Supplemental Figure 1
(Corresponding to Figure 2)


## Supplemental Figure 1

Subcellular localization of mTOR in p18 knockout MPC-5 podocytes.
Double staining of mTOR and LAMP2 (lysosomal membrane protein) was performed under amino acid-deprived or -replenished conditions in control and p18 knockout MPC-5 podocyte.

## Supplemental Figure 2

 (Corresponding to Figure 3)

## Supplemental Figure 2

Characterization of the mTOR signaling in p18 knockout MPC-5 podocytes.
(A) Control and p18 knockout MPC-5 podocytes were harvested in normal growing condition.

The expression of $\mathrm{p} 18, \mathrm{mTORC} 1$, and mTORC 2 components were determined by western blotting using the indicated antibodies.
(B) Time course of mTORC1 activation by serum in control and p18
knockout MPC-5 podocytes. Cells were serum starved, then stimulated with serum ( $2 \%$ ) for the indicated times. Levels of mTORC1 (pS6K1 and p4EBP1) and mTORC2 activity (pAKT (S473)) were monitored by western blotting using the indicated antibodies.

A


B


## Supplemental Figure 3

Characterization of the mTOR signaling in p18 knockout primary podocytes.
(A) Control (F/F) and p18 knockout (KO) primary podocytes were serum starved, then stimulated with the indicated concentrations of serum for 15 min . Levels of mTORC1 and mTORC2 activity were monitored.
(B) Similar experiments were performed by using insulin.


## Supplemental Figure 4

Characterization of the mTOR signaling in p18 knockout MEF.
(A) p18 knockout ( p 18 KO ) and p18 expressing ( p 18 WT ) MEFs were incubated with amino acid free media containing
$10 \%$ dialyzed serum for 60 min , then stimulated with amino acids ( 1 x ) for the indicated times.
(B) Cells were starved with serum-free media for 10 hrs , then stimulated with $2 \%$ serum for the indicated times. Levels of mTORC1 ( $\mathrm{pS6K1}$ and p 4 EBP 1 ) and mTORC2 activity ( $\mathrm{pAKT}(S 473)$ ) were monitored by western blotting using the indicated antibodies.

Supplemental Figure 5 (Corresponding to Figure 5)


Supplemental Figure 5
Ragulator is important for active Rheb-induced mTORC1 activation and podocyte injury in MPC-5 podocytes.
(A) TSC1 is knocked down in control and p18 knockout MPC-5 podocytes. Cells were harvested under normal growing conditions. Levels of mTORC1 activity were monitored by western blotting using the indicated antibodies.
(B) Levels of cleaved or non-cleaved caspase3 and Akt phosphorylation were monitored.


B



Wild type TSC1 KO
TSC1/p18 DKO

## Supplemental Figure 6

Examination of podocyte apoptosis and detachment in podo-TSC1 KO and podo-TSC1/p18 DKO mice.
(A) Glomeruli were isolated from 8 week-old wild type, podo-TSC1 KO, and podo-TSC1/p18 double KO mice.

Levels of non-cleaved and cleaved caspase 3 were monitored.
(B) 24-hours-urine were collected from the indicated mice. mRNAs were purified from the urinary sediments and levels of Nephrin, PPIA (Peptidyl-prolyl cis-trans isomerase), and AQP2 (Aquaporin-2) mRNAs were measured. The amounts of nephrin and PPIA (used as a house-keeping) mRNA were normalized by AQP2 mRNA.


## Supplemental Figure 7

An illustration of mTORC1 signaling pathway. Ragulator, the GEF complex for RagA, stimulates RagA in response to amino acids thereby recruiting mTORC1 to the lysosomal membrane. On the lysosomal membrane, mTORC1 is directly activated by Rheb, which is stimulated by growth factors, glucose, and/or amino acids through the inhibition of the TSC complex. Acivated mTORC1 inhibits the activity of PI3K through negative feedback inhibiton from S6K.

