**Supplementary data**

# 3D organoid-derived human glomeruli for personalised podocyte disease modelling and drug screening.

Lorna J. Hale, Sara E. Howden, Belinda Phipson, Andrew Lonsdale, Pei X. Er, Irene Ghobrial, Salman Hosawi, Sean Wilson, Kynan Lawlor, Shahnaz Khan, Alicia Oshlack, Catherine Quinlan , Rachel Lennon, Melissa H. Little

**Contents**

***Supplementary Table 1.***

| Symbol | Gene name | logFC | *P* Value |
|---|---|---|---|
| CDKN1A | Cyclin dependent kinase inhibitor 1A | 4.085346 | 4.60E-14 |
| PDZK1IP1 | PDZK1 interacting protein 1 | 5.862841 | 6.45E-13 |
| TRIM22 | Tripartite motif containing 22 | 3.559167 | 1.02E-10 |
| TNFRSF10B | TNF receptor superfamily member 10b | 2.509024 | 1.46E-10 |
| C3 | Complement C3 | 8.140257 | 1.48E-10 |
| CLDN1 | Claudin 1 | 3.55235 | 2.66E-10 |
| PHLDA1 | Pleckstrin homology like domain family A member 1 | 3.311057 | 8.52E-10 |
| PBXIP1 | PBX homeobox interacting protein 1 | 2.216353 | 2.12E-09 |
| EDA2R | Ectodysplasin A2 receptor | 4.138096 | 2.47E-09 |
| TMEM159 | Transmembrane protein 159 | 3.193489 | 1.08E-08 |
| FUCA1 | Fucosidase, alpha-L- 1, tissue | 2.381889 | 1.22E-08 |
| NRP2 | Neuropilin 2 | 2.440631 | 3.27E-08 |
| CSF1 | Colony stimulating factor 1 | 2.518191 | 5.04E-08 |
| SUSD6 | Sushi domain containing 6 | 2.583393 | 5.78E-08 |
| SOWAHB | Sosondowah ankyrin repeat domain family member B | 3.912102 | 1.26E-07 |
| GDF15 | Growth differentiation factor 15 | 6.631471 | 1.47E-07 |
| IL6 | Interleukin 6 | 5.364979 | 1.83E-07 |
| DRAM1 | DNA damage regulated autophagy modulator 1 | 3.06241 | 2.11E-07 |
| BTG2 | BTG anti-proliferation factor 2 | 3.541625 | 2.32E-07 |
| ATG16L2 | Autophagy related 16 like 2 | 3.300185 | 2.37E-07 |
| TPCN1 | Two pore segment channel 1 | 2.927092 | 2.94E-07 |
| TNFSF10 | TNF superfamily member 10 | 5.100832 | 2.98E-07 |
| SEL1L3 | SEL1L family member 3 | 2.515535 | 3.13E-07 |
| TRANK1 | Tetratricopeptide repeat and ankyrin repeat containing 1 | 3.54394 | 3.61E-07 |
| SULF2 | Sulfatase 2 | 7.209275 | 6.50E-07 |
| PLAU | Plasminogen activator, urokinase | 3.675696 | 7.17E-07 |
| MANBA | Mannosidase beta | 2.464472 | 7.60E-07 |
| CD82 | CD82 molecule | 4.16917 | 1.22E-06 |
| JUP | Junction plakoglobin | 2.868247 | 1.23E-06 |
| CDKN1A | Cyclin dependent kinase inhibitor 1A | 4.085346 | 4.60E-14 |

Footnote: The top 30 most differentially upregulated genes following induced differentiated at 37°C.

***Supplementary Table 2.***

| UNIPROT | Description | Gene Symbol | OrgGlom (C4) ion intensity | OrgPT (C4) ion intensity | Max FC | Anova (*p*) | Division | Category |
|---|---|---|---|---|---|---|---|---|
| O00468 | Agrin | AGRN | 89.87 | 40.52 | 2.22 | 0.08692 | Core Matrisome | ECM Glycoproteins |
| O00468 | Agrin (fragment) | AGRN | 5.93 | 5.21 | 1.14 | 0.93235 | Core Matrisome | ECM Glycoproteins |
| P39060 | Collagen, type XVIII, alpha 1, isoform CRA_d | COL18A1 | 215.94 | 232.26 | 0.93 | 0.74107 | Core Matrisome | Collagens |
| P02452 | Collagen, type I, alpha 1, isoform CRA_a | COL1A1 | 18.76 | 17.13 | 1.09 | 0.70141 | Core Matrisome | Collagens |
| P02452 | Collagen alpha-1(I) chain | COL1A1 | 182.72 | 265.91 | 0.69 | 0.25455 | Core Matrisome | Collagens |
| P08123 | Collagen alpha-2(I) chain | COL1A2 | 14.95 | 24.48 | 0.61 | 0.21043 | Core Matrisome | Collagens |
| P02458 | Collagen alpha-1(II) chain | COL2A1 | 20.05 | 36.08 | 0.56 | 0.08036 | Core Matrisome | Collagens |
| P02462 | Collagen alpha-1(IV) chain | COL4A1 | 185.21 | 3.36 | 55.19 | 0.00004 | Core Matrisome | Collagens |
| P08572 | Collagen, type IV, alpha 2, isoform CRA_a | COL4A2 | 152.12 | 73.31 | 2.07 | 0.04782 | Core Matrisome | Collagens |
| Q01955 | Collagen alpha-3(IV) chain | COL4A3 | 13.06 | 13.53 | 0.96 | 0.80129 | Core Matrisome | Collagens |
| P29400 | Collagen alpha-5(IV) chain | COL4A5 | 269.55 | 409.16 | 0.66 | 0.02797 | Core Matrisome | Collagens |
| Q14031 | Collagen alpha-6(IV) chain | COL4A6 | 117.94 | 107.53 | 1.10 | 0.80216 | Core Matrisome | Collagens |
| P12109 | Collagen alpha-1(VI) chain | COL6A1 | 6.06 | 7.36 | 0.82 | 0.22733 | Core Matrisome | Collagens |
| P12110 | Collagen alpha-2(VI) chain | COL6A2 | 15.08 | 3.90 | 3.86 | 0.07267 | Core Matrisome | Collagens |
| P12111 | Collagen alpha-3(VI) chain | COL6A3 | 25.68 | 79.46 | 0.32 | 0.13462 | Core Matrisome | Collagens |
| P07339 | Cathepsin D | CTSD | 1.29 | 3.84 | 0.34 | 0.00250 | Matrisome-Assoc. | ECM Regulators |
| Q86XX4 | Extracellular matrix protein FRAS1 | FRAS1 | 25.91 | 7.81 | 3.32 | 0.20262 | Core Matrisome | ECM Glycoproteins |
| Q5SZK8 | FRAS1-related extracellular matrix protein 2 | FREM2 | 34.23 | 3.40 | 10.06 | 0.00503 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P43026 | GDF5 (fragment) | GDF5 | 115.28 | 96.54 | 1.19 | 0.37924 | Matrisome-Assoc. | Secreted Factors |
| P10915 | Hyaluronan and proteoglycan link protein 1, isoform, CRA_a | HAPLN1 | 46.68 | 79.82 | 0.58 | 0.38245 | Core Matrisome | Proteoglycans |
| P51610 | Host cell factor 1 | HCFC1 | 15.64 | 34.72 | 0.45 | 0.03141 | Matrisome-Assoc. | Secreted Factors |
| Q8NDA | Hemicentin-2 | HMCN2 | 32.36 | 8.71 | 3.72 | 0.08496 | Core Matrisome | ECM Glycoproteins |
| P98160 | Heparan sulfate proteoglycan 2 (Perlecan), isoform CRA_b | HSPG2 | 35.00 | 16.86 | 2.08 | 0.03036 | Core Matrisome | Proteoglycans |
| Q92743 | HTRA1 protein (fragment) | HTRA1 | 1.31 | 1.48 | 0.88 | 0.44501 | Matrisome-Assoc. | ECM Regulators |
| Q16270 | Insulin-like growth factor binding protein 7 (Fragment) | IGFBP7 | 52.27 | 2.49 | 21.02 | 0.00139 | Core Matrisome | ECM Glycoproteins |
| P25391 | Laminin subunit alpha-1 | LAMA1 | 9.42 | 5.74 | 1.64 | 0.16871 | Core Matrisome | ECM Glycoproteins |
| Q16787 | Laminin subunit alpha-3 | LAMA3 | 21.12 | 11.43 | 1.85 | 0.12774 | Core Matrisome | ECM Glycoproteins |
| O15230 | Laminin subunit alpha-5 | LAMA5 | 115.69 | 4.46 | 25.93 | 0.00001 | Core Matrisome | ECM Glycoproteins |
| P07942 | Laminin subunit beta-1 | LAMB1 | 30.1 | 0.61 | 49.03 | 0.01163 | Core Matrisome | ECM Glycoproteins |
| P55268 | Laminin, beta 2 (Laminin S), isoform CRA_a | LAMB2 | 74.18 | 2.09 | 35.51 | 0.00242 | Core Matrisome | ECM Glycoproteins |
| P11047 | Laminin subunit beta-4 | LAMB4 | 4.42 | 7.45 | 0.59 | 0.39654 | Core Matrisome | ECM Glycoproteins |
| A4D0S4 | Laminin, gamma 1 (Formerly LAMB2), isoform CRA_a | LAMC1 | 143.38 | 2.91 | 49.30 | 0.00007 | Core Matrisome | ECM Glycoproteins |
| Q08397 | Lysyl oxidase homolog 1 | LOXL1 | 14.18 | 13.37 | 1.06 | 0.65604 | Matrisome-Assoc. | ECM Regulators |
| P55081 | Microfibrillar-associated protein 1 | MFAP1 | 14.31 | 20.20 | 0.71 | 0.15588 | Core Matrisome | ECM Glycoproteins |
| Q08431 | Milk fat globule-EGF factor 8 protein, isoform CRA_a | MFGE8 | 24.11 | 11.77 | 2.05 | 0.05612 | Core Matrisome | ECM Glycoproteins |
| P14543 | Nidogen 1 | NID1 | 87.75 | 1.90 | 46.25 | 0.00002 | Core Matrisome | ECM Glycoproteins |
| Q8IVN8 | Somatomedin-B and thrombospondin type-1 domain containing protein | SBSPON | 28.20 | 1.14 | 24.78 | 0.00017 | Core Matrisome | ECM Glycoproteins |
| P50454 | Serpin peptidase inhibitor, clade H (HSP47), member 1, (CBP1) | SERPINH1 | 249.18 | 26.06 | 9.56 | 0.00152 | Matrisome-Assoc. | ECM Regulators |
| O94813 | Slit homolog 2 protein | SLIT2 | 25.58 | 20.82 | 1.23 | 0.54663 | Core Matrisome | ECM Glycoproteins |
| P21980 | Protein-glutamine gamma-glutmyltransferase 2 | TGM2 | 6.21 | 2.77 | 2.24 | 0.02147 | Matrisome-Assoc. | ECM Regulators |
| O43548 | Protein-glutamine gamma-glutmyltransferase 5 | TGM5 | 8.41 | 4.54 | 1.85 | 0.50593 | Matrisome-Assoc. | ECM Regulators |
| Q9GZM7 | Tubulointerstitial nephritis antigen-like | TINAGL1 | 0.84 | 0.09 | 9.05 | 0.21190 | Core Matrisome | ECM Glycoproteins |

Footnote: Enriched extracellular matrix (C4 fraction) enriched proteins identified by mass spectrometry.

***Supplementary Table 3.***

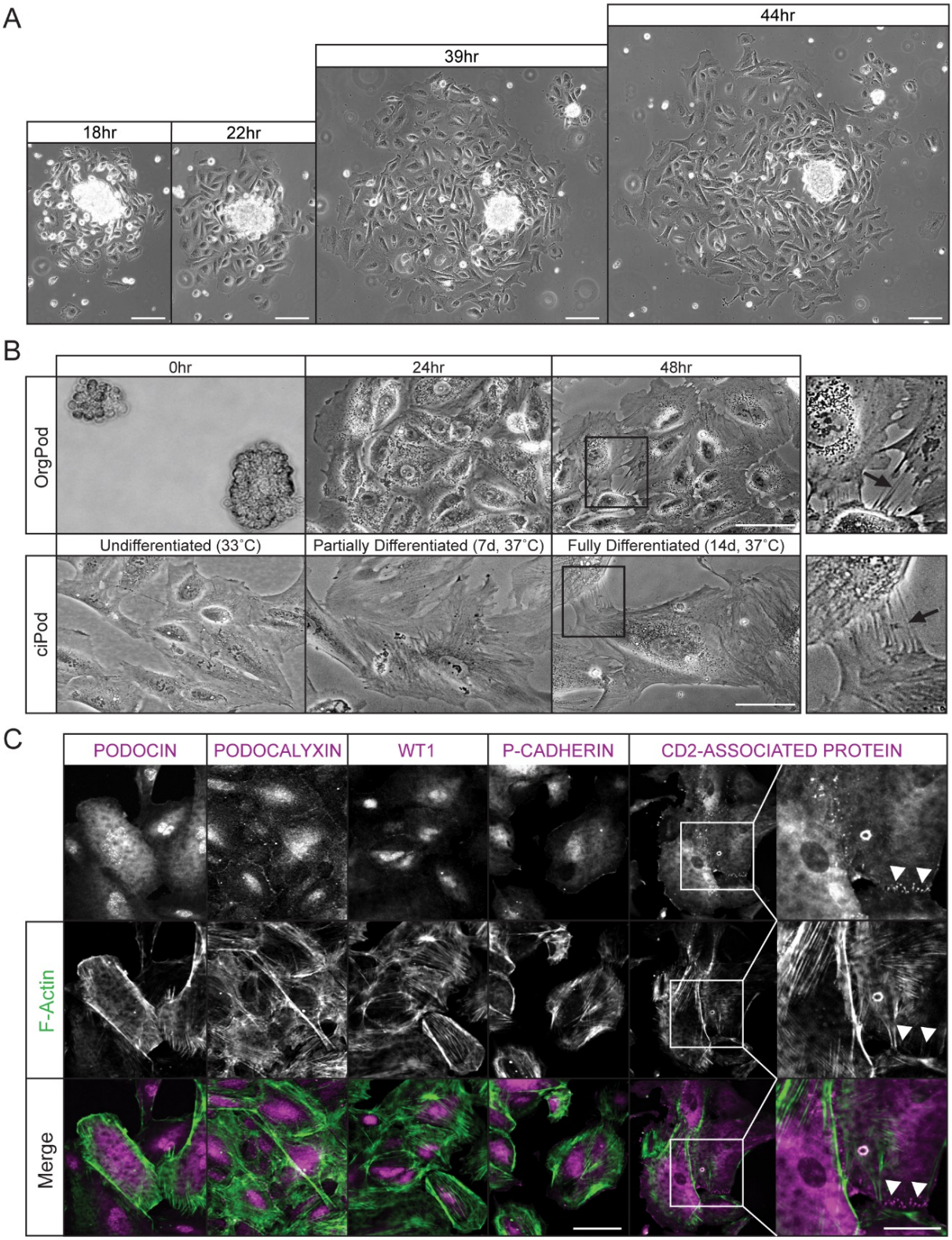
| UNIPROT | Description | Gene Symbol | OrgGlom (C4) ion intensity | OrgPT (C4) ion intensity | Max FC | Anova (*p*) | Division | Category |
|---|---|---|---|---|---|---|---|---|
| O14672 | ADAM metallopeptidase domain 10, isoform CRA_b | ADAM10 | 13.51 | 16.47 | 0.82 | 0.19109 | Matrisome-Assoc. | ECM Regulators |
| O00468 | Agrin | AGRN | 13.71 | 3.10 | 4.42 | 0.04029 | Core Matrisome | ECM Glycoproteins |
| P04083 | Annexin A1 | ANXA1 | 21.07 | 51.37 | 4.13 | 0.00029 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P07355 | Annexin | ANXA2 | 376.20 | 178.74 | 2.10 | 0.00007 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P09525 | Annexin A4 | ANXA4 | 106.96 | 154.44 | 0.69 | 0.00041 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P08758 | Annexin A5 | ANXA5 | 123.11 | 85.33 | 1.44 | 0.06088 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P12109 | Collagen alpha-1(VI) chain | COL6A1 | 10.43 | 15.67 | 0.67 | 0.37976 | Core Matrisome | Collagens |
| A8TX70 | Collagen alpha-5(VI) chain | COL6A5 | 36.67 | 20.25 | 1.81 | 0.0088 | Core Matrisome | Collagens |
| Q02388 | Collagen alpha-1(VII) chain | COL7A1 | 10.18 | 12.07 | 0.84 | 0.52353 | Core Matrisome | Collagens |
| P53634 | Dipeptidyl peptidase 1 | CTSC | 66.91 | 49.81 | 1.34 | 0.80944 | Matrisome-Assoc. | ECM Regulators |
| P53634 | Dipeptidyl peptidase 1 (fragment) | CTSC | 4.52 | 1.82 | 2.48 | 0.25719 | Matrisome-Assoc. | ECM Regulators |
| P07339 | Cathepsin D | CTSD | 280.35 | 227.53 | 1.23 | 0.00218 | Matrisome-Assoc. | ECM Regulators |
| Q9UBR2 | Cathepsin Z | CTSZ | 21.82 | 24.20 | 0.90 | 0.08967 | Matrisome-Assoc. | ECM Regulators |
| P51610 | Host cell factor 1 | HCFC1 | 36.52 | 51.98 | 0.70 | 0.17547 | Matrisome-Assoc. | Secreted Factors |
| P98160 | Basement membrane-specific heparan sulfate proteoglycan core protein | HSPG2 | 16.69 | 10.29 | 1.62 | 0.01327 | Core Matrisome | Proteoglycans |
| Q16270 | Insulin-like growth factor binding protein 7, isoform CRA_a (fragment) | IGFBP7 | 6.54 | 6.67 | 0.98 | 0.78312 | Core Matrisome | ECM Glycoproteins |
| P19823 | Inter-alpha-trypsin inhibitor heavy chain H2 | ITIH2 | 22.99 | 25.44 | 0.90 | 0.58236 | Matrisome-Assoc. | ECM Regulators |
| O15230 | Laminin subunit alpha-5 | LAMA5 | 2456.96 | 2831.03 | 0.87 | 0.20977 | Core Matrisome | ECM Glycoproteins |
| P55268 | Laminin, beta 2 (Laminin S), isoform CRA_a | LAMB2 | 10.49 | 15.37 | 0.68 | 0.13858 | Core Matrisome | ECM Glycoproteins |
| P11047 | Laminin, gamma 1 (Formerly LAMB2), isoform CRA_a | LAMC1 | 59.22 | 10.48 | 5.65 | 0.00060 | Core Matrisome | ECM Glycoproteins |
| P49257 | Lectin, mannose-binding 1, isoform CRA_b | LMAN1 | 0.45 | 44.13 | 0.01 | 0.02840 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P14543 | Nidogen-1 | NID1 | 43.75 | 3.64 | 12.02 | 0.00004 | Core Matrisome | ECM Glycoproteins |
| Q14112 | Nidogen-2 | NID2 | 6.07 | 1.66 | 3.66 | 0.00264 | Core Matrisome | ECM Glycoproteins |
| P13674 | Procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha polypeptide I | P4HA1 | 39.42 | 60.43 | 0.65 | 0.01261 | Matrisome-Assoc. | ECM Regulators |
| O15460 | Prolyl 4-hydroxylase subunit alpha-2 | P4HA2 | 5.65 | 3.95 | 1.43 | 0.25057 | Matrisome-Assoc. | ECM Regulators |
| Q02809 | Procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1, isoform CRA_a | PLOD1 | 20.33 | 17.1 | 1.19 | 0.05547 | Matrisome-Assoc. | ECM Regulators |
| O00469 | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 | PLOD2 | 96.00 | 76.96 | 1.25 | 0.05608 | Matrisome-Assoc. | ECM Regulators |
| O60568 | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3 | PLOD3 | 20.41 | 25.65 | 0.80 | 0.09117 | Matrisome-Assoc. | ECM Regulators |
| O15031 | Plexin B-2 | PLXNB2 | 35.33 | 34.24 | 1.03 | 0.67752 | Matrisome-Assoc. | ECM-affiliated Proteins |
| P35237 | Serpin peptidase inhibitor, clade B, member 6, isoform CRA_a | SERPINB6 | 18.30 | 9.30 | 1.97 | 0.00529 | Matrisome-Assoc. | ECM Regulators |
| P50453 | Serpin peptidase inhibitor, clade B, member 9, isoform CRA_a | SERPINB9 | 99.45 | 62.04 | 1.60 | 0.64945 | Matrisome-Assoc. | ECM Regulators |

Footnote: Enriched cellular and vesicular proteins (C1 fraction) identified by mass spectrometry.

***Supplementary Table 4.***

| Symbol | Gene name | Max FC | *P* Value | Association |
|---|---|---|---|---|
| KDR | Kinase insert domain receptor | 23.93 | 1.94E-03 | Podocyte / Endothelial |
| COL4A4 | Collagen Type IV alpha 4 chain | 15.89 | 1.72E-04 | GBM |
| MMP2 | Matrix metallopeptidase 2 | 8.61 | 1.65E-05 | GBM / Mesangium |
| CXCL12 | Chemokine (C-X-C motif) ligand 12 | 8.38 | 8.87E-03 | Renal vasculature |
| COL4A3 | Collagen Type IV alpha 3 chain | 7.71 | 1.73E-03 | GBM |
| ITGA2 | Integrin, alpha 2 | 6.85 | 4.49E-03 | GBM / matrices |
| TEK | TEK receptor tyrosine kinase (TIE2) | 6.34 | 1.31E-04 | Endothelial |
| FN1 | Fibronectin 1 | 6.26 | 3.10E-03 | Glomerular |
| ANGPTL2 | Angiopoietin like 2 | 5.41 | 1.76E-03 | Endothelial / vessels |
| IGFBP3 | Insulin like growth factor binding protein 3 | 4.25 | 1.45E-05 | Podocyte |
| SHISA3 | Shisa homolog 3 | 4.19 | 5.16E-05 | Nephron progenitors |
| MME | Membrane metallo-endopeptidase (CD10) | 3.97 | 8.52E-05 | Podocyte |
| IGFBP5 | Insulin like growth factor binding protein | 3.61 | 19.7E-05 | Mesangium |
| EMCN | Endomucin | 3.21 | 3.27E-05 | Endothelial |
| UCHL1 | Ubiquitin C-terminal hydrolase L1 | 2.74 | 5.13E-03 | Podocyte / PEC |
| TAGLN | Transgelin 2 | 2.74 | 1.94E-04 | Mesangium |
| CDH5 | Cadherin 5 | 2.66 | 9.40E-02 | Endothelial |
| GJA5 | Gap junction protein alpha 5 | 2.57 | 1.78E-03 | Endothelial / vessels |
| SMAD7 | SMAD family member 7 | 2.38 | 4.26E-05 | Glomerular |
| CX3CL1 | C-X3-C motif chemokine ligand 1 | 2.32 | 3.46E-05 | Glomerular |
| FGF2 | Fibroblast growth factor 2 | 2.30 | 1.18E-02 | Glomerular |
| GATA3 | GATA binding protein 3 | 2.25 | 1.35E-02 | Glomerular |
| DES | Desmin | 2.19 | 8.66E-03 | Mesangium |
| FOXD1 | Forkhead box D1 | 2.01 | 8.68E-03 | Mesangium |
| C1QTNF12 | C1q and TNF related 12 | 2.00 | 1.49E-02 | Podocyte |

Footnote: Represents the most differentially expressed glomerular-specific genes [1] identified between MAFB-mTAGBFP sorted cells isolated at d7+10 and organoid glomeruli isolated at d7+19.

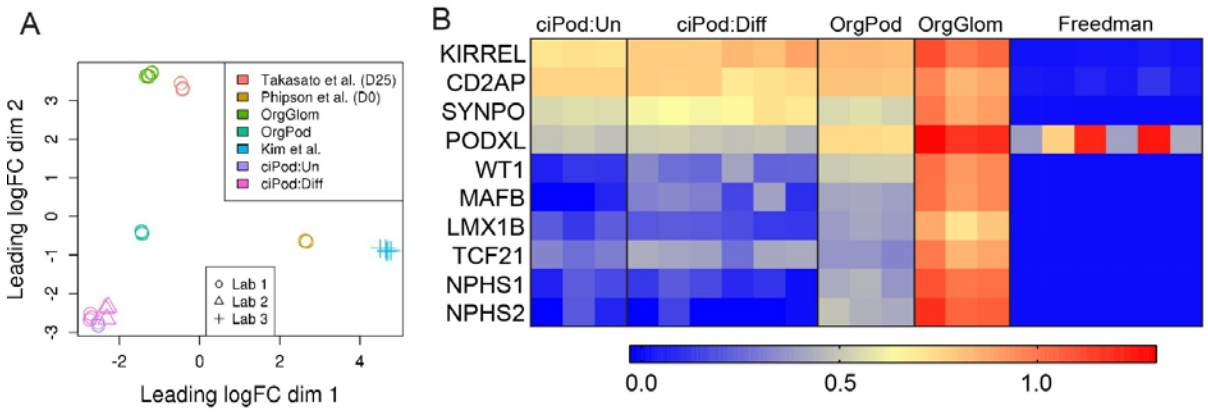



***Supplementary Figure 1. Morphology and identity of primary podocytes isolated from organoid glomeruli.*** **A.** Organoid-derived podocytes (OrgPods) actively migrate from organoid glomeruli (OrgGloms) over time, forming a progressively more arborized monolayer.

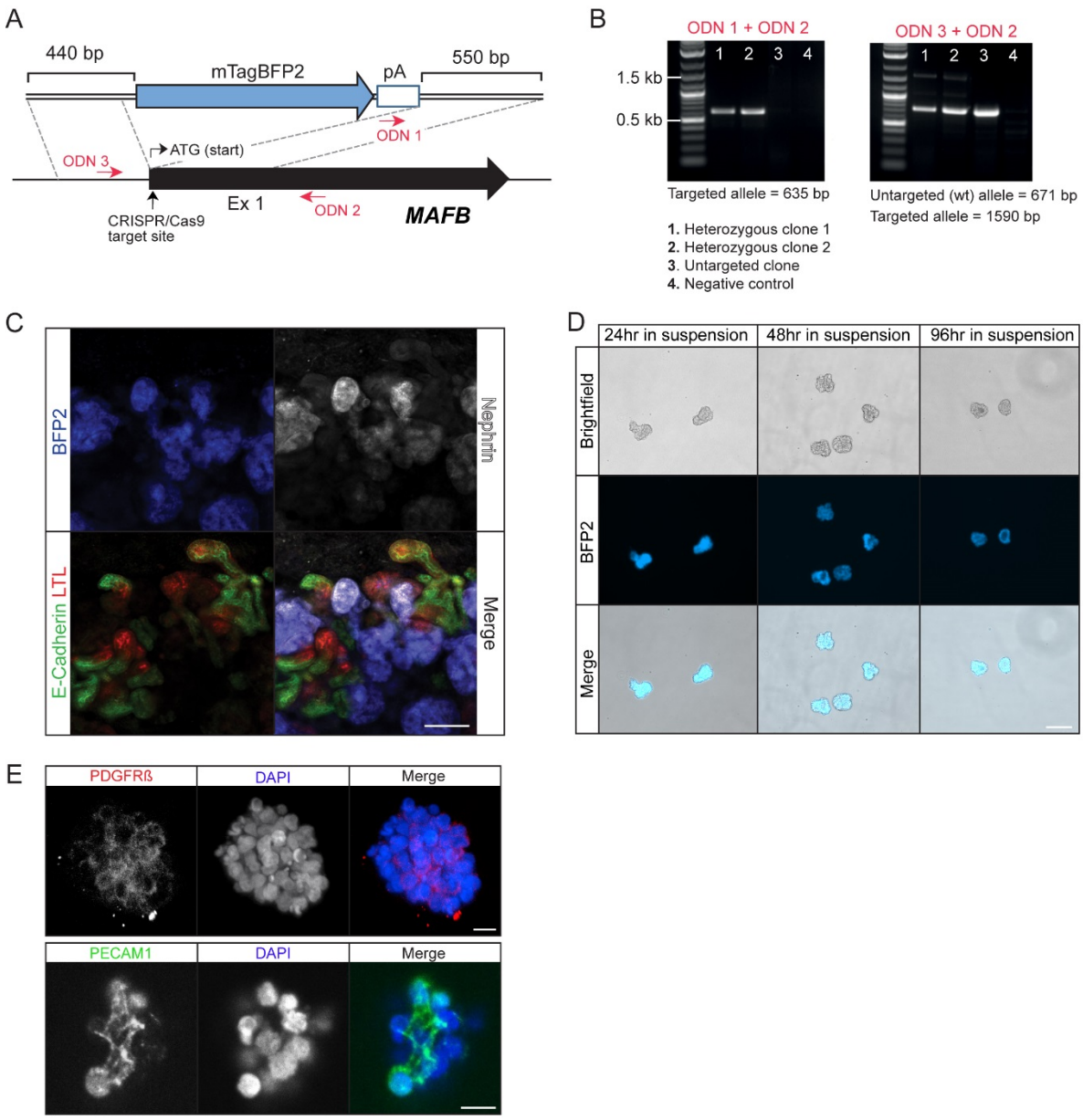
Scale bars 100µm. **B.** Organoid-derived podocytes (OrgPods) in culture show comparable morphology to the gold-standard conditionally immortalised human podocyte cell line (ciPod) [2], displaying a flat arborized morphology with processes connecting adjacent cells. Scale bars 50µm. **C.** Fluorescent visualisation of classical podocyte markers alongside phalloidin to mark F-actin showed appropriate expression and localisation of these proteins within the OrgPod cell population (arrowheads mark areas of interest). PODOCIN showed a cytoplasmic distribution with increased levels at cell edges; PODOCALYXIN showed a punctuated distribution across the cell with focussed expression at cell junctions. WILMS' TUMOUR was predominately nuclear, as anticipated, whilst P-CADHERIN, another marker of differentiated cells, was cytoplasmic, perinuclear and also present at the cell surface. SYNAPTOPODIN was found in close association with stress fibres of the actin cytoskeleton whilst CD2-ASSOCIATED PROTEIN showed mature expression in areas of cell-cell contact at the edges of F-actin tips. Representative immunofluorescence images shown in greyscale for single channels, merged images in colour. Scale bar 50µm.

***Supplementary Figure 2. Transcriptional comparison of organoid glomeruli with podocytes cultured in 2D.*** **A.** Gene Ontology (GO) enrichment analysis of the top 100 differentially

expressed genes upregulated between differentiated and undifferentiated ciPods. The top 10 significant GO terms based on *P* value are presented. *P* value of 0.05 shown as dotted line. **B.** The reproducibility of the OrgGlom model using clones derived from 3 different iPSC lines was highlighted by podocyte gene expression levels in a targeted gene panel. Clone 1 represents the line used for all other subsequent analyses. Two way ANOVA, between clones $p$=0.1943; error bars = S.E.M.; significant difference between clones assessed by Tukey's multiple comparisons test; F-value=1.684; DF=2; biological replicates n=3.

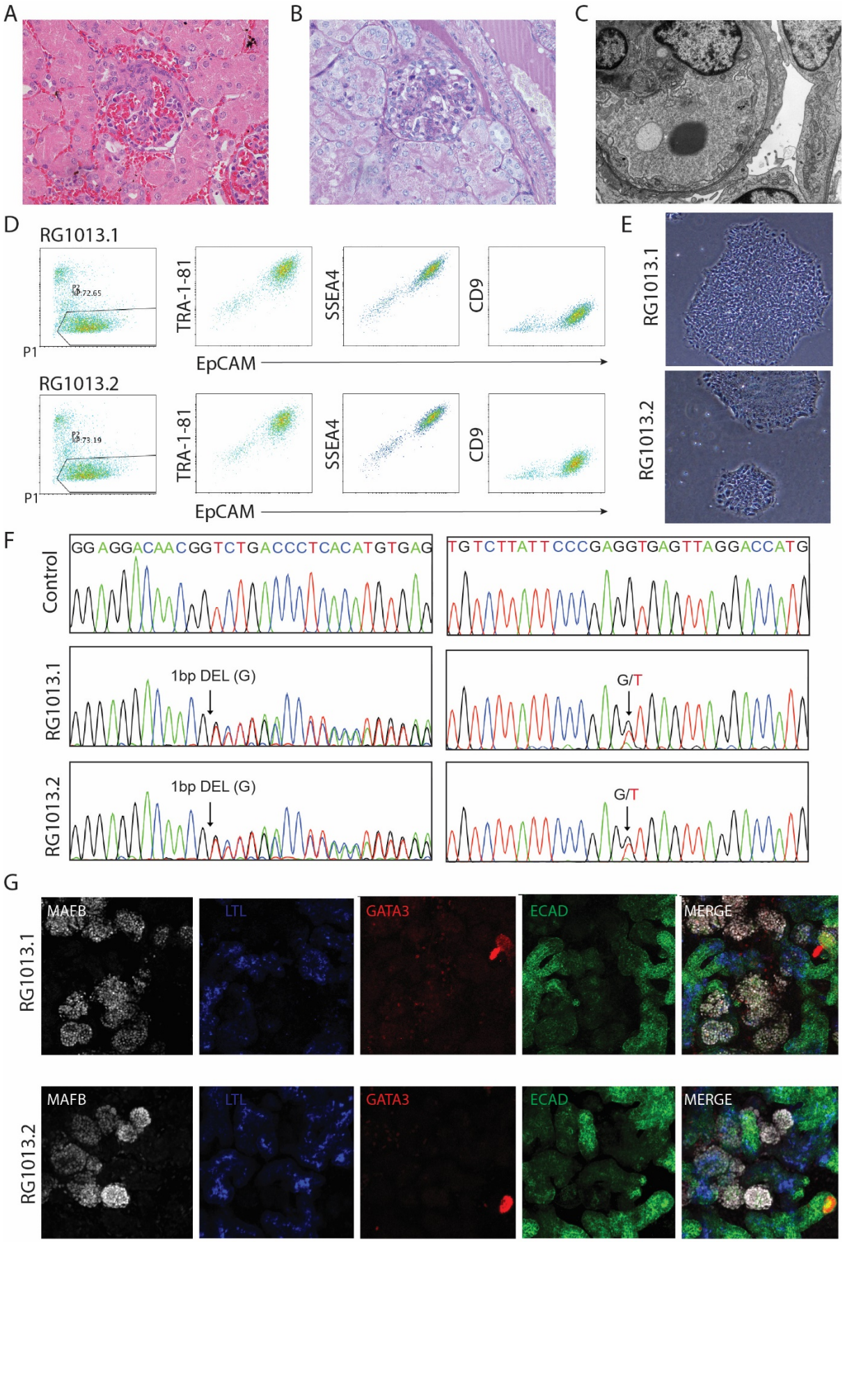



***Supplementary Figure 3. Transcriptional comparison of organoids, OrgGloms, ciPods and undifferentiated iPSC.*** **A.** Principle component analysis of RNA sequencing (RNA-Seq) data was performed on data from Day 7+18 (Day 25) total kidney organoids [3], undifferentiated iPSC lines [4], OrgGloms, OrgPods and ciPods in both differentiated and undifferentiated states (our laboratory and a previously published ciPod data (Lab 2, [5]), in addition to data collected from Kim et al (Lab 3, [6]). All biological replicates are indicated as individual points. **B.** Heatmap illustrating relative expression of key podocyte genes in OrgGloms and undifferentiated iPSC [6].

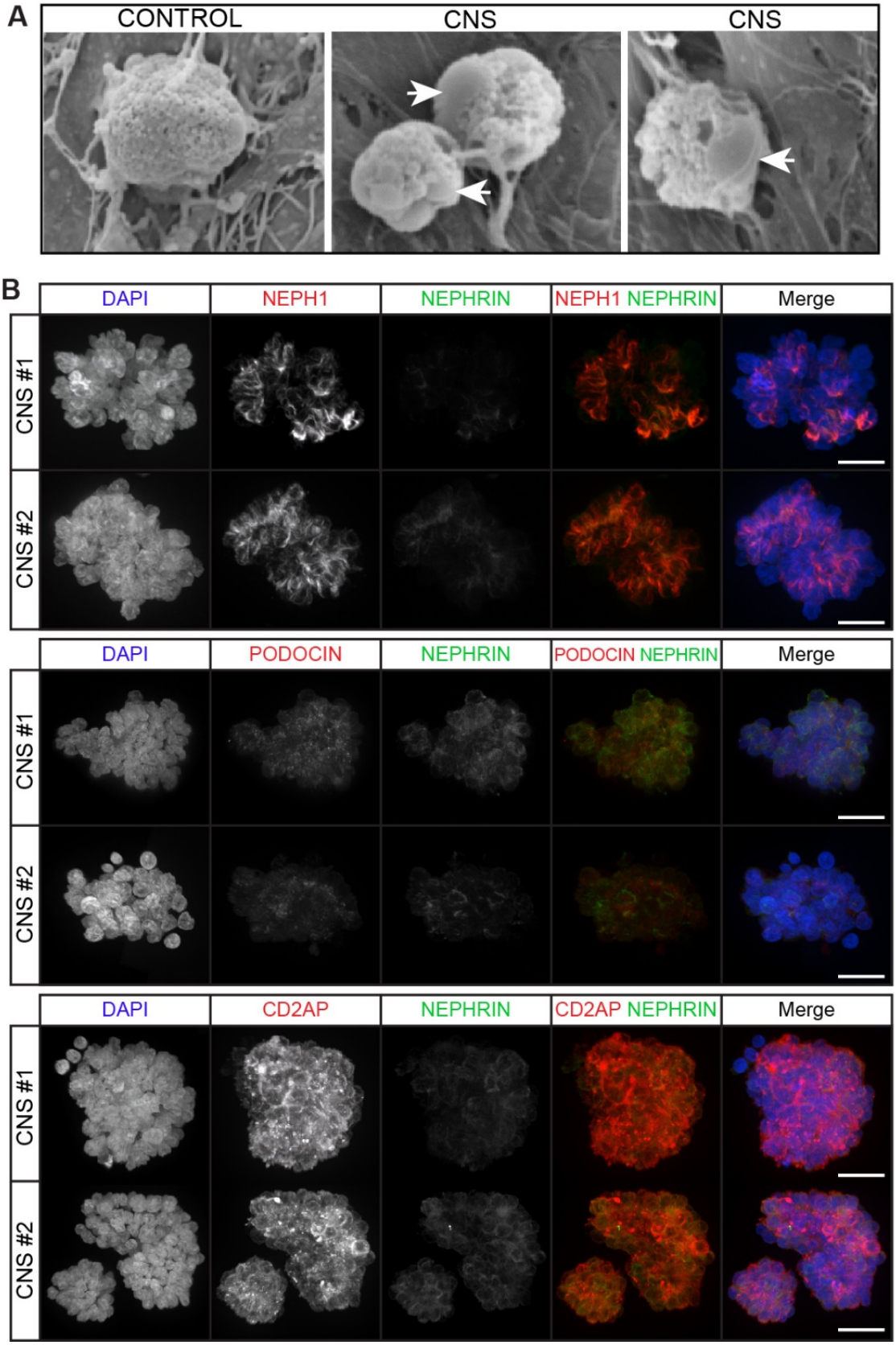



***Supplementary Figure 4. Use of a human MAFB reporter iPSC line to analyse organoid-derived podocytes and glomeruli.*** **A.** Design of human MAFB-BFP2 reporter iPSC lines. Schematic diagram of the MAFB locus and the homologous template used for gene targeting. The oligonucleotides (ODNs) used for identifying targeted clones by PCR are indicated. pA, polyA signal; Ex, exon. **B.** Gel analysis of the PCR products amplified from correctly targeted MAFB clones. Primers ODN-1 and ODN-2 flank the recombination site with ODN-3 binding within the reporter cassette and ODN-2 binding outside the region of homology. **C.** MAFB-BFP2 iPSC reporter cell line was successfully directly differentiated into kidney organoid. Immunostaining of fixed organoids showed appropriate nephron segmentation with lotus tetragonolobus lectin (LTL) marking proximal tubule and E-CADHERIN marking maturing

proximal tubule, distal tubule and collecting duct. MAFB-BFP2 signal was found to overlay with NEPHRIN in the glomeruli confirming the specificity of the MAFB-tagged line. Scale bar 200µm. **D.** Live imaging of MAFB-BFP2 reporter expression over time in sieved OrgGloms shows robust gene expression when in suspension up to 96 hours post-isolation. **E.** Immunostaining of isolated OrgGloms shows low level expression of the endothelial marker PLATELET ENDOTHELIAL CELL ADHESION MOLECULE (*PECAM1*) and mesangial marker PLATELET-DERIVED GROWTH FACTOR RECEPTOR BETA (*PDGFRβ*). Expression levels of single channels shown in greyscale to preserve maximum contrast, merged images shown in colour. Scale bars 10µm.


A
B
C
D
RG1013.1
72.65
P1
TRA-1-81
SSEA4
CD9
EpCAM
RG1013.2
73.19
P1
TRA-1-81
SSEA4
CD9
EpCAM
E
RG1013.1
RG1013.2
F
Control
GGAGGACAACGGTCTGACCCTCACATGTGAG
TGTCTTATTCCCGAGGTGAGTTAGGACCATG
RG1013.1
1bp DEL (G)
G/T
RG1013.2
1bp DEL (G)
G/T
G
RG1013.1
MAFB
LTL
GATA3
ECAD
MERGE
RG1013.2
MAFB
LTL
GATA3
ECAD
MERGE

***Supplementary Figure 5. Clinical characterisation of congenital nephrotic syndrome patient and derivation and validation of patient iPSC clones.*** **A, B.** Haematoxylin and eosin (**A**) and PAS (**B**) stained sections of kidney tissue from congential nephrotic syndrome patient. **C.** TEM of podocyes from congenital nephrotic syndrome patient RG1013 kidney tissue revealing evidence for podocyte effacement and loos of microvilli. **D.** FACS analysis of pluripotency cell surface markers for patient-derived iPSC clones RG1013.1 and RG1013.2. SNP analysis was performed to validate the chromosomal integrity of both clones. No aneuploidies were detected. **E.** Brightfield images of patient-derived iPSC clones RG1013.1 and RG1013.2 in culture showing appropriate morphology for a pluripotent stem cell population. **F.** DNA sequencing was performed on both RG1013.1 and 1013.2 to validate the presence of the original patient mutations in exons 10 and 27. Chromatograms are presented for both regions showing clear evidence of a heterozygous mutation in these iPSC clones. **G.** Generation of appropriately patterned kidney organoids from both RG1013.1 and RG1013.2 iPSC. Immunofluorescence staining revealed the presence of appropriately patterned nephrons as evidenced by the presence of podocytes (MAFB, white), proximal segments (LTL, blue), distal segments (CDH1, green) and collecting duct (GATA3/CDH1, red/green).




***Supplementary Figure 6. Characterisation of organoid glomeruli from congenital nephrotic syndrome patient lines.*** **A.** Scanning electron microscopy of glomeruli within organoids derived from a control iPSC line (MAFBmTAGBFP reporter line) and a congenital nephrotic

syndrome (CNS) patient-derived iPSC line. Arrows indicate broad, flattened podocytes within patient glomeruli. Magnification 8000x. **B.** Immunostaining of OrgGloms isolated from CNS patient organoids derived from both iPSC clones (CNS#1: RG1013.1, CNS#2: RG1013.2) show comparable protein intensity, representative images shown. Scale bars 10µm.

***Supplementary Video 1. Three-dimensional video of immunostained whole sieved organoid glomerulus.*** Confocal z-stack images were reconstructed to form a 3D image of the organoid glomerulus, immunostained for the podocyte proteins NEPHRIN (green), NEPH1 (red) and Podocalyxin (magenta) in addition to nuclei marked with DAPI (blue). Co-localisation of NEPHRIN and NEPH1 can clearly be observed at the basal junction between cells.

***Supplementary references:***


1. Lindenmeyer, M.T., et al., *Systematic analysis of a novel human renal glomerulus-enriched gene expression dataset.* PLoS One, 2010. **5**(7): p. e11545.
2. Saleem, M.A., et al., *A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression.* J Am Soc Nephrol, 2002. **13**(3): p. 630-8.
3. Takasato, M., et al., *Generation of kidney organoids from human pluripotent stem cells.* Nat Protoc, 2016. **11**(9): p. 1681-92.
4. Phipson, B., et al., *Transcriptional evaluation of the developmental accuracy, reproducibility and robustness of kidney organoids derived from human pluripotent stem cells.* Nature Methods, 2018. **In Press**.
5. Jiang, L., et al., *RNA sequencing analysis of human podocytes reveals glucocorticoid regulated gene networks targeting non-immune pathways.* Sci Rep, 2016. **6**: p. 35671.
6. Kim, Y.K., et al., *Gene-Edited Human Kidney Organoids Reveal Mechanisms of Disease in Podocyte Development.* Stem Cells, 2017.