SUPPLEMENTAL MATERIAL

Supplemental Methods

Supplementary Figure 1. Data description.

Supplementary Figure 2. Generation of *in-silico* IF images for training the pix2pix network.

Supplementary Figure 3: Automation of Wiggins method for estimating podocyte nuclear caliper diameter.

Supplementary Figure 4: Performance of the proposed deep learning network model in detecting and segmenting podocyte nuclei in human biopsy WSIs with respect to the number of podocyte nuclei per glomerulus and number of glomerular profiles per WSI.

Supplementary Figure 5. Comparison of pix2pix results with manual annotation of podocyte nuclei.

Supplementary Figure 6. Pixel-wise class imbalance of training data.

Supplementary Table 1. STZ dose per mouse for DKD data.

Supplementary Table 2. Extracted features per PAS image patch from podocyte nuclei (as identified by ground-truth, CNN, and pix2pix) and glomeruli, averaged over each dataset, per disease state.

Supplementary Table 3: Comparison of *d*, *D*, and *CF* values extracted from IF and PAS images.

Supplementary Table 4: Comparison of *D* and *CF* values extracted from mouse, rat, and human IF/IHC ground-truth images with values reported by Venkatareddy *et al*., *JASN*, 2014.

Supplementary Table 5: Comparison of *d*, *D*, and *CF* values, and podocyte volume density estimates extracted from ground-truth IF/IHC images and computationally predicted podocyte nuclei in PAS images from the hold-out WSIs.

Supplementary Table 6. Comparison of conditional probability of class assignment for central and peripheral podocyte nuclei, and non-podocyte glomerular nuclei between the expert annotators and the GAN.

SUPPLEMENTAL METHODS:

I. PodoSighter plugin execution

In order to run the PodoSighter plugin on your PAS-stained renal section, follow these steps:

1 - Register/log in at https://athena.ccr.buffalo.edu/.

2 - Upload your slide and the respective glomerulus annotation file (.xml) via the upload button on the top right corner of the screen. The trained models for the respective species and podocyte markers (p57, WT1) can be downloaded from https://buffalo.app.box.com/folder/136316130144.

3 - Open your slide using 'Sarder lab slide analyzer'.

4 - On the top right corner of the screen, select the 'Analyses' button, and from the drop-down menu, select 'sarderlab/podosighter>V1>PodoSighter_cnn' or 'sarderlab/podosighter>V1> PodoSighter_pix2pix', depending on which pipeline (CNN or pix2pix) is to be used.

5 - Once you select the plugin, a user input section is displayed on the left. For user inputs, follow the instructions below.

PodoSighter_CNN user inputs

Data Folder: Select the folder containing the slide and xml annotations.

Input PAS whole slide image: Select the whole slide image (WSI) to be analyzed.

Input Glomerular Annotation File: Select the xml file containing glomerulus annotations (either manually annotated or extracted using the HAIL pipeline.

Model: Select the trained model (e.g., mou_wt1_model.ckpt-50000.data-00000-of-00001).

Model checkpoint: Select the latest checkpoint of the trained model (e.g., mou_wt1_checkpoint).

Model idx: Select the index file of the trained model (e.g., mou_wt1_model.ckpt-50000.index).

JSON format podocyte nuclei annotation filename: Select the name of output (podocyte) json file (e.g., "abc_json").

XML format podocyte nuclei annotation filename: Select the name of output (podocyte) xml file (e.g., "abc_xml").

CSV output filename: Select the name of output csv file containing podocyte metrics (e.g., "abc_csv").

PodoSighter_pix2pix user inputs:

Data Folder: Select the folder containing the slide and xml annotations.

Input PAS whole slide image: Select the whole slide image (WSI) to be analyzed.

Input Glomerular Annotation File: Select the xml file containing glomerulus annotations (either manually annotated or automatically extracted using the HAIL pipeline).

Trained Generator Model: Select the trained generator model. (e.g., mou_wt1_net_G.pth).

Trained Discriminator Model: Select the trained discriminator model. (e.g., mou_wt1_net_D.pth).

JSON format podocyte nuclei annotation filename: Select the name of output (podocyte) json file (e.g., "abc_json").

XML format podocyte nuclei annotation filename: Select the name of output (podocyte) xml file (e.g., "abc_xml").

CSV output filename: Select the name of output csv file containing podocyte metrics (e.g., "abc_csv").

User parameters for both plugins:

Since each WSI differs in staining, imaging, resolution, etc., we provide the option to adjust the parameters to generate optimal results for your WSIs. Listed below are the different parameters and their definitions:

Tissue thickness: Enter your tissue thickness (in microns).

PAS nuclei intensity threshold: Selects the threshold for segmenting hematoxylin-stained nuclei (ranging from 0 to 1).

Gaussian filter size parameter: Blurs the PAS image prior to application of the threshold. Stands for "Gaussian filter size".

Morphological disc size: Refers to size of structuring element to perform morphological opening of segmented nuclei.

Species: Specify mouse, rat, or human.

Gpu_id: Setting this parameter to 0, would enable the code to run on the GPU (-1 for CPU: not recommended).

resolution: This parameter specifies whether the analysis should be done in high resolution (0) or as a downsampled (1) version of the WSI, to save time.

Size threshold: Removes unwanted noise from the segmented nuclei. Stands for "size threshold".

watershed_thre: Sets the distance parameter for the watershed segmentation of segmented nuclei (ranging from 0 to 1). Stands for "watershed (distance) threshold".

Listed below are the parameters used in our study.

Dataset	Species	PAS nuclei intensity threshold	Gaussian filter size parameter	Morpho-logical disc size	resolution	Size threshold	watershed_ thre
Mouse WT1	Mouse	0.3	4	6	0	1800	0.2
Mouse p57	Mouse	0.5	5	6	0	800	0.2
Rat WT1	Rat	0.5	5	4	0	400	0.2
Rat p57	Rat	0.5	5	4	0	400	0.2
Human autopsy WT1	Human	0.5	5	6	0	400	0.2
Human autopsy p57	Human	0.5	5	6	0	400	0.2
Human pediatric WT1	Human	0.4	5	6	0	400	0.2
Human pediatric p57	Human	0.4	5	6	0	400	0.2

Once the plugin is done running, select the 'Analyses' button again, and from the drop-down menu, select ‘sarderlab/podosighter>V1>TranslateXMLtoJson' to convert the xml file into json.

II. Network performance with respect to preanalytical errors.

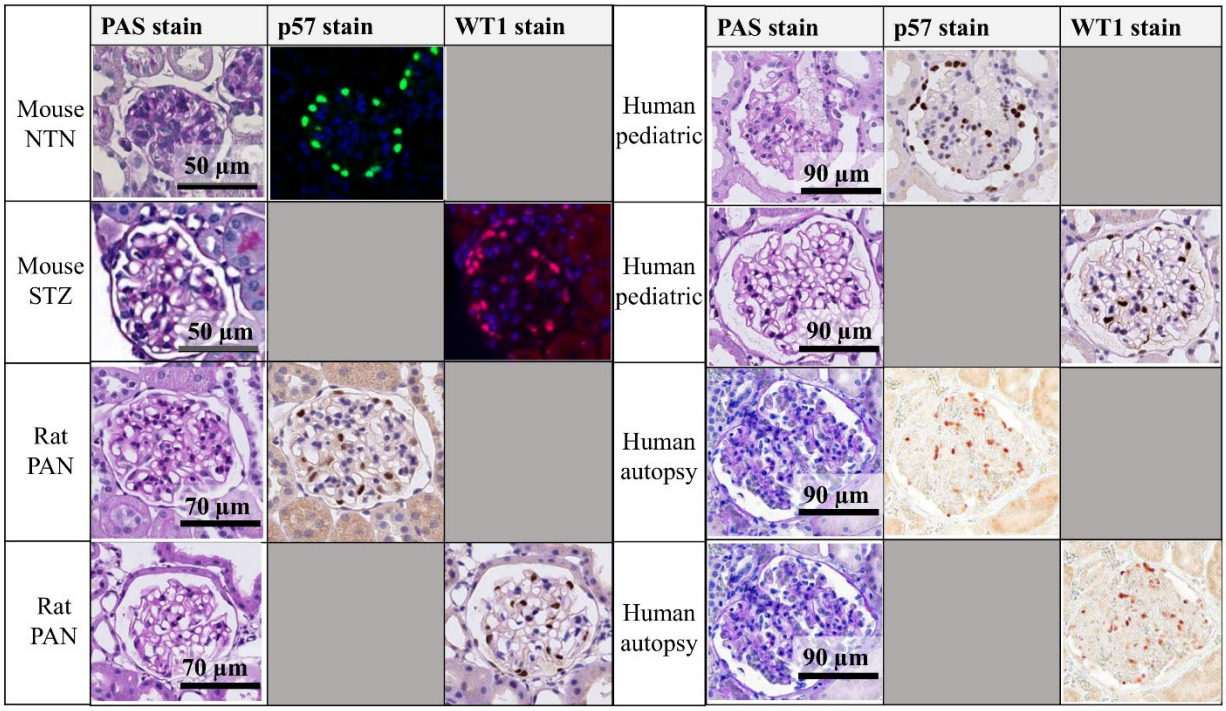
The three renal pathologists identified ~100 glomerular image patches (obtained from the mouse, rat, and human WSI cohorts used in this work) containing one or more of the preanalytical errors listed below. The performance of the network (as indicated by the average dice coefficients) in these image patches are shown below, averaged with respect to the identified artifacts.

The glomerular image patches containing the artifacts are available at https://bit.ly/3e6XZzs. Additionally, images with each of the 31 artifacts listed below has been segregated into separate folders, named accordingly, with the respective identification codes listed below.

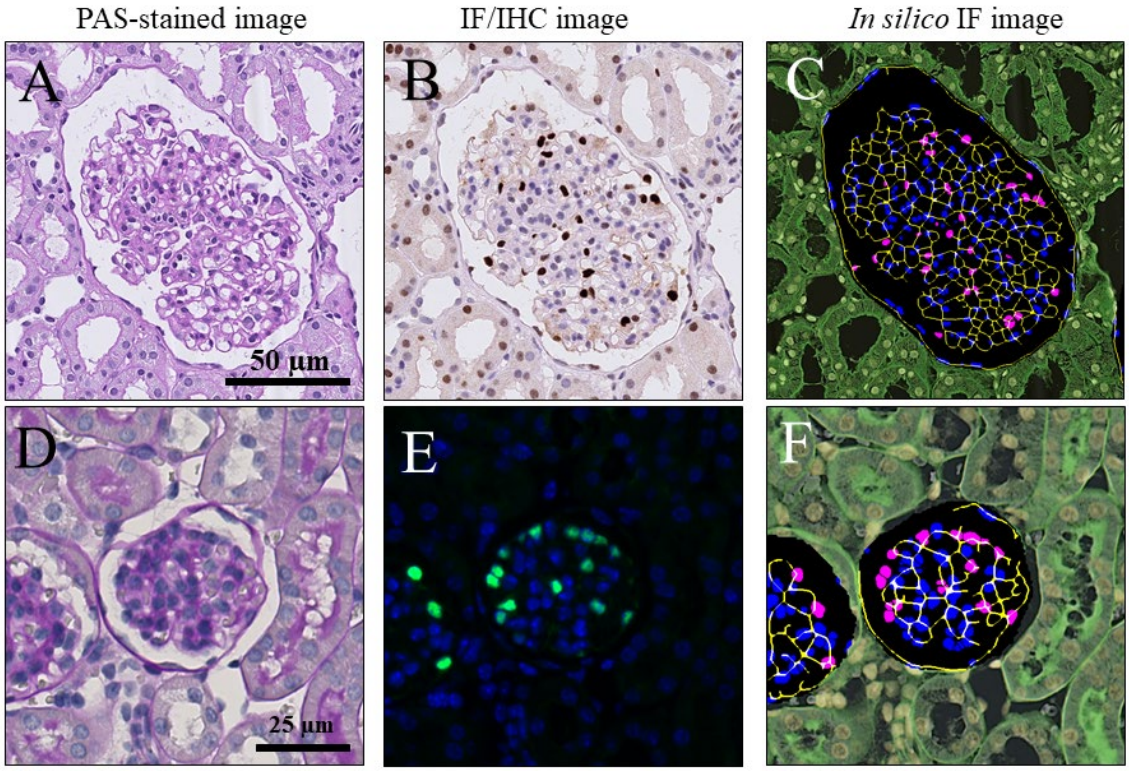
Pathologist confidence	**Identification code**	**Artifact**	**Average dice coefficients**
Definitely	A01	Blurry	0.49
Maybe	A02	Blurry	0.58
Definitely	A03	Presence of foreign material	0.52
Definitely	A04	Cutting artifact	0.34
Definitely	A05	Crush artifact	0.54
Definitely	A06	Tissue fold	0.63
Maybe	A07	Tissue fold	0.67
Definitely	A08	Variable nuclear basophilia	0.65
Maybe	A09	Variable nuclear basophilia	0.58
Definitely	A10	PAS label very light, hard to orient nuclei	0.54
Definitely	A11	Blurring and light staining on one pole of glomerulus	0.57
Definitely	A12	Partial blurring not on glomerulus	0.76

Definitely	A13	One segment of globally sclerotic glomerulus	0.70
Definitely	A14	Blurring and light nuclei at one pole of globally sclerotic glomerulus	0.36
Definitely	A15	Foreign material obscures one pole of glomerulus	0.71
Definitely	A16	Blurring in region of fold	0.46
Definitely	A17	Fold not on glomerulus	0.58
Definitely	A18	Dye aggregate segmentally obscures glomerulus	0.64
Maybe	A19	Center of glomerulus looks thicker (possible early fold)	0.51
Definitely	A20	Only affects one pole of glomerulus	0.77
Definitely	A21	Blurring in area of fold but does not affect glomerulus	0.84
Definitely	A22	Only partial obstruction of glomerulus by foreign material	0.41
Definitely	A23	Foreign material outside glomerulus	0.70
Maybe	A24	Section thickening; PAS label incomplete making orientation of nuclei to GBM difficult in	0.33
Maybe	A25	Segmental thickening	0.50
Maybe	A26	Thick section leading to early segmental blurring	0.58
Definitely	A27	Segmental changes	0.46
Definitely	A28	Partial glomerular profile with only two capillary loops	0.74

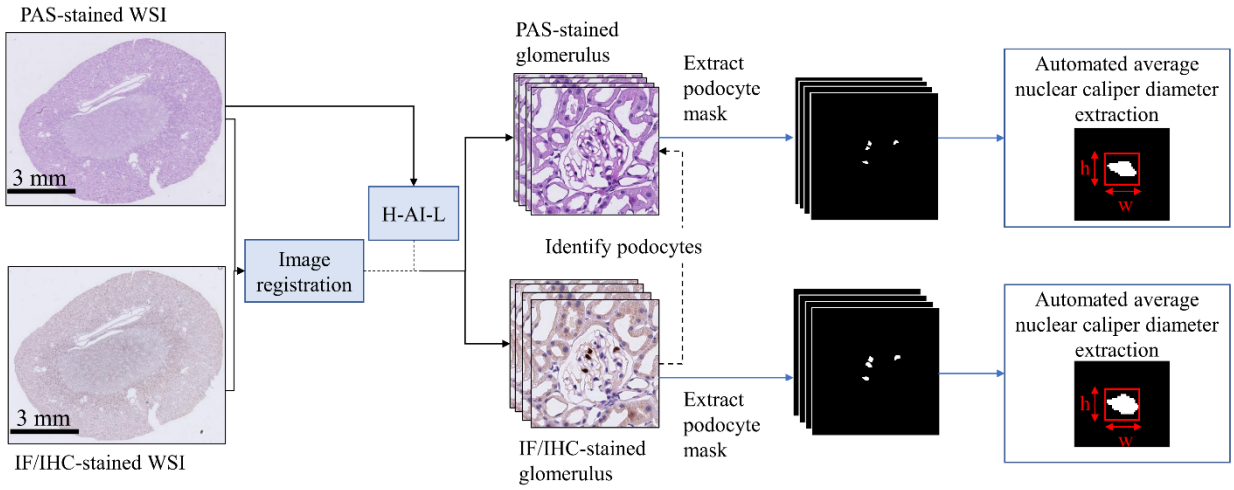
Maybe	A29	Cellular crescent with foreign material only obscuring a small portion of crescent. Minimal blurring from thickened section	0.40
Maybe	A30	Limited segmental obscuring of glomerulus by foreign material	0.28
Maybe	A31	Nuclear staining variable	0.56



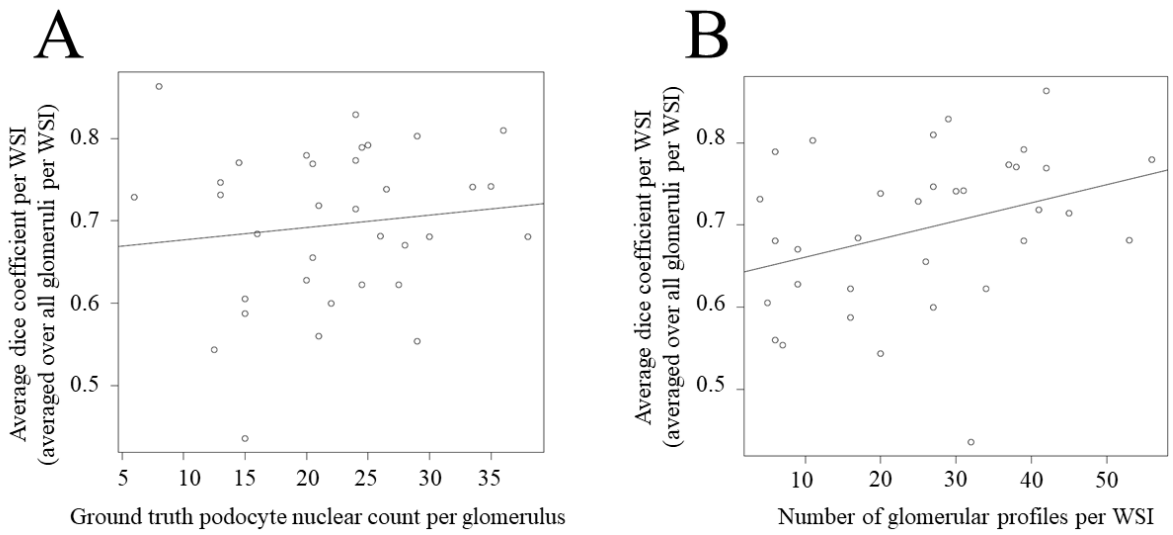
Supplementary Figure 1: Data description. The different datasets and podocyte nuclei markers used for this study.



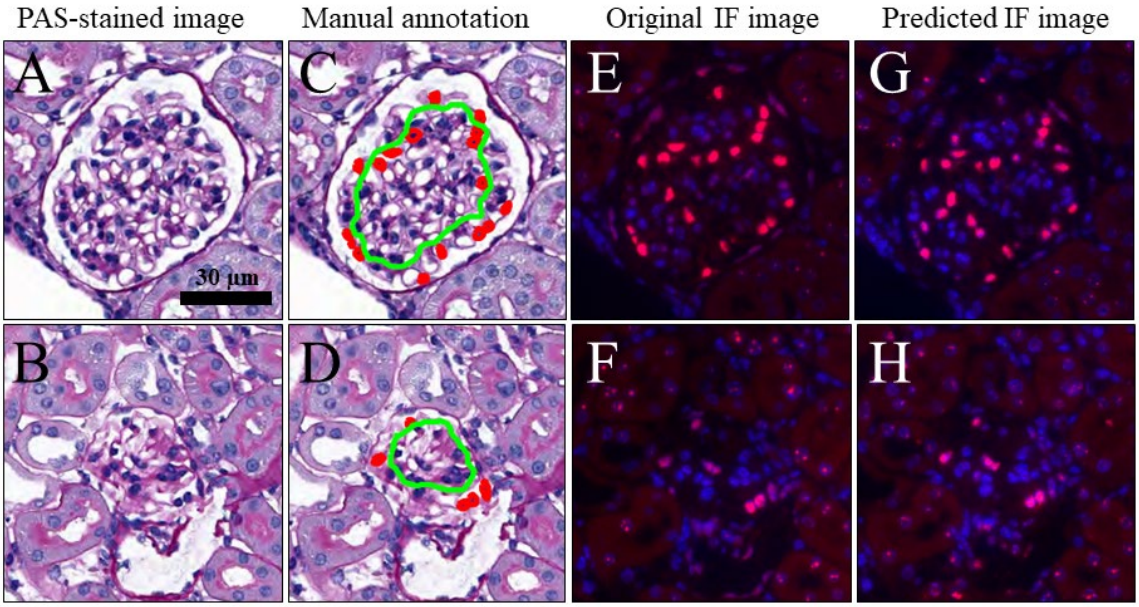
Supplementary Figure 2: Generation of *in-silico* IF images for training the pix2pix network. Example PAS-stained images of glomeruli, their corresponding IF/ IHC ground truth images marking podocyte nuclei and generated *in-silico* IF images for training the pix2pix network.



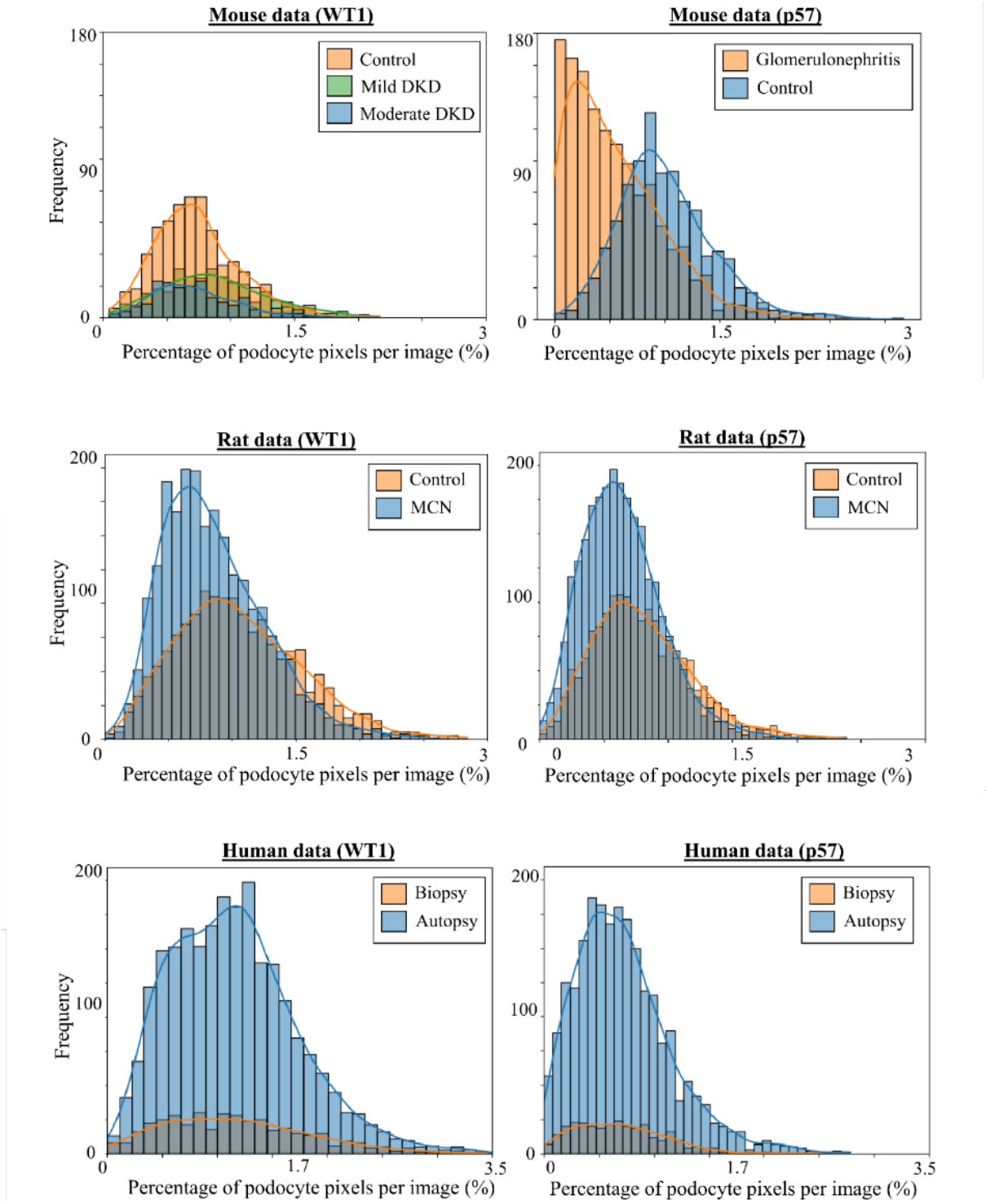
Supplementary Figure 3: Automation of Wiggins method for estimating podocyte nuclear caliper diameter. The Wiggins method was automated for the extraction of podocyte nuclear mean caliper diameters. First, the PAS and the corresponding IF/IHC images were registered. Next, the H-AI-L tool[1, 2] was used to extract the corresponding glomeruli from both sets of WSIs and the IF/IHC images were used to identify podocyte nuclei in the PAS images. Lastly, the height and width of the podocyte nuclei were averaged to extract the apparent mean averaged nuclear caliper diameter per WSI.



Supplementary Figure 4: Performance of the proposed deep learning network model in detecting and segmenting podocyte nuclei in human biopsy WSIs with respect to the number of podocyte nuclei per glomerulus and number of glomerular profiles per WSI. A) The regression plot indicates that our method remained unaffected by the number of podocyte nuclei (R^2 = 0.01, p = 0.49). B) The regression plot shows that with an increasing number of glomerular profiles per WSI, the performance of the network showed improvement (R^2 = 0.11, p = 0.05).



Supplementary Figure 5: Comparison of pix2pix results with manual annotation of podocyte nuclei. a-b) Glomerulus image patches, c-d) Manual annotation of podocyte nuclei by one of the expert annotators is shown in red. The green line separates the peripheral from the central podocyte nuclei, e-f) ground truth IF images, and g-h) pix2pix-predicted IF images detecting podocyte nuclei irrespective of their location within the glomerulus.



Supplementary Figure 6. Pixel-wise class imbalance of training data. The histograms depict the percentage of podocyte nuclei pixels per glomerulus image patch for each dataset. The data displays the class imbalance and skewness per training set. Particularly in the mouse p57 data, the training images demonstrate a right-skewed distribution, which explains the sub-optimal performance of the GAN in this particular dataset.

Supplementary Table 1: STZ dose per mouse for DKD data.

Mouse	STZ Dose (50mg/kg)
1	1.1
2	1.15
3	1.2
4	1
5	1.15
6	1.15
7	1.25
8	0
9	0
10	0
11	0
12	0
13	0
14	0

Mice #8 - #14 were control cases.

Supplementary Table 6: Comparison of conditional probability of class assignment for central and peripheral podocyte nuclei, and non-podocyte glomerular nuclei between the expert annotators and the GAN.

Conditional probability of class assignment	**Peripheral podocytes (*n* = 548)**	**Central podocytes ($n = 131$)**	**Other nuclei ($n = 1705$)**
Pr(pix2pix|GT)	0.93 [0.90 0.95]	0.86 [0.79 0.92]	0.94 [0.93 0.95]
Pr(Annotator#1| GT)	0.88 [0.85 0.91]	0.24 [0.17 0.33]	0.96 [0.95 0.97]
Pr(Annotator#2| GT)	0.84 [0.80 0.87]	0.41 [0.33 0.50]	0.94 [0.93 0.95]
Pr(Annotator#3| GT)	0.82 [0.78 0.85]	0.42 [0.33 0.51]	0.96 [0.95 0.97]
Pr(Avg. of Annotators| GT)	0.84 [0.81 0.87]	0.36 [0.28 0.44]	0.95 [0.94 0.96]

Each value reported as observed value [lower 95% confidence interval, upper 95% confidence interval].

References

1. Lutnick B, Ginley B, Govind D, McGarry SD, LaViolette PS, Yacoub R, et al. An integrated iterative annotation technique for easing neural network training in medical image analysis. Nature machine intelligence. 2019;1(2):112-9.
2. Lutnick B, Manthey D, Sarder P. A tool for user friendly, cloud based, whole slide image segmentation. arXiv preprint arXiv:210107222. 2021.