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Supplemental Methods

Coomassie blue staining and Western Blotting for purified IgA1-immune complexes

IgA1-immune complexes derived from patients and healthy controls were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and then, the gels were stained with Coomassie blue staining solution. For Western blotting, after SDS-PAGE, the IgA1-immune complexes were transferred to polyvinylidene fluoride membranes (PVDF membranes, Millipore). After blocking, the membranes were immunoblotted with rabbit monoclonal anti-human IgA1 antibody (ab193187, Abcam, UK), rabbit polyclonal anti-human collectin11 antibody (Proteintech, USA), rabbit polyclonal anti-human C3c antibody (Dako, Denmark) and mouse monoclonal anti-human IgG antibody (Abcam, UK), followed by anti-mouse/rabbit IgG-HRP antibodies (Jackson ImmunoReseach, USA). Finally, the membranes were developed with a chemiluminescent HRP substrate (Millipore) according to the manufacturer's instructions.

Independent validation population

For validation, another 24 patients of IgA nephropathy and 20 age- and sex-matched healthy controls were additionally recruited. Plasma samples of enrolled patients and healthy controls were pooled for IgA1-immune complexes purification. And purified IgA1-immune complexes were then used for mesangial cells treatment.

Human mesangial cells treated with IgG

IgG were purified from plasma of patients with IgA nephropathy using protein G Agarose column (Thermo, USA). In brief, acidic buffer (0.5M acetic acid adjusted to pH 3.0 with ammonium hydroxide) was used to elute IgG fractions from protein G affinity column. Then, the IgG fractions were neutralized to pH 7.0 immediately and

dialyzed against PBS. For cell experiments, human mesangial cells were first serum deprived overnight and then treated with 100 μg/ml purified IgG.

RT-qPCR

RNA was extracted using TRIzol and then reverse-transcribed to cDNA. Then, the expression of collectin11 was quantified by qPCR using TaqMan gene expression assays (Applied Systems, USA).

Proximity ligation assay

The Duolink In Situ Red Starter Kit (Sigma, USA) was used to perform the proximity ligation assay, and the assay was conducted according to the manual instructions. Primary antibodies used were as mentioned above.

Immunofluorescence microscopy and image analysis

A Nikon Eclipse 90i microscope was used for the observation of the kidney tissues. For cell staining, a confocal microscope (Zeiss LSM 710) was used. ImageJ software was used for quantitative analysis. From each coverslip of stained mesangial cells, at least 8 individual fields were photographed. The background signal was subtracted using the same threshold setting, and then the total fluorescence intensity was measured and adjusted for the area of the nuclei.

Validation of galactose-deficient IgA1

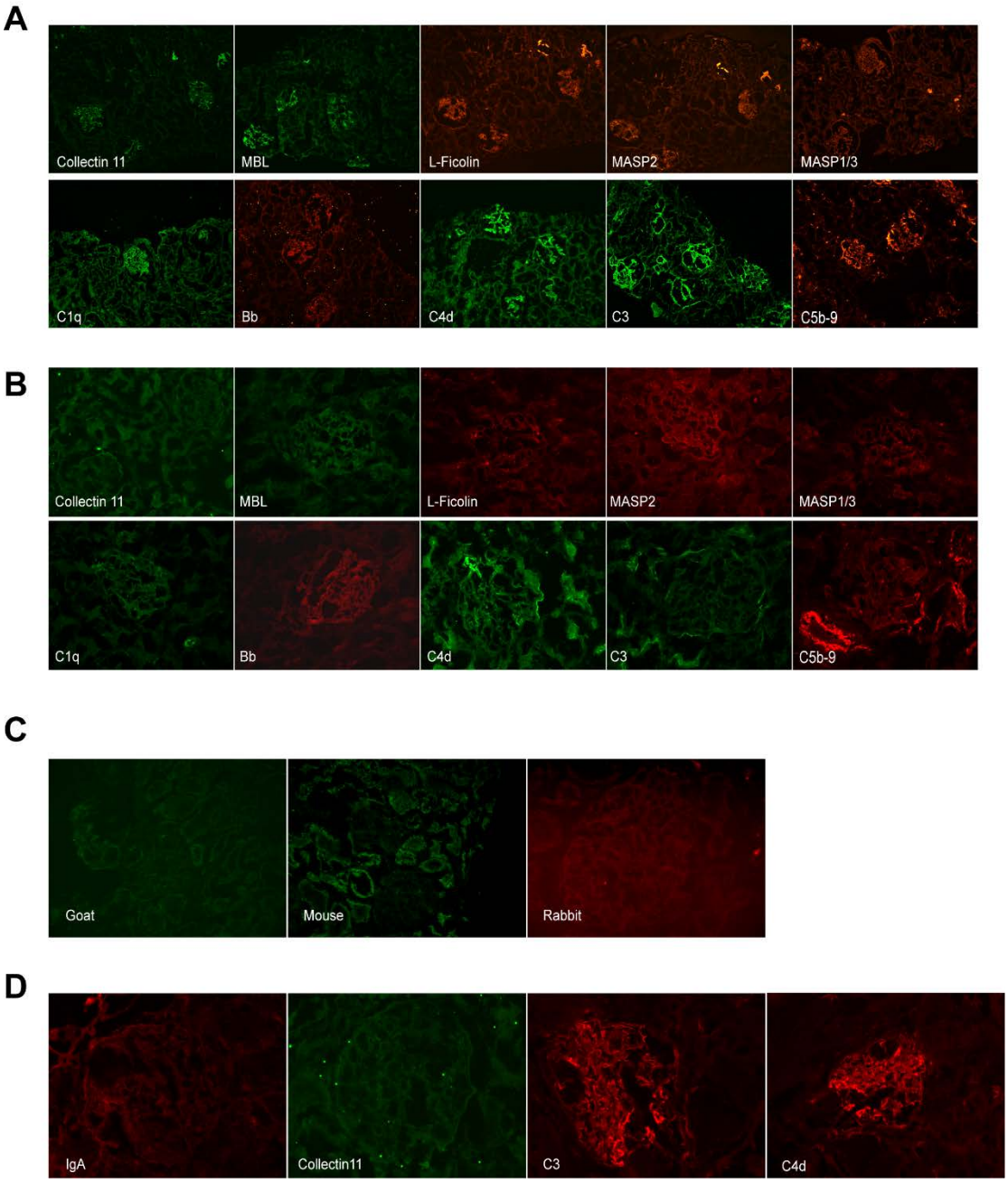
The enzyme digestion efficiency of momomeric IgA1 treated with neuraminidase and galactosidase was validated using ELISA, in which microplates were coated with 2 μg/ml prepared Gd-IgA1 and then detected with the lectin Helix pomatia agglutinin (HPA) (Sigma, USA), which recognizes core glycan α-N-acetyl galactosamine (GalNac) after galactose deficiency.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 software (SPSS, Chicago, USA).

Patients with missing data were excluded from the analysis. Normally distributed quantitative variables are expressed as the means ± standard deviation; nonnormally distributed variables are presented as the medians (interquartile ranges); categorical data are expressed as absolute frequencies and percentages. The unpaired Student's t test was used for normally distributed data, and the Mann-Whitney test was used for nonnormally distributed data. Data were considered statistically significant when a two-tailed p value <0.05.

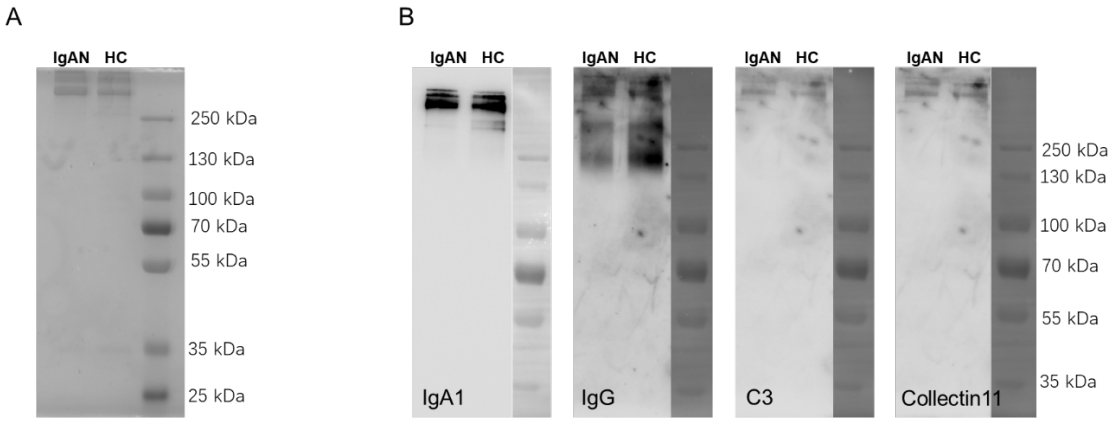
Supplemental Figure 1



Representative immunostaining images of complement proteins in kidney tissue. **A,** Representative low-powered images show the deposition of complement proteins in the kidney tissue of patients with IgA nephropathy; **B,** Para-carcinoma kidney tissue specimens from a patient of renal cell carcinoma were used as non-disease control and the background staining for our detected markers was weak; **C**, The isotype control antibodies were used for immunofluorescent staining and presented with no or trace

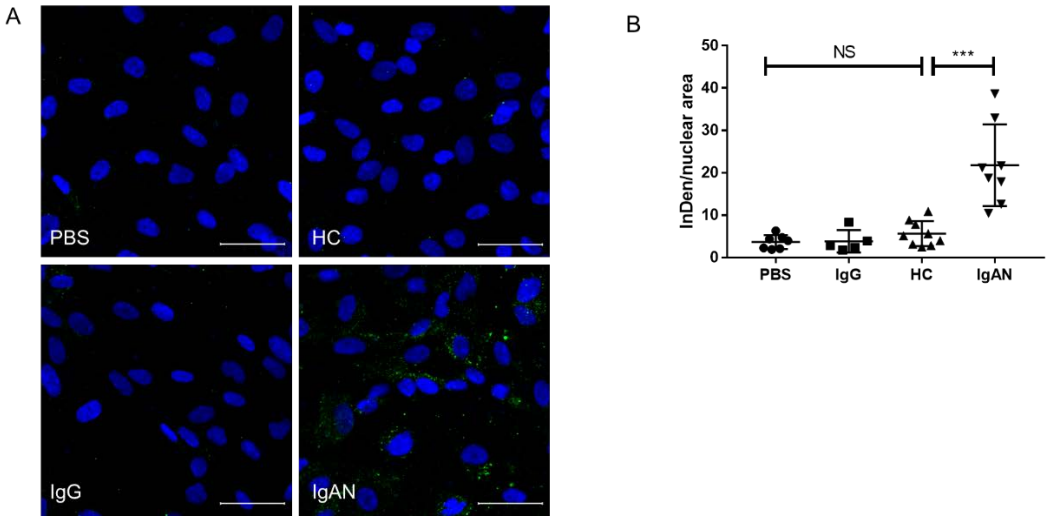
background signal; **D**, Representative images show the positive staining of C3 and C4d and negative staining of collctin11 and IgA1 in the glomeruli of patients with FSGS. MBL, Mannose-binding lectin; MASP, Mannose-binding lectin Associated Serine Protease.

Supplemental Figure 2



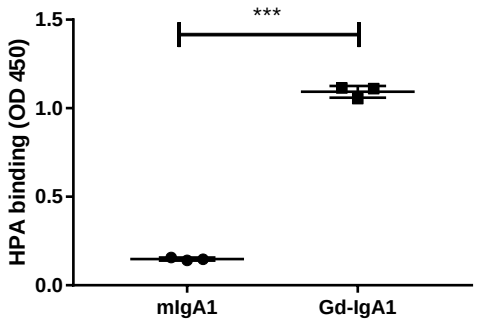
Purity and composition of IgA1-immune complexes analyzed by SDS-PAGE and Western blot. A, Analysis by SDS-PAGE of IgA1-immune complexes purified from both patients of IgA nephropathy and healthy controls; **B**, The composition of IgA1-immune complexes was examined by western blot. IgA1, IgG, C3 and collectin11 were detected. All samples were ran under nonreducing condition. IgAN. IgA nephropathy; HC, healthy controls.

Supplemental Figure 3



Independent validation of the promoting effect the of IgA1-immune complexes from patients with IgA nephropathy on the expression of collectin11. A, 100 μg/ml IgA1-immune complexes derived from patients or controls or 100 μg/ml pure IgG were incubated with human mesangial cells for 24 hours, then intracellular collectin11 expression were detected by immunofluorescent staining. Scale bar: 50μm; B, Fluorescence intensity quantification of intracellular collectin11 shown in A. IgAN. IgA nephropathy; HC, healthy controls.

Supplemental Figure 4



Validation of the effective removal of terminal sialic acid and galactose from monomeric IgA1. Gd-IgA1 showed increased binding with HPA, a lectin that recognizes GalNac residues, compared with monomeric IgA1. Gd-IgA1, galactose-deficient IgA1; HPA, Helix pomatia agglutinin.