α– and β–Adducin polymorphisms affect podocyte proteins and proteinuria in rodents and renal function decline in human IgA nephropathy

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Supplementary Material

Supplementary Methods

Immunogold electron microscopy. The analysis was performed on rat renal tissue, fixed in a mixture of formaldehyde, glutaraldehyde, and phosphate buffer, soaked in glucose and frozen, or alternatively embedded in Lowicril K4M resin (Electron Microscopy Sciences, Societa` Italiana Chimici, Rome, Italy), as previously described (Regele HM, Fillipovic E, Langer B, Poczewki H, Kraxberger I, Bittner RE, Kerjaschki D: Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. J Am Soc Nephrol 2000, 11:403–412). An indirect immunogold labeling procedure was performed on ultrathin sections.
Briefly, after blocking, the material was incubated with the primary antibody, then by the secondary 10-nm gold-conjugated goat anti-mouse secondary antibody (Aurion, DBA, Milan, Italy). Specificity of antibody labelling was demonstrated by the lack of staining after substituting proper control immunoglobulins (Invitrogen) for the primary antibody.

Figure S1

Immunogold analysis in rat kidneys

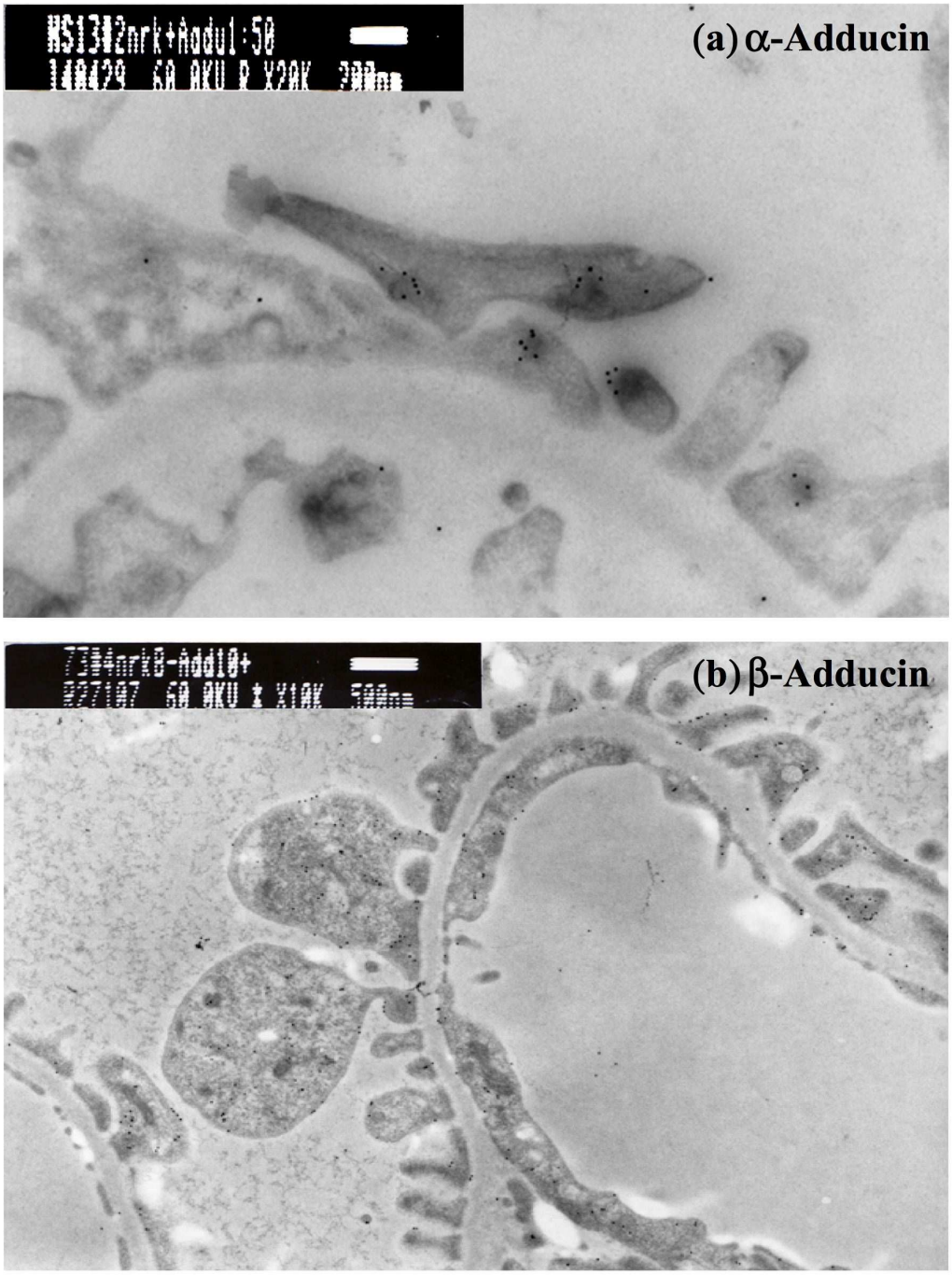

Figure S1 Immunogold electron microscopy for α-Adducin and β-Adducin localization in rat glomeruli.
(a) α-Adducin looks localized around vesicles present in podocyte foot processes; (b) β-Adducin is either dispersed in the cytoplasm and localized along the surface of podocyte foot processes.

Figure S2

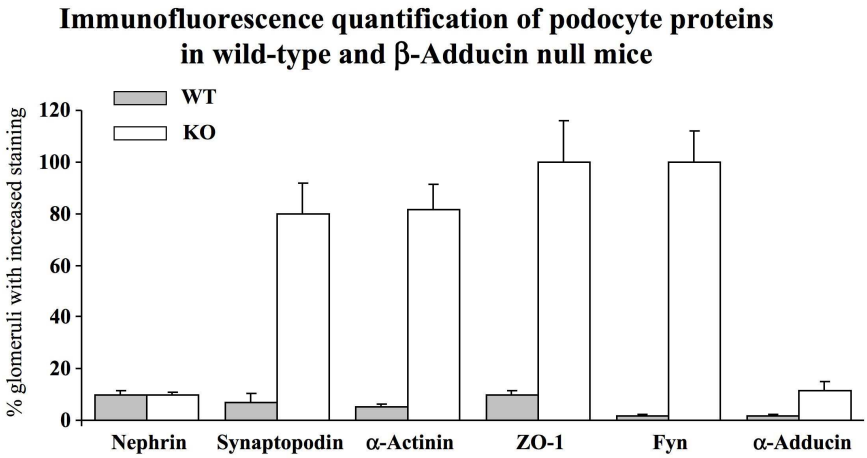

Figure S2 Immunofluorescence quantification of podocyte proteins and Adducin in mouse kidney sections from β-Adducin knockout mice. The results refer to the immunofluorescence analysis showed in Figure 3 of the manuscript. Five wild-type (WT) and β-Adducin knockout mice (KO) have been analyzed. Forty glomeruli per mouse were evaluated and quantification has been expressed as percentage of glomeruli with increased staining. β-Adducin is absent in KO mice. Data are mean ± sem.

Figure S3

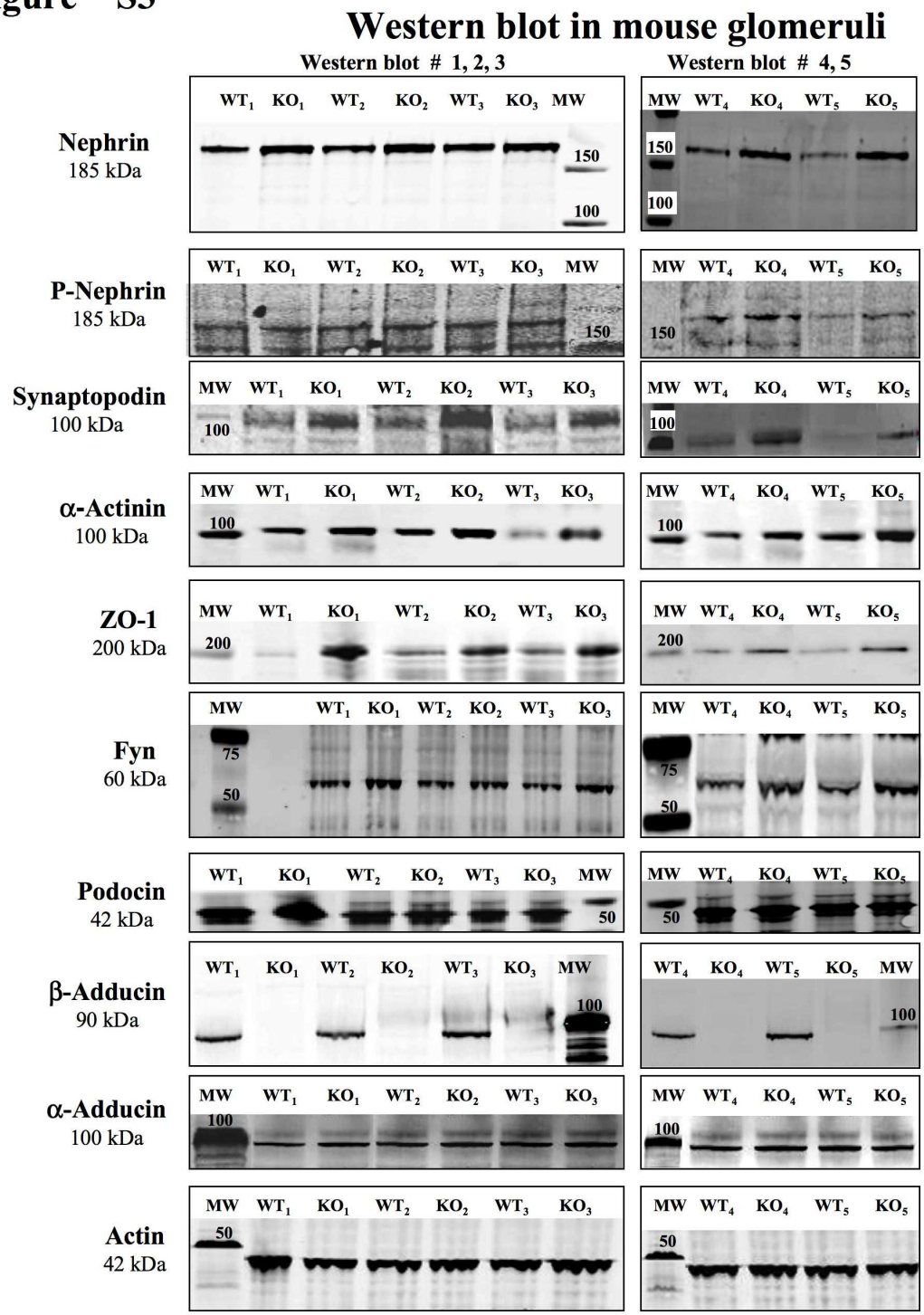

Figure S3 Western blot analysis of podocyte proteins in isolated glomeruli from β-Adducin knockout mice. The Western blot analysis refers to the data reported in Figure 4 of the manuscript. The figure shows the Western blots (10 µg protein/lane) for podocyte protein quantification on the individual preparations of isolated glomeruli from wild-type (WT) and β-Adducin knockout mice (KO) (n=5 preparations for each group). Standard molecular weights (MW) are indicated.

Figure S4

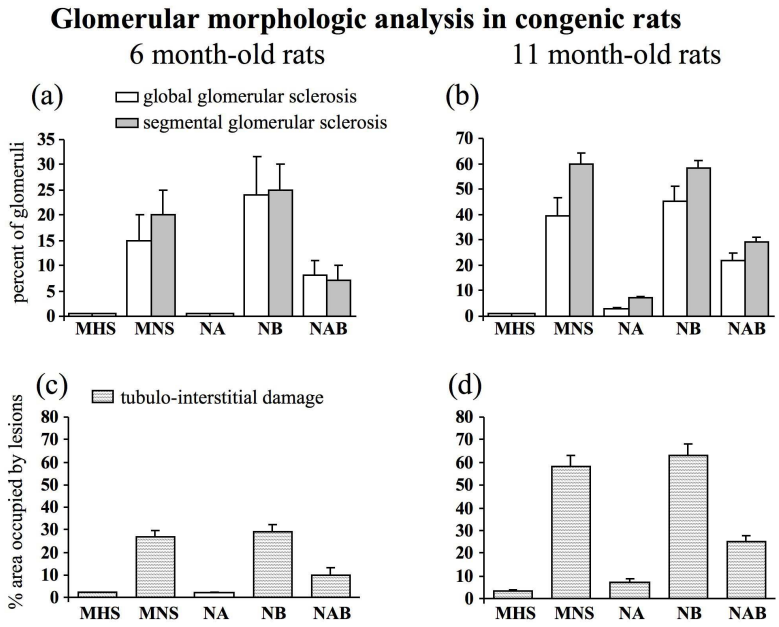

Figure S4 Glomerular morphologic analysis in kidneys from congenic rat substrains. The analysis refers to the images reported in Figure 6 of the manuscript. The analysis has been performed in MHS, MNS and congenic substrains for α–Adducin (NA), β-Adducin (NB) and αβ-Adducin (NAB) (n=5 per group) at 6 and 11 months of age. Forty glomeruli per sample have been analyzed for each rat. The figure shows the percentage of glomeruli affected by global and segmental sclerosis in rats at 6 (a) and 11 months (b) and the percentage of area occupied by tubulo-interstitial damage in rats at 6 (c) and 11 months (d). Data are mean ± sem.

Figure S5

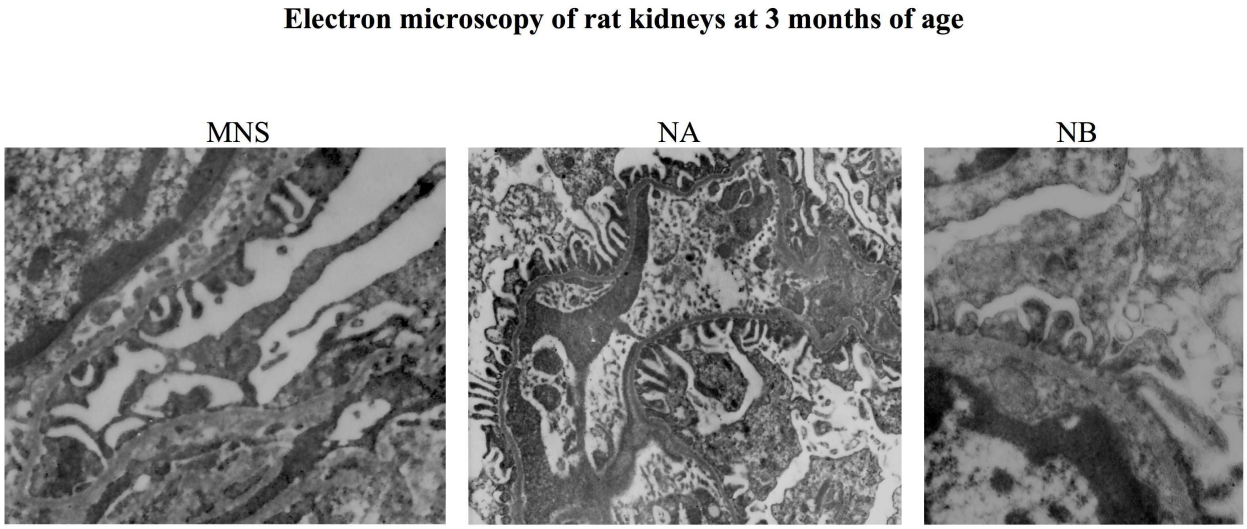

Figure S5 Electron microscopy in rat kidneys from congenic rat substrains. The analysis has been performed in kidneys of MNS, NA and NB rats at 3 months of age (n=5 per group). MNS and NB glomeruli display podocyte foot process effacement, while NA appears protected. Magnification: 8000X (MNS, NB), 6000X (NA).

Figure S6

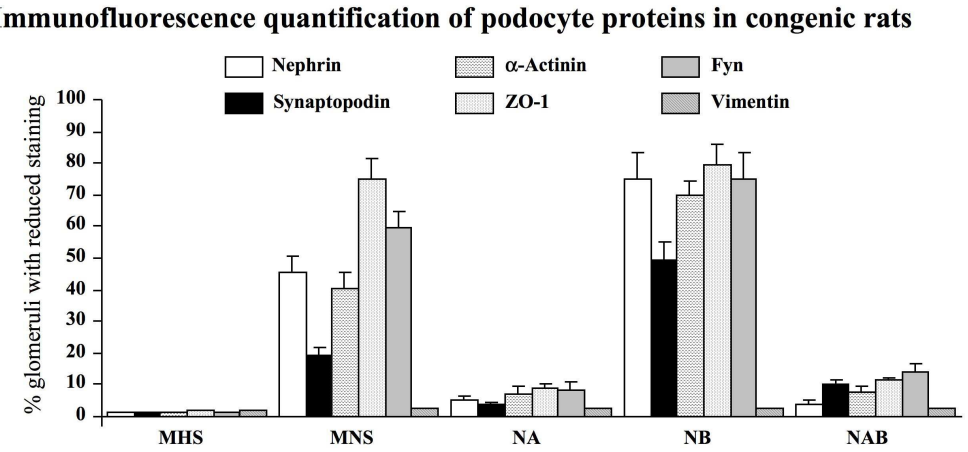

Figure S6 Quantitative evaluation of immunofluorescence analysis for podocyte proteins in congenic rats. The results refer to the immunofluorescence analysis showed in Figure 7-8 of the manuscript. The analysis has been performed in MHS, MNS and congenic substrains for α–Adducin (NA), β-Adducin (NB) and αβ-Adducin (NAB) (n=5 per group) at 1.5 months of age. Forty glomeruli per animal have been analyzed. The quantification has been expressed as percentage of glomeruli with reduced staining of podocyte proteins. Data are mean ± sem.

Figure S7

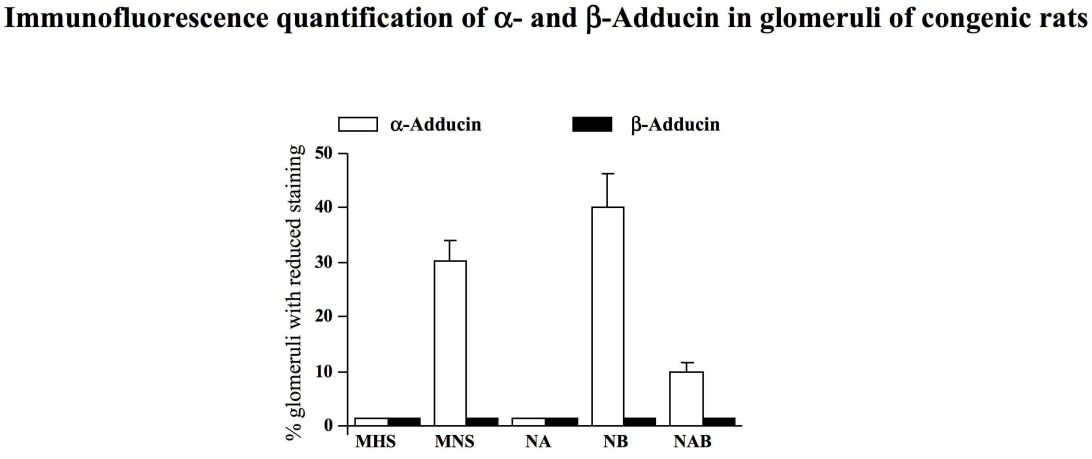

Figure S7 Quantitative evaluation of immunofluorescence analysis for Adducin in glomeruli from congenic rat substrains. The results refer to the immunofluorescence analysis showed in Figure 9 of the manuscript. The analysis has been performed in MHS, MNS and congenic substrains for α–Adducin (NA), β-Adducin (NB) and αβ-Adducin (NAB) (n=5 per group) at 1.5 months of age. Forty glomeruli have been analyzed for each rat. The quantification has been expressed as percentage of glomeruli with reduced staining of α– and β-Adducin. Data are mean ± sem.

Figure S8

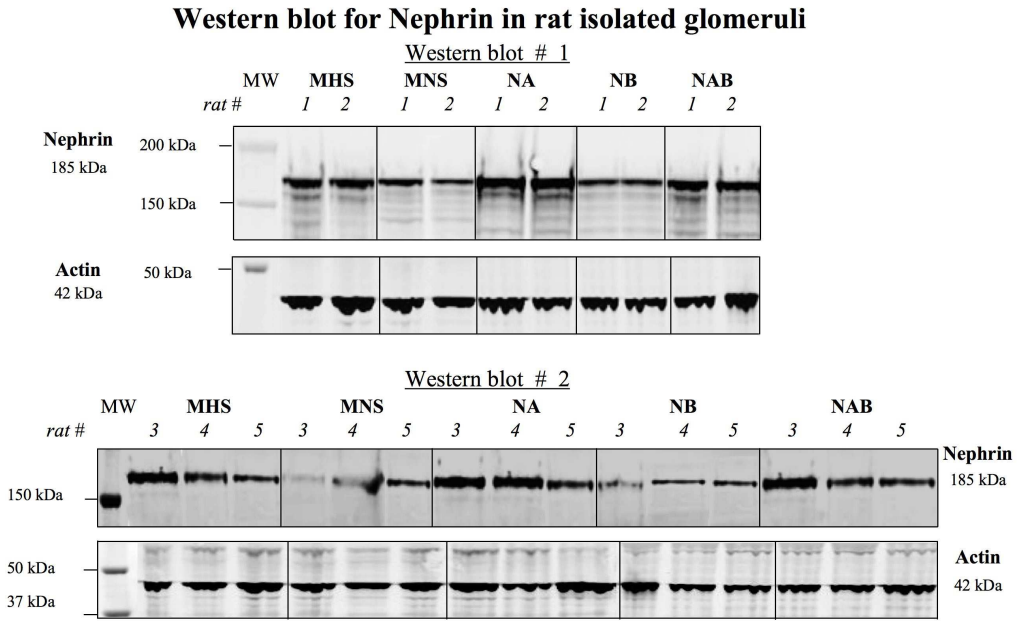

Figure S8 Western blot analysis of Nephrin and Actin in isolated glomeruli from congenic rat substrains. The Western blot analysis refers to the data reported in Figure 10 of the manuscript. The figure shows the Western blots of the individual glomerular preparations from MHS, MNS and congenic substrains for α–Adducin (NA), β-Adducin (NB) and αβ-Adducin (NAB) (n=5 per group), probed with Nephrin and Actin antibodies. No difference in actin was detected among all groups. Standard molecular weights (MW) are indicated.

Figure S9

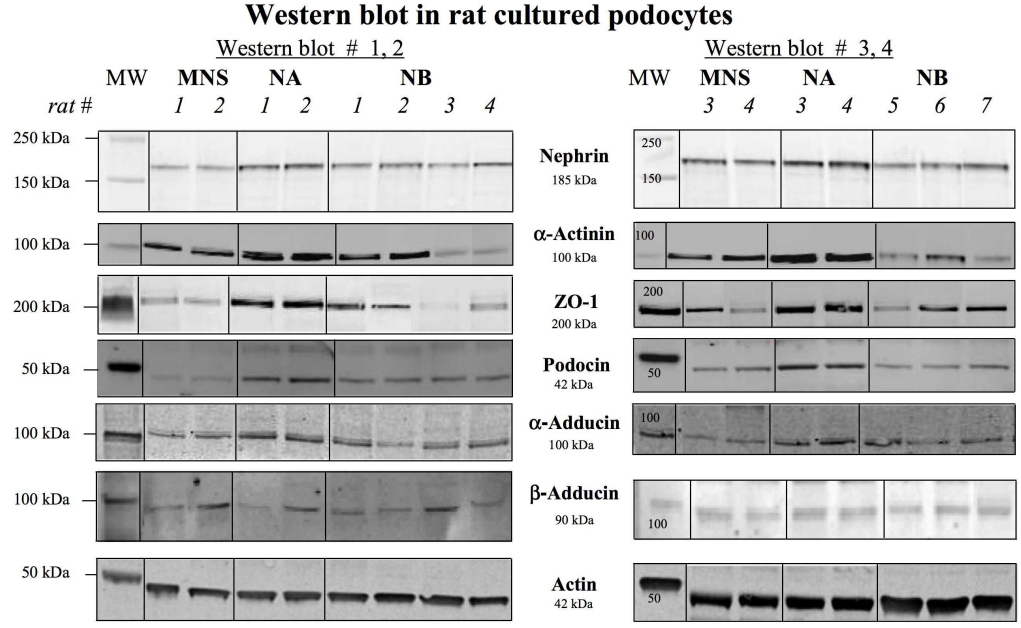

Figure S9 Western blot analysis of podocyte proteins in primary cultures of glomerular podocytes from congenic rat substrains. The Western blot analysis refers to the data reported in Figure 11 of the manuscript. The figure shows the Western blots of the individual podocyte preparations obtained from MNS (n=4) and congenic substrains for α–Adducin (NA) (n=4) and β-Adducin (NB) (n=7) probed with antibodies against podocyte proteins. Standard molecular weights (MW) are indicated.

Figure S10

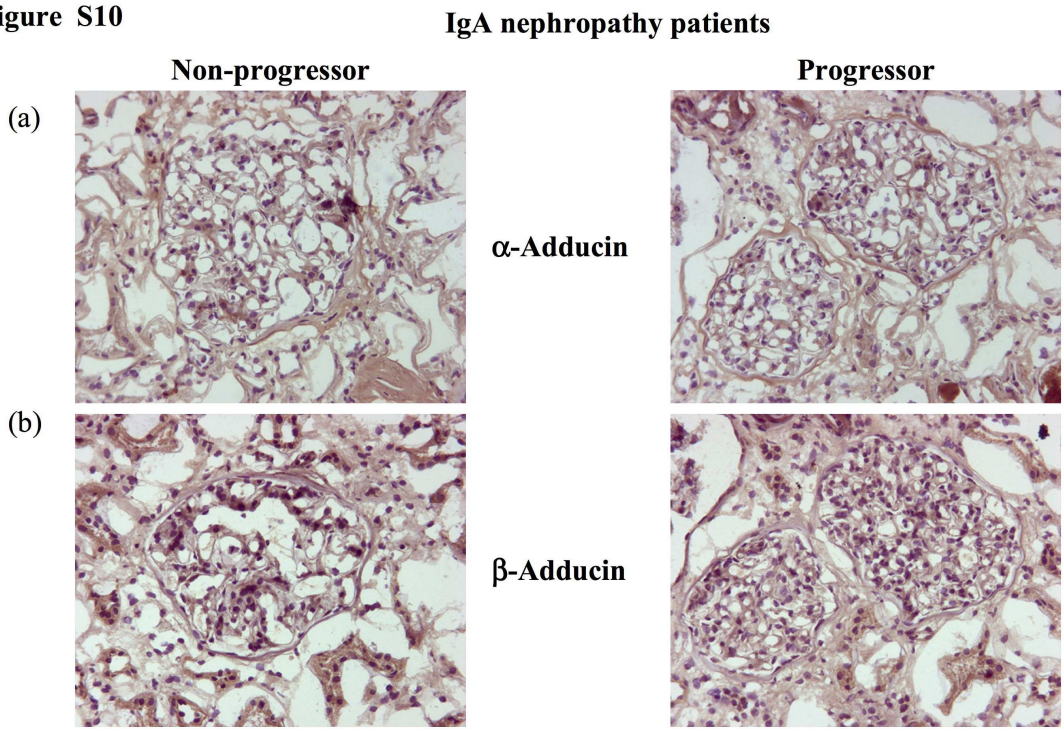

Figure S10 IgA nephropathy patients. Twenty IgAN patients have been analyzed and divided into two groups according to the rate of renal function decline: stable renal function (non-progressors) or rapidly progressing towards ESRF (progressors). Renal images were obtained from renal biopsies of two representative patients belonging to the non-progressor and progressor group. No difference in α–Adducin (a) and β-Adducin (b) glomerular expressions was detected between the two groups. Magnification: 250X