Spiny mice activate unique transcriptional programs after severe kidney injury regenerating organ function without fibrosis

Highlights

Acomys fully regenerate kidney structure and function without fibrosis after injury

Unique gene clusters rapidly activated in surviving cells align with regeneration

Acomys genome appears poised at the time of kidney injury to initiate regeneration

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Spiny mice activate unique transcriptional programs after severe kidney injury regenerating organ function without fibrosis

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SUMMARY
Fibrosis-driven solid organ failure is an enormous burden on global health. Spiny mice (Acomys) are terrestrial mammals that can regenerate severe skin wounds without scars to avoid predation. Whether spiny mice also regenerate internal organ injuries is unknown. Here, we show that despite equivalent acute obstructive or ischemic kidney injury, spiny mice fully regenerate nephron structure and organ function without fibrosis, whereas C57Bl/6 or CD1 mice progress to complete organ failure with extensive renal fibrosis. Two mechanisms for vertebrate regeneration have been proposed that emphasize either extrinsic (pro-regenerative macrophages) or intrinsic (surviving cells of the organ itself) controls. Comparative transcriptome analysis revealed that the Acomys genome appears poised at the time of injury to initiate regeneration by surviving kidney cells, whereas macrophage accumulation was not detected until about day 7. Thus, we provide evidence for rapid activation of a gene expression signature for regenerative wound healing in the spiny mouse kidney.

INTRODUCTION
Solid organ failure is the result of dysregulated wound healing that leads to progressive loss of tissue function, fibrosis, and eventual organ failure (Duffield et al., 2013; Humphreys, 2018). The global health burden for loss of vital organ function due to progressive fibrosis is enormous (Gurtner et al., 2008). There are currently very few treatment options for patients with end-stage kidney disease or similar degenerative fibrotic conditions of the heart, lungs, liver, or reproductive organs (Hill et al., 2016; Rockey et al., 2015). Looking at nature for a possible solution, it was reported that adult rodents of the genus Acomys (spiny mice) can shed their dorsal skin as a deterrent to avoid predators and fully regenerate the lost tissue (Seifert et al., 2012). This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
unprecedented findings for adult mammalian kidney represent the first step to understand how wound healing evolved to be uncoupled from a robust fibrotic response to injury and redirected toward regeneration/complete repair of complex vital organ function in a terrestrial mammal, the spiny mouse.

RESULTS

Spiny mice fail to develop fibrosis after UUO injury

Tubulointerstitial fibrosis is the final common pathway of many forms of kidney disease (Duffield et al., 2013; Duffield, 2014; Humphreys, 2018). UUO is a reliable and aggressive model of chronic kidney injury that produces robust interstitial fibrosis. In previously reported studies where the contralateral kidney was removed after 7 days of obstruction in Mus, UUO kidneys appear less damaged than B6 UUO kidneys after 14 days of obstruction. Upon gross inspection, contralateral kidneys (NK) from the two species are similar in length but Acomys UUO kidneys appear less damaged than B6 UUO kidneys after 14 days of obstruction.

(C) Panel demonstrates preservation of parenchymal thickness (greater distance between arrows) in Acomys kidney with renal pelvis noted by dotted white line.

(D) The graph demonstrates the best fit line of the ratio of UUO:NK kidneys for each time point, and the slopes were analyzed by linear regression (n = 6-10/time point).

(E) Total collagen content was measured by micrograms hydroxyproline per mg wet kidney weight. Graph summarizes total collagen measurement for B6 and Acomys NK and UUO kidneys (n = 6-8/time point for each group).

(F) Graph summarizes image analysis of picrosirius red staining for each UUO time point (n = 6/time point for each group).

(G) UUO was performed on outbred CD-1 mice (green) (n = 3-4/time point), and the development of fibrosis was compared with B6 (blue) and Acomys (red).

(H) Graph summarizes image analysis of picrosirius red staining with (H) representative digital images (400x). Glomeruli are outlined (dotted green). Arrows demonstrate Sirius red staining of interstitial matrix.

(I) Dilated tubular area was measured in Masson trichrome sections; graph summarizes image analysis of tubular dilation area in B6 and Acomys after UUO (n = 6-7/time point for each group). Data are represented as mean ± SEM. 400x field = 388 µm². B6 versus Acomys: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; CD-1 versus Acomys: |p < 0.0001; B6 versus CD-1: ¥ p < 0.0001.

Figure 1. Absence of fibrosis after severe obstructive injury in Acomys

(A and B) Upper) The ureter from the left kidney was ligated (arrow) to produce obstructive injury in both Acomys and C57BL/6J (B6) mice. (Lower) Upon gross inspection, contralateral kidneys (NK) from the two species are similar in length but Acomys UUO kidneys appear less damaged than B6 UUO kidneys after 14 days of obstruction.

Methods

Figure 1C demonstrates preservation of tissue structure with renal pelvis noted by dotted white line.

EXPERIMENTAL DESIGN

Methods

Measurements

Figure 1D demonstrates the best fit line of the ratio of UUO:NK kidneys for each time point, and the slopes were analyzed by linear regression (n = 6-10/time point).

Figure 1E demonstrates total collagen content was measured by micrograms hydroxyproline per mg wet kidney weight. Graph summarizes total collagen measurement for B6 and Acomys NK and UUO kidneys (n = 6-8/time point for each group).

Figure 1F demonstrates image analysis of picrosirius red staining for each UUO time point (n = 6/time point for each group).

Figure 1G demonstrates UUO was performed on outbred CD-1 mice (green) (n = 3-4/time point), and the development of fibrosis was compared with B6 (blue) and Acomys (red).

Figure 1H demonstrates image analysis of picrosirius red staining with (H) representative digital images (400x). Glomeruli are outlined (dotted green). Arrows demonstrate Sirius red staining of interstitial matrix.

Figure 1I demonstrates dilated tubular area was measured in Masson trichrome sections; graph summarizes image analysis of tubular dilation area in B6 and Acomys after UUO (n = 6-7/time point for each group). Data are represented as mean ± SEM. 400x field = 388 µm². B6 versus Acomys: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; CD-1 versus Acomys: |p < 0.0001; B6 versus CD-1: ¥ p < 0.0001.
was confirmed by the maintenance of relatively normal kidney weights in obstructed Acomys kidneys compared with rapid declines in kidney weights in Mus as a result of progressive renal fibrosis (Figure 1D; ratio of slopes: \( m_{\text{Mus}}/m_{\text{Acomys}} = -7.5; p = 0.03 \)). There were no significant differences in uninjured contralateral kidney weights between Acomys and Mus (data not shown).

Progression of fibrosis was monitored by three assays. Total collagen levels were determined biochemically as total hydroxyproline content. Kidney collagen levels increased rapidly in Mus whereas Acomys exhibited no significant change from the uninjured contralateral kidney (NK) (\( n = 6–8 \); Figure 1E). Remarkably, even out to 21 days of obstruction, there were no significant differences in total collagen levels between UUO kidney and uninjured NK in Acomys (\( n = 6–8 \); Figure 1E). Computer-assisted image analysis of picrosirius red-stained kidney tissue sections demonstrated a nearly complete absence of interstitial matrix fibrosis at each time point after UUO injury in Acomys even out to 21 days of obstruction (Acomys: NK versus 3d–21d, \( n = 6–8 \); Figure 1E). We found that IFTA scores were markedly reduced in Acomys compared with Mus (B6) despite chronic obstructive injury in both species (Figure S2). In order to test our finding in an outbred strain of Mus, we performed UUO surgeries on CD1 mice and measured fibrosis severity by picrosirius red staining.

Of interest, we found even more dramatic increases in interstitial fibrosis in CD1 mice, producing even greater differences in fibrotic tissue areas when compared with Acomys (Figures 1G and 1H; \( p < 0.0001 \)). All together, these results demonstrate that, in contrast to either inbred or outbred Mus strains, Acomys preserved renal mass and did not develop fibrotic tissue in response to severe and chronic kidney injury.

**Acomys maintains tubular integrity and represses myofibroblast accumulation after UUO injury**

To quantify the extent of obstructive tubular injury, we measured the dilated tubular area in Acomys and Mus at day 3 through day 14 after UUO. As expected from the obvious hydronephrosis seen in Figures 1A and 1B, we found that tubular dilation increased significantly in both species following UUO compared with the contralateral uninjured kidney and peaked at day 7 (Figure 1F; \( p < 0.05 \)). Importantly, the extent of tubular dilation was not significantly different between Acomys and Mus throughout the time course examined (Figure 1F). Activated myofibroblasts (positive for smooth muscle \( \alpha \)-actin, Acta2/SM\( \alpha \)A) are a major source of collagen-rich extracellular matrix produced during kidney fibrosis. Chronic tubular injury is known to promote the production of intrarenal profibrotic cytokines that activate myofibroblasts (Grergic et al., 2014). Although SM\( \alpha \)A immunolabeling increased after UUO in both species, Mus exhibited higher levels of SM\( \alpha \)A + myofibroblasts compared with Acomys (Figures 2A and 2B; \( p < 0.01 \)). In contrast to the lack of fibrosis in Acomys after UUO (Figures 1D and 1E), there was a significant increase in SM\( \alpha \)A + myofibroblasts at day 14 after UUO compared with NK and day 3 time points (Figure 2B; \( p < 0.05 \)). These results suggest that the absence of interstitial matrix deposition in Acomys after UUO injury is not due to the absence of myofibroblasts but rather species-specific differences in myofibroblast phenotypes. Chronic inflammation with a predominance of macrophages is a characteristic finding in organ injury and is strongly correlated with tissue fibrosis (Duffield, 2014). Similar to Mus, F4/80 + macrophages infiltrated Acomys kidneys after UUO (Figure 2C). To quantify macrophage infiltration, whole kidneys were enzymatically digested into single-cell suspensions and analyzed for F4/80 expression by flow cytometry (Figure 2D). As expected, the number of F4/80 macrophages increased in Mus UUO kidneys with advancing obstruction (days 7 and 14) compared with contralateral NK (\( p < 0.001 \)). In comparing Acomys with Mus kidneys, significant reductions in F4/80 + macrophage content were found at each time point examined (\( p < 0.05 \)) but less dramatic than seen in the fibrosis (Figures 1D and 1E) and myofibroblast (Figure 2B) data.

These results suggest that the unique absence of fibrosis in Acomys is not due to a complete absence of a chronic inflammatory response or an absence of myofibroblasts in injured kidney tissue but suggests evolutionary adaptations of these cell types in regenerative wound repair. Tubular integrity is strongly correlated with nephrin function and can serve as a histological surrogate of whole kidney function (Liu et al., 2013; Chaabane et al., 2013). Cdh1 (E-cadherin) is an indicator of tubular cell integrity and polarity whose expression is lost with ongoing obstructive injury, leading to loss of tubular architecture (Zheng et al., 2016). As expected for Mus, there was progressive loss of Cdh1 expression with each time point after UUO compared with the contralateral normal kidney (Figures 2E and 2F). However, in Acomys no significant changes in Cdh1 protein levels with advancing obstructive injury were observed until day 14 compared with the
contralateral uninjured kidney. There were no differences in Cdh1 expression levels in uninjured contralateral kidneys between Acomys and Mus (Figure 2F). Thus, despite severe chronic obstruction, Cdh1 protein levels were maintained in Acomys while becoming significantly decreased in Mus. Altogether, these results demonstrate that despite equivalent tubular dilation with obstruction (Figure 1I), significant attenuations in myofibroblast activation, macrophage infiltration, and Cdh1 protein loss were found in Acomys kidneys that correlated with preservation of tubular integrity and absence of interstitial fibrosis when compared with Mus.

**Initial injury response transcriptomes in Acomys correlate with regeneration**

In order to further delineate the differences in initial responses to tissue injury between Acomys and Mus, we carried out RNA seq analysis on whole kidneys. We compared transcriptional responses at days 2 and 5 in Acomys kidneys versus Mus kidneys after UUO. We aligned our Acomys dataset to existing spiny mouse transcriptomes (Mamrot et al., 2017) and identified 1700–2500 Acomys differentially regulated loci across sham and injured groups, which principal component analysis (PCA) revealed as distinct datasets (Figure S3A). We successfully mapped 77% of Acomys differentially regulated transcripts between day...
Comparative analysis of differentially expressed RNA Seq transcripts with an FDR <0.05, and a 1.5 linear fold change (logFC >0.585) from B6 and Acomys demonstrated overlapping and nonoverlapping gene subsets (Figure S3C).

(A) Heatmaps between both species revealed similar overall patterns of expression at days 2 and 5 after UUO despite dramatically different fibrosis outcomes.

(B) Overlapping genes between B6 and Acomys demonstrated expected upregulated injury response, matrix, and inflammation genes and downregulated transport channels and enzymes. For nonoverlapping genes, we performed an interaction analysis on differentially expressed transcripts between species based on its mean group expression level (FDR <0.05 and a 1.5 linear fold change) to indicate a distinct species-specific gene expression change.

(C) Interaction analyses revealed 6 clusters of genes that were differentially expressed with UUO injury (Sham:UUO) between Mus and Acomys at days 2 and 5 datasets.

(D) Representative graphs of cluster genes from day 2 and day 5 datasets indicate the most significant gene in each cluster at the top with their pattern of expression below. Notable and novel genes for the cluster are noted on the sides of the graph (green: secreted factors and matrix proteins; red: transcription factors or DNA binding protein; black: other; *5d only).

2/sham and day 5/sham UUO datasets to identifiable mouse genes (Figure S3B), which were used in subsequent analyses. Comparisons between species for day 2 and day 5 datasets revealed extensive overlap between M musculus and Acomys initial transcriptional responses to injury, predominantly in upregulated genes (Figure S3C). Within the overlapping dataset (Figure S3) there were largely conserved transcriptional responses to tissue injury between Mus and Acomys at both day 2 and day 5 (Figure 3A). Further analysis of
genes (>1 log CPM) in this conserved response to injury identified the upregulation of typical markers of kidney injury, including Haver1 (Kim1), Lcn2 (Ngal), Timp1, Sox9, Vcam1, Serpine1, and Icam1, after UUO (Figure 3B). Although fewer downregulated genes were identified in Acomys compared with Mus, they showed a similar pattern affecting ion transport genes in the distal segments of the nephron (Clcnka, Aqp3, ATP6v1b1, Slc26a7, Slc12a1, and Slc4a1) and enzymes (Dnase1, Gpx6) (Figure 3B). These data demonstrated there were many conserved responses to kidney injury between Mus and Acomys despite the divergent outcomes for interstitial fibrosis and kidney function.

To identify potential differences in transcriptional responses to kidney injury leading to scarless regenerative repair in Acomys, we considered whether any gene in the nonoverlapping dataset (Figure S3C) exhibited a statistical interaction difference from its mean group expression level (FDR < 0.05 and a 1.5 linear fold change) that could indicate a distinct species-specific change in gene expression (Acomys relative to Mus). This analysis resulted in 843 genes between day 2 and day 5 groups (Figure 3C). Interaction analyses of day 2 and day 5 datasets revealed 6 clusters of genes that were differentially regulated with UUO injury (sham:UOO) between Mus and Acomys (Figure 3C). For example, cluster 1 contains a set of injury response genes that are upregulated after UUO in Mus but in Acomys exhibit increased expression in sham but do not significantly change after UUO. Hence, clusters 1 and 4 represent Mus-specific genomic responses to UUO injury (i.e. upregulated genes), whereas the response to injury in Acomys is to maintain expression of these genes at homeostatic levels (equal to sham). Examination of the expression level for the top gene in cluster 1 (Lig2) and cluster 4 (Acat3) illustrate the general pattern for other cluster members, many of which are secreted factors, cytokines, transcription factors, or DNA-binding chromatin modifiers (Figure 3D).

Interestingly, profibrotic and myofibroblast-inducing factors upregulated by UUO in Mus in cluster 4 (Tgb3, Dkk3, Runx1, Gli3, Nkd2) were not significantly changed from sham levels in Acomys. In addition, a concerted upregulation of inflammatory mediators in Mus in cluster 1 (Atf3, Nhl3, Nfkβ2, Relb) and cluster 4 (Nfkbid, Nfkβe, Cita) were also unchanged from sham in Acomys. These clusters suggest that the response to kidney injury in Acomys involves downregulation of the responsiveness of critical profibrotic and inflammatory mediators that drive myofibroblast accumulation and interstitial fibrosis in Mus (Figure 3D). In contrast, clusters 2 and 3 represent Acomys-specific kidney response gene sets (upregulated with UUO), as they are unchanged or downregulated in Mus. In order to investigate the drivers of the transcriptional responses in Acomys, we performed a transcription factor enrichment analysis (Keenan et al., 2019) on the genes from clusters 2 and 3 (Table S1). Of the top 10 transcription factors from this analysis, many were involved in regulation of cell-cycle activity and five were significantly upregulated in Acomys at day 2 (Cenpa, Myb12, Dnmt1, Foxm1, Znf367). Ingenuity pathway analysis (IPA) was performed on day 2 differentially expressed transcripts between Mus and Acomys (Figure 4). Although the analysis is somewhat limited by the incomplete identification of the full set of differentially regulated genes in Acomys, the pathways were markedly distinct. We examined the top 15 canonical pathways identified by IPA and categorized them for both species (Table S2); this demonstrates the difference in response to chronic injury
between the species: a regenerative response (cell-cycle regulation and DNA damage response) predominated in Acomys compared with inflammation and fibrosis in Mus (Figures 4A and 4B). Consistent with our tissue analysis, common pathways between both species included fibrosis/myofibroblast activation, acute phase response, and fibrosis signaling and confirmed that the key processes associated with fibrogenesis are present in Acomys at the RNA level (Figure S4). However, the pathways that are unique to Acomys are strongly activated (Figures 4B and S5) and, in combination with our functional outcome of no fibrosis, suggested that they are critical to the regenerative response.

Based on the regenerative potential of Acomys, we investigated the expression of established nephrogenic genes (Hendry et al., 2011; O’Brien and McMahon, 2011; Oxburgh, 2018; Adam et al., 2017) in clusters 1–6. We found several differentially regulated nephron progenitor genes selectively induced in Acomys (Figure 5A). Especially interesting were the genes from cluster 3 (Cdh1, Cdh6, Osr1, Ror1, Ror2, and Stmn1) that are upregulated in Acomys after UUO but not in Mus. Furthermore, cluster 2, the other Acomys kidney response gene cluster, contained genes Igf2 and H19 that have been linked to persistence of nephron progenitors (Yermalovich et al., 2019) and to angiogenesis (Haddad et al., 2021). To validate the RNA seq analysis, we chose Cdh6 because it was the most highly differentially regulated among the nephron progenitor genes (Figure 5B). Cdh6 is strongly expressed in nephron progenitor cells during kidney development and then downregulated after birth (Mah et al., 2000). During nephrogenesis, Cdh6 is expressed in mesenchymal aggregates, renal vesicle, and proximal tubule progenitors (Cho et al., 1999). Cdh6 is strongly expressed in the nephrogenic zone where mesenchymal-to-epithelial transition is occurring and becomes downregulated in mature proximal tubular cells (Cho et al., 1998). At day 3 after UUO, Cdh6 is confined to a small subset of tubules in Mus (Figures 5C and 5G); however, there was striking reexpression of Cdh6 in mosaic patches throughout the proximal tubular network in Acomys with little or no overlap with Cdh1-positive tubular cells (Figures 5D and 5D′ arrows). Western blot analysis confirmed significantly increased protein levels of Cdh6 in UUO kidneys with a 5-fold increase at day 14 in Acomys compared with Mus (Figures 5E and 5F). Taken together, the reactivation of nephrogenic progenitor gene expression after injury in Acomys suggests a mechanistic basis for regeneration of kidney mass without fibrosis. Thus, despite the activation of typical injury response genes in Acomys, the net effect is a reprogramming of the cellular response to injury toward cell-cycle control and DNA damage responses that drives a regenerative program, leading to a completely different wound healing outcome when compared with that observed in Mus.

Assessment of kidney structure and function after ischemia-reperfusion injury

Although the UUO model is useful in the study of experimental renal fibrosis in animals, it is of limited relevance to human kidney disease. Ischemia-reperfusion is a more relevant model of acute necrotic kidney injury followed by maladaptive repair typically observed in humans. In order to provide a stringent test
of Acomys capacity for scarless, regenerative repair of kidney injury, we produced severe ischemic injury by clamping the renal vasculature for 40 min followed by reperfusion of the ischemic kidney (Liu et al., 2017). To quantify the extent of initial injury, we performed uni-IRI with a simultaneous contralateral nephrectomy (uni-IRI-Nx) on Acomys and Mus and sacrificed them at 24 h after surgery in order to correlate whole organ kidney function with histology after severe acute injury. We found dramatic elevations in blood urea nitrogen (BUN) levels 24 h after uni-IRI + Nx in both species, indicating acute loss of kidney function (Figure 6A). Importantly, these elevated BUN levels were not significantly different between Acomys and Mus. Instead, there was a trend toward higher BUN levels in Acomys (BUN: Mus versus Acomys, 102 ± 16 versus 129 ± 24 mg/dL). We also measured serum creatinine and found this independent assay of kidney function was also acutely elevated after IRI compared with control with no difference between species after IRI (Figure 6B). H&E sections on the Uni-IRI-Nx kidneys from Acomys and Mus at 24 h were analyzed for tubular cell necrosis, tubular casts, and dilation and assigned cumulative tubular injury scores. Consistent with our kidney function data at 24 h, we found equivalent and extensive damage to tubular structures in both species at 24 h after IRI (Figures 6C and 6D). Therefore, consistent with our transcriptome data, both species experience acute kidney injury and tissue damage to equal extents after prolonged renal ischemia as assessed both histologically and functionally.

Regeneration of tubular damage after ischemia-reperfusion injury in Acomys
As a first step to investigate the ability of Acomys to regenerate kidney structure and function after acute kidney injury, we performed uni-IRI without contralateral nephrectomy to allow long-term survival in both species and then sacrificed animals at day 14 to assess kidney structure by histology. Similar to our UUO study, there was a robust preservation of kidney tissue mass following severe ischemic injury by IRI in Acomys compared with progressive decline in kidney tissue mass in Mus (Figure 7A). At day 14 after IRI, ischemic/contralateral kidney weight ratios were maintained in Acomys (0.92 ± 0.01) compared with almost 40% loss of renal parenchymal mass to fibrosis in Mus (0.64 ± 0.05). Remarkably, despite severe acute ischemic injury, we found a complete absence of fibrosis by picrosirius red staining in Acomys compared with either B6 or CD1 strains of M. musculus (Figures 7B and 7C). Acute tubular injury leads to activation and accumulation of myofibroblasts. In Mus, Acta2/SMαA immunolabeling progressively increased with time after IRI, whereas in Acomys, SMαA+ myofibroblasts plateaued at day 7 and were significantly less abundant at day 14 compared with Mus (Figures 7D and 7E; p < 0.0001). Interestingly, despite similar ischemic injury F4/80 + macrophage infiltration was significantly decreased in Acomys compared with Mus (Figure S6). Consistent with an equivalent initial injury after IRI, there was a similar loss of Cdh1 in both species at day 7 (Figures 7F and 7G). However, at day 14, Acomys was able to regenerate mature tubular epithelium with Cdh1 immunostaining returning to baseline levels, whereas Cdh1 expression...
progressively declined by day 14 in Mus (Figures 7F and 7G; p < 0.0001). Likewise, staining for the tubular basement membrane protein laminin showed progressive shrinking and thickening by day 14 in Acomys kidney consistent with tubular atrophy, whereas basement membrane structures at day 14 in B6 strongly resembled normal uninjured kidney (Ctrl) (Figure 7H). The removal of necrotic and cellular debris after

Figure 7. Regeneration of nephron architecture after severe ischemia reperfusion injury in Acomys
In order to determine effect of acute injury on regeneration, uni-IRI was performed on B6, CD-1, and Acomys.
(A) Graph summarizes data on IRI/contralateral (NK) kidney weight ratio at time of sacrifice (n = 4–6/group): *p < 0.05, **p < 0.01.
(B) Graph summarizes image analysis of picrosirius red staining for each IRI time point (n = 5–6/time point for each group); B6 (blue), CD-1 (green), Acomys (red): *p < 0.05, ****p < 0.0001.
(C) Representative picrosirius red digital images (400x). Glomeruli are outlined (dotted green). Arrows demonstrate Sirius red staining of interstitial matrix.
(D) Smooth muscle alpha actin (Acta2/SMαA) expression was investigated by immunofluorescence-confocal microscopy at days 7 and 14 after uni-IRI. Representative digital images (400x) of SMαA expression (green) for B6 and Acomys at days 7 and 14 after uni-IRI.
(E) Graph summarizes image analysis for SMαA at each time point (n = 4–5/time point for each group).
(F) Tubular integrity was examined by confocal microscopy for Cdh1. Representative digital images (400x) of Cdh1 (red) expression for B6 and Acomys.
(G) Graph summarizes image analysis results for Cdh1 levels at each time point (n = 4–5/time point for each group).
(H) Laminin (red) immunostaining of tubular epithelial basement membrane architecture at day 0, 24 h, and 14 days after IRI injury (400x; scale bars, 100µm). Note Acomys kidney at day 14 (D14 IRI) strongly resembles uninjured kidney (Ctrl) in tubular basement membrane architecture, whereas B6 basement membranes demonstrate collapse and thickening with advancing fibrosis. Scale bars, 100 µm. Tubular casts and debris were identified on Periodic acid Schiff (PAS) stain in unilateral IRI kidneys at 24 h, 72 h, and 7 days in B6 and Acomys (I, J–O).
(I–O) (I) Graph summarizes image analysis results for tubular casts and intraluminal debris after IRI. Representative fields from 200x images demonstrate representative tubular casts and intraluminal debris (arrow) in IRI kidneys at 24 h (H and K), 72 h (L and M), and 7 days (N and O) in B6 and Acomys kidneys. Arrows demonstrate areas of intraluminal debris/casts. Data are represented as mean ± SEM. 400x field = 388 µm². B6 (blue) versus Acomys (red): *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; between time points #p < 0.05, ##p < 0.01, ###p < 0.001, ####p < 0.0001.
injury is an important component of wound repair and tissue regeneration (Duffield, 2014). Intraluminal casts and debris were quantitated from kidney sections. This analysis revealed that both species exhibited equivalent levels of tubular casts/debris at 24 h after IRI (Figures 7I–7K, arrows). At later time points, Mus retained this intraluminal debris, whereas Acomys efficiently cleared it from the tubular network (Figures 7I, 7L, and 7O, arrows).

We then employed light sheet fluorescence microscopy (LSFM) to determine whether Acomys kidneys exhibited anatomical defects 14 days after IRI. We took advantage of the inherent autofluorescence signal generated by formaldehyde-based protein cross-linking to analyze structural changes throughout the entire kidney and especially the tubular casts (Leischner et al., 2015; Dodt et al., 2007). In undamaged Mus and Acomys kidneys, high-density protein-protein cross-linked structures generated similar high-contrast structure signals (tubules, ECM) compared with lower density structures (glomeruli, renal pelvis) and empty blood vessel lumens (Figure S7A). Consistent with our tubular cast data (Figure 7I), there was a persistence of tubular casts within the whole kidney in Mus at 14 days after IRI compared with a near-complete resolution of tubular casts in Acomys (Figure S7B). Although there were similar levels of tubular necrosis and tubular casts seen in the corticomedullary junction at 24 h after severe IRI in both species (Figure 6C), what was strikingly different in Acomys was the abundance of polymorphonuclear cells and other nucleated cells within intraluminal tubular casts that was seen much less frequently in Mus (Figures S8A–S8E, arrows). Consistent with our quantitative data (Figure 7I), by 72 h tubular casts, dilation, and interstitial inflammation progressed in Mus (Figures S8F and S8G), whereas in Acomys the intraluminal cellular debris had been removed and replaced by highly nuclear, somewhat disorganized tubular structures (Figure S8H), with flattened epithelial cells suggesting progression to/from a more dedifferentiated state (Figures S8I and S8J, arrows). By day 7, tubular damage and interstitial fibrosis continue to progress in Mus (Figures S8K and S8L) compared with the appearance of defined tubular structures with open lumens in Acomys (Figures S8M and S8N) and reestablishment of brush border structures in more mature differentiated tubular epithelial cells (Figures S8O) consistent with the increase in Cdh1 (Figures 7F and 7G).

**Complete regeneration of kidney function after IRI in Acomys**

To compare the degree of nephron function with structural regeneration in the uni-IRI damaged kidney, we performed uni-IRI followed by a contralateral nephrectomy at day 14 and then measured kidney function over the next 2 days. Importantly, we found striking and reproducible differences in day 16 BUN levels between Acomys and Mus (Figure 8A). Consistent with a complete absence of interstitial fibrosis (Figure 7B) and restoration of mature tubular epithelium (Figures 7F and 7G) by day 14, we found that BUN levels had returned to normal values in Acomys indicative of complete regeneration of kidney function by 16 days after IRI (compare Figures 7B–7O and 8A). By contrast, Mus BUN levels were substantially increased indicative of progressive renal failure (Figure 8A). Relative changes in serum creatinine were also severely elevated in Mus after IRI and were restored to control levels in Acomys after IRI (Figure 8B).

Finally, in order to investigate the cellular basis of the regenerative response after severe IRI, we performed pulse labeling with EdU nucleoside every 2 days in Mus and Acomys with sacrifice at day 14. Surprisingly, there were no differences in total proliferating cells between Acomys and Mus at day 14 (Figures 8C–8E and 8I). However, consistent with the regeneration of nephron structure and function in Acomys, we found a 2.4-fold increase in proliferation in the proximal tubular compartments (Cdh1-positive) of the kidney (Figures 8H and 8I). In striking contrast to Mus, there was a substantial 20-fold increase in the number of ZO-1/Tjp1-positive glomerular cells (podocytes) after IRI in Acomys compared with Mus (Figures 8E, 8F, and 8I). The proliferation of podocytes strongly suggested that Acomys regenerated the glomerular compartment in addition to the tubular epithelium. In contrast, in Mus the predominant proliferating compartment emanated from the Cdh1- and Tjp-negative (other) subpopulation, which was primarily composed of interstitial cells and likely myofibroblasts. Both Cdh6 and Osr1 were significantly increased in the Acomys IRI kidney early after injury compared with little expression in Mus (Figures S9A–S9J). Interestingly, Cdh6 and Osr1 appear to be co-expressed in the same cells in the Acomys IRI kidney (Figure S9H). These unprecedented findings confirmed that the response to severe acute kidney injury in Acomys does not lead to the progressive, degenerative fibrotic response characteristic of mouse and human kidneys, but instead strikingly results in the complete regeneration of nephron structure and function with no fibrosis.
DISCUSSION

Restoration of solid organ architecture and function after injury or disease remains the holy grail of regenerative medicine. Using two different and highly aggressive forms of experimental kidney injury that produce organ failure due to renal fibrosis in Mus, we show that there was an absence of fibrotic tissue and a remarkable regeneration of kidney morphology and nephron function in Acomys compared with either inbred (B6) or outbred (CD1) strains of Mus. These striking differences in wound healing responses were not due to the failure of our injury models to produce acute organ damage in Acomys kidneys because histological and functional assays along with conserved injury response transcriptomes demonstrated equivalent tissue injuries in the first 24–48 h after UUO or IRI surgeries in both species. Yet within the milieu of common tissue damage, we observed that unique gene clusters were rapidly activated in surviving resident cells in Acomys kidney that included embryonic nephrogenic and angiogenic pathways that aligned with the subsequent regeneration of kidney architecture and function without fibrosis; this suggests that the spiny mouse genome is poised to rapidly initiate scarless wound healing in the adult kidney.

Figure 8. Restoration of nephron function after severe ischemia reperfusion injury through regeneration of glomerular and tubular compartment

In order to determine functional recovery, uni-IRI was performed, the contralateral kidney was removed at day 14, and kidney function was monitored until sacrifice at day 16. (A) Graph summarizes BUN levels were determined in uninjured animals (Ctrl) and those sacrificed at 1day 6, 2 days after contralateral nephrectomy (IRI). Note the complete recovery of nephron function in the injured Acomys kidneys at day 16 compared with the high BUN levels indicative of kidney failure in B6 injured kidneys. (B) Graph summarizes relative creatinine levels in B6 and Acomys at day 16 confirming findings in graph (A). In order to identify proliferating subpopulations after IRI, animals were pulsed with EdU every 2 days starting at the time of surgery. (C–H) Representative digital images (400x) of proliferating populations, EdU+ (gray), (400x, scale bars, 100 μm) (E and F) podocytes/glomerular, ZO-1/Tjp1 (green) (scale bars 50 μm), and (G and H) proximal tubular compartment, Cdh1 (red) (scale bars, 50 μm). Note podocytes proliferating in Acomys after injury but not in B6. (I) Graph summarizes image analysis results for total proliferating cells (EdU+), proliferating podocytes (EdU + Tjp1+), proximal tubular cells (EdU + Cdh1+), and other (EdU + Tjp1− Cdh1−). Data are represented as mean ± SEM. 400x field = 388 μm2. B6: blue, Acomys: red; B6 versus Acomys: NS: not significant, **p < 0.01, ***p < 0.001, ****p < 0.0001.
Muroid rodents of the genus *Acomys* (spiny mice) have evolved the ability to shed their dorsal skin to avoid predation and then to completely regenerate the lost skin tissue without fibrosis or scar formation (Seifert et al., 2012; Brant et al., 2015; Gawniluk et al., 2016; Matias Santos et al., 2016; Jiang et al., 2019). Mechanical and histological assays showed that *Acomys* skin is specialized to be structurally fragile and prone to tear under low tensile forces (Seifert et al., 2012). It, therefore, cannot be assumed *a priori* that the regenerative response to tissue injury in the skin, the first target of predatory attacks in the wild, necessarily extends to internal organs in *Acomys* species. A similar ear skin regenerative response was previously reported for the MRL/MpJ strain of mice (Clark et al., 1998). However, multiple attempts to determine if regenerative wound healing extended to internal organs, including kidneys, of these mice provided little or no support for this idea (Oh et al., 2004; Robey and Murry, 2008). Thus, our results on the striking absence of fibrotic tissue formation and the complete regeneration of nephron function in *Acomys* kidney suggest that the regenerative wound healing response previously described in the skin (Seifert et al., 2012; Brant et al., 2015; Gawniluk et al., 2016; Matias Santos et al., 2016; Jiang et al., 2019) may indeed be a systemic property in spiny mice that extends to critical internal organs as well. Similar to our transcriptome data, *Acomys* regenerative renogeneration in skeletal muscle also demonstrated transcriptional activation of developmental pathways (Maden et al., 2018; Brant et al., 2019).

Our IPA analysis suggested that regulatory factors controlling cell cycle and DNA damage responses act to differentiate regenerative wound healing in *Acomys* from fibrogenic wound healing in *Mus*. Recent studies show that epigenetic factors can modulate nephron progenitor cell renewal and differentiation during development (Liu et al., 2018, 2020; Wanner et al., 2019). Furthermore, progression of acute kidney injury (AKI) and chronic kidney disease (CKD) can be modulated by epigenetic factors such as DNA methylation and histone modifications, some of which persist after AKI and likely promote fibrosis and CKD (Liu et al., 2017; Chawla et al., 2014; Ferenbach and Bonventre, 2015; Sharfian et al., 2018; Soofi et al., 2020). Recently, activating chromatin marks such as histone K4 trimethylation were shown to be dynamically regulated after kidney injury and play key roles in activating reparative programs such as Sox9 expression in mice (Soofi et al., 2020). Our data suggest that the *Acomys* genome is poised to initiate scarless wound healing at the time of kidney injury. Whether and to what extent epigenetic pathways orchestrate this activation of poised genomic sites and set into motion pathways for scarless and regenerative wound healing pathways is an ongoing subject of investigation.

Our comparative transcriptomic analysis identified 843 genes in six unique gene clusters that are differentially regulated in the *Acomys* early cohort compared with *Mus*. Among these were cell-cycle control genes, particularly at the G2-M transition, DNA damage response and checkpoint regulation genes, S-phase entry genes, and cell survival genes. EdU labeling studies verified the increased cell-cycle progression and DNA synthesis in glomerular and epithelial cells, whereas, of particular interest, reduced cell proliferation was found in interstitial cells implicated in producing fibrotic tissue in mice and humans (Duffield, 2014; Humphreys, 2018; Kuppe et al., 2021). In addition, we found selective upregulation of nephrogenic genes including Osr1, Ror1/2, and Cdhd6. Immunofluorescence and western blot studies verified the increased expression of Cdhd6 in expanding patches of tubular epithelial cells in *Acomys*. RNA scope in situ hybridization analysis showed co-expression of Osr1 and Cdhd6 in the same cells within these expanding epithelial patches in *Acomys* but not *Mus*. Thus, the *Acomys* genome response to kidney injury included rapid activation of genes linked to cell-cycle progression, DNA damage response, developmental pathways, angiogenesis, and metabolism.

AKI initiates a fibrogenic cascade that leaves patients at high risk for developing CKD and progressive loss of renal function (Chawla et al., 2014; Ferenbach and Bonventre, 2015). Although elegant studies in *Mus* have produced substantial insights into the pathogenesis of renal fibrosis, translating these findings into therapeutic solutions has been disappointing. Wound healing in most adult mammals, including humans, is a process of repair that frequently replaces functional tissue, with a collagen-rich extracellular matrix resulting in maintenance of tissue integrity but often with a corresponding loss of organ function. By contrast, some fish and amphibian species can fully regenerate damaged tissue and restore organ function after amputation or severe tissue injuries without fibrosis (Kragl et al., 2009; Poss, 2010). In the zebrafish kidney, for example, there is evidence of formation of new nephrons after gentamicin nephrotoxicity (McCannell and Wingert, 2014). However, in adult mammals there are no reports of nephron formation de novo after kidney injury. We now provide evidence for a potentially transformative new mammalian model for kidney disease that has evolved a distinctly different wound healing response to kidney injury than the currently
studied mouse, rat, or human models. Whether spiny mice can completely restore kidney function after obstructive or ischemic injuries by forming new nephrons or by rapid and efficient repair of damaged nephrons with restoration of mature tissue structure is yet to be determined. In either case, our data suggest that an in-depth analysis of the molecular basis for scar-free regenerative wound healing in Acomys species could be a gateway for development of novel antifibrotic therapies for kidney disease.

Limitations of the study
The injury models we used, while common to the field, are biased toward endothelial and tubular injury and not specifically targeted to glomeruli or other elements of kidney function. In addition, we did not surgically resect kidney mass to address the question of complete de novo tissue regeneration, but experiments are currently planned to investigate this aspect of regeneration. Another limitation is that the gene expression studies were bulk RNA seq approaches that limit our understanding of how individual cell types respond to kidney injury. Although these methods have allowed us to identify a gene expression signature associated with kidney regeneration, more work is needed to delineate the functional mechanisms by which wound healing is redirected from fibrosis to regeneration in the spiny mouse kidney. Finally, because of the historical use of male mice in studies of kidney fibrosis with the injury models used, we only used male animals for these experiments.

STAR METHODS
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Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103269.

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AUTHOR CONTRIBUTIONS
DMO, CMB, NB, KB, AT, XS, JO, AMP, and BRN performed the experiments. PW and KJM established and maintained breeding colony and IACUC protocols. DMO, DRB, and MWM designed the experiments. JWM, TXB, BRN, AET, EDN, and DMO performed the bioinformatics analysis for the transcriptome data.
Physiol. Ren. Physiol. are arrested but not restored by release of functional decline and glomerulotubular injury.

Adam, M., Potter, A.S., and Potter, S.S. (2017). Psychrophilic proteases dramatically reduce single cell RNA-seq artifacts: a molecular atlas of kidney development. Development 144, 3625–3632. https://doi.org/10.1242/dev.151142.

Brant, J.O., Lopez, M.C., Baker, H.V., Barbazuk, W.B., and Maden, M. (2015). A comparative analysis of gene expression profiles during skin regeneration in mus and acomys. PLoS One 10, e0142931.

Brant, J.O., Boatwright, J.L., Davenport, R., Sandoval, A.G.W., Maden, M., and Barbazuk, W.B. (2019). Comparative transcriptomic analysis of dermal wound healing reveals de novo skeletal muscle regeneration in Acomys cahirinus. PLoS One 14, e0216228. https://doi.org/10.1371/journal.pone.0216228.

Chaabane, W., Praddaude, F., Buleon, M., Vallet, M., Rischmann, P., Galarreta, C.I., Chaabane, W., Praddaude, F., Buleon, M., Jaafar, et al. (2019). Comparative transcriptomic analysis of gene expression profiles during skin regeneration in mus and acomys. PLoS One 14, e0216228. https://doi.org/10.1371/journal.pone.0216228.

Duffield, J.S., Pathak, M., Thannickal, V.J., and Wynn, T.A. (2013). Host responses in tissue repair and fibrosis. Annu. Rev. Pathol. 8, 241–276. https://doi.org/10.1146/annurev-pathol-020712-163930.

Ferenbach, D.A., and Bonventre, J.V. (2015). Mechanisms of maladaptive repair after AKI leading to accelerated kidney aging and CKD. Nat. Rev. Nephrol. 11, 264–276. https://doi.org/10.1038/nrneph.2015.3.

Gawriluk, T.R., Simkin, J., Thompson, K.L., Biswas, S.K., Clarke-Salzler, Z., Kimani, J.M., Kama, S.G., Smith, J.J., Ezenwa, V.O., and Seifert, A.W. (2016). Comparative analysis of ear-hole closure identifies epimorphic regeneration as a discrete trait in mammals. Nat. Commun. 7, 11164. https://doi.org/10.1038/ncomms11164.

Grajc, I., Krautzberger, A.M., Hofmeister, A., Lalli, M., DiRocco, D.P., Fleig, S.V., Liu, J., Duffield, J.S., McMahon, A.P., Aronow, B., and Humphreys, B.D. (2014). Translational profiles of medul lary myofibroblasts during kidney fibrosis. J. Am. Soc. Nephrol. 25, 1979–1990. https://doi.org/10.1667/ASN.2013101143.

Gurtner, G.C., Werner, S., Brandon, Y., and Longaker, M.T. (2008). Wound repair and regeneration. Nature 453, 314–321. https://doi.org/10.1038/nature06709.

Haddad, G., Kölling, M., Wegmann, U.A., Dettling, A., Seeher, H., Schmitt, R., Soerensen-Zender, I., Haller, H., Kistler, A.D., Ducek, A., et al. (2021). Renal AAV2-mediated overexpression of long non-coding RNA H19 attenuates ischemic acute kidney injury through sponging of microRNA-30a-5p. J. Am. Soc. Nephrol. 32, 323–341. https://doi.org/10.1681/ASN.2020060775.

Hendry, C., Rumballe, B., Moritz, K., and Little, M.H. (2011). Defining and redefining the nephron progenitor population. Pediatr. Nephrol. 26, 1395–1406. https://doi.org/10.1007/s00467-010-1750-4.

Hill, N.R., Fatoba, S.T., Oke, J.L., Hirst, J.A., O’Callaghan, C.A., Lasserson, D.S., and Hobbs, F.D.R. (2016). Global prevalence of chronic kidney disease – a systematic review and meta-analysis. PLoS One 11, e0158765. https://doi.org/10.1371/journal.pone.0158765.

Humphreys, B.D. (2018). Mechanisms of renal fibrosis. Annu. Rev. Physiol. 80, 309–326. https://doi.org/10.1146/annurev-physiol-022516-034227.

Jiang, T.X., Han, H.I.C., Ou, K.L., Lei, M., and Chuong, C.M. (2019). Comparative regenerative biology of spiny (Acomys cahirinus) and laboratory (Mus musculus) mouse skin. Exp. Dermatol. 28, 442–449. https://doi.org/10.1111/exd.13899.

Keenan, A.B., Torre, D., Lachmann, A., Leong, A.K., Wojciechowicz, M.L., Uti, V., Jagodnik, K.M., Kropiwinicki, i., Wang, Z., and Ma’ayan, A. (2019). CHEA3: transcription factor enrichment analysis by orthogonal omics integration. Nuclear Acid Res. 47, W222–W224. https://doi.org/10.1093/nar/gkz446.

Kragl, M., Knapp, D., Nacu, E., Khattak, S., Maden, M., Epperlein, H.H., and Tanaka, E.M. (2009). Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature 460, 60–65. https://doi.org/10.1038/nature08152.

Kuppe, C., Ibrahim, M.M., Kranz, J., Zhang, X., Ziegler, S., Perales-Patón, J., Jensen, J., Reimer, K.C., Smith, J.R., Dobie, R., et al. (2021). Decoding myofibroblast origins in human kidney fibrosis. Nature 589, 281–286. https://doi.org/10.1038/s41586-020-2941-1.

Leischner, U., Schirol, A., Zieglaßgänsberger, W., and Dott, H.U. (2015). Formalin-induced fluorescence reveals cell shape and morphology in biological tissue samples. PLoS One 5, e10391. https://doi.org/10.1371/journal.pone.010391.

Liu, W., Li, X., Xiao, Y., Meng, X.M., Wan, C., Yang, B., Lan, H.Y., Lin, H.Y., and Xia, Y. (2013). Dragon (reductive guidance molecule RGMb) inhibits E-cadherin expression and induces apoptosis in renal tubular epithelial cells. J. Biol. Chem. 288, 31528–31539. https://doi.org/10.1074/jbc.M113.517573.

Liu, J., Kumar, S., Dolzenko, E., Alvarado, G.F., Guo, J., Lu, C., Chen, Y., Li, M., Dessing, M.C., Farve, R.K., et al. (2017). Molecular characterization of the transition from acute to chronic kidney injury following ischemia/reperfusion. JCI Insight 2, e94716. https://doi.org/10.1172/jci.insight.94716.

Liu, H., Chen, S., Yao, X., Li, Y., Chen, C.H., Liu, J., Saifudeen, Z., and El-Dahr, S.S. (2018). Histone deacetylases 1 and 2 regulate the transcription programs of nephron progenitors and renal vesicles. Development 145, dev153619. https://doi.org/10.1242/dev.153619.

Liu, H., Hilliard, S., Kelly, E., Chen, C.H., Saifudeen, Z., and El-Dahr, S.S. (2020). The polycomb proteins EZH1 and EZH2 co-regulate...
chromatin accessibility and nephron progenitor cell lifespan in mice. J. Biol. Chem. 295, 11542–11558. https://doi.org/10.1074/jbc.RA120.013348.

Lund, S.P., Nettleton, D., McCarthy, D.J., and Smyth, G.K. (2012). Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. Stat. Appl. Genet. Mol. Biol. 11, B. https://doi.org/10.1515/1544-6115-1826.

Maden, M., Brant, J.O., Rubiano, A., Sandoval, A.G.W., Simmons, C., Mitchell, R., Collin-Hooper, H., Jacobson, J., Omani, S., and Patel, K. (2018). Perfect chronic skeletal muscle regeneration in adult spiny mice, Acomys cahirinus. Sci. Rep. 8, 8920. https://doi.org/10.1038/s41598-018-1756-6.

Mah, S.P., Saueressig, M., Goulding, M., Kintner, C., and Dressler, G.R. (2000). Kidney development in cadherin-6 mutants: delayed mesenchyme-to-epithelial conversion and loss of nephrons. Dev. Biol. 223, 38–53. https://doi.org/10.1006/dbio.2000.9786.

Mamrot, J., Legaie, R., Ellery, S.J., Wilson, T., Seeman, T., Powell, D.R., Gardner, D.K., Walker, D.W., Temple-Smith, P., Papenfuss, A.T., and Dickinson, H. (2017). De novo transcriptome assembly for the spiny mouse (Acomys cahirinus). Cell. Mol. Biol. 103, 70–80. https://doi.org/10.1111/cmm.12851.

Matias Santos, D., Rita, A.M., Casanellas, I., Brito Ova, A., Araujo, I.M., Power, D., and Tiscornia, G. (2018). DNA methyltransferase 1 controls nephron progenitor cell renewal and tissue regeneration in African spiny mice, Acomys cahirinus. Sci. Rep. 8, 17870. https://doi.org/10.1038/s41598-018-35943-x.

Okamura, D.M., Pennathur, S., Pasichnyk, K., López-Guisa, J.M., Collins, S., Febbraio, M., Heinecke, J., and Eddy, A.A. (2009). CD36 regulates oxidative stress and inflammation in hypercholesterolemic CKD. J. Am. Soc. Nephrol. 20, 492–505. https://doi.org/10.1681/ASN.2008010009.

Okamura, D.M., Bahrami, N.M., Ren, S., Pasichnyk, K., Williams, J.M., Ganggoiti, J.A., López-Guisa, J.M., Yamaguchi, I., Barshop, B.A., Duffield, J.S., and Eddy, A.A. (2014). Cysteine modulates oxidative stress and blocks myofibroblast activity in CKD. Am. Soc. Nephrol. 25, 43–54. https://doi.org/10.1681/ASN.2012090962.

Oxburgh, L. (2018). Kidney nephron determination. Annu. Rev. Cell Dev. Biol. 34, 427–450. https://doi.org/10.1146/annurev-cellbio-100616-060647.

Pennathur, S., Pasichnyk, K., Bahrami, N.M., Zeng, L., Febbraio, M., Yamaguchi, I., and Okamura, D.M. (2015). The macrophage phagocytic receptor CD36 promotes fibrogenic pathways on removal of apoptotic cells during chronic kidney disease. Am. J. Pathol. 185, 2232–2245. https://doi.org/10.1016/j.ajpath.2015.04.016.

Poss, K.D. (2010). Advances in understanding tissue regenerative capacity and mechanisms in animals. Nat. Rev. Genet. 11, 710–722. https://doi.org/10.1038/nrg2879.

Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessler-Lavigne, M. (2014). iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. Cell. 159, 896–910. https://doi.org/10.1016/j.celrep.2014.10.010.

Robey, T.E., and Munro, C.E. (2008). Absence of regeneration in the MRL/MpJ mouse heart following infarction or cryoinjury. Cardiovasc. Pathol. 17, 6–13. https://doi.org/10.1016/j.carpath.2007.01.005.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 26, 139–140. https://doi.org/10.1093/bioinformatics/btp516.

Rockey, D.C., Bell, P.D., and Hill, J.A. (2015). Fibrosis: a common pathway to organ injury and failure. N. Engl. J. Med. 372, 1138–1149. https://doi.org/10.1056/NEJMra1300575.

Seifert, A.W., Kama, S.G., Seifert, M.G., Goheen, J.R., Palmer, T.M., and Maden, M. (2012). Skin shedding and tissue regeneration in African spiny mice (Acomys). Nature 489, 561–565. https://doi.org/10.1038/nature11499.

Sharifian, R., Okamura, D.A., Denisenko, O., Zager, R.A., Johnson, A., Gharib, S.A., and Bombszyk, K. (2018). Distinct patterns of transcriptional and epigenetic alterations characterize acute and chronic kidney injury. Sci. Rep. 8, 17870. https://doi.org/10.1038/s41598-018-35943-x.

Simkin, J., Gawriluk, T.R., Gensel, J.C., and Seifert, A.W. (2017). Macrophages are necessary for epimorphic regeneration in African spiny mice. eLife 6, e24623. https://doi.org/10.7554/eLife.24623.

Soofi, A., Kutschat, A.P., Azam, M., Laszczyk, A.M., and Dressler, G.R. (2020). Repression of Lin28 and let-7 is a key mechanism for chronic kidney disease. Proc. Natl. Acad. Sci. U.S.A. 117, 394–403. https://doi.org/10.1073/pnas.1911486117.

Tapmeier, T.T., Brown, K.L., Tang, Z., Sacks, S.H., Sheerin, N.S., and Wong, W. (2008). Reimplantation of the ureter after unilateral ureteral obstruction provides a model that allows functional evaluation. Kidney Int. 73, 885–889. https://doi.org/10.1038/sj.ki.5002797.

Wanner, N., Vormweg, J., Combesc, A., Wilson, S., Plappert, J., Raffenbeul, G., Puelles, V.G., Rahman, R.U., Liwinski, T., Lindner, S., et al. (2019). DNA methyltransferase 1 controls nephron progenitor cell renewal and differentiation. J. Am. Soc. Nephrol. 30, 63–78. https://doi.org/10.1681/ASN.2018070736.

Yermakovich, A.V., Osborne, J.K., Sousa, P., Han, A., Kinney, M.A., Chen, M.J., Robinton, D.A., Montie, H., Pearson, D.S., Wilson, S.B., et al. (2019). Lnc28 and let-7 regulate the timing of cessation of murine nephrogenesis. Nat. Commun. 10, 168. https://doi.org/10.1038/s41467-018-08127-4.

Zheng, G., Zhang, J., Zhao, H., Wang, H., Pang, M., Qiao, X., Lee, S.R., Hsu, T.T., Tan, T.K., Lyons, J.G., et al. (2016). α3 Integrin of cell-cell contact mediates kidney fibrosis by integrin-linked kinase in proximal tubular E-cadherin deficient mice. Am. J. Pathol. 186, 1847–1860. https://doi.org/10.1016/j.ajpath.2016.03.015.
# STAR★METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse monoclonal anti-Actin, alpha Smooth Muscle (clone 1A4) (Acta2) | Sigma-Aldrich | Cat# A2547; RRID: AB_476701 |
| Rabbit polyclonal pan-Laminin | Abcam | Cat# ab11575; RRID: AB_298179 |
| Rat monoclonal F4/80 | Thermo Fisher Scientific | Cat# MF48000; RRID: AB_10376289 |
| Mouse monoclonal E cadherin (Cdh1) | BD Biosciences | Cat# 610181; RRID: AB_397581 |
| Rabbit monoclonal ZO-1 | Abcam | Cat# ab221547; RRID: AB_2892660 |
| Mouse monoclonal anti-Vinculin | Sigma-Aldrich | Cat# V9131; RRID: AB_477629 |
| Donkey polyclonal anti-Rabbit IgG (H+L), 488 | Jackson ImmunoResearch | Cat# 711-545-152; RRID: AB_2313584 |
| Donkey polyclonal anti-Rabbit IgG (H+L), 594 | Jackson ImmunoResearch | Cat# 711-585-152; RRID: AB_2340621 |
| Donkey polyclonal anti-Rat IgG (H+L), Cy3 | Jackson ImmunoResearch | Cat# 712-165-153; RRID: AB_2340667 |
| Donkey polyclonal anti-Rat (H+L), HRP | ThermoFisher | Cat# A18739; RRID: AB_2535516 |
| Goat polyclonal anti-Rabbit IgG (H+L), HRP | Jackson ImmunoResearch | Cat# 111-035-003; RRID:AB_2313567 |
| Goat polyclonal anti-Rat (H+L), Cross adsorbed Cy3 | ThermoFisher | Cat# A10522; RRID:AB_2534031 |
| Goat polyclonal anti-Mouse IgG1 Secondary Antibody, Alexa Fluor 488 conjugate | Thermo Fisher Scientific | Cat# A-21121; RRID:AB_2535764 |
| Goat polyclonal anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | Thermo Fisher Scientific | Cat# A-21131; RRID:AB_2535771 |
| Goat polyclonal anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 | Thermo Fisher Scientific | Cat# A-21135; RRID:AB_2535774 |
| Goat polyclonal anti-Rabbit IgG (H+L)Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | Thermo Fisher Scientific | Cat# A-11008; RRID:AB_143165 |
| Goat polyclonal anti-Rabbit IgG (H+L)Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 | Thermo Fisher Scientific | Cat# A-11012; RRID:AB_2534079 |
| IRDye® 680RD Goat anti-Mouse IgG (H+L) | LI-COR Biosciences | Cat# 925-68070; RRID:AB_2651128 |
| IRDye® 800CW Goat anti-Rabbit IgG (H+L) | LI-COR Biosciences | Cat# 925-32211; RRID:AB_2651127 |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| Normal Donkey serum | Jackson ImmunoResearch | Cat# 017-000-001; RRID: AB_2337254 |
| Normal Goat serum | Jackson ImmunoResearch | Cat# 005-000-121; RRID: AB_2336990 |
| Alexa Fluor 555 Tyramide Reagent | ThermoFisher | Cat# B40955 |
| Alexa Fluor 488 Tyramide Reagent | ThermoFisher | Cat# B40953 |
| **Critical commercial assays** |        |            |
| RNAScope Multiplex Fluorescent V2 | ACD Bio | custom |
| Mus and custom Acomys cahirinus RNA probes | | |
| Urea nitrogen (BUN) colorimetric detection kit | Arbor Assays | Cat# K024-H1 |
| Mouse creatinine assay kit | Crystal Chem | Cat# 80350 |
| **Deposited data** |        |            |
| UUO Acomys and Mus RNA Seq transcriptome | GEO | GSE168876 |
| **Experimental models: Organisms/strains** |        |            |
| Acomys cahirinus: African Spiny Mouse | This study | N/A |
| Mus musculus: Crl:CD1(ICR) | Charles River | RRID:IMSR_CRL:22 |
| Mus musculus: C57BL6/J | Jackson | RRID:IMSR_CRL:22 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and reasonable requests for resources and reagents should be directed to and will be fulfilled by the lead contact Dr. Mark W. Majesky (mwm84@uw.edu).

Materials availability
Materials used in this study are available from the lead contact upon reasonable request.

Data and code availability
● RNA-seq data presented in Figure 3 have been deposited at GEO and are publicly available at #GSE168876.
● This paper does not report original code.
● Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal husbandry
The Seattle Children’s Research Institute’s (SCRI) Institutional Animal Care and Use Committee (IACUC) approved all animal procedures. Adult male Mus musculus (C57BL/6J (B6), CD-1 – source - The Jackson Laboratory) and Acomys cahirinus (colony at SCRI) were maintained within the Seattle Children’s Research Institute’s onsite vivarium. B6 and CD1 mice were housed in a pathogen-free room maintained on 12:12 (Light:Dark) lighting schedule, Acomys were housed in a separate room maintained on 14:10 (Light:Dark) schedule, and all animals received food and water ad libitum.

Experimental models
We utilized two models of kidney injury to investigate the differences in wound healing and fibrosis: Unilateral ureteral obstruction (UUO) and ischemia reperfusion injury (IRI). B6 and CD-1 mice were used as inbred and outbred strains of Mus, respectively. Surgery was performed on male animals between 3 and 6 months of age, male littermates were randomly assigned to experimental groups. UUO surgeries were performed (Ökamura et al., 2009, 2014) (n = 6–8/group), and animals were sacrificed at 3, 7, 14,
and 21 days after surgery. Mice received isoflurane anesthesia (5% induction; 1-3% maintenance) in oxygen through a precision vaporizer (Portable Anesthesia Machine, PAM; Molecular Imaging Products, Bend, OR, USA). Acornys received isoflurane anesthesia at 3-4% due to some sensitivity to larger doses of isoflurane. The incision site was shaved and sterile prepped with Betadine and ethanol. A 0.5 cm anterior vertical incision was made in the left lower quadrant. The left ureter was identified and tied with two 4-0 silk sutures placed close to the pelvis of the kidney. Sham animals had their ureter manipulated without placement of silk suture. Warm saline was given intraperitoneal. The muscle layer was closed with 4-0 Webcryl absorbable sutures (Patterson Veterinary Supply, #V397) and the skin was closed with 4-0 Weblon nylon sutures (Patterson Veterinary Supply, #662BL). Animals received buprenorphine (Patterson Veterinary Supply, #07-892-5235) 0.1 mg/kg perioperatively and postoperatively per IACUC guidelines for pain control.

Unilateral IRI (uni-IRI) surgeries were performed (Okamura et al., 2014; Pennathur et al., 2015) and that the vascular pedicle was clamped for 40 minutes (n = 5-6/group), and animals were sacrificed at 24 h, 72 h, 7 days, and 14 days after surgery. Animals received isoflurane induction as noted above. The incision site was shaved and sterile prep with Betadine and ethanol. Animals were kept at a constant temperature (37 °C) with TCAT-2 temperature controller (Braintree Scientific, #TCAT2DF) during the entire procedure. A 1 cm anterior horizontal incision was made in the mid right quadrant. The left renal vascular pedicle was identified and a vascular clamp (Braintree Scientific, #MVC02) was placed for 40 minutes, with confirmation of ischemia and reperfusion. Sham animals had their vascular pedicle manipulated without placement of clamp. Warm saline was given intraperitoneal. The muscle layer was closed with 4-0 Webcryl absorbable sutures (Patterson Veterinary Supply, #V397) and the skin was closed with 4-0 Weblon nylon sutures (Patterson Veterinary Supply, #662BL). Animals received buprenorphine (Patterson Veterinary Supply, #07-892-5235) 0.1 mg/kg perioperatively and postoperatively per IACUC guidelines for pain control. In select cases, in order to assess initial injury, the contralateral kidney was removed at the time of uni-IRI surgery and sacrificed at 24 h after surgery. In select cases, in order to quantify kidney function in the uni-IRI kidney after initial injury, a contralateral nephrectomy was performed 14 days post-surgery (Okamura et al., 2014). Blood was drawn daily until sacrifice at 2 days post-nephrectomy. All procedures were performed in accordance with the guidelines established by National Research Council Guide for the Care and Use of Laboratory Animals and approval of our Institute Animal Care and Use Committee (IACUC). Contralateral, UUO, and IRI kidneys were harvested and processed for RNA and protein extraction and histological studies (Okamura et al., 2014; Pennathur et al., 2015). Frozen tissue samples were stored at -80 °C.

**METHOD DETAILS**

**Collagen content**

Hydroxyproline content of kidney tissue (µg of hydroxyproline per mg of wet weight kidney section) was measured by acid hydrolysis of the tissue section (Okamura et al., 2009, 2014). Frozen kidney tissue was weighed and placed in a 16 mm glass tube with sealed cap with 500 µl 12N HCl. Samples were boiled in a heating block for 2-3 minutes at 100 °C and vortex intermittently to dissolve tissue completely. Temperature was increased to 110 °C for acid hydrolysis of proteins overnight in hood. Caps were screwed on tightly. Temperature was decreased to 75 °C and caps opened to allow sample to dry thoroughly overnight. Dried samples were solubilized in 500 µl of Collagen buffer (Citric acid, pH 6.1). Vortex and pipette to mix. Particulates were removed with a 0.45 µm centrifugal filter unit (Ultrafree MC Millipore #UFC30HVNB). Chloramine T solution and DMB solution were made on the day of assay. Samples were heated to 65 °C. Collagen (hydroxyproline) standards (100 ng, 200 ng, 500 ng, 1000 ng, and 2000 ng) were used; 10 µl of sample was placed in a 96 well microtiter plate and 100 µl of fresh Chloramine T solution was added to start oxidation reaction. Plate was kept at room temperature for 15 minutes, then 100 µl of fresh DMB was added. Samples were placed at 65 °C for 20 minutes. Reaction was stopped by placing on ice for 3 minutes. Samples were read on a microtiter plate reader at 550 nm. Conversion factor for ng of hydroxyproline in sample of total collagen is 7.83.

**Histological examination**

Immunohistochemical staining was performed on sections of paraffin-embedded tissue or cryosections of snap-frozen tissue using procedures established in our laboratory with VECTASTAIN Elite ABC Kits (Vector Laboratories, Inc.) and AEC Substrate Chromogen K3464 (Dako Corp.). Sections were blocked with Avidin/Biotin blocking kit (Vector Laboratories, Inc.). Computer-assisted image analysis was performed on 6 randomly selected 400x magnified images of slides from individual animals with Image-Pro Plus software (Mediatech). The investigator was blinded to the experimental groups at the time of analysis. Picrosirius
red staining was performed (Okamura et al., 2014; Pennathur et al., 2015). Paraffin slides were rehydrated. Slides were stained with 0.1% picrosirius red for one hour at room temperature and washed in two changes of acidified water. Slides were dehydrated and mounted with VectaMount permanent mounting medium. Quantification of interstitial staining of picrosirius red (SR) staining was performed in a blinded manner using Image-Pro/ImageJ software with randomly selected cortical fields. SR glomerular staining was subtracted and net SR area was normalized to net tubulointerstitial area of 400x field (Net area = Total – glomerular area - empty space). Masson Trichrome and hematoxylin eosin stains were performed on paraffin sections by standard protocols. Interstitial fibrosis and tubular atrophy (IFTA) scores were analyzed on 6 randomly selected 400x Masson Trichrome stained images. The following IFTA scores were assigned, in a blinded manner, based on the estimated percent area affected with tubular atrophy, loss of tubular structure, interstitial inflammation, and interstitial fibrosis in the field: 1 (normal); 2 (<10%), 3 (10-25%); 4 (26-50%); or 5 (>50%). Dilated tubular area was measured using Image-Pro software on 400x Masson trichrome stained images. Tubular casts and intraluminal debris area was measured using Image-Pro software on 200x PAS-stained images and normalized to nettubulointerstitial area for 6 randomly selected cortical fields. Secondary antibodies were shown to be non-reactive with tissue sections stained without the primary antibody.

**F4/80 macrophage quantification**

Animals were perfused with cold normal saline and contralateral and UUO kidneys were placed on ice, minced, digested with Liberase TL (Roche) with 1% DNase (Sigma-Aldrich), then placed at 37°C for 10 minutes and vortexed intermittently. An equal volume of ice-cold HBSS+10%FBS was added to stop Liberase/DNase. Glomeruli were removed by passing cell suspension through a 40 μm Nylon filter. Cells were stained per protocol with DAPI, PE-Cy7-anti-CD45, PE-anti-CD11b, APC-eFluor780-F4/80 from BD Sciences. Cells were blocked with mouse Fc Block (BD Biosciences). Leukocytes were identified and gated based on their positive F4/80 expression. Data was acquired on the LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Inc).

**RNAScope in situ hybridization**

RNAScope was performed on B6 and Acomys kidney tissue by ACD Bio-Techne. Unique multiplex fluorophore control (Polr2a, Ppib, Ubc, Hprt) and target (Cdh6, Osr1) probes for both Mus and Acomys were designed by Advanced Cell Diagnostics (ACDBio). Freshly cut cryosections from day 3 IRI tissue were sent and sample quality control and optimization was performed by ACDBio on kidney tissue. Multiplex RNAScope was performed on cryosections from individual animals (n = 3/group) with both control and target probes at ACDBio and returned to our lab. Samples were imaged on a Leica SP5 confocal microscope (six 400x images/slide). Image analysis was performed on Cdh6 and Osr1 and analyzed by GraphPad Prism.

**Kidney function**

Serum was analyzed for blood urea nitrogen (BUN) using the Urea Nitrogen (BUN) Reagent Set kit (Arbor Assays). Serum was analyzed for creatinine content using the Mouse Creatinine Kit, Enzymatic (Crystal Chem, Inc.). Samples were processed according to manufacturer’s protocol. All samples were performed in triplicate.

**Immunofluoresence**

For cryosectioning, excised tissue was embedded and flash-frozen in O.C.T medium (Tissue Tech) using a dry-ice slurry/2-methylbutanol mixture and cryosectioned between 8-10um. Tissue cryosections were washed with PBS and fixed with 4% PFA for 10 min. Post fixation, slides were washed three times for 5 min each with PBS followed by permeabilization using 0.2% Triton-X100 in PBS (PBT) for 10 min. Slides were then blocked (5% BSA, 2% normal goat serum in PBT) at room temperature for 1hr. Post block, tissue sections were then incubated in primary antibody overnight at 4°C in blocking solution (3% BSA, 0.2% Triton-X100 in PBS). Primary antibodies used include pan-Laminin (Abcam, #ab11575), Acta2 (Sigma, #A2547), F4/80 (Invitrogen, #MF48000), Cdh1 (BD Bioscience, #610181), and ZO-1/Tjp1 (Abcam, #ab221547). After overnight incubation, slides were washed with PBS, and then incubated with goat ALEXA-Fluor 594- or ALEXA-Fluor 488- conjugated antibodies (Thermo Fisher Scientific) for 2 h at room temperature in blocking solution. Cell nuclei were counterstained with DAPI (Molecular Probes) and mounted in 4% (w/v) propyl gallate anti-fade solution. Immunofluorescent images were obtained using
an SP5 confocal microscope (Leica). Acta2 and Cdh1 confocal image quantification analysis was performed as noted above (Okamura et al., 2014; Pennathur et al., 2015).

### RNA sequencing analysis

Age-matched adult male C57BL/6J (B6) and Acomys (2-4 months) were subjected to unilateral ureter obstruction (UUO) procedure at the left ureter. Kidneys from sham operated controls and UUO-treated animals after 2 and 5 days were collected after perfusion with ice-cold normal saline and flash frozen in liquid nitrogen. Total RNA was extracted from kidney using Trizol (Invitrogen) and Direct-zol RNA Kit (R2072, Zymo Research) according to manufacturer instructions. RNA samples were analyzed with Agilent 6000 RNA Nano Kit at Bioanalyzer 2100 for quality assurance and then submitted to Novogene (Chula Vista, CA) for oligo-dT based mRNA enrichment, cDNA library generation with random hexamers, and Illumina sequencing at 50M reads in paired end 100bp sequencing conditions. The Acomys transcript data was aligned to the Tr2aacs transcriptome (19), and re-annotated by blasting against the mouse transcriptome. We obtained an average of 57 million reads from both Mus and Acomys libraries, mapped >75% of reads to a transcript in mouse transcriptome and Acomys Tr2aacds transcriptome for homology-based identification (Table S3).

Bioconductor edgeR package was used to filter out genes with consistently low levels of expression, and to linear model, fit and count data (Robinson et al., 2010). We made comparisons between within-species groups using quasi-likelihood F-tests at a log fold change of 0.585, which is equal to a linear fold change of 1.5, or a 50% difference in expression between groups, and a false discovery rate (FDR) of 0.05 (Lund et al., 2012). This approach generated approximately 3900-5300 Mus and approximately 1700-2500 Acomys differentially expressed transcripts between UUO injured and sham operated control kidneys in the same species. Genes with conserved increased or decreased differential expression profiles between injured and sham operated kidneys were identified. Non-conserved species-specific gene expression differences were also identified by using limma voom function to fit data to a linear model, first considering within species differences between time points to obviate potential technical differences between transcriptome assemblies and completeness, followed by empirical Bayes adjusted interaction tests for the d2 vs sham, d5 vs sham and d5 vs d2 comparisons, such as (d2Acomys – shamAcomys) – (d2Mus – shamMus). This resulted in identification of approximately 500-800 statistically significant transcript interactions between species that exhibited a non-conserved change in expression level at an FDR < 0.05 and a 1.5 linear fold change (logFC > 0.585) within one of the 4 groups. To visualize interaction data, we averaged the expression level for a given gene across each comparison, calculated the difference from the mean, and color-coded gene z-scores to reflect the magnitude of expression differences.

### Pathway analysis

Ingenuity Pathway Analysis (version 01-19-02, QIAGEN) was used to identify the most significant canonical pathways based on differentially expressed transcripts between UUO and sham as determined by RNA sequencing, using linear fold change of expression -2 to +2 and FDR>0.05 as cutoffs for the analysis. Here we increased the absolute fold change from 1.5 to 2 in order to more stringently identify important pathways. The top 15 most significant differentially regulated canonical pathways by p-value for B6 and Acomys are reported along with the activation z-score. The z-score was calculated by IPA and was based on the direction of effect of each differentially regulated gene within a pathway and predicted whether a pathway was up or down regulated after day 2 UUO. Transcription factor enrichment analysis was performed using ChIP-X Enrichment Analysis Version 3 (ChEA3), which applied the Fisher’s Exact Test to compare genes from clusters 2 and 3 of the RNA sequencing analysis to transcription factor – target interactions from published ChIP-Seq and co-expression databases (Keenan et al., 2019).

### Lightsheet ultramicroscopy

B6 and Acomys UUO and IRI and contralateral control kidneys were collected from animals after 14 days of injury. Animals were perfused with PBS and then 4% PFA-PBS solution, kidneys were removed and post-fixed in 4%PFA overnight at 4°C. Kidneys were dehydrated through a methanol series, delipidated, and cleared using the iDISCO protocol (Renier et al., 2014). Cleared kidneys were imaged with an UltraMicroscope (LaVision Biotech), using 488 nm lightsheet with a Z-step of 3 μm, across multiple magnifications (μm to mm). Image volumes were assembled and analyzed using Imaris (Bitplane) and FIJI. Cortex region of interest (ROI) was manually defined (kidney outer surface to cortex/medulla boundary) using Surfaces. High-
contrast regions within the cortex ROI were automatically/semi-automatically rendered and segmented using Surfaces in Imaris.

**Thymidine pulse labeling**
Age-matched adult male C57BL/6J (B6) and Acomys (2-4 months) normal/uninjured (n = 4) and uni-IRI (n = 6) received EdU (50 mg/kg) intraperitoneally starting on the day of surgery and every 2 days until sacrifice at day 14. Cryosections were stained using the Click-IT kit S94 (ThermoFisher) along with Cdh1 and ZO-1/Tjp1, imaged by confocal microscopy. Digital images were obtained and image analysis performed on six images per animal.

**QUANTIFICATION AND STATISTICAL ANALYSIS**
All data are presented as the mean and standard error. All statistical analyses were performed using GraphPad PRISM 7.0 (GraphPad Software) and STATA 14 (StataCorp LP) software. Two-way analysis of variance (ANOVA) was performed for all parametric data including computer-assisted image analysis data for time and species. For image analysis data, the arithmetic mean of six randomly selected images of slides for each animal was used for the two-way ANOVA. Sidak’s and Tukey’s multiple comparison post-tests were utilized for time and species, respectively. Nonparametric data (IFTA and tubular injury scores) were analyzed using the Mann-Whitney U test. A p value < 0.05 was considered statistically significant. UUO kidney weights were analyzed by linear regression.