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Supplemental Material

The Kidney-Related Effects of Polystyrene Microplastics on Human Kidney Proximal Tubular Epithelial Cells HK-2 and Male C57BL/6 Mice

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Figure S1. PS-MPs characterization. The diameter of PS-MPs was detected with TEM.

Figure S2. Representative Western blots evaluating Bax, Bad, and Bcl2 in HK-2 cells treated with PS-MPs Bad, Bcl2 and Bax were assessed after PS-MPs treatment at a concentration of 0.8 mg/ml for 0, 5, 10, 20, 30, 60 min. The mean and SD summary data for quantification of Western blots are shown in Table S3.

Figure S3. Quantification of Western blots evaluating IRE1 α , ATF6, and p-EIF2 α in HK-2 cells treated with PS-MPs. ER stress-related proteins IRE1 α , ATF6, p-EIF2 α , and EIF2 α , were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) IRE1 α /GAPDH ratio. (B) ATF6/GAPDH ratio. (C) p-EIF2 α / EIF2 α ratio. N=3. Data are presented as the mean \pm SD. *P < 0.05 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test, IRE1 α /GAPDH, 0 mg/ml group vs 0.8 mg/ml group. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results are shown in Table S4.

Figure S4. Quantification of Western blots evaluating the phosphorylation of MAPK signaling pathway components ERK1/2, JNK, and p38 in HK-2 cells treated with PS-MPs. MAPK signaling pathway components, such as p-ERK1/2, ERK1/2, p-JNK, JNK, p-p38 and, p38, were assessed after PS-MPs treatment at a concentration of 0.8 mg/ml for 0, 5, 10, 20, 30, 60 min. The Western blotting results were graphed and statistically analyzed. (A) p-ERK1/2/ERK1/2 ratio, N=3. (B) p-JNK/JNK ratio, N=3. (C) p-p38/ p-p38 ratio, N=2. Data are presented as the mean \pm SD. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S5. Quantification of Western blots evaluating cPLA2 and COX-1 in HK-2 cells treated with PS-MPs. Inflammation-related proteins cPLA2 and COX-1, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) cPLA2/GAPDH ratio. (B) COX-1/GAPDH ratio. N=3. Data are presented as the mean \pm SD. *P < 0.05 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S6. Quantification of Western blots evaluating the phosphorylation of mTOR and Akt in HK-2 cells treated with PS-MPs. AKT/mTOR signaling pathway components, such as p-mTOR, mTOR, p-AKT, and AKT, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 1 h. The Western blotting results were graphed and statistically analyzed. (A) p-mTOR/mTOR ratio. (B) p-AKT/AKT ratio. N=2. Data are presented as the mean \pm SD. *P < 0.05 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S7. Quantification of Western blots evaluating the expression of p62, Beclin 1, and LC3 in HK-2 cells treated with PS-MPs. Autophagy-related proteins p62, Beclin 1, and LC3, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) p62/GAPDH ratio. (B) Beclin 1/GAPDH ratio. (B) LC3-II/LC3-I ratio. N=3. Data are presented as the mean \pm SD. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S8. Quantification of Western blots evaluating the expression Bad, IRE1 α , p-ERK1/2, p-mTOR, and LC3-II/LC3-I ratio in HK-2 cells treated with PS-MPs alone, MitoTEMPO alone or in combination. (A) Cells were pretreated for 1 h with MitoTEMPO (100 μ M) then exposed to PS-MPs (0.8 mg/ml) for 20 min. Mitochondrial-mediated apoptosis protein Bad was assessed. (B) Cells were pretreated for 1 h with MitoTEMPO and then exposed to PS-MPs for 24 h. ER stress-related protein IRE1 α was assessed. (C) Cells were pretreated for 1 h with MitoTEMPO for 12 h and exposed to PS-MPs for 30 min. MAPK signaling pathway component p-ERK1/2 and ERK1/2 was assessed. (D) Cells were pretreated for 1 h with MitoTEMPO and then exposed to PS-MPs for 1 h. AKT/mTOR pathway components p-mTOR and mTOR were assessed. (E) Cells were pretreated for 1 h with MitoTEMPO for 1 h and then exposed to 0.8 mg/ml PS-MPs for 24 h. Autophagy-related protein LC3 was assessed. The Western blotting results were graphed and statistically analyzed. N=2. Data are presented as the mean \pm SD. *P < 0.05 compared with control group as determined by t test. PS-MPs 0.8 mg/ml group vs MitoTEMPO (0 μ M) group, (A) P=0.0462. (B) P= 0.0325. (C) P= 0.4509. (D) P= 0.0107. (E) P= 0.0475. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S9. Quantification of western blot analysis of ATG5 knockdown cells treated with PS-MPs for expression of ATG5, LC3 and COX-1. Inflammation-related proteins were evaluated after PS-MPs treatment at concentrations of 0.4 and 0.8 mg/ml for 48 h in ATG5^{KD} HK-2 cells. The Western blotting results were graphed and statistically analyzed. (A) ATG5/GAPDH ratio, N=3 (B) LC3-II/LC3-I ratio, N=3 (C) COX-1/GAPDH ratio, N=2. Data are presented as the mean \pm SD. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control group as determined by two-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S10. The effects of PS-MPs on mouse muscle and grip strength. Six-week-old C57BL/6 male mice without and with 0.2 mg/day and 0.4 mg/day PS-MPs 2 times per week were examined, and the leg muscles of mice were harvested at 8 weeks. (A) Hematoxylin and eosin (H&E) staining, Masson's trichrome staining (MTS), and IHC staining of dystrophin in the muscular sections from mice with or without oral gavage of PS-MPs. Hematoxylin-stained cell nuclei were blue and eosin-stained the extracellular matrix and cytoplasm were pink. MTS-stained collagen fiber was blue and muscle fiber was red. Muscle fiber and IHC staining of dystrophin were quantified and presented % area. The mean and SD summary data for quantification are shown in Table S5. (B) Handgrip strength in a single-blind test of mice with oral gavage of 0.4 mg/day PS-MPs for 8 weeks before the mice were sacrificed. Data are presented as the mean \pm SD. N=7, ***P < 0.001 compared with sham group of mice as determined by t test (Sham group vs PS-MPs group: P<0.001). The mean and SD summary data for handgrip strength are shown in Table S3. Scale bar=60 μ m.

Figure S11. Protein expression in mouse urine after treatment with PS-MPs. (A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of urine from the mice was collected after oral gavage of 0.4 mg/day PS-MPs for 4 weeks. The red frame shows the difference between the groups treated with oral gavage of 0.4 mg/day PS-MPs or the sham group. Bovine serum albumin (BSA) is a serum albumin protein derived from cows. N=3. (B) Immunoblotting of urine samples from 3 different mice with albumin at 8 weeks. (C) The Western blotting results were graphed and statistically analyzed. N=3. Data are presented as the mean \pm SD. ***P < 0.001 compared with sham group as determined by t test. P < 0.001. The mean and SD summary data for quantification of Western blots are shown in Table S3.