Supplementary Materials for

Recessive NOS1AP variants impair actin remodeling and cause glomerulopathy in humans and mice

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The PDF file includes:

Figs. S1 to S10

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/1/eabe1386/DC1)

Movies S1 and S2
Human Podocyte

Glomerular podocytes express NOS1AP (Fig. 2A-B, S7A-B).

Discovery of NOS1AP mutations in NS patients (Fig. 1, Table 1).

NOS1AP localizes to filopodia and podosomes (Fig. 2C-D).

WT NOS1AP, but not NS mutants, promotes filopodia and podosomes (Fig. 2C-D, 3A-B, S5).

WT NOS1AP, but not NS mutants, increases active CDC42 (Fig. 3C) with opposite effects on RAC1 (Fig. S6A).

CDC42 inhibitor CASIN blocks NOS1AP-induced filopodia (Fig. 3D, S6B).

Formin inhibitor SMIFH2 blocks NOS1AP-induced filopodia (Fig. 3E, S6C).

WT Nos1ap, but not NS mutants, rescues PMR reduced by NOS1AP knockdown (Fig. 3F).

Active CDC42 rescues PMR reduced by NOS1AP knockdown (Fig. 3G).

DIAPH3 rescues PMR reduced by NOS1AP knockdown (Fig. 3H).

NOS1AP c.428G>A mutant organoids exhibit aberrant glomerulogenesis (Fig. 4, S7-S8).

Nos1apEx3-/Ex3- mice develop proteinuric kidney disease (Fig. 5, S9-S10).

WT NOS1AP, but not NS mutants, promotes migration upstream of DIAPH3 [19-24].

CDC42 promotes filopodia through DIAPH3 and podosomes through NWASP [19-24, 41].

NWASP localizes to and induces podosomes [41].

Nwasp deletion cause NS in mice [14].

Nos1ap deletion cause murine NS [14].

Human recessive mutations in CDC42 GEFs ITSN1 and ITSN2 cause NS [8].

Itsn2 and Cdc42 deletion causes murine NS [8,10,12].

WT NOS1AP, but not NS mutants, promotes podosomes (Fig. 2C-D).

CDC42 promotes migration upstream of DIAPH3 [19-24].
**Figure S1. NOS1AP mutations impair the CDC42 actin regulatory pathway in nephrotic syndrome.**

This diagram delineates the functional aspects of *NOS1AP* loss-of-function symbolized in a human podocyte. We identified recessive mutations in *NOS1AP* as a novel cause of monogenic NS. Based on our findings, *NOS1AP* mutations impair the activation of the established actin regulator CDC42 and its effector DIAPH3. This leads to defective filopodia and podosome formation and decreased podocyte migration rate (PMR), resulting in aberrant glomerulogenesis in a human kidney organoid model and proteinuric kidney disease in mice. Established knowledge is listed on the left, while novel NS pathway steps based on our findings are on the right. Proteins encoded by human or mouse monogenic NS genes are encoded accordingly. Abbreviations: NS, nephrotic syndrome; PMR, podocyte migration rate.
**A**

B1018 (H)  
c.428G>A  
p.C143Y  

B1018 Mother (h)  
c.428G>A  
p.C143Y  

**B**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A1018** | Autosomes | **A5106** | Autosomes |
| ![NPL Score](image) | ![NPL Score](image) |

**C**

c.345-3T>G  
Splice  

**D**

![Image A1018](image)  
![Image A5106](image)
Figure S2. Sanger sequence confirms NOS1AP mutations in A1018 and A5106.

A. Homozygosity mapping identified recessive candidate loci. Profiles of nonparametric lod (NPL) scores across genome were generated based on WES variant data using Homozygosity Mapper for A5106 (left) and based on homozygous SNPs for A1018 (right). Maximum NPL peaks indicate candidate regions of homozygosity by descent as recessive candidate loci. Black circles demonstrate the peak regions in which NOS1AP variants were identified in chromosome 1.

B. Sanger tracings for affected subject A1018 (left) and his mother (right) are shown, which confirm the NOS1AP mutation c.428G>A and demonstrate maternal segregation.

C. Sanger tracings for affected subject A5106 confirms the homozygous splice variant in NOS1AP.

D. 2nd biopsy of patient A1018 was performed at 6 years of age. Micrographs prepared from the obtained tissue demonstrate flattened podocyte foot processes (asterisks) and thickened glomerular basement membrane (hashtags).

H, homozygous; h, heterozygous
Figure S3. Paralog analysis reveals strong conservation of cysteine 143 in phosphotyrosine binding (PTB) domains and structural modeling of Cys143Tyr mutation predicts instability.

A. Ribbon structure of NUMB PTB domain (top) shows Cysteine 122 (red arrow), which is paralogous to NOS1AP cysteine 143. This cysteine residue is present on β-pleated sheet 7 in the NUMB PTB domain. A higher magnification image of the ball-and-stick representation is shown (bottom).

B. Frequency diagram of the primary amino acid sequence of 7th NOS1AP β-pleated sheet is shown. The absolute frequency of amino acids is shown below each NOS1AP residue. Cysteine 143 (red arrow) is conserved in 86/101 (85%) of PTB domains. Red shading indicates conservation in >75% PTB domains. Orange highlighting indicates conservation across 50-75% of PTB domains.

C. Frequency diagram shows the conservation of each amino acid in the NOS1AP PTB domain across 101 other PTB domains by multiple sequence alignment. Blue arrows indicate β-pleated sheets, while red bars denote α-helices. Cysteine 143 (red arrow) is only one of 4 amino acids in the 152 amino acid NOS1AP PTB domain that is highly conserved in >75% of PTB domains.

D. Matrix displays 5 structures (designated by protein data bank identifier) with >30% similarity to NOS1AP PTB sequence. The bottom left part of the table shows root-mean-square deviation, while the top right part indicates the number of residues aligned using PyMol.

E. Ribbon representation of the overlapping structures from (D) shows that these structures have high three-dimensional similarity and that the paralogous cysteines (stick representation) to NOS1AP cysteine 143 (red arrow) have similar localization in the paralogous structures.

F. The stability score output from Site Directed Mutator (pseudo ΔΔG) is shown. The negative values yielded for the cysteine-to-tyrosine mutation across all 5 structures suggests this mutation destabilizes the protein tertiary structure.

G. Expresso amino acid alignment is shown of structures from (D) to NOS1AP PTB domain. Cys143 and paralogous cysteines are colored in red. All residues that have at least one atom (excluding hydrogens) 5Å maximum away from the C_b or S_g atoms of the aligned cysteine are in yellow.

H. PyMol ribbon structures of paralogous PTB domain structural models are shown. In each structure, the mutated tyrosine residue (grey stick representation) clashes with neighboring amino acid residues (red spheres) relative to the wildtype cysteine residue (black/gold stick representation).
Figure S4. NOS1AP antibody validation effect of NOS1AP NS mutations (p.C143Y, p.I116Afs*4) on protein overexpression and localization in a human podocyte cell line.

A. NOS1AP protein domain structure is shown in relation to the immunogen against which the polyclonal rabbit NOS1AP antibody NBP2-38758 was generated.
B. Immunoblot shows that NBP2-38758 identifies overexpressed myc-tagged NOS1AP in a human podocyte cell line at the same molecular weight (kDa) as anti-myc antibody. β-actin levels demonstrate equivalent protein loading.
C. Co-immunofluorescence with NBP2-38758 reveals that this antibody identifies GFP-tagged NOS1AP upon overexpression in a human podocyte cell line (right 3 columns) by overlapping staining (yellow/orange overlap in composite row). Secondary only and non-transfected cell controls show no background signal.
D. Western blotting of Myc-tagged wild-type (WT) and NS mutation containing NOS1AP constructs expressed in a podocyte cell line shows comparable expression of WT and C143Y mutant at the full length size (FL), while the I116Afs*4 mutant yields a smaller protein at the expected 15 kDa size of the truncated protein (TR). Blots were interrogated with antibodies against MYC, NOS1AP and β-actin.
E. Upon overexpression of wildtype and NS mutant NOS1AP constructs, immunofluorescence and confocal microscopy imaging was performed with the ER marker BiP and the Golgi marker GOLGB. No constructs co-localize with BiP or GOLGB. WT and C143Y mutants similarly localize to cytoplasm and podosomes (arrowheads). The I116Afs*4 mutant construct co-localizes with DAPI staining in nucleus.
F. Immunofluorescence and confocal microscopy imaging of rat kidney sections demonstrates NOS1AP signal was abrogated by blocking peptide preadsorption (NOS1AP+PEP). Glomerular podocyte slit diaphragm marker nephrin co-staining was performed. Scale bar: 25 μm.
G. By immunofluorescence and confocal microscopy, Z-stack confirmed as in (E) that MYC-tagged NOS1AP I116Afs*4 mutant does not co-localize with the ER marker BiP.
H. Immunofluorescence and confocal microscopy was performed as in (E). Z-stack shows that the MYC-tagged NOS1AP I116Afs*4 mutant co-localizes to DAPI stained nuclei encircled by the NUP153 nuclear pore protein. Abbreviations: FL, full-length NOS1AP; TR, truncated NOS1AP. (Scale bar: 7.5 μm)
**A**

PHALL DAPI

MYC_MOCK  MYC_Hs_NOS1AP_WT  MYC_Hs_NOS1AP_C143Y

(MYC_Hs_NOS1AP_I116A*fs4 (SPLICE SITE))

**B**

PODOSOME PER TRANSFECTED CELL DISTRIBUTION

% OF MYC(+) TRANSFECTED CELLS
(N = 100 cells)

| F-actin(+) Podosomes per Cell | MYC_MOCK | MYC_NOS1AP_WT | MYC_NOS1AP_C143Y | MYC_NOS1AP_I116A*fs4 |
|-------------------------------|----------|---------------|-------------------|-----------------------|
| 0                             | 71       | 43            | 57                | 61                    |
| 1-10                          | 12       | 13            | 13                | 26                    |
| 11-20                         | 11       | 12            | 11                | 10                    |
| 21+                           | 6        | 32            | 19                | 3                     |

**D**

MYC-NOS1AP DIAPH3 DAPI

INSET
Figure S5. Wild-type NOS1AP increases podosome frequency and surrounds endogenous CDC42 effector DIAPH3 in podocytes, whereas human NOS1AP NS patient mutations have reduced effect

A. Human immortalized podocytes were transfected with MYC MOCK, MYC-tagged wildtype NOS1AP or NS mutants. Using immunofluorescence and confocal microscopy imaging, transfected cells (N = 100 cells per group) were scored for number of podosomes, defined as F-actin(+) peripheral rings. Representative images of cells from each transfection group are shown with podosomes indicated by white arrows. (Scale bar: 7.5 μm)

B. While only 6% of MOCK transfected cells had >21 podosomes, wildtype NOS1AP overexpression led 32% of cells with >21 podosomes. In contrast, the two mutant constructs p.C143Y and p.I116Afs*4 induced only 19% and 3% of cells with >21 podosomes, respectively.

C. Table shows scoring results from (B) by transfection group and cells with number of podosomes. There is a higher frequency of wild-type NOS1AP transfected cells with >21 podosomes when compared to the other transfected groups, as described in (A) (red outline).

D. Overexpressed MYC-NOS1AP (red) localizes to podosomes (based on NWASP localization in Fig. 2D) in a human podocyte cell line, in which the actin regulatory factor and CDC42 effector DIAPH3 (green) is present at the center of podosomes. (Scale bar: 2.5 μm)
Figure S6. Effect of overexpressed NOS1AP on active RHOA and RAC1 levels and of pharmacological inhibition on NOS1AP induced filopodia formation, and NOS1AP knockdown and Nos1ap, CDC42, and DIAPH3 cDNA rescue in human immortalized podocytes.

A. HEK293T cells were transfected as in Figure 3C. Active RAC1 levels were measured by G-LISA RAC1 assay. Wildtype NOS1AP did not significantly increase active RAC1 levels, while NS mutant constructs did (1.59 and 1.77-fold increases) (1-way ANOVA). Active RHOA levels were measured by G-LISA RHOA assay. Wildtype and mutant constructs had no significant effect on active RHOA levels (1-way ANOVA). Each dot represents an independent biological replicate.

B. A human podocyte cell line expressing MOCK-GFP or GFP tagged WT NOS1AP was treated with increasing doses of CDC42 inhibitor CASIN or the vehicle DMSO. Filopodia formation was quantified as in Figure 3B at 13 hours after transfection. Representative images for Figure 3D are shown with white arrows pointing to cells bearing ≥2 filopodia (scale bar: 100 μM).

C. A human podocyte cell line expressing MOCK GFP or GFP tagged WT NOS1AP was treated with increasing doses of formin inhibitor SMIFH2 or vehicle DMSO. Filopodia formation was quantified as in Figure 3B at 14 hours after transfection. Representative images for Figure 3E are shown with white arrows pointing to cells bearing filopodia (scale bar: 100 μM).

D. siRNA Knockdown of YAP1 with two independent siRNA in a podocyte cell line was validated by western blot relative to non-transfected (NT) and scrambled siRNA transfected (Scr) controls.

E. Human immortalized podocyte cells were transfected with either scrambled or YAP1 siRNA and, at 24 hours, with Mock-GFP or GFP tagged WT NOS1AP. Filopodia formation was quantified as in Figure 3B at 10 hours after plasmid transfection. There was no statistically significant difference in filopodia formation with YAP1 knockdown by 1-way ANOVA.

F. As in (E), human podocytes transfected with either Mock-GFP or GFP tagged WT NOS1AP were subsequently treated with NOS1 inhibitor and arginine analog L-NAME versus vehicle control (PBS). Filopodia formation was quantified as in Figure 3B at 13 hours. There was no difference in NOS1AP-induced filopodia formation between L-NAME and vehicle groups.

G. Quantitative RT-PCR of NOS1AP mRNA expression was performed in scrambled shRNA expressing versus NOS1AP-specific shRNA expressing human immortalized podocytes. Mean NOS1AP knockdown was 75% relative to scrambled control cells.

H. Knockdown of NOS1AP was assessed by western blot, showing reduced protein expression in knockdown cells relative to scrambled control (black arrowhead).

I. Cell proliferation assay (XTT) was performed in human immortalized podocytes. Graph shows absorbance readings (475 nm – 660 nm) in arbitrary units for negative control (full media only), two independent scrambled shRNA control lines and three independent lines expressing NOS1AP-specific shRNA. No discernible difference in proliferation behavior was observed in between the different cell lines.

J. Immunoblotting for apoptosis marker cleaved caspase 3 was performed in scrambled shRNA expressing versus NOS1AP-specific shRNA expressing human immortalized podocytes. Long exposure (3000 s) reveals faint band at the size of (uncleaved) procaspase 3 (black arrowhead) but no detectable signal at the expected size of cleaved caspase 3 (grey arrowhead).

K. cDNA constructs of myc-tagged wildtype mouse Nos1ap (WT) and constructs based on human NOS1AP mutations (C143Y, I116Afs*4) were over-expressed in a NOS1AP shRNA expressing human podocyte cell line. Protein lysates at 12 hours post-transfection were evaluated by immunoblotting, showing comparable protein levels of the wildtype and C143Y construct (FL, full length). The I116Afs*4 construct has lower protein levels at a smaller molecular weight (TR, truncated).

L. As in (K), a NOS1AP shRNA podocyte cell line was transfected with cDNA of myc-tagged wildtype human CDC42 (WT) and cDNA constructs of the hypomorphic variant T17N and constitutively active variant Q61L. Protein lysates at 24 hours post-transfection were evaluated by immunoblotting, showing comparable protein levels.

M. As in (K), a NOS1AP shRNA podocyte cell line was transfected with a myc-tagged wildtype human DIAPH3 cDNA construct. Protein lysates at 24 hours post-transfection were evaluated by immunoblotting, showing expression of this construct.

N. Knockdown of NOS1AP (red) caused reduced PMR compared with scrambled shRNA (black). This was rescued by overexpression of WT Nos1ap (green) but not by wildtype CDC42 (purple) nor the hypomorphic CDC42 mutant T17N (pink).
Figure S7. Generation of human iPSC-derived and kidney organoids harboring the recessive NOS1AP NS patient mutation c.428G>A.

A. Single cell RNA sequencing data of wild type kidney organoids illustrates enrichment of NOS1AP mRNA expression in the podocyte cluster, which is marked by enriched expression of NPHS1 and NPHS2.

B. Bulk RNA sequencing data illustrates increased NOS1AP mRNA expression in sieved 3D organoid glomeruli than in 2D cultures of immortalized podocytes.

C. The CRISPR guide RNA and DNA repair template is shown, which was employed to mutate the wildtype (WT) G nucleotide (green) for missense variant A (red) for G. In addition, the synonymous 3bp change (blue) upstream was employed for ease of identification of edited clones.

D. Sanger sequencing chromatograms from WT and NOS1AP mutant (c.428G>A) iPSC clones are shown, demonstrating knock-in of the c.428G>A missense variant (red) and the synonymous 3bp change (blue) in the mutant clone.

E. Kidney organoid differentiation schema is shown. The organoid culture was performed as described before (62, 63). The following doses were employed for each reagent: CHIR99021 7um, FGF9 200ng/mL, and ATRA 2uM. No GF (growth factors) were administered after day 14.

F. Immunofluorescence images of bioprinted kidney organoids from both iPSC clones showing expected staining for podocytes (Nephrin in white), proximal tubule cells (LTL in blue), distal tubule cells (ECAD in green) and urothelial precursor cells (positive for both ECAD in green and GATA3 in red). (Low power scale bar 500 μm, inset scale bar 100 μm.)
Figure S8. Kidney organoids harboring the recessive NOS1AP NS patient mutation c.428G>A show comparable NOS1AP protein levels but exhibit aberrantly formed glomeruli and increased apoptosis.

A. Immunofluorescence of NOS1AP demonstrates localization to podocytes in organoid glomeruli adjacent to the podocyte marker synaptopodin (SYNPO; merge image shown in Figure 4A) and basement membrane protein laminin A5 (LAMA5). (Scale Bars left panel 20 μm, right panel 10 μm.)

B. Quantification of NOS1AP mRNA levels normalized to MAFB mRNA levels by quantitative RT-PCR shows no significant difference between wildtype and knock-in organoids.

C. Quantitation of NOS1AP protein levels normalized to MAFB protein levels by SimpleWes capillary Western Blot shows no significant difference between wildtype and knock-in organoids.

D. Schematic overview of human NOS1AP protein shows the position of patient A1018 missense mutation as well as the immunogens used to generate two different NOS1AP antibodies. Antibody 1, Novus NBP2-38758; Antibody 2, Novus NBP2-38151.

E. By whole-mount immunofluorescence staining with NOS1AP antibody 1, wild type organoids demonstrate NOS1AP localization to glomeruli (SYNPO) with no signal evident in proximal (HNF4A, nuclear) or distal (ECAD, cell wall) tubules. NOS1AP c.428G>A organoids demonstrated no detectable NOS1AP signal. (Scale bars: 50 μm). This was validated across multiple differentiations in two independent iPSC backgrounds.

F. Whole-mount immunofluorescence using NOS1AP antibody 2, which was generated against a more C-terminal immunogen of NOS1AP, shows comparable signal intensity and glomerular localization both for wild type and NOS1AP c.428G>A organoids.

G. Whole-mount immunofluorescence of organoids derived from iPSC cell line PCS201010 (iPSC cell line CRL1502 shown in Figure 4F) for apoptotic marker cleaved capase 3 (CASP3) is shown. CASP3 signal is increased in glomeruli (NPHS1 positive area) of NOS1AP mutant organoid glomeruli, relative to wildtype organoids. CASP3 signal in tubular segments (HNF4A positive area) is not increased. (Scale Bar 100 μm.)

H. Additional images of wildtype (WT) and NOS1AP c.428G>A mutant organoids (PAS staining) are shown as in Figure 4B, where glomerular tufts (within white lines) were defined as linear podocyte monolayers organized bilaterally about established extracellular matrix (black lines) and were reduced in NOS1AP mutant organoids. Mutant organoids also demonstrate increased pyknotic nuclei (arrowheads), indicative of cell death. (Scale Bar 20 μm.)
### Table: Serum Albumin by Age (months)

| Age (months) | Serum Albumin (g/dL) |
|--------------|----------------------|
| 3-4          |                      |
| 5-6          |                      |
| 7-8          |                      |
| 9-10         |                      |
| 11-12        |                      |
| 13-15        |                      |

**Legend**
- Nos1apEx3-/+ (n=10)
- Nos1apEx3-/Ex3- (n=10)
- Wild Type (n=5)

### Graphs

#### A
- **X-axis**: Age (months)
- **Y-axis**: Serum BUN (mg/dL)
- **Legend**:
  - WT
  - Het
  - HOM

#### B
- **X-axis**: Age (months)
- **Y-axis**: Urine Albumin / Creatinine Ratio (mg/mg)
- **Legend**:
  - Albuminuria - female Nos1apEx3-/Ex3- vs all controls
  - Albuminuria - female Nos1apEx3-/Ex3- vs male & female WT

#### C
- **Image**: Protein gel analysis
- **Markers**: 60 kDA, 80 kDA

#### D
- **X-axis**: Age (months)
- **Y-axis**: Serum Albumin (g/dL)
- **Legend**:
  - Wild Type (N=5)
  - Nos1apEx3+/+ (N=10)
  - Nos1apEx3-/Ex3- (N=10)

#### E
- **X-axis**: Age (months)
- **Y-axis**: Urine Albumin / Creatinine Ratio (mg/mg)
- **Legend**:
  - female (N=5)
  - male (N=5)

#### F
- **X-axis**: Age (months)
- **Y-axis**: Urine Albumin / Creatinine Ratio (mg/mg)
- **Legend**:
  - female Nos1apEx3-/Ex3- (N=5)
  - male & female WT (N=5)

#### G
- **X-axis**: Treatment time (weeks)
- **Y-axis**: Urine Albumin / Creatinine Ratio (mg/mg)
- **Legend**:
  - Nos1apEx3-/+ untreated (het, n=4)
  - Nos1apEx3-/+ Dexa (het, n=6)
  - Nos1apEx3-/Ex3- untreated (hom, n=5)
  - Nos1apEx3-/Ex3- Dexa (hom, n=5)
Figure S9. Nos1ap<sup>Ex3-/Ex3-</sup> mice develop glomerular proteinuria but not hypoalbuminemia which is not ameliorated by dexamethasone treatment.

A. Amino acid sequence conservation of PTB region encoded by Nos1ap exon 3 shows 12/31 amino acids are identical from vertebrate to invertebrate species down to C. elegans.

B. Urinary albumin / creatinine ratios (3-11 months) for 5 wild type, 10 heterozygous Nos1ap<sup>Ex3-/+</sup> and 10 homozygous Nos1ap<sup>Ex3-/Ex3-</sup> mice are depicted. Each line represents an individual animal. Nos1ap<sup>Ex3-/Ex3-</sup> mice develop significant albuminuria across the displayed time-course.

C. Coomassie blue staining of acrylamide gel is shown, in which urine proteins are visualized from homozygous Nos1ap<sup>Ex3-/Ex3-</sup> (HOM) and heterozygote mice (Het). ~60 kDA protein bands, consistent with albumin, are noted in homozygote urine but not heterozygote urine.

D. Blood urea nitrogen (BUN) levels and Serum albumin levels (7-15 months) for 5 wild type, 10 heterozygous Nos1ap<sup>Ex3-/+</sup> and 10 homozygous Nos1ap<sup>Ex3-/Ex3-</sup> mice are depicted. Nos1ap<sup>Ex3-/Ex3-</sup> mice developed significant albuminuria (Figure 5B, S19A) but no renal failure or significant hypoalbuminemia as indicated by normal-range BUN levels or serum albumin levels respectively across the displayed time-course.

E. Urinary albumin / creatinine ratios (3-11 months) for 5 male and 5 female homozygote Nos1ap<sup>Ex3-/Ex3-</sup> mice are depicted. Male mice exhibit more albuminuria than females. Wilcoxon test, *p<0.05.

F. Urinary albumin / creatinine ratios (3-11 months) for 5 wild type, 10 heterozygous Nos1ap<sup>Ex3-/+</sup> and 5 female homozygous Nos1ap<sup>Ex3-/Ex3-</sup> mice are depicted. Female Nos1ap<sup>Ex3-/Ex3-</sup> mice develop significant albuminuria when compared to control male and female mice. Friedman test, **p<0.01.

G. Urinary albumin / creatinine ratios (3-5 months) for heterozygous Nos1ap<sup>Ex3-/+</sup> and homozygous Nos1ap<sup>Ex3-/Ex3-</sup> mice treated with dexamethasone (Dexa) or vehicle (untreated) are depicted. Dexamethasone increases albuminuria in both heterozygotes and homozygotes.
A

Nos1ap$^{Ex3/+}$

Nos1ap$^{Ex3/-Ex3-}$

B

PAS Glomerular Cross-sectional Area

C

Collagen positive area in Masson’s Trichrome stained sections

D

Nos1ap$^{Ex3/+}$

Nos1ap$^{Ex3/-Ex3-}$
Figure S10. Nos1apEx3-/Ex3- mice exhibit increased mesangial expansion, glomerular size, and glomerular sclerosis.

A. (A) Representative PAS stained kidney sections for Nos1apEx3-/+ and Nos1apEx3-/Ex3- mice are shown (5 animals per genotype, 11 months old (3) and 16 months old (2)) (Scale Bar: 200 μm). Homozygote mice have increased matrix expansion and glomerular size.

B. (B) Box Plot shows glomerular cross-sectional area (each dot represents one glomerulus from animals in (A)). Mann-Whitney test, *p<0.05.

C. (C) Box Plot shows fraction of collagen deposition in glomeruli based on Masson's Trichrome staining in (D) (each dot represents one glomerulus from animals as in (A)). Kruskal-Wallis test, ***p<0.001

D. (D) Representative Masson's Trichrome stained kidney sections for Nos1apEx3-/+ and Nos1apEx3-/Ex3- mice are shown (animal numbers as in (A), Scale Bar: 200 μm). Homozygote mice have increased sclerosis.