

Appendix for:

PARK15/FBXO7 is dispensable for PINK1/Parkin mitophagy in iNeurons and HeLa cell systems

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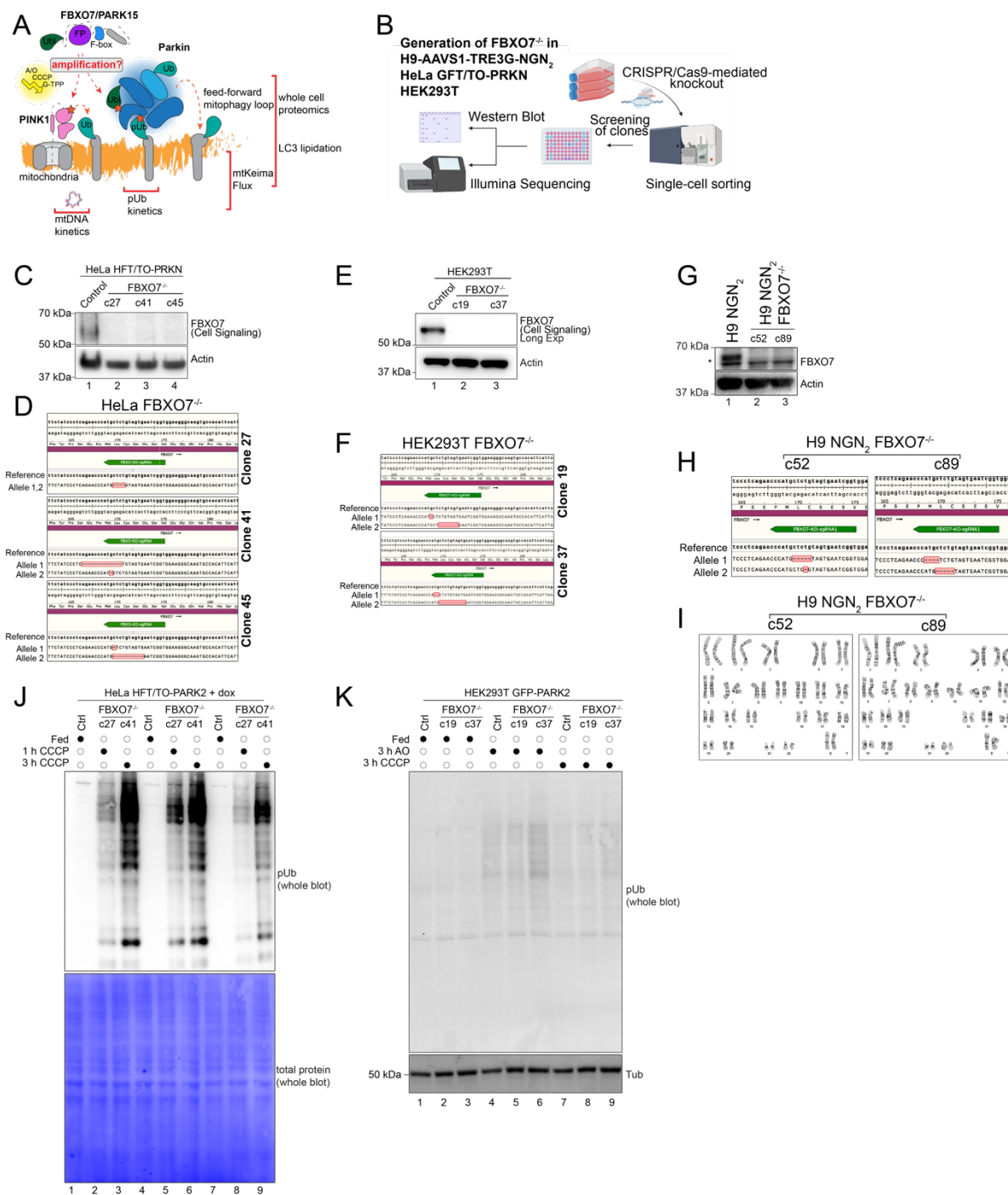
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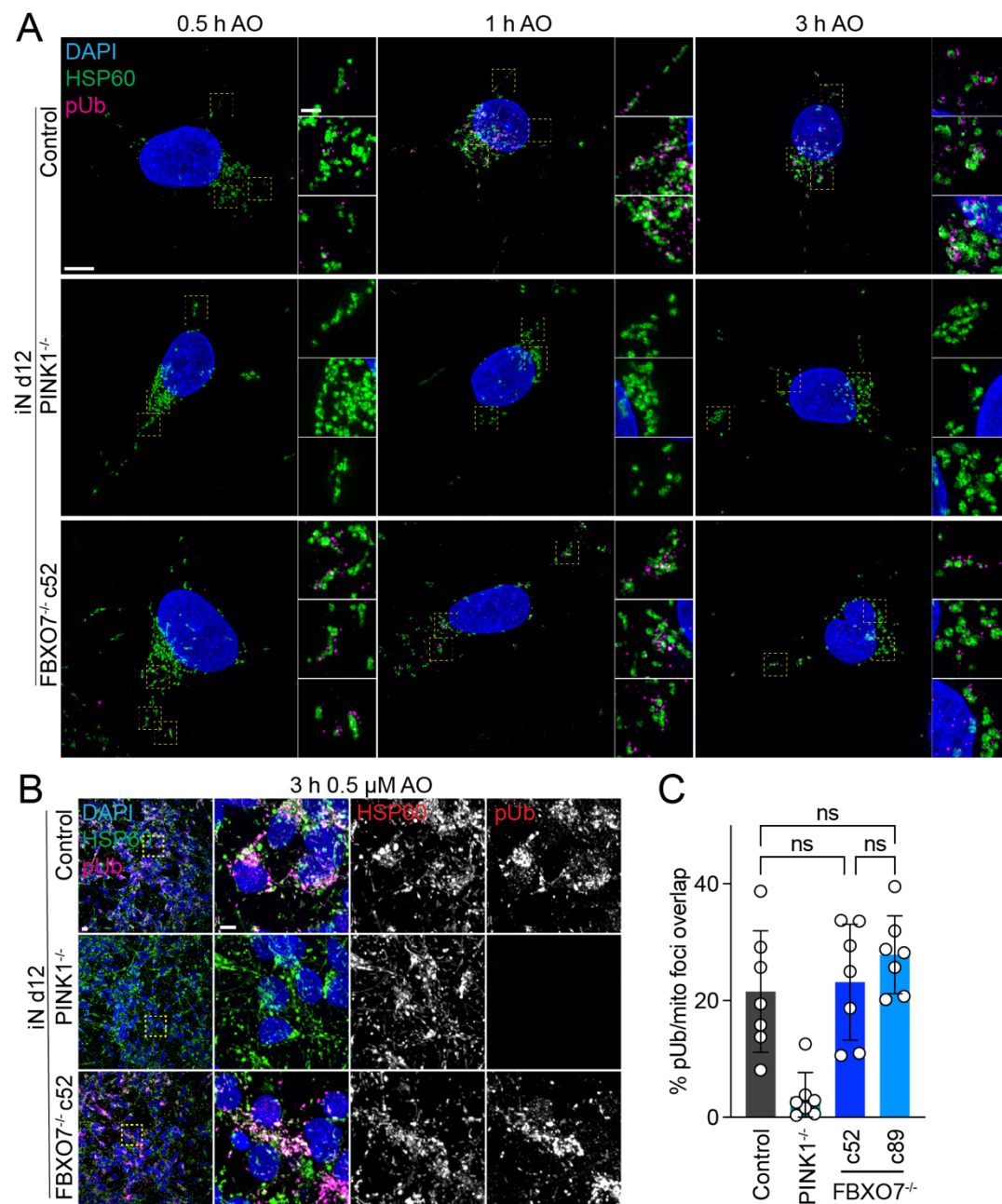
Appendix Figure S1



Appendix Figure S1: Tool kit for analysis of HeLa cells and iNeurons lacking FBXO7. (A) Working model summarizing the suggested modes of action of FBXO7 in PINK1/Parkin mitophagy. (B) Schematic for generation of FBXO7^{-/-} in HeLa and hESC cell lines. (C) Western Blot analysis on HeLa control and FBXO7^{-/-} whole cell lysate. (D) Targeting of gRNA sequence within FBXO7 gene along with the sequences of alleles A and B. MiSeq analysis of the three HeLa clones (c27, c41, c45) used for this study are shown. (E) Western Blot analysis of HEK293T control and FBXO7^{-/-} whole cell lysate. (F) MiSeq analysis of the two HEK293T clones (c19 and c37). (G) Western Blot analysis on hESC control and FBXO7^{-/-} whole cell lysate. Asterisk indicates non-specific band. (H) Targeting of gRNA sequence within FBXO7 gene along with the sequences of alleles A and B. MiSeq analysis of the two clones (c52, c89) used for this study are shown. (I) Karyotype analysis of FBXO7^{-/-} c52 and c89 hESCs. (J)

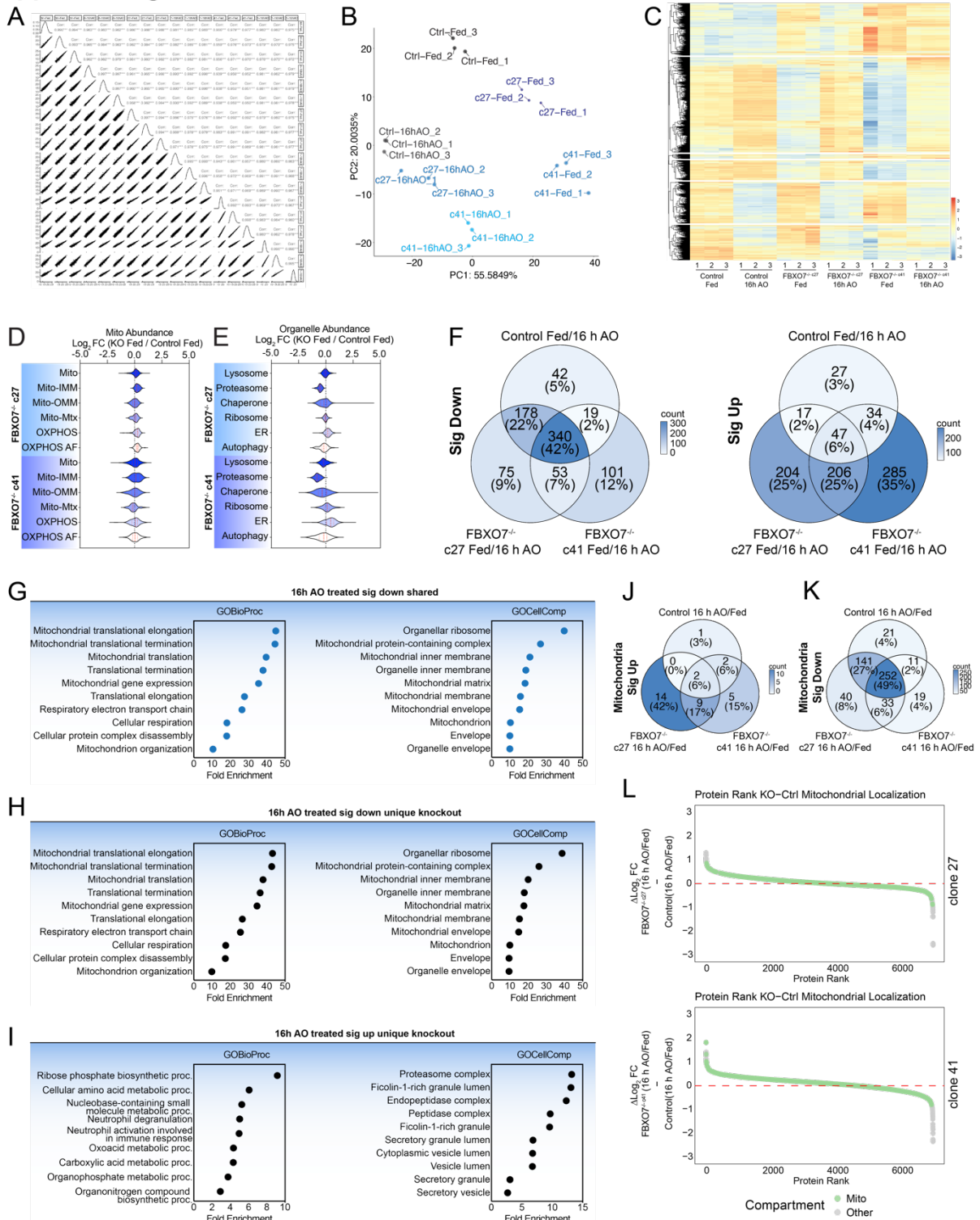
Western Blot of HeLa Control and FBXO7^{-/-} cells after mitophagy induction using CCCP for indicated times. (K) Western Blot of HEK293T Control and FBXO7^{-/-} (expressing GFP-Parkin) after mitophagy induction after indicated times.

Appendix Figure S2



Appendix Figure S2. Super-resolution pUb detection in iNeurons in response to mitochondrial depolarization. (A) 3D-SIM images of iNeurons day 12 control, PINK1^{-/-} and FBXO7^{-/-} cell lines after AO-induced mitophagy. Cells were stained for nuclear DNA (DAPI), mitochondria (HSP60) and pUb. Related to **Figure 2D**. Scale bar = 5 μ m or 1 μ m. (B) Confocal images of iNeurons day 12 control, PINK1^{-/-} and FBXO7^{-/-} cell lines after AO-induced mitophagy with lower AO concentrations. Cells were stained for nuclear DNA (DAPI), mitochondria (HSP60) and pUb. (C) Evaluation of images depicted in **B**. Error bars depict S.D. from 12 image stacks measured per condition. One-way ANOVA with multiple comparisons. Scale bar = 10 μ m and 5 μ m.

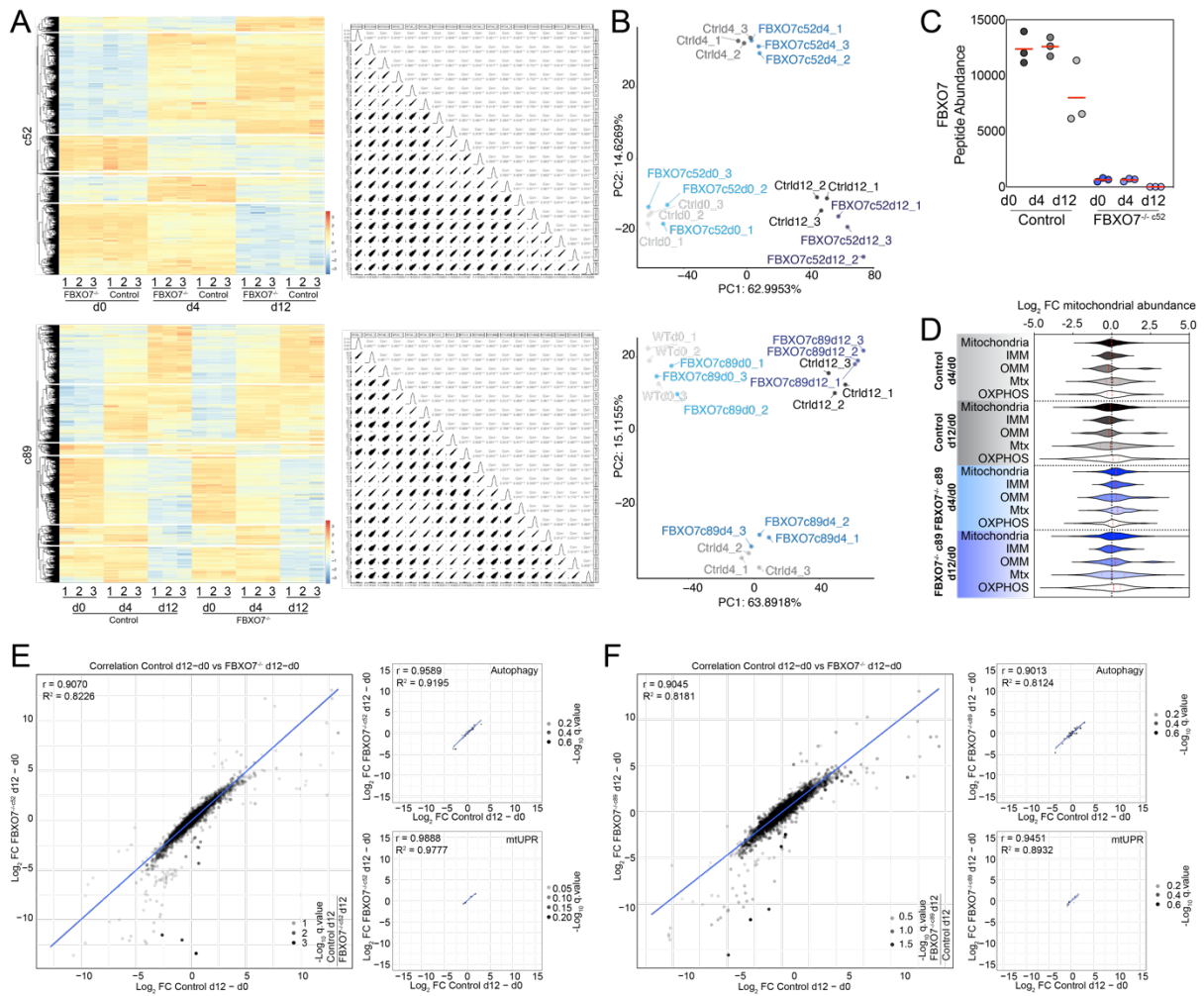
Appendix Figure S3



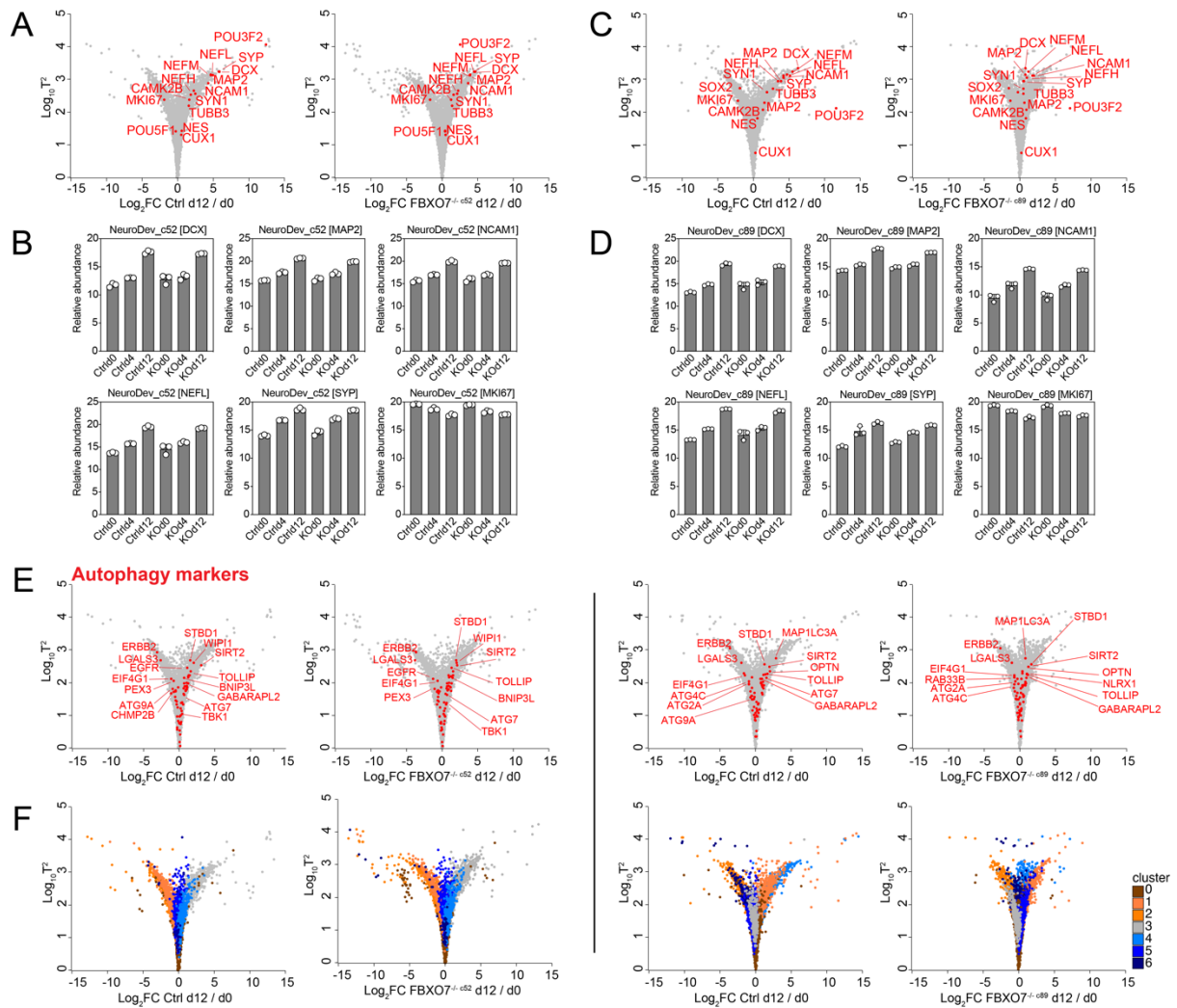
Appendix Figure S3. Proteomic analysis of HeLa cells lacking FBXO7 during mitophagy. (A,B) Correlation plots (panel A) and PCA analysis (panel B) for ~8000 proteins quantified by TMT proteomics in individual replicates for the experiment outlined in **Figure 4A**. (C) Hierarchical clustering of 8000 proteins quantified in the experiment outlined in **Figure 4A**. (D,E) Violin plots [$\text{Log}_2(\text{control} / \text{FBXO7}^{-/-})$] for FBXO7^{-/-} c27 and c41 cells depicting alterations in the abundance of mitochondrial proteins (left panel) or specific organelles or protein complexes (right panel). (F) Venn diagrams depicting the overlap in the number of proteins whose levels are reduced (left panel) or increased (right panel) for the data shown in **Figure 4B** are shown. Proteins whose abundance is significantly reduced upon AO treatment are

enriched for the mitochondrial organelle (right panel), as expected for cells undergoing mitophagy. **(G)** GO-term enrichment analysis of genes significantly down in both Control and FBXO7^{-/-} after 16 h AO mitophagy. **(H,I)** GO-term enrichment analysis of unique genes significantly down (panel H) or up (panel I) in FBXO7^{-/-} after 16 h AO mitophagy. **(J,K)** Venn diagrams depicting the overlap in the number of mitochondrial proteins whose levels are significantly increased **(J)** or decreased **(K)** are shown. The plurality of proteins which are significantly decreased, are shared in all cell lines. **(L)** Ranked protein abundance of ΔLog_2 (KO[16 h AO/Fed] – Control [16 h AO/Fed]) for FBXO7^{-/-} clone 27 (top) and clone 41 (bottom). Mitochondrial annotated proteins are depicted green, all other detected proteins in grey.

Appendix Figure S4



Appendix Figure S4. Proteomic analysis of human ES cells lacking FBXO7 during neurogenesis. (A) Hierarchical clustering and correlation plots of control and FBXO7^{-/-} c52 (top) or c89 (bottom) at indicated days of differentiation in the experiment outlined in Figure 5A. **(B)** PCA analysis of whole cell proteomic samples from control and FBXO7^{-/-} cells. Triplicates for each timepoint and genotype were used. **(C)** FBXO7 peptide abundance in control and FBXO7^{-/-} cells at indicated days of differentiation. **(D)** Violin plots [Log₂ (control / FBXO7^{-/-})] for FBXO7^{-/-} c89 cells depicting alterations in the abundance of mitochondrial proteins. **(E,F)** Correlation plot of d12 / d0 of Control vs FBXO7^{-/-} cells (**E**: clone 52; **F**: clone 89). Autophagy and mtUPR proteins are plotted on the right. Pearson's *r* correlation and R² of KO vs Ctrl are indicated on the top left corners.



Appendix Figure S5. Comparison of human ES cell neurogenesis with or without FBXO7. (A) Hotelling plots ($\text{Log}_{10} T^2$ statistic versus $\text{Log}_2 \text{FC}$ day 12/day 0) of control (left panel) or $\text{FBXO7}^{-/-}$ c52 hES cells undergoing differentiation. Selected neurogenesis factors are indicated. (B) The relative abundance of selected proteins is shown in the lower histograms at day 0, 4 and 12 of differentiation. (C,D) Hotelling plots similar to A,B for control and $\text{FBXO7}^{-/-}$ c89. (E) Patterns of changes in the abundance of autophagy proteins in control (left panel) and $\text{FBXO7}^{-/-}$ (right panel) day 12 iNeurons relative to day 0 cells displayed on a Hotelling plot. (F) Cluster analysis of control and $\text{FBXO7}^{-/-}$ cells comparing day 12 of differentiation to day 0. Individual clusters are displayed on Hotelling plots for each genotype.