Appendix:

p38 MAPK-dependent phosphorylation of TFEB promotes monocyte to macrophage differentiation

José A. Martina, Eutteum Jeong and Rosa Puertollano
Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.

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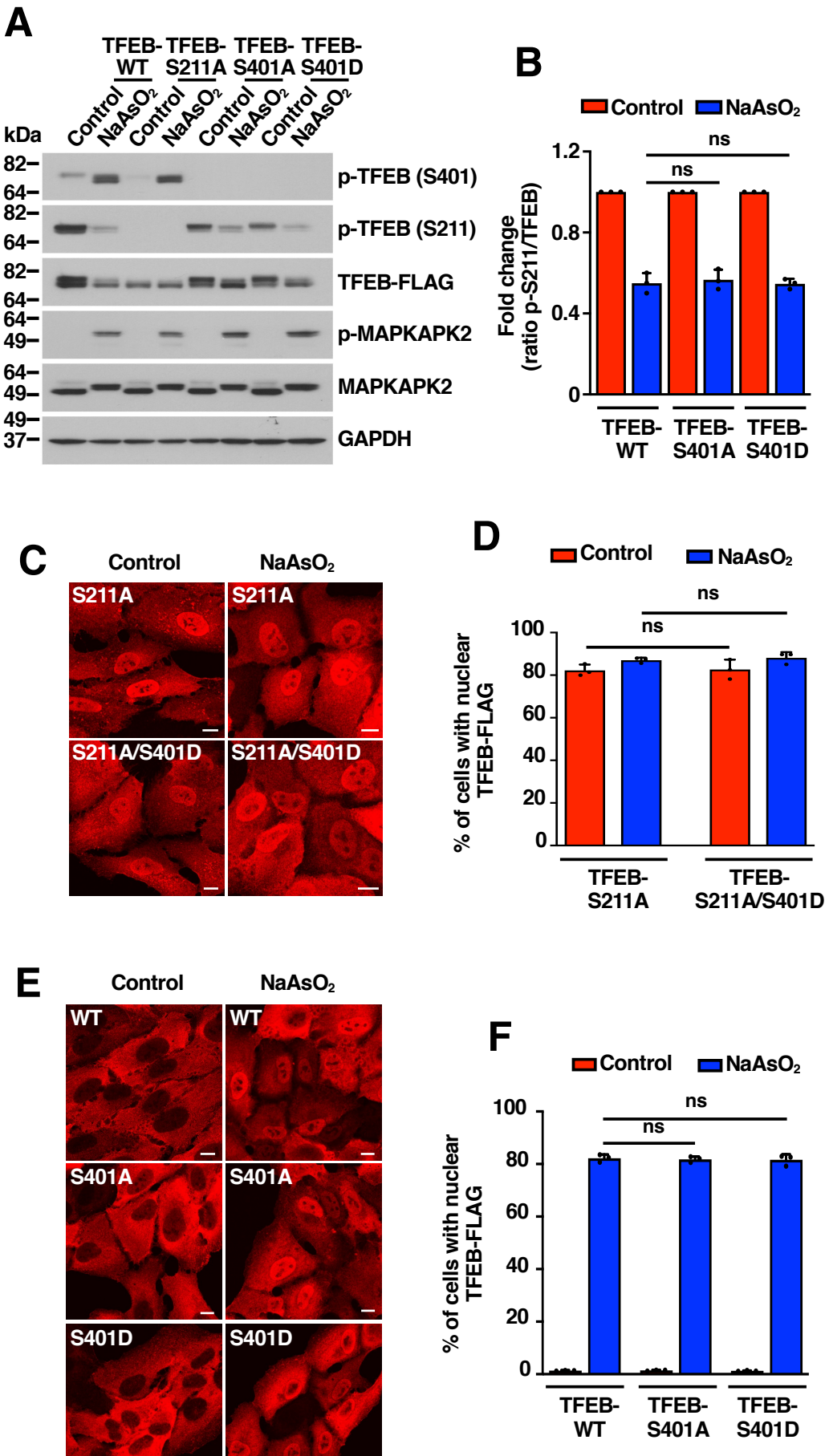

A
TFEB-WT
TFEB-S211A
TFEB-S401A
TFEB-S401D
Control
NaAsO2
kDa
82–
64–
p-TFEB (S401)
p-TFEB (S211)
TFEB-FLAG
p-MAPKAPK2
MAPKAPK2
GAPDH
49–
37–
B
Control
NaAsO2
ns
Fold change (ratio p-S211/TFEB)
1.2
0.8
0.4
0
TFEB-WT
TFEB-S401A
TFEB-S401D
C
Control
NaAsO2
S211A
S211A/S401D
D
% of cells with nuclear TFEB-FLAG
100
80
60
40
20
0
TFEB-S211A
TFEB-S211A/S401D
E
WT
S401A
S401D
F

Appendix Figure S1. Subcellular distribution of TFEB is not affected in S401 mutants upon $NaAsO_2$ treatment.

A. Immunoblot analysis of protein lysates from ARPE-19 cells expressing TFEB-WT-FLAG, TFEB-S211A-FLAG, TFEB-S401A-FLAG or TFEB-S401D-FLAG treated with 200 μM $NaAsO_2$ for 1 h.

B. Quantification of immunoblot data shown in (A). Data are presented as mean ± SD using one-way ANOVA, (ns) not significant from three independent experiments.

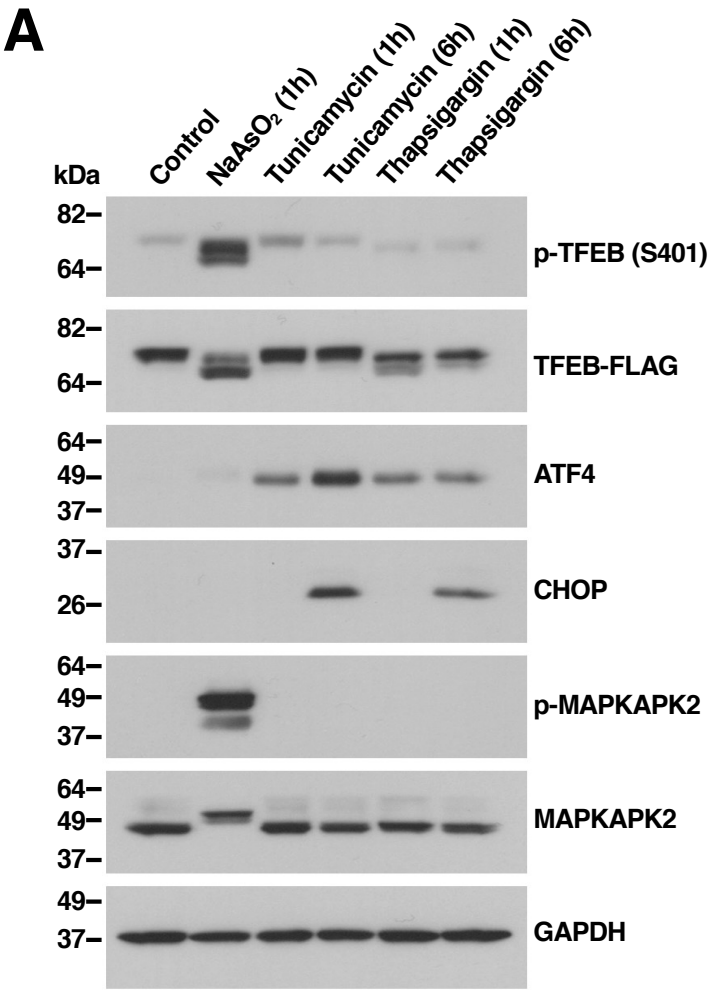
C. Immunofluorescence confocal microscopy of ARPE-19 cells overexpressing TFEB-S211A-FLAG and TFEB-S211A/S401D-FLAG showing the subcellular distribution of recombinant TFEB in response to treatments with 200 μM $NaAsO_2$ for 1 h. Scale bars, 10 μm.

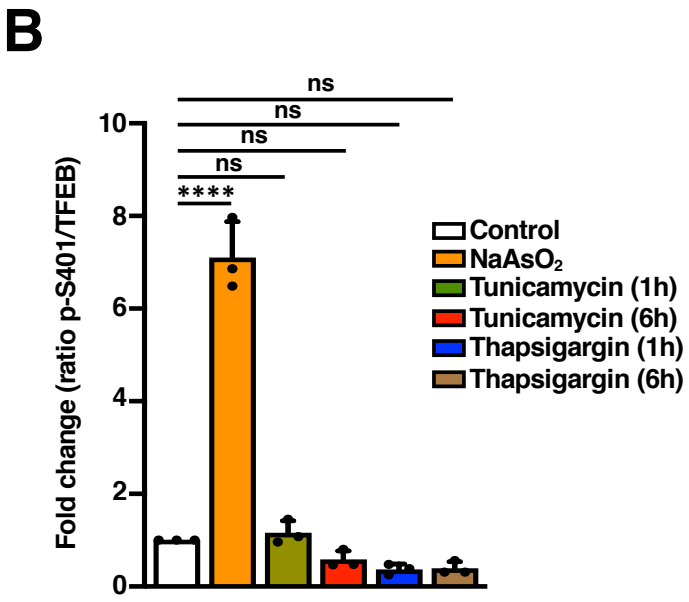
D. Quantification of the nuclear localization of recombinant TFEB in ARPE-19 cells shown in (C). Data are presented as mean ± SD using one-way ANOVA, (ns) not significant as compared to the same treatment in TFEB-S211A-FLAG overexpressing cells, with >150 cells counted per trial from three independent experiments.

E. Immunofluorescence confocal microscopy of ARPE-19 cells overexpressing TFEB-WT-FLAG, TFEB-S401A-FLAG or TFEB-S401D-FLAG showing the subcellular distribution of recombinant TFEB in response to treatments with 200 μM $NaAsO_2$ for 1 h. Scale bars, 10 μm.

F. Quantification of the nuclear localization of recombinant TFEB in ARPE-19 cells shown in (E). Data are presented as mean ± SD using one-way ANOVA, (ns) not significant as compared to the same treatment in TFEB-WT-FLAG overexpressing cells, with >200 cells counted per trial from three independent experiments.

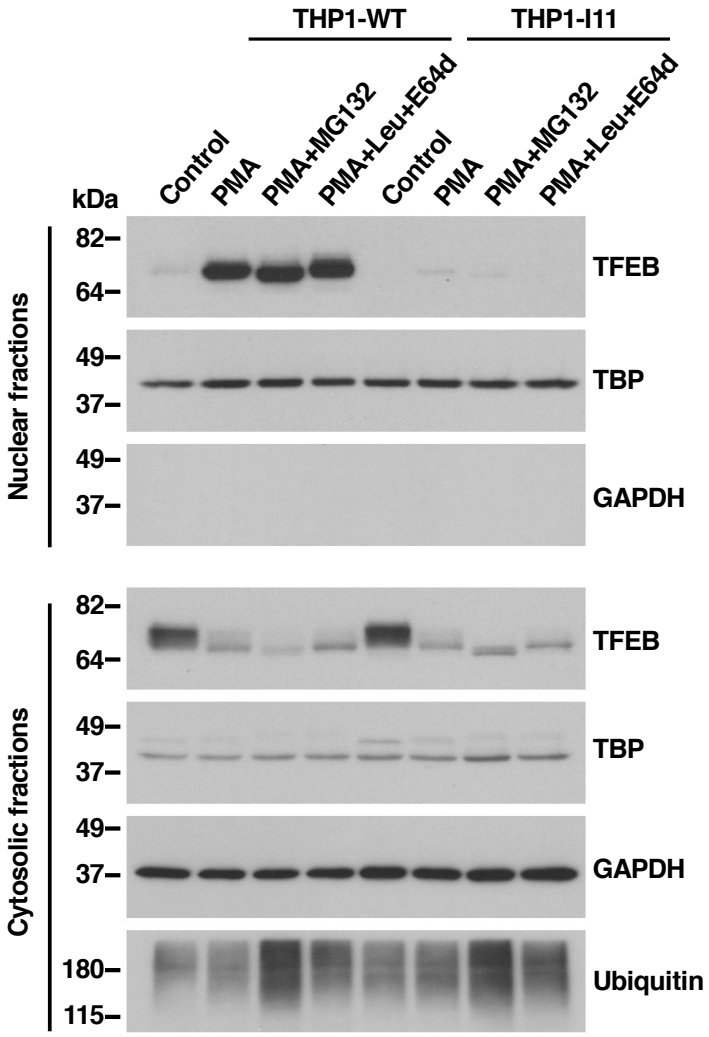
Data information: n = 3 biological replicates (each dot represents a biological replicate).





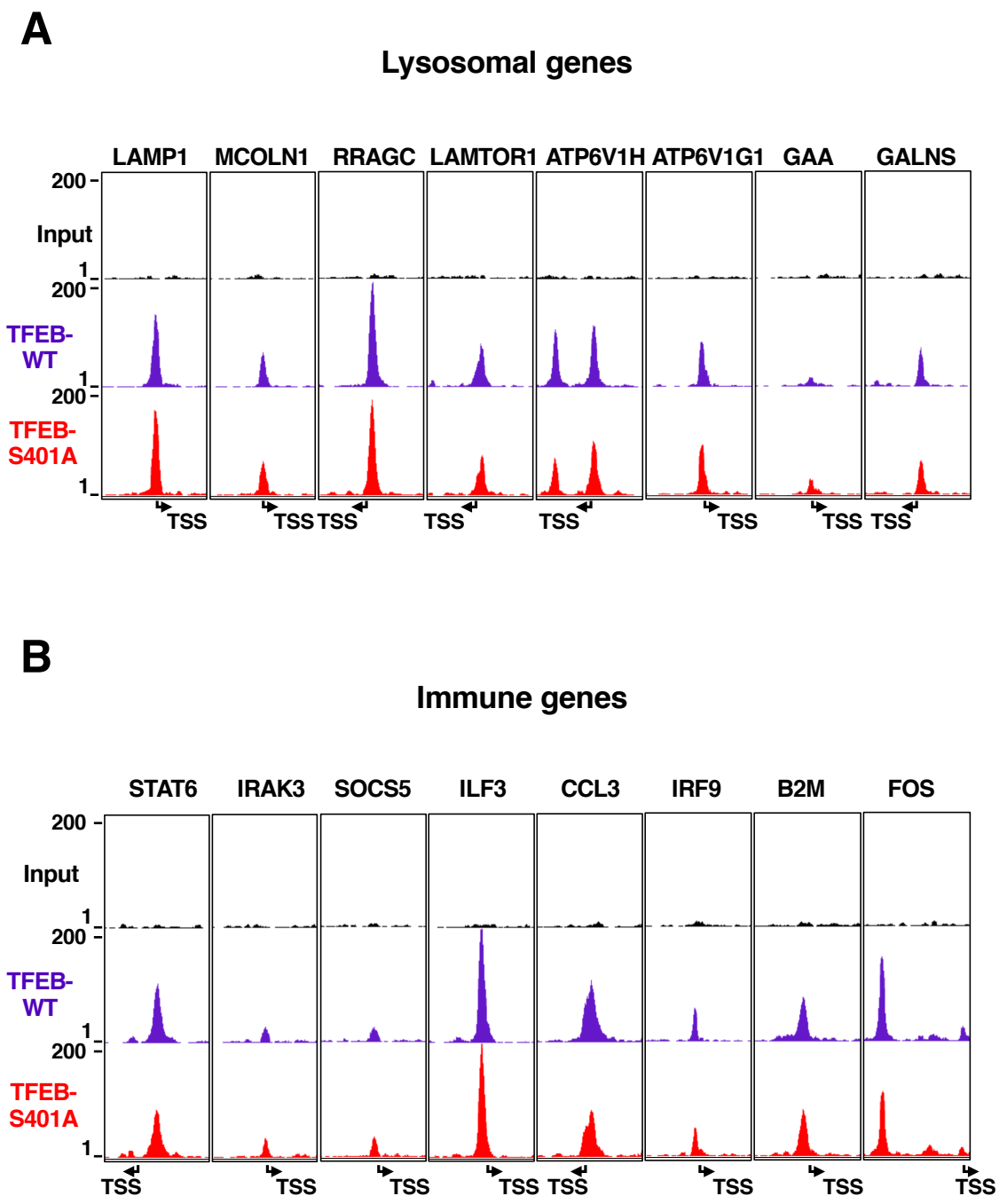
Appendix Figure S2. ER stress is not responsible for TFEB serine 401 phosphorylation upon $NaAsO_2$ treatment.

A. Immunoblot analysis of protein lysates from HeLa cells stably expressing TFEB-WT-FLAG incubated with either 200 µM $NaAsO_2$ for 1 h or 5 µg/ml Tunicamycin or 100 nM Thapsigargin for 1h and 6h.
B. Quantification of immunoblot data shown in (A). Data are presented as mean ± SD using one-way ANOVA, (ns) not significant, and (****)p<0.0001 from three independent experiments (each dot represents a biological replicate).



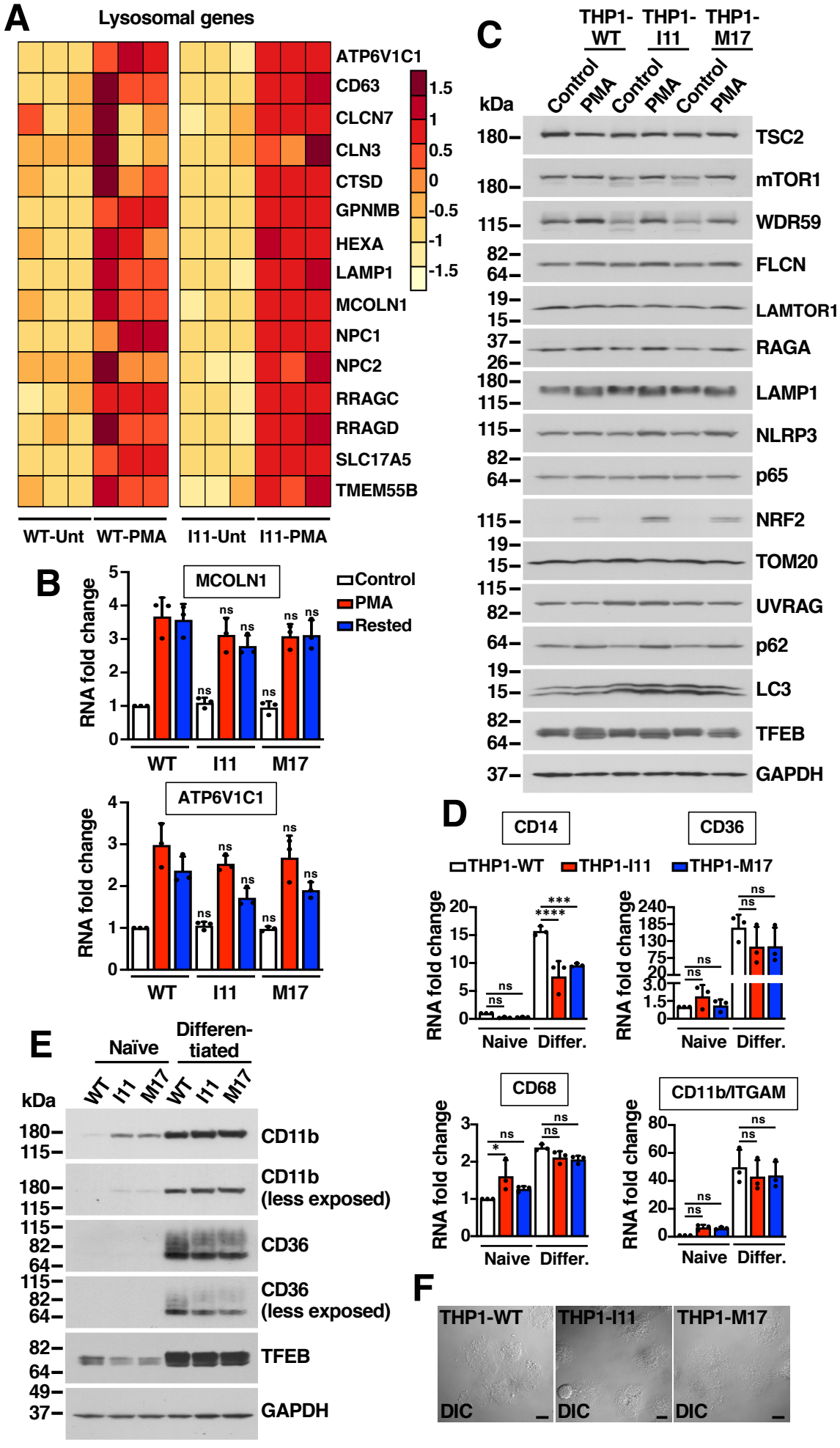
Appendix Figure S3. Decreased nuclear accumulation of TFEB-S401A is not caused by proteasomal or lysosomal degradation.

Immunoblot analysis of proteins from nuclear and cytosolic fractions from naïve THP1-WT or TFEB-S401A knock-in (clone I11) cells incubated with either 10 μM GM132 or 100 μg/ml Leupeptin (Leu) and 10 μM E64d for 3 h prior to the addition of 50 ng/ml PMA for 1 h.



Appendix Figure S4. TFEB-WT and TFEB-S401A bind to the promoter of lysosomal and immune genes with similar affinity.

ChIP-seq analysis of lysosomal (A) and immune genes (B) from THP1-WT and TFEB-S401A knock-in (clone I11) cells treated with 50 ng/ml PMA for 12 h. Arrows indicate the transcription start site (TSS) for each gene analyzed.


A
Lysosomal genes
ATP6V1C1
CD63
CLCN7
CLN3
CTSD
GPNMB
HEXA
LAMP1
MCOLN1
NPC1
NPC2
RRAGC
RRAGD
SLC17A5
TMEM55B
1.5
1
0.5
0
-0.5
-1
-1.5
WT-Unt
WT-PMA
I11-Unt
I11-PMA
B
MCOLN1
RNA fold change
Control
PMA
Rested
WT
I11
M17
ATP6V1C1
C
THP1-WT
THP1-I11
THP1-M17
Control
PMA
kDa
TSC2
mTOR1
WDR59
FLCN
LAMTOR1
RAGA
LAMP1
NLRP3
p65
NRF2
TOM20
UVRAG
p62
LC3
TFEB
GAPDH
D
CD14
CD36
CD68
CD11b/ITGAM
THP1-WT
THP1-I11
THP1-M17
RNA fold change
Naive
Differ.
E
Naïve
Differen-tiated
WT
I11
M17
CD11b
CD11b (less exposed)
CD36
CD36 (less exposed)
TFEB
GAPDH
F
THP1-WT
THP1-I11
THP1-M17
DIC

Appendix Figure S5. PMA-dependent phosphorylation of TFEB-S401 does not result in lysosomal biogenesis in THP1 cells.

A. Heat map of 15 known TFEB lysosomal target genes from RNA-Seq analysis of naïve THP1-WT cells (Control, WT-Unt) or naïve TFEB-S401A knock-in (Control, I11-Unt) cells versus the corresponding cells incubated with 50 ng/ml PMA for 6 h (WT-PMA or I11-PMA).

B. Relative quantitative RT-PCR analysis of the mRNA expression of TFEB lysosomal target genes (MCOLN1 and ATP6V1C1) in naïve THP1-WT or TFEB-S401A knock-ins (clones I11 and M17) cells incubated with 50 ng/ml PMA for 24 h as well as PMA-differentiated cells (Rested). Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, (ns) not significant from three independent experiments.

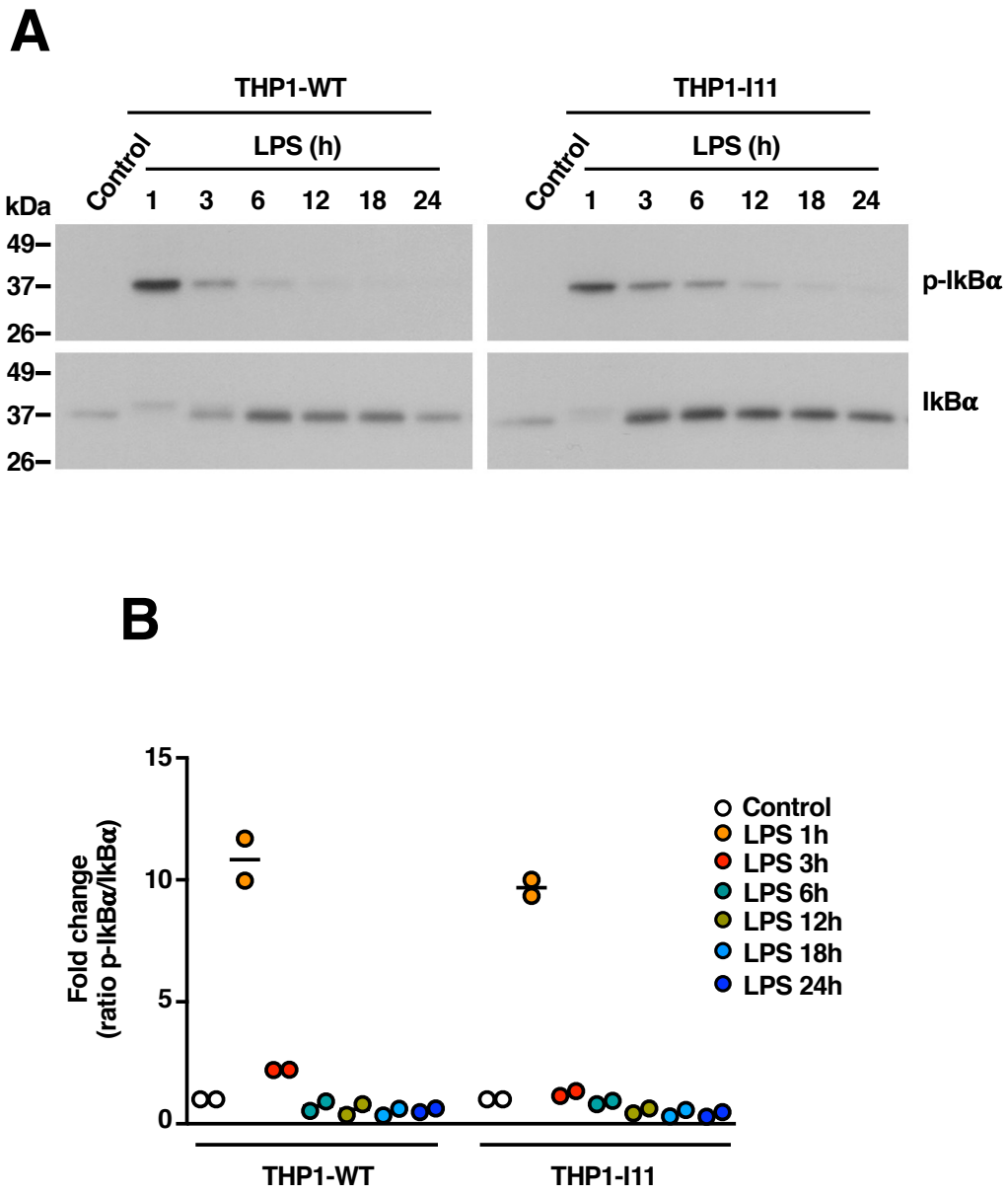
C. Immunoblot analysis of protein lysates from naïve THP1-WT or TFEB-S401A knock-ins (clones I11 and M17) cells treated with 50 ng/ml PMA for 24 h.

D. Relative quantitative RT-PCR analysis of the mRNA expression of macrophage surface marker genes in naïve THP1-WT or TFEB-S401A knock-ins (clones I11 and M17) as well as in PMA-differentiated cells (Differ., Rested). Data are presented as mean ± SD using one-way ANOVA (unpaired) followed by Tukey's multiple comparisons test, (ns) not significant, (*)$p<0.05$, (***)$p< 0.001$ and (****)$p< 0.0001$ from three independent experiments.

E. Immunoblot analysis of protein lysates from naïve THP1-WT or TFEB-S401A knock-ins (clones I11 and M17) cells as well as PMA-differentiated cells. Immunoblots are representative of at least three independent experiments.

F. Differential interference contrast microscopy (DIC) of PMA-differentiated (Rested) THP1-WT or TFEB-S401A knock-ins (clones I11 and M17) cells. Scale bars, 10 μm.

Data information: $n = 3$ biological replicates (each dot represents a biological replicate).



Appendix Figure S6. Normal activation of NF-KB **pathway in THP1 S401A mutant cells.**

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

B. Quantification of immunoblot data shown in (A). Data are presented as mean, and dots represent individual biological replicates.

Appendix Table S1: Top 10 most significant differentially expressed gene sets between PMA-treated THP1-WT and PMA-treated THP1-I11 cells (MSigDB Hallmark 2020)

Term	p-value	q-value	Overlap genes
TNF-alpha Signaling via NF-kB	2.0E-26	9.6E-25	[CDKN1A, BTG1, SDC4, TNFAIP6, RNF19B, CXCL1, SLC2A3, CXCL3, AREG, TNF, CXCL2, ICAM1, DRAM1, NFIL3, CCND1, CCL5, CCRL2, PHLDA1, IER2, IER3, TGIF1, EGR1, DUSP2, JAG1, DUSP1, IFNGR2, PLAUR, FOS, TRAF1, ATP2B1, NFKBIA, YRDC, SPSB1, BCL6, IL1B, BCL3, PTX3, FJX1, CD44, HBEGF]
Inflammatory Response	2.3E-17	5.5E-16	[CDKN1A, CALCRL, CXCL8, TNFAIP6, PTGER2, AQP9, AHR, ICAM1, RGS1, CCL5, PDPN, CCRL2, CLEC5A, CD55, MSR1, IFNGR2, PLAUR, ATP2B1, ACVR2A, EREG, NFKBIA, MMP14, P2RX4, ADORA2B, IL1B, LTA, CD48, ITGA5, MET, CHST2, HBEGF]
IL-2/STAT5 Signaling	8.0E-14	1.3E-12	[ECM1, PTGER2, GPR65, SLC2A3, FURIN, AHR, ALCAM, PNP, SOCS1, NFIL3, SPP1, PHLDA1, EOMES, PRNP, SPRY4, EMP1, TRAF1, DHRS3, HOPX, CKAP4, ARL4A, CDCP1, P2RX4, TLR7, CD48, CD44, TNFRSF21]
Epithelial Mesenchymal Transition	5.7E-09	6.9E-08	[OXTR, ECM1, CXCL8, SERPINE2, SDC4, LAMA2, PRRX1, IGFBP3, TFPI2, PLAUR, CXCL1, AREG, FGF2, MMP14, COL4A2, DPYSL3, SPP1, PTX3, ITGA5, FERMT2, CD44]
p53 Pathway	7.2E-07	6.9E-06	[CDKN1A, BTG1, PDGFA, RNF19B, FOS, TM4SF1, ZFP36L1, RAP2B, SOCS1, DRAM1, DDIT3, PLXNB2, STOM, TRIB3, UPP1, IER3, S100A10, HBEGF]
Hypoxia	3.2E-06	2.6E-05	[CDKN1A, BTG1, SDC4, ANXA2, DUSP1, TGFB3, IGFBP3, PLAUR, SLC2A3, ENO1, FOS, EXT1, NFIL3, ADORA2B, DDIT3, CHST2, IER3]
Allograft Rejection	5.3E-05	3.6E-04	[IFNGR2, GPR65, GCNT1, TNF, ACVR2A, ICAM1, EREG, SOCS1, IL1B, CCL5, BCL3, EIF3J, ST8SIA4, APBB1, CCR5]
Apoptosis	7.8E-05	4.7E-04	[CDKN1A, ANXA1, EMP1, TNF, EREG, KRT18, CCND1, ANKH, DDIT3, IL1B, LMNA, CD44, IER3]
IL-6/JAK/STAT3 Signaling	1.5E-04	8.2E-04	[SOCS1, IFNGR2, IL1B, CXCL1, CXCL3, A2M, TNF, TNFRSF21, CD44]
KRAS Signaling Up	1.9E-04	9.2E-04	[PRRX1, IGFBP3, PLAUR, EMP1, TRAF1, GNG11, EREG, HSD11B1, YRDC, ANKH, IL1B, SPP1, PLEK2, HBEGF]

Appendix Table S2: primary and secondary antibodies

Target	Company; Catalogue Number	Dilution
4E-BP1	Cell Signaling Technology; 9644	1:5000 (WB)
ATF4	Cell Signaling Technology; 11815	1:1000 (WB)
ASC	AdipoGen; AG-25B-0006	1:2000 (IF)
c-JUN	Cell Signaling Technology; 9165	1:1000 (WB)
CD11b/ITGAM	Cell Signaling Technology; 49420	1:1000 (WB)
CD14	Cell Signaling Technology; 56082	1:1000 (WB)
CD16	Cell Signaling Technology; 80006	1:1000 (WB)
CD36	Cell Signaling Technology; 14347	1:1000 (WB)
CD68	Cell Signaling Technology; 86985	1:1000 (WB)
CD71	Cell Signaling Technology; 13113	1:1000 (WB)
CHOP	Cell Signaling Technology; 2895	1:1000 (WB)
CLEAVED GASDERMIN D	Cell Signaling Technology; 36425	1:500 (WB)
eIF4E	Cell Signaling Technology; 2067	1:1000 (WB)
FLAG	Sigma-Aldrich; F1804	1:50000 (WB), 1:6000 (IF)
FLCN	Cell Signaling Technology; 3697	1:1000 (WB)
GAPDH	Thermo-Fisher; AM4300	1:50000 (WB)
GASDERMIN D	Cell Signaling Technology; 39754	1:500 (WB)
IκBα	Cell Signaling Technology; 4814	1:1000 (WB)
IL18	Cell Signaling Technology; 54943	1:500 (WB)
IL1β	GeneTex; GTX130021	1:2000 (WB)
JNK1	Cell Signaling Technology; 3708	1:1000 (WB)
JNK2	Cell Signaling Technology; 9258	1:1000 (WB)
LAMP-1	DSHB, University of Iowa; H4A3	1:3000 (WB)
LAMTOR1	Sigma-Aldrich; HPA002997	1:1000 (WB)
LC3	Sigma-Aldrich; L7543	1:2000 (WB)
MAPKAPK-2	Cell Signaling Technology; 12155	1:5000 (WB)
MAPKAPK-3	Cell Signaling Technology; 7421	1:500 (WB)
MSK1	Bethyl Laboratories; A302-747A	1:1000 (WB)
MSK2	Bethyl Laboratories; A302-746A	1:500 (WB)
mTOR	Cell Signaling Technology; 2983	1:1000 (WB)
NF-κB p65	Cell Signaling Technology; 8242	1:5000 (WB)
NLRP3	Cell Signaling Technology; 15101	1:1000 (WB)
NRF2	Cell Signaling Technology; 12721	1:250 (WB)
p38 MAPK	Cell Signaling Technology; 9212	1:2000 (WB)
p38α MAPK	Cell Signaling Technology; 9218	1:1000 (WB)
p38β MAPK	Cell Signaling Technology; 2339	1:1000 (WB)
p38γ MAPK	Cell Signaling Technology; 2307	1:1000 (WB)
p38δ MAPK	Cell Signaling Technology; 2308	1:1000 (WB)
p44/42 MAPK (Erk1/2)	Cell Signaling Technology; 9102	1:2000 (WB)
Phospho-4E-BP1 (Ser65)	Cell Signaling Technology; 9451	1:500 (WB)
Phospho-c-JUN (Ser73)	Cell Signaling Technology; 3270	1:1000 (WB)
Phospho-eIF4E (Ser209)	Cell Signaling Technology; 9741	1:500 (WB)
Phospho-IκBα (Ser32)	Cell Signaling Technology; 2859	1:500 (WB)
Phospho-MAPKAPK-2 (Thr334)	Cell Signaling Technology; 3007	1:1000 (WB)
Phospho-p38 MAPK (Thr180/Tyr182)	Cell Signaling Technology; 9211	1:2000 (WB)
Phospho-p44/42 MAPK (Thr202/Tyr204)	Cell Signaling Technology; 4377	1:2000 (WB)
Phospho-TFEB (Ser211)	Cell Signaling Technology; 37681	1:500 (WB)
RagA	Cell Signaling Technology; 4357	1:1000 (WB)
SQSTM1/p62	Abcam; ab155686	1:3000 (WB)
TBP	Cell Signaling Technology; 44059	1:5000 (WB)
TFE3	Sigma-Aldrich; HPA023881	1:2000 (WB)
TFEB	Cell Signaling Technology; 4240	1:3000 (WB)
TFEB	Cell Signaling Technology; 37785	2-4ug (ChIP)
TFEB	Bethyl Laboratories; A303-673A	1:3000 (WB), 1:3000 (IF)
TNF-α	Cell Signaling Technology; 8184	1:500 (WB)
TOM20	Cell Signaling Technology; 42406	1:2000 (WB)
TUBERIN/TSC2	Cell Signaling Technology; 4308	1:2000 (WB)
UVRAG	Cell Signaling Technology; 5320	1:1000 (WB)
WDR59	Cell Signaling Technology; 53385	1:2000 (WB)
Goat anti-rabbit IgG-Alexa Fluor 568	Thermo-Fisher; A-11036	1:1000 (IF)
Goat anti-mouse IgG-Alexa Fluor 568	Thermo-Fisher; A-11008	1:1000 (IF)
Horse anti-mouse IgG-HRP	Cell Signaling Technology; 7076	1:8000 (WB)
Goat anti-rabbit IgG-HRP	Cell Signaling Technology; 7074	1:8000 (WB)

WB: western blot, IF: immunofluorescence, ChIP: Chromatin immunoprecipitation

Appendix Table S3: qRT-PCR primers

Gene Symbol	Company; Catalogue Number
ATP6V1C1	QIAGEN; QT00015022
CCL5	QIAGEN; QT00090083
CD11b/ITGAM	QIAGEN; QT00031500
CD14	QIAGEN; QT00208817
CD36	QIAGEN; QT01674008
CD68	QIAGEN; QT00037184
CTSD	QIAGEN; QT00020391
CXCL1	QIAGEN; QT00199752
CXCL3	QIAGEN; QT00015442
CXCL5	QIAGEN; QT00203686
CXCL6	QIAGEN; QT00211155
CXCL8	QIAGEN; QT00000322
EREG	QIAGEN; QT00019194
GABARAPL1	QIAGEN; QT00096509
GAPDH	QIAGEN; QT01192646
HEXA	QIAGEN; QT00079877
IFNGR2	QIAGEN; QT00089278
IL10	QIAGEN; QT00041685
IL18	QIAGEN; QT00014560
IL1R	QIAGEN; QT00081263
IL1β	QIAGEN; QT00021385
IL23A	QIAGEN; QT00204078
IL33	QIAGEN; QT00041559
LAMP1	QIAGEN; QT00070994
LIF	QIAGEN; QT00001442
MCOLN1	QIAGEN; QT00094234
RRAGC	QIAGEN; QT00086527
TFEB	QIAGEN; QT00069951
TNFα	QIAGEN; QT00029162