

Bone marrow mesenchymal stem cell-derived exosomal miR-425-5p attenuates diabetic nephropathy by suppressing podocyte WTAP/MET/GSK3 β signalling

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Abstract

Diabetic nephropathy is driven in part by podocyte injury, but the contribution of N⁶-methyladenosine (m⁶A) regulation remains unclear. Here we show that WT1-associated protein (WTAP) is increased in diabetic kidneys and high-glucose-treated podocytes, where it stabilizes MET mRNA through m⁶A modification, activates MET/glycogen synthase kinase 3 beta (GSK3 β) signalling and promotes podocyte injury. WTAP knockdown reduces apoptosis, restores podocin and ameliorates renal injury in diabetic mice. Integrated microRNA profiling identifies miR-425-5p as a direct suppressor of WTAP. Hypoxia-preconditioned bone marrow mesenchymal stem cell-derived exosomes are enriched in miR-425-5p and inhibit WTAP/MET/GSK3 β signalling in podocytes and diabetic mice; inhibition of miR-425-5p attenuates these effects. These findings define a WTAP-dependent m⁶A pathway in diabetic podocyte injury and establish exosomal miR-425-5p delivery as a therapeutic strategy.

Introduction

Diabetic nephropathy (DN) remains a leading cause of progressive chronic kidney disease and end-stage renal disease, characterized by persistent albuminuria and a progressive decline in renal filtration capacity ¹. During DN progression, hyperglycemia-induced metabolic stress, oxidative damage, inflammation, and haemodynamic abnormalities collectively disrupt the glomerular filtration barrier ². Podocytes are particularly susceptible to diabetic injury because they maintain the slit diaphragm and have limited regenerative capacity ^{3,4}. Defining the molecular mechanisms that govern podocyte dysfunction under diabetic conditions is therefore essential for developing strategies to halt DN progression.

N⁶-methyladenosine (m⁶A) is the most abundant internal modification of eukaryotic messenger RNA and regulates RNA processing, nuclear export, stability, translation, and degradation ^{5,6}. WT1-associated protein (WTAP), a core regulatory subunit of the METTL3/METTL14 m⁶A methyltransferase complex, is essential for substrate recognition, transcript-selective m⁶A deposition, and subsequent RNA fate determination ^{7,8}. Dysregulated m⁶A modification contributes to renal pathological processes including diabetic renal injury, ischemia–reperfusion injury, and renal fibrosis ^{9–11}. However, m⁶A research in kidney disease has largely focused on tubular and fibrotic responses; the role of WTAP-mediated m⁶A remodeling in diabetic podocytes and the identity of downstream methylated transcripts remain largely unexplored.

The MET/GSK3 β signalling axis may contribute to podocyte injury. Canonical hepatocyte growth factor (HGF)-induced MET activation maintains podocyte homeostasis and reduces proteinuria by regulating autophagy through the PI3K/Akt–GSK3 β –TFEB pathway ^{12,13}. In contrast, aberrant GSK3 β activation has been implicated in podocyte stress, inflammation, and diabetic kidney disease progression ^{14–16}. Studies in nonrenal systems further suggest functional interplay between MET signalling and GSK3 β activity ¹⁷. Whether WTAP-mediated maintenance of MET abundance under diabetic conditions preserves the

classical ligand-dependent cytoprotective MET program or promotes a noncanonical MET signalling state associated with GSK3 β -driven injury remains unknown. This distinction is critical because increased total MET expression does not necessarily reflect receptor activation or downstream functional output. Defining how WTAP regulates MET transcript fate and signalling consequences is therefore essential for understanding the context-specific role of MET in diabetic podocyte injury.

Extracellular vesicles (EVs) mediate intercellular transfer of proteins, lipids, and noncoding RNAs and are promising cell-free effectors of tissue repair and regeneration^{18,19}. Because the therapeutic effects of mesenchymal stem cells (MSCs) are largely mediated through paracrine signalling, MSC-derived EVs provide an acellular platform for delivering bioactive molecules without administering viable cells. This strategy reduces risks associated with long-term cellular persistence, uncontrolled proliferation, and unintended differentiation. Compared with MSC therapy, EVs are non-self-replicating, have lower immunogenicity, and offer manufacturing advantages including purification, formulation, potency-based quality control, and cargo or surface engineering^{20–23}. Comparative proteomic analyses of exosomes from bone marrow, adipose tissue, and umbilical cord MSCs have identified conserved functions and source-specific signatures, with BMSC-Exos showing greater enrichment of regeneration-associated proteins²⁴. We therefore selected BMSC-Exos as a biologically relevant and experimentally tractable vehicle for podocyte-targeted microRNA (miRNA) delivery. Engineered BMSC-Exos can efficiently deliver therapeutic miRNAs to injured renal cells and alleviate renal fibrosis *in vivo*²⁵. Because miRNAs repress target mRNAs through sequence-specific recognition²⁶, we hypothesized that an exosome-enriched miRNA targeting WTAP links BMSC-Exos treatment to m⁶A-dependent podocyte protection. The identity and functional significance of this miRNA in DN remained unknown.

Here, we integrated analyses of human renal tissues, *in vitro* high-glucose-treated podocyte models, WTAP loss-of-function approaches, streptozotocin (STZ)-induced diabetic mice, mechanistic studies of the MET/GSK3 β interaction, BMSC-Exos intervention, and miRNA target validation to investigate WTAP-mediated m⁶A regulation in diabetic podocyte injury. Specifically, we tested the hypothesis that BMSC-Exos alleviate podocyte injury by delivering miR-425-5p, which suppresses WTAP expression and modulates the WTAP/MET/GSK3 β signalling axis, thereby preserving podocyte integrity under diabetic conditions.

Results

RNA m⁶A methylation is involved in podocyte injury under high-glucose conditions

To profile RNA modifications in renal tissues from patients with DN, we used LC–MS, which detected up to 56 nucleosides. Multiple-reaction-monitoring peak areas for individual modified nucleosides were extracted from the LC–MS/MS data and normalized for quantitative analysis. Fourteen RNA modifications had peak areas > 2,000. DN tissues showed a higher normalized m⁶A/A peak-area signal

than control tissues, and m⁶A/A was among the most prominently increased modifications measured (Fig. 1A). In parallel, m⁶A dot blotting showed stronger global m⁶A signals in high-glucose-treated MPC-5 cells than in NG controls across serial RNA inputs (Fig. 1B).

We next assessed m⁶A-modifying enzymes in renal specimens from patients with DN using Human m⁶A mRNA and lncRNA epitranscriptomic microarrays. Among the methyltransferases and demethylases screened, WTAP expression was significantly increased in DN tissues and was selected for mechanistic analysis (Fig. 1C). WTAP immunohistochemistry showed stronger nuclear staining in glomerular podocytes from DN kidneys, and qPCR confirmed higher WTAP mRNA abundance in DN samples than in controls (Fig. 1D,E). WTAP expression correlated positively with 24-h urinary protein excretion and negatively with estimated glomerular filtration rate (Fig. 1F,G). Collectively, these results reveal a DN-associated increase in m⁶A modification and WTAP expression and support a critical epitranscriptomic role for WTAP in podocyte injury.

WTAP knockdown attenuates high-glucose- and streptozotocin-induced podocyte injury

We next examined whether WTAP suppression alleviated podocyte injury under HG conditions. In HG-treated MPC-5 cells, WTAP knockdown reduced cleaved caspase-3 expression and increased podocin expression (Fig. 2A,B). Annexin V/PI flow cytometry showed a parallel reduction in apoptosis: total apoptosis was 0.20 ± 0.06% in NG cells, 24.91 ± 0.79% in HG cells, 25.21 ± 0.72% in HG + LV-shNC cells, and 3.19 ± 0.40% in HG + LV-shWTAP-2 cells (Fig. 2C).

We then investigated WTAP function in vivo using an STZ-induced diabetic mouse model with podocyte-specific WTAP knockdown driven by nephrin promoter-controlled AAV9-shWTAP (Fig. 2D). STZ markedly increased UACR, and AAV9-mediated WTAP knockdown significantly attenuated this increase relative to the STZ+AAV9-shNC group (Fig. 2E). Glomerular immunoblotting showed reduced cleaved caspase-3 and restored podocin expression after AAV9-shWTAP intervention (Fig. 2F,G). Representative TUNEL, PAS/Masson, and transmission electron microscopy images showed less apoptosis, glomerular injury, and podocyte ultrastructural damage in AAV9-shWTAP-treated diabetic mice (Fig. 2H,I). Collectively, these in vitro and in vivo results demonstrate that WTAP knockdown protects against podocyte injury.

WTAP regulates MET expression in an m⁶A-dependent manner

To investigate how upregulated WTAP contributes to DN, we established and validated WTAP-knockout HPCs (Supplementary Fig. 1A). MeRIP-seq in control and WTAP-knockout HPCs showed that m⁶A peaks were enriched within coding sequences (Fig. 3A,C) and characterized by the RRACH motif (Fig. 3B). The hyper-upregulated subset identified using the human m⁶A mRNA and lncRNA epitranscriptomic microarray comprised 317 qualified features corresponding to 303 unique genes, whereas WTAP knockout produced 1,074 sites with decreased m⁶A modification across 758 unique genes. Intersection

of these gene sets identified 17 candidates (Fig. 3D). MeRIP-seq revealed global m⁶A alterations after WTAP depletion, including reduced m⁶A peaks on MET (Fig. 3E). In human DN renal tissues, MET mRNA abundance and m⁶A methylation signal were both elevated and positively correlated (Fig. 3F,G). This association indicated that increased MET m⁶A methylation is linked to enhanced MET expression in diabetic kidneys and prompted further analysis of WTAP-mediated MET methylation.

SRAMP analysis predicted three candidate sites in mouse Met mRNA (Supplementary Fig. 1B), and m⁶A-RIP-qPCR in MPC-5 cells validated significant enrichment at positions 718, 837, and 1233 in the m⁶A-immunoprecipitated fraction relative to IgG controls (Fig. 3H–J). These results establish WTAP as a direct regulator of MET m⁶A methylation. We then examined whether WTAP-dependent MET methylation affects mRNA stability. Among three lentiviral shRNAs targeting WTAP, LV-shWTAP-2 showed the highest knockdown efficiency by immunoblotting and qPCR (Supplementary Fig. 1C,D). WTAP knockdown accelerated MET mRNA decay and reduced its half-life from 3.647 h in LV-shNC cells to 2.045 h in LV-shWTAP-2 cells (Fig. 3K). MET protein expression was significantly increased in HG-treated MPC-5 cells relative to NG controls and remained elevated in the HG + LV-shNC group, whereas WTAP knockdown markedly reduced MET protein abundance under HG conditions (Fig. 3L,M). Thus, WTAP sustains MET mRNA stability through m⁶A modification and contributes to increased MET expression in podocytes under HG conditions.

MET interacts with GSK3β to form a functional protein complex

To determine how WTAP-mediated MET transcript stabilization contributes to downstream podocyte injury signalling, we investigated MET-associated signalling partners. Given the established role of GSK3β in diabetic podocyte stress responses, we tested whether MET directly associates with GSK3β. Rigid-body protein–protein docking predicted a stable MET–GSK3β complex, with a buried interface area of 1,717.8 Å² and an estimated binding free energy of – 14.2 kcal/mol by PDBePISA analysis (Fig. 4A–D). LigPlot+ identified multiple interacting residues and hydrogen-bond networks within the predicted interface, supporting the structural feasibility of MET–GSK3β association. Reciprocal co-IP in MPC-5 podocytes experimentally validated the interaction: GSK3β was detected in MET immunoprecipitates, and MET was enriched in GSK3β immunoprecipitates, whereas no comparable signal was observed in IgG controls (Fig. 4E). These results identify MET as a previously unrecognized GSK3β-interacting protein in podocytes and support formation of a MET–GSK3β protein complex.

miR-425-5p is a key miRNA targeting WTAP

To identify upstream regulators of WTAP, we performed renal miRNA profiling in control and DN samples. Unsupervised hierarchical clustering robustly separated control and DN tissues, revealing a distinct miRNA expression signature. Using a prespecified exploratory threshold of nominal $P < 0.05$ and

[fold change] >2, we identified 359 differentially expressed small noncoding RNAs, including 198 upregulated and 161 downregulated candidates (Fig. 5A,B).

Among the downregulated candidates, 87 were annotated as unique mature miRNAs and subjected to integrative target-prediction analysis. Intersecting DN-downregulated miRNAs with WTAP-target predictions from ENCORI, miRmap, and TargetScan identified miR-425-5p, miR-132-3p, and miR-181c-5p (Fig. 5C). Based on differential expression, statistical prioritization, and target-prediction confidence, miR-425-5p was selected as the leading WTAP regulator for functional validation (Supplementary Fig. 3).

TargetScan identified a conserved miR-425-5p seed-matching sequence at nucleotides 26–32 of the WTAP 3' UTR (Fig. 5D). Luciferase reporter constructs containing the wild-type WTAP 3' UTR or a seed-disrupted mutant were generated (Fig. 5E). In MPC-5 cells, miR-425-5p mimics significantly reduced luciferase activity from the wild-type WTAP 3' UTR reporter, whereas the inhibitory effect was abolished in the seed-mutant reporter (Fig. 5F). These findings demonstrate that miR-425-5p directly binds the predicted seed-matching region of the WTAP 3' UTR and establish WTAP as a direct post-transcriptional target of miR-425-5p.

Characterization of hypoxia-conditioned BMSC-Exos and miR-425-5p enrichment

Mouse BMSCs were isolated and characterized as the source of therapeutic exosomes. The cells exhibited adipogenic, osteogenic, and chondrogenic differentiation potential. Flow cytometry confirmed expression of canonical mesenchymal markers (CD29, CD73, CD90, CD105, and CD166), whereas hematopoietic, endothelial, and immunological exclusion markers (CD11b, CD31, CD34, CD45, and HLA-DR) were absent or minimally expressed (Supplementary Fig. 4), confirming the mesenchymal identity and purity of the isolated BMSCs.

BMSC-derived exosomes were then isolated and characterized. TEM showed the typical cup-shaped vesicular morphology, NTA confirmed the expected size distribution, and immunoblotting demonstrated CD9, CD81, and TSG101 expression with no detectable calnexin, confirming successful isolation of BMSC-Exos (Fig. 6A–C). PKH26-labelled BMSC-Exos were efficiently internalized by MPC-5 podocytes under NG and HG conditions. DiR-labelled exosomes showed broad systemic distribution after intravenous administration, supporting their suitability for in vivo delivery (Fig. 6D,E).

Because hypoxic preconditioning can regulate the biological activity and cargo composition of BMSC-derived exosomes, BMSCs were cultured under hypoxic conditions to enhance miR-425-5p loading. Hypoxia caused no apparent morphological changes in BMSCs but significantly increased miR-425-5p abundance in secreted exosomes relative to normoxic controls (Fig. 6F,G). Collectively, these findings demonstrate the generation of hypoxia-conditioned, miR-425-5p-enriched BMSC-derived exosomes for subsequent functional studies.

BMSC-Exos alleviate diabetic nephropathy through miR-425-5p in vitro and in vivo

We next investigated whether exosomal miR-425-5p mediates the protective effects of BMSC-Exos. In HG-treated MPC-5 podocytes, BMSC-Exos reduced WTAP, MET, GSK3 β , and cleaved caspase-3 expression while restoring podocin expression (Fig. 7A,B). Silencing exosomal miR-425-5p with anti-mmu-miR-425-5p partially abolished these molecular effects, whereas anti-miR-NC had no significant effect. Flow cytometry likewise showed that BMSC-Exos markedly reduced HG-induced podocyte apoptosis; this protection was partially attenuated by anti-mmu-miR-425-5p but unchanged by anti-miR-NC (Fig. 7C). These findings indicate that BMSC-Exos-mediated protection against HG-induced podocyte injury is largely dependent on exosomal miR-425-5p.

We further validated exosomal miR-425-5p in vivo using STZ-induced diabetic mice with renal delivery of LV-NC or LV-anti-mmu-miR-425-5p (Fig. 7D). BMSC-Exos significantly reduced UACR in diabetic mice, whereas miR-425-5p inhibition markedly attenuated this effect (Fig. 7E). Consistent with the in vitro findings, BMSC-Exos decreased glomerular WTAP, MET, GSK3 β , and cleaved caspase-3 expression while increasing podocin; these effects were partially reversed by LV-anti-mmu-miR-425-5p (Fig. 7F,G). TUNEL staining, PAS/Masson staining, and transmission electron microscopy showed that miR-425-5p inhibition attenuated the beneficial effects of BMSC-Exos on podocyte apoptosis, glomerular pathology, and ultrastructural damage (Fig. 7H,I). Collectively, these findings demonstrate that exosomal miR-425-5p is a critical mediator of BMSC-Exos-induced renoprotection through regulation of the WTAP/MET/GSK3 β injury-response axis.

Discussion

Podocyte injury and depletion are central drivers of albuminuria and progressive glomerular dysfunction in DN ^{27–31}. Although epitranscriptomic regulation increasingly appears to contribute to diabetic kidney disease, the mechanisms linking m⁶A modification to podocyte damage remain incompletely understood. Our study identifies transcript-selective m⁶A regulation as a molecular bridge between diabetic stress and podocyte injury. Increased renal m⁶A abundance, elevated WTAP expression, and their associations with proteinuria and reduced eGFR indicate that WTAP dysregulation is clinically relevant rather than merely a consequence of HG exposure. These findings are consistent with studies showing that m⁶A regulators contribute to diabetic renal injury in a cell- and transcript-specific manner ^{32,33}.

METTL14-dependent m⁶A modification of SIRT1 has been implicated in podocyte injury and glomerulopathy, whereas METTL3-dependent modification of TIMP2 has been linked to podocyte injury in DN ^{34,35}. WTAP has also been implicated in diabetic renal injury through TRIM22, DKK3, and NLRP3 in predominantly tubular models ^{36–38}. Here, integrated analyses identified MET as a downstream WTAP-regulated transcript in podocytes. However, the role of MET in diabetic podocyte injury requires careful

interpretation. Although HGF/MET activation has been reported to protect podocytes by maintaining mitochondrial homeostasis³⁹, our data indicate that increased MET expression is involved in WTAP-induced podocyte injury. Reciprocal co-IP demonstrated the MET–GSK3 β interaction. This finding is notable because elevated GSK3 β activity consistently drives podocyte oxidative stress, inflammation, and insulin resistance in diabetes^{40,41}. Nevertheless, our findings do not imply that MET is universally pathogenic or that canonical HGF/MET signalling should be therapeutically inhibited. Further assessment of phospho-MET and GSK3 β phosphorylation, together with pathway-specific rescue experiments, is required to define the precise relationship between MET and GSK3 β .

Our study further identifies miR-425-5p as an upstream regulator of the WTAP-mediated injury pathway. Renal miRNA profiling, bioinformatic prediction, and seed-mutant reporter assays demonstrate that miR-425-5p directly suppresses WTAP through post-transcriptional regulation. Reduced renal miR-425-5p in DN may therefore contribute to WTAP accumulation. Although miR-425-5p has context-dependent effects in diabetes-associated endothelial dysfunction and in adipocyte differentiation and lipid accumulation during SGLT2 inhibitor treatment^{42,43}, our findings establish its podocyte-specific function as a suppressor of WTAP-mediated injury signalling. This context dependence likely reflects differences in cellular origin, disease stage, and downstream target availability.

Importantly, these findings translate the regulatory relationship into a therapeutic strategy using BMSC-Exos. In HG-treated podocytes and diabetic mice, BMSC-Exos reduced WTAP/MET/GSK3 β -associated injury responses, restored podocyte markers, and improved renal functional and structural abnormalities. Inhibition of exosomal miR-425-5p partially weakened these protective effects, demonstrating that miR-425-5p is an important mediator of BMSC-Exos-induced renoprotection. The incomplete reversal further suggests that additional exosomal cargo molecules contribute to the therapeutic response, consistent with the multifunctional nature of MSC-derived EVs^{44–47}. Hypoxic conditioning enhanced miR-425-5p enrichment in BMSC-Exos, supporting modification of donor-cell culture conditions to improve EV function.

This study has several limitations. First, although the STZ-induced diabetic model provides valuable mechanistic insight, it does not fully recapitulate the complexity of human type 2 diabetes and advanced DN. A multimodel strategy integrating complementary DN models will be needed to enhance translational relevance. For example, a high-fat diet plus STZ model better reflects insulin resistance and metabolic dysregulation, whereas genetic models such as db/db or eNOS-deficient mice can reproduce progressive fibrosis and haemodynamic changes. Second, further studies are required to identify the responsible m⁶A reader proteins and clarify the phosphorylation-dependent signalling hierarchy within the MET/GSK3 β module. Third, clinical translation of EV-based therapies will require standardized manufacturing, robust potency assays, comprehensive dose optimization, and safety evaluation. Despite these limitations, our findings support an epitranscriptomic regulatory circuit in diabetic podocyte injury that can be modulated by extracellular vesicle-mediated miRNA delivery.

In conclusion, our findings identify the WTAP/MET/GSK3 β regulatory axis as a critical mechanism underlying diabetic podocyte injury. WTAP-dependent m⁶A remodeling enhances MET mRNA stability and promotes a GSK3 β -associated injury phenotype, whereas miR-425-5p-enriched BMSC-Exos restore this imbalance and confer podocyte protection. These results uncover a previously unrecognized epitranscriptomic–extracellular vesicle regulatory pathway in DN.

Methods

Human renal tissue samples

Human renal tissue specimens were collected from patients treated at the First Affiliated Hospital of Zhengzhou University between May 2020 and May 2021. Control renal tissues were obtained from macroscopically normal kidney tissue located at least 5 cm from the tumour margin in patients undergoing nephrectomy for localized renal tumors. DN was confirmed by percutaneous renal biopsy. Patients were excluded if they met any of the following criteria: (i) coexistence of another renal disease identified by pathological examination; (ii) a history of hypertension, cardiovascular or cerebrovascular disease, or another endocrine disorder; (iii) use of medications known to affect glucose metabolism, lipid metabolism, or renal function; or (iv) pregnancy or lactation. Demographic characteristics, clinical laboratory data, and renal histopathological findings were systematically collected for all participants.

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval no. KY-2018-LW-66), and all procedures complied with the Declaration of Helsinki. All participants provided written informed consent before enrollment and tissue collection.

Renal miRNA expression profiling

DN and control renal tissue samples ($n = 3$ per group) underwent miRNA expression profiling using the Affymetrix GeneChip miRNA 4.0 platform with miRBase Release 20.0 annotation. Raw data were preprocessed using the robust multi-array average method and analysed with limma. miRNAs meeting the prespecified exploratory criteria of nominal $P < 0.05$ and $|\text{fold change}| > 2$ were retained. DN-downregulated miRNAs were intersected with ENCORI, miRmap, and TargetScan predictions to identify candidate WTAP-targeting miRNAs^{48–50}.

Liquid chromatography–mass spectrometry analysis of RNA modifications

DN and control renal tissue samples ($n = 3$ per group) underwent liquid chromatography–mass spectrometry (LC–MS) analysis of RNA modifications. mRNA nucleosides were quantified using a digestion and multiple-reaction-monitoring workflow adapted from established m⁶A quantification

protocols⁸. Total RNA quality was evaluated by agarose gel electrophoresis and NanoDrop, and mRNA was enriched from approximately 5 µg of total RNA using the NEBNext Poly(A) mRNA Magnetic Isolation Module. Purified mRNA was digested to nucleosides with Benzonase, phosphodiesterase I, and alkaline phosphatase. Clarified filtrates were analysed using an Agilent 6460 QQQ/1260 HPLC system equipped with an SB-Aq column. Modified-nucleoside signals were extracted using Agilent Qualitative Analysis software and normalized to purified mRNA input.

Human m⁶A mRNA and long noncoding RNA epitranscriptomic microarrays

DN and control renal tissue samples ($n = 3$ per group) were analysed using Human m⁶A mRNA and long noncoding RNA (lncRNA) epitranscriptomic microarrays. Transcript abundance and m⁶A modification were profiled with the Arraystar Human mRNA and lncRNA Epitranscriptomic Microarray (8 × 60K) according to the manufacturer's protocol. Normalized immunoprecipitated and supernatant intensities were used to calculate methylation level, methylation quantity, and RNA abundance. Probes flagged as "Present" or "Marginal" in at least three of the six samples were retained. Transcripts meeting the exploratory criteria of nominal $P < 0.05$ and fold change ≥ 1.5 were retained. Hyper-up transcripts were defined by concurrent increases in m⁶A signal and mRNA abundance in DN versus control tissues.

Cell culture and establishment of stable cell lines

Conditionally immortalized human podocytes (HPCs; RRID: CVCL_W186), obtained from Jinling Clinical Medical College of Nanjing Medical University, were maintained in DMEM/F-12 supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. WTAP-deficient HPCs were generated by CRISPR/Cas9-based lentiviral editing, and knockout was confirmed by immunoblotting.

MPC-5 mouse podocytes (Shanghai QuiCell Biotechnology; catalog no. QuiCell-M357) were maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂. For high-glucose (HG) stimulation, cells were cultured for 24 h in glucose-free DMEM supplemented with 30 mM D-glucose; normal-glucose (NG) controls were maintained in medium containing 5.6 mM D-glucose. WTAP knockdown was achieved using lentiviral vectors encoding WTAP-targeting short hairpin RNA (shRNA). MPC-5 cells were transduced at the optimized multiplicity of infection, and transduction efficiency was monitored by fluorescence microscopy. Knockdown efficiency was verified by quantitative real-time PCR (qRT-PCR) and immunoblotting. LV-shWTAP-2 showed the highest knockdown efficiency and was selected for subsequent experiments. The shRNA sequences are provided in Supplementary Table 1.

Methylated RNA immunoprecipitation sequencing

Methylated RNA immunoprecipitation sequencing (MeRIP-seq) was performed using established principles^{51,52}. mRNA was purified from WTAP-knockout and negative-control HPCs and sequenced by Cloudseq Biotech Inc. on an Illumina HiSeq 4000 platform. Reads passing Q30 quality assessment were trimmed with Cutadapt, aligned to hg38 using HISAT2, and analysed for methylated peaks with MACS2 using input RNA as the control. Exonic peaks were retained for mRNA-focused analysis, differential peaks were defined from WTAP-knockout versus control outputs, and representative signals were visualized in Integrative Genomics Viewer.

m⁶A dot blot

Global m⁶A levels in MPC-5 cells were evaluated by dot blotting. Poly(A)+ mRNA was isolated and spotted onto positively charged nylon membranes, followed by ultraviolet crosslinking. Membranes were stained with methylene blue to verify equal RNA loading and then incubated with an anti-m⁶A antibody. Immunoreactive signals were visualized using enhanced chemiluminescence.

m⁶A-RIP-qPCR

Candidate m⁶A sites in mouse Met mRNA (NM_001410124.1) were predicted using SRAMP⁵³. Regions centred at positions 718, 837, and 1233 were selected for m⁶A RNA immunoprecipitation followed by qPCR (m⁶A-RIP-qPCR). RNA from each biological replicate was divided into input, m⁶A-IP, and IgG fractions. Enrichment was calculated by the percent-input method after adjustment of the 5% input fraction to 100%. Primer pairs are listed in Supplementary Table 1.

mRNA stability analysis

MET mRNA stability was evaluated using an actinomycin D transcriptional inhibition assay. MPC-5 cells transduced with LV-shNC or LV-shWTAP-2 were treated with actinomycin D (10 µg/mL), and total RNA was isolated at 0, 2, 4, and 8 h. MET mRNA levels were quantified by qRT-PCR, normalized to GAPDH, and fitted to a one-phase exponential decay model to estimate mRNA half-life.

Quantitative real-time PCR

For mRNA expression analysis, complementary DNA was synthesized from total RNA by reverse transcription, followed by SYBR Green-based qRT-PCR according to the manufacturer's instructions. Target-gene expression was normalized to GAPDH and quantified using the 2^{-ΔΔCt} method.

For exosomal miRNA analysis, total RNA was extracted from isolated exosomes and supplemented with synthetic *Caenorhabditis elegans* cel-miR-39-3p as an external spike-in control before poly(A) tailing and

reverse transcription. Exosomal miR-425-5p abundance was quantified by qRT-PCR and normalized to cel-miR-39-3p.

Protein–protein docking and reciprocal co-immunoprecipitation

The potential MET–GSK3 β interaction was first evaluated by protein–protein docking. Crystal structures of MET and GSK3 β (Protein Data Bank ID: 6AE3) were subjected to molecular docking using ClusPro^{54,55}. The model with the highest cluster occupancy under balanced scoring was selected for further analysis. Protein–protein interface interactions were characterized using PDBePISA⁵⁶, interacting residues were annotated with LigPlot+, and the docking complex was visualized in PyMOL.

To validate the predicted interaction, reciprocal co-immunoprecipitation (co-IP) assays were performed in MPC-5 cells. Cell lysates were immunoprecipitated with anti-MET or anti-GSK3 β antibodies; normal immunoglobulin G (IgG) served as the negative immunoprecipitation control. An aliquot of total cell lysate was retained as the input control. Immunoprecipitated proteins were analysed by immunoblotting with antibodies against the reciprocal binding partner.

Animal experiments

Male C57BL/6J mice (*Mus musculus*), aged 6–8 weeks, were maintained in a specific pathogen-free facility with unrestricted access to standard chow and water. All animal experiments were approved by the Ethics Committee of Zhengzhou University (approval no. ZZU-LAC20240607[04]). After a 1-week acclimatization period, mice were randomly assigned to experimental groups according to baseline body weight. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ; 55 mg/kg/day) for 5 consecutive days. Control mice received an equivalent volume of citrate buffer using the same procedure. After diabetes was confirmed by two consecutive fasting blood glucose measurements ≥ 16.7 mmol/L, mice were maintained for an additional 8 weeks before intervention.

For podocyte-specific WTAP knockdown, mice received a recombinant adeno-associated virus serotype 9 (AAV9) vector expressing Wtap-targeting shRNA under the nephrin promoter. Eight weeks after STZ administration, diabetic mice received tail-vein injections of AAV9-shWTAP or the corresponding negative-control vector at approximately $2\text{--}3 \times 10^{11}$ vector genomes per mouse. At the study endpoint, renal injury was evaluated by integrated analyses of renal function, molecular alterations, histopathology, apoptosis, and ultrastructure.

Bone marrow mesenchymal stem cell isolation and characterization

Bone marrow mesenchymal stem cells (BMSCs) were harvested from the femoral and tibial marrow cavities of 4–6-week-old C57BL/6J mice and cultured in MesenCult Expansion Kit medium according to the manufacturer's instructions. For hypoxic culture, cells were maintained at 5% O₂ and 5% CO₂; normoxic controls were cultured under atmospheric oxygen (20–21% O₂) with 5% CO₂. BMSCs at passages 3–5 were used for differentiation assays, immunophenotypic characterization, and exosome isolation.

BMSC multipotency was evaluated by adipogenic, osteogenic, and chondrogenic induction followed by Oil Red O, Alizarin Red S, and Alcian Blue staining, respectively. For immunophenotyping, cultured BMSCs were gently dissociated into single-cell suspensions, passed through a 40-μm cell strainer, and stained for MSC-positive markers (CD29, CD73, CD90, CD105, and CD166) and hematopoietic or non-MSC lineage markers (CD11b, CD31, CD34, CD45, and HLA-DR). Flow cytometric analysis excluded debris using forward-scatter area (FSC-A) and side-scatter area (SSC-A). Marker positivity was determined using thresholds established with the corresponding isotype-matched controls.

Exosome isolation and intervention assays

Conditioned medium from BMSCs at passages 3–5 underwent sequential differential centrifugation to remove cells and debris, followed by filtration through a 0.22-μm filter. After ultracentrifugation at 200,000 × g for 120 min at 4 °C, the exosome pellet was resuspended in phosphate-buffered saline, and protein content was measured using a bicinchoninic acid assay. Exosome morphology, particle-size distribution, and marker expression were evaluated by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and immunoblotting for CD9, CD81, and TSG101, respectively; calnexin served as a negative marker.

For in vitro intervention, high-glucose-treated MPC-5 cells were incubated with BMSC-Exos at 60 μg/mL. Where indicated, cells were transfected with an anti-mmu-miR-425-5p inhibitor or negative-control inhibitor (anti-miR-NC) using Lipofectamine 3000 before exosome treatment. After treatment, protein expression was analysed by immunoblotting, apoptosis was assessed by Annexin V/propidium iodide (PI) staining and flow cytometry, and WTAP, MET, GSK3β, and podocin expression was evaluated by immunofluorescence.

For in vivo intervention, STZ-induced diabetic mice were randomly assigned to the STZ, STZ+BMSC-Exos, STZ+BMSC-Exos+LV-NC, or STZ+BMSC-Exos+LV-anti-mmu-miR-425-5p groups. Eight weeks after STZ administration, mice received bilateral renal subcapsular injections of LV-anti-mmu-miR-425-5p or the corresponding negative-control vector (LV-NC). Two weeks later, BMSC-Exos were administered by tail-vein injection at 100 μg per mouse twice weekly for 6 consecutive weeks. Renal tissues and blood samples were collected at the study endpoint for biochemical, histological, and molecular analyses.

Dual-luciferase reporter assay

The putative miR-425-5p binding site within the WTAP 3' untranslated region (3' UTR) was predicted using TargetScanMouse 8.0 ⁴⁸. Wild-type (WT) and seed-mutant (MUT) WTAP 3' UTR fragments were cloned into the pmirGLO dual-luciferase reporter vector (GP-miRGLO/pmirGLO). MPC-5 cells were co-transfected with reporter constructs and either a miR-425-5p mimic or negative-control mimic (miR-NC). Forty-eight hours after transfection, dual-luciferase activity was measured according to the manufacturer's instructions. Relative activity was calculated as the firefly-to-Renilla luciferase ratio. All experiments were independently repeated three times.

Biochemical and histological analyses

Urine was collected in metabolic cages, and the urinary albumin-to-creatinine ratio (UACR) was measured using a urine microalbumin assay kit. Mouse glomeruli were isolated by Dynabead perfusion, collagenase digestion, filtration, and magnetic collection.

Immunohistochemistry, immunofluorescence, terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL), periodic acid–Schiff (PAS) staining, Masson trichrome staining, and transmission electron microscopy were performed using standard fixation, blocking, antibody incubation, staining, and imaging procedures. Antibody and kit details are provided in Supplementary Table 2.

Immunoblotting

Proteins were extracted from cells, isolated glomeruli, BMSCs, or exosome preparations using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Equal amounts of protein were resolved by SDS–PAGE, transferred to PVDF membranes, sequentially incubated with primary and horseradish peroxidase-conjugated secondary antibodies, and visualized using enhanced chemiluminescence.

Flow cytometry

Apoptosis in MPC-5 cells was assessed by Annexin V/PI double staining and flow cytometry using a CytoFLEX flow cytometer. Cells were harvested, gently dissociated, passed through a cell strainer to generate uniform single-cell suspensions, and stained with Annexin V and PI according to the manufacturer's protocol. Debris and noncellular events were excluded using FSC-A and SSC-A. Cells were categorized as viable (Annexin V⁻/PI⁻), early apoptotic (Annexin V⁺/PI⁻), late apoptotic (Annexin V⁺/PI⁺), or necrotic (Annexin V⁻/PI⁺). Total apoptosis was calculated as the sum of early and late apoptotic fractions. The gating strategy is shown in Supplementary Figure 2.

Statistics and reproducibility

Statistical analyses were performed using GraphPad Prism 8.0 unless otherwise specified. Quantitative data are presented as mean $\pm$ SD. Two-group comparisons used Student's t test or Welch's t test according to variance homogeneity; paired m⁶A-RIP-qPCR comparisons used two-sided paired t tests. Multiple-group comparisons used one-way ANOVA with Tukey post hoc testing or Welch ANOVA with Dunnett T3 testing. Luciferase assays used two-way ANOVA with Sidak testing. Actinomycin D decay curves were fitted by nonlinear regression. Spearman or Pearson correlation analysis was used according to data distribution. $P < 0.05$ was considered statistically significant; figure panels denote * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Biological sample sizes and numbers of independent experiments are specified in the relevant Methods subsections and figure legends. Mice were randomized to experimental groups according to baseline body weight, and dual-luciferase reporter assays were independently repeated three times.

Declarations

Data availability

The MeRIP-seq, miRNA array, and m⁶A microarray datasets generated in this study have been deposited in the NCBI Gene Expression Omnibus under Series accession numbers GSE342411, GSE343128, and GSE342221, respectively, and will be released upon publication. Other data supporting the findings are included in the article and its Supplementary Information or are available from the corresponding authors upon reasonable request.

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Author contributions

X.P.Z.: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. X.H.S.: Investigation. Y.Q.L.: Investigation. W.Q.C.: Investigation. M.C.: Methodology, Writing – review and editing. S.J.Z.: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, Supervision, Funding acquisition, Writing – original draft, Writing – review and editing. Y.F.L.: Conceptualization, Methodology, Investigation, Data curation,

Formal analysis, Visualization, Resources, Supervision, Funding acquisition, Writing – original draft, Writing – review and editing. All authors reviewed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Figures

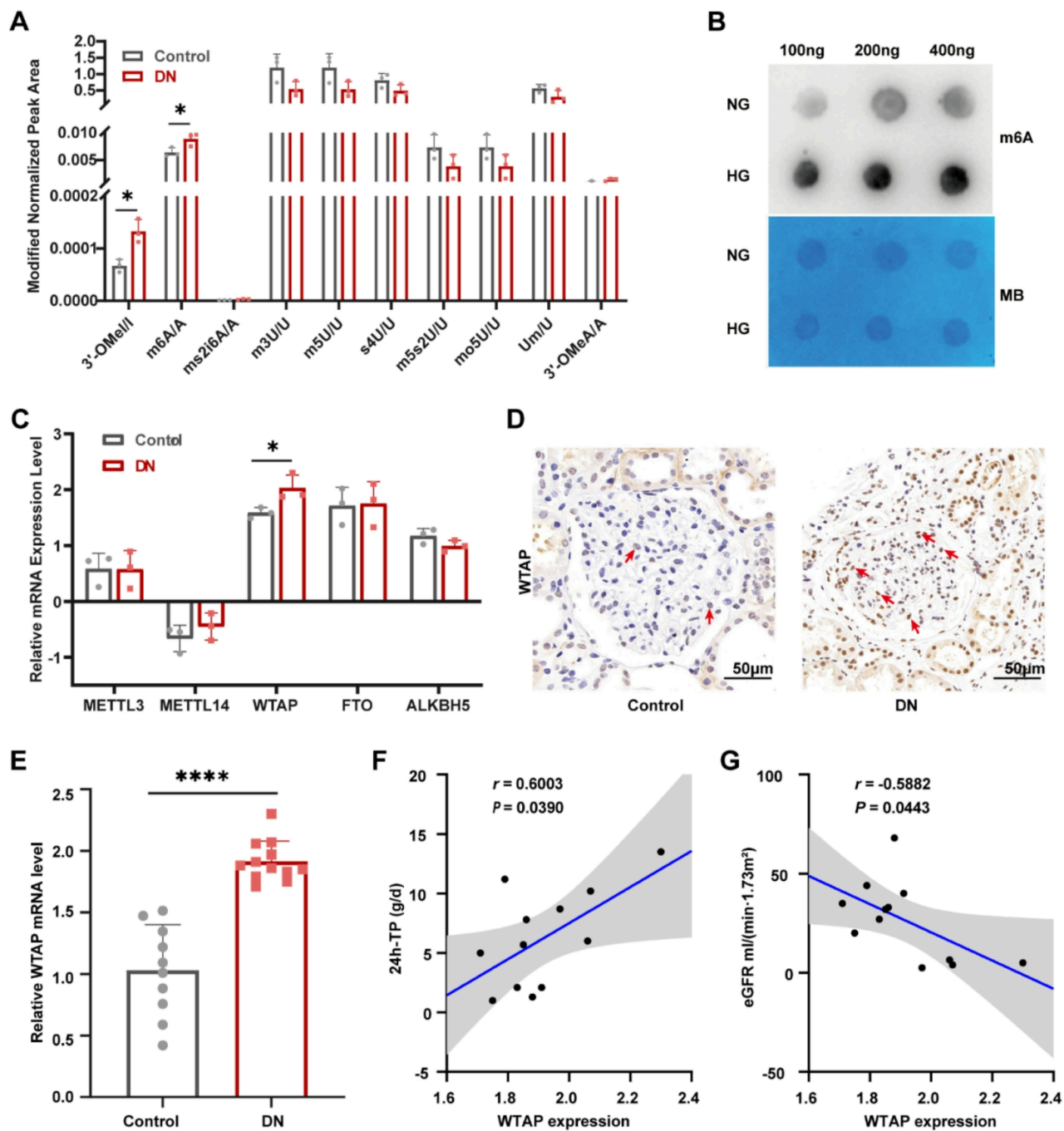


Figure 1

RNA m⁶A methylation is involved in high-glucose-induced podocyte injury. (A) LC–MS quantification of RNA modifications shows an increased normalized m⁶A/A peak-area signal in DN samples ($n = 3$ per group). (B) m⁶A dot blotting shows increased global m⁶A signals in HG-treated MPC-5 cells at RNA inputs of 100, 200, and 400 ng; methylene blue served as the loading control. (C) Human m⁶A mRNA and lncRNA epitranscriptomic microarray analysis shows increased WTAP mRNA expression in DN renal

tissues ($n = 3$ per group). (D) WTAP immunohistochemistry shows enhanced nuclear staining in glomerular podocytes from DN kidneys (red arrows); scale bar, 50 μm . (E) qPCR shows significantly increased WTAP mRNA in DN tissues (control, $n = 10$; DN, $n = 12$). (F) WTAP expression correlates positively with 24-h urinary protein excretion in DN samples ($n = 12$). (G) WTAP expression correlates negatively with eGFR in DN samples ($n = 12$). Data are mean $\pm$ SD. $*P < 0.05$; $****P < 0.0001$. DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; HG, high glucose; LC–MS, liquid chromatography–mass spectrometry; lncRNA, long noncoding RNA; m⁶A, N⁶-methyladenosine; qPCR, quantitative PCR; SD, standard deviation; WTAP, WT1-associated protein.

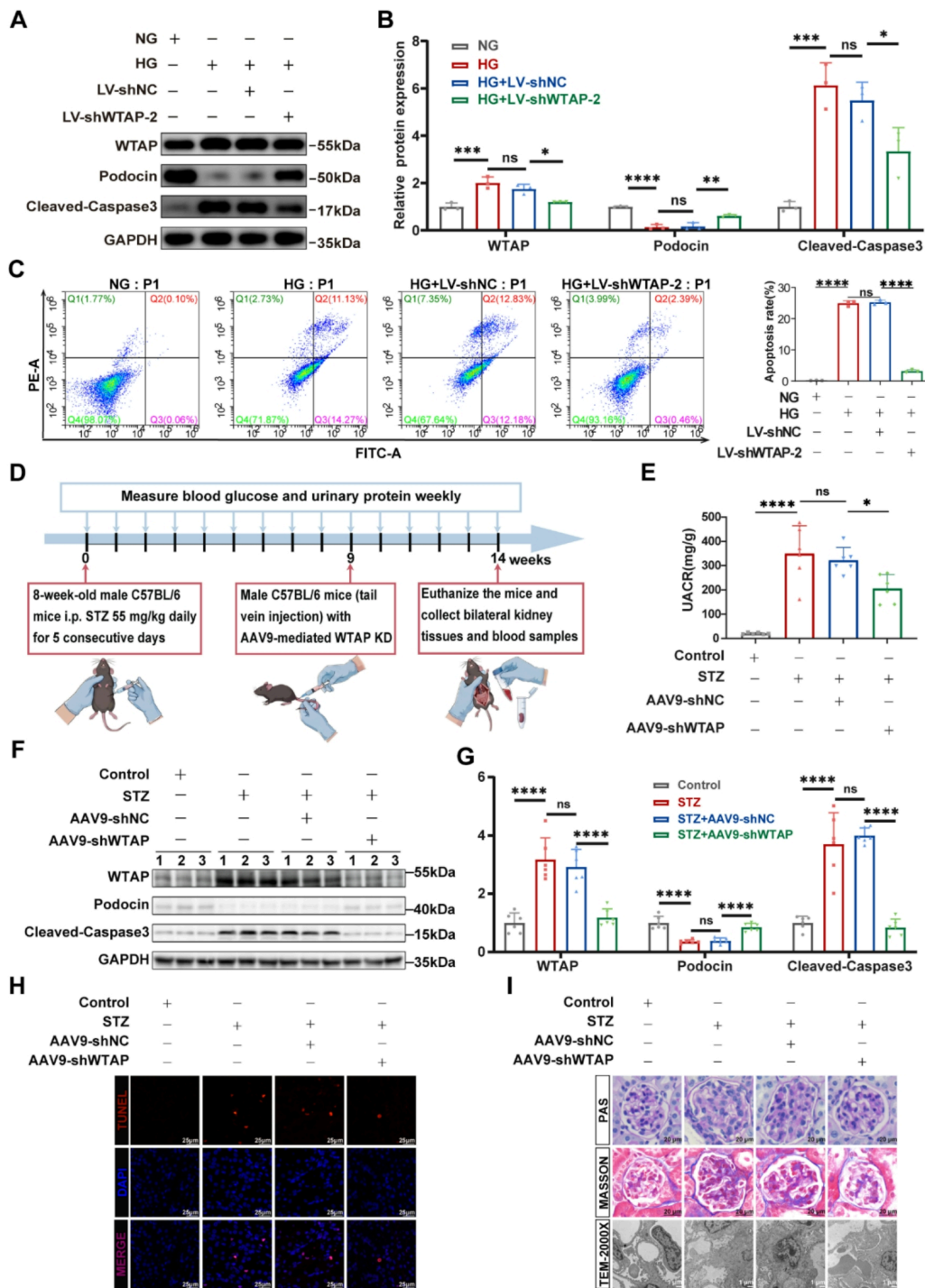


Figure 2

WTAP knockdown attenuates high-glucose- and streptozotocin-induced podocyte injury. (A,B) Representative immunoblots (A) and densitometric quantification (B) of WTAP, podocin, and cleaved caspase-3 in MPC-5 cells under NG or HG conditions with or without LV-shWTAP-2. (C) Representative Annexin V/PI flow cytometry plots (left) and quantitative analysis (right) of podocyte apoptosis; three data points are shown per group. (D) Experimental design for STZ-induced diabetes and podocyte-

directed AAV9-shWTAP treatment in male mice. (E) UACR in the indicated mouse groups ($n = 6$ per group). (F,G) Representative immunoblots (F) and densitometric quantification (G) of WTAP, podocin, and cleaved caspase-3 in glomeruli from control, STZ, STZ+AAV9-shNC, and STZ+AAV9-shWTAP groups ($n = 6$ per group). (H) Representative TUNEL images of renal apoptosis; nuclei were counterstained with DAPI (blue); scale bar, 25 μm . (I) PAS and Masson trichrome staining (scale bars, 20 μm) show glomerular mesangial expansion and collagen deposition, respectively; TEM images (scale bar, 1 μm) show ultrastructural changes. Data are mean $\pm$ SD; each symbol represents one data point or mouse. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$; ns, not significant. AAV9, adeno-associated virus serotype 9; DAPI, 4',6-diamidino-2-phenylindole; HG, high glucose; NG, normal glucose; PAS, periodic acid–Schiff; PI, propidium iodide; SD, standard deviation; STZ, streptozotocin; TEM, transmission electron microscopy; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labelling; UACR, urinary albumin-to-creatinine ratio; WTAP, WT1-associated protein.

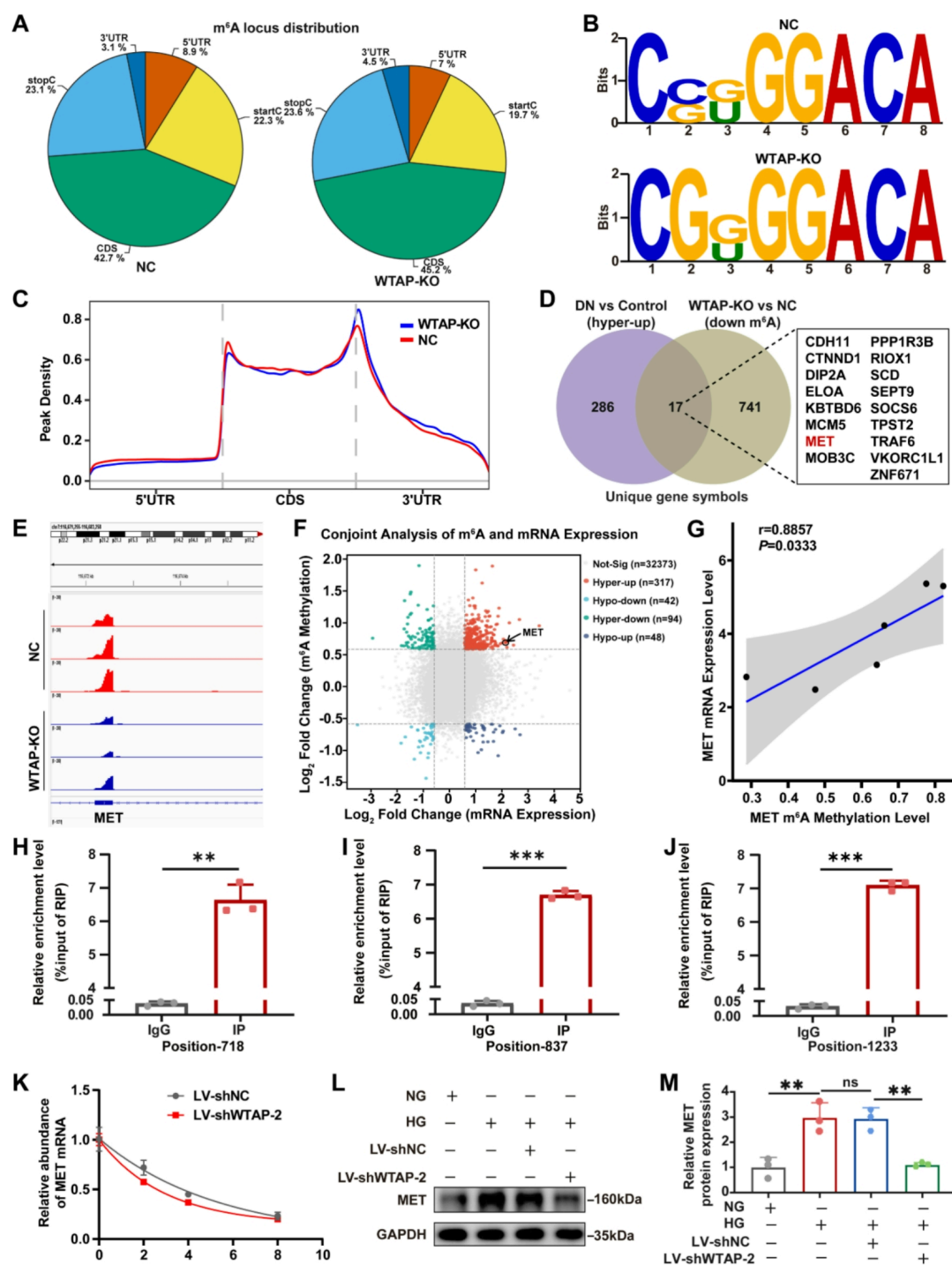


Figure 3

WTAP regulates MET expression through m⁶A modification. (A) Distribution of m⁶A loci in control and WTAP-knockout HPCs. (B) DREME analysis in MEME Suite version 5.3.0 identifies enrichment of the RRACH consensus motif among m⁶A peaks. (C) Metagene distribution of m⁶A-enriched peaks generated using the metaPlot R package. (D) Integration of the human m⁶A mRNA/lncRNA epitranscriptomic microarray and MeRIP-seq datasets identifies 17 overlapping genes. (E) Integrative Genomics Viewer

tracks show decreased m⁶A peak abundance on MET in WTAP-knockout cells. (F) Joint analysis of m⁶A and mRNA changes in human DN versus control renal tissues (*n* = 3 per group); MET is indicated among hypermethylated, upregulated transcripts. (G) Pearson correlation analysis shows a positive correlation between MET mRNA expression and m⁶A methylation level (*n* = 6). (H–J) m⁶A-RIP-qPCR shows relative m⁶A enrichment at positions 718, 837, and 1233 of the Met transcript compared with IgG controls in MPC-5 cells. (K) MET mRNA stability after actinomycin D treatment; half-life was 3.647 h in the LV-shNC group and 2.045 h in the LV-shWTAP-2 group. (L) Representative MET immunoblots and (M) densitometric quantification in MPC-5 cells under NG or HG conditions with or without LV-shWTAP-2. Data are mean ± SD. ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001; ns, not significant. DN, diabetic nephropathy; HG, high glucose; HPC, human podocyte cell; IgG, immunoglobulin G; lncRNA, long noncoding RNA; m⁶A, N⁶-methyladenosine; MeRIP-seq, methylated RNA immunoprecipitation sequencing; NG, normal glucose; qPCR, quantitative PCR; SD, standard deviation; WTAP, WT1-associated protein.

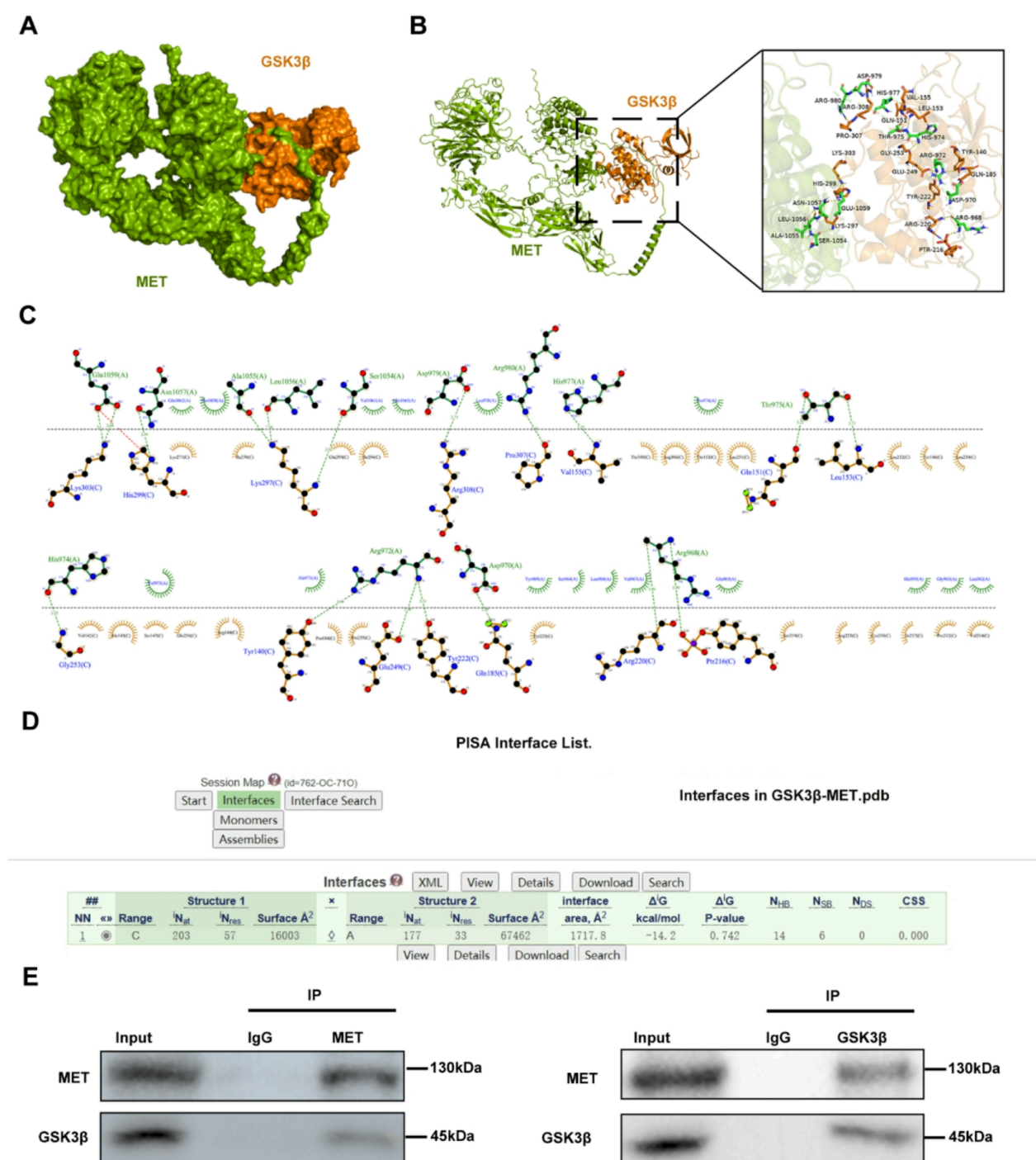


Figure 4

MET interacts with GSK3 β to form a functional protein complex. (A) Three-dimensional surface representation of the molecular docking model showing the overall interface between MET (green) and GSK3 β (orange). (B) Ribbon representation of the MET–GSK3 β complex; the enlarged boxed region shows interacting amino acid residues and intermolecular bonds. (C) Two-dimensional schematic of intermolecular interactions between MET (top) and GSK3 β (bottom), including hydrogen bonds (dashed

lines) and hydrophobic contacts. (D) Thermodynamic and structural parameters of the MET–GSK3β interface calculated using PDBePISA, showing an interface area of 1,717.8 Å² and predicted ΔiG of –14.2 kcal/mol. (E) Endogenous MET–GSK3β interaction validated by reciprocal co-IP. Whole-cell lysates were immunoprecipitated with anti-MET (left) or anti-GSK3β (right); species-matched IgG served as the negative control. co-IP, co-immunoprecipitation; GSK3β, glycogen synthase kinase 3 beta; IgG, immunoglobulin G.

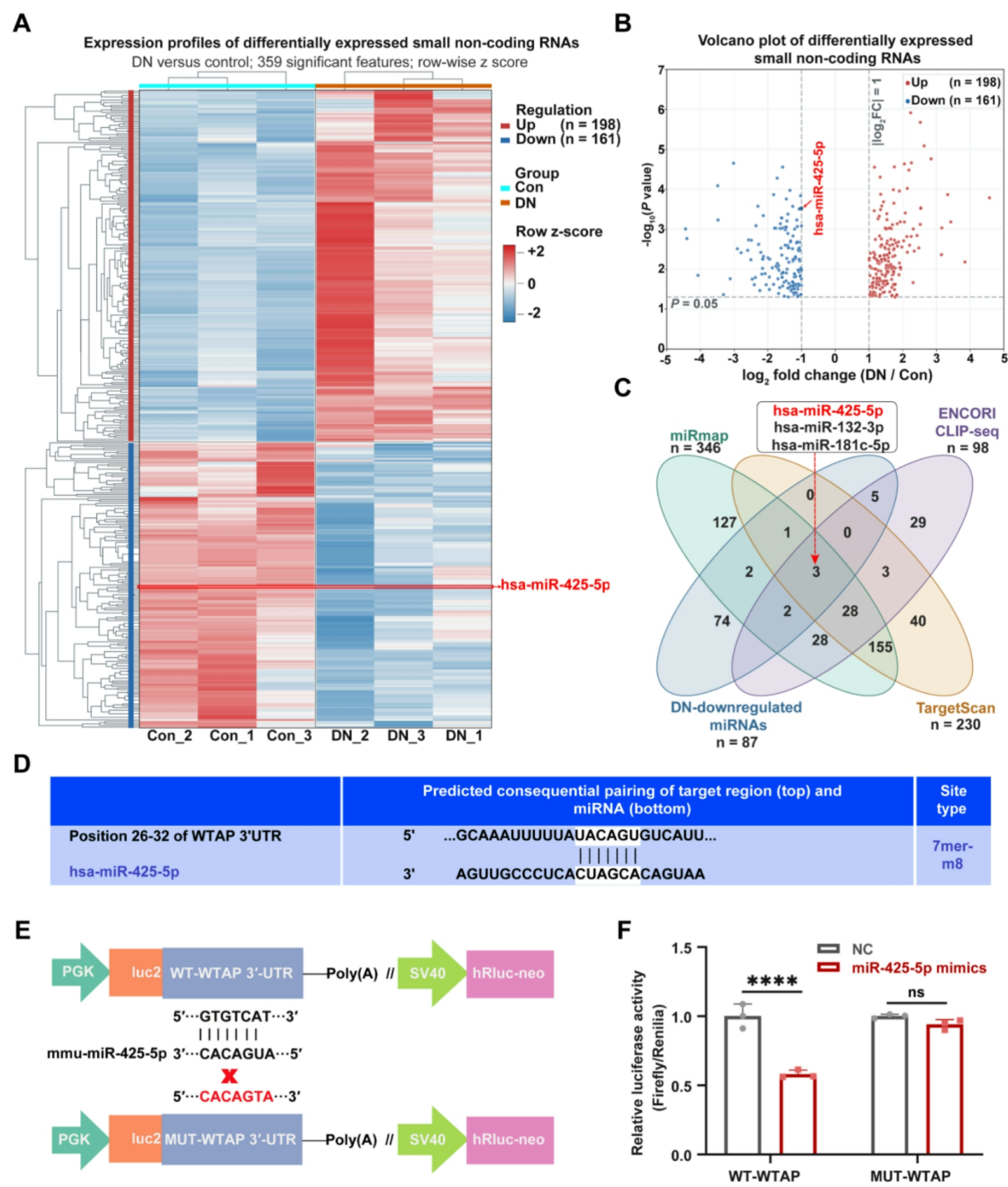


Figure 5

miR-425-5p is a key miRNA targeting WTAP. (A) Hierarchical clustering heatmap of differentially expressed small noncoding RNAs in DN versus control samples ($n = 3$ per group); hsa-miR-425-5p is highlighted. (B) Volcano plot of differentially expressed small noncoding RNAs. Red dots indicate significantly upregulated RNAs and blue dots indicate downregulated RNAs; hsa-miR-425-5p is labelled. (C) Venn diagram showing the intersection of DN-downregulated miRNAs with predicted WTAP-targeting miRNAs from miRmap, ENCORI CLIP-seq, and TargetScan; hsa-miR-425-5p is among the leading candidates. (D) TargetScan prediction of the 7mer-m8 complementary binding site between hsa-miR-425-5p and the WTAP 3' UTR. (E) Construction of wild-type (WT-WTAP 3' UTR) and seed-mutant (MUT-WTAP 3' UTR) luciferase reporters; mutated nucleotides are shown in red. (F) Dual-luciferase reporter assay in MPC-5 cells co-transfected with WT-WTAP or MUT-WTAP reporters and a miR-425-5p mimic or negative-control mimic (miR-NC). Firefly luciferase activity was normalized to Renilla luciferase activity ($n = 3$ independent experiments). Data are mean $\pm$ SD. **** $P < 0.0001$; ns, not significant. CLIP-seq, cross-linking immunoprecipitation sequencing; DN, diabetic nephropathy; miRNA, microRNA; SD, standard deviation; UTR, untranslated region; WTAP, WT1-associated protein.

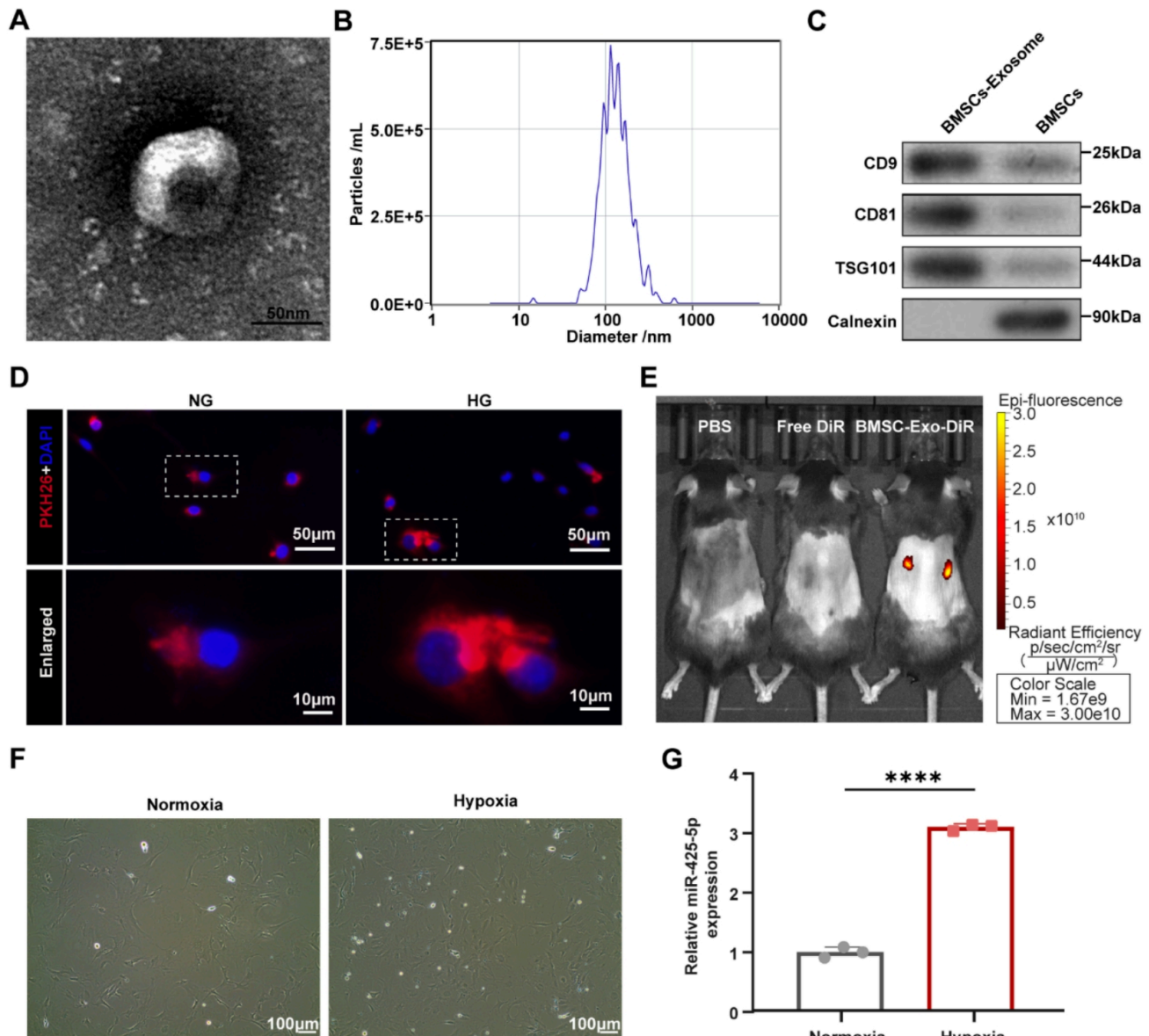


Figure 6

Characterization of hypoxia-conditioned BMSC-Exos and miR-425-5p enrichment. (A) Representative TEM image showing the cup-shaped morphology of BMSC-derived exosomes; scale bar, 50 nm. (B) NTA of particle-size distribution. D_{50} was 127.4 nm, mean diameter was 142.5 nm, and particle concentration was approximately 1.7×10^{10} particles/mL. (C) Immunoblotting for exosome-positive markers CD9, CD81, and TSG101 and the negative marker calnexin in BMSC-Exos and parental BMSCs. (D) Uptake of PKH26-labelled BMSC-Exos (red) by MPC-5 cells under NG and HG conditions; nuclei were counterstained with DAPI (blue). Scale bars, 50 μ m (top) and 10 μ m (bottom, enlarged views). (E) Whole-body fluorescence 24 h after tail-vein administration of PBS, free DiR, or DiR-labelled BMSC-Exos. (F) Representative BMSC morphology under normoxia and hypoxia; scale bars, 100 μ m. (G) Exosomal miR-

425-5p abundance after normoxic or hypoxic culture, measured by qPCR and normalized to the cel-miR-39-3p spike-in control ($n = 3$). Data are mean $\pm$ SD. **** $P < 0.0001$. BMSC, bone marrow mesenchymal stem cell; BMSC-Exos, BMSC-derived exosomes; DAPI, 4',6-diamidino-2-phenylindole; HG, high glucose; NG, normal glucose; NTA, nanoparticle tracking analysis; PBS, phosphate-buffered saline; qPCR, quantitative PCR; SD, standard deviation; TEM, transmission electron microscopy.

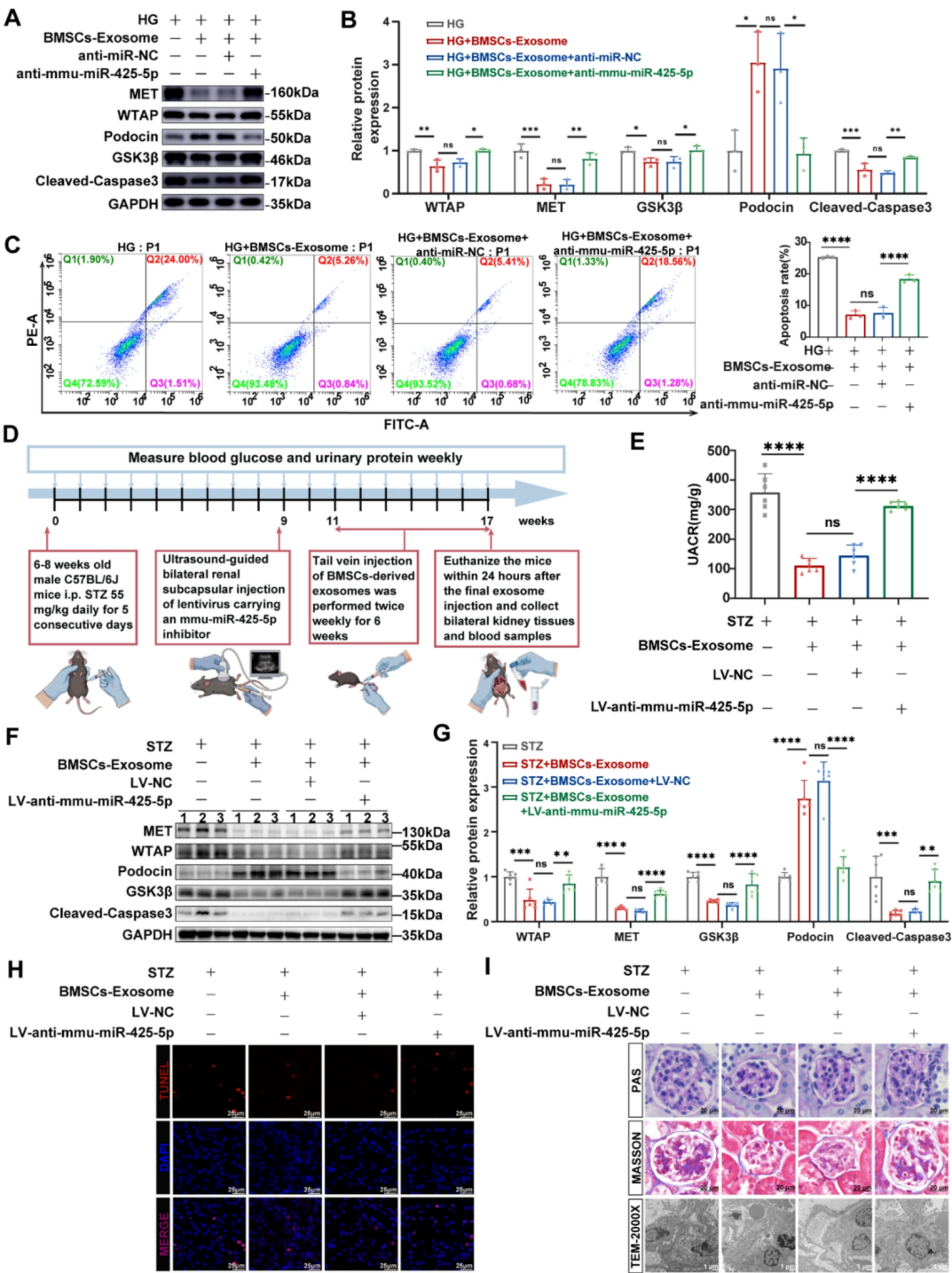


Figure 7

BMSC-Exos alleviate podocyte injury in diabetic nephropathy in vitro and in vivo through miR-425-5p. (A,B) Representative immunoblots (A) and densitometric quantification (B) of MET, WTAP, podocin, GSK3 β , and cleaved caspase-3 in HG-cultured podocytes treated with BMSC-Exos with or without anti-mmu-miR-425-5p. (C) Representative flow cytometry plots (left) and quantitative analysis (right) of podocyte apoptosis; three data points are shown per group. (D) Experimental design for STZ-induced diabetes, renal delivery of LV-anti-mmu-miR-425-5p or LV-NC, and systemic BMSC-Exos treatment in male mice. (E) UACR in the indicated mouse groups. (F,G) Representative immunoblots (F) and densitometric quantification (G) of WTAP, MET, GSK3 β , podocin, and cleaved caspase-3 in glomeruli from STZ mice receiving the indicated treatments. (H) Representative TUNEL images of renal apoptosis; scale bar, 25 μ m. (I) PAS and Masson trichrome staining (scale bars, 20 μ m) show glomerular mesangial expansion and collagen deposition, respectively; TEM images (scale bar, 1 μ m) show ultrastructural changes. Data are mean $\pm$ SD; each symbol represents one data point or mouse. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns, not significant. BMSC-Exos, bone marrow mesenchymal stem cell-derived exosomes; GSK3 β , glycogen synthase kinase 3 beta; HG, high glucose; PAS, periodic acid–Schiff; SD, standard deviation; STZ, streptozotocin; TEM, transmission electron microscopy; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labelling; UACR, urinary albumin-to-creatinine ratio; WTAP, WT1-associated protein.

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