



# THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

### **A Kinome-wide screen identifies a CDKL5-SOX9 regulatory axis in epithelial cell death and kidney injury**

**Citation for published version:**

Kim, JY, Bai, Y, Jayne, LA, Hector, R, Persaud, AK, Ong, SS, Rojesh, S, Feng, MJHH, Chung, S, Cianciolo, RE, Christman, JW, Campbell, MJ, Gardner, DS, Baker, SD, Sparreboom, A, Govindarajan, R, Singh, H, Chen, T, Po, M, Susztak, K, Cobb, S & Pabla, NS 2020, 'A Kinome-wide screen identifies a CDKL5-SOX9 regulatory axis in epithelial cell death and kidney injury', *Nature Communications*.  
<https://doi.org/10.1038/s41467-020-15638-6>

**Digital Object Identifier (DOI):**

[10.1038/s41467-020-15638-6](https://doi.org/10.1038/s41467-020-15638-6)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Nature Communications

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



1 **Peer Review Information:** *Nature Communications* thanks William Brian Reeves and other, anonymous,  
2 reviewers for their contribution to the peer review of this work.

3

4 **A Kinome-wide screen identifies a CDKL5-SOX9 regulatory axis in**  
5 **epithelial cell death and kidney injury**

6

7 Ji Young Kim<sup>1#</sup>, Yuntao Bai<sup>1#</sup>, Laura A. Jayne<sup>1</sup>, Ralph D. Hector<sup>2</sup>, Avinash K. Persaud<sup>1,3</sup>, Su Sien Ong<sup>4</sup>,  
8 Shreshtha Rojesh<sup>5</sup>, Radhika Raj<sup>1</sup>, Mei Ji He Ho Feng<sup>1</sup>, Sangwoon Chung<sup>6</sup>, Rachel E. Cianciolo<sup>7</sup>, John W.  
9 Christman<sup>6</sup>, Moray J. Campbell<sup>1</sup>, David S. Gardner<sup>8</sup>, Sharyn D. Baker<sup>1</sup>, Alex Sparreboom<sup>1</sup>, Rajgopal  
10 Govindarajan<sup>1</sup>, Harpreet Singh<sup>9</sup>, Taosheng Chen<sup>4</sup>, Ming Poi<sup>1,3</sup>, Katalin Susztak<sup>5</sup>, Stuart R. Cobb<sup>2</sup>, Navjot  
11 Singh Pabla<sup>1\*</sup>

12

13 <sup>1</sup>Division of Pharmaceutics & Comprehensive Cancer Center, the Ohio State University, Columbus, OH,  
14 USA. <sup>2</sup> Simons Initiative for the Developing Brain & Patrick Wild Centre, Centre for Discovery Brain  
15 Sciences, University of Edinburgh, Edinburgh, UK. <sup>3</sup>Division of Pharmacy Practice and Science, College  
16 of Pharmacy, the Ohio State University, Columbus, OH, USA. <sup>4</sup>Department of Chemical Biology &  
17 Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA. <sup>5</sup>Renal Electrolyte and  
18 Hypertension Division, Department of Medicine and Genetics, University of Pennsylvania, Philadelphia,  
19 PA, USA. <sup>6</sup>Pulmonary, Sleep and Critical Care Medicine, Wexner Medical Center, Davis Heart and Lung  
20 Research Institute, <sup>7</sup>Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio  
21 State University, Columbus, OH, USA. <sup>8</sup>School of Veterinary Medicine and Science, University of  
22 Nottingham, Loughborough, Leicestershire, UK., and <sup>9</sup>Department of Physiology and Cell Biology and  
23 Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA.

24

25 <sup>#</sup>these authors contributed equally to this work: Ji Young Kim and Yuntao Bai.

26 <sup>\*</sup>Correspondence and requests for materials should be addressed to N.P. (email: [pabla.2@osu.edu](mailto:pabla.2@osu.edu)).

27

28 **ABSTRACT**

29 Renal tubular epithelial cells (RTECs) perform the essential function of maintaining the constancy of body  
30 fluid composition and volume. Toxic, inflammatory, or hypoxic-insults to RTECs can cause systemic fluid  
31 imbalance, electrolyte abnormalities and metabolic waste accumulation- manifesting as acute kidney injury  
32 (AKI), a common disorder associated with adverse long-term sequelae and high mortality. Here we report  
33 the results of a kinome-wide RNAi screen for cellular pathways involved in AKI-associated RTEC-  
34 dysfunction and cell death. Our screen and validation studies reveal an essential role of Cdkl5-kinase in  
35 RTEC cell death. In mouse models, genetic or pharmacological Cdkl5 inhibition mitigates nephrotoxic and  
36 ischemia-associated AKI. We propose that Cdkl5 is a stress-responsive kinase that promotes renal injury  
37 in part through phosphorylation-dependent suppression of pro-survival transcription regulator Sox9. These  
38 findings reveal a surprising non-neuronal function of Cdkl5, identify a pathogenic Cdkl5-Sox9 axis in  
39 epithelial cell-death, and support CDKL5 antagonism as a therapeutic approach for AKI.

40

41

42

43

44

45

46

47

48

49

50

51

52 **Introduction**

53           The ability of vertebrates to maintain a stable, relatively constant 'internal milieu' is inextricably  
54 linked to the function of the kidneys<sup>1</sup>. Through a continuous filtration-reabsorption process, kidneys  
55 regulate the fluid and molecular composition of blood. Within the kidneys, the renal tubular epithelial cells  
56 (RTECs) carry out the enormous task of selective reabsorption of water, ions, and essential nutrients as  
57 well as excretion of metabolic waste, thereby converting the glomerular filtrate into a concentrated urine  
58 whose composition is constantly fine-tuned to maintain organismal homeostasis. RTEC dysfunction can  
59 thus lead to systemic electrolyte and fluid imbalances along with accumulation of metabolic and toxic  
60 waste triggering deleterious systemic effects and multi-organ failure.

61           Numerous clinical conditions such as sepsis, cardiac surgery, drug toxicities, cancer therapy and  
62 rhabdomyolysis are associated with inflammatory, toxic, and hypoxic insults to RTECs<sup>2-6</sup>. The resulting  
63 RTEC dysfunction and cell-death<sup>7</sup> are the hallmarks and underlying cause of acute kidney injury (AKI), a  
64 common disorder that predominantly develops in hospitalized patients<sup>8</sup>. Due to the lack of treatment  
65 options, annually an estimated two million people worldwide die of AKI<sup>9</sup>. Importantly, the patients that  
66 recover from an AKI episode are at increased risk of developing chronic kidney disease, end-stage renal  
67 disease and cardiovascular dysfunction- disorders associated with significant morbidity and mortality<sup>10,11</sup>.  
68 Over the past decade, it has become apparent that the pathophysiology of AKI is exceedingly complex<sup>12</sup>.  
69 Multiple molecular and cellular pathways are involved in RTEC dysfunction and cell-death<sup>7</sup>. Vascular and  
70 immune cells also contribute to renal impairment<sup>13-15</sup>. Recent advancements in our understanding of the  
71 pathophysiological basis of AKI have however not yet resulted in clinical benefits, in part, due to the non-  
72 druggable nature of several identified molecular targets and associated pathways. One possible way to  
73 transcend these difficulties is to utilize unbiased functional genomic screening to systematically uncover  
74 the role of 'druggable genes' in AKI.

75           Of the estimated ~20,000 protein-coding genes in the human genome, ~10% encode proteins that  
76 can currently be targeted by small-molecule drugs, a group defined as 'druggable genome'<sup>16</sup>. Protein

77 kinases<sup>17</sup> are one of the largest family in the ‘druggable genome’, along with G-coupled protein receptors.  
78 Due to the potential wide-spread role of kinases in disease pathogenesis as well as suitable  
79 pharmacological properties and clinical safety profile of kinase inhibitors, protein kinases have emerged as  
80 attractive therapeutic targets<sup>18,19</sup>. Nevertheless, the underlying biology of the majority of kinases remains  
81 yet to be fully elucidated. Moreover, the role of protein kinases in the pathogenesis of non-oncological  
82 diseases, especially AKI remains underexplored.

83 Here, we have used a kinome-wide screening approach to identify kinases that contribute to RTEC  
84 cell-death in order to reveal therapeutic targets for AKI. Initial *in vitro* RNAi-based screening and  
85 subsequent *in vivo* validation experiments identified cyclin-dependent kinase-like 5 (Cdkl5) also known as  
86 serine/threonine kinase 9 (Stk9)<sup>20</sup> as a key regulator of renal cell-death and injury. *CDKL5* has mostly been  
87 studied for its role in human neuronal development since mutations in this X-linked gene are associated  
88 with neurodevelopmental disorders including early-onset seizures<sup>21,22</sup>. Surprisingly, we have uncovered a  
89 previously unrecognized function of Cdkl5 as a crucial regulator of renal injury and have identified the  
90 transcription factor Sox9 as one of its crucial downstream target.

91

## 92 **RESULTS**

93 ***Identification of kinases involved in RTEC cell-death.*** We performed a kinome-wide small interfering  
94 RNA (siRNA) screen in BUMPT cells in order to identify protein kinases that regulate renal epithelial cell-  
95 death. High transient transfection efficiency (~95%) of this murine RTEC cell-line makes it a suitable model  
96 for high-throughput (siRNA) screening assays. For the primary screen, BUMPT cells were transfected with  
97 either control siRNAs (non-targeting, *Pkcδ* and *Plk1*) or siRNAs targeting protein kinases, phosphatases  
98 and related targets (780 genes, Dharmacon), followed by induction of cell-death by treatment with cisplatin  
99 and assessment of cellular viability by cell-titer glo assay (**Fig. 1a**). Cisplatin-induced cell-death in BUMPT  
100 cells partially mimics conditions observed during cisplatin-associated AKI<sup>23</sup>. The *in vitro* screening assay  
101 involved the treatment of BUMPT cells with 15 μM cisplatin, which reduced the cell viability by ~75% in 48  
102 hours in the un-transfected and control siRNA (non-targeting) transfected cells (**Supplementary Figure**

103 **1a-b**). Cisplatin-induced cell-death was partially ameliorated by protein kinase c  $\delta$  (*Pkc $\delta$* ) knockdown  
104 (positive control), which is an established<sup>24</sup> pro-apoptotic gene and significantly increased by polo-like  
105 kinase 1 (*Plk1*) knockdown (negative control).

106 The primary screen was carried out in triplicate and subsequent data analysis (**Fig. 1b-c**) yielded  
107 seven hit candidates (**Supplementary Table 1**) that mitigated cell-death to an extent that was significantly  
108 ( $p < 0.05$ , 1-way ANOVA followed by Dunnett's test) greater than the positive control (*Pkc $\delta$*  siRNA). For  
109 stringent validation of these identified-hits, we performed confirmatory experiments by employing distinct  
110 siRNAs/shRNAs, cell lines and assay systems. In the secondary screening, we utilized dissimilar siRNAs  
111 from a different source (Sigma) and used different cell viability and cell-death assays (MTT, Trypan Blue  
112 and Caspase assay). Secondary screening in BUMPT cells (**Fig. 1d and Supplementary Figure 1c-d**)  
113 validated three out of seven hits obtained in the primary screen. Similar studies in HK-2 (human kidney-2)  
114 cells, a human RTEC cell-line showed that *CDKL5* knockdown significantly reduced cisplatin-induced cell-  
115 death (**Fig. 1e and Supplementary Figure 1e-f**). *Cdkl5* was the top-hit in both the primary and secondary  
116 screens and hence we selected it for further confirmation.

117 The CDKL-family (CDKL1-5) comprises five members that share structural similarities with cyclin-  
118 dependent kinases (CDKs) as well as mitogen-activated protein kinases (MAPKs), however, their  
119 biological functions and linked signal transduction pathways remain obscure<sup>25,26</sup>. *CDKL5* is highly  
120 expressed in the brain and *CDKL5* loss-of-function mutations are associated with neurodevelopmental  
121 disorders in humans, although the underlying mechanisms are incompletely understood<sup>27</sup>. It also remains  
122 unknown if CDKL5 kinase controls any biological processes in non-neuronal tissues, such as testes and  
123 kidneys, where it is known to be expressed<sup>20,28</sup>.

124 Mechanisms underlying CDKL5 activation also remain unclear. However, similar to MAPKs,  
125 CDKL5 contains the TEY sequence within its activation loop (**Fig. 1f**). The TEY motif in the extracellular  
126 signal-regulated kinases (ERKs) undergoes dual phosphorylation resulting in kinase activation. This  
127 mechanism of activation is in most cases initiated by other upstream kinases or in some cases via auto-  
128 phosphorylation as has been proposed for ERK7 and CDKL5<sup>29</sup>. To confirm the role of *Cdkl5* kinase in  
129 RTEC cell-death, we carried out tertiary screening where we silenced *Cdkl5* expression in BUMPT cells

130 using a shRNA targeting the 3' UTR (untranslated region) of *Cdkl5* gene and carried out 'add-back'  
131 experiments by over-expressing shRNA-resistant *Cdkl5* constructs including wild-type, kinase-dead and  
132 TEY mutants (**Fig. 1g-h and Supplementary Figure 1g-h**). We found that shRNA-mediated *Cdkl5*  
133 knockdown reduces cisplatin-induced cell-death and importantly this phenotype was reversed by wild-type  
134 but not kinase-dead or TEY-mutant *Cdkl5* overexpression. Of note, overexpression of WT *Cdkl5* in the  
135 control cells did not influence RTEC cell-death. This may be due to limiting upstream activation signals,  
136 since unlike the wild-type *Cdkl5*, overexpression of catalytically active *Cdkl5* (lacking the regulatory  
137 domain) increases cisplatin-associated RTEC cell death (**Supplementary Figure 1i-k**). Collectively, our  
138 siRNA screening and validation studies identified *Cdkl5* kinase (**Fig. 1h**) as a crucial, previously unknown  
139 regulator of renal epithelial cell-death.

140

#### 141 ***Cdkl5* kinase activity increases in RTECs during AKI.**

142 While we used a cisplatin-based *in vitro* screening method to identify putative regulators of RTEC  
143 cell-death and dysfunction, our overall goal was to identify and validate targets that contribute to the  
144 pathogenesis of AKI associated with multiple etiologies. Hence confirmatory *in vivo* studies were carried  
145 out in two distinct and widely-used models of AKI namely, ischemia-reperfusion injury and cisplatin-  
146 associated AKI<sup>30</sup>. In these mouse models, the onset of AKI was determined by three diverse indicators of  
147 renal structure and function: accumulation of nitrogenous waste (blood urea nitrogen and serum  
148 creatinine), biomarkers (kidney injury molecule-1 [*Kim-1*]<sup>31</sup> and neutrophil gelatinase-associated lipocalin  
149 [*Ngal*]<sup>32</sup>) and histological analysis (H&E staining and renal damage score) (**Fig. 2 a-g**). In the ischemic  
150 injury model, AKI onset occurs 24-hours post-surgery, while in the cisplatin-associated renal injury model,  
151 renal impairment is seen 72-hours post-injection. We found that *Cdkl5* protein levels showed significant  
152 variations, but overall we observed marginal increase during the early phases of AKI, followed by reduction  
153 at later time-points (**Fig. 2h**). To examine the *Cdkl5* phosphorylation status in the activation loop, we  
154 generated a phospho-threonine-169 antibody that recognizes phosphorylated threonine within the TEY  
155 motif (**Supplementary Figure 2**). Western-blot analysis showed that *Cdkl5* phosphorylation increased

156 during AKI (**Fig. 2h**). Subsequently, kinase assays showed increased Cdkl5 activity in renal tissues during  
157 the early stages of AKI (**Fig. 2i-k**).

158 We next investigated whether the increased Cdkl5 activity is localized in the RTECs- the major cell-  
159 type that are impacted during AKI<sup>7</sup>. In order to label and isolate RTECs from murine kidneys, we crossed  
160 the *ROSA<sup>mT/mG</sup>* strain with the renal tubular epithelial cell-specific *Ggt1-Cre* (gamma-glutamyltransferase-1)  
161 mice to generate transgenic mice that express membrane-localized GFP (green fluorescent protein) in the  
162 tubular epithelial cells (**Fig. 2i and Supplementary Figure 3**). We then isolated GFP positive cells from  
163 the kidneys of untreated and cisplatin-treated mice (**Fig. 2m**), followed by examination of Cdkl5 kinase  
164 activity (**Fig. 2n**). These studies confirmed that Cdkl5 activity increases in RTECs (GFP positive cells) early  
165 during the development of AKI. Furthermore, increased Cdkl5 kinase activity was also observed in murine  
166 models of rhabdomyolysis and folic acid-associated AKI as well as in a previously described<sup>33</sup> porcine-  
167 model of ischemic AKI (**Supplementary Figure 4a-g**). In support of the *in vivo* studies, increased Cdkl5  
168 activity was also observed in primary RTECs under multiple stress conditions, including cisplatin, hydrogen  
169 peroxide, hypoxia and hemin treatments (**Supplementary Figure 4h-i**). Under these conditions, increased  
170 Cdkl5 activity seemed to be independent of the cell cycle phase. In summary, these results show that  
171 irrespective of the nature of the initial injury, increase in Cdkl5 kinase activity is a common phenomenon  
172 during AKI, signifying a potential functional role in disease pathogenesis.

173

174 ***Cdkl5* gene ablation in epithelial cells mitigates AKI.** We next sought to examine the consequence of  
175 *Cdkl5* gene deletion on the severity of AKI. Germline *Cdkl5* knockout mice are viable<sup>27</sup>, although they  
176 exhibit certain non-lethal neuronal phenotypes. We found that *Cdkl5* knockout mice do not have any overt  
177 renal abnormalities under normal conditions (**Supplementary Figure 5a-b**), which gave us the opportunity  
178 to examine the effect of *Cdkl5* deficiency on the severity of AKI. We found that as compared to wild-type  
179 littermates, *Cdkl5<sup>-/-</sup>* mice showed protection from ischemia-associated AKI as revealed by multiple  
180 indicators: BUN, Creatinine, *Kim1* expression, and histological analysis (**Supplementary Figure 6a-e**).  
181 Likewise, *Cdkl5<sup>-/-</sup>* mice displayed resistance to cisplatin-associated AKI (**Supplementary Figure 6f-i**).



182 To probe the RTEC-specific role of Cdkl5 in the pathogenesis of AKI, we generated *Cdkl5*  
183 conditional knockout mice (*Cdkl5*<sup>PT-/-</sup>) by crossing the *Cdkl5*-floxed mice with the *Ggt1-Cre* mice. In *Ggt1-*  
184 *Cre* mice, *Cre* recombinase is expressed in RTECs 7-10 days after birth, such that *Cre* expression most  
185 likely occurs after the completion of renal development<sup>34</sup>. We found that normal renal function  
186 (**Supplementary Figure 5c-d**) is un-affected by *Cdkl5* deficiency (**Fig. 3a**). Importantly, *Cdkl5* gene  
187 ablation in RTECs provided significant protection from ischemia-associated (**Fig. 3b-e**) and cisplatin-  
188 mediated (**Fig. 3f-i**) AKI. To investigate the effect of *Cdkl5* deficiency on renal cell-death and to exclude the  
189 possibility of non-specific compensatory changes, we cultured primary RTECs from the *Cdkl5*-floxed mice  
190 and carried out *Cdkl5* deletion under *in vitro* conditions using lentivirus-mediated *Cre* expression (**Fig. 3j-**  
191 **k**). We found that *Cdkl5* deletion provides significant protection from cisplatin-mediated cell-death.  
192 Collectively, these studies suggested that Cdkl5 kinase plays a pathogenic role during the development of  
193 AKI.

194

195 ***Cdkl5 phosphorylates Sox9 during AKI.*** We next pursued the Cdkl5-dependent mechanisms that  
196 contribute to renal dysfunction. CDKL5 regulates several neuronal functions; however, the downstream  
197 signaling pathways remain incompletely understood. Previous reports have described functional  
198 interactions of CDKL5 with other proteins with important neuronal functions<sup>25,35-39</sup>. Whether these  
199 interactions are relevant in renal epithelial cells is however unclear. Therefore, in an attempt to understand  
200 the mechanistic basis of Cdkl5-dependent renal injury, we sought to identify Cdkl5 interacting proteins. To  
201 this end, we immunoprecipitated (IP) endogenous Cdkl5 from ischemic renal tissues and found that a ~65  
202 kDa protein was associated with Cdkl5. Mass spectrometric analysis identified this protein as the  
203 transcription factor Sox9 (Sex-determining Region Y (SRY) box 9) (**Fig. 4a**). Sox9 is a member of Sox  
204 family, which are a group of transcription factors that have essential roles in cell-fate determination during  
205 embryonic development and adult tissue homeostasis<sup>40</sup>. Interestingly, Sox9 is also known to suppress cell-  
206 death during development, adult tissue homeostasis and oncogenesis<sup>41,42</sup>.

207 We confirmed that Cdkl5 interacts with Sox9, by Cdkl5-IP and reverse IP (Sox9-IP) experiments  
208 (**Supplementary Figure 7**). Notably, Sox9 protein is expressed at low amounts in control kidneys and its

209 expression is induced during AKI (input blots, **Supplementary Figure 7**). Given the physical association  
210 between Cdkl5 and Sox9 in renal tissues, we considered if Sox9 is a previously unknown Cdkl5 substrate.  
211 Based on sequence analysis and global phospho-proteomics data<sup>43</sup>, we identified 5 putative  
212 phosphorylation sites in the Sox9 protein. We then tested the ability of purified Cdkl5 to phosphorylate  
213 wild-type and Ser-to-Ala Sox9 mutants. We found that Cdkl5 could phosphorylate wild-type Sox9 (**Fig. 4b**).  
214 Importantly, Ser-199 was found to be the major site of phosphorylation since Ser-to-Ala mutation at this  
215 site significantly abolished Cdkl5-mediated Sox9 phosphorylation (**Fig. 4b**). The Ser-199 site is  
216 evolutionarily conserved (**Fig. 4c**), however the functional consequence of phosphorylation at this site has  
217 not been previously studied.

218 To ascertain the functional consequence of Cdkl5-mediated Sox9 phosphorylation, we investigated  
219 the potential effect of phosphorylation at Ser-199 site on Sox9 localization and stability. We generated  
220 S199A (non-phosphorylatable) and S199D (phospho-mimetic) Sox9 mutants and then examined their  
221 localization and stability in BUMPT cells. Sox9 sub-cellular localization was predominantly nuclear and was  
222 unaffected by S199A or S199D mutation (**Supplementary Figure 8a**). Interestingly, cycloheximide (CHX)  
223 pulse-chase experiments showed that S199A mutant was more stable than the wild-type Sox9, while the  
224 phospho-mimetic S199D mutant had significantly reduced stability (**Supplementary Figure 8b-c**). Based  
225 on these studies, we hypothesized that Cdkl5-dependent phosphorylation at Ser-199 suppresses Sox9  
226 function during AKI.

227 To test our hypothesis and observe Sox9 phosphorylation *in vivo*, we generated an anti-phospho-  
228 Ser-199 specific antibody (**Supplementary Figure 9**), and then examined the levels of total and  
229 phosphorylated Sox9 in renal tissues. In the wild-type mice, total Sox9 protein level were low in control  
230 kidneys, however, its expression increased during both ischemia-reperfusion and cisplatin-associated AKI  
231 (**Fig. 4d-f**). Intriguingly, AKI-induced increase in the Sox9 protein expression had strikingly different  
232 dynamics in the *Cdkl5*<sup>PT-/-</sup> mice. Firstly, as compared to wild-type mice, AKI-associated Sox9 induction  
233 occurred at a much earlier time-point in the *Cdkl5*<sup>PT-/-</sup> mice and secondly, the magnitude of Sox9 induction  
234 was higher in the *Cdkl5*<sup>PT-/-</sup> mice. Interestingly, phospho-Ser-199 -Sox9 levels also increased during AKI in  
235 the wild-type mice, however, Sox9 phosphorylation in the *Cdkl5*<sup>PT-/-</sup> kidneys was pointedly suppressed

236 (Fig. 4e & g). We also examined total and phosphorylated Cdk15 protein levels in these tissues  
237 (Supplementary Figure 10). Importantly, the levels of Sox9 mRNA induction during AKI was not  
238 significantly different in the wild-type and *Cdk15*<sup>PT-/-</sup> mice (Supplementary Figure 11). Based on these  
239 findings, we postulated that Cdk15 activation might contribute to AKI, in part through phosphorylation-  
240 dependent regulation of Sox9 function.

241

242 **Sox9 plays a protective role during AKI.** In the murine kidneys, Sox9 facilitates recovery of renal  
243 function after the onset of AKI<sup>44,45</sup>. After the initial injury phase, Sox9 expressing RTECs contribute to  
244 regeneration and recovery, however the role of Sox9 in the initial injury phase remains unclear. To study  
245 the role of Sox9 in the early acute phase of AKI we generated RTEC-specific Sox9 deficient (*Sox9*<sup>PT-/-</sup>)  
246 mice (Fig. 5a), which had normal renal function under baseline conditions (Supplementary Figure 5e-f).  
247 Importantly, Sox9 deficiency markedly increased renal damage in both the ischemia (Fig. 5b-e) and  
248 cisplatin-associated (Fig. 5f-i) AKI. Primary RTECs with Sox9 gene ablation were also sensitive to  
249 cisplatin-mediated cell-death (Fig. 5j-k). Interestingly, unlike the normal untreated kidneys (which have  
250 very low Sox9 expression); the primary RTECs expressed clearly detectable levels of Sox9 and were used  
251 for further studies. We carried out 'add-back' experiments in the *Sox9*<sup>-/-</sup> primary RTECs and found that  
252 S199A mutation provided significantly higher protection than the WT Sox9, while S199D mutant had  
253 minimal effects, which could be partly due to reduced S199D stability during cisplatin treatment  
254 (Supplementary Figure 12). These results suggest that Sox9 plays a protective role during the early  
255 phase of AKI and Cdk15 mediated phosphorylation at S199 site likely reduces its functional activity.

256 To elucidate the underlying mechanisms, we next carried out chromatin immunoprecipitation  
257 (ChIP) based analysis of Sox9 target genes in normal and injured kidneys (Supplementary Figure 13a).  
258 Targets were selected based on ChIP-seq analysis in a previous study<sup>46</sup> and included genes known to be  
259 differentially regulated during AKI<sup>47</sup>. Our results show that during ischemic injury, Sox9 binds to the  
260 promoter region ( $\pm 2$  kb of transcription start sites) of several genes (*Wwp2*, *Ap2 $\beta$* , *Pi3kca*, *Myof*, *sema3e*  
261 and *Gadd45a*). For *Wwp2*, *myof*, *Sema3e* and *Gadd45a* these findings were confirmed in three distinct  
262 models of AKI (Supplementary Figure 13b-e). For further confirmation, gene expression analysis was

263 carried out, which showed that as compared to the littermate controls, renal tissues of Sox9<sup>PT-/-</sup> mice have  
264 diminished mRNA expression of *Wwp2*, *Myof* and *Sema3e*, while *Gadd45a* expression is elevated  
265 (**Supplementary Figure 14**). In the *Cdkl5*<sup>PT-ly</sup> mice, which had elevated levels of Sox9 protein during AKI,  
266 the mRNA levels of Sox9-dependent pro-survival genes (*Wwp2*, *Myof* and *Sema3e* ) was significantly  
267 increased, while *Gadd45a* gene expression was reduced (**Supplementary Figure 15**). Luciferase based  
268 reporter assays confirmed Sox9 binding within the promoter regions of *Wwp2*, *Myof* and *Sema3e* genes  
269 (**Supplementary Figure 16**). Finally, functional studies show that *Wwp2*, *Myof* and *Sema3e* knockdown  
270 sensitizes RTECs to injury, while *Gadd45a* knockdown provides protection from cell-death  
271 (**Supplementary Figure 17**). Thus by increasing the expression of pro-survival genes like *Wwp2*, *myof*  
272 and *sema3e*, Sox9 likely promotes cellular survival during AKI. These genes are known to regulate  
273 phosphoinositide 3-kinase (PI3K)- phosphatase and tensin homolog (PTEN) signaling (*Wwp2*)<sup>48</sup>,  
274 membrane and mitochondrial functions (*Myoferlin*)<sup>49,50</sup> and cell-death (*Sema3e*)<sup>51</sup> in non-renal epithelial  
275 cells. Whether these genes regulate RTEC dysfunction and cell-death *in vivo* through similar mechanisms  
276 remains unknown. Notably, along with *Wwp2*, *Myof* and *Sema3e*, Sox9-dependent renal protective  
277 transcriptional program likely involves multiple target genes that would require further exploration.  
278 However, our results support the notion that by suppressing Sox9 function, *Cdkl5* subdues and delays a  
279 Sox9-dependent protective transcriptional program, contributing to epithelial cell-death and AKI.

280

281 **Targeted *Cdkl5* inhibition mitigates renal injury in vivo.** Genetic *Cdkl5* ablation alleviated renal injury,  
282 raising the prospect that a targeted *Cdkl5*-kinase inhibitor might prevent and or reduce renal injury. While  
283 CDKL5-specific inhibitors have not been specifically pursued, several known protein kinase inhibitors have  
284 been tested for their ability to inhibit CDKL5 in global kinome-wide assays<sup>52</sup>. Based on these studies, we  
285 compiled a panel of small-molecules with demonstrated CDKL5 inhibition activity. We then tested these  
286 compounds for their ability to inhibit *Cdkl5* function using *in vitro* kinase assays (**Fig. 6a**). Among these  
287 inhibitors, AST-487 was found to be the most potent *Cdkl5* inhibitor (EC50=87 nM). AST-487 also inhibited  
288 *Cdkl5* activity in BUMPT cells and provided protection from cisplatin-induced cell-death (**Supplementary**  
289 **Figure 18a-d**). While AST-487 potently inhibited *Cdkl5* activity, similar to most kinase inhibitors, AST-487

290 likely inhibits multiple kinases including RET kinase<sup>53</sup>. To examine the role of Cdkl5 inhibition in the renal  
291 protective effect of AST-487, we thus utilized a chemical genomics approach<sup>54,55</sup>. To this end, we  
292 generated a *Cdkl5* construct with a gatekeeper mutation (F89A), which confers resistance to AST-487-  
293 mediated kinase inhibition (**Supplementary Figure 18e**). Importantly, overexpression of *Cdkl5*-gate-  
294 keeper mutant abrogated AST-487-mediated protection from cisplatin-induced cell-death (**Supplementary**  
295 **Figure 18f-h**). Since an AST-487 resistant Cdkl5 mutant is able to reverse the cytoprotective effects of  
296 AST-487, these studies provide compelling evidence that AST-487 mediated Cdkl5 inhibition is at least  
297 partly responsible for its renal protective effects.

298 To ascertain the potential efficacy of AST-487 *in vivo*, we performed pilot assessment studies. Oral  
299 administration of a single dose of 25 mg/kg AST-487 reduced Cdkl5 kinase activity in the kidneys by ~90%  
300 (**Fig. 6b**). Remarkably, AST-487 treatment (single dose of 25 mg/kg, 6 hours after cisplatin injection or  
301 ischemic surgery) significantly reduced cisplatin and ischemia-associated AKI in the wild-type mice (**Fig.**  
302 **6c-h**). We then carried out further studies in both control and *Cdkl5*-deficient mouse models. We found that  
303 AST-487 treatment reduced Cdkl5 phosphorylation and kinase activity (**Supplementary Figure 19a-b**).  
304 Importantly, AST-487 treatment did not afford protective effects in the *Cdkl5*-deficient mice  
305 (**Supplementary Figure 19c-e**). Furthermore, AST-487 treatment in wild-type mice resulted in blunted  
306 Sox9 phosphorylation and markedly increased accumulation of Sox9 during AKI (**Fig. 6i and**  
307 **Supplementary Figure 20**). Even though AST-487 treatment conferred protection in the wild-type mice,  
308 we questioned if Cdkl5 inhibition just delays the development of kidney injury or it has long-term  
309 protective effects. Indeed, long-term survival experiments showed that AST-487 treatment reduces  
310 cisplatin-associated mortality (**Supplementary Figure 21a**). In further support, genetic *Cdkl5*-deficiency  
311 also provides long-term protection and survival benefits (**Supplementary Figure 21b**).

312

313 **Sox9 dependent and independent regulation of AKI.** To examine the dependence of Sox9 pathway in  
314 Cdkl5-associated renal injury, we initially examined the effect of Cdkl5 inhibition in control and Sox9-  
315 deficient mice challenged with ischemic injury. We found that Cdkl5 inhibition provides protection in both  
316 WT and Sox9<sup>PT-/-</sup> mice; however, the extent of protection is much lower in the Sox9<sup>PT-/-</sup> mice (**Fig. 7a-c**).

317 Mice treated with cisplatin showed a similar phenotype (**Supplementary Figure 22a-c**). We confirmed  
318 these results in primary RTECs, where Cdkl5 inhibition protected both WT and Sox9<sup>-/-</sup> cells; however, the  
319 extent of protection was lower in the Sox9<sup>-/-</sup> cells (**Supplementary Figure 23a-c**).

320 To corroborate these findings, we next used the genetic knockout approach and performed similar studies  
321 in control, single and double knockout mice (dKO<sup>PT</sup>) (**Fig. 7d**). As compared to the Cdkl5<sup>PT-/-</sup> mice, the  
322 dKO<sup>PT</sup> mice showed higher injury when challenged with ischemia, while as compared to the Sox9<sup>PT-/-</sup> mice,  
323 the dKO<sup>PT</sup> mice showed lower injury (**Fig. 7d-g**). We observed similar results in the cisplatin-toxicity model  
324 (**Supplementary Figure 22d-f**). Studies with primary RTECs with single or double gene ablation also  
325 confirmed the *in vivo* findings (**Supplementary Figure 23d-g**). These results suggest that Cdkl5 regulates  
326 renal injury in both Sox9 dependent and independent manner. Furthermore, it is likely that regulation of  
327 Sox9 function during AKI occurs in both Cdkl5 dependent and independent manner.

328 Finally, we performed series of studies in female mice. We found that similar to male mice, Cdkl5 activity  
329 increases during AKI in females and genetic or pharmacological inhibition of Cdkl5 function provides  
330 protection from ischemia and cisplatin-associated AKI (**Supplementary Figure 24 and 25**). Cdkl5-  
331 dependent Sox9 phosphorylation was also confirmed in female mice (**Supplementary Figure 26**).  
332 Collectively, these proof-of-principle experiments in multiple AKI mouse models showed robust therapeutic  
333 effects of Cdkl5 inhibition.

334

## 335 **DISCUSSION**

336 Here we have found that cyclin-dependent kinase-like 5 (Cdkl5) also known as serine/threonine  
337 kinase 9 (Stk9) is a stress responsive kinase that controls epithelial cell fate during acute kidney injury. We  
338 propose that Cdkl5 activation promotes renal dysfunction through phosphorylation-mediated functional  
339 suppression of pro-survival transcription factor Sox9.

340 Very little is known about the five members of the CDKL family (CDKL1-5), though they have been  
341 linked to certain neuronal functions<sup>56</sup>. In humans, mutations in the X-linked *CDKL5* gene are associated  
342 with neurodevelopmental disorders characterized by infantile seizures and developmental delay<sup>22,35,35,57-60</sup>.

343 Some of these phenotypes have been recapitulated in the germline *Cdkl5* knockout mice<sup>27</sup>. Most studies  
344 on CDKL5 function remain predominantly focused on its role in neuronal development. Interestingly,  
345 CDKL5 expression is not restricted to the brain, but is also detected in peripheral organs, particularly testes  
346 and kidney<sup>20</sup>. Our studies demonstrates *Cdkl5* expression in RTECs and reveals its functional activation  
347 during AKI. It is noteworthy that germline or renal epithelial-cell specific *Cdkl5* deficiency did not have any  
348 overt effect on normal kidney structure or function. Importantly, germline or RTEC-specific *Cdkl5* deletion  
349 conferred significant protection from AKI. Primary RTECs with *Cdkl5* deficiency were also resistant to  
350 cellular injury. These studies suggest that *Cdkl5* is not required for normal renal development or function,  
351 however, under stress conditions, *Cdkl5* contributes to renal cell-death and dysfunction.

352 The CDKL-family shares structural features with CDKs (cyclin-dependent kinases) as well as  
353 MAPKs (mitogen-activated protein kinases) and GSKs (Glycogen synthase kinases)<sup>56</sup>. Although their  
354 nomenclature suggests similarity with CDKs, CDKLs have several features that distinguish them from  
355 CDKs, including the lack of evidence that CDKLs require cyclin binding, the presence of variant PSTAIRE  
356 motifs within the C-helix and large C-terminal regulatory domains with nuclear localization signals<sup>56</sup>.  
357 Moreover, there is no clear evidence that CDKLs are involved in cell cycle regulation. Interestingly, our  
358 studies suggest that *Cdkl5* might be a cell-cycle-independent stress-responsive kinase in RTECs, with  
359 much more functional similarity with MAPKs than CDKs. In support of this notion, our studies show *Cdkl5*  
360 activation under markedly distinct conditions of cellular stress both *in vitro* and *in vivo*. In this regard, *Cdkl5*  
361 seems to share functional similarities with MAPKs, which are known components of cellular stress  
362 response pathways<sup>61</sup>.

363 While the upstream signaling remains unknown, we have identified the transcription factor Sox9 as  
364 a bona fide *Cdkl5* substrate and a key downstream target in renal epithelial cells. The endogenous  
365 substrates of CDKL5 have been previously investigated to understand its function in neurons<sup>25,35-39</sup>.  
366 Whether these previously identified substrates are involved in *Cdkl5*-dependent renal cell-death remains  
367 unclear. However, through a pull-down experiment, we identified Sox9 as a *Cdkl5* substrate in RTECs.  
368 Sox9 is a transcription factor that controls cell-fate decisions during embryonic development and  
369 homeostasis of a broad range of adult tissues<sup>62-64</sup>. Moreover, in cancer cells, SOX9 inhibits apoptosis and

370 promotes proliferation, invasion, and metastasis<sup>65-67</sup>. Interestingly, two recent studies<sup>44,45</sup> have shown that  
371 transcriptional up-regulation of Sox9 is an early cellular response to renal injury and Sox9 is essential for  
372 repair and recovery post AKI. After the initial injury phase, Sox9 expressing renal epithelial cells play a  
373 crucial role in the subsequent repair processes. Here we show that renal tubule specific conditional Sox9  
374 knockout mice are hypersensitive to AKI, indicating that along with its role in recovery and repair, Sox9  
375 plays a pro-survival role in the early phase of AKI.

376 We also found that Cdkl5 phosphorylates Sox9 at Ser-199 residue during kidney injury *in vivo*.  
377 Cdkl5-mediated phosphorylation seems to reduce the stability of Sox9 protein. Indeed, while the injury-  
378 induced transcriptional up-regulation of Sox9 was similar in the control and *Cdkl5*-null mice, *Cdkl5* deletion  
379 in RTECs both hastened and markedly increased the accumulation of Sox9 protein (**Fig. 4**).  
380 Pharmacological inhibition of Cdkl5 kinase also resulted in increased accumulation of Sox9 during AKI  
381 (**Fig. 6**). Importantly, examination of the protein stability of various Sox9 mutants (S199A>WT>S199D)  
382 indicated that Sox9 phosphorylation at Ser-199 likely causes increased proteasomal degradation resulting  
383 in diminished half-life. However, we cannot rule out the possibility that Sox9 phosphorylation at Ser-199  
384 might have other biological effects, including changes in dimerization or altered binding to partner proteins.  
385 Ser-199 phosphorylation might also alter the affinity of Sox9 for target genes, a possibility that we cannot  
386 currently examine due to the inability to perform chromatin-immunoprecipitation with the phospho-Sox9  
387 antibody. However, these studies have revealed robust Cdkl5-dependent Sox9 phosphorylation in RTECs  
388 as part of cellular stress response to distinct injuries.

389 AKI is associated with a high risk for mortality, development of chronic kidney disease, and multi-  
390 organ dysfunction<sup>2,10</sup>. Currently, no specific treatments or prophylactic approaches are available to treat or  
391 prevent AKI. We provide proof-of-principle studies showing that targeted Cdkl5 inhibition can provide  
392 protection from renal injury. The small molecule Cdkl5-inhibitor AST-487 mitigated renal injury in multiple  
393 mouse models of AKI. While these studies provide promising proof-of-concept data, clinical translation of  
394 these studies would depend on the development and or identification of Cdkl5 inhibitors with much more  
395 specificity than AST-487. Our study also raises three important questions that require further exploration.  
396 Firstly, in adults, could systemic Cdkl5 inhibition cause toxicity in the central nervous system? While Cdkl5



397 is clearly important for early neuronal development, it is unclear if it has any essential function in the adult  
398 brain and so, it remains unknown whether short-term pharmacological Cdkl5 inhibition would have any  
399 CNS toxicities. However, we propose that the likelihood of any neuronal side effects could be easily  
400 reduced by selecting Cdkl5-inhibitors that do not cross the blood-brain barrier. Secondly, could systemic  
401 Cdkl5 inhibition cause toxicity in other peripheral organs or influence renal recovery, regeneration and  
402 fibrosis? Future studies would be required to examine these possibilities, however, we have found that  
403 Cdkl5 inhibition not just delays renal injury, but also confers long-term survival benefits, without overt  
404 systemic toxicities (**Supplementary Figure 21**). Thirdly, it would be critical to examine if Cdkl5-inhibition  
405 dependent Sox9 stabilization has any detrimental long-term effects in the kidneys.

406 Our study also raises the possibility that the Cdkl5-Sox9 axis might have important biological  
407 functions in other non-renal cell types, especially neurons and cancer cells. An essential question that  
408 merits further investigation is whether disruption of CDKL5-SOX9 axis underlie some of the neuronal  
409 phenotype observed in humans and mice with loss-of-function *CDKL5* mutations. Moreover, SOX9 has  
410 emerged as an essential regulator of cancer cell stem-ness, differentiation and apoptosis. We find that  
411 CDKL5 is widely expressed in cancer cell lines (**Supplementary Figure 27**); raising the possibility, that  
412 CDKL5 might regulate SOX9 function in cancer cells. CDKL5 might be a crucial nuclear kinase that  
413 suppresses SOX9 function under conditions of cellular stress. Future studies will likely provide insights into  
414 these important questions and provide a better understanding of the biological function of the enigmatic  
415 CDKL family of kinases.

416

417

## 418 **METHODS**

419 **Cell Culture and reagents.** Boston University mouse proximal tubule cells (BUMPT; clone 306; originally  
420 from Drs. Wilfred Lieberthal and John Schwartz, Boston University School of Medicine, Boston, MA and  
421 obtained from Dr. Zheng Dong, Augusta University, Augusta, GA) were grown at 37°C in Dulbecco's  
422 modified Eagle's medium with 10% fetal bovine serum (FBS). The human renal tubular cell line, HK-2 cells

423 (ATCC, CRL-2190) were grown in keratinocyte media (K-SFM) according to the provider's instructions.  
424 Protein kinase inhibitors were obtained from Sigma-Aldrich or Selleckchem. Radiolabelled compounds  
425 were obtained from American Radiochemicals or Moravek Biochemicals.

426 **Primary tubular cell culture and transduction.** Anti-GFP antibody and MACS columns (Miltenyi Biotec)  
427 were used to isolate GFP positive tubular epithelial cells. For primary cell culture, tubular epithelial cells  
428 were isolated from 6-8 weeks old male mice<sup>24</sup>. Briefly, mice were euthanized by carbon dioxide  
429 asphyxiation, kidneys were excised and renal cortical tissues were minced thoroughly and digested with  
430 0.75 mg/ml collagenase IV (Thermo Fisher Scientific). Renal tubular epithelial cells were then purified by  
431 centrifugation at 2,000 g for 10 min in DMEM/F-12 medium with 32% Percoll (Amersham). After washes  
432 with serum-free media, the cells were plated in collagen-coated dishes and cultured in DMEM/F-12  
433 medium supplemented with 5 µg/ml transferrin, 5 µg/ml insulin, 0.05 µM hydrocortisone, 50 µM vitamin C  
434 (Sigma-Aldrich). Fresh media was supplemented every alternate day and after 5–7 days of growth, the  
435 isolated proximal tubular cells were trypsinized and re-plated at  $1 \times 10^5$  cells per well in 24-well plates. For  
436 Cre mediated gene excision, cultured primary tubular cells were transduced with high titer ( $1 \times 10^8$  CFU/ml)  
437 LV-CMV-Cre-GFP lentivirus (Kerafast), followed by cisplatin treatment 48 hours later. Microscopic  
438 examinations were carried out to ensure that greater than 90% cells were GFP (Cre) positive before  
439 proceeding with cisplatin treatment. For Sox9 'add-back' experiments, proximal tubular cells from WT and  
440 Sox9<sup>PT-/-</sup> cells were transduced with either lentivirus (pLenti-C-Myc-DDK-P2A-Puro, Origene) encoding WT  
441 or Sox9 mutants (S199A and S199D). To induce cell death, primary RTECs were incubated with 50 µM  
442 cisplatin (Sigma-Aldrich) in fresh culture medium for 24 hours, followed by viability and caspase assays.

443 **siRNA kinome screening.** BUMPT cells were used for the siRNA kinome screening using methods  
444 similar to our previous study<sup>55</sup>. Briefly, the Dharmacon mouse siRNA library targeting protein kinases and  
445 related genes (780 genes) containing four pooled siRNAs for each gene was utilized in the primary screen.  
446 Briefly, the BUMPT cells were plated in 96-well plates and reverse transfected with 25 nM siRNA using  
447 Lipofectamine RNAiMAX reagent (Life Technologies). At 48 hours post-transfection, cells were treated with  
448 15 µM cisplatin in fresh media. Subsequently, 48 hours post-treatment, CellTiter-Glo luminescent cell  
449 viability assay (Promega) was carried out to determine cellular viability. The siRNAs that protected BUMPT

450 cells from cisplatin-induced cell death greater than the positive control (*Pkcδ* siRNA) were selected for  
451 secondary screening. The primary screen was carried out in triplicate samples and data analysis was  
452 performed according to established methods<sup>55</sup>.

453 **Cell Viability and Caspase assays.** Cellular viability was examined using three different assays, namely  
454 MTT, CellTiter-Glo, and trypan blue staining. MTT assays were performed using 3-(4,5-dimethylthiazol-2-  
455 yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma-Aldrich). BUMPT cells or RTECs were seeded  
456 in 96-well plates, followed by cisplatin treatment for 24-48 hours. After treatment, 10  $\mu$ L of MTT reagent (5  
457 mg/mL MTT in PBS) was added to each well and plates were incubated at 37°C with 5 % CO<sub>2</sub> for 4 hours,  
458 followed by addition of 100  $\mu$ l acidified isopropanol (Sigma-Aldrich) and measurement of absorbance at  
459 590 nm. The half maximal inhibitory concentration (IC<sub>50</sub>) was evaluated by nonlinear regression analysis  
460 using GraphPad Prism. Similar to MTT assays, CellTiter-Glo (Promega) assays were performed according  
461 to established methods followed by luminescence measurement. Cell viability was also measured by  
462 trypan blue exclusion method. Briefly, cell were harvested, followed by trypan blue staining and manual cell  
463 counting with a hemocytometer and/or by using Countess Automated Cell Counter (Thermo Fischer);  
464 translucent cells were considered as viable and blue-stained cells were counted as dead. Cell viability was  
465 calculated by dividing the number of viable cells by total cell number; each sample was done in triplicate.

466 Caspase activity was measured in cell lysates using an in vitro assay<sup>68</sup>. Briefly, RTECs were lysed in a  
467 buffer containing 1% Triton X-100 and 10  $\mu$ g protein from cell lysates was added to an enzymatic assay  
468 buffer containing 50  $\mu$ M DEVD-AFC for 60 minutes at 37°C. Fluorescence at excitation 360 nm/emission  
469 535 nm was measured and free AFC was used to plot a standard curve, and using the standard curve, the  
470 fluorescence reading from the enzymatic reaction was converted into the nM AFC liberated per mg protein  
471 per hour as a measure of caspase activity.

472 **Mice Breeding.** All animals were housed and handled in accordance with approved Institutional Animal  
473 Care and Use Committee procedures. All animal studies were conducted according to protocols approved  
474 by the Institutional Animal Care and Use Committees of The Ohio State University (2017R00000006). Mice  
475 used in the current study were housed in a temperature-controlled environment with a 12 hour light cycle

476 and given a standard diet and water ad libitum. Germline *Cdkl5*-deficient mice (stock no. 021967) were  
477 obtained from Jackson Laboratories and heterozygous mice were bred in-house to obtain wild-type and  
478 knock-out littermates. Conditional gene knock-out in renal tubular epithelial cells was achieved through  
479 breeding of *Cdkl5* floxed mice (Jackson Laboratory, stock no. 030523) and *Sox9* floxed mice (Jackson  
480 Laboratory, stock no. 013106) with *Ggt1-Cre* mice (Jackson Laboratory, stock no. 012841). Double  
481 Knockout mice (dKO<sup>PT</sup>) were generated by crossing Cre positive *Cdkl5* and *Sox9* floxed mice. mT/mG  
482 mice which express cell membrane-targeted, two-color fluorescent Cre-reporter allele were obtained from  
483 Jackson Laboratories (stock no. 007676). In these mice prior to Cre recombination, cell membrane-  
484 localized tdTomato (mT) fluorescence expression is widespread in cells/tissues and Cre recombinase  
485 expression induces cell membrane-localized EGFP (mG) fluorescence expression replacing the red  
486 fluorescence. The mT/mG mice were bred with *Ggt1-Cre* strain. For all mouse colonies, the pups were ear  
487 tagged and genotyped at 3 weeks of age.

488 ***Animal models of Acute Kidney Injury.*** For all experiments, age-matched (8–12 week) male or female  
489 mice were used. Littermates were used in studies with germline, mutant or conditional knockout mice. For  
490 experiments where only wild-type mice were used, 8- to 12-wk-old male C57BL/6J or FvB mice were  
491 obtained from Jackson Laboratories.

492 For cisplatin nephrotoxicity experiments, cisplatin (15-30 mg/kg) was administered by i.p. injection<sup>24</sup>.  
493 Optimal cisplatin dose was determined for each strain by dose-response experiments. After cisplatin  
494 injection, blood was collected on days 0–3 by submandibular vein bleed or on day 3 via cardiac puncture  
495 after carbon dioxide asphyxiation. Renal tissues were collected and processed for Western blot and  
496 histological analysis.

497 For ischemia-reperfusion experiments, mice were anesthetized by isoflurane and placed on a surgical  
498 platform where the body temperature was monitored throughout the procedure. The skin was disinfected,  
499 kidneys were exposed and bilateral renal pedicles were clamped for 28-35 minutes. Subsequently, the  
500 clamps were released to initiate the reperfusion followed by suturing to close the muscle and skin around  
501 the incision. To compensate for the fluid loss, 0.5 ml warm sterile saline was administered via intra-

502 peritoneal injection. Blood was collected on days 0–2 by submandibular vein bleed or on day 2 via cardiac  
503 puncture after carbon dioxide asphyxiation. Renal tissues were collected and processed for Western blot  
504 and histological analysis. For Cdk15 pharmacological inhibition studies, vehicle (1:10 v/v N-  
505 methylpyrrolidone/PEG300) or AST-487 were administered by oral gavage (25 mg/kg) six hours post-  
506 cisplatin injection or ischemic surgery.

507 To induce rhabdomyolysis, 8-12 weeks old male C57BL/6J mice were injected with 7.5 ml/kg 50% glycerol  
508 intramuscularly to the two hind-legs or injected with saline as a control, followed by blood and tissue  
509 collection on day 0-2. To induce folic acid (FA) mediated kidney injury, male FvB wild-type mice (~25 g, 10  
510 weeks old) were purchased from Jackson Laboratory and administered with FA (250 mg/kg, dissolved in  
511 300 mM NaHCO<sub>3</sub>) through intraperitoneal injection.

512 **Assessment of renal damage.** Renal damage was assessed by serum analysis (blood urea nitrogen and  
513 creatinine), histological examination (H&E staining) and analysis of renal expression of injury biomarkers  
514 (*Kim-1* and *Ngal*). Mouse blood samples were collected at indicated time-points, followed by blood urine  
515 nitrogen and creatinine measurement by QuantiChrom™ Urea Assay Kit (DIUR-100) and Creatinine  
516 Colorimetric Assay Kit (Cayman Chemical). For histological analysis, mouse kidneys were harvested and  
517 embedded in paraffin at indicated time-points before and after AKI induction. Tissue sections (5 μm) were  
518 stained with hematoxylin and eosin by standard methods. Histopathologic scoring was conducted by in a  
519 blinded fashion by examining ten consecutive 100x fields per section from at least three mice per group.  
520 Tubular damage was scored by calculation of the percentage of tubules that showed dilation, epithelium  
521 flattening, cast formation, loss of brush border and nuclei, and denudation of the basement membrane.  
522 The degree of tissue damage was scored based on the percentage of damaged tubules as previously<sup>24</sup>  
523 described: 0: no damage; 1: <25%; 2: 25–50%; 3: 50–75%; 4: >75%.

524 **Gene expression analysis.** Total RNA was extracted from cell lines and murine kidneys using the  
525 RNeasy Mini Kit (Qiagen). NanoDrop was used to measure RNA quality and quantity. 1 μg total RNA was  
526 then reverse transcribed using the high capacity cDNA Reverse Transcription Kit (Thermo Fischer  
527 Scientific). qPCR analysis was then performed using the SYBR green master mix with sequence-specific

528 predesigned primers (Sigma). The sequences of qPCR primers are shown in **Supplementary Table 2**. For  
529 quantitative analysis, target gene values were normalized to  $\beta$ -actin gene expression using the  $\Delta\Delta$ CT  
530 value method.

531 **Protein analysis.** Whole cell lysates from RTECs, cell lines and renal cortical tissues were made in  
532 modified RIPA buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na<sub>2</sub>EDTA, 1 mM EGTA, 1% NP-40,  
533 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, protease and phosphatase inhibitors)  
534 supplemented with 1% SDS. Cellular lysates for CDKL5 immunoprecipitation and kinase assay were made  
535 in modified RIPA buffer supplemented with 0.1% SDS. For co-immunoprecipitation experiments, cell  
536 lysates were made in modified RIPA buffer supplemented with 0.2%  $\beta$ -maltoside. Immunoprecipitations  
537 were carried out as described previously<sup>55</sup> using anti-FLAG (EZview Red ANTI-FLAG M2 Affinity Gel,  
538 Sigma-Aldrich), anti-CDKL5 (Millipore, MABS1132) and anti-SOX9 antibodies (Abcam, ab3697). Invitrogen  
539 Bis-tris gradient mini or midi-gels were used for western blot analysis, followed by detection by ECL  
540 reagent (Cell Signaling). Primary antibodies used for western blot analysis were from Cell Signaling: FLAG  
541 (14793), Histone H3 (4499), GAPDH (5174), and Santa Cruz Biotech:  $\beta$ -actin (47778), NGAL (50351),  
542 Myoferlin (376879), Sema3e (74554), Gadd45a (6850), Abcam: SOX9 (EPR14335-78), and CDKL5  
543 (ab22453). All primary antibodies were used at 1:1,000 dilution. Secondary antibodies were from Jackson  
544 Immunoresearch and used at 1:2,000 dilutions. Uncropped images of western blots are shown in **Source**  
545 **Data File**. Protein lysates used to determine CDKL5 expression in cancer cell lines were obtained from  
546 the DCTD Tumor Repository, National Cancer Institute at Frederick and the list of cell lines is provided in  
547 **Supplementary Table 3**.

548 **Protein kinase assay.** Protein kinase assays of purified proteins and immuno-precipitated kinases were  
549 carried by in vitro assays<sup>55,68</sup>. For assays with purified proteins, CDKL5 recombinant human protein was  
550 obtained from Life technologies (A30493). To purify Sox9 wild-type and mutant proteins, FLAG-tagged  
551 Sox9 constructs were sub-cloned into pT7CFE1-CHis plasmid (Thermo Fischer). These constructs were  
552 then used for *in vitro* translation using a HeLa cell lysate-based Kit (1-Step Human Coupled IVT Kit – DNA;  
553 88881, Life Technologies). The *in vitro* translated proteins were then purified using His Pur cobalt spin  
554 columns (Thermo Scientific). For *in vitro* kinase assays, recombinant CDKL5 and purified Sox9 proteins

555 were incubated in a kinase buffer (Cell Signaling, 9802) supplemented with [gamma-P32] Adenosine 5'-  
556 triphosphate (ATP) at 30°C for 30 min. After the incubation period, the reaction was terminated, followed by  
557 auto-radiographic examination of phosphorylated proteins and subsequent western blot analysis to  
558 determine the level of input proteins. For assays used to examine multiple kinase inhibitors, purified  
559 kinases (CDK2, CDK4, CDK6, and CDKL5) were incubated with 1 µM concentration of kinase inhibitors for  
560 30 minutes followed by kinase assays using ADP-Glo Kinase Assay kit (Promega).

561 Renal tissues and cells were lysed with a buffer containing 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1%  
562 (vol/vol) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 10  
563 µg/ml leupeptin, 10 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 50 mM NaF, 0.2% (wt/vol)  
564 dodecyl β-d-maltoside, and 20 mM Tris (pH 7.5). The soluble extracts were then subjected to Cdkl5  
565 immunoprecipitation. Briefly, 500 µg protein lysate was incubated with 2 µg IgG or anti-Cdkl5 antibody at  
566 4°C overnight, followed by addition of 30 µl of agarose protein A/G beads. Bead-bound immunoprecipitates  
567 were washed and collected by centrifugation. Immunoprecipitates were added to a protein kinase reaction  
568 buffer containing 20 µM ATP and myelin basic protein (Millipore) as substrate and incubated at 30°C for  
569 30 min. The ADP-Glo™ Kinase Assay (promega) kit was then used to measure kinase activity. This is a  
570 luminescent ADP detection assay that provides a method to measure kinase activity by quantifying the  
571 amount of ADP produced during a kinase reaction. After the reaction was terminated western blot analysis  
572 was carried out to determine the level of immunoprecipitated proteins. Relative kinase activity was  
573 calculated by normalizing the kinase activity (luminescence) to the amount of immunoprecipitated protein  
574 (densitometry of Cdkl5 signal). The specificity of Cdkl5 kinase assay was verified by conducting assays  
575 using wild type and *Cdkl5*<sup>-/-</sup> tissues, which demonstrated undetectable activity in the *Cdkl5* deficient tissues  
576 (**Supplementary Figure 19 a-b**).

577 **Mass spectrometry analysis.** Mass spectrometric analysis was performed at the Taplin Biological Mass  
578 Spectrometry Facility (Harvard University). Excised gel bands were cut into approximately 1 mm<sup>3</sup> pieces.  
579 Gel pieces were then subjected to a modified in-gel trypsin digestion procedure<sup>69</sup>. Gel pieces were washed  
580 and dehydrated with acetonitrile for 10 min. followed by removal of acetonitrile. Pieces were then  
581 completely dried in a speed-vac. Rehydration of the gel pieces was with 50 mM ammonium bicarbonate

582 solution containing 12.5 ng/μl modified sequencing-grade trypsin (Promega, Madison, WI) at 4°C. After 45  
583 min., the excess trypsin solution was removed and replaced with 50 mM ammonium bicarbonate solution  
584 to just cover the gel pieces. Samples were then placed in a 37°C room overnight. Peptides were later  
585 extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution  
586 containing 50% acetonitrile and 1% formic acid. The extracts were then dried in a speed-vac (~1 hr) and  
587 reconstituted in 5 - 10 μl of HPLC solvent A (2.5% acetonitrile, 0.1% formic acid). A nano-scale reverse-  
588 phase HPLC capillary column was created by packing 2.6 μm C18 spherical silica beads into a fused silica  
589 capillary (100 μm inner diameter x ~30 cm length) with a flame-drawn tip. After equilibrating the column  
590 each sample was loaded via a Famos auto sampler (LC Packings, San Francisco CA) onto the column. A  
591 gradient was formed and peptides were eluted with increasing concentrations of solvent B (97.5%  
592 acetonitrile, 0.1% formic acid). As peptides eluted they were subjected to electrospray ionization and then  
593 entered into an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo Fisher Scientific, Waltham,  
594 MA). Peptides were detected, isolated, and fragmented to produce a tandem mass spectrum of specific  
595 fragment ions for each peptide. The peptides were fragmented using CID (collision induced  
596 disassociation). A high resolution scan was done at 60,000 resolution, followed by 20 low-resolution  
597 MS/MS scans in the ion-trap. Peptide sequences (and protein identity) were determined by matching  
598 protein databases (Uniprot) with the acquired fragmentation pattern by the software program, Sequest  
599 Version 3.2 (ThermoFisher, San Jose, CA). The database was indexed based on a trypsin digestion, with  
600 two missed cleavages. Fixed modification of 57.0214 Da on cysteine (iodoacetamide) and a variable  
601 modification of 15.9949 Da on methionine were considered. The MS1 mass tolerance was 50 ppm and the  
602 MS2 tolerance was 1.0 Da. The peptide mass range used was 600–6000 Da. All accepted peptides have a  
603 cross-correlation (Xcorr) score of at least 0.5. All databases include a reversed version of all the  
604 sequences and the data was filtered to between a one and two percent peptide false discovery rate (FDR).  
605 For analysis, we applied a cutoff of five unique peptides per protein. The peptides used for identification of  
606 Sox9 are shown in **Supplementary Table 4**.

607 ***Generation of phospho-Ser-199-SOX9 and Phospho-Thr-169-Cdk15 antibodies.*** Phospho-specific  
608 antibodies was generated and characterized by established methods<sup>70</sup>. Briefly, the rabbit anti-phospho-



609 antibodies was generated by using the 118-day protocol (Covance). Peptide surrounding the Ser-199 of  
610 Sox9 and Thr-169 region of Cdkl5 was used for immunization. Immunoblot and ELISA-based method were  
611 used to test the bleeds for antibody production, followed by purification of phospho- antibody by affinity  
612 purification. The specificity of the purified antibody was confirmed *in vitro* kinase assays and tissues from  
613 knockout mice. De-phosphorylation assays were carried out by incubation of cell lysates with recombinant  
614 lambda phosphatase (New England Biolabs, P0753) at 30°C for 2 hours, followed by western blot analysis  
615 with phospho- and total Sox9 and Cdkl5 antibodies.

616 **Chromatin immunoprecipitation–qPCR.** Chromatin immunoprecipitation (ChIP) assays were performed  
617 using the Pierce Magnetic ChIP Kit according to the manufacturer’s instructions<sup>70</sup>. Briefly, cross-linking  
618 with 1% formaldehyde was carried out in RTECs or renal tissues, followed by quenching with glycine, cell  
619 harvesting and DNA fragmentation by sonication. Lysates were precleared for 1 hour with Protein A+G  
620 magnetic beads (EMD Millipore). Precleared lysates were then incubated with 5 µg of anti-SOX9  
621 antibodies (Abcam, ab3697) overnight at 4°C, followed by addition of Protein A+G magnetic beads and  
622 incubation for 4 hours at 4°C. Subsequently, the beads were repeatedly washed, followed by elution of the  
623 protein-DNA complexes, reversal of cross-links, and DNA purification. Standard qPCR analysis was then  
624 carried out using primers spanning the promoters of target genes. The sequences of primers are shown in  
625 **Supplementary Table 2.**

626 **Plasmids and site-directed mutagenesis.** The *Cdkl5* and *Sox9* plasmids with pCMV6-entry backbone  
627 were obtained from Origene. The QuikChange II XL Site-Directed Mutagenesis Kit (Agilent) was utilized to  
628 generate mutants, according to suggested methods. The QuikChange primer design program was  
629 employed to design mutagenesis primers<sup>55</sup>. Primers were synthesized by Integrated DNA Technologies.  
630 All constructs were sequenced to confirm successful mutagenesis. The mutagenesis primer sequences are  
631 shown in **Supplementary Table 2.**

632 **Promoter Luciferase Assay** HEK293 cells were stably transfected with either empty vector (pCMV6) or  
633 Sox9 expression vector (Origene). These cells were then utilized for promoter luciferase reporter assays<sup>70</sup>.  
634 Briefly,  $5 \times 10^3$  cells were plated overnight on white poly-l-lysine–coated 96-well plates, followed by

635 transient transfection with either promoter constructs (Switchgear Genomics, encoding 2kb sequence  
636 upstream of transcription start sites of following genes: Gadd45a, Wwp2, Sema3e and Myof) or empty  
637 promoter construct at 30 ng in combination with the Cypridina TK control construct (Switchgear Genomics)  
638 at 1 ng, according to the manufacturer's protocol (Switchgear Genomics, Lightswitch Dual Assay kit,  
639 DA010). The promoter construct encodes a Renilla luminescent reporter gene, called RenSP, while the  
640 transfection and normalization vector encodes a Cypridina luciferase. The Renilla luciferase activity was  
641 normalized with the Cypridina luciferase activity.

642 **Statistical considerations.** Data are presented as mean with s.e.m, unless stated otherwise. Statistical  
643 calculations (Student's *t*-test or analysis of variance) were carried out using GraphPad Prism.  $p < 0.05$  was  
644 considered statistically significant. To calculate statistical significance between two groups, two-tailed  
645 unpaired Student's *t* test was performed. One-way ANOVA followed by Tukey's or Dunnett's multiple-  
646 comparisons test was used for comparisons among three or more groups. For all the experimental data  
647 presented in the manuscript, no sample outliers were excluded.

#### 648 **DATA AVAILABILITY**

649 The source data underlying figures (1b, 1d, 1e-h, 2a-f, 2h-k, 2n, 3a-k, 4a-b, 4d-f, 5a-k, 6a-l, and 7a-g) and  
650 supplementary figures (1a-k, 2a-b, 4a-i, 5a-f, 6a-l, 7, 8a-c, 9a-b, 10a-d, aa, 12a-e, 13a-e, 14a-h, 15a-h, 16,  
651 17a-p, 18a-h, 19a-e, 20, 21a-b, 22a-f, 23a-g, 24a-g, 25a-g, 26a-c, and 27) are provided as a Source Data  
652 file. A reporting summary for this Article is available as a Supplementary Information file. All data  
653 supporting the findings of this study are available from the corresponding author on reasonable request.

#### 654 **ACKNOWLEDGEMENTS**

655 We thank Drs. Christopher Coss and Christina Drenberg (Ohio State University) for critical reading of the  
656 manuscript prior to submission. We thank Dr. Zheng Dong (Augusta University) for providing the BUMPT  
657 cell line, which was originally obtained from Drs. Wilfred Lieberthal and John Schwartz, Boston University  
658 School of Medicine, Boston, MA. We thank the DCTD Tumor Repository, National Cancer Institute at  
659 Frederick for providing the cellular lysates used for protein analysis in various cancer cell lines. This study  
660 was supported by funds from the Ohio State University Comprehensive Cancer Center, Pelotonia

661 foundation, American Heart Association (17SDG33440070) and National Cancer Institute (NCI R01  
662 CA215802). N.S.P was supported by a Scientist Development Grant from the American Heart Association.  
663 Y.B. was supported by a postdoctoral fellowship from American Heart Association.

664

## 665 **AUTHOR CONTRIBUTIONS**

666 N.S.P., J.Y.K., and Y.B. developed the concepts for the manuscript, designed and performed the  
667 experiments and analyzed the results. N.S.P., S.S.O., and T.C were involved with the kinome-wide siRNA  
668 screen. L.A.J carried out the experiments with GFP mice and carried out mouse colony management.  
669 M.J.F, A.K.P., M.P., J.Y.K., R.D.H., S.R.C., M.J.C., H.S., and N.P. performed, analyzed results and or  
670 provided expertise with cell viability, gene expression studies and bioinformatics analysis. S.R. and K.S.  
671 were involved with studies with folic acid mediated AKI and provided expertise with renal SOX9 regulation.  
672 Y.B., R.R., and R.G. performed experiments and or analyzed CDKL5 protein expression in cancer cell  
673 lines. N.S.P., M.J.F., and R.E.C. carried out histological analysis of kidney damage. D.S.G. was involved  
674 with the porcine model of AKI. S.D.B and A.S. provided reagents and expertise with pharmacology of  
675 kinase inhibitors. N.S.P. prepared the manuscript and all authors contributed to editing the paper.

676

## 677 **COMPETING INTERESTS**

678 The authors declare no competing interests.

## 679 **REFERENCES**

- 680 1. Smith, H. W. *From fish to philosopher; the story of our internal environment*. (1959).
- 681 2. Zuk, A. & Bonventre, J. V. Acute Kidney Injury. *Annu. Rev. Med.* **67**, 293–307 (2016).
- 682 3. Okubo, K. *et al.* Macrophage extracellular trap formation promoted by platelet activation is a key  
683 mediator of rhabdomyolysis-induced acute kidney injury. *Nat. Med.* **24**, 232–238 (2018).
- 684 4. Rosner, M. H. & Perazella, M. A. Acute Kidney Injury in Patients with Cancer. *New England Journal of*  
685 *Medicine* **376**, 1770–1781 (2017).

- 686 5. Schrier, R. W. & Wang, W. Acute renal failure and sepsis. *N. Engl. J. Med.* **351**, 159–169 (2004).
- 687 6. Lam, A. Q. & Humphreys, B. D. Onco-Nephrology: AKI in the Cancer Patient. *Clin J Am Soc Nephrol* **7**,
- 688 1692–1700 (2012).
- 689 7. Linkermann, A. *et al.* Regulated cell death in AKI. *J. Am. Soc. Nephrol.* **25**, 2689–2701 (2014).
- 690 8. Bellomo, R., Kellum, J. A. & Ronco, C. Acute kidney injury. *Lancet* **380**, 756–766 (2012).
- 691 9. Murugan, R. & Kellum, J. A. Acute kidney injury: what's the prognosis? *Nat Rev Nephrol* **7**, 209–217
- 692 (2011).
- 693 10. Chawla, L. S., Eggers, P. W., Star, R. A. & Kimmel, P. L. Acute kidney injury and chronic kidney
- 694 disease as interconnected syndromes. *N. Engl. J. Med.* **371**, 58–66 (2014).
- 695 11. Bock, J. S. & Gottlieb, S. S. Cardiorenal syndrome: new perspectives. *Circulation* **121**, 2592–2600
- 696 (2010).
- 697 12. Bonventre, J. V. & Yang, L. Cellular pathophysiology of ischemic acute kidney injury. *J. Clin.*
- 698 *Invest.* **121**, 4210–4221 (2011).
- 699 13. Li, L. & Okusa, M. D. Macrophages, dendritic cells, and kidney ischemia-reperfusion injury. *Semin.*
- 700 *Nephrol.* **30**, 268–277 (2010).
- 701 14. Ramesh, G. & Reeves, W. B. TNF-alpha mediates chemokine and cytokine expression and renal
- 702 injury in cisplatin nephrotoxicity. *J. Clin. Invest.* **110**, 835–842 (2002).
- 703 15. Ferenbach, D. A. & Bonventre, J. V. Kidney tubules: intertubular, vascular, and glomerular cross-
- 704 talk. *Curr. Opin. Nephrol. Hypertens.* **25**, 194–202 (2016).
- 705 16. Hopkins, A. L. & Groom, C. R. The druggable genome. *Nature Reviews Drug Discovery* **1**, 727–
- 706 730 (2002).
- 707 17. Manning, G., Whyte, D. B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase
- 708 complement of the human genome. *Science* **298**, 1912–1934 (2002).
- 709 18. Levitzki, A. Tyrosine kinase inhibitors: views of selectivity, sensitivity, and clinical performance.
- 710 *Annu. Rev. Pharmacol. Toxicol.* **53**, 161–185 (2013).
- 711 19. Gross, S., Rahal, R., Stransky, N., Lengauer, C. & Hoeflich, K. P. Targeting cancer with kinase
- 712 inhibitors. *J. Clin. Invest.* **125**, 1780–1789 (2015).

- 713 20. Montini, E. *et al.* Identification and characterization of a novel serine-threonine kinase gene from  
714 the Xp22 region. *Genomics* **51**, 427–433 (1998).
- 715 21. Kalscheuer, V. M. *et al.* Disruption of the serine/threonine kinase 9 gene causes severe X-linked  
716 infantile spasms and mental retardation. *Am. J. Hum. Genet.* **72**, 1401–1411 (2003).
- 717 22. Tao, J. *et al.* Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene are  
718 associated with severe neurodevelopmental retardation. *Am. J. Hum. Genet.* **75**, 1149–1154 (2004).
- 719 23. Pabla, N. & Dong, Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney*  
720 *Int.* **73**, 994–1007 (2008).
- 721 24. Pabla, N. *et al.* Inhibition of PKC $\delta$  reduces cisplatin-induced nephrotoxicity without blocking  
722 chemotherapeutic efficacy in mouse models of cancer. *J. Clin. Invest.* **121**, 2709–2722 (2011).
- 723 25. Muñoz, I. M. *et al.* Phosphoproteomic screening identifies physiological substrates of the CDKL5  
724 kinase. *EMBO J.* **37**, (2018).
- 725 26. Bahi-Buisson, N. *et al.* Recurrent mutations in the CDKL5 gene: genotype-phenotype relationships.  
726 *Am. J. Med. Genet. A* **158A**, 1612–1619 (2012).
- 727 27. Wang, I.-T. J. *et al.* Loss of CDKL5 disrupts kinome profile and event-related potentials leading to  
728 autistic-like phenotypes in mice. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 21516–21521 (2012).
- 729 28. Hector, R. D. *et al.* Characterisation of CDKL5 Transcript Isoforms in Human and Mouse. *PLoS*  
730 *ONE* **11**, e0157758 (2016).
- 731 29. Bertani, I. *et al.* Functional Consequences of Mutations in CDKL5, an X-linked Gene Involved in  
732 Infantile Spasms and Mental Retardation. *J. Biol. Chem.* **281**, 32048–32056 (2006).
- 733 30. de Caestecker, M. *et al.* Bridging Translation by Improving Preclinical Study Design in AKI. *J. Am.*  
734 *Soc. Nephrol.* **26**, 2905–2916 (2015).
- 735 31. Ichimura, T. *et al.* Kidney Injury Molecule-1 (KIM-1), a Putative Epithelial Cell Adhesion Molecule  
736 Containing a Novel Immunoglobulin Domain, Is Up-regulated in Renal Cells after Injury. *J. Biol. Chem.*  
737 **273**, 4135–4142 (1998).
- 738 32. Paragas, N. *et al.* The Ngal reporter mouse detects the response of the kidney to injury in real time.  
739 *Nat. Med.* **17**, 216–222 (2011).

- 740 33. Gardner, D. S. *et al.* Remote effects of acute kidney injury in a porcine model. *Am. J. Physiol.*  
741 *Renal Physiol.* **310**, F259-271 (2016).
- 742 34. Iwano, M. *et al.* Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J. Clin.*  
743 *Invest.* **110**, 341–350 (2002).
- 744 35. Mari, F. *et al.* CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for  
745 the early-onset seizure variant of Rett syndrome. *Hum. Mol. Genet.* **14**, 1935–1946 (2005).
- 746 36. Ricciardi, S. *et al.* CDKL5 ensures excitatory synapse stability by reinforcing NGL-1-PSD95  
747 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. *Nat. Cell*  
748 *Biol.* **14**, 911–923 (2012).
- 749 37. Zhu, Y.-C. *et al.* Palmitoylation-dependent CDKL5-PSD-95 interaction regulates synaptic targeting  
750 of CDKL5 and dendritic spine development. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 9118–9123 (2013).
- 751 38. Kameshita, I. *et al.* Cyclin-dependent kinase-like 5 binds and phosphorylates DNA  
752 methyltransferase 1. *Biochem. Biophys. Res. Commun.* **377**, 1162–1167 (2008).
- 753 39. Baltussen, L. L. *et al.* Chemical genetic identification of CDKL5 substrates reveals its role in  
754 neuronal microtubule dynamics. *EMBO J.* **37**, (2018).
- 755 40. Jo, A. *et al.* The versatile functions of Sox9 in development, stem cells, and human diseases.  
756 *Genes Dis* **1**, 149–161 (2014).
- 757 41. Larsimont, J.-C. *et al.* Sox9 Controls Self-Renewal of Oncogene Targeted Cells and Links Tumor  
758 Initiation and Invasion. *Cell Stem Cell* **17**, 60–73 (2015).
- 759 42. Akiyama, H., Chaboissier, M.-C., Martin, J. F., Schedl, A. & de Crombrughe, B. The transcription  
760 factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is  
761 required for expression of Sox5 and Sox6. *Genes Dev.* **16**, 2813–2828 (2002).
- 762 43. Hornbeck, P. V. *et al.* PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids*  
763 *Res.* **43**, D512-520 (2015).
- 764 44. Kumar, S. *et al.* Sox9 Activation Highlights a Cellular Pathway of Renal Repair in the Acutely  
765 Injured Mammalian Kidney. *Cell Rep* **12**, 1325–1338 (2015).

- 766 45. Kang, H. M. *et al.* Sox9-Positive Progenitor Cells Play a Key Role in Renal Tubule Epithelial  
767 Regeneration in Mice. *Cell Rep* **14**, 861–871 (2016).
- 768 46. Kadaja, M. *et al.* SOX9: a stem cell transcriptional regulator of secreted niche signaling factors.  
769 *Genes Dev.* **28**, 328–341 (2014).
- 770 47. Liu, J. *et al.* Molecular characterization of the transition from acute to chronic kidney injury following  
771 ischemia/reperfusion. *JCI Insight* **2**, (2017).
- 772 48. Maddika, S. *et al.* WWP2 is an E3 ubiquitin ligase for PTEN. *Nat. Cell Biol.* **13**, 728–733 (2011).
- 773 49. Davis, D. B., Delmonte, A. J., Ly, C. T. & McNally, E. M. Myoferlin, a candidate gene and potential  
774 modifier of muscular dystrophy. *Hum. Mol. Genet.* **9**, 217–226 (2000).
- 775 50. Rademaker, G. *et al.* Myoferlin controls mitochondrial structure and activity in pancreatic ductal  
776 adenocarcinoma, and affects tumor aggressiveness. *Oncogene* **37**, 4398–4412 (2018).
- 777 51. Eissa, N. *et al.* Semaphorin 3E regulates apoptosis in the intestinal epithelium during the  
778 development of colitis. *Biochem. Pharmacol.* **166**, 264–273 (2019).
- 779 52. Davis, M. I. *et al.* Comprehensive analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **29**, 1046–  
780 1051 (2011).
- 781 53. Akeno-Stuart, N. *et al.* The RET kinase inhibitor NVP-AST487 blocks growth and calcitonin gene  
782 expression through distinct mechanisms in medullary thyroid cancer cells. *Cancer Res.* **67**, 6956–6964  
783 (2007).
- 784 54. Li, J. *et al.* A chemical and phosphoproteomic characterization of dasatinib action in lung cancer.  
785 *Nat. Chem. Biol.* **6**, 291–299 (2010).
- 786 55. Sprowl, J. A. *et al.* A phosphotyrosine switch regulates organic cation transporters. *Nat Commun* **7**,  
787 10880 (2016).
- 788 56. Canning, P. *et al.* CDKL Family Kinases Have Evolved Distinct Structural Features and Ciliary  
789 Function. *Cell Rep* **22**, 885–894 (2018).
- 790 57. Weaving, L. S. *et al.* Mutations of CDKL5 cause a severe neurodevelopmental disorder with  
791 infantile spasms and mental retardation. *Am. J. Hum. Genet.* **75**, 1079–1093 (2004).

- 792 58. Lin, C., Franco, B. & Rosner, M. R. CDKL5/Stk9 kinase inactivation is associated with neuronal  
793 developmental disorders. *Hum. Mol. Genet.* **14**, 3775–3786 (2005).
- 794 59. Huppke, P., Ohlenbusch, A., Brendel, C., Laccone, F. & Gärtner, J. Mutation analysis of the HDAC  
795 1, 2, 8 and CDKL5 genes in Rett syndrome patients without mutations in MECP2. *Am. J. Med. Genet. A*  
796 **137**, 136–138 (2005).
- 797 60. Evans, J. C. *et al.* Early onset seizures and Rett-like features associated with mutations in CDKL5.  
798 *Eur. J. Hum. Genet.* **13**, 1113–1120 (2005).
- 799 61. Zhang, W. & Liu, H. T. MAPK signal pathways in the regulation of cell proliferation in mammalian  
800 cells. *Cell Res.* **12**, 9–18 (2002).
- 801 62. Prior, H. M. & Walter, M. A. SOX genes: architects of development. *Mol. Med.* **2**, 405–412 (1996).
- 802 63. Koopman, P. Sry and Sox9: mammalian testis-determining genes. *Cell. Mol. Life Sci.* **55**, 839–856  
803 (1999).
- 804 64. Barrionuevo, F. & Scherer, G. SOX E genes: SOX9 and SOX8 in mammalian testis development.  
805 *Int. J. Biochem. Cell Biol.* **42**, 433–436 (2010).
- 806 65. Tsuda, M. *et al.* The BRG1/SOX9 axis is critical for acinar cell-derived pancreatic tumorigenesis. *J.*  
807 *Clin. Invest.* **128**, 3475–3489 (2018).
- 808 66. Zhu, Z., Dai, J., Liao, Y. & Wang, T. Sox9 Protects against Human Lung Fibroblast Cell Apoptosis  
809 Induced by LPS through Activation of the AKT/GSK3 $\beta$  Pathway. *Biochemistry Mosc.* **82**, 606–612  
810 (2017).
- 811 67. Kawaguchi, Y. Sox9 and programming of liver and pancreatic progenitors. *J. Clin. Invest.* **123**,  
812 1881–1886 (2013).
- 813 68. Wang, J. *et al.* Caspase-mediated cleavage of ATM during cisplatin-induced tubular cell apoptosis:  
814 inactivation of its kinase activity toward p53. *Am. J. Physiol. Renal Physiol.* **291**, F1300–1307 (2006).
- 815 69. Shevchenko, A., Wilm, M., Vorm, O. & Mann, M. Mass spectrometric sequencing of proteins silver-  
816 stained polyacrylamide gels. *Anal. Chem.* **68**, 850–858 (1996).
- 817 70. van Oosterwijk, J. G. *et al.* Hypoxia-induced upregulation of BMX kinase mediates therapeutic  
818 resistance in acute myeloid leukemia. *J. Clin. Invest.* **128**, 369–380 (2018).



819

820

821

822

823

824

825

826

827

## 828 **FIGURE LEGENDS**

829 **Figure 1: A Kinome-wide screen uncovers protein kinases involved in RTEC cell-death.** (a) Scheme  
830 depicting the assay conditions used in the primary siRNA screen. BUMPT cells were transfected with  
831 Kinome-wide siRNA library (Dharmacon), followed by cisplatin treatment and cell-titer-glo based viability  
832 assay. (b) Results of primary RNAi screening, shown by plotting the relative survival post-cisplatin  
833 treatment of individual siRNA-targeted genes obtained from triplicate samples. (c) Kinome map (KinMap)  
834 depicting kinases identified in the primary screen. (d) Validation of primary hits by distinct siRNAs (Sigma)  
835 in BUMPT cells. Survival data (MTT assay) are presented as individual data points (n = 4 biologically  
836 independent samples), from one out of three independent experiments, all producing similar results. (e)  
837 Further secondary screening was carried out in HK-2 cells, by RNAi mediated knockdown of indicates  
838 genes, followed by MTT-based cellular viability assay. Data are presented as individual data points (n = 4  
839 biologically independent samples), from one out of three independent experiments, all producing similar  
840 results. (f) Schematic representation of CDKL5, the top hit and other members of CMGC kinase family. (g-  
841 h) Tertiary screening was carried for the top hit (*Cdkl5*) by shRNA mediated knockdown in BUMPT cells  
842 and 'add back' of wild-type and mutant *Cdkl5*. Cellular viability assays (MTT) showed that shRNA mediated

843 *Cdkl5* knockdown protects BUMPT cells from cisplatin-mediated cell-death, an effect that was reversed by  
844 re-introduction of wild-type but not mutant *Cdkl5*. Data are presented as individual data points (n = 4  
845 biologically independent samples), from one out of three independent experiments, all producing similar  
846 results. Representative western blot results demonstrating shRNA mediated CDKL5 kinase knockdown  
847 and introduction of un-tagged wild-type, kinase dead (KD), and TEY/AEF *Cdkl5* constructs. Data is  
848 representative of three independent experiments. In all the bar graphs, experimental values are presented  
849 as mean  $\pm$  s.e.m. The height of error bar=1 s.e. and  $p < 0.05$  was indicated as statistically significant. 1-way  
850 ANOVA followed by Dunnett's (d and e) or Tukey's multiple-comparisons test (h) was carried out and  
851 statistical significance is indicated by \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data are provided as a  
852 Source Data file.

853

854 **Figure 2: CDKL5 activity increases in renal tubular epithelial cells during AKI.** (a-c) Bilateral renal  
855 ischemia was induced in male wild-type (C57BL/6) mice for 30 minutes followed by reperfusion for  
856 indicated time-points. Blood urea nitrogen, serum creatinine and histological analysis (H&E staining) were  
857 used to examine renal function and damage. (d-f) C57BL/6 mice were treated with cisplatin (30 mg/kg,  
858 intra-peritoneal injection) and BUN, serum creatinine and histological analysis were conducted at the  
859 indicated time-points. (g) Representative H&E staining depicting tubular damage (indicated by asterisk) in  
860 both ischemic and cisplatin treated mice. The graphs (a-f) represent data from a single experiment (n = 5  
861 biologically independent samples), from one out of three independent experiments, all producing similar  
862 results. (h) Renal tissues from control, ischemic and cisplatin treated mice were used for western blot  
863 analysis of indicated proteins. Data presented is representative of five independent experiments, which  
864 yielded similar results. (i-k) *Cdkl5* was immuno-precipitated from the kidneys of control, ischemic and  
865 cisplatin treated mice, followed by *in vitro* kinase assays. The representative western blots show the levels  
866 of *Cdkl5* immuno-precipitated from tissue samples. The graphs represent data from a single experiment  
867 (n = 6 biologically independent samples), from one out of four independent experiments, all producing  
868 similar results. (l) *Ggt1-Cre* mice were crossed with *ROSA<sup>mT/mG</sup>* mice to generate transgenic mice that  
869 express membrane localized EGFP in renal tubular epithelial cells. Representative image shows EGFP

870 expression in renal tubular cells. Arrows with dotted lines indicate tubular cells, while arrows with solid line  
871 shows the glomerulus. (m) Schematic representation of procedure used to isolate EGFP positive renal  
872 epithelial cells. (n) Cdkl5 immunoprecipitation and *in vitro* kinase assay from indicated cells. The graph  
873 (n=4) is representative of two independent experiments. In all the bar graphs, experimental values are  
874 presented as mean  $\pm$  s.e.m. The height of error bar=1 s.e. and  $p < 0.05$  was indicated as statistically  
875 significant. 1-way ANOVA followed by Dunnett's (a-f and i-j) or Tukey's multiple-comparisons test (n) was  
876 carried out and statistical significance is indicated by \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Scale bar (g & i):  
877 100  $\mu$ m. Source data are provided as a Source Data file.

878

879

880 **Figure 3: RTEC specific Cdkl5 deletion provides protection from AKI.** To generate mice with RTEC  
881 specific *Cdkl5* knockout, *Ggt1-Cre* mice were crossed with *Cdkl5* floxed mice. (a) Representative western  
882 blots showing successful knockout in the renal tissues. Littermate control and *Cdkl5* conditional knockout  
883 male mice (indicated by *Cdkl5*<sup>PT-ly</sup>) were then challenged with bilateral renal ischemia or cisplatin  
884 treatment. Bilateral renal ischemia was induced in wild-type and *Cdkl5*<sup>PT-ly</sup> mice for 30 minutes followed by  
885 examination of renal structure and function. (b) Blood urea nitrogen (c) Serum creatinine (d) renal *Kim1*  
886 mRNA expression (e) renal histological analysis (H&E) showed that tubular epithelial-specific *Cdkl5*  
887 deficiency confers protection from ischemia-associated AKI. Data presented (b-e) is cumulative of two  
888 independent experiment (n=6). Wild-type and *Cdkl5*<sup>PT-/-</sup> mice were treated with cisplatin (25 mg/kg)  
889 followed by examination of renal function. (f) Blood urea nitrogen (g) Serum creatinine (h) renal *Kim1*  
890 mRNA expression (i) renal histological analysis (H&E) showed that *Cdkl5* contributes to cisplatin-mediated  
891 AKI. Data presented (f-i) is cumulative of two out of four independent experiment (n=8), that showed  
892 similar results. (j) Primary renal tubular cells were cultured from female wild-type and *Cdkl5* floxed mice.  
893 One week later, lentiviral transductions (Cre) were carried out to ablate *Cdkl5* gene. Western blot analysis  
894 confirmed CDKL5 ablation. Blots are representative of two independent experiments. (k) Primary renal  
895 tubular cells with indicated genotype were treated with 50  $\mu$ M Cisplatin, followed by cell viability

896 assessment using trypan blue staining. Data are presented as individual data points (n = 4 biologically  
897 independent samples), from one out of three independent experiments, all producing similar results. In all  
898 the bar graphs, experimental values are presented as mean  $\pm$  s.e.m. The height of error bar=1 s.e. and  
899  $p < 0.05$  was indicated as statistically significant. 1-way ANOVA followed by Tukey's multiple-comparisons  
900 test was carried out and statistical significance is indicated by \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source  
901 data are provided as a Source Data file.

902

903

904

905

906 **Figure 4: Cdkl5 phosphorylates Sox9 at Serine 199 site.** (a) Bilateral renal ischemia was induced in  
907 C57BL/6 mice for 30 minutes followed by reperfusion for one day. Renal cortical lysates were then used to  
908 immunoprecipitate Cdkl5, while IgG was used as negative control. Immunoprecipitates were then run on a  
909 4-12% gradient SDS-PAGE gel followed by protein visualization with SYPRO Ruby Protein Gel Stain. The  
910 ~65 Kda Cdkl5-interacting protein was then identified by mass spectrometric analysis as Sox9 as  
911 described in the Methods section (b) Purified wild-type Cdkl5 and wild-type and mutant Sox9 proteins were  
912 co-incubated in a kinase assay buffer with [ $\gamma$ - $^{32}$ P]-ATP for 30 minutes. Samples were then run on  
913 SDS-PAGE gel followed by transfer to PVDF membrane. Radiolabeled Sox9 was examined by  
914 autoradiography, followed by western blot analysis to examine the input proteins. Blots are representative  
915 of two independent experiments. (c) Schematic representation of Sox9 protein (modified from Ref. 64).  
916 Protein sequence analysis showed that the sequence surrounding the Ser-199 site is highly conserved.  
917 HMG, indicates high mobility group box DNA binding domain, CD, indicates Conserved domain and, PQA  
918 indicates proline-glutamine-alanine rich domain. (d) Control, cisplatin and ischemic renal tissues from  
919 control and *Cdkl5*<sup>PT-ly</sup> mice were subjected to immunoblot analysis of indicated proteins. Blots are  
920 representative of at least three independent experiments. (e-f) Densitometric analysis of Sox9 and p-Ser-  
921 199 Sox9 protein levels. Graph represents cumulative results (n=5 independent biological samples) from

922 three independent experiments. Densitometric analysis was carried out using Image J and the signals of  
923 indicated proteins were normalized by actin levels in the same samples. In all the bar graphs, experimental  
924 values are presented as mean  $\pm$  s.e.m. The height of error bar=1 s.e. and  $p < 0.05$  was indicated as  
925 statistically significant. 1-way ANOVA followed by Tukey's multiple-comparisons test was carried out and  
926 statistical significance is indicated by \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data are provided as a  
927 Source Data file.

928

929

930

931

932

933 **Figure 5: SOX9 plays a protective role during AKI.** To generate mice with renal tubule specific Sox9  
934 knockout, *Ggt1-Cre* mice were crossed with Sox9 floxed mice. (a) Representative western blots showing  
935 successful knockout in the renal tissues. Littermate control and Sox9 conditional knockout mice (indicated  
936 by Sox9<sup>PT-/-</sup>) were used to study the role of SOX9 in AKI. Bilateral renal ischemia was induced in wild-type  
937 and Sox9<sup>PT-/-</sup> mice for 30 minutes followed by examination of renal structure and function. (b) Blood urea  
938 nitrogen (c) Serum creatinine (d) renal *Kim1* mRNA expression (e) renal histological analysis (H&E)  
939 showed that tubular epithelial-specific Sox9 deficiency exacerbates ischemia-associated AKI. Data  
940 presented (b-e) is cumulative of three independent experiment (n=6-7). Wild-type and Sox9<sup>PT-/-</sup> mice were  
941 treated with cisplatin (30 mg/kg) followed by examination of renal function. (f) Blood urea nitrogen (g)  
942 Serum creatinine (h) renal *Kim1* mRNA expression (i) renal histological analysis (H&E) showed that SOX9  
943 regulates cisplatin-mediated AKI. Data presented (f-i) is cumulative of two out of four independent  
944 experiment (n=8), that showed similar results. (j) Primary renal tubular cells were cultured from wild-type  
945 and Sox9 floxed mice. One week later, lentiviral transductions (Cre) were carried out to delete Sox9 gene.  
946 Western blot analysis confirmed SOX9 deletion. Blots are representative of two independent experiments.

947 (k) Primary renal tubular cells with indicated genotype were treated with 50  $\mu$ M Cisplatin, followed by cell  
948 viability assessment using trypan blue staining. Data are presented as individual data points (n=4  
949 biologically independent samples), from one out of three independent experiments, all producing similar  
950 results. In all the bar graphs, experimental values are presented as mean  $\pm$  s.e.m. The height of error  
951 bar=1 s.e. and p<0.05 was indicated as statistically significant. 1-way ANOVA followed by Tukey's  
952 multiple-comparisons test was carried out and statistical significance is indicated by \*p < 0.05, \*\*p < 0.01,  
953 \*\*\*p < 0.001. Source data are provided as a Source Data file.

954

955

956

957

958

959 **Figure 6. A small molecule Cdkl5 inhibitor mitigates AKI.** (a) *In vitro* kinase assays were carried out  
960 for cell cycle-related kinases and CDKL5 for the indicated inhibitors at a single concentration of 1 $\mu$ M.  
961 Kinase activity is presented as a heat map, where blue indicates no inhibition (high kinase activity), while  
962 red indicates kinase inhibition (low kinase activity). AST-487 was found to inhibit CDKL5, without affecting  
963 the activity of cell cycle related kinases. Data presented here is the mean of three independent  
964 experiments. (b) C57BL/6 mice were treated with either vehicle or AST-487 through oral administration  
965 followed by examination of Cdkl5 activity in renal tissues. Data are presented as individual data points  
966 (n=5 biologically independent samples), from one out of two independent experiments, all producing  
967 similar results. (c-e) Bilateral renal ischemia was induced in wild-type C57BL/6 mice for 30 minutes  
968 followed by reperfusion for indicated time-points. Mice were treated with either vehicle or AST-487 (25  
969 mg/kg, oral gavage) 6 hours post-ischemia, followed by assessment of renal function and damage. (c)  
970 Blood urea nitrogen (d) Serum creatinine (e) renal histological analysis (H&E) Data presented (c-e) are  
971 cumulative of three independent experiment (n=8). (f-h) Wild-type C57BL/6 mice were injected with

972 cisplatin (30 mg/kg, i.p.) followed by treatment with either vehicle or AST-487 (25 mg/kg, oral gavage) 6  
973 hours later, followed by assessment of renal function and damage at indicated time-points. Data presented  
974 (e-h) are cumulative of two out of four independent experiment (n=8), that showed similar results. (i)  
975 Western blot analysis of renal tissues indicated that AST-487 suppress Sox9 phosphorylation and  
976 increases Sox9 stability *in vivo*. Blots are representative of three independent experiments. In all the bar  
977 graphs, experimental values are presented as mean  $\pm$  s.e.m. The height of error bar=1 s.e. and  $p < 0.05$   
978 was indicated as statistically significant. 1-way ANOVA followed by Dunnett's (b) or Tukey's multiple-  
979 comparisons test (c-h) was carried out and statistical significance is indicated by \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p$   
980  $< 0.001$ . Source data are provided as a Source Data file.

981

982

983

984

985

986 **Figure 7: Cdkl5 regulates AKI in a Sox9 dependent and independent manner.** Bilateral renal ischemic  
987 surgery was carried out in littermate control and Sox9<sup>PT-/-</sup> mice, followed by administration of either vehicle  
988 or AST-487 (25 mg/kg, oral gavage, 6 hours post-IR). At 48 hours renal function and damage were  
989 assessed through measurement of (a) Blood urea nitrogen (b) Serum creatinine and (c) renal histological  
990 analysis (H&E). Age-matched WT, Cdkl5<sup>PT-/y</sup>, Sox9<sup>PT-/-</sup>, and Cdkl5<sup>PT-/y</sup> Sox9<sup>PT-/-</sup> (double knock out mice  
991 indicated as dKO<sup>PT</sup>) underwent bilateral renal ischemia for 30 minutes, followed by (d) Western blot  
992 analysis of renal tissues at 24 hours post-reperfusion (one out of two independent experiments) and  
993 assessment of renal structure and function at 48 hours through measurement of (e) Blood urea nitrogen (f)  
994 Serum creatinine and (g) renal histological analysis (H&E). Data presented (a-c, e-g) are cumulative of  
995 three independent experiment (n=6). In all the bar graphs, experimental values are presented as mean  $\pm$   
996 s.e.m. The height of error bar=1 s.e. and  $p < 0.05$  was indicated as statistically significant. 1-way ANOVA

997 followed by Tukey's multiple-comparisons test was carried out and statistical significance is indicated by \*p  
998 < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. Source data are provided as a Source Data file.

999

1000

1001















