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Tau reduction impairs nephrocyte function in *Drosophila*

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Tau, a microtubule-associated protein, is known for its significant involvement in neurodegenerative diseases. While various molecular and immunohistochemical techniques have confirmed the presence of Tau in podocytes, its precise function within these cells remains elusive. In this study, we investigate the role of Tau in kidney podocytes using *Drosophila* pericardial nephrocytes as a model. We found that knockdown of *Drosophila* Tau in nephrocytes resulted in apoptotic cell death and the disruption of nephrocyte structure. Furthermore, we observed that decreased Tau levels induced genomic damage and abnormal distribution of γ-H2Av, altering nuclei architecture in nephrocytes, and affecting the nuclear membrane structure by interfering with lamin with aging. Additionally, *Tau* knockdown led to a reduction in lipid droplets in *Drosophila* fat body tissues, suggesting a potential role of Tau in inter-organ communication. These findings underscore the importance of Tau in the nephrocytes of *Drosophila*, and advocate further research to broaden our understanding of podocyte biology in kidney diseases. [BMB Reports 2025; 58(4): 169-174]

INTRODUCTION

The intricate architecture of renal podocytes constitutes a fundamental component of the glomerular filtration barrier, and is essential for maintaining kidney function and homeostasis (1). Podocytes are highly specialized epithelial cells that enwrap the glomerular capillaries, and play a pivotal role in regulating the passage of solutes and water from the bloodstream into the urinary space, while preventing the loss of essential proteins and cells (2, 3). The structural integrity of podocytes is maintained by a complex interplay of cytoskeletal elements, including actin filaments, intermediate filaments, and microtubules, which contribute to their unique morphology and dynamic behavior (4-6). Profound alterations in the cellular architecture of podocytes, notably the flattening of foot processes and subsequent loss of podocytes, are induced by severe filtration deficiencies, ultimately manifesting as increased levels of proteinuria.

Tau is a microtubule-associated protein that plays an important role in various neurodegenerative diseases, such as tauopathies (7). Originally characterized for its role in stabilizing microtubules and facilitating axonal transport in neurons, Tau is present in non-neuronal tissues, including the kidneys (8). The key regulators of microtubule dynamics, Tau, were abundantly expressed in the glomerular podocytes of mice, and it is regulated by glycogen synthase kinase−3β *in vivo* (9). Furthermore, single-cell RNA sequencing has identified approximately 90 candidate genes for podocyte-specific expression, including Tau (10). Thus, although various molecular and immunohistochemical techniques have confirmed the presence of Tau in podocytes, its exact role in these cells remains unclear. Slight attention has been paid to Tau distribution in the podocytes of kidney tissues, and the functional significance of Tau in non-neural tissues under non-pathological conditions is largely unknown. Moreover, understanding the function of Tau in kidney podocytes has significant implications for deciphering the mechanisms underlying podocyte biology, and the pathogenesis of glomerular diseases.

Drosophila renal system has a highly specialized filtering structure, the nephrocyte diaphragm, which is similar to the slit diaphragm of mammalian glomerular cells. There are two types of nephrocytes in *Drosophila*: pericardial nephrocytes, located adjacent to the heart tube, and garland nephrocytes, which surround the esophagus (11). Pericardial nephrocytes in *Drosophila* are podocyte-like cells with additional properties shared with the mammalian proximal tubules of the kidney. Therefore, the structural and functional similarities between the *Drosophila* nephrocyte and the human podocyte indicated it could provide a powerful model to study podocyte dysfunction and kidney diseases. Here, we used *Drosophila* pericardial nephrocytes as models for mammalian glomerular podocytes and proximal tubule cells, to study the function of Tau in kidney tissue. We first showed that the nephrocyte-specific knockdown of conserved

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endogenous *Drosophila* Tau (dTau) decreased the number of nephrocytes and disrupted nephrocyte structure by inducing apoptotic cell death. Importantly, we found that the downregulation of *dTau* affected the structure of the nuclear membrane in nephrocytes by interfering with lamin expression in an age-dependent manner, which was confirmed in the renal podocytes of mice (8). Moreover, we showed that the nephrocyte-specific knockdown of *dTau* promoted the reduction of lipid droplets in other organs and fat body tissues in *Drosophila*. This phenotype in *dTau* knockdown flies is likely to regulate interorgan communication between nephrocytes and fat bodies. Therefore, our results indicate that the *dTau* loss-of-function has a strong impact on the structure and function of nephrocytes in *Drosophila*. These findings also suggest that the role of dTau in animal podocytes of kidney tissues is evolutionarily conserved, and that *Drosophila* may be used to identify novel pathways relevant to the regulation and role of Tau in human.

RESULTS

dTau downregulation in nephrocytes induces apoptotic cell death

To investigate the physiological role of Tau in kidney, we utilized *Drosophila* nephrocytes as a model for podocytes in kidney tissues. To assess whether dTau affects nephrocyte function in *Drosophila*, we labeled nephrocytes using a membrane-bound red fluorescent protein (mCD8−RFP) reporter driven by the nephrocyte-specific driver *sns-GCN-Gal4*. Interestingly, the average size of nephrocytes was significantly decreased in *dTau* RNAi-expressing flies, compared to that in control flies (Fig. 1A, arrowheads). Moreover, we observed the longevity of nephrocyte-specific *dTau* RNAi-expressing flies. Downregulation of *dTau* in nephrocytes shortened the late stages of lifespan in *Drosophila*, with the mean lifespan slightly decreasing by approximately 12% (Fig. 1B). Additionally, we measured the expression levels of *Drosophila* death caspase-1 (DCP−1), a homolog of animal caspase-3, which is an effector caspase inducing apoptotic cell death after activation by cleavage by an initiator caspase (12). Immunohistochemistry with antibodies against activated DCP−1 has been widely used to quantify apoptosis in *Drosophila*. We therefore stained the nephrocytes of control and *dTau* RNAi-expressing flies of different ages with anti-DCP−1 antibodies to determine whether apoptosis increased. The number of DCP−1 signals significantly increased in the nephrocytes of 3-day *dTau* RNAi-expressing flies, compared to age-matched controls (Fig. 1C, D). This measure of apoptotic cell death dramatically increased with age in experimental flies at 20 days (d) of age. To assess the importance of Tau proteins in nephrocyte function, we investigated the role of pericardial nephrocytes in toxin uptake and sequestration (13). Silver nitrate ($AgNO_3$) is taken up by nephrocytes, and sequestered intracellularly. However, when nephrocytes are dysfunctional, they are unable to absorb $AgNO_3$ (14). Ingested $AgNO_3$ is normally sequestered in pericardial nephrocytes. We also expressed *dTau* RNAi in larval nephrocytes, and compared the $AgNO_3$ levels with those of the controls (Fig. 1E, F). A significant reduction of approximately 30% in $AgNO_3$ levels was observed in larval nephrocytes following *dTau* knockdown (Fig. 1F). These results indicate that the downregulation of *dTau* caused severe morphological and functional abnormalities in the nephrocytes of *Drosophila*.

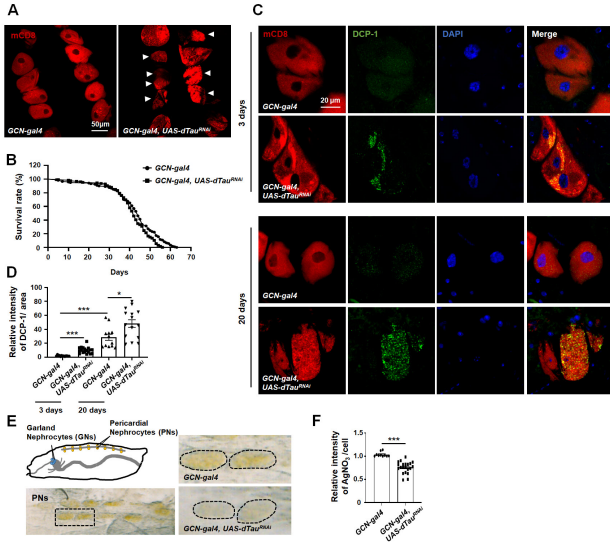

Fig. 1. Knockdown of *dTau* disrupts nephrocyte function in *Drosophila*. (A) Pericardial nephrocytes in the abdomen from mCD8−RFP-expressing flies under the control of *snsGCN−Gal4*. The RNAi-mediated knockdown of *dTau* caused severe morphological abnormalities in Drosophila nephrocytes (arrowheads). (B) The RNAi-mediated genetic knockdown of *dTau* in nephrocytes decreased lifespan ($n > 150$ each). (C) Pericardial nephrocytes (red) in the abdomen from mCD8−RFP-expressing flies under the control of *snsGCN−Gal4*. Nephrocytes dissected from (3 or 20)-d-old *dTau* RNAi-expressing flies were stained with anti-cleaved DCP−1 antibody (green) and DAPI (blue). Increased DCP−1 signals, a marker for apoptosis, were observed in the nephrocytes of *dTau* RNAi-expressing flies. (D) Quantification of the number of DCP−1 positive puncta in the nephrocytes of adult flies at (3 and 20)-d-old. A significant increase in the number of puncta was observed in the *dTau* RNAi-expressing nephrocytes, compared to the control. Error bars represent the mean ± standard deviation ($n \geq 10$ nephrocytes for each genotype). Statistical significance was determined using Student's *t*-test (*$P < 0.05$, ***$P < 0.001$). (E) Photomicrograph showing ingested $AgNO_3$ sequestered in larval nephrocytes. The pericardial nephrocytes in control flies contained silver, but not when *dTau* had been silenced (brown pigment). Dashed lines indicate the borders of pericardial nephrocytes. (F) $AgNO_3$ levels were determined from photomicrographs, and expressed relative to control nephrocytes in *snsGCN−Gal4* transgenic flies. For quantification, 20 nephrocytes were analyzed from each of three female flies per genotype. Experimental significance was determined using Student's *t*-test (***$P < 0.001$).

Downregulation of *dTau* disrupts nuclear structure by regulating Lamin B in the nephrocytes of *Drosophila*

We observed fragmented or small nuclei stained with DAPI in

the nephrocytes of 20-d-old flies expressing *dTau* RNAi (Fig. 2A, B). Cellular senescence is often accompanied by changes in chromatin structure, leading to the formation of senescence-associated heterochromatic foci (SAHFs) (15). When stained with DAPI, normal cells typically display a uniform and diffuse distribution of DNA throughout the cell nucleus. However, nephrocyte expressing *dTau* RNAi leads to chromatin condensation, resulting in the formation of characteristic heterochromatin structures known as SAHFs (Fig. 2A, arrowheads). These notable phenotypes in nephrocyte nuclei appear to result from SAHF formation induced by cellular senescence. To investigate genomic damage

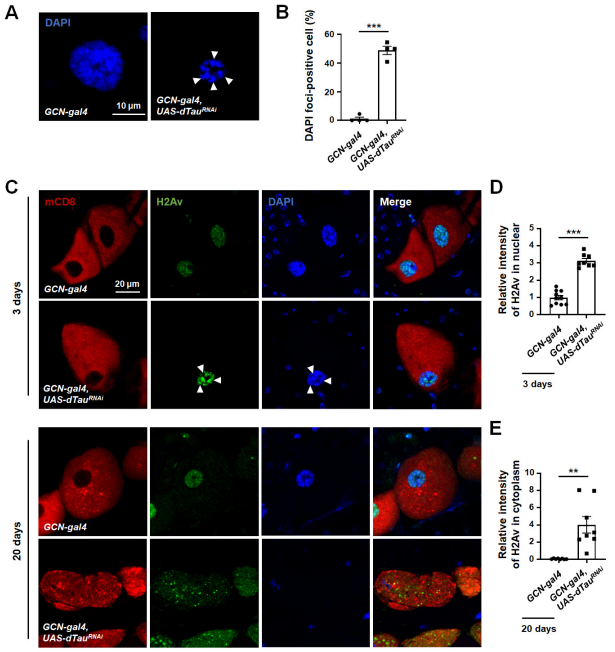

Fig. 2. Knockdown of *dTau* disrupts nuclear structure and forms senescence-associated heterochromatin foci (SAHF) in nephrocytes. (A) Nephrocytes were stained with DAPI (blue) to visualize DNA. SAHF, a marker of cellular senescence, was observed in the nephrocytes of *dTau* RNAi-expressing flies (arrowheads). (B) Quantification of DAPI foci-positive nephrocytes in adult flies. A significant increase in the number of DAPI foci-positive nephrocytes was observed in the *dTau* RNAi-expressing nephrocytes, compared to the control. Error bars represent the mean ± standard deviation (n = 4 adult flies for each genotype). Statistical significance was determined using Student's *t*-test (***P < 0.001). (C) Pericardial nephrocytes (red) in the abdomen of mCD8−RFP-expressing flies under the control of *snsGCN−Gal4*. Nephrocytes dissected from (3 or 20)-d-old *dTau* RNAi-expressing flies were stained with anti-histone H2Av antibody (green) and DAPI (blue). *dTau* knockdown exhibits increased frequencies of γ-H2Av foci in the nephrocytes of 3-d-old flies (arrowheads). Dispersed γ-H2Av foci in 20-d-old *dTau* RNAi-expressing flies were detected in the cytoplasm of nephrocytes. (D, E) Quantification of nuclear and cytoplasmic γ-H2Av fluorescent signal in the nephrocytes. Error bars represent the mean ± standard deviation (n ≥ 8 nephrocytes for each genotype). Statistical significance was determined using Student's *t*-test (**P < 0.01, ***P < 0.001).

in nephrocytes with fragmented or disrupted nuclei, we examined double-strand breaks (DSBs). One of the initial responses to DNA DSBs is the phosphorylation of the histone variant H2A, referred to as H2AX in animals, and H2Av in *Drosophila* (16, 17). Senescent cells typically exhibit DNA damage response components, such as γ-H2Av foci (18, 19). Immunofluorescence analysis of nephrocytes from *dTau* RNAi-expressing flies using an antibody against the histone variant γ-H2Av, an early and specific marker of DNA damage, revealed the accumulation of γ-H2Av-positive foci in 20-d-old flies, confirming *dTau* knockdown-induced genomic instability (Fig. 2C, D, arrowheads). Interestingly, dispersed γ-H2Av labeling concentrated in the cytoplasm of nephrocytes was detected in *dTau* RNAi-expressing flies (Fig. 2C, E). These results align with a previous reported loss of Tau of altered distribution of γ-H2AX in the hippocampus of adult mice lacking Tau (20). Thus, our findings suggest that Tau downregulation induces the genomic damage or abnormal distribution of γ-H2Av by altering the nuclear architecture in *Drosophila* nephrocytes, implicating Tau protein in the regulation of cellular senescence.

Increasing evidence indicates that Tau regulates nuclear morphology. Alterations in nuclear structure have been observed in neuronal cells overexpressing human Tau in the cytosol, leading to rearrangement of the filamentous lamin nucleoskeleton (21). Morphological changes in the lamin nucleoskeleton

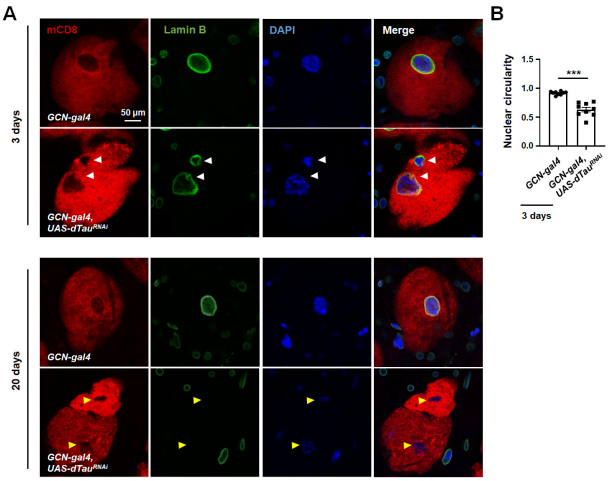

Fig. 3. Knockdown of *dTau* causes disrupted nuclear morphology by regulating lamin in nephrocytes. (A) The pericardial nephrocytes (red) in the abdomen from mCD8−RFP-expressing flies under the control of *snsGCN−Gal4*. Nephrocytes dissected from (3 or 20)-d-old *dTau* RNAi-expressing flies were stained with anti-lamin B antibody (green) and DAPI (blue). Abnormal nuclear morphology was observed in the nephrocytes of 3-d-old *dTau* RNAi-expressing flies (arrowheads). Knockdown of *dTau* caused loss of lamin in the nephrocytes of 20-d-old flies (yellow arrowheads). (B) Quantification of the nuclear circularity in the nephrocytes of adult flies at 3-d-old. Error bars represent the mean ± standard deviation (n ≥ 9 nephrocytes for each genotype). Statistical significance was determined using Student's *t*-test (***P < 0.001).

have also been observed in the brains of flies overexpressing Tau^{R406W} (22). Tau interacts with nuclear pore complexes, and regulates nuclear morphology and integrity (23). Furthermore, nuclear lamin B1 (LMNB1) depletion occurs during senescence, and LMNB1 knockdown facilitates spatial relocalization of perinuclear H3K9me3–positive heterochromatin, promoting SAHF formation (24). To investigate the role of dTau in nuclear envelope morphology in nephrocytes, we examined adult nephrocytes stained with an anti-lamin B antibody using fluorescence microscopy. Three-d-old *dTau* RNAi-expressing flies displayed defective nuclear structures in nephrocytes. Moreover, *dTau* RNAi led to nuclear lamin depletion in the nephrocytes of 20-d-old flies (Fig. 3A, B, arrowheads). In *Drosophila*, damage to lamins and changes in nuclear envelope structure in nephrocytes caused by dTau become increasingly severe with aging. These findings suggest that Tau plays a critical role in regulating the nuclear architecture associated with lamin in nephrocyte nuclei.

Nephrocyte-specific knockdown of dTau decreased lipid droplets (LDs) in the fat body

Our findings demonstrate that the nephrocyte-specific knockdown of *dTau* led to a decrease in LDs within the fat body. This reduction in LDs was associated with a decrease in adult body weight (Fig. 4A), suggesting a potential role of dTau in fat storage. In *Drosophila*, fat is primarily stored as LDs in the fat body, which functions akin to the adipose tissue in mammals, and exhibits liver-like detoxification activity (25). To assess fat body morphology, we employed the neutral lipid stain Oil Red O. Notably, staining of adult fat bodies in the abdomen revealed a significantly lower staining intensity and smaller LD size in *dTau* RNAi-expressing flies, compared to control animals (60% smaller) (Fig. 4B, C), indicating impaired LD formation. Similarly, analysis of larval fat bodies also showed smaller LDs in *dTau* RNAi-expressing larvae, compared to controls (Fig. 4D, 4E). These findings suggest that dTau expression in nephrocytes is crucial for LD size and storage in the adipose tissue of *Drosophila*.

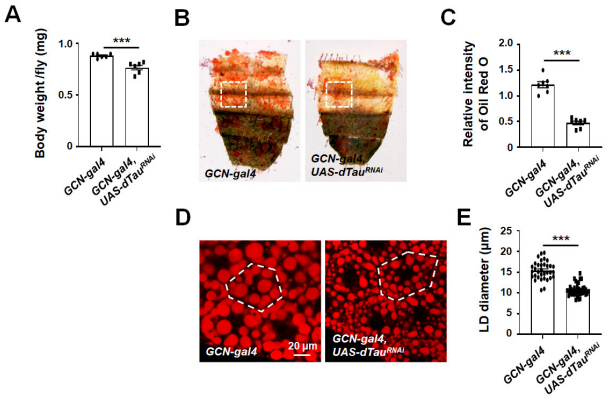

Fig. 4. Nephrocyte-specific knockdown of *dTau* leads to smaller lipid droplets (LDs) in fat body tissues. (A) Body weight of *dTau* RNAi-expressing flies compared to control *snsGCN−Gal4* flies. Error bars represent the mean ± standard deviation (n = 6 replicates; each replicate contained (7-10) flies). Experimental significance was determined using Student's *t*-test (***P < 0.001). (B) LDs labeled by Oil red O (red) in adult fat bodies from *dTau* RNAi-expressing flies. Knockdown of *dTau* exhibited lipid storage defects with small LDs (dotted box). (C) Graphs showing quantification of fluorescence intensity of LDs stained with Oil red O. Error bars represent the mean ± standard deviation (n = 7 abdomens for each genotype). Experimental significance was determined using Student's *t*-test (***P < 0.001). (D) LDs labeled by Oil red O (red) in larval fat bodies from *dTau* RNAi-expressing flies. Knockdown of *dTau* exhibited a small size of LDs. Dashed lines indicate the borders of cells. (E) Quantification of LD diameter in (D). Quantification of the diameter of the three largest LDs per cell in 10 cells from each genotype. Error bars represent the mean ± standard deviation. Experimental significance was determined using Student's *t*-test (***P < 0.001).

DISCUSSION

Podocytes, highly specialized epithelial cells characterized by distinct structures, possess numerous foot processes that encircle the glomerular capillaries, forming a filtration barrier. Many patients with glomerular diseases exhibit podocyte damage (26). Moreover, podocyte loss results in severe filtration defects, ultimately leading to proteinuria (27, 28). Given these observations, investigating the unique architecture and function of podocytes, as well as identifying novel podocyte viability regulators, has become crucial in various experimental models.

Tau, originally recognized for its role in stabilizing microtubules and facilitating axonal transport in neurons, has been detected in non-neuronal tissues, including the kidneys. Recent studies have demonstrated the presence of Tau in the glomeruli of the renal cortex and podocytes of kidney tissues (8). The authors proposed that Tau may influence glomerular morphology by modulating microtubule dynamics (8). However, its precise role in podocytopathy and glomerular repair remains elusive, and in this study was explored using a *Drosophila* model of nephrocyte defects and nephropathy. Taking advantage of the high degree of conservation of filtration structures between mammalian podocytes and *Drosophila* nephrocytes, we investigated the role of Tau in this model system, and the consequences of silencing its expression.

In this study, we discovered that the nephrocyte-specific knockdown of conserved endogenous *Drosophila* Tau resulted in a decrease in the number of nephrocytes, and disrupted nephrocyte structure by inducing apoptotic cell death. Additionally, we evaluated the impact of dTau knockdown on the physiological functions of nephrocytes in the presence of dietary $AgNO_3$. Pericardial nephrocytes in *Drosophila* play a crucial role in the uptake and sequestration of toxins (11, 13, 29). Normally, ingested $AgNO_3$ is sequestered in pericardial nephrocytes; however, dTau knockdown in flies abolishes $AgNO_3$ sequestration in nephrocytes. Furthermore, the downregulation of dTau leads to the reduction and disorganization of lamins in nephrocytes in an age-dependent manner, facilitating the formation of heterochromatic SAHFs in the

nucleus, subsequent DNA damage, cellular senescence, and apoptosis. Therefore, a decrease in the level of *dTau* has a physiological function that regulates the organization and structure of lamins, known to play a pivotal role in regulating genomic stability and gene expression directly involved in nephrocyte function and structure. Consequently, defects in and loss of *dTau* result in nephrocyte dysfunction and depletion in *Drosophila*.

Interestingly, we demonstrated that the nephrocyte-specific knockdown of *dTau* promoted the reduction of lipid droplets in other organs and fat body tissues in *Drosophila*. Our findings suggest that dTau may regulate interorgan communication between nephrocytes and fat tissue in maintaining lipid metabolism in *Drosophila*. A recent study reported that unpaired 3, a cytokine released from pericardial nephrocytes in *Drosophila*, can target tissues, such as fat cells, to control the fat body (30). This corroborates our results, indicating the potential regulation of fat metabolism by Tau, with crosstalk between nephrocytes and fat cells.

In conclusion, our findings indicate that Tau regulates nephrocyte viability and lipid metabolism in fat tissues via interorgan communication. The data obtained from our investigation into Tau function in *Drosophila* nephrocytes strongly advocate further research endeavors aimed at elucidating podocyte biology in renal diseases, and at unraveling the physiological significance of Tau in kidney function. Additional studies are warranted to gain a deeper understanding of this association.

MATERIALS AND METHODS

Drosophila stocks

All stock flies were reared at 25°C under standard humidity conditions with standard food. Crosses were performed using conventional procedures. The nephrocyte-specific driver, *snsGCN-Gal4* line, was provided by Dr. Ross L. Cagan (University of Pennsylvania, Philadelphia, PA, USA). The *UAS-dTau RNAi* line was obtained from the Bloomington *Drosophila* Stock Center.

Lifespan assay

For the lifespan assay, 20 male flies were grouped per vial (a total of 10 vials per genotype were used in this assay) and maintained at 25°C. On each subsequent day, flies from all groups were transferred to fresh vials, and the number of decreased flies was recorded daily. Lifespan data from more than 150 flies were collected.

Immunohistochemistry

To analyze nephrocytes, the abdomens of adult male flies were dissected in phosphate-buffered saline (PBS) and fixed with 4% formaldehyde in fixative buffer (100 mM 1,4-piperazinediethanesulfonic acid (PIPES), 1 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100, and 2 mM $MgSO_4$, pH 6.9) for 30 min. Tissues were washed with PBST (PBS containing 0.1% Triton X-100) for 10 min and blocked with 5% bovine serum albumin in PBST. Subsequently, the samples were incubated with the primary antibodies: rabbit anti-DCP-1 antibody (1:100; Cell Signaling Technology, Cat#: 9578, Danvers, MA, USA), mouse anti-Histone H2Av antibody (1:100; DSHB, Cat#: UNC93-5.2.1, Iowa City, IA, USA), and mouse anti-Lamin B (Dm0) antibody (1:100; DSHB, Cat#: ADL67.10, Iowa City, IA, USA) for 12 h at 4°C. Afterward, samples were incubated with Alexa 488-conjugated secondary antibodies (1:200; Invitrogen, Cat#: A11034, A11001, Carlsbad, CA, USA) and 4′,6-diamidino-2-phenylindole (DAPI, 1:500; Sigma-Aldrich, Cat#: D9564, St. Louis, MO, USA) in blocking buffer. Tissues were mounted using SlowFade™ Gold antifade reagent (Invitrogen, Cat#: S36936, Carlsbad, CA, USA). Imaging was performed using a Leica fluorescence microscope (MZ10F) (Leica Microsystems, Wetzlar, Hessen, Germany) and a Carl Zeiss confocal microscope (LSM710) (Carl Zeiss, Oberkochen, Baden-Württemberg, Germany) at the Soonchunhyang Biomedical Research Core Facility of the Korea Basic Science Institute (KBSI).

$AgNO_3$ uptake assay

Flies of the appropriate genotype were allowed to lay eggs on standard apple juice agar plates with yeast for 24 h. Freshly emerged first-instar larvae were then transferred to agar plates supplemented with yeast paste containing $AgNO_3$ (2.0 g yeast in 3.5 ml 0.0005% $AgNO_3$ solution). The plates were maintained at 25°C until adulthood. $AgNO_3$ uptake by pericardial nephrocytes was assessed in adult flies at 1-day post-emergence by dissecting heart tissues into *Drosophila* Schneider's Medium (Thermo Fisher Scientific) and examining cells by phase-contrast microscopy after 10 minutes of fixation in 4% paraformaldehyde in PBS. For quantification, more than 10 nephrocytes were analyzed from each of the three female flies per genotype.

Lipid droplet staining assay

For lipid droplet staining, adult flies or larvae were dissected in PBS and fixed with 4% formaldehyde in a fixative buffer (100 mM PIPES, 1 mM EGTA, 1% Triton X-100, and 2 mM $MgSO_4$, pH 6.9) for 30 min at 25°C. The tissues were then washed in PBST and incubated for 30 min in 0.1% Oil Red O (Sigma-Aldrich, Cat#: 09755, St. Louis, MO, USA) solution or Nile Red (Sigma-Aldrich, Cat#: M3013, St. Louis, MO, USA) staining solution (1 mg/ml in dimethyl sulfoxide, 1:2000 in PBST). Subsequently, the samples were washed with PBST and mounted using SlowFade™ Gold antifade reagent (Invitrogen, Cat#: S36936, Carlsbad, CA, USA). Imaging was conducted using a confocal microscope (LSM710) (Carl Zeiss, Oberkochen, Germany).

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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