

Supplementary information

Cell cycle analysis by flow cytometry

Cell cycle from HEK293T control and CLN3-KO cells were synchronize through an overnight starvation using HBSS. After the synchronization, HBSS was replaced by complete medium for 0 and 9 hours, and then washed and fixed with cold ethanol (70%) for 15 min on ice. Cells were next washed and treated with ribonuclease. Propidium iodide (PI) was added with a final concentration of 30 µg/mL.

Data were acquired in a BD FACSCalibur flow cytometer (Becton Dickinson): PI fluorescence was measured in the PI channel (585/42 BP). The software for acquisition was BD FACSDiva, and data from at least 5000 cells were analysed with FlowJo.

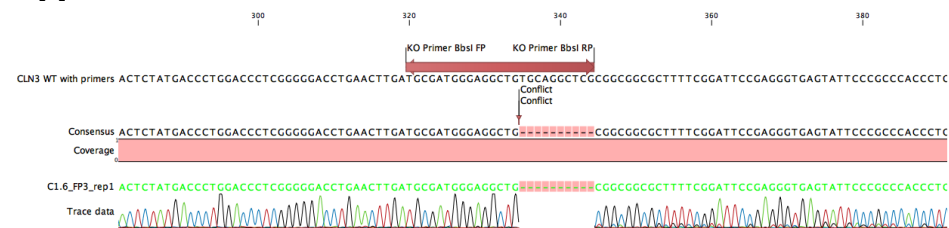
Lysosomal pH and degradative activity

For lysosome pH, cells were loaded with dextran conjugated with FITC (100 µg/mL, Merck, 60842-46-8) and dextran conjugated with Alexa Fluor 647 (60 µg/mL, ThermoFisher, D22914) overnight, followed by a 4h chase before treatment with 100 nM bafilomycin for 1h. Before cells imaging, cells were treated for 15 min with Magic-red (MR, Bio-Rad, ICT937) at 37°C. Cells were live-imaged by confocal microscopy in presence of 1 mM of HEPES in complete DMEM without phenol-red.

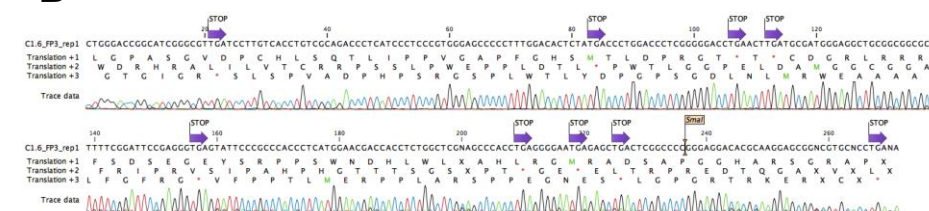
To measure the fluorescence intensity ratio between FITC/ Alexa Fluor 647-dextran and MR/ Alexa Fluor 647, a threshold was applied to Alexa Fluor 647-dextran channel to define the lysosomes through the analyse particle macro from ImageJ Fiji software. The background was removed from the obtained fluorescence values.

Figure S1 – Characterization of CLN3KO cell line

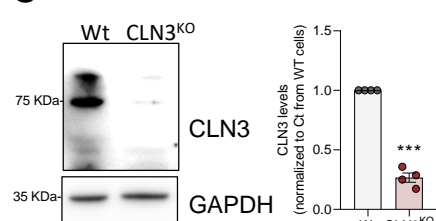
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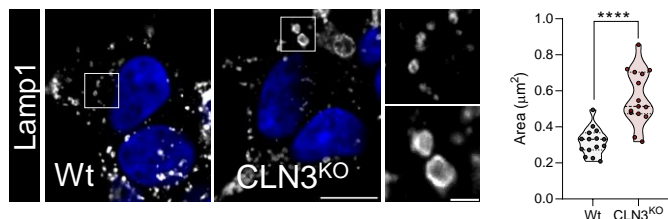
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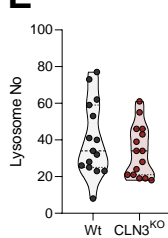
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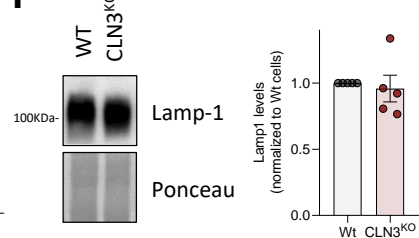
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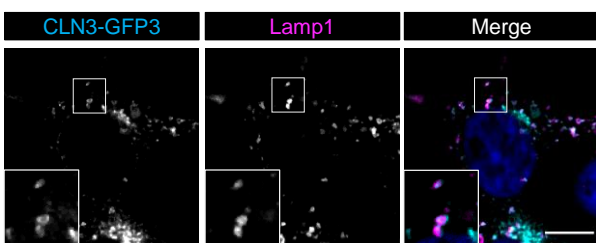
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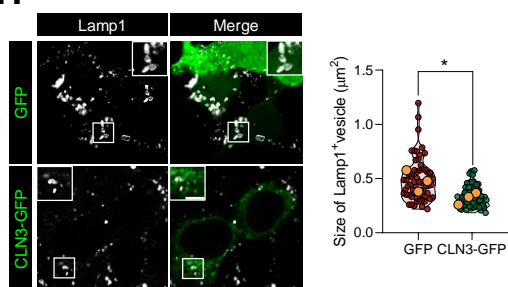
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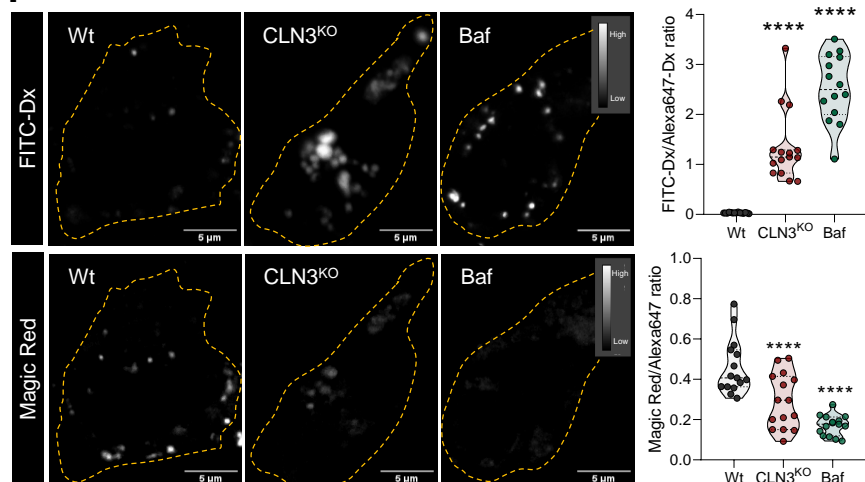
G



H



I



J

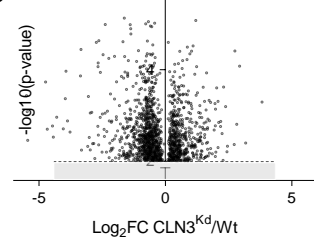


Figure S1 Characterization of HEK293T CLN3-KO cell line.

(A) Schematic representation of sequence deleted in CLN3 encoding gene. **(B)** Putative translation sequences showing the premature stop codons. **(C)** Representative immunoblot image and quantification of CLN3 protein levels in Wt and CLN3^{KO} cells. GAPDH and ponceau were used as loading controls. **(D)** Confocal fluorescence images of HEK293T Wt and CLN3^{KO} cells immunostained for Lamp1 protein and the respective quantification of lysosomal area. Scale bar 10 μ m and 2 μ m in the inset images. **(E)** Quantification of total number per cell in HEK293T Wt and CLN3^{KO} cells. **(F)** Representative immunoblot image and quantification of Lamp1 protein levels in Wt and CLN3^{KO} cells. Ponceau were used as loading controls. **(G)** Confocal fluorescence images of cells HEK293T parental line transfected with CLN3-GFP and immunostained for Lamp1. Scale bar 10 μ m and 2 μ m in the inset images. **(H)** Confocal fluorescence images of cells HEK293T CLN3^{KO} transfected with CLN3-GFP and immunostained for Lamp1. Quantification of lysosomal size from at least 50 HEK293T CLN3^{KO} cells transfected with GFP or CLN3-GFP. **(I)** Representative fluorescence images of Wt and CLN3^{KO} cells loaded with FITC-dextran (Dx), sensitive to pH, and magic red fluorescence, a substrate of cathepsin B. Scale bar 10 μ m. The graphs represent the quantification of FITC-dextran (on the top) and magic red fluorescence (on the bottom) normalized to Alexa647-dextran, not pH sensitive. At least 15 cells were analysed. All the results are mean \pm SEM of at least three independent experiments. * p<0.05; ***p<0.001; **** p<0.0001; using unpaired t-test or Dunnett's multiple comparisons test. **(J)** Differentially expressed genes (DEG) between HEK293T wild type (Wt) and CLN3 knockdown (CLN3^{KO}) are represented as a volcano plot: log p-value adjTtest in the y-axis and log₂FC in the x-axis. The transcripts overexpressed in KO are to the right of the plot, and on the left, the repressed genes.

Figure S2

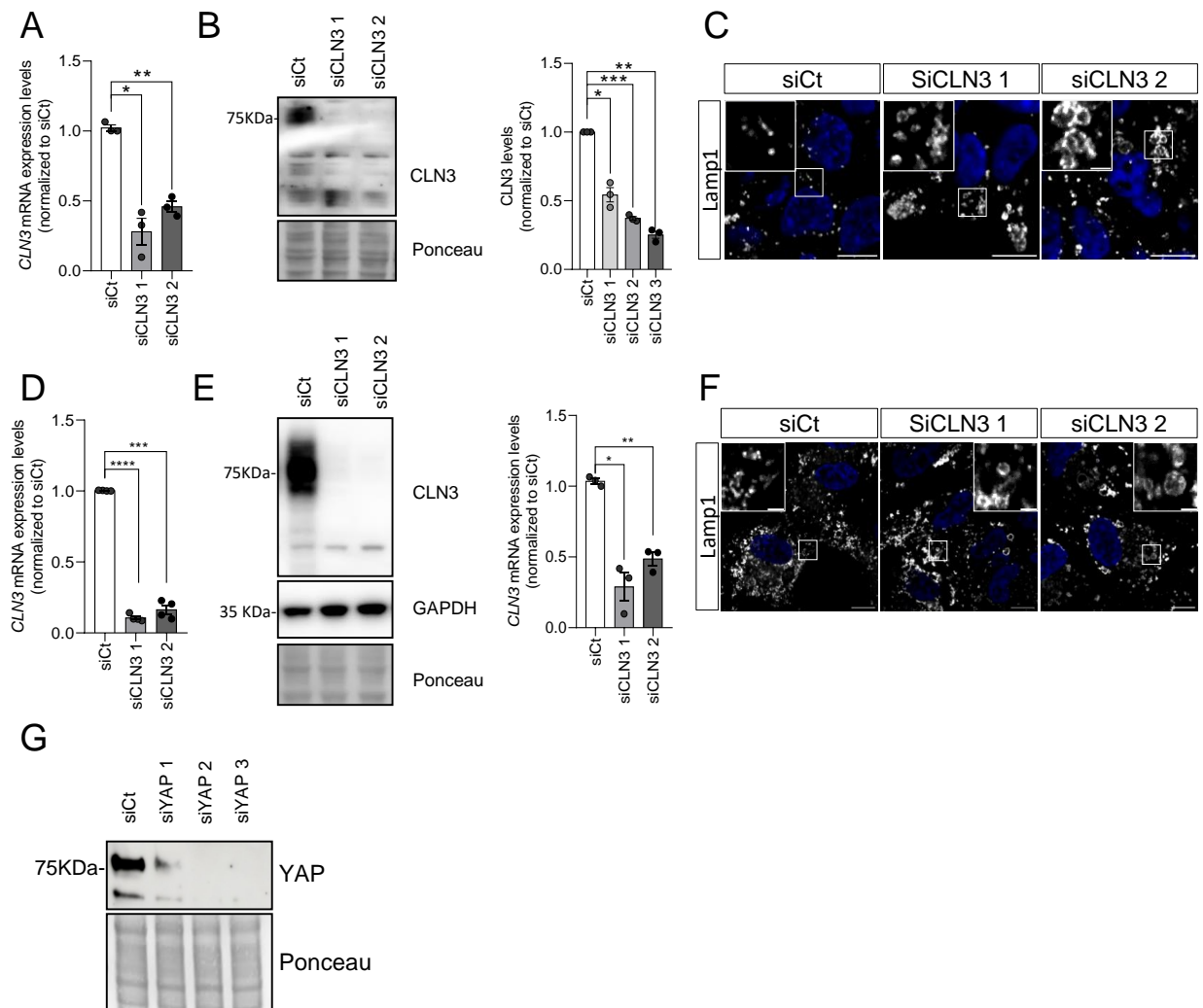


Figure S2 Characterization of transient modulation of CLN3 and YAP levels in HEK293T parental line cell line.

(A) mRNA levels of CLN3 gene in HEK293T parental line transfected with siCt or two different siRNA against CLN3 transcript. (B) Representative immunoblot image and quantification of CLN3 protein levels in the conditions described in (A). Ponceau was used as loading controls. (C) Confocal fluorescence images of cells under the conditions described in (A). Scale bar 10µm and 2µm in the inset images. (D) mRNA levels of CLN3 gene in ARPE19 parental line transfected with siCt or two different siRNA against CLN3 transcript. (E) Representative immunoblot image and quantification of CLN3 protein levels in the conditions described in (D). Ponceau was used as loading controls. (F) Confocal fluorescence images of cells under the conditions described in D. Scale bar 10µm and 2µm in the inset images. (G) Representative immunoblot image YAP protein levels from HEK293T parental line transfected with siCt or three different siRNA against YAP transcript. Ponceau was used as loading controls. All the results are mean±SEM of at least three independent experiments. * p<0.05; **p<0.01; ***p<0.001; **** p<0.0001; using Dunnett's multiple comparisons test.

Figure S3

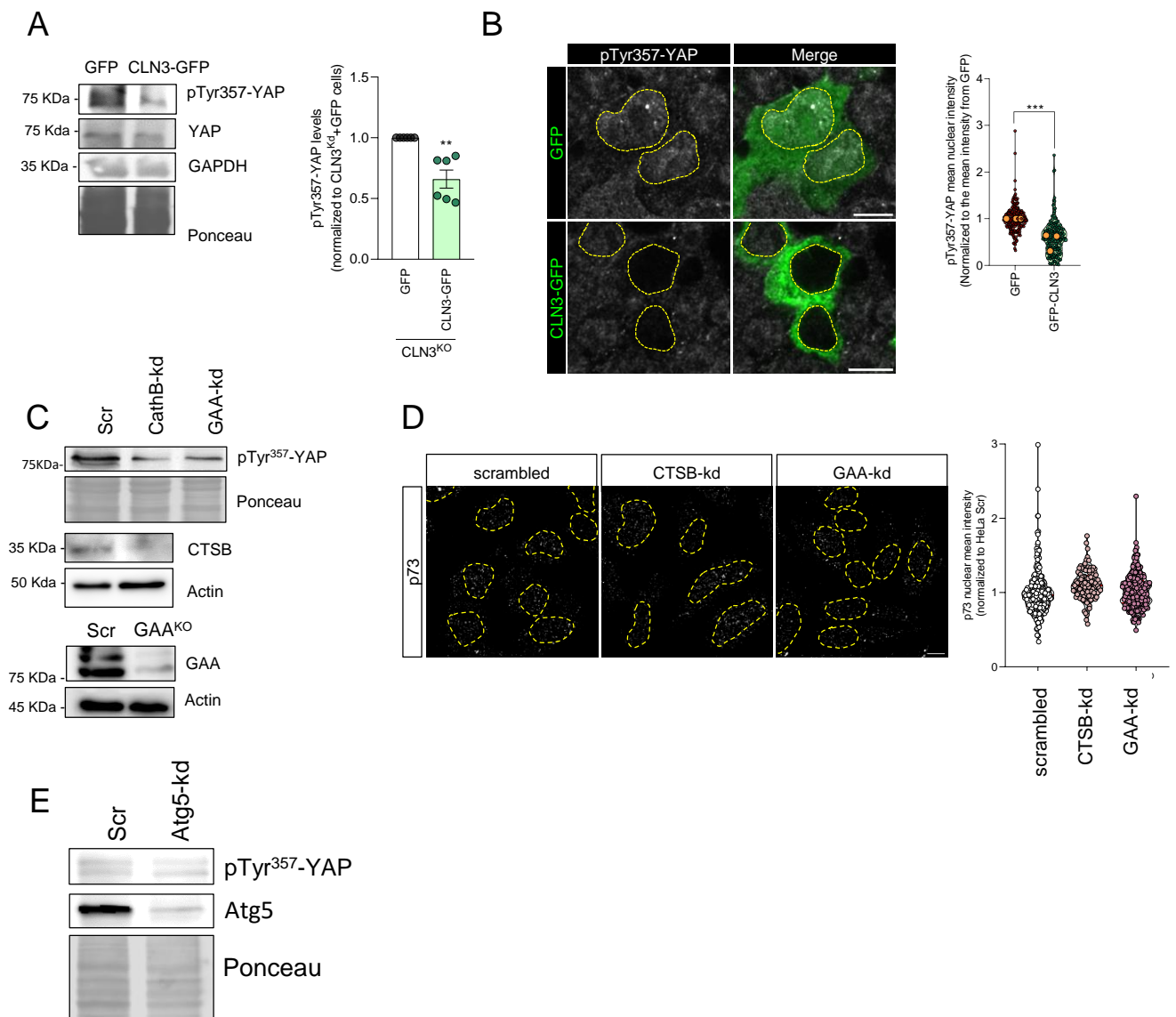


Figure S3 Restoring CLN3 levels in HEK293T CLN3-KO cells led to a decrease in YAP phosphorylation at the Tyr357 residue.

(A) Representative immunoblot image and quantification of pTyr357-YAP in CLN3^{KO} cells transfected with GFP or CLN3-GFP treated. GAPDH and Ponceau were used as loading controls. (B) Confocal fluorescence images and quantification of cells under the conditions described in A immunostained for pTyr357-YAP. Scale bar 10 μ m. At least 150 nucleus were analysed. The results are mean \pm SEM of at least three independent experiments. ** $p < 0.01$; *** $p < 0.001$ using t-test. (C) Representative immunoblot image of pTyr357-YAP in control, Cathepsin-B knock-down (CTSB-kd) and acid alpha-glucosidase knock-down (GAA-kd) HeLa cells. Protein depletion was also confirmed by CTSB and GAA immunoblotting. Actin, GAPDH or Ponceau were used as loading controls. (D) Confocal fluorescence images and quantification of cells under the conditions described in C immunostained for p73. (E) Representative immunoblot image of pTyr357-YAP in control and Atg5 knock-down (Atg5-kd) HeLa cells.

Figure S4

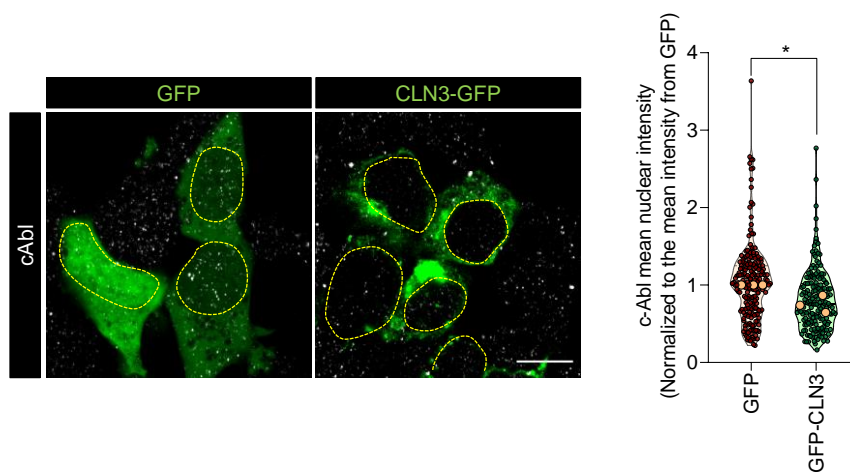


Figure S4 Restoring CLN3 levels in HEK293T CLN3-KO cells led to a decrease in c-Abl recruitment to the nucleus.

(A) Confocal fluorescence images and quantification of CLN3^{KO} cells transfected with GFP or CLN3-GFP treated and immunostained for c-Abl. Scale bar 10 μ m. At least 150 nucleus were analysed. All the results are mean \pm SEM of at least three independent experiments. * $p < 0.05$; ** $p < 0.01$; using t-test.

Figure S5

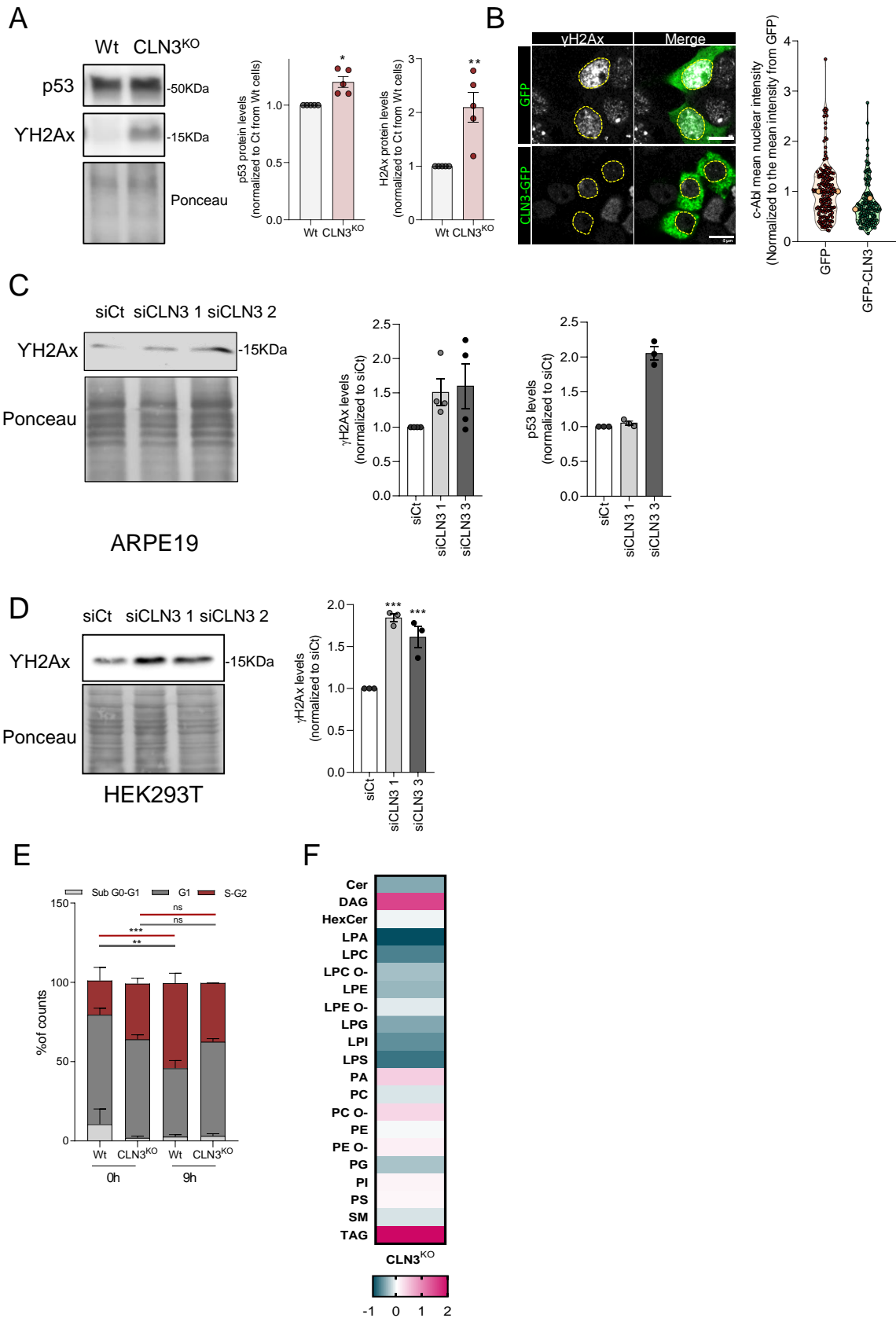


Figure S5 Loss of CLN3 function induces increase in DNA damage and cell cycle arrest possible by affecting nuclear lipidome.

(A) Representative immunoblot image and quantification of p53 and YH2AX in nuclear extracts from HEK293T parental and CLN3-KO cell line. Ponceau was used as loading controls. (B) Confocal fluorescence images and quantification of CLN3^{KO} cells transfected with GFP or CLN3-GFP treated and immunostained for YH2AX. Scale bar 10µm. At least 150 nucleus were analysed. (C-D) Representative immunoblot image and quantification of p53 and YH2AX in ARPE19 (C) or HEK293T (D) parental cell line transfected with siCt or two siRNA against CLN3 transcripts. Ponceau was used as loading controls. (E) Cell cycle quantification of Wt and CLN3^{KO} cells stained with propidium iodide (PI) after cells synchronization with an overnight starvation. The percentage of cells in each cycle phase was quantified by flow cytometry (three independent experiments). (F) Heat map of lipid classes affected in the nucleus of CLN3-KO when compared with HEK293T parental cell line. All the results are mean±SEM of at least three independent experiments. * p<0.05; **p<0.01; ***p<0.001; using t-test or Dunnett's multiple comparisons test.

Figure S6

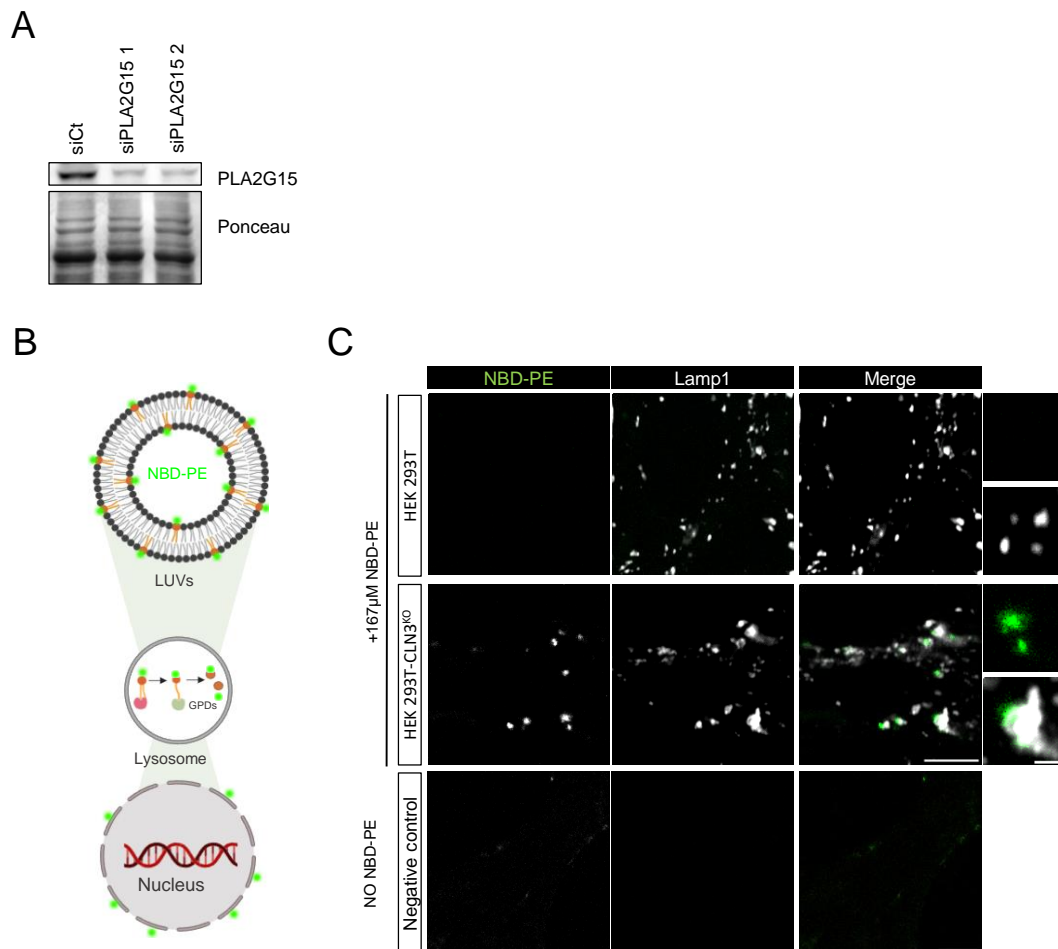


Figure S6 Confirmation of PLA2G15 depletion and NBD-PE distribution in CLN3-KO cells.

(A) Representative immunoblot image of PLA2G15 in HEK293T parental cell line transfected with siCt or two siRNA against PLA2G15 transcripts. Ponceau was used as loading controls. (B) Schematic representation of the experimental approach using large unilamellar vesicles (LUVs) containing NBD-PE fluorescent lipids. LUVs will be internalized by endocytosis and lipids degraded by lysosomal lipases. We hypothesised that products resulting from fluorescent lipids degradation will be detected at nuclear envelope. (C) Representative confocal images of Wt and CLN3-KO cells loaded with NBD-PE-containing liposomes and immunostained for Lamp1. Scale bar 10µm and 2 µm in the insets.

Table 1 – List of human primers and siRNA sequences.

<i>Primers</i>	<i>Sequences (5'-3')</i>
h_PUMA_F	ATCAATCCCATTGCATAGGTTTAG
h_PUMA_R	ACTAAGGCTGGGGCGCTTC
h_TP53AIP1_F	GGCTCAGACACACACACCT
h_TP53AIP1_R	GGCCTGTCTCTAAGCACTGT
h_Bax_F	ATGTTTTTCTGACGGCAACTTC
h_Bax_R	ATCAGTTCGGCAACCTTG
h_TP73_F	CCCACCACTTTGAGGTCCT
h_TP73_R	GGCGATCTGGCAGTAGAGTT
h_DR5_F	GTGATTCAGGTGAAGTGGAGC
h_DR5_R	CGACCTTGACCATCCCTCTG
hYAP1_F	TAGCCCTGCGTAGCCAGTTA
hYAP1_R	TCATGCTTAGTCCACTGTCTGT
<i>siRNA</i>	<i>Sequences (5'-3')</i>
h_siCLN3_1	UUGUUCUUUCAAGGUCUAUUCUUUAUAGACCUUGAA
h_siCLN3_2	GCAGUACCGAUGGUACCAUAGCAUCUGGUACCAUCG
h_siYAP_1	ACGGUAGAUUUACUGACAAUUCAUCAGAUAAUUAU
h_siYAP_2	GCUGCCACCAAGCUAGAUUUUCUUUAUCUAGCUUGG
h_siPLA2G15_1	GUAUCUGGAUUCUGGCAAACUUUAUUGCCAGAAUC
h_siPLA2G15_2	AGACCGAAAGCUACUUCACAGAUUGUGAAGUAGCUU

Table 1 – List of murine primers.

S16-F	AGGAGCGATTTGCTGGTGTGG
S16-R	GCTACCAGGGCCTTTGAGATG
Cycloph-F	GGCAAATGCTGGACCAAACACAA
Cycloph-R	GTAAAATGCCCGCAAGTCAAAAG
mYAP1-RT-F	CGGCAGTCCTCCTTTGAGAT
mYAP1-RT-R	GGTCCTGCCATGTTGTTGTC
mTP53-RT-F	AACTATGGCTTCCACCTGGG
mTP53-RT-R	TGAGGGGAGGAGAGTACGTG
mBBC3-RT-F	GGATGGCGGACGACCTCAA
mBBC3-RT-R	TCGGTGTGATGCTGCTCTT
mBAX-RT-F	ATCCAAGACCAGGGTGGCTG
mBAX-RT-R	TCACTGTCTGCCATGTGGGG
mTrp73-RT-F	TCACCTTCCAGCAGTCGAGC
mTrp73-RT-R	TGGATGGGGCATGTCTTAGCA
mDr5-RT-F	AGCCCATCAAGAGGACCCTG
mDr5-RT-R	AGGCTTGCAGTTCCTTCTGA
mcd68-RT-F	TCAGCTGCCTGACAAGGGAC
mcd68-RT-R	GCAGCAAGAGGGACTGGTCA
mCln3ACFOR	CCTCCAGGAAAGGTGGACAG
mCln3ACREV	GCTCGAAAAGTCCCTGGTTG