Expanded View Figures

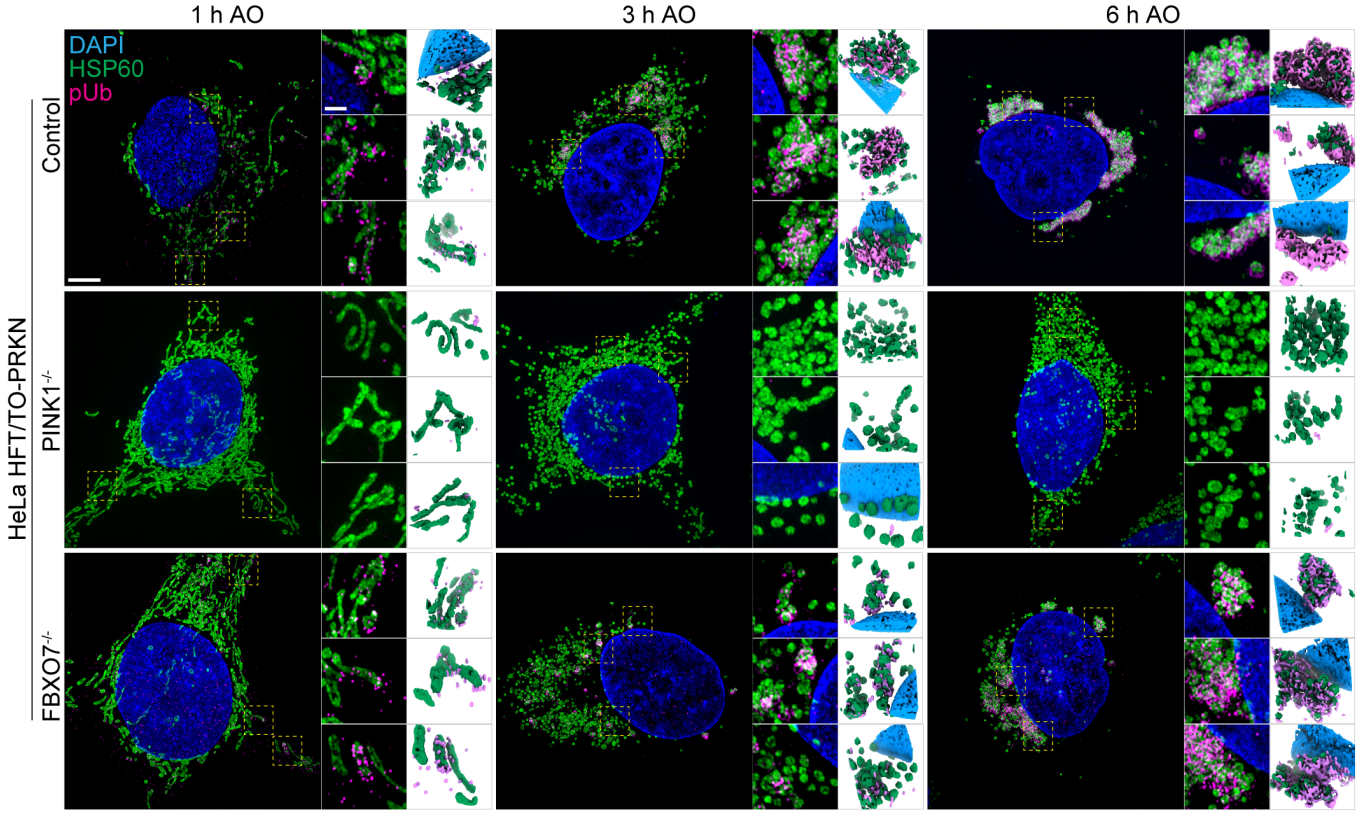

Figure EV1. Super-resolution pUb detection in HeLa cells in response to mitochondrial depolarization.

3D-SIM images of HeLa control, PINK1$^{-/-}$ and FBXO7$^{-/-}$ cell lines after 1 h, 3 h, or 6 h, AO-induced mitophagy (related to Fig 2A and B). Cells were stained for nuclear DNA (DAPI), mitochondria (HSP60) and pUb. Note that 1 h AO panel is reused from Fig 2A for side-by-side comparison. Scale bar = 5 μm or 1 μm.

Figure EV2. Parkin and autophagy regulator recruitment in FBXO7$^{-/-}$ cells.

A Overview of methods for analysis of Parkin recruitment to damaged mitochondria.

B Cells were imaged for GFP-Parkin, nuclei, and mitochondria and segmented to facilitate analysis of Parkin recruitment to mitochondria in single cells/mitochondrial organelles.

C Std mitochondrial signal vs. Parkin intensity and Std Parkin vs. mitochondria with or without 1 h AO-induced mitophagy are shown. Data from three replicates, including a total of 238 image stacks, containing 16,888 cells and 237,848 mitochondrial objects.

D Recruitment of p62 to mitochondria (not stained) in control or FBXO7$^{-/-}$ HeLa cells with or without treatment with AO (16 h) was examined by confocal imaging. Scale bar = 10 and 5 μm.

E, F Quantification of cells in panel (D). Assays were performed in biological triplicate with 4 image stacks taken per repeat. n = 6,712 cells. Error bars depict SD from triplicate experiments from two independent clones. One-way ANOVA with multiple comparisons; ***P = 0.0002, *P = 0.0355, **P = 0.0029, ****P < 0.0001.

G Quantification of number of p62 foci normalized per cell in control, PINK1$^{-/-}$ and FBXO7$^{-/-}$ cells with or without 3 h AO treatment. Assays were performed in biological triplicate with 4 image stacks taken per repeat. n = 12,646 cells. Error bars depict SD from triplicate experiments from two independent clones. One-way ANOVA with multiple comparisons; ****P < 0.0001.

H Control or FBXO7$^{-/-}$ HeLa cells expressing Parkin were either left untreated or incubated with AO for 1 or 6 h and extracts subjected to Western Blotting with α-LC3B and α-Actin as a loading control.

I The ratio of LC3B lipidation (LC3B-II/LC3B-I) was quantified from triplicate experiments from three independent clones. One-way ANOVA with multiple comparisons. *P = 0.0104.

J Immunofluorescence staining of WIPI2 (magenta) and Tom20 (gray) in HeLa Control and FBXO7$^{-/-}$ cells after mitophagy induction. Arrows depict WIPI2 foci on the mitochondrial staining. Scale bar = 10 μm (overview) and 10 μm (insets).

K Evaluation of mitochondrially localized WIPI2 foci per cell based on images shown in (J). Data based on 15 image stacks from three technical replicates of two independent clones. One-way ANOVA with multiple comparisons. *P = 0.0331.

L Immunofluorescence staining of HEK293T Control and FBXO7$^{-/-}$ cells expressing GFP-Parkin for HSP60 (mitochondria, magenta), pUb (gray) and GFP (Parkin, green) after mitophagy induction for indicated times. Scale bar = 10 μm. Number of individual cells analyzed per condition is indicted in the right bottom of each image.

M Analysis of pUb intensity (left) and Parkin intensity (right) in the mitochondrial mask. Error bars depict SEM from three biological replicates from two independent clones. 2-way ANOVA with multiple comparisons; **P = 0.001, ****P < 0.0001.

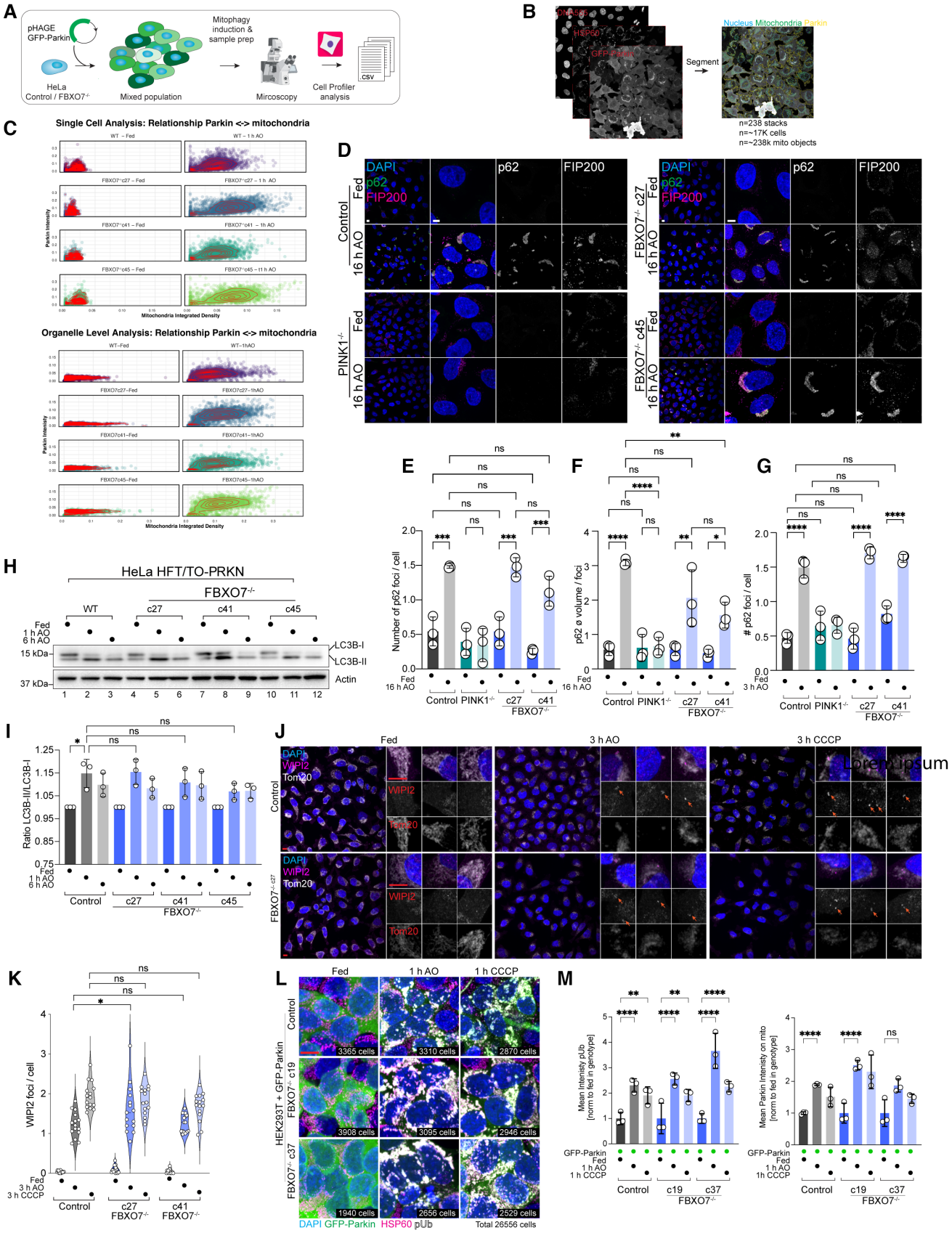
A
pHAGE GFP-Parkin
HeLa Control / FBXO7-/-
Mixed population
Mitophagy induction & sample prep
Mircoscopy
Cell Profiler analysis
csv
B
Segment
Nucleus Mitochondria Parkin
n=238 stacks
n=~17K cells
n=~238k mito objects
C
Single Cell Analysis: Relationship Parkin <-> mitochondria
Organelle Level Analysis: Relationship Parkin <-> mitochondria
Mitochondria Integrated Density
Parkin Intensity
D
DAPI
p62
FIP200
Control
PINK1-/-
FBXO7-/- c27
FBXO7-/- c45
Fed
16 h AO
E
Number of p62 foci / cell
F
p62 ø volume / foci
G
p62 foci / cell
Control PINK1-/- c27 c41
FBXO7-/-
H
HeLa HFT/TO-PRKN
FBXO7-/-
WT c27 c41 c45
Fed
1 h AO
6 h AO
15 kDa
37 kDa
LC3B-I
LC3B-II
Actin
I
Ratio LC3B-II/LC3B-I
Control c27 c41 c45
FBXO7-/-
J
Fed 3 h AO 3 h CCCP
DAPI
WIPI2
Tom20
Control
FBXO7-/- c27
Lorem ipsum
K
WIPI2 foci / cell
Fed 3 h AO 3 h CCCP
Control c27 c41
FBXO7-/-
L
Fed 1 h AO 1 h CCCP
HEK293T + GFP-Parkin
Control
FBXO7-/- c19
FBXO7-/- c37
3365 cells
3310 cells
2870 cells
3908 cells
3095 cells
2946 cells
1940 cells
2656 cells
2529 cells
DAPI GFP-Parkin HSP60 pUb
Total 26556 cells
M
Mean Intensity pUb [norm to fed in genotype]
Mean Parkin Intensity on mito [norm to fed in genotype]
GFP-Parkin
Fed
1 h AO
1 h CCCP
Control c19 c37
FBXO7-/-

Figure EV2.

Figure EV3. Interaction analysis of FBXO7.

A Interaction network analysis of PINK1 (left) and FBXO7 (right) based on interaction proteomics data from our BioPlex Interactome (Huttlin *et al*, 2021) (see Materials and Methods for details). Figure key can be found beneath panel.
B Summary of major interactions observed for FBXO7 in the context of the relevant protein complexes.
C Size-exclusion chromatography traces of indicated FBXO7 complexe on Superdex 200 Increase 10/300 GL.
D SDS–PAGE gel analysis of fractions of SKP1-FBXO7 complexes. Box colors match traces from (C).
E Ridgeline plots of mtKeima-shift analysis by flow cytometry in HeLa control, PINK1$^{-/-}$ and FBXO7$^{-/-}$ cell lines treated with AO at the indicated times. All lines are normalized to the BafA sample.

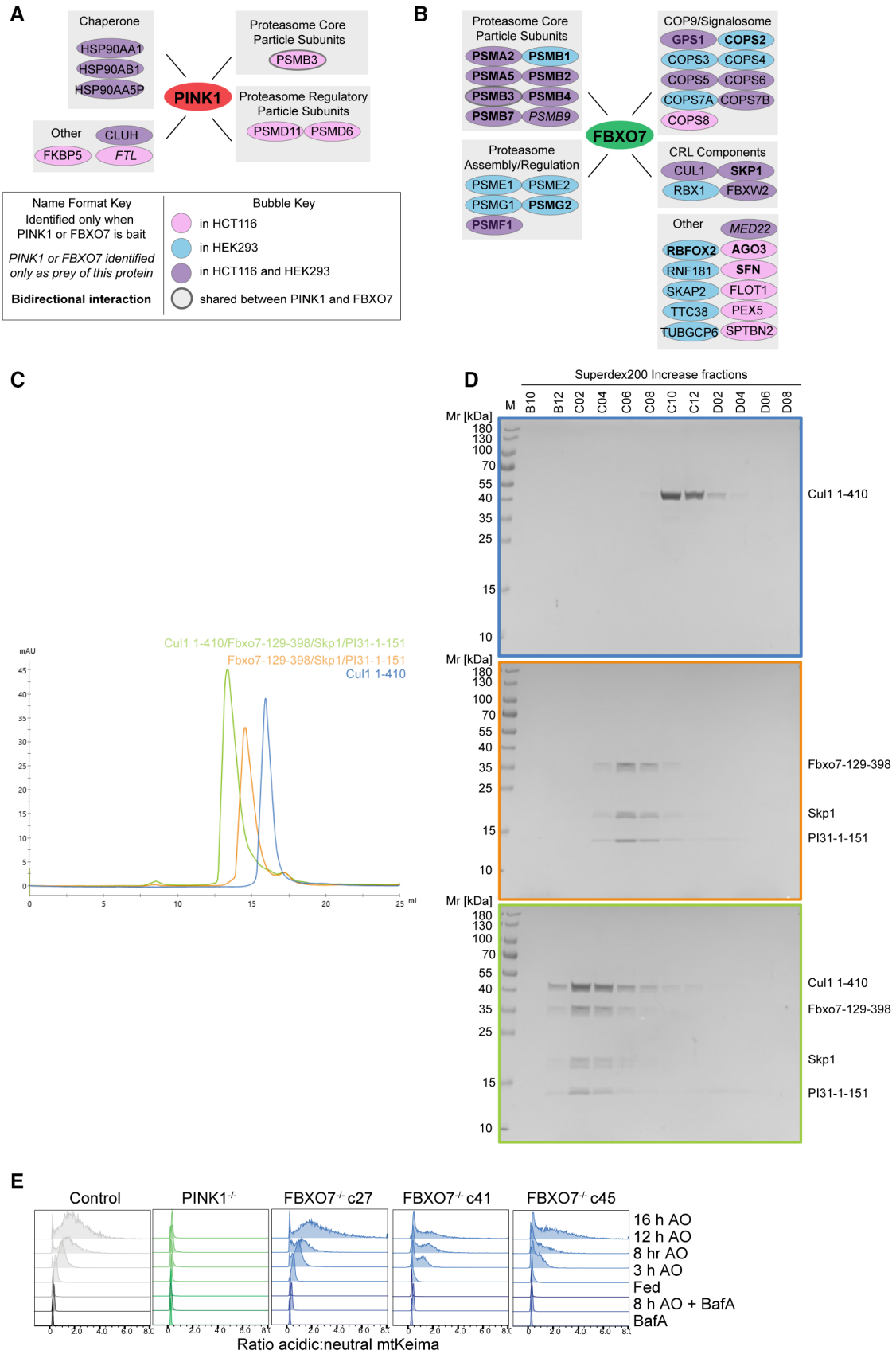
A
Chaperone
HSP90AA1
HSP90AB1
HSP90AA5P
Other
CLUH
FKBP5
FTL
PINK1
Proteasome Core Particle Subunits
PSMB3
Proteasome Regulatory Particle Subunits
PSMD11
PSMD6
Name Format Key
Identified only when PINK1 or FBXO7 is bait
PINK1 or FBXO7 identified only as prey of this protein
Bidirectional interaction
Bubble Key
in HCT116
in HEK293
in HCT116 and HEK293
shared between PINK1 and FBXO7
B
Proteasome Core Particle Subunits
PSMA2 PSMB1
PSMA5 PSMB2
PSMB3 PSMB4
PSMB7 PSMB9
Proteasome Assembly/Regulation
PSME1 PSME2
PSMG1 PSMG2
PSMF1
FBXO7
COP9/Signalosome
GPS1 COPS2
COPS3 COPS4
COPS5 COPS6
COPS7A COPS7B
COPS8
CRL Components
CUL1 SKP1
RBX1 FBXW2
Other
MED22
RBFOX2 AGO3
RNF181 SFN
SKAP2 FLOT1
TTC38 PEX5
TUBGCP6 SPTBN2
C
Cul1 1-410/Fbxo7-129-398/Skp1/PI31-1-151
Fbxo7-129-398/Skp1/PI31-1-151
Cul1 1-410
mAU
ml
D
Superdex200 Increase fractions
M B10 B12 C02 C04 C06 C08 C10 C12 D02 D04 D06 D08
Mr [kDa]
180 130 100 70 55 40 35 25 15 10
Cul1 1-410
Fbxo7-129-398
Skp1
PI31-1-151
E
Control
PINK1-/-
FBXO7-/- c27
FBXO7-/- c41
FBXO7-/- c45
16 h AO
12 h AO
8 hr AO
3 h AO
Fed
8 h AO + BafA
BafA
Ratio acidic:neutral mtKeima

Figure EV3.

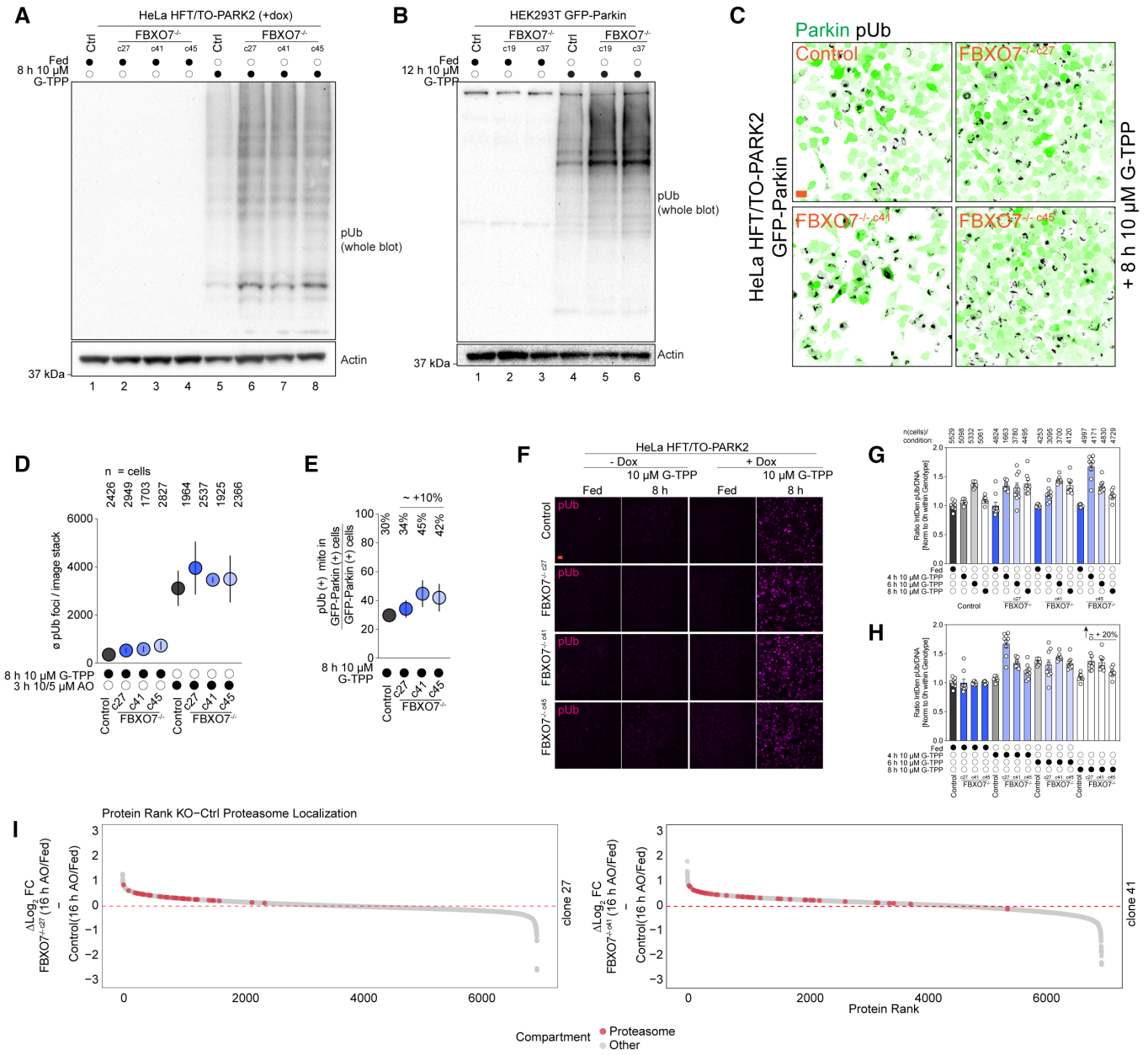

Figure EV4. Analysis of FBXO7$^{-/-}$ response to G-TPP.

A Western Blot for pUb of HeLa Control and FBXO7$^{-/-}$ cells after mitophagy induction using G-TPP for indicated times.

B Western Blot for pUb of HEK293T Control and FBXO7$^{-/-}$ cells after mitophagy induction using G-TPP for indicated times.

C Immunofluorescence images of HeLa Control and FBXO7$^{-/-}$ cells after G-TPP treatment. Staining for Parkin (GFP, green) and pUb (gray) are shown. Scale bars = 10 µm.

D, E Evaluation of mitophagic response to G-TPP in Hela Control and FBXO7$^{-/-}$ cells, based on images from (C). Ø (average) number of pUb per image stack are plotted. Number of analyzed cells for each condition is indicted in (D). Error bars depict SEM. (E) Ratio of pUb-positive mitochondria in GFP-Parkin-positive cells divided by total number of GFP-Parkin-positive cells as proxy for mitophagy induction rate is plotted. Ø (average) % numbers for each genotype are indicted above the plot. Error bars depict SEM.

F Immunofluorescence images of HeLa Control and FBXO7$^{-/-}$ cells after G-TPP timecourse treatment ± doxycycline (= Parkin expression). Staining for pUb (magenta) is shown. Scale bars = 25 µm.

G, H Quantification of pUb/DNA integrated density normalized to 0 h treatment within genotype. Panel (H) depicts the measurements sorted by treatment time. *N* (cells) analyzed per condition is indicated at the bottom of the graph. Error bars depict SEM.

I Ranked protein abundance of Δ log2 (KO[16 h AO/Fed]—Control [16 h AO/Fed]) for FBXO7$^{-/-}$ clone 27 (top) and clone 41 (bottom). Proteasomal annotated proteins are depicted red, all other detected proteins in gray.