Supplemental Figures

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              Exon 1    Signal peptide    Exon 2                    Domain I
Isoform1   1  MGLPRRLLLLLLLATTCVPASQGLQCMQCESNQSCLVEECALGQDLCRTTVLREWQDDRE
Isoform2   1  MGLPRRLLLLLLLATTCVPASQGLQCMQCESNQSCLVEECALGQDLCRTTVLREWQDDRE

              Exon  3              Domain I                   Exon 4
Isoform1  61  LEVVTRGCAHSEKTNRTMSYRMGSMIISLTETVCATNLCNRPRPGARGRAFPQGRYLECA
Isoform2  61  LEVVTRGCAHSEKTNRTMSYRMGSMIISLTETVCATNLCNRPRPGARGRAFPQGRYLECA

                                        Domain II      Exon 5
Isoform1 121  SCTSLDQSCERGREQSLQCRYPTEHCIEVVTLQSTERSLKDEDYTRGCGSLPGCPGTAGF
Isoform2 121  SCTSLDQSCERGREQSLQCRYPTEHCIEVVTLQSTESKLPSAGQLLVEIFKSWEQSASKR

                 Domain II     Exon 6               Domain III
Isoform1 181  HSNQTFHFLKCCNYTHCNGGPVLDLQSFPPNGFQCYSCEGNNTLGCSSEEASLINCRGPM
Isoform2 181  QLNPHTVTGPTFSVTGSSRSLDQLGSDQEPSYLIMSPILLSF------------------

                    Exon 7               Domain III
Isoform1 241  NQCLVATGLDVLGNRSYTVRGCATASWCQGSHVADSFPTHLNVSVSCCHGSGCNSPTGGA
Isorform2 223 ------------------------------------------------------------

                 Region for GPI anchor
Isoform1 301  PRPGPAQLSLIASLLLTLGLWGVLLWT
Isoform2 223  ---------------------------
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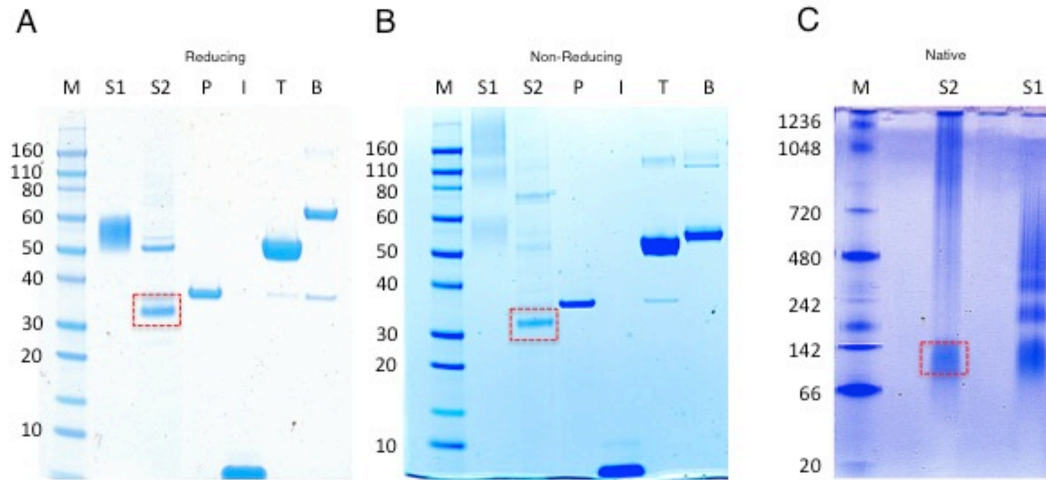
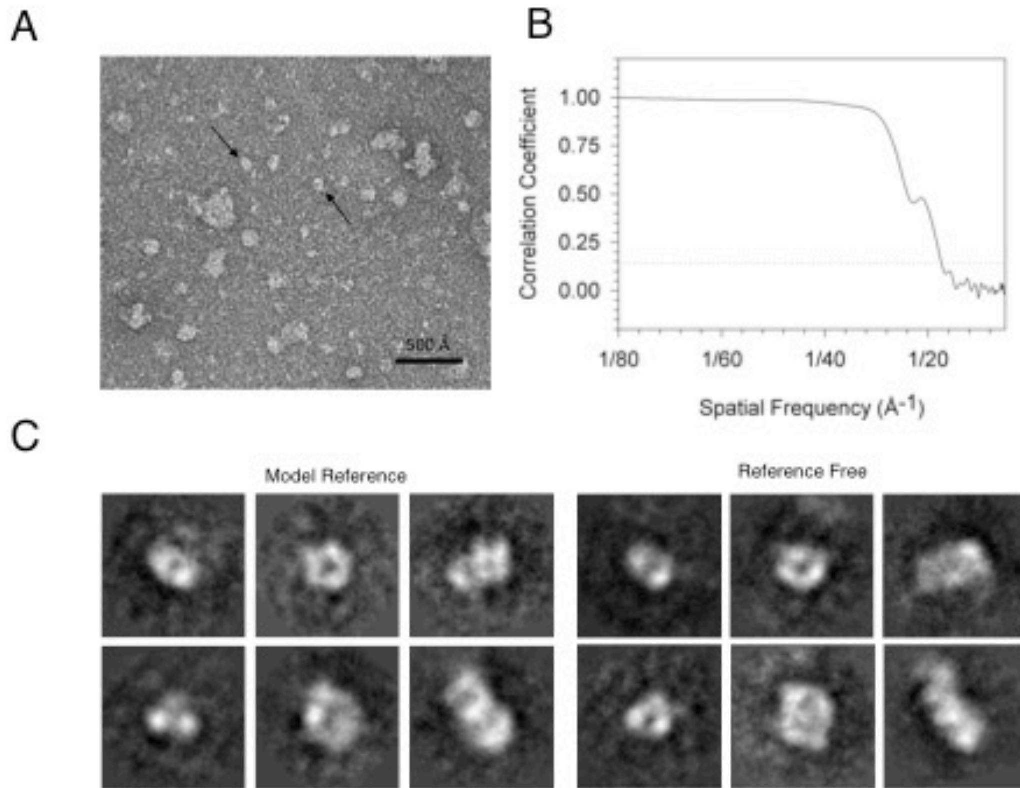
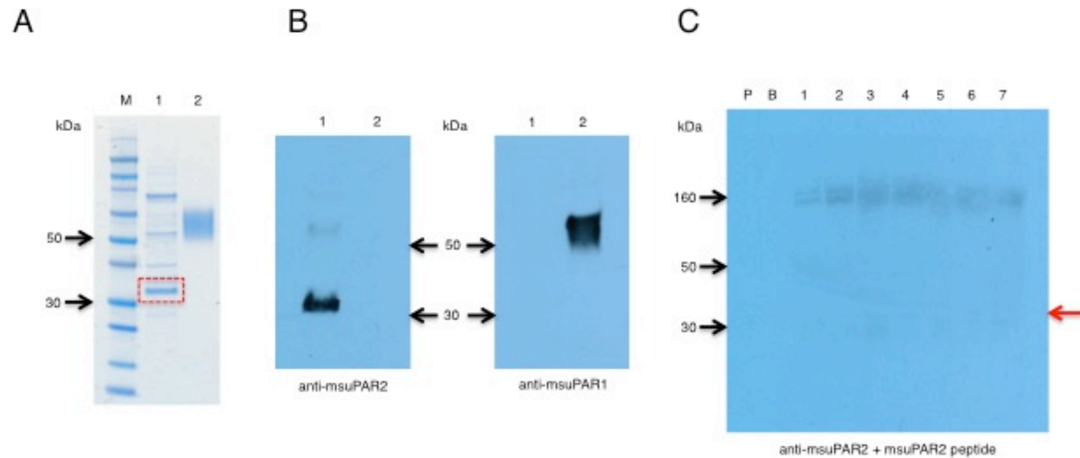
Supplemental Figure 1. Alignment of protein sequences for muPAR isoform 1 and 2. The locations of domains I-III are labeled above the sequences and indicated with black lines. The starting positions of the exons are indicated with dotted lines. The blue region shows the region in isoform 2 generated through alternative splicing. The signal peptide is indicated with grey text and the region for GPI attachment is highlighted in green.



Supplemental Figure 2. Characterization of mouse suPAR recombinant proteins by electrophoresis. Equal amount of purified recombinant msuPAR1 or msuPAR2 proteins was incubated, (**A**) with reducing sample buffer (LDS + DTT), or (**B**) with non-reducing sample buffer (LDS) and heated at 70 degree for 10 min before running NuPAGE gels. (**C**) Blue native PAGE was performed to characterize msuPAR proteins in native condition. S1, msuPAR1; S2, msuPAR2; P, PNGase F; I, insulin; T, tubulin; B, BSA. P, I, T and B are served as migration controls. Images shown are representatives of three separate experiments. msuPAR2 is highlighted in red rectangle.

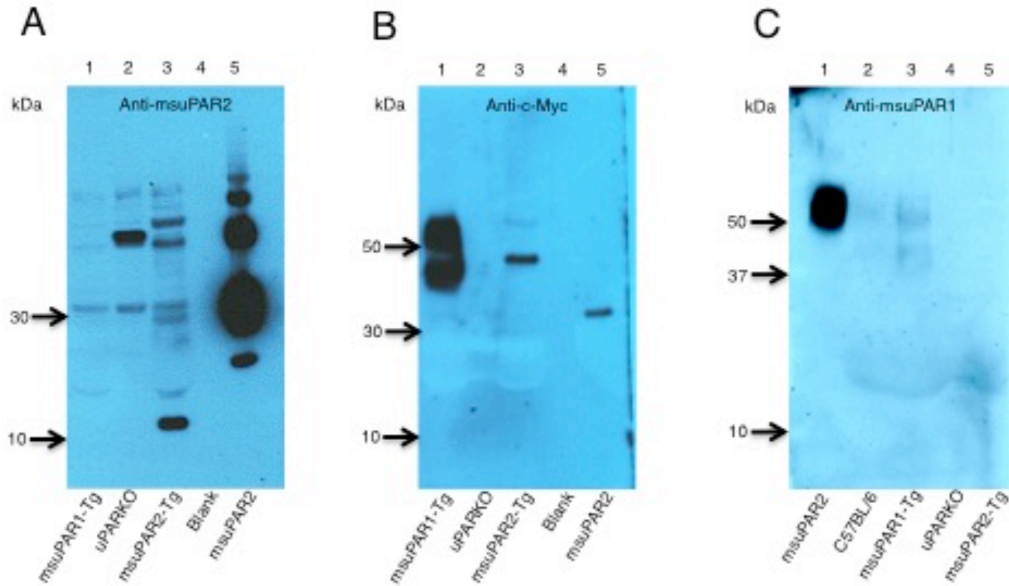


Supplemental Figure 3. Electron microscopy analysis of msuPAR2 recombinant protein. (**A**). The arrows indicate two example particles selected for the analysis. The scale bar is 500Å (upper panel, left). (**B)**. Fourier-shell correlation curve for the independent, split data set refinement. The dotted line indicates the resolution at 0.142 correlation ("Gold standard" resolution) (upper panel, right). (**C**). On the lower left panel are class averages generated from the final round of refinement. On the lower right panel are class averages generated from preliminary reference-free classification and averaging. The reference-free class averages were paired with the model-based classes using the automated multi-reference alignment routines in EMAN.

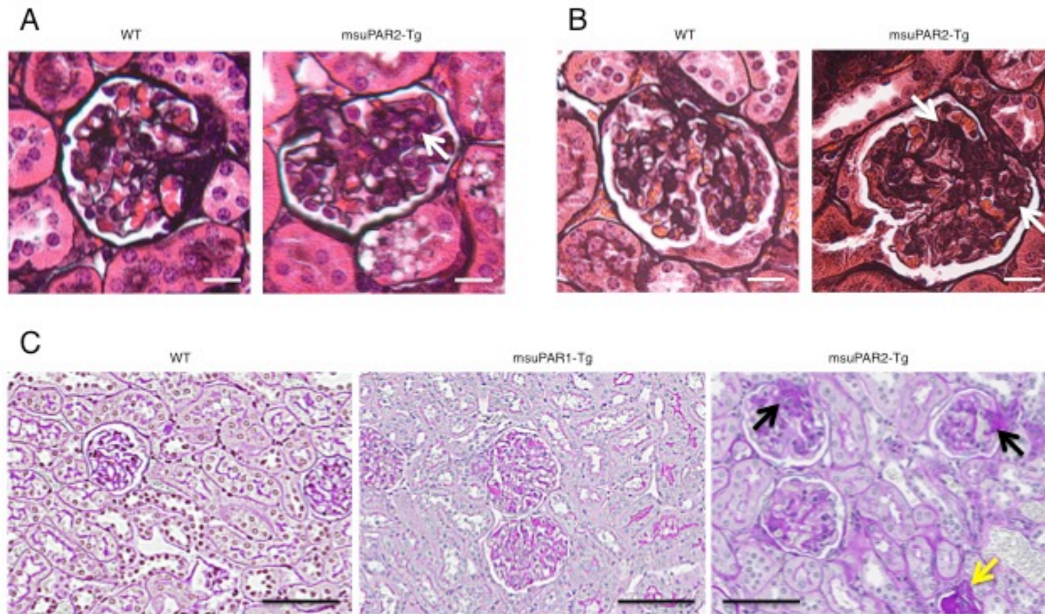


Supplemental Figure 4. Verification of rabbit anti-msuPAR2 antibody and detection of msuPAR2 in serum. (**A**). SDS-PAGE gel showing loading of 1 μg of recombinant protein msuPAR1 (lane 2) and msuPAR2 (lane 1). msuPAR2 is highlighted in red rectangle. (**B**) 100 ng of msuPAR2 and msuPAR1 protein were loaded into lane 1 and land 2 respectively and processed for blotting. Customized rabbit anti-msuPAR2 antibody was verified as it detected msuPAR2 in lane 1, but not msuPAR1 in lane 2 (Left panel). In contrast, a goat anti-msuPAR1 antibody detected msuPAR1 protein in lane 2, but not msuPAR2 protein in lane 1 (Right panel). (**C**) msuPAR2 peptide blocked the detection of msuPAR2 in msuPAR2-Tg mouse sera. To control for Fig 2D, duplicated mouse serum protein blots were incubated with msuPAR2 peptide and anti-msuPAR2 antibody mixture simultaneously as with anti-msuPAR2 antibody alone. As shown here, msuPAR2 peptide abolished the detection of msuPAR2 either with purified msuPAR2 protein as positive control (P), or with msuPAR2 in msuPAR2-

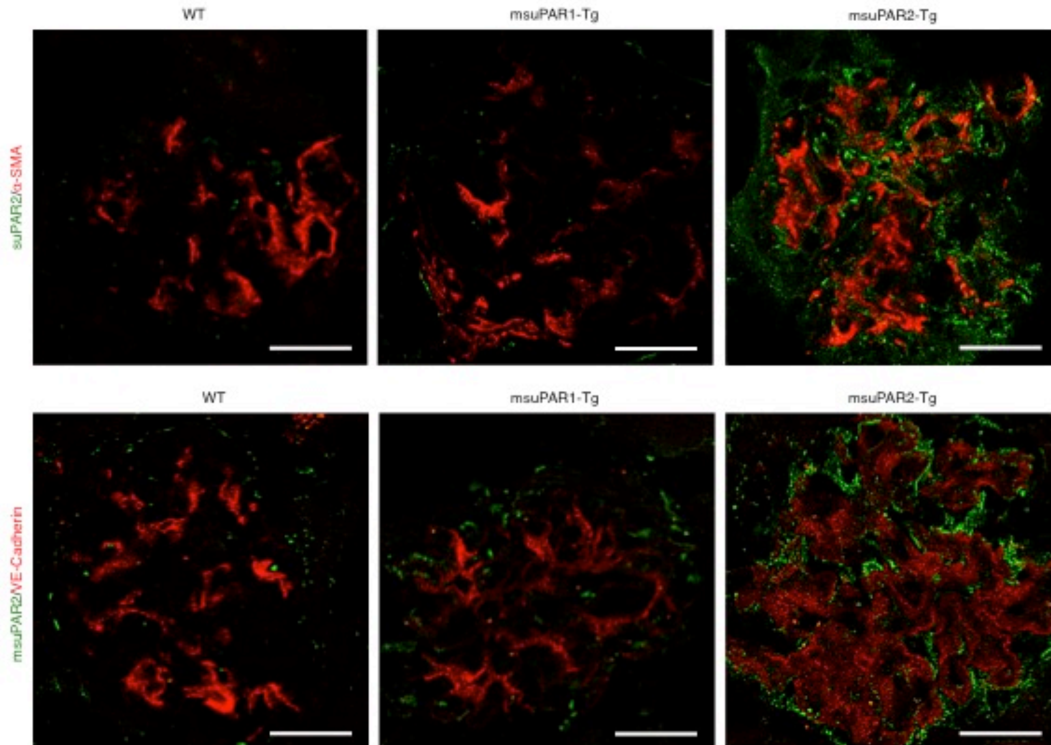
Tg mouse sera. B, blank; Lane 1 represents uPAR KO sera; Lane 2, msuPAR1-Tg sera; Lane 3 to Lane 7, sera from different msuPAR2-Tg mice.



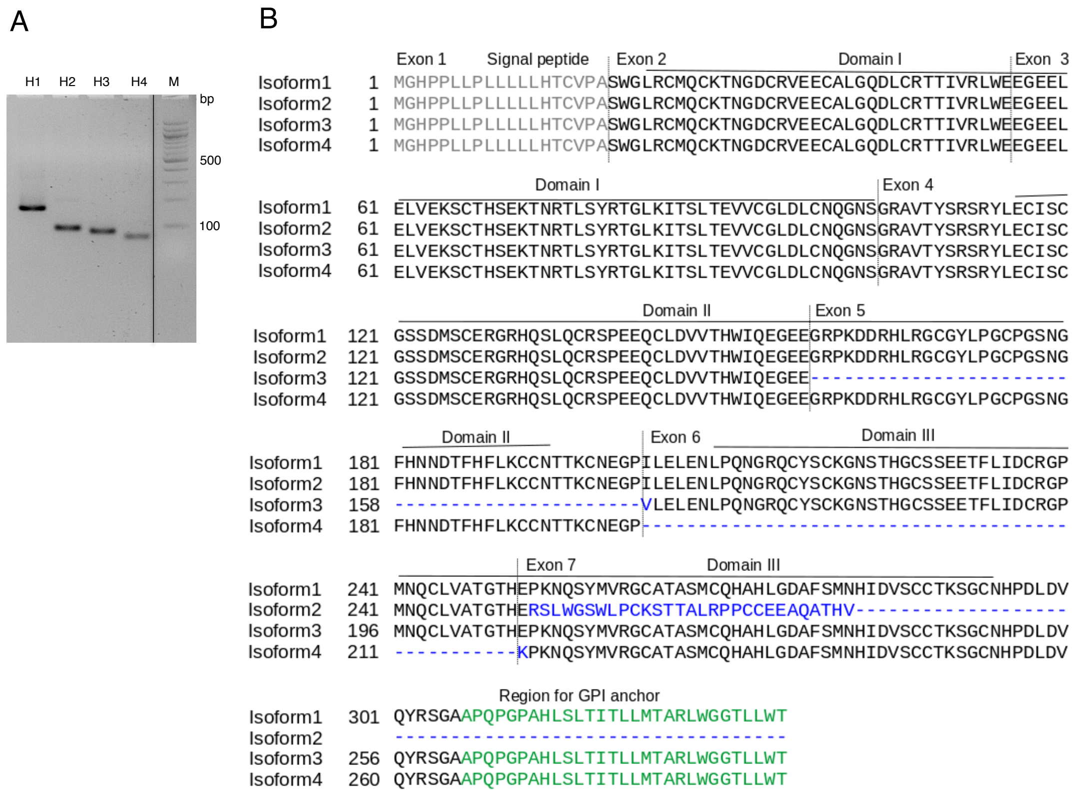
Supplemental Figure 5. msuPAR2 fragment is detected in urine. Urine samples were blotted with anti-msuPAR2 antibody (**A**), and anti-c-Myc antibody (**B**) and anti-msuPAR1 antibody (**C**) respectively. msuPAR2 fragment was recognized by anti-msuPAR2 antibody, but not by anti-c-Myc antibody that detected msuPAR1 in msuPAR1-Tg mice. The fragment was not detected in the urine samples form msuPAR1 transgenic mice or LPS treated C57BL/6 mice by msuPAR1 antibody, which is raised against msuPAR1 protein.



Supplemental Figure 6. Kidney histology. (**A**) Jones silver stain of the kidneys from regular chow fed mice. Scale bar, 20 μm. WT, wild type littermate controls. (**B**). Jones silver stain of the kidneys from HFD treated mice. Scale bar, 20 μm. (**C**). Representative PAS staining of kidney sections from WT, msuPAR1-Tg and msuPAR2-Tg mice at low magnification. All mice received HFD treatment for 6 months starting at 2 months of age. Typical FSGS-like changes were appreciated in msuPAR2-Tg mice but not in msuPAR1-Tg mice, nor in WT mice. Scale bar, 100 μm. Arrows (white in **A**, and **B**, black in **C**) show focal glomerular changes in kidney sections of msuPAR2-Tg mice. Yellow arrow in **C** shows a globally sclerotic glomerulus.



Supplemental Figure 7. Glomerular localization of msuPAR2 in msuPAR2-Tg mice. Double immunofluorescence staining with kidney cryosections shows no co-localization of msuPAR2 with the mesangial cell marker α-SMA or the glomerular endothelial cell marker VE-Cadherin. Scale bar, 20 μm. Contrast this with co-localization of msuPAR2 with the podocyte marker, synaptopodin (Figure 4E).



Supplemental Figure 8. mRNA expression and sequence alignment of of human uPAR isoforms. (**A**) Four human uPAR isoforms were detected in peripheral blood mononuclear cells harvested from normal subjects at mRNA level. H1 to H4 representing human uPAR isoform 1, 2, 3 and 4 respectively. The DNA markers (M) were run on the same gel in a noncontiguous lane**.** (**B**) Alignment of protein sequences of human uPAR variants. The locations of domains I-III are labeled above the sequences and indicated with black lines. The starting positions of the exons are indicated with dotted lines. Blue color is used to indicate mutations introduced by alternative splicing. The signal peptide is indicated with grey text and the region for GPI attachment is highlighted in green.

Patent information

a. Patent numbers for JR and SS

i. Role of Soluble uPAR in the Pathogenesis of Proteinuric Kidney Disease

1. JP 5798037 B2 (Granted 8/28/2015)

2. JP 6254125 (Granted 12/8/2017)

3. EP 2352503 A1 (Granted 7/19/2017)

4. EP 7180935.3

5. CA 2779958 A1

6. US 20110212083

ii. REDUCING SOLUBLE UROKINASE RECEPTOR IN THE CIRCULATION

1. JP 2014517750 A (Granted)

2. EP 2707055 A2 (Granted)

3. US 9,867,923 (Granted)

iii. NON-GLYCOSYLATED suPAR BIOMARKERS AND USES THEREOF

1. US20160169910

2. EP3028044

3. CA2919687

4. JP2016530510

iv. (INTEGRIN PATENT) Methods/Compositions for the Treatment of Proteinuric Diseases

1. JP 5637855 B2(GRANTED)

2. EP 2217238 B1 (GRANTED)

3. EP 2730282

4. US 9,345,739 A1 (GRANTED)

5. US 20160296592

b. Patent application number for CW: US20140302065