## Supplemental Materials and Methods.

*Immunofluorescence staining of TLR7 proteins in renal tissues of IgAN patients.*

Paraffin-embedded kidney specimens were prepared as 4 μM slides, and subjected to rehydration and antigen retrieval. The sections were blocked in PBS containing 3% BSA and 0.03% Triton-X100, then they were stained with different primary antibodies, including rabbit anti-TLR7 antibodies (Abcam, ab45371; Thermo Fisher Scientific, PA5-23126; Thermo Fisher Scientific, PA1-28109) and rabbit normal IgG (Cell Signaling Technology, 2729S). Secondary antibodies conjugated with Alexa-Fluor 594 were used to detect the primary antibodies. Finally, nuclei were stained by DAPI and slides were washed extensively before mounting. Digital images were obtained using confocal laser scanning microscopy Zeiss 880 (Leica). Periodic acid-Schiff staining was performed with a PAS kit (BASO, BA-4080B) and observed using a microscope.

*Immunofluorescence staining of B cells and T cells infiltrates in renal tissues of IgAN patients.*

Paraffin-embedded kidney specimens were prepared as described above, then incubated with anti-CD19 antibody (Lifespan Bioscience, LSB-13077) and rat anti-CD3 antibody (Abcam, ab11089), Mouse anti-IgA (Santa Cruz Biotechnology, sc-271913), Rabbit anti-TLR7 (Thermo Fisher Scientific, PA1-28109). Frozen sections of renal biopsy samples from IgAN patients were stained with antibodies for TLR7 (R&D systems, MAB5875), IL6 (Affinity Biotechnology, DF6087), IL12 (Affinity Biotechnology, AF5133), and CD19 (Lifespan Bioscience, LSB-13077). Primary antibodies were detected by different fluorescein-conjugated secondary antibodies. Finally, nuclei were stained by DAPI and slides were washed extensively before mounting. At least 5 fields were taken with confocal laser scanning microscopy Zeiss 880 (Leica) for each sample, and

representative image was used for presentation.

*Immortalization of B cells from PBMCs with Epstein-Barr virus (EBV).*

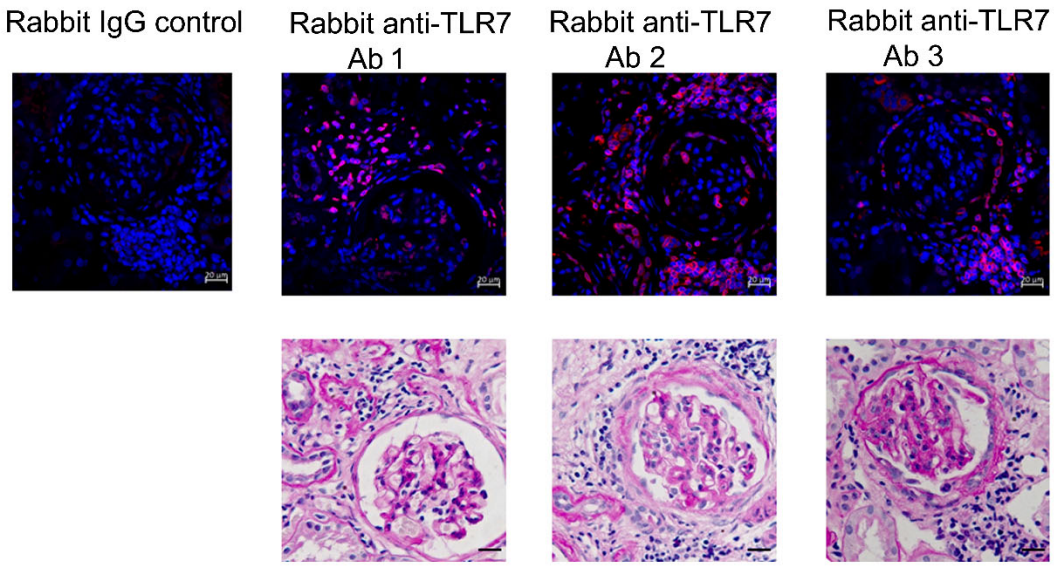
PBMCs from 5 mL venous blood were enriched by Ficoll-paque density centrifugation. After washing with PBS, cells were suspended in 2.5 ml of 1640 complete medium plus 2.5 ml culture supernatant from B95-8 cells, then incubated at 37 °C for 4 h. 5 mL of fresh medium containing 1 μg/mL cycloporin A (Solarbio, C8781) was added into culture. The culture was then incubated in $CO_2$ incubator at 37°C for 21 days without changing medium. At day 22, 10 mL of fresh complete medium was added into culture and cells were split into two bottles. At day 28, cells were collected for phenotype identification and culture passage. Fluorescein-conjugated antibodies for CD19, CD38, and CD20 (eBioscience) were incubated with EBV-immortalized cells and analyzed in flow cytometry.

*In vitro PBMCs culture with TLR7/8 ligands.*

Plates for PBMCs culture were pre-coated with anti-IgM antibodies (Jackson ImmunoResearch, 109-005-129) at a concentration of 5 μg/mL, and incubated at 4°C overnight. PBMCs from patients or controls were isolated with ficoll gradient centrifugation and suspended in complete 1640 medium. The cells were stimulated in the presence or absence of TLR7/8 ligand-R848 (Enzo life science, ALX-420-038-M005). At day 4, the cells were collected, subjected to surface labeling of CD19, and analyzed by flow cytometry. Culture supernatant was collected at different time points for measurement of IgA1 by ELISA.

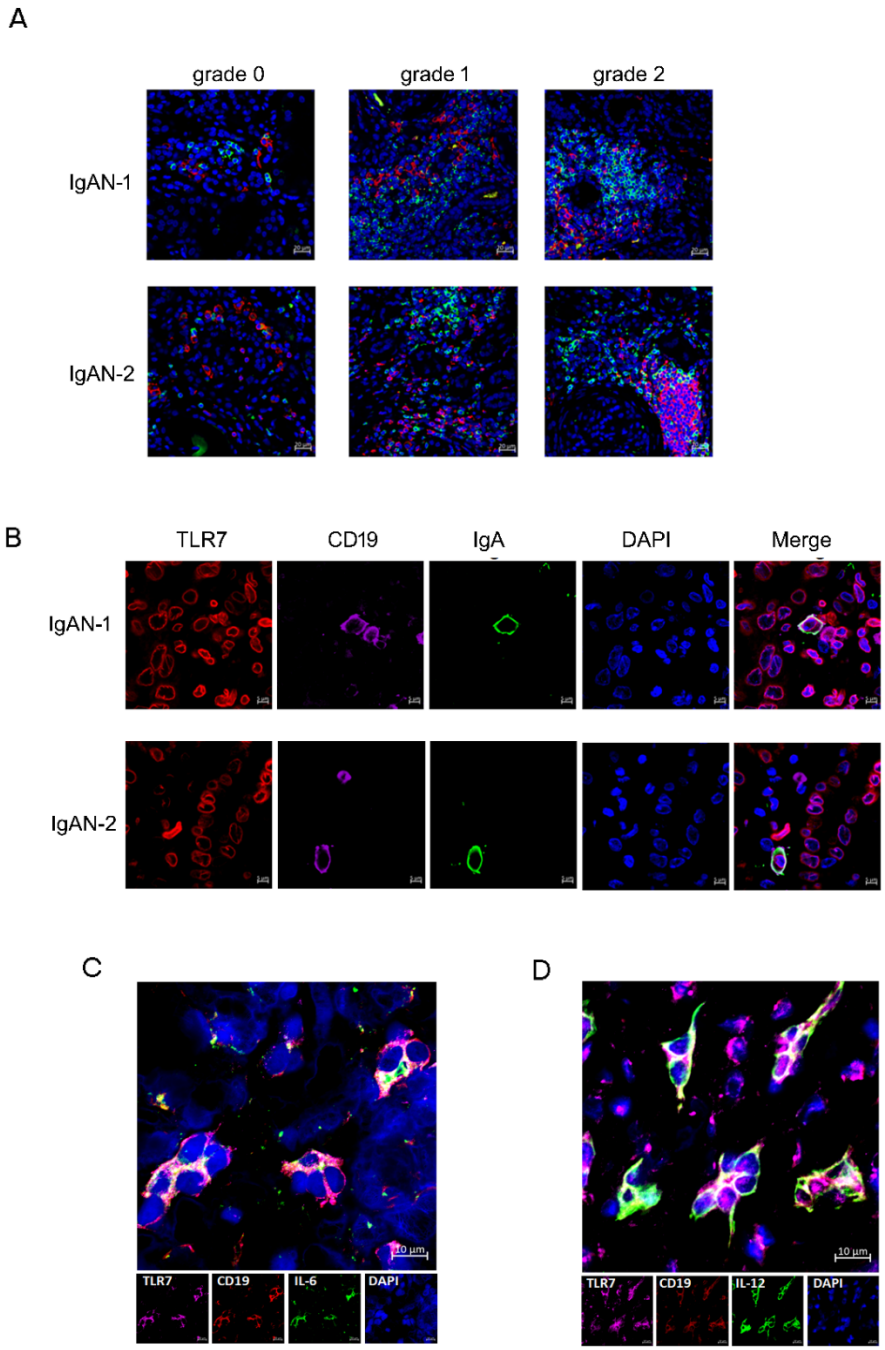
*Expression of O-glycosyltransferases in PBMCs of IgAN after TLR7/8 activation.*

PBMCs were prepared and cultured in complete medium with TLR7/8 ligand-R848 (Enzo life science, ALX-420-038-M005) as described above. At day 4, cells were collected for RNA extraction and gene expression analysis with real-time PCR. At day 13, cells were collected for western blot analysis.




**Supplemental Figure 1 Immunofluorescence staining of TLR7 proteins in kidney biopsy specimens of IgAN patients.**

Representative immunofluorescence images of TLR7 proteins in kidneys of patients with IgAN, as detected with three different rabbit anti-human TLR7 antibodies or normal rabbit IgG control antibodies. TLR7 positive signal was presented in red and nuclei was presented in blue (upper panel). Images of PAS staining of the same area were demonstrated in lower panel. *n* = 3. Scale bar, 20 μM.




**Supplemental Figure 2 Cytokine synthesis in infiltrating B cells in kidneys of patients with IgAN.** (A) Representative confocal images for the presence of T cell ($CD3^+$, green) and B cells ($CD19^+$, red) in renal tissue of patients with IgAN (*n* = 8) of different grades: scarce infiltration (grade 0), mild infiltration (grade 1), dense population (grade 2). Scale bar presents 20 µM. (B) Representative confocal images for IgA in intrarenal $CD19^+$ B cells of patients with IgAN (*n* = 6). Scale bar presents 5 µM. (C) Representative confocal images for inflammatory cytokines IL6 in TLR7 positive B cells in kidney specimens of patients with IgAN (*n* = 3). Scale bar presents 10 µM. (D) Representative confocal images for IL12 in TLR7 positive B cells in kidney specimens of patients with IgAN (*n* = 3). Scale bar presents 10 µM.

**Supplemental Figure 3 Selection criteria for candidates with chronic kidney diseases**

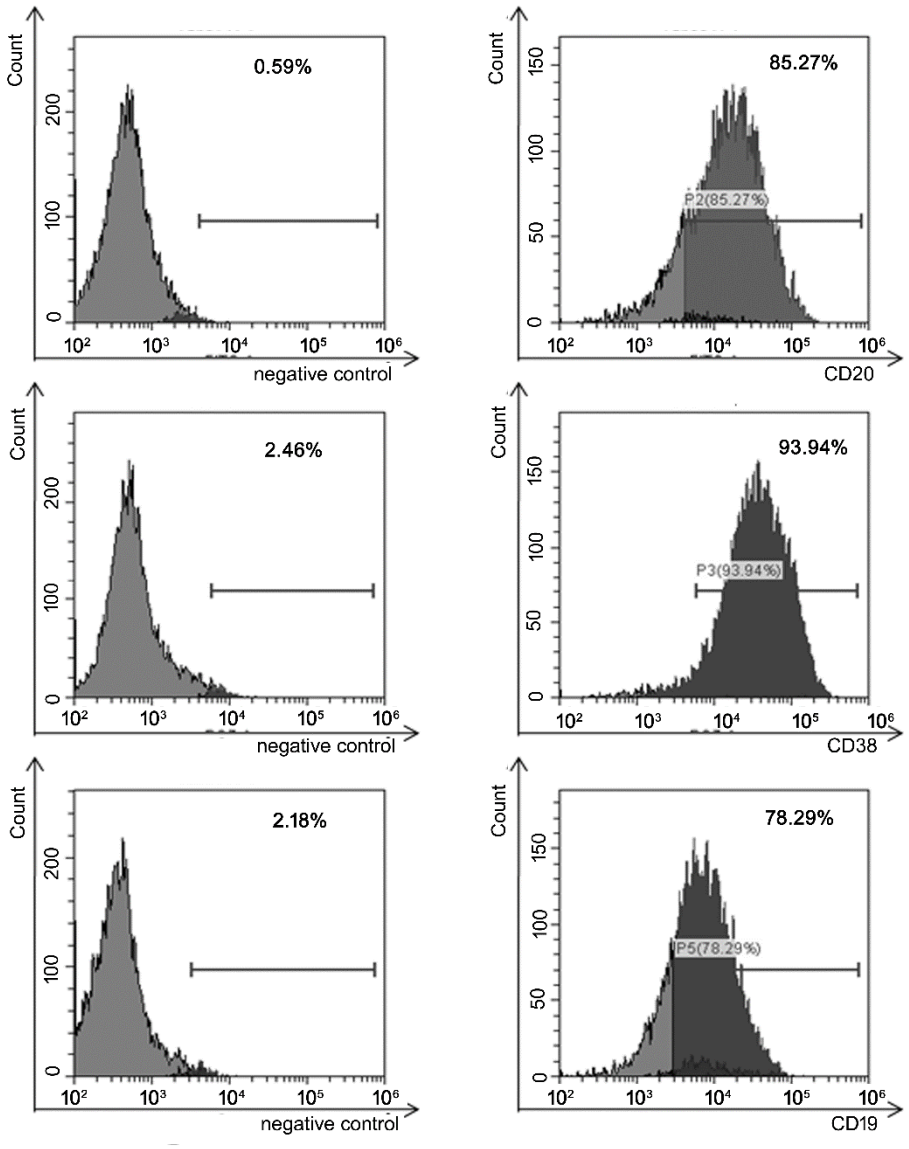
Inclusion criteria

- Age: 14~60 years, regardless of gender.
- Clinical evaluation and renal biopsy diagnostic for primary IgA nephropathy, membranous nephropathy, minimal change disease.
- No steroid therapy or immunosuppressive drug treatment within one year.
- Estimated GFR ≥ 15ml/min/1.73 $m^2$.

Exclusion criteria

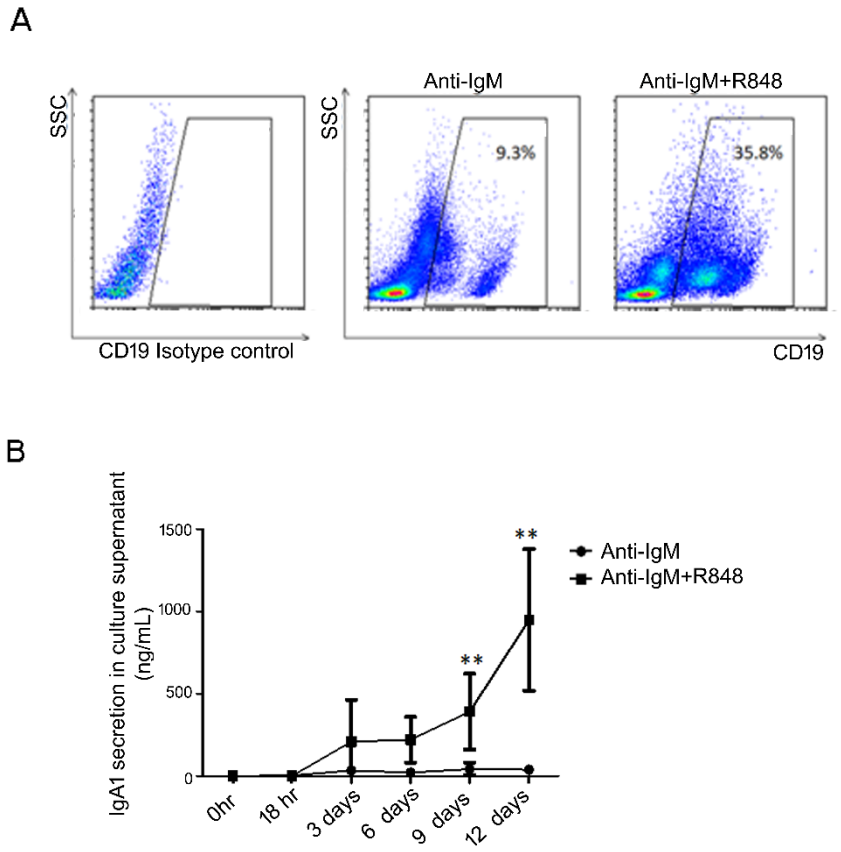
- Active clinical infection symptoms when blood sample was taken*; Systemic infection within one month.
- Autoimmune disease (SLE、Type I diabetes).
- Secondary IgAN such as lupus, purpuric nephritis, HBV-related IgAN;
- Acute or chronic virus infection (HBV, HCV, Syphilis, HIV); Hepatic dysfunction (ALT、AST increasement ≥ 2.5 fold).
- Malignant Tumor.
- Pregnancy or lactation period.

*All included donors did not show clinical infection symptoms 3 days before till 3 days after the day of blood sample taken.



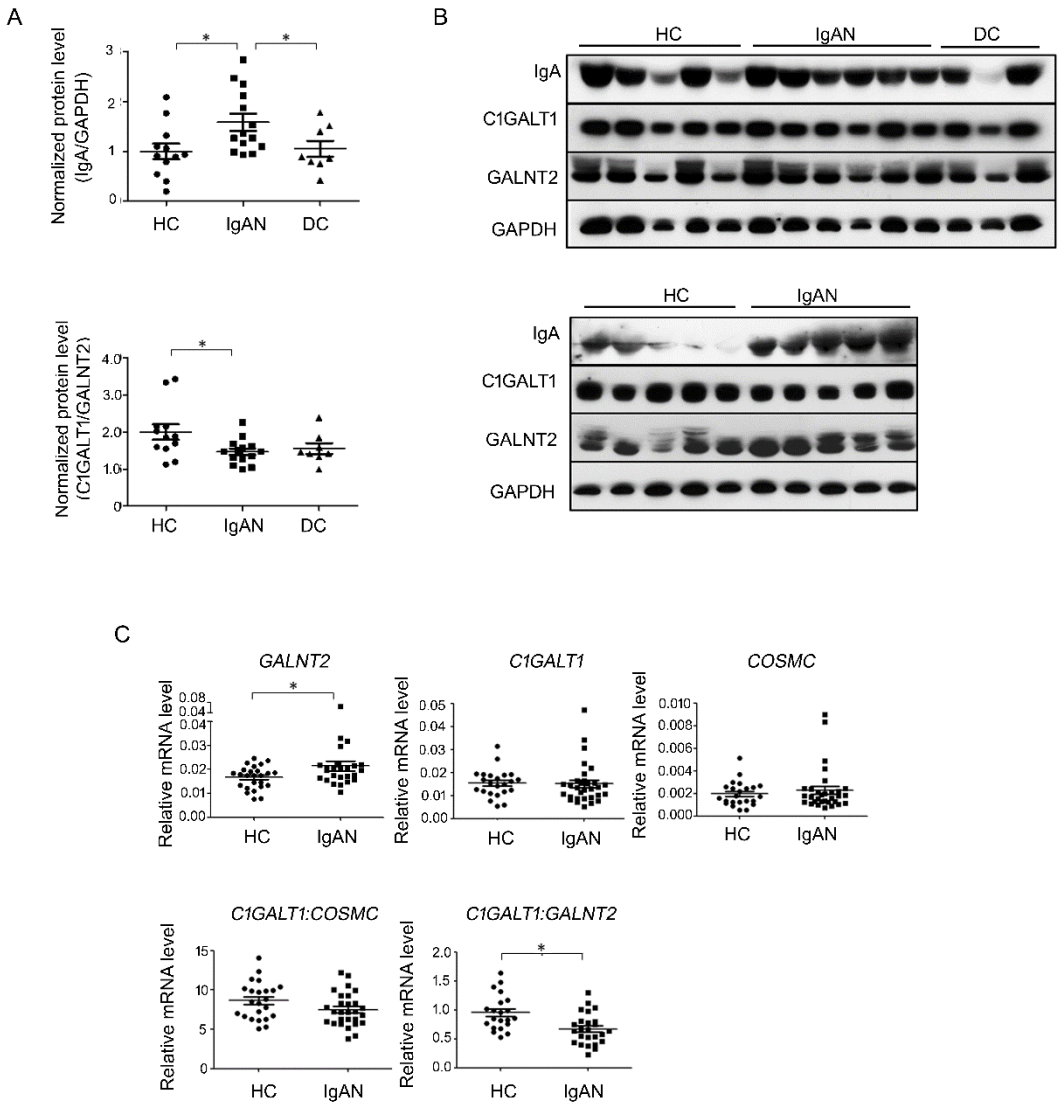

**Supplemental Figure 4 Phenotype characterization of EBV-immortalized B cells from patients with IgAN.**

Representative cytometry images of surface CD20, CD38, CD19 molecules on EBV-immortalized B cells from PBMCs of patients with IgAN (n = 3) after 4 weeks incubation with culture supernatant of B95-8 cells.




**Supplemental Figure 5 In vitro PBMCs culture with TLR7/8 ligand R848.**

PBMCs from healthy donors (*n* = 3) were isolated and cultured with anti-IgM stimulation in the presence or absence of R848. (A) Representative cytometry images of $CD19^+$ B cells after 3 days of R848 stimulation, as analyzed by flow cytometry. (B) The secretion of IgA1 in culture supernatant at different time points after R848 stimulation, measured by sandwich ELISA. Data were presented as mean ± SEM and analyzed by two-tailed unpaired Student's *t* test. ** $p < 0.01$ for treatment (Anti-IgM plus R848) versus treatment (Anti-IgM only).




**Supplemental Figure 6 Expression of IgA and GALNT2 in PBMCs of patients with IgAN after TLR7/8 activation.**

PBMCs were prepared and cultured with R848 for 12 days. Proteins were extracted from PBMCs and analyzed by western blot. (A) Protein levels of IgA, C1GALT1, and GALNT2 in cultured PBMCs and representative immunoblot images (B). Data were analyzed by One-way ANOVA with Newman-keuls multiple comparison test. HC, healthy controls (*n* = 29, combined as 12 samples); IgAN, IgAN patients (*n* = 27, combined as 14 samples), and DC, disease controls (MCD/MN) (*n* = 20, combined as 8 samples). (C) Real-time PCR analysis for mRNA levels of *O*-glycosyltransferases in PBMCs after stimulation with R848 for 3 days. HC, Healthy controls (*n* = 23); IgAN, IgAN patients (*n* = 30). Data were presented as mean ± SEM and analyzed by two-tailed unpaired Student's *t* test. * $p < 0.05$.

**Supplemental Figure 7 Primers designed for real-time PCR analysis**

*C1GALT1* (NM_020156)

Forward 5'AATGCTGCCTTCACAAGGTT3', Reverse 5'GGTGAGTGGAAGAACCCAA3';

*COSMC* (NM_152692)

Forward 5'CGGATGAAGTGCAAAACAAA3', Reverse 5'CACCCTTCAAAAAGGA GCTG3';

*GALNT2* (NM_004481.3):

Forward 5'AAGGAGAAGTCGGTGAAGCA3', Reverse 5'TTGAGCGTGAACTTCCACTG3';

*TLR7* (NM_016562.3)

Forward 5'GATGCCTTCCAGTTGCGATA3', Reverse 5'TCCGTATGGTTAACCCACCA3';

*TLR8* (NM_138636.5)

Forward 5' CTCATGCAGAGCATC AACCA3', Reverse 5'ACACTGGCTCCAGCAGGATA3';

*GAPDH* (NM_002046.4)

Forward 5'GAGTCAAC GGATTTGGTCGT3', Reverse 5'GACAAGCTTCCCGTTCTCAG 3'.