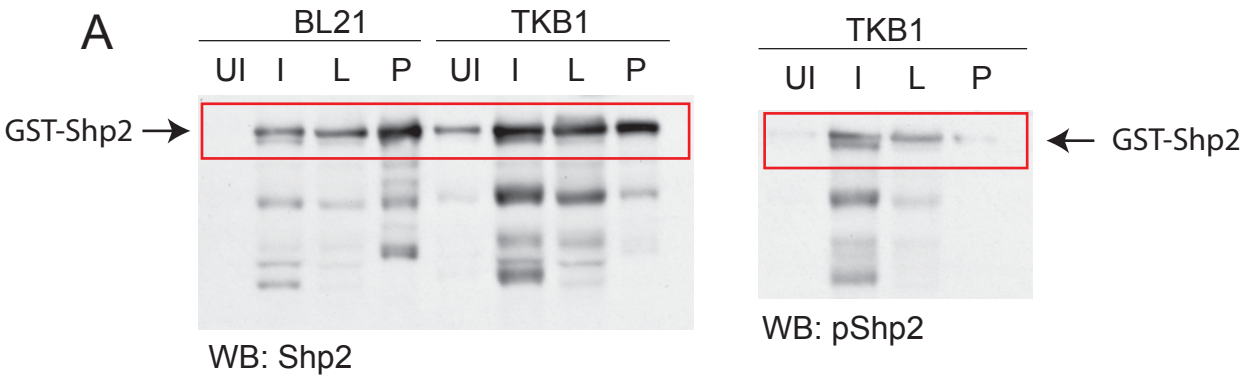
Supplementary Data

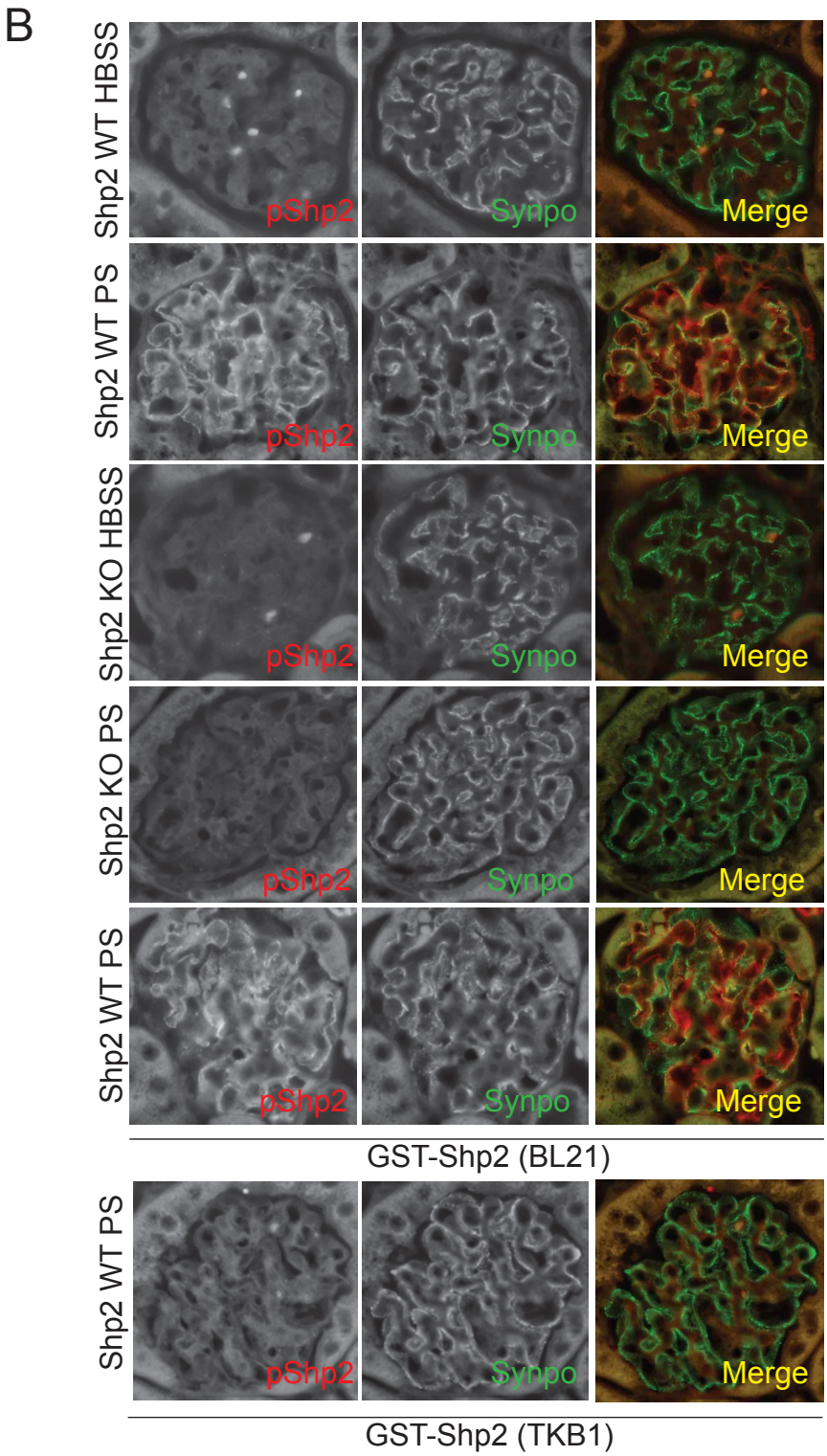
Shp2 associates and enhances nephrin tyrosine phosphorylation and is necessary for foot process spreading in mouse models of podocyte injury.

Rakesh Verma, Madhusudan Venkatareddy, Anne Kalinowski, Sanjeevkumar Patel, David J. Salant and Puneet Garg

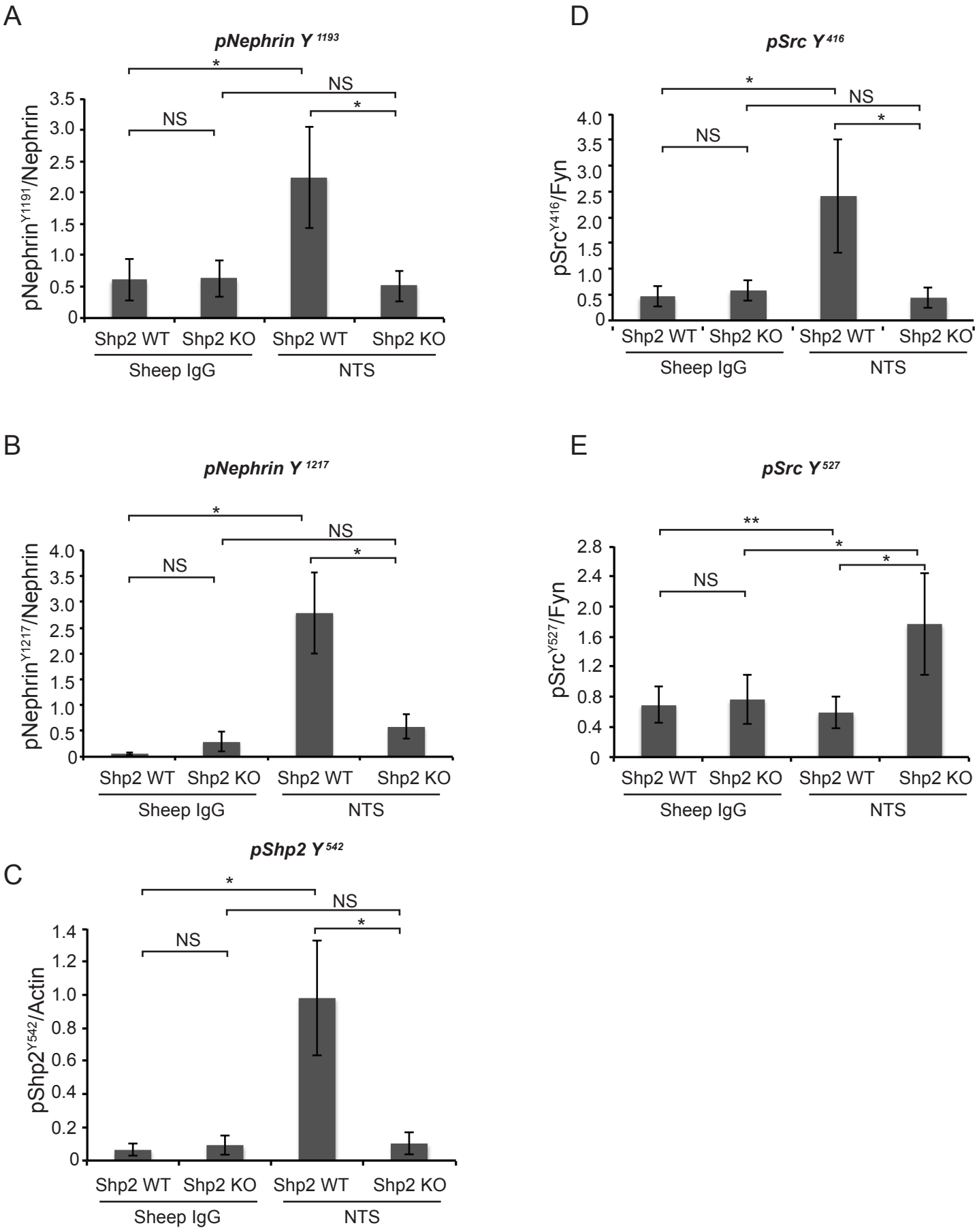
Supplementary Figure S1. *Characterization of phosph-Shp2 antibody.* (A) Full length GST-tagged Shp2 was expressed in BL21 E.coli or TKB1 E.coli. Left panel shows blotting using Shp2 antibody for un-induced (UI), induced (I), bacterial lysate (L) and purified (P) GST-Shp2 in both BL21 and TKB1 E. coli. Right panel shows blotting with phospho-Shp2 Y^{542} (pShp2) antibody. (B) Immunostaining of mouse kidney sections following HBSS and protamine sulfate infusion. There is increase in pShp2 staining following protamine sulfate infusion that is quenched by prior incubation with tyrosine phosphorylated GST-Shp2 (Bottom panel).

Supplementary Figure S2. *Quantification of nephrin, Shp2 and Src tyrosine phosphorylation in* $Shp2^{fl/fl,Cre+}$ *and* $Shp2^{fl/fl,Cre-}$ *mouse glomerular lysates following NTS injection.* Mouse glomerular lysates from $Shp2^{fl/fl,Cre+}$ and $Shp2^{fl/fl,Cre-}$ mouse kidneys 48 hrs following NTS injection were resolved using SDS-PAGE were blotted with indicated antibodies. ImageJ software was used to quantify the density of the bands. Values were normalized to the total protein. There is increase in nephrin Y1193 and Y1217, Shp2 Y542 and Src Y416 tyrosine phosphorylation following NTS injection in $Shp2^{fl/fl,Cre+}$ mouse that is abrogated in the $Shp2^{fl/fl,Cre-}$ mouse glomerular lysates. Data are mean ± SEM. *P<0.001, **P<0.01.





Supplementary Figure 1



Supplementary Figure S2