Supplementary

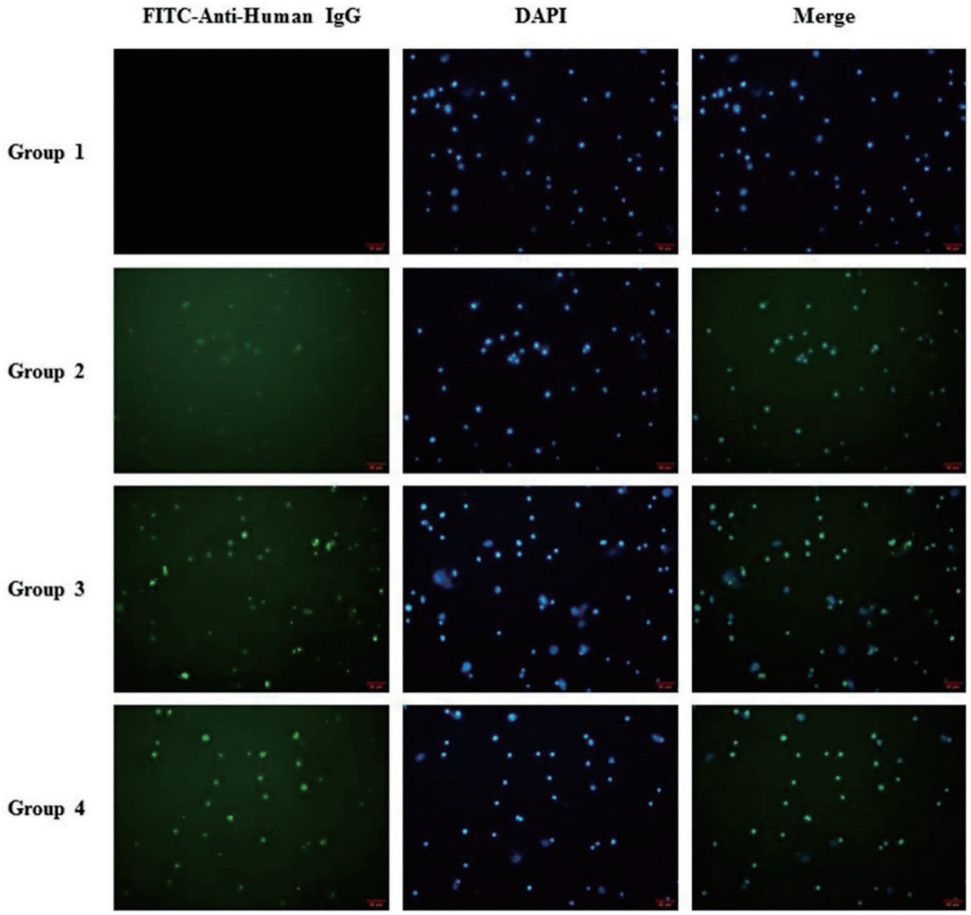

Figure S1 IgG-type autoantibody specific against podocytes can be found in the serum of children with PNS. IgG antibodies purified from the serum of 20 children with PNS, serving as the primary antibody, were incubated with podocytes and then rinsed with PBS. FITC-labeled monkey anti-human IgG (1:100 dilution) was added as the secondary antibody, followed by incubation for 1 hour at room temperature, rinsing, and observation and photographing under an inverted fluorescent microscope. Group 1: blank control (primary antibody: no serum added); group 2: negative serum control (primary antibody: healthy human serum); group 3: serum from a child with PNS with IgG-type autoantibody specific against podocytes as the primary antibody (initial concentration); group 4: serum from a child with PNS with IgG-type autoantibody specific against podocytes as the primary antibody (1:10 dilution). Scale bar: 50 μm. Indirect immunofluorescence method was used for staining.

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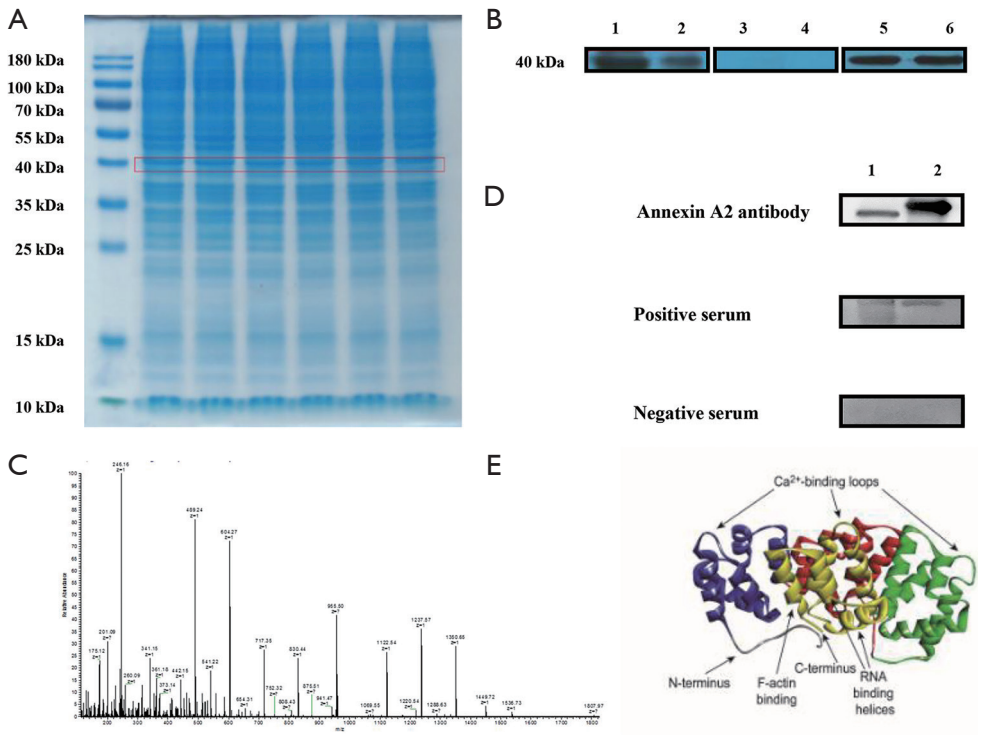

Figure S2 Annexin A_2 in podocytes is the main target antigen of the autoantibody in children with PNS. For the total protein of podocytes, two sets of SDS-PAGE were conducted in parallel, one of which was used for Coomassie Brilliant Blue staining (A) and the other transferred to a nitrocellulose membrane after gel electrophoresis, to which serum from children with PNS was added (the presence of IgG autoantibody against podocytes had been verified via mouse podocyte immunofluorescence) for western blotting detection. Coomassie Brilliant Blue-stained bands corresponding to western blotting positive bands (B) were selected for mass spectrometry to identify Annexin A_2 protein (C). The commercial recombinant Annexin A_2 protein specifically reacted with serum from children with PNS containing Annexin A_2 antibody, and the commercial Annexin A_2 antibody also specifically reacted with the Annexin A_2 protein band isolated from podocyte total protein after SDS-PAGE (D). (B) (1: 1,000-fold diluted IgG antibodies purified from the serum of PNS children; 2: 2000-fold diluted IgG antibodies purified from the serum of PNS children; 3: 1,000-fold diluted IgG antibodies purified from the serum of healthy people; 4: 2000-fold diluted IgG antibodies purified from the serum of healthy people; 5-6: β-actin). (D) (1: Annexin A_2 protein band isolated from podocyte total protein after SDS-PAGE; 2: Recombinant Annexin A_2 protein; Positive serum: Serum containing Annexin A_2 antibody collected from children with PNS; Negative serum: serum collected from healthy people). (E) The three-dimensional structure of the Annexin A_2 protein.

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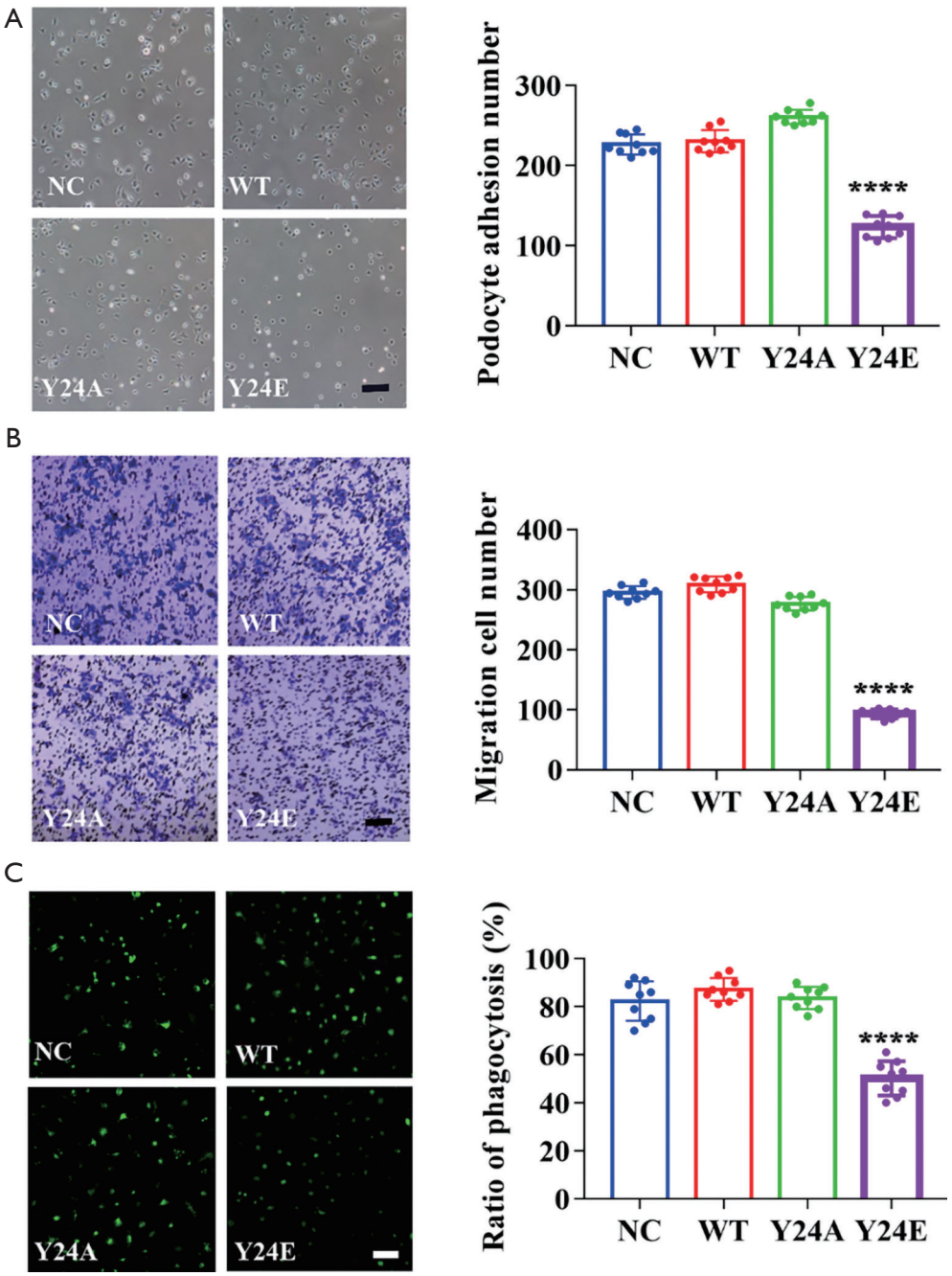

Figure S3 Tyr24 hyperphosphorylation of Annexin A_2 in podocytes can affect podocyte function. Tyr24 hyperphosphorylation of Annexin A_2 in podocytes can reduce the adhesion (A), migration (B) and phagocytosis (C) ability of podocytes. Scale bar: 100 μm. ****, P<0.0001. Migration ability was detected by Coomassie Brilliant Blue staining. Phagocytosis ability was detected by indirect immunofluorescence staining.

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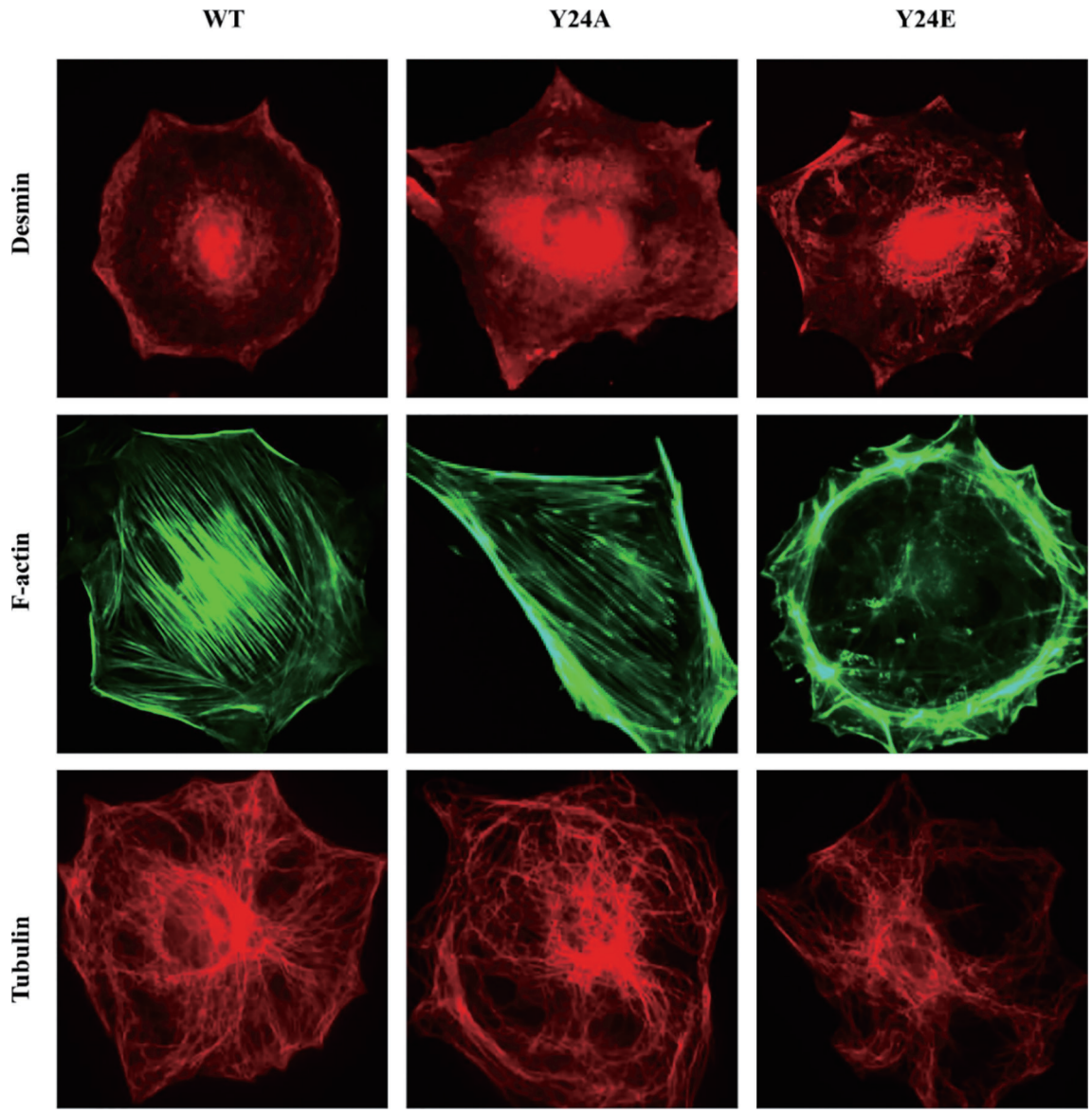

Figure S4 Tyr24 hyperphosphorylation of Annexin A_2 in podocytes can cause podocyte cytoskeletal changes. Indirect immunofluorescence method was used for staining. 600 X optical magnification.

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