

Snapshots of Nascent RNA Reveal Cell- and Stimulus-Specific Responses to Acute Kidney Injury

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Supplemental Figures and Figure Legends

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Figure S1. **Patterning of Gene Expression.** (A) Human Kidney Biopsy. Nephrectomy- 18 minutes of warm ischemia followed by cold ischemia. *LCN2* (NGAL) is expressed in large tubular ducts in excess of surrounding proximal tubules. RNAscope Red-NGAL probe (Cat. No. 559441-C2) was diluted 1:3000 (5 times the dilution used in Figure 1). (B) Acute tubular injury in the setting of pyelonephritis. *LCN2* (NGAL) is expressed in tubules. RNAscope Green-NGAL probe (Cat. No. 559441). Bar = 20 μ m.

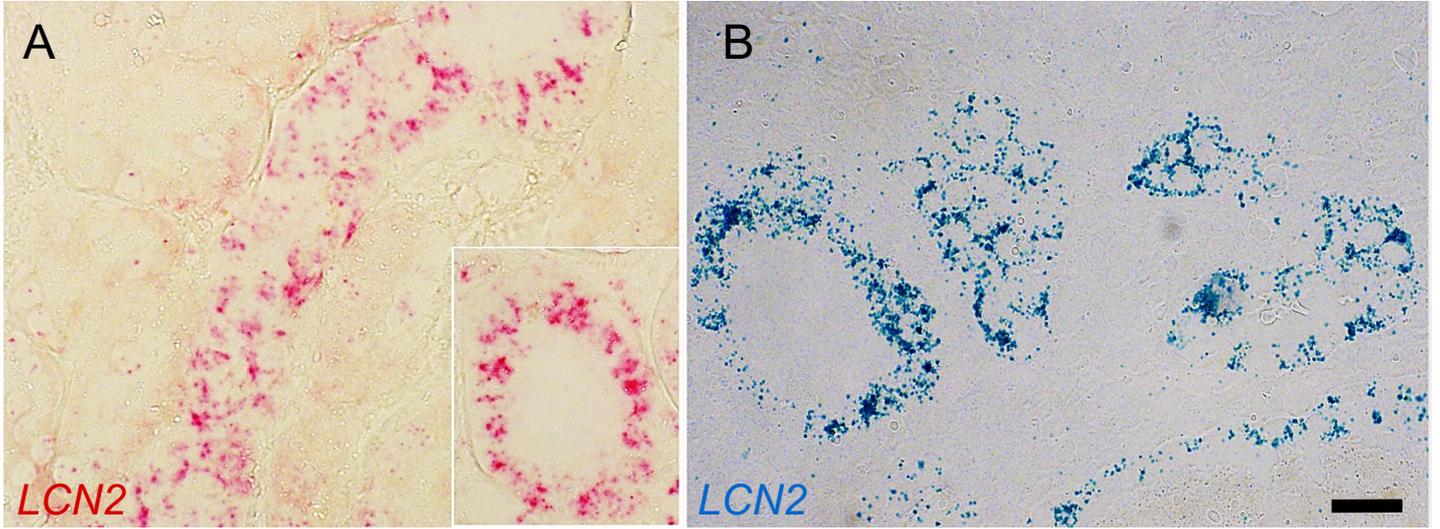


Figure S1

Figure S2. **Optimization of the pulldown of 4-TU labeled RNA.** Total RNA extracts from kidneys of 3 month old mice *EIIACre; Rosa^{Uprtf/+}* were thio-biotinylated and thio-RNA recovered using avidin-bead precipitation. A range of Biotin HPDP (0%, 50%, 100%, Cx-L), and a range of avidin beads (0, 50%, 100%, 200%) were evaluated by BioAnalyzer detection of RNA. Note that the pulldown was optimal at 100% crosslinking reagent and 50% or 100% beads. 100% refers to recommendations of Gay et al (28).

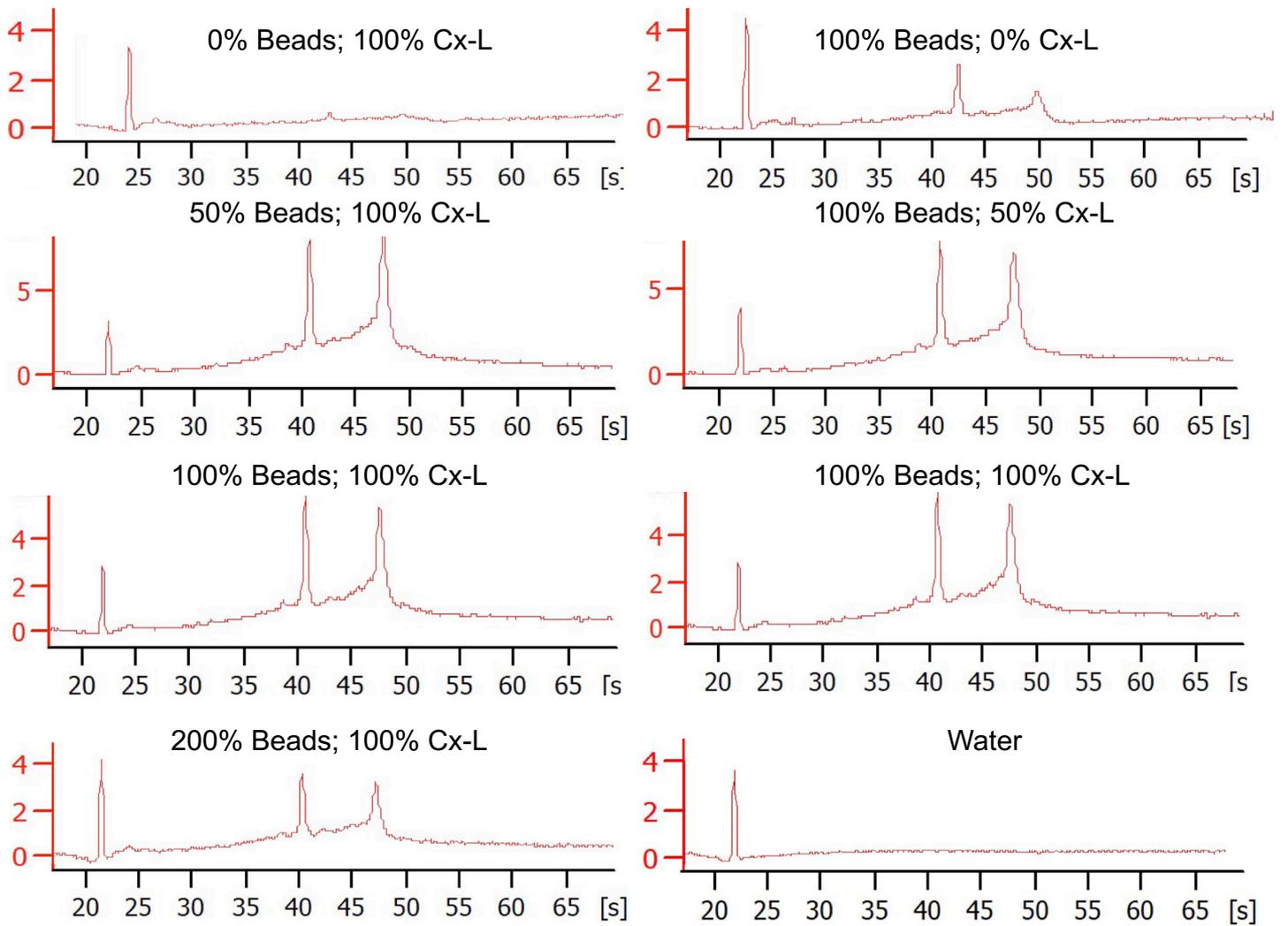
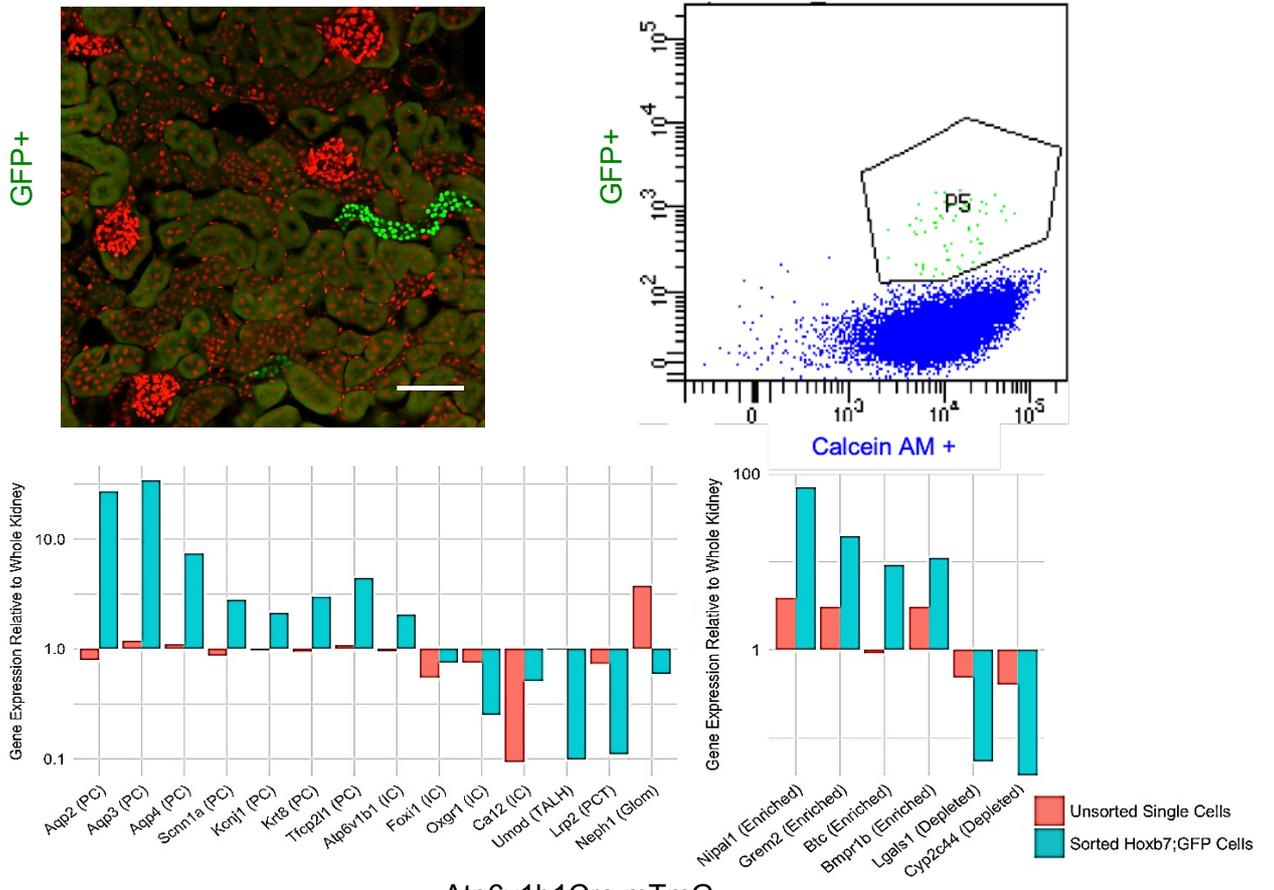


Figure S2

Figure S3. Validation of Cell Specific Cre Driven RNA Isolation. *Hoxb7Cre;Rosa^{nT-nG/nT-nG}* and *Atp6v1b1Cre;Rosa^{mTmG/mTmG}* mice revealed collecting duct specific GFP staining. The GFP⁺ cells were isolated from 3 month old *Hoxb7Cre;Rosa^{nT-nG/nT-nG}* and *Atp6v1b1Cre;Rosa^{mTmG/mTmG}* mice by FACS (gated for Calcein AM⁺ live cells) from dissociated kidneys and then subjected to RT-qPCR. Note that *Hoxb7Cre* enriches for PC genes while *Atp6v1b1Cre* enriches for IC genes, despite overlapping Cre domains. Scale Bar = 100 μ m.

Hoxb7Cre nTnG



Atp6v1b1Cre mTnG

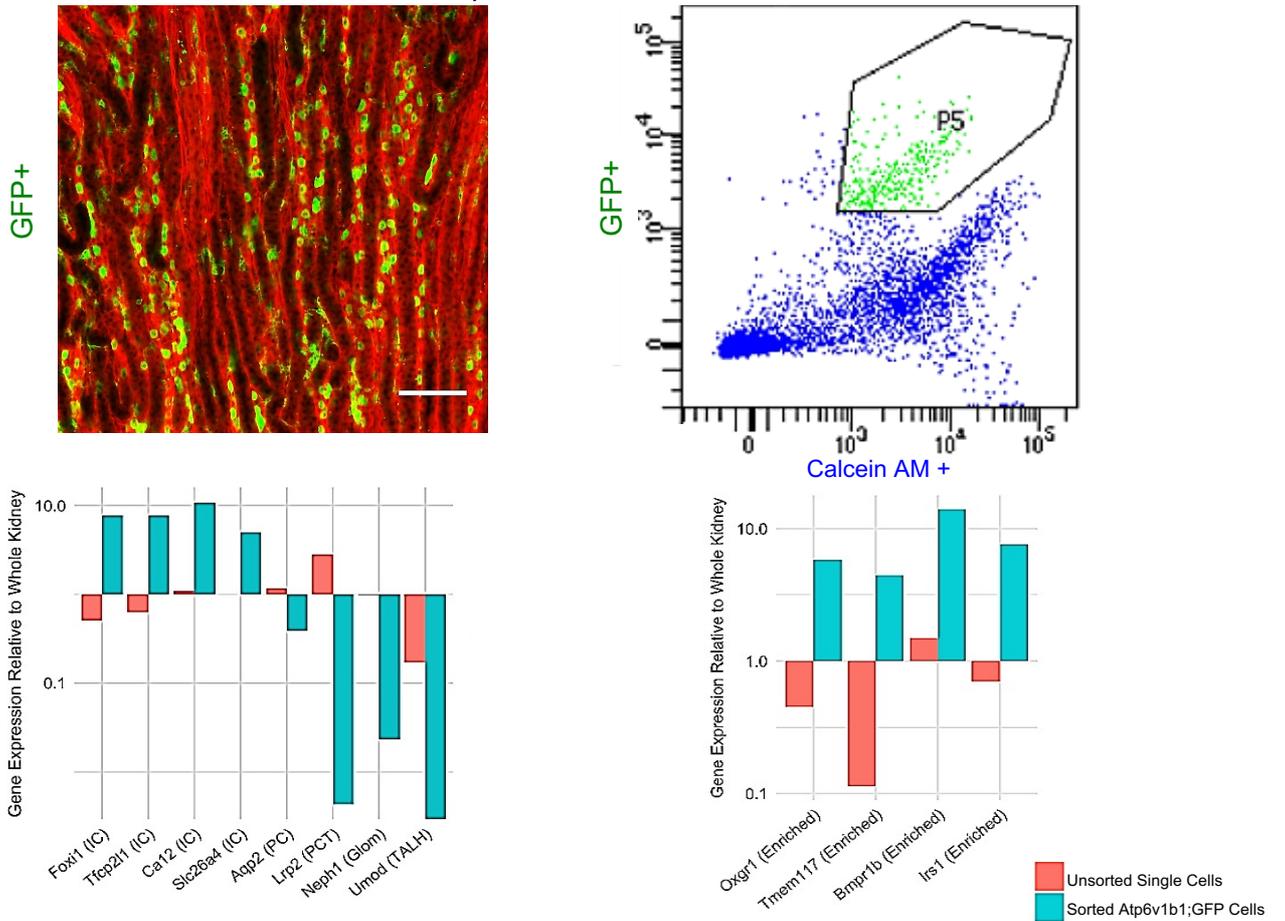
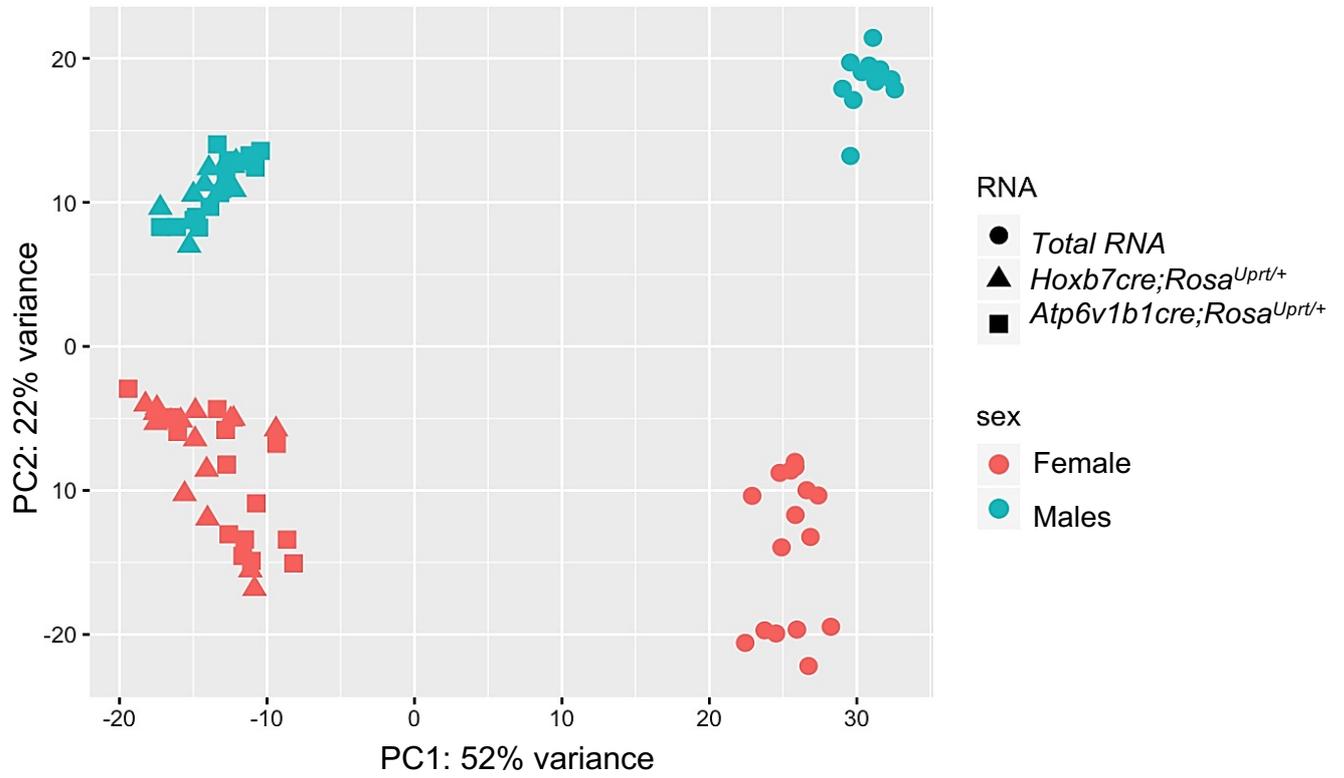


Figure S3

Figure S4. **Principal Component Analysis.** (A) Inspection of variance across PC1 and PC2 revealed that PC1 distinguished total kidney RNA vs *Hoxb7Cre;Rosa^{Uprt/+}* and *Atp6v1b1Cre;Rosa^{Uprt/+}* pulldowns while PC2 distinguished male from female mice at baseline. (B) Inspection of variance across PC1 and PC2 revealed that PC1 distinguished volume depletion from ischemia and PC2 distinguished *Hoxb7Cre;Rosa^{Uprt/+}* and *Atp6v1b1Cre;Rosa^{Uprt/+}* pulldowns. Volume Depleted mice, n=7; Ischemic mice, n=7; Control mice, n=8.

A Pulldown vs Total RNA and Male vs Female Mice



B Male Mice

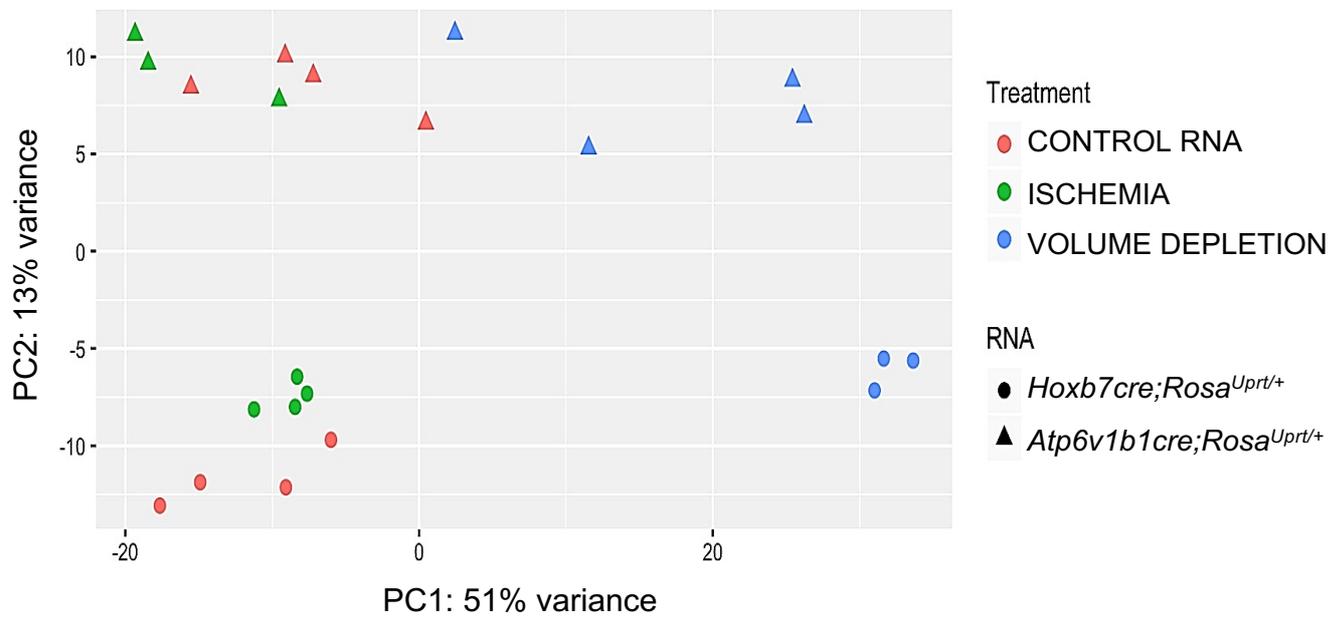


Figure S4

Figure S5. **Generation of *Lrp2*^{CreERT2/+}**: *Lrp2* translational STOP (TAG) was replaced with the “P2a-CreERT2-frt-neo-frt” cassette, permitting the expression of both *Lrp2* and *CreERT2* from the same allele. The Neo cassette was removed by crossing Actin-FIpe mice.

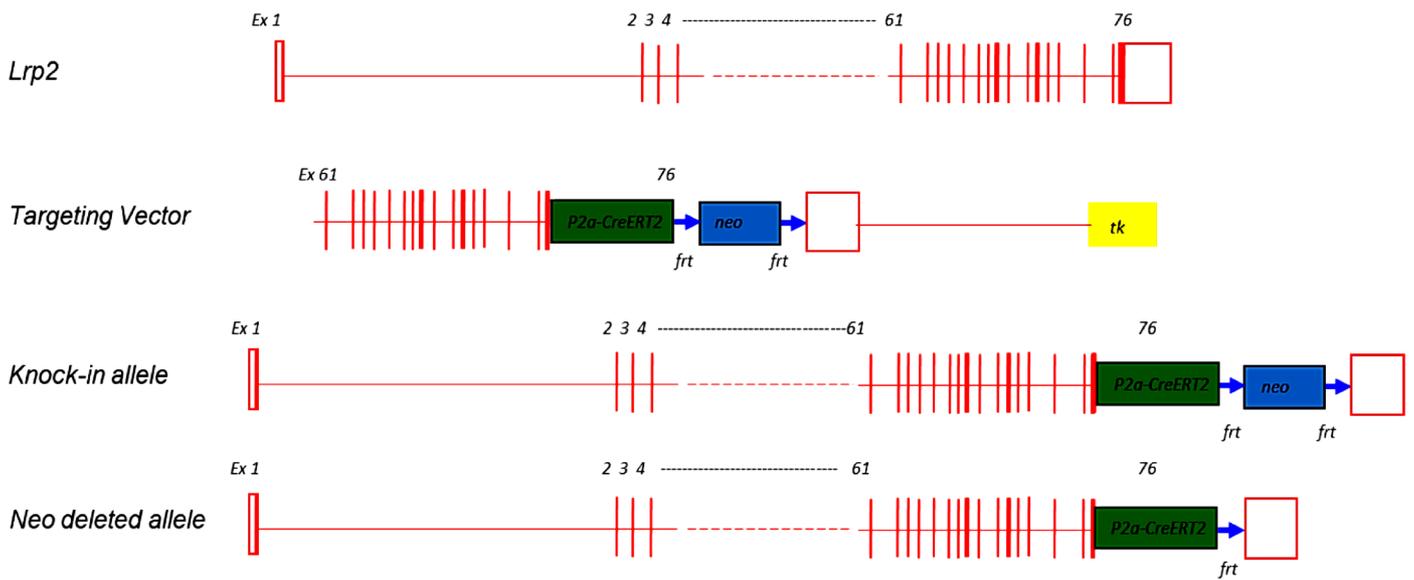


Figure S5

Figure S6. **Time-dependent gene expression in mouse model of volume depletion.** GFP labeled intercalated cells were isolated by FACS from *Atp6v1b1Cre;Rosa^{mTmG/mTmG}* 3 month old mice subject to volume depletion for 0, 1, 2, and 3 days and assayed by RT-qPCR using *Actb* as reference. mRNA expression at day 0 (control) was set as 1. The asterisks indicate Bonferroni adjusted significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, respectively compared to day 0 control (two tailed t test); ns indicates no significance. $n=8$. Data should be compared to Figure 2E.

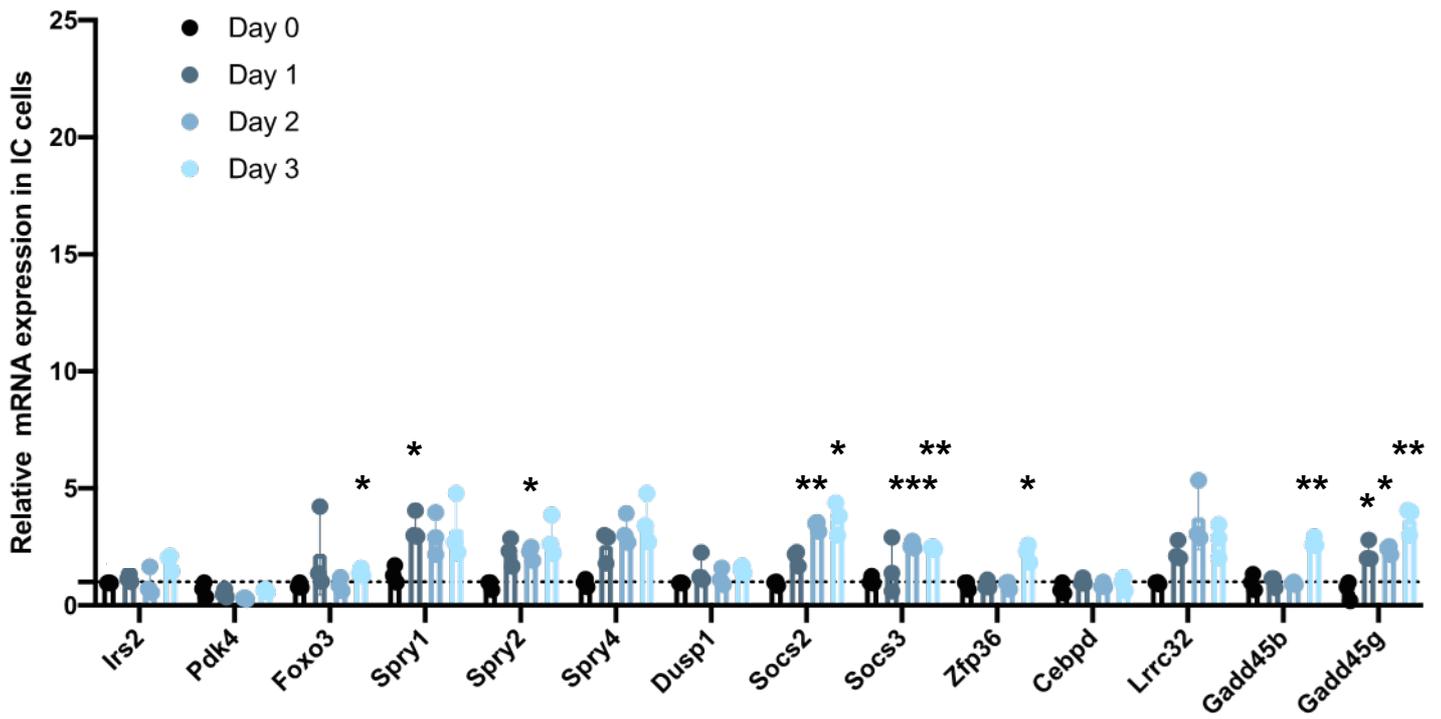


Figure S6

Figure S7. **Strengths and weaknesses of different transcriptomic methods.**

Method	Strengths	Weaknesses
UPRT Labeling Sequencing Nascent RNA	<p>Labels RNA in a specific cell type within intact tissue, preserving cellular interactions and organ physiology.</p> <p>Labels kinetically active nascent transcription at a chosen time point</p> <p>Labels several types of RNA: mRNA, noncoding RNA, miRNA, and rRNA.</p> <p>Isolates adequate amounts of nascent RNA even derived from rare or dispersed cells without batch pooling.</p> <p>Isolates nascent RNA without organ dissection or tissue dissociation.</p> <p>No effect on mouse viability. No variation in the expression of UPRT.</p>	<p>Depends on Cre drivers. Useful only in species that can express Cre.</p> <p>UPRT measures nascent transcripts which do not fully map to steady state transcriptomic databases.</p> <p>May over-represent uracil-rich RNA, impacting pulldown efficiency and hence quantitative comparisons across all members of a gene-set.</p>
Fluorescence activated cell sorting (FACS) Sequencing Steady State RNA	<p>Isolates different cell types on the basis of surface markers, gene expression, cellular size and granularity.</p> <p>Isolates a quantifiable number of cells from a specific population (ranging from a single cell to millions of cells).</p> <p>Isolates cells for analysis of steady state RNA by RNA-seq.</p>	<p>Tissue dissociation into a single cell suspensions results in selective cell isolation, cell stress and death.</p> <p>Flow cytometry contributes to cell stress and cell death, yet requires living cells for successful sorting.</p> <p>Cell recovery can vary 50-70%, potentially limiting rare cell types.</p> <p>Depends on the availability of antibodies targeted to the cell surface antigens.</p>
Single cell scRNA-seq Sequencing Steady State RNA	<p>Provides gene expression at single cell resolution</p> <p>Categorizes heterogeneous cell types independently of predefined markers or assumptions regarding cell hierarchies</p> <p>Able to discover novel cell types</p> <p>Enables bioinformatic reconstruction of a dynamic cellular process such as development, differentiation, cell states and disease progression.</p>	<p>Depends on RNA preservation, including freezing protocols</p> <p>Depends on tissue dissection or FACS purification to enrich for a specific cell types.</p> <p>Tissue dissociation into single cell suspensions results in selective cell isolation and contributes to cell stress and cell death, yet living cells are required for successful analysis.</p> <p>Low RNA capture efficiency, low sequencing coverage, low detection of specific transcripts, high dropout rates, high level of technical noise, and complex informatics</p> <p>Contamination by dying or dead cells or by mixed multiple cells (i.e. doublets)</p>

Figure S7

Figure S8. Two Hits: Volume Depletion and Chronic Inflammation (Zfp36 KO). (A) *Zfp36* expression in *Zfp36*^{+/+} vs *Zfp36*^{-/-} 3 month mice *p<0.05; ***p<0.001 (B) *Pappa2* expression marked volume depletion in both wild type and *Zfp36*^{-/-} mice. ***p<0.001; WT Control vs WT volume depletion; *Zfp36* knockout control vs *Zfp36* knockout volume depletion. Significance was determined by Student's two tailed t-test. Data represent mean ± SD. (C) Urine immunoblot for Ngal reveals limited expression of Ngal in wild type mice even after 72 hours of volume depletion. In contrast, volume depletion stimulated Ngal expression in mice with chronic inflammatory disease due to *Zfp36* deletion (3 month old littermates). Cloned purified Ngal proteins are standards. (D) Induction of Kim-1 in proximal tubules of *Zfp36*^{-/-} mice exposed to volume depletion. In contrast, wild type control mice, *Zfp36*^{-/-} control mice, and wild type (all 3 month old littermates) volume depleted mice all failed to express this marker of ischemic nephropathy. sCr was elevated equivalently in wild type and *Zfp36*^{-/-} volume depletion; n=8 mice; WT Control vs WT volume depletion: p= 0.02; *Zfp36* knockout control vs *Zfp36* knockout volume depletion: p= 0.025. Bars= 25µM.

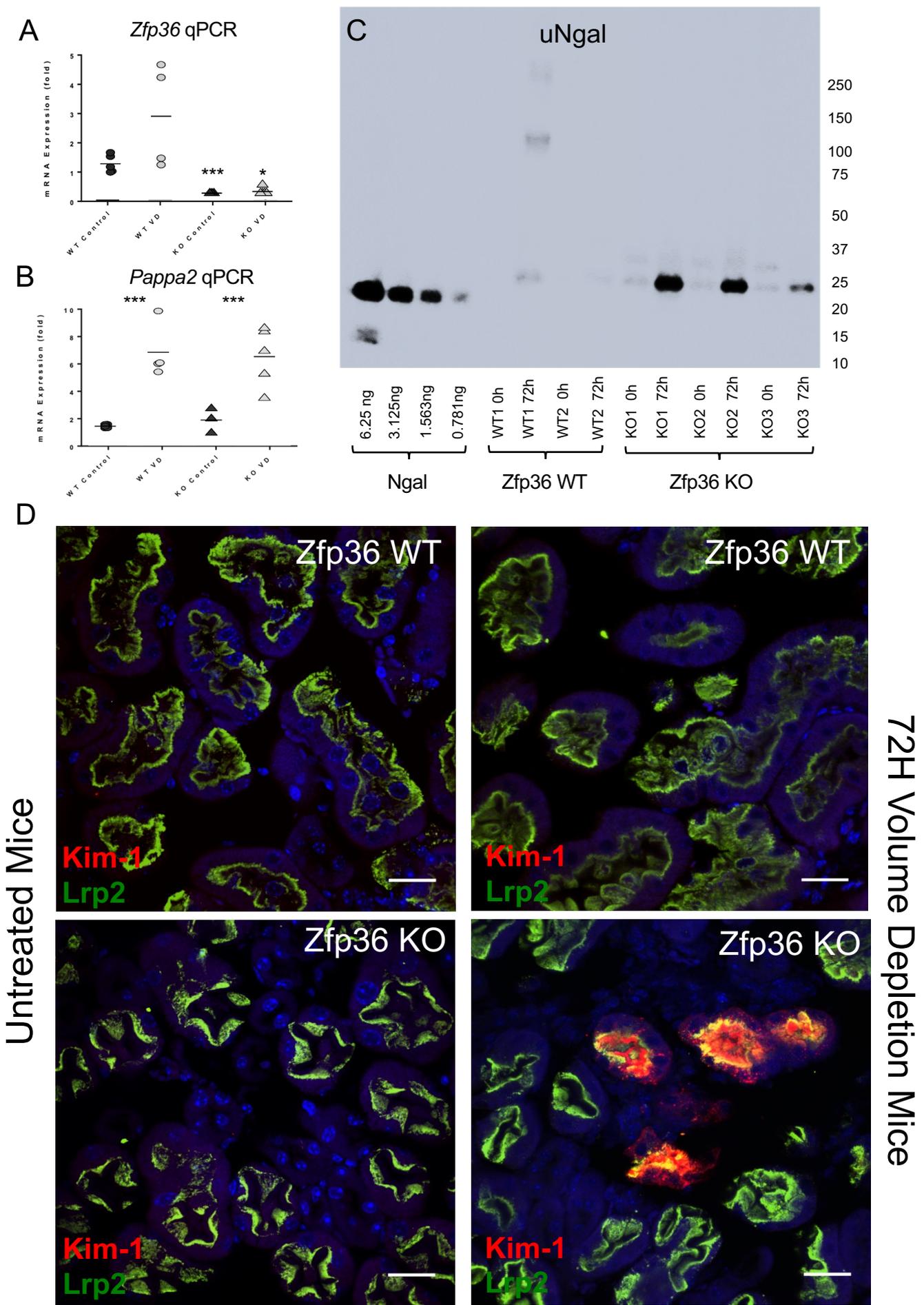


Figure S8

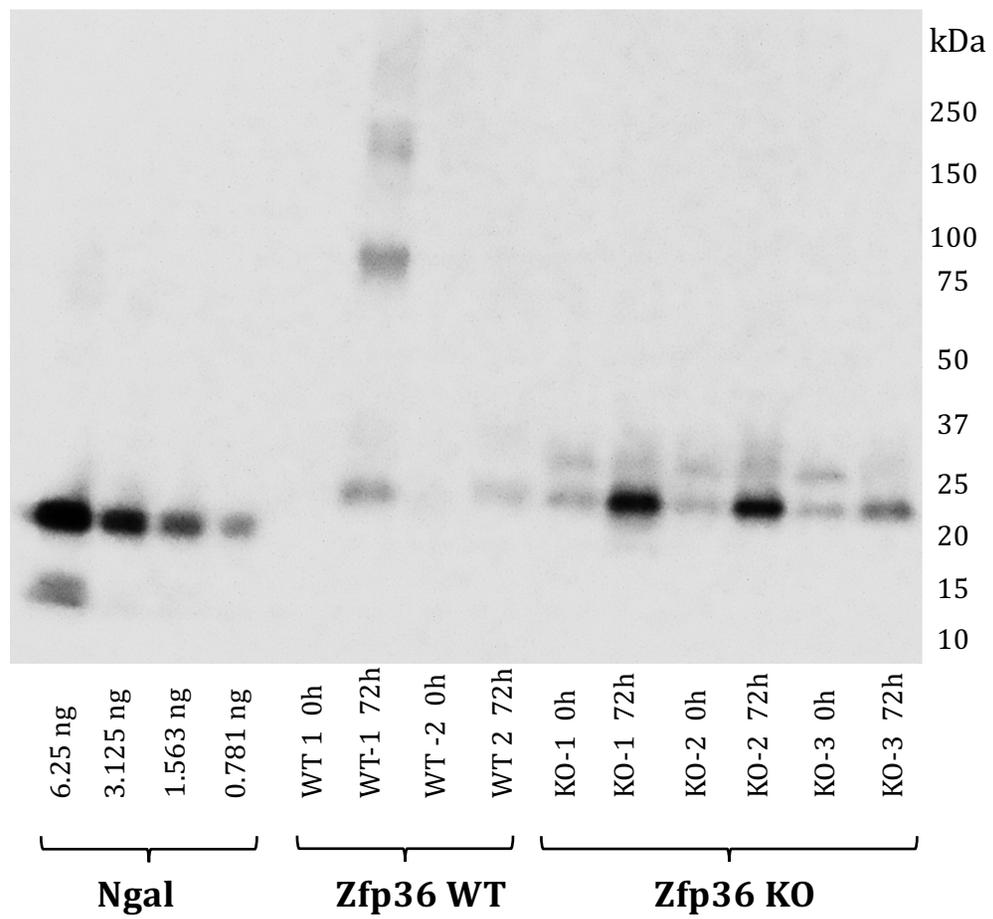


Figure S9

Supplemental Tables: Control, Ischemic and Volume Depleted Mice Datasets.

Supplemental Table 1. Hoxb7Cre pulldown vs Total RNA values from male and female controls; $p < 0.05$; $n = 21$ mice

Supplemental Table 2. Atp6v1b1Cre pulldown vs Total RNA values from male and female controls; $p < 0.05$; $n = 21$ mice.

Supplemental Table 3. Hoxb7Cre pulldown ischemia vs Hoxb7Cre pulldown from control kidneys; $p < 0.05$; male mice; $n = 7$.

Supplemental Table 4. Hoxb7Cre pulldown volume depleted vs Hoxb7Cre pulldown from control kidneys; $p < 0.05$; male mice; $n = 7$.

Supplemental Table 5. Hoxb7Cre pulldown from ischemic vs volume depleted kidneys; $p < 0.05$; \log_2 fold > 1

Supplemental Table 6. Atp6v1b1Cre pulldown ischemia vs Atp6v1b1Cre pulldown from control kidneys; male; $p < 0.05$; $n = 7$.

Supplemental Table 7. Atp6v1b1Cre pulldown volume depletion vs Atp6v1b1Cre pulldown from control kidneys; $p < 0.05$; male; $n = 8$.

Supplemental Table 8. Atp6v1b1Cre pulldown from ischemic vs volume depleted kidneys; $p < 0.05$; \log_2 fold > 1

Supplemental Table 9. Atp6v1b1Cre vs Hoxb7Cre pulldowns from ischemia kidneys; $p < 0.05$; \log_2 fold > 1

Supplemental Table 10. Atp6v1b1Cre vs Hoxb7Cre pulldowns from volume depleted kidneys. $p < 0.05$; \log_2 fold > 1

Supplemental Table 11. Genes coding for secreted proteins comparing volume depletion and ischemic kidneys; $p < 0.05$; \log_2 difference > 1 .

Supplemental Table 12. Primers

Supplemental Table 13. Probes and Antibodies