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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 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 ± 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 ± 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 ± 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 ± 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, IRE1α, 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 μM) 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 IRE1α 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 ± SD. *P < 0.05 compared with control group as determined by t test. PS-MPs 0.8 mg/ml group vs MitoTEMPO (0 μM) 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-2cells. 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 ± 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 ± 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 μm.

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 ± 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.

Supplemental material

Table S1. Physical characteristics of PS-MPs.

Characterization	Mean	SD
Hydrodynamic diameter (nm)	1878	67.7
Zeta potential (mV)	-76	1.2
PDI[a]	0.189	-

PDI[a] is the polydispersity index

Table S2. The estimated concentrations of PS-MPs in each kidney.

Sample	Raman intensity (count)	Estimated concentration ($mg/g_{[kidney]}$)
Sham group-1	288	0.47
Sham group-2	290	0.48
Sham group-3	317	0.71
Sham group-4	319	0.73
Sham group-5	323	0.76
PS-MPs group-1	582	2.94
PS-MPs group-2	588	2.99
PS-MPs group-3	594	3.04
PS-MPs group-4	596	3.06
PS-MPs group-5	627	3.32

Table S3. The mean and SD summary data for quantification.

Figure 1D	1h		2h			
	Mean	SD	Mean	SD		
0 mg/ml	100.00	4.74	100.00	4.35		
0.05 mg/ml	122.50	16.55	131.50	6.59		
0.1 mg/ml	141.70	20.35	152.40	7.78		
0.2 mg/ml	161.60	25.55	202.40	19.30		
0.4 mg/ml	206.80	35.39	274.80	43.72		
0.8 mg/ml	271.70	60.18	355.20	64.07		
Figure 2A	**1 day**		**2 day**		**3 day**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
0 mg/ml	100.00	0.94	100.00	3.55	100.00	0.74

0.025 mg/ml	99.18	1.45	99.89	4.05	104.13	1.93
0.05 mg/ml	100.58	2.09	99.05	4.14	101.81	1.85
0.1 mg/ml	101.45	3.71	99.27	4.78	102.65	1.30
0.2 mg/ml	102.77	3.78	101.24	2.47	102.76	3.87
0.4 mg/ml	102.79	7.67	98.99	2.37	104.87	2.67
0.8 mg/ml	98.87	2.23	101.29	4.30	108.04	0.67
Figure 2C	**Necrosis index**		**Apoptosis index**			
	Mean	**SD**	**Mean**	**SD**		
0 mg/ml	3.71	1.00	9.76	1.87		
0.05 mg/ml	3.75	0.52	8.97	3.96		
0.1 mg/ml	4.22	1.09	9.30	3.47		
0.2 mg/ml	5.38	1.54	10.78	4.28		
0.4 mg/ml	6.92	0.45	9.89	3.62		
0.8 mg/ml	8.38	2.07	8.94	4.29		
Figure 2E	**Mean**	**SD**				
0 mg/ml	100.00	16.25				
0.05 mg/ml	122.46	21.63				
0.1 mg/ml	149.52	14.20				
0.2 mg/ml	200.53	16.48				
0.4 mg/ml	333.54	62.88				
0.8 mg/ml	301.13	63.93				
Figure 3G	**Mean**	**SD**				
0 mg/ml	2.81	0.77				
0.05 mg/ml	4.77	1.06				
0.1 mg/ml	8.33	1.32				
0.2 mg/ml	11.49	2.57				
0.4 mg/ml	18.97	3.48				
0.8 mg/ml	22.62	3.80				
Figure 4B	**Mean**	**SD**				
PS-MPs 0 mg/ml	100.00	3.34				
PS-MPs 0.8 mg/ml + MitoTEMPO (0 μM)	295.50	21.51				

PS-MPs 0.8 mg/ml + MitoTEMPO (40 μM)	262.80	52.73				
PS-MPs 0.8 mg/ml + MitoTEMPO (80 μM)	239.20	3.85				
PS-MPs 0.8 mg/ml + MitoTEMPO (100 μM)	169.40	9.63				
Figure 5B	**Void**		**ATG5^{KD}#1**		**ATG5^{KD}#2**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
0 mg/ml	100.00	4.73	100.00	9.15	100.00	5.12
0.4 mg/ml	102.41	2.95	79.87	3.68	66.85	16.71
0.8 mg/ml	95.64	4.57	75.68	2.24	66.66	13.61
Figure 5D	***Atg5*$^{+/+}$ MEF cells**		***Atg5*$^{-/-}$ MEF cells**			
	Mean	**SD**	**Mean**	**SD**		
0 mg/ml	100.00	3.30	100.00	4.57		
0.4 mg/ml	97.80	7.76	84.50	4.97		
0.8 mg/ml	90.80	1.97	65.60	7.40		
Figure 5F	***Atg5*$^{+/+}$ MEF cells**		***Atg5*$^{-/-}$ MEF cells**			
Necrosis index	**Mean**	**SD**	**Mean**	**SD**		
0 mg/ml	3.29	0.67	2.03	1.05		
0.4 mg/ml	6.02	1.97	3.68	1.60		
0.8 mg/ml	6.20	2.54	4.43	2.12		
	***Atg5*$^{+/+}$ MEF cells**		***Atg5*$^{-/-}$ MEF cells**			
Apoptosis index	**Mean**	**SD**	**Mean**	**SD**		
0 mg/ml	12.70	4.69	9.34	3.18		
0.4 mg/ml	19.78	2.45	27.60	2.39		
0.8 mg/ml	25.16	1.81	36.96	4.50		
Figure 6A	**sham group**		**0.2 mg/day group**		**0.4 mg/day group**	
4 weeks group	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0 week	23.17	1.54	23.36	1.32	23.36	1.32
1 week	24.64	2.17	24.75	1.92	24.89	1.15
2 weeks	25.39	2.40	25.64	1.93	25.82	1.22

3 weeks	26.44	2.35	26.36	1.85	26.50	1.23
4 weeks	26.81	2.39	26.94	1.82	26.44	1.15
	sham group		**0.2 mg/day group**		**0.4 mg/day group**	
8 weeks group	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0 week	23.19	0.62	23.29	1.32	23.34	1.42
1 week	24.16	0.29	24.43	1.78	24.21	1.92
2 weeks	24.94	0.46	25.21	1.74	25.18	1.80
3 weeks	26.00	0.65	25.86	1.46	25.91	1.70
4 weeks	26.06	1.02	26.31	1.53	26.19	1.67
5 weeks	26.69	0.84	26.50	1.31	26.69	1.94
6 weeks	27.13	0.95	27.00	1.65	27.81	2.09
7 weeks	27.38	0.74	27.06	1.59	27.81	2.07
8 weeks	27.75	1.10	27.81	1.73	28.38	1.94
Figure 6B	**4 weeks**		**8 weeks**			
BUN	**Mean**	**SD**	**Mean**	**SD**		
sham group	30.50	1.80	34.00	3.10		
0.2 mg/day group	28.70	2.10	33.40	1.80		
0.4 mg/day group	27.90	1.50	31.50	3.00		
	4 weeks		**8 weeks**			
CREA	**Mean**	**SD**	**Mean**	**SD**		
sham group	0.240	0.021	0.458	0.008		
0.2 mg/day group	0.198	0.011	0.424	0.017		
0.4 mg/day group	0.202	0.011	0.386	0.022		
Figure 6D	**4 weeks**		**8 weeks**			
	Mean	**SD**	**Mean**	**SD**		
sham group	0.51	0.10	0.56	0.10		
0.2 mg/day group	0.83	0.15	1.23	0.30		
0.4 mg/day group	1.05	0.05	1.31	0.32		
Figure 7D	**Mean**	**SD**				
sham group	0.63	0.14				
PS-MPs group	3.07	0.15				
Figure S2	**0 min**		**5 min**		**10 min**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**

(A) Bad/GAPDH ratio	1.0	0.00	1.35	0.23	1.40	0.10
(B) Bcl2/GAPDH ratio	1.0	0.00	1.16	0.10	1.00	0.08
(C) Bax/GAPDH ratio	1.0	0.00	1.03	0.05	1.01	0.08
	20 min		**30 min**		**60 min**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) Bad/GAPDH ratio	1.55	0.10	1.60	0.14	1.40	0.17
(B) Bcl2/GAPDH ratio	0.58	0.10	0.69	0.20	0.72	0.04
(C) Bax/GAPDH ratio	1.04	0.05	1.05	0.06	1.09	0.11
Figure S3	**0 mg/ml group**		**0.05 mg/ml group**		**0.1 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) IRE1α/GAPDH ratio	1.00	0.00	1.20	0.09	1.36	0.26
(B) ATF6/GAPDH ratio	1.00	0.00	1.09	0.19	1.02	0.06
(C) p-EIF2α/ EIF2α ratio	1.00	0.00	1.27	0.32	1.13	0.31
	0.2 mg/ml group		**0.4 mg/ml group**		**0.8 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) IRE1α/GAPDH ratio	1.51	0.35	1.56	0.23	1.57	0.18
(B) ATF6/GAPDH ratio	0.95	0.09	0.99	0.14	0.95	0.16
(C) p-EIF2α/ EIF2α ratio	1.27	0.25	1.30	0.40	1.47	0.21
Figure S4	**0 min**		**5 min**		**10 min**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) p-ERK1/2/ERK1/2 ratio	1.00	0.00	1.20	0.06	1.40	0.14
(B) p-JNK/JNK ratio	1.00	0.00	1.33	0.12	1.39	0.09
(C) p-p38/ p-p38 ratio	1.00	0.00	1.49	0.23	1.61	0.11
	20 min		**30 min**		**60 min**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) p-ERK1/2/ERK1/2 ratio	1.53	0.18	1.21	0.13	1.34	0.13
(B) p-JNK/JNK ratio	1.54	0.33	1.70	0.29	1.07	0.06
(C) p-p38/ p-p38 ratio	1.51	0.03	1.48	0.04	0.88	0.33
Figure S5	**0 mg/ml group**		**0.05 mg/ml group**		**0.1 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) cPLA2/GAPDH ratio	1.00	0.00	0.79	0.14	0.80	0.17
(B) COX-1/GAPDH ratio	1.00	0.00	1.07	0.08	1.21	0.07
	0.2 mg/ml group		**0.4 mg/ml group**		**0.8 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**

(A) cPLA2/GAPDH ratio	0.84	0.21	1.11	0.22	1.47	0.14
(B) COX-1/GAPDH ratio	1.15	0.13	1.28	0.15	1.29	0.14
Figure S6	**0 mg/ml group**		**0.05 mg/ml group**		**0.1 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) p-mTOR/mTOR ratio	1.00	0.00	0.86	0.03	0.88	0.02
(B) p-AKT/AKT ratio	1.00	0.00	0.93	0.19	0.77	0.16
	0.2 mg/ml group		**0.4 mg/ml group**		**0.8 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) p-mTOR/mTOR ratio	0.66	0.08	0.68	0.19	0.59	0.10
(B) p-AKT/AKT ratio	0.74	0.10	0.70	0.10	0.59	0.09
Figure S7	**0 mg/ml group**		**0.05 mg/ml group**		**0.1 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) p62/GAPDH ratio	1.00	0.00	1.05	0.11	1.07	0.11
(B) Beclin 1/GAPDH ratio	1.00	0.00	1.12	0.13	1.21	0.03
(C) LC3-II/LC3-I ratio	1.00	0.00	1.27	0.18	1.62	0.26
	0.2 mg/ml group		**0.4 mg/ml group**		**0.8 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
(A) p62/GAPDH ratio	1.09	0.26	1.15	0.09	1.40	0.26
(B) Beclin 1/GAPDH ratio	1.21	0.11	1.12	0.03	1.02	0.11
(C) LC3-II/LC3-I ratio	2.21	0.52	4.69	1.99	6.55	1.87
Figure S8	**PS-MPs 0 mg/ml**		**MitoTEMPO (0 μM)**			
	Mean	**SD**	**Mean**	**SD**		
(A) Bad/GAPDH ratio	1.00	0.00	1.18	0.12		
(B) IRE1α/GAPDH ratio	1.00	0.00	1.29	0.07		
(C) p-ERK1/2/ERK1/2 ratio	1.00	0.00	1.03	0.01		
(D) p-mTOR/mTOR ratio	1.00	0.00	1.03	0.02		
(E) LC3-II/LC3-I ratio	1.00	0.00	0.74	0.54		
	PS-MPs 0.8 mg/ml		**PS-MPs 0.8 mg/ml + MitoTEMPO (100 μM)**			
	Mean	SD	Mean	SD		

(A) Bad/GAPDH ratio	1.46	0.26	0.91	0.21		
(B) IRE1α/GAPDH ratio	1.40	0.12	1.11	0.08		
(C) p-ERK1/2/ERK1/2 ratio	5.21	5.23	4.11	3.90		
(D) p-mTOR/mTOR ratio	0.75	0.03	1.26	0.07		
(E) LC3-II/LC3-I ratio	14.55	6.60	9.87	6.10		
Figure S9	**0 mg/ml group**		**0.4 mg/ml group**		**0.8 mg/ml group**	
(A) ATG5/GAPDH ratio	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Void	1.00	0.00	1.07	0.17	1.01	0.33
$ATG5^{KD}$#1	0.55	0.17	0.44	0.20	0.32	0.08
$ATG5^{KD}$#2	0.47	0.08	0.42	0.07	0.34	0.05
(B) LC3-II/LC3-I ratio	**0 mg/ml group**		**0.4 mg/ml group**		**0.8 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Void	1.00	0.00	5.38	0.97	8.42	4.15
$ATG5^{KD}$#1	0.71	0.54	3.34	1.59	3.98	1.20
$ATG5^{KD}$#2	0.45	0.46	3.06	0.70	4.68	1.25
(C) COX-1/GAPDH ratio	**0 mg/ml group**		**0.4 mg/ml group**		**0.8 mg/ml group**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Void	1.00	0.00	1.03	0.24	1.15	0.17
$ATG5^{KD}$#1	1.15	0.03	1.27	0.18	1.47	0.15
$ATG5^{KD}$#2	0.92	0.18	1.28	0.25	1.28	0.05
Figure S10B	**Mean**	**SD**				
Sham group	0.128389	0.0071273				
PS-MPs group	0.111361	0.006699				
Figure S11C	**Mean**	**SD**				
Sham group	1.00	0.00				
PS-MPs group	1.37	0.07				

Table S4. P-values for non-statistically and statistically significant results.

Figure 1D		
Sidak's multiple comparisons test	**P Value**	**Summary**
1h		
0 mg/ml group vs 0.05 mg/ml group	0.9262	ns
0 mg/ml group vs 0.1 mg/ml group	0.5023	ns

0 mg/ml group vs 0.2 mg/ml group	0.1391	ns
0 mg/ml group vs 0.4 mg/ml group	0.0025	**
0 mg/ml group vs 0.8 mg/ml group	<0.001	***
2h		
0 mg/ml group vs 0.05 mg/ml group	0.7598	ns
0 mg/ml group vs 0.1 mg/ml group	0.2685	ns
0 mg/ml group vs 0.2 mg/ml group	0.0039	**
0 mg/ml group vs 0.4 mg/ml group	<0.001	***
0 mg/ml group vs 0.8 mg/ml group	<0.001	***
Figure 2A		
Dunnett's multiple comparisons test	**P Value**	**Summary**
1 day		
0 mg/ml group vs 0.05 mg/ml group	0.9996	ns
0 mg/ml group vs 0.1 mg/ml group	0.9997	ns
0 mg/ml group vs 0.2 mg/ml group	0.9878	ns
0 mg/ml group vs 0.4 mg/ml group	0.8045	ns
0 mg/ml group vs 0.8 mg/ml group	0.7999	ns
0 mg/ml group vs 0.05 mg/ml group	0.9960	ns
2 day		
0 mg/ml group vs 0.05 mg/ml group	>0.9999	ns
0 mg/ml group vs 0.1 mg/ml group	0.9980	ns
0 mg/ml group vs 0.2 mg/ml group	0.9996	ns
0 mg/ml group vs 0.4 mg/ml group	0.9943	ns
0 mg/ml group vs 0.8 mg/ml group	0.9978	ns
0 mg/ml group vs 0.05 mg/ml group	0.9932	ns
3 day		
0 mg/ml group vs 0.05 mg/ml group	0.4578	ns
0 mg/ml group vs 0.1 mg/ml group	0.9635	ns
0 mg/ml group vs 0.2 mg/ml group	0.8315	ns
0 mg/ml group vs 0.4 mg/ml group	0.8083	ns
0 mg/ml group vs 0.8 mg/ml group	0.2989	ns
0 mg/ml group vs 0.05 mg/ml group	0.0241	*

Figure 2C		
Dunnett’s multiple comparisons test	**P Value**	**Summary**
Necrosis index		
0 mg/ml group vs 0.05 mg/ml group	>0.9999	ns
0 mg/ml group vs 0.1 mg/ml group	0.9796	ns
0 mg/ml group vs 0.2 mg/ml group	0.3830	ns
0 mg/ml group vs 0.4 mg/ml group	0.0319	*
0 mg/ml group vs 0.8 mg/ml group	0.0026	**
Apoptosis index		
0 mg/ml group vs 0.05 mg/ml group	0.9986	ns
0 mg/ml group vs 0.1 mg/ml group	0.9998	ns
0 mg/ml group vs 0.2 mg/ml group	0.9962	ns
0 mg/ml group vs 0.4 mg/ml group	>0.9999	ns
0 mg/ml group vs 0.8 mg/ml group	0.9985	ns
Figure 2E		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs 0.05 mg/ml group	0.9269	ns
0 mg/ml group vs 0.1 mg/ml group	0.4328	ns
0 mg/ml group vs 0.2 mg/ml group	0.0331	*
0 mg/ml group vs 0.4 mg/ml group	<0.001	***
0 mg/ml group vs 0.8 mg/ml group	<0.001	***
Figure 3G		
Dunnett’s multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs 0.05 mg/ml group	0.7149	ns
0 mg/ml group vs 0.1 mg/ml group	0.0265	*
0 mg/ml group vs 0.2 mg/ml group	<0.001	***
0 mg/ml group vs 0.4 mg/ml group	<0.001	***
0 mg/ml group vs 0.8 mg/ml group	<0.001	***
Figure 4B		
Dunnett's multiple comparisons test	**P Value**	**Summary**
PS-MPs 0.8 mg/ml group vs MitoTEMPO (0	0.0019	**

μM) group		
PS-MPs 0.8 mg/ml group vs PS-MPs 0.8 mg/ml group + MitoTEMPO (40 μM) group	0.5755	ns
PS-MPs 0.8 mg/ml group vs PS-MPs 0.8 mg/ml group + MitoTEMPO (80 μM) group	0.2123	ns
PS-MPs 0.8 mg/ml group vs PS-MPs 0.8 mg/ml group + MitoTEMPO (100 μM) group	0.0135	*
Figure 5B		
Dunnett's multiple comparisons test	**P Value**	**Summary**
Control		
0 mg/ml group vs 0.4 mg/ml group	0.9156	ns
0 mg/ml group vs 0.8 mg/ml group	0.7564	ns
$ATG5^{KD}$#1		
0 mg/ml group vs 0.4 mg/ml group	0.0174	*
0 mg/ml group vs 0.8 mg/ml group	0.0047	**
$ATG5^{KD}$#2		
0 mg/ml group vs 0.4 mg/ml group	<0.001	***
0 mg/ml group vs 0.8 mg/ml group	<0.001	***
Figure 5D		
Sidak's multiple comparisons test	**P Value**	**Summary**
Atg5$^{+/+}$ MEF cells vs *Atg5*$^{-/-}$ MEF cells		
0 mg/ml	>0.9999	ns
0.4 mg/ml	0.0321	*
0.8 mg/ml	<0.001	***
Figure 5F		
Sidak's multiple comparisons test	**P Value**	**Summary**
Atg5$^{+/+}$ MEF cells vs *Atg5*$^{-/-}$ MEF cells		
Necrosis index		
0 mg/ml	0.7873	ns
0.4 mg/ml	0.3460	ns
0.8 mg/ml	0.5692	ns

Apoptosis index		
0 mg/ml	0.5657	ns
0.4 mg/ml	0.0426	*
0.8 mg/ml	0.0030	**
Figure 6A		
4 weeks		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 week		
sham group vs 0.2 mg/day group	0.9678	ns
sham group vs 0.4 mg/day group	0.9678	ns
1 week		
sham group vs 0.2 mg/day group	0.9896	ns
sham group vs 0.4 mg/day group	0.9464	ns
2 weeks		
sham group vs 0.2 mg/day group	0.9426	ns
sham group vs 0.4 mg/day group	0.8434	ns
3 weeks		
sham group vs 0.2 mg/day group	0.9939	ns
sham group vs 0.4 mg/day group	0.9964	ns
4 weeks		
sham group vs 0.2 mg/day group	0.9855	ns
sham group vs 0.4 mg/day group	0.8787	ns
Figure 6A		
8 weeks		
Dunnett’s multiple comparisons test	**P Value**	**Summary**
0 week		
sham group vs 0.2 mg/day group	0.9866	ns
sham group vs 0.4 mg/day group	0.9725	ns
1 week		

sham group vs 0.2 mg/day group	0.9079	ns
sham group vs 0.4 mg/day group	0.9974	ns
2 weeks		
sham group vs 0.2 mg/day group	0.9038	ns
sham group vs 0.4 mg/day group	0.9234	ns
3 weeks		
sham group vs 0.2 mg/day group	0.9725	ns
sham group vs 0.4 mg/day group	0.9882	ns
4 weeks		
sham group vs 0.2 mg/day group	0.9197	ns
sham group vs 0.4 mg/day group	0.9791	ns
5 weeks		
sham group vs 0.2 mg/day group	0.9538	ns
sham group vs 0.4 mg/day group	>0.9999	ns
6 weeks		
sham group vs 0.2 mg/day group	0.9791	ns
sham group vs 0.4 mg/day group	0.5481	ns
7 weeks		
sham group vs 0.2 mg/day group	0.8779	ns
sham group vs 0.4 mg/day group	0.7773	ns
8 weeks		
sham group vs 0.2 mg/day group	0.9947	ns
sham group vs 0.4 mg/day group	0.6056	ns
Figure 6B		
Tukey’s multiple comparisons test	**P Value**	**Summary**
BUN		
4 weeks		
sham group vs 0.2 mg/day group	0.4435	ns
sham group vs 0.4 mg/day group	0.1952	ns
0.2 mg/day vs 0.4 mg/day group	0.8475	ns

8 weeks		
sham group vs 0.2 mg/day group	0.9109	ns
sham group vs 0.4 mg/day group	0.2191	ns
0.2 mg/day vs 0.4 mg/day group	0.4057	ns
CREA		
4 weeks		
sham group vs 0.2 mg/day group	0.001	***
sham group vs 0.4 mg/day group	0.0026	**
0.2 mg/day vs 0.4 mg/day group	0.9169	ns
8 weeks		
sham group vs 0.2 mg/day group	0.0068	**
sham group vs 0.4 mg/day group	<0.001	***
0.2 mg/day vs 0.4 mg/day group	0.0026	**
Figure 6D		
Dunnett's multiple comparisons test	**P Value**	**Summary**
4 weeks		
sham group vs 0.2 mg/day group	0.0342	*
sham group vs 0.4 mg/day group	<0.001	***
8 weeks		
sham group vs 0.2 mg/day group	<0.001	***
sham group vs 0.4 mg/day group	<0.001	***
Figure S2 (A) Bad/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 min vs. 5 min	0.0426	*
0 min vs. 10 min	0.0187	*
0 min vs. 20 min	0.0019	**
0 min vs. 30 min	0.001	***
0 min vs. 60 min	0.0177	*
(B) Bcl2/GAPDH ratio		

Dunnett's multiple comparisons test	**P Value**	**Summary**
0 min vs. 5 min	0.2896	ns
0 min vs. 10 min	>0.9999	ns
0 min vs. 20 min	0.0016	**
0 min vs. 30 min	0.014	*
0 min vs. 60 min	0.0262	*
(C) Bax/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 min vs. 5 min	0.9788	ns
0 min vs. 10 min	0.9997	ns
0 min vs. 20 min	0.9289	ns
0 min vs. 30 min	0.8328	ns
0 min vs. 60 min	0.3755	ns
Figure S3 (A) IRE1α/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.6975	ns
0 mg/ml group vs. 0.1 mg/ml group	0.2046	ns
0 mg/ml group vs. 0.2 mg/ml group	0.0503	ns
0 mg/ml group vs. 0.4 mg/ml group	0.0301	*
0 mg/ml group vs. 0.8 mg/ml group	0.0277	*
(B) ATF6/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.8567	ns
0 mg/ml group vs. 0.1 mg/ml group	0.9997	ns
0 mg/ml group vs. 0.2 mg/ml group	0.9755	ns
0 mg/ml group vs. 0.4 mg/ml group	>0.9999	ns
0 mg/ml group vs. 0.8 mg/ml group	0.9755	ns
(C) p-EIF2α/ EIF2α ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.6628	ns
0 mg/ml group vs. 0.1 mg/ml group	0.9622	ns
0 mg/ml group vs. 0.2 mg/ml group	0.6628	ns

0 mg/ml group vs. 0.4 mg/ml group	0.5674	ns
0 mg/ml group vs. 0.8 mg/ml group	0.2066	ns
Figure S4 (A) p-ERK1/2/ERK1/2 ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 min vs. 5 min	0.2169	ns
0 min vs. 10 min	0.0063	**
0 min vs. 20 min	0.0007	***
0 min vs. 30 min	0.1889	ns
0 min vs. 60 min	0.0197	*
(B) p-JNK/JNK ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 min vs. 5 min	0.186	ns
0 min vs. 10 min	0.097	ns
0 min vs. 20 min	0.0186	*
0 min vs. 30 min	0.003	**
0 min vs. 60 min	0.9833	ns
(C) p-p38/ p-p38 ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 min vs. 5 min	0.0976	ns
0 min vs. 10 min	0.0407	*
0 min vs. 20 min	0.0816	ns
0 min vs. 30 min	0.1012	ns
0 min vs. 60 min	0.9217	ns
Figure S5 (A) cPLA2/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.427	ns
0 mg/ml group vs. 0.1 mg/ml group	0.4607	ns
0 mg/ml group vs. 0.2 mg/ml group	0.6585	ns
0 mg/ml group vs. 0.4 mg/ml group	0.8875	ns
0 mg/ml group vs. 0.8 mg/ml group	0.0171	*

(B) COX-1/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.8594	ns
0 mg/ml group vs. 0.1 mg/ml group	0.1201	ns
0 mg/ml group vs. 0.2 mg/ml group	0.3394	ns
0 mg/ml group vs. 0.4 mg/ml group	0.0284	*
0 mg/ml group vs. 0.8 mg/ml group	0.023	*
Figure S6 (A) p-mTOR/mTOR ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.4861	ns
0 mg/ml group vs. 0.1 mg/ml group	0.6201	ns
0 mg/ml group vs. 0.2 mg/ml group	0.0428	*
0 mg/ml group vs. 0.4 mg/ml group	0.0553	ns
0 mg/ml group vs. 0.8 mg/ml group	0.019	*
(B) p-AKT/AKT ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.9618	ns
0 mg/ml group vs. 0.1 mg/ml group	0.3052	ns
0 mg/ml group vs. 0.2 mg/ml group	0.2308	ns
0 mg/ml group vs. 0.4 mg/ml group	0.1477	ns
0 mg/ml group vs. 0.8 mg/ml group	0.0476	*
Figure S7		
(A)p62/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.9946	ns
0 mg/ml group vs. 0.1 mg/ml group	0.9769	ns
0 mg/ml group vs. 0.2 mg/ml group	0.9467	ns
0 mg/ml group vs. 0.4 mg/ml group	0.7221	ns
0 mg/ml group vs. 0.8 mg/ml group	0.0501	ns
(B) Beclin 1/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.3355	ns

0 mg/ml group vs. 0.1 mg/ml group	0.0386	*
0 mg/ml group vs. 0.2 mg/ml group	0.0354	*
0 mg/ml group vs. 0.4 mg/ml group	0.2911	ns
0 mg/ml group vs. 0.8 mg/ml group	0.9985	ns
(C) LC3-II/LC3-I ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.05 mg/ml group	0.9982	ns
0 mg/ml group vs. 0.1 mg/ml group	0.9383	ns
0 mg/ml group vs. 0.2 mg/ml group	0.582	ns
0 mg/ml group vs. 0.4 mg/ml group	0.0077	**
0 mg/ml group vs. 0.8 mg/ml group	<0.001	***
Figure S9 (A) ATG5/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group		
Void vs. $ATG5^{KD}$#1	0.005	**
Void vs. $ATG5^{KD}$ #2	0.0013	**
0.4 mg/ml group		
Void vs. $ATG5^{KD}$ #1	<0.001	***
Void vs. $ATG5^{KD}$ #2	<0.001	***
0.8 mg/ml group		
Void vs. $ATG5^{KD}$ #1	<0.001	***
Void vs. $ATG5^{KD}$ #2	<0.001	***
(B) LC3-II/LC3-I ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group		
Void vs. $ATG5^{KD}$#1	0.9659	ns
Void vs. $ATG5^{KD}$ #2	0.8892	ns
0.4 mg/ml group		
Void vs. $ATG5^{KD}$#1	0.2524	ns

Void vs. ATG5^{KD} #2	0.1786	ns
0.8 mg/ml group		
Void vs. ATG5^{KD}#1	0.0076	**
Void vs. ATG5^{KD} #2	0.0233	*
(C) COX-1/GAPDH ratio		
Dunnett's multiple comparisons test	**P Value**	**Summary**
0 mg/ml group vs. 0.4 mg/ml group	0.1785	ns
0 mg/ml group vs. 0.8 mg/ml group	0.0295	*

Table S5. The quantification of IHC or staining analysis.

IRE1α	**sham group**		**0.2 mg/day group**		**0.4 mg/day group**	
% Area	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
4W	12.84	5.35	14.85	4.79	26.80*	5.98
8W	12.98	4.86	18.18	3.48	26.19	3.10
COX-1	**sham group**		**0.2 mg/day group**		**0.4 mg/day group**	
% Area	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
4W	8.25	6.29	11.63	5.75	19.14	4.39
8W	9.99	6.82	15.68	3.04	25.54*	1.67
LC3	**sham group**		**0.2 mg/day group**		**0.4 mg/day group**	
% Area	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
4W	11.27	4.34	13.73	7.82	15.94	1.34
8W	7.63	4.78	13.73	5.23	21.45*	1.13
MTS	**sham group**		**0.2 mg/day group**		**0.4 mg/day group**	
% Area	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
8W	91.00	0.16	80.15	10.12	66.53*	11.56
Dystrophin	**sham group**		**0.2 mg/day group**		**0.4 mg/day group**	
% Area	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
8W	1.14	0.39	0.14*	0.03	0.19*	0.15

Data are presented as the mean ± SD. N=2, 10 fields of view per kidney. *P < 0.05, compared with sham group as determined by two-way ANOVA with Dunnett's multiple comparison test.

Supplementary figures

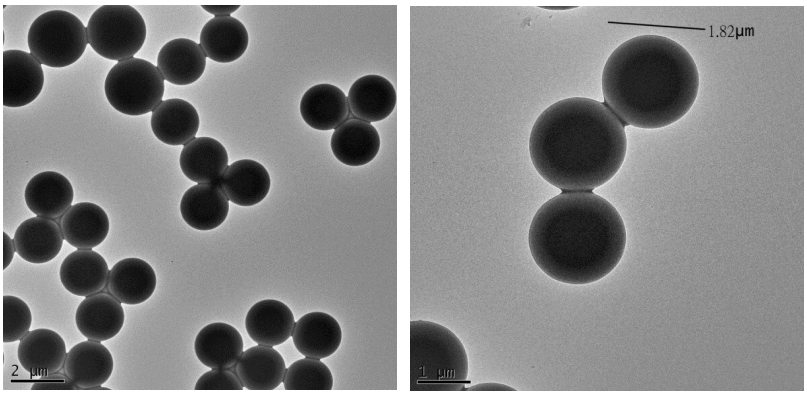

Figure S1. PS-MPs characterization. The diameter of PS-MPs was detected with TEM.

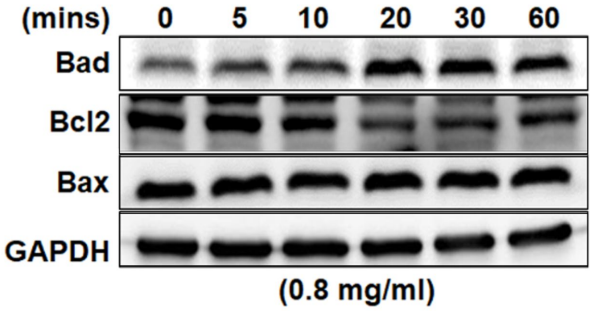

Figure S2. Representative Western blots evaluating Bax, Bad, and Bcl2 in HK-2 cells treated with PS-MPs Bad, Bcl2 and Bax were assessed after PS-MPs treatment at a concentration of 0.8 mg/ml for 0, 5, 10, 20, 30, 60 min. The mean and SD summary data for quantification of Western blots are shown in Table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

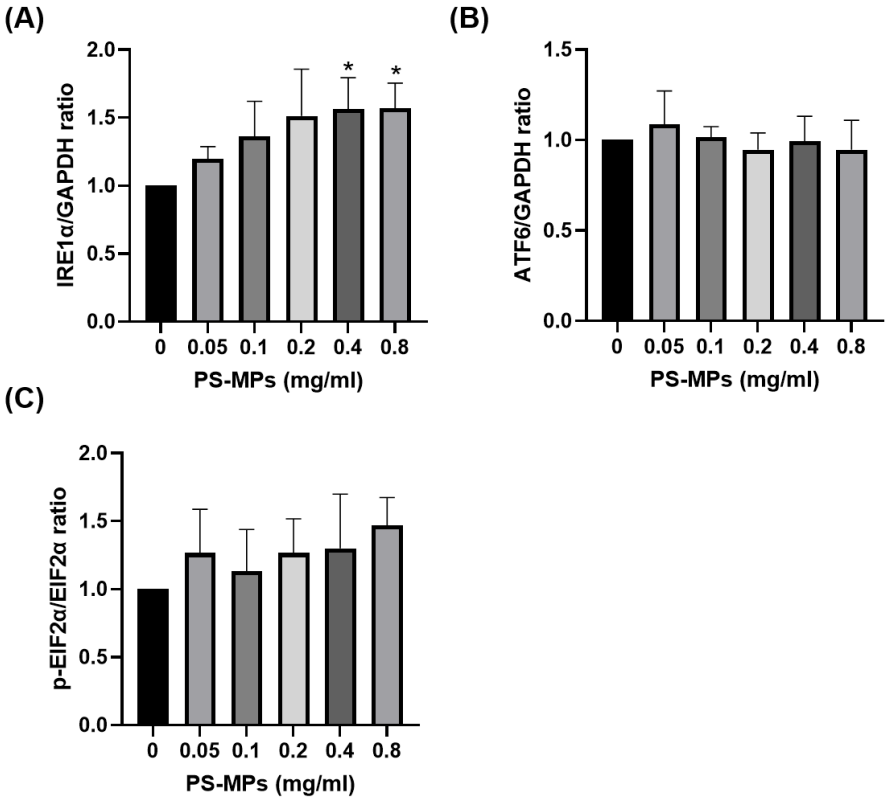

Figure S3. Quantification of Western blots evaluating IRE1α, ATF6, and p-EIF2α in HK-2 cells treated with PS-MPs. ER stress-related proteins IRE1α, ATF6, p-EIF2α, and EIF2α, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) IRE1α/GAPDH ratio. (B) ATF6/GAPDH ratio. (C) p-EIF2α/ EIF2α ratio. N=3. Data are presented as the mean ± SD. *P < 0.05 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test, IRE1α/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.

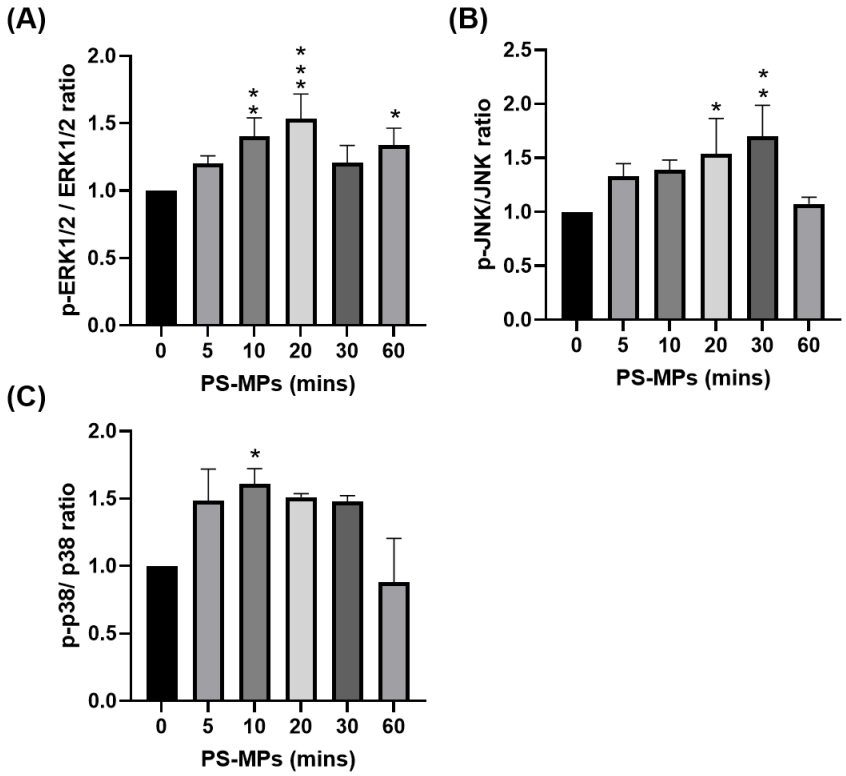

Figure S4. Quantification of Western blots evaluating the phosphorylation of MAPK signaling pathway components ERK1/2, JNK, and p38 in HK-2 cells treated with PS-MPs. MAPK signaling pathway components, such as p-ERK1/2, ERK1/2, p-JNK, JNK, p-p38 and, p38, were assessed after PS-MPs treatment at a concentration 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 ± 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.

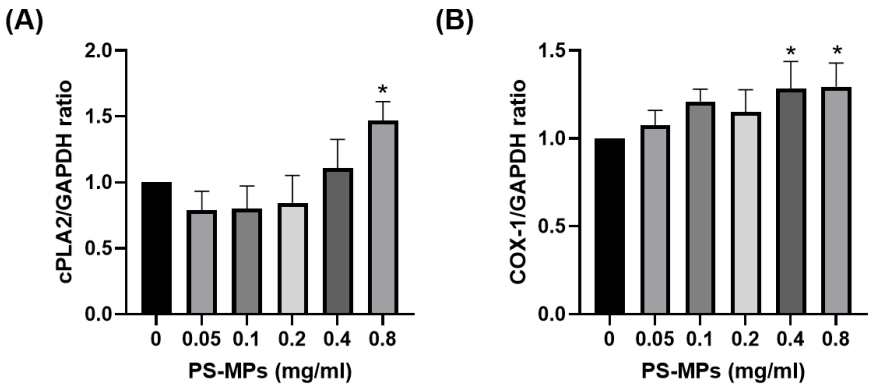

Figure S5. Quantification of Western blots evaluating cPLA2 and COX-1 in HK-2 cells treated with PS-MPs. Inflammation-related proteins cPLA2 and COX-1, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) cPLA2/GAPDH ratio. (B) COX-1/GAPDH ratio. N=3. Data are presented as the mean ± 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.

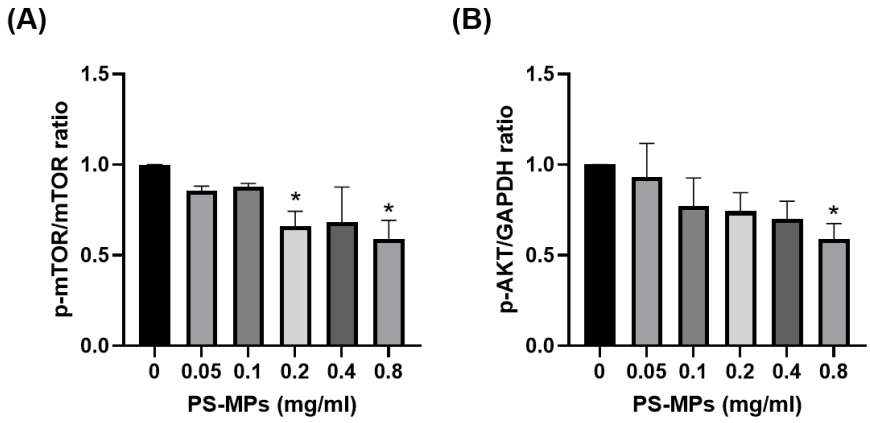

Figure S6. Quantification of Western blots evaluating the phosphorylation of mTOR and Akt in HK-2 cells treated with PS-MPs. AKT/mTOR signaling pathway components, such as p-mTOR, mTOR, p-AKT, and AKT, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 1 h. 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 ± 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.

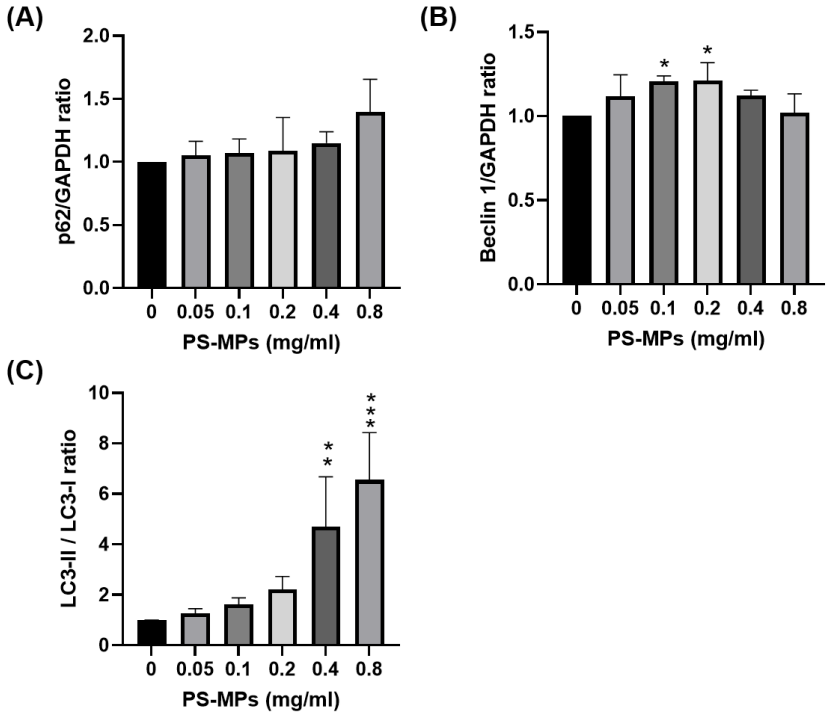

Figure S7. Quantification of Western blots evaluating the expression of p62, Beclin 1, and LC3 in HK-2 cells treated with PS-MPs. Autophagy-related proteins p62, Beclin 1, and LC3, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting 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 ± 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.

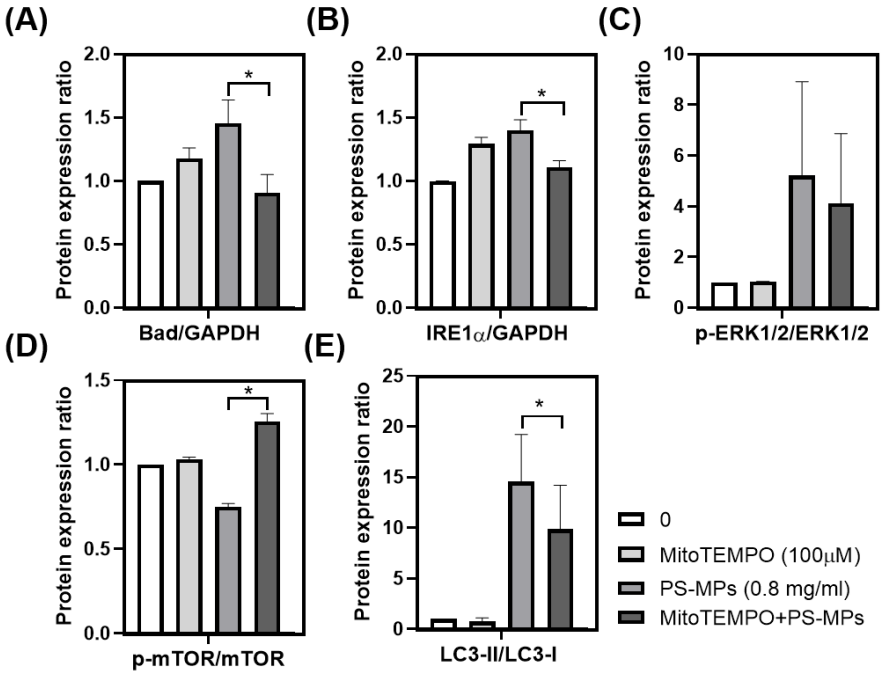

Figure S8. Quantification of Western blots evaluating the expression Bad, IRE1α, p-ERK1/2, p-mTOR, and LC3-II/LC3-I ratio in HK-2 cells treated with PS-MPs alone, MitoTEMPO alone or in combination. (A) Cells were pretreated for 1 h with MitoTEMPO (100 μM) then exposed to PS-MPs (0.8 mg/ml) for 20 min. 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 IRE1α 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 ± SD. *P < 0.05 compared with control group as determined by t test. PS-MPs 0.8 mg/ml group vs MitoTEMPO (0 μM) 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.

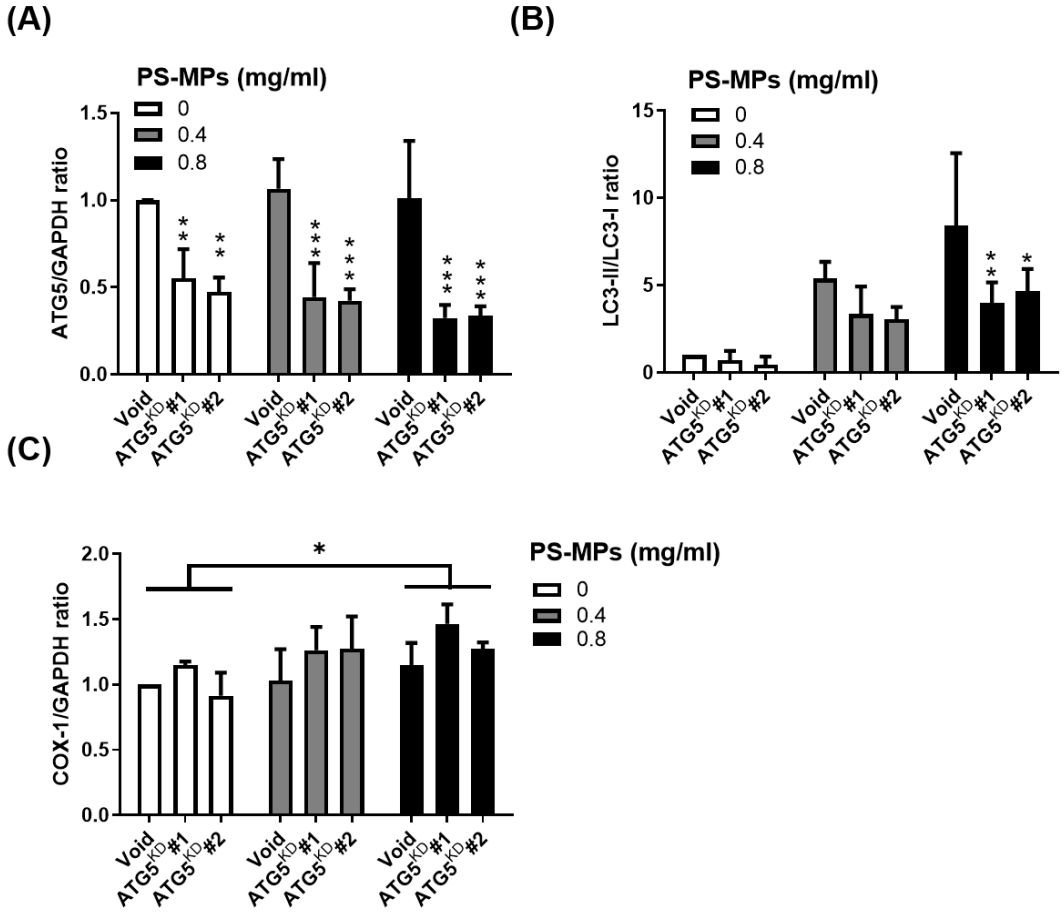

Figure S9. Quantification of western blot analysis of ATG5 knockdown cells treated with PS-MPs for expression of ATG5, LC3 and COX-1. Inflammation-related proteins were evaluated after PS-MPs treatment at concentrations of 0.4 and 0.8 mg/ml for 48 h in $ATG5^{KD}$ HK-2cells. The Western blotting results 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 ± 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.

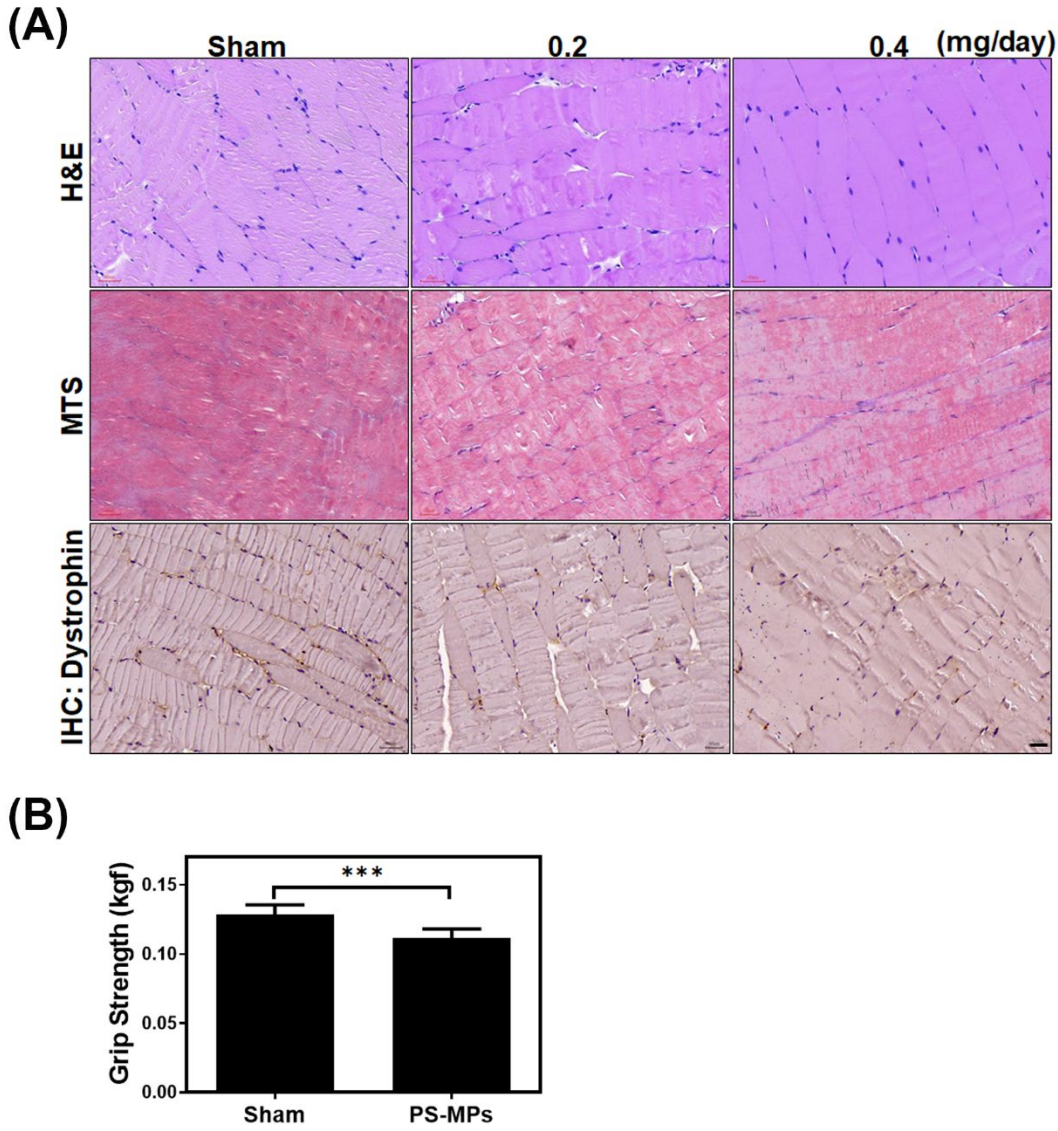

Figure S10. The effects of PS-MPs on mouse muscle and grip strength. Six-week-old C57BL/6 male mice without and with 0.2 mg/day and 0.4 mg/day PS-MPs 2 times per week were examined, and the leg muscles of mice were harvested at 8 weeks. (A) Hematoxylin and eosin (H&E) staining, Masson's trichrome staining (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 ± 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 μm.

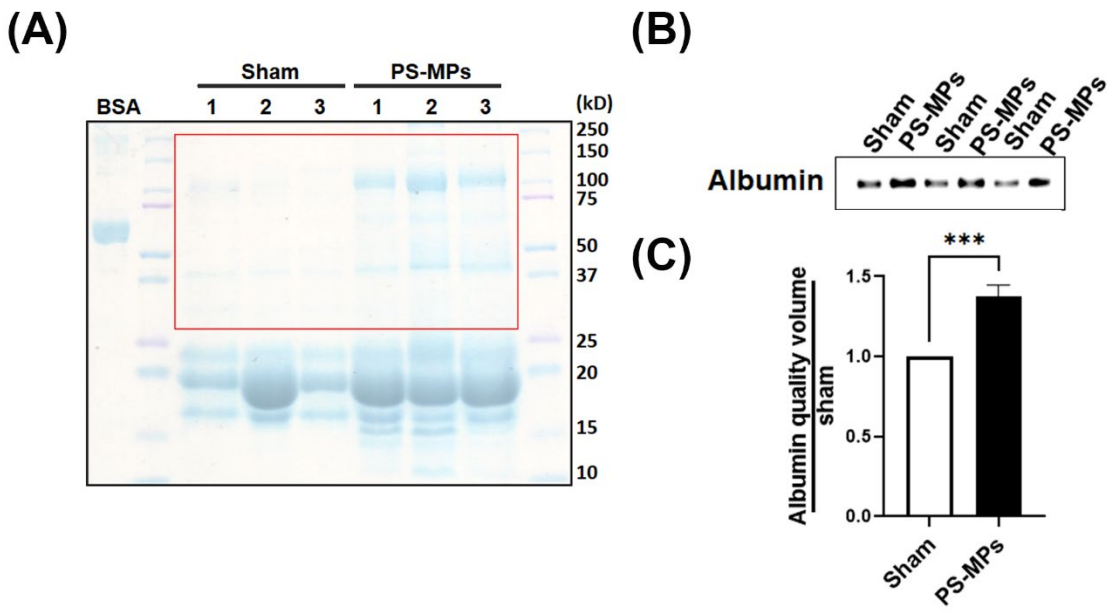

Figure S11. Protein expression in mouse urine after treatment with PS-MPs. (A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of urine from the mice was collected after oral gavage of 0.4 mg/day PS-MPs for 4 weeks. The red frame shows the difference between the groups treated with 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 ± 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.