

**Spatiotemporal transcriptomic analysis during cold ischemic injury to the murine kidney reveals
compartment-specific changes**

Supplemental Figures:

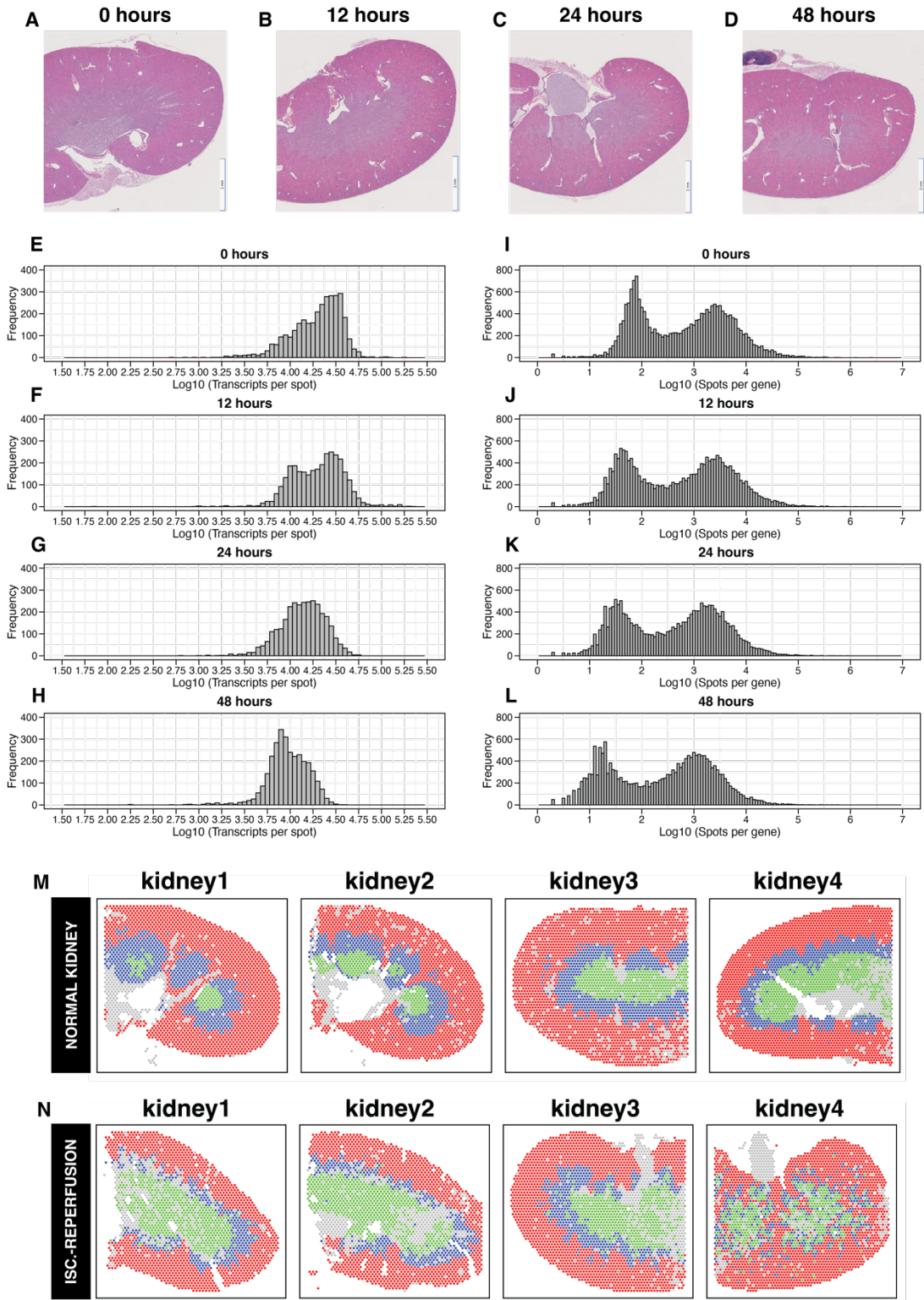


Fig. S1. Tissue specimens and quality control of cold ischemia injury kidneys. H&E-stained images of kidney tissue specimens subjected to **(A)** 0 hours **(B)** 12 hours **(C)** 24 hours and **(D)** 48 hours of cold ischemia injury. Histograms depicting distribution of **(E-H)** transcripts per spot and **(I-L)** spots per gene at time points 0 hours, 12 hours, 24 hours and 48 hours of cold ischemia injury respectively. Annotations highlighting the different compartments namely cortex (red), outer medulla (blue), inner medulla (green) and other (gray), within the normal control (CTRL) kidneys **(M)** and 24-hours warm ischemia reperfusion (AKI24) injury **(N)** tissue specimens.

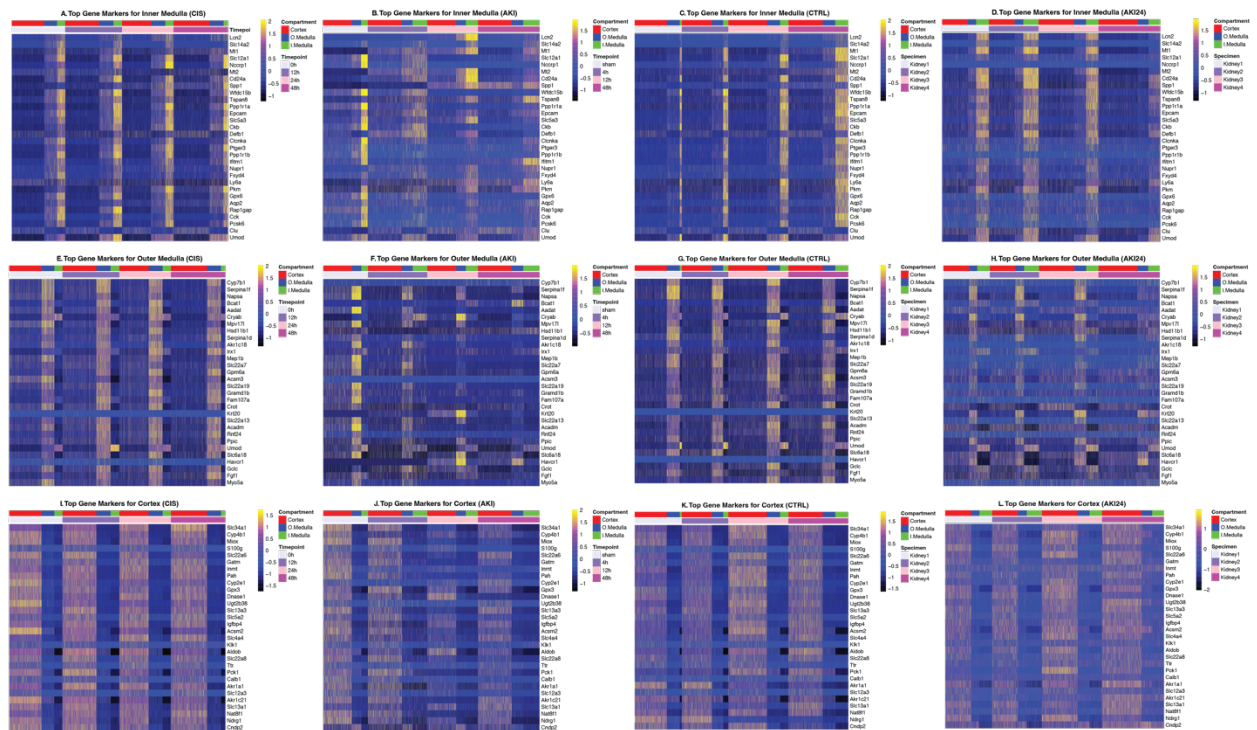


Fig. S2. Heatmap of top compartment-specific marker genes for cold ischemia (CIS), warm ischemia-reperfusion-induced acute kidney injury (AKI), normal control kidneys (CTRL) and 24-hours warm ischemia-reperfusion-induced acute kidney injury (AKI24) injury datasets. Heatmap of top compartment-specific marker genes expression within the different kidney tissue compartments namely (A,B,C,D) inner medulla (I. medulla (green)), (E,F,G,H) outer medulla (O. medulla (blue)) and (I,J,K,L) cortex (red) belonging to different timepoints of cold ischemia (CIS) and warm ischemia-reperfusion (AKI) injury, normal control kidneys (CTRL) and 24-hours warm ischemic-reperfusion injury (AKI24) datasets respectively.

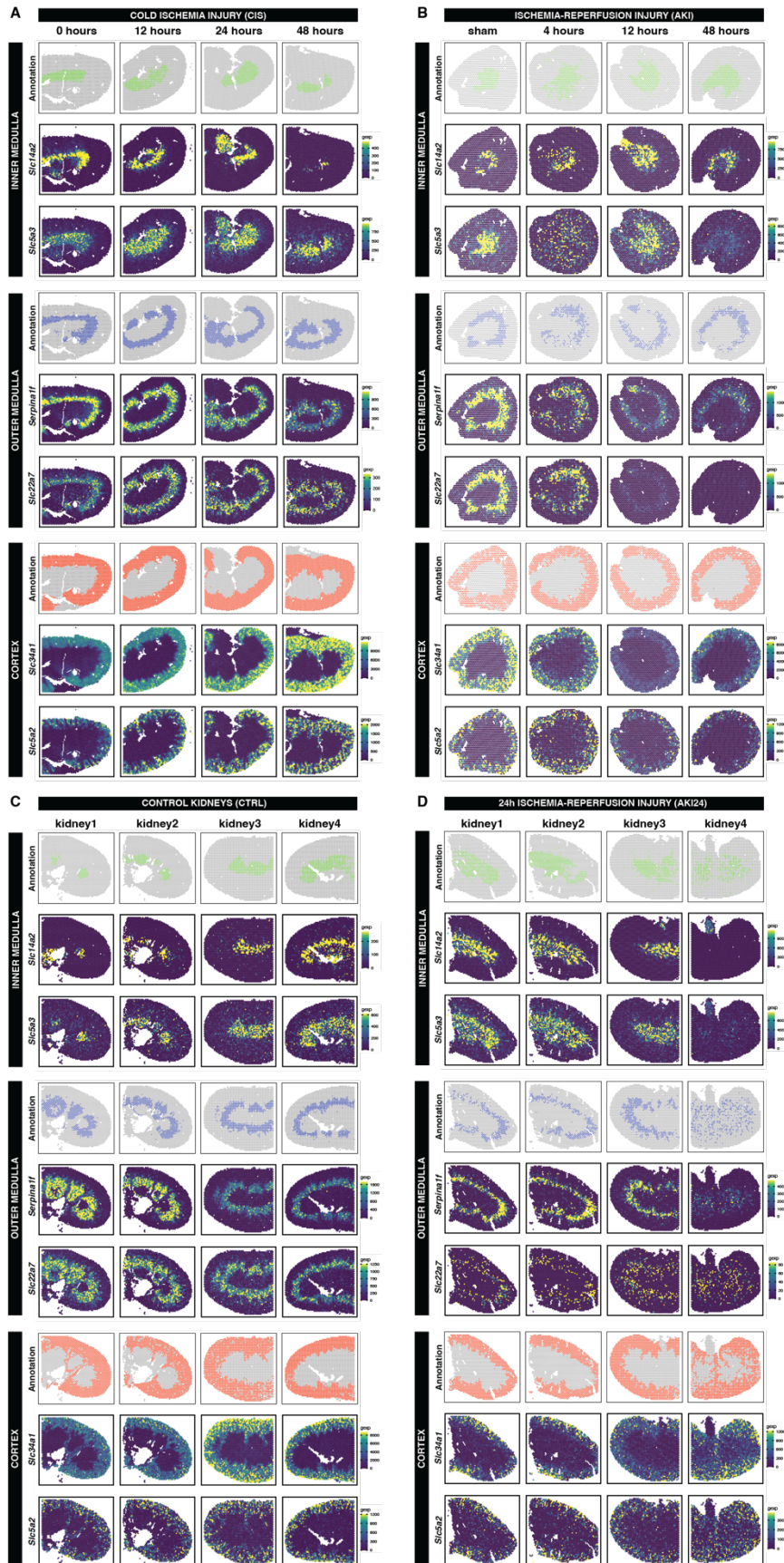


Fig. S3. Spatiotemporal expression patterns of compartment-specific marker genes in different experimental groups. Presentation of inner medulla marker genes (*Slc14a2*, *Slc5a3*); outer medulla marker genes (*Serpina1f*, *Slc22a7*); cortex marker genes (*Slc34a1*, *Slc5a2*) within the **(A)** cold ischemia injury (CIS), **(B)** warm ischemia-reperfusion injury (AKI), **(C)** normal control kidneys (CTRL) and **(D)** 24-hours warm ischemia-reperfusion injury (AKI24) kidney tissue specimens. Color coded annotations: red (cortex), blue (outer medulla), green (inner medulla) and other (gray), highlight the location of the different compartments within respective tissue specimens.

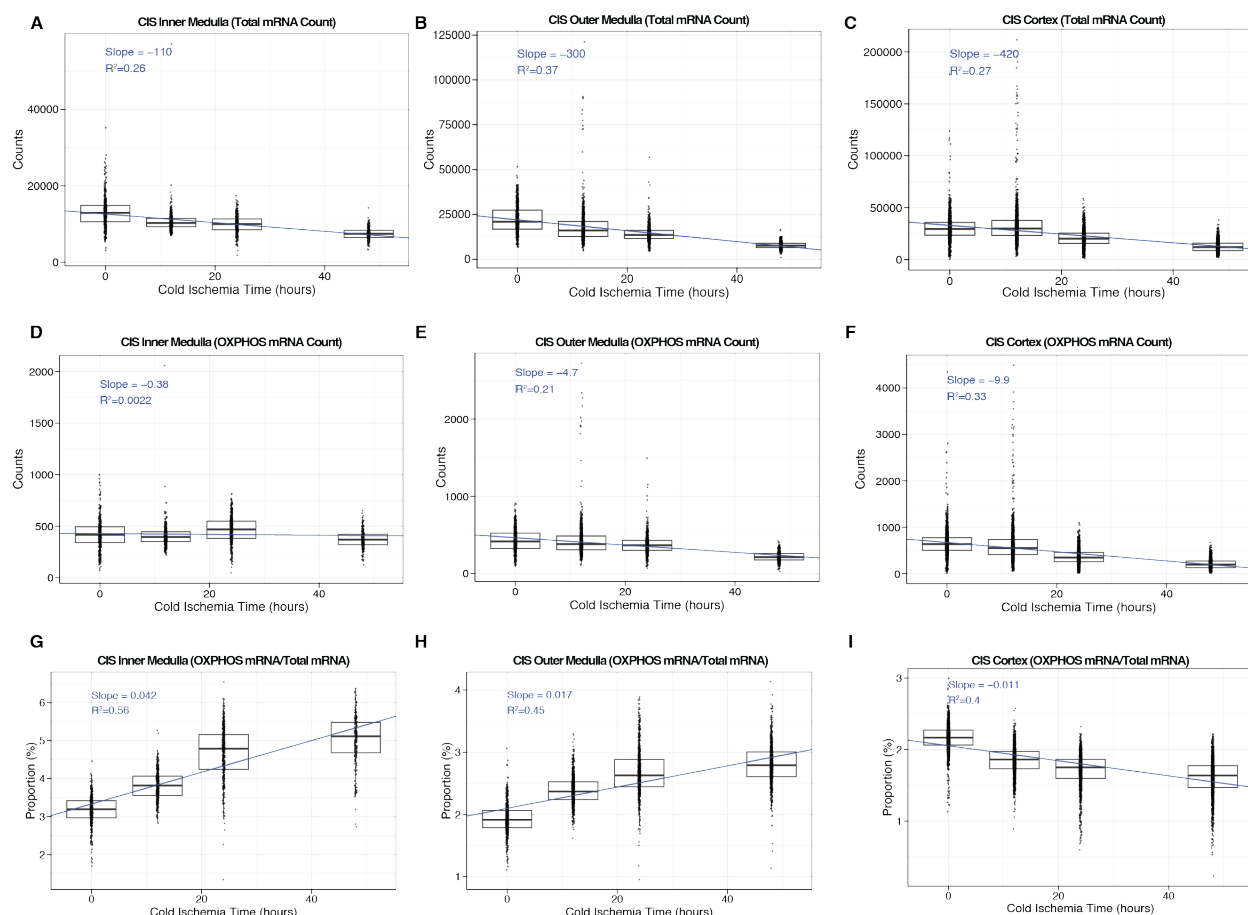


Fig. S4. Compartment-specific proportional enrichment or depletion of OXPHOS genes within cold ischemia kidney tissue. Temporal change in total mRNA count within the **(A)** inner medulla **(B)** outer medulla and **(C)** cortex. Temporal change in OXPHOS related genes within the **(D)** inner medulla **(E)** outer medulla and **(F)** cortex. Temporal change in proportion of OXPHOS related genes with **(G)** inner medulla **(H)** outer medulla and **(I)** cortex. The regression slopes (Slope) and goodness of fit (R^2) of each of the plots is provided at the upper left corner of the respective plots.

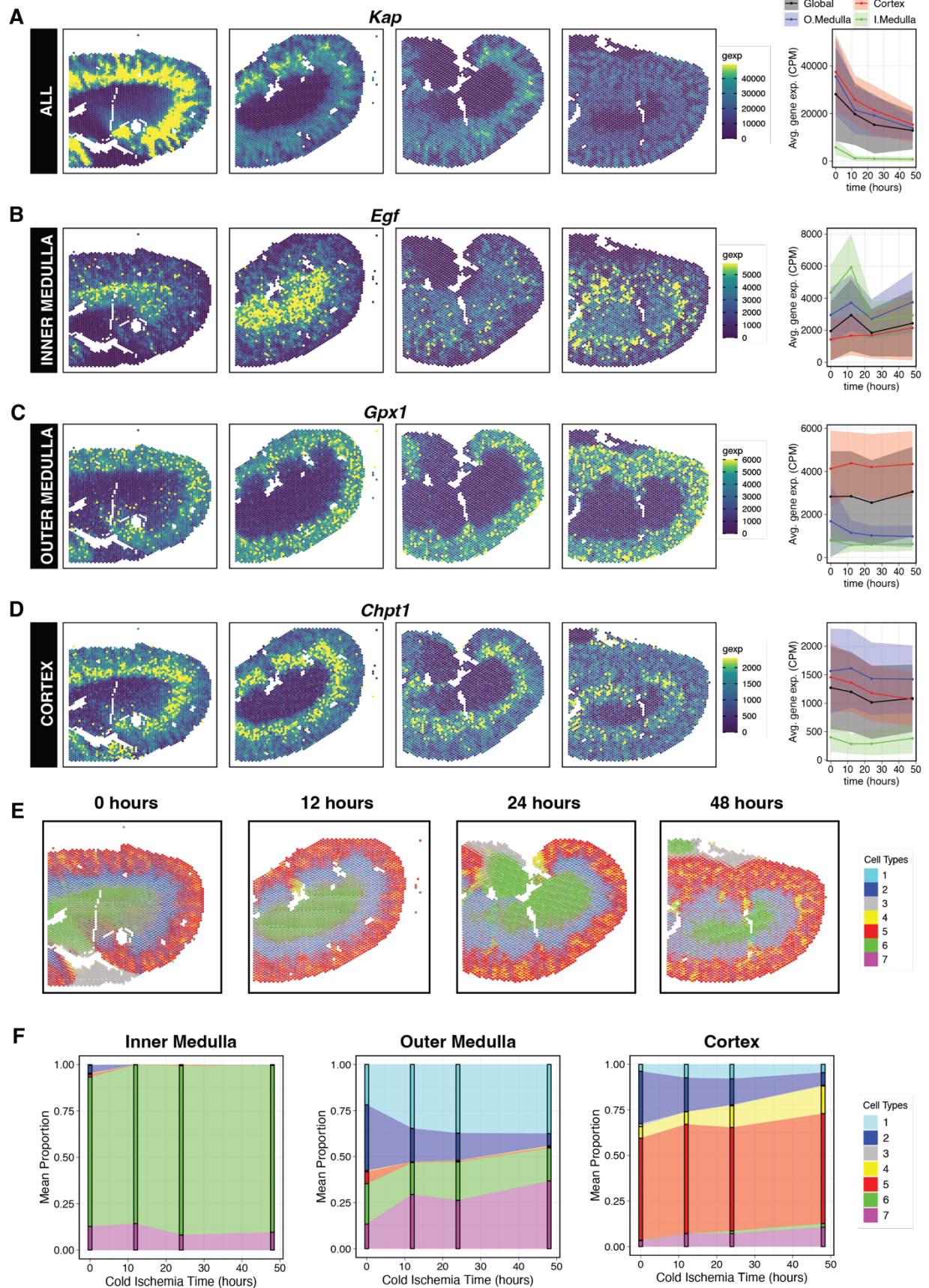
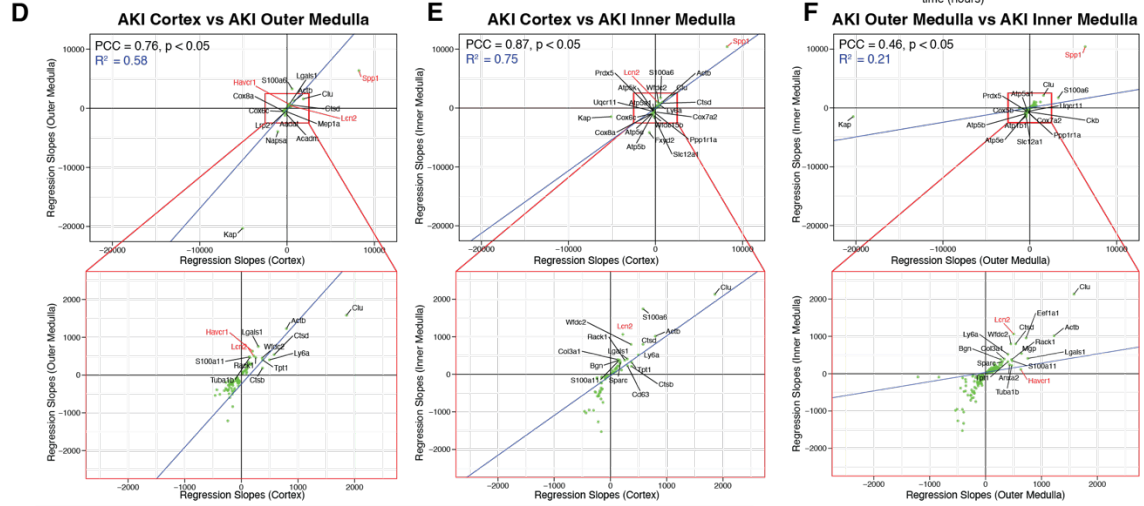
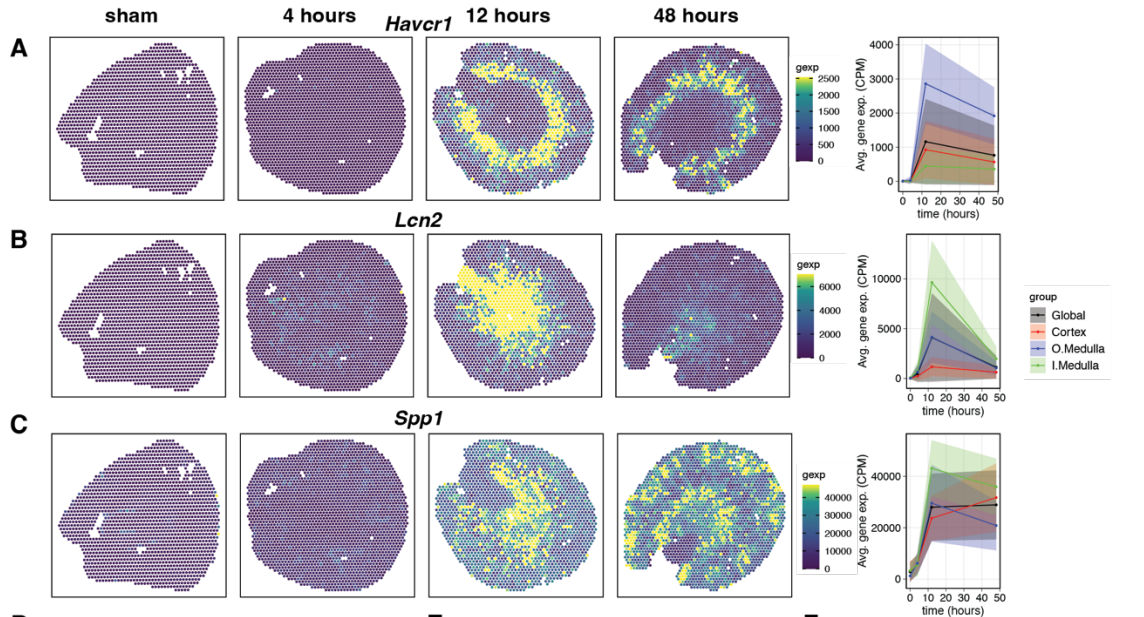


Fig. S5. Representative genes demonstrating compartment-specific downward temporal trend in cold ischemic kidneys and deconvolved cell type changes. While **(A)** *Kap* demonstrated temporally downward trend in all three compartments with varying degrees, **(B)** *Egf*, **(C)** *Gpx1* and **(D)** *Chpt1* were confined to the inner medulla, outer medulla, and cortex respectively. The ribbon plots demonstrate the mean $\pm$ s.d. expression (CPM normalized) of the respective genes within different compartments of the corresponding cold ischemic tissue at different time points. **(E)** Spatial gene expression plots demonstrating spatiotemporal expression of deconvolved cell types (1-7) within cold ischemic kidney tissues. **(F)** Compartment-specific deconvolved cell type composition changes during cold ischemia injury in kidneys.

ISCHEMIA REPERFUSION INJURY (AKI) IN FEMALE MICE



24h ISCHEMIA REPERFUSION INJURY (AKI24) IN MALE MICE

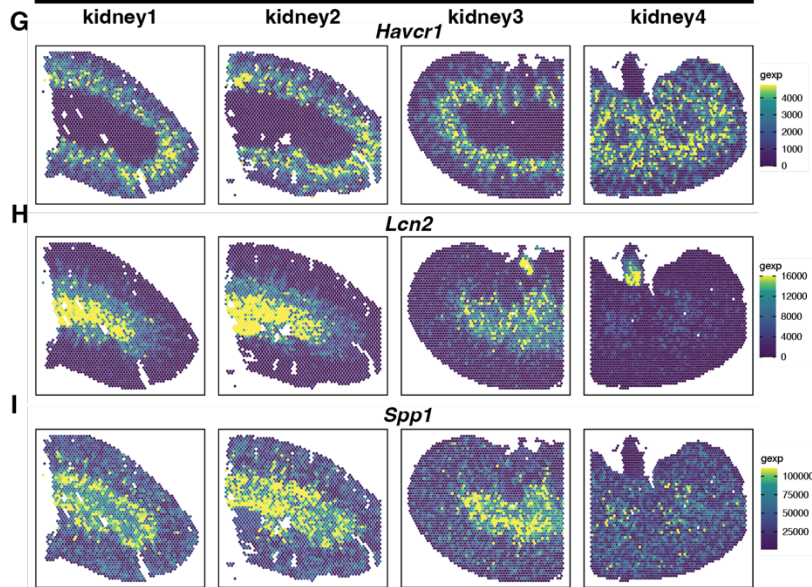
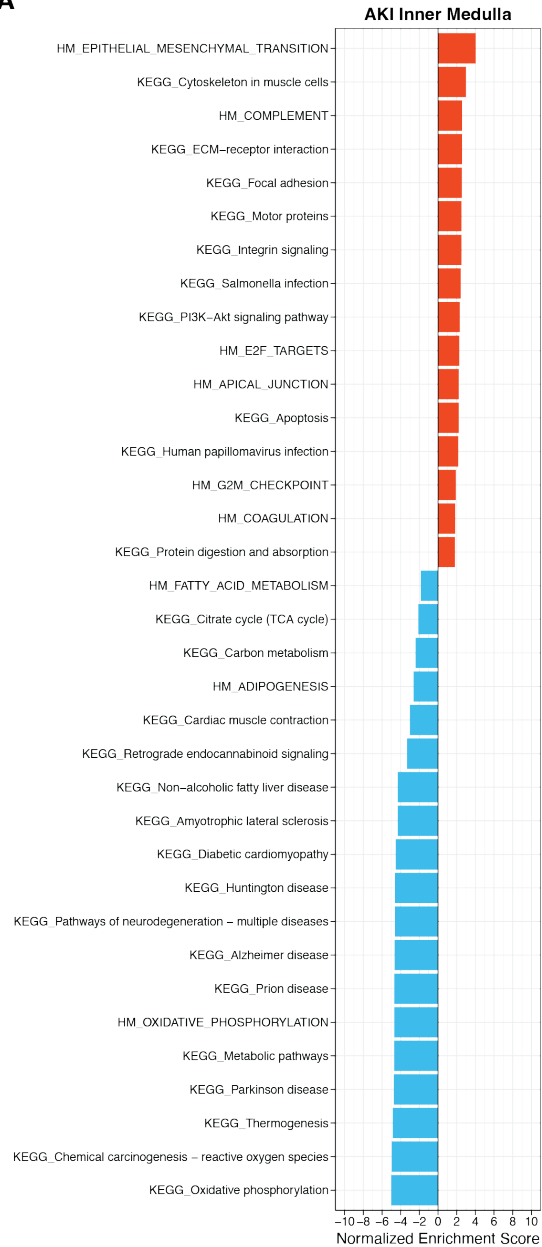
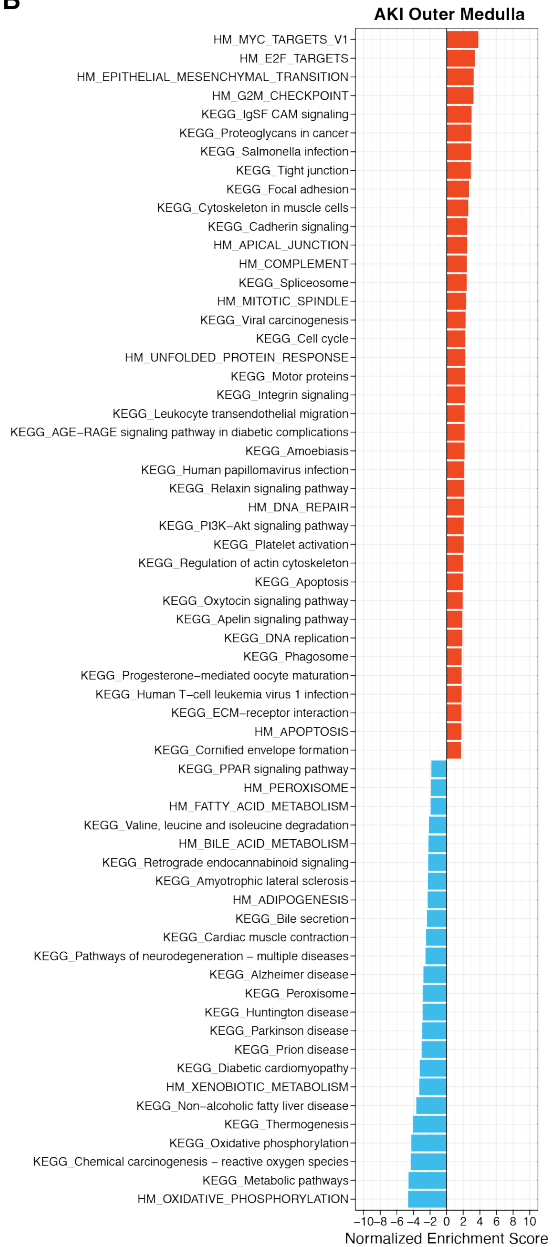


Fig. S6. Spatiotemporal transcriptional dynamics within female and male experimental models of warm ischemia-reperfusion injury (i.e., AKI and AKI24). Spatial plots depicting the spatiotemporal expression of putative AKI marker genes like **(A)** *Havcr1*, **(B)** *Lcn2* and **(C)** *Spp1* within warm ischemia- reperfusion injury (AKI) kidney tissue specimens from female murine models. Scatter plots depicting the correlation analysis of the temporal transcriptional dynamics between the different compartments: **(D)** cortex vs outer medulla **(E)** cortex vs inner medulla and **(F)** outer medulla vs inner medulla of the acute kidney injury (AKI) tissue. Genes exhibiting covarying (green) or divergent (purple) temporal trend within these compartments can be visualized along with their Pearson correlation coefficients (PCC) and linear regression slopes (blue line). AKI marker genes like *Spp1*, *Havcr1* and *Lcn2* within these compartments have been highlighted red. Spatial plots depicting the spatiotemporal expression of **(G)** *Havcr1*, **(H)** *Lcn2* and **(I)** *Spp1* within 24-hours warm ischemia-reperfusion injury (AKI24) kidney tissue specimens from male murine models.

A



B



C

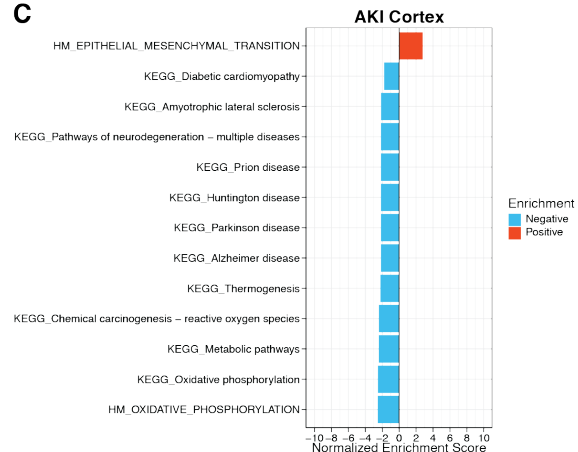


Fig. S7. Compartment-specific enrichment of molecular pathways within acute kidney injury (AKI) tissues. Bar plots highlighting all the enriched molecular pathways (Hallmark and KEGG) that demonstrated similar temporal dynamics (enrichment: positive (red), negative (cyan)) within the corresponding **(A)** inner medulla, **(B)** outer medulla and **(C)** cortex of warm ischemia-reperfusion injury (AKI) kidney tissue. Similar, bar plots highlighting all the enriched molecular pathways (KEGG) that demonstrated similar temporal dynamics (enrichment: positive (red), negative (cyan)) within the corresponding **(D)** inner medulla, **(E)** outer medulla and **(F)** cortex of warm ischemia-reperfusion injury (AKI) kidney tissue. (Note: Hallmark pathways have a prefix “HM_” and are in uppercase whereas KEGG pathways have a prefix “KEGG_”.)

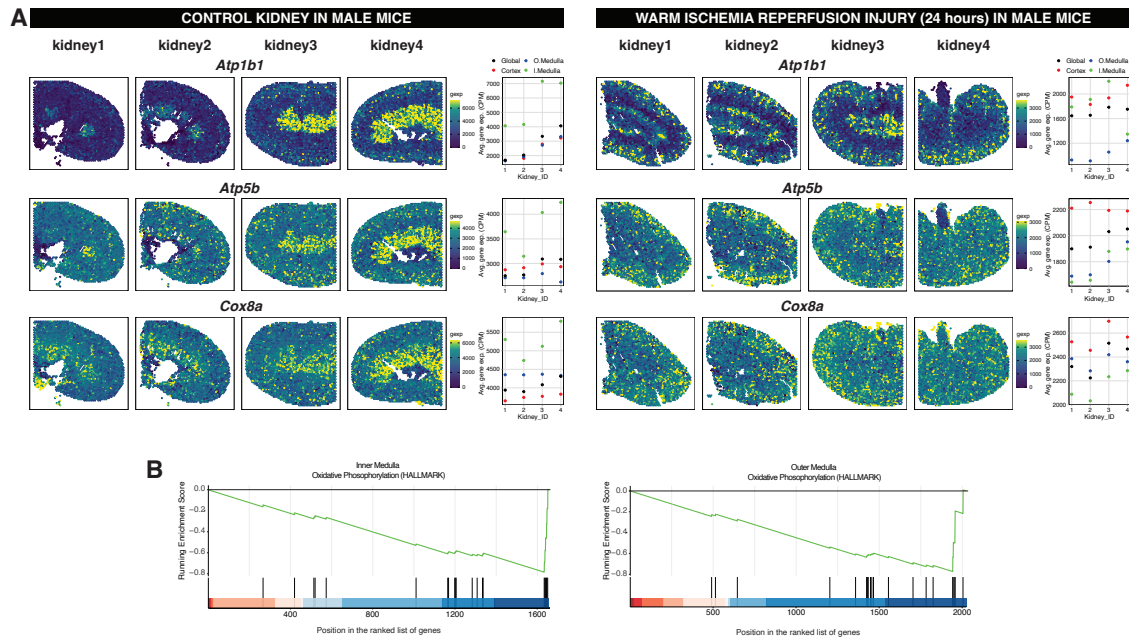


Fig. S8. Differential spatiotemporal expression of OXPHOS genes between the control (CTRL) and warm ischemia injury (24 hours) male kidney. (A) Spatial plots depicting the differential spatiotemporal expression of OXPHOS genes like *Atp1b1*, *Atp5b* and *Cox8a* between the control and warm ischemia-reperfusion injury (AKI24) kidney tissue specimens from male murine model. **(B)** Running enrichment score plots highlighting differential negative enrichment of OXPHOS pathway (HALLMARK) within the inner medulla of warm ischemia-reperfusion injury (AKI24) male kidney tissue as compared to control male kidney.