Supplemental Data

Blocking rpS6 Phosphorylation Is Detrimental to *Tsc1* Deletion-induced Kidney Growth

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Running Title: Tsc1 and rpS6 in Renal Growth

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Wu et al _ Figure S1

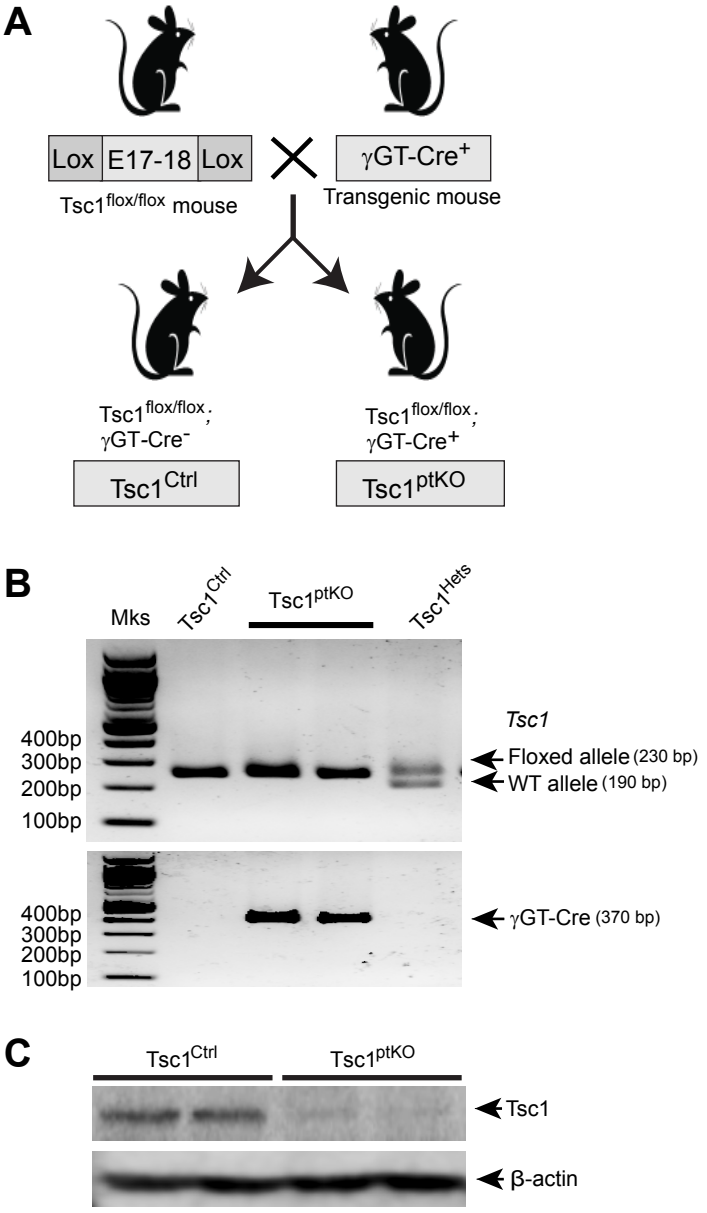

Figure S1. Generation of renal proximal tubule cell-specific *Tsc1* gene knockout mice. (A) A schematic depicting the generation of renal proximal tubule cell-specific Tsc1 knockout ($Tsc1^{ptKO}$) mice. (B) PCR genotyping detected only the 230-bp floxed *Tsc1* allele in both $Tsc1^{Ctrl}$ and $Tsc1^{ptKO}$ mice but detected both the 230-bp floxed *Tsc1* allele and the 190-bp wild type (WT) *Tsc1* allele in heterozygous-floxed ($Tsc1^{Hets}$) mice; however, only $Tsc1^{ptKO}$ mice were detected positive for the *γGT-Cre* transgene, which defines Cre-mediated deletion of floxed alleles. (C) Immunoblotting of renal cortices enriched with proximal tubules confirmed γGT-Cre-mediated effective deletion of Tsc1 protein. Shown are representative gels from at least three separate experiments with similar results.

Wu et al _ Figure S2

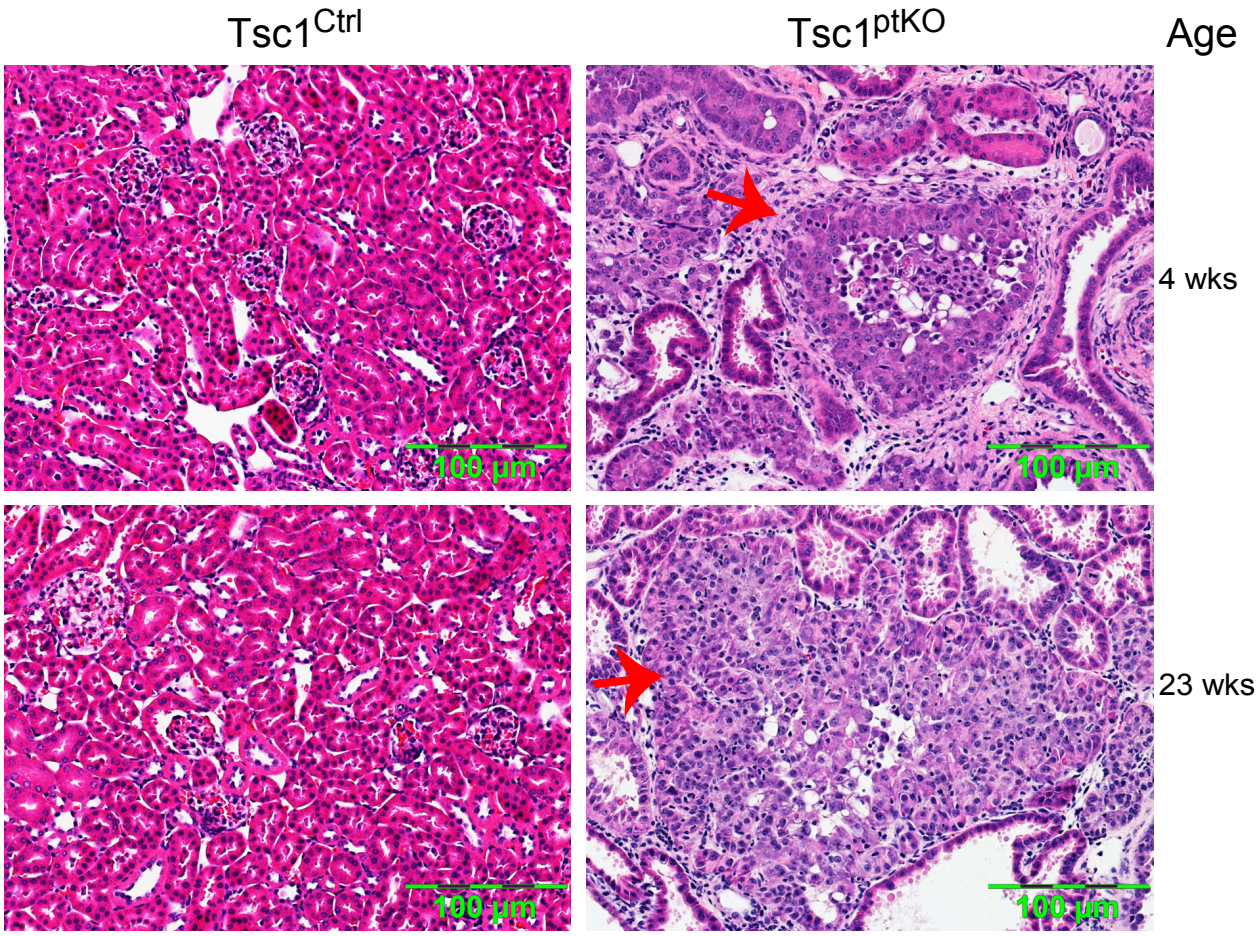

Figure S2. Deletion of Tsc1 in renal proximal tubules caused occasional tumorous lesions (red arrows) seemingly resulted from dysregulated tubular epithelial cell proliferation filling up the lumens of enlarged renal tubules to form microscopic hamartomatous renal tumors. By 23 weeks of age, $Tsc1^{ptKO}$ mice had markedly larger hamartomas than those observed at 4 weeks of age. Shown are representative images from *n* of at least five mice per genotype group with similar results. (Scale bar, 100 µm.)

Wu et al _ Figure S3

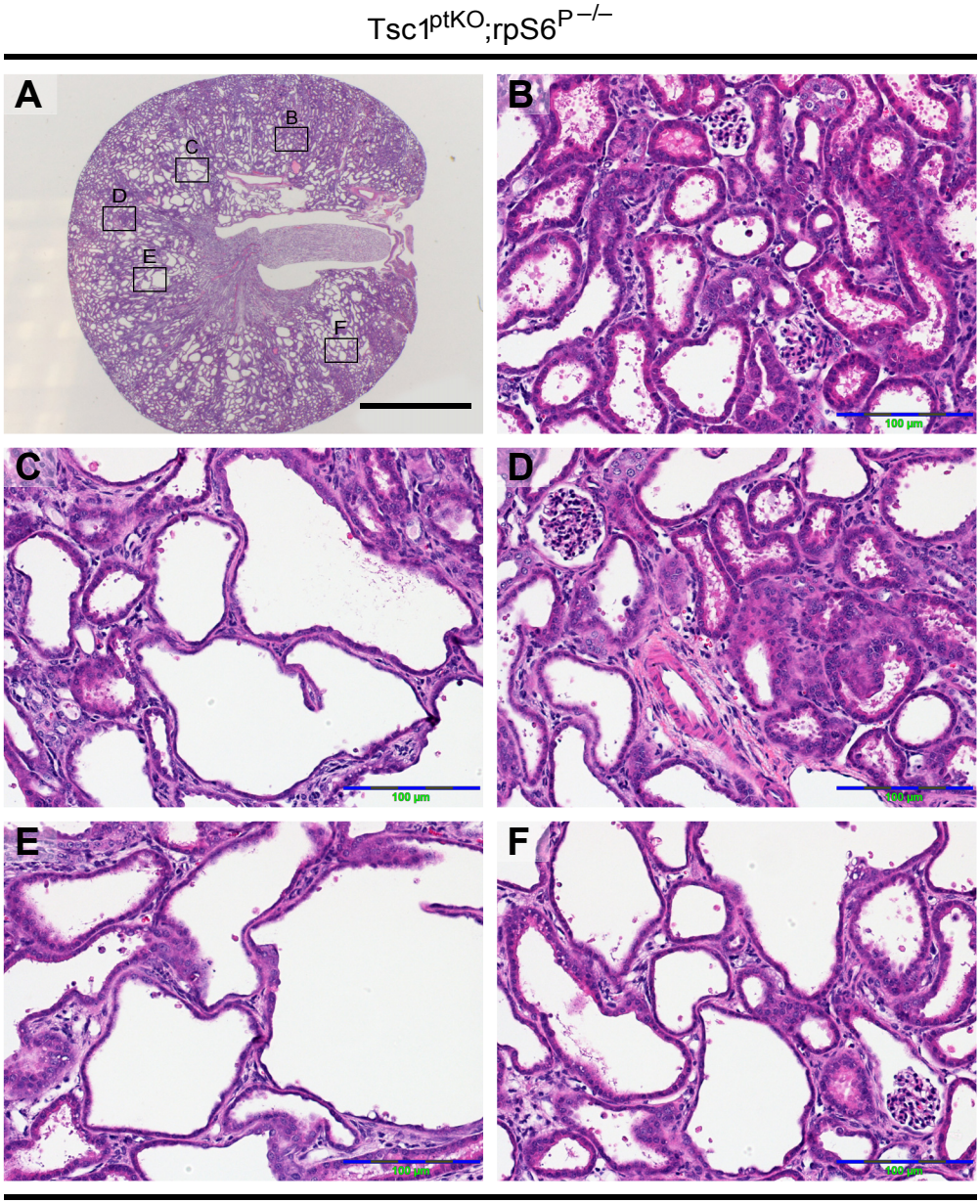

Figure S3. Genetic deletion of rpS6 phosphorylation on the background of $Tsc1^{ptKO}$ mice produced the double mutant ($Tsc1^{ptKO}$;$rpS6^{P-/-}$) mice carrying enormously enlarged kidneys with a pale appearance due to exacerbated cystogenesis (as shown in Figure 4C). (A) The full cross-sectional kidney picture of the cystic kidneys from $Tsc1^{ptKO}$;$rpS6^{P-/-}$ mice (H&E staining). (B-F) Higher magnification images from various areas indicating the exacerbated cystogenesis and nephron damage in the double mutant $Tsc1^{ptKO}$;$rpS6^{P-/-}$ mice by 4 weeks of age (compare with the images of $Tsc1^{ptKO}$ mice in Figure S5). Shown are representative images from 5 mice per group with similar results. (Scale bars: 2 mm in A; 100 µm in B-F.)

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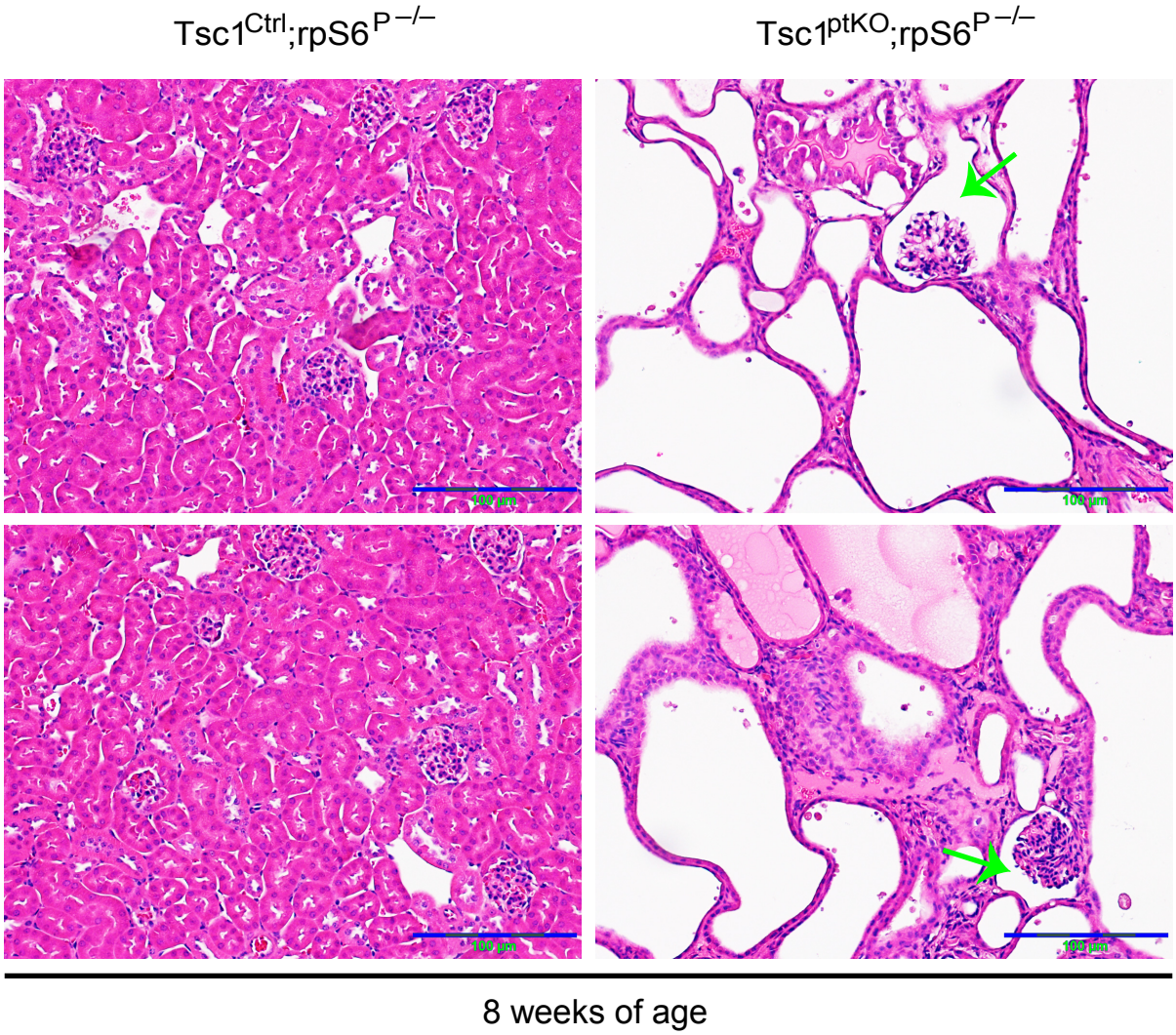

Figure S4. Genetic deletion of rpS6 phosphorylation on the background of $Tsc1^{ptKO}$ mice produced the double mutant ($Tsc1^{ptKO}$;$rpS6^{P-/-}$) mice exhibiting glomerular cysts (*green arrows*) in the areas with massive enlarged renal cysts (most cysts were lined with flattened monolayer of epithelial cells) by 8 weeks of age (H&E staining); there was no cyst formation when rpS6 phosphorylation was genetically deleted on the background of $Tsc1^{Ctrl}$ mice ($Tsc1^{Ctrl}$;$rpS6^{P-/-}$). (Scale bar, 100 μm.)

Wu et al _ Figure S5

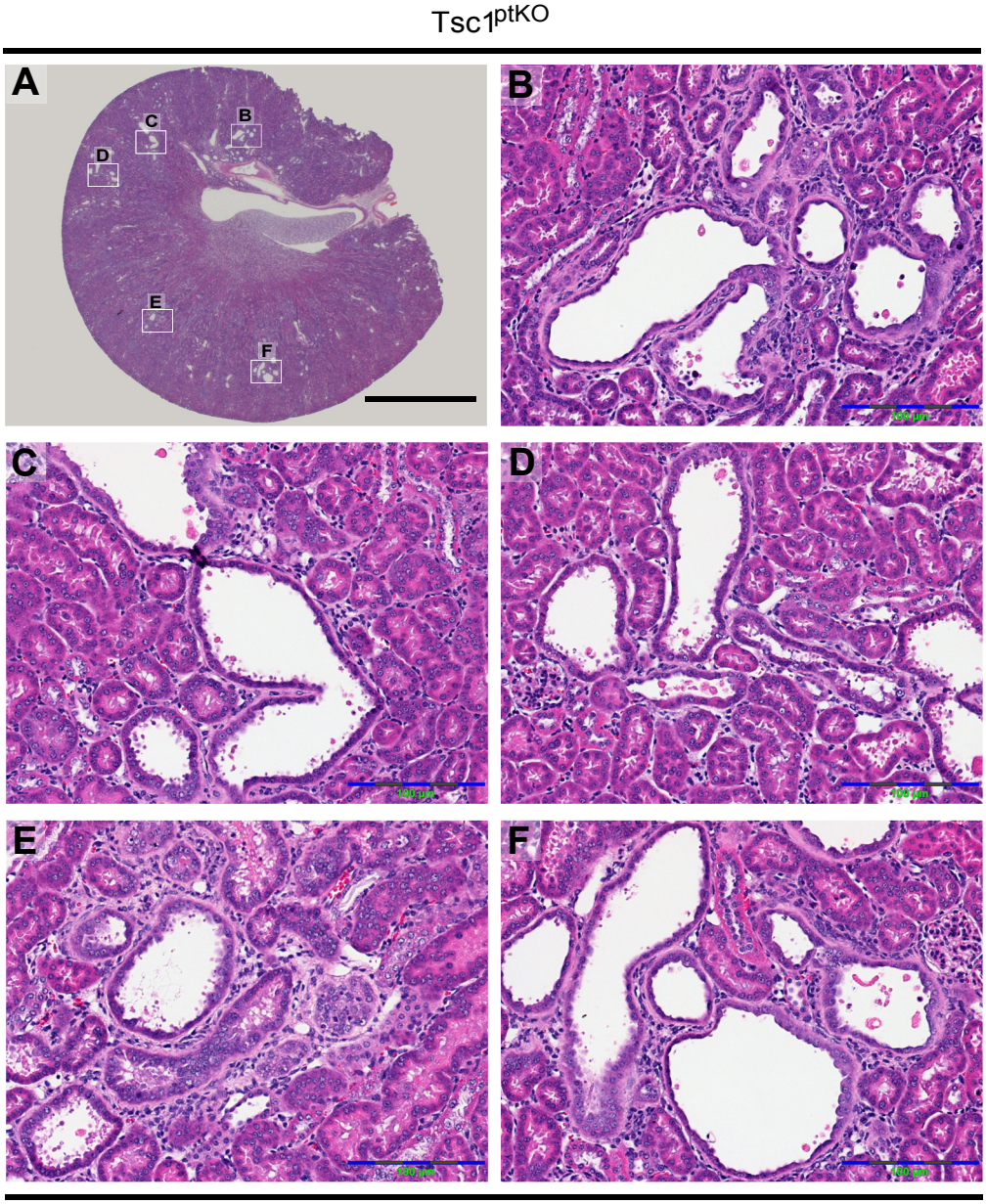

Figure S5. Deletion of Tsc1 in mouse renal proximal tubules resulted in enormously enlarged kidneys (as shown in Figure 4C). (A) The full cross-sectional kidney picture of the enormously hypertrophied kidneys of $Tsc1^{ptKO}$ mice revealing a few focal microscopic renal cysts**.** (B-F) Higher magnification kidney section images highlighting the focal areas that have the most prominent renal cysts, in contrast to the exacerbated cystogenesis seen in the double mutant ($Tsc1^{ptKO}$;$rpS6^{P-/-}$) mice by 4 weeks of age (compare with the images in Figure S3). Shown are representative images from 5 mice per group with similar results. (Scale bars: 2 mm in A; 100 μm in B-F.)

Wu et al _ Figure S6

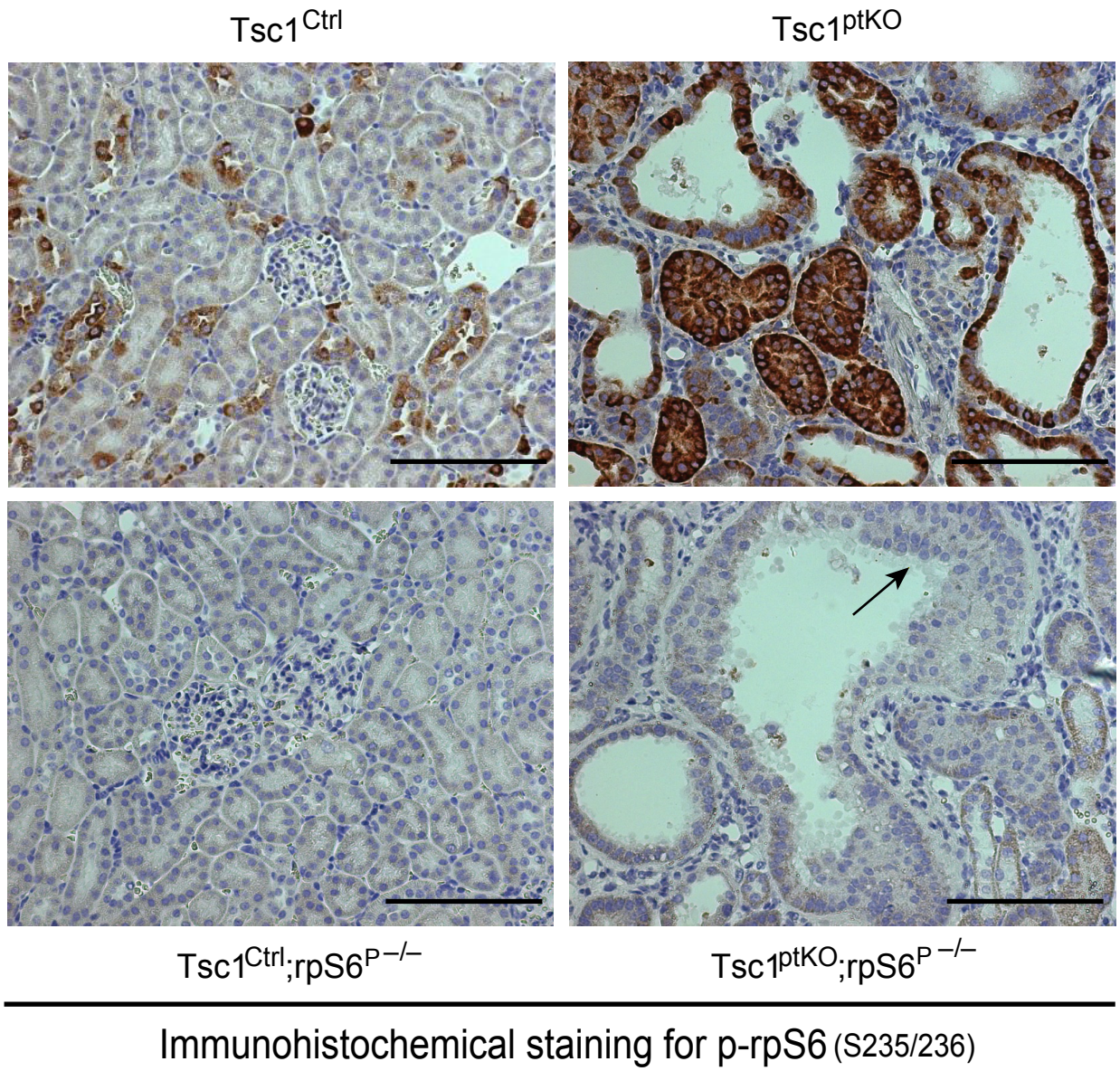

Figure S6. Immunohistochemistry staining for rpS6 phosphorylation (p-rpS6) indicated that rpS6 phosphorylation was markedly activated in $Tsc1^{ptKO}$ mice compared with $Tsc1^{Ctrl}$ mice but was completely deleted in both $Tsc1^{Ctrl}$;$rpS6^{P-/-}$ and $Tsc1^{ptKO}$;$rpS6^{P-/-}$ mice, as further confirmed by immunoblotting analysis (as shown in Figure 8, A and B); however, complete deletion of rpS6 phosphorylation did not inhibit the proliferation of cyst-lining epithelial cells in $Tsc1^{ptKO}$;$rpS6^{P-/-}$ mice at 4 weeks of age (with a black arrow pointing to aberrantly proliferated multi-layers of epithelial cells lining an enlarged renal tubule or cyst). (Scale bar, 100 μm.)

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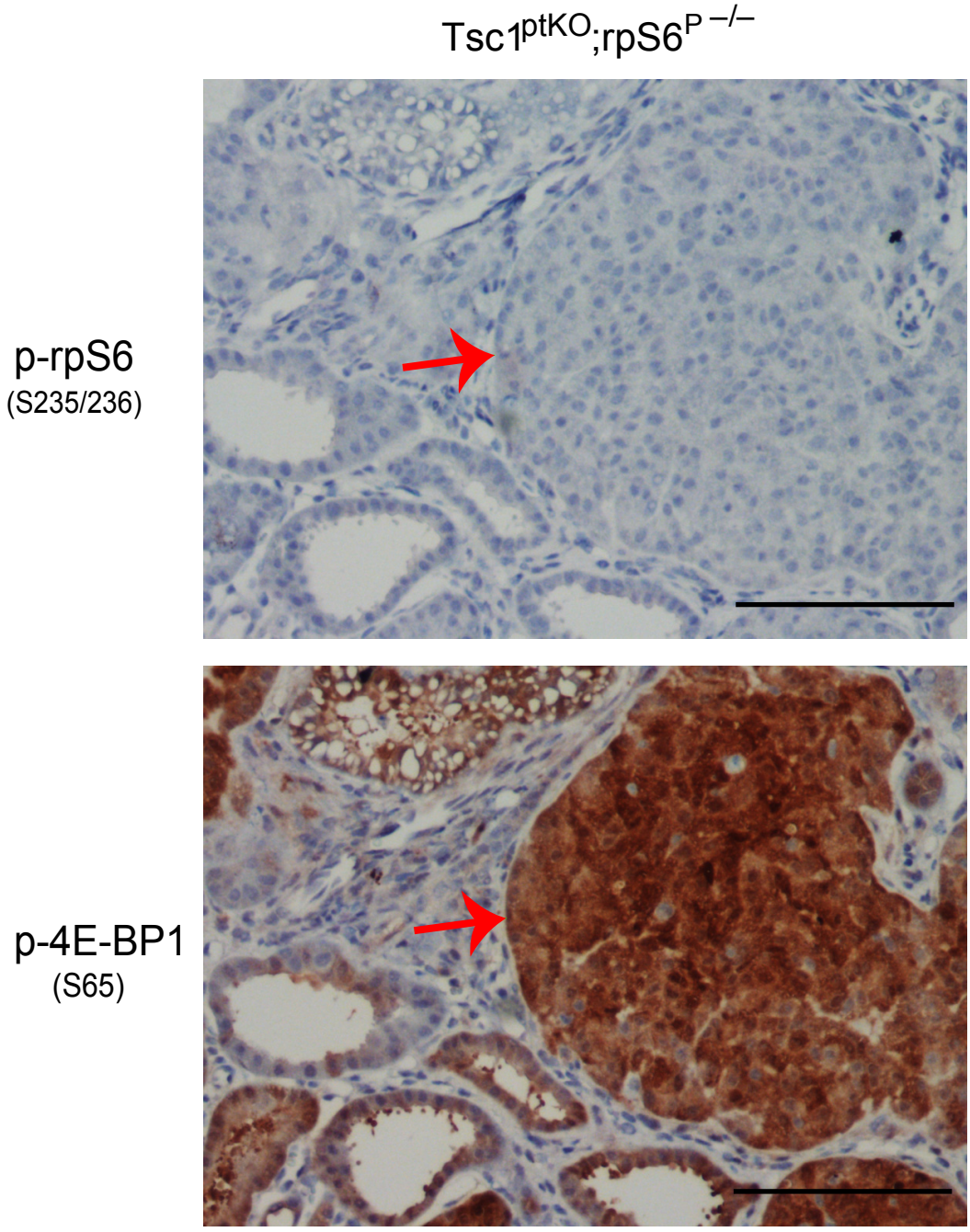

Figure S7. Immunohistochemical staining of consecutive kidney sections for phosho-rpS6 (p-rpS6) and phospho-4E-PB1 (p-4E-BP1), respectively, confirmed complete deletion of rpS6 phosphorylation and aberrantly heightened 4E-BP1 phosphorylation in the proliferated epithelial cells that form microscopic renal tumors (*red arrow*) in $Tsc1^{ptKO};rpS6^{P-/-}$ mice. (Scale bar, 100 μm.)