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LncRNA SOX2OT improves insulin resistance in type 2 diabetes with sleep disorders by activating IRS/PI3K/AKT and suppressing oxidative stress and inflammation through the miR-552-5p/SOX2 axis

Yalan Wang[1], Li Zheng[1], Jing Dai[1], Xiaoshi Yang[1], Feng Tang[1], Yan Li[1] and Yu Lu[2]

[1] *Department of Endocrinology, Wuchang Hospital Affiliated to Wuhan University of Science and Technology, Wuchang District, Wuhan, China*
[2] *Wuhan Mental Health Center, Qiaokou District, Wuhan, China*

Abstract. Obstructive sleep apnoea (OSA) and type 2 diabetes mellitus (T2DM) frequently co-occur, with long noncoding RNAs (lncRNAs) implicated in their shared pathophysiology (T2DM-OSA). LncRNA sex determining region Y-box protein 2 (SOX2) overlapping transcript (SOX2OT) is downregulated in diabetic models. This study investigated SOX2OT's regulatory roles using *in vivo* and *in vitro* T2DM-OSA models. Intermittent hypoxia (IH) and high glucose (HG) significantly suppressed SOX2OT expression in murine systems and HepG2 hepatocytes. SOX2OT overexpression enhanced glucose consumption and uptake in HG/IH-treated cells by activating insulin receptor substrate/phosphatidylinositol 3-kinase/protein kinase B (IRS/PI3K/AKT) signaling. Concurrently, SOX2OT attenuated oxidative stress (reactive oxygen species (ROS), malondialdehyde (MDA)) while elevating antioxidant activity (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px)), and suppressed inflammatory cytokines (tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β)). Mechanistically, SOX2OT functioned as a competing endogenous RNA for microRNA-552-5p (miR-552-5p) to depress SOX2 expression. Crucially, SOX2 knockdown abolished SOX2OT-mediated cytoprotection. Thus, SOX2OT ameliorates insulin resistance in T2DM-OSA through IRS/PI3K/AKT activation and miR-552-5p/SOX2-mediated suppression of oxidative stress and inflammation.

Key words: SOX2OT — miR-552-5p — Insulin resistance — Oxidative stress — Intermittent hypoxia

Introduction

Type 2 diabetes mellitus (T2DM), a prevalent chronic metabolic disorder characterized by hyperglycemia, affects approximately 400 million people globally (Mlynarska et al. 2025). Obstructive sleep apnoea (OSA), characterized by sleep-associated breathing disturbances, causes intermittent hypoxia (IH) and sleep fragmentation (Ji et al. 2022). Compelling evidence indicates a bidirectional correlation between OSA and T2DM. OSA is an independent risk factor for T2DM development, with 15–30% of OSA patients having comorbid T2DM (Marshall et al. 2009; Pamidi and Tasali 2012). Increasing OSA severity correlates with elevated insulin resistance, impaired glucose tolerance, and higher T2DM incidence (Punjabi et al. 2004; Kent et al. 2014), while T2DM prevalence is significantly higher in OSA patients (Huang and Chen 2025). Conversely, patients with established T2DM exhibit substantially higher OSA rates (Foster et al. 2009; Subramanian et al. 2019; Qie et al. 2020). This interconnection involves shared risk factors like obesity and pathological mechanisms

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Correspondence to: Yan Li, Department of Endocrinology, Wuchang Hospital Affiliated to Wuhan University of Science and Technology, No. 116 Yangyuan Street, Wuchang District, Wuhan 430061, China
E-mail: 15107112931@163.com
Yu Lu, Wuhan Mental Health Center, No. 93, Youyi Road, Qiaokou District, Wuhan 430061, China
E-mail: 842682974@qq.com

including insulin resistance, inflammation, and oxidative stress (Orru et al. 2020; Weinberg Sibony et al. 2024). OSA remains frequently undiagnosed in T2DM populations, and untreated OSA exacerbates metabolic dysregulation and increases cardiovascular risk (Davies et al. 2022; Chang et al. 2023). While continuous positive airway pressure (CPAP) is the primary OSA treatment, its efficacy in T2DM patients remains limited (Labarca et al. 2018). Early detection of OSA-comorbid T2DM is therefore clinically imperative. Long noncoding RNAs (lncRNAs) – transcripts > 200 nucleotides without protein-coding potential (Zhang X et al. 2019) – represent emerging biomarkers and therapeutic targets in T2DM and OSA. For instance, lncRNA LINC01018 protects against high glucose (HG)-induced β-cell dysfunction by sequestering miR-499a-5p (Liu et al. 2023). In OSA, lncRNA XR_596701 protects cardiomyocytes from IH-induced injury through regulation of miR-344b-5p and Fas apoptotic inhibitory molecule 3 (FAIM3) (Chen et al. 2022). Additionally, lncRNAs metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and nuclear paraspeckle assembly transcript 1 (NEAT1) contribute to T2DM-OSA pathogenesis (Du et al. 2020; Chen et al. 2024). Despite these advances, the functional roles and regulatory mechanisms of lncRNAs in T2DM-OSA comorbidity remain underexplored. The sex determining region Y-box protein 2 (SOX2) overlapping transcript (SOX2OT), an evolutionarily conserved lncRNA which contains the SOX2 gene in its intronic region (Amaral et al. 2009), is significantly downregulated in diabetic models (Zhang et al. 2018; Chen et al. 2021). SOX2OT overexpression attenuates HG-induced oxidative stress and apoptosis in renal cells (Zhang Y et al. 2019; Ye et al. 2023), suggesting protective roles in diabetic complications. However, its function in T2DM-OSA remains unknown. As lncRNAs often regulate pathogenesis *via* microRNA (miRNA) interactions – key post-transcriptional regulators of gene expression implicated in T2DM and OSA (Pasquinelli 2012; Ha and Kim 2014; Kraczkowska et al. 2022; Moriondo et al. 2022) – we hypothesized that SOX2OT modulates T2DM-OSA through miRNA-dependent mechanisms.

This study investigates SOX2OT's functional roles and regulatory mechanisms in T2DM-OSA comorbidity using complementary animal and cellular models. Our findings reveal novel insights into lncRNA-mediated pathology and identify potential diagnostic/therapeutic targets for T2DM-OSA.

Materials and Methods

Animals

Male T2DM model KKAγ mice (12 weeks old, 36–39 g) and age-matched control C57BL/6J mice were obtained from Charles River Laboratories (Beijing, China). Mice were housed under a 12-h light/dark cycle at 23°C and 55% humidity (4 *per* cage) with *ad libitum* access to water and a standard laboratory chow containing 4–6% fat (Harlan Teklad). After one week of acclimatization, mice underwent IH modeling simulating OSA in customized chambers (60×30×25 cm) using rapid cycles of 5% O_2 (30 s) and 21% O_2 (90 s) *per* established protocols (Du et al. 2020; Chen et al. 2024). Rapid nitrogen/oxygen infusion reduced chamber O_2 to 5% within 30 s, immediately followed by 21% O_2 maintenance for 90 s. This 120-s cycle was repeated 30 times *per* hour (30 cycles/h), totaling 8 h daily for 28 days. Control mice received normoxic air (21% O_2; 8 h/day × 4 weeks). Post-IH, mice were anesthetized (1–2% isoflurane), subjected to fasting blood glucose measurement, and euthanized *via* pentobarbital overdose (60 mg/kg). Terminal procedures included: 1) arterial blood collection (1 ml; cardiac puncture), and 2) liver tissue harvest (snap-frozen in liquid nitrogen; −80°C storage). All protocols followed National Institutes of Health guidelines and were approved by the Institutional Animal Ethics Committee of Wuchang Hospital.

Biochemistry test

Serum isolation was achieved by centrifuging blood samples at 1,000×*g* for 15 min at 4°C. Systemic inflammation markers tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) were quantified in both serum and liver homogenates using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Wuhan, China), with tissue concentrations normalized to total protein content *via* bicinchoninic acid (BCA) assay. Oxidative stress parameters malondialdehyde (MDA) and superoxide dismutase (SOD) activity were concurrently measured in serum and liver specimens using standardized colorimetric kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Insulin resistance was calculated using the validated homeostatic model assessment (HOMA-IR) formula: [fasting blood glucose (mmol/l) × fasting insulin (μIU/ml)]/22.5. Technical validation included triplicate measurements for all assays, utilization of calibrated microplate readers for absorbance detection, and strict adherence to manufacturer-specified temperature and incubation parameters to ensure inter-assay consistency across biological matrices.

Quantitative real-time PCR (RT-qPCR)

Total RNA from liver tissues or HepG2 cells was extracted using a TRIzol Reagent (Beyotime, Shanghai, China). RNA concentration and purity were quantified *via* NanoDrop™ spectrophotometry (Thermo Fisher). First-strand cDNA synthesis utilized 1 μg total RNA with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher). Quan-

titative PCR amplification was performed in triplicate using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher) on a QuantStudio™ 5 system (Applied Biosystems) under standardized cycling: 50°C/2 min → 95°C/10 min → [95°C/15 s → 60°C/60 s] × 40 cycles. All reactions maintained 20 μl volumes with 200 nM primer concentrations (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the endogenous control, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with melt curve validation.

Cell culture

HepG2 cells (Procell, Wuhan) were maintained in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Beyotime) at 37°C in a 5% CO_2 humidified incubator. For experimental treatments, cells at 70% confluency were assigned to four groups: Control: 5.5 mM glucose + normoxic cycles (21% O_2); HG: 30 mM glucose + normoxic cycles; IH: 5.5 mM glucose + IH cycles; HG + IH: 30 mM glucose + IH cycles. Glucose stimulation (16 h) preceded hypoxia exposure. IH cycles consisted of 30 s at 1.5% O_2 followed by 90 s at 21% O_2, administered for 8 continuous hours using a ProOx C21 chamber controller (BioSpherix) *per* established protocols (Guo et al. 2019; Chen et al. 2024). Normoxic controls received constant 21% O_2.

Cell transfection

Small interfering RNA of sex determining region Y-box protein 2 (SOX2) (siSOX2), miR-522-5p mimics, and miR-676-3p mimics as well as negative controls (siNC and NC mimics) were provided by GenePharma (Shanghai), and SOX2OT overexpression plasmid and empty vector were provided by Ribobio (Guangzhou, China). HepG2 cells were seeded at 1×10^5 cells/well in 6-well plates and cultured until 70% confluency. Transfection was performed using Lipofectamine 2000 reagent (Thermo Fisher) according to manufacturer specifications.

Glucose consumption measurement

After transfection, the medium was removed and replaced with fresh medium containing 11 mM glucose. Following 12-h incubation, glucose consumption was calculated by subtracting glucose concentrations in plated wells from blank wells. To normalize glucose uptake to cell viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Beyotime) was added for 4-h incubation, dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm to normalize glucose consumption to cell viability.

Intracellular glycogen measurement

Intracellular glycogen was quantified using a Glycogen Assay Kit (Nanjing Jiancheng Bioengineering). After cell lysis, samples underwent chromogenic reaction according to the manufacturer's instructions. Absorbance was measured at 620 nm, and glycogen concentration was determined using a standard curve generated from known glycogen concentrations.

Intracellular reactive oxygen species (ROS) detection

Intracellular ROS levels were quantified using the fluorogenic probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Beyotime). Briefly, HepG2 cells were seeded at 6×10^4 cells/well in 24-well plates and subjected to experimental treatments. After interventions, cells were incubated with 10 μM DCFH-DA diluted in serum-free medium for 1 h at 37°C under dark conditions. Following three washes with ice-cold phosphate buffer saline (PBS, pH 7.4), fluorescence images were captured using an Olympus BX51 fluorescence microscope. Quantitative analysis was performed using Image-ProPlus6.0 software.

Glucose uptake analysis

For glucose uptake quantification, HepG2 cells were seeded in 24-well plates at 2×10^5 cells/well. Following experimental

Table 1. Sequences of primers used for transcription-quantitative PCR

Gene (mouse)	Sequence (5'→3')
SOX2OT	F: ACGGGCACACATCAAGCATA
	R: AGCCTCAGCCCCCTATACAA
SOX2	F: AGGAGAGAAGTTTGGAGCCC
	R: TCTGGCGGAGAATAGTTGGG
GAPDH	F: TGAGGCCGGTGCTGAGTATGTCG
	R: CCACAGTCTTCTGGGTGGCAGTG
Gene (human)	**Sequence (5'→3')**
SOX2OT	F: TCACTTACAAGACAGCTCTGTTCAGT
	R: CAGCATTGCCACGACCTTCCA
TNF-α	F: GAGGCCAAGCCCTGGTATG
	R: CGGGCCGATTGATCTCAGC
IL-1β	F: CGATGCACCTGTACGATCAC
	R: TCTTTCAACACGCAGGACAG
IL-6	F: ACAGGGAGAGGGAGCGATAA
	R: GAGAAGGCAACTGGACCGAA
SOX2	F: AGGACTGAGAGAAAGAAGAGGAG
	R: CGCCGCCGATGATTGTTAT
GAPDH	F: CCAGGTGGTCTCCTCTGA
	R: GCTGTAGCCAAATCGTTGT

F, forward; R, reverse.

treatments, cells were incubated with 10 nM 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG; Beyotime), a fluorescent D-glucose analog, in serum-free medium for 30 min at 37°C. After three washes with ice-cold PBS to remove unincorporated probe, fresh serum-free medium was added. Fluorescence intensity was measured using a Synergy H1 microplate reader (Thermo Fisher). Data were normalized to total protein content (BCA assay) and expressed as percentage of control (untreated cells defined as 100%).

Western blotting

Total protein from liver tissues or HepG2 cells was isolated using Radio-Immunoprecipitation Assay Lysis Buffer (Sigma-Aldrich) and quantified *via* BCA assay. Proteins (40 μg) were separated by 10% Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred on a polyvinylidene fluoride (PVDF) membrane (0.45 μm; Millipore). After blocked using 5% skimmed milk for 2 h, membranes were incubated overnight at 4°C with primary antibodies against phospho-insulin receptor substrate-1 (p-IRS-1) (Thy612), IRS-1, phosphatidylinositol 3-kinase (PI3K) p85α, phospho-protein kinase B (p-AKT) (Ser473), AKT, SOX2, and GAPDH. Following Tris-Buffered Saline with Tween 20 (TBST) washes, membranes were incubated with Horseradish Peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. Protein bands were visualized using enhanced chemiluminescence reagent (Beyotime) and quantified with ImageLab3.0 software.

Intracellular MDA, SOD, glutathione peroxidase (GSH-Px) detection

Intracellular oxidative stress biomarkers – MDA, SOD, and GSH-Px – were quantified in HepG2 cell lysates using manufacturer-validated kits (Nanjing Jiancheng Bioengineering Institute). Cells were lysed with 0.2% Triton X-100 (Sigma-Aldrich) in ice-cold PBS, centrifuged at 10,000×*g* (4°C, 10 min), and supernatants transferred to 96-well plates for analysis.

Luciferase reporter assays

Wild-type (Wt) SOX2OT or SOX2 3'untranslated region (3'UTR) fragments containing microRNA-552-5p (miR-552-5p) or miR-676-3p complementary sequences and their mutated (Mut) counterparts were cloned into the pmirGLO dual-luciferase vector (Promega) to generate SOX2OT-Wt-pmirGLO, SOX2OT-Mut-pmirGLO, SOX2-Wt-pmirGLO, and SOX2-Mut-pmirGLO plasmids. HEK293T cells were co-transfected with either miR-552-5p mimics, miR-676-3p mimics, or NC mimics alongside wild-type or mutant plasmids using Lipofectamine 2000. After 48-h incubation, firefly and Renilla luciferase activities were quantified using a dual-luciferase assay system (Beyotime), with relative activity calculated as firefly luciferase luminescence normalized to Renilla luminescence *per* experimental group.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9.0, with all data presented as mean ± standard deviation (SD); biological replication was rigorously implemented – *in vitro* studies utilized three independent experiments, while *in vivo* investigations employed eight mice *per* group as biological replicates. Inter-group comparisons employed one-way analysis of variance (ANOVA) with Tukey's *post hoc* test for multi-group analyses and unpaired Student's *t*-test for pairwise comparisons. Statistical significance was uniformly defined as $p < 0.05$.

Results

SOX2OT downregulation in T2DM-OSA murine models and associated cellular phenotypes in HepG2 cells

The T2DM-OSA comorbidity model was successfully established through IH exposure in diabetic KKAγ mice, which exhibited significantly enhanced body weight gain compared to control mice (Fig. S1A in Supplementary Material). Biochemical analyses revealed significant hyperglycemia and insulin resistance in T2DM-OSA mice compared to controls, evidenced by elevated fasting blood glucose, fasting serum insulin, and HOMA-IR index (Fig. S1B–D). Serum lipid profiling demonstrated pronounced dyslipidemia, with marked increases in TC and TG (Fig. S1E,F). Systemic inflammation was robustly elevated as indicated by heightened serum TNF-α and IL-1β levels, while oxidative stress was confirmed through increased MDA and reduced SOD activity (Fig.1G–J). Critically, liver-specific analyses were expanded to address pathological mechanisms: Hepatic TNF-α, IL-1β, MDA, and SOD measurements revealed severe intrahepatic inflammation and oxidative damage (Fig. S1K–N), with Western blotting confirming impaired insulin signaling *via* suppression of p-IRS-1, PI3K, and p-AKT protein expression (Fig. S1O,P). These integrated findings establish the liver as a pivotal site for multi-organ dysregulation in T2DM-OSA comorbidity. Parallel molecular profiling identified significant downregulation of lncRNA SOX2OT in both serum and liver compartments of T2DM-OSA mice (Fig. 1A,B). This molecular signature was recapitulated *in vitro* through a rigorously validated cellular T2DM-OSA model, where HG and IH exposure induced progressive metabolic impairment evidenced by inhibited glucose consumption (Fig. 1C) and

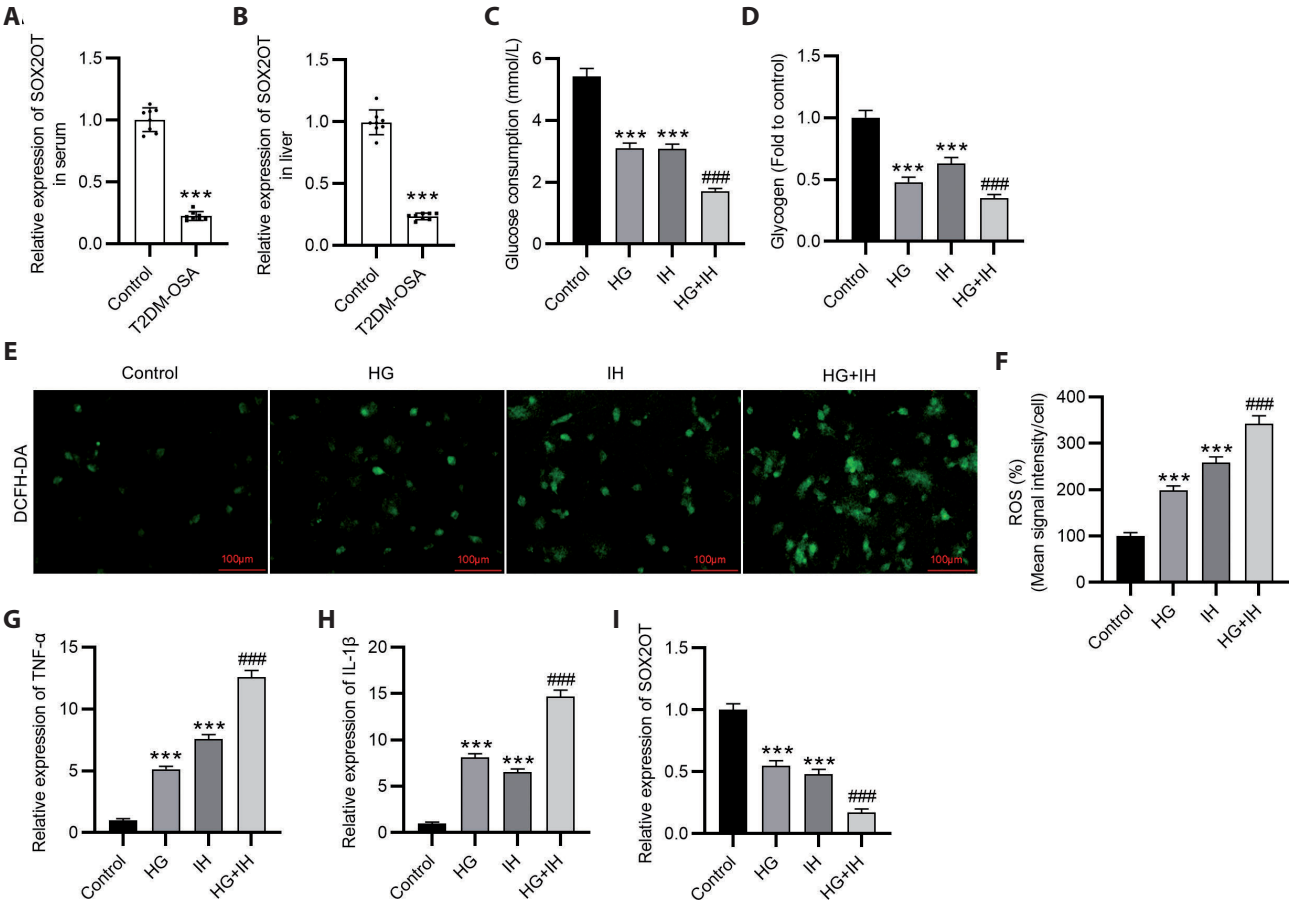

Figure 1. SOX2OT downregulation in T2DM-OSA murine models and associated cellular phenotypes in HepG2 cells. Murine studies: RT-qPCR of SOX2OT serum level (**A**) and SOX2OT mRNA expression in the liver (**B**) in T2DM-OSA and normal mice. HepG2 cellular studies: Glucose consumption (**C**) and intracellular glycogen content (**D**) in HepG2 cells under HG/IH. DCFH-DA staining (**E**) and quantification of intracellular ROS level (**F**) in HepG2 cells under HG/IH. RT-qPCR of TNF-α (**G**), IL-1β (**H**) and SOX2OT (**I**) mRNA levels in HepG2 cells under HG/IH. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* IH group. SOX2OT, sex determining region Y-box protein 2 overlapping transcript; T2DM-OSA, type 2 diabetes mellitus with obstructive sleep apnoea; HG high glucose; IH, intermittent hypoxia; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α; IL-1β, interleukin-1β.

reduced glycogen synthesis (Fig. 1D), alongside exacerbated oxidative stress manifested through elevated ROS generation (Fig. 1E,F) and amplified proinflammatory cytokine expression (Fig. 1G,H). Critically, the combined HG + IH treatment consistently elicited the most severe phenotypic disruptions across all parameters, culminating in maximal suppression of SOX2OT expression (Fig. 1I).

SOX2OT overexpression improves glucose consumption and uptake abilities of HG/IH-treated HepG2 cells through IRS-1/PI3K/AKT activation

Given the downregulation of SOX2OT observed in HG/IH-treated HepG2 cells, we implemented SOX2OT overexpression *via* a specific vector to investigate its functional consequences (Fig. 2A). Utilizing the fluorescent glucose analog 2-NBDG to monitor cellular glucose uptake, we observed that co-exposure to HG and IH dramatically suppressed 2-NBDG internalization; however, SOX2OT overexpression significantly rescued this deficit (Fig. 2B,C). Similarly, HG/IH treatment substantially reduced glucose consumption, an effect counteracted by SOX2OT upregulation (Fig. 2D). Intracellular glycogen content, which was markedly depleted under combined HG/IH conditions, was also restored following SOX2OT overexpression (Fig. 2E). At the molecular level, western blot analysis demonstrated that SOX2OT overexpression effectively reversed the HG/IH-induced suppression of p-IRS-1 and AKT phosphorylation as well as PI3K protein expression (Fig. 2F), indicating reactivation of insulin signaling pathways.

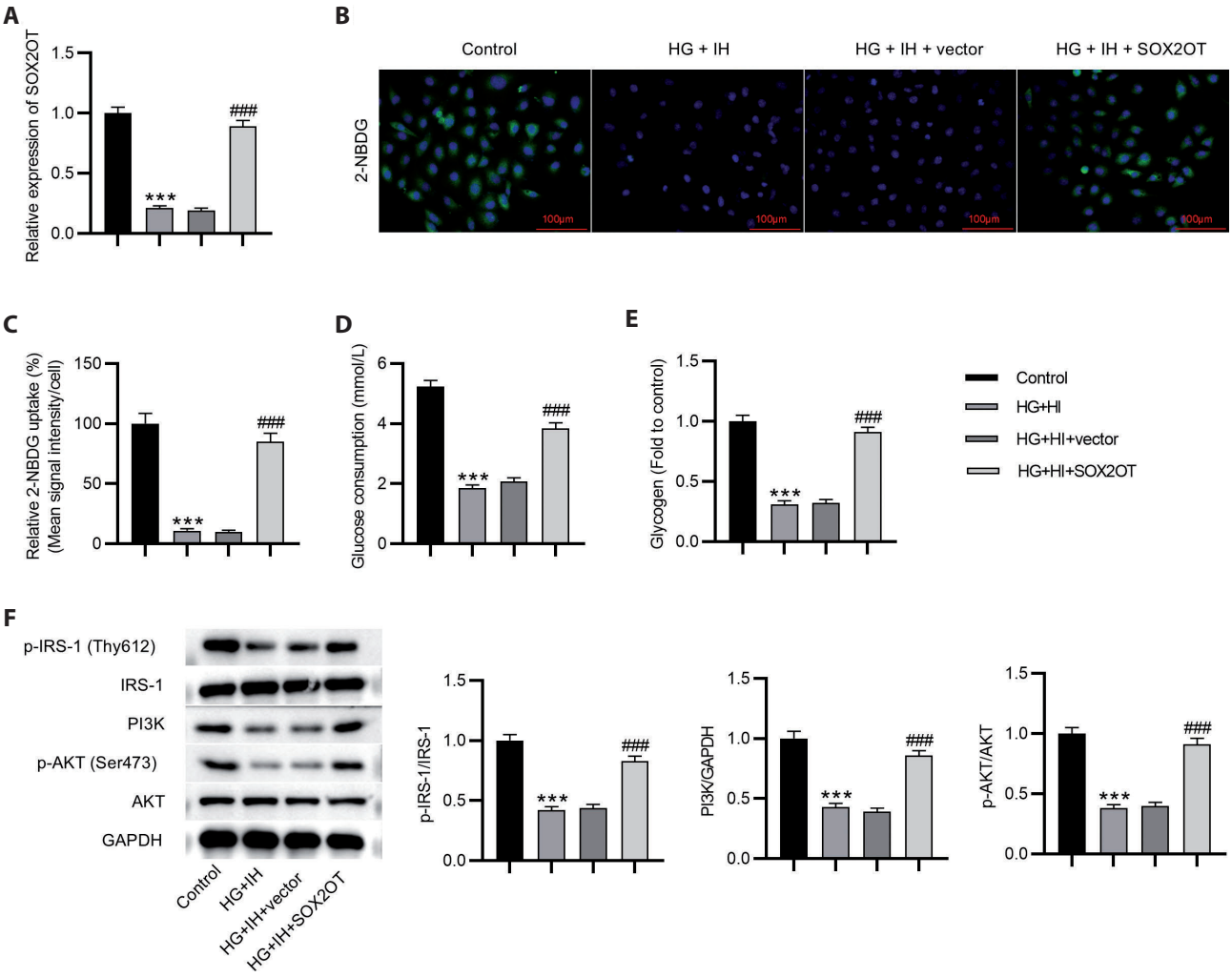

Figure 2. SOX2OT overexpression improves glucose consumption and uptake abilities of HG/IH-treated HepG2 cells by activating IRS-1/PI3K/AKT signaling. **A.** RT-qPCR of mRNA expression in HepG2 cells of control, HG+IH, HG+IH+vector, and HG+IH+SOX2OT groups. 2-NBDG test (**B**) and quantification (**C**) of glucose uptake in HepG2 cells. Glucose consumption (**D**) and intracellular glycogen content (**E**) in HepG2 cells. **F.** Western blotting of p-IRS-1 (Thy612), IRS-1, PI3K, p-AKT (Ser473), and AKT protein levels in HepG2 cells. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* HG+IH group. 2-NBDG, 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose; p-IRS-1, phospho-insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; p-AKT, phospho-protein kinase B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. For more abbreviations, see Figure 1.

SOX2OT overexpression ameliorates oxidative stress and inflammation in HG/IH-treated HepG2 cells

DCFH-DA staining demonstrated that SOX2OT overexpression effectively reversed the HG/IH-induced elevation in intracellular ROS levels (Fig. 3A,B). Concurrently, SOX2OT restoration significantly attenuated oxidative stress in HG/IH-treated HepG2 cells, evidenced by reduced MDA content alongside enhanced SOD and GSH-Px activities compared to vector-transfected controls (Fig. 3C–E). Moreover, SOX2OT upregulation substantially mitigated HG/IH-triggered proinflammatory responses, as indicated by downregulated mRNA expression of TNF-α, IL-1β, and IL-6 (Fig. 3F).

SOX2OT interacts with miR-552-5p to regulate SOX2 expression

RT-qPCR analysis demonstrated that HG and/or IH treatment, particularly their combination, downregulated SOX2 in HepG2 cells (Fig. 4A), while SOX2OT overexpression markedly increased SOX2 mRNA levels (Fig. 4B). Consistently, T2DM-OSA mice showed significantly reduced SOX2 expression in both serum and liver tissues *versus* controls (Fig.

4C,D). Bioinformatic prediction *via* Starbase and TargetScan databases identified miR-676-3p and miR-552-5p as miRNAs with binding sites on both SOX2OT and SOX2 (Fig. 4E), with specific binding sequences illustrated (Fig. 4F and Fig. S2A). Luciferase reporter assays confirmed that miR-552-5p mimics substantially reduced luciferase activity when co-transfected with wild-type SOX2OT or SOX2 3'UTR constructs (Fig. 4G,H), whereas mutations in the binding sites abrogated this effect. In contrast, miR-676-3p exhibited no significant regulatory activity on these targets (Fig. S2B,C), leading us to prioritize miR-552-5p for further investigation. Critically, although SOX2OT overexpression rescued the HG/IH-induced suppression of SOX2 expression at both mRNA and protein levels (Fig. 4I,J), concurrent miR-552-5p overexpression completely abolished this protective effect, confirming the miR-552-5p/SOX2 axis as the mechanistic core of SOX2OT-mediated regulation in T2DM-OSA pathology.

SOX2 knockdown reverses SOX2OT overexpression-mediated protection against HG/IH-induced cytotoxicity

Western blotting confirmed successful SOX2 knockdown in HepG2 cells (Fig. 5A), which consequently attenuated the SOX2OT overexpression-mediated enhancement of glucose uptake monitored *via* 2-NBDG fluorescence (Fig. 5B). SOX2 depletion further reduced glucose consumption in HG/IH-treated cells despite SOX2OT restoration, and counteracted SOX2OT-induced glycogen synthesis recovery (Fig. 5C). Mechanistically, SOX2 knockdown reversed the SOX2OT-driven upregulation of phosphorylated IRS-1 and AKT alongside PI3K protein expression (Fig. 5D). Crucially, SOX2 ablation reinstated pathological oxidative stress and inflammation, elevating ROS, MDA, TNF-α, and IL-1β levels while suppressing SOD activity (Fig. 5E–H), collectively establishing SOX2 as the indispensable executor of SOX2OT's cytoprotective functions.

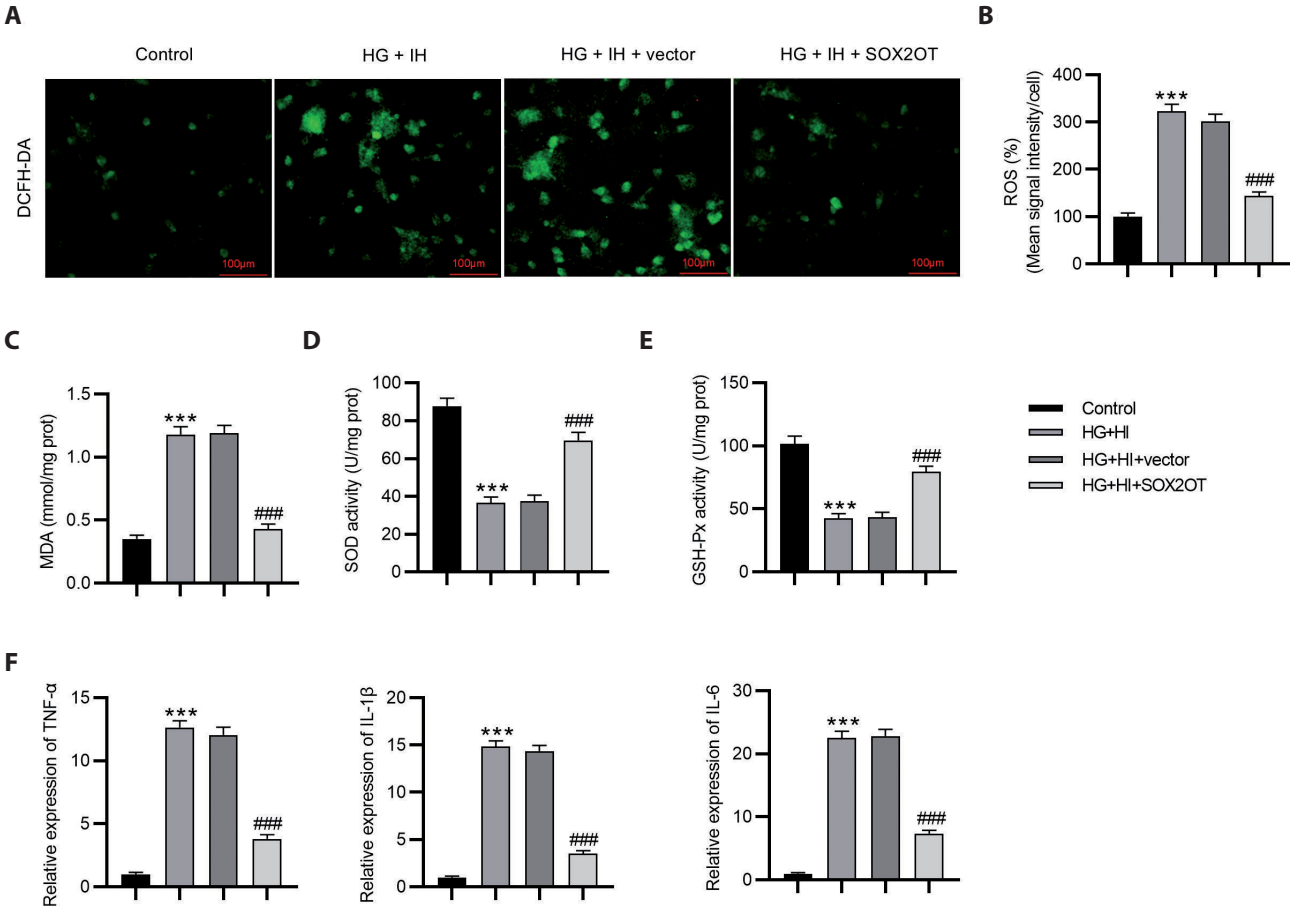

Figure 3. SOX2OT overexpression ameliorates oxidative stress and inflammation in HG/IH-treated HepG2 cells. DCFH-DA staining (**A**) and quantification of ROS level (**B**) in HepG2 cells of Control, HG+IH, HG+IH+vector, and HG+IH+SOX2OT groups. MDA content (**C**), SOD activity (**D**) and GSH-Px activity (**E**) in all groups. **F.** RT-qPCR of TNF-α, IL-1β, and IL-6 mRNA levels. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* HG+IH group. MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. For more abbreviations, see Figure 1.

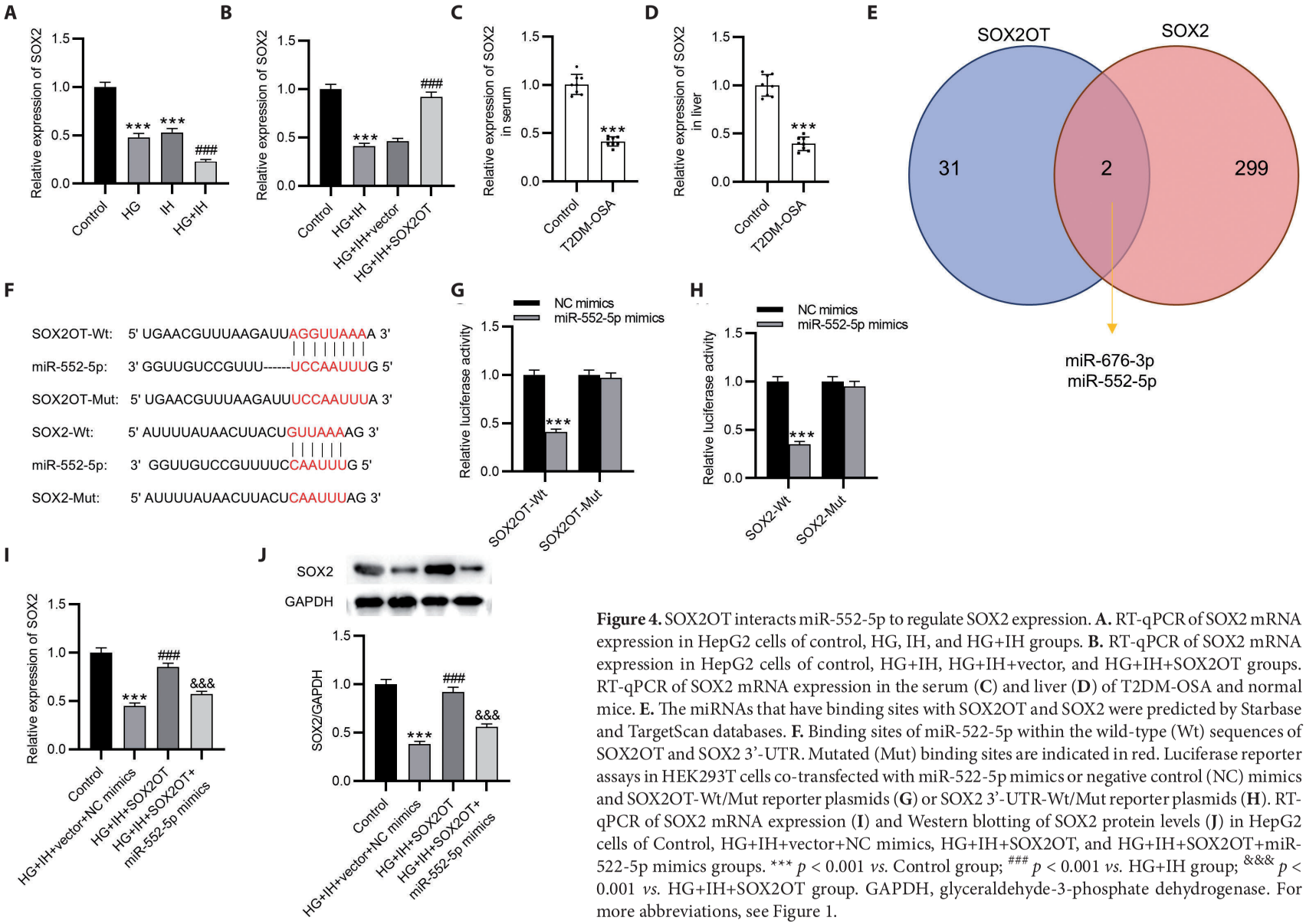

Figure 4. SOX2OT interacts miR-552-5p to regulate SOX2 expression. **A.** RT-qPCR of SOX2 mRNA expression in HepG2 cells of control, HG, IH, and HG+IH groups. **B.** RT-qPCR of SOX2 mRNA expression in HepG2 cells of control, HG+IH, HG+IH+vector, and HG+IH+SOX2OT groups. RT-qPCR of SOX2 mRNA expression in the serum (**C**) and liver (**D**) of T2DM-OSA and normal mice. **E.** The miRNAs that have binding sites with SOX2OT and SOX2 were predicted by Starbase and TargetScan databases. **F.** Binding sites of miR-522-5p within the wild-type (Wt) sequences of SOX2OT and SOX2 3'-UTR. Mutated (Mut) binding sites are indicated in red. Luciferase reporter assays in HEK293T cells co-transfected with miR-522-5p mimics or negative control (NC) mimics and SOX2OT-Wt/Mut reporter plasmids (**G**) or SOX2 3'-UTR-Wt/Mut reporter plasmids (**H**). RT-qPCR of SOX2 mRNA expression (**I**) and Western blotting of SOX2 protein levels (**J**) in HepG2 cells of Control, HG+IH+vector+NC mimics, HG+IH+SOX2OT, and HG+IH+SOX2OT+miR-522-5p mimics groups. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* HG+IH group; &&& $p < 0.001$ *vs.* HG+IH+SOX2OT group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. For more abbreviations, see Figure 1.

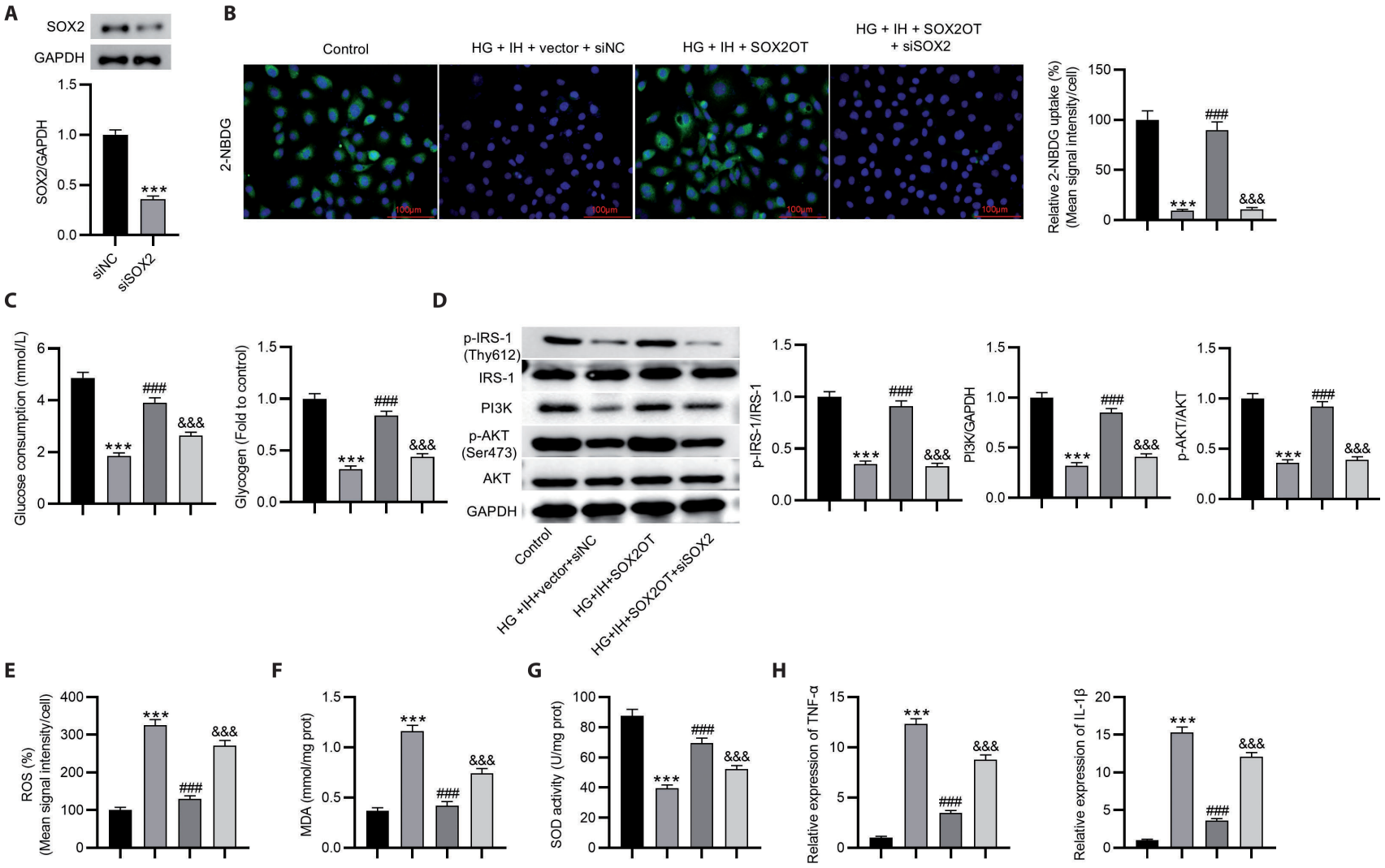

Figure 5. SOX2 knockdown reverses the protective effect of SOX2OT overexpression against HG/IH-induced cell injury. **A.** Western blotting of SOX2 protein levels in HepG2 cells of siNC and si-SOX2 groups. **B.** 2-NBDG test of glucose uptake in HepG2 cells of control, HG+IH+vector+siNC, HG+IH+SOX2OT, and HG+IH+SOX2OT+siSOX2 groups. **C.** Glucose consumption and glycogen content. **D.** Western blotting of p-IRS-1 (Thy612), IRS-1, PI3K, p-AKT (Ser473), and AKT protein levels. **E.** ROS level. **F.** MDA content. **G.** SOD activity. **H.** RT-qPCR of TNF-α and IL-1β mRNA levels. *** $p < 0.001$ *vs.* Control group; ### $p < 0.001$ *vs.* HG+IH+vector+siNC group; &&& $p < 0.001$ *vs.* HG+IH+SOX2OT group. SOX2, sex determining region Y-box protein 2; 2-NBDG, 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose; p-IRS-1, phospho-insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; p-AKT, phospho-protein kinase B; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor α; IL-1β, interleukin-1β. For more abbreviations, see Figure 1.

Discussion

Extensive studies have elucidated the interplay between T2DM and OSA, where severe OSA exacerbates insulin resistance and increases T2DM risk in affected patients, while untreated OSA significantly compromises glycemic control and worsens prognosis in comorbid T2DM-OSA (Devaraj 2020). Notably, T2DM patients exhibit a higher prevalence of OSA compared to nondiabetic individuals (Lavrentaki et al. 2019), with insulin-treated T2DM particularly correlated with elevated OSA risk, likely due to suboptimal glycemic management (Huang et al. 2018). The pathophysiological link involves IH and sleep fragmentation from OSA, which directly contribute to metabolic dysregulation characterized by insulin resistance, glucose intolerance, and T2DM progression (Aurora and Punjabi 2013). LncRNAs have emerged as key regulators in metabolic diseases (Ji et al. 2020), with accumulating evidence implicating specific lncRNAs in T2DM-OSA pathogenesis. Focusing on lncRNA SOX2OT, we note its documented dysregulation in diabetic models (Zhang et al. 2019; Chen et al. 2021) and roles in diabetic complications (Zhang et al. 2019; Ye et al. 2023). However, the functional significance of SOX2OT in the clinically prevalent T2DM-OSA comorbidity – a distinct entity characterized by synergistic pathological interactions between hyperglycemia and IH – remains unexplored.

This study provides the first mechanistic evidence that SOX2OT attenuates insulin resistance specifically in the T2DM-OSA milieu through a novel miR-552-5p/SOX2-dependent axis. Prior work indicates SOX2OT overexpression mitigates oxidative stress and apoptosis in diabetic nephropathy (Zhang et al. 2019; Ye et al. 2023), suggesting broader protective roles in metabolic contexts. Our findings extend this understanding by demonstrating that in the context of combined hyperglycemic and hypoxic stress – a hallmark of T2DM-OSA – SOX2OT uniquely coordinates IRS/PI3K/AKT-mediated insulin sensitization with suppression of oxidative stress and inflammation *via* the previously unreported mechanism involving miR-552-5p sponging and SOX2 derepression. These results position SOX2OT as a critical molecular mediator specifically in T2DM-OSA pathology.

Hyperglycemia, a hallmark of T2DM (Majety et al. 2023), primarily stems from insulin resistance – manifested by impaired glycogen synthesis, reduced cellular glucose uptake, heightened gluconeogenesis, and disrupted insulin signaling (Bornfeldt and Tabas 2011). OSA independently associates with abnormal glucose metabolism and insulin resistance, accelerating T2DM development (Ip et al. 2002). The liver plays a central role in glucoregulation, governing glycogenesis, glycogenolysis, glycolysis, and gluconeogenesis (Han et al. 2016). Insulin enhances hepatic glycogen storage while suppressing gluconeogenic enzyme expression (Kubota et al. 2025). It is essential to contextualize that hepatocytes (including HepG2 cells) express constitutively active glucose transporter 2 (GLUT2) glucose transporters. Unlike insulin-sensitive GLUT4 transporters in muscle or adipose tissue, GLUT2 mediates basal glucose uptake in an insulin-independent manner, primarily driven by the portal blood glucose concentration gradient (Leturque et al. 2009).

Consequently, the IRS/PI3K/AKT pathway – while critical for insulin signaling – does not directly regulate GLUT2-dependent glucose influx in hepatocytes. Instead, its activation primarily governs post-uptake glucose utilization, including glycogenesis and suppression of gluconeogenesis. In our cellular T2DM-OSA model (HG/IH-treated HepG2 hepatocytes), SOX2OT was significantly downregulated, mirroring observations in serum and liver tissues of diabetic mice exposed to IH. Crucially, SOX2OT restoration enhanced glucose consumption (reflecting metabolic utilization) and glycogen content, indicating improved insulin sensitivity. Notably, while SOX2OT did not directly modulate GLUT2-mediated influx (as GLUT2 operates independently of insulin signaling), it potently activated IRS-1/PI3K/AKT signaling. Specifically, SOX2OT reversed HG/IH-induced reductions in IRS-1 phosphorylation (Tyr612), PI3K expression, and AKT phosphorylation (Ser473). This indicates that SOX2OT ameliorates hepatic insulin resistance by enhancing insulin signaling downstream of glucose uptake, thereby promoting glycogen synthesis and inhibiting gluconeogenesis through PI3K/AKT-dependent mechanisms (e.g., activation of glycogen synthase and suppression of PEPCK/G6Pase).

Oxidative stress and inflammation are key pathological drivers in T2DM-OSA. Elevated ROS and oxidative biomarkers are well-documented in OSA patients (Prabhakar et al. 2007), and antioxidant interventions improve vascular function in this population (Grebe et al. 2006). Rodent models confirm IH-induced ROS overproduction (Peng et al. 2012), while in diabetes, chronic hyperglycemia fuels oxidative damage (Luc et al. 2019). Key indicators like MDA (lipid peroxidation marker), SOD (free radical scavenger), and GSH-Px (antioxidant enzyme) reflect oxidative stress severity (Jomova and Valko 2011). Our data show SOX2OT overexpression counteracted HG/IH-induced ROS/MDA accumulation while restoring SOD/GSH-Px activity, aligning with its known antioxidative role in diabetic complications (Ye et al. 2023). As reported, inflammatory markers are elevated in OSA patients compared with control subjects, primarily due to IH and sleep fragmentation (Zdravkovic et al. 2022). Concurrently, this inflammation promotes the development of insulin resistance, glucose tolerance, and ultimately T2DM (Storgaard et al. 2014), with elevated inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) consistently observed in diabetic patients (Barone and Menna-Barreto 2011; Golbidi et al. 2012). Herein, we found that SOX2OT overexpression suppressed TNF-α, IL-1β, and IL-6 pro-

duction in HG/IH-treated HepG2 cells, further mitigating insulin resistance.

Mechanistically, we identified the SOX2OT/miR-552-5p/SOX2 axis as central to this regulation. LncRNAs often function as competing endogenous RNAs (ceRNAs), sequestering miRNAs to derepress target gene expression (Xu et al. 2022). Bioinformatics and luciferase assays confirmed SOX2OT directly binds miR-552-5p, which concurrently targets the 3'UTR of SOX2. SOX2 downregulation in diabetic patients (Potdar and D'Souza 2011) and its reported anti-inflammatory/antioxidant functions in diabetes (Li et al. 2023) support this axis. Critically, SOX2 knockdown abolished SOX2OT-mediated improvements in glucose metabolism, insulin signaling, and cytoprotection, establishing SOX2 as the indispensable effector.

While this study establishes SOX2OT's role *via* IRS/PI3K/AKT activation, we acknowledge that: 1) The precise relationship between hepatic GLUT2 activity and the IRS/PI3K/AKT pathway in T2DM-OSA warrants direct investigation. Future studies should quantify GLUT2-mediated glucose flux alongside post-uptake metabolic endpoints to fully dissect SOX2OT's impact on hepatic glucose handling; 2) SOX2OT's effects may involve off-target pathways beyond the identified axis; 3) *In vivo* validation using SOX2OT-knockout/transgenic models is essential to confirm physiological relevance.

In conclusion, lncRNA SOX2OT emerges as a promising prognostic biomarker and therapeutic target for T2DM-OSA. Its upregulation coordinately improves insulin sensitivity – primarily through enhancing post-uptake glucose utilization in hepatocytes *via* IRS/PI3K/AKT activation – while suppressing oxidative stress and inflammation through the miR-552-5p/SOX2 axis (Fig. S3). This multifaceted mechanism offers novel insights for diagnosing and treating T2DM-OSA comorbidity.

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Conflict of interest. The authors declare that they are no conflicts of interests.

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Ethical Approval. All animal experiments were approved by the Ethics Committee on Animal Experimentation of Wuchang Hospital Affiliated to Wuhan University of Science and Technology.

Authors' contributions. YW conceived and designed the experiments. YW, LZ, JD, XY, FT, YL and YL carried out the experiments, analyzed the data and drafted the manuscript. All authors agreed to be accountable for all aspects of the work. All authors have read and approved the final manuscript.

Data availability statement. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary Material

LncRNA SOX2OT improves insulin resistance in type 2 diabetes with sleep disorders by activating IRS/PI3K/AKT and suppressing oxidative stress and inflammation through the miR-552-5p/SOX2 axis

Yalan Wang[1], Li Zheng[1], Jing Dai[1], Xiaoshi Yang[1], Feng Tang[1], Yan Li[1] and Yu Lu[2]

[1] *Department of Endocrinology, Wuchang Hospital Affiliated to Wuhan University of Science and Technology, Wuchang District, Wuhan, China*
[2] *Wuhan Mental Health Center, Qiaokou District, Wuhan, China*

Supplementary Figure

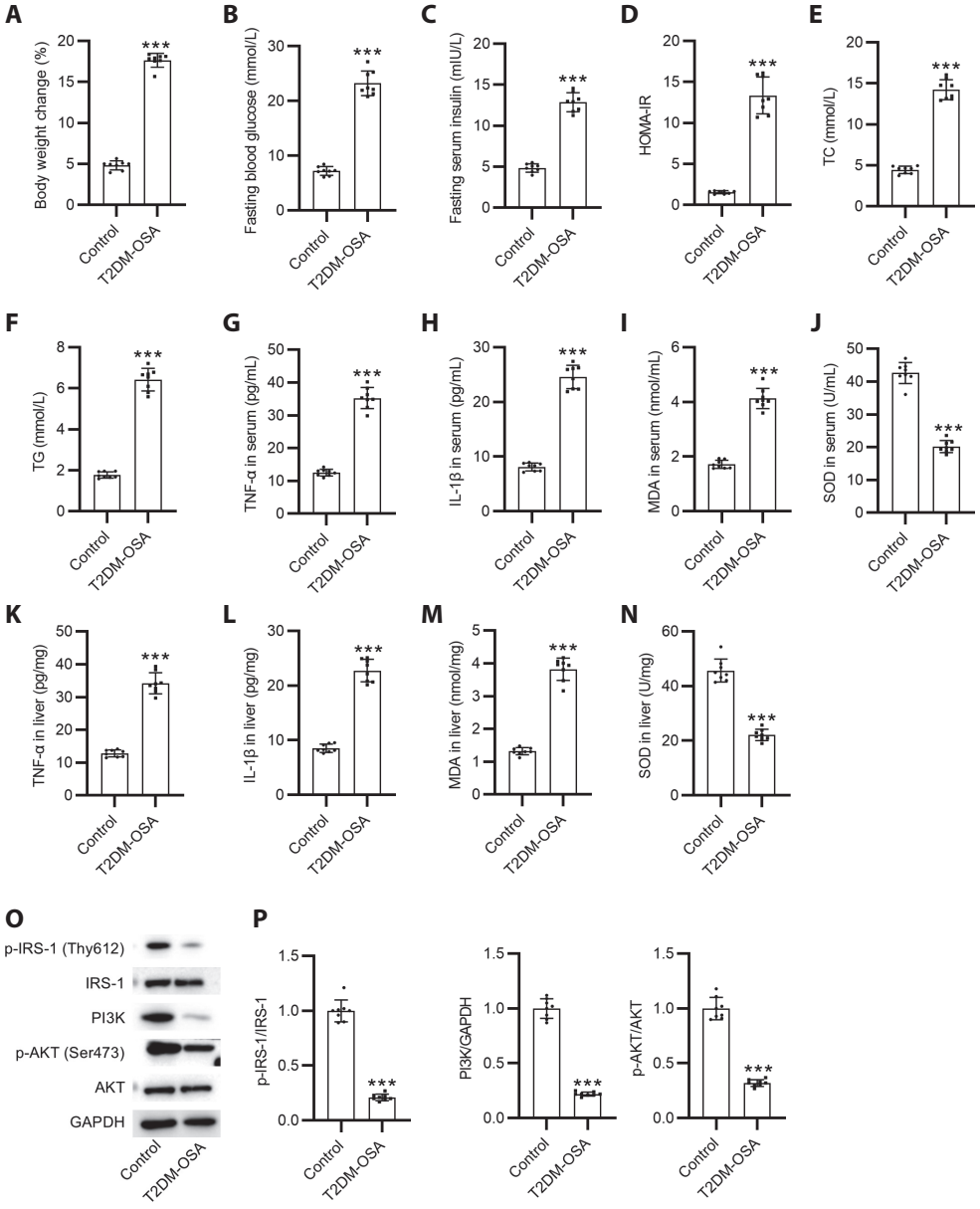

Figure S1. Biochemical parameters of T2DM-OSA mice. **A.** Body weight. **B.** Fasting blood glucose. **C.** Fasting serum insulin. **D.** HOMA-IR index. **E.** TC level. **F.** TG level. **G.** Serum TNF-α level. **H.** Serum IL-1β level. **I.** Serum MDA level. **J.** Serum SOD activity. **K.** Hepatic TNF-α level. **L.** Hepatic IL-1β level. **M.** Hepatic MDA level. **N.** Hepatic SOD activity. **O,P.** Western blotting of p-IRS-1 (Thy612), IRS-1, PI3K, p-AKT (Ser473), and AKT protein levels in the liver of T2DM-OSA and normal mice. *** $p < 0.001$ *vs.* Control group. T2DM-OSA, type 2 diabetes mellitus with obstructive sleep apnoea; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor α; IL-1β, Interleukin-1β; MDA, malondialdehyde; SOD, superoxide dismutase; p-IRS-1, phospho-insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; p-AKT, phosphoprotein kinase B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

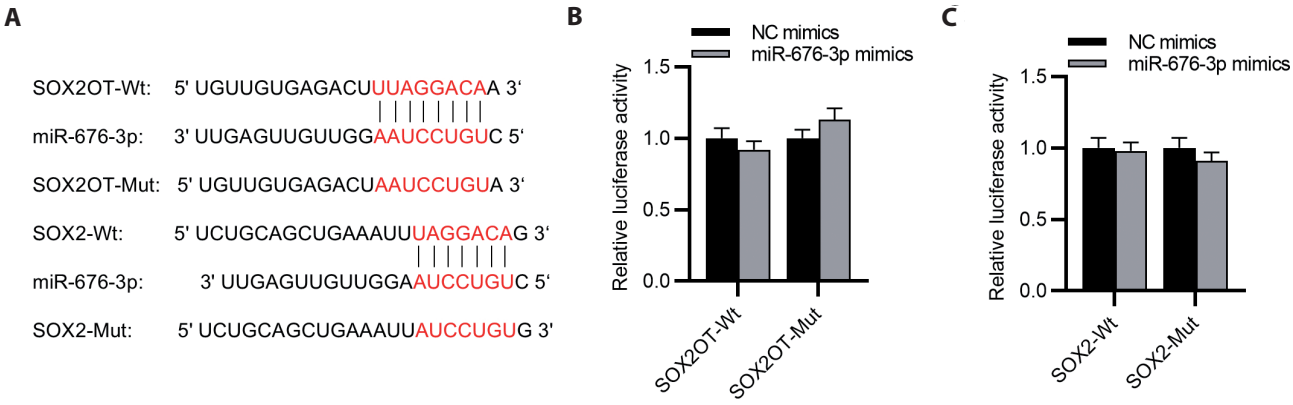

Figure S2. Interaction between SOX2OT or SOX2 and miR-676-3p. **A.** Binding sites of miR-676-3p within the wild-type (Wt) sequences of SOX2OT and SOX2 3'-UTR. Mutated (Mut) binding sites are indicated in red. **B,C.** Luciferase reporter assays in HEK293T cells co-transfected with miR-676-3p mimics or negative control (NC) mimics and SOX2OT-Wt/Mut reporter plasmids or SOX2 3'-UTR-Wt/Mut reporter plasmids. SOX2, sex determining region Y-box protein 2; SOX2OT, sex determining region Y-box protein 2 overlapping transcript.

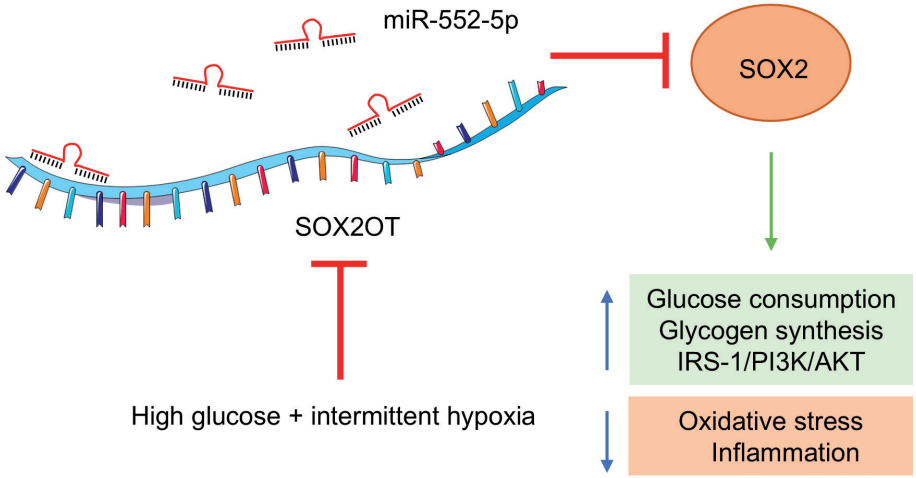

Figure S3. Proposed mechanism by which SOX2OT upregulation mitigates insulin resistance, oxidative stress, and inflammation in T2DM-OSA.