

Supplementary Fig. 2. Immunofluorescence images of podocytes treated with nephrotic plasma for different durations stained for vinculin (focal adhesion complexes) and phalloidin (actin cytoskeleton) to assess podocyte cytoskeleton integrity. Differentiated podocytes were incubated in medium containing 5% plasma from healthy control, SSNS, and SRNS (without mutation in *NPHS2* and *WT1*) for different durations (1, 3, 6, and 12 hours). After incubation these cells were fixed with 4% formaldehyde and incubated in antivinculin antibody, phalloidin antibody, and nuclei were stained with 4',6-diamidino-2- phenylindole (DAPI; blue) and imaged using direct fluorescence microscope (Olympus, Tokyo, Japan) at ×40 magnification. In a healthy podocyte (untreated and incubated with healthy control), focal adhesion complexes can be visualized as green punctate marks at ends of red parallel strands of actin fibers representing the cytoskeleton. Podocytes treated with SSNS and SRNS plasma showed loss of focal adhesion complexes and disorganization of cytoskeleton (loss of parallel arrangement of actin fibers) at 6 hours as opposed to untreated cells and those treated with healthy plasma. SSNS, steroid-susceptible nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome.