

**Supplemental information**

**Current methodological challenges of single-cell and single-nucleus RNA-sequencing in glomerular diseases**

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## Supplementary tables

**Table S1:** Overview of all studies reporting on scRNA-seq and/or snRNA-seq on mouse tissue

| Author                                       | Protocol                                     | Age         | Disease?   | T°                                  | Cells            | Depth  | Genes                   | Glom.   | Remarks  |
|--|--|-------------|--|-------------------------------------|------------------|--|-------------------------|---------|--|
| Adam <i>et al.</i> <sup>1</sup> , 2017       | Drop-seq                                     | N           | Healthy (n=12)                                   | (+), C<br>(+), W                    | 20,424           | ?  | > 1,000                 | P       | First use of CAP on renal tissue (4853 cells, remaining cells with warm dissociation)<br>P°: not mentioned                             |
| Chen <i>et al.</i> <sup>2</sup> , 2017       | Fluidigm C1<br>SMART-seq                     | A           | Healthy (n=?)                                    | (+), W                              | 184              | > 10 <sup>7</sup>  | 2,855                   | -       | scRNA-seq on FACS-isolated collecting duct cells (A-ICs, B-ICs, PCs)   |
| Lu <i>et al.</i> <sup>3</sup> , 2017         | Fluidigm C1<br>SMART-seq                     | A           | Healthy (n=1)                                    | (+), W                              | 14*              | ±23<br>x10 <sup>6</sup>                                    | 6,220                   | M       | scRNA-seq on magnetic bead-isolated glomeruli<br>*: only mesangial cells   |
| Lu <i>et al.</i> <sup>4</sup> , 2017         | Fluidigm C1<br>SMART-seq                     | A           | Healthy (n=?)                                    | (+), W                              | 20*              | ±17<br>x10 <sup>6</sup>                                    | 3,737                   | P       | scRNA-seq on magnetic bead-isolated glomeruli<br>*: only podocytes<br>P°: 20 cells (100%)  |
| Wang <i>et al.</i> <sup>5</sup> , 2018       | SMART-seq2                                   | C           | Healthy (n=?)                                    | (+), W<br>Met.                      | 502              | ?  | ±1,900                  | -       | scRNA-seq on FACS-isolated kidney immune cells (CD45+)<br>Study on preservation fidelity of Hypothermosol FRS                          |
| Karaiskos <i>et al.</i> <sup>6</sup> , 2018  | Drop-seq                                     | A           | Healthy (n=8)                                    | (-), W<br>Met.                      | 12,954           | 9,400  | 630                     | P, E, M | scRNA-seq on magnetic bead-isolated glomeruli<br>P°: 10,364 cells (80.01%)   |
| Park <i>et al.</i> <sup>7</sup> , 2018       | 10x<br>Chromium                              | A<br>(?)    | Healthy (n=7)                                    | (+), W                              | 43,745           | 38,588   | 940                     | P, M    | P°: 78 cells (0.18%)   |
| Wu <i>et al.</i> <sup>8</sup> , 2018         | Drop-seq                                     | A           | Healthy (n=1)                                    | (-), W<br>Met.                      | 3,531            | ±5,500   | ±1,000                  | -       | *: data from validation study with sn10x platform<br>P°: 227 cells (2.4%) <sup>9</sup> (mean of sNuc-Drop-Seq, DroNC-seq and sNuc-10x) |
|  | sNuc-Drop-Seq<br>DroNC-seq<br>sNuc-10x*      | A           | Healthy (n=3)<br>UUOS (n=1)*                     | (-), C<br>Snap.                     | 7,860;<br>6,147* | ±7,000   | ±850-<br>1,100;<br>763* | P, E, M |  |
| Kramann <i>et al.</i> <sup>10</sup> , 2018   | SMART-seq                                    | A           | UUOS (n=3)                                       | (+), W                              | 357              | ?  | ?                       | -       | scRNA-seq on FACS-isolated myofibroblasts (PDGFRβ+)  |
| Cao <i>et al.</i> <sup>11</sup> , 2018       | Sci-CAR*                                     | A           | Healthy (n=2)                                    | (-), C<br>Snap.                     | 13,893           | 140,000<br>;1,011**  | ±500                    | P       | *: =sci-RNA-seq+sci-ATAC-seq<br>**: mUMIs per cell<br>P°: not mentioned  |
| Schaum <i>et al.</i> <sup>12</sup> , 2018    | SMART-seq2<br>10x<br>Chromium                | A           | Healthy (n=6)*<br>Healthy (n=3)**                | (+), W                              | 519*;<br>2,781** | 10 <sup>4</sup> -<br>10 <sup>6</sup> *;<br>4,000**,<br>*** | ±1,250*<br>;±1,800**    | M       | Study on mouse atlas (Tabula Muris)<br>*: SMART-seq2<br>**: droplet-based<br>***: mUMIs per cell                                       |
| Fu <i>et al.</i> <sup>13</sup> , 2019        | Fluidigm C1<br>mRNA Seq<br>HT                | A           | streptozotocin-induced DM (n=3)<br>Healthy (n=3) | (+), W                              | 644              | 40,000   | 3,457                   | P, E, M | scRNA-seq on magnetic bead-isolated glomeruli<br>P°: 66 cells (10.25%)   |
| Zimmerman <i>et al.</i> <sup>14</sup> , 2019 | 10x<br>Chromium<br>Fluidigm C1<br>SMART-seq* | A           | Healthy (n=3)                                    | (+), W                              | 3,013            | 80,508   | 952                     | -       | scRNA-seq on FACS-isolated immune cells (CD45+, exclusion of lymphocytes)<br>*: validation study on macrophages                        |
| Ransick <i>et al.</i> <sup>15</sup> , 2019   | 10x<br>Chromium                              | A           | Healthy (n=4)                                    | (+), C                              | 31,265           | 70,446   | 1,395                   | P       | Three kidney regions were dissected before dissociation<br>P°: 24 cells (0.08%) <sup>9</sup>   |
| Barry <i>et al.</i> <sup>16</sup> , 2019     | Drop-seq*                                    | F<br>N<br>A | Healthy (n=?)                                    | (+), W                              | 4,552            | ?  | 200-<br>2,500           | E       | *: ddSEQ by Illumina<br>scRNA-seq on FACS-isolated endothelial cells (CD31+)   |
| Dumas <i>et al.</i> <sup>17</sup> , 2020     | 10x<br>Chromium                              | A           | Healthy (n=?)<br>Total of 60 kidneys             | (+), W                              | 40,662           | 63,692   | 1,141                   | E       | Cortex and medulla dissected before separate dissociation. scRNA-seq on FACS/MACS-isolated endothelial cells (CD31+)                   |
| Denisenko <i>et al.</i> <sup>18</sup> , 2020 | 10x<br>Chromium                              | A           | Healthy (n=18)*                                  | (+), W/C<br>(-), W/C<br>Met., Cryo. | 77,656           | 52,000   | 981                     | P, M    | Comparison of different dissociation and storage techniques<br>P°: 3 cells (0.03%) in W<br>P°: 330 cells (2.78%) in C                  |

|  |                         |   |  |              |                    |                                  |                     |         |  |
|--|-------------------------|---|--|--------------|--------------------|----------------------------------|---------------------|---------|--|
|  | 10x Chromium            | A | Healthy (n=18)*  | (-), C Snap. | 98,303             | 52,000                           | 1,819               | P, M    | P°: 0.7%<br>*: total amount of mice (scRNA-seq/snRNA-seq/bulk)   |
| Chung <i>et al.</i> <sup>9</sup> , 2020            | 10x Chromium            | A | Healthy (n=?)<br>Nephritis (n=?)<br>DM (n=?)<br>Toxic (n=?)<br>CD2AP def. (n=?)        | (+), W       | 74,149             | ?                                | 2,878               | P, E, M | scRNA-seq on magnetic bead-isolated glomeruli<br>P°: 11,431 cells (15.42%)   |
| Kreiman <i>et al.</i> <sup>19</sup> , 2020         | 10x Chromium            | A | Healthy (n=3)<br>IRI (n=3)   | (+), W       | ?                  | ?                                | ?                   | ?       | Exclusive focus on cluster of CXCR5+ cells   |
| Conway <i>et al.</i> <sup>20</sup> , 2020          | 10x Chromium SMART-seq2 | A | Sham (n=3*,1**)<br>UUO-2 (n=3*,1**)<br>UUO-7 (n=3*,1**)<br>R-UUO (n=3*,1**)            | (+), W       | 16,967*; 362**     | 87,500*; 41,000***; ±4,600* ,*** | 1,218*; 3,156**     | - ****  | *: 10x<br>**: SMART-seq2<br>***: mUMIs per cell<br>****: very few podocytes, too few for cluster   |
| Kirita <i>et al.</i> <sup>21</sup> , 2020          | 10x Chromium            | A | IRI (n=15)<br>Sham (n=3)   | (-), C Snap. | 126,578            | ?                                | 150-8,000           | P, M    | P°: not mentioned  |
| Zhao <i>et al.</i> <sup>22</sup> , 2020            | 10x Chromium            | A | IRI ± XJB-5-131 (n=5)*<br>Sham (n=5)**   | (+), W       | 7,581*; 6,069**    | 27,920* ; 39,310* *              | 2,369               | -       | *: ischemia/reperfusion mice<br>**: sham mice  |
| Ge <i>et al.</i> <sup>23</sup> , 2020              | Fluidigm C1 mRNA Seq HT | A | Healthy (n=12)   | (+), W       | 326                | 40,000                           | 3,417               | P, E, M | scRNA-seq on magnetic bead-isolated glomeruli<br>P°: 50 cells (15.3%)  |
| Do Valle Duraes <i>et al.</i> <sup>24</sup> , 2020 | 10x Chromium            | A | Healthy (n=2*+2**)<br>IRI + CN (n=2*+2**)<br>IRI - C (n=4*+4**)                        | (+), W       | 28,767*; 22,851* * | ?                                | 1,500*; ?**         | -       | *: scRNA-seq on FACS-isolated CD45+<br>**: scRNA-seq on FACS-isolated CD4+   |
| Hyndman <i>et al.</i> <sup>25</sup> , 2020         | 10x Chromium            | A | Healthy (n=2)<br>HDAC-KO mouse (n=2)   | (-), C Snap. | 25,075             | 23,000                           | 1,804               | P       | Study focusing on Hdac1/2 KO mice<br>P°: 311 cells (1.2%)  |
| Rudman-Melnick <i>et al.</i> <sup>26</sup> , 2020  | Drop-seq                | A | Healthy (n=?)<br>IRI (n=21)<br>+ mice for validation study                             | (+), W       | 54,730             | ?                                | >500                | P       | P°: not mentioned  |
| Kalucka <i>et al.</i> <sup>27</sup> , 2020         | 10x Chromium            | A | Healthy (n=6)  | (+), W       | 4,003              | 60,000; 1,580*                   | 898                 | E       | scRNA-seq on FACS/MACS-isolated endothelial cells (CD31+)<br>*: mUMIs per cell   |
| Legouis <i>et al.</i> <sup>28</sup> , 2020         | 10x Chromium            | A | Healthy (n=?)<br>64h after IRI (n=?)<br>96h after IRI (n=?)<br>28d after IRI (n=?)     | (+), C       | 6,086*; 11,274* *  | ?                                | 400-3,000           | P       | snRNA-seq on FACS-isolated GFP+-nuclei (with GFP labelling renal tubule cells)<br>*: healthy mice (GFP+ and - nuclei)<br>**: IRI-mice (GFP+ nuclei)<br>P°: not mentioned                             |
| Dangi <i>et al.</i> <sup>29</sup> , 2020           | 10x Chromium            | A | Untransplanted (n=2)<br>Transplanted, rejecting (n=2)<br>Transplanted, tolerated (n=2) | (+), W       | 30,053             | ?                                | ?                   | -       | Study using a murine kidney transplant model. Allografts retrieved at 15d post-transplant.   |
| Marshall <i>et al.</i> <sup>30</sup> , 2020        | HyPR-seq                | A | Healthy (n=2)*<br>DKD (n=2)**  | (+), W       | 14,288*; 14,837* * | 203***                           | Probing of 32 genes | P, M    | New targeted scRNA-seq technique using specific DNA probes (HyPR-seq)<br>*: BTBR <i>wt/wt</i> mice<br>**: BTBR <i>ob/ob</i> mice<br>***: mUMIs per cell<br>P°: 132 cells (0.9%)*; 12 cells (0.08%)** |
| Omori <i>et al.</i> <sup>31</sup> , 2020           | 10x Chromium            | A | p16-Cre <sup>ERT2</sup> -tdTomato mouse (n=?)  | (+), W       | 2,403              | > 800*                           | > 200               | -       | New mouse model on cell senescence; Td-tomato labelling of p16-high cells as a marker for cell senescence<br>*: UMIs per cell  |
| Ni <i>et al.</i> <sup>32</sup> , 2021              | 10x Chromium            | A | Healthy (n=?)  | (+), W       | ?                  | ?                                | 200-3,000           | -       | Study focusing on phosphate metabolism and FGF23-mediated pathways   |
| Sidhom <i>et al.</i> <sup>33</sup> , 2021          | 10x Chromium            | A | Pdss2 <sup>hdkd</sup> mice (n=3)*  | (-), C Snap. | 20,441*; 16,119*   | ±1,000                           | ±1,500              | P       | *: Mice with homozygous mutation in Pdss2-gene (CoQ-   |

|  |              |   |   |              |                       |         |           |      |  |
|--|--------------|---|---|--------------|-----------------------|---------|-----------|------|--|
|  |              |   | Healthy (n=3)**   |              | *                     |         |           |      | pathway)<br>**: controls<br>P°: 61 cells (0.3%)*, 41 cells (0.25%)**   |
| Hinze <i>et al.</i> <sup>34</sup> , 2021     | Drop-seq     | A | Healthy (n=2)   | (-), C Met.  | 5,675*; 6,327**       | 772***  | 456       | P    | Study incorporates NovoSpaRc<br>Grhl2 <sup>CD2-/-</sup> mice are a model for lower corticomedullary osmolality gradient<br>*: 'whole kidney'<br>**: dissected kidney regions before dissociation<br>***: mean transcripts per cell<br>P°: not mentioned<br>P°: not mentioned |
|  | 10x Chromium | A | Healthy (n=2)<br>Grhl2 <sup>CD2-/-</sup> (n=1 mouse, 2 kidneys) | (-), C Snap. | ?                     | ?       | 500-5,000 | P    |  |
| Dhillon <i>et al.</i> <sup>35</sup> , 2021   | 10x Chromium | A | Healthy (n=6)*<br>FAN mice (n=2)**                              | (+), W       | 37,361*; 27,730*<br>* | ?       | 200-3,000 | P, E | Study also performed scRNA-seq on UUO-mice and human kidney organoids<br>*: Healthy controls<br>**: CKD/fibrosis mouse model<br>P°: 97 cells (0.15%)   |
| Janosevic <i>et al.</i> <sup>36</sup> , 2021 | 10x Chromium | A | Endotoxin treated mice (n=7 mice, 14 kidneys)                   | (+), W       | 63,287                | ±50,000 | 200-3,000 | -    | Study of murine endotoxemia model. Analysis on 7 timepoints after LPS-injection (0h, 1h, 4h, 16h, 27h, 36h, 48h), 1 mouse per timepoint.   |

**Legend:** scRNA-seq experiments are shown in blue, snRNA-seq experiments are shown in red; '**Age**': age of mice (F = fetal, N = newborn, C = child, A = adult); '**Disease**': healthy or pathological kidney tissue (with n = number of mice used); '**T°**': fresh vs. frozen tissue and warm vs. cold dissociation ((+) = fresh tissue, (-) = frozen tissue, W = warm dissociation, C = cold dissociation, 'Met'=methanol-fixation, 'Cryo'=cryopreservation, 'Snap'=snap-frozen tissue); '**Cells**': total number of cells isolated and analyzed after quality control; '**Depth**': sequencing depth defined as 'mean reads per cell'; 'mean transcripts per cell' or 'mean UMIs per cell' are reported with an '\*'; '**Genes**': mean number of genes per cell; '**Glom.**': isolation of glomerular cells ('-' = no glomerular cells isolated, 'P' = podocytes, 'E' = glomerular endothelial cells, 'M' = mesangial cells; '?' is used when data could not be found in the published paper. The 'sequencing depth' or 'mean genes per cell' may be written as a range or '>' or '±' when no exact figure could be extracted from the published studies. '**P°**' refers to the absolute and relative number of podocytes isolated in scRNA-seq or snRNA-seq studies.

### Abbreviations:

CAP: cold active protease; FACS: fluorescence-activated cell sorting; A-ICs: A-intercalated cells; B-ICs: B-intercalated cells; PCs: principal cells; UUOS: unilateral ureteral obstruction surgery; UMI: unique molecular identifier; mUMI: mean UMIs (per cell); MACS: magnetic-activated cell sorting; DKD: diabetic kidney disease; CD2AP def.: CD2AP deficiency; IRI: ischemia reperfusion injury; UUO-2: two days after unilateral ureteral obstruction surgery; UUO-7: seven days after unilateral ureteral obstruction surgery; R-UUO: reversible unilateral ureteral obstruction (surgery); sham: sham surgery; XJB-5-131: a synthetic anti-oxidant; IRI ± CN: unilateral ischemia reperfusion injury with or without immediate contralateral nephrectomy; HDAC-KO mouse: Hdac1/2 knockout mouse; GFP: green fluorescent protein; Grhl2<sup>CD2-/-</sup>: mouse lacking Grhl2 transcription factor in collecting ducts; FAN: Folic acid nephropathy.

**Table S2:** Overview of all studies reporting on scRNA-seq and/or snRNA-seq on human tissue

| Author                                       | Protocol                            | Age       | Tx?    | Disease?   | Biopsy  | T°                  | Cells                | Depth                                     | Genes                        | Glom.         | Remarks   |
|--|-------------------------------------|-----------|--------|--|---|---------------------|----------------------|---|------------------------------|---------------|---|
| Der <i>et al.</i> <sup>37</sup> , 2017       | Fluidigm C1 SMART-seq               | A         | N      | SLE (n=10)   | CNB (n=16)                                    | (+), W              | 899*                 | 500,000                                   | 700                          | -             | *: pooling of renal, skin and PBMCs   |
| Gillies <i>et al.</i> <sup>38</sup> , 2018   | 10x Chromium                        | A         | N      | Healthy (T) (n=3)  | Neph (n=3)                                    | (-), W Cryo         | 4,734                | ?   | ?                            | P, M          | Study on eQTL and integration with scRNA-seq<br>P°: 49 cells (1%)   |
| Wu <i>et al.</i> <sup>39</sup> , 2018        | inDrops                             | A         | T      | Acute TCMR (n=1),  | CNB (n=1)                                     | (+), W              | 4,487                | 50,000                                    | 827                          | -             | P°: not mentioned   |
|  | inDrops                             | A         | N      | Healthy (D) (n=1)  | Neph. (n=1)                                   | (+), C              | 4,259                | 50,000                                    | >400                         | P             |   |
| Young <i>et al.</i> <sup>40</sup> , 2018     | 10x Chromium                        | F, C, A   | N      | Fetal (n=2)<br>Healthy (D) (n=1)<br>Healthy (T)* (n=8)<br>Tumor* (n=8) | Neph. (n=20)<br>Whole (n=2)<br>Other** (n=16) | (+), W              | 72,501               | ?   | ?                            | P, E          | *: same patients<br>**: tissue from renal pelvis, ureter and tumor<br>P°: 259 cells (0.3%)  |
| Wilson <i>et al.</i> <sup>41</sup> , 2019    | 10x Chromium                        | A         | N      | Healthy (T) (n=3)<br>DKD (T) (n=3)                                     | Neph. (n=6)                                   | (-), C Snap.        | 23,980               | 6,894*                                    | 2,541                        | P, E, M       | *: mUMIs per cell<br>P°: 663 cells (2.76%)  |
| Der <i>et al.</i> <sup>42</sup> , 2019       | Fluidigm C1 mRNA Seq HT             | A         | N      | SLE (n=21)<br>Healthy (P) (n=3)  | CNB (n=24)                                    | (-), W Cryo.        | 4,019*               | 200,000                                   | ?                            | M             | *: pooling of renal and skin cells  |
| Arazi <i>et al.</i> <sup>43</sup> , 2019     | CEL-seq2*<br>10x Chromium**         | A         | N      | SLE (n=24)<br>Healthy (P) (n=10)                                       | CNB (n=34)                                    | (-), W Cryo         | 2,838*;<br>122**     | ± 10 <sup>6</sup> *;<br>?*                | 1,000-5,000*;<br>250-3,500** | -             | scRNA-seq on FACS-isolated leukocytes (CD45+) vs. epithelial cells (CD45-, CD10+)<br>*: CEL-seq2<br>**: 10x on 2 healthy donor biopsies |
| Lake <i>et al.</i> <sup>44</sup> , 2019      | snDrop-seq                          | A         | N      | Healthy (T) (n=14)<br>Healthy (D) (n=2)                                | Neph. (n=19)                                  | (+), C (-), C Snap. | 17,659               | 1,082*                                    | 589                          | P, E, M       | *: mean transcripts per cell, not raw reads<br>P°: 859 cells (4.86%)  |
| Stewart <i>et al.</i> <sup>45</sup> , 2019   | 10x Chromium                        | F, C, A   | N      | Fetal (n=6)<br>Healthy (T) (n=10)<br>Healthy (D) (n=3)                 | Neph. (n=14)<br>Whole (n=6)                   | (+), W              | 40,268*;<br>27,203** | ?   | ?                            | P, E          | *: mature kidneys<br>**: fetal kidneys<br>P°: 126 cells (0.3%) for F & C  |
| Zimmerman <i>et al.</i> <sup>14</sup> , 2019 | 10x Chromium Fluidigm C1 SMART-seq* | A         | N      | Healthy (T) (n=1)  | Neph. (n=1)                                   | (+), W              | 2,868                | 112,080                                   | 878                          | -             | scRNA-seq on FACS-isolated immune cells (CD45+, exclusion of lymphocytes)<br>*: validation study on macrophages                         |
| Menon <i>et al.</i> <sup>46</sup> , 2020     | 10x Chromium                        | A         | N<br>T | Healthy (T) (n=16)<br>Healthy (P) (n=3)<br>Healthy (S) (n=5)           | CNB (n=8)<br>Neph. (n=16)                     | (-), W Cryo.        | 7,524*;<br>14,744**  | 3,971* <sup>***</sup> ;<br>3,089**<br>*** | 1,339*;<br>1,134**           | P, E, M       | *: CNB<br>**: nephrectomies<br>***: mUMIs per cell<br>P°: 11 cells (0.14%)*, 159 cells (1.08%)**  |
| Liao <i>et al.</i> <sup>47</sup> , 2020      | 10x Chromium                        | A         | N      | Healthy (T) (n=3)  | Neph. (n=3)                                   | (+), W              | 23,366               | ±30,000                                   | ±800                         | -             |   |
| Malone <i>et al.</i> <sup>48</sup> , 2020    | 10x Chromium                        | A         | T      | ABMR (n=3)<br>Non-rejection AKI (n=2)                                  | CNB (n=5)                                     | (+), W              | 81,139               | 2,497*                                    | 1,124                        | -             | *: mean transcripts per cell, not raw reads   |
| Liu <i>et al.</i> <sup>49</sup> , 2020       | 10x Chromium                        | A         | T      | Chronic rejection (n=2)  | Neph* (n=2)                                   | (+), W              | 27,197               | ±2,500**                                  | 200-2,500                    | -             | *: reason for nephrectomy unclear<br>**: mUMIs per cell   |
| Han <i>et al.</i> <sup>50</sup> , 2020       | Microwell-seq                       | F*<br>A** | N      | Fetal (n=4)*<br>Healthy (D) (n=1)**<br>Healthy (T) (n=2)**             | Whole* (n=4)<br>Neph** (n=3)                  | (+), W              | 22,439*;<br>22,692** | ±858* <sup>***</sup> ;<br>±1,251**<br>*** | ?                            | P*, M*<br>E** | *: fetal kidney<br>**: adult kidney<br>***: mUMIs per cell  |

|  |              |   |   |                                     |                          |                |                      |                      |                    |        |  |
|--|--------------|---|---|-------------------------------------|--------------------------|----------------|----------------------|----------------------|--------------------|--------|--|
|  |              |   |   |                                     |                          |                |                      |                      |                    |        | Large study constructing the 'human cell landscape'<br>P°: not mentioned   |
| Deng <i>et al.</i> <sup>51</sup> , 2020  | 10x Chromium | A | N | Healthy (T) (n=1)                   | Neph (n=1)               | (+), W         | 6,138                | ?                    | ?                  | ?      | Study also performed nested PCR and Sanger sequencing on FACS-isolated PTs   |
| Menon <i>et al.</i> <sup>52</sup> , 2020 | 10x Chromium | A | N | Healthy (P) (n=18)<br>DKD (n=44)    | CNB (n=62)               | (-), W<br>Cryo | 25,163*;<br>85,872** | ?                    | 500-5000           | P, M   | *: healthy<br>**: DKD<br>P°: not mentioned   |
| Zheng <i>et al.</i> <sup>53</sup> , 2020 | STRT-seq     | A | N | IgAN (n=13)*<br>Healthy (T)** (n=6) | CNB (n=13)<br>Neph (n=6) | (+), W<br>***  | 2,022*;<br>763**     | 37,875*;<br>38,391** | 3,348*;<br>3,105** | P, M   | *: IgAN<br>**: healthy<br>***: Stepwise dissociation:<br>1) glomeruli isolation (pipetting),<br>2) MACS CD14+ cells,<br>3) MACS CD326+ cells,<br>4) unselected cells<br>P°: 22 cells (1%)*, 4 cells (0.52%)**                                |
| Kuppe <i>et al.</i> <sup>54</sup> , 2021 | 10x Chromium | A | N | CKD aHT (n=6)<br>Healthy (T) (n=7)  | Neph (n=13)              | (+), W         | 53,672*;<br>33,690** | ?                    | ?                  | P*, E* | scRNA-seq on FACS-sorted CD10+ and CD10- cells<br>*: CD10- cells (11 pts)<br>**: CD10+ cells (8 pts)<br>P°: 44 cells (0.08%)*<br>Study also performed scRNA-seq on FACS-isolated PDGFRα+, PDGFRβ+ cells in mouse kidney fibrosis experiments |

**Legend:** scRNA-seq experiments are shown in blue, snRNA-seq experiments is shown in red; **‘Age’:** age of patients (F = fetal, N = newborn, C= child, A =adult); **‘Tx’:** tissue from native kidneys or transplant kidneys (N = native, T = transplant); **‘Disease’:** healthy or pathological kidney tissue (‘(D)’ = healthy tissue from discarded kidneys after prelevation for potential transplantation, ‘(T)’ = tumor-free tissue at maximal distance of mass or tumor, ‘(P)’= preperfusion or pretransplant core needle biopsy of living donor kidney, ‘(S)’=surveillance kidney transplant biopsy, ‘Tumor’ = tumorally invaded tissue, with n = number of patients); **‘Biopsy’:** biopsy technique (CNB = core needle biopsy, Neph. = biopsy from partial/total nephrectomy, Whole = dissection of whole fetal kidney, n = biopsy samples taken); **‘T°’:** fresh vs. frozen tissue and warm vs. cold dissociation ((+) = fresh tissue, (-) = frozen tissue, W = warm dissociation, C = cold dissociation, ‘Met’=methanol-fixation, ‘Cryo’=cryopreservation, ‘Snap’=snap-frozen tissue); **‘Cells’:** total number of cells isolated and analyzed after quality control (QC); **‘Depth’:** sequencing depth defined as ‘mean (raw) reads per cell’; ‘mean transcripts per cell’ or ‘mean UMIs per cell’ are reported with an ‘\*’; **‘Genes’:** mean number of genes per cell; **‘Glom.’:** isolation of glomerular cells (‘-’ = no glomerular cells isolated, ‘P’= podocytes, ‘E’ = glomerular endothelial cells, ‘M’ = mesangial cells); ‘?’ is used when data could not be found in the published paper. The ‘sequencing depth’ or ‘mean genes per cell’ may be written as a range or ‘>’ or ‘±’ when no exact figure could be extracted from the published studies. **‘P°’** refers to the absolute and relative number of podocytes isolated in scRNA-seq or snRNA-seq studies.

**Abbreviations:**

PBMC: peripheral blood mononuclear cells; eQTL: expression quantitative trait loci; SLE: systemic lupus erythematosus; TCMR: T-cell mediated rejection; DKD: diabetic kidney disease; FACS: fluorescence-activated cell sorting; ABMR: acute antibody-mediated rejection; AKI: acute kidney injury; PTs: proximal tubule cells; IgAN: IgA nephropathy; MACS: magnetic-activated cell sorting; CKD aHT: 'hypertensive nephrosclerosis'; pts: patients.

**Table S3:** scRNA-seq studies on methanol-fixed or cryopreserved tissue of mouse kidneys

| Author                                       | Protocol     | Age | Disease?        | T°                            | Cells           | Depth  | Genes  | Glom.   | Remarks   |
|--|--------------|-----|-----------------|-------------------------------|-----------------|--------|--------|---------|---|
| Karaikos <i>et al.</i> <sup>6</sup> , 2018   | Drop-seq     | A   | Healthy (n=8)   | (-), W Met.                   | 12,954          | 9,400  | 630    | P, E, M | scRNA-seq on magnetic bead-isolated glomeruli<br>P°: 10,364 cells (80.01%)  |
| Wu <i>et al.</i> <sup>8</sup> , 2018         | Drop-seq     | A   | Healthy (n=1)   | (-), W Met.                   | 3,531           | ±5,500 | ±1,000 | -       | Study also performed snRNA-seq on healthy tissue (Table S1)   |
| Denisenko <i>et al.</i> <sup>18</sup> , 2020 | 10x Chromium | A   | Healthy (n=18)* | (+), W/C (-), W/C Met., Cryo. | 77,656          | 52,000 | 981    | P, M    | Comparison of different dissociation and storage techniques<br>P°: 3 cells (0.03%) in W<br>P°: 330 cells (2.78%) in C<br>*: total of 18 mice for all experiments including bulk RNA-seq and snRNA-seq |
| Hinze <i>et al.</i> <sup>34</sup> , 2021     | Drop-seq     | A   | Healthy (n=2)   | (-), C Met.                   | 5,675*; 6,327** | 772*** | 456    | P       | Study incorporates NovoSpaRc<br>*: 'whole kidney'<br>**: dissected kidney regions before dissociation<br>***: mean transcripts per cell<br>P°: not mentioned  |

**Legend:** ‘Age’: age of mice (A =adult); ‘Disease’: healthy or pathological kidney tissue (with n = number of mice used); ‘T°’: fresh vs. frozen tissue and warm vs. cold dissociation ((+) = fresh tissue, (-) = frozen tissue, W = warm dissociation, C = cold dissociation, ‘Met’=methanol-fixation, ‘Cryo’=cryopreservation); ‘Cells’: total number of cells isolated and analyzed after quality control; ‘Depth’: sequencing depth defined as ‘mean reads per cell’; ‘Genes’: mean number of genes per cell; ‘Glom.’: isolation of glomerular cells (‘-’ = no glomerular cells isolated, ‘P’ = podocytes, ‘E’ = glomerular endothelial cells, ‘M’ = mesangial cells. The ‘sequencing depth’ or ‘mean genes per cell’ may be written as ‘±’ when no exact figure could be extracted from the published studies. ‘P°’ refers to the absolute and relative number of podocytes isolated in scRNA-seq studies.



**Table S4:** scRNA-seq studies on cryopreserved human renal tissue

| Author                                     | Protocol                 | Age | Tx? | Disease?   | Biopsy                 | T°           | Cells             | Depth                    | Genes                     | Glom.   | Remarks   |
|--|--------------------------|-----|-----|--|------------------------|--------------|-------------------|--------------------------|---------------------------|---------|---|
| Gillies <i>et al.</i> <sup>38</sup> , 2018 | 10x Chromium             | A   | N   | Healthy (T) (n=3)                                      | Neph (n=3)             | (-), W Cryo  | 4,734             | ?                        | ?                         | P, M    | Study on eQTL and integration with scRNA-seq P°: 49 cells (1%)  |
| Der <i>et al.</i> <sup>42</sup> , 2019     | Fluidigm C1 mRNA Seq HT  | A   | N   | SLE (n=21) Healthy (P) (n=3)                           | CNB (n=24)             | (-), W Cryo. | 4,019*            | 200,000                  | ?                         | M       | *: pooling of renal and skin cells  |
| Arazi <i>et al.</i> <sup>43</sup> , 2019   | CEL-seq2* 10x Chromium** | A   | N   | SLE (n=24) Healthy (P) (n=10)                          | CNB (n=34)             | (-), W Cryo  | 2,838*; 122**     | ± 10 <sup>6</sup> *; ?** | 1,000-5,000*; 250-3,500** | -       | scRNA-seq on FACS-isolated leukocytes (CD45+) vs. epithelial cells (CD45-, CD10+) *: CEL-seq2 **: 10x on 2 healthy donor biopsies |
| Menon <i>et al.</i> <sup>46</sup> , 2020   | 10x Chromium             | A   | N T | Healthy (T) (n=16) Healthy (P) (n=3) Healthy (S) (n=5) | CNB (n=8) Neph. (n=16) | (-), W Cryo. | 7,524*; 14,744**  | 3,971*;***; 3,089**,*    | 1,339*; 1,134**           | P, E, M | *: CNB **: nephrectomies ***: mUMIs per cell P°: 11 cells (0.14%)*, 159 cells (1.08%)**   |
| Menon <i>et al.</i> <sup>52</sup> , 2020   | 10x Chromium             | A   | N   | Healthy (P) (n=18) DKD (n=44)                          | CNB (n=62)             | (-), W Cryo  | 25,163*; 85,872** | ?                        | 500-5000                  | P, M    | *: healthy **: DKD P°: not mentioned  |

**Legend:** ‘Age’: age of patients (A =adult); ‘Tx’: tissue from native kidneys or transplant kidneys (N = native, T = transplant); ‘Disease’: healthy or pathological kidney tissue (‘P’= preperfusion or pretransplant core needle biopsy of living donor kidney, ‘S’=surveillance kidney transplant biopsy, n = number of patients); ‘Biopsy’: biopsy technique (CNB = core needle biopsy, Neph. = biopsy from partial/total nephrectomy, n = biopsy samples taken); ‘T°’: fresh vs. frozen tissue and warm vs. cold dissociation ((+) = fresh tissue, (-) = frozen tissue, W = warm dissociation, ‘Cryo’=cryopreservation); ‘Cells’: total number of cells isolated and analyzed after quality control; ‘Depth’: sequencing depth defined as ‘mean (raw) reads per cell’; ‘mean transcripts per cell’ or ‘mean UMIs per cell’ are reported with an ‘\*’; ‘Genes’: mean number of genes per cell; ‘Glom.’: isolation of glomerular cells (‘-’ = no glomerular cells, ‘P’ = podocytes, ‘E’ = glomerular endothelial cells, ‘M’ = mesangial cells). ‘?’ is used when data could not be found in published paper. ‘P°’ refers to the absolute and relative number of podocytes isolated in scRNA-seq studies.

**Abbreviations:** eQTL: expression quantitative trait loci; SLE: systemic lupus erythematosus; DKD: diabetic kidney disease.

**Table S5:** scRNA-seq or snRNA-seq studies on unsorted mouse renal tissue

| Author  | Protocol                                | Age      | Disease?  | T°                                   | Cells                    | Depth  | Genes                        | Glom.     | Remarks  |
|---|---|----------|---|--------------------------------------|--------------------------|--|------------------------------|-----------|--|
| Adam <i>et al.</i> <sup>1</sup> , 2017            | Drop-seq                                | N        | Healthy (n=12)  | (+), C<br>(+), W                     | 20,424                   | ?  | > 1,000                      | P         | First use of CAP on renal tissue (4853 cells, remaining cells with warm dissociation)<br>P°: not mentioned   |
| Park <i>et al.</i> <sup>7</sup> , 2018            | 10x Chromium                            | A<br>(?) | Healthy (n=7)   | (+), W                               | 43,745                   | 38,588   | 940                          | P, M      | P°: 78 cells (0.18%)   |
| Wu <i>et al.</i> <sup>8</sup> , 2018              | Drop-seq                                | A        | Healthy (n=1)   | (-), W<br>Met.                       | 3,531                    | ±5,500   | ±1,000                       | -         | *: data from validation study with sn10x platform<br>P°: 227 cells (2.4%) <sup>9</sup> (mean of sNuc-Drop-Seq, DroNC-seq and sNuc-10x)   |
|   | sNuc-Drop-Seq<br>DroNC-seq<br>sNuc-10x* | A        | Healthy (n=3)<br>UUOS (n=1)*  | (-), C<br>Snap.                      | 7,860;<br>6,147*         | ±7,000   | ±850-<br>1,100;<br>763*      | P, E, M   |  |
| Cao <i>et al.</i> <sup>11</sup> , 2018            | Sci-CAR*                                | A        | Healthy (n=2)   | (-), C<br>Snap.                      | 13,893                   | 140,000<br>;1,011**  | ±500                         | P         | *: =sci-RNA-seq+sci-ATAC-seq<br>**: mUMIs per cell<br>P°: not mentioned  |
| Schaum <i>et al.</i> <sup>12</sup> , 2018         | SMART-seq2<br>10x Chromium              | A        | Healthy (n=6)*<br>Healthy (n=3)**   | (+), W                               | 519*;<br>2,781**         | 10 <sup>4</sup> -5-<br>10 <sup>6</sup> *;<br>4,000**;<br>*** | ±1,250*<br>;<br>±1,800*<br>* | M         | Study on mouse atlas (Tabula Muris)<br>*: SMART-seq2<br>**: droplet-based<br>***: mUMIs per cell   |
| Ransick <i>et al.</i> <sup>15</sup> , 2019        | 10x Chromium                            | A        | Healthy (n=4)   | (+), C                               | 31,265                   | 70,446   | 1,395                        | P         | Three kidney regions were dissected before dissociation<br>P°: 24 cells (0.08%) <sup>9</sup>   |
| Denisenko <i>et al.</i> <sup>18</sup> , 2020      | 10x Chromium                            | A        | Healthy (n=18)*   | (+), W/C<br>(-), W/C<br>Met., Cry o. | 77,656                   | 52,000   | 981                          | P, M      | Comparison of different dissociation and storage techniques<br>P°: 3 cells (0.03%) in W<br>P°: 330 cells (2.78%) in C<br>P°: 0.7%  |
|   | 10x Chromium                            | A        | Healthy (n=18)*   | (-), C<br>Snap.                      | 98,303                   | 52,000   | 1,819                        | P, M      | *: total amount of mice (scRNA-seq/snRNA-seq/bulk)   |
| Conway <i>et al.</i> <sup>20</sup> , 2020         | 10x Chromium<br>SMART-seq2              | A        | Sham (n=3*,1**)<br>UUO-2 (n=3*,1**)<br>UUO-7 (n=3*,1**)<br>R-UUO (n=3*,1**) | (+), W                               | 16,967*<br>; 362**       | 87,500*<br>;<br>41,000<br>***;<br>±4,600*<br>***             | 1,218*;<br>3,156**           | -<br>**** | *: 10x<br>**: SMART-seq2<br>***: mUMIs per cell<br>****: very few podocytes, too few for cluster   |
| Kirita <i>et al.</i> <sup>21</sup> , 2020         | 10x Chromium                            | A        | IRI (n=15)<br>Sham (n=3)  | (-), C<br>Snap.                      | 126,578                  | ?  | 150-<br>8,000                | P, M      | P°: not mentioned  |
| Zhao <i>et al.</i> <sup>22</sup> , 2020           | 10x Chromium                            | A        | IRI ± XJB-5-131 (n=5)*<br>Sham (n=5)**                                      | (+), W                               | 7,581*;<br>6,069**       | 27,920*<br>;39,310<br>**                                     | 2,369                        | -         | *: ischemia/reperfusion mice<br>**: sham mice  |
| Hyndman <i>et al.</i> <sup>25</sup> , 2020        | 10x Chromium                            | A        | Healthy (n=2)<br>HDAC-KO mouse (n=2)  | (-), C<br>Snap.                      | 25,075                   | 23,000   | 1,804                        | P         | Study focusing on Hdac1/2 KO mice<br>P°: 311 cells (1.2%)  |
| Rudman-Melnick <i>et al.</i> <sup>26</sup> , 2020 | Drop-seq                                | A        | Healthy (n=?)<br>IRI (n=21)<br>+ mice for validation study                  | (+), W                               | 54,730                   | ?  | >500                         | P         | P°: not mentioned  |
| Dangi <i>et al.</i> <sup>29</sup> , 2020          | 10x Chromium                            | A        | Healthy (n=2)<br>Tx, rejecting (n=2)<br>Tx, tolerized (n=2)                 | (+), W                               | 30,053                   | ?  | ?                            | -         | Study using a murine kidney transplant model. Allografts retrieved at 15d post-transplant.   |
| Marshall <i>et al.</i> <sup>30</sup> , 2020       | HyPR-seq                                | A        | Healthy (n=2)*<br>DKD (n=2)**   | (+), W                               | 14,288*;<br>14,837*<br>* | 203***   | Probing of 32 genes          | P, M      | New targeted scRNA-seq technique using specific DNA probes (HyPR-seq)<br>*: BTBR wt/wt mice<br>**: BTBR ob/ob mice<br>***: mUMIs per cell<br>P°: 132 cells (0.9%)*; 12 cells (0.08%)** |
| Omori <i>et al.</i> <sup>31</sup> , 2020          | 10x Chromium                            | A        | p16-Cre <sup>ERT2</sup> -tdTomato mouse (n=?)                               | (+), W                               | 2,403                    | > 800*   | > 200                        | -         | New mouse model on cell senescence; Td-tomato labelling of p16-high cells as a marker for cell senescence<br>*: UMIs per cell  |

|                                       |              |   |   |              |                     |         |           |      |  |
|---------------------------------------|--------------|---|---|--------------|---------------------|---------|-----------|------|--|
| Ni et al. <sup>32</sup> , 2021        | 10x Chromium | A | Healthy (n=?)   | (+), W       | ?                   | ?       | 200-3,000 | -    | Study focusing on phosphate metabolism and FGF23-mediated pathways   |
| Sidhom et al. <sup>33</sup> , 2021    | 10x Chromium | A | Pdss2 <sup>kd/kd</sup> mice (n=3)*<br>Healthy (n=3)**           | (-), C Snap. | 20,441*;<br>16,119* | ±1,000  | ±1,500    | P    | *: Mice with homozygous mutation in Pdss2-gene (CoQ-pathway)<br>**: controls<br>P°: 61 cells (0.3%)*, 41 cells (0.25%)**   |
| Hinze et al. <sup>34</sup> , 2021     | Drop-seq     | A | Healthy (n=2)   | (-), C Met.  | 5,675*;<br>6,327**  | 772***  | 456       | P    | Grhl2 <sup>CD2-/-</sup> mice are a model for lower corticomedullary osmolality gradient<br>*: 'whole kidney'<br>**: dissected kidney regions before dissociation<br>***: mean transcripts per cell<br>P°: not mentioned<br>P°: not mentioned |
|                                       | 10x Chromium | A | Healthy (n=2)<br>Grhl2 <sup>CD2-/-</sup> (n=1 mouse, 2 kidneys) | (-), C Snap. | ?                   | ?       | 500-5,000 | P    |  |
| Dhillon et al. <sup>35</sup> , 2021   | 10x Chromium | A | Healthy (n=6)*<br>FAN mice (n=2)**                              | (+), W       | 37,361*;<br>27,730* | ?       | 200-3,000 | P, E | *: Healthy controls<br>**: CKD/fibrosis mouse model<br>P°: 97 cells (0.15%)  |
| Janosevic et al. <sup>36</sup> , 2021 | 10x Chromium | A | Endotoxin treated mice (n=7 mice, 14 kidneys)                   | (+), W       | 63,287              | ±50,000 | 200-3,000 | -    | Study of murine endotoxemia model. Analysis on 7 timepoints after LPS-injection (0h, 1h, 4h, 16h, 27h, 36h, 48h), 1 mouse per timepoint.   |

**Legend:** scRNA-seq experiments are shown in blue, snRNA-seq experiments are shown in red; ‘Age’: age of mice (N = newborn, A =adult); ‘Disease’: healthy or pathological kidney tissue (with n = number of mice used); ‘T°’: fresh vs. frozen tissue and warm vs. cold dissociation ((+) = fresh tissue, (-) = frozen tissue, W = warm dissociation, C = cold dissociation, ‘Met’=methanol-fixation, ‘Cryo’=cryopreservation, ‘Snap’=snap-frozen tissue); ‘Cells’: total number of cells isolated and analyzed after quality control; ‘Depth’: sequencing depth defined as ‘mean reads per cell’; ‘Genes’: mean number of genes per cell; ‘Glom.’: isolation of glomerular cells (‘-’ = no glomerular cells isolated, ‘P’ = podocytes, ‘E’ = glomerular endothelial cells, ‘M’ = mesangial cells, green color = podocytes identified, red color = no podocytes identified); ‘P°’ refers to the absolute and relative number of podocytes isolated in scRNA-seq studies.

**Abbreviations:** CAP: cold active protease; UUOS: unilateral ureteral obstruction surgery; UUO-2: two days after unilateral ureteral obstruction surgery; UUO-7: seven days after unilateral ureteral obstruction surgery; R-UUO: reversible unilateral ureteral obstruction (surgery); sham: sham surgery; IRI: ischemia reperfusion injury; XJB-5-131: a synthetic anti-oxidant; HDAC-KO mouse: Hdac1/2 knockout mouse; Tx: mice receiving kidney transplant; DKD: diabetic kidney disease; Grhl2<sup>CD2-/-</sup>: mouse lacking Grhl2 transcription factor in collecting ducts; FAN: Folic acid nephropathy.

## Supplementary figures

### Search strategy

#### Aim:

- The following search strategy was used to compile the available scRNA-seq and snRNA-seq studies on human and mouse kidney tissue, as outlined in Tables S1-S2.

#### Sources:

- An electronic search on the database of PubMed was performed:
  - o last literature search was done on 2th of March 2021; studies were screened from 2009 (= first publication of a scRNA-seq technique by Tang *et al.*<sup>1</sup>) up until 2th of March 2021; included studies ranged from August 2017 up until January 2021.
- Additional searches:
  - o Handsearching of references lists of primary studies and reviews.

#### Study eligibility criteria:

- Publication language: studies in English were included
- Only peer reviewed studies were included, pre-prints were excluded
- Study characteristics:
  - o Inclusion criteria:
    - Primary studies reporting on single-cell transcriptomics (scRNA-seq and/or snRNA-seq) experiments
    - Experiments should be performed on human or mouse renal tissue
  - o Exclusion criteria:
    - Review articles/editorials without primary data on experiments
    - Articles exclusively using previously published single-cell transcriptomics databases
    - Studies on kidney organoids and stem cells (incl. hESC and iPSC)
    - Studies exclusively on urinary cells
    - Studies exclusively on developing fetal renal tissue (human or animal)
    - Studies on commercially available cultured kidney cells
    - Studies exclusively on renal tumors
    - Studies on other animals (e.g. zebrafish, hamster)
    - Reviews without scRNA-seq or snRNA-seq experiments were excluded

#### Data collection process:

- DD reviewed the literature, screened the studies and included the eligible studies.

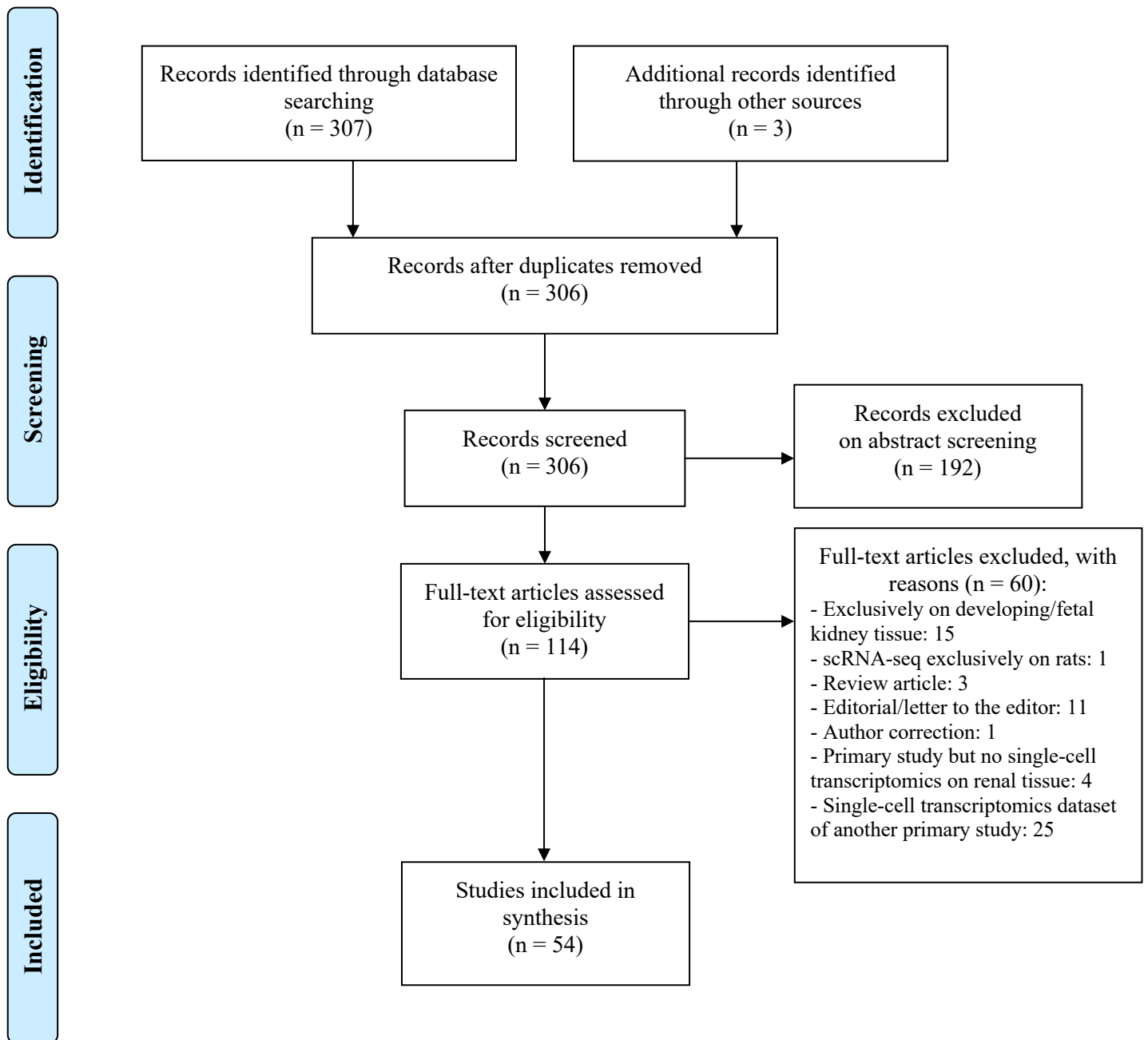
#### Search terms/strategy:

- DD used a combined scRNA-seq and snRNA-seq 'query'.
- Combined Pubmed search query:

("Single-Cell Analysis"[Mesh] OR "scRNA-seq"[tiab] OR "single cell RNA seq\*"[tiab] OR "single cell RNA"[tiab] OR "single cell mRNA"[tiab] OR "scRNA"[tiab] OR "snRNA-seq"[tiab] OR "single nucleus RNA seq\*"[tiab] OR "single nucleus RNA"[tiab] OR "single nuclei RNA"[tiab] OR "snRNA"[tiab] OR "single cell transcript\*"[tiab]) AND ("Kidney"[Mesh] OR "Nephrology"[Mesh] OR kidney\*[tiab] OR renal\*[tiab] OR neph\*[tiab])

**Figure S1:** Literature search strategy

<sup>1</sup>= literature reference<sup>55</sup>



**Figure S2:** PRISMA 2009 flow diagram of the literature search strategy<sup>56</sup>

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