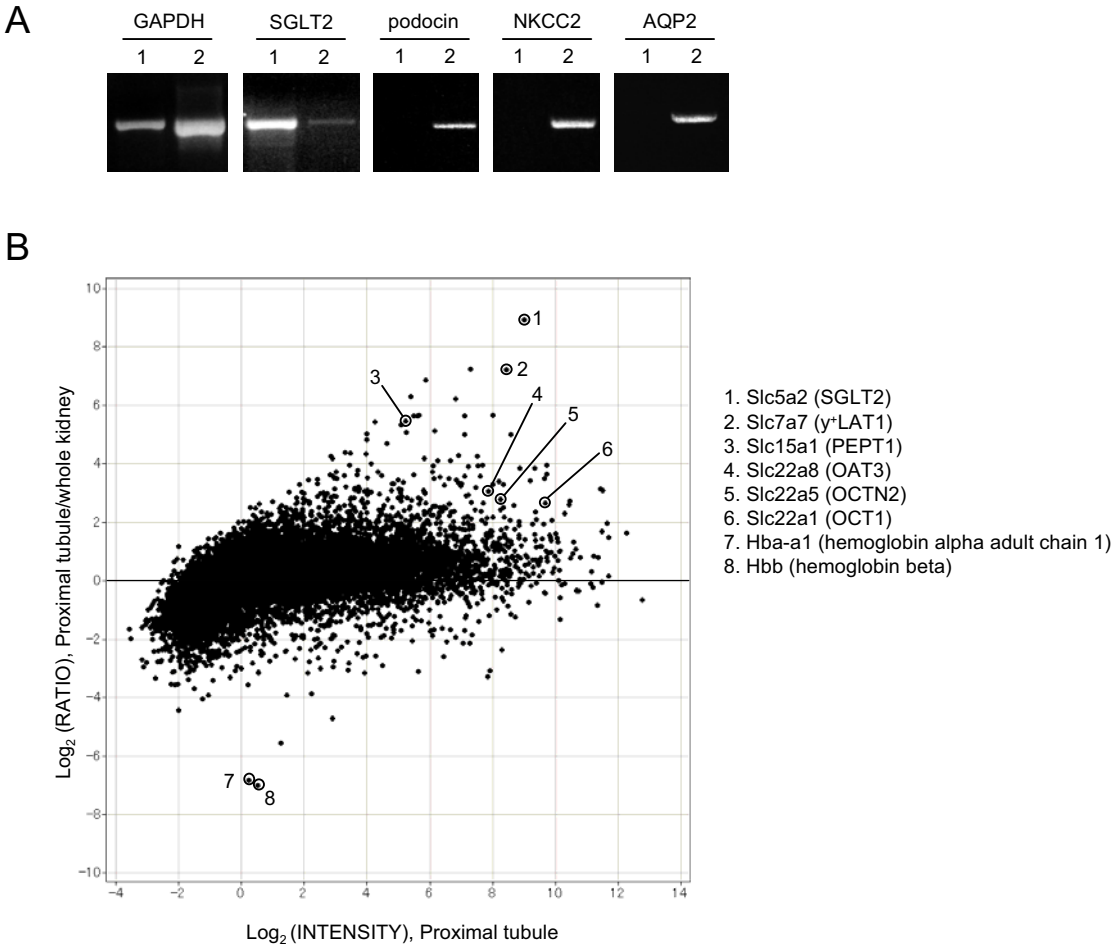
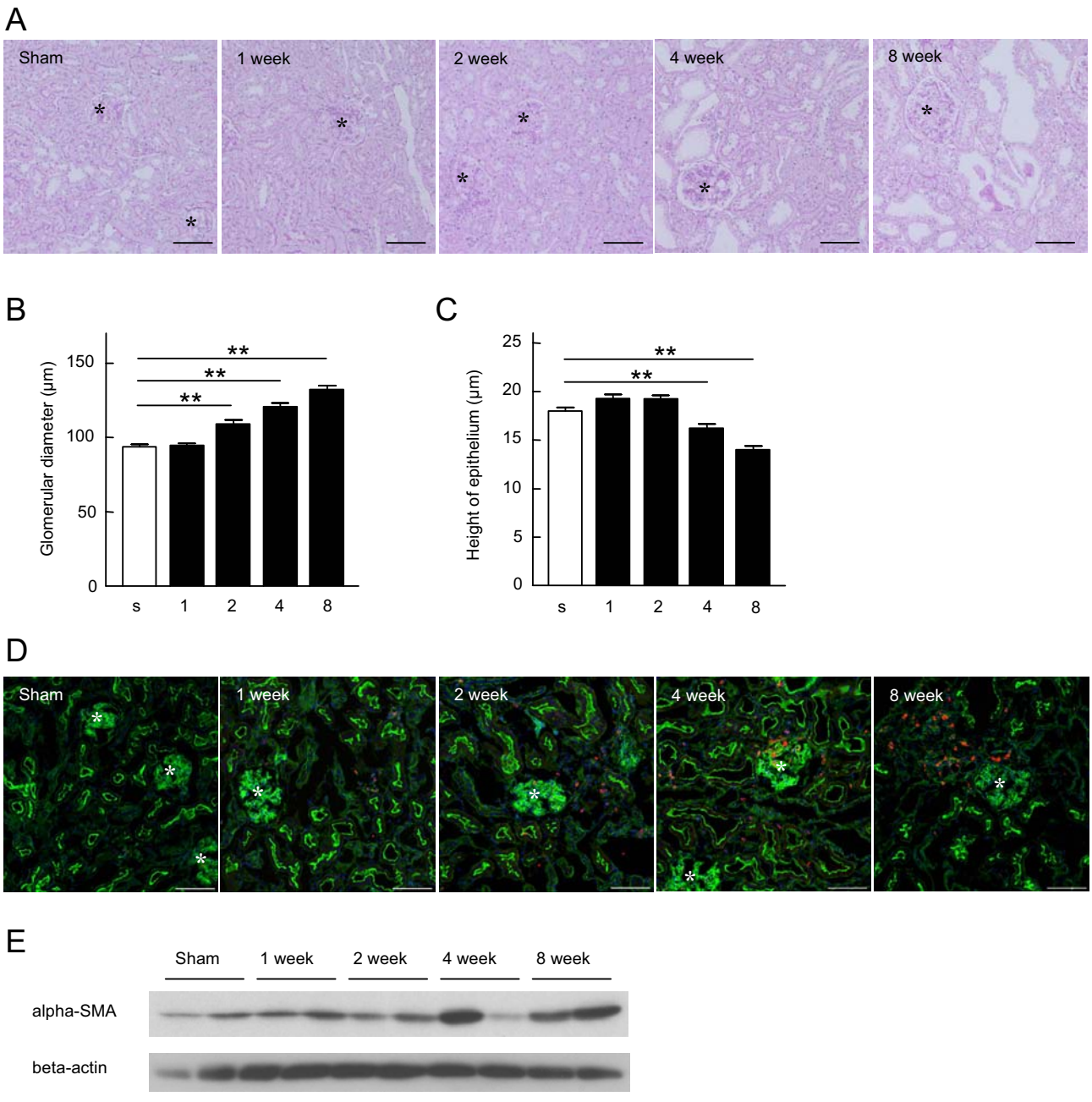
Supplementary Figure 1



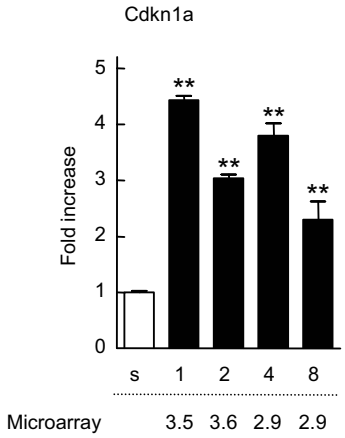
Supplementary Figure 1. Examination of the purity of the isolated proximal tubules. The expression of SGLT2, podocin, NKCC2 and AQP2 was examined by RT-PCR using the cDNA from isolated proximal tubules (1) or whole kidney (2) (**A**). The expression of SGLT2, Podocin, NKCC2 and AQP2 was used as a marker for proximal tubules, glomeruli, thick ascending limbs and collecting ducts, respectively. Microarray analysis was performed using the isolated proximal tubules and whole kidney spedimens from normal rats (**B**). The ratio of the intensity of proximal tubules to whole kidney were calculated. The genes that were reported to be expressed at the proximal tubules showed high ratio (1-6), while the genes that were not contained in the proximal tubules showed low value (7 and 8). SGLT, Na^+/glucose cotransporter; NKCC, Na^+-K^+-Cl^- cotransportery; AQP, aquaporin; y^+LAT, y(+)L-type amino acid transporter; PEPT, H^+/peptide transporter; OAT, organic anion transporter; OCTN, organic cation/carnitine transporter; OCT, organic cation transporter.

Supplementary Figure 2



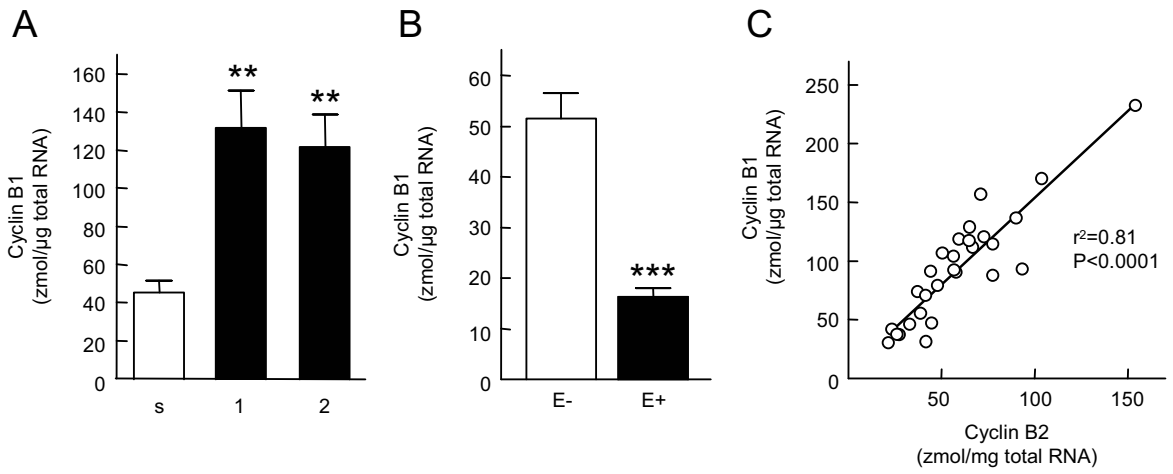
Supplementary Figure 2. Evaluation of progressive renal failure in Nx rats. The kidney was fixed in ethyl Carnoy's solution and stained with periodic acid-Schiff's reagent (**A**). *, glomeruli. Scale bar; 100 μm. Measurement of glomerular diameter in sham-operated and Nx rats at 1, 2, 4 and 8 weeks after surgery (**B**). The glomerular diameter was not changed at 1 week after Nx compared to sham-operated rats, and it was significantly increased in Nx rats at 2, 4 and 8 weeks after surgery with time after surgery. Multiple comparisons were performed with Dunnett's two-tailed test after a one-way ANOVA. ** $P < 0.01$, significantly different from sham-operated rats. The height of epithelial cells were measured (**C**). It was tended to increase in 1 and 2 weeks after Nx, but it was significantly decreased in 4 and 8 weeks after Nx. Immunofluorescent analysis of ED1 (**D**). No signal was observed in sham-operated rats. The signals were gradually increased in tubulointerstitial space in Nx rats at 1 and 2 weeks after surgery. Strong signals for ED1 in the glomeruli and tubulointerstitial space were observed in Nx rats at 4 and 8 weeks after surgery. Immunoblotting of alpha-SMA in sham-operated and Nx rats (**E**). The levels of alpha-SMA were comparable between sham-operated and Nx rats in 1 and 2 weeks after Nx, while those were markedly increased in 4 and 8 weeks after Nx.

Supplementary Figure 3



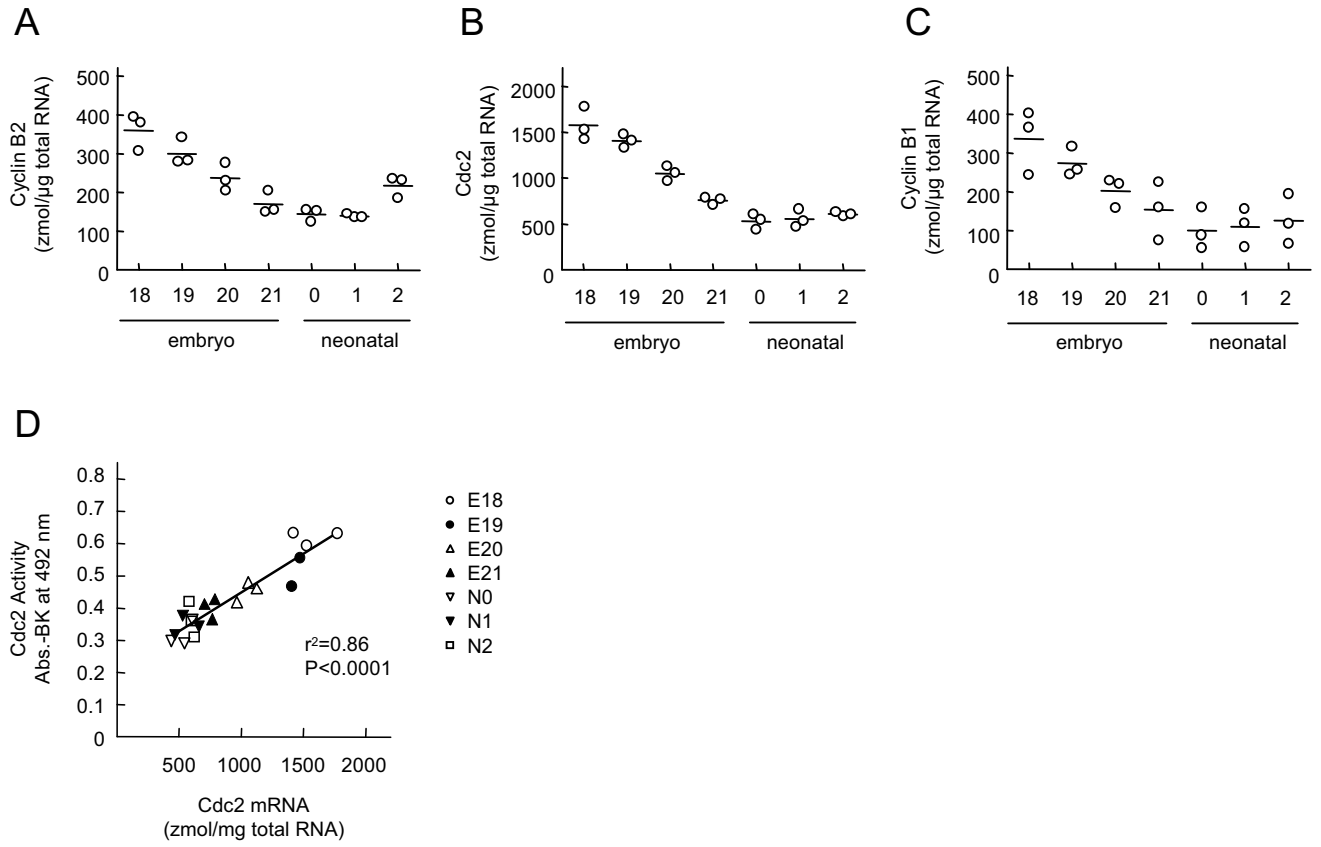
Supplementary Figure 3. Expression profile of cyclin-dependent kinase inhibitor 1a (Cdkn1a/p21$^{WAF1/Cip1}$). An equal amount of cDNA was pooled from the remnant kidney of each rat, and the expressional changes of mRNA were measured by real-time PCR and analyzed by the delta-delta Ct method. The numbers below each column show the fold change in the microarray analysis. s, sham-operated rats; 1, 2, 4, and 8, Nx rats at 1, 2, 4, and 8 weeks after surgery. Multiple comparisons were performed with Dunnett's two-tailed test after a one-way ANOVA. ** $P < 0.01$, significantly different from sham-operated rats.

Supplementary Figure 4



Supplementary Figure 4. Detection of the mRNA level of Cyclin B1 in the kidney. The effect of subtotal nephrectomy on the mRNA level of Cyclin B1 (**A**). s, sham-operated rats; 1 and 2, Nx rats at 1 and 2 weeks after surgery. Multiple comparisons were performed with Dunnett's two-tailed test after a one-way ANOVA. ** $P < 0.01$, significantly different from sham-operated rats. The effect of everolimus on the mRNA level of Cyclin B1 in Nx rats (**B**). E–, Nx rats treated with the vehicle; E+, Nx rats treated with everolimus. *** $P < 0.001$, significantly different from vehicle treated (E–) rats. Correlation between the mRNA levels of Cyclin B2 and Cyclin B1 in sham-operated and Nx rats (**C**). Linear regression analysis was performed and the correlation coefficient (r) was calculated.

Supplementary Figure 5



Supplementary Figure 5. Expression of Cyclin B2, Cdc2 and Cyclin B1 and the activity of Cdc2 in the embryonic and neonatal kidney. Wistar/ST rats at various stages of gestation were purchased and the kidneys were collected. We used three separate batches of maternal rats. The mRNA levels of Cyclin B2 (**A**), Cdc2 (**B**) and Cyclin B1 (**C**) were measured by real-time PCR. The levels of Cyclin B2, Cdc2 and Cyclin B1 were high in the embryonic and neonatal kidney and decreased with growth. The correlation between the activity and mRNA expression of Cdc2 in embryonic and neonatal kidney (**D**). The linear regression analysis was performed and the correlation coefficient (r) was calculated. E, embryonic day; N, neonatal day.

Supplementary methods

Examination of the purity of the isolated proximal tubules by RT-PCR and microarray analysis

Total RNA was extracted from the isolated proximal tubules or whole kidney specimens of normal rats using RNeasy Mini Kit (QIAGEN). The total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and subjected to digestion with RNase H (Invitrogen Co., Carlsbad, CA). After dilution of reaction mixture, the expression of SGLT2, podocin, NKCC2, AQP2 and GAPDH was examined by RT-PCR using the aliquots of cDNA. Following the denaturing of the single-strand DNA at 95°C for 3 min, RCR was performed: 94°C for 1 min, 55°C for 1 min (podocin, NKCC2 and AQP2) or 65°C for 1 min (SGLT2) or 60°C for 1 min (GAPDH), 72°C for 1 min for 30 cycles. The primer sets used for RT-PCR were referred to previous reports (SGLT2 and GAPDH (3); podocin (1); NKCC2 and AQP2 (2)). The expression of GAPDH was examined as internal control. The expressional profiles in isolated proximal tubules and whole kidney were compared by microarray analysis. The labeling reaction, array hybridization, chemiluminescence detection, and image acquisition were performed as described in **Materials and Methods** section.

Immunoblot analysis

The kidney was homogenized in a lysis buffer containing 1% NP-40, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM NaF, 1 mM sodium pyrophosphate, 1 mM Na_3VO_4, and protease inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). The whole-kidney lysate was centrifuged at 10,000 × g at 4°C for 15 min. The supernatant

was collected, and the protein concentration was determined. The lysate (50 μg) was separated by SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. After blocking the membrane, the blots were incubated with antisera against alpha-SMA or beta-actin (Sigma-Aldrich). The bound antibody was detected on X-ray film by enhanced chemiluminescence (ECL) with horseradish peroxidase-conjugated secondary antibodies and cyclic diacylhydrazides (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). The levels of beta-actin were examined as internal control.

Real-time RCR in embryonic and neonatal kidney

In the case of embryonic and neonatal kidney, Wistar/ST rats at various stages of gestation were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan); the kidneys were then collected from 9 to 12 embryos of the same maternal rat. We used three separate batches of maternal rats.

References

1. **Kawachi H, Koike H, Kurihara H, Sakai T, Shimizu F**. Cloning of rat homologue of podocin: Expression in proteinuric states and in developing glomeruli. *J Am Soc Nephrol* 13: 46-56, 2003.
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