

1 **Lactylation-dependent DDX18 nucleolar escape regulates *CD44* mRNA stability to drive fibrotic**
2 **progression in kidney injury**

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Abstract

Following acute kidney injury (AKI), a substantial subset of patients experiences an irreversible progression to chronic kidney disease (CKD), yet the molecular determinants governing this maladaptive transition remain elusive, and effective clinical interventions are lacking. Here, we identify lactate as a key metabolic determinant orchestrating the transition from AKI to CKD. Analysis of the UK Biobank cohort reveals that elevated circulating lactate independently predicts CKD development in AKI patients and correlates with fibrotic progression. Using murine ischemia-reperfusion injury models, we demonstrate that lactate drives sustained renal damage through post-translational lactylation of the RNA helicase DDX18. Mechanistically, p300-mediated lactylation of DDX18 at lysine 116 disrupts its nucleolar retention, causing redistribution to the nucleoplasm where it acquires enhanced binding affinity for *CD44* mRNA. This subcellular relocation stabilizes *CD44* mRNA through altered RNA-protein interactions, thereby amplifying fibrotic signaling pathways. Therapeutically, we developed a kidney-targeted, cell-penetrating peptide that specifically inhibits DDX18 K116 lactylation, effectively attenuating fibrotic progression in injured kidneys. Our findings establish protein lactylation as a regulatory mechanism governing RNA helicase nucleolar localization and subsequent control of mRNA stability, revealing a potential therapeutic target for interrupting fibrotic processes in chronic kidney disease.

Introduction

Kidney disease encompasses acute and chronic disorders that progressively impair renal function. Acute kidney injury (AKI), a common clinical syndrome associated with substantial morbidity and mortality, is caused by ischemia, nephrotoxins, and sepsis-related inflammation (1-3). Emerging evidence reveals that AKI often initiates complex pathophysiological cascades with diverse clinical trajectories, including complete recovery, partial resolution, recurrent episodes, or progression to chronic kidney disease (CKD). The AKI-to-CKD transition is a critical event in the patient's clinical trajectory and is characterized by persistent inflammation, tubular atrophy, interstitial fibrosis, and progressive loss of renal function (4, 5). Understanding the molecular mechanisms governing this transition remains a critical clinical challenge.

Tubulointerstitial fibrosis is the common pathway driving kidney dysfunction, characterized by pathological accumulation of extracellular matrix (ECM) components (6, 7). Beyond structural support, ECM functions as a platform regulating cellular behavior (8). During AKI-to-CKD transition, excessive ECM synthesis and impaired degradation cause pathological deposition of collagens, fibronectin, and proteoglycans (7, 9), whereas dysregulated remodeling enzymes disrupt matrix architecture and drive fibrotic progression (6, 8). Central to this process are renal tubular epithelial cells (TECs), which exhibit remarkable phenotypic plasticity following injury (10, 11). Rather than serving as passive victims or bystanders, TECs actively shape disease trajectories through context-dependent activation of regenerative or maladaptive repair programs (12, 13). However, the molecular mechanisms governing TEC fate decisions in CKD remain incompletely understood.

RNA binding proteins (RBPs) are post-transcriptional regulators that orchestrate RNA metabolism from processing and localization to stability and translation (14). Accumulating evidence

67 implicates the role of RBPs in AKI, diabetic kidney disease, and glomerular disorders (15). For
68 instance, Hu antigen R (HuR) promotes septic AKI-to-CKD transition by stabilizing CD147 mRNA
69 (16). Similarly, AU-rich element RNA-binding protein (AUF1) promotes fibrotic progression through
70 destabilization of NEDD4L mRNA and overactivating of TGF- β signaling (17). These findings
71 position RBPs as attractive therapeutic targets, yet how to interface with RBP function during CKD
72 progression remains poorly explored.

73 Metabolic reprogramming is a fundamental driver of kidney disease progression. Alterations in
74 circulating metabolites, including uric acid, sugars, amino acids, lipids, and organic acids, correlate
75 with and potentially predict CKD onset and progression (18-20). However, the metabolic mechanisms
76 linking AKI to fibrosis remain unclear. Our biobank cohort analysis identified lactate as a key
77 metabolic driver of kidney fibrotic progression following AKI, with elevated circulating lactate
78 independently predicting subsequent CKD development. Using a murine renal ischemia-reperfusion
79 model, lactate was found to drive persistent kidney injury by promoting post-translational lactylation
80 of RNA helicase DDX18, a RBP that belongs to DEAD-box protein family. Specifically, p300-
81 mediated lactylation of DDX18 at lysine 116 (K116) triggered its redistribution from the nucleolus to
82 the nucleoplasm, enhancing its binding to and stabilization of *CD44* mRNA and thereby accelerating
83 fibrosis. We further developed a kidney-targeted, cell-penetrating peptide that selectively inhibited
84 DDX18 K116 lactylation and attenuated fibrosis in injured kidneys. These findings define a lactate–
85 DDX18–*CD44* axis linking metabolic reprogramming to maladaptive AKI-to-CKD progression and
86 provide a potential therapeutic strategy for progressive kidney disease.

Results

Elevated lactate predicts the progression of AKI-to-CKD

To delineate the metabolic factors driving the AKI-to-CKD transition, we performed plasma metabolomic profiling in a longitudinal UK Biobank cohort *via* the Nightingale Health NMR platform, focusing on plasma lactate measured in participants with AKI within the 1 year preceding recruitment (identified through Hospital Episode Statistics). Patients who progressed to CKD displayed elevated plasma lactate compared to non-progressing counterparts (**Fig. 1A, B**). Elevated lactate predicted higher cumulative risk of CKD progression (**Fig. 1C**), independent of initial renal function, as serum creatinine levels were comparable between the low- and high-lactate groups (**Tab. S1**). Parallel proteomic analysis revealed enhanced fibrogenesis in patients progressing from AKI to CKD, characterized by ECM components (**Fig. 1D**). This progression signature featured upregulation of structural ECM proteins, including collagens and matrix metalloproteinases (MMPs), alongside fibroblast markers such as α -smooth muscle actin (encoded by ACTA2) (**Fig. 1E**). Collectively, these data establish lactate as a potential mechanistic link between metabolic dysregulation and progressive renal fibrogenesis following AKI.

Next, we investigated exogenous lactate administration in a mouse model of bilateral ischemia-reperfusion injury (BIRI) (**Fig. 1F**). Lactate exacerbated kidney dysfunction, increasing the serum levels of creatinine, uric acid, and blood urea nitrogen (BUN) (**Fig. 1G**). Histological analyses revealed tubular damage and interstitial collagen deposition (**Fig. 1H**). Molecular analyses showed increased expression of fibrotic markers, including fibronectin, α -smooth muscle actin (α -SMA), and collagen 1, at mRNA (**Fig. 1I**) and protein levels (**Fig. 1J, K**). We next assessed whether lactate accumulation was associated with injury progression. Acute lactate administration transiently raised renal lactate

109 levels (**Fig. S1A**). Chronic lactate treatment did not alter renal/plasma lactate levels or plasma pH in
110 sham-operated mice, but increased renal/plasma lactate and reduced plasma pH in BIRI mice (**Fig.**
111 **S1B-D**), indicating selective amplification of injury-associated metabolic disturbances. LDHA and
112 LDHB expression was markedly increased in BIRI kidneys (**Fig. S1E, F**), and in hypoxia-exposed
113 HK-2 cells (**Fig. S1G-I**). Pharmacological inhibition of LDH using (R)-GNE-140 could alleviate AKI-
114 to-CKD progression (**Fig. 1L**), reduced kidney dysfunction (**Fig. 1M**), alleviated histopathological
115 damage (**Fig. 1N**), and suppressed renal fibrosis markers (**Fig. 1O-Q**). Consistently, (R)-GNE-140
116 also reduced lactate accumulation in BIRI kidneys and TECs (**Fig. S1J, K**), and prevented hypoxia-
117 induced fibrotic responses *in vitro* (**Fig. S1L-N**). Collectively, these data suggest that lactate
118 accumulation may be involved in the progression from AKI to CKD.

119

120 **Aberrant protein lactylation marks injured TECs**

121 Protein lysine lactylation (Kla), a lactate-derived post-translational modification, regulates diverse
122 cellular processes (21). We hypothesized that this modification may contribute to the AKI-to-CKD
123 progression. To test this, we stratified human AKI biopsies by proteinuria, a robust indicator of CKD
124 development (**Tab. S2**) (22). Biopsies from AKI patients with persistent proteinuria exhibited a marked
125 upregulation of global protein lactylation compared to non-proteinuric controls (**Fig. 2A**). Elevated
126 lactylation was recapitulated in kidney tissue from BIRI mice, predominantly localized to renal TECs
127 (**Fig. 2B**). Lactylation intensity increased progressively after kidney injury (**Fig. 2C**) and was further
128 potentiated by lactate administration (**Fig. 2D**).

129 Lactylation proteomics using a specific Kla antibody (**Fig. S2**) identified 143 proteins with
130 increased Kla modifications and 39 proteins with decreased Kla modifications following hypoxic

injury in TECs, revealing 207 upregulated and 73 downregulated lactylation sites (**Fig. 2E, F and Fig. S3A**). Most lactylated proteins localized to the nucleus (**Fig. 2G**) and were implicated in RNA metabolic and processing pathways (**Fig. 2H and Fig. S3B**). Sequence motif analysis revealed that lactylation sites preferentially cluster near lysine residues (**Fig. S3C, D**). Higher lactylation enrichment was observed particularly in RNA helicase proteins, key regulators of RNA metabolism involved in ribosome biogenesis, translation, RNA export, and stability (23, 24). We focused on DDX18, an ATP-dependent RNA helicase critical for rRNA transcription, which presented increased lactylation (**Fig. 2I, Tab. S3**).

Interestingly, renal DDX18 expression remained stable in human renal disease (**Fig. 2J**) and mouse kidney injury models (**Fig. 2K**), whereas DDX18 lactylation was increased in BIRI kidneys (**Fig. 2L**), particularly with lactate administration (**Fig. 2M**). *In vitro* studies corroborated these findings, demonstrating elevated DDX18 lactylation in H/R-treated TECs (**Fig. 2N**). Thus, DDX18 lactylation, rather than expression, appears critical in the transition toward chronic disease states.

DDX18 orchestrates fibrogenic processes during the progression of kidney disease

To investigate the role of TEC-expressed DDX18 during AKI-to-CKD progression, TEC-specific *DDX18* knockout mice were generated *via* a *Ksp* promoter-driven AAV9 vector (**Fig. 3A**). DDX18 deletion was restricted to the kidneys, with no off-target reduction in other tissues (**Fig. S4**). TEC-specific DDX18 depletion protected against BIRI-induced renal dysfunction and structural deterioration, with reduced tubular injury and interstitial collagen deposition (**Fig. 3B, C**). Protection was observed in both male and female mice (**Fig. S5**). DDX18 deficiency blunted the upregulation of profibrotic markers at both the transcript and protein levels following BIRI (**Fig. 3D-F**). RNA-seq

analysis of microdissected tubulointerstitial tissue from patients with kidney disease (GSE175759) revealed a positive correlation between *DDX18* expression and fibrotic gene signatures (**Fig. S6**), supporting a role for DDX18 in human fibrotic progression. To further elucidate the role of DDX18 in fibrogenesis, *DDX18* was silenced in hypoxia-challenged TECs. DDX18 depletion attenuated hypoxia-induced TEC migration (**Fig. 3G**), accompanied by reduced fibrotic marker expression (**Fig. 3H-J**). Together, these findings identify DDX18 as a critical regulator of fibrogenic reprogramming during kidney disease progression.

DDX18 lactylation at lysine K116 mediates its pro-fibrogenic function

Lactylation occurs on specific lysine residues that are essential for protein folding and function (25). Structural mapping of DDX18 identified five conserved lysine residues (**Fig. 4A**), among which K116 exhibited the hypoxia-induced lactylation in injured TECs (**Fig. 4B**), within the missed-cleavage peptide KMVNDAEPDTK. To functionally validate this site, we generated a lysine-to-arginine substitution series (K→R mutants). Compared with the DDX18^{WT} and other mutants, the DDX18^{K116R} mutant presented decreased DDX18 lactylation (**Fig. 4C**), identifying K116 as the major lactylation site. K116 is highly conserved from *Danio rerio* to *Homo sapiens* (**Fig. 4D**).

We then generated an antibody against DDX18 lactylated at K116 (DDX18-K116la) (**Fig. 4E and Fig. S7**). DDX18 K116 lactylation was increased in TECs treated with lactate (**Fig. 4F**) or in response to hypoxic conditions (**Fig. 4G**). DDX18 K116 lactylation in TECs induced by lactate treatment was dose-dependent (**Fig. S8A**) and time-dependent (**Fig. S8B**). In addition, serum starvation, CoCl₂, or cisplatin treatment increased DDX18 K116 lactylation in TECs (**Fig. S8C-E**). Conversely, blunting lactate metabolism with the LDH inhibitor oxamate or the pyruvate dehydrogenase activator dichloroacetate (DCA) attenuated DDX18 K116 lactylation (**Fig. S8F, G**). Increased K116 lactylation

175 was seen in primary TECs from BIRI mice (**Fig. 4H**), proteinuric versus non-proteinuric AKI kidneys
176 (**Fig. 4I**), and AKI-to-CKD mice (**Fig. 4J**). Reconstituting DDX18-deficient cells with DDX18^{WT} or
177 DDX18^{K116R} mutant showed that DDX18^{K116R} cells had lower migratory capacity (**Fig. 4K**) and less
178 fibrotic marker levels (**Fig. 4L-N**) than DDX18^{WT} cells, indicating DDX18 K116 lactylation drives
179 TEC fibrogenesis under pathological conditions.

180

181 **p300 mediates DDX18 lactylation to drive fibrotic signaling in injured TECs**

182 To identify potential lactylation regulators of DDX18, we performed immunoprecipitation coupled
183 with mass spectrometry (MS) in TECs (**Fig. S9A**). Among the identified proteins, the established
184 lactyltransferases AARS1 and p300 have emerged as promising candidates (**Fig. S9B**).
185 Coimmunoprecipitation confirmed an interaction of DDX18 and p300, but not AARS1 (**Fig. S9C, D**
186 **and Fig. 5A**), which increased following BIRI injury (**Fig. 5B**), coinciding with H/R-induced p300
187 upregulation in HK-2 cells (**Fig. 5C**). p300 overexpression increased DDX18 K116 lactylation under
188 H/R conditions without altering total DDX18 levels (**Fig. 5D**). Conversely, CRISPR/Cas9-mediated
189 p300 knockdown attenuated DDX18 K116 lactylation in injured TECs (**Fig. 5E**). P300 deficiency
190 eliminated lactylation differences between DDX18^{WT}- and DDX18^{K116R}-expressing cells (**Fig. 5F**),
191 confirming p300 as the primary enzyme mediating this modification. We then analyzed whether the
192 nucleolar localization of DDX18 depends on p300. P300 depletion impaired DDX18 translocation
193 from the nucleolus to the nucleoplasm following H/R stimulation (**Fig. 5G**), and abrogated the distinct
194 fibrotic responses of DDX18^{WT}- and DDX18^{K116R}-expressing cells (**Fig. 5H-J**). The p300 inhibitor
195 C646 similarly abolished differences in fibrotic marker expression between DDX18^{WT} and
196 DDX18^{K116R}-expressing cells (**Fig. S10**). These data suggest that metabolic stress promotes p300-

197 mediated DDX18 K116 lactylation, enabling DDX18 to promote fibrotic signaling.

198

199 **DDX18 K116 lactylation regulates *CD44* mRNA stability**

200 Given the emerging role of DDX18 in nucleolar liquid-liquid phase separation (26), we explored how
201 lactylation influences its cellular distribution. H/R treatment markedly decreased the intensity of
202 DDX18 condensates within HK-2 nucleoli (**Fig. 6A**), whereas the DDX18^{K116R} resisted H/R-induced
203 nucleolar displacement (**Fig. 6B**), indicating that K116 lactylation directly regulates DDX18 nucleolar
204 distribution.

205 RNA immunoprecipitation sequencing (RIP-seq) showed DDX18 targets enriched in RNA
206 metabolic processes (**Fig. 6C**). RNA-seq comparing DDX18^{WT} and DDX18^{K116R}-expressing cells
207 identified differential expression of genes involved in cell adhesion, adherens junctions, cell-substrate
208 junctions, and focal adhesions (**Fig. 6D**). Integrating both datasets identified 481 common targets
209 responsive to DDX18 binding and H/R treatment (**Fig. 6E**). Cell adhesion-related genes were
210 downregulated in DDX18^{K116R} cells following H/R exposure (**Fig. 6F**). Among these, CD44, a
211 multifunctional cell surface receptor implicated in cell-matrix adhesion (27), showed pronounced
212 downregulation (**Fig. 6G**). CD44 also plays a critical role in modulating fibroblast behavior through
213 regulating ECM expression (28). *CD44* expression positively correlated with fibrotic signatures (**Fig.**
214 **S11**) and *DDX18* expression (**Fig. 6H**) in kidney disease samples (GSE175759), supporting a
215 functional *DDX18-CD44* link in renal fibrosis.

216 Immunoblotting (**Fig. 6I**) and immunofluorescence (**Fig. 6J**) confirmed reduced CD44 protein in
217 DDX18^{K116R}-expressing cells under H/R. CD44 depletion eliminated the differential fibrotic response
218 between DDX18^{WT} and DDX18^{K116R}-reconstituted cells (**Fig. 6K-M**), establishing CD44 as a key

219 downstream effector in DDX18 lactylation-mediated fibrogenesis. RNA stability assays showed that
220 DDX18^{K116R} accelerated *CD44* mRNA degradation compared with DDX18^{WT} (**Fig. 6N**), indicating
221 that K116 lactylation stabilizes *CD44* transcripts. RIP analysis demonstrated enrichment of *CD44*
222 mRNA in DDX18 immunoprecipitates relative to IgG controls (**Fig. 6O**), and deletion mapping
223 confirmed the DDX18-binding region on *CD44* mRNA (**Fig. 6P**). In cells expressing a *CD44* variant
224 lacking this region, differences in *CD44* mRNA stability (**Fig. 6Q**) and protein expression (**Fig. 6R**)
225 between DDX18^{WT}- and DDX18^{K116R}-overexpressing cells were eliminated. Together, these results
226 establish that the lactylation-induced redistribution of DDX18 from the *CD44* transcript regulates
227 *CD44* mRNA stability, a critical factor driving fibrotic progression in kidney disease.

228

229 **Targeted inhibition of DDX18 K116 lactylation mitigates the progression of AKI-to-CKD in mice**

230 Because p300 inhibition or blockade of lactate metabolism affects multiple pathways, we sought a
231 selective strategy to inhibit DDX18 K116 lactylation. Synthetic peptides based on post-translational
232 modification motifs can inhibit specific protein modifications (29). Based on the sequence surrounding
233 DDX18 K116, we designed and synthesized five cell-penetrating peptides (CPPs)-conjugated
234 inhibitors (**Fig. 7A**). Among them, DDX18 K116-peptide-2# (hereafter K116-pe) exhibited the
235 strongest inhibitory effect on lactylation (**Fig. 7B**), a K116R substitution served as the specificity
236 control (K116R-pe). K116-pe attenuated the hypoxia-induced expression of fibrosis-related proteins
237 in TECs (**Fig. 7C-E**), supporting its therapeutic potential.

238 We then engineered a kidney-targeted delivery platform using L-serine-modified poly (lactic-co-
239 glycolic acid) (PLGA) to facilitate the selective renal delivery of the K116-pe in BIRI mice (**Fig. 7F**,
240 **Fig. S12**). Biodistribution studies using mCherry-labeled carriers demonstrated the preferential renal

241 accumulation and extended retention of L-serine-modified PLGA-K116-pe (PLGA-Ser-MOF-K116-
242 pe) (**Fig. 7G**), with tubule-specific localization verified by immunofluorescence and electron
243 microscopy (**Fig. S13**). The delivery system showed a favorable safety profile, with no effects on
244 survival or organ function (**Fig. S14**). BIRI mice treated with PLGA-Ser-MOF-K116-pe, but not the
245 K116R control, on day 1 post-surgery showed alleviated AKI-to-CKD transition (**Fig. 7H-J**), an effect
246 reproduced in female mice (**Fig. S15**). PLGA-Ser-MOF-K116-pe treatment markedly decreased renal
247 fibrogenic progression in BIRI mice (**Fig. 7K-M**), and reduced CD44 expression in the kidneys of
248 BIRI mice (**Fig. 7N, O**). Together, these results suggest that the selective inhibition of DDX18 K116
249 lactylation represents a promising therapeutic strategy to mitigate AKI-to-CKD progression.

Discussion

The pathological transition from AKI to CKD is a major clinical challenge, predominantly driven by maladaptive molecular crosstalk within TECs (4). Here, we identify a lactate-dependent regulatory axis in which p300-mediated lactylation of DDX18 at K116 promotes its nucleolar redistribution, enhances *CD44* mRNA stability, and activates profibrotic programs in TECs. Renal-targeted delivery of cell-penetrating lactylation inhibitors using L-serine-functionalized PLGA nanoparticles reduced DDX18 K116 lactylation and attenuated AKI-to-CKD progression in preclinical models.

The kidney plays a crucial role in gluconeogenesis, with lactate as the primary substrate under physiological conditions (30). Elevated lactate is an independent risk factor for patients with sepsis-induced AKI (31). Both renal glucose release and lactate uptake are diminished in human and experimental AKI, correlating with increased mortality (32). A previous clinical cohort study linked urinary lactate to tubular injury biomarkers and albuminuria (33), while serum lactate dehydrogenase-to-albumin ratio predicts poor AKI prognosis (34). Our biobank analysis extends these findings, showing that elevated serum lactate independently predicts CKD progression after AKI. In murine BIRI, exogenous lactate accelerated kidney dysfunction, whereas inhibiting lactate production attenuated fibrosis. TECs have high metabolic demands and are particularly susceptible to injury during AKI (10). While transient TEC migration is a physiologically essential feature of early epithelial repair (35, 36), our findings indicate that enhanced TEC migration is accompanied by increased expression of profibrotic mediators and ECM proteins. Whether hypermigration itself directly contributes to fibrotic progression or instead represents a parallel consequence of broader maladaptive epithelial reprogramming remains to be determined. Recent studies reported elevated lactate promotes fibrogenic responses in DKD mice and AGE-stimulated TECs (37). Our findings reveal a context-

272 dependent role of lactate during AKI progression. Systemic lactate in sham mice did not alter baseline
273 function, but post-ischemic lactate accumulation exacerbated fibrosis, indicating lactate acts as a
274 disease-associated signaling molecule amplifying injury rather than a primary nephrotoxin.

275 RNA helicases regulate diverse cellular processes through interactions among RNA, DNA, and
276 proteins (24, 38). Our proteomic analysis revealed extensive lactylation of nuclear RNA helicases in
277 injured TECs. Lactate has emerged as an important signaling molecule that regulates cellular
278 phenotypes through lysine lactylation (39). Wang et al. demonstrated that lactate promotes kidney
279 fibrosis through histone lactylation (H4K12la)-mediated NF- κ B activation (40). In contrast, our study
280 identifies a complementary post-transcriptional mechanism via non-histone DDX18 lactylation,
281 suggesting histone and non-histone lactylation cooperate during AKI-to-CKD transition. Protein
282 lactylation also contributes to tubular inflammation, cell death, and fibrosis (41), representing an
283 upstream link between metabolic stress and fibrogenic signaling (42). DDXs promote RNA processing
284 and are essential for rearranging RNA structures to promote RNA-protein interactions in an ATP-
285 dependent manner (43). They are crucial for genome protection, with dysfunction implicated in cancer
286 development, viral infections, and tissue differentiation (24, 44, 45). DDX24 stabilizes fanconi anemia
287 complementation group A (FANCA) mRNA to maintain cell cycle progression and DNA repair (46).
288 DDX18 interacts with RNA/DNA hybrids to maintain genome integrity (47, 48). Additionally, DDX18
289 enhances STAT1 expression, leading to increased PD-L1 expression (49). Our study demonstrates that
290 lactylation modulates the RNA-binding properties of DDX18, expanding understanding of post-
291 translational regulation of DDX function. Hypoxia-treated immortalized TECs enabled detailed
292 analysis of DDX18 lactylation and RNA interactions but did not fully reproduce the metabolic
293 complexity of ischemic kidney injury, including acidosis and the accumulation of multiple

intermediates. Future studies using kidney organoids or other physiologically relevant models are needed to refine these findings.

Progressive fibrosis, a hallmark of AKI-to-CKD transition, is characterized by excessive ECM production and dysregulated turnover (9). CD44 is a multifunctional receptor integrating extracellular signals with growth factor pathway (28, 50). Upon activation, this transmembrane receptor transduces signals via membrane-associated cytoskeletal proteins to drive transcriptional programs governing cell-matrix adhesion, migration, proliferation, differentiation, and survival (27, 28). In renal diseases, CD44 is markedly increased on injured TECs, contributing to tubular damage, apoptosis, and fibrosis (51-53), partly through HGF and TGF- β signaling pathways (52, 54). Our data establish CD44 as a downstream effector of lactylated DDX18. Mechanistically, lactylated DDX18 directly binds to and stabilizes *CD44* mRNA, boosting its expression and driving profibrotic responses in TECs. Although RIP-seq confirmed this interaction, the precise binding motifs remain unknown, and this mechanism links altered lactate metabolism to RNA regulation and ECM remodeling.

Protein lactylation is catalyzed by lactyltransferases including p300, GCN5, TIP60, and KAT8 (21), as well as alanyl-tRNA synthetases AARS1 and AARS2 (55-57). We demonstrated that p300 directly lactylates DDX18 and that lactate facilitates this modification by strengthening the p300-DDX18 interaction. As acetyltransferases, p300 has been reported to acetylate both free and nucleosomal histones (58). P300-mediated acetylation of copper metabolism murr1 domain 1 (COMMD1) triggers RelA ubiquitylation and nucleolar translocation (59). Similarly, acetylation of the WRN helicase by p300 promotes its translocation from the nucleolus to nucleoplasmic foci (60). Our findings extend this paradigm by demonstrating that p300-mediated lactylation dictates the nucleolar-nucleoplasmic shuttling of DDX18, revealing a mechanism regulating RNA helicase localization and

316 function. Pharmacological inhibition of p300 reduced DDX18 lactylation and attenuated hypoxia-
317 induced profibrotic responses in TECs. CPPs targeting DDX18 K116 lactylation inhibited TEC
318 fibrogenic activation. To improve renal delivery, we engineered L-serine-functionalized PLGA-MOF
319 nanoparticles. Nanoparticle biodistribution is influenced by physicochemical properties, including
320 particle size (61-63). The PLGA-Ser-MOF base framework measured approximately 65 nm, while the
321 mCherry-conjugated construct approximately 70 nm, both within the mesangial-targeting range. These
322 properties support preferential renal accumulation and TEC uptake, thereby attenuating renal
323 fibrosis *in vivo*. Although AAV9 exhibits broad tissue tropism, Cas9 expression in our study was
324 restricted to renal tubular epithelial cells by the Ksp-cadherin promoter. Therefore, the kidney-specific
325 reduction of DDX18 reflects promoter-driven control of Cas9 activity rather than differences in editing
326 efficiency between kidney and liver tissues.

327 In summary, DDX18 K116 lactylation acts as a pivotal regulator of AKI-to-CKD progression.
328 Lactate accumulation and p300-dependent lactylation trigger DDX18 nucleolar redistribution, enhance
329 *CD44* mRNA stability, and drive ECM remodeling and fibrosis. Peptide-mediated targeting of DDX18
330 lactylation significantly reduces renal fibrosis in preclinical models. These findings identify
331 lactylation-mediated regulation of RNA-helicase function as a potential link between metabolic stress
332 and fibrotic responses and warrant investigation in other fibrotic diseases.

333 **Methods**

334 **Sex as a biological variable**

335 Our study examined male and female animals, and similar findings are reported for both sexes.

336 **Prospective cohort study in the UK Biobank**

337 We analyzed UK Biobank data from approximately 500,000 participants aged 40–69 years
338 recruited across the United Kingdom between 2006 and 2010, with linked hospital, mortality, and
339 baseline omics records. AKI and CKD were identified using ICD-10 codes N17 and N18, respectively.

340 Participants with pre-recruitment AKI were followed for CKD progression, defined as the first post-
341 recruitment N18 diagnosis. Those with CKD before or at recruitment were excluded, and participants
342 without subsequent CKD formed the non-progression group. Baseline EDTA plasma underwent
343 proteomic and metabolomic profiling. Proteomics used the Olink Explore 3,072 platform, covering
344 cardiometabolic, inflammation, neurology, and oncology panels. Randomized samples were analyzed
345 by proximity extension assay, sequenced on NovaSeq 6,000 systems, and processed into quality-
346 controlled, rank-based inverse-normal-transformed NPX values (64). Metabolomics was performed in
347 118,019 baseline samples using Nightingale Health’s NMR platform and a 500 MHz Bruker AVANCE
348 IIIHD spectrometer, quantifying 249 biomarkers. Quality control included duplicates, coefficients of
349 variation <5% for most biomarkers, and cross-batch consistency checks. Participants were stratified
350 by median lactate into low (≤ 4.135 mmol/L) and high (> 4.135 mmol/L) groups, and baseline NMR-
351 measured creatinine was compared. Cox models estimated adjusted hazard ratios for CKD progression,
352 controlling for age, sex, body mass index, smoking, and alcohol intake. Kaplan-Meier curves were
353 compared by log-rank test. All participants provided informed consent. Ethical approval was granted
354 by the North West Research Ethics Committee (06/MRE08/65), and data were accessed under UKB

355 application 90369.

356 **Patient samples**

357 Human kidney biopsy specimens were obtained from Nanfang Hospital, Southern Medical
358 University. Tissues were formaldehyde-fixed and paraffin-embedded using standard procedures. The
359 study was approved by the Institutional Ethics Committee of Nanfang Hospital and conducted in
360 accordance with the Declaration of Helsinki (NFEC-2025-057). Written informed consent was
361 obtained from all participants.

362 **Animals**

363 Eight-week-old C57BL/6J mice (20–24 g) were obtained from the Experimental Animal Center
364 of Southern Medical University. TEC-specific *DDX18* knockout mice were generated at 6 weeks by
365 tail-vein injection of an AAV9-CRISPR/Cas9 system driven by the TEC-specific Ksp promoter. Exons
366 2-4 of mouse *DDX18* (NM_025860.3) were excised using sgRNA-A1
367 (GGAACAGCCACGGCCAAGTG, targeting upstream of exon 2) and sgRNA-A2
368 (AGTCTTCAGTATAGCCAAAC, targeting downstream of exon 4), inducing a frameshift mutation.
369 Due to AAV packaging constraints, two vectors were used: AAV-U6>sgRNA-A1–U6>sgRNA-A2–
370 CMV>EGFP and AAV-Ksp>SaCas9, with Ksp promoter restricting SaCas9 expression to TECs.
371 Recombinant AAV9 particles were produced in HEK293T cells by triple transfection, purified by
372 iodixanol-gradient ultracentrifugation, and quantified by qPCR. Each vector was administered at $2 \times$
373 10^{11} viral genomes per mouse. Renal *DDX18* deletion was confirmed by immunoblotting and
374 immunofluorescence. All procedures were approved by the Laboratory Animal Care and Ethical
375 Committee of Southern Medical University (D2022054).

376 **Bilateral ischemia–reperfusion injury model**

377 For the bilateral ischemia–reperfusion injury AKI-to-CKD model, renal pedicles were clamped
378 for 30 min under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia. Body temperature was
379 maintained at 37°C, and incisions were closed after reperfusion. Sham-operated mice underwent
380 identical procedures without clamping. For pharmacological treatment, the LDH inhibitor (R)-GNE-
381 140 (Merck, HY-100742A) was administered orally at 100 mg/kg every 48 h. Sodium lactate, prepared
382 at 200 mg/mL in PBS, was slowly injected intraperitoneally at 2 g/kg every 48 h for 14 days.

383 **Lactate and pH measurement**

384 Tissue and serum lactate were measured using a Lactate Assay Kit (Sigma-Aldrich, MAK064).
385 Kidney tissue (50 mg) was homogenized in 200 µL assay buffer and centrifuged at $13,000 \times g$ for 10
386 min at 4°C, with values normalized to tissue weight. Human plasma lactate was obtained from UK
387 Biobank NMR data. Blood pH was measured at 37°C using a Radiometer capillary electrode and PHM
388 73 analyzer.

389 **Serum biochemistry**

390 Mouse blood was centrifuged at $1,000 \times g$ for 20 min at 4°C to isolate serum. Serum creatinine
391 and blood urea nitrogen were measured using an automated biochemical analyzer (Mindray BS-240)
392 according to the manufacturer's instructions.

393 **Histological analysis and injury scoring**

394 Kidneys were fixed in 4% paraformaldehyde, paraffin-embedded, sectioned at 4–5 µm, and
395 stained with Sirius Red (Merck, 365548) to assess tissue architecture and fibrosis. Tubular injury in
396 the corticomedullary region was scored as 0 (none), 1 (<25%), 2 (25–50%), 3 (50–75%), or 4 (>75%).
397 At least nine fields per sample were independently evaluated by two investigators blinded to group
398 allocation.

399 **Cell culture and hypoxia–reoxygenation model**

400 HK-2 cells (ATCC CRL-2190) and HEK293T cells (ATCC CRL-3216) were obtained from the
401 American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in DMEM (Thermo
402 Fisher Scientific, 11965092) supplemented with 10% fetal bovine serum (A5256701), 100 U/mL
403 penicillin, and 100 mg/mL streptomycin (Merck, P4333) at 37°C in 5% CO₂. For hypoxia–
404 reoxygenation experiments, cells were incubated in glucose- and serum-free DMEM under 1% O₂ for
405 24 h, followed by reoxygenation under normoxia.

406 **Isolation of mouse renal tubular cells**

407 Kidneys were excised, minced, and digested with collagenase IV and dispase (Roche) at 37°C for
408 30–45 min. The tissue was mechanically dissociated, filtered through a fine mesh, and centrifuged to
409 remove debris. Isolated renal tubular cells were cultured in serum-supplemented DMEM/F-12.

410 **Cell migration assay**

411 HK-2 cells were seeded in 6-well plates at 1×10^5 cells/well and grown to confluence. A scratch
412 was made with a sterile 200-μL pipette tip, followed by PBS washing and serum-free DMEM
413 replacement. Wound closure at 0 and 48 h was imaged by phase-contrast microscopy and quantified
414 using ImageJ.

415 **4D label-free quantitative lactylproteomic analysis**

416 HK-2 cells were sonicated in 8 M urea buffer with protease inhibitors, centrifuged at $12,000 \times g$
417 for 10 min at 4°C, and quantified by BCA assay. Proteins were reduced with 5 mM dithiothreitol at
418 56°C for 30 min, alkylated with 11 mM iodoacetamide for 15 min in darkness, diluted with 100 mM
419 TEAB to <2 M urea, and digested with trypsin at 1:50 overnight and 1:100 for 4 h. Lactylated peptides
420 were enriched overnight at 4°C in NETN buffer using anti-L-lactyl-lysine antibody beads (PTM Bio,

PTM-1404), eluted with 0.1% trifluoroacetic acid, and desalted using C18 ZipTips. Peptides were analyzed on a nanoElute UHPLC–timsTOF Pro system using a 60-min, 6–80% acetonitrile gradient at 450 nL/min. PASEF settings included a 1.70-kV electrospray voltage, 100–1,700 m/z range, 10 MS/MS scans per cycle, and 24-s dynamic exclusion. Functional enrichment and network analyses used Metascape, Cytoscape, and MCODE. Subcellular localization was assigned using WoLF PSORT. Analyses were performed by Jingjie PTM Biolabs (Hangzhou, China).

RNA immunoprecipitation sequencing analysis

RNA immunoprecipitation was performed using the BersinBio kit (Bes5101). DDX18-deficient HK-2 cells expressing DDX18^{WT} or DDX18^{K116R} were subjected to hypoxia–reoxygenation. Cell lysates were incubated overnight at 4°C with an anti-DDX18 antibody or control IgG. Protein–RNA complexes were captured with A/G magnetic beads for 1 h at 4°C, washed six times, and RNA was extracted using TRIzol (Thermo Fisher Scientific, 15596026CN). RNA quality was assessed using an Agilent 2100 Bioanalyzer and SimpleNano spectrophotometer. Reads were quality-checked with FastQC, trimmed using Trimmomatic, aligned to GRCh38/hg38 with STAR, and quantified using featureCounts with Ensembl annotations. Differential enrichment between immunoprecipitated and matched input or IgG samples was analyzed using DESeq2. Transcripts with adjusted $P < 0.05$ and $|\log_2 \text{fold change}| \geq 1$ were considered significant interactors. GO and KEGG enrichment analyses were performed for functional annotation.

RNA sequencing analysis

Total RNA was extracted with TRIzol from hypoxia–reoxygenation-treated DDX18-deficient HK-2 cells expressing DDX18^{WT} or DDX18^{K116R}. RNA integrity was assessed using an Agilent 2100 Bioanalyzer. Poly(A)⁺ mRNA was isolated, fragmented, and reverse-transcribed. Double-stranded

443 cDNA underwent end repair, A-tailing, adapter ligation, and selection of approximately 200-bp
444 fragments using AMPure XP beads (Beckman, A63881). Libraries were amplified, quality-controlled,
445 and sequenced on an Illumina HiSeq platform by Novogene. Reads were assessed with FastQC,
446 trimmed using Trimmomatic, aligned to GRCh38/hg38 with STAR, and quantified using
447 featureCounts with Ensembl annotations. DESeq2 identified differentially expressed genes at adjusted
448 $P < 0.05$ and $|\log_2 \text{fold change}| \geq 1$. Principal component analysis and hierarchical clustering were used
449 to assess reproducibility, followed by GO and KEGG analyses.

450 **Quantitative RT-PCR analysis**

451 Total RNA was extracted from kidney tissues or cells using TRIzol. RNA (1 μg) was reverse-
452 transcribed using TranScript All-in-One SuperMix (TransGene Biotech, AE341-02), and qPCR was
453 performed with TransScript Green SuperMix (AQ211-01) on a 7900HT system. Target-gene
454 expression was normalized to β -actin using the $2^{-\Delta\Delta C_t}$ method.

455 **RNA stability analysis**

456 Cells were treated with actinomycin D (Merck, A4262), and RNA was collected at 0, 2, 4, 6, and
457 8 h. *CD44* mRNA levels were measured by RT-qPCR and expressed relative to baseline. mRNA half-
458 life was calculated by one-phase decay regression.

459 **Immunoblot analysis**

460 Tissues and cells were lysed in strong RIPA buffer (Beyotime, P0013B) containing protease
461 inhibitors (Merck, P9599). After sonication and centrifugation, protein concentrations were
462 determined by BCA assay. Equal protein amounts were separated by 10% SDS-PAGE and transferred
463 to PVDF membranes. Membranes were blocked with 1% BSA, incubated with primary antibodies
464 including anti-DDX18 antibody (Proteintech, 28502-1), fibronectin polyclonal antibody (Proteintech,

465 15613-1-AP), collagen type 1 monoclonal antibody (Proteintech, 66761-1-Ig, clone 3D5E8), α -SMA
466 polyclonal antibody (Proteintech, 14395-1-AP), β -actin monoclonal antibody (Proteintech, 66009-1-
467 Ig, clone 2D4H5), CD44 Polyclonal antibody (Proteintech, 15675-1-AP), and anti-L-lactyl lysine
468 rabbit mAb (PTM Bio, PTM-1401) overnight at 4°C and HRP-conjugated secondary antibodies for 1
469 h at room temperature, and developed by enhanced chemiluminescence.

470 **Immunoprecipitation and LC–MS/MS analysis**

471 Cells were lysed for 20 min at 4°C in weak RIPA buffer (Beyotime, P0013D) containing protease
472 inhibitors and clarified by centrifugation. Total protein (0.1 mg) was incubated with the indicated
473 antibody and 100 μ L protein A/G magnetic beads (MCE, HY-K0202) for 4 h at 4°C. Beads were
474 washed three times, and bound proteins were collected. LC–MS/MS was performed on a Bruker
475 timsTOF Pro 2 mass spectrometer with a CaptiveSpray source. Data were acquired in DIA mode over
476 100–1,700 m/z using a 1.4-kV spray voltage, 180°C drying gas, 100-ms ramp time, and 100% duty
477 cycle. Raw data were analyzed with DIA-NN v1.8.1 against the UniProt human proteome database.

478 **Immunohistochemistry and immunofluorescence**

479 For immunohistochemistry, kidneys were fixed in 4% paraformaldehyde, paraffin-embedded, and
480 sectioned at 3 μ m. After deparaffinization, rehydration, hydrogen peroxide treatment, and citrate-
481 buffer antigen retrieval (Beyotime, P0081), sections were incubated with primary antibodies overnight
482 at 4°C, followed by HRP-conjugated secondary antibodies and Dako REAL EnVision detection. For
483 tissue immunofluorescence, antigen-retrieved sections were permeabilized with 0.2% Triton X-100 for
484 10 min, blocked with 5% BSA for 1 h, and incubated with primary antibodies overnight at 4°C.
485 Fluorophore-conjugated secondary antibodies were applied for 1 h, followed by DAPI staining (Merck,
486 D9542). For cellular immunofluorescence, chamber-slide cultures were fixed with 4%

487 paraformaldehyde for 15 min, permeabilized with 0.3% Triton X-100 for 3 min, blocked, and
488 incubated with primary antibodies overnight at 4°C and secondary antibodies (Proteintech, SA00003-
489 2 and SA00013-3) for 1 h. Images were acquired using a Nikon AX-SHR confocal microscope under
490 identical settings. DDX18 and nuclear structures were further examined by HIS-SIM microscopy with
491 a 100×/1.5 NA oil objective.

492 **Preparation of the DDX18-K116 lactylation-specific antibody**

493 The DDX18-K116 lactylation-specific antibody was generated in-house using New Zealand
494 rabbits immunized with the synthetic lactylated peptide KKRK(Lac)MVNDAEPDTK. Rabbits were
495 immunized six times according to the immunization schedule, and antiserum titers were monitored by
496 indirect ELISA after the third to sixth immunizations. Serum was collected when antibody titers
497 exceeded 1:32,000, followed by antigen-specific affinity purification. Purified antibodies were
498 required to reach titers >1:64,000 and demonstrated selective recognition of the lactylated peptide by
499 dot-blot analysis.

500 **Generation of DDX18- and p300-knockout cells**

501 HK-2 cells were transfected with 2 µg of DDX18 or p300 CRISPR/Cas9 knockout plasmids
502 (Santa Cruz Biotechnology, sc-406057 and sc-400055) using UltraCruz reagent (sc-395739). Protein
503 expression was assessed by immunoblotting 24 h after transfection.

504 **Generation of DDX18^{WT}- and DDX18^{K116R}-expressing cells**

505 DDX18-knockout HK-2 cells were generated using the DDX18 CRISPR/Cas9 plasmid and
506 validated by immunoblotting. Verified cells were reconstituted with DDX18^{WT} or DDX18^{K116R}
507 plasmids using Lipofectamine 2000 (Invitrogen, 11668019), and exogenous expression was confirmed
508 before subsequent experiments.

509 **Peptide synthesis**

510 Peptides were synthesized by solid-phase methods, purified to >98% by reverse-phase HPLC,
511 and verified by mass spectrometry. D-amino acid variants were used for in vivo studies. Lyophilized
512 peptides were dissolved in sterile PBS at 10 mM, filter-sterilized, aliquoted, stored at -80°C , and
513 rechecked by analytical HPLC before use. The inhibitory peptide sequences were: Peptide-1#:
514 HLYVSPWGGKKKKRKMANTAGPD, Peptide-2#: HLYVSPWGGKKKKKKKKRKMANT, Peptide-
515 3#: HLYVSPWGGKKKKKKRKMANTAG, K116-Peptide-4#: HLYVSPWGGGEVKKKKKKKKRKM
516 Peptide-5#: HLYVSPWGGKKRKMANTAGPDTK.

517 **Synthesis and biodistribution of PLGA-Ser-MOF-K116-pe**

518 PLGA-Ser-MOF-K116-pe nanocomposites were synthesized in three steps. PLGA and serine
519 separately dissolved in DMF, were conjugated after serine activation with equimolar EDC·HCl/DMAP
520 for 30 min. After 8 h, PLGA-Ser was water-precipitated, washed five times, and lyophilized.
521 Zirconium-based MOFs were sonicated in anhydrous DMF and activated with 10 mM EDC·HCl and
522 5 mM NHS for 30 min, then reacted overnight under nitrogen with K116-targeting peptide at a 1:10
523 MOF-to-peptide molar ratio. The resulting peptide-loaded MOFs (MOFPs) were washed and
524 lyophilized. PLGA-Ser and MOFPs (50 mg each) were probe-sonicated in 10 mL DMF and stirred for
525 24 h for electrostatic assembly, then sequentially washed with DMF, ethanol, and water before
526 lyophilization. For biodistribution analysis, mice received intravenous mCherry-labeled PLGA-Ser-
527 MOF-K116-pe (10 mg/kg) and were euthanized 12 h later. Organ fluorescence was quantified using
528 an In-Vivo FX Pro system. Renal targeting was further evaluated by ICP-MS measurement of
529 zirconium, tubular immunofluorescence, and electron microscopy.

530 **Statistical analysis**

531 Statistical analyses were performed using GraphPad Prism 9.0. Data are presented as mean $\pm$
532 SEM. Two-group comparisons used unpaired two-tailed Student's t tests, whereas multiple-group
533 comparisons used two-way ANOVA. Pearson's correlation assessed associations between variables.
534 $P < 0.05$ was considered statistically significant.
535

536 **Data availability**

537 Raw RNA-seq and RIP-seq sequencing data have been deposited in the NCBI Gene Expression
538 Omnibus (GEO) and are publicly available under accession numbers GSE333635 for RNA-seq and
539 GSE333824 for RIP-seq. The raw data from the 4D label-free quantitative lactylproteomic analysis
540 and the immunoprecipitation–LC–MS/MS analysis have been deposited in the ProteomeXchange
541 Consortium under accession numbers PXD078736 and PXD079002, respectively. The Supporting
542 Data Values file is provided as a supplement to this manuscript. All other data supporting the finding
543 of this study are available upon request from the corresponding authors.

544

545 **Study approval**

546 All animal procedures were approved by the Laboratory Animal Care and Ethical Committee of
547 Southern Medical University and complied with all relevant ethical regulations (NO. D2022054).

548

549 **Author contributions**

550 LD, JX, and MT contributed equally to this work. DZ, TT, JA, HH, and LD conceptualized and planned
551 all experiments, methodology, and analysis. Formal analysis of all data was performed by LD, MT,
552 and JX. Funding acquisition was done by DZ and LD. Original drafting of the manuscript was done
553 by DZ and LD, with review and editing done by DZ, LD, JX, MT, XZ, and JZ (Jianbo Zhao).
554 Sequencing experiments were performed by SL, YL, TW, and JZ (Jia Zhou). In vivo experiments were
555 done by LD, JX, MT, and SL. In vitro experiments were performed by LD, JX, MT, YL, and QC
556 Human samples were obtained from JG and JA. Project management and oversight were performed
557 by DZ.

558 **Conflict of interest**

559 The authors have declared that no conflict of interest exists.

560

561 **Funding support**

562 National Natural Science Foundation of China (Nos. 82471852 and 82271782, DZ).

563 National Natural Science Foundation of China (Nos. 82370744, JA)

564 National Natural Science Foundation of China (Nos. 82402129, LD).

565 Science and Technology Planning Project of Guangdong Province (NO. 2024A0505040013, DZ),

566 Provincial Key Laboratory of Immune Regulation and Immunotherapy (NO. 2022B1212010009, DZ).

567

568 **Acknowledgments**

569 We thank Nanfang Hospital of Southern Medical University for providing the tissue used in this study.

570 We thank CSR Biotech (Guangzhou) Co., Ltd. for imaging by using their commercial super-resolution
571 microscope (Cell Xpanse), data acquisition, SR image reconstruction.

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Figure legends

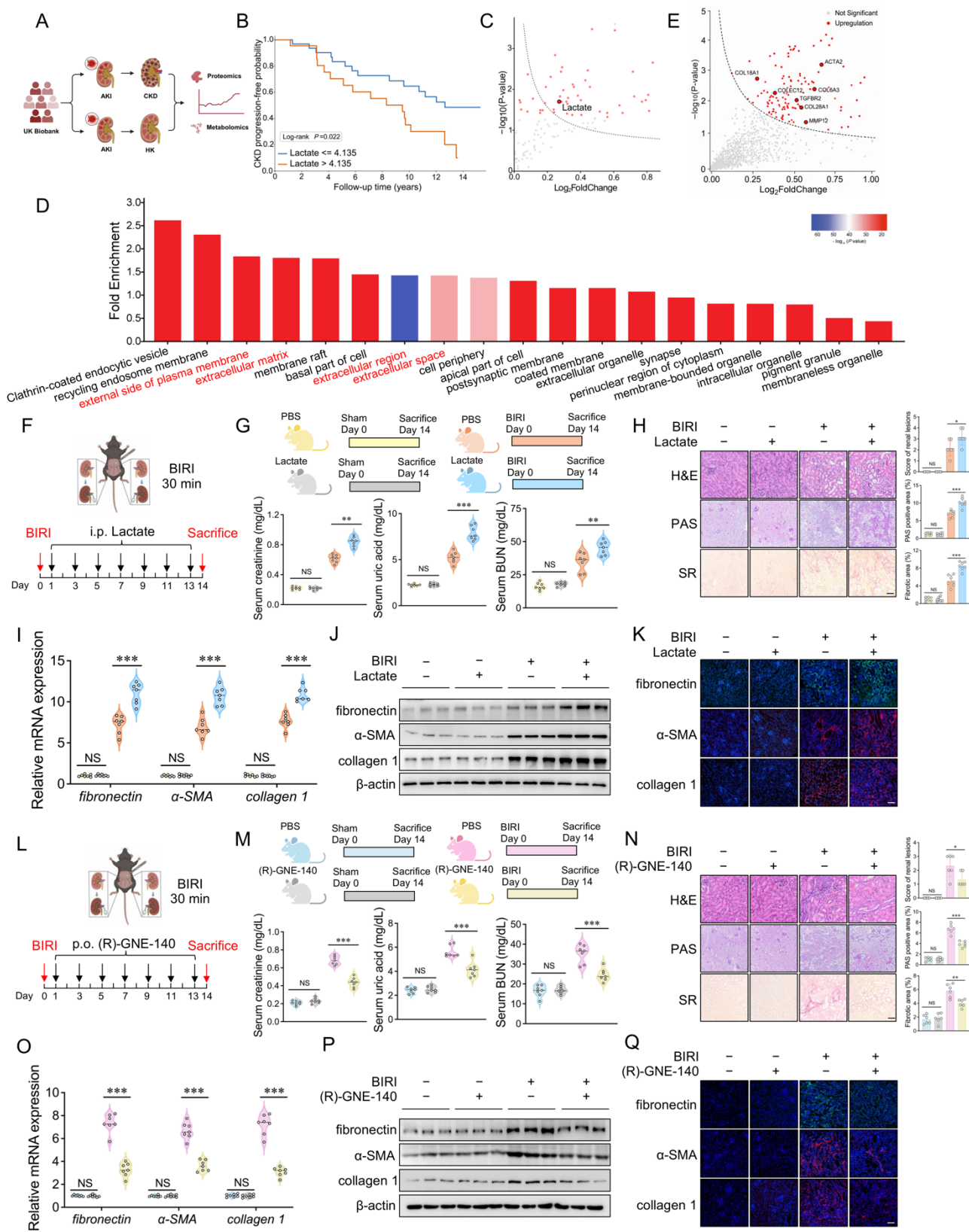


Figure 1. Elevated lactate levels accelerate the progression from AKI to CKD

A-E, UK Biobank cohort analysis. **(A)** Study workflow for a nested case-control analysis within the UK Biobank. Cases comprised baseline AKI patients who progressed to CKD, whereas non-progressing AKI patients served as controls. Baseline proteomic and metabolomic profiles were analyzed. **(B)** Kaplan-Meier curve of cumulative CKD incidence among baseline AKI patients stratified by high (>4.135 mmol/L) and low (≤ 4.135 mmol/L) circulating lactate levels. **(C)** Upregulated metabolites in AKI patients progressing to CKD, with a marked increase in lactate. **(D)** Gene Ontology (GO) enrichment of upregulated proteins in patients who progressed to CKD, highlighting ECM remodeling pathways. **(E)** Volcano plot illustrating proteomic alterations between AKI patients with and without CKD progression. **F-K**, Lactate exacerbates renal fibrosis in a murine BIRI model. **(F)** Experimental design for lactate administration (2 g/kg, i.p.) in BIRI mice. **(G)** Serum creatinine, uric acid, and BUN were assessed on day 14 postinjury (n=7 per group). **(H)** Representative H&E, PAS, Sirius Red and quantitative scoring showing enhanced tubular injury and collagen deposition (scale bar=100 μ m). Fibrotic markers (fibronectin, α -SMA, and collagen 1) analyzed by **(I)** qPCR, **(J)** western blotting, and **(K)** immunofluorescence. Scale bar=100 μ m. **L-Q**, LDHA inhibition attenuates AKI-to-CKD progression. **(L)** Timeline for (R)-GNE-140 (100 mg/kg) treatment. **(M)** Improved renal function parameters in the inhibitor-treated group (n=7 per group). **(N)** Representative histopathology (H&E, PAS, Sirius Red) and quantitative scoring showing reduced tubular injury and collagen deposition. Histopathological amelioration of fibrosis features. scale bar=100 μ m. Decreased mRNA **(O)**, protein **(P)**, and histological levels **(Q)** of fibrotic markers following LDHA inhibition. Representative data from one of three independent experiments are shown. Data in **G-I** and **M-O** were analyzed by two-way ANOVA. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS: not significant.

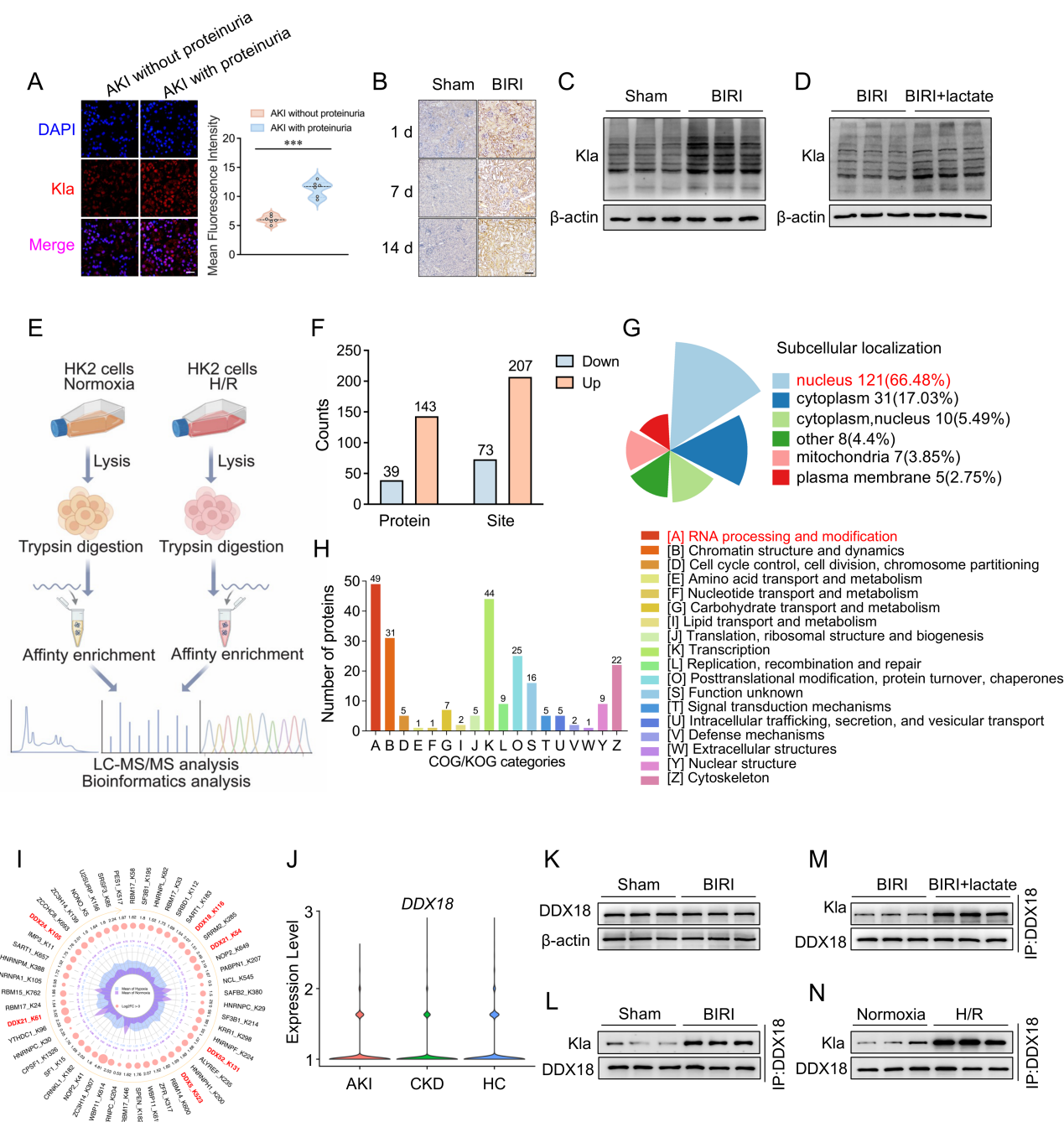
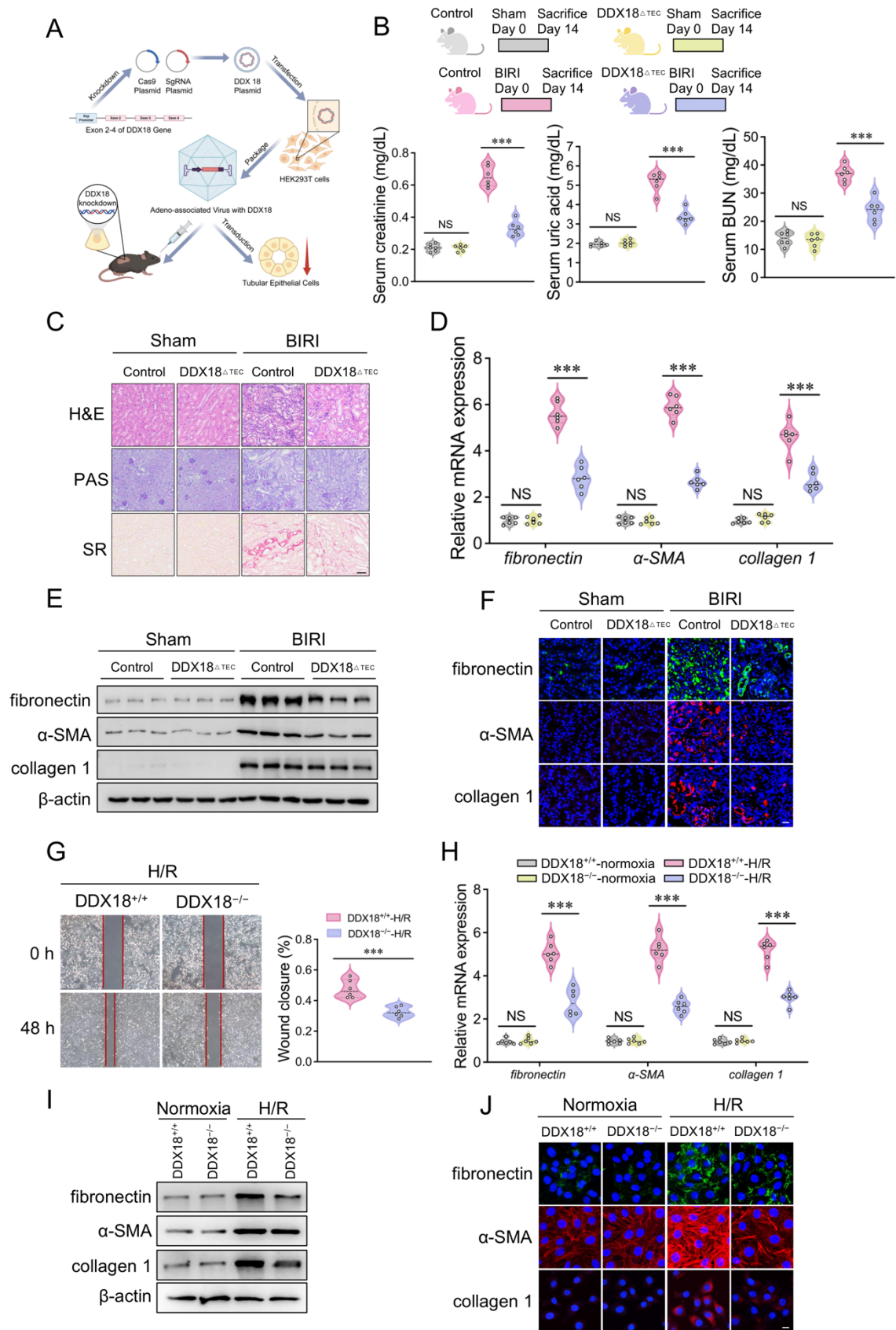


Figure 2. Protein lactylation dynamics in kidney injury and TECs under hypoxic conditions

A, Renal immunofluorescence showing increased global lysine lactylation (pan-Kla) in AKI patients with versus without proteinuria (scale bar=100 μ m). **B–D**, Temporal changes in murine BIRI. **(B)** Pan-Kla immunohistochemistry showing progressive lactylation in renal TECs after injury. **(C)** Immunoblotting confirming sustained renal lactylation on day 14. **(D)** Lactate supplementation (2 g/kg,

751 intraperitoneally) further increased renal lactylation. **E–I**, Lactylomic profiling of HK-2 cells under
752 H/R. **(E)** Experimental workflow (n=3). **(F)** Quantitative analysis identified 280 lactylation sites across
753 182 differentially lactylated proteins. **(G)** Subcellular distribution of these proteins. **(H)** COG/KOG
754 analysis showing enrichment in RNA-processing pathways. **(I)** Radar plot of proteins enriched in RNA
755 processing and modification; blue and purple values indicate relative abundance under normoxia and
756 H/R, black values indicate log₂ fold change. **J, K**, DDX18 expression remained unchanged during
757 renal injury. **(J)** Single-nucleus RNA-sequencing data (GSE183277) showing comparable *DDX18*
758 expression in AKI, CKD, and healthy controls. **(K)** Renal DDX18 protein levels in BIRI mice. **L–N**,
759 BIRI increases DDX18 lactylation. **(L)** Renal DDX18-K1a was elevated after BIRI. **(M)** Lactate
760 supplementation further enhanced DDX18 lactylation. **(N)** H/R induced DDX18 lactylation in primary
761 tubular epithelial cells without altering total DDX18 protein. Representative data from one of three
762 independent experiments are shown. Data in A were analyzed using an unpaired two-tailed Student's
763 t-test. ***P<0.001.



766 **Figure 3. TEC-specific ablation of *DDX18* mitigates AKI-to-CKD progression in BIRI mice**

767 **A**, Schematic of TEC-specific *DDX18* knockout using a Ksp promoter-driven AAV9 vector. **B-F**,

768 Control and TEC-specific *DDX18* knockout mice underwent BIRI, and kidneys were collected on day

769 14. **(B)** Improved renal function, indicated by reduced serum creatinine, uric acid, and BUN. **(C)**

770 Representative H&E, PAS, and Sirius red staining. Scale bar = 100 μ m. Downregulation of fibrotic

771 markers at **(D)** mRNA, **(E)** protein, and **(F)** histological expression levels in TEC-specific *DDX18*

772 knockout BIRI mice. Scale bar=100 μ m. **G-J**, *In vitro* validation using *DDX18*-deficient TECs under

773 H/R conditions. **(G)** Scratch wound assay showing impaired migration of *DDX18*^{-/-} versus *DDX18*^{+/+}

774 HK-2 cells under H/R, with wound closure (%) quantified at 48 hours post-scratch. Decreased fibrotic

775 marker expression by **(H)** qRT-PCR, **(I)** western blotting, and **(J)** immunofluorescence. Scale bar=20

776 μ m. Representative data from one of three independent experiments are shown. Two-way ANOVA was

777 used for data **B**, **D**, and **H**. Unpaired two-tailed Student's t-test for **G**. ***p<0.001, NS: not significant.

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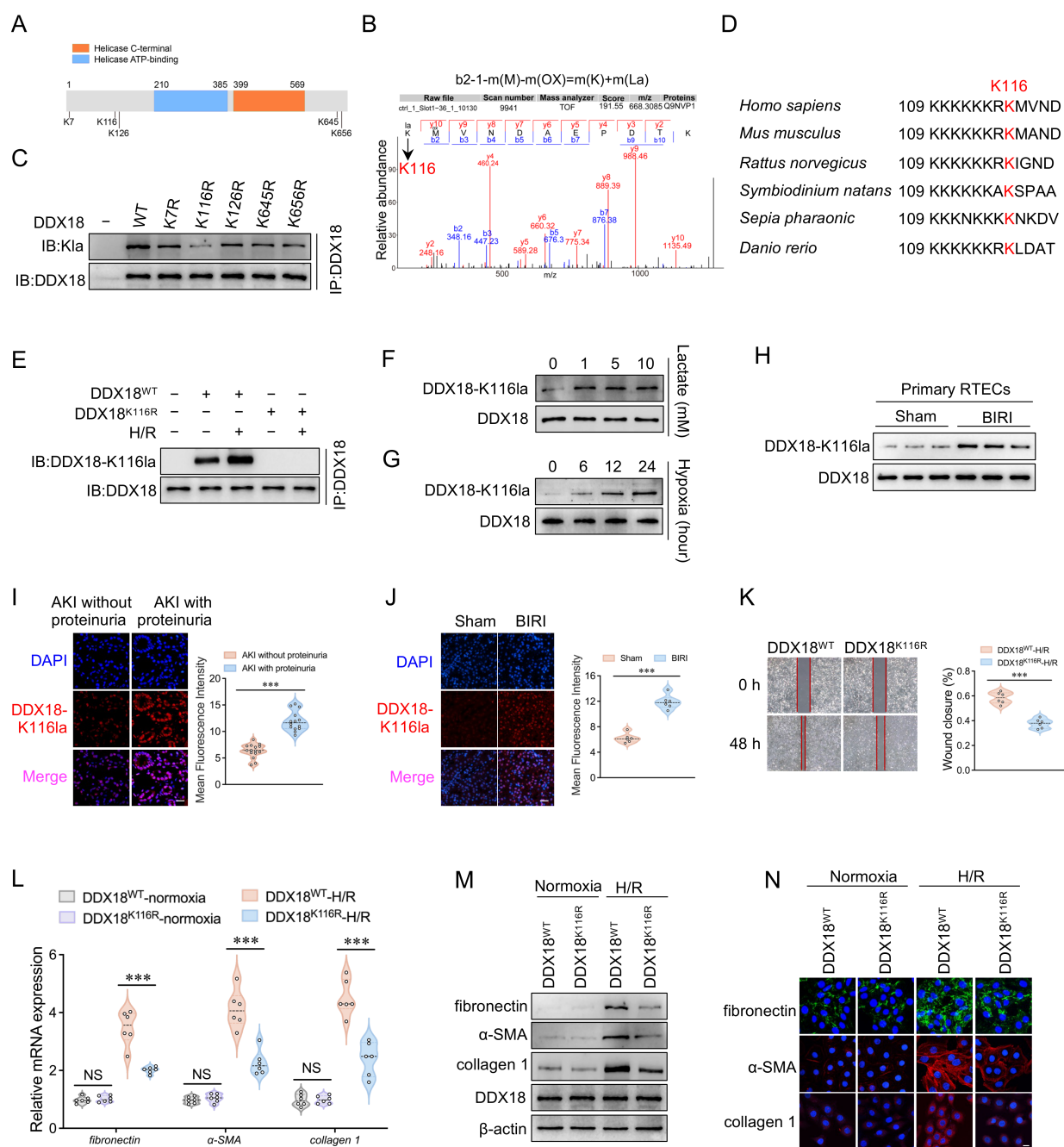
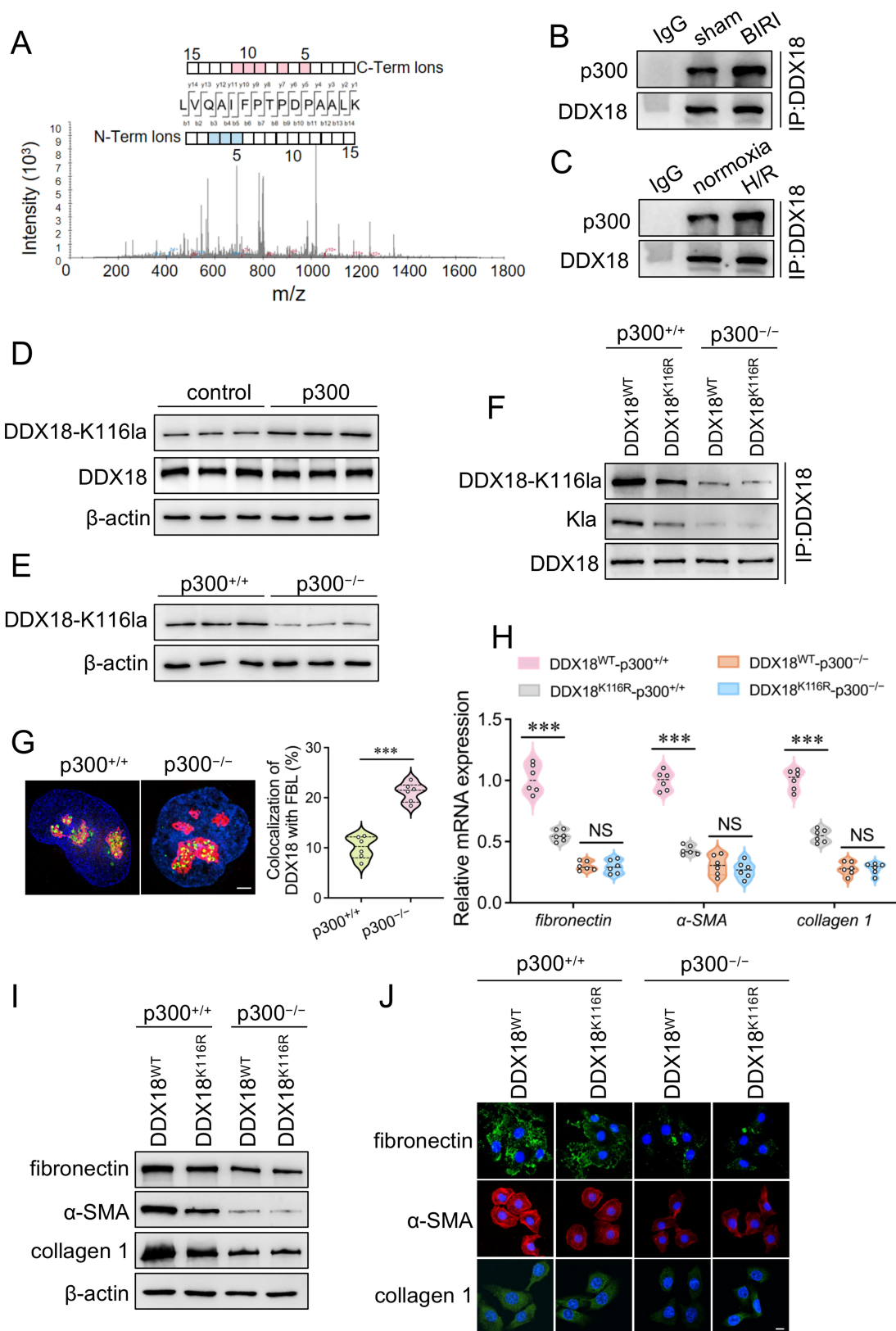


Figure 4. DDX18-K116 lactylation drives the AKI-to-CKD transition in BIRI mice

A, Domain architecture of DDX18 highlighting putative lactylation sites. **B**, MS analysis confirming lactylation at K116. The x-axis represents the mass-to-charge ratio (m/z), corresponding to the molecular mass divided by the charge state of the peptide. The y-axis represents the intensity of the fragment ion peaks. Red peaks correspond to b-ions and blue peaks to y-ions. For sequencing, b-ions

are counted from the N-terminus, while y-ions are counted from the C-terminus. The calculation formula is $b2-1-m(M)-m(OX)=m(K)+m(La)$. **C**, DDX18 lactylation in HK-2 cells expressing DDX18^{WT} or multiple lysine-to-arginine (K→R) mutants of DDX18. **D**, Cross-species alignment showing conserved K116 (red) across seven species. **E**, DDX18-K116 Lactylation in HK-2 cells expressing DDX18^{WT} or the DDX18^{K116R} mutant under H/R stress. **F**, DDX18-K116 lactylation in HK-2 cells treated with lactate. **G**, Hypoxia treatment induced lactylation of DDX18-K116. **H**, DDX18 K116 lactylation was significantly increased in primary renal tubular epithelial cells from BIRI mice. **I-J**, Spatial distribution of DDX18-K116 lactylation *in vivo*. **(I)** Increased DDX18-K116 lactylation in renal tissues from AKI patients with proteinuria versus without proteinuria. Scale bar=100 μm. **(J)** Immunofluorescence staining of DDX18-K116 lactylation in kidney sections from BIRI mice after 24 hours reperfusion. Scale bar=100 μm. **K-N**, *In vitro* validation using HK-2 cells expressing DDX18^{K116R} under H/R stress. **(K)** Wound healing assay showing the migration capacity of DDX18^{WT}- or the DDX18^{K116R}- expressing HK-2 cells, with wound closure (%) quantified at 0 and 48 hours. Fibrotic marker expression **(L)** was determined by RT-qPCR analysis. The levels of fibrosis-related proteins were determined by **(M)** western blotting and **(N)** immunofluorescence. Scale bar=20 μm. Representative data from one of three independent experiments are shown. Unpaired two-tailed Student's t-test was used for data **I**, **J**, and **K** statistical analysis. Two-way ANOVA was used for data **L** statistical analysis. ***p<0.001, NS: not significant.



803

804

Figure 5. p300 mediates hypoxia-induced DDX18 lactylation, which drives fibrotic responses

A-C, p300 interacts with DDX18 under hypoxia. **(A)** Proteomic interactome analysis of DDX18 immunoprecipitates from HK-2 cells with mass spectrometry confirmed p300 association. **(B)** Coimmunoprecipitation validating the endogenous DDX18-p300 interaction in kidney tissues from sham and BIRI mice. **(C)** Coimmunoprecipitation confirming the DDX18-p300 interaction in HK-2 cells under H/R conditions. **D-F**, Regulation of DDX18 lactylation by p300. **(D)** Increased K116-specific lactylation of DDX18 following p300 overexpression in H/R-treated cells. **(E)** Reduced K116-specific DDX18 lactylation in CRISPR/Cas9-mediated p300 knockout cells under H/R. **(F)** Immunoblot analysis of DDX18 lactylation in p300^{+/+} or p300^{-/-} HK-2 cells expressing DDX18^{WT} or DDX18^{K116R} under H/R conditions. **G**, Immunofluorescence showing the subcellular localization of DDX18 (red) relative to the nucleolar marker FBL (green) in WT and p300 knockout cells under H/R conditions. Scale bar=20 μ m. **H-J**, Fibrotic marker expression was determined by quantitative RT-qPCR (**H**), western blotting (**I**), and immunofluorescence (**J**). Scale bar=20 μ m. Representative data from one of three independent experiments are shown. Unpaired two-tailed Student's t-test was used for data **G** statistical analysis. Two-way ANOVA was used for statistical analysis in data **H**. ***p<0.001. NS, not significant.

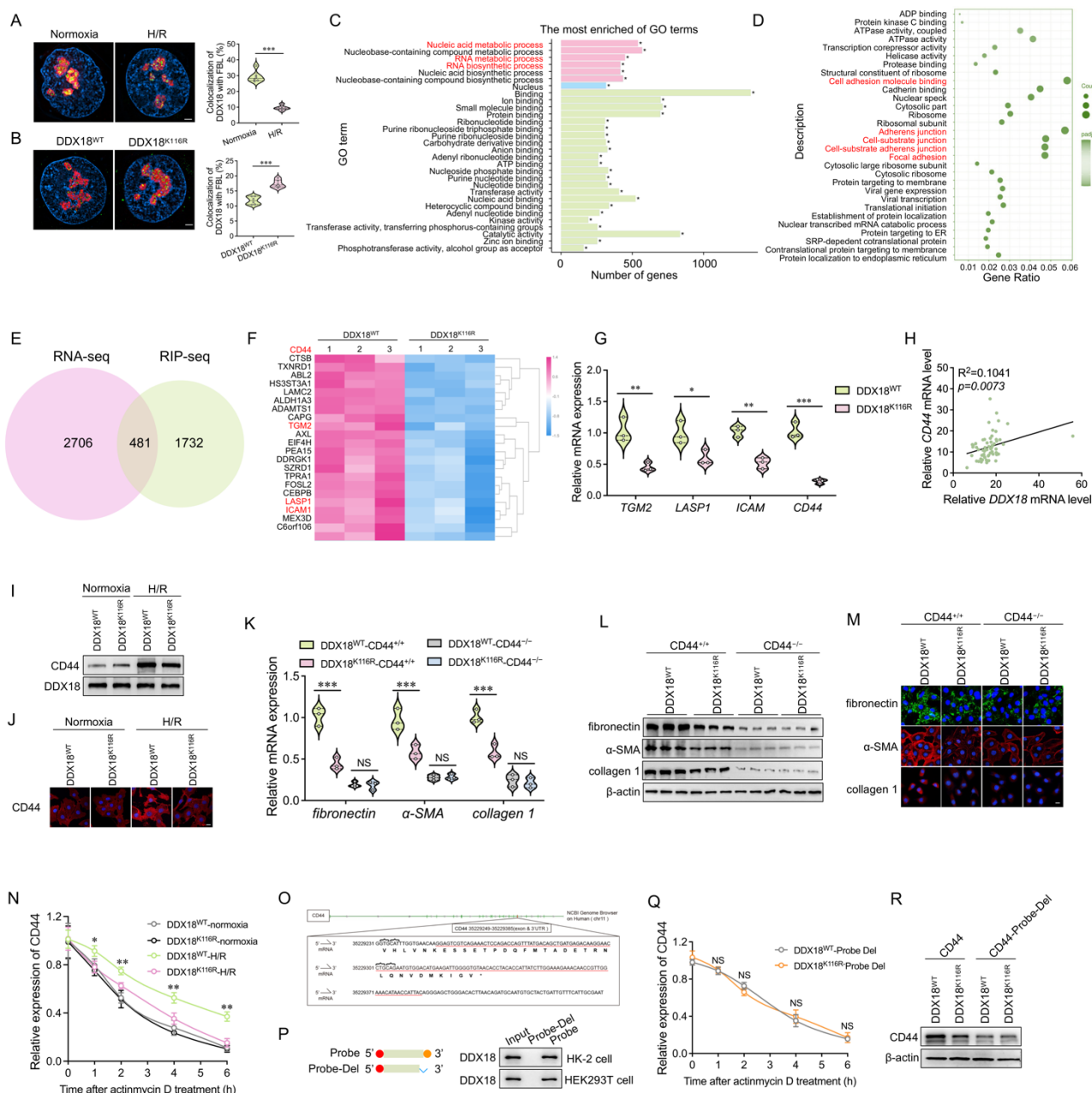


Figure 6. DDX18-K116 lactylation facilitates TEC activation through *CD44* RNA binding

A, Immunofluorescence showing the subcellular localization of DDX18 (red) relative to the nucleolar marker fibrillar (FBL, green) in HK-2 cells under normoxia or H/R conditions. **B**, Subcellular localization of DDX18 (red) with nucleolar (green) in HK-2 cells expressing the DDX18^{WT} or DDX18^{K116R} mutant under H/R. **C**, GO analysis (RIP-seq, base mean ≥ 500 , IP/input ≥ 2 , $p < 0.05$) of

DDX18-bound transcripts in H/R-treated cells. **D**, GO analysis (RNA-seq, fold change>2, p<0.05) of genes differentially expressed between DDX18^{WT}- and DDX18^{K116R}-expressing HK-2 cells under H/R. **E-G**, Integrative multiomics identifies *CD44* as a key target. **(E)** Venn diagram of overlapping genes from the RNA-seq and RIP-seq. **(F)** Heatmap analysis of overlapping downregulated genes in DDX18^{K116R} mutant cells, with cell adhesion-associated genes highlighted. (Padj<0.0001, |log2FoldChange|>0.2) **(G)** DDX18-deficient HK-2 cells were reconstituted with either DDX18^{WT} or the DDX18^{K116R} before exposure to H/R conditions. Relative mRNA levels of candidate genes associated with adhesion function were quantified *via* RT-qPCR. **H**, Transcriptomic data (GEO: GSE175759) identified the correlation between *CD44* and *DDX18* expression levels in microdissected tubulointerstitial tissue from patients with kidney disease. **I, J**, The protein level of CD44 was determined by **(I)** western blotting and **(J)** immunofluorescence. Scale bar=20 μm. **K-M**, CD44^{+/+} and DDX18^{-/-} HK-2 cells were transfected with DDX18^{WT} or the DDX18^{K116R} mutant and subsequently subjected to H/R. Fibrotic gene expression **(K)** was determined by RT-qPCR. The levels of fibrosis-related proteins were determined by **(L)** western blotting and **(M)** immunofluorescence. Scale bar=20 μm. **N**, *CD44* mRNA stability in DDX18^{WT} or DDX18^{K116R}-expressing HK-2 cells under hypoxic conditions was detected by actinomycin D assay. **O**, Schematic diagrams showing the DDX18-binding site on the *CD44* transcript by RIP-seq. **P**, Biotinylated RNA probe and its deletion mutant (probe-Del, 35229249--35229385 nt deficiency) were incubated with HK-2 or HEK293T cell lysates, pulled down with streptavidin beads, and associated proteins analyzed by western blotting. **Q-R**, CD44 and DDX18 double-knockout HK-2 cells were cotransfected with DDX18^{WT} or DDX18^{K116R} and a CD44-Del plasmid (CD44 region 35229249-35229385 deletion) or CD44 plasmid. **(Q)** *CD44* mRNA stability in HK-2 cells was detected by actinomycin D assay. **(R)** CD44 protein level was determined by western

849 blot analysis. Unpaired two-tailed Student's t-test was used for data **A**, **B**, **G**, and **Q** statistical analysis.
850 Two-way ANOVA was used for statistical analysis in data **K**, and **N**. Representative data from one of
851 three independent experiments are shown. *** $p < 0.001$. NS, not significant. Pearson's correlation
852 analysis was used for statistical analysis in data **H**.
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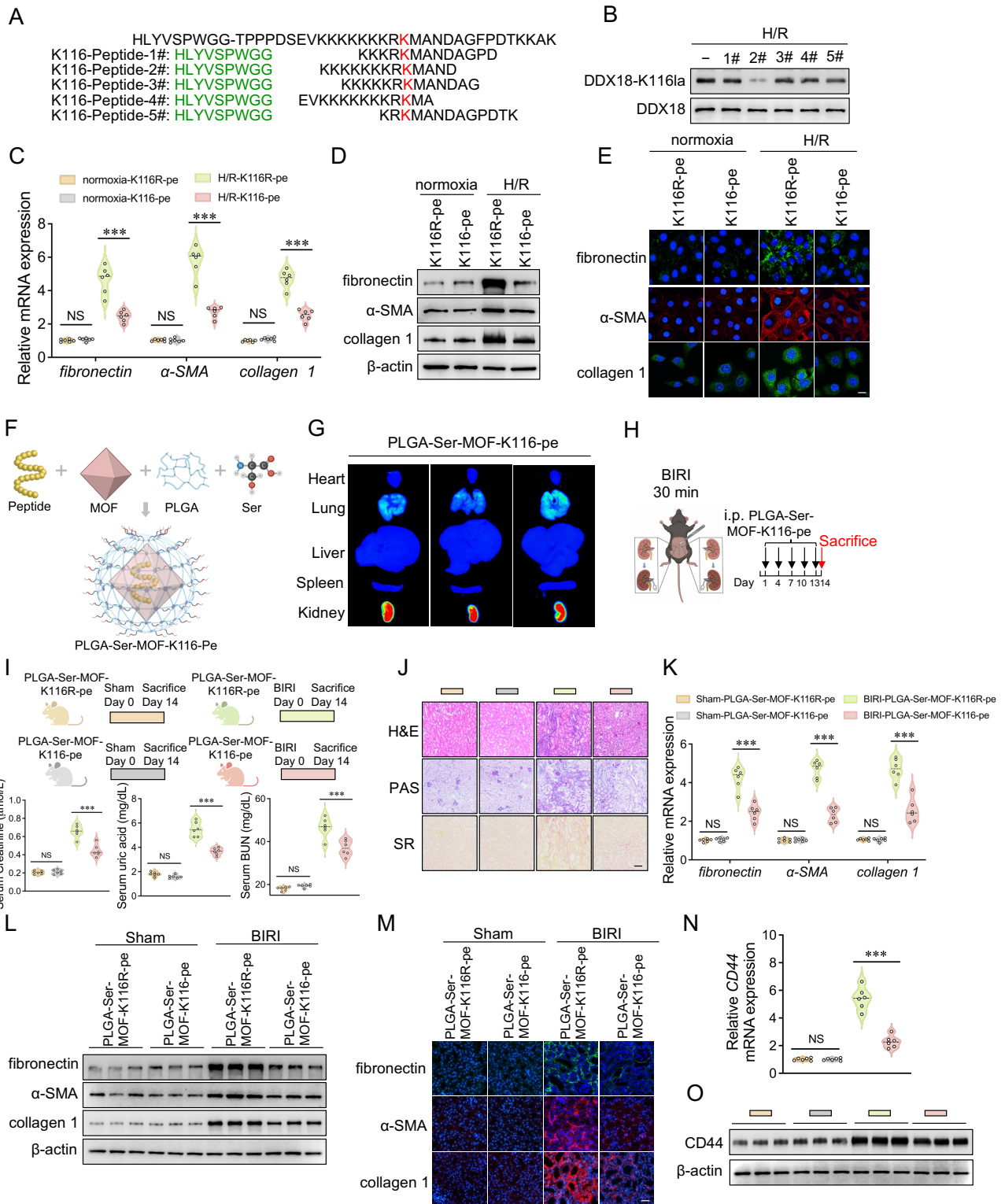


Figure 7. K116-targeted peptide inhibits DDX18 lactylation and attenuates AKI-to-CKD progression

A-D, Development and *in vitro* validation of DDX18-K116-targeting peptides. **(A)** Structural

858 schematic of synthetic peptides designed to competitively inhibit DDX18-K116 lactylation. **(B)**
859 Western blot validation of peptide specificity: five candidate peptides were screened for their ability
860 to inhibit DDX18-K116 lactylation in HK2 cells under H/R. **C-E**, *In vitro* validation using HK-2 cells
861 under H/R stress with K116-pe. **(C)** Suppression of fibrotic gene expression was determined by RT-
862 qPCR. A concordant reduction in fibrosis-related proteins was demonstrated by **(D)** western blot and
863 **(E)** immunofluorescence. Scale bar=20 μ m. **F, G**, Engineering and biodistribution of PLGA-Ser-
864 MOF-K116-pe nanoparticles. **(F)** Schematic of PLGA-Ser-MOF-K116-pe nanoparticle synthesis. **(G)**
865 Ex vivo fluorescence imaging of mCherry-labeled PLGA-Ser-MOF-K116-pe nanoparticles in major
866 organs 12 hours postinjection. **H-O**, Therapeutic efficacy of PLGA-Ser-MOF-K116-pe in murine AKI-
867 to-CKD progression. **(H)** Treatment schedule for targeted delivery to injured renal tubules in BIRI
868 mice. **(I)** Improved renal function in PLGA-Ser-MOF-K116-pe-treated BIRI mice, shown by reduced
869 serum creatinine, uric acid, and BUN levels at experiment end. **(J)** Histopathological assessment using
870 H&E, PAS, and Sirius red staining. Scale bar=100 μ m. Downregulation of fibrotic markers across **(K)**
871 mRNA, **(L)** protein, and **(M)** histological expression levels in PLGA-Ser-MOF-K116-pe-treated BIRI
872 mice. Reduced CD44 expression in kidney tissues from PLGA-Ser-MOF-K116-pe-treated BIRI mice
873 was detected at both the **(N)** mRNA and **(O)** protein levels. Representative data from one of three
874 independent experiments are shown. Two-way ANOVA was used for data in **C, I, K**, and **N** statistical
875 analysis. ** $p < 0.01$, *** $p < 0.001$. NS, not significant.