**Supplemental File 1.** Overview of KPMP technologies- supplementary methods

*Transcriptomics.* Several technologies are generating gene expression data at bulk, regional and single cell level for comprehensive coverage of the transcriptome for the reference (relatively healthy or pathologically normal) and disease atlases (AKI and CKD subtypes). Single nucleus (sn) (UCSD/WU) and single cell (sc) (UCSF and Premiere -Michigan/Broad/Princeton sites) RNA-seq technologies are being used for cataloging molecularly-defined cell populations for the generation a single cell kidney atlas.(4, 7, 9) Each of these technologies uses different approaches in sample preparation, dissociation or processing that have unique advantages and disadvantages (see TIS protocol on <https://kpmp.org/researcher-resources/>). Given the potential to introduce artifacts or miss cell types due to processing of these specimens, concurrent bulk RNA expression analysis on undissociated tissue or regional laser microdissection (LMD) (IU/OSU) is performed.(5, 6, 10) Using the LMD technology, transcriptomic signatures are identified for seven sub-segments of the nephron including the proximal tubule (PT), thick ascending loop of Henle (TAL), distal convoluted tubule (DCT), collecting duct (CD), as well as compartment-specific signatures for glomeruli, the tubulo-interstitium (TI), the interstitium (without glomeruli or tubules), as well as a bulk cross-section of the entire biopsy. LMD transcriptomic signatures can serve as an important independent validation measure that provides regional spatial context to cell populations discovered using single cell technologies. To complement the mRNA signatures obtained from the single event technologies and regional transcriptomics, miRNA sequencing of bulk cross-sections from the same OCT (optimum cutting temperature embedding media)-embedded core are sequenced specifically for small RNA (IU/OSU).(8)

*Proteomics.* Two different approaches were planned in the KPMP to generate reference and diseased kidney proteome. The regional/segmental approach by the IU/OSU group combines the collection of specific regions of the kidney using LMD with quantitative proteome analysis using state-of-the-art high performance liquid chromatography (HPLC)/mass spectrometry (MS) instrumentation to generate agnostic global profiles of the glomerular and tubulo-interstitial compartments with high sensitivity and reproducibility.(13) The UCSF group is employing recently developed nanoscale proteomics analysis in which a few cells can be processed to generate cell-specific protein profiles.(18) Combining this technology with regions isolated by LMD provides regional and spatial definitions of generated proteome data. Both technologies additionally provide bulk proteomics data on tissue sections allowing cross platform/site analysis.

*Metabolomics.* The UTHSA/PNNL group will generate spatial metabolomics measurements by using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI), an approach that has been utilized previously for kidney molecular imaging.(2, 12) Here, fresh-frozen tissues are cryosectioned, mounted on optically transparent and electrically conductive slides, coated with an organic matrix that assists in facilitating desorption and ionization of endogenous molecules, and serially probed with a laser to attain mass spectral information at predefined locations. They employ two different platforms to generate metabolite profiles that are designed for cross validation and complementarity. An important aspect is that data are processed using the METASPACE platform (a tool developed by the EMBL portion of this group),(1, 11) which is an automated molecular annotation engine that enables data visualization and co-registration with other optical images.

*2D and 3D Imaging*. There are several imaging technologies within KPMP that inform on precise 2D and 3D expression relationships of biomolecules, cells and structures with varying degree of multiplexing, spatial resolution and tissue preservation conditions **(Fig. 2).** The UCSD/WU group will develop Decoding Amplified taRgeted Transcripts with Fluorescence In-Situ Hybridization (DART-FISH) to delineate single cell mRNA expression of several hundred transcripts in each cell at high resolution in 3D using high resolution confocal microscopy. The UCSF and PREMIERE sites will use miFISH to generate simultaneous gene and protein expression data at the single cell level in 2D space.(14) The IU/OSU site will utilize large scale 3D confocal imaging of longitudinal kidney thick sections probing concurrently for 8 different targets using antibodies or fluorescent small molecules, followed by tissue cytometry analysis.(16, 17) Prior to any staining, this technology uses label-free imaging to determine tissue integrity using endogenous fluorescence and quantify collagen deposition using second harmonic generation. The resulting image volumes undergo cytometry analysis with a customized Volumetric Tissue Cytometry and Analysis (VTEA) software. This approach allows the interactive exploration of the image volumes, as well as quantitative analysis of the abundance, distribution, and other 3D spatial features of interest for various cell types, based on supervised and unsupervised analytical approaches.(15) The UCSF site will use Co-detection by indexing (CODEX),(3) an orthogonal approach to perform 2D profiling *in situ* on up to 30 antigens at single-cell resolution on a single tissue section using antibodies labeled with unique oligonucleotide tags (“barcodes”). The labeling and detection is done in an iterative manner in groups of two or three targets per cycle by fluorescent dyes labeled with oligonucleotide sequences (“reporters”) corresponding to a given subset of antibody barcodes; in each cycle the previous reporters are removed and a new set is introduced. The montage image consisting of all signals from each cycle is analyzed for marker intensity distribution across the tissue section, for cell count of a given cell population defined by marker sets and for spatial relationship between cell populations of interest in the specimen.(3)

**References for supplementary methods**

1. **Alexandrov T, Ovchinnikova K, Palmer A, Kovalev V, Tarasov A, Stuart L, Nigmetzianov R, and Fay D**. METASPACE: A community-populated knowledge base of spatial metabolomes in health and disease. *bioRxiv* 539478, 2019.

2. **Darshi M, Van Espen B, and Sharma K**. Metabolomics in Diabetic Kidney Disease: Unraveling the Biochemistry of a Silent Killer. *Am J Nephrol* 44: 92-103, 2016.

3. **Goltsev Y, Samusik N, Kennedy-Darling J, Bhate S, Hale M, Vazquez G, Black S, and Nolan GP**. Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging. *Cell* 174: 968-981 e915, 2018.

4. **Harder JL, Menon R, Otto EA, Zhou J, Eddy S, Wys NL, O'Connor C, Luo J, Nair V, Cebrian C, Spence JR, Bitzer M, Troyanskaya OG, Hodgin JB, Wiggins RC, Freedman BS, Kretzler M, European Renal c DNAB, and Nephrotic Syndrome Study N**. Organoid single cell profiling identifies a transcriptional signature of glomerular disease. *JCI Insight* 4: 2019.

5. **Hato T, Zollman A, Plotkin Z, El-Achkar TM, Maier BF, Pay SL, Dube S, Cabral P, Yoshimoto M, McClintick J, and Dagher PC**. Endotoxin Preconditioning Reprograms S1 Tubules and Macrophages to Protect the Kidney. *J Am Soc Nephrol* 29: 104-117, 2018.

6. **LaFavers KA, Macedo E, Garimella PS, Lima C, Khan S, Myslinski J, McClintick J, Witzmann FA, Winfree S, Phillips CL, Hato T, Dagher PC, Wu XR, El-Achkar TM, and Micanovic R**. Circulating uromodulin inhibits systemic oxidative stress by inactivating the TRPM2 channel. *Sci Transl Med* 11: 2019.

7. **Lake BB, Chen S, Hoshi M, Plongthongkum N, Salamon D, Knoten A, Vijayan A, Venkatesh R, Kim EH, Gao D, Gaut J, Zhang K, and Jain S**. A single-nucleus RNA-sequencing pipeline to decipher the molecular anatomy and pathophysiology of human kidneys. *Nat Commun* 10: 2832-2832, 2019.

8. **Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, Rheinheimer S, Meder B, Stahler C, Meese E, and Keller A**. Distribution of miRNA expression across human tissues. *Nucleic Acids Res* 44: 3865-3877, 2016.

9. **Menon R, Otto EA, Kokoruda A, Zhou J, Zhang Z, Yoon E, Chen YC, Troyanskaya O, Spence JR, Kretzler M, and Cebrian C**. Single-cell analysis of progenitor cell dynamics and lineage specification in the human fetal kidney. *Development* 145: 2018.

10. **Micanovic R, Khan S, and El-Achkar TM**. Immunofluorescence laser micro-dissection of specific nephron segments in the mouse kidney allows targeted downstream proteomic analysis. *Physiol Rep* 3: 2015.

11. **Palmer A, Phapale P, Chernyavsky I, Lavigne R, Fay D, Tarasov A, Kovalev V, Fuchser J, Nikolenko S, Pineau C, Becker M, and Alexandrov T**. FDR-controlled metabolite annotation for high-resolution imaging mass spectrometry. *Nat Methods* 14: 57-60, 2017.

12. **Prentice BM, Caprioli RM, and Vuiblet V**. Label-free molecular imaging of the kidney. *Kidney Int* 92: 580-598, 2017.

13. **Rovin BH, and Klein JB**. Proteomics and autoimmune kidney disease. *Clin Immunol* 161: 23-30, 2015.

14. **Vasquez JJ, Hussien R, Aguilar-Rodriguez B, Junger H, Dobi D, Henrich TJ, Thanh C, Gibson E, Hogan LE, McCune J, Hunt PW, Stoddart CA, and Laszik ZG**. Elucidating the Burden of HIV in Tissues Using Multiplexed Immunofluorescence and In Situ Hybridization: Methods for the Single-Cell Phenotypic Characterization of Cells Harboring HIV In Situ. *J Histochem Cytochem* 66: 427-446, 2018.

15. **Winfree S, Dagher PC, Dunn KW, Eadon MT, Ferkowicz M, Barwinska D, Kelly KJ, Sutton TA, and El-Achkar TM**. Quantitative Large-Scale Three-Dimensional Imaging of Human Kidney Biopsies: A Bridge to Precision Medicine in Kidney Disease. *Nephron* 140: 134-139, 2018.

16. **Winfree S, Ferkowicz MJ, Dagher PC, Kelly KJ, Eadon MT, Sutton TA, Markel TA, Yoder MC, Dunn KW, and El-Achkar TM**. Large-scale 3-dimensional quantitative imaging of tissues: state-of-the-art and translational implications. *Transl Res* 189: 1-12, 2017.

17. **Winfree S, Khan S, Micanovic R, Eadon MT, Kelly KJ, Sutton TA, Phillips CL, Dunn KW, and El-Achkar TM**. Quantitative Three-Dimensional Tissue Cytometry to Study Kidney Tissue and Resident Immune Cells. *J Am Soc Nephrol* 28: 2108-2118, 2017.

18. **Zhu Y, Piehowski PD, Zhao R, Chen J, Shen Y, Moore RJ, Shukla AK, Petyuk VA, Campbell-Thompson M, Mathews CE, Smith RD, Qian WJ, and Kelly RT**. Nanodroplet processing platform for deep and quantitative proteome profiling of 10-100 mammalian cells. *Nat Commun* 9: 882, 2018.