

# The spatially resolved transcriptome signatures of glomeruli in chronic kidney disease

## Supplementary Results

### DSP reveals transcriptional programs in adult AS and FSGS glomeruli

Because some cases of familial FSGS present with *Col4V* gene mutations (1-3), and share some common pathological features with AS, we determined if AS and FSGS glomeruli share patterns of gene expression. As shown in the PCA in Figure 4A, major differences between AS #3 and non-diseased glomeruli #9, #10 based on the top 10% of genes in PC2 and PC3 are associated with focal adhesions and G protein-coupled receptor signaling pathway, but little difference in PC1. In contrast, the glomeruli from the FSGS biopsies #4, #5 separated from controls predominantly in PC1, with the top 10% of genes driving the PC1 separation associated with focal adhesion, selenocysteine synthesis and SRP-dependent co-translational protein targeting to membrane (Suppl. Dataset 2).

Our analysis revealed 930 common genes that were differentially expressed (97 upregulated and 833 downregulated) in the glomeruli of AS and FSGS vs non-diseased glomeruli. We performed enrichment analysis on the 97 genes that were commonly upregulated and found pathways related to mitotic cell cycle phase transition, angiogenesis and Hippo signaling (Suppl. Figure 6A). We also performed enrichment analysis on the 833 genes that were commonly downregulated and found pathways associated with G protein-coupled receptor signaling pathway (Suppl. Figure 6B; Suppl. Dataset 5). Complete results of the enrichment analysis of those genes differentially up or downregulated between each disease and non-diseased control, as shown in the Venn diagram in Suppl. Figure 6A and B are reported in Suppl. Dataset 5).

### DSP reveals transcriptional programs in young and adult AS

We compared the transcriptional profiles of glomeruli in the young AS to that in the adult AS patients to determine potential similarities and differences. PCA analysis clearly revealed that young and adult AS glomeruli have small but measurable transcriptional differences (Suppl. Figure 6C). Enrichment analysis based on the top 10% of genes in PC1 and PC2 revealed pathways associated with cytoplasmic translation, selenocystein synthesis, and SRP-dependent co-translational protein targeting to membrane among others (Suppl. Dataset 11). Analysis of the genes differentially upregulated in young AS vs adult AS glomeruli showed enrichment in gene ontology terms associated with regulation of apoptosis and transcriptional pathway (Suppl. Figure 6D, Suppl. Dataset 12). Our analysis found no enrichment for the downregulated genes. These results may suggest potential differences in the

glomeruli between young and adult AS patients in the cellular mechanisms related to protein production and processing, such as selenocysteine synthesis (4,5).

### **DSP reveals transcriptional programs in adult AS glomeruli**

We performed DSP on an adult AS patient (#3) separately from the young AS patients because of the age difference and other CKDs to reveal patient specific transcriptional features and used non-diseased glomeruli from two adult patients as a reference control (Table 1).

We analyzed all the detected genes, and transcripts commonly expressed (n=1,058) in all glomeruli (n=11) showed a very similar enrichment pattern to that observed in the young AS patients, including L13a-mediated translational silencing of ceruloplasmin expression, selenocysteine synthesis and ECM-receptor interactions (Suppl. Figure 7A, Suppl. Dataset 13).

We compared the adult AS glomeruli with non-diseased glomeruli from adult patient nephrectomy specimens (n=12, derived from two different patients #9, #10; 6 glomeruli per biopsy. PCA partitioned the AS and non-diseased glomeruli into distinct clusters (Suppl. Figure 7B, Suppl. Dataset 13). GO enrichment analysis performed on the top 10% of genes contributing to PC1 and PC2 revealed that the functions separating the AS glomeruli and non-diseased glomeruli are associated with extracellular matrix, focal adhesions and AGE-RAGE signaling. These pathways were also found in the young AS PCA analysis (Figure 6B), indicating potential similarities.

Transcripts with higher expression in AS #3 relative to non-diseased (#9, #10) glomeruli were enriched in pathways commonly observed in CKD, such as Hippo and VEGF signaling. In addition, pathways associated with focal adhesion, vasculogenesis, kidney development and PI3K-Akt signaling were also highly enriched. Transcripts with lower expression were enriched for RNA polymerase dependent transcriptional regulation, G-protein coupled receptor signaling and pathways not previously associated to AS, such as cellular response to zinc, cadmium and copper ions and as well as olfactory receptor activity (Suppl. Figure 7C, Suppl. Dataset 13).

### **DSP reveals transcriptional programs in FSGS glomeruli**

We investigated the transcriptional changes in FSGS glomeruli following the same analysis scheme as with the AS glomeruli. Genes commonly expressed in all glomeruli (n=1,205 genes in FSGS #4, and n=636 genes in FSGS #5) were enriched in pathways associated with amino acid and protein metabolism including selenocysteine synthesis and L13a-mediated translational silencing of ceruloplasmin expression, cell-ECM interactions, and actin cytoskeleton organization. In addition, glomeruli in FSGS #5 were enriched in gene ontology terms linked to podocyte differentiation and glomerulus development (Suppl. Figure 8A-B; Suppl. Dataset 14).

We next compared the transcriptomes between FSGS and age-matched non-diseased glomeruli. The expression profiles of the two groups clearly separated on the PCA (Suppl. Figure 8C). GO enrichment analysis performed on the top 10% of genes contributing to PC1 revealed that the functions driving the separation were mainly related to selenocysteine synthesis, SRP-dependent co-translational protein targeting to membrane and L13a-mediated translational silencing of Ceruloplasmin expression. Minimal separation was detected in PC2 with functions related to vasculature development and function, cell-ECM interactions and G protein-coupled receptor signaling pathway (Suppl. Figure 8C, Suppl. Dataset 14). It is noticeable that FSGS biopsies displayed greater interglomerular-heterogeneity when compared to the non-diseased controls (Suppl. Figure 8C). Transcripts with higher expression in FSGS glomeruli (both biopsies combined) relative to non-diseased (#9, #10) glomeruli were predominantly enriched in pathways related to ECM remodeling and collagen biosynthesis. Transcripts with lower expression were enriched for expression of olfactory receptors (Suppl. Figure 8D, Suppl. Dataset 14).

### **DSP reveals transcriptional programs in MN glomeruli**

We also analyzed glomeruli affected by MN from a PLA2R-autoantibody-negative (#6) and a PLA2R-autoantibody-positive (#7) patient. PLA2R is defined as the dominant glomerular (podocyte) target epitope for autoantibodies leading to membranous nephropathy (6). Patients negative for anti-PLA2R autoantibodies are more likely to undergo spontaneous remission of nephropathy.

In the biopsy of the PLA2R (+) patient, genes common to all glomeruli (n=2,981) were highly enriched in transcriptional programs related to mitochondrial metabolic processes, vascular development, ECM organization and signaling by NOTCH and WNT pathways. (Suppl. Figure 9A, Suppl. Dataset 15). In the PLA2R (-) patient, genes common to all glomeruli (n=1,010) were highly enriched in transcriptional program similar to that of PLA2R (+) biopsy but also include pathways such as AGE-RAGE and selenocysteine synthesis pathways (Figure 9B, Suppl. Dataset 15).

We compared the transcriptome in the MN glomeruli with age-matched non-diseased glomeruli (n=12 glomeruli, #9, #10): glomeruli from the PLA2R (+) patient #7 clustered together with the non-diseased glomeruli, while the glomeruli from the PLA2R (-) patient #6 clustered separately with greater dispersion along both the PC1 and PC2 axes (Suppl. Figure 9C). GO enrichment analysis performed on the top 10% of genes contributing to PC1 and PC2 revealed that the functions driving the separation between the MN glomeruli in #6 and non-disease glomeruli were related to regulation of integrin mediated cell adhesion and interferon-mediated signaling pathway in PC1, and Rap1, Ras, PI3K-Akt signaling pathways among others in PC2 (Suppl. Figure 9C, Suppl. Dataset 15).

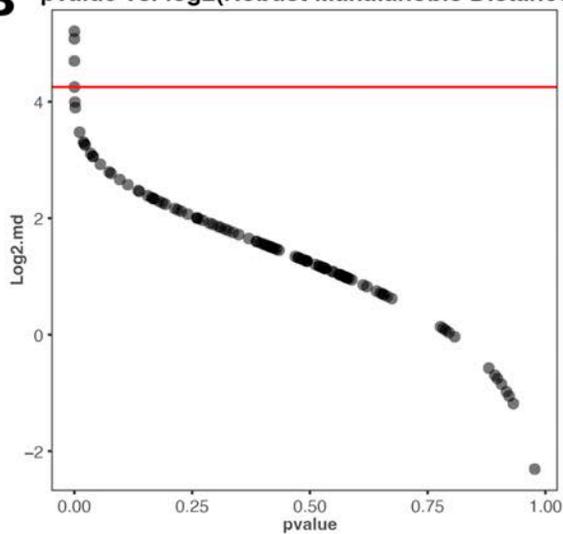
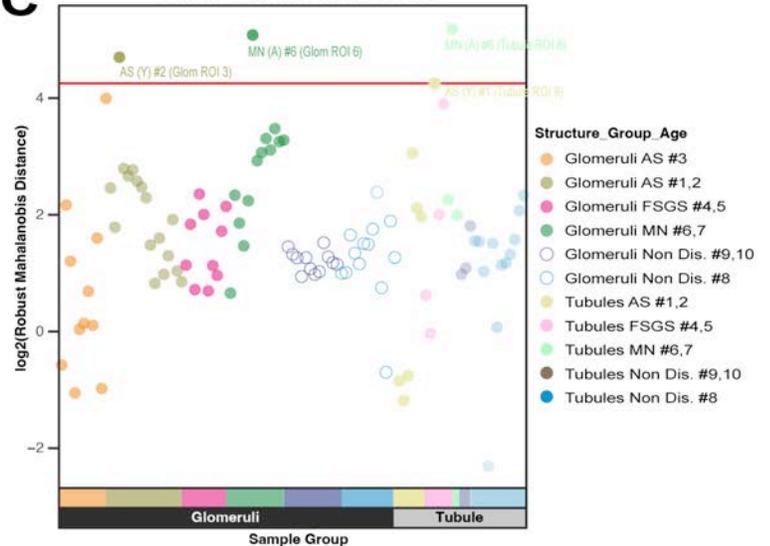
Transcripts with higher expression in MN (#6, #7) relative to non-diseased (#9, #10) glomeruli were enriched in transcriptional program related to VEGF signaling and vasculogenesis. In addition, pathways associated with PI3K-Akt, MAPK, IL17, Hippo and apelin signaling were also highly enriched. Transcripts with lower expression were enriched for selenocysteine synthesis, SRP-dependent cotranslational protein targeting to membrane, L13a-mediated translational silencing of Ceruloplasmin expression and pathways not previously associated to AS, such as cellular response to zinc, cadmium and copper ions activity (Suppl. Figure 9D, Suppl. Dataset 15).

## Bibliography

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**A**

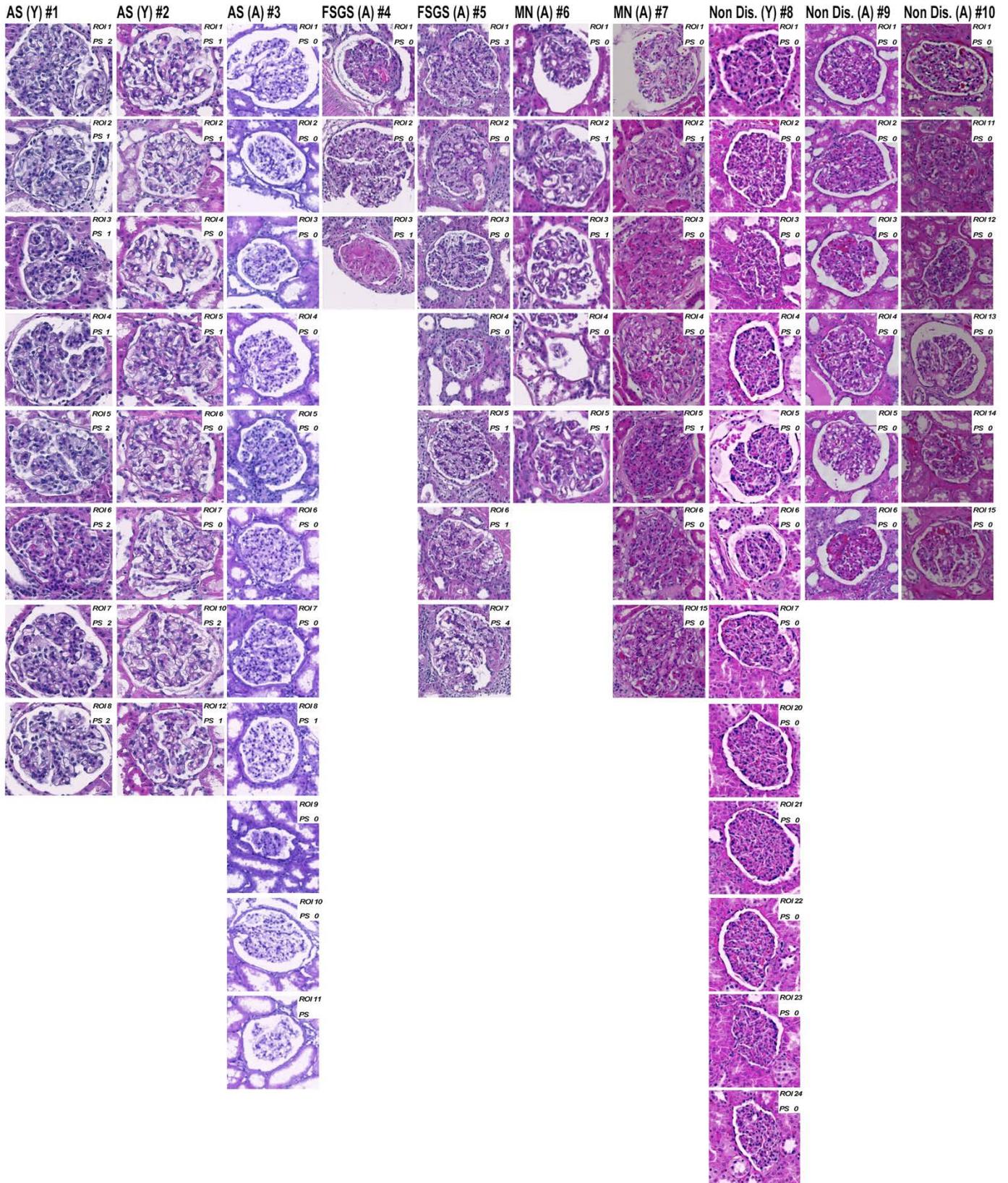
Sample ID #		1	2	3	4	5	6	7	8	9	10	
Outlier test	ROI	AS	AS	AS	FSGS	FSGS	MN	MN	Ctrl	Ctrl	Ctrl	Total ROI
Before	Glomerular	8	9	11	3	7	6	7	12	6	6	75
	Tubular	4	3	1	2	3	3	0	12	3	0	31
After	Glomerular	8	8	11	3	7	5	7	12	6	6	73
	Tubular	3	3	1	2	3	2	0	12	3	0	29

**B** pvalue vs. log2(Robust Mahalanobis Distance)**C** Sample outlier result ( $p < 1e-05$ )

## Supplementary Figure 1: Quality control assessment and elimination of glomerular and tubular ROI.

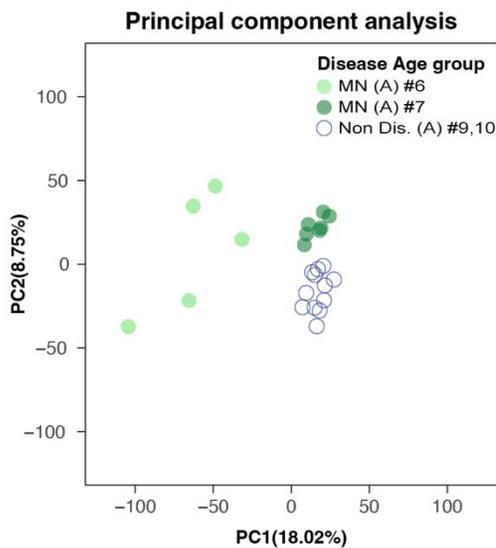
**A.** Summary table showing the number of glomerular and tubular ROI before and after the outlier detection for each disease and each patient based on a robust Mahalanobis distance (rMd) with a p value threshold of 0.0001 (see methods for further details). **B.** A scatter plot showing the distribution of glomerular and tubular ROI based on the log2 of the Mahalanobis distance (log2.md). The red line indicates the  $P = 0.00001$  value threshold. **C.** A scatter plot showing the outlier glomerular ( $n=2$ ) and tubular ROI ( $n=1$ ) above the red line based on Mahalanobis distance. The red line defines the p value threshold described in (A).

# Glomerular ROI from AS, FSGS, MN, and Non-Diseased biopsies



## Supplementary Figure 2: Histology of all glomerular ROI.

All the kidney biopsy samples that were processed for the DSP analysis (#1-10) were stained with hematoxylin and eosin for morphological characterization and assessment blindly by a pathologist. Representative images of all the glomeruli from all the patients that passed the outlier test are shown. The number of each corresponding ROI and pathology score (PS) are shown on every glomerular image.



## Supplementary Figure 3: Principal component analysis of MN

Unsupervised principal component analysis (PCA) based on label free quantification of the transcripts expressed in MN (#6, light green circles; #7, dark green circles), and adult non-diseased glomerular ROI (#9,10, blue circles), based on PC1 and PC2. Percentage of total variance explained by each component is indicated after each principal component.

## A TOP 25 upregulated genes

GENES	AS (A) #3		FSGS (A) #4,5		MN (A) #6,7	
	log2 FC	FDR	log2 FC	FDR	log2 FC	FDR
<i>TNS1</i>	0.971	5.20E-05	1.318	1.42E-06	0.616	1.04E-02
<i>CCND1</i>	0.960	1.12E-04	0.621	5.45E-03	0.877	5.52E-03
<i>GJA5</i>	0.946	2.87E-03	0.867	2.74E-03	1.332	3.79E-04
<i>UNC5B</i>	0.937	1.17E-04	0.575	4.65E-02	0.432	4.68E-02
<i>LAMP1</i>	0.901	1.72E-03	0.765	4.67E-03	0.795	1.14E-02
<i>MYO1E</i>	0.867	4.64E-04	0.453	4.23E-02	0.718	4.39E-03
<i>NOTCH3</i>	0.864	1.02E-03	0.885	3.49E-05	0.653	1.19E-03
<i>TP53INP2</i>	0.857	2.05E-03	0.703	7.59E-05	0.442	2.42E-02
<i>PALLD</i>	0.811	4.60E-05	0.784	3.02E-04	0.530	3.10E-02
<i>CLIP3</i>	0.792	2.67E-04	0.311	2.32E-02	0.693	8.26E-03
<i>PDGFRB</i>	0.774	4.75E-03	0.984	3.18E-05	0.493	1.87E-02
<i>TRIM8</i>	0.770	1.04E-03	0.548	9.27E-03	0.663	2.68E-03
<i>MECOM</i>	0.736	4.60E-05	0.705	7.33E-05	0.494	3.61E-02
<i>TM9SF2</i>	0.731	6.67E-04	0.565	8.85E-03	0.581	8.90E-03
<i>PRRC2B</i>	0.727	1.81E-04	0.377	1.26E-02	0.386	8.98E-03
<i>TBX3</i>	0.725	3.82E-03	0.608	1.56E-02	0.768	4.39E-03
<i>LEPROT</i>	0.692	1.82E-03	0.536	3.23E-02	0.530	1.93E-02
<i>DYNLRB1</i>	0.692	9.06E-05	0.622	2.23E-04	0.904	1.55E-07
<i>TMTCT1</i>	0.681	1.19E-02	0.914	1.43E-04	0.525	1.79E-02
<i>RFXP1</i>	0.655	4.03E-02	0.664	1.60E-03	0.463	2.79E-02
<i>PLOD1</i>	0.643	8.75E-03	0.448	3.32E-02	0.619	7.40E-03
<i>PIM3</i>	0.642	8.58E-03	0.515	2.22E-02	0.507	5.52E-03
<i>PTPN12</i>	0.633	3.05E-04	0.517	2.09E-04	0.545	7.78E-04
<i>ZNF460</i>	0.614	1.34E-02	0.430	9.40E-03	0.434	4.61E-02
<i>MEIS2</i>	0.594	2.36E-02	0.498	2.08E-02	0.705	6.04E-03

## B TOP 25 downregulated genes

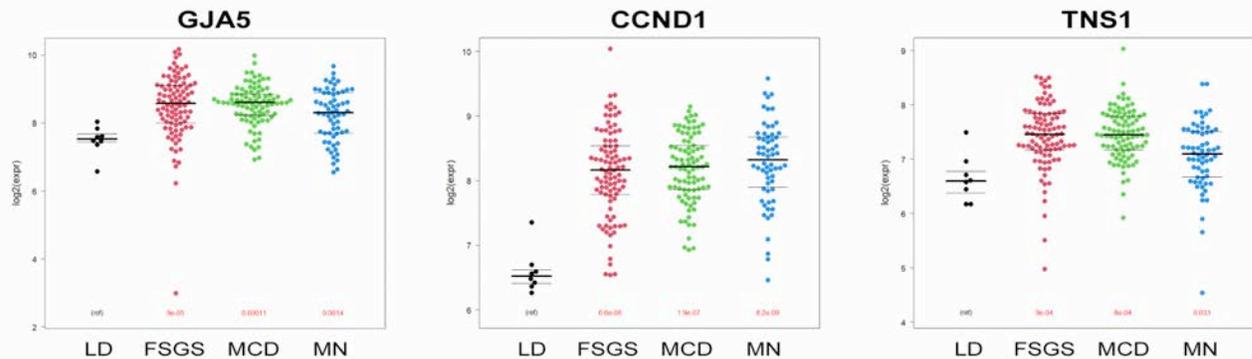
GENES	AS (A) #3		FSGS (A) #4,5		MN (A) #6,7	
	log2FC	FDR	log2FC	FDR	log2FC	FDR
<i>TMEM30A</i>	-3.605	2.48E-14	-3.805	5.13E-07	-0.907	4.50E-02
<i>XKR6</i>	-3.599	9.11E-15	-2.312	4.43E-11	-1.029	4.27E-05
<i>TTC31</i>	-3.528	1.84E-06	-3.024	1.03E-04	-1.559	3.07E-02
<i>ADAMTS13</i>	-3.500	1.47E-12	-4.166	3.55E-14	-1.740	1.05E-09
<i>TMA7</i>	-3.368	2.38E-22	-2.971	1.02E-08	-1.338	4.04E-04
<i>HOXB8</i>	-3.217	4.57E-10	-4.177	1.09E-18	-1.593	1.21E-08
<i>ZNF346</i>	-3.187	1.33E-11	-3.168	1.73E-05	-1.253	4.63E-03
<i>TRAF3IP3</i>	-3.175	1.04E-03	-2.384	1.96E-06	-1.818	7.28E-04
<i>KLRD1</i>	-3.052	3.13E-12	-3.165	1.07E-04	-1.072	2.79E-02
<i>PLEKHN1</i>	-2.940	1.60E-11	-3.207	2.40E-14	-0.929	2.09E-05
<i>CIQTNF4</i>	-2.807	1.82E-11	-2.341	2.38E-13	-0.568	1.04E-03
<i>ZNF468</i>	-2.780	1.21E-11	-4.780	4.98E-05	-1.535	4.12E-02
<i>SEMA6C</i>	-2.766	2.48E-14	-2.578	4.06E-10	-0.516	1.12E-02
<i>INHBB</i>	-2.661	6.73E-07	-2.644	1.31E-06	-0.883	3.41E-02
<i>SARDH</i>	-2.444	5.42E-12	-2.281	1.12E-02	-0.815	4.79E-02
<i>SOX4</i>	-2.322	4.50E-14	-2.758	1.76E-14	-0.352	3.10E-02
<i>PSMG3</i>	-2.274	2.64E-06	-1.985	6.39E-06	-1.002	3.06E-02
<i>IL1RAPL2</i>	-2.205	9.74E-13	-2.057	1.55E-09	-0.619	8.13E-04
<i>ATP5MG</i>	-2.161	4.25E-10	-2.179	6.56E-11	-1.057	2.19E-04
<i>HSPB8</i>	-2.132	1.41E-13	-4.129	1.09E-06	-0.955	5.43E-04
<i>PRAMEF1</i>	-2.112	1.07E-10	-2.852	8.55E-05	-0.897	3.95E-02
<i>MAGEA4</i>	-2.111	1.14E-01	-1.896	2.04E-03	-1.106	7.78E-04
<i>RCHY1</i>	-2.110	5.18E-12	-2.689	2.62E-05	-1.120	3.27E-02
<i>CCDC13</i>	-2.083	2.09E-04	-1.917	1.10E-04	-0.326	1.82E-02
<i>ZNF43</i>	-1.965	2.36E-09	-4.093	1.74E-05	-0.995	4.31E-02

**Supplementary Figure 4: Top upregulated and downregulated genes common to all the adult glomeruli in AS, FSGS and MN patients.**

**A-B.** Tables showing the 25 most highly upregulated (A) and downregulated (B) genes showing log2 fold change (log2FC), false discovery rate (FDR) for AS (#3), FSGS (#4,5) and MN (#6,7) patients compared to non-diseased controls (#9,10).

**A**

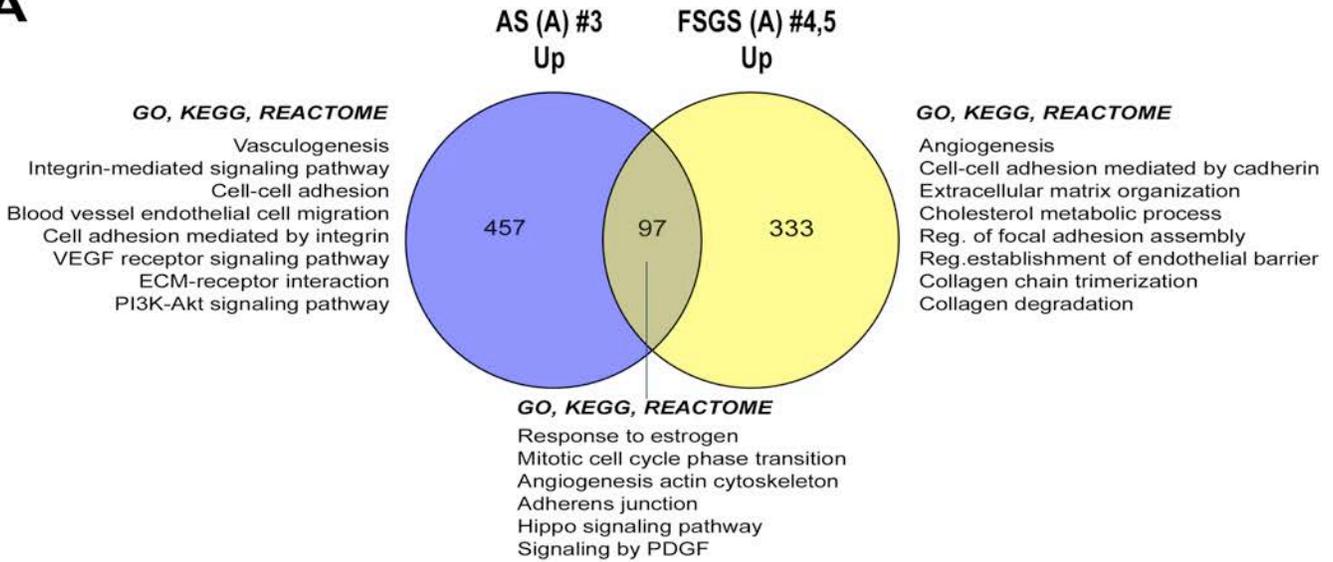
Neptune Glomerular Data Set	Samples
Living Donor (LD, control)	8
FSGS (focal segmental glomerulosclerosis)	93
MCD (minimal change disease)	89
MN (membranous nephropathy)	62

**B**

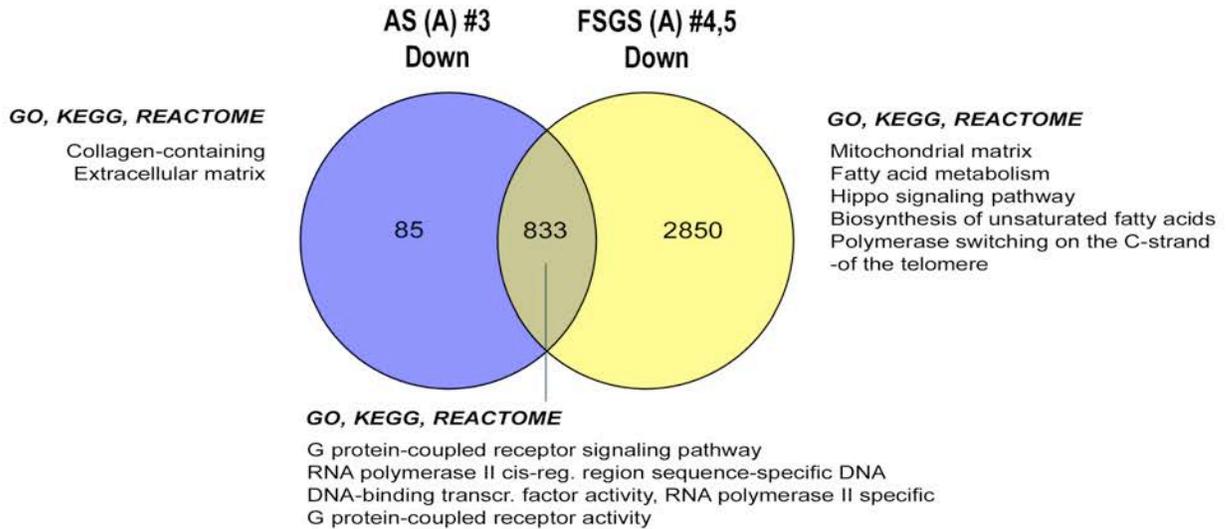
### Supplementary Figure 5: Neptune glomerular data set

**A.** Number of samples of microdissected glomeruli from living donor (LD), FSGS, MCD (minimal change disease), and MN. Bulk RNA-seq was performed and data normalized by Limma-Voom and DE analyzed by Limma. **B.** Dot plots showing the relative expression of GJA5, CCND1, TNS1 in LD, FSGS, MCD and MN patients. P values are based on the moderated t-statistic estimated by the limma R package (7).

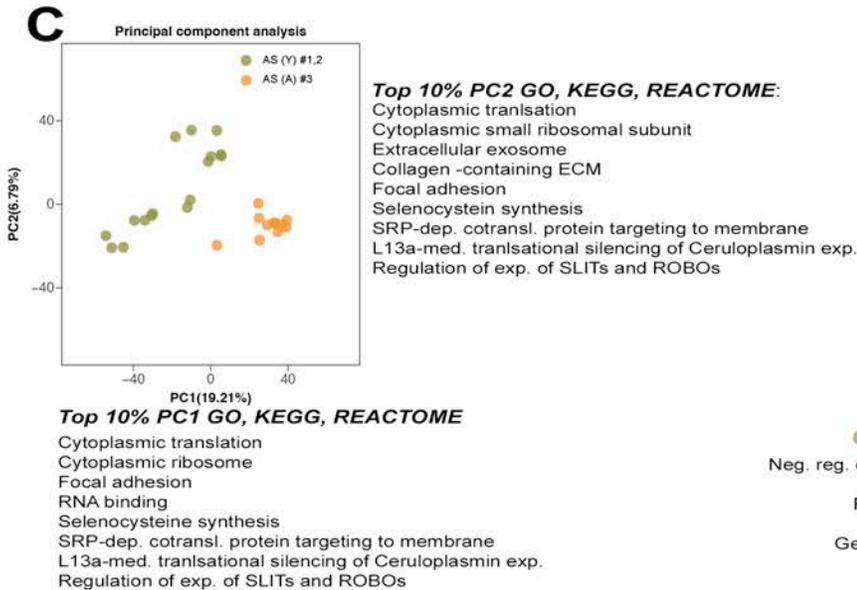
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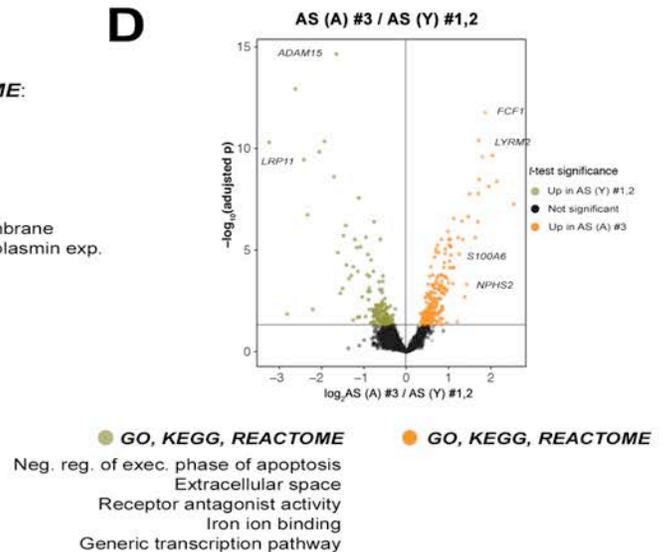
**B**



**C**

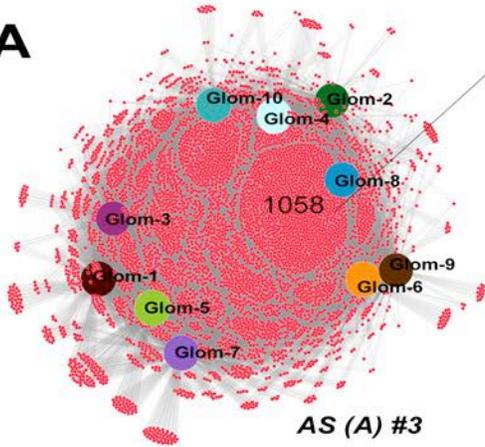


**D**



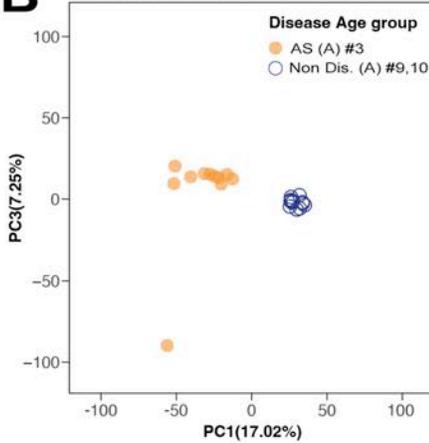
## Supplementary Figure 6: Transcriptional program of glomeruli in adult AS compared with adult FSGS and young AS.

**A.** Venn diagram showing the total number of differentially upregulated genes (Student t-test, and binomial test BH adjusted- $P < 0.05$ ) in AS (#3) and FSGS (#4-5). A list of significantly enriched GO terms, KEGG and REACTOME pathways (EASE modified Fisher's exact  $p < 0.05$ ) for the genes commonly upregulated ( $n=97$  genes), upregulated only in AS (#3,  $n=457$ ) and only in FSGS (#4,5,  $n=333$ ) are depicted next to the Venn diagram. **B.** Venn diagram showing the total number of differentially downregulated genes (Student t-test, and binomial test BH adjusted- $P < 0.05$ ) in AS (#3) and FSGS (#4-5). A list of significantly enriched GO terms, KEGG and REACTOME pathways (EASE modified Fisher's exact  $p < 0.05$ ) for the genes commonly downregulated ( $n=833$  genes), downregulated only in AS (#3,  $n=85$ ) and only in FSGS (#4,5,  $n=2,850$ ) are depicted next to the Venn diagram. **C.** Unsupervised principal component analysis based on label free quantification of the transcripts expressed in glomeruli of AS (#3) versus AS (#1,2) patients analyzed based on principal components PC1 and PC2. Percentage of total variance is indicated on each PC axis. A list of significantly enriched GO terms, KEGG and REACTOME pathways (EASE modified Fisher's exact  $p < 0.05$ ) for the list of 10% transcripts significantly higher or lower in abundance in each PC are shown next to the plot. **D.** Volcano plot representing the result of the Student's  $t$ -test (BH adjusted- $P < 0.05$ ) comparison of differential gene expression between glomeruli of AS (#3) versus AS (#1,2) patients. A select list of GO terms, and KEGG pathways significantly enriched (EASE modified Fisher exact,  $P < 0.05$ ) for genes expressed higher in each group (Student's  $t$ -test and binomial test) is depicted on each side of the volcano plot.

**A****GO, KEGG, REACTOME**

Cadherin binding  
 Integrin binding  
 ECM binding  
 Cytoplasmic translation  
 Blood vessel morphogenesis  
 Nephron development  
 Vasculogenesis  
 Oxidative phosphorylation  
 Kidney development  
 Nephron epithelium development  
 Glomerulus development  
 Kidney endothelium development  
 Endothelial cell migration

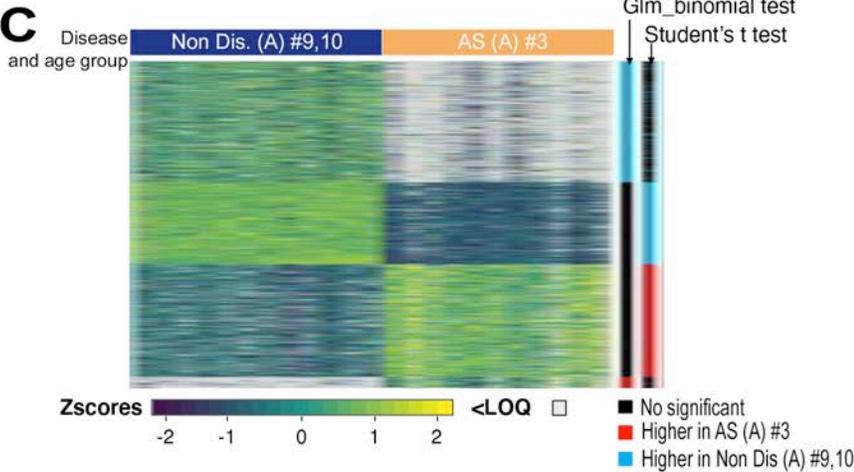
Cell-matrix adhesion  
 Neg. regulation of cell projection organiz.  
 Extracellular exosome  
 Oxidative phosphorylation  
 Focal adhesion  
 Regulation of actin cytoskeleton  
 L13a-meidated translational silencing of Ceruloplasmin expression  
 Peptide chain elongation  
 Selenocysteine synthesis  
 NMD independent of the Exon Junction Complex  
 SRP-dependent cotranslational protein targeting to membrane  
 Selenoamino acid metabolism  
 Regulation of expression of SLITs and ROBOs

**B****Principal component analysis****Top 10% PC1 GO, KEGG, REACTOME**

Cell differentiation  
 Extracellular region  
 RNA polymerase II cis-regulatory region sequence specific DNA binding  
 Metal ion binding  
 Generic transcription pathway

**Top 10% PC2 GO, KEGG, REACTOME:**

Positive regulation of angiogenesis  
 Cell migration  
 ECM disassembly  
 Regulation of oxidative phosphorylation  
 Extracellular exosome  
 Focal adhesion  
 Clathrin-coated pit  
 Cytoskeleton  
 Endoplasmic reticulum  
 Cadherin binding  
 PI3K-Akt signaling pathway  
 AGE-RAGE signaling pathway in diabetic complications

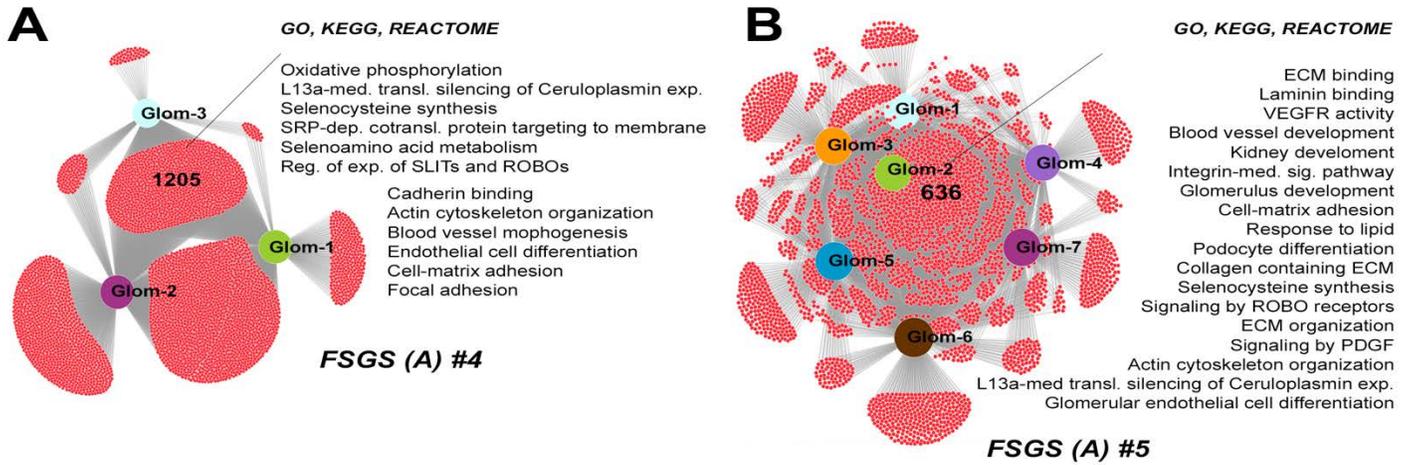
**C****GO, KEGG, REACTOME**

Vasculogenesis  
 Reg. of stem cell proliferation  
 Cell-cell adhesion  
 Integrin mediated signaling pathway  
 VEGFR signaling pathway  
 Kidney development  
 Blood vessel endothelial cell migration  
 Focal adhesion  
 Actin cytoskeleton  
 RNA poly. II cis-reg. region sequence specific DNA binding  
 Cadherin binding  
 Hippo signaling pathway  
 PI3K-Akt signaling pathway  
 Focal adhesion  
 MET activates PTK2 signaling

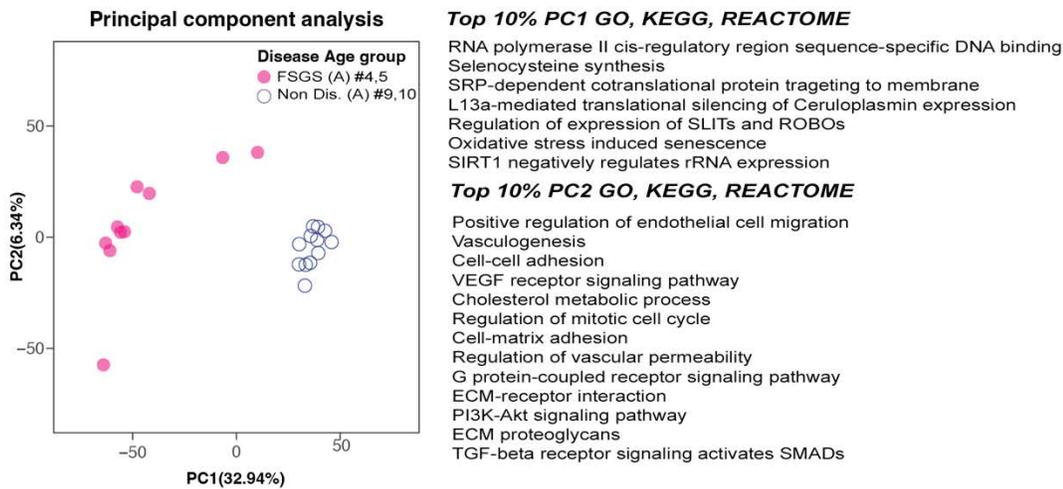
G-protein-coupled receptor signaling pathway  
 Cellular response to zinc, cadmium, copper ion  
 RNA poly. II cis-reg. region sequence specific DNA binding  
 G protein-coupled receptor activity  
 Olfactory receptor activity

## Supplementary Figure 7: Gene expression signature in adult AS glomeruli.

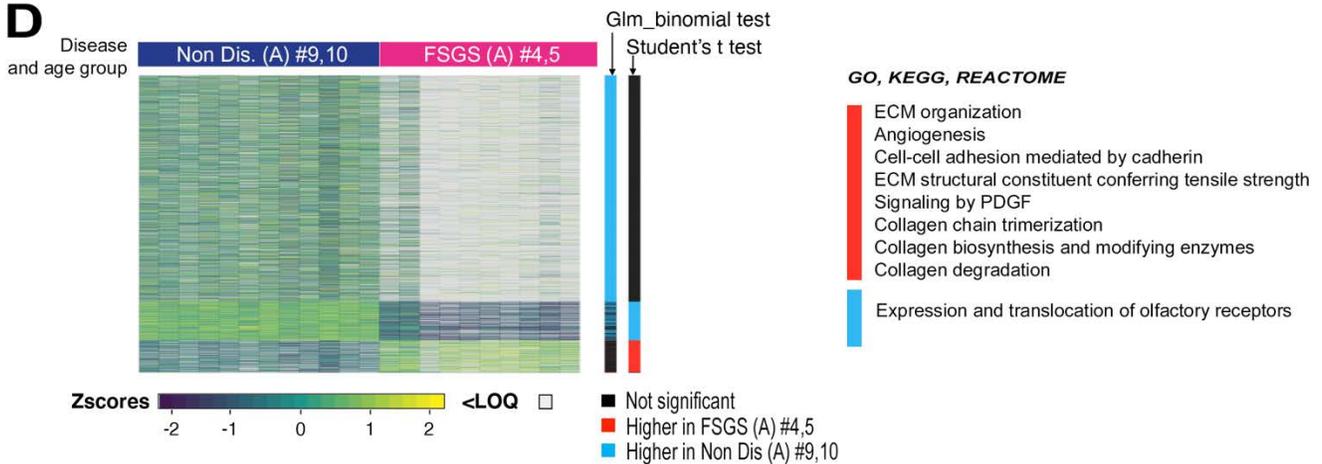
**A.** Venn diagram of TMM normalized expression of transcripts detected above LOQ in the glomeruli (n=11) of adult AS patient (#3; note the Venn diagram is showing the maximum of 10 gloms), showing the distribution of the number of genes commonly expressed in all AS #3 glomeruli (n= 1,058 genes). The black line points to the enriched pathways (GO, KEGG, REACTOME) for the commonly expressed genes between all glomeruli. **B.** Unsupervised principal component analysis (PCA) based on label free quantification of the transcripts expressed in young AS (#3, orange circles), and adult non-diseased glomerular ROI (#9,10, blue circles), based on PC1 and PC2. Percentage of variance explained by each principal component is indicated after the principal components. A select list of significantly enriched GO terms, KEGG and REACTOME pathways (EASE modified Fisher's exact  $p < 0.05$ ) for the list of 10% transcripts significantly higher or lower in abundance in PC1 and PC2 are shown next to the plot. **C.** Heatmap depicting the transcripts significantly modulated between AS (#3), and non-diseased (#9,10); analysis of variance Student's t-test and binomial test, BH adjusted- $P < 0.05$ . Transcripts less than LOQ in value are shown in white. A select list of GO terms, KEGG and REACTOME pathways significantly enriched (EASE modified Fisher exact,  $P < 0.05$ ) for the upregulated and downregulated genes in AS (#3) versus non-diseased (#9,10) are shown to the right of the heatmap.



**C**

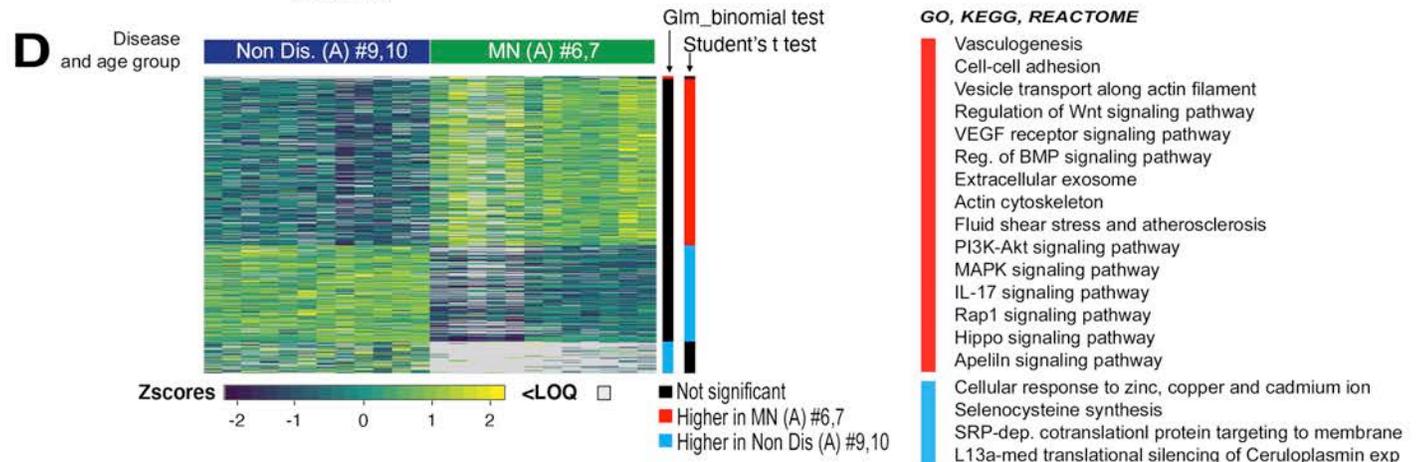
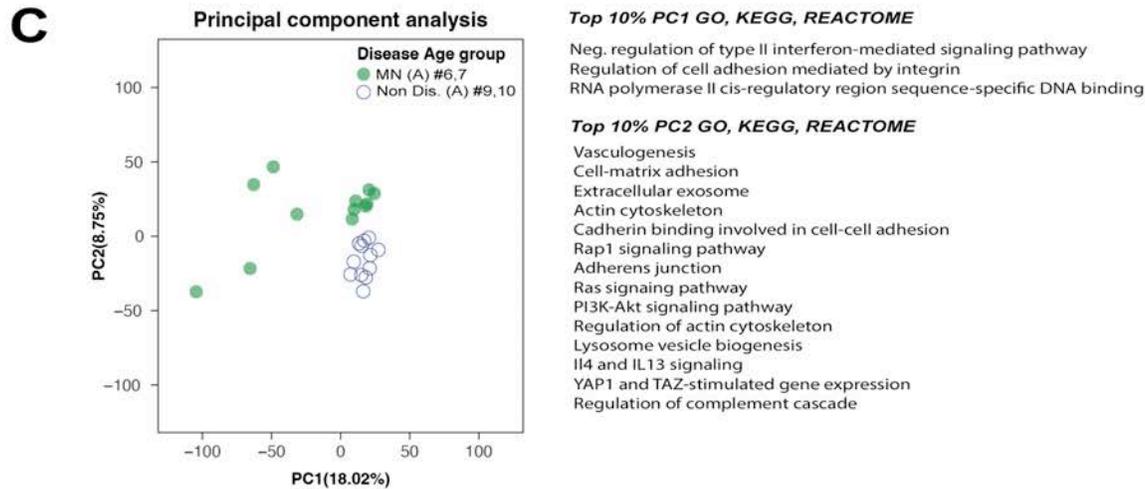
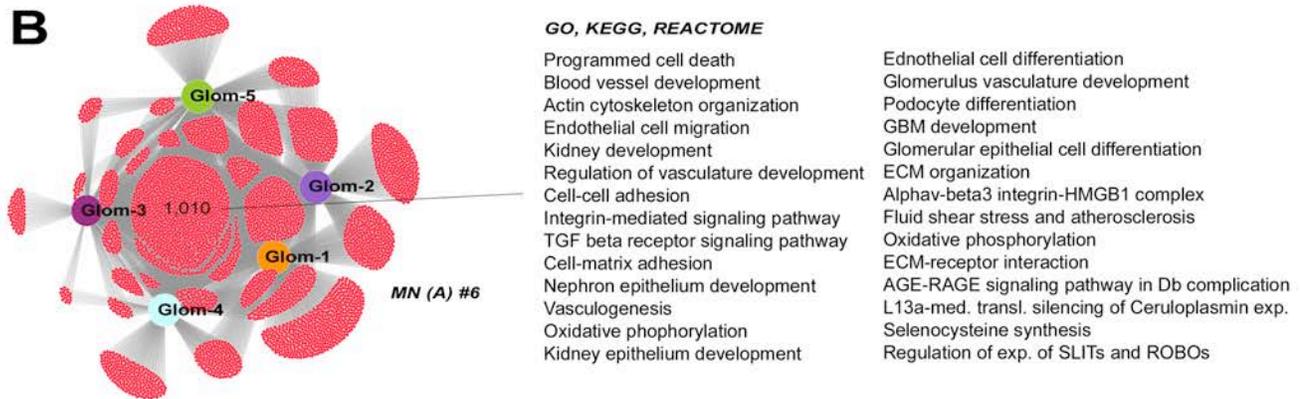
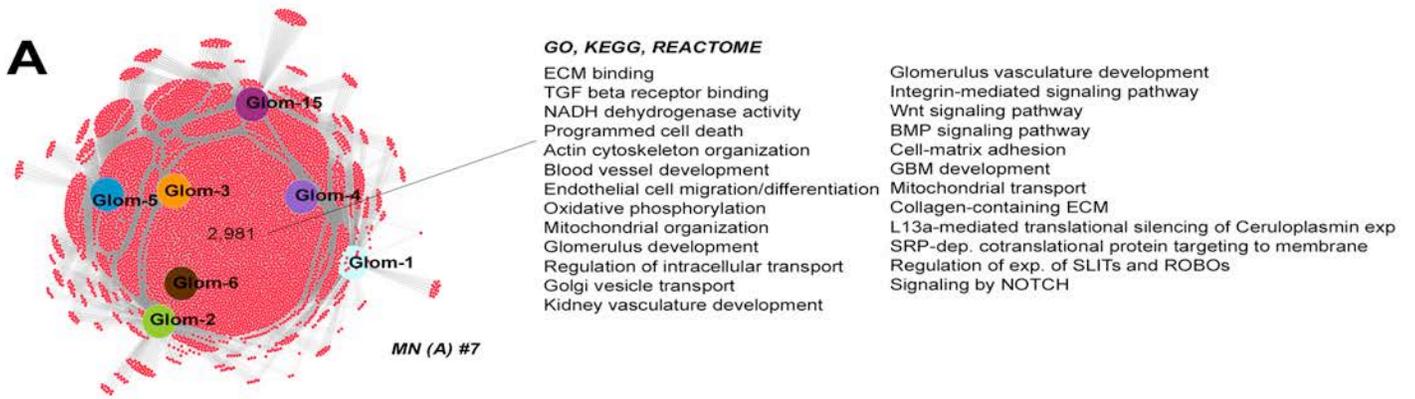


**D**



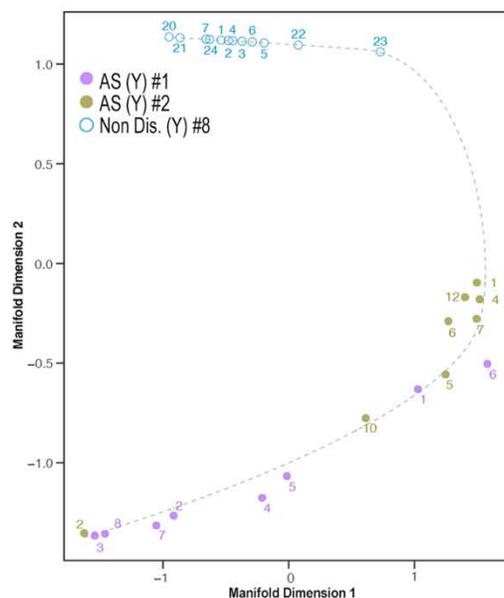
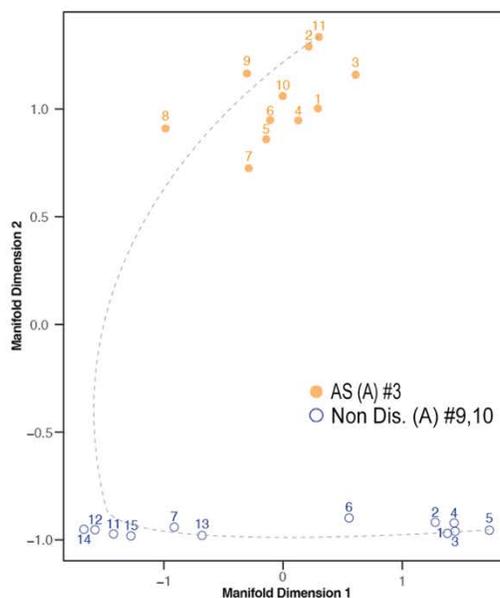
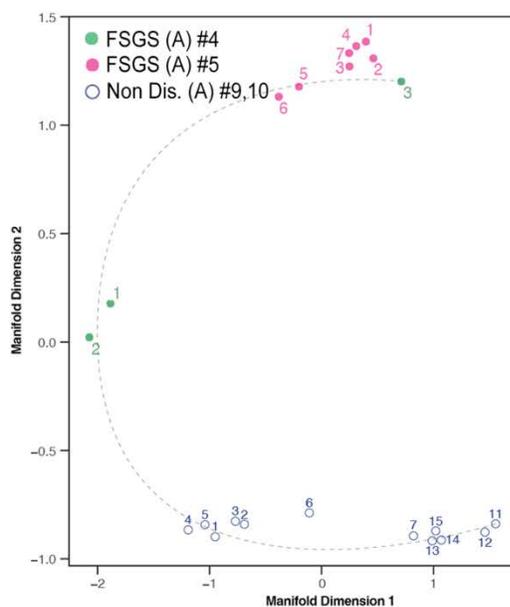
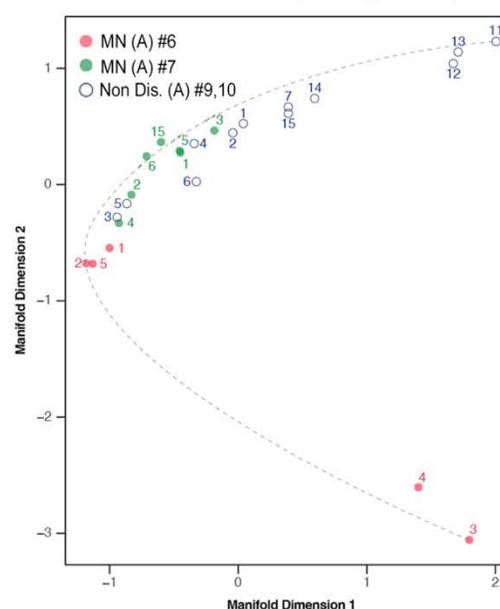
### Supplementary Figure 8: Gene expression signature in adult FSGS glomeruli.

**A-B.** Venn diagrams of TMM normalized expression of genes detected above LOQ in the adult FSGS biopsies (sample #4,5), showing the distribution of the number of genes commonly expressed in FSGS #4 glomeruli (n=1,205 genes; A), and FSGS #5 (n=656 genes; B). The black lines point to the enriched pathways (GO, KEGG, REACTOME) for the commonly expressed genes in FSGS #4 and #5. **C.** Unsupervised principal component analysis (PCA) based on label free quantification of the transcripts expressed in adult FSGS (#4,5, pink circles), and adult non-diseased glomerular ROI (#9,10, blue circles), based on PC1 and PC2. Percentage of variance explained by each principal component is indicated after the principal components. A select list of significantly enriched GO terms, KEGG and REACTOME pathways (EASE modified Fisher's exact  $p < 0.05$ ) for the list of 10% transcripts significantly higher or lower in abundance in PC1 and PC2 are shown next to the plot. **D.** Heatmap depicting the transcripts significantly modulated between FSGS (#4,5), and non-diseased (#9,10); analysis of variance Student's t-test and binomial test, BH-adjusted  $P < 0.05$ . Transcripts less than LOQ in value are shown in white. A selection of GO terms, KEGG and REACTOME pathways significantly enriched (EASE modified Fisher exact,  $P < 0.05$ ) for the upregulated and downregulated genes in FSGS (#4,5) versus non-diseased (#9,10) are shown to the right of the heatmap.



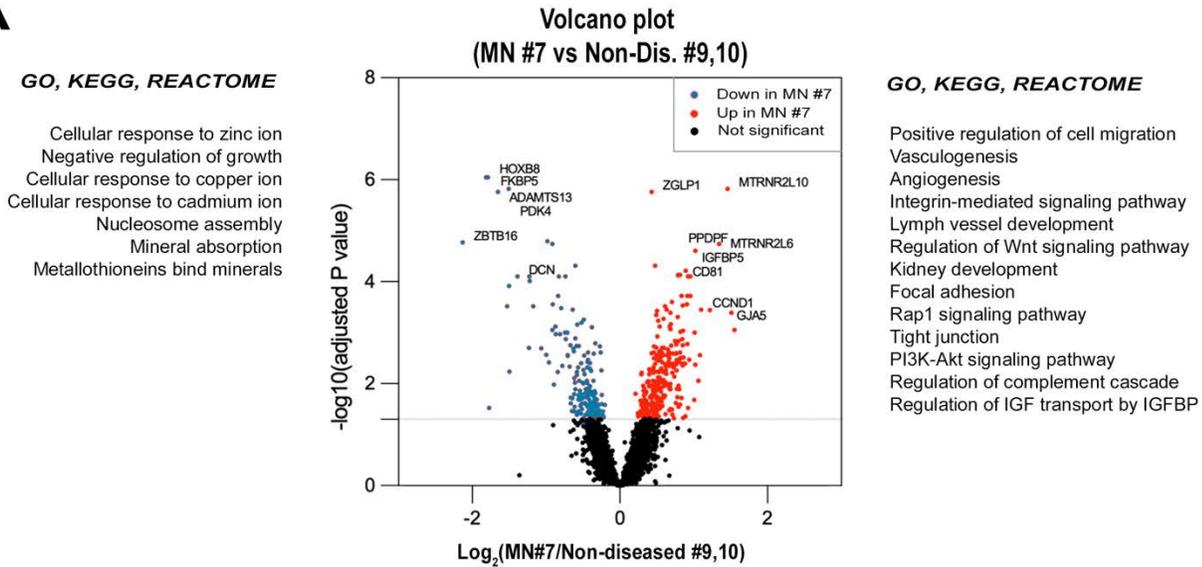
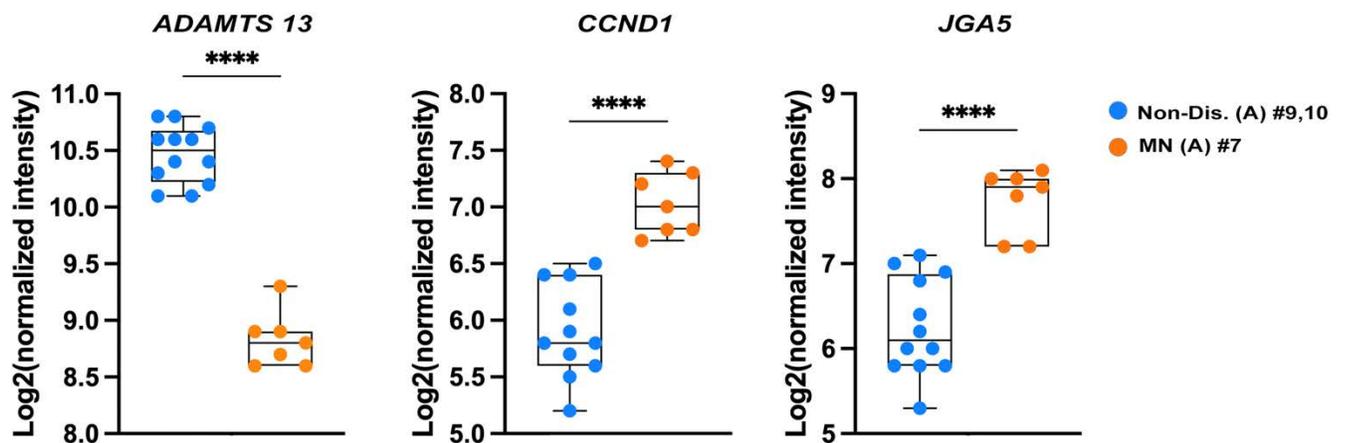
### Supplementary Figure 9: Gene expression signature in adult MN glomeruli.

**A-B.** Venn diagrams of TMM normalized expression of genes detected above LOQ in the adult MN biopsies (sample #6,7), showing the distribution of the number of genes commonly expressed in MN #7 glomeruli (n=2,981 genes; A), and MN #6 (n=1,010 genes; B). The black lines point to the enriched pathways (GO, KEGG, REACTOME) for the commonly expressed genes in MN #7 (A) and #6 (B). **C.** Unsupervised principal component analysis (PCA) based on label free quantification of the transcripts expressed in adult MN (#6,7 green), and adult non-diseased glomerular ROI (#9,10, blue circles), based on PC1 and PC2. Percentage of explained variance is indicated after each principal component. A select list of significantly enriched GO terms, KEGG and REACTOME pathways (EASE modified Fisher's exact  $p < 0.05$ ) for the list of the top 10% transcripts contributing to PC1 and PC2 are shown next to the plot. **D.** Heatmap depicting the transcripts significantly modulated between MN (#6,7), and non-diseased (#9,10); analysis of variance Student's t-test and binomial test, BH adjusted- $P < 0.05$ . Transcripts less than LOQ in value are shown in white. A select list of GO terms, KEGG and REACTOME pathways significantly enriched (EASE modified Fisher exact,  $P < 0.05$ ) for the upregulated and downregulated genes in MN (#6,7) versus non-diseased (#9,10) are shown to the right of the heatmap.

**A** SLICER LLE trajectory analysis**B** SLICER LLE trajectory analysis**C** SLICER LLE trajectory analysis**D** SLICER LLE trajectory analysis

### Supplementary Figure 10: Trajectory analysis.

**A-F.** Mapping of gene expression perturbation data to the inferred trajectories by SLICER from non-diseased control (#8, blue) and young AS (#1 purple, #2 grey-green; A), non-diseased control (#9-10) and adult AS (#3, orange; B), adult FSGS (#4 green, #5 pink; C), adult MN (#6 red, #7, green; D). The number of the glomerular ROI are depicted next to each data point. The dotted lines represent fitting curves that indicate the relationship between different glomeruli on the trajectory path.

**A****B**

**Supplementary Figure 11: Glomerular expression of *ADAMTS13*, *CCND1* and *GJA5* in PLA2R positive MN patient. A.** Volcano plot showing the transcript modulated in MN versus non-diseased controls, Student's *t*-test BH adjusted-*P* were used to generate this plot. A select list of GO terms, and KEGG pathways significantly enriched (EASE modified Fisher exact,  $P < 0.05$ ) for genes expressed higher in each group (Student's *t*-test and binomial test) is depicted on each side of the volcano plot. Boxplots of normalized intensities for *ADAMTS13*, *CCND1*, *GJA5* in glomeruli of PLA2R positive MN patient #7 versus non-diseased control (#9,10). Each dot represents a glomerular ROI. Student's *t*-test \*\*\*\* $P < 0.0001$ .