Flow-enhanced vascularization and maturation of kidney organoids in vitro

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Kidney organoids derived from human pluripotent stem cells have glomerular- and tubular-like compartments that are largely avascular and immature in static culture. Here we report an in vitro method for culturing kidney organoids under flow on millifluidic chips, which expands their endogenous pool of endothelial progenitor cells and generates vascular networks with perfusable lumens surrounded by mural cells. We found that vascularized kidney organoids cultured under flow had more mature podocyte and tubular compartments with enhanced cellular polarity and adult gene expression compared with that in static controls. Glomerular vascular development progressed through intermediate stages akin to those involved in the embryonic mammalian kidney's formation of capillary loops abutting foot processes. The association of vessels with these compartments was reduced after disruption of the endogenous VEGF gradient. The ability to induce substantial vascularization and morphological maturation of kidney organoids in vitro under flow opens new avenues for studies of kidney development, disease, and regeneration.

The kidney continuously filters blood and maintains fluid homeostasis. These functions rely on specialized glomerular and tubular tissue compartments integrated with a complex vascular network. Although kidney organoids have such compartments1–8, their vasculature development—for example, the formation of PECAM1+ vascular networks with luminal architecture—is limited in static culture9–12. Further, gene expression in podocytes and tubular epithelial cells in static organoids is reflective of less mature renal tissue compared with that of human adult kidneys described in published work1–8. To date, researchers have depended on animal transplantation to produce kidney organoids with a perfusable vasculature that facilitates nephron epithelial maturation10–12. However, the reliance on an animal host limits both the scalability and the translation of organoid-based approaches, particularly for in vitro applications.

Given that multilineage communication with vasculature is implicated in epithelial maturation in vivo13, we posit that enhanced vascularization and maturation may be promoted in human pluripotent stem cell (hPSC)-derived human kidney tissue in vitro when it is subjected to environmental cues. To test our hypothesis, we developed a millifluidic culture system to probe the effects of extracellular matrix (ECM), media composition, fluidic shear stress (FSS), and coculture with human endothelial cells on the in vitro development of kidney organoids.

Results

Developing kidney organoids exhibit enhanced vascularization under flow. In our 3D-printed millifluidic chips, we subjected organoids to superfusion (flow over their top surface) with a controlled wall shear (i.e., FSS; Fig. 1a (top), Supplementary Fig. 1). The developing kidney organoids adhered to and became partially embedded in an ~1-mm-thick layer of gelatin–fibrin (gelbrin) ECM coating the bottom of the printed chip14,15, which allowed fluid to freely flow through the gap (2.6 mm in height) between the ECM surface and the upper chip housing (Supplementary Fig. 1). The adherent gelbrin matrix led to enhanced peripheral expression of the vascular marker PECAM1 and its precursor, MCAM16, within 1 week in static conditions, compared with expression in nonadherent matrices (e.g., glass, plastic, fibrin with or without collagen type 1) (Fig. 1b, Supplementary Fig. 2a–f). We tested several media compositions, as well as coculture with primary human endothelia with or without fibroblasts, and found that most conditions inhibited nephron formation or did not enhance vascularization under fluid flow (Supplementary Figs. 2g–i and 3). A low FBS concentration of 1.5%, typically used in endothelial culture media, permitted nephrogenesis and enhanced vascular network formation in developing kidney organoids under static conditions (Supplementary Fig. 2h, i).

To determine the effects of fluid flow, we placed developing kidney organoids on the adherent gelbrin layer and superfused them overnight with basal organoid media supplemented with 1.5% FBS in a closed-loop system over a range of flow rates from 0.04 ml/min (low FSS; ~0.0001 dyn/cm²) to 1–4.27 ml/min (high FSS; 0.008–0.035 dyn/cm²) while continuing the published differentiation protocol12 (Fig. 1a, bottom). We observed enhanced formation of MCAM+ and PECAM1+ vascular networks in organoids cultured in vitro under high FSS after 10 d of perfusion (differentiation day 21), with nephrons forming over time (Fig. 1c–i, Supplementary Video 1). To quantify their vascularization, we evaluated confocal images of whole organoids using the AngioTool plugin in ImageJ17. We found that the PECAM1+ vasculature of organoids cultured under high FSS showed a fivefold increase in vessel percent area compared with that of organoids cultured under low FSS (Fig. 1m, Supplementary Video 1).
Supplementary Fig. 4). Similarly, we found that the PECAM1+ vasculature showed a tenfold increase in junctional density (i.e., branch points per unit area) and average vessel length (i.e., interjunctional distance) under high FSS compared with that of organoids cultured in static or low-FSS conditions (Fig. 1m). Concomitantly, PECAM1 transcripts were fivefold upregulated under high FSS relative to expression in controls (Fig. 1n). Note that such differences cannot be attributed solely to variations in media volume, as organoids cultured under high FSS with either 0.2 ml or 1 ml of media per organoid in our closed-loop system did not show statistically...
significant differences in their vasculature ($P > 0.05$, two-way analysis of variance (ANOVA) with Tukey's multiple-comparisons test; Supplementary Fig. 5). Together, these observations suggest that FSS is a critical environmental cue that facilitates the vascularization of kidney organoids in vitro.

**Perfusable vessels form in developing kidney organoids under flow.** During kidney development, vascular formation is believed to arise via a combination of vasculogenesis (i.e., de novo formation of blood vessels through the differentiation and coalescence of endothelial progenitor cells (EPCs)) and angiogenesis (i.e., the formation of blood vessels from pre-existing vessels). In the context of organoid culture, FSS has been shown to promote the formation of perfusable vessels. The ability of organoids to develop vessels that can sustain perfusion is critical for their physiological relevance and functional analysis.

**Fig. 2 | Intra- and interorganoid vascular networks with perfusable lumens supported by mural cells are observed for kidney organoids cultured under high flow in vitro.** a, Diagram of endothelial maturation in developing kidneys in vivo, from progenitor cells to sustained terminal marker expression. b, Flow cytometry of dissociated whole organoids on day 21. EPCs were FLK1$^+$, c-d, qPCR of endothelial cell markers in developing organoids at day 21. e, qPCR of PDGFRβ in developing organoids at day 21. Individual symbols in c-e represent three technical replicates on RNA pooled from 6 organoids (biological replicates) per condition. All data are plotted as mean ± s.d. f, Immunostaining for PODXL, PECAM1, and PDGFRβ in organoids under high FSS. Scale bars, 15 μm. g, Confocal 3D renderings of vascular markers within whole-mount organoids. Scale bars, 30 μm. White arrows highlight areas that are PECAM1$^+$MCAM$^-$ h, A single z-slice from g in which white arrows highlight open lumens. Scale bar, 30 μm. i-k, Transmission electron microscopy images showing circular openings encompassed by a thin membrane that reflect vascular lumens for kidney organoids subjected to high FSS (j) and E14.5 mouse embryonic kidney in vivo (k). Red plus signs indicate vascular lumens. Scale bars, 10 μm (i, k) or 2 μm (j). l-m, Z-slice at the base of a kidney organoid cultured under high FSS, showing the vascular network (l) and the accumulation of fluorescent beads within the vascular network (m). Scale bars, 100 μm. n, Confocal 3D rendering of bridging between two adjacent whole organoids (outlined by dashed white lines). Scale bar, 100 μm. DAPI, 4′,6-diamidino-2-phenylindole; PECAM1, CD31; MCAM, CD146; KDR, FLK1; PODXL, podocalyxin; PDGFRβ, platelet-derived growth factor receptor-β. Statistical significance (c-e) was attributed to values of $P < 0.05$ as determined by one-way ANOVA with Tukey's multiple-comparisons test. **$P < 0.01$, ***$P < 0.001$.
Fig. 3 | Tubular epithelia mature and undergo morphogenesis to become a polarized, ciliated compartment in contact with vasculature in response to high-flow conditions on chip. a. Expression of collagen IV and LTL in tubule cross-sections under static and high-flow conditions at day 21. Scale bars, 5 μm. The plots in yellow at the bottom show the intensity of LTL across a line scan denoted by yellow arrows in the images above. b. Tubule cross-sections showing the ciliary marker TUBA4A and expression of ATP1A1 (Na/K ATPase subunit-a1) under static versus high-flow conditions on chip on day 21. Scale bars, 5 μm. c. The percentage of ciliated cells under static (n = 101 cells) and high-flow (n = 64 cells) conditions. Samples were determined as a field of view in an LTL+ tubule where at least 8 cells could be viewed in cross-section. d-f, qPCR of ciliary markers, solute transporters, drug transporters, and adult transcription factors on day 21 under the indicated growth conditions. g, Whole-organoid 3D confocal imaging stacks (i) of a representative high-flow condition. The plots in yellow at the bottom show the intensity of LTL across a line scan denoted by yellow arrows in the images above. h, The average distance in 3D between the vasculature and the tubules. Data in h,i represent biological replicates, or whole organoids, of 6, 5, 4, and 6 per condition (static, high flow (–VEGF), high flow (+VEGF), and high flow, respectively). j,k, Immunostaining for the indicated markers in transverse (j) and longitudinal (k) planes in high flow at day 21. Scale bars, 20 μm. DAPI, 4',6-diamidino-2-phenylindole; LTL, Lotus tetragonolobus lectin; PECAM1, CD31; PODXL, podocalyxin; TUBA4A, tubulin-α4A; AQPI, aquaporin 1; SLC34A1, Na/P cotransporter; ATP1A1, Na/K ATPase; ABCB1, MDR1; LRP2, megalin; BNC2, basonuclin 2; Npas2, neuronal PAS domain protein 2; TRPS1, transcription repressor GATA binding 1; PKD1, polycystin 1; PKD2, polycystin 2; NPHP1, nephrocystin 1; NPHP6, nephrocystin 6; PKHD1, fibrocystin. All data are plotted as mean ± s.d. For c, statistical significance was attributed to values of P < 0.05 as determined by unpaired t-test with Welch’s correction. Individual symbols in d-f represent three technical replicates on RNA pooled from 6 organoids (biological replicates) per condition. Statistical analysis for d-f,h,i was determined at a value of P < 0.05 as determined by one-way ANOVA with Tukey’s multiple-comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001.
Fig. 4 | Flow-enhanced glomerular vascularization and morphogenesis of kidney organoids in vitro mirrors stages of glomerular development in vivo. 

**a** A 3D-rendered confocal image of vascular invasion in a PODXL+ cluster showing afferent and efferent vessels. Scale bar, 40 µm. 

**b** The percentage of PODXL+ clusters that exhibited vascular wrapping or invasion under static and high FSS conditions with or without VEGF (addition or inhibition), representing whole organoids of sample size 16, 6, 7, and 14 for static, high-flow VEGF addition, high-flow VEGF inhibition, and high-flow conditions, respectively. 

**c** qPCR of VEGF at day 21. 

**d** A 3D-rendered confocal image of capillary invasion in an SSB in a vascularized organoid under high FSS in vitro (day 21). Scale bar, 10 µm. 

**e** Single confocal z-slice showing capillary invasion with a PECAM1+MCAM+ cell (white arrow) and MCAM+ vascular precursors (CD146+ cells). Scale bar, 10 µm. 

**f** MCAM+PECAM1+ glomerular tuft-like formation shown as a single z-slice from a confocal image. Scale bar, 10 µm. 

**g–i** Transmission electron microscopy (TEM) images of structures correlating with the immunofluorescence images in kidney organoids (day 21). Scale bars, 4 µm (g, h) or 10 µm (i). 

**j–l** Corresponding stages in E14.5 mouse kidneys. Red dashed lines depict clefts, white arrows denote capillary invasion, and red plus signs denote RBCs. B, Bowman’s capsule–like structure. Scale bars, 8 µm (j, k) or 50 µm (l). 

**m** TEM images of a glomerulus-like structure in organoids cultured under high FSS (day 21) showing a parietal membrane enclosing a visceral cluster of cells (left), which manifest interdigitating cytoplasmic projections extending across and into the plane of field under higher magnification (right). Scale bars, 10 µm (left) or 1 µm (right). 

**n** TEM images of a glomerulus-like compartment in organoids cultured under high FSS (day 21) (left) in which higher magnification shows podocyte foot processes abutting a glomerular tuft-like formation (right). Scale bars, 2 µm (left) or 200 nm (right). 

**o, p** qPCR for podocyte foot process proteins (o) and an adult transcription factor (p). DAPI, 4′,6-diamidino-2-phenylindole; MCAM, CD146; PECAM1, CD31; PODXL, podocalyxin; SYNPO, synaptopodin; NPHS1, nephrin; PDGFRβ, platelet-derived growth factor receptor-β; VEGFA, vascular endothelial growth factor A; CASZ1, castor zinc finger 1. All data are plotted as mean ± s.d. For b, statistical significance was attributed to values of \( P < 0.05 \) as determined by two-way ANOVA with Tukey’s multiple-comparisons test. Individual symbols in o, p represent three technical replicates on RNA pooled from 6 organoids (biological replicates) per condition. Statistical analysis for o, p was determined at a value of \( P < 0.05 \) as determined by one-way ANOVA with Tukey’s multiple-comparisons test. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).
of new blood vessels that sprout from pre-existing vessels [14, 15]. In the developing mammalian kidney in vivo, fate mapping shows that KDR+ (FLK1) cells serve as EPCs that transform into intermediate MCAM+ cells and, ultimately, PECAM1+ mature endothelia [12, 21] (Fig. 2a). Akin to metanephric mesenchyme in vivo [15], KDR+ EPCs were concurrently induced in vitro with SIX2PAX2SALL1+ nephron progenitor cells that differentiate into the epithelial cell types of the nephron (Supplementary Figs. 6 and 7). Under high FSS, KDR+ EPCs increased in number to 11.2% of the cell population in whole organoids, compared with 4.25% in static culture on chip (Fig. 2b, Supplementary Fig. 8). Moreover, transcripts for KDR and MCAM were upregulated after 10 d of high flow (differentiation day 21) (Fig. 2c, d), further indicating that FSS is an important environmental cue to expand vascular potential. Meanwhile, the expression of PDGFbR was upregulated under high flow (Fig. 2e), where recruitment of PDGFbR+ pericyte-like cells to PECAM1+ networks was consistent with vessel maturation (Fig. 2f). As the vasculature within these kidney organoids evolved in vitro, we noted heterogeneous expression of vascular precursor and mature markers, including areas that were MCAM+, PECAM1+, or MCAM+PECAM1+ after 10 d of perfusion (differentiation day 21) (Fig. 2g, Supplementary Fig. 9). We observed open circular structures that lacked nuclei (DAPI) and were surrounded by MCAM+ and PECAM1+ regions, which are indicative of lumens with varying diameter, reflecting multiscale vessel formation (Fig. 2h). Notably, we observed analogous structures in both hPSC-derived kidney organoids exposed to high FSS (Fig. 2i,j) and kidneys from mice aged embryonic day 14.5 (E14.5; Fig. 2k). To explore whether this embedded vasculature was perfusable, we carried out time-lapse live-cell monitoring of kidney organoids cultured in vitro under high FSS, and stained the vasculature with the lectin Ulex europaeus I lectin. These experiments, carried out within minutes after the introduction of fluorescent beads (100-nm diameter) to the flowing media, confirmed luminal perfusion in a subset of peripheral vessels (Supplementary Fig. 10, Supplementary Video 2). To determine the extent of perfusable vasculature in whole kidney organoids, we first superperfused fluorescent beads in media for several hours, then acquired live confocal images (z-stack) at the base of the organoid embedded in ECM. The organoids were then fixed, immunostained for PECAM1, and coregistered with fiduciary markers. We observed the presence of beads in locations coincident with larger PECAM1+ vessels, several hundred micrometers from the superperfused surface (Fig. 2l,m, Supplementary Fig. 11). We also observed branched PECAM1+ networks that contained terminal sprout-like structures, which lacked a PDGFbR+ cell lining, suggestive of angiogenesis (Supplementary Fig. 12a,b). We found that kidney organoids fused in as little as 24 h in culture under high FSS (Supplementary Video 3) and that robust vascular networks arose between adjacent organoids on chip (Fig. 2n). The association of PECAM1+ networks with ACTA2+ smooth-muscle-like cells suggests maturation of arterial lineage cells [15], whereas the venous marker EMCN stains presumptive venous lineage cells (Supplementary Fig. 12c,d). Thus, kidney organoids subjected to the appropriate combination of adherent ECM, culture media, and FSS in vitro form increasingly mature, perfusable vasculature of varying size and lineage.

**Tubular epithelia exhibit enhanced maturation under flow.** Under static conditions, kidney organoids develop more limited vasculature, and tubular epithelia often have immature gene expression profiles with morphology analogous to that of first-trimester kidney [14, 15]. After subcapsular transplantation to a mouse kidney, progressive morphogenesis of tubular structures occurs, as evident by polarization, the formation of a brush border, and ciliary assembly in vivo [14]. Native kidneys rely on extensive fluid flow both within and between tubular, interstitial, and vascular compartments to facilitate reabsorption of ~99% of the glomerular filtrate. We hypothesized that a similar progressive morphogenesis and maturation of gene expression profiles may occur in hPSC-derived tubular cells in vitro when they are subjected to high FSS. Indeed, we found that the polarity of tubules formed in vitro was enhanced, with apical enrichment of the brush border marker *Lotus tetragonolobus* lectin (LTL) (Fig. 3a, Supplementary Fig. 13a–d) after 10 d (differentiation day 21) of culture under high FSS. Similarly, we found that primary cilia were apically enriched from 50% in static culture to 89% of LTL+ cells under high FSS (Fig. 3b, Supplementary Fig. 13e–j). Consistent with polarization and maturation-associated ciliary assembly, the expression of ciliary proteins (PKD1, PKD2, NPHP1, NPHP6, PKHD1) was upregulated (Fig. 3d). Concurrently, expression of tubular epithelial transporters including AQP1, solute transporters (SLC34A1, ATP1A1, SLC6A19, SLC9A3, SLC2A2), and drug transporters (ABCB1, LR2) was upregulated compared with that in static controls and undifferentiated hPSCs (Fig. 3e, Supplementary Fig. 14), indicative of enhanced functional potential. The maturation of tubular epithelial cells was evident from the upregulation of adult transcription factors (NAN2, NPA52, TRPS1) (Fig. 3f), which are reported as mature proximal tubule markers by single-cell RNA-seq in adult human kidneys [15]. We quantified the association between PECAM1+ networks and LTL+ tubules by using confocal imaging rendered with Imaris surface functions, distance transformation, and masking tools (Fig. 3g), and found that for organoids cultured under high FSS, the percentage of vascular surface area in contact with tubules was increased by nearly threefold compared with that in organoids in static culture (Fig. 3h, Supplementary Fig. 15). Additionally, the mean distance between a given LTL+ tubule and the nearest blood vessel decreased ~70% under high FSS (Fig. 3i). We observed this enhanced tubule–vascular association in both transverse and longitudinal orientations (Figs. 3j, k, Supplementary Video 4). Notably, we found that the maintenance of endogenous VEGF gradients in organoids cultured in vitro was crucial for vascular–tubular interactions. For example, the close association of LTL+ tubules and blood vessels was disrupted when 100 ng/mL of VEGF or a VEGF inhibitor (bevacizumab) at 250 μg/mL was added to the media during the first 10 d of high-FSS culture, causing reversion to the tubule–vascular association observed for organoids in static culture (Fig. 3j, k, Supplementary Fig. 15). In short, organoid culture under fluid flow in vitro supports the maturation and morphogenesis of tubular epithelia in kidney organoids, probably as a result of interlineage endothelial–epithelial communication [15].

**Glomerular vascularization and maturation in vitro mirror early stages of development in vivo.** The glomerular structures of kidney organoids in static culture are largely avascular [14, 15]. After transplantation of a kidney organoid into an animal, host-derived vascularization promotes glomerular vascularization [15]. To determine whether FSS-induced vascularization of organoids in vitro extends to glomerular compartments, we used confocal imaging to quantify PODXL+ podocyte clusters invaded by MCAM+ and PECAM1+ vascular structures in static and high-FSS conditions (Fig. 4a, b, Supplementary Fig. 16). Under high FSS, MCAM+ and PECAM1+ vascular invasion and wrapping of PODXL+ clusters was significantly increased (MCAM, *P* < 0.01; PECAM1, *P* < 0.001; two-way ANOVA with Tukey’s multiple-comparisons test; Fig. 4b). Notably, invasion was greater than 60% in high-FSS samples, compared with 10–20% in static controls (Fig. 4b), consistent with the requirement of vascular flow for glomerular assembly in animal studies [16]. Given that we observed significant upregulation of VEGF-A expression in organoids cultured under high FSS (*P* < 0.001, one-way ANOVA with Tukey’s multiple-comparisons test; Fig. 4c), we postulated that these samples could be used to elucidate the role of VEGF in glomerular vascularization in vitro. Both VEGF inhibition (250 μg/mL bevacizumab for 10 d on chip) and VEGF addition (100 ng/mL for 10 d on chip) significantly reduced the incidence of invasion of
PODXL⁺ glomeruli-like compartments by PECAM⁺ and MCAM⁺ vascular networks under high FSS (P<0.001, two-way ANOVA with Tukey’s multiple-comparisons test; Fig. 4b). As the vessel percent area under high-flow conditions remained unchanged between endogenous VEGF, VEGF-addition, and VEGF-inhibition conditions, the difference in glomerular vascularization cannot be attributed to an increased abundance of vasculature (Supplementary Fig. 17, Supplementary Table 3). Unlike the random sporoadic growth observed for organoids cultured in exogenously disrupted gradients, the reduced junctional density and increased average vessel length seen in unperturbed media conditions suggest that vessels grow toward an endogenous VEGF gradient (Supplementary Fig. 17b,c). The endogenous upregulation of VEGF under high FSS (Fig. 4c) appears to generate gradients that allow vessels to reach glomerulus-like compartments in time to invade rather than wrap Bowman’s capsule-like structures within these kidney organoids in vitro.

Vascularization of glomeruli in vitro commences with the invasion of an S-shaped body (SSB) by a single capillary loop around which podocytes coalesce, with the formation of a primitive Bowman’s capsule (capillary loop stage (CLS)), followed by vascular expansion to form nascent glomerular tufts in early corpuscles. After 10 d of organoid culture under high FSS (differentiation day 21), glomeruli in vitro varied in a spectrum between SSBs, CLS, and early-corpuscle-like structures. Consistent with vascular invasion of an SSB, a PECAM⁺ vessel invaded a cleft in an SSB-like structure (Fig. 4d, Supplementary Video 5a). Meanwhile, the ‘luminal’ feature of an MCAM⁺PECAM⁺ vessel invading a PODXL⁺ cellular cluster, surrounded by a putative Bowman’s capsule, suggests CLS-like developing glomeruli (Fig. 4e, Supplementary Video 5). Renal-corpuscle-like structures contained MCAM⁺PECAM⁺ vasculature suggestive of capillary loops (Fig. 4f, Supplementary Fig. 16b, Supplementary Video 6). Transmission electron microscopy showed analogous structures between developing glomeruli in high-FSS organoids and E14.5 mouse kidneys, revealing that in vitro organoids may follow in vivo glomerular development through SSB, capillary loop, and early corpuscle stages (Fig. 4g–l). Capillary-loop-like structures in organoid glomeruli in vitro showed open lumens without red blood cells, which are present in capillary loops in mice (Fig. 4h–l). Scanning electron microscopy showed a thin-layered capsular structure suggestive of a parietal layer of epithelial cells (Bowman’s capsule) that contained round cellular bodies with elongated cytoplasmic projections indicative of a visceral layer of epithelium (podocytes) (Fig. 4m). Similar to E14.5 embryonic mouse kidneys and human adult kidneys, grapelike clusters of visceral epithelial cells in organoids under high flow consisted of round cellular bodies with interdigitating foot-process-like structures (Supplementary Fig. 18). The cytoplasmic projections, consisting of primary stalks and secondary side branches, appeared to be polarized and abutting thin-layered membranes of capillary-loop-like structures (Fig. 4n, Supplementary Fig. 19), consistent with foot processes. Compared with those under static conditions, the foot-process-like structures seemed more prominent under high FSS, with significant upregulation of NPHS1 (nephrin) and SYNPO (synaptopodin), encoding foot-process-associated proteins (P<0.001, one-way ANOVA with Tukey’s multiple-comparisons test; Fig. 4o, Supplementary Fig. 20a–d). Further, these structures fused in response to doxorubicin, as observed for foot processes in mammalian kidneys (Supplementary Fig. 20e). Concurrently, transcriptional maturation was evident from enhanced expression of podocyte adult transcription factors (WT1, CASZ1, CUX1, TEAD1) (Fig. 4p, Supplementary Fig. 14b) with accompanying appropriate expression of the housekeeping gene GAPDH among all PCR samples (Supplementary Fig. 14c,d). These observations indicate that organoids cultured under high FSS show enhanced glomerular vascularization and foot process maturation, which is required to facilitate functional morphogenesis of podocytes in vitro.

Discussion
Kidney organoids subjected to high FSS during development in vitro showed a notable enhancement in the abundance of vascular and increased maturity in their tubular and glomerular compartments, along with concomitant morphogenesis of tubular epithelial cells and podocytes. FSS is the key parameter driving our biological findings; however, other chemomechanical cues arising from the underlying ECM, flow profile, or media composition can work collectively. Although our method does not yet ensure that the microvascular networks present in these kidney organoids will be readily perfusable, the ability to promote their flow-enhanced development in vitro opens new avenues for investigating organogenesis, nephrotoxicity, tubular and glomerular disease, and kidney regeneration with a simple millifluidic chip. Our findings could be applicable to other organoid types that might similarly benefit from controlled fluid flow during development from embryonic to more functional organ equivalents in vitro.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41592-019-0325-y.

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Author contributions
K.A.H., N.G., J.A.L., R.M., D.B.K., and J.V.B. conceived the project. K.A.H., N.G., and K.T.K. designed the research, and R.M. and J.A.L. supervised the research. K.A.H., N.G., K.T.K., R.M., and D.B.K. designed, performed, and analyzed all experiments. M.T.V. provided critical insights into embryonic development, cell sources, and mouse embryonic kidneys. M.S.-S. designed and built the silicone millifluidic chips, interfacing with perfusion pumps, and analyzed the fluid flow profiles on chip. D.M. and T.M. sourced and validated antibodies, optimized staining protocols, and provided invaluable cell culture analysis and support. T.F. developed methodology for quantifying vascular and tubule features in confocal imaging stacks. All authors contributed to manuscript writing.

Competing interests
J.V.B. and R.M. are co-inventors on patents (PCT/US16/52350) on organoid technologies that are assigned to Partners Healthcare. J.V.B. or his family has received income for consulting from companies interested in biomarkers: Sekisui, Millennium, Johnson & Johnson, and Novartis. J.V.B. is a co-founder of, is a consultant to, and owns equity in Goldfisch Bio. K.A.H. is a co-founder and chairwoman of NanoHybrids Inc. J.A.L. is a co-founder of and owns equity in Voxel8 Inc.

Additional information
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Methods
Millifluidic chip fabrication. We used a silicone-based ink to 3D-print customized perfusion gaskets (Supplementary Fig. 1), in which developing kidney organoids were placed on an engineered ECM layer (1 mm thick) and subjected to a controlled FSS environment. The ink is composed of a two-part elastomer (SE 17100, Dow Chemical) with a 10:1 base-to-catalyst ratio (by weight) and was homogenized with a centrifugal mixer for 2 min (2,000 rpm; AE-310, Thinky Corp, Japan). The silicone ink was printed within 2 h of being mixed with catalyst. This ink was loaded in a syringe (EFD Inc., East Providence, RI, USA) and carefully pipetted to remove any air bubbles before printing at room temperature. The chips were fabricated using a custom-designed, multimaterial 3D bioprinter equipped with four independently addressable printheads mounted on a three-axis, motion-controlled gantry with a build volume of 725 × 650 × 125 mm (AGB 10000, Aerotech Inc., Pittsburgh, PA, USA). The silicone (PDMS) ink was housed in a syringe barrel to which a 410-m diameter nozzle was attached via a luer lock (EFD Inc., East Providence, RI, USA) and extruded through the nozzles by applied air pressure (800 Ultra dispensing system, EFD Inc., East Providence, RI, USA) ranging from 10 to 90 p.s.i., corresponding to print speeds between 1 mm/s and 5 cm/s. The customized perfusion chip gasket was printed by deposition of the silicone ink through a tapered 410-µm nozzle onto 50 × 75 mm glass slides. The glass slide setup was created with a custom MATLAB script that generates G-code for a final gasket structure. After printing, the perfusion chip was cured at 80 °C in an oven for >1 h, stored at room temperature, and autoclaved before use. The organoid chamber was 15 mm wide by 3.6 mm high and 60 mm long; the ECM was placed on the base of the perfusion gasket and was 1 mm thick. The organoids (between 4 and 20 per chip) were placed centrally in an area 8 mm wide by 3.6 mm high and 20 mm long as shown in Supplementary Fig. 1d.

Engineered extracellular matrix preparation and rheology. The ECM comprised a gelatin network. To prepare the ECM components, the user first produces a 15% (w/v) gelatin solution (Type A, 300 bloom from porcine skin; Sigma) by adding gelatin powder to a warm solution (70 °C) of DPBS (1× Dulbecco’s PBS without Ca++ and Mg++). The gelatin is stirred for 12 h at 70 °C, and the pH is then adjusted to 7.5 with 1 M NaOH. The solution is sterile-filtered and stored at 4 °C in aliquots for later usage (<3 months). A fibrinogen solution (50 mg/ml) is made by lyophilized bovine blood plasma protein (Millipore) dissolved at 37 °C in sterile DPBS without Ca++ and Mg++. The solution is held at 37 °C for 45 min to allow complete dissolution. The transglutaminase solution (60 mg/ml) is made from lyophilized powder (Moo Gloo, TI) dissolved in DPBS without Ca++ and Mg++ and gently mixed for 20 s. The solution is then held at 37 °C for 20 min and sterile-filtered before use. A CaCl2 stock solution (250 mM) is prepared from CaCl2 pellets dissolved in sterile water. For the preparation of stock solutions of thrombin, lyopholized thrombin (Sigma Aldrich) is reconstituted at 500 U/ml in sterile water and stored at −20 °C. Thrombin aliquots are thawed immediately before use.

Before a layer of engineered ECM is cast in the 3D-printed chip, several components are mixed in advance at appropriate concentrations, including 10 mg/ml fibrinogen, 2% wt gelatin, 2.5 mM CaCl2, and 0.2% wt transglutaminase. This solution is then equilibrated at 37 °C for 15–20 min before use to improve the optical clarity of the ECM. Next, the solution is rapidly mixed with stock thrombin solution at a ratio of 250:1, resulting in a final thrombin concentration of 2 U/ml. Within 2 min at 37 °C, soluble fibrinogen cures to a fibrin gel. For this reason, the ECM solution must be cast onto the base of the perfusion chip immediately after mixed with thrombin. The mixture is then placed in a sterile container and kept in the incubator for a minimum of 30 min before assembly with housing, media, and perfusate aggregate integration.

A controlled stress rheometer (DHR-3, TA Instruments, New Castle, DE, USA) with a 40-mm-diameter, 2° cone and plate geometry was used to measure the rheological properties of the ECM. The shear storage (G’) and loss (G”) moduli were measured at 1 Hz and an oscillatory strain (ε) of 0.1%. For time sweeps, a premixed ECM solution containing thrombin was rapidly placed onto the Peltier plate held at 37 °C. The G’ of the final cured engineered ECM was approximately 800 Pa.

To prepare the ECM formulations shown in Supplementary Fig. 2, we used fibrinogen in either 10 mg/ml or 25 mg/ml with thrombin at 2 U/ml and 2.5 mM CaCl2. The fibrin–Col I ECM was prepared from fibrinogen solution mixed at a final concentration of 25 mg/ml with collagen I (rat tail collagen I, Corning; 1 mg/ml) at pH 7.5, a thrombin concentration of 2 U/ml, and 2.5 mM CaCl2, Matrigel (Corning) was diluted by 50% with sterile PBS and cured at 37 °C.

We also made and evaluated another ECM formulation that included fibrin along with human umbilical vein endothelial cells (HUVECs) and human neonatal dermal fibroblasts cells (HNFDCs) (Supplementary Fig. 3b). We prepared the preformed network of HUVECs and HNFDCs by combining HUVECs and HNFDCs at a 5:1 ratio at a concentration of 2 million cells/ml in 10 mg/ml fibrin gel. The cells were cultured in 1:1 DMEM:endothelial growth medium 2 (ECM2); Lonza) plus 5% FBS for 3 d. After 3 d, the spontaneous tissue-like aggregates were loaded on top of the fibrin gel supporting the HUVEC/HNFDC network. At this point, the media was changed to 1:1 ECM2:ARPM1 (Advanced Roswell Park Memorial Institute medium + 1× GlutaMAX) and held in static conditions for 7 d.

Organoid assembly and perfusion on printed millifluidic chips. For assembly of the kidney organoids-on-chip, perfusate aggregates (with ages between 11 and 14 d) in media were pipetted onto the top of the ECM on gasket in the window region highlighted in Supplementary Fig. 1d. A large aggregate can fit on chip, and we typically used between 4 and 20 per run, but upward of 100 or more can fit if needed. The organoids were randomly spaced within the window of 8 × 20 mm; note that the distance between the organoid surface on the ECM and the upper acrylic lid was 2.6 mm. The gasket was then placed into a machined stainless steel base. Stainless steel tubes (MicroGroup, Inc.; grade 304, 3.04 mm × 2.54 mm × 125 mm) were used for assembly with housing, media, and perfusate aggregate integration.

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Supplementary Fig. 11). Although we found that the beads nonspecifically bound to the ECM, we added 100-nm fluorescent beads to the media (FluoSpheres from Thermo Fisher) because it is an easy way to perfuse in all small-cell fractions of organoids. High-FSS conditions had already been used up to passage 9. HUVECs expressing RFP (Angio-Proteomie) were cultured with EGM2 media (Lonza) and used up to passage 10. Human glomerular microvascular endothelial cells (GMVECs) expressing RFP (Angio-Proteomie) were cultured with EGM2 medium (Lonza) and used up to passage 9. HUVECs expressing RFP (Angio-Proteomie) were cultured with EGM2 media (Lonza) and used up to passage 9. HNDFs expressing GFP (Angio-Proteomie) were cultured per the supplier's instructions and used up to passage 15.

Organoid preparation and culture. Organoid preparation is covered in detail elsewhere, but briefly, hPSCs were differentiated into metanephric mesenchyme cells that included SIX2+ nephron progenitor cells with approximately 80–90% efficiency by a three-step directed differentiation protocol (Fig. 1a, bottom). Metanephric mesenchyme cells were differentiated into tubulogenic aggregates in suspension culture, and then the aggregates were transferred onto the chip (Fig. 1a, top) anytime between days 11 and 14 of differentiation (Supplementary Fig. 2g). Further differentiation into kidney organoids was stimulated by the same directed differentiation protocol, reported previously, except that 1.5% FBS (heat inactivated; Gibco) was added. This same process can be used to massively scale up kidney organoids. An organoid from E14.5 (Kuray) (Supplementary Fig. 21). With traditional methods, kidney organoids will contain roughly 100,000 cells per aggregate. However, within the same footprint as a single well in a six-well plate, approximately 1,000 mini-organoids can be produced, containing approximately 5,000 cells per aggregate. Notably, when these mini-organoids were placed within our engineered microenvironment on chip, they behaved similarly to larger organoids and developed enhanced vascularization under high-FSS conditions.

We explored several experimental conditions that did not lead to enhanced vascularization. In particular, the addition of adult human primary GMECs, either by aggregation with nephron progenitor cells at day 8 or placement of small-cell fractions of organoids cultured on this chip showed enhancements in vascularity and in tubular and glomerular maturation comparable to those of organoids subjected to an FSS ranging from 8 × 10⁻³ to 3.5 × 10⁻² dyne/cm².

Cell culture. h9 human embryonic stem cells (ESC; WiCell) and BJFF human induced PSCs (provided by Prof. Sanjay Jain at Washington University) were maintained in feeder-free culture with StemFit Basic02 (Ajinomoto Co., Inc.) supplemented with 10 ng/ml FGF2 (Peprotech) as previously reported. Human glomerular microvascular endothelial cells (GMVECs) expressing RFP (Angio-Proteomie) were cultured with EGM2 medium (Lonza) and used up to passage 9. HUVECs expressing RFP (Angio-Proteomie) were cultured with EGM2 media (Lonza) and used up to passage 9. HNDFs expressing GFP (Angio-Proteomie) were cultured per the supplier's instructions and used up to passage 15.

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color) was washed out. Organoids and mouse kidneys were placed in a solution of 30% sucrose in PBS for 1 h. Then that solution was removed and replaced with a 1:1 mixture of 30% sucrose solution in PBS/optimal cutting temperature (OCT) freezing medium (Electron Microscopy Science) for 30–45 min at room temperature. Organoids were then set in a cryomold for freezing, excess fluid was removed, and OCT was placed on top to fill in the mold. The organoids were frozen and placed in the –20 °C freezer overnight. The samples were then cut in a cryotome in 5-μm sections (Thermo Fisher), mounted on glass slides, and stained with hematoxylin and eosin. Once the opening to Bowman’s capsules was visible, sectioning was ceased. We then unembded the organoids from the OCT by heating the samples to 40 °C and physically removing them from OCT, washed them extensively with water, and dehydrated the tissue. Subsequent dehydration in varying grades of ethanol was required (20 min each; 30%, 50%, 70%, 90%, 3 × 20 min 100%). The samples were then placed in 50% ethanol and 50% hexamethyldisilazane (HMDS) for 30 min followed by 100% HMDS for three rounds of 30 min each. All steps were performed in a closed glass container. After the final washing with HMDS, the samples were removed and placed in an open container in the fume hood to dry. Dried samples were mounted to aluminum pin mounts with conductive carbon tape, sputter-coated with 5 nm of gold or platinum, and imaged with an UltraPlus field emission scanning electron microscope (Zeiss) at 1 keV.

**Immunostaining.** We used immunostaining followed by confocal microscopy to assess the localization of cellular or extracellular proteins within or adjacent to organoids. Prior to immunostaining, each organoid sample was washed with PBS and then fixed for 1 h with 10% buffered formalin. The fixative was removed via several washes in PBS for several hours, and samples were then blocked overnight with 1% wt% donkey serum in PBS with 0.125 wt% Triton X-100. Primary antibodies to the protein or biomarker of interest were incubated with the constructs for 2 d at 4 °C at the dilutions listed in Supplementary Table 2 in a solution of 0.5 wt% BSA and 0.125 wt% Triton X-100. Removal of unbound primary antibodies was accomplished via a wash step against a solution of PBS or 0.5 wt% BSA and 0.125 wt% Triton X-100 in PBS for 1 d. Secondary antibodies were incubated with the constructs for several hours at 1:500 dilution in a solution of 0.5 wt% BSA and 0.125 wt% Triton X-100 in PBS. Samples were counterstained with DAPI and then washed for at least several hours in PBS before imaging. A full antibody list can be found in Supplementary Table 2.

**Image rendering and analysis.** Phase contrast microscopy was carried out on an inverted Leica DM IL scope with objectives ranging from 1.25× to 40×. Confocal microscopy was done on an upright Zeiss LSM 710 with water-immersion objectives ranging from 10× to 40× and spectral lasers at wavelengths of 405, 488, 514, 561, and 633 nm. Image reconstructions of z-stacks were generated in Imaris using the z-projection function with the maximum pixel intensity setting. Any increases in brightness were applied uniformly across an entire z-projected volume. Confocal z-stacks of PECAM1 were taken of fixed whole-mount organoids, both iPSC- and ESC-derived, in the various culture conditions. The z-stacks were taken at the limit of the confocal depth with each sample, nearly 250 μm per sample, which represents approximately the same volume per organoid analyzed. Those z-stacks were then flattened in ImageJ to a 2D maximum-intensity projection (as required by the AngioTool for input). The default settings were used on the AngioTool for analysis, and vessel diameters of 4, 7, 10, and 14 were analyzed for each organoid. In all cases, the whole organoid was used for analysis.

**Imaris analysis.** Confocal z-stacks of PECAM1 and LTL were taken of fixed whole-mount organoids, both iPSC- and ESC-derived, in the various culture conditions. The z-stacks were taken at the limit of the confocal depth with each sample, roughly 250 μm per sample, which represents approximately the same volume per organoid analyzed. Those z-stacks were then opened in Imaris imaging software. As shown in Supplementary Fig. 15, the confocal 3D rendering was turned into a vascular surface and a tubule surface. Then we used the Imaris surface area XTension to quantify the percent overlap between the vascular and tubular surfaces in 3D; values are reported in Fig. 3h. For assessment of the average distance from the vasculature to a tubule, first the Imaris distance transformation XTension was run on the tubule surface. The resulting channel was then masked by the vascular surface to create a new masked distance transformation channel. That masked distance transformation channel was sent to Fiji (ImageJ) to retrieve a histogram of the masked voxels, from which we obtained a geometric mean for each sample, reported in Fig. 3l.

**Statistics and reproducibility.** Data in all bar charts and dot plots are expressed as mean ± s.d. Statistical analysis was done in MATLAB and GraphPad Prism 7, and statistical significance was attributed to values of P < 0.05 as determined by ANOVA analysis, as described in the figure legends. Different significance levels (P values) are indicated in each figure with asterisks: *P < 0.05, **P < 0.01, ***P < 0.001. P values for Supplementary Fig. 17c are summarized in Supplementary Table 3. For transparency, we state here the number of times experiments were repeated independently, with similar results obtained, to produce the data shown: Fig. 1b, 6; Fig. 1c–e, 15; Fig. 1f, 1; Figs. 1g, 1; Fig. 2b, 2; Fig. 2c–e, 1; Fig. 2f–h, 15; Fig. 2i, 5; Fig. 2l, 15; Fig. 2m, 9; Fig. 2n, 10; Fig. 3a,b, 3; Fig. 3c, 3; Fig. 3d–f, 1; Fig. 3g, 15; Fig. 3h, 2; Fig. 3j,k, 5; Fig. 4a, 15; Fig. 4b, 3; Fig. 4c, 1; Fig. 4d–l, 15; Fig. 4m, 15; Fig. 4n, 15; Fig. 4o,p, 1; Supplementary Fig. 1f,g, 2; Supplementary Fig. 2a–f, 6; Supplementary Fig. 2h,i, 15; Supplementary Fig. 3a,b, 2; Supplementary Fig. 4a–l, 4; Supplementary Figs. 5–8, 2; Supplementary Fig. 9, 15; Supplementary Figs. 10 and 11, 9; Supplementary Fig. 12a,b, 15; Supplementary Fig. 12c,d, 2; Supplementary Fig. 13a–j, 3; Supplementary Fig. 14a–d, 1; Supplementary Fig. 15a,b, 15; Supplementary Fig. 17a, 3; Supplementary Fig. 17b,c, 4; Supplementary Fig. 18a–d, 15; Supplementary Fig. 19, 15; Supplementary Fig. 20, 3; Supplementary Fig. 21, 10; Supplementary Fig. 22, 3; Supplementary Video 2, 9; Supplementary Videos 3–6, 15.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The data generated in this study are available from the corresponding authors upon request.

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| ✗  | State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

No specific codes were used to collect data. Data was collected using standard brightfield and confocal imaging, qrtPCR, and flow cytometry machines.

Data analysis

Data analysis of the data collected (see above) was performed using standard commands in Excel 2016 and plotting/statistics were performed in Graphpad Prism 7 software. qPCR primers were designed using Primer3 (version 4.1.0). For millifluidic chip fabrication, the gasket tool-path was created using a custom MATLAB script. Flow modeling was performed using COMSOL Multiphysics simulation software. G code was used to program an Aerotech 3D stage to print the silicon gaskets. The Fiji (ImageJ) plugin Angiotool was used to analyze abundance and character of vasculature. Imaris software was used to analyze in 3D the vascular and tubule surfaces and quantify their relative overlap and distance from each other using confocal z stack images as an input.

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Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Regarding the availability of data, no publicly available datasets were used, supplementary figures 7 and 8 include raw flow cytometry data with gating strategy, and there are no restrictions to data availability - please directly contact the corresponding author.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size was determined by number of independent kidney organoids used per sample. No statistical method was used to pre-determine sample size, rather mirrored animal experiments with each independent organoid treated as a littermate. The sample size was sufficient for our claims based on statistical significance.

Data exclusions
No data was excluded from the analyses.

Replication
Measures taken to verify the reproducibility of the experimental findings include analyses based on individual organoids, all organoids across samples generated from the same batch of nephron progenitor cells and immunostained simultaneously for each experiment, and then repeating experiments across organoids generated from multiple batches of nephron progenitor cells were performed. All attempts at replication were successful.

Randomization
Samples (organoids) were allocated to the different experimental groups completely randomly with each new batch of nephron progenitor cells.

Blinding
Investigators were not blinded to group allocation during data collection and analysis. As identical gains and exposure times were used in fluorescent imaging during data collection when quantitative comparisons were made, and identical thresholds were applied during unbiased data analysis using Fiji/ImageJ/Imaris, blinding was not required for the data generated from immunostaining. The gating strategies for flow cytometry are depicted in the raw data provided in supplemental figures.

Reporting for specific materials, systems and methods

Materials & experimental systems

| Materials & experimental systems | n/a Involved in the study |
|---------------------------------|--------------------------|
| Unique biological materials     | ☒                        |
| Antibodies                      | ☒                        |
| Eukaryotic cell lines           | ☒                        |
| Palaeontology                   | ☒                        |
| Animals and other organisms     | ☒                        |
| Human research participants     | ☒                        |

Methods

| Methods | n/a Involved in the study |
|---------|--------------------------|
| ChIP-seq | ☒                        |
| Flow cytometry | ☒              |
| MRI-based neuroimaging | ☒           |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

The unique material used in this study relates to human pluripotent stem cell-derived kidney organoids. The kidney organoids were generated from the H9 human embryonic stem cell line available from WiCell and the directed differentiation protocol from human stem cells to kidney organoids has been published in Nature Biotech (Morizane et al, 2015) and Nature Protocols.
(Morizane et al, 2017). We also used the iPSC line BJFF provided by Sanjay Jain as discussed in the methods section of the manuscript. All unique materials are readily available from the authors.

### Antibodies

| Antibodies used | Antibody, Source Catalog #, Host Species, Reactivity Concentration |
|-----------------|---------------------------------------------------------------|
| MCAM, Abcam, ab75769, Rabbit, anti-human, 1:250 |
| PECAM1, Abcam, ab9498, Mouse, anti-human, 1:250 |
| EMCN, Sigma-Aldrich, SA4502163, Rabbit, anti-human, 1:250 |
| PDGFRB, Abcam, ab32570, Rabbit, anti-human, 1:250 |
| Col IV, Abcam, ab52235, Rabbit, anti-human, 1:250 |
| ATP1A1, Abcam, ab76020, Rabbit, anti-human, 1:250 |
| TUBA4A, Abcam, ab24610, Mouse, anti-human, 1:250 |
| SIX2, Proteintech, 11562, Rabbit, anti-human, 1:500 |
| SALL1, R&D Systems, PP-K9814-00, Mouse, anti-human, 1:100 |
| PAX2, Covance, PRB-276P, Rabbit, anti-human, 1:500 |
| aSMA, Abcam, Ab5964, Rat, anti-human, 1:250 |
| FLK1-555, Bioss, bs-10412R, anti-Human, 1:10 |

### Validation

All antibodies used were validated for the application used (IHC, ICC, flow cytometry) according to manufacturers' websites. Validation statements for the species and application on the manufacturer's website are listed below. Antibodies were further chosen based on having references in the literature, and include many antibodies used and reported in our labs prior work published in Nature affiliated journals.

- ab75769: Reacts with: Mouse, Rat, Human, Suitable for ICC/IF, IHC-Fr, WB, IHC-P, Flow Cyt.
- AF1658: Reacts with Human, Suitable for WB, Flow Cyt., IHC
- ab9498: Reacts with Mouse, Human, Cynomolgus monkey, Suitable for IHC-Fr, ICC/IF, Flow Cyt, WB, IHC-P.
- SAB4502163: Reacts with rat, mouse, human, Suitable for ELISA, IF.
- ab32570: Reacts with Mouse, Rat, Human, Suitable for ICC-Fr, Flow Cyt, IHC, ICC-FrFl.
- ab52235: Reacts with mouse, human, Suitable for IHC, WB, ELISA.
- ab76020: Reacts with mouse, rat, human, tilapia, Suitable for ICC/IF, WB, Flow Cyt, IHC-P.
- ab24610: Reacts with Mouse, Rat, Sheep, Human, Monkey, Sea urchin, Suitable for Flow Cyt, WB, IHC-P, ICC/IF, IP, IHC-FrFl.
- ab1562: Reacts with human, mouse, rat, American alligator, Suitable for WB, IP, IHC.
- PP-K9814-00: Reacts with human, Suitable for WB, ELISA. IHC application was reported in previous publications such as PMID: 24332837.
- PRB-276P: Reacts with human, mouse, chicken, xenopus, zebrafish, Suitable for WB, IHC.
- Ab5964: Reacts with human, Suitable for IHC, WB.
- bs-10412R: Reacts with human, mouse, rat, pig, Suitable for WB, FCM, IHC.

### Eukaryotic cell lines

**Cell line source(s)**

- H9 human embryonic stem cells are available from WiCell. BJFF iPSC line is available from Sanjay Jain upon request and MTA. GMECs, HUVECs, and HDNFS are available from Angio-Proteomie.

**Authentication**

- The H9 cell line is authenticated by WiCell, who distributes it commercially. Washington University in Saint Louis authenticated the BJFF line. GMECs, HUVECs, and HDNFS are authenticated by Angio-Proteomie.

**Mycoplasma contamination**

- Our H9 cell line has repeatedly tested negative for mycoplasma contamination. We run tests monthly. GMECs, HUVECs, and HDNFS are checked by Angio-Proteomie.

**Commonly misidentified lines**

- No commonly misidentified cell line was used.

### Animals and other organisms

**Laboratory animals**

All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by Institutional Animal Care and Use Committees at Brigham and Women's Hospital. Embryonic kidneys at stage E14.5 (day of plug=EO.5) were isolated from timed pregnant females (Charles River).

**Wild animals**

- The study did not involve wild animals.

**Field-collected samples**

- The study did not involve samples collected from the field.
Flow Cytometry

Plots

Confirm that:
☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☒ All plots are contour plots with outliers or pseudocolor plots.
☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation | Samples were prepared from experimentally treated kidney organoids as outlined in materials and methods. Briefly, single cell dissociation of kidney organoids was performed using a combination of enzymatic (collagenase type IV, Stemcell Technologies x30 min total incubation) and interval manual dissociation using serially reduced sizes of pipette tips.

Instrument | A BD LSR Fortessa was used in the Baer Core at Harvard University.

Software | FloJo was used to collect and analyze the flow cytometry data.

Cell population abundance | Cells were not sorted such that no post-sort fractions existed.

Gating strategy | Rather than a description, we have included the gating strategy used for all relevant experiments in the supplementary figures, which include the preliminary FSC/SSC gates of the starting cell populations and later indicate boundaries between “positive” and “negative” staining cell populations with percentages labeled.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.