Supplementary data and methods

Title: Role of endothelial micronuclei-contained DNA in atherosclerosis

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Methods

Cell culture and treatments

HAoECs (passage 2,3; C-12271, PromoCell) and MAoECs (passage 3; PELOBiotech) cell lines were cultured in endothelial cell complete growth medium (Promocell) containing gentamicin (0.05 mg/ml; ThermoFisher). All cells were grown at 37°C and 5% CO_2 in a humified incubator. MAoECs and HAoECs were treated or not with 25, 50, or 100µg/ml of human oxLDL (437644, Sigma-Aldrich) for 4, 24, and 48h, unless stated otherwise. Notably, MAoEC response to treatments or transfection requires 6-12h compared to HAoECs, which require almost 24-48h (7, 23). MAoECs and MAoECs were treated 4, 24, and 48h with 25, 50, or 100µg/ml oxLDL. To inhibit DNA-PKCs activity, LTURM34 (S8427) or AZD7648 (S8843, both Biozol) chemical inhibitors were used alone or in combination with 50µg/ml oxLDL at different, serial-diluted, concentrations (5, 2.5, 1, 0.5, 0.09, and 0.025 µM).

Viral cell transduction for live track of cell cycle

To obtain ECs expressing fluorescently labelled cell cycle markers, HAoECs (3.5 x10^5/ml) were cultured in gelatin-coated 8-well chambers (80841, Ibidi) and infected with 40 viral particle/ cell (PPC) of a second generation BacMam virus containing the Premo FUCCI cell cycle sensor Cdt1-*TagRFP* and geminin-*emGFP* genomic constructs, concomitantly with BacMam Enhancer. After an overnight, cells were incubated 2h with NucBlue Live ReadyProbes Reagent (R37605, ThermoFisher) and live tracked to follow MN formation, cell cycle phases, and DNA synthesis using a Leica TCS SP8 Confocal microscope equipped with a 37°C and 5% CO_2 chamber.

Simulation of low and high shear stress

HAoECs and MAoECs were cultured in collagen-coated perfusion chambers (µ-Slides VI$^{0.4}$, ibidi GmbH) and exposed to low shear stress (LSS, 5 dyne cm^2) or high shear stress (HSS, 10 dyne cm^2) for 48h generated by a pump system (Ibidi) for the perfusion with EC complete medium containing 10 µM 5-ethynyl-2'-deoxyuridine (EdU, Click-iT® EdU Alexa Fluor® 488 Imaging Kit, Life Technologies).

Cell culture staining

HAoECs were treated as described before. At the end of the experiments, cells were fixed and permeabilized using the ViewRNA cell plus fixation/permeabilization buffer set (00-19001, Invitrogen) according to manufacturer's instruction. Cells were blocked with VieWRNA Cell Plus Blocking/Antibody diluent for 30 min and antibodies (**STable1**) incubated using the same diluent overnight in a humified chamber. For Nup17 and Nu153 staining (**STable1**), cells were fixed 30 min in 2% PFA, permeabilized 25 min with 0.3% Triton X-100, and incubated with primary antibodies overnight in a humified chamber. Cells were incubated with appropriate fluorescently labelled secondary antibodies (**STable1**) and mounted with mounting media with DAPI (50011, Ibidi).

Animal models

Apoe$^{-/-}$ mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used for experiments. Male and female littermates were equally used for experiments. Eight to ten-week-old mice were fed a chow-diet (normal diet, ND), comprising 3.3% crude fat and 19% protein (CD.88137, ssniff Spezialdiäten GmbH), or a high fat diet (western diet, WD), comprising 22% crude fat, 1% corn oil, 34.4% sugar, and 0.21% cholesterol (D12079, ssniff Spezialdiäten GmbH) for 4 or 12 weeks. All animal experiments were reviewed and approved by the local authorities (State Agency for Nature, Environment and Consumer Protection of North Rhein-Westphalia and District Government of Upper Bavaria) in accordance with German animal protection laws. At the end of the experiments, mice were anesthetized with ketamine (150 mg/kg) and Xylazin (30 mg/kg). After perfusion with PBS from the left ventricle, all tissues were collected and fixed with paxgene or 4% paraformaldehyde (PFA), and paraffin embedded for further experiments.

En face immunostaining of murine arches and thoracic aortae

To analyse MN formation, DNA replication and damage, DDR markers, and cGAS at predilection and non-predilection atherosclerosis sites, aortic arches, and thoracic aortae from 4- and 12-week WD- or ND-fed mice were fixed in paxgene and *en face* prepared. After antigen retrieval, tissues were cooled down, blocked with Blocking solution and incubated with selective antibodies (**STable1**). Tissues were embedded in VECTASHIELD® Antifade Mounting Medium with DAPI (H-1200-10, Vector Labotatories). *Z*-stacks images were acquired using a Leica TCS SP8 Confocal microscope equipped with a 63x oil objective. At least 4 mice per group were used.

Data regarding MN formation were expressed as number of CD31+ cells containing MN, in percentage. Double positive staining was always normalized on total CD31^{+} cells first, then on the appropriate fraction. The analysis of the staining was performed in a blinded manner.

3D image reconstruction and Imaris spatial triple co-localization with heterochromatin

For detailed study of γH2AX and H3K9me3 co-localizaton with Dapi bright or weak foci at the MN periphery, three-dimensional confocal laser scanning microscopy (CLSM) was performed with a Leica SP8 3X microscope equipped with a 63×/1.40 (Leica) oil immersion objective. Optical zoom was used where applicable. A UV laser (405 nm) was used for excitation of DAPI or NucBlue. A tuneable white light laser for selective excitation of Star635P, AlexaFluor488, and Cy3 fluorochromes was used for the detection of γH2AX, H3K9me3, and Lamin B1. All data were acquired in three dimensions and voxel size was determined according to Nyquist sampling criterion. Image reconstructions were performed using the LAS X software package v.3.0.2 (Leica) and deconvolution was performed combining the Huygens Professional software package v.19.10 (Scientific Volume, Hilversum, The Netherlands) using the unsupervised CLSM algorithms. Co-localization analysis were performed using the Imaris 8.4.2 software equipped with the imaging processing toolbox MATLAB. Spatial distribution of automatically calculated protein voxels (1.4µm spots) was defined according to the voxel localization to the nuclear surface, which corresponded to the nuclear membrane surface and total nuclear volume calculated using the spatial distribution of the Lamin B1 and DAPI, respectively (1.328µm surface, 14µm volume thresholds). A voxel value of 0.5 µm was used as threshold to discriminate closed versus far from membrane γH2AX, H3K9me3, and DAPI bright foci. The number of DAPI weak foci was estimated by calculating the number of γH2AX and H3K9me3 foci closed to the surface lacking a co-localization with DAPI bright foci. The analysis of the staining was performed in a blind manner from 4 independent experiments.

Size gated FACS sorter for MN and PN isolation

To set up the FACS-sorter, HAoECs were seeded on gelatin-coated 75 cm^2 flasks and treated or not with Nocodazole (6h, 200 ng/ml), a chemical inhibitor of microtubule polymerization and of the mitotic spindle that blocks cells in cytokinesis, synchronizes the cell cycle, and induces MN formation. At the end of treatments, cells were enzymatically collected with Accutase (Sigma-Aldrich), cell pellet washed with PBS, and resuspended in Lysis 1. Cells were immediately pulse vortexed for 15sec and incubated 1h at room temperature (RT). Cell suspension was then incubated 1h with Lysis 2. Appropriate fluorescent molecules were added in Lysis 1 and/or 2 according to the experiment: 7-AAD (1:1000, cat nr. Abcam), Hoechst 33342 (1:1000, 62249), SYTOX Orange (1:2000, S34861, ThermoFisher) for dead cell discrimination; SYTO 13 (S7575), SYTO 85 (S11366), or SYTO 40 (S11351) (all 1:2000, ThermoFisher), for intact cells/nuclei selection. Size gates were defined using nonfluorescent microspheres with the following diameters: 1, 2, 4, 6, 10, and 15 µm (Flow Cytometry Size Calibration Kit, F13838, ThermoFisher). A 200 threshold was used to include 1-2 µm size, but 1 µm were excluded. Identity of MN and PN was confirmed by 3D stack confocal microscopy before and after sorting.

Live track and staining of MN engulfment

Recipient HAoECs were treated 48h with or without 50µg oxLDL, alone or in combination with cGAS LNA-GapmeRs or CMV-cGAS vector. Donor HAoECs were treated 48h with or without 50µg oxLDL, alone or in combination with cGAS LNA-GapmeRs. MN from donor cells were labelled with SYTO 13 and FACS sorted as described before. Recipient cells were incubated with NucBlue and Cell Tracking Red Dye (ab269446, Abcam) for Live imaging 2h before the ending of the treatment. At the end, media of recipient cells was replaced and incubated with SYTO 13 MN (300 MN/µl) and cells live tracked to record the entrance and fate of "donor" MN using a Leica TCS SP8 Confocal microscope equipped with a 37°C and 5% CO_2 chamber. The data points oh 1,2,4, and 6h were considered according to MN engulfment and reincorporation in the PN observed at 6h. At 12 and 24h cells incubated with oxLDL-deriving MN showed significant morphological changes like apoptotic cells. Therefore, cells were incubated with donor MN till 6h. Subsequently, recipient cells were incubated with NucBlue 2h before the ending of the treatments, then incubated with donor MN till 1,2,4, and 6h. At the end of each incubation time, cells were fixed and permeabilized with ViewRNA cell plus fixation/permeabilization buffer set and incubated with β-catenin (cell membrane marker), cGAS, and Lamin B1 antibodies as indicated before. Z stack images were acquired using a Leica TCS SP8 Confocal microscope equipped with a 63×/1.40 (Leica) oil immersion objective. Images were processed with Huygens and visualised with Imaris Viewer (v9.9.1) as indicated above.

Caspase 3/7 enzymatic assay during MN engulfment

Recipient HAoECs were cultured on gelatin-coated white flat 96-well plates, treated as indicated above, and incubated or not with donor MN as described before. The activity of caspase-3 was assessed by incubating cells at 37°C with a luminogenic caspase-3 substrate containing the tetrapeptide sequence DEVD (Caspe-Glo 3/7 Assay System, G8091, Promega). Luminescence was measured after 1,2,4, and 6h and is proportional to the amount of caspase activity present. Luminescence was measured in a plate luminometer (Infinite® 200 PRO, Tecan). Six technical replicates per condition were performed for each of the 3 biologically independent experiments.

Reactive oxygen species (ROS) formation

ROS production was measured using the OxiSelect Intracellular ROS Assay Kit (STA-342, Cell Biolabs, San Diego, CA, USA) according to manufacturer's instructions. Briefly, HAoECs were seeded on gelatin-coated 96-well plates and treated or not 4, 24, and 48h with 25, 50, or 100 µg/ml oxLDL. Then, cells were incubated with 2', 7'-Dichlorodihydrofluorescin diacetate (DCFH-DA) during the last 30 min of treatment. Cells were washed with PBS to remove any DCFH-DA excess and fluorescence analysed at 485 nm excitation/530 nm emission using a fluorescent plate reader (Infinite® 200 PRO, Tecan, Männedorf, Switzerland). ROS levels were expressed as DCFH-DA relative fluorescence intensity and reported as fold change of the control.

Protein isolation from ECs incubated with MN

Recipient HAoECs were treated and incubated with donor MN for 6 or 24h as described before. At the end of the experiment, cells were washed once with PBS and lysed in RIPA Buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease (cOmplete™ ULTRA Tablets, Mini, EDTA-free, 05892791001, Roche) and phosphatase (PhosSTOP, 4906837001, Roche) inhibitor cocktails. Cell lysates were incubated on ice for 30 min and supernatants collected by centrifugation of 15min at 30000 x g 4°C. Protein determination was performed using the DC Protein Assay Kit (5000111, BioRad). Protein lysates were then mixed with 4x LDS (NuPAGE™ LDS Sample Buffer 4X, NP0007, ThermoFisher) and 0.5 mM DTT (D0632, Sigma-Aldrich), and boiled for 10 min at 95°C. The same procedure was applied for isolation of total proteins.

De novo MNeic RNA synthesis in vitro and in mice

HAoECs were seeded and transfected as indicated before. One hour before the ending of treatments, cells were incubated with 1mM 5-ethynyl uridine (EU), then fixed 30 min with 2% PFA, and permeabilized 15 min with 0.5% Triton X-100. EU labelling was made according to manufacturer's instruction, followed by co-staining with Lamin B1 and γH2AX antibodies (**STable1**) to detect whether RNA synthesis occurred in DNA damage-enriched MN and PN. Nuclei were stained using a mounting media with DAPI (Ibidi). Images were acquired using a Leica TCS SP8 Confocal microscope.

To analyse *de novo* MNeic RNA synthesis *in vivo*, ApoE$^{-/-}$ mice were fed a WD for 12 weeks and intravenously injected with 100µl of a 20mg/ml solution of EU in PBS. Arches were harvested 5h after injection and fixed in paxgene. *En face* tissues were stained with TMR-azide according to manufactures instructions, with some modifications. Tissues were co-stained with CD31, Lamin B1, γH2AX (**STable1**), and mounted with Vectashield + DAPI. *Z*-stack images were acquired by Leica TCS SP8 Confocal microscope.

WGS of FACS-sorted MN and PN

HAoECs and MAoECs were seeded on gelatin-coated 75 cm^2 flasks and treated or not for 24 and 48h with 50µg oxLDL. At the end of the treatment, cells were processed as described before to isolate MN and PN by FACS-sorter, with some modifications. Briefly, SYTO 13 was used as fluorescent marker to isolate total MN and PN for DNA, whereas fluorescently labelled Ki67 or γH2AX were supplemented to Lysis 2 to isolate MN and PN with an active DNA synthesis (24h) or damage (48h), respectively. For DNA isolation, the fractions from 2 to 8 µm (MN) and from 15 to 20 µm (PN) were pooled into two sub-fractions. DNA was isolated using the QIAamp DNA micro kit (56304, Qiagen) and quality analysed by agarose gel. WGS was performed by BGI Genomics (Hong Kong, China) that generated the cDNA library using a low input WGS library preparation kit (Kapa Hyper Library Prep kit), followed by a DNBseq Low input WGS (PE100bp, 90Gb). Due to low DNA amount obtained from Ki67$^+$ and γH2AX$^+$ MN and PN, DNA was pre-amplified through a MDA amplification step, followed by Low

input WGS library preparation (Kapa Hyper Library Prep kit) and a DNBseq Low input WGS (PE100bp, 90Gb).

Bioinformatic WGS analysis of chromosome segregation, mutations, and pathway enrichment

Identification of functional annotation for Human Copy Number Variants (CNVs) was made using the InCAS tool from Mendel Institute (https://incas.css-mendel.it). Structural variant (SV) prediction to discover, genotype and visualize deletions, tandem duplications, inversions and translocations at single-nucleotide resolution in short-read and long-read massively parallel WGS data was made using Delly (24). Pathway identification of genes containing SVs was performed using the Enrichr tool (25-27).

Nucleus and cytoplasm protein isolation

For nucleus and cytoplasm protein isolation, cells were lysed in ice-cold Buffer A supplemented with protease and phosphatase inhibitors and incubated on ice for 5 min. Cytoplasm fraction was collected by centrifugation at 800 x g for 10 min 4°C. Pellet was resuspended in *Buffer B* containing protease and phosphatase inhibitor cocktails, followed by 300mM NaCl addition and sonication 3 times for 5 sec at Amplitude 10%. Nuclear proteins were incubated 30 min on ice and collected by centrifugation at 30000 x g for 40 min at 4°C. Proteins were quantified by DC Protein Assay, and boiled for 10 min at 95°C.

Protein isolation from FACS-sorted MN and PN

MN and PN were sorted, and sub-fractions pooled as described before. MN and PN proteins isolated using an approach like nuclear proteins, with some modifications. Proteins were concentrated using the Protein-Concentrate Micro Kit (2100, Millipore), resuspended with LDS (NuPAGE™ LDS Sample Buffer 4X, NP0007, ThermoFisher), 0.5 mM DTT (D0632, Sigma-Aldrich), and boiled for 10 min at 95°C. Cooled proteins were directly separated on SDS-PAGE as described below. Densitometry analysis was performed with ImageJ (v1.53t) and data normalized on total number of MN and PN (data obtained during FACS sorting).

Immunoblotting

Twenty-to-thirty µg of total extracts, and 10 µg of nucleus and cytoplasm extracts, were separated on NuPAGE™ 4-12% Bis-Tris Protein Gels. For detection of high molecular weight (HMW) proteins, like P-DNAPKCs and DNA-PKCs, 25-30µg of proteins were loaded on NuPage 3-8% Tris-Acetate Protein Gels using a NuPAGE Tris-Ace SDS Running Buffer (20x). Proteins were transferred onto polyvinylidene difluoride (PVDF) 0.45µm (HMW) or 0.2µm (low MW) membranes (Millipore). Membranes were blocked 2h at room temperature with 10% milk or 10% BSA in TBS for total or phosphorylated proteins, respectively. Blots were incubated with primary antibodies at 4° overnight. Information on primary and secondary antibodies is provided in **STable1**. Membranes were washed 3 times (10 min each) with TBS-T (TBS plus 0.1% Tween-20). HRP-conjugated secondary antibodies were incubated 1h at room temperature. Membranes were washed 3 times with TBS-T and protein signals visualized with SuperSignal™ West Femto Maximum Sensitivity Substrate (34094, ThermoFisher) using an ImageQuant LAS 4000 scanner (GE Healthcare). Images were analysed using ImageJ. Densitometries were normalized on β-actin (total), Lamin B1 (nuclear), or α-Tubulin (Cytoplasm) housekeepers, unless stated otherwise. Data are represented as fold change of the control groups from 4 independent experiments.

Cytotoxicity assay of DNA-PKCs chemical inhibitors

HAoECs were treated 4, 24, and 48h with 50µg/ml oxLDL, alone or in combination with LTURM34 or AZD7648 at different, serial-diluted, concentrations (5, 2.5, 1, 0.5, 0.09, and

0.025 µM). Cytotoxicity of DNA-PKCs inhibitors was evaluated using the CyQUANT™ LDH Cytotoxicity Assay (fluorescence, C20302, Invitrogen) according to manufacturer's instructions (n=3 per group). Fluorescence was analysed using a fluorescent plate reader (Infinite® 200 PRO, Tecan, Männedorf, Switzerland), and data analysed according to manufacturer's instruction.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.5.0 (GraphPad Software, Inc., San Diego, CA, USA). Data were initially evaluated for normal distribution with the Shapiro-Wilk test. Comparisons between two groups with normally distributed variables were analysed by Student's unpaired *t* test. Not normally distributed variables were compared by the Mann–Whitney test. Analysis of variance (ANOVA) was used to determine the significant differences between more than two groups. In all cases, p values < 0.05 were considered significant.

STable1. Primary and secondary antibodies used for *in vitro* and *in vivo* applications

Antibody	**Cat. Nr.**	**Brand**	**Dilution**
gamma H2AX, pAb	A300-081A-M	Bethyl	1:500
Ki67 pAb	ab15580	Abcam	1:1000
PECAM-1 (H-3)	sc-376764	Santa Cruz	1:100
Lamin B1 (C-5)	sc-365962	Santa Cruz	1:200
Lamin B1 (B-10)	sc-374015	Santa Cruz	1:200
C6orf150 antibody (cGAS)	orb2965	Biorbyt	1:300
53BP1	NB100-305SS	Novus Biologicals	1:200
Phospho-ATM (Ser1981)	651201	Biolegend	1:500
Anti-DNA PKcs (phospho S2056) pAb	ab18192	Abcam	1:600
BRCA1 (1472) mAb	NBP2-53177	Novus Biologicals	1:200
H3K9me3	39161	Active motif	1:1000
Nup107	ABIN6990942	Antibodies-online	1:200
Nup153 (R4C8)	ab81463	Abcam	1:300
TRAF1		Abcam	1:600
CENP-B		Abcam	1:600

Antibody	**Cat. Nr.**	**Brand**	**Dilution**
pH2AX (Ser140)		Bethyl	1:600
Lamin B1 (A-11)	sc-374015	Santa Cruz	1:1000
Rad51 (14B4)	NB100-148	Novus Biologicals	1:500
Ku80 (111)	MA5-12933	ThermoFisher	1:500
NFATC2IP	PA5-99664	ThermoFisher	1:1000
SMC6 (A-3) mAb	sc-365742	Santa Cruz	1:500
beta-Actin (C4) HRP, mIgG1	sc-47778 HRP	Santa Cruz	1:4000
Phospho-DNA-PKcs (Ser2056) (E9J4G)	68716S	Cell Signalling	1:500
DNA-PKcs	4602T	Cell Signalling	1:500
Anti-rabbit IgG, HRP-linked	7074S	Cell Signalling	1:2000
Anti-mouse IgG, HRP-linked	7076S	Cell Signalling	1:2000

Antibody	**Cat. Nr.**	**Brand**	**Dilution**
anti-H2A.X Phospho (Ser139)	613408	Biolegend	1:300
Ki67	151204	Biolegend	1:200
Lamin B1 (B-10)	374015 AF594	SantaCruz	1:500

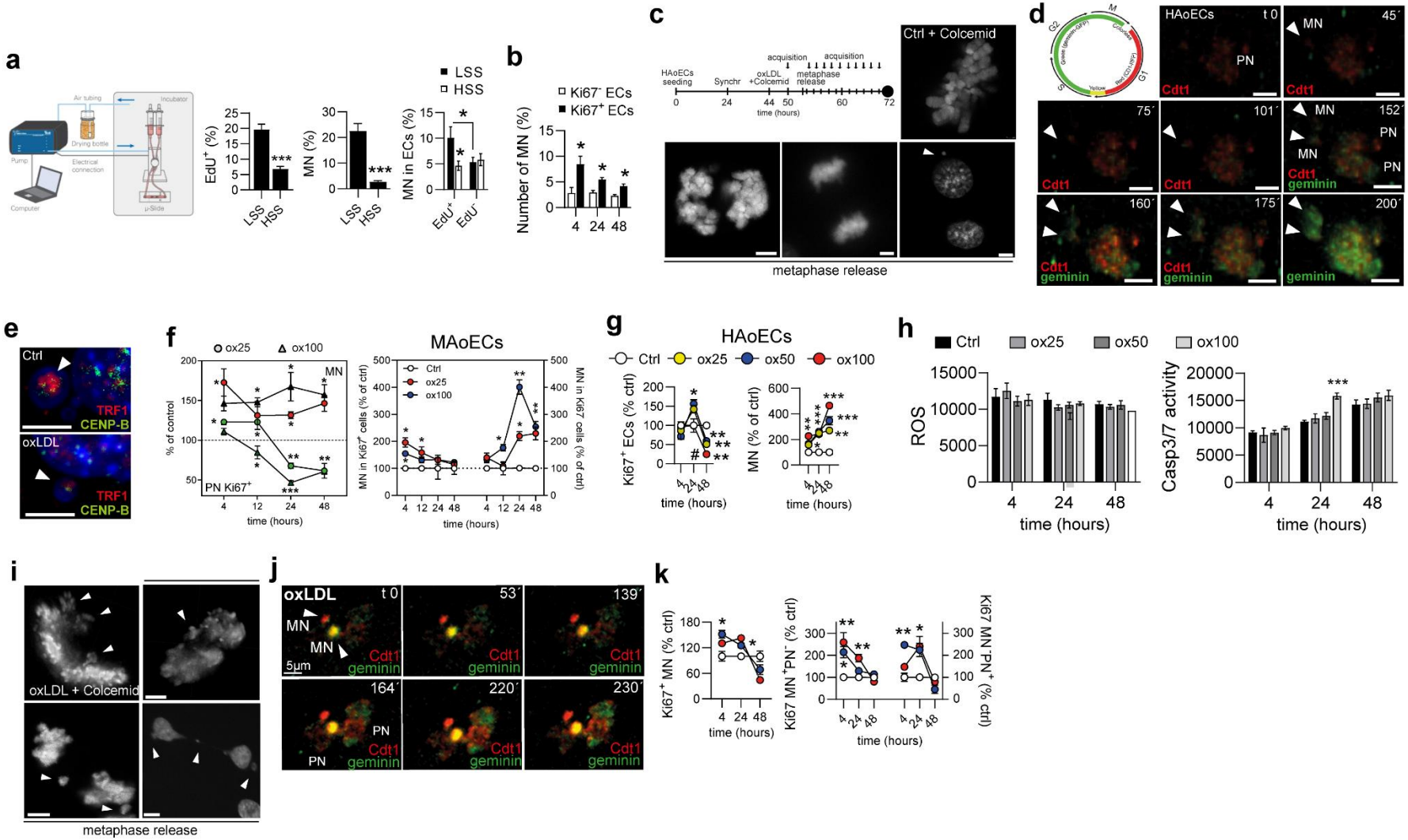
Suppl. Fig1. (a) MAoECs were cultured under LSS (5 dyne cm^{-2}) or HSS (10 dyne cm^{-2}) and incubated with 5-ethynyl-2´-deoxyuridine (EdU) to evaluate the percentage of proliferating ECs containing MN. Data are expressed as percentage of total ECs (n= 6-9 samples per group). **(b)** MAoECs were let grown for 4, 24, and 48h and stained for immunofluorescence detection and quantification of $Ki67^+$ ECs, and to analyse MN content. Data are normalized on total ECs. **(c)** Extended data on HAoECs synchronization and metaphase release with Colcemid to analyse the cell cycle phase or **(d)** the expression of cell cycle indicators of DNA synchronization. Images are representatives of 3 independent experiments. Scale bar: 10µm. **(e)** Representative images of telomerase (TRF1) and centromers (CENP-B) markers in HAoECs treated or not 48h with 50 µg/ml oxLDL. Images are representative of 3 independent experiments. **(f)** MAoECs and **(g,k)** HAoECs treated 4, 24, and 48h with 25, 50 or 100µg/ml oxLDL to analyse the number of Ki67+MN and the asynchronized DNA duplication of MN compared with their respective PN (Ki67 MN^+PN^- and MN^-PN^+) normalized on the total number of $Ki67^-$ or $Ki67^+$ PN, respectively (n= 10 per group), or **(h)** to measure the ROS and Caspase 3/7 activity (n=6 per group), or the cell cycle phase as indicated in **(c)** and **(d)**. Scale bar: 10 µm. *p<0.05; **p<0.01; ***p<0.001.

Suppl. Fig2. (a) MN and DD in HAoECs treated or not with oxLDL and transfected with lncWDR59 GapmeRs for 48h (n=3) **(b)** Representative workflow of FACS sorting strategy optimized to separate PN and MN from oxLDL-treated murine and human AoECs. ECs were lysate and PN and MN sorted according to their size. Beads were used to set up the gates. Efficiency of the lysate and of the sorting was tested using three different nuclei markers, **(c)** by analysing the size of PN and MN of adherent ECs, **(d)** by FACS confirmation of IF data on damage and proliferation (γH2AX, Ki67) and **(e)** using a 3D stacks images acquired with a Confocal microscope (images are representatives of 3 independent sorters). *p<0.05.

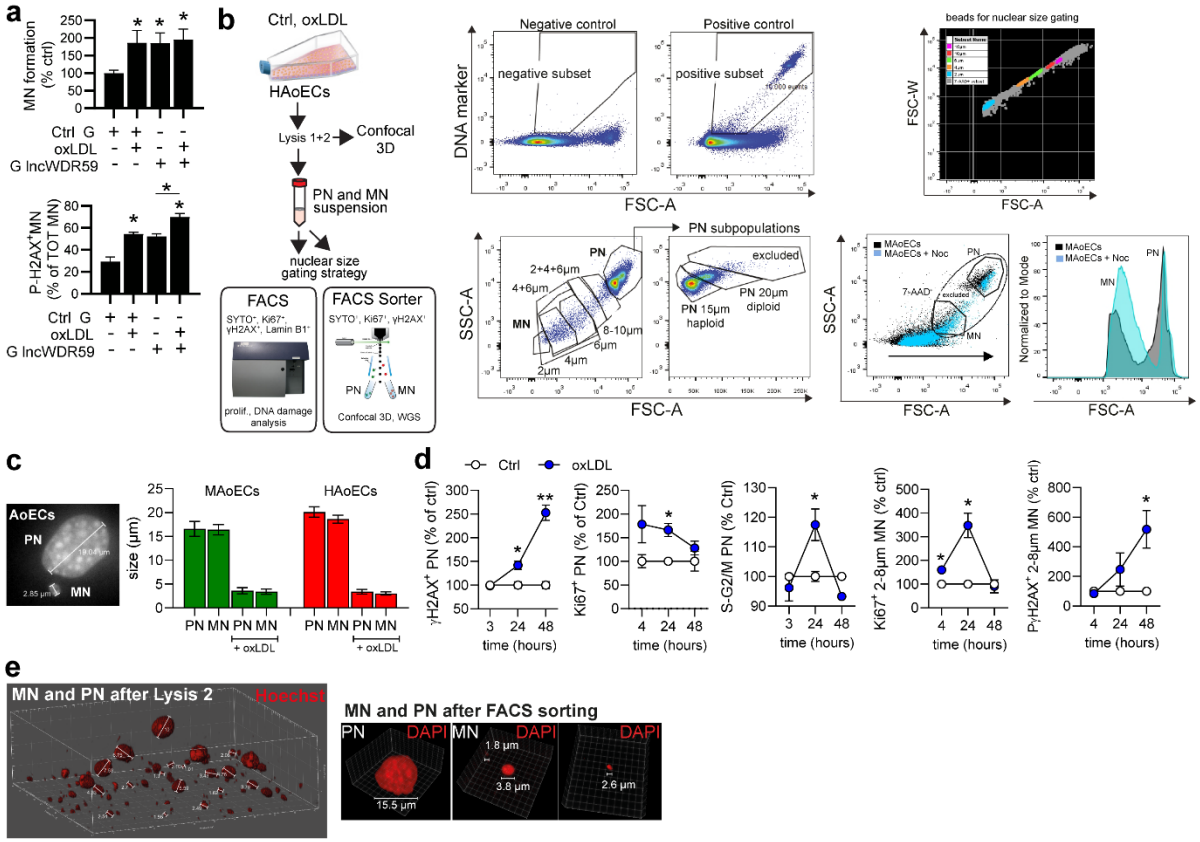
Suppl. Fig3. Gene pathways enrichment analysis of oxLDL MN vs Ctrl MN and oxLDL PN vs Ctrl PN genes. (a,b) Graphical representation of the FACS sorted MN and principal nuclei (PN) from HAoECs treated or not with oxLDL for 24h. Analysis and comparison of enriched and mutated genes has been done between: MN vs. PN from untreated (group A) or oxLDL treated (group B) HAoECs, MN vs. MN and PN vs. PN from untreated vs. oxLDL treated ECs (group C and D, respectively). Coverage 30x. **(c-d)** MN from HAoECs and MN from oxLDL treated ECs (group C), or PN from ECs compared to PN from oxLDL treated ECs (group D). Scatter plots of groups C and D is generated from the Enrichr analysis of mutated genes showing an insertion (ins), deletions (del), onversions (inv), or duplicated inversions (dup inv) in the PN after oxLDL treatment. **(e)** Graphical representation of isolated MN and PN labelled with Ki67 or DNA damage marke and **(f)** relative mutations in MN vs PN (ctrl or oxLDL). Selective mutations in genes related to DDR are in **(i-k)**. Scatter plots represents the most significant pathways. Odds ratio colour and bubble graphs represent the number of genes within the same pathway. Genes are graphed according to their odds ratio and the logarithmic negative p value. The pathways identified within all Enrichr databases are in bold. **(g,h)** HAoECs were treated with 50µg/ml oxLDL for 24 or 48h, alone or in combination with an alkyne-modified nucleoside, 5-ethynyl uridine (EU), for 1 or 24h, to detect newly synthetized RNA and RNA levels. Moreover, cells were co-stained with γH2AX to analyse the RNA synthesis (1h incubation of EU) in MN with damaged DNA. z stacks images were collected with a Confocal microscope. RNA transcription has been analysed and compared with the etherochromatin state using the DAPI staining. Moreover, the number of RNA^+ $γH2AX^+$ MN has been analysed and data normalized on total number of MN and ECs and expressed in percentage (n= 6 per group). Scale bar: 5 µm. *p<0.05; **p<0.01; ***p<0.001.

Suppl. Fig4. NHEJ markers and inhibition of DNA-PKCs. HAoECs were treated or not with 50µg/ml oxLDL for the time indicated to analyse **(a)** the levels of Smc6 in total and nuclear fractions, **(b)** and the levels of 53BP1 in ECs and MN containing damaged DNA. **(c,d)** OxLDL treated or not ECs were co-treated with serial dilutions of DNA-PKCs inhibitors (AZD7648 or LTURM34) for 4, 24, or 48h. DNA-PKCs cytotoxicity and efficiency were measured by fluorescence or western blot (n=3 per group).

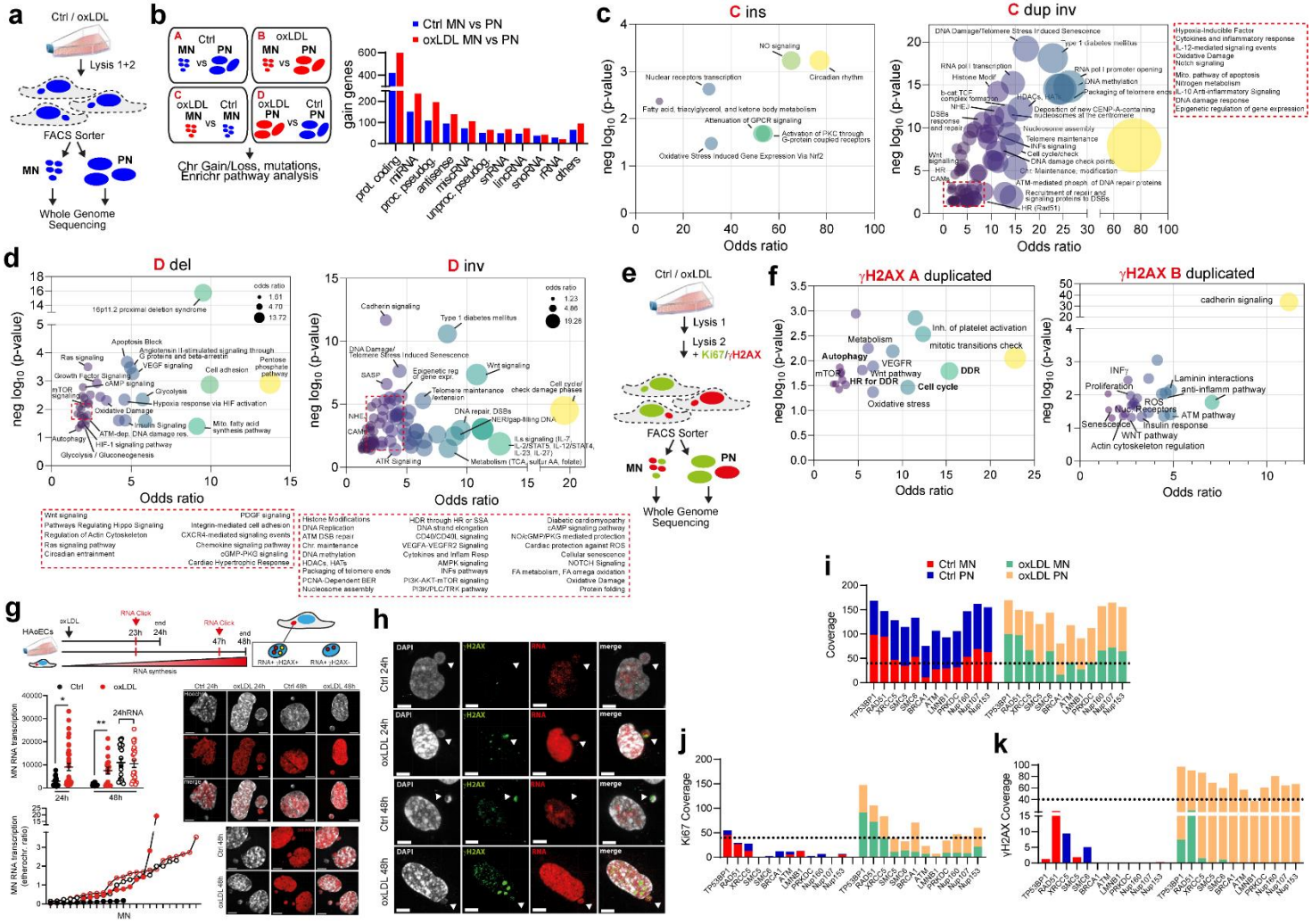
Suppl. Fig5 Effect of oxLDL and hyperlipidaemia on MN lamina assembly. (a) Linear intensity analysis of Lamin B1**,** γH2AX, DAPi, and H3K9me3 multiple localization in MN and PN from HAoECs treated with 50µg/ml oxLDL for 48h. **(b)** FACS sorting of MN and PN to analyse **(b)** Lamin B1 **(d)** Ki67, **(e)** γH2AX, and **(f)** double positive events. MN were analysed according to their Lamin B1 integrity as MN with an intact (Lamin $B1^+$ MN) or disrupted lamina (Lamin $B1^-$MN), and by immunofluorescence according to their **(c)** DNA synthesis ($Ki67^+$ MN) and **(d)** damage (DP, $Ki67^+\gamma H2AX^+$ MN) (n= 4 per group). **(e)** Aortic arches of $ApoE^{-/-}$ fed 4 and 12 weeks of normal or high fat diet (ND, HFD) were *en face* stained for Ki67, γH2AX, and Lamin B1, to analyse the effect of HFD on DNA damage on MN Lamin B1 disruption (Lamin $B1^+$ or Lamin $B1^-$ $CD31^+$ ECs), and containing duplicating DNA ($Ki67^+$ MN), or double positive (DP, $Ki67^+\gamma H2AX^+$ MN). CD31 was used as EC marker. Nuclei were stained with DAPI (n = 4-5 mice per group). (f) HAoECs treated 4, 24, and 48h with 50µg/ml oxLDL were stained with Nup107 and γH2AX to analyse the localization of Nup107 in MN with an intact (Lamin $B1^+$) or disrupted lamina (Lamin $B1^-$) (n=3 per group). Scale bar: 1µm. *p<0.05; **p<0.01; ***p<0.001.



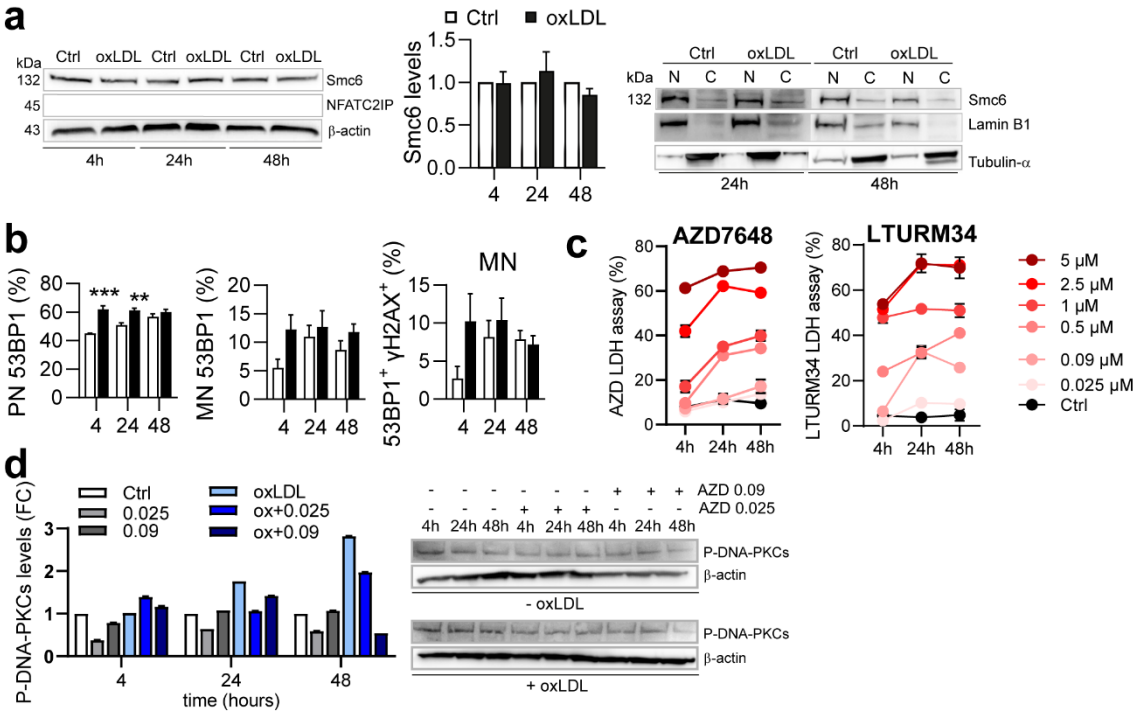
Supplementary Figure 1



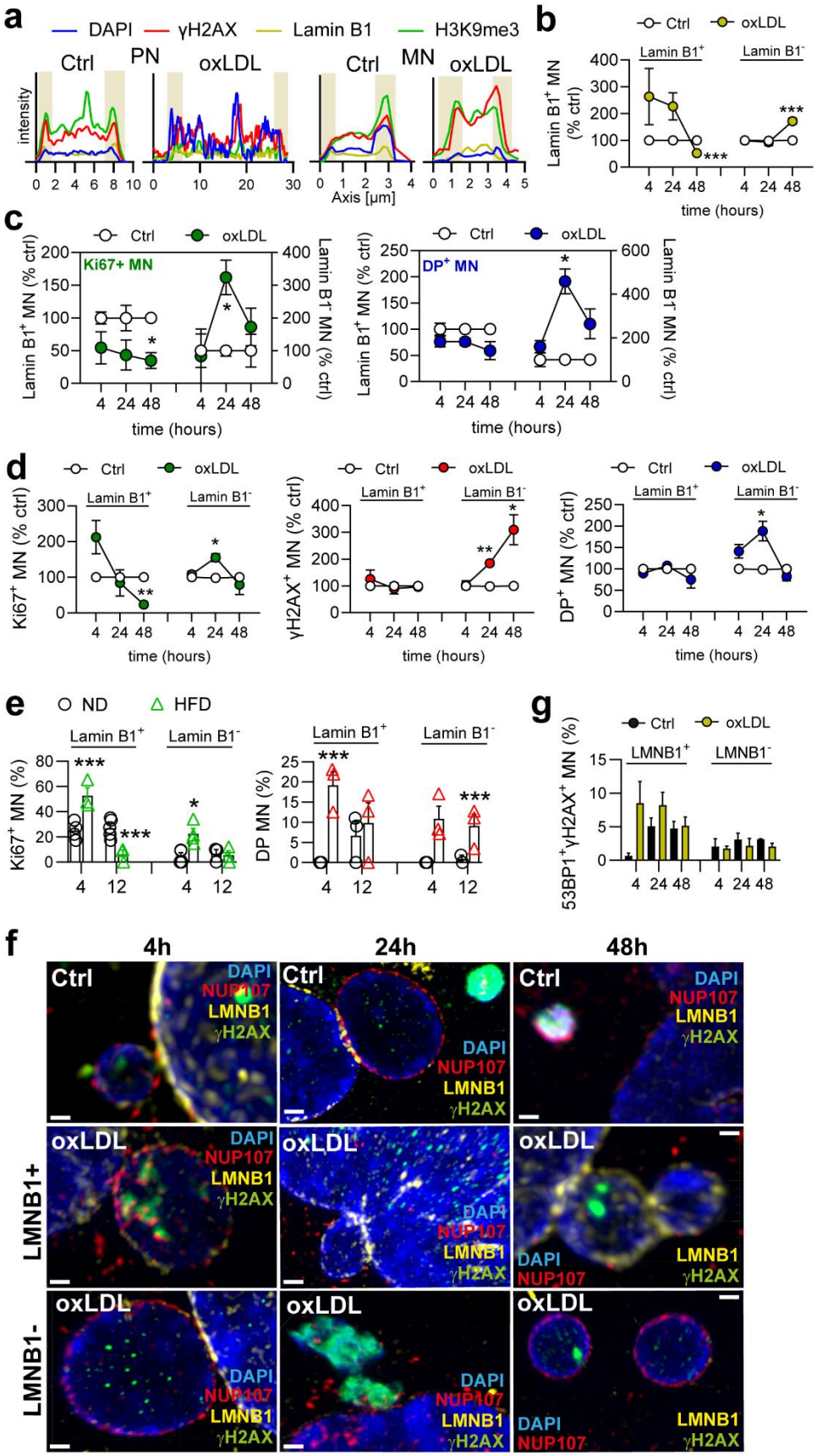
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5