

Targeting phospholipase *Pla2g15* reduces long-chain BMP phospholipids abundance and senescence in kidney cells

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ABSTRACT

Chronic kidney disease (CKD), a major age-related pathology, is driven by the accumulation of chronic senescent proximal tubular epithelial cells (TECs), promoting fibrosis and functional decline. Although senescence involves metabolic reprogramming, the role of complex lipid metabolism in TECs senescence remains poorly understood. Here, we identify a critical role for lysosomal phospholipid metabolism in senescent TECs. We show that the lysosomal phospholipid bis(monoacylglycerol)phosphate (BMP) increases during kidney aging and in senescent TECs. Genetic knockout of the BMP hydrolase *Pla2g15* in TECs elevated total lysophosphatidylglycerol (LPG) and BMP, with a notable shift in BMP species. Long-chain unsaturated BMPs, which accumulate during doxorubicin-induced senescence in TECs, were specifically reduced in *Pla2g15*-deficient cells, whereas wild-type TECs showed increased levels upon senescence induction. *Pla2g15* knockout also provided significant protection against doxorubicin-induced senescence. Proteomic profiling revealed that this protection was linked to enhanced mitochondrial metabolism, including NAD⁺ metabolism and fatty acid oxidation. These findings demonstrate that PLA2G15 contributes to TEC senescence through selective BMP lipid remodelling and regulation of mitochondrial function. Targeting PLA2G15 therefore may represent a promising therapeutic strategy to prevent senescence and the associated burden of CKD progression.

INTRODUCTION

Aging is a primary risk factor for chronic kidney disease (CKD), a condition whose global prevalence is rising at an alarming rate [1]. Central to the decline of kidney function with age and CKD is the accumulation of chronic senescent cells, particularly in the highly metabolically active proximal tubular epithelial cells (TECs) [2, 3]. While acute senescence can be protective, chronic senescent TECs drive tissue dysfunction, fibrosis, and the progression to CKD through the secretion of pro-inflammatory and pro-fibrotic factors [3]. Consequently, understanding the molecular drivers of TEC senescence presents a critical opportunity to develop novel therapeutic strategies to address the growing burden of CKD.

Cellular senescence is characterized by a state of profound metabolic rewiring, driven by the dysfunction of key organelles [4, 5]. While mitochondrial impairment is a well-recognized hallmark, equally critical are the alterations in lysosomes. Senescent cells undergo a notable expansion in lysosomal number and size, a phenomenon often accompanied by a decline in degradative function [6]. Lysosomal dysfunction during aging disrupts lipid homeostasis, leading to an accumulation of sphingosine and ceramides [7]. Additionally, aging cells accumulate complex lipids such as phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) species [8, 9]. However, the functional consequences of this lipid accumulation during aging remain poorly defined, and their specific roles in establishing or maintaining the senescent state are largely unknown.

Our previous work identified the lysosomal phospholipid bis(monoacylglycerol)phosphate (BMP) as a key molecule that accumulates in the aging kidney and in the urine of patients with CKD [10, 11]. BMP is a unique lysophospholipid localized within the endolysosomal system, where it plays a crucial role in maintaining lysosomal function, membrane dynamics, and cargo degradation [12]. It is synthesized through a two-step process: phosphatidylglycerol (PG) is first converted into lysophosphatidylglycerol (LPG), which is then further processed into BMP. For a long time, the enzyme responsible for converting LPG into BMP remained unidentified. Recently, ceroid-lipofuscinosis neuronal protein 5 (CLN5) was reported to catalyze this conversion, linking it to BMP synthesis and lysosomal integrity [13]. However, additional studies suggest that other enzymes are also involved in BMP metabolism, indicating a more complex and redundant regulatory network [14]. Phospholipase A2 group XV (PLA2G15) is the only lysosomal phospholipase of the PLA2 family, and its distinct localization points to a role in

BMP remodelling [15]. Indeed, recent work has demonstrated that PLA2G15 in mammals contributes to the hydrolysis of BMP, further connecting it to lysosomal lipid dynamics and signaling.

Given its subcellular localization, PLA2G15 is uniquely positioned at the intersection of BMP metabolism, lysosomal homeostasis, and aging. Therefore, in this study, we sought to investigate the role of the lysosomal phospholipid BMP and its hydrolase, PLA2G15, in senescent murine TECs and to elucidate its contribution to age-associated CKD decline. To directly assess the functional contribution of PLA2G15 to these processes, we generated *Pla2g15* knockout TECs using CRISPR/Cas9. This loss-of-function approach enabled us to determine how PLA2G15 deficiency alters BMP metabolism and lysosomal homeostasis under senescence-inducing conditions. We subsequently evaluated the effects of PLA2G15 deficiency in doxorubicin-induced TECs senescence using a multi-omic approach.

RESULTS

Generation and validation of *Pla2g15* KO cells

The BMP synthesis pathway involves the sequential conversion of PG to LPG, and then to BMP by CLN5. BMP is then hydrolysed by PLA2G15 (Figure 1A) [13, 16]. To define the contribution of PLA2G15 to TECs senescence, we established a *Pla2g15* knockout in murine immortalized proximal TECs using CRISPR/Cas9. Validation in two independent clones confirmed a sustained reduction in PLA2G15 protein expression (Figure 1B). KO1 shows a 2-base pair deletion, and KO2 a 11-base pair deletion, both causing a frame shift mutation. and a corresponding decrease in PLA2G15 enzyme activity (Figure 1C).

We next performed untargeted LC-MS-based lipidomics to profile BMPs and other lipids belonging to the BMPs synthesis pathway in the absence of *Pla2g15* in TECs (Supplementary Table 1). In total, 2737 unique lipids were measured. *Pla2g15* loss had a widespread impact on the lipid landscape in TECs. Compared to wild-type cells, *Pla2g15* knockout clones showed a similar number of lipid abundance (wild-type versus KO1 showed 472 lipids decreased and 794 lipids increased, while wild-type versus KO2, 604 lipids decreased and 555 lipids increased) (Figure 1D, 1E). This included an accumulation of the BMP precursor, LPG, in both the *Pla2g15* knockout cell lines (Figure 1F) and a significant increase in total BMP lipids (Figure 1G). Volcano plot of BMP species shows that out of 102 BMP species, 59 BMP species are increased (Figure 1H). Interestingly, mostly the long-chain

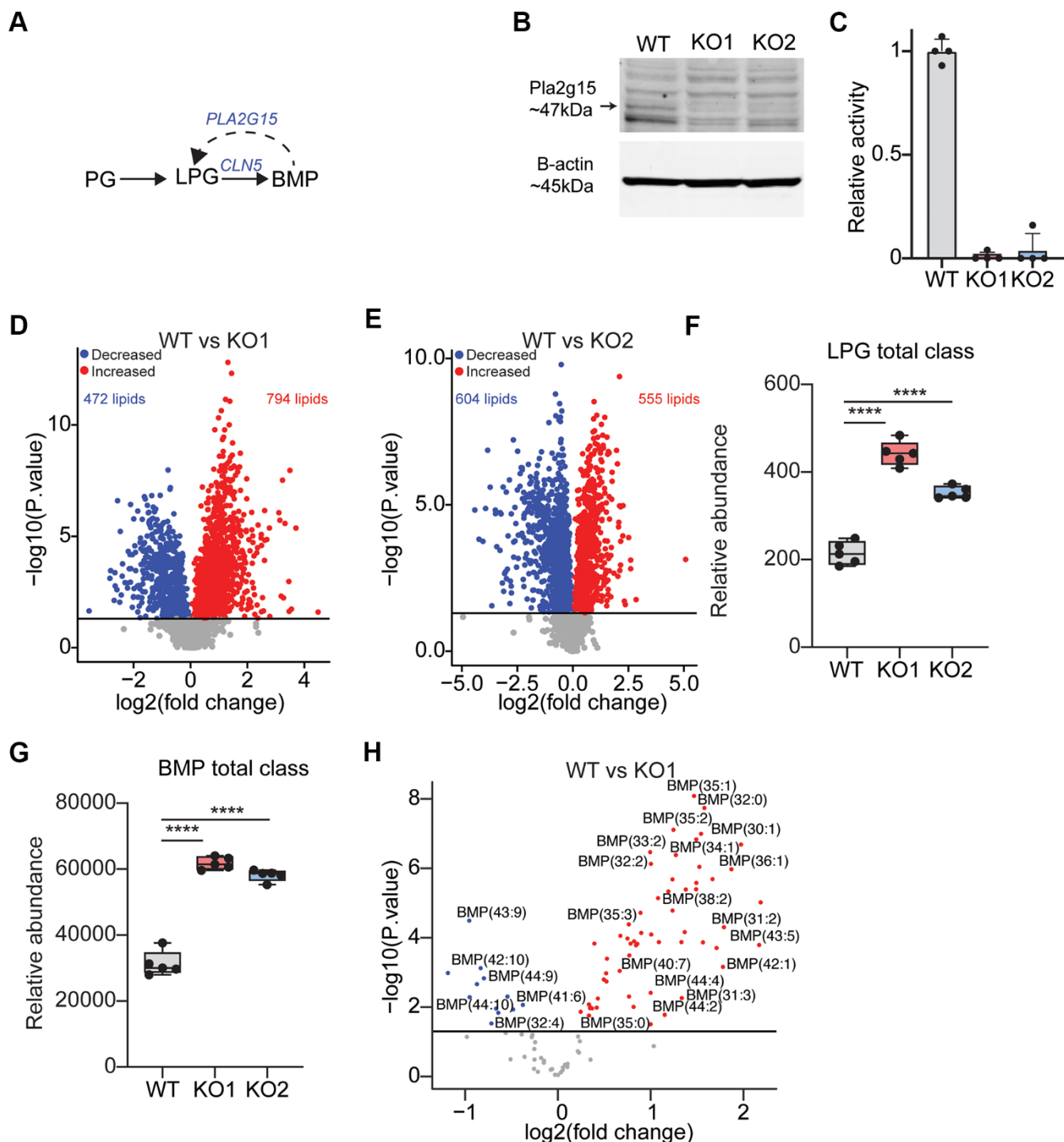


Figure 1. CRISPR/Cas9 knockout of *Pla2g15* in proximal tubular epithelial cells. (A) Bis(monoacylglycerol)phosphate (BMP) synthesis pathway, in which phosphatidylglycerol (PG) is converted into lysophosphatidylglycerol (LPG) and LPG is converted into BMP by CLN5. BMP is then hydrolyzed by PLA2G15, reforming LPG. (B) Confirmation of *Pla2g15* knockout (KO) via PLA2G15 protein expression with Western Blot and (C) via enzyme activity of PLA2G15. Relative activity of the cell lysate. The y-axis shows the fluorescent activity per minute per gram of protein and subsequently normalized to wild-type cells. (D) Volcano plot of the lipid species, comparing wild-type cells versus KO1 cells. (E) Volcano plot of the lipid species, comparing wild-type cells versus KO2 cells. (F) Total lipid class of LPG in wild-type and *Pla2g15* KO cells. (G) Total lipid class of BMP in wild-type and *Pla2g15* knockout cells. (H) Volcano plot of BMP lipid species in wild-type versus KO1 cells. Statistical comparison was determined by using the One-way ANOVA test for more than two groups; *** $p < 0.001$, and **** $p < 0.0001$.

unsaturated lipids are decreased in *Pla2g15* knockout cells compared to wild-type (Figure 1H). This lipid profile confirms that our *Pla2g15* knockout was successful and functionally interferes with the BMP metabolism, leading to both LPG and BMP accumulation, consistent with previous studies showing that disruption of PLA2G15 function impairs BMP hydrolysis [15, 16].

***Pla2g15* knockout prevents doxorubicin-induced senescence**

TEC senescence progressively accumulates with age and contributes to the functional decline of the kidney. To investigate whether PLA2G15 is involved in this process, we examined markers of senescence and PLA2G15 expression in kidneys from young (12 weeks) and aged (24 months) male mice. Senescent TECs accumulation in aged kidneys was confirmed by increased SA- β -gal staining, which was predominantly localized to TECs (Supplementary Figure 1A). In addition, aged kidneys showed reduced Lamin B1 expression in TECs as assessed by immunostaining, and this observation was further supported by gene expression analysis (Supplementary Figure 1B, 1C). Consistent with this senescent phenotype, *Cdkn1a/p21* expression was significantly elevated in aged kidneys compared to young controls (Supplementary Figure 1D). Importantly, we previously demonstrated that increased p21 expression in aged murine kidneys is predominantly localized to TEC [17]. In parallel, PLA2G15 expression was significantly increased in aged kidneys (Figure 2A), and accompanied by a marked accumulation of BMPs (Figure 2B), suggesting altered lysosomal phospholipid metabolism during renal aging. Since TECs are a major site of lipid accumulation and senescence in aging and CKD [2, 10], we next investigated whether PLA2G15 directly contributes to the senescent phenotype in these cells. To model stress-induced TEC senescence *in vitro*, we treated TECs with doxorubicin, a well-established inducer of DNA damage and cellular senescence in epithelial cells. Using this controlled TEC-specific model in wild-type and *Pla2g15* knockout cells allowed us to determine how loss of PLA2G15 affects senescence-associated alterations in BMP metabolism and lysosomal homeostasis. Doxorubicin exposure (24h) induced a robust senescence response in wild-type cells, characterized by increased P21 protein expression (Figure 2D) and G2/M cell cycle arrest assessed by BrdU incorporation (Figure 2E, 2G). G2/M cell cycle arrest is a feature of stress-induced cellular senescence. In contrast, doxorubicin-exposed *Pla2g15* knockout cells showed a significant reduction in P21 protein and G2/M arrest compared to senescent wild-type cells. (Figure 2G). Altogether, this suggests that *Pla2g15*

knockout rescues doxorubicin-induced senescence in TECs.

***Pla2g15* knockout cells show reduced long-chain BMP lipids**

To further explore the relationship between PLA2G15, BMP metabolism, and senescence, we performed lipidomic profiling of doxorubicin-induced senescence in wild-type and knockout cells. Interestingly, our lipidomics data revealed a biphasic regulation of BMP species in response to doxorubicin (Supplementary Table 1). In wild-type cells, doxorubicin stimulation led to an accumulation of several long-chain polyunsaturated BMPs (for instance, BMP42:6, BMP42:8, and BMP44:8). Strikingly, the absence of *Pla2g15* exhibited an inverted BMP lipid profile, with elevated short-chain BMPs characterized and a reduction in long-chain lipids. However, doxorubicin exposure in knockout cells did not further impact these changes (Figure 3A, 3B). Overall, *Pla2g15* knockout senescent cells displayed a consistent increase in short-chain BMPs and a decrease in long-chain BMPs compared to wild-type cells (Figure 3C). Furthermore, the more polyunsaturated BMPs are decreased in *Pla2g15* knockout cells, indicating lipid remodeling in *Pla2g15* knockout cells with a shift towards smaller and monounsaturated BMPs. Together, these findings indicate that accumulation of long-chain BMP lipids is linked to tubular senescence and that loss of *Pla2g15* prevents this maladaptive lipid shift, thereby conferring protection against senescence.

Proteomics on senescent *Pla2g15* knockout cells reveals increased mitochondrial metabolism

To uncover the mechanisms by which loss of *Pla2g15* reduces TEC senescence, we performed proteomic profiling on wild-type and *Pla2g15* knockout TECs following doxorubicin exposure (Supplementary Table 2). In total, 3411 proteins were identified. Principal component analysis showed a clear separation between the experimental groups (Figure 4A). 910 proteins showed increased protein expression, while 498 proteins were decreased in expression of proteins in *Pla2g15* knockout cells compared to wild-type cells upon doxorubicin exposure (Figure 4B). Gene ontology (GO) analysis identified a significant enrichment of mitochondrial-related pathways, including NADH regeneration, succinate metabolism, mitochondrial acetyl-CoA biosynthesis, fatty acid oxidation, and NAD biosynthetic processes (Figure 4C). In contrast, pathways related to DNA replication and cell cycle progression were downregulated, such as DNA strand elongation, mitotic sister chromatid cohesion, nuclear DNA replication initiation, and mitotic DNA replication

initiation (Figure 4D). These processes are essential for cell proliferation and are commonly activated during the early stages of senescence, as cells undergo a final round of replication before cell cycle arrest [18]. Additionally, pathways related to ribosomal RNA

processing and modification were significantly reduced. Consistent with these findings, proteomic analysis confirmed the reduction in P21 abundance in senescent *Pla2g15* knockout cells, in line with our Western blot analysis (Figure 4E, 2G). In addition, the

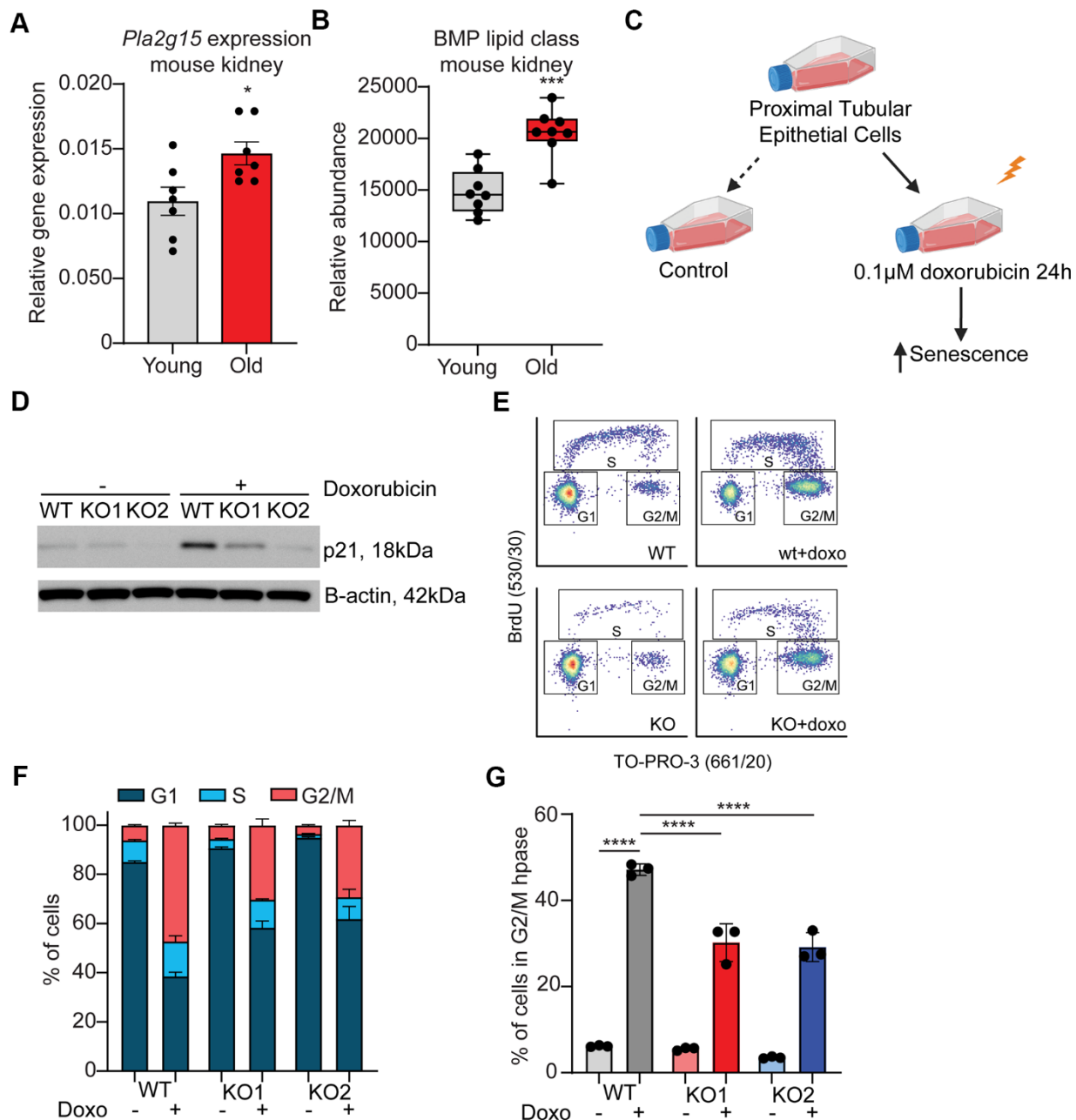


Figure 2. Reduced senescence in *Pla2g15* knockout tubular epithelial cells. (A) Gene expression of *Pla2g15* measured by RT-PCR relative to the housekeeping gene *Ppia*. In young (12 weeks old) vs. aged (24 months old) mice kidneys. (B) BMP class measured by LC-MS lipidomics in the kidneys of young (12 weeks) vs. old (24 months) mice. (C) In the senescence model, TECs are exposed to 0.1 μM doxorubicin. (D) Western blot of wild-type and knockout cells treated with and without doxorubicin, probed with antibodies against P21 and β-actin. (E) Dot plot of the cell cycle analysis of wild-type and knockout cells treated with or without doxorubicin. (F) Quantification of the cell cycle analysis. Showing reduced G2/M arrest in doxorubicin-exposed cells. (G) Bar plot of the G2/M phase of the cell cycle. Statistical comparison was determined by using the unpaired t-test for two groups, or the One-way ANOVA test for more than two groups; ***p < 0.001, and ****p < 0.0001.

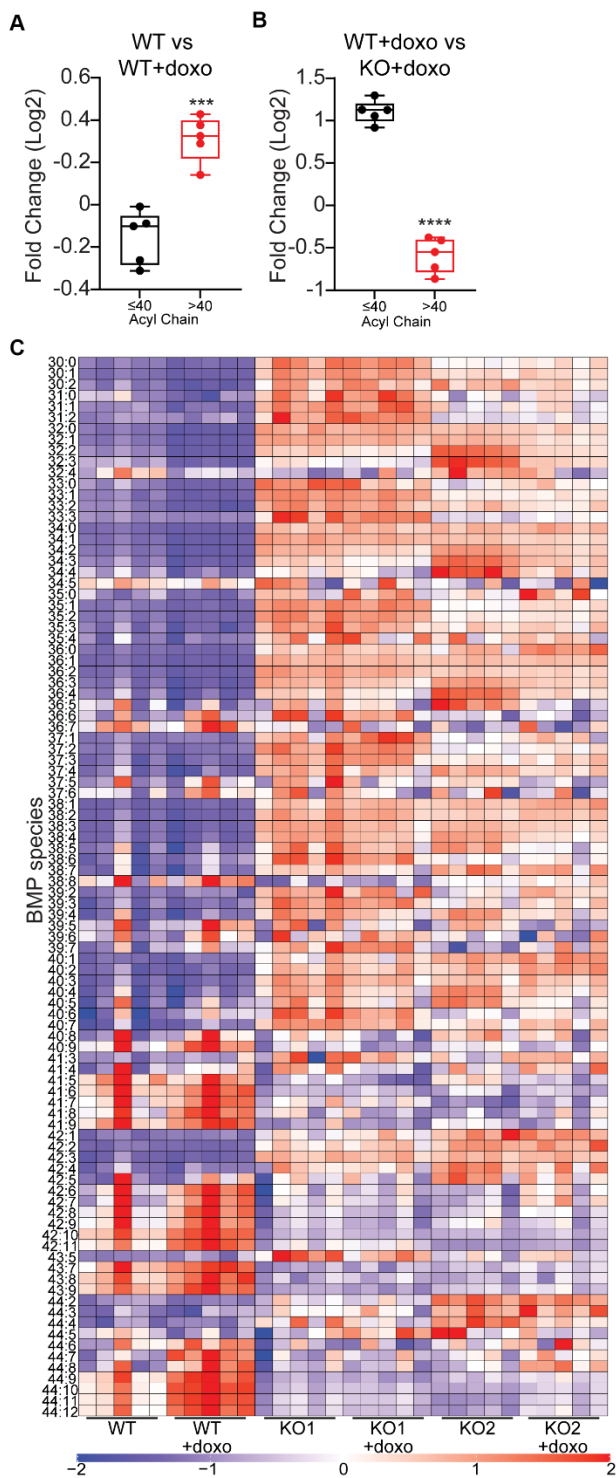


Figure 3. Reduced abundance of long-chain BMP species in *Pla2g15* KO cells. (A) Fold-change (Log_2) of BMP abundance in wild-type vs. doxorubicin-exposed wild-type cells, separated into short (≤ 40) and longer (> 40) lipids. (B) Fold-change (Log_2) of BMP abundance doxorubicin-treated wild-type and KO1 cells, separated in short (≤ 40) and longer (> 40) lipids. (C) Heatmap of BMP species. Statistical comparison was determined by using the unpaired t-test for two groups; *** $p < 0.001$, **** $p < 0.0001$.

expression of cell cycle regulator Cyclin-dependent kinase 1 (CDK1) was decreased in *Pla2g15* knockout cells exposed to doxorubicin, while being increased in wild-type cells (Figure 4F). Loss of *Pla2g15* also resulted in increased expression of Lysosomal-associated membrane protein 1 (LAMP1), suggesting increased lysosomal abundance (Figure 4G). Importantly, multiple mitochondrial proteins were downregulated in wild-type cells but are upregulated in the *Pla2g15* knockout cells (Figure 4H, 4I). Specifically, proteins involved in NAD⁺ metabolism, such as Nicotinamide phosphoribosyltransferase (NAMPT) and Nicotinamide mononucleotide adenylyl-transferase 3 (NMNAT3), were significantly increased in *Pla2g15* knockout cells (Figure 4J, 4K). Fatty acid oxidation proteins, including Carnitine palmitoyl-transferase 2 (CPT2), also showed higher expression in *Pla2g15* knockout cells exposed to doxorubicin (Figure 4L).

Altogether, these results indicate that loss of *Pla2g15* appears to favor a metabolic profile that alleviates lysosomal lipid stress, preserves mitochondrial function, and protects against TEC senescence (Figure 5), highlighting *Pla2g15* as a potential therapeutic target to counteract kidney aging and CKD progression.

DISCUSSION

In this study, we identified a previously underexplored link between the BMP hydrolase, PLA2G15, BMP metabolism and cellular senescence in kidney proximal TECs. Previous work from our group showed that BMP levels are elevated in aged mice and human muscle and reduced upon exercise [11]. Given the observed increase in *Pla2g15* expression and BMP accumulation in aged kidneys, where chronic senescence of TECs is observed, we hypothesized that *Pla2g15* may act as a critical modulator of lysosomal phospholipid signalling in the context of TECs senescence. In the aging kidney, senescent cells, particularly within the tubular compartment, accumulate progressively and contribute to functional decline [17, 19, 20]. Our findings demonstrate that loss of *Pla2g15* protects TECs from doxorubicin-induced senescence. This protection is accompanied by a reduction in long-chain BMP lipids and a metabolic shift towards enhanced mitochondrial metabolism. Furthermore, the downregulation of cell cycle and ribosome biogenesis pathways further supports a disrupted senescence program in *Pla2g15* knockout cells [18, 21].

We have recently demonstrated that the predicted orthologue of *Pla2g15* in *C. elegans*, known as *plag-15*, does not directly regulate BMP but instead modulates lysophospholipid metabolism. Remarkably, and in line

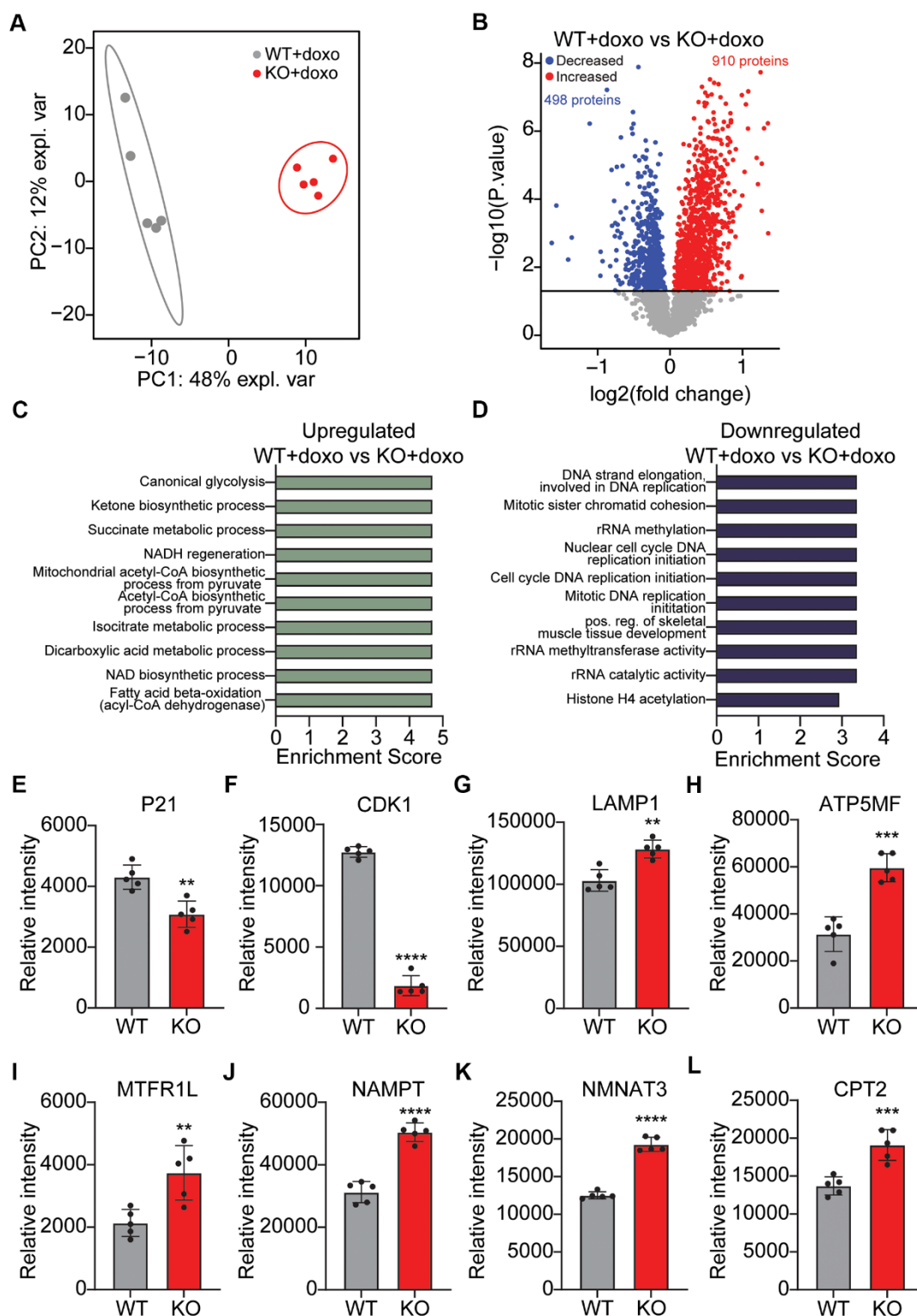


Figure 4. Proteomics of *Pla2g15* KO cells. (A) Principal Component Analysis (PCA) plot of the proteome, showing group separation based on the proteome in *Pla2g15* KO cells exposed to doxorubicin versus wild-type cells treated with doxorubicin. (B) Volcano plot of proteomics results, comparing wild-type cells versus KO1 exposed to doxorubicin. Pathway enrichment analysis of upregulated (C) and downregulated (D) transcripts (non-adjusted p-value: $p < 0.01$) in *Pla2g15* KO cells compared to the wild-type cells, after exposure to doxorubicin. Protein expression of (E) P21, (F) CDK1 and (G) LAMP1 and mitochondrial proteins (H) ATP5MG, (I) MTFR1L, (J) NAMPT, (K) NMNAT3 and (L) CPT2 in wild-type and *Pla2g15* KO cells exposed or not exposed to doxorubicin. Knockout shown in this figure is KO1. Statistical comparison was determined by using the unpaired t-test for two groups; ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

with the results described here, knockdown of *plag-15* in *C. elegans* improved healthspan and lifespan by upregulating several lysosomal-related genes, reinforcing a causal link between phospholipid remodeling and lysosomal function in aging [22]. A similar phospholipid-lysosome axis emerges in mammalian kidney disease. In CKD and diabetic kidney disease, TEC exhibit lipotoxic remodeling with impaired fatty acid oxidation (FAO), accompanied by the accumulation of toxic lipids, ceramides, lysophospholipids and phosphatidylinositols all of which are known drivers of TEC dysfunction [23, 24]. Notably, BMP, a signature lipid of late endosomes/lysosomes, is consistently elevated in diseased and aged kidneys and is increasingly recognized as a functional readout of lysosomal stress rather than an inert byproduct [10, 11].

Recent studies clarified PLA2G15 function, identifying it as a BMP hydrolase [16]. Knocking out *Pla2g15* *in vitro* resulted in elevated levels of total BMP. Despite the overall increase of BMP, their results show a reduction in longer-chain BMP lipids in the *Pla2g15* knockout cells. This observation is consistent with our findings and supports the idea that PLA2G15 plays a role in the degradation of long-chain unsaturated BMP lipids [16]. *Pla2g15* knockout cells not only showed a decrease in these longer BMP lipids but also a striking reduction in several shorter, highly unsaturated BMPs, including BMP (32:4), BMP (34:5), and BMP (38:8). This raises the possibility that PLA2G15 exhibits substrate selectivity, preferentially hydrolyzing unsaturated BMP lipids over saturated or longer-chain species. These findings also suggest that additional BMP hydrolases may exist, and identifying such enzymes will be critical for our complete understanding

of BMP lipid metabolism, its turnover, and how disruptions in this pathway contribute to disease.

Our findings point to PLA2G15 as a critical regulator of senescence through its role in lysosomal lipid remodelling and mitochondrial metabolism. Senescence is characterized by changes in the mitochondria, such as mass, dynamics, structure, and functions [25]. Our proteomics analysis showed a significant increase in mitochondrial proteins. First of all, our GO terms revealed the upregulation of many mitochondrial biological processes. This is in line with previous research, showing that in models of diabetic kidney disease where senescence occurs primarily in TECs or other *in vitro* models of TECs senescence, there is a reduction in oxidative phosphorylation in mitochondria, reduced ATP production and thus less energy [2, 17, 26]. During AKI and CKD, cells have reduced mitochondrial biogenesis, reduced oxidative phosphorylation, and increased glycolysis [27]. In contrast, *Pla2g15* knockout cells displayed increased mitochondrial function, further supporting their protection against senescence. Furthermore, our GO terms showed downregulation of genes involved in ribosomal metabolism. Similarly, pathways related to ribosomal RNA processing and modification, such as rRNA methylation, rRNA methyltransferase activity, and rRNA catalytic activity, were significantly down-regulated. These findings suggest a suppression of ribosome biogenesis [28]. Inhibition of ribosome biogenesis can trigger senescence through nucleolar stress and p53 activation [29], and in our knockout cells this downregulation likely reflects a failure to engage the biosynthetic and chromatin programs typically associated with the senescence response [21].

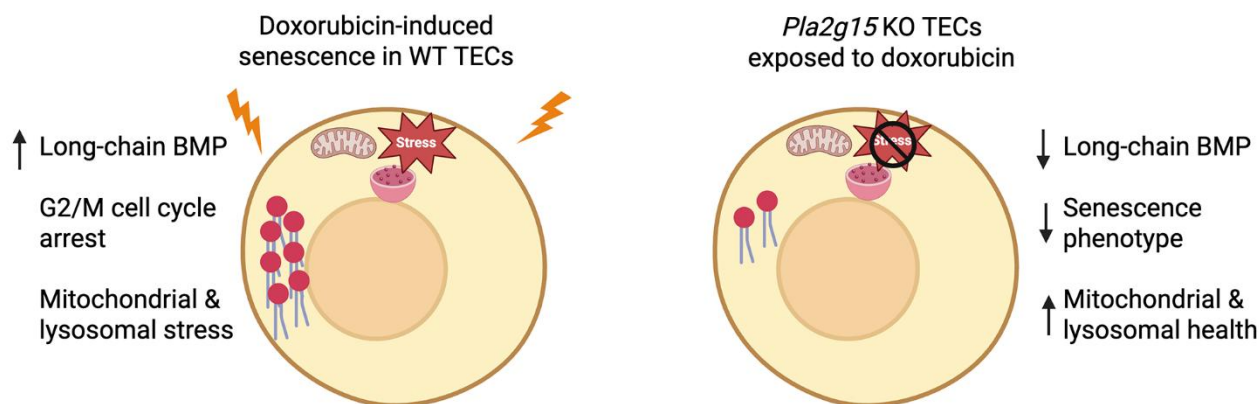


Figure 5. Mechanism of *Pla2g15* knockout. Doxorubicin-induced senescence is characterized by elevated long-chain BMP species, increased G2/M cell-cycle arrest, and pronounced mitochondrial and lysosomal stress. In contrast, *Pla2g15*-deficient cells exposed to doxorubicin show reduced accumulation of long-chain BMP lipids, improved mitochondrial and lysosomal health, and an overall attenuation of the senescence phenotype. Created with BioRender.com.

Nonetheless, our study has several limitations. First, while we observe an association between altered BMP species, PLA2G15 expression, and senescence in aged kidneys, these findings are correlative and do not establish causality *in vivo*. Functional validation in *Pla2g15* knockout mouse models will be required to determine whether PLA2G15 directly drives senescence and BMP remodelling in the aging kidney. Second, we used doxorubicin-induced senescence as an *in vitro* model. While this is a robust and widely used approach to induce stress-associated cellular senescence, it does not fully recapitulate the complexity of TECs aging *in vivo*. Alternative stressors and experimental systems may further help to generalize and extend our findings. Additionally, our conclusions are based on *in vitro* experiments combined with genetic deletion of *Pla2g15*. It remains unclear whether pharmacological inhibition of PLA2G15 would reproduce the observed effects and whether these findings translate to *in vivo* disease settings. Therefore, *in vivo* and pharmacological validation will be important next steps.

Although proteomic analysis suggests alterations in mitochondrial pathways in *Pla2g15*-deficient cells, these findings are indirect and not supported by functional metabolic assays. Similarly, lysosomal function was inferred from lipidomic and proteomic changes rather than directly measured. More detailed functional studies will be required to clarify these mechanisms. Lastly, only male mice were included in this study. Given well-established sex-specific differences in kidney disease, future studies should examine whether these findings are applicable to female mice.

In conclusion, our work uncovers a mechanistic link between lysosomal lipid remodeling and TEC senescence, identifies PLA2G15 as a regulator of BMP lipid composition and mitochondrial and lysosomal integrity, and positions PLA2G15 as a promising target to modulate maladaptive senescence in aging and CKD.

MATERIALS AND METHODS

Cell culture and senescence model

TECs isolated from immortomice were maintained in DMEM/F12 medium (Thermo Fisher) with 10% fetal bovine serum (Thermo Fisher), 5 µg/ml insulin and transferrin, 5 ng/ml sodium selenite (Thermo Fisher), 40 pg/ml triiodothyronine (Sigma-Aldrich), 36 ng/ml hydrocortisone (Sigma-Aldrich), 20 ng/ml epidermal growth factor (Sigma-Aldrich), 2 mM L-glutamine (Thermo Fisher) and 1% penicillin/streptomycin (Thermo Fisher) and IFN γ (10 ng/ml, Prospec) at 33° C, 5% CO $_2$. Before conducting experiments, cells were

grown at 37° C without IFN γ for a week, to reduce the SV40 large T antigen activity [30].

Pla2g15 KO generation

Single guide RNAs (sgRNAs) targeting *Pla2g15* were designed and chosen for high predicted on-target activity and minimal off-target effects. The sgRNA are: KO1 fwd: CACCgCCGGAGCTGGGTCTCGCGAC, rev: AAACGTCGCGAGACCCAGCTCCGGC. KO2: CACCgGACGTTGGGCCGGGAGCGTC, rev: AAACGACGCTCCCGGCCCAACGTCC. Oligonucleotides encoding each sgRNA were cloned into a GFP-expressing CRISPR-Cas9 vector PX458.

TECs were seeded in 6-well plates, one day before transfection. Plasmid DNA was delivered using jetPRIME® transfection reagent (Polyplus Transfection) according to the manufacturer's instructions, using 5µg of the sgRNA. Forty-eight hours post-transfection, GFP-positive cells were single-cell sorted into 96-well plates using a BD FACSAria™ to isolate successfully transfected cells. Clones were expanded and screened for *Pla2g15* knockout by sequencing.

Enzyme activity

To measure the enzyme activity of PLA2G15, cells were homogenized by sonication in MilliQ water on ice. 0.5 mg/ml of protein was used in the Lysosomal Phospholipase A2 (LPLA2) Activity Assay for measuring enzyme activity (K-7000A, Echelon Biosciences Inc.).

Western blot

Cell pellets were resuspended in 300 µl RIPA buffer with cOmplete™, Mini Protease Inhibitor Cocktail (Roche). Protein quantification was performed with a BCA assay. Cell homogenates (30 µg) were subjected to electrophoresis on a NuPAGE™ Bis-Tris Mini Protein Gels, 4–12%, 1.0 mm, and transferred to a nitrocellulose sheet. After blocking of non-specific binding sites with 3% BSA in phosphate-buffered saline plus 0.1% (w/v) Tween-20 for 30 minutes, the blot was incubated overnight at 4° C with rabbit polyclonal antibodies raised against PLA2G15 (PA5-31701, Thermo Fisher). As a loading control, the membranes were re-probed with a monoclonal antibody against β -actin (A5441, Sigma-Aldrich) using a 1:10000 dilution (incubation 2h). Antigen-antibody complexes were visualized with IRDye 800CW GAR IgG goat anti-rabbit secondary antibody (926-32211, LI-COR Biosciences) for PLA2G15, and IRDye® 680RD donkey anti-mouse IgG secondary antibody (926-68072, LI-COR Biosciences) for β -actin using the

Odyssey Infrared Imaging System (LI-COR Biosciences). Anti-P21 antibody [HUGO291] was used for P21 (ab107099, Abcam).

qRT-PCR

Sections were cut from snap-frozen renal mouse tissue and RNA was isolated using Trizol. RNA was reverse transcribed into cDNA using oligo dT primers. Cells were harvested by adding TRIzol. After RNA isolation, RNA was quantified with Nanodrop and 1 µg of extracted RNA was reverse transcribed into cDNA according to manufacturer's instructions using the QuantiTect Reverse Transcription Kit (QIAGEN; Venlo, The Netherlands). Quantitative gene expression analysis was performed by using the SensiFAST SYBR No-ROX (GC Biotech) and the quantitative gene expression was measured by the LightCycler 480 Instrument II (Roche). Primers used are: *Pla2g15* forward: CTCTGGCTGAATCTGGAAGT, *Pla2g15* reverse: GCCCGAGATGTTCTGTTGTAA, *Lmnbl* forward: GGGAAGTTTATTCGCTTGAAGA *Lmnbl* reverse: ATCTCCAGCCTCCATT. Housekeeping gene *Ppia* forward: TGCCAGGGTGGTGACTTTAC, Housekeeping gene *Ppia* reverse: GATGCCAGGAC CTGTATGCT.

Mouse studies

C57BL/6J male mice, either young (3 months) or aged (24 months), were used in this study. These animals were previously used in another study and were handled, housed, and treated in the same manner as described therein [11]. All experiments were conducted in accordance with local and national ethical regulations and were approved by the Amsterdam UMC ethical committee (license GMZ102816-1).

SA-β-galactosidase staining

Frozen kidney sections were cut (5µM thickness) and allowed to dry at room temperature. The senescence beta-galactosidase staining kit (Cell signaling #9860) was used and sections were fixed and stained according to the manufacturer's protocol and the images were made with Olympus BX51. Quantification was performed by QuPath-0.6.0.

Immunohistochemistry staining

Paraffin-embedded mouse kidney sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with methanol/H₂O₂ for 15 minutes. Antigen retrieval was performed in citrate buffer (pH 6.0) for 20 minutes. Sections were incubated overnight at 4° C with anti-Lamin B1 (1:1000; Abcam,

ab16048), followed by HRP-conjugated goat anti-rabbit secondary antibody (BrightVision, DVPR110HRP) for 30 minutes at room temperature. Signal was developed using DAB (Immunologic, BrightDAB BS04) for 8 minutes. Sections were then dehydrated and mounted. Immunofluorescent images were made with Leica DM500B.

FACS - Cell cycle assay with BrdU incorporation

Cells were incubated with 20 mM BrdU (Invitrogen) for 1 hour, washed with PBS, trypsinized, and transferred to FACS tubes. After incubation with FACS buffer (2% FCS in PBS), cells were resuspended in PBS and cold absolute ethanol was added dropwise while vortexing. Samples were stored at 4° C for at least one week. Following fixation, cells were centrifuged at 2000 rpm, washed with cold 0.1% BSA in PBS while vortexing, and resuspended in pepsin-HCl solution (0.4 mg/ml pepsin [Thermo Scientific] in 0.1 N HCl). Cells were incubated for 30 minutes at 37° C in a water bath, followed by staining in 2 N HCl for 25 minutes at 37° C. Subsequently, cells were washed with PBT (PBS containing 0.05% Tween-20) and PBT_B (PBT supplemented with 2% BSA), then stained with 0.5 µg/ml anti-BrdU-FITC in PBT_B for 30 minutes. After sequential washes with PBT and PBT_B, cells were resuspended in 243.75 µl FACS buffer, followed by the addition of 10 µl diluted TO-PRO-3 stock and 2.5 µl RNase stock. Samples were incubated for 15 minutes at 37° C. Flow cytometry was performed on a BD FACSCanto, measuring BrdU and TO-PRO-3 staining. The APC channel (TO-PRO-3) was set to linear scale with APC-H and APC-W activated, and data were acquired at a low flow rate.

Lipidomics

Lipidomics was performed as previously described, with minor adjustments [31]. In a 2 ml tube, the following amounts of internal standards dissolved in 1:1 (v/v) methanol:chloroform were added to each sample: Bis(monoacylglycero)phosphate BMP(14:0)₂ (0.2 nmol), Ceramide-1-phosphate C1P (d18:1/12:0) (0.125 nmol), D7-Cholesteryl Ester CE(16:0) (2.5 nmol), Ceramide Cer(d18:1/12:0) (0.125 nmol), Ceramide Cer(d18:1/25:0) (0.125 nmol), Cardiolipin CL(14:0)₄ (0.1 nmol), Diacylglycerol DAG(14:0)₂ (0.5 nmol), Glucose Ceramide GlcCer(d18:1/12:0) (0.125 nmol), Lactose Ceramide LacCer(d18:1/12:0) (0.125 nmol), Lysophosphatidic acid LPA(14:0) (0.1 nmol), Lysophosphatidylcholine LPC(14:0) (0.5 nmol), Lysophosphatidylethanolamine LPE(14:0) (0.1 nmol), Lysophosphatidylglycerol LPG(14:0) (0.02 nmol), Phosphatidic acid PA(14:0)₂ (0.5 nmol),

Phosphatidylcholine PC(14:0)₂ (2 nmol), Phosphatidylethanolamine PE(14:0)₂ (0.5 nmol), Phosphatidylglycerol PG(14:0)₂ (0.1 nmol), Phosphatidylinositol PI(8:0)₂ (0.5 nmol), Phosphatidylserine PS(14:0)₂ (5 nmol), Sphinganine 1-phosphate SIP(d17:0) (0.125 nmol), Sphinganine-1-phosphate SIP(d17:1) (0.125 nmol), Ceramide phosphocholines SM(d18:1/12:0) (2.125 nmol), Sphingosine SPH(d17:0) (0.125 nmol), Sphingosine SPH(d17:1) (0.125 nmol), Triacylglycerol TAG(14:0)₂ (0.5 nmol). Then, 1.5 ml 1:1 (v/v) methanol:chloroform was added before thorough mixing. Each sample was centrifuged for 10 minutes at 14,000 rpm and the supernatant was transferred to a clean 1.5 ml tube and evaporated under a stream of nitrogen at 30° C. The residue was dissolved in 100 µl of 1:1 (v/v) methanol:chloroform. Lipids were analyzed using a Thermo Scientific Ultimate 3000 binary HPLC coupled to a Q Exactive Plus Orbitrap mass spectrometer. For normal phase separation, 2 µl of each sample was injected onto a Phenomenex® LUNA silica, 250 * 2 mm, 5 µm 100Å. Column temperature was held at 25° C. Mobile phase consisted of (A) 85:15 (v/v) methanol:water containing 0.0125% formic acid and 3.35 mmol/l ammonia and (B) 97:3 (v/v) chloroform:methanol containing 0.0125% formic acid. Using a flow rate of 0.3 ml/min, the LC gradient consisted of: Dwell at 10% A 0-1 min, ramp to 20% A at 4 min, ramp to 85% A at 12 min, ramp to 100% A at 12.1 min, dwell at 100% A 12.1-14 min, ramp to 10% A at 14.1 min, dwell at 10% A for 14.1-15 min. For reversed phase separation, 5 µl of each sample was injected onto a Waters HSS T3 column (150 x 2.1 mm, 1.8 µm particle size). Column temperature was held at 60° C. Mobile phase consisted of (A) 4:6 (v/v) methanol:water and B 1:9 (v/v) methanol:isopropanol, both containing 0.1% formic acid and 10 mmol/l ammonia. Using a flow rate of 0.4 ml/min, the LC gradient consisted of: Dwell at 100% A at 0 min, ramp to 80% A at 1 min, ramp to 0% A at 16 min, dwell at 0% A for 16-20 min, ramp to 100% A at 20.1 min, dwell at 100% A for 20.1-21 min. MS data were acquired using negative and positive ionization using continuous scanning over the range of m/z 150 to m/z 2000. Data were analyzed using an in-house developed metabolomics pipeline written in the R (<https://www.r-project.org/>) and MATLAB (<https://www.mathworks.com/>) programming languages. All reported lipids were normalized to corresponding internal standards according to lipid class. Lipid identification has been based on a combination of accurate mass, (relative) retention times, fragmentation spectra, analysis of samples with known metabolic defects, and the injection of relevant standards.

Proteomics

Proteomics sample preparation was performed using a Thermo Scientific™ EasyPep™ MS Sample Prep Kit (A40006), according to the kit's instructions. Briefly, 200 µl lysis buffer was added to each sample and a Thermo Scientific™ Pierce™ BCA Protein Assay (23225) was performed according to kit instructions to determine protein content. For each sample, 100 µg of protein was transferred to a new 2 ml tube. Samples were reduced and alkylated at 95° C for ten minutes, followed by a two-hour incubation at 37° C with a Trypsin/Lys-C protease mixture. After sample clean-up with the Peptide Clean-up Plate, samples were dried under nitrogen at 60° C, before being resuspended in a 100 µL mixture of 97:3 (v/v) water:acetonitrile, containing 0.1% formic acid.

Samples were kept at 12° C during analysis, and 10 µl of each sample was injected. The injection order for samples was random, with injections of a pooled sample at the start and end, as well as at varying intervals throughout the series. Chromatographic separation was achieved on a Waters™ Acquity UPLC, using a Waters™ Acquity UPLC BEH C18 Column (130Å, 1.7 µm, 2.1 mm X 50 mm) (186002350), equipped with a Waters™ Acquity UPLC Vanguard BEH C18 precolumn (186003975). Column temperature was held at 60° C. Mobile phase consisted of (A) water and (B) acetonitrile, both containing 0.1% formic acid. Using a starting flow rate of 0.5 ml/min, the LC gradient consisted of: Dwell at 3% B for 0-0.1 min; Ramp to 40% B at 4.3 min; Ramp to 80% B at 4.31 min with a flow rate of 0.85 ml/min; Dwell at 80% B for 4.31-4.40 min with a flow rate of 0.85 ml/min; Ramp to 3% B at 4.50 min with a flow rate of 0.6 ml/min; Dwell at 3% B for 4.5-5 min with a flow rate of 0.5 ml/min.

MS data were acquired with a Bruker timsTOF Pro 2 using positive ionization in DIA-PASEF mode as previously reported [32]. A detailed report of the MS method is provided in Supplementary Method.

DIA-PASEF data files were processed using DIA-NN version 1.8.1 [33]. The following DIA-NN options were enabled: Reannotate, Contaminants, FASTA digest for library-free search/library generation, N-term M excision, C carbamidomethylation, MBR, No shared spectra. Other settings were as follows: Missed cleavages: 1; Peptide length range: 7-30; Precursor charge range: 1-4; maximal number of variable modifications: 0.

Precursor m/z range: 300-1800; Fragment ion m/z range: 200-1800; Precursor FDR (%): 1; Mass accuracy: 0.0; MS1 accuracy: 0.0; Scan window: 0;

Protein names (from FASTA); Double-pass mode; Quant UMS (high precision); RT-dependent; IDs, RT & IM profiling; Optimal results. Data were normalized in DIA-NN using MaxLFQ [34].

Quantification and statistical analysis

Comparisons between more than two groups were performed by using One-way ANOVA test, while for comparisons with two groups, a student t-test was used. The log-rank test was used for lifespan analysis. Prism 9 software was used for statistical analysis. P-values below 0.05 were significant (**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant).

Data availability

The lipidomics data is available via Metabolights: MTBLS15090. The Proteomics data is available via PXD: PXD074442.

AUTHOR CONTRIBUTIONS

S.v.d.R., A.W.G., R.H.H., G.E.J., and A.T. designed the project. S.v.d.R., S.W.D., L.M.B. and R.O. performed experiments. S.v.d.R., B.V.S., M.v.W., and F.M.V. performed and/or interpreted lipidomics experiments. A.D.D. performed the bioinformatics analysis of the lipidomics data. S.v.d.R., B.V.S., S.R.P. and C.R.J. developed, performed and analyzed the proteomics. S.v.d.R., A.W.G., R.H.H., G.E.J. and A.T. wrote the manuscript with the contribution of all the other authors.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

The Institutional Animal Care and Use Committee of the Amsterdam UMC approved all animal procedures (Approval Number: GMZ102816-1).

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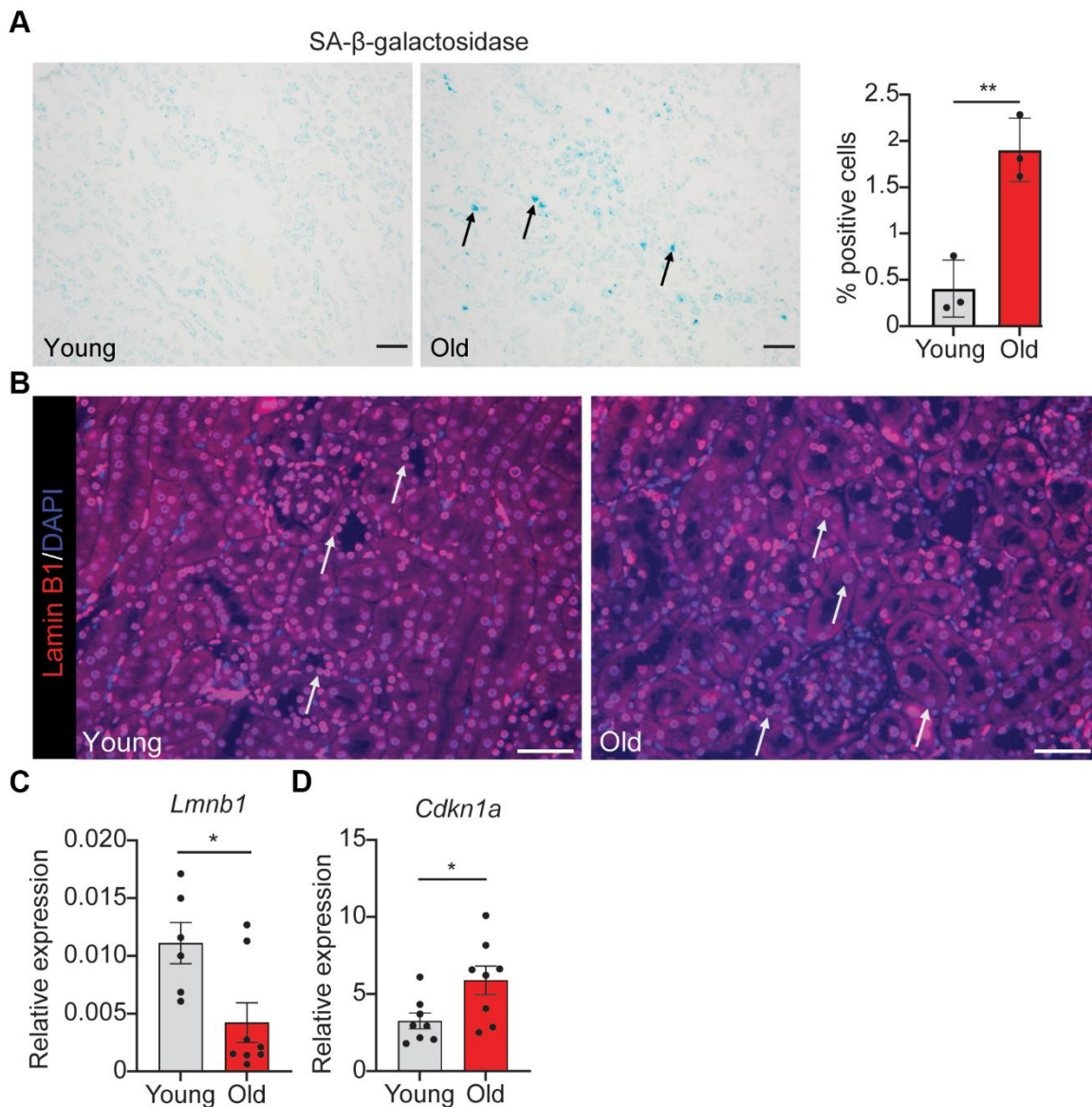
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SUPPLEMENTARY MATERIALS

Supplementary Figure



Supplementary Figure 1. Senescence phenotype in aged mouse kidney. (A) Representative images and quantification of SA- β -galactosidase staining in frozen mouse kidney tissues in young and old mice, showing increased SA- β -galactosidase in the tubular compartment. Scale bar is 100 μ M. (B) Immunofluorescence staining of Lamin B1 and DAPI in frozen mouse kidney tissue of young and aged mice, showing reduced Lamin B1 expression in aged kidneys. Arrows pointing towards Lamin B1 positive tubules (young) and Lamin B1 loss in tubules of aged mouse. (C) Gene expression of *Lmnbl1*, relative to housekeeping gene *Ppia*, demonstrating decreased expression in aged mice. (D) Gene expression of *Cdkn1a*, relative to the housekeeping gene *Ppia*, showing increased expression in aged mice. Statistical comparison was determined by using the unpaired t-test for two groups; * $p < 0.05$ and ** $p < 0.01$.

Supplementary Tables

Please browse Full Text version to see the data of Supplementary Tables 1, 2.

Supplementary Table 1. Excel file containing lipidomics data.

Supplementary Table 2. Excel file containing proteomics data.

Supplementary Method

Please browse Full Text version to see the data of Supplementary Method 1.

Supplementary Method 1. Supplementary methods for proteomics.