Chronic exercise protects against the progression of renal cyst growth and dysfunction in rats with polycystic kidney disease

Jiahe Qiu, MSc[1], Yoichi Sato, MSc[1], Lusi Xu, PhD[1,2], Takahiro Miura, MD PhD[1], Masahiro Kohzuki, MD PhD[1], Osamu Ito, MD PhD[1,2]

[1]Department of Internal Medicine and Rehabilitation Science, Tohoku University Graduate School of Medicine, Sendai, Japan

[2]Division of General Medicine and Rehabilitation, Tohoku Medical and Pharmaceutical University Faculty of Medicine, Sendai, Japan

Short Title: Exercise is renoprotective in PCK rats

Corresponding author: Osamu Ito, MD, PhD

Division of General Medicine and Rehabilitation, Tohoku Medical and Pharmaceutical University Faculty of Medicine

1-15-1 Fukumoto, Miyagino-ku, Sendai 983-8536, Japan

Tel.: +81-22-259-1221

E-mail: oito@hosp.tohoku-mpu.ac.jp

Word counts: 3365 words

Abstract

Introduction: Polycystic kidney disease (PKD) is a genetic disorder characterized by the progressive enlargement of renal epithelial cysts and renal dysfunction. Previous studies have reported the beneficial effects of chronic exercise on chronic kidney disease. However, the effects of chronic exercise have not been fully examined in PKD patients or models. The effects of chronic exercise on the progression of PKD were investigated in a polycystic kidney (PCK) rat model.

Methods: Six-week-old male PCK rats were divided into a sedentary group and an exercise group. The exercise group underwent forced treadmill exercise for 12 weeks (28 m/min, 60 min/day, 5 days/week). After 12 weeks, kidney function and histology were examined, protein expressions were analyzed, and signaling cascades of PKD were examined.

Results: Chronic exercise reduced the excretion of urinary protein, liver-type fatty acid-binding protein, plasma creatinine, urea nitrogen, and increased plasma irisin and urinary arginine vasopressin (AVP) excretion. Chronic exercise also slowed renal cyst growth, glomerular damage, and interstitial fibrosis, and led to reduced Ki-67 expression. Chronic exercise had no effect on cAMP content but decreased the renal expression of B-Raf and reduced the phosphorylation of extracellular signal-regulated kinase (ERK), mammalian target of rapamycin (mTOR), and S6.

Conclusion: Chronic exercise slows renal cyst growth and damage in PCK rats, despite increasing AVP, with down-regulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in the kidney of PCK rats.

Abstract word count: 230/275 words

Keywords

Polycystic kidney disease; PCK rats; chronic exercise; cyst growth; renal protection

Introduction

Polycystic kidney disease (PKD) is the most prevalent of all genetic disorders, which is characterized by progressive enlargement of epithelial cysts in the kidney. Autosomal dominant PKD (ADPKD) is caused by mutations in *PKD1* (encoding polycystin-1) or *PKD2* (encoding polycystin-2), whereas autosomal recessive PKD (ARPKD) is caused by mutations in *PKHD1* (encoding fibrocystin). Polycystin-1, polycystin-2, and fibrocystin are all localized in the primary cilia and are required for the regulation of Ca^{2+} influx in response to ciliary bending. Primary cilia abnormalities are associated with lowered intracellular Ca^{2+} (1). Low intracellular Ca^{2+}-related abnormal signaling leads to the induction of cyst epithelial cell proliferation, which is a key feature of cyst growth (2).

Low intracellular Ca^{2+} activates adenylyl cyclase and increases intracellular cAMP levels. Next, cAMP and protein kinase A signaling upregulates the B-Raf and extracellular signaling-regulated kinase (ERK) pathway in renal cyst epithelial cells (3). The finding that increased cAMP signaling is a crucial driver of cyst growth has led to the development of arginine vasopressin (AVP) type 2 receptor (V2R)-based therapy. Antagonists of V2R, including tolvaptan, reduce renal cAMP content by inhibiting V2 receptors which coupled with the stimulatory G protein (Gs) and slow cyst growth

and the decline of renal function in ADPKD patients and rodent PKD models (4). In addition, it has been reported that the mammalian target of rapamycin (mTOR) and S6 pathway promotes cyst growth by enhancing the proliferation, size, and metabolism of renal tubular cells (5).

Lifestyle modifications that slow the progression of chronic kidney disease (CKD) have long been a topic of research interest. Clinical studies have reported that chronic exercise slows the decline in glomerular filtration rate (6, 7), decreases albuminuria (8), delays the initiation of dialysis, and diminishes overall mortality in CKD patients (9). We have also reported that chronic exercise at moderate intensity has renal protective effects in CKD model rats with 5/6 nephrectomy, diabetic nephropathy, and salt-sensitive hypertension (10-14). With respect to the mechanisms of the beneficial effects of chronic exercise, a newly discovered exercise-induced myokine, irisin, has been reported to have renal protective effects (15). Both endurance and resistance exercises increase irisin in skeletal muscles and plasma (16). Moreover, recombinant irisin administration prevents renal damage and fibrosis in mice with folic acid nephropathy, unilateral ureteral obstruction, and 5/6 nephrectomy (15).

ADPKD patients with a glomerular filtration rate $\geq$ 60 (mL/min/1.73 m^2) have a low exercise capacity (17). Similarly, we recently reported a low exercise capacity in polycystic kidney (PCK)

rats (18), which have polycystic kidney and liver diseases and resemble human ADPKD (19, 20). Chronic exercise at a moderate intensity for 12 weeks improved the low exercise capacity and, unexpectedly, slowed liver cyst growth and fibrosis in PCK rats (18). However, it is controversial whether chronic exercise has renal protective effects in PKD patients and/or models, because acute or chronic exercise stimulates the posterior pituitary gland to secrete AVP, thus increasing AVP levels (21). Therefore, we examined the effects of chronic exercise on the progression of PKD, as well as on the signaling cascades responsible for cellular proliferation in PCK rats.

Methods

Experimental animals

Five-week-old male PCK and Sprague-Dawley rats were obtained from Charles River Laboratories Japan (Yokohama, Japan). All rats had free access to tap water and were fed a standard rat diet (Labo MR Stock, Nosan Kogyo Co., Yokohama, Japan). All animal experiments were approved by the Tohoku University Committee for Animal Experiments and were performed in accordance with the Guidelines for Animal Experiments and Related Activities of Tohoku University (permit no. 2018-084).

Exercise protocol

After 1 week of acclimatization, PCK rats were divided into the sedentary group (Sed-PCK, *n*=10) or the exercise group (Ex-PCK, *n*=10). The Sprague-Dawley (SD) rats were set as a control group (Con-SD, *n*=10). The Ex-PCK group underwent forced treadmill exercise with moderate intensity, using treadmills (KN-73; Natsume Industries, Tokyo, Japan) for 12 weeks with the following protocol: 28 m/min, 60 min/day, and 5 days/week (11).

Plasma and urinary parameters

The rats were housed individually in metabolic cages (Model ST; Sugiyama-General, Tokyo, Japan) for 3 days to acclimatize to the conditions. Food and water intake were measured, and urine was collected on ice for 24h. Systolic blood pressure was measured using the tail-cuff method (MK-2000A; Muromachi Kikai, Tokyo, Japan). The rats were euthanized with sodium pentobarbitone (100 mg/kg, i.p.) and blood samples were collected from the ventral aorta. Urine and blood samples were centrifuged for 10 min at 2 000 × g, and the supernatant was collected. Plasma and urine aliquots were rapidly frozen and stored at –80°C until analysis.

Urinary protein and plasma glucose, total cholesterol, triglyceride, urea nitrogen, and creatinine were measured using standard auto-analysis techniques (SRL Inc., Tokyo, Japan). L-FABP was

measured using a highly sensitive enzyme-linked immunosorbent assay (CMIC, Tokyo, Japan) (22). Plasma AVP levels are fluctuated by anesthetics or stress (23), and the indwelling catheter into the femoral artery may affect treadmill running. Therefore, we measured AVP concentration in the 24h urine by radioimmunoassay (SRL, Tokyo, Japan) and calculated urinary AVP excretion for 24h described previously (24, 25). Plasma irisin was measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA).

Histological analysis

After the rats were sacrificed, kidneys were excised and decapsulated. The left kidney was immediately frozen in liquid nitrogen and the right kidney was sliced perpendicularly to the sagittal axis at approximately 5 mm intervals. Slices from the midportion of the kidneys were fixed in 10% buffered formalin overnight, and the tissue was then embedded in paraffin. Sections (3 μm thick) were stained with hematoxylin and eosin (HE), periodic acid–Schiff (PAS), and Masson's trichrome (MT) following standard protocols. The whole kidney area and the cyst area in the HE-stained sections were determined using ImageJ analysis software (National Institutes of Health, Bethesda, MD) (26). Glomerular injury was evaluated in PAS-stained glomeruli using the index of glomerular

sclerosis (13). The percentage of interstitial fibrosis area was estimated in MT-stained tissue, except for the cyst areas, glomeruli, and blood vessels, as described previously (11, 14).

Immunohistochemical analysis

Deparaffinized kidney sections (5 μm thick) were immunostained with antibodies against desmin (ab8470, Abcam, Cambridge, UK), Ki-67 (#418071, Nichirei Biosciences, Tokyo, Japan), p-mTOR (#293133, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and p-ERK (#4376, Cell Signaling Technology, Danvers, MA, USA) according to the instructions for analyzing under a light microscope (Eclipse 80i microscope, Nikon, Tokyo, Japan). For each section, 30 randomly chosen fields were photographed using a digital color camera (DS-Fi2-U3 color camera, Nikon). Using ImageJ, the stained percentage of the target area was then estimated after selecting a glomerular area with desmin staining(13). The percentage of cells positive for Ki-67, was calculated from the total number of cells containing epithelial cysts and non-cystic tubules from each kidney section using ImageJ, as described previously (27).

Western blot analysis

The frozen kidney of each rat was thawed, dissected into the cortex and medulla, and then homogenized in 100 mmol/L potassium buffer (pH 7.25) containing 30% glycerol, 1 mmol/L

dithiothreitol, and 0.1 mmol/L phenylmethylsulfonyl fluoride (14). Protein expression and phosphorylation were examined using western blot analysis, as described previously (18). Antibodies against Raf-B (#5284; Santa Cruz), ERK (#4695; Cell Signaling Technology), p-ERK (#4376; Cell Signaling Technology), mTOR (#2983; Cell Signaling Technology), p-mTOR (#2971; Cell Signaling Technology), S6 (#2217; Cell Signaling Technology), and p-S6 (#2211; Cell Signaling Technology) were used. Secondary HRP-conjugated mouse anti-rabbit (#2357; Santa Cruz) and rabbit anti-mouse (#516102; Santa Cruz) antibodies were then used. Relative band intensities were quantified using ImageJ and normalized using β-actin (A2228; Sigma-Aldrich, St. Louis, MO, USA) as an internal standard.

cAMP assay

The frozen kidneys were ground to a fine powder with liquid nitrogen in a stainless-steel mortar. After the liquid nitrogen had evaporated, the tissues were assayed for cAMP using an enzyme-linked immunosorbent assay kit (Enzo Life Sciences Inc., Farmingdale, NY, USA) (28). Results are expressed in pmol/mg of tissue protein.

Statistical analysis

Data are expressed as the mean ± SEM. Statistical comparisons between the groups were performed using the two-tailed unpaired *t*-test or one-way ANOVA. All analyses were carried out using GraphPad Prism software (version 8.4; GraphPad Inc., La Jolla, CA, USA). *P*-values of <0.05 were considered statistically significant.

Results

General parameters and urinary parameters

PCK rats as a slow progression model of PKD and Sprague-Dawley (SD) rats as a control model, were used to assess general parameters and urinary parameters in the kidney. Bodyweight was similar between the control SD rats (Con-SD) and sedentary PCK rats (Sed-PCK) groups, but was significantly lower in the exercise PCK rats (Ex-PCK) group than in the Sed-PCK group after 10 weeks of age ($P<0.05$) (Figure 1A). There were no differences in food or water intake among the three groups (Figure 1B and 1C). Urine output was similar between the Con-SD and Sed-PCK groups, but was significantly lower in the Ex-PCK group than in the Sed-PCK group at the end of the experiment ($P<0.05$) (Figure 1D). Urinary protein and liver-type fatty acid-binding protein (L-FABP) excretions were significantly increased in the Sed-PCK group after 14 weeks of age

compared with the beginning of the experiment, and were significantly higher in the Sed-PCK group than in the Ex-PCK group by the end of the experiment ($P<0.01$ and $P<0.01$, respectively) (Figure 1E and 1F).

Plasma parameters

Table 1 shows the plasma parameters of the groups. Total cholesterol and creatinine were significantly higher in the Sed-PCK group than in the Con-SD group, and plasma glucose was significantly lower in the Sed-PCK group than in the Con-SD group. Glucose, total cholesterol, triglyceride, urea nitrogen, and creatinine were significantly lower in the Ex-PCK group than in the Sed-PCK group. Plasma irisin was similar between the Con-SD and Sed-PCK groups, but was significantly higher in the Ex-PCK group than in the Sed-PCK or Con-SD group ($P<0.01$ and $P<0.05$, respectively).

Kidney weight and morphology

Figure 2A shows representative images of the HE-stained kidney from the three groups. Renal cysts were observed in the outer medulla of both the Sed-PCK and Ex-PCK groups, and cyst sizes were smaller in the Ex-PCK group than in the Sed-PCK group. Total kidney weight was significantly lower in the Ex-PCK group than in the Sed-PCK group ($P<0.01$) (Figure 2B), but the kidney-to-

body weight ratio was not significantly different between the two PCK groups (Figure 2C). The cystic index was significantly higher in the Sed-PCK group than in the Con-SD group ($P<0.01$), and significantly lower in the Ex-PCK group than in the Sed-PCK group ($P<0.05$) (Figure 2D).

Glomerular damage and renal interstitial fibrosis

Figure 3A shows representative images of PAS-stained and desmin-immunostained glomeruli and MT-stained kidneys in each group. Glomerular sclerosis, podocyte injury, and renal interstitial fibrosis were observed in the Sed-PCK group. The index of glomerular sclerosis was significantly higher in the Sed-PCK group than in the Con-SD group ($P<0.01$), and significantly lower in the Ex-PCK group than in the Sed-PCK group ($P<0.05$) (Figure 3B). The desmin-positive staining area in the glomeruli was significantly larger in the Sed-PCK group than in the Con-SD group ($P<0.01$), and significantly smaller in the Ex-PCK group than in the Sed-PCK group ($P<0.01$) (Figure 3C). The renal interstitial fibrosis area was significantly higher in the Sed-PCK group than in the Con-SD group ($P<0.01$), and smaller in the Ex-PCK group than in the Sed-PCK group (Figure 3D).

Cell proliferation and signaling cascades

Figure 4A shows representative images of the kidney immunostained for Ki-67 from the Sed-PCK and Ex-PCK groups. Ki-67-positive cells were highly expressed in the cyst-lining epithelium,

159 interstitium, and non-cystic tubules of the Sed-PCK group. Chronic exercise led to fewer Ki-67-
160 positive cells. The Ki-67 labeling index was significantly lower in the cyst-lining epithelium and
161 non-cystic tubules in the Ex-PCK group compared with the Sed-PCK group (P<0.01 and P<0.01,
162 respectively) (Figure 4B and 4C).

163 Urinary AVP excretion was significantly higher in the Sed-PCK group than in the Con-SD group
164 (P<0.05), and was considerably higher in the Ex-PCK group than in the Sed-PCK group (P<0.05)
165 (Figure 5A). Renal cAMP content was significantly higher in the Sed-PCK group than in the Con-
166 SD group (P<0.05), but it was not significantly different between the Sed-PCK and Ex-PCK groups
167 (Figure 5B). Renal B-Raf expression was significantly higher in the Sed-PCK group than in the
168 Con-SD (P<0.01), and significantly lower in the Ex-PCK group than in the Sed-PCK group
169 (P<0.01) (Figure 5C).

170 Figure 6A and 6B show representative images of kidneys immunostained for phosphorylated
171 (p-) ERK and p-mTOR, respectively, from each group. The p-ERK and p-mTOR proteins were
172 highly expressed in the cyst-lining epithelium and non-cystic tubules in the Sed-PCK group, and
173 chronic exercise decreased their expressions (Figure 6A and 6B). Renal ERK and mTOR
174 phosphorylation were significantly higher in the Sed-PCK group than in the Con-SD group (P<0.01

and $P<0.01$, respectively), and S6 phosphorylation also tended to be higher in the Sed-PCK group compared with the Con-SD group (Figure 6C, 6D, and 6E). Renal ERK, mTOR, and S6 phosphorylation was significantly lower in the Ex-PCK group than in the Sed-PCK group ($P<0.01$, $P<0.01$, and $P<0.01$, respectively).

Discussion

Chronic exercise has renal protective effects in CKD patients and models (10-14); however, the renal protective effects of chronic exercise have not yet been reported in PKD patients or models. The present study revealed that chronic exercise at a moderate intensity slowed the progression of renal cyst growth, glomerular damage, interstitial fibrosis, and renal dysfunction in PCK rats, despite increasing AVP. Chronic exercise also inhibited excessive cell proliferation, with down-regulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in renal epithelial cells. To the best of our knowledge, the present study is the first to report that chronic exercise has therapeutic potential against cyst growth and renal dysfunction in PKD.

We chose the exercise protocol in the present study based on our previous study of CKD model rats with 5/6 nephrectomy (11), in which proteinuria and glomerular sclerosis were significantly

attenuated after 12 weeks of chronic exercise. We confirmed that when PCK rats run at a speed of 28 m/min on the treadmill, oxygen consumption ($\dot{V}O_2$) corresponds to approximately 65% of the maximal $\dot{V}O_2$, which is assumed to be aerobic exercise at a moderate intensity (18). In contrast to the present results, Darnley et al. reported that treadmill exercise (14 m/min, 30 min/day, 3 days/week) for 6 weeks did not lead to any changes in serum urea nitrogen or creatinine in Han:SPRD-*cy* rats (19). Similarly, in our pilot studies, chronic exercise for 8 weeks did not significantly affect renal cyst growth in PCK rats (data not shown). Thus, the intensity, time, frequency, and duration of the exercise protocol may be important to obtain benefits in PKD models. In agreement with our previous studies (10-13), chronic exercise lowered proteinuria and plasma creatinine and attenuated glomerular sclerosis and podocyte injury in PCK rats. Chronic exercise for 8 weeks significantly decreased urinary protein excretion (Figure 1E) without significant effects on renal cyst growth in PCK rats (data not shown). Therefore, glomerular protection may be a primary effect of chronic exercise, rather than being secondary to slowing renal cyst growth. Urinary L-FABP excretion, a biomarker of proximal tubular stress and tubulointerstitial disorder, increases linearly with age and reflects the progression of tubulointerstitial disorder in PCK rats (29). Chronic exercise might therefore strongly attenuate proximal tubular stress and tubulointerstitial disorder in

PCK rats. As an indicator of cell proliferation, chronic exercise decreased the number of Ki-67-positive cells in the kidneys of PCK rats, indicating the inhibition of excessive cell proliferation. As well as in the kidney, chronic exercise has also been recently reported to slow the progression of cyst growth and fibrosis in the liver of PCK rats (18).

The present study indicates that chronic exercise increases AVP in PCK rats. In agreement with these results, AVP synthesis and secretion have been previously reported to increase during exercise (30). Sustained moderate exercise (at an intensity threshold of 40%–65% of $\dot{V}O_{2max}$) increased plasma AVP (31, 32). Furthermore, chronic exercise with a treadmill for 5 weeks increased plasma AVP in Wistar rats (33). The present study also indicates that chronic exercise did not change renal cAMP content and did decrease the cAMP-inducible B-Raf expression in PCK rats, despite increasing AVP, suggesting that chronic exercise might inactivate adenylate cyclase via the inhibitory G protein (Gi). Previous studies indicate that norepinephrine and α2-adrenergic receptor (α2-AR) agonists inhibit the AVP-activated adenylate cyclase, cAMP content, and water transport in the collecting ducts (34-36). Therefore, it is possible that chronic exercise might stimulate renal sympathetic activity and activate α2-AR in the collecting ducts to slow the progression of renal cyst growth with reducing the renal cAMP content in PCK rats. In this regard, our preliminary study

223 indicates that chronic treatment of the α2-AR agonist, clonidine slows the progression of renal cyst
224 growth in PCK rats (data not shown).

225 Previous studies indicate that even normal plasma AVP levels increase B-Raf expression and
226 ERK phosphorylation in the kidneys of PCK rats, and that inhibition of AVP by V2R antagonists
227 and hydration can down-regulate the B-Raf/ERK pathway (24, 37). The present study indicates that
228 chronic exercise down-regulates not only the B-Raf/ERK pathway but also the mTOR/S6 pathway
229 in the kidneys of PCK rats. Both mTOR and ERK are involved in excessive cell proliferation and
230 cyst growth in the renal tubules and cholangiocytes of PCK rats (20). However, neither tolvaptan
231 nor an ERK inhibitor, AEZ-131, affected S6 phosphorylation in the kidney of PCK rats, and the
232 suppressive effects of tolvaptan and an mTOR inhibitor, rapamycin, on renal cyst growth were
233 additive (38). The suppressive effects of chronic exercise on excessive cell proliferation and renal
234 cyst growth in the present study might therefore be mediated by down-regulation of both the B-
235 Raf/ERK and mTOR/S6 pathways in the kidneys of PCK rats. Several types of exercise affect
236 mTOR and ERK in the skeletal muscle, fat, liver and vasculature (39-41). However, the effects of
237 exercise on mTOR or ERK have not previously been reported in the kidney, especially in the renal
238 tubules. We recently reported that chronic exercise down-regulates mTOR and ERK

239 phosphorylation in the liver and cholangiocytes in PCK rats (18). In agreement with the results from
240 PCK rats, chronic exercise with a treadmill inactivated mTOR and suppressed excessive cell
241 proliferation in hepatocellular carcinoma in PTEN-deficient mice (42) and carcinoma-implanted
242 rats (43).

243 The present study also demonstrates that chronic exercise increases plasma irisin in PCK rats.
244 Irisin mediates the beneficial effects of exercise, such as by promoting the brown adipose formation
245 and improving the metabolism, and also has a beneficial role in kidney and heart diseases (15, 44-
246 46). In one study, plasma irisin levels were significantly decreased in CKD patients and were
247 inversely correlated with blood urea nitrogen and creatinine levels (47). In another study, skeletal
248 muscle-specific PGC-1α overexpression increased irisin production and plasma irisin levels and
249 attenuated renal damage in mice with folic acid nephropathy, unilateral ureteral obstruction, and 5/6
250 nephrectomy (15). Moreover, recombinant irisin administration attenuated renal damage in the
251 mouse kidney disease models (15). Although it is unknown whether irisin can inhibit cyst growth,
252 irisin has been reported to inhibit mTOR, ERK, and cell proliferation in cultured cardiomyocytes,
253 cardiomyoblasts, and pancreatic cancer cells (48, 49). Additionally, irisin increased intracellular

254 Ca^{2+} in cultured cardiomyoblasts and endothelial cells (50). Future study is necessary to examine
255 whether irisin directly acts on renal epithelial cells and inhibits cyst growth in PCK rats.
256 In conclusion, chronic exercise slows the progression of PKD pathologies, such as renal
257 dysfunction, renal cyst growth, glomerular damage, and renal interstitial fibrosis in PCK rats.
258 Despite increasing AVP, chronic exercise also inhibits excessive cell proliferation, with down-
259 regulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in the kidney of PCK rats. Although
260 the results of the present study may not be directly applicable to humans, chronic exercise may be
261 a novel therapeutic approach against cyst growth and renal dysfunction in PKD patients.
262

263 **Acknowledgments**

264 This work was supported by Grants-in-Aid for Scientific Research from Japan Society for the
265 Promotion of Science grants 15K12573, 17H02119, 20H04054, 20J12732 and 20K19338. We
266 greatly appreciate the technical support received from the Biomedical Research Unit of Tohoku
267 University Hospital, Animal Pathology Platform and Biomedical Research Core of Tohoku
268 University Graduate School of Medicine for the histopathological analysis. The results of the study
269 are presented clearly, honestly, and without fabrication or inappropriate data manipulation. The

results of the present study do not constitute endorsement by the American College of Sports Medicine.

Conflict of Interest

The authors declare no conflicts of interest associated with this manuscript.

References

1. Torres VE, Harris PC. Strategies targeting cAMP signaling in the treatment of polycystic kidney disease. *J Am Soc Nephrol.* 2014;25(1):18-32.

2. Yamaguchi T, Hempson SJ, Reif GA, Hedge AM, Wallace DP. Calcium restores a normal proliferation phenotype in human polycystic kidney disease epithelial cells. *J Am Soc Nephrol.* 2006;17(1):178-87.

3. Yamaguchi T, Pelling JC, Ramaswamy NT, et al. cAMP stimulates the in vitro proliferation of renal cyst epithelial cells by activating the extracellular signal-regulated kinase pathway. *Kidney Int.* 2000;57(4):1460-71.

4. Torres VE. Therapies to slow polycystic kidney disease. *Nephron Exp Nephrol.* 2004;98(1):e1-7.

5. Margaria JP, Campa CC, De Santis MC, Hirsch E, Franco I. The PI3K/Akt/mTOR pathway in polycystic kidney disease: A complex interaction with polycystins and primary cilium. *Cell Signal.* 2020;66:109468.

6. Castaneda C, Gordon PL, Uhlin KL, et al. Resistance training to counteract the catabolism of a low-protein diet in patients with chronic renal insufficiency. A

290 randomized, controlled trial. *Ann Intern Med.* 2001;135(11):965-76.

291 7. Greenwood SA, Koufaki P, Mercer TH, et al. Effect of exercise training on
292 estimated GFR, vascular health, and cardiorespiratory fitness in patients with CKD:
293 a pilot randomized controlled trial. *Am J Kidney Dis.* 2015;65(3):425-34.

294 8. Hellberg M, Hoglund P, Svensson P, Clyne N. Randomized Controlled Trial of
295 Exercise in CKD-The RENEXC Study. *Kidney Int Rep.* 2019;4(7):963-76.

296 9. Chen IR, Wang SM, Liang CC, et al. Association of Walking with Survival and
297 RRT Among Patients with CKD Stages 3-5. *Clin J Am Soc Nephro.* 2014;9(7):1183-
298 9.

299 10. Kohzuki M, Kamimoto M, Wu XM, et al. Renal protective effects of chronic
300 exercise and antihypertensive therapy in hypertensive rats with chronic renal
301 failure. *J Hypertens.* 2001;19(10):1877-82.

302 11. Kanazawa M, Kawamura T, Li L, et al. Combination of exercise and enalapril
303 enhances renoprotective and peripheral effects in rats with renal ablation. *Am J*
304 *Hypertens.* 2006;19(1):80-6.

305 12. Tufescu A, Kanazawa M, Ishida A, et al. Combination of exercise and losartan

306 enhances renoprotective and peripheral effects in spontaneously type 2 diabetes
307 mellitus rats with nephropathy. *J Hypertens.* 2008;26(2):312-21.
308 13. Ito D, Cao P, Kakihana T, et al. Chronic Running Exercise Alleviates Early
309 Progression of Nephropathy with Upregulation of Nitric Oxide Synthases and
310 Suppression of Glycation in Zucker Diabetic Rats. *PLoS One.* 2015;10(9):e0138037.
311 14. Ogawa Y, Takahashi J, Sakuyama A, et al. Exercise training delays renal
312 disorders with decreasing oxidative stress and increasing production of 20-
313 hydroxyeicosatetraenoic acid in Dahl salt-sensitive rats. *J Hypertens.*
314 2020;38(7):1336-46.
315 15. Peng H, Wang Q, Lou T, et al. Myokine mediated muscle-kidney crosstalk
316 suppresses metabolic reprogramming and fibrosis in damaged kidneys. *Nat*
317 *Commun.* 2017;8(1):1493.
318 16. Tsuchiya Y, Ando D, Takamatsu K, Goto K. Resistance exercise induces a greater
319 irisin response than endurance exercise. *Metabolism.* 2015;64(9):1042-50.
320 17. Fischer MJ, O'Hare AM. Epidemiology of hypertension in the elderly with
321 chronic kidney disease. *Adv Chronic Kidney Dis.* 2010;17(4):329-40.

18. Sato Y, Qiu J, Miura T, Kohzuki M, Ito O. Effects of Long-Term Exercise on Liver Cyst in Polycystic Liver Disease Model Rats. *Med Sci Sports Exerc.* 2020;52(6):1272-9.

19. Darnley MJ, DiMarco NM, Aukema HM. Safety of chronic exercise in a rat model of kidney disease. *Med Sci Sports Exerc.* 2000;32(3):576-80.

20. Nagao S, Kugita M, Yoshihara D, Yamaguchi T. Animal models for human polycystic kidney disease. *Exp Anim.* 2012;61(5):477-88.

21. Hew-Butler T. Arginine vasopressin, fluid balance and exercise: is exercise-associated hyponatraemia a disorder of arginine vasopressin secretion? *Sports Med.* 2010;40(6):459-79.

22. Kamijo A, Sugaya T, Hikawa A, et al. Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. *Am J Pathol.* 2004;165(4):1243-55.

23. Ivanyi T, Wiegant VM, de Wied D. Differential effects of emotional and physical stress on the central and peripheral secretion of neurohypophysial hormones in male rats. *Life Sci.* 1991;48(13):1309-16.

338 24. Nagao S, Nishii K, Katsuyama M, et al. Increased water intake decreases
339 progression of polycystic kidney disease in the PCK rat. *J Am Soc Nephrol.*
340 2006;17(8):2220-7.
341 25. Wang X, Wu Y, Ward CJ, Harris PC, Torres VE. Vasopressin directly regulates
342 cyst growth in polycystic kidney disease. *J Am Soc Nephrol.* 2008;19(1):102-8.
343 26. Shibazaki S, Yu Z, Nishio S, et al. Cyst formation and activation of the
344 extracellular regulated kinase pathway after kidney specific inactivation of Pkd1.
345 *Hum Mol Genet.* 2008;17(11):1505-16.
346 27. Kapoor S, Rodriguez D, Riwanto M, et al. Effect of Sodium-Glucose Cotransport
347 Inhibition on Polycystic Kidney Disease Progression in PCK Rats. *PLoS One.*
348 2015;10(4):e0125603.
349 28. Nagao S, Kugita M, Kumamoto K, Yoshimura A, Nishii K, Yamaguchi T. Increased
350 salt intake does not worsen the progression of renal cystic disease in high water-
351 loaded PCK rats. *PLoS One.* 2019;14(3):e0207461.
352 29. Watanabe S, Ichikawa D, Sugaya T, et al. Urinary Level of Liver-Type Fatty Acid
353 Binding Protein Reflects the Degree of Tubulointerstitial Damage in Polycystic

354 Kidney Disease. *Kidney Blood Press Res.* 2018;43(6):1716-29.

355 30. Antunes-Rodrigues J, de Castro M, Elias LL, Valenca MM, McCann SM.
356 Neuroendocrine control of body fluid metabolism. *Physiol Rev.* 2004;84(1):169-
357 208.

358 31. Freund BJ, Shizuru EM, Hashiro GM, Claybaugh JR. Hormonal, electrolyte, and
359 renal responses to exercise are intensity dependent. *J Appl Physiol (1985).*
360 1991;70(2):900-6.

361 32. Convertino VA, Keil LC, Bernauer EM, Greenleaf JE. Plasma volume, osmolality,
362 vasopressin, and renin activity during graded exercise in man. *J Appl Physiol Respir*
363 *Environ Exerc Physiol.* 1981;50(1):123-8.

364 33. Ghaemmaghami F, Gauquelin G, Gharib C, et al. Effects of treadmill running and
365 swimming on plasma and brain vasopressin levels in rats. *Eur J Appl Physiol Occup*
366 *Physiol.* 1987;56(1):1-6.

367 34. Krothapalli RK, Suki WN. Functional characterization of the alpha adrenergic
368 receptor modulating the hydroosmotic effect of vasopressin on the rabbit cortical
369 collecting tubule. *J Clin Invest.* 1984;73(3):740-9.

35. Umemura S, Marver D, Smyth DD, Pettinger WA. Alpha2-adrenoceptors and cellular cAMP levels in single nephron segments from the rat. *Am J Physiol.* 1985;249(1 Pt 2):F28-33.

36. Hawk CT, Schafer JA. Clonidine, but not bradykinin or ANP, inhibits Na+ and water transport in Dahl SS rat CCD. *Kidney Int.* 1993;44(1):30-5.

37. Wang X, Gattone V, 2nd, Harris PC, Torres VE. Effectiveness of vasopressin V2 receptor antagonists OPC-31260 and OPC-41061 on polycystic kidney disease development in the PCK rat. *J Am Soc Nephrol.* 2005;16(4):846-51.

38. Sabbatini M, Russo L, Cappellaio F, et al. Effects of combined administration of rapamycin, tolvaptan, and AEZ-131 on the progression of polycystic disease in PCK rats. *Am J Physiol Renal Physiol.* 2014;306(10):F1243-50.

39. Watson K, Baar K. mTOR and the health benefits of exercise. *Semin Cell Dev Biol.* 2014;36:130-9.

40. Widegren U, Ryder JW, Zierath JR. Mitogen-activated protein kinase signal transduction in skeletal muscle: effects of exercise and muscle contraction. *Acta Physiol Scand.* 2001;172(3):227-38.

386 41. Kojda G, Hambrecht R. Molecular mechanisms of vascular adaptations to
387 exercise. Physical activity as an effective antioxidant therapy? *Cardiovasc Res.*
388 2005;67(2):187-97.
389 42. Piguet AC, Saran U, Simillion C, et al. Regular exercise decreases liver tumors
390 development in hepatocyte-specific PTEN-deficient mice independently of
391 steatosis. *J Hepatol.* 2015;62(6):1296-303.
392 43. Saran U, Guarino M, Rodriguez S, et al. Anti-tumoral effects of exercise on
393 hepatocellular carcinoma growth. *Hepatol Commun.* 2018;2(5):607-20.
394 44. Cunha A. Basic research: Irisin--behind the benefits of exercise. *Nat Rev*
395 *Endocrinol.* 2012;8(4):195.
396 45. Kuloglu T, Aydin S, Eren MN, et al. Irisin: a potentially candidate marker for
397 myocardial infarction. *Peptides.* 2014;55:85-91.
398 46. Yu Q, Kou W, Xu X, et al. FNDC5/Irisin inhibits pathological cardiac
399 hypertrophy. *Clin Sci (Lond).* 2019;133(5):611-27.
400 47. Wen MS, Wang CY, Lin SL, Hung KC. Decrease in irisin in patients with chronic
401 kidney disease. *PLoS One.* 2013;8(5):e64025.

48. Liu J, Song N, Huang Y, Chen Y. Irisin inhibits pancreatic cancer cell growth via the AMPK-mTOR pathway. *Sci Rep.* 2018;8(1):15247.

49. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol.* 2008;70:23-49.

50. Xie C, Zhang Y, Tran TD, et al. Irisin Controls Growth, Intracellular Ca2+ Signals, and Mitochondrial Thermogenesis in Cardiomyoblasts. *PLoS One.* 2015;10(8):e0136816.

Figure legends

Figure 1. Effects of chronic exercise on general parameters and urinary parameters in PCK rats. Time courses of **(A)** body weight, **(B)** food intake, **(C)** water intake, **(D)** urine volume, **(E)** urinary protein excretion, and **(F)** urinary L-FABP excretion were compared among the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups (n=10 in each group). Data are presented as the mean ± SEM. *P<0.05, **P<0.01 compared with the Con-SD group; #P<0.05, ##P<0.01 compared with the Sed-PCK group.

Figure 2. Effects of chronic exercise on kidney cysts in PCK rats. (A) Representative images of kidney specimens stained with HE in the Con-SD, Sed-PCK, and Ex-PCK groups. **(B)** Total kidney weight, **(C)** kidney-to-body weight ratio, and **(D)** cystic index were compared among the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups (n=10 in each group). Data are presented as the mean ± SEM. **P<0.01 compared with the Con-SD group; #P<0.05 compared with the Sed-PCK group; ns: no significant difference.

Figure 3. Effects of chronic exercise on glomerular sclerosis, podocyte injury, and renal interstitial fibrosis in PCK rats. (A) Representative images of periodic acid–Schiff (PAS)-stained, desmin-immunostained glomeruli and Masson's trichrome stained kidneys in the Con-SD, Sed-PCK, and Ex-PCK groups. **(B)** Index of glomerular sclerosis, **(C)** desmin-positive staining area (%), and **(D)** interstitial fibrosis area (%) in the Con-SD (rectangle dots), Sed-PCK (closed dots) and Ex-PCK (round dots) groups (n=10 in each group). Data are presented as the mean ± SEM. *P<0.05, **P<0.01 compared with the Con-SD group; #P<0.05, ##P<0.01 compared with the Sed-PCK group.

Figure 4. Effects of chronic exercise on cell proliferation in the kidneys of PCK rats. (A) Representative images of kidney specimens immunostained for Ki-67 in the Sed-PCK and Ex-PCK groups. **(B)** Ki-67 labeling index in the cyst-lining epithelium of the Sed-PCK (closed dots) and Ex-PCK (round dots) groups (n=10 in each group). **(C)** Ki-67 labeling index in the non-cystic tubules of the Sed-PCK (closed dots) and

Ex-PCK (round dots) groups (*n*=10 in each group). Data are presented as the mean ± SEM. ##*P*<0.01 compared with the Sed-PCK group.

Figure 5. Effects of chronic exercise on urinary AVP excretion, renal cAMP content, and renal B-Raf expression in PCK rats. (A) Urinary AVP excretion in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups. **(B)** Renal cAMP content in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups (*n*=10 in each group). **(C)** Western blotting analysis of B-Raf expression in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups (*n*=8 in each group). Top panels show representative immunoblotting. Each lane was loaded with a protein sample prepared from four different rats per group. The ratio in the Con-SD group was assigned a value of 1. Data are presented as the mean ± SEM. **P*<0.05, ***P*<0.01 compared with the Con-SD group; #*P*<0.05, ##*P*<0.01 compared with the Sed-PCK group; ns: no significant difference.

Figure 6. Effects of chronic exercise on the phosphorylation of ERK, mTOR, and S6 in PCK rats. Representative images of kidney specimens immunostained for **(A)** p-ERK and **(B)** p-mTOR in the Con-SD, Sed-PCK, and Ex-PCK groups. Western blotting analysis of **(C)** p-ERK, **(D)** p-mTOR, and **(E)** p-S6 expression in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups (n=8 in each group). Top panels show representative immunoblotting. Each lane was loaded with a protein sample prepared from four different rats per group. Ratios of the relative band intensity of the phosphorylated protein to that of the total protein were calculated. The ratio in the Con-SD group was assigned a value of 1. Data are presented as the mean ± SEM. **P<0.01 compared with the Con-SD group; ##P<0.01 compared with the Sed-PCK group; ns: no significant difference.

Table

Table 1. Blood pressure and plasma parameters

	Con-SD	Sed-PCK	Ex-PCK
Systolic Blood Pressure (mmHg)	106 ± 7	104 ± 3	96 ± 4
Glucose (mg/dL)	169.1 ± 6.8	139.7 ± 7.8*	125.5 ± 20.1*
Total cholesterol (mg/dL)	67.9 ± 5.1	148.2 ± 8.6*	114.1 ± 7.6*#
Triglyceride (mg/dL)	59.7 ± 6.0	69.3 ± 5.3	49.8 ± 3.7#
Urea nitrogen (mg/dL)	16.8 ± 0.5	18.2 ± 0.9	15.9 ± 0.3#
Creatinine (mg/dL)	0.28 ± 0.01	0.35 ± 0.02*	0.30 ± 0.01#
Irisin (ng/dL)	1134.8 ± 29.7	1070.0 ± 41.0	1578.1 ± 106.0*##

Con-SD, control Sprague-Dawley rats; Sed-PCK, sedentary polycystic kidney rats; Ex-PCK, exercise polycystic kidney rats.

Data are presented as means±SEM. n=10 in each group. *P<0.05 compared with the Con-SD group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 compared with the Sed-PCK group.

Figure1

Figure 1

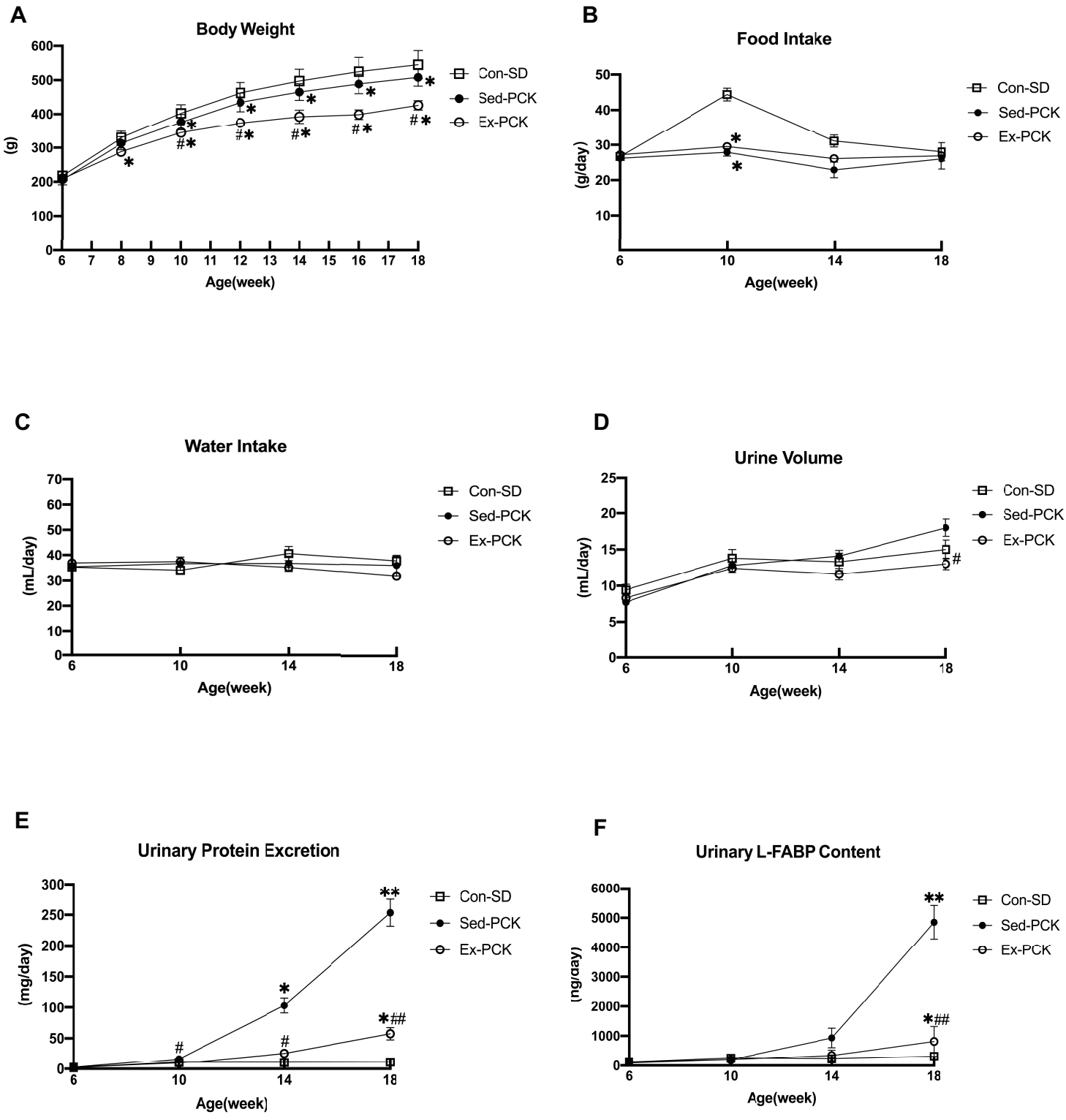


Figure 2

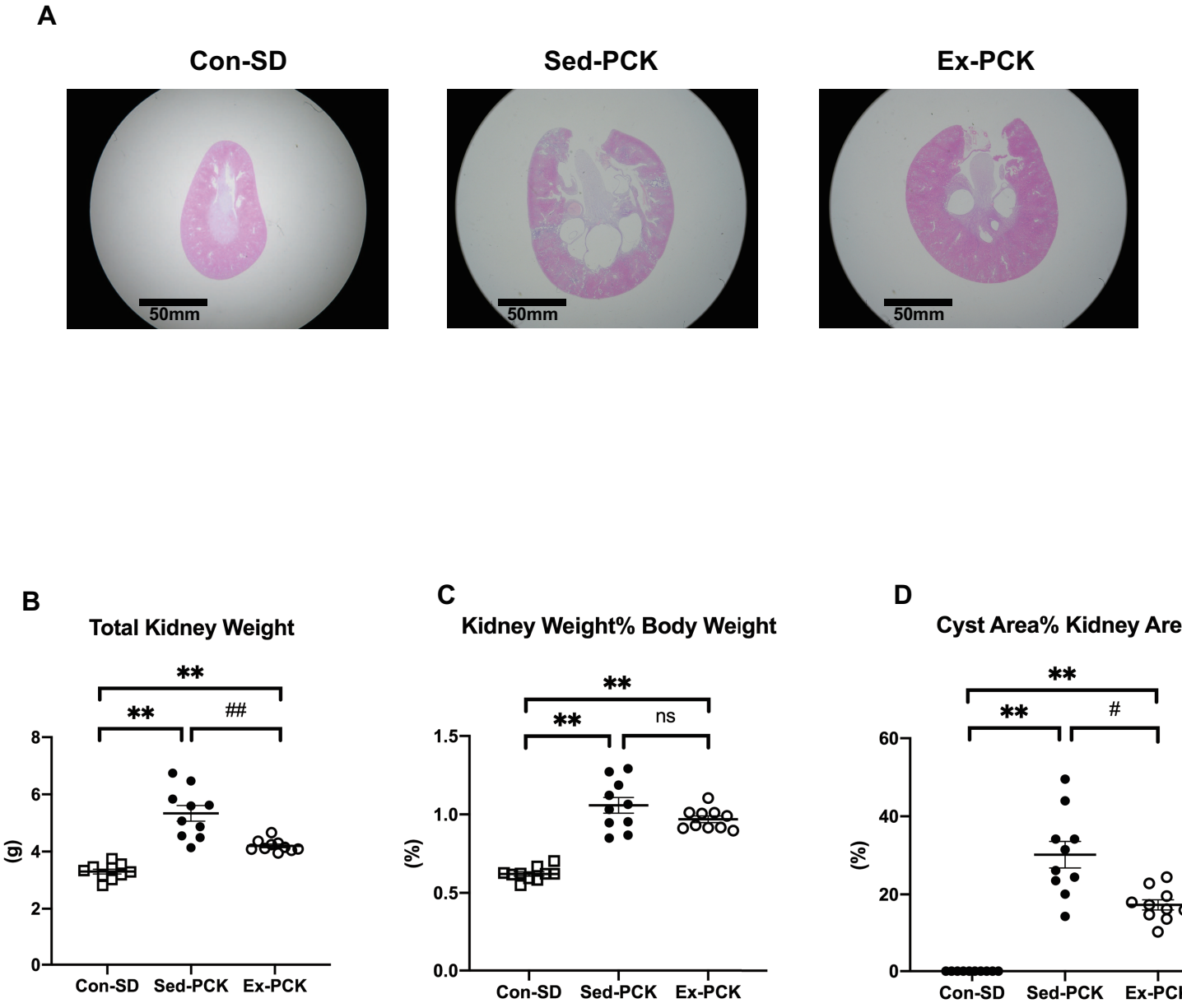
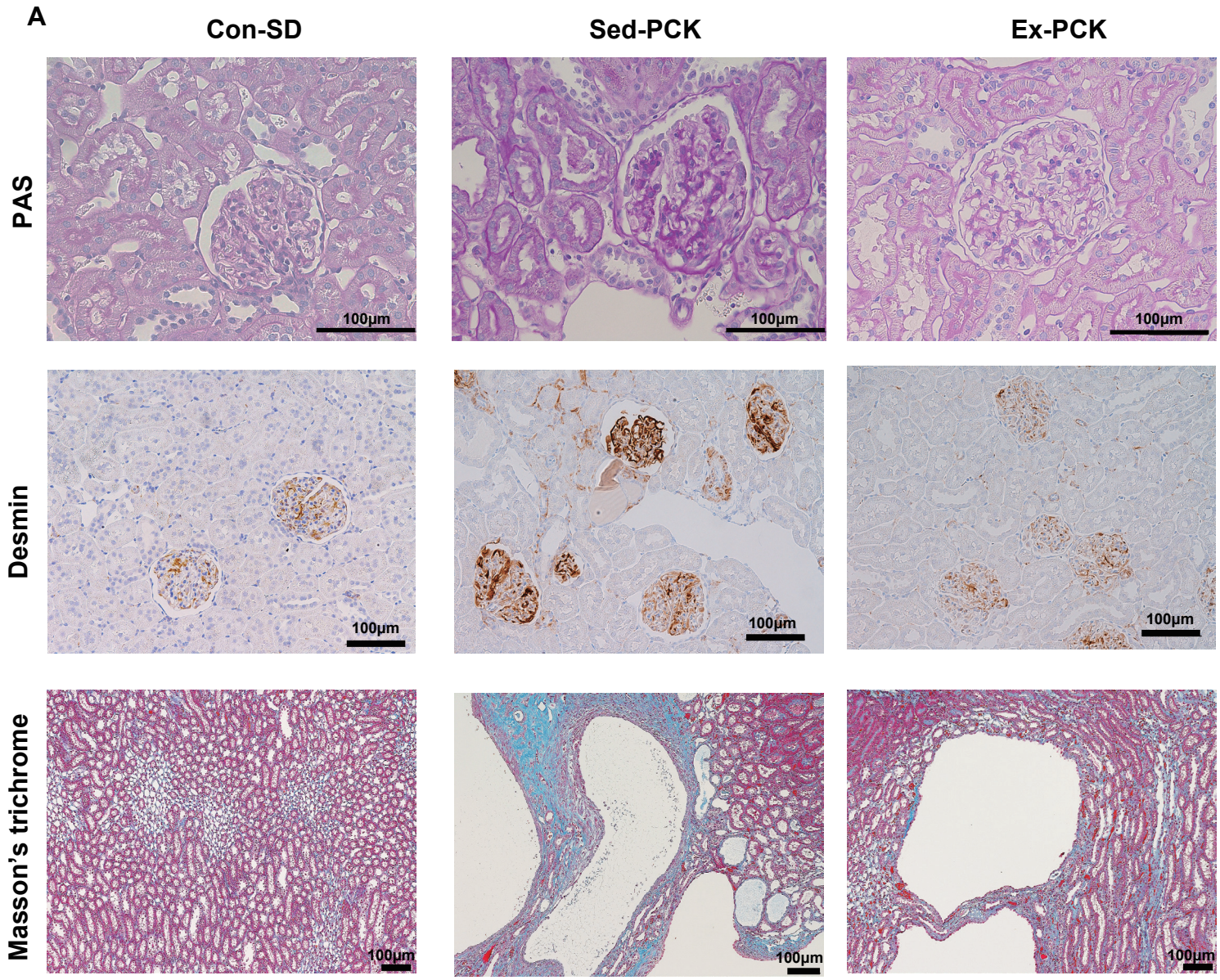
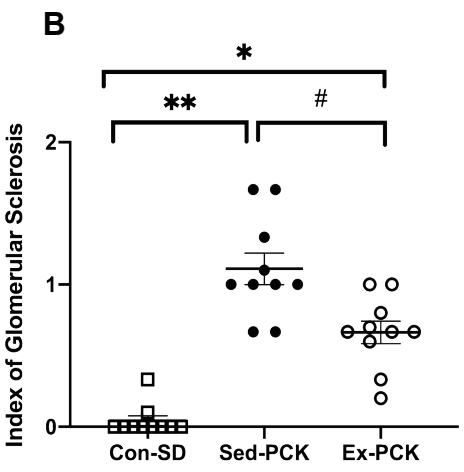
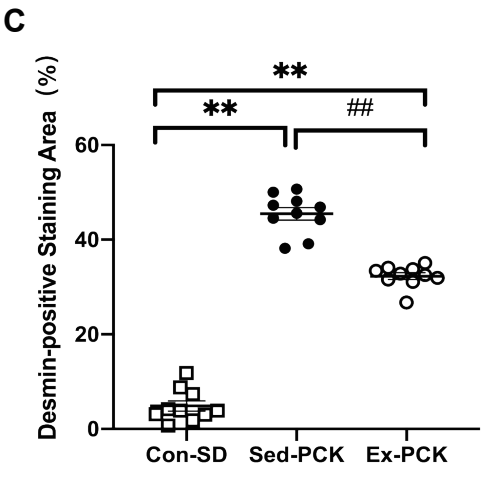

A
Con-SD
Sed-PCK
Ex-PCK
50mm
50mm
50mm
B
Total Kidney Weight
**
**
##
(g)
8
6
4
2
0
Con-SD
Sed-PCK
Ex-PCK
C
Kidney Weight% Body Weight
**
**
ns
(%)
1.5
1.0
0.5
0.0
Con-SD
Sed-PCK
Ex-PCK
D
Cyst Area% Kidney Area
**
**
#
(%)
60
40
20
0
Con-SD
Sed-PCK
Ex-PCK

Figure 3







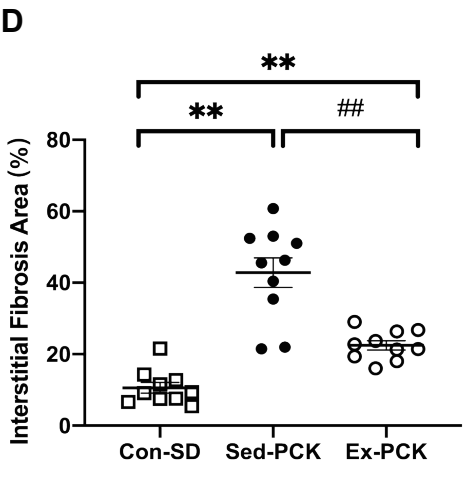
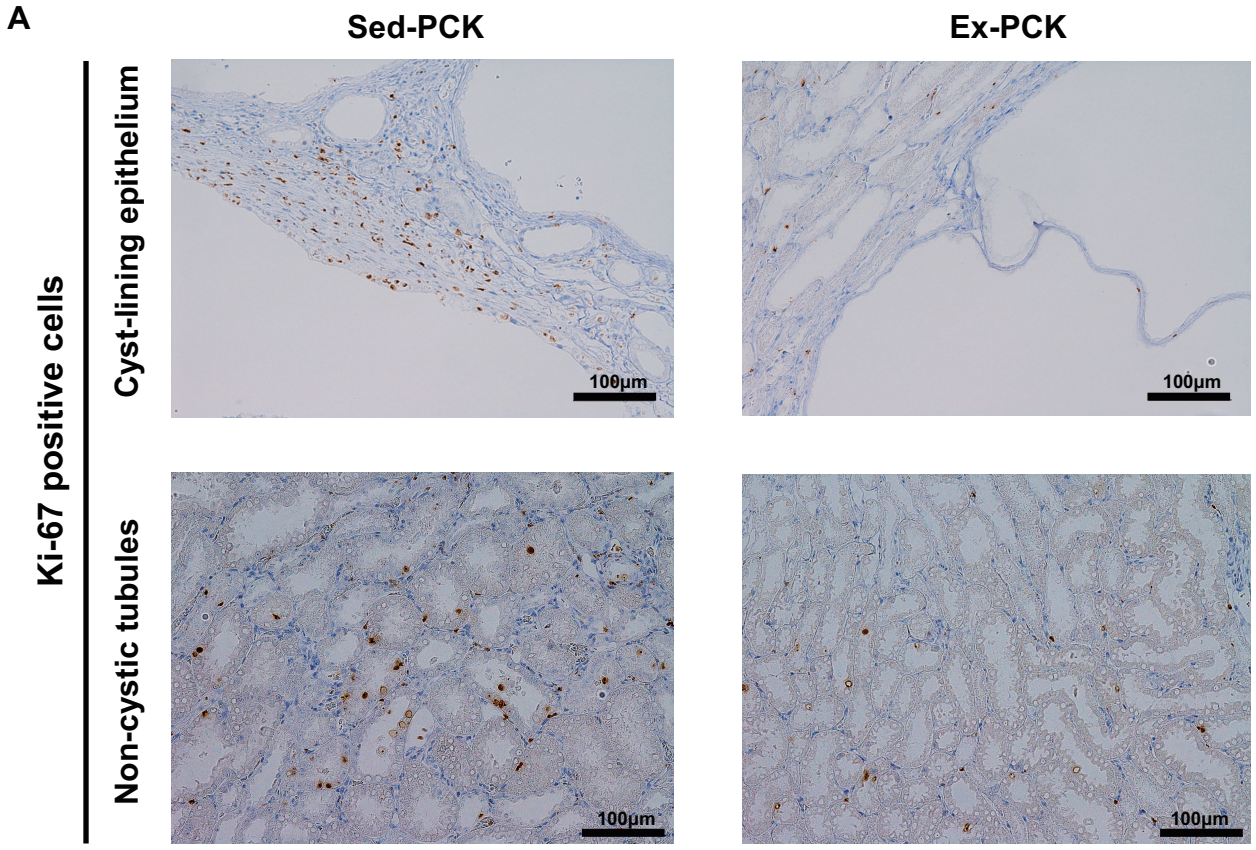
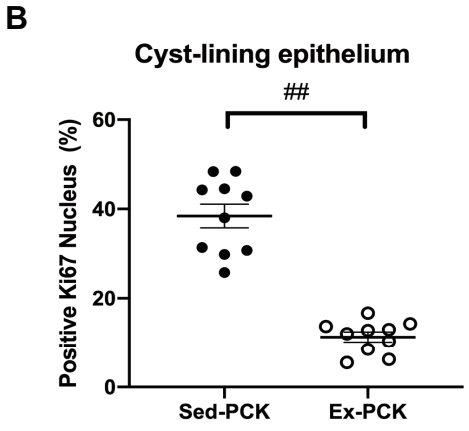


Figure 4


A
Sed-PCK
Ex-PCK
Ki-67 positive cells
Cyst-lining epithelium
Non-cystic tubules
100μm
100μm
100μm
100μm


B
Cyst-lining epithelium
##
Positive Ki67 Nucleus (%)
60
40
20
0
Sed-PCK
Ex-PCK

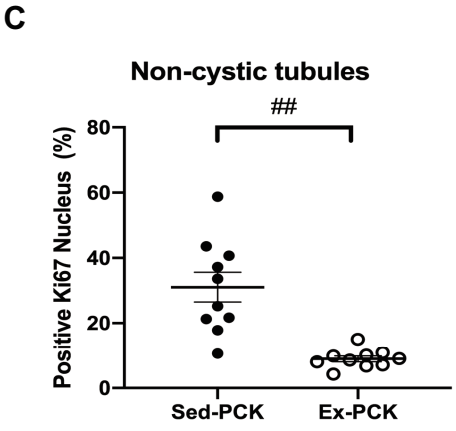

C
Non-cystic tubules
##
Positive Ki67 Nucleus (%)
80
60
40
20
0
Sed-PCK
Ex-PCK

Figure5

Figure 5

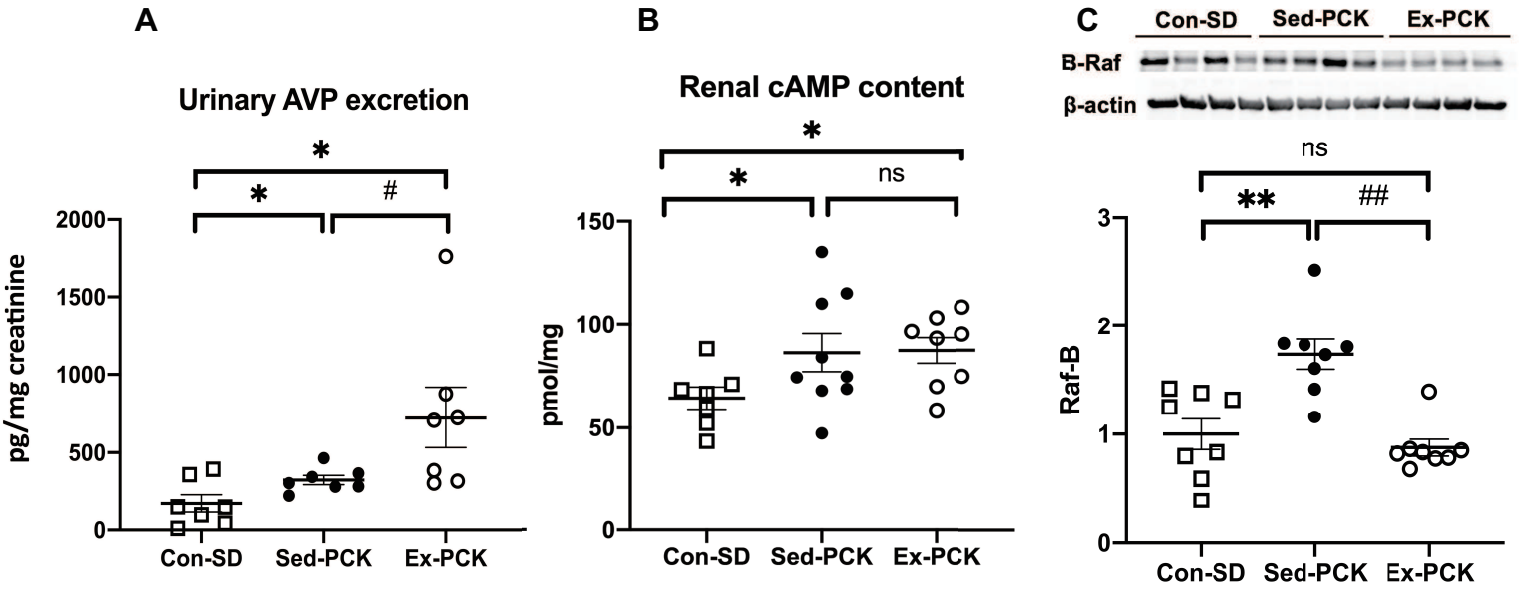
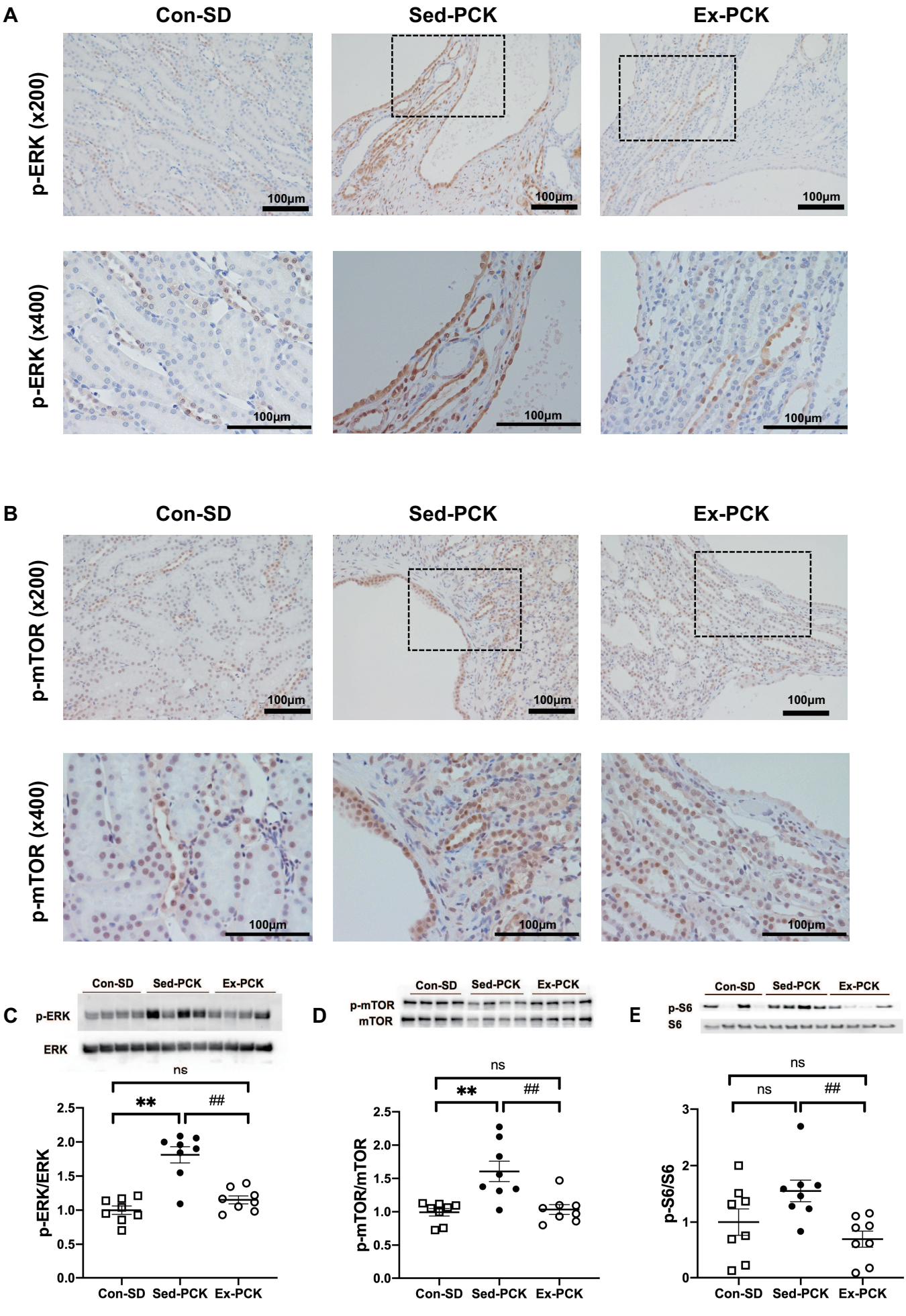


Figure 6


A
Con-SD
Sed-PCK
Ex-PCK
p-ERK (x200)
p-ERK (x400)
100μm
B
p-mTOR (x200)
p-mTOR (x400)
C
p-ERK
ERK
p-ERK/ERK
D
p-mTOR
mTOR
p-mTOR/mTOR
E
p-S6
S6
p-S6/S6
ns
**
##