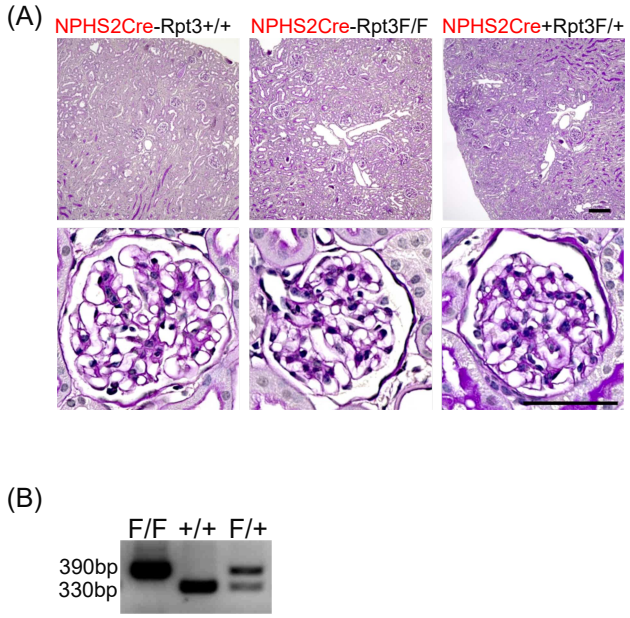
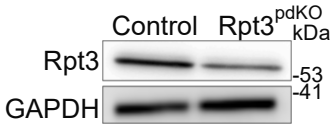
Supplemental material Table of Contents

Supplemental Figure 1

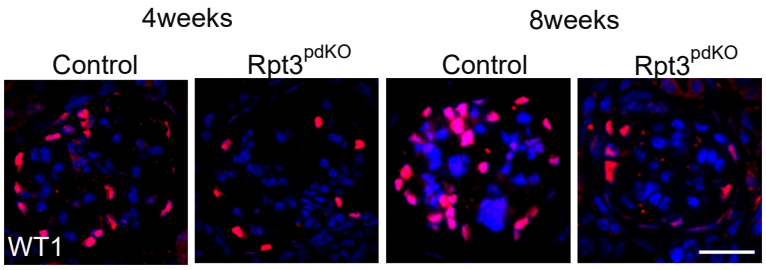


Supplemental Figure 1. Periodic acid–Schiff (PAS) staining of NPHS-Cre-Rpt3 flox/flox and NPHS-Cre+Rpt3 flox/WT (A) NPHS-Cre-Rpt3 flox/flox and NPHS-Cre+Rpt3 flox/WT showed no pathologic phenotypes at 2 months of age when examined using histologic methods. (B) Rpt3 flox/flox, Rpt3 WT/WT and Rpt3 flox/+WT were distinguished by genotyping with cut tails. Scale bars: 100 μm (upper), 50 μm (lower).



Supplemental Figure 2. The expression of Rpt3 was decreased in glomeruli of Rpt3^{pdKO} mice at 4 weeks of age.

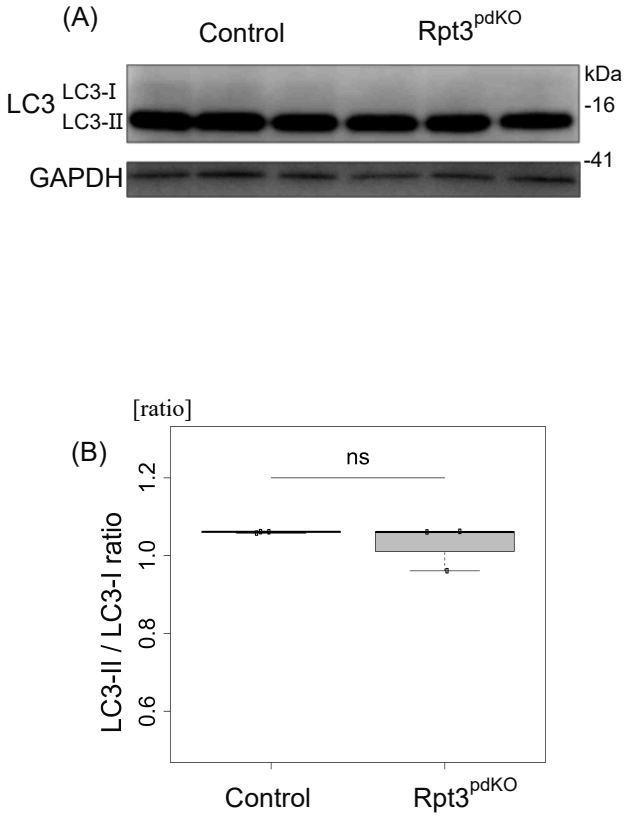
Supplemental Figure 3



Supplemental Figure 3. The number of WT1 was decreased in the glomeruli of $Rpt3^{pdKO}$ kidneys compared with $Rpt3^{Control}$ kidneys.

In IF staining, the number of WT1 which is a nuclear marker for podocytes was decreased in the kidney of $Rpt3^{pdKO}$ mice than in the kidney of $Rpt3^{Control}$ mice at 4 and 8 weeks of age. Scale bars: 20 μm.

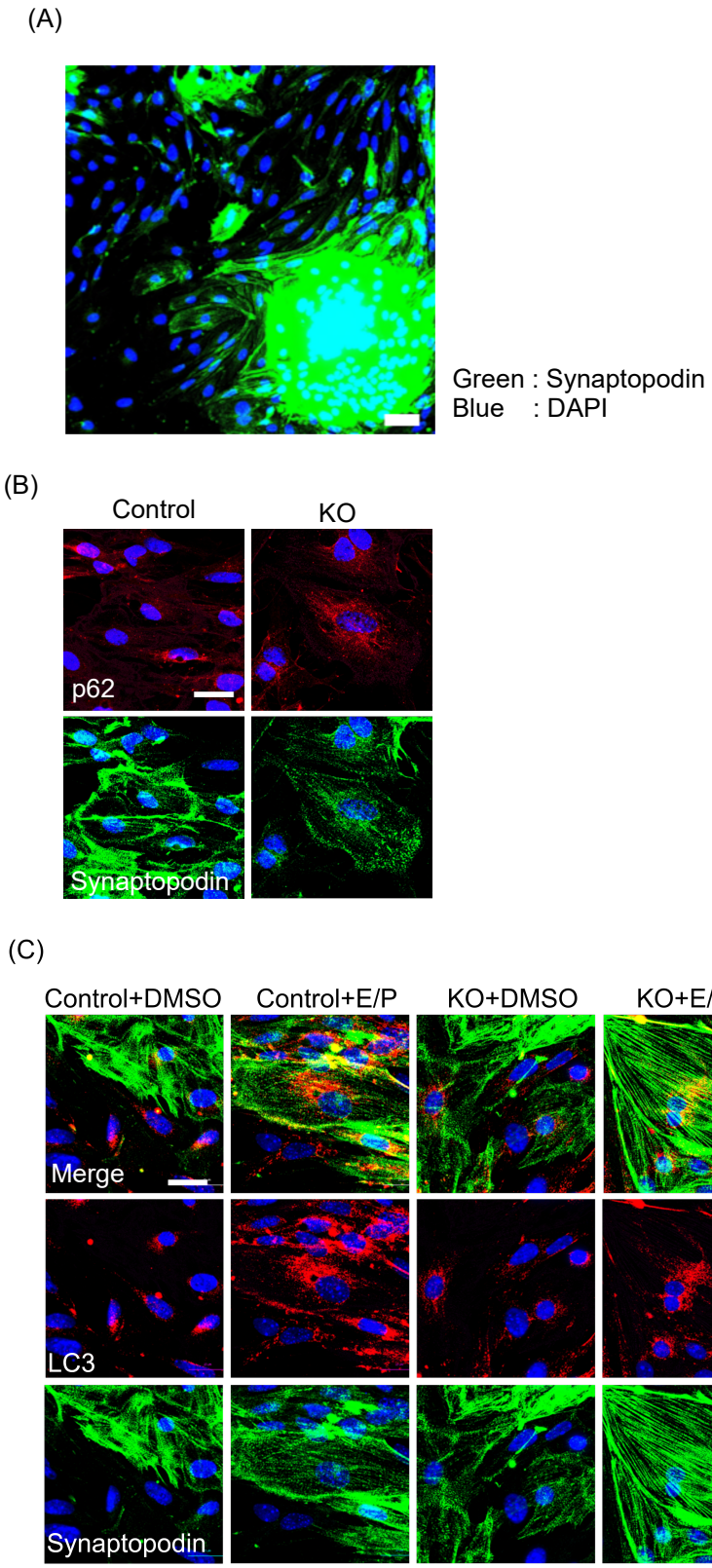
Supplemental Figure 4



Supplemental Figure 4. There was no significant change in the expression of LC3 between $Rpt3^{Control}$ and $Rpt3^{pdKO}$ mice.

(A and B) There was no significant change in the LC3-II / LC3-I ratio between $Rpt3^{Control}$ and $Rpt3^{pdKO}$ kidneys at 4 weeks of age in WB by glomerular lysate (n=3, P=0.39, Welch's test).

Supplemental Figure 5



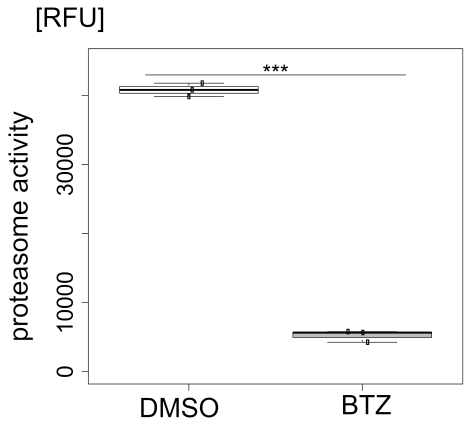
Supplemental Figure 5. The expressions of p62 and LC3 were analyzed using primary culture podocytes from 4 weeks of age mice.

(A) Synaptopodin-positive cellular outgrowths from glomeruli were distinguished as podocytes. (B) The accumulation of p62 was detected in primary cultured podocytes from $Rpt3^{pdKO}$ mice. (C) Three days after primary culture, podocytes were treated with lysosomal protease inhibitors E64d (10 μg/ ml) and pepstatin A (25 μg/ ml) (E/P) for 24 hours. Under the effect of E/P, the expression of LC3 was lower in the podocytes from $Rpt3^{pdKO}$ mice than from $Rpt3^{Control}$ mice. Scale bars: 100 μm (A), 25 μm (B and C).

Supplemental Figure 6

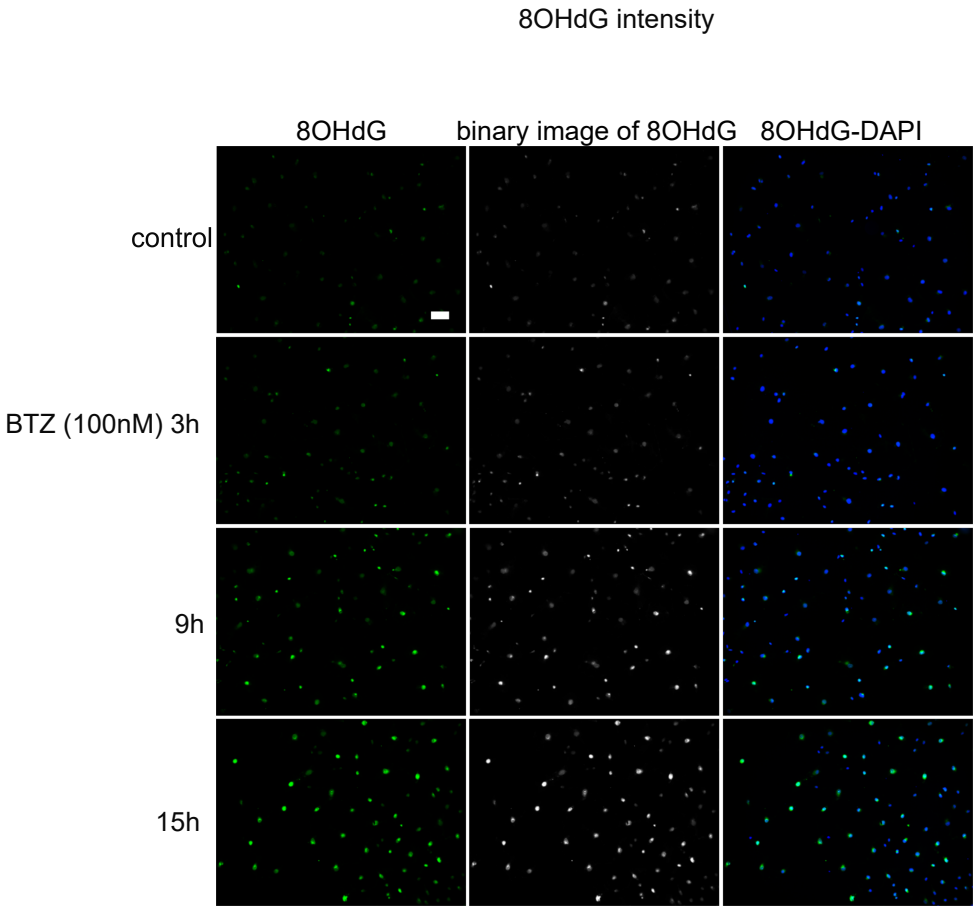
Supplemental Figure 6. Rhodamine phalloidin staining showed that the treatment of BTZ altered the actin formation of cultured podocytes. Bortezomib (BTZ) was used at concentration of 100nM. Scale bars: 20 μm

Supplemental Figure 7



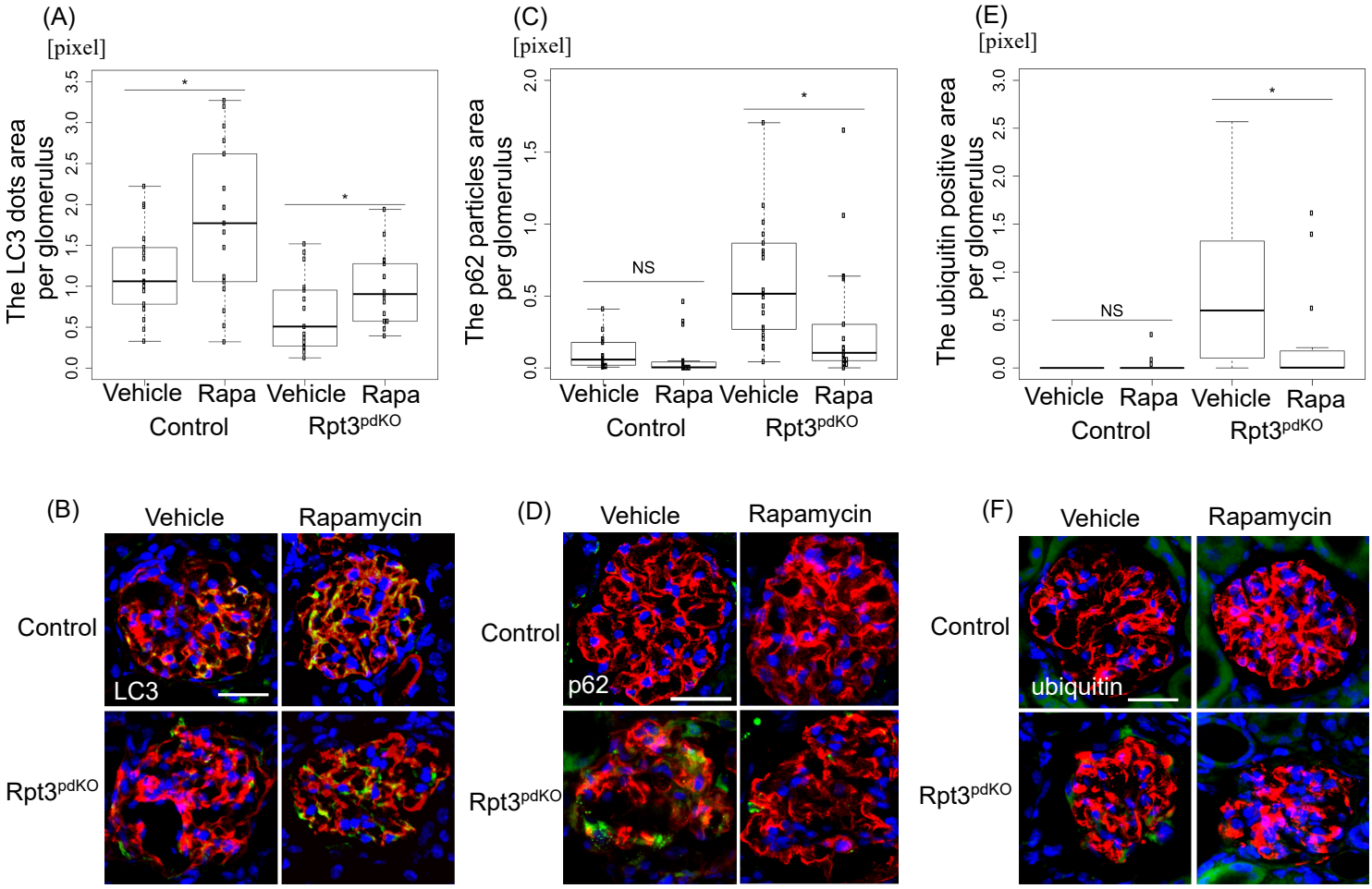
Supplemental Figure 7. Proteasome activity was decreased by proteasome inhibition in podocytes at 6 hours after treatment of bortezomib to cultured podocytes. The proteasome activity of podocytes was reduced to about 13% compared with that of DMSO treated podocytes.

Supplemental Figure 8



Supplemental Figure 8. Immunofluorescence intensity of 8-OHdG was increased in podocytes in a time dependent manner after treatment wtih 100nM of BTZ. Left side shows the 8-OHdG staining, middle shows the binary image of 8-OHdG staining and right side shows co-staining of 8-OHdG and nuclei; Green : 8HdG, Blue : DAPI. Scale bars: 50 μm

Supplemental Figure 9



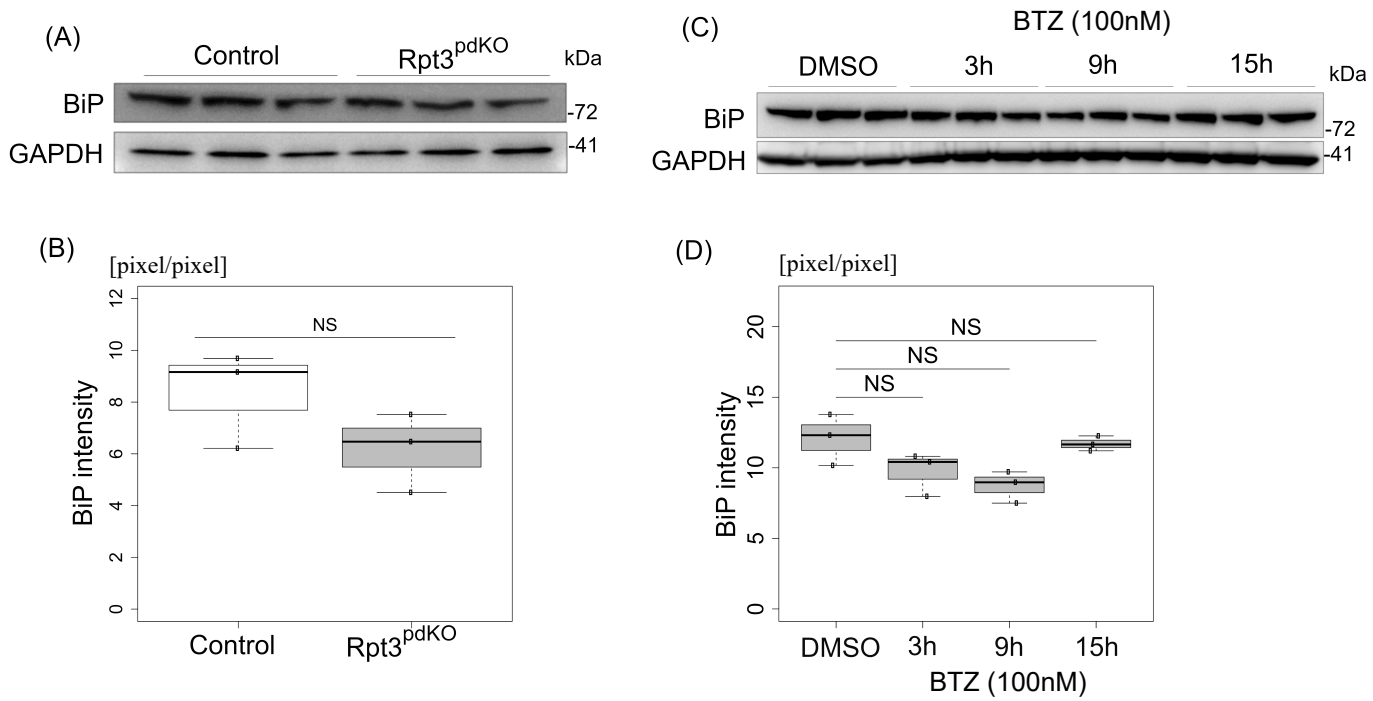
Supplemental Figure 9. Rapamycin induced autophagic activity of podocytes in Rpt3^{pdKO} mice.

(A and B) The LC3 dots in podocytes increased by rapamycin treatment in control and Rpt3^{pdKO} mice.

(C and D) The p62 particles in podocytes decreased by rapamycin treatment in Rpt3^{pdKO} mice.

(E and F) The ubiquitin accumulation in podocytes decreased by rapamycin treatment in Rpt3^{pdKO} mice.

($P<0.05$ by Mann-Whitney's U test)



Supplemental Figure 10. ER stress did not participate in podocyte injury via the impariment of proteasome function.

(A) There was no significant change in the expressions of BiP between $Rpt3^{Control}$ and $Rpt3^{pdKO}$ mice in WB by glomerular lysate (n=3). (B) The densitometry quantification of BiP expression in WB shown in (A) (n=3). (C) There was no significant change in the expression of BiP in cultured podocytes after the treatment with 100nM of BTZ in vitro (n=3). (D) The densitometry quantification of BiP expression in WB shown in (C) (n=3). *P<0.05 ; **P<0. 01 ; ***P<0. 001 by Welch's t-test.