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Supplemental Information

c-Abl Inhibition Activates TFEB and Promotes Cellular Clearance in a Lysosomal Disorder

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Figure S1

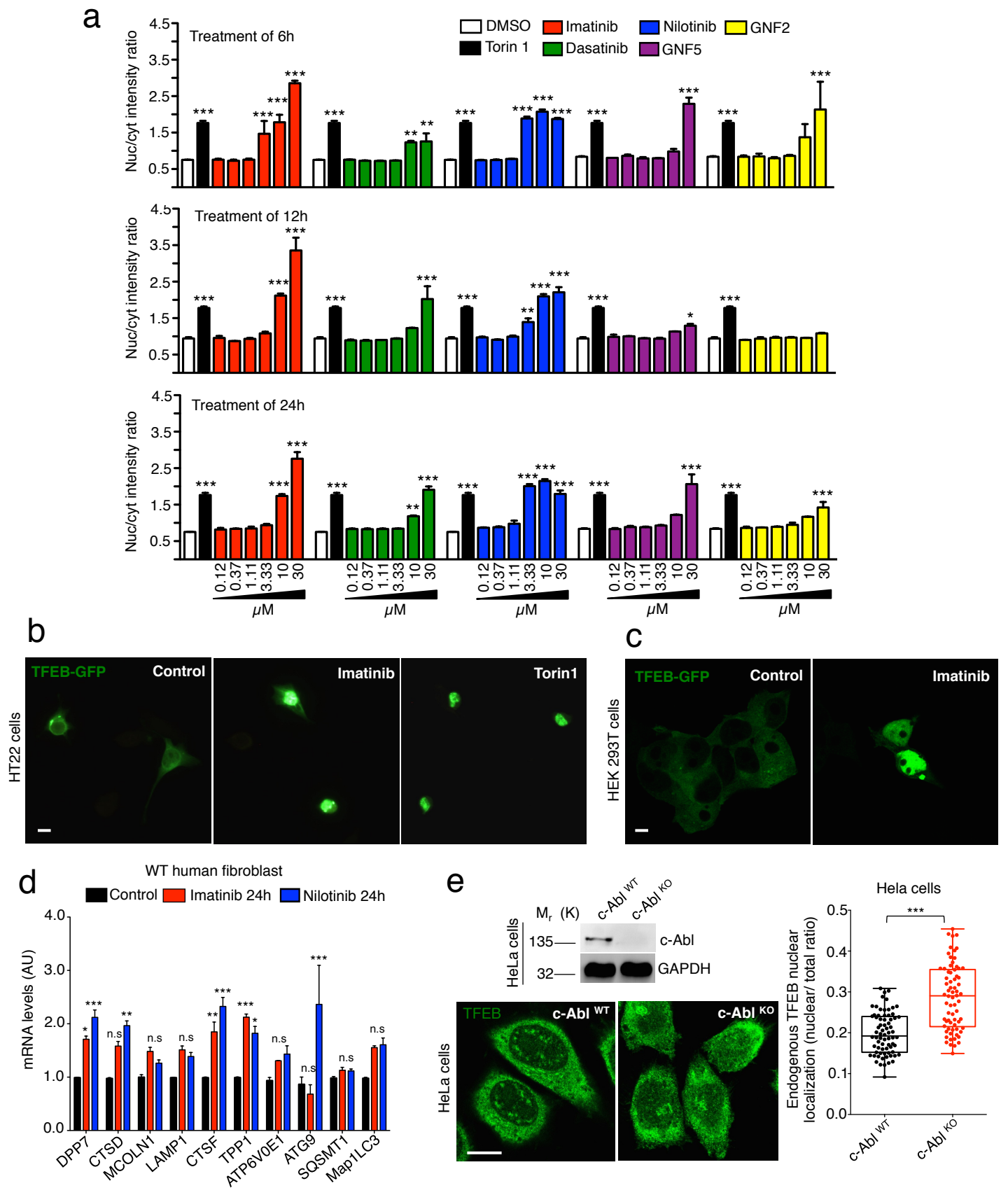
a
DMSO
Imatinib
Nilotinib
GNF2
Torin 1
Dasatinib
GNF5
Treatment of 6h
Treatment of 12h
Treatment of 24h
Nuc/cyt intensity ratio
0.12
0.37
1.11
3.33
10
30
μM
b
TFEB-GFP
Control
Imatinib
Torin1
HT22 cells
c
TFEB-GFP
Control
Imatinib
HEK 293T cells
d
WT human fibroblast
Control
Imatinib 24h
Nilotinib 24h
mRNA levels (AU)
DPP7
CTSD
MCOLN1
LAMP1
CTSF
TPP1
ATP6V0E1
ATG9
SQSMT1
Map1LC3
n.s
e
Mr (K)
c-Abl WT
c-Abl KO
HeLa cells
135
32
c-Abl
GAPDH
TFEB
HeLa cells
Hela cells
Endogenous TFEB nuclear localization (nuclear/ total ratio)

Figure S1. c-Abl inhibitors promote TFEB nuclear translocation. Related to Figure 1. HeLa TFEB-GFP cells were treated with DMSO (control), Torin1 0.3 μM (positive control) for 3h and c-Abl inhibitors at different concentrations for 6h, 12h and 24h. Then the cells were fixed and stained with DAPI. (a) The graph shows the ratio value resulting from the average intensity of nuclear TFEB–GFP fluorescence divided by the average intensity of cytosolic TFEB–GFP fluorescence. Black bars represent Torin1 treatment (positive control). Differences are statistically significant compared with the control conditions (DMSO). For each condition 450-800 cells were analyzed (7 images per sample) *n*=4 biological independent samples. (b) Representative images of HT22 or HEK293T cells (c) transfected transiently with a TFEB-GFP plasmid for 24h and treated with Imatinib 10μM for 24h and Torin1 0.3 μM for 3h as a positive control. *n*=3 independent experiments. Scale bars, 10 μM. (d) The graph shows the results of q- PCR analysis of mRNA levels of different TFEB target genes in wild type human fibroblasts treated with Imatinib or Nilotinib 10μM for 24h. (e) Representative western blot of c-Abl and images of endogenous TFEB in Hela WT and Hela c-Abl KO cells. The right graph represents the quantification of endogenous TFEB nuclear localization. *n*=70 cells were analyzed per condition. Scale bars, 10 μM. Statistical analysis with one-way ANOVA followed by Tukey's post test and Student's *t*-tests when comparing two experimental groups. *$p < 0.05$, **$p < 0.01$,***$p < 0.001$. Data represent mean ± SEM.

Figure S2

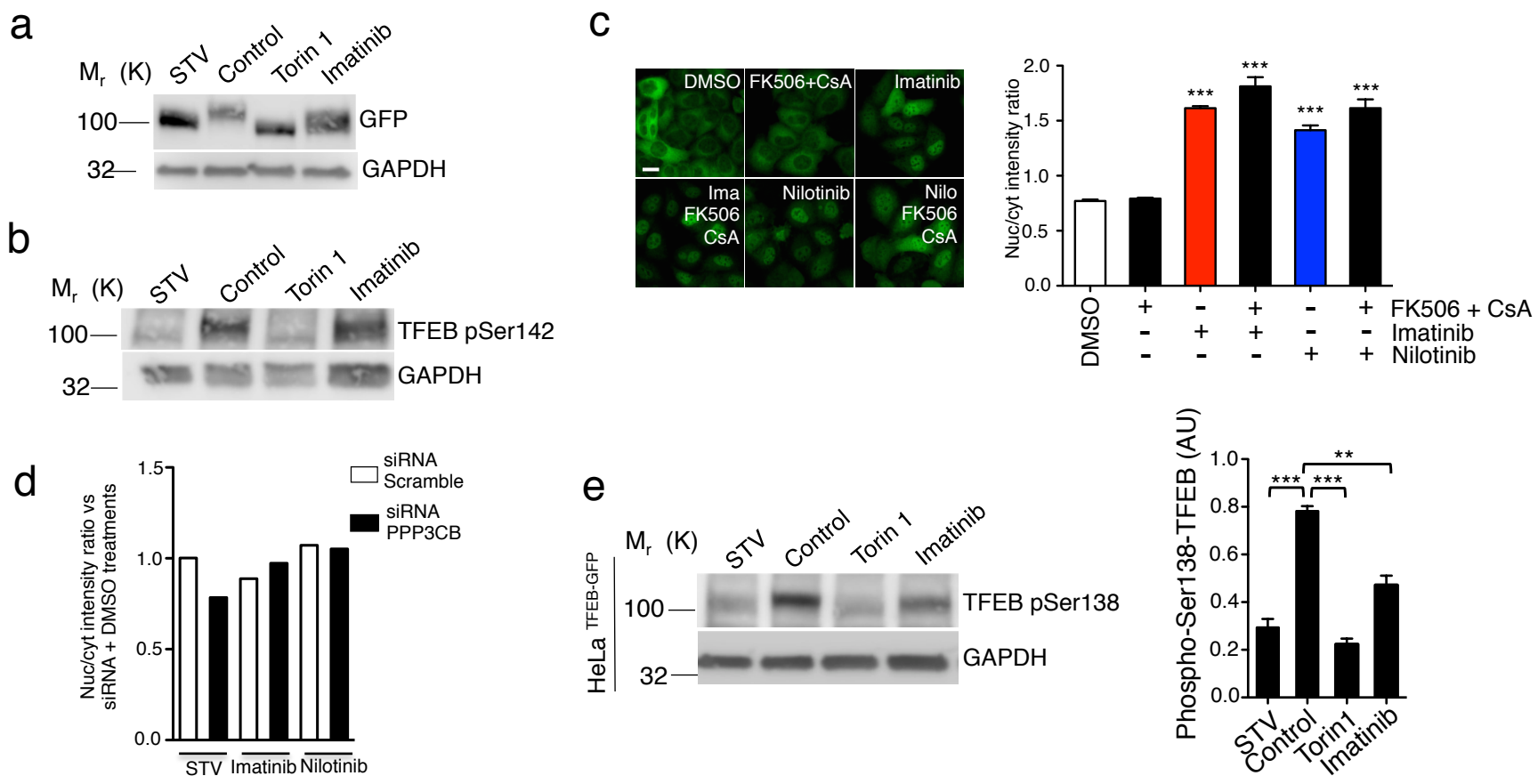
a
M$_r$ (K)
STV
Control
Torin 1
Imatinib
100
GFP
32
GAPDH
b
M$_r$ (K)
STV
Control
Torin 1
Imatinib
100
TFEB pSer142
32
GAPDH
c
DMSO
FK506+CsA
Imatinib
Ima FK506 CsA
Nilotinib
Nilo FK506 CsA
Nuc/cyt intensity ratio
2.0
1.5
1.0
0.5
0.0
***
DMSO
FK506 + CsA
Imatinib
Nilotinib
d
Nuc/cyt intensity ratio vs siRNA + DMSO treatments
siRNA Scramble
siRNA PPP3CB
1.5
1.0
0.5
0.0
STV
Imatinib
Nilotinib
e
HeLa TFEB-GFP
M$_r$ (K)
STV
Control
Torin 1
Imatinib
100
TFEB pSer138
32
GAPDH
Phospho-Ser138-TFEB (AU)
1.0
0.8
0.6
0.4
0.2
0.0
***
**
STV
Control
Torin1
Imatinib
f
HeLa TFEB-GFP
M$_r$ (K)
STV
Control
Torin 1
Imatinib
Nilotinib
63
phospho-S6K
63
Total-S6K
32
GAPDH
g
M$_r$ (K)
STV
Control
Torin 1
Imatinib 3h
Imatinib 6h
Imatinib 24h
63
phospho-S6K
63
Total-S6K
32
GAPDH

Figure S2. c-Abl inhibition does not affect mTORC1 activity. Related to Figure 3. (a) Representative Western blot showing TFEB- GFP in Hela TFEB-GFP cells treated with Imatinib 10μM for 3h. Torin1 0.3μM and starvation media (STV) for 3h was used as positive controls. (b) Representative Western blot showing TFEB phosphorylated on S142 in Hela TFEB-GFP cells treated with Imatinib 10μM for 3h. Torin1 0.3μM and starvation media (STV) for 3h was used as positive control. (c) Hela TFEB-GFP cells were treated with calcineurin inhibitors cyclosporine A (CsA) 10 μM and FK506 5μM for 1h. Then, cells were treated with Imatinib or Nilotinib 10μM for 3h. Representative images of the TFEB-GFP translocation assay and quantification n=3 independent experiments. Scale bars, 10 μM. (d) Quantification of TFEB-GFP translocation assay. Hela TFEB-GFP cells were transfected with a scramble siRNA and PPP3CB siRNA for 72h. Then, cells were treated with DMSO for 3h, starvation media (STV) for 3h and Imatinib or Nilotinib 10μM for 3h. Values of each treatment presented in the plot were normalized with DMSO treatment. n=2 independent experiments. (e) Representative Western blot and quantification of TFEB phosphorylated on S138 in Hela TFEB- GFP cells treated with Imatinib 10μM for 3h and also in Hela TFEB-GFP cells treated with siRNA against c-Abl for 48h. Torin1 0.3μM and starvation media (STV) for 3h was used as positive control. n=3 independent experiments. (f) Representative Western blot of phospho p70-S6K in Hela TFEB-GFP cells treated with Imatinib and Nilotinib 10μM for 3h. Torin1 0.3μM and STV media for 3h were used as positive controls. n=3 independent experiments. (g) Representative western blot of phospho p70-S6K in Hela cells treated with Imatinib 10μM for 3h, 6h and 24h. Torin1 0.3μM and STV media for 3h were used as positive controls. n=3 independent experiments. Statistical analysis with one-way ANOVA followed by Tukey's post test. *$p < 0.05$, **$p < 0.01$,***$p < 0.001$. Data represent mean ± SEM.

Figure S3

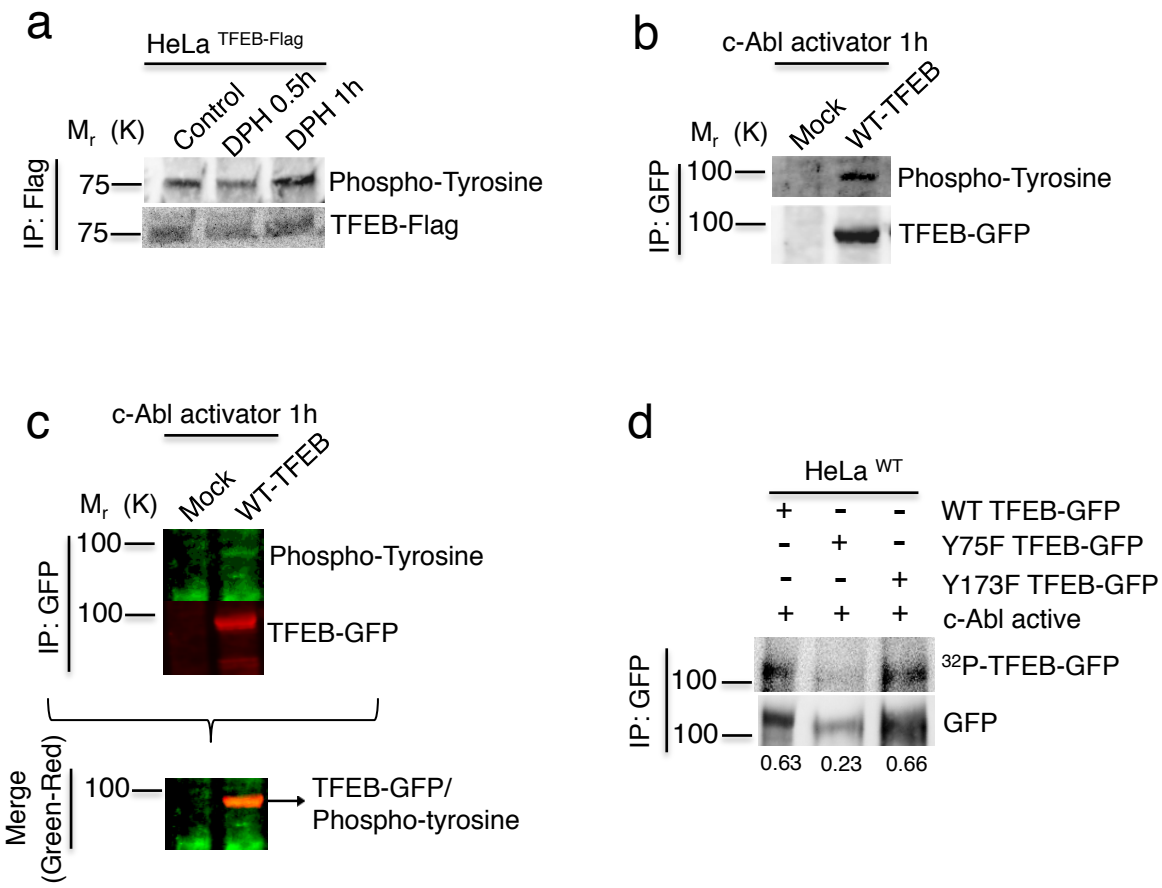
a
HeLa TFEB-Flag
Control
DPH 0.5h
DPH 1h
Mr (K)
IP: Flag
75
75
Phospho-Tyrosine
TFEB-Flag
b
c-Abl activator 1h
Mock
WT-TFEB
Mr (K)
IP: GFP
100
100
Phospho-Tyrosine
TFEB-GFP
c
c-Abl activator 1h
Mock
WT-TFEB
Mr (K)
IP: GFP
100
100
Phospho-Tyrosine
TFEB-GFP
Merge
(Green-Red)
100
TFEB-GFP/
Phospho-tyrosine
d
HeLa WT
+ - - WT TFEB-GFP
- + - Y75F TFEB-GFP
- - + Y173F TFEB-GFP
+ + + c-Abl active
IP: GFP
100
100
32P-TFEB-GFP
GFP
0.63
0.23
0.66

Figure S3. c-Abl activation by DPH induces TFEB tyrosine phosphorylation. Related to Figure 4. (a) Hela TFEB-Flag cells were treated with DPH 1μM for 0.5h and 1h. Flag was immunoprecipitated using beads-anti-Flag and then used a anti-phospho- tyrosine antibody. *n*=2 independent experiments. (b) Hela TFEB-GFP cells were treated with DPH 1μM for 1h. GFP was immunoprecipitated using beads-anti-GFP and then used a anti-phospho-tyrosine antibody. *n*=2 independent experiments. (c) Merge of the western blot images showed in (b). (d) Autoradiography of an *in vitro* phosphorylation assay. Site- directed Y75F and Y173F TFEB-GFP mutants were transfected into Hela cells. TFEB-GFP IP was incubated with human recombinant c-Abl active and ATP-γ-32P for 2h.

Figure S4

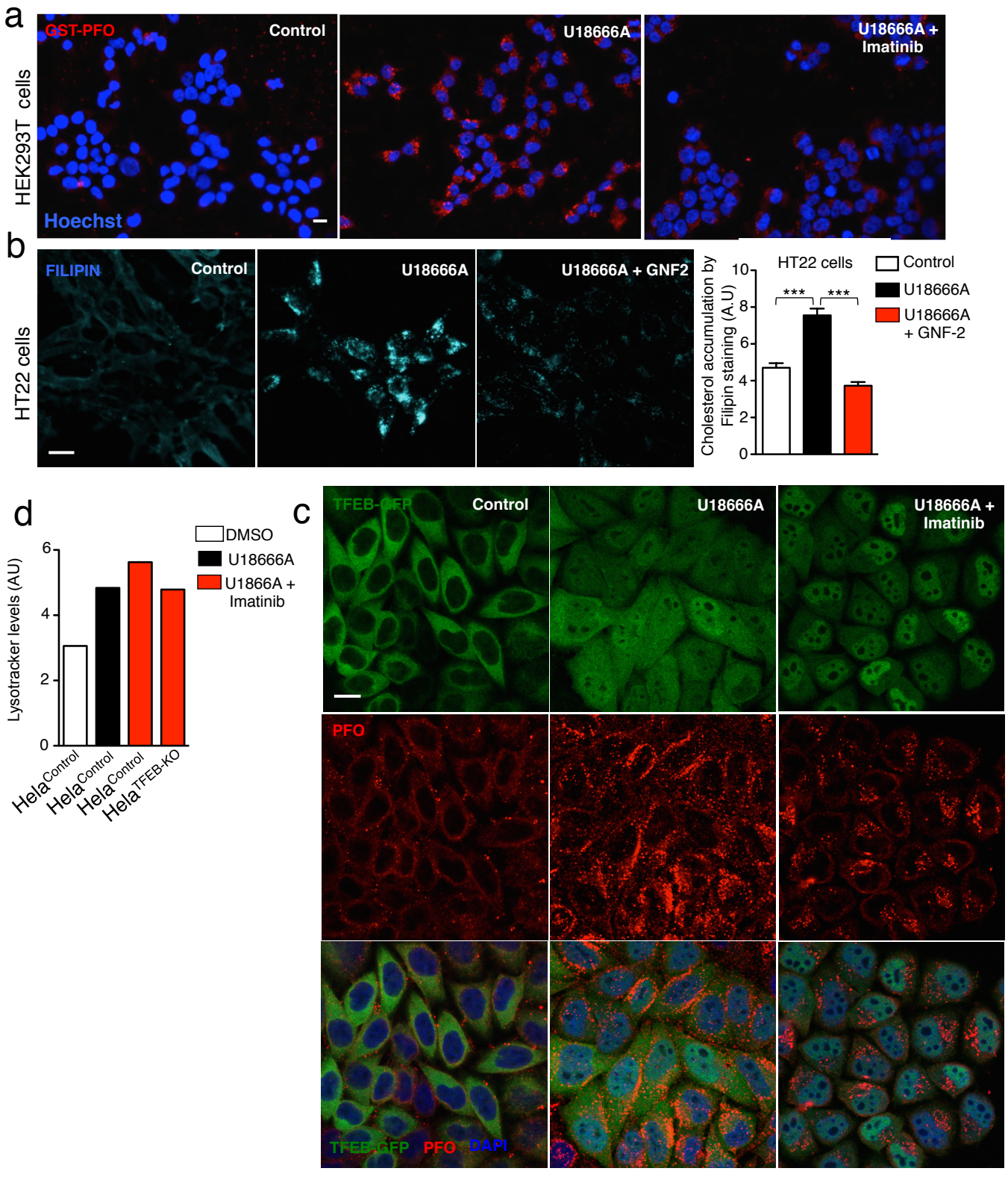
a
HEK293T cells
GST-PFO
Control
U18666A
U18666A + Imatinib
Hoechst
b
HT22 cells
FILIPIN
Control
U18666A
U18666A + GNF2
HT22 cells
Control
U18666A
U18666A + GNF-2
Cholesterol accumulation by Filipin staining (A.U)
***
***
d
DMSO
U18666A
U1866A + Imatinib
Lysotracker levels (AU)
Hela Control
Hela Control
Hela Control
Hela TFEB-KO
c
TFEB-GFP
Control
U18666A
U18666A + Imatinib
PFO
TFEB-GFP
PFO
DAPI

Figure S4. c-Abl inhibitors reduce cholesterol accumulation in U18666A-treated cells. Related to Figure 5.

(a) Representative images of cholesterol accumulation. HEK293T cells were treated with U18666A 0.5µg/mL and/or Imatinib 10µM for 24h. Then GST-PFO (red) immunofluorescence and Hoechst (blue) staining were performed. Scale bars, 10 µM. (b) Representative images and quantification of cholesterol accumulation using filipin staining on HT22 cells treated with U18666A 0.5µg/mL and/or GNF2 10µM for 24h. *n*=3 independent experiments. Scale bars, 10 µM. (c) Representative confocal microscopy images showing cholesterol accumulation and TFEB-GFP localization. Hela TFEB-GFP cells were treated with U18666A 0.5µg/ mL and/or Imatinib 10µM for 24h. Then GST-PFO immunofluorescence (red) and DAPI staining (blue) were performed. Scale bars, 10 µM. (d) Flow cytometry quantitative analysis of lysotracker in Hela cells and Hela TFEB-KO cells treated with U18666A and/or Imatinib and Nilotinib 10µM for 24h. *n*=10,000 cells per conditions. Statistical analysis with one-way ANOVA followed by Tukey's post. *$p < 0.05$, **$p < 0.01$,***$p < 0.001$. Data represent mean ± SEM.

Figure S5

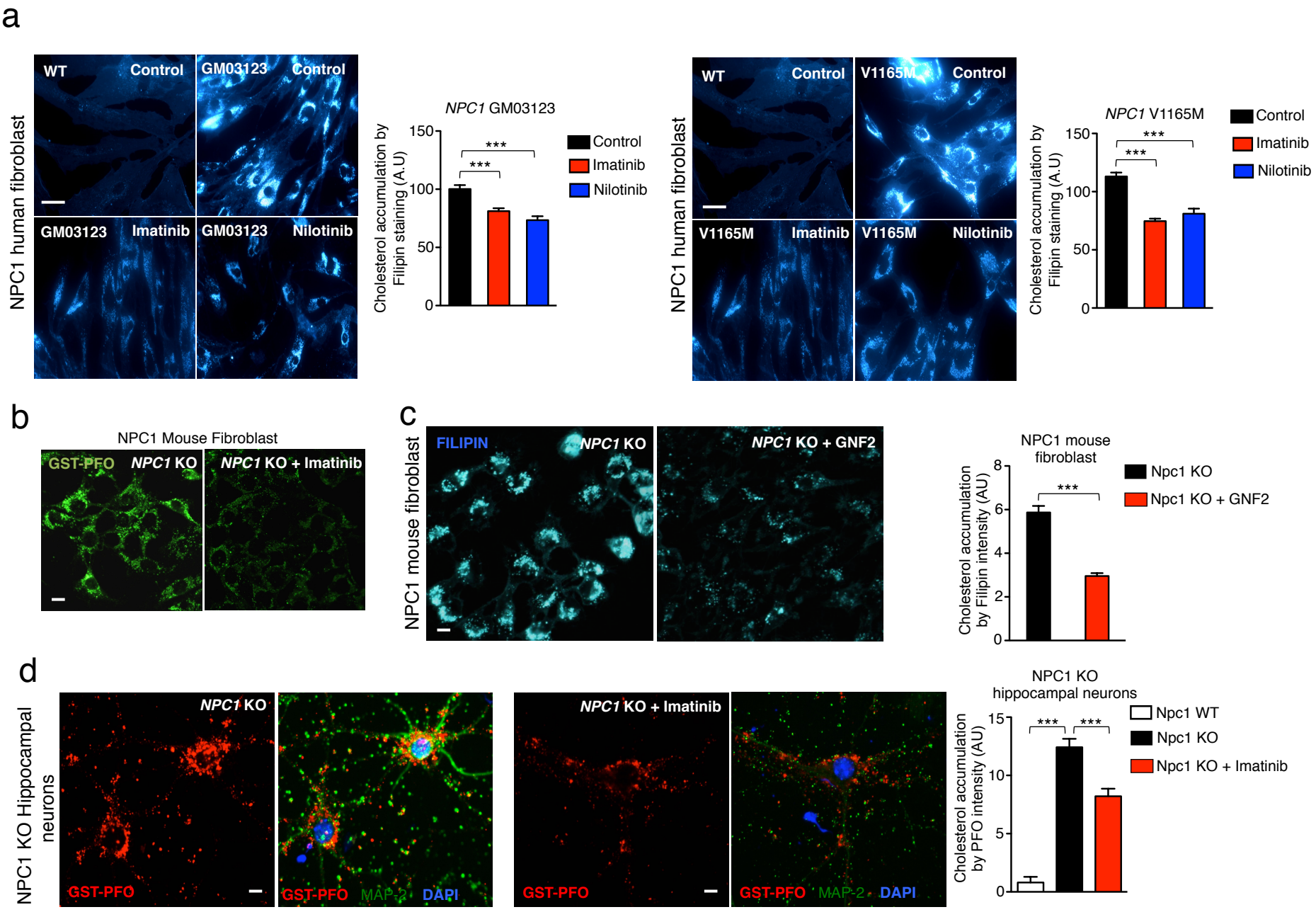
a
NPC1 human fibroblast
WT
Control
GM03123
Control
GM03123
Imatinib
GM03123
Nilotinib
NPC1 GM03123
Cholesterol accumulation by Filipin staining (A.U)
150
100
50
0
***
***
Control
Imatinib
Nilotinib
NPC1 human fibroblast
WT
Control
V1165M
Control
V1165M
Imatinib
V1165M
Nilotinib
NPC1 V1165M
Cholesterol accumulation by Filipin staining (A.U)
150
100
50
0
***
***
Control
Imatinib
Nilotinib
b
NPC1 Mouse Fibroblast
GST-PFO
NPC1 KO
NPC1 KO + Imatinib
c
NPC1 mouse fibroblast
FILIPIN
NPC1 KO
NPC1 KO + GNF2
NPC1 mouse fibroblast
Cholesterol accumulation by Filipin intensity (AU)
8
6
4
2
0
***
Npc1 KO
Npc1 KO + GNF2
d
NPC1 KO Hippocampal neurons
NPC1 KO
GST-PFO
GST-PFO
MAP-2
DAPI
NPC1 KO + Imatinib
GST-PFO
GST-PFO
MAP-2
DAPI
NPC1 KO hippocampal neurons
Cholesterol accumulation by PFO intensity (AU)
15
10
5
0
***
***
Npc1 WT
Npc1 KO
Npc1 KO + Imatinib

Figure S5. Cholesterol-lowering effect of c-Abl inhibitors in different NPC1 *in vitro* models. Related to Figure 6. (a) Representative images of NPC1 human fibroblast stained with filipin and quantification of cholesterol accumulation. NPC1 cells were treated with Imatinib 10μM and Nilotinib 10μM for 48h. *n*=3 independent experiments. Scale bars, 50 μM. (b) Representative images of cholesterol accumulation visualized by PFO staining (green) in NPC1 KO mice fibroblasts treated with Imatinib 10μM for 24h. Scale bars, 10 μM. (c) Representative images and quantification of cholesterol accumulation using filipin staining in NPC1 mouse fibroblast cells treated with GNF2 10μM for 24h. *n*=3 independent experiments. Scale bars, 10 μM. (d) Representative images and quantification of cholesterol accumulation using GST-PFO immunofluorescence, MAP2 (green) and DAPI (blue) on hippocampal neurons cultures (7 DIV) from NPC mice treated with Imatinib 10μM for 24h. *n*=3 independent experiments. Scale bars, 10 μM. Statistical analysis with one-way ANOVA followed by Tukey's post test and Sutdent's *t*-tests when comparing two experimental groups *$p < 0.05$, **$p < 0.01$,***$p < 0.001$. Data represent mean ± SEM.

TRANSPARENT METHODS

KEY RESOURCES TABLE. Related to Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
TFEB	Cell Signaling Technology	Cat# 4240, RRID:AB_11220225
c-Abl	Cell Signaling Technology	Cat# 2862, RRID:AB_2257757
Histone H3	Cell Signaling Technology	Cat# 9715, RRID:AB_331563
Phospho-P70 S6 Kinase	Cell Signaling Technology	Cat# 9205, RRID:AB_330944
P70 S6 Kinase	Cell Signaling Technology	Cat# 9202, RRID:AB_331676
Phospho-(Ser) 14-3-3 Binding Motif (Detection of TFEB phosphorylation at Ser211)	Cell Signaling Technology	Cat# 9601, RRID:AB_330306
Phospho-CrkII	Cell Signaling Technology	Cat# 3491, RRID:AB_2229920
Mouse TFEB	Proteintech Group	Cat# 13372-1-AP, RRID:AB_2199611
TFEB	Betyl Laboratories	Cat# A303-672A,RRID:AB_11204598
GFP	Molecular Probes	Cat# A-11122, RRID:AB_221569
Hoechst 33342	Cell Signaling Technology	Cat# 4082, RRID:AB_10626776
LysoTracker red DND-99	Thermo Fischer Scientific	L7528
Fluoromont-G with DAPI	Thermo Fischer Scientific	00-4959-52
GST	GE Healthcare	Cat# 27-4577-01, RRID:AB_771432
c-Abl	Santa Cruz Biotechnology	Cat# sc-23, RRID:AB_626775
GAPDH	Santa Cruz Biotechnology	Cat# sc-32233, RRID:AB_627679
Lamp1-D4B	Santa Cruz Biotechnology	Cat# sc-19992, RRID:AB_2134495
Lamp1	Abcam	Cat# ab25630, RRID:AB_470708
TFEB	Sigma-Aldrich	Cat# AV100809, RRID:AB_1857934
phospho-c-Abl	Sigma-Aldrich	Cat# C5240, RRID:AB_262088
ANTI-FLAG M2 affinity gel	Sigma-Aldrich	Cat# A2220, RRID:AB_10063035
FLAG	Sigma-Aldrich	Cat# F7425, RRID:AB_439687

Agarose anti-GFP	Vector Laboratories	Cat# MB-0732, RRID:AB_2336839
Phospho-tyrosine	Merck Millipore	Cat# 05-321, RRID:AB_309678
MAP2	Merck Millipore	Cat# MAB3418, RRID:AB_94856
2hosphor-Ser-142-TFEB	Andrea Ballabio Lab, TIGEM	N/A
2hosphor-Ser-138-TFEB	Andrea Ballabio Lab, TIGEM	N/A
2hosphor-Ser-211-TFEB	Andrea Ballabio Lab, TIGEM	N/A
Chemicals, Peptides, and Recombinant Proteins		
Filipin	Sigma-Aldrich	F4767
Tamoxifen	Sigma-Aldrich	T5648
Imatinib mesylate	Cayman Chemical Company	13139
Nilotinib	Cayman Chemical Company	10010422
Dasatinib	Cayman Chemical Company	11498
Torin 1	Tocris	4247
GNF2	NIH	N/A
GNF5	NIH	N/A
U18666A	Enzo Life Sciences	BML-S200
GST-PFO	Andrzej Sobota Lab, Nencki Institute of Experimental Biology.	N/A
Critical Commercial Assays		
TransIT-LT1	Mirus Bio LCC	MIR 2300
Lipofectamine 2000	Thermo Fischer Scientific	12566014
RNAiMAX	Thermo Fischer Scientific	13778150
Rneasy mini kit	Qiagen	74104
QuantiTect Reverse Transcription Kit	Qiagen	205313
Experimental Models: Cell Lines		
Hela	Andrea Ballabio Lab, TIGEM	N/A
Hela TFEB-GFP	Andrea Ballabio Lab, TIGEM	N/A
Hela-3x-Flag	Andrea Ballabio Lab, TIGEM	N/A
TFEB-KO	Andrea Ballabio Lab, TIGEM	N/A
TSC2 KO	Andrea Ballabio Lab, TIGEM	N/A
Hepa 1-6	ATCC	N/A
HEK293T	ATCC	N/A
NPC1 mouse fibroblast	Peter Lobel Lab, Rutgers University	N/A

NPC1 human fibroblast V1165M (c.2795+1G>C c.3493G>A p.V1165M)	Andrea Dardis (Coordinator Centre for Rare Diseases, Italy)	N/A
NPC1 human fibroblast GM03123 1018 (c.709C>T p.P237S c. 3182T>C p. I1061T)	Coriell Institute	N/A
HT22	Elena Pasquale Lab, (Sanford-Burnham Medical Research Institute)	N/A
H4 GFP-mRFP-LC3	Patricia Burgos Lab, (Universidad Austral de Chile and Universidad San Sebastián, Chile)	N/A
c-Abl KO	Alejandra Alvarez Lab, (Pontificia Universidad Católica de Chile)	N/A
Experimental Models: Organisms/Strains		
$Npc1^{+/-}$ BALB/c	Jackson laboratory	N/A
$c\text{-}Abl^{loxp}/c\text{-}Abl^{loxp}$	Jackson laboratory	N/A
$Nestin\text{-}Cre^{+}$	Jackson laboratory	N/A
Oligonucleotides		
Non-targeting siRNA Pool (scramble)	Dharmacon	N/A
On-Target plus Human siRNA anti-c-Abl	Dharmacon	N/A
Recombinant DNA		
TFEB-GFP	Andrea Ballabio Lab, TIGEM	N/A
TFEB-3xFlag	Andrea Ballabio Lab, TIGEM	N/A
c-Abl-ERT2	Douglas R. Green lab (St. Jude Children's Research Hospital, Memphis, USA)	N/A
c-Abl-ERT2 KD	Douglas R. Green lab (St. Jude Children's Research Hospital, Memphis, USA)	N/A
sgRNA clone for Human c-Abl gene, Target site: GTCTGAGTGAAGCCGCTCGT	Gene Copeia TM	N/A
Software and Algorithms		
netphos 2.0	Bloom et al., 1999	http://www.cbs.dtu.dk/services/NetPhos-2.0/
GPS 2.1.2	Xue et al., 2011	N/A

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines culture:
Hela cells, Hela TFEB-GFP cells, TFEB-3xFlag cells, TFEB-KO cells, H4 cells, TSC2 KO cells and c-Abl KO cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 IU/mL penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum.

Mice hippocampal neuron cultures:
Hippocampi from c-Abl$^{floxo/floxo}$ Nestin Cre (c-Abl KO) and c-Abl$^{floxo/floxo}$ (wild-type) mice were removed on postnatal day 1 (1 day later, the genotype of the animals (c-Abl$^{+/+}$ (WT) or c-Abl$^{-/-}$ (c-Abl KO) was determined) or from NPC1$^{-/-}$ (NPC) mice, were dissected in Ca^{2+}/Mg^{2+}-free Hank's balanced salt solution (HBSS) and washed with HBSS. Then, the tissue was incubated for 15 min at 37°C with HBSS and 0.25% trypsin. Then, the tissue was washed with HBSS and mechanically dissociated in DMEM plus 100 µg/mL streptomycin, 100 U/mL penicillin and 10% horse serum (Invitrogen, Waltham, MA, USA). After this, hippocampal neurons were seeded onto poly-lysine coated coverslips and maintained at 37°C in 5% CO_2. After 2h, culture medium was replaced with Neurobasal growth medium (Invitrogen) supplemented with 100 U/mL penicillin, and 100 µg/mL streptomycin, B27 (Invitrogen) and 2 mM L-glutamine. Next day, neurons were treated with AraC 2µM for 24 h.

Animals:
Npc1$^{+/-}$ BALB/c mice carrying a heterozygous mutation in the NPC1 gene were obtained from Jackson laboratory (Sacramento, CA, USA). Genotypes were identified using a PCR-based screening (Amigo et al., 2002). c-Abl KO mice were bred from c-Abl^{loxp}/c-Abl^{loxp} and Nestin-Cre^{+} was purchased from Jackson laboratory. The animal protocols used were reviewed and approved by the animal studies board at our institution.

METHODS DETAILS

Imatinib, Nilotinib, Dasatinib, GNF2, GNF5, U18666A (U18), Torin1, treatments: All the cell lines were treated as indicated in the manuscript. Cells were treated with Imatinib 10µM or Nilotinib 10µM and later, cells were treated with U18 0.5 µg/mL for 24 h when is indicated. c-Abl KO hippocampal neurons were treated with U18 0.5 µg/mL for 24h.

Plasmids and siRNA: TFEB-GFP and TFEB-3xFlag were obtained from Andrea Ballabio's Lab (TIGEM, Italy). Non-targeting siRNA Pool (scramble), On-Target plus Human siRNA anti-c-Abl were purchased from Dharmacon (GE Healthcare, USA). PPP3CB siRNA Ambion 4390824. c-Abl ERT2 and c-Abl ERT2 KD, were generated in the Douglas R. Green lab (St. Jude Children's Research Hospital, Memphis, USA). TFEB-GFP, TFEB-3xFLag, c-Abl ERT2 and c-Abl ERT2 KD were transfected into cells using: TransIT-LT1 transfection reagent was purchased from Mirus Bio LCC (USA) and Lipofectamine 2000 reagent Thermo Fischer Scientific (Waltham, Massachusetts, USA). siRNA scramble, On-Target plus Human siRNA anti-c-Abl and PPP3CB were transfected into cells using Lipofectamine RNAiMAX transfection reagent Thermo Fischer Scientific (Waltham, Massachusetts, USA). 48h or 72h after transfection, cells were lysed in RIPA buffer and used for Immunoprecipitation and/or immunoblot analysis.

TFEB tyrosine phosphorylation assay: Hela cells were transfected with c-Abl ERT2 and c-Abl ERT2 KD plasmid. The cells were then treated with Tamoxifen 0.1 µM for 6h. Then cells were lysed in immunoprecipitation buffer plus protease and phosphatase inhibitors. We used agarose beads anti-GFP for the immunoprecipitations. TFEB tyrosine phosphorylation was evaluated with an anti-phospho-Tyr antibody (4G10, Millipore).

Immunofluorescence procedures: Cell lines and hippocampal neurons were seeded in coverslips in 24-well culture plates. After treatment, cells were washed with PBS and fixed in 4% paraformaldehyde in PBS for 20 min. Then, cells were permeabilized in 0.2% Triton X-100 in PBS for 10 min and after two washes, cells were incubated in 3% BSA in PBS for 30 min at room temperature. Then, cell were incubated with primary antibodies against Lamp1 or TFEB at 4°C overnight. For the GST-PFO assay, cells were incubated

(2 times) with 3% fish gelatin in PBS 1X (30 min each incubation). Then, cells were treated with 5 µg/ml GST-PFO for 45 min at RT and washed five times in 0.2% fish gelatin/PBS 1X followed by incubation with primary antibodies against GST goat at 4°C overnight. The cells were washed four times with PBS and then incubated with anti-mouse/anti-goat Alexa 488 and anti-rabbit/anti-goat Alexa 555 antibodies (Life Technologies, Carlsbad, United States of America) at room temperature for 1h. The fluorescence images were captured with Opera high content system; Perkin-Elmer. For confocal imaging, the samples were examined under a Zeiss LSM 700 confocal microscope or with an Olympus BX51 microscope and analyzed and quantified with the ImageJ software

Luminal Lamp1: Cells were seeded in a coverslips. After 24 hours were washed with MEM + 10mM HEPES and Treated with Imatinib 10 µM for 24 hours followed by incubation with primary antibody (Lamp1-D4B) in MEM + 10 mM Hepes +1% BSA for 20 min at 4°C. Then PFA 4% for 20 min and Washed once in PBS followed by incubation with secondary antibody (Alexa Fluor 488) in PBS + 1% BSA for 1h at RT. The cells were washed, incubated with Hoechst for 10 min at RT. Images were obtained with Zeiss a LSM 700 confocal microscope.

High-Content TFEB-GFP Translocation, GST-PFO and GFP-mRFP-LC3 assays: Hela cells, HeLa TFEB-GFP cells, H4 cells, NPC1 human fibroblast were seeded in 96- or 384-well plates and treated as indicated in the text, washed, fixed and stained with DAPI or Hoechst. The acquisition of the images was by using confocal automated microscopy (Opera high-content system; Perkin-Elmer). A dedicated script was developed to perform the analysis of TFEB localization, GST-PFO and GFP-mRFP-LC3 puncta on the different images (Harmony and Acapella software; Perkin-Elmer) (Medina et al., 2015).

In vitro phosphorylation assay: Kinase assay samples were performed as follow; TFEB-Flag or GST–CrkII, 5 µM ATP, and 0.5 µCi of [γ^{32}P]ATP, 1 mM sodium orthovanadate, 100 ng/µL of bovine serum albumin, 25 mM HEPES, pH 7.25, 100 mM NaCl, 5 mM MgCl2 and 5% glycerol. Samples were preincubated 5 minutes at at 30°C and then 25 µL reactions were initiated by the addition of 10 nM of c-Abl T315I kinase. Then we performed a pull down of TFEB-Flag or GST-CrkII, washed and analyzed by autoradiography.

Flow Cytometry: For GST-PFO analysis, confluent cells transfected were trypsinized and washed with PBS before incubation with PFA 4% for 20 min. Then cells were washed with PBS, centrifuged and re-suspended in PBS/Triton X-100 0.05% (v/v) for 5 min. Then cells were centrifuged and re-suspended in gelatin fish 3% for 15 min. Cells were centrifuged and re-suspended in GST-PFO 10 µg/mL in gelatin fish 1% for 45 min. Then, cells were treated with Anti-GST Alexa fluor-647 in gelatin fish 1% for 1 h. Finally, cells were resuspended in 0.3 mL PBS and analyzed on a FACS (BD FACSCanto II Flow Cytometer). For lysotracker red analysis, confluent cells were trypsinized for 5 min, centrifuged and re-suspended in DMEM 1% FBS. Then cells were incubated with Lysotracker red DND-99 for 5 min, washed and then analyzed on a FACS (BD FACSCanto II Flow Cytometer).

Nuclei-cytoplasmic fractions: Cells were seeded and treated as indicated in the text, washed and scraped gently. Then, cells were centrifuged and supernatant was discard and pellet was diluted an incubated for 2 minutes at RT with buffer A (20 mM Tris-HCL 7.6; 0.1 mM EDTA; 2 mM MgCl2 6H20; 0.5 mM NaF; 0.5 mM Na3 OV4) and then was incubated for 10 minutes on ice. Then NP-40 1% was added and samples were passes trough a syringe 20G 3 times. After this, samples were centrifuged 500 g for 3 minutes at 4ºC. Supernatant correspond to cytoplasmic fraction. Cell pellet was washed 3 times with buffer A + 1% NP-40 centrifuging at 500 g for 3 minutes at 4ºC. Then were treated with buffer B (20 mM Hepes pH 7.9; 400 mM NaCl; 2.5% (V/V) Glicerol; 1 mM EDTA; 0.5 mM DTT; 0.5 mM NaF; 0.5 mM Na3 OV4) and incubated in liquid nitrogen and then at 37ºC. Then samples were incubated on ice for 20 minutes, centrifuged at 20.000 g for 20 minutes and the supernatant corresponded to nuclear fraction.

Filipin staining: Cells lines or hippocampal neurons were fixed in 4% paraformaldehyde in PBS for 30 min. Then, cells were washed in PBS and treated with 1.5 mg/mL glycine for 20 min. After this, cells were treated with 25 µg/mL Filipin (Sigma) for 30 min and washed with PBS. Images were captured with an

Olympus BX51 microscope and analyzed with the ImageJ software.

Immunoblot analysis: Cells were lysed in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with cocktail of protease inhibitors (Roche) and Na_3VO_4. The homogenates were maintained on ice for 10 min and then they were centrifuged at 10,000g for 10 min. The supernatant was recovered, and protein concentration was determined with the Pierce BCA protein assay kit (23225) purchased from Thermo Scientific (Waltham, United States of America). Proteins were resolved in SDS-PAGE, transferred to Nitrocellulose membranes (Thermo Scientific), and probed with primary antibodies against TFEB, GFP, c-Abl, p-c-Abl, p-CRKII, GAPDH and p-P70 S6 Kinase, total-P70 S6 Kinase. The reactions were followed by incubation with HRP labeled secondary antibodies and developed using the ECL technique (Thermo Scientific).

Quantitative Real-Time PCR: Total RNA from Hela cells and human fibroblasts was extracted using an RNeasy Mini Kit (Qiagen), and reverse-transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time quantitative PCR assays were performed in triplicate using LightCycler 480 SYBR Green I Master in a LightCycler System 2.0 (Rocher Applied Science). The oligonucleotides used were as follows: GAPDH; fw: 5'-attgttcgtcatgggtgtgaa-3', rev: 5'-aggggtgctaagcagttggt-3', DPP7; fw: 5'-gattcggaggaacctgagtg-3', rev: 5'-cggaagcaggatcttctgg-3', CTSD; fw: 5'-cttcgacaacctgatgcagc-3', rev: 5'-tacttggagtctgtgccacc-3', MCOLN1; fw: 5'-gagtccctgcgacaagtttc-3', rev: 5'-tgttctcttcccggaatgtc-3', LAMP1; fw: 5'-ccaacttctctgctgccttc-3', rev: 5'-agcaatcaacgagactgggg-3'. CTSF; fw: 5'-acagaggaggagttccgcacta-3', rev: 5'-gcttgcttcatcttgttgcca-3', TPP1; fw: 5'-gatcccagctctcctcaatacg-3', rev: 5'-gccatttttgcaccgtgtg-3', ATP6V0E1; fw: 5'-cattgtgatgagcgtgttctgg-3', rev: 5'-aactccccggttaggaccctta-3', ATG9; fw: 5'-ttcctttgcccttatgcatg-3', rev: 5'-aaccgcatcaaagaaagctc-3', SQSTM1; fw: 5'-caggtcttccaaggttgagg-3', rev: 5'-ataaaaacacggccatttgc-3', ABL1; fw: 5'-ctgcaaatccaagaaggggct-3', rev: 5'-ctgaggctcaaagtcagatgcta-3', Map1LC3; fw: 5'- agcgctacaagggtgagaa-3', rev: 5'-gttcaccagcaggaagaag-3'. Comparative CT method was used to calculate the relative quantities of cDNA.

Site-directed mutagenesis: TFEB mutants in Tyrosine were generated by PCR using the proofreading Pfu polymerase (Stratagene, Santa Clara, United States of America), followed by DpnI (New England Biolabs, Massachusetts, United States of America) digestion of the methylated parental plasmid. Oligonucleotides used were as follows: Y75F; fw: 5'-gttgaaggtgcagtccttcctggagaatcccac-3', rev: 5'-gtgggattctccaggaaggactgcaccttcaac-3', Y173F; fw: 5'-gacgatgtccttggcttcatcaatcctgaaatgc-3', rev: 5'-gcatttcaggattgatgaagccaaggacatcgtc-3'. Each clone was verified by automated sequencing.

Histological analysis: Mice perfusion was performed with 4% paraformaldehyde in PBS. Then, brains were post-fixed overnight at 4°C and after this, placed in 20% sucrose in PBS at 4°C overnight. Then brains were cut in 25 µM thick sagittal sections by cryostat at (Leika) at -20°C. Permeabilized slices with 0.1% Triton X-100 were blocked in 5% BSA or Gelatin in PBS and incubated overnight with the antibody rabbit anti-TFEB and GST in 5% BSA in PBS. For GST-PFO staining, tissues from five males and three females were treated with GST-PFO after gelatin block. The primary antibody was visualized with anti-rabbit Alexa-Fluor 555 or Alexa-Fluor 488.

Beam test: 4 males and 5 females mice were trained to cross a beam as quickly as possible. The, animals were placed at the end of a beam (100 cm long; 2.5 cm wide) and the number of falls during the test was counted.

Hela c-Abl KO cells: Hela cells were transfected a plasmid containing all-in-one sgRNA clone for Human c-Abl gene, Target site: GTCTGAGTGAAGCCGCTCGT (Gene Copeia TM). After 48 h of transfection, cells were incubated with G418 0.5 mg/mL for 48 h. Then, cells were washed and incubated with G418 0.5 mg/mL for 2 weeks. Then we select colonies and incubated in a 96 wells plate. Then each clone was tested by western blot. Endogenous TFEB was analyzed using Betyl laboratories antibody.

Statistical analysis: Mean and SEM values and the number of experiments are indicated in each figure legend. One-way ANOVA tests were performed followed by Bonferroni post-test using the Prisma Software.

Statement of ethics: All protocols were approved and followed local guidance documents generated by the *ad hoc* committee of the Chilean Science and Technology Council (CONICYT) and were approved by the Scientific Ethics Committee for the care of animals and the environment, Pontificia Universidad Católica de Chile #160321008 (exCEBA 14-038). Protocols are in agreement with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals recommended by the Institute for Laboratory Animal Research in its Guide for Care and Use of Laboratory Animals.

Supplemental References

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