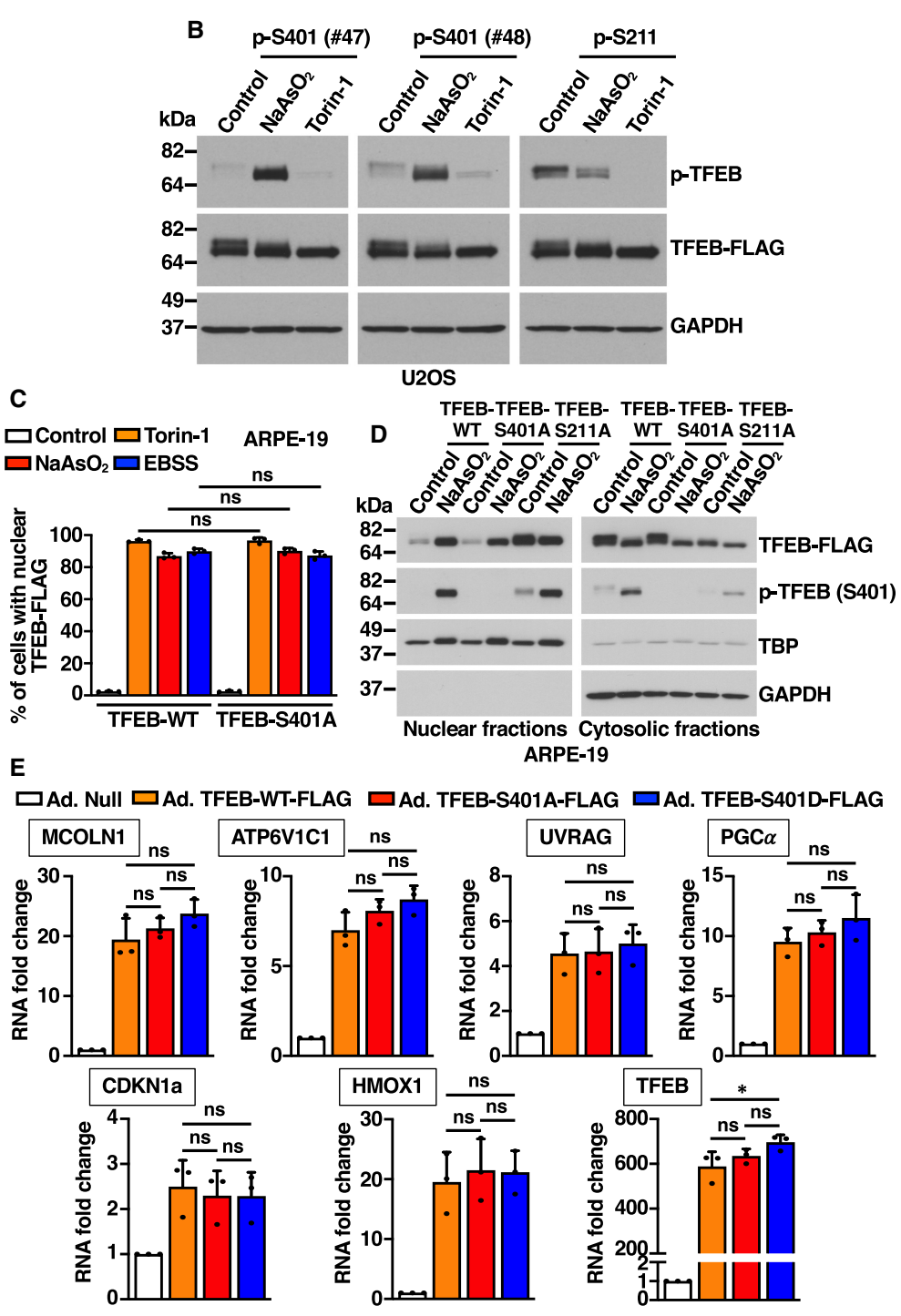

Figure EV1.

Figure EV2. Phosphorylation of TFEB S401 by p38 MAPK. Related to Fig 2.

A Immunoblot analysis of protein lysates from ARPE-19 cells expressing TFEB-WT-FLAG incubated with 15 mM NAC, 20 μM SB203580, 250 nM Torin-1 for 1 h or EBSS for 4 h prior to the addition of 250 μM $NaAsO_2$ for 1 h.

B Immunoblot analysis of protein lysates from ARPE-19 cells expressing TFEB-WT-FLAG incubated with 250 μM $NaAsO_2$ for 1 h. Then, cells were washed with HBSS and incubated in fresh medium without $NaAsO_2$ for the indicated times.

C Immunoblot analysis of protein lysates from HeLa and ARPE-19 cells.

D Immunoblot analysis of protein lysates from HeLa cells stably expressing TFEB-WT-FLAG depleted of p38 MAPK (α), p38 MAPK (β), or p38 MAPK (α + β) and incubated with 200 μM $NaAsO_2$ for 1 h or irradiated with UV-C light and recovered for 30 min. Immunoblots are representative of at least three independent experiments.

E Immunoblot analysis of protein lysates from HeLa cells stably expressing TFEB-WT-FLAG depleted of MAPKAPK2, MAPKAPK3, MSK1, and MSK2 and incubated with 200 μM $NaAsO_2$ for 1 h. Immunoblots are representative of at least three independent experiments.

F Immunoblot analysis of protein lysates from HeLa cells stably expressing TFEB-WT-FLAG incubated with the indicated kinase inhibitors for 1 h prior to the addition of 200 μM $NaAsO_2$ for 1 h.

Source data are available online for this figure.

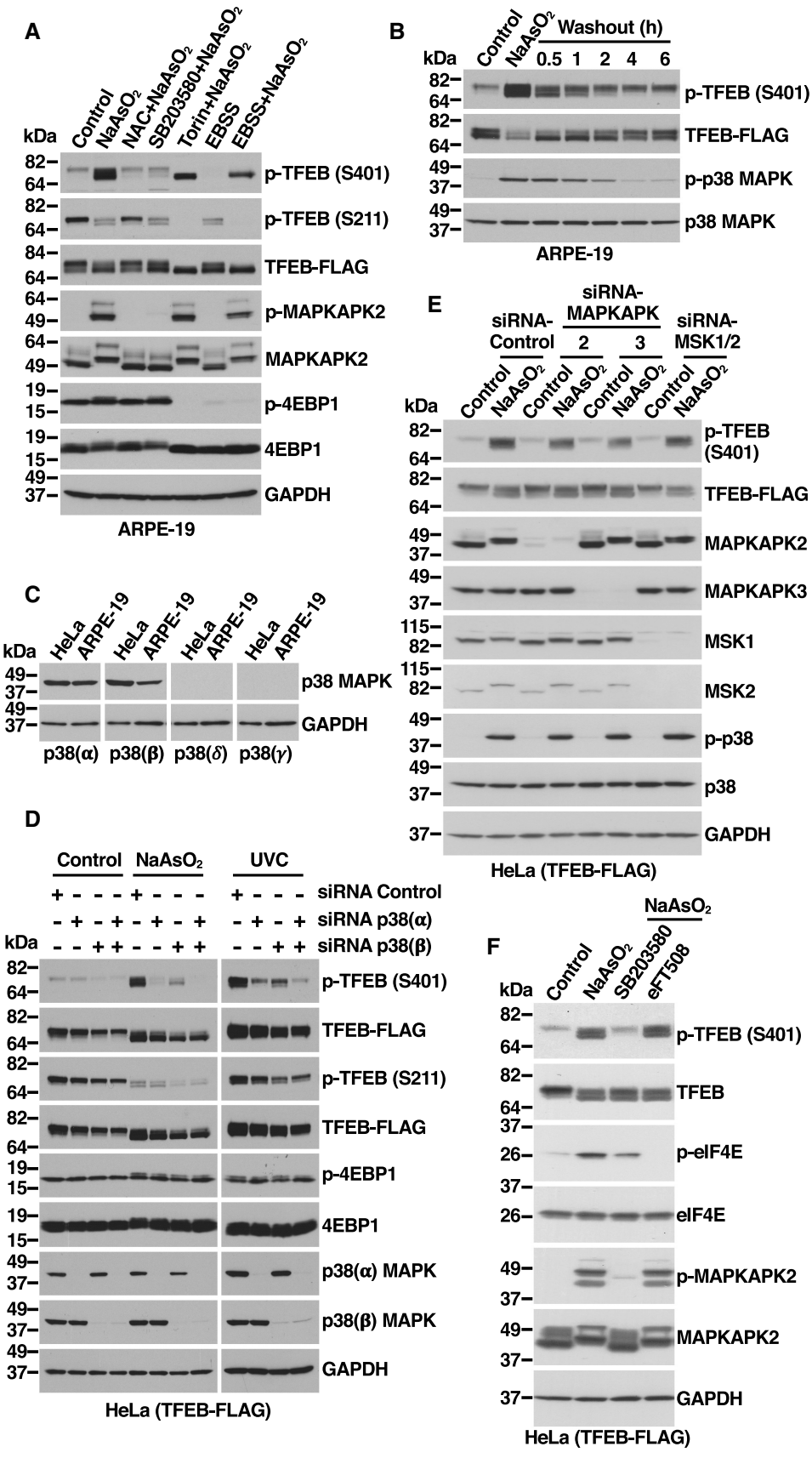
A
Control
NaAsO2
NAC+NaAsO2
SB203580+NaAsO2
Torin+NaAsO2
EBSS
EBSS+NaAsO2
kDa
p-TFEB (S401)
p-TFEB (S211)
TFEB-FLAG
p-MAPKAPK2
MAPKAPK2
p-4EBP1
4EBP1
GAPDH
ARPE-19
B
Control
NaAsO2
Washout (h)
0.5 1 2 4 6
p-TFEB (S401)
TFEB-FLAG
p-p38 MAPK
p38 MAPK
ARPE-19
C
HeLa
ARPE-19
p38 MAPK
GAPDH
p38(α) p38(β) p38(δ) p38(γ)
D
Control
NaAsO2
UVC
siRNA Control
siRNA p38(α)
siRNA p38(β)
p-TFEB (S401)
TFEB-FLAG
p-TFEB (S211)
TFEB-FLAG
p-4EBP1
4EBP1
p38(α) MAPK
p38(β) MAPK
GAPDH
HeLa (TFEB-FLAG)
E
siRNA-Control
siRNA-MAPKAPK
2
3
siRNA-MSK1/2
Control
NaAsO2
p-TFEB (S401)
TFEB-FLAG
MAPKAPK2
MAPKAPK3
MSK1
MSK2
p-p38
p38
GAPDH
HeLa (TFEB-FLAG)
F
NaAsO2
Control
NaAsO2
SB203580
eFT508
p-TFEB (S401)
TFEB
p-eIF4E
eIF4E
p-MAPKAPK2
MAPKAPK2
GAPDH
HeLa (TFEB-FLAG)

Figure EV2.

Figure EV3. TFEB-S401 phosphorylation induced by $NaAsO_2$ and LPS treatments is absent in S401A knock-in THP1 cells. Related to Fig 3.

A Flowchart indicating the different steps followed to differentiate primary human monocytes into macrophage cells.
B Immunoblot analysis of protein lysates from primary human monocytes undifferentiated (Naïve) or GM-CSF-differentiated primary macrophages. Samples are from three independent experiments.
C Immunoblot analysis of protein lysates from primary human macrophages incubated with 1 μg/ml LPS for the indicated times. Immunoblots are representative of at least four independent experiments.
D Quantification of immunoblot data shown in (C). Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Dunnett's multiple comparisons test, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ as compared to untreated cells from at least four independent experiments.
E Immunoblot analysis of protein lysates from THP1 cells WT or TFEB-S401A knock-ins (clones I11 and M17) incubated with 100 μM $NaAsO_2$ for 1 h.
F Immunoblot analysis of protein lysates from PMA-differentiated THP1 cells WT or TFEB-S401A knock-ins (clones I11 and M17) incubated with 1 μg/ml LPS for the indicated times. Immunoblots are representative of at least three independent experiments.

Data information: $n \geq 3$ biological replicates (each dot represents a biological replicate).
Source data are available online for this figure.

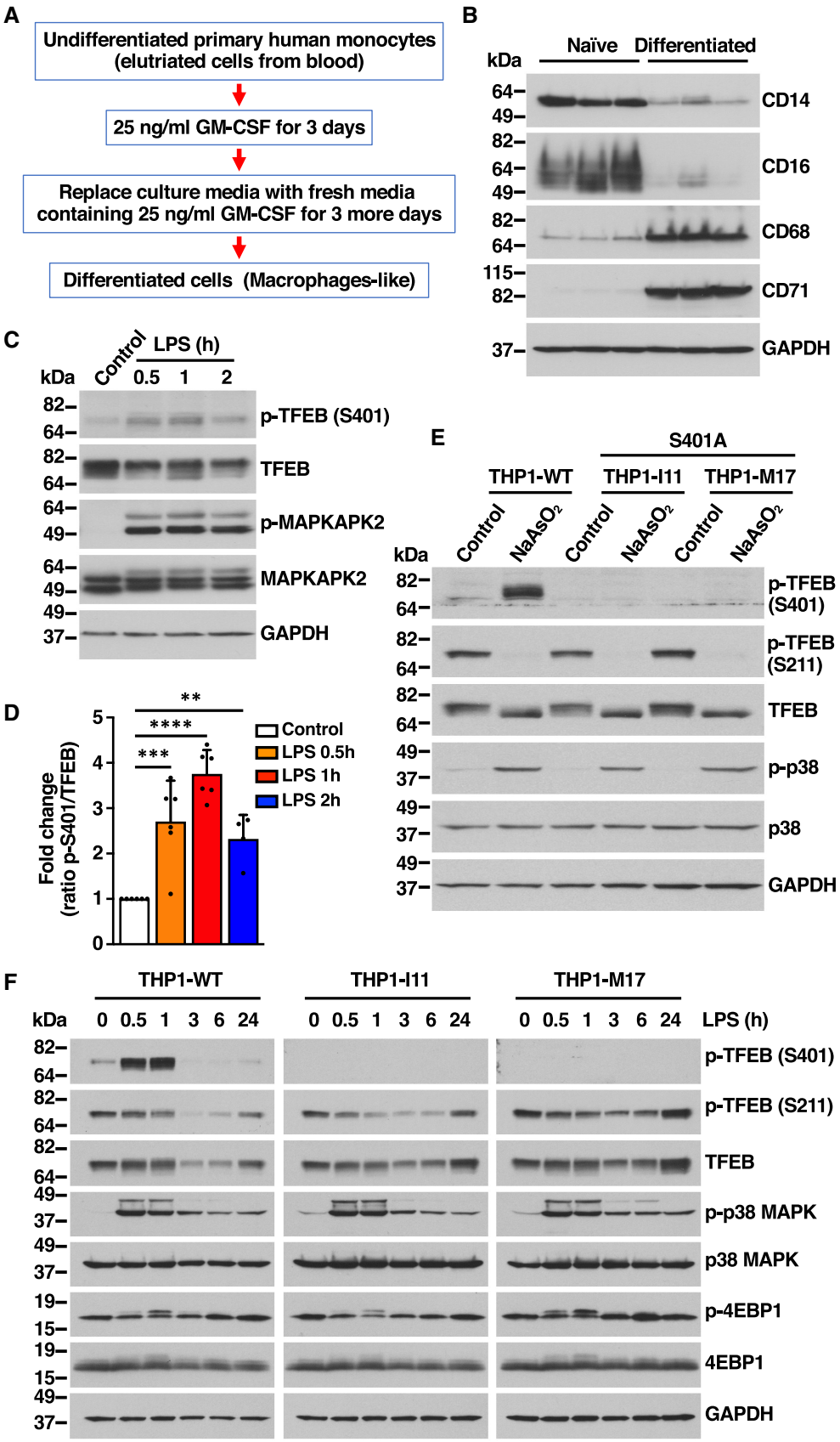
A
Undifferentiated primary human monocytes (elutriated cells from blood)
25 ng/ml GM-CSF for 3 days
Replace culture media with fresh media containing 25 ng/ml GM-CSF for 3 more days
Differentiated cells (Macrophages-like)
B
Naïve
Differentiated
kDa
CD14
CD16
CD68
CD71
GAPDH
C
Control
LPS (h)
0.5
1
2
p-TFEB (S401)
TFEB
p-MAPKAPK2
MAPKAPK2
GAPDH
D
Fold change (ratio p-S401/TFEB)
Control
LPS 0.5h
LPS 1h
LPS 2h
E
S401A
THP1-WT
THP1-I11
THP1-M17
NaAsO2
p-TFEB (S401)
p-TFEB (S211)
TFEB
p-p38
p38
GAPDH
F
THP1-WT
THP1-I11
THP1-M17
0 0.5 1 3 6 24
LPS (h)
p-TFEB (S401)
p-TFEB (S211)
TFEB
p-p38 MAPK
p38 MAPK
p-4EBP1
4EBP1
GAPDH

Figure EV3.

Figure EV4. PMA-dependent activation of TFEB in THP1 cells. Related to Fig 4.

A Immunoblot analysis of protein lysates from naïve THP1-WT or TFEB-S401A knock-in (clone I11) cells treated with 50 ng/ml PMA for the indicated times, and PMA-differentiated THP1 (Rested) cells.

B Immunoblot analysis of protein lysates from naïve THP1-WT cells treated with 50 ng/ml PMA for 30 min and with 100 μM $NaAsO_2$ for 1 h.

C Quantification of immunoblot data shown in (B). Data are presented as mean ± SD using unpaired Student's *t*-test, ****$P < 0.0001$ from three independent experiments.

D Immunoblot analysis of protein lysates from naïve THP1-WT cells treated with 5 μM CRT0066101(CRT, PKD inhibitor) or 5 μM Bisindolylmaleimide IV (BIS, PKC inhibitor) for 1 h prior to the addition of 50 ng/ml PMA for 1 h.

E Immunoblot analysis of proteins from nuclear and cytosolic fractions from naïve THP1-WT treated with 5 μM CRT0066101(CRT, PKD inhibitor) or 5 μM Bisindolylmaleimide IV (BIS, PKC inhibitor) for 1 h prior to the addition of 50 ng/ml PMA for 1 h.

F Quantification of the nuclear localization of recombinant TFEB in HeLa cells expressing TFEB-WT-FLAG treated with 5 μM CRT0066101(CRT, PKD inhibitor) or 5 μM Bisindolylmaleimide IV (BIS, PKC inhibitor) for 1 h prior to the addition of 500 ng/ml PMA for 45 min. Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, ***$P < 0.001$ as compared to PMA-treated cells, with > 200 cells counted per trial from three independent experiments.

G Immunoblot analysis of proteins from nuclear and cytosolic fractions from naïve THP1-WT or TFEB-S401A knock-in (clone I11) cells nutrients starved with EBSS for 12 h. The antibody directed against the central region of TFEB was obtained from Cell Signaling Technology (CST).

H Relative quantitative RT–PCR analysis of the mRNA expression of lysosome- (MCOLN1, ATP6V1C1, CTSD, LAMP1, HEXA, RAGC) and autophagy-related (GABARAPL1) genes in naïve THP1-WT or TFEB-S401A knock-in (clone I11) cells nutrients starved with EBSS for 12 h. Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, *$P < 0.05$ and **$P < 0.01$ from three independent experiments.

I Immunoblot analysis of proteins from nuclear and cytosolic fractions from naïve THP1-WT or TFEB-S401A knock-in (clone I11) cells or PMA-differentiated THP1 (Rested) cells treated with either 50 ng/ml PMA or 250 nM Torin-1 for 1 h.

Data information: $n = 3$ biological replicates (each dot represents a biological replicate).
Source data are available online for this figure.

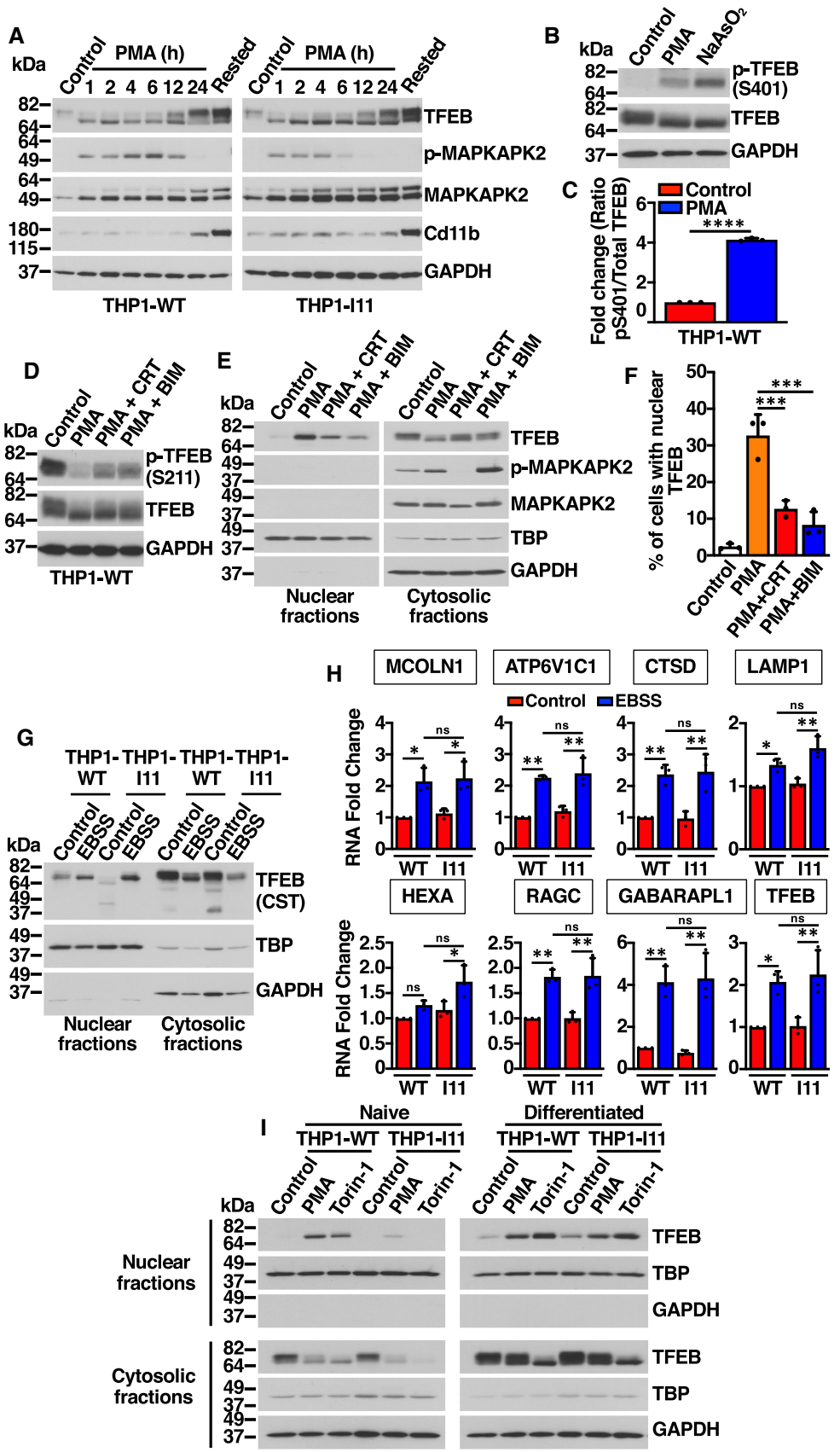
A
kDa
Control
PMA (h)
1 2 4 6 12 24
Rested
TFEB
p-MAPKAPK2
MAPKAPK2
Cd11b
GAPDH
THP1-WT
THP1-I11
B
Control
PMA
NaAsO2
p-TFEB (S401)
TFEB
GAPDH
C
Control
PMA
Fold change (Ratio pS401/Total TFEB)
****
THP1-WT
D
Control
PMA
PMA + CRT
PMA + BIM
p-TFEB (S211)
TFEB
GAPDH
THP1-WT
E
Nuclear fractions
Cytosolic fractions
TBP
F
% of cells with nuclear TFEB
***
Control
PMA
PMA+CRT
PMA+BIM
G
THP1-WT
THP1-I11
Control
EBSS
TFEB (CST)
TBP
GAPDH
H
MCOLN1
ATP6V1C1
CTSD
LAMP1
HEXA
RAGC
GABARAPL1
TFEB
RNA Fold Change
WT
I11
I
Naive
Differentiated
Torin-1
Nuclear fractions
Cytosolic fractions

Figure EV4.

Figure EV5. Macrophage polarization is affected in THP1 TFEB-S401A mutant cells in response to LPS. Related to Fig 6.

A Immunoblot analysis of protein lysates from PMA-differentiated (Rested) THP1-WT or TFEB-S401A knock-ins (clones I11 and M17) cells incubated with 1 μg/ml LPS for the indicated times.

B Quantification of immunoblot data shown in (A). Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, $*P < 0.05$ and $***P < 0.001$ as compared to the same treatment condition in THP1-WT cells from three independent experiments.

C Immunoblot analysis of proteins from nuclear and cytosolic fractions from PMA-differentiated (Rested) THP1-WT or TFEB-S401A knock-in (clone I11) cells incubated with 1 μg/ml LPS for the indicated times or 250 nM Torin-1 for 1 h.

D Quantification of immunoblot data shown in (C). Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, (ns) not significant as compared to the same treatment condition in THP1-WT cells from three independent experiments.

E Immunofluorescence confocal microscopy of PMA-differentiated (Rested) THP1-WT or TFEB-S401A knock-in (clones I11 and M17) cells incubated with 0.1 μg/ml LPS for 4 h prior to the addition of 15 μM Nigericin for 45 min. Arrows point to the position of ASC specks indicative of inflammasome activation. Scale bars, 10 μm.

F Quantification of the percentage of cells with ASC specks shown in (E). Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, $***P < 0.001$ and $****P < 0.0001$ as compared to THP1-WT cells treated with LPS + Nigericin with > 200 cells counted per trial from three independent experiments.

Data information: $n \geq 3$ biological replicates (each dot represents a biological replicate).
Source data are available online for this figure.

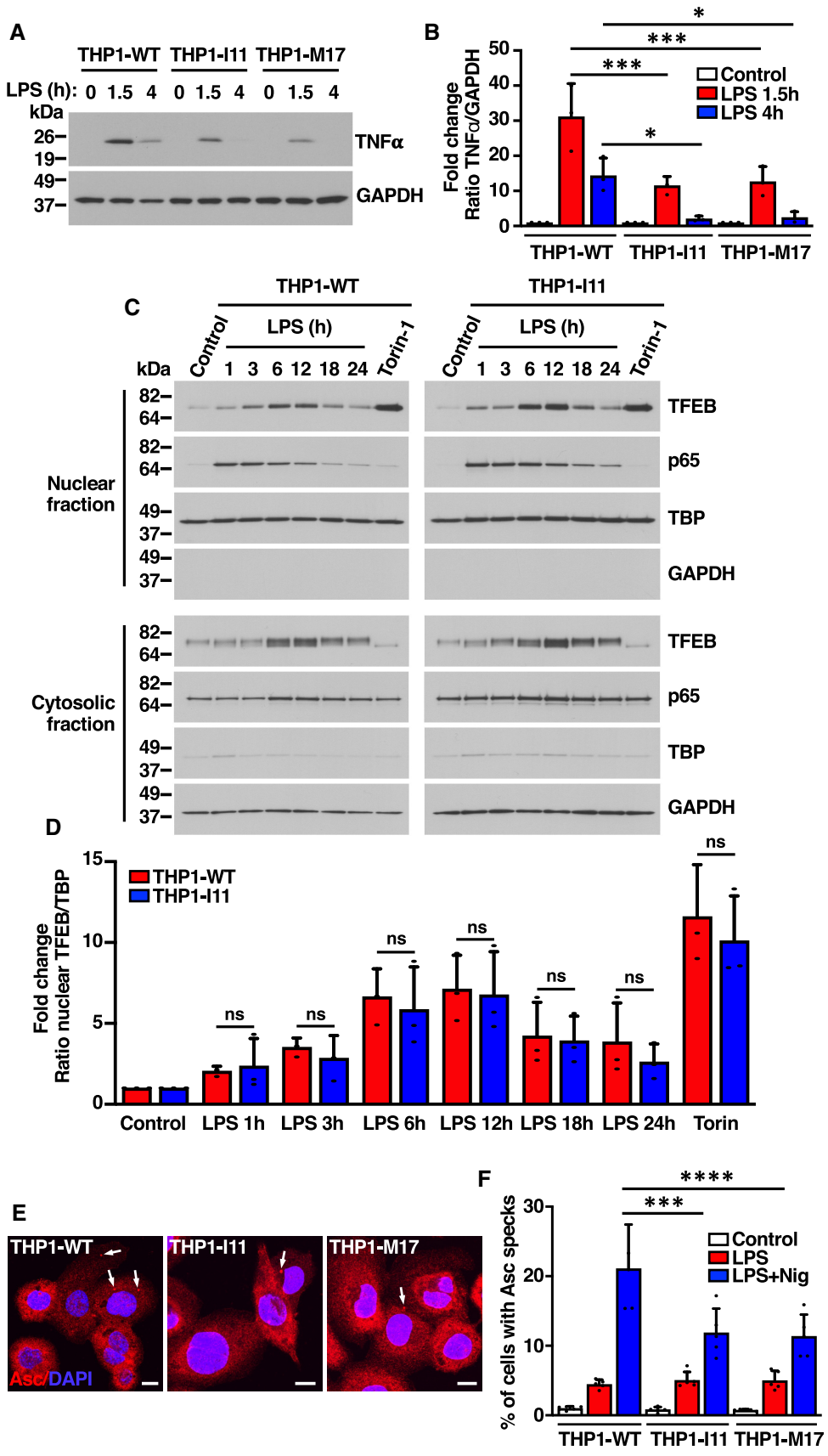
A
THP1-WT THP1-I11 THP1-M17
LPS (h): 0 1.5 4 0 1.5 4 0 1.5 4
kDa
26–
19–
49–
37–
TNFα
GAPDH
B
Fold change Ratio TNFα/GAPDH
50
40
30
20
10
0
*
***
***
*
Control
LPS 1.5h
LPS 4h
THP1-WT THP1-I11 THP1-M17
C
THP1-WT
THP1-I11
Control
LPS (h)
Torin-1
kDa
1 3 6 12 18 24
82–
64–
49–
37–
Nuclear fraction
Cytosolic fraction
TFEB
p65
TBP
GAPDH
D
Fold change Ratio nuclear TFEB/TBP
15
10
5
0
THP1-WT
THP1-I11
ns
Control LPS 1h LPS 3h LPS 6h LPS 12h LPS 18h LPS 24h Torin
E
THP1-WT
THP1-I11
THP1-M17
Asc/DAPI
F
% of cells with Asc specks
30
20
10
0
****
***
Control
LPS
LPS+Nig
THP1-WT THP1-I11 THP1-M17

Figure EV5.