Figure S1: UM based visualization and quantification of Isolectin signal intensity. Alexa Fluor 647-coupled Isolectin GS-IB4-perfused kidney of C57BL/6 mice. **(A)** UM 3D volume rending or **(B)** representative 2D section showing glomeruli and capillaries (Isolectin, green). **(A)** For illustration of the different signal intensities, the brightness of Isolectin signal intensity (green) was chosen optimally for the 3DISCO dataset and then also applied to the BABB, ECI and iDISCO+ data set. Data are shown with identical visualization settings. Scale bar represents 200 µm. **(B)** For illustration of the image quality in each method, the brightness of the Isolectin signal intensity (green) was chosen optimally for each clearing method individually. Glomeruli, capillary and background are shown. Scale bar represents 150 µm. **(C)** Quantification of glomeruli, capillary parenchyma fluorescent signal intensity. **(D)** Relative fluorescent signal intensity values are shown for glomeruli or capillaries, normalized to parenchyma. Data are shown as mean ± SEM (n=3). One-way ANOVA followed by Newman-Keuls post-hoc test was used; *p<0.05, **p<0.01. 3DISCO: three-dimensional imaging of solvent-cleared organs; BABB: benzyl alcohol, benzyl benzoate; ECi: ethyl cinnamate or iDISCO+: immunostaining 3DISCO.

Figure S2: UM based visualization and quantification of aSMA signal intensity. (A-B) Whole-mount-stained kidney of C57BL/6 mice. **(A)** Representative UM 3D volume rending or **(B)** 2D section showing aSMA positive vessel (red). **(A)** For illustration of the different signal intensities, the brightness of aSMA signal intensity (red) was chosen optimally for iDISCO+ dataset and then also applied to the BABB, ECi and 3DISCO data set. Scale bar represents 200 µm. **(B)** For illustration of the image quality in each method, the brightness of aSMA signal intensity (red) was chosen optimally for each clearing method individually. aSMA positive vessel and background is shown. Scale bar represents 150 µm. **(C)** Quantification of aSMA fluorescent signal intensity and background. **(D)** Relative fluorescent signal intensity values are shown for aSMA, normalized to parenchyma. Data are shown as mean ± SEM (n=3), One-way ANOVA followed by Newman-Keuls post-hoc test was used; *p<0.05. 3DISCO: three-dimensional imaging of solvent-cleared organs; BABB: benzyl alcohol, benzyl benzoate; ECi: ethyl cinnamate or iDISCO+: immunostaining 3DISCO.

Figure S3: UM based visualization and quantification of renal vessel. 500 µm × 500 µm × 600 µm kidney cortex of C57BL/6 mice are shown: endothelial cells are stained with Isolectin GS-IB4 A647. **(A1)** Original channel: aSMA positive vessels (red), glomeruli and capillaries (green). **(A2)** masked and segmented glomeruli (blue). **(A3)** αSMA-positive vessels (red), capillaries (green) and glomeruli (blue) are visualized in surface mode. The surface of the capillaries is 85% transparent. Scale bar represents 70 µm.

Figure S4: Representative micro-CT 2D image of a MicroFil-perfused matrigel plug. In vivo matrigel angiogenesis assay in SCID mice (n=2). MicroFil-positive staining (light grey, marked with a dot) and the outer region of the matrigel plug are marked (dashed line). Scale bar represents 1 mm. PBS: phosphate buffered saline.

Figure S5: UM based visualization of HUVEC integrated into murine blood vessel system. HUVEC stained by Vybrant dil (red) were injected into SCID mice. Endothelial cells are stained with Isolectin GS-IB4 A647 (green). **(A1-3)** HUVEC (red) building the glycocalyx in different z-levels. **(A4-6)** HUVEC is detected by the Imaris algorithm (blue). Scale bar represents 50 µm. HUVEC: human umbilical vein endothelial cell.

Figure S6: Organs after the 3DISCO or EtOH tissue clearing as seen by visible light. (A1,) Matrigel isolated from SCID mouse,**(A2)** heart and **(A3)** piece of kidney after 3DISCO clearing isolated from C57BL/6 mouse and **(A4)** common carotid artery isolated from ApoE$^{-/-}$ mouse after EtOH clearing.

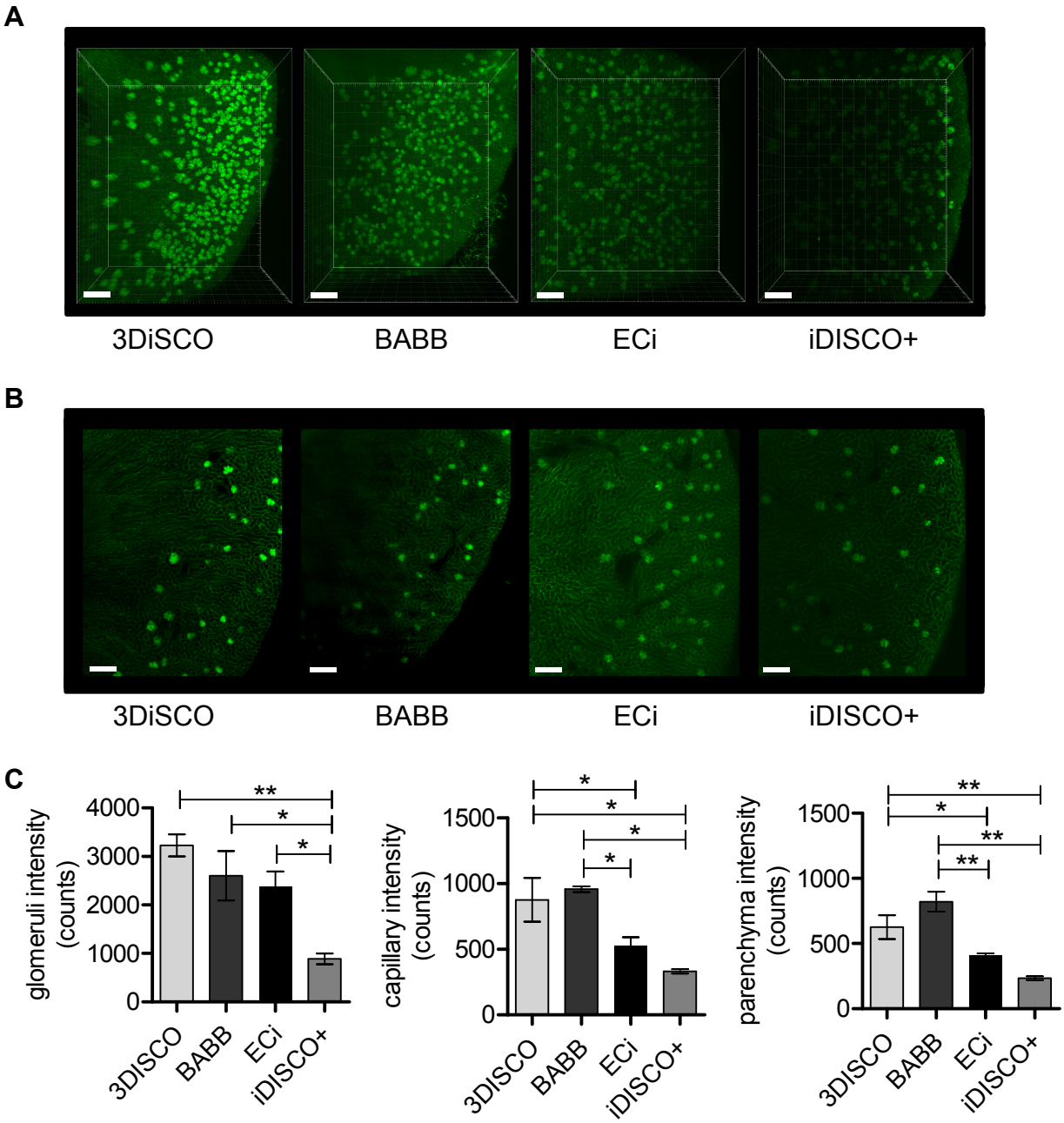
A
3DiSCO
BABB
ECi
iDISCO+
B
3DiSCO
BABB
ECi
iDISCO+
C
glomeruli intensity (counts)
4000
3000
2000
1000
0
**
*
*
3DISCO
BABB
ECi
iDISCO+
capillary intensity (counts)
1500
1000
500
0
*
*
*
*
3DISCO
BABB
ECi
iDISCO+
parenchyma intensity (counts)
1500
1000
500
0
**
*
**
**
3DISCO
BABB
ECi
iDISCO+
D
relativ glomeruli intensity (glomeruli/ parenchyma)
8
6
4
2
0
3DISCO
BABB
ECi
iDISCO+
relativ capillary intensity (capillary/ parenchyma)
2.0
1.5
1.0
0.5
0.0
3DISCO
BABB
ECi
iDISCO+

Figure S1

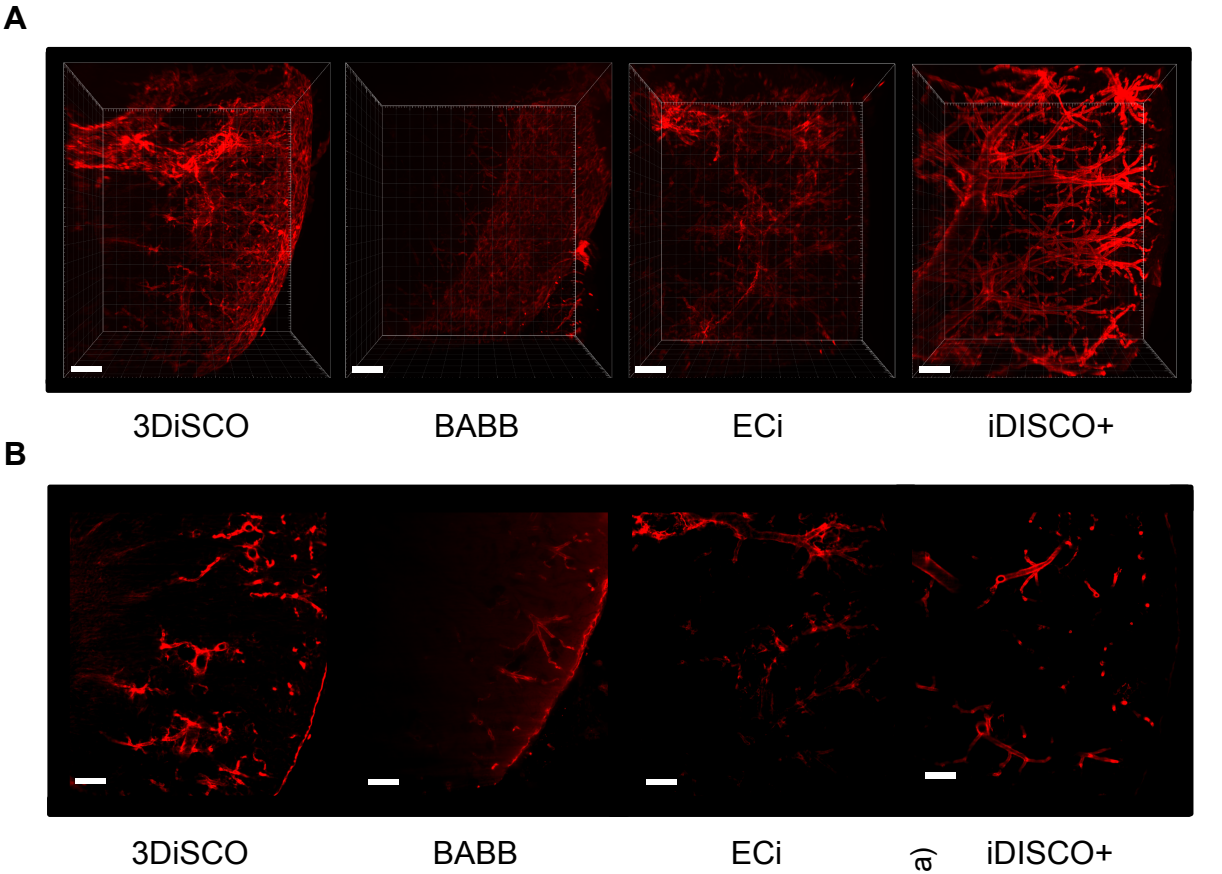
A
3DiSCO
BABB
ECi
iDISCO+
B
3DiSCO
BABB
ECi
iDISCO+
C
aSMA intensity (counts)
25000
20000
15000
10000
5000
0
3DISCO
BABB
ECi
iDISCO+
parenchyma intensity (counts)
1500
1000
500
0
3DISCO
BABB
ECi
iDISCO+
D
relativ aSMA intensity (aSMA pos. vessel/ parenchyma)
50
40
30
20
10
0
*
3DISCO
BABB
ECi
iDISCO+

Figure S2

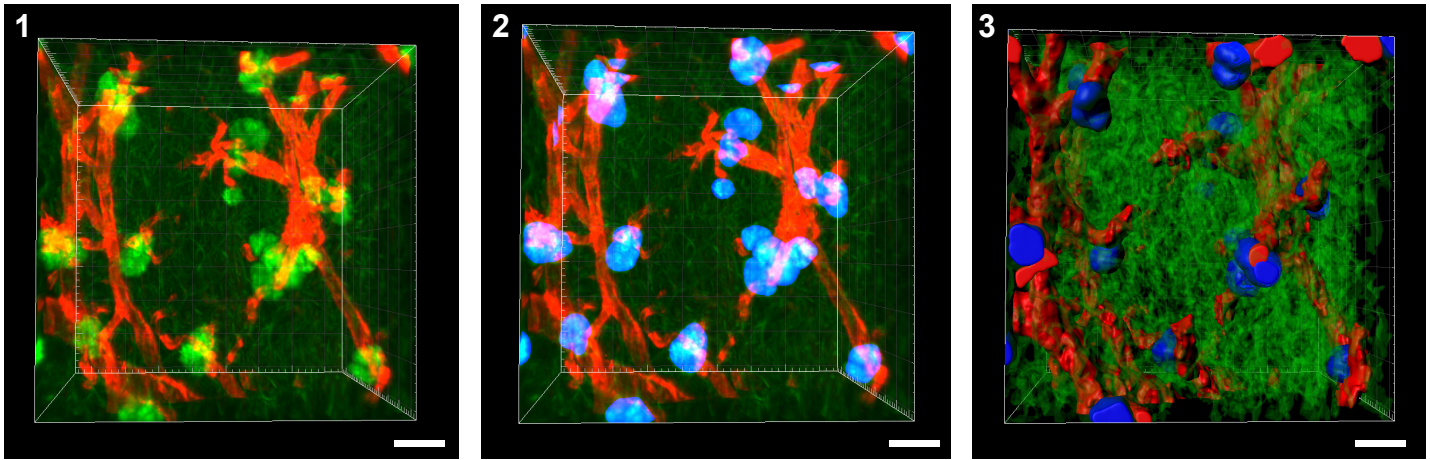

Figure S3

A

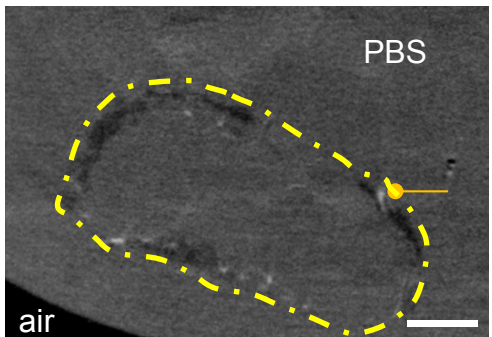

Figure S4

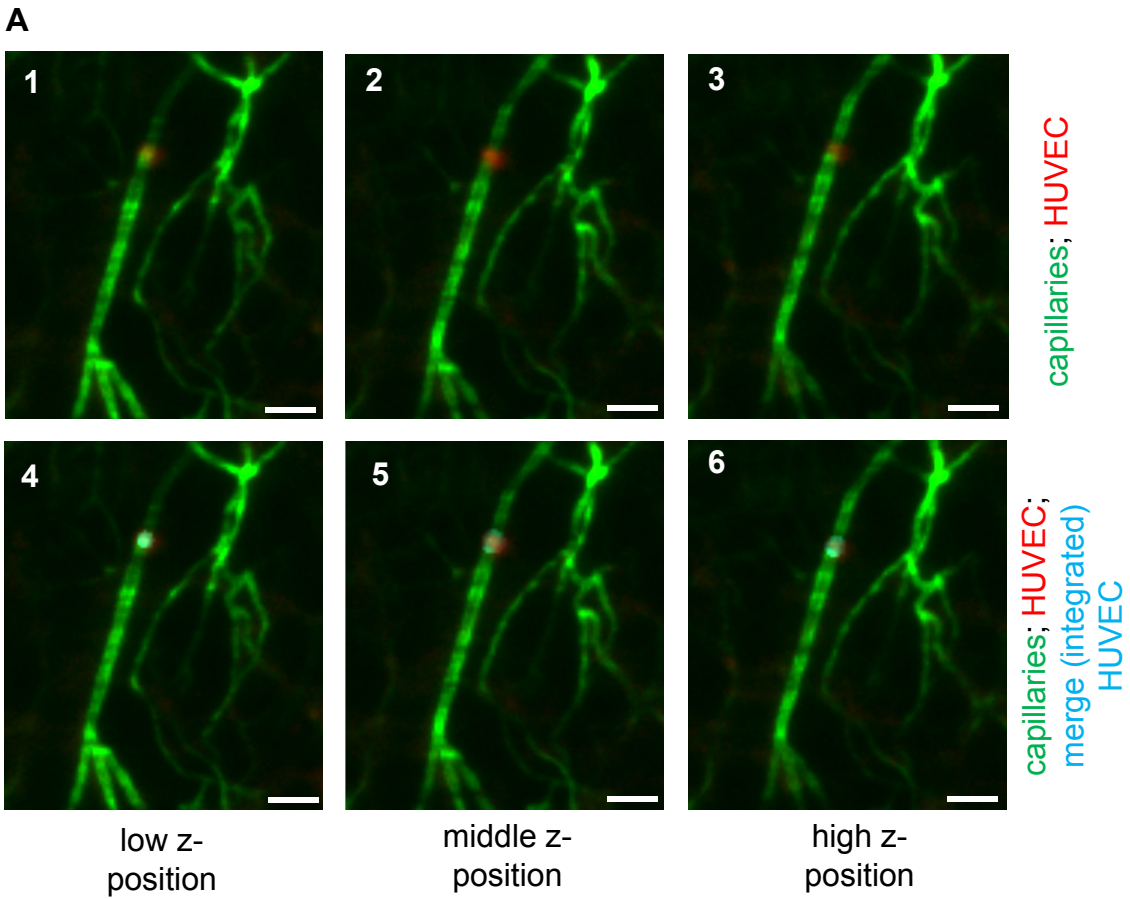
A
1
2
3
4
5
6
capillaries; HUVEC
capillaries; HUVEC; merge (integrated) HUVEC
low z-position
middle z-position
high z-position

Figure S5

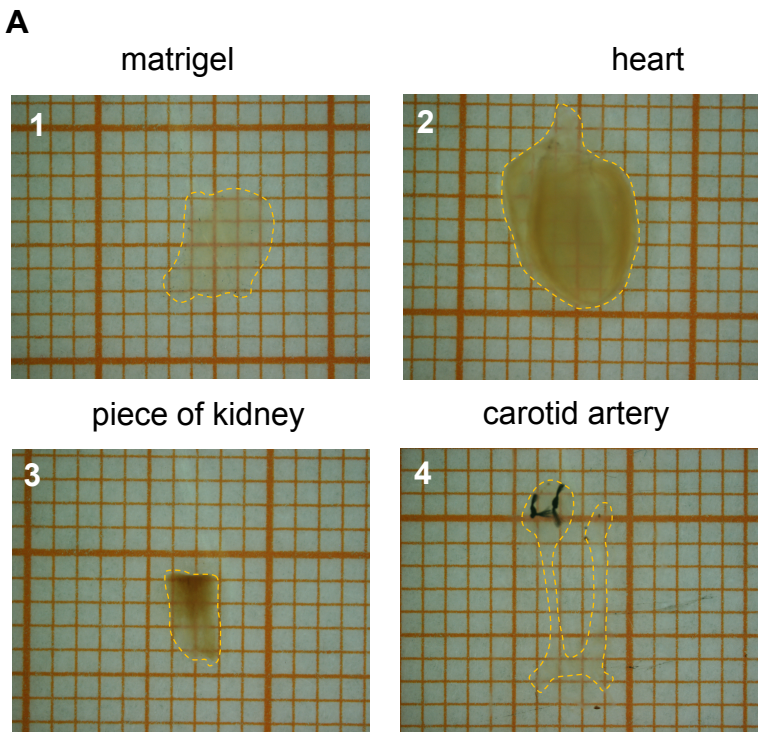

Figure S6

Supplementary Methods

Imaris was used for the following quantification.

Definitions: The Imaris FilamentTracer (Filament) offers features to segment, track, edit, draw, display, and measure Filaments. The FilamentTracer processes one channel at a time and extract objects that qualify as Filaments (e.g length and average thickness comply with criteria). The IsoSurface visualization is a computer-generated representation of a specified gray value range in the data set. It creates an artificial solid object in order to visualize the range of interest of a volume object (Imaris Reference Manual V 6.0).

Matrigel plug study: 2D quantification of vessel diameter and density. In detail "IsoSurface Algorithm" was used to mask capillaries. "Surface Gain Size" was set to 1.00 µm to get a sharp and detailed surface of the channel. "Duplicate Channel" was enabled to maintain the original channel. "Mask All" function was used to homogenize the fluorescent signal inside the surface. Mask settings were set to "Constant Inside/Outside" and "Set Voxel Inside Surface to 200". Thereby the "Filament Algorithm" "Autopaths Loops" could be used on the newly created capillaries channel. An approximate diameter of 1.95 µm was chosen. "Fill Cavities" and "Connected BaseLine" with 0.000 as low and 10.000 as high threshold was applied. To avoid wrong branches "Branch Length Ratio" was set to 3.000.

Matrigel plug and spheroid-based matrigel plug study: 3D quantification of vessel diameter and density. Vascular tree-analysis was performed for vascularized matrigel plugs. The dataset was cropped and sampled down [edit, resample 3d, fixed ratio x/y/z] by a factor of 2 to run the "IsoSurface Algorithm" [surface grain size = 1 µm; Enable Eliminate Background = true; Diameter Of Largest Sphere = 48.8 um; Enable Automatic Threshold = false; Manual Threshold Value = 45.5135; Active Threshold = true; Enable Automatic Threshold B = true; Manual Threshold Value B = 1487.55; Active Threshold B = false]. The plug was divided into four or eight parts using the "Crop 3D Function" and analyzed. The "IsoSurface Algorithm" was used and the blood vessels were masked (channel selection: channel "x"; duplicate channel before mask = check; set voxels outside surface to: 0.000; set voxels inside surface to: 200). On the newly created blood vessel channel, the "Filament Algorithm" was applied (Name = Threshold (loops); enable preprocessing= false; approximate diameter = 10µm; Preserve edges = false; Fill cavities = true; connective baseline = true; branch length ratio = 5.000; find dendrite beginning point = false; delete working channel = true). Finally the volume of the entire plug was analyzed using the "IsoSurface Algorithm" in the down sampled image using the auto fluorescence signal.

Spheroid-based matrigel plug study: Quantification of single cell integration into vascular networks. The auto fluorescence signals were deleted manually with the surface function. Background subtraction was performed with the "Background Subtraction Algorithm" ("Filter Width": 2000 µm). Vybrant dil-stained HUVECs were detected and counted using the "Spots Algorithm" ("Estimated Diameter": 8 µm; "Background Subtraction": true; "Intensity Center" Ch=3 above 720; Region Growing Type: local contrast). The lower threshold was chosen according to the background signal. Cells integrated into the vascular network of the mouse were detected with a "Filter setup". Here the "Threshold Intensity Max" function (channel 2 above 581) was used to detect spots/cells containing an Isolectin GS-IB4 A647 signal higher than the background signal.

Kidney 1 study: Quantification of glomeruli diameter.. Glomeruli were masked with the "IsoSurface Algorithm" and analyzed with the "Spots Algorithm". Within the "Spots Algorithm" different spot sizes ("Region Growing") were used (estimated diameter XY = 60 µm; estimated diameter Z = 70 µm). The filter setting "Distance to Image Boarder XYZ" was used to exclude half imaged glomeruli at the

image boarders. **Quantification of the vascular volume fraction of the kidney cortex.** In the 3D UM Imaris dataset a region in the cortex with a volume of 500 µm x 500 µm x 600 µm was cropped out and analyzed **(Fig. S3)**. Background subtraction on the Isolectin GS-IB4 A647 positive blood vessel channel was performed using a filter width = 2000 µm. Smooth muscle positive vessels, capillaries and glomeruli were segmented and analyzed separately as described earlier using the "IsoSurface Algorithm". Glomeruli in the cortex were counted with the spots algorithm as described earlier and were normalized to the analyzed volume.

Kidney 2 study: Quantification of fluorescent signal intensity. The 3D UM Imaris datasets were sampled down by a factor of 2 and analyzed without cropping out a region of interest. Quantification of Isolectin fluorescent signal intensity: Glomeruli were detected using the "Spots Algorithm" (Estimated XY Diameter = 60.0 µm; Estimated Z Diameter = 70.0 µm; Background Subtraction = true, Detect Ellipsoids = true; Classify Spots with "Quality" above automatic Threshold-Filter; Region Growing Type = Local Contrast; Region Growing Automatic Threshold = true). Spots have been used to mask the Isolectin channel and delete the glomeruli signal mask (set voxel inside surface 0.00). Capillaries and parenchymal were detected with a threshold based "IsoSurface Algorithm" (Enable Smooth = true; Surface Grain Size = 2.60 µm; Enable Eliminate Background = true; Diameter Of Largest Sphere = 9.75 µm). The threshold was calculated depending on the mean intensity of the glomeruli of each sample generated by the spot-algorithm. The surface of the capillaries was used to mask the Isolectin channel to delete also the capillaries signal (set voxel inside surface 0.00). The threshold for every clearing method was calculated on the basis of the mean glomeruli intensity. The parenchymal signal represents the remaining mean signal intensity ("IsoSurface Algorithm", Enable Smooth = true; Surface Grain Size = 20 µm; Enable Eliminate Background = true; Diameter of Largest Sphere = 1000 µm). Quantification of aSMA fluorescent signal intensity: The Imaris tool "Measurement Points" was used to measure the signal intensity of aSMA positive vessel and background. The fluorescent intensity of five aSMA positive vessel and three parenchymal signals were collected for every sample.