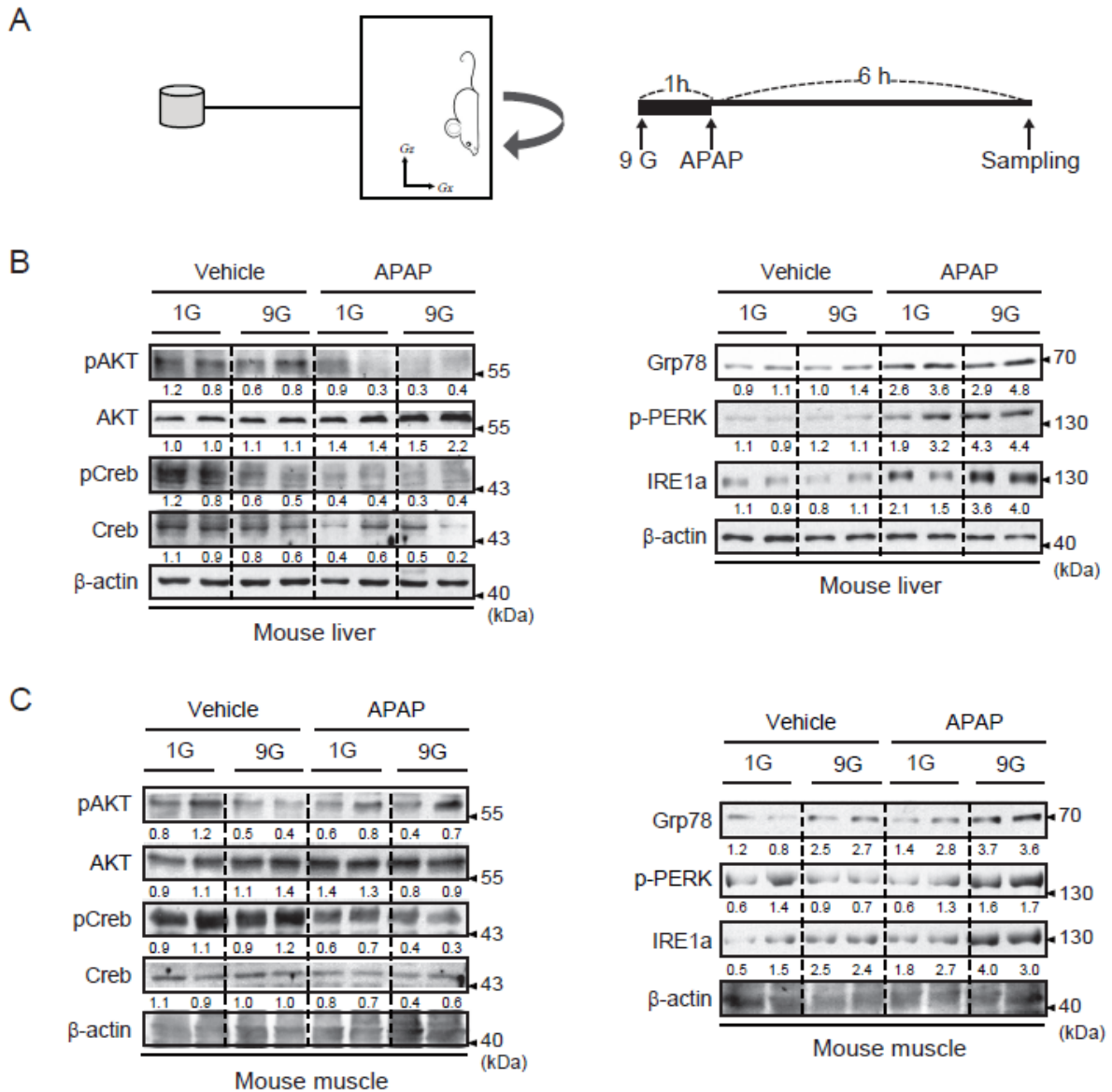


## **Supporting Information**

Hypergravity load modulates acetaminophen nephrotoxicity via endoplasmic  
reticulum stress in association with hepatic microRNA-122 expression

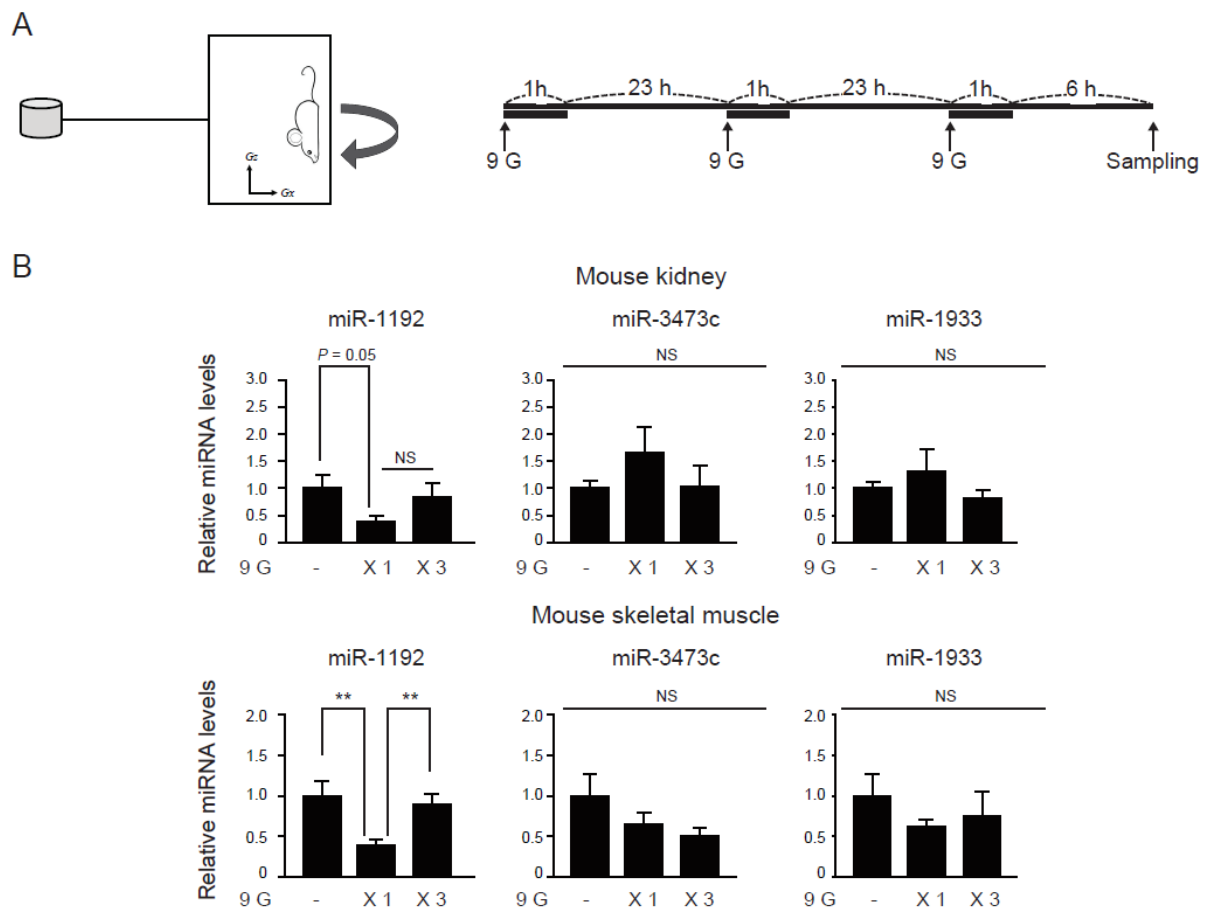
## Supporting Results



**Supplementary Figure 1. Effects of combined treatment with hypergravity and APAP on cell viability and ER stress markers**

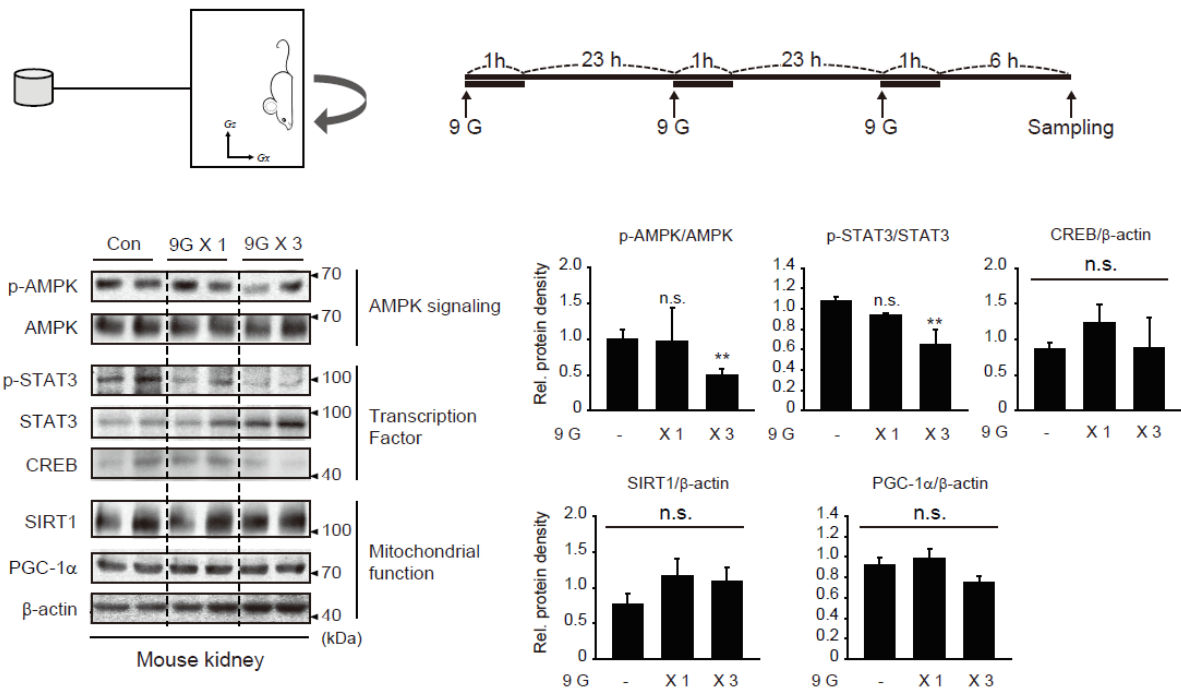
(A) Schematic of hypergravity and APAP treatments. (B, C) Immunoblots for the pAKT, pCreb, and ER stress markers in the liver and tibialis anterior muscle homogenates. Male C57BL/6 mice were exposed to a single +9 Gx load for 1 h, followed by APAP treatment (500 mg/kg BW, i.p.), then

sacrificed 6 h afterward. Immunoblots were assessed from two samples per condition (randomly selected from 6 samples). The values represent fold changes relative to the control ( $\beta$ -actin).



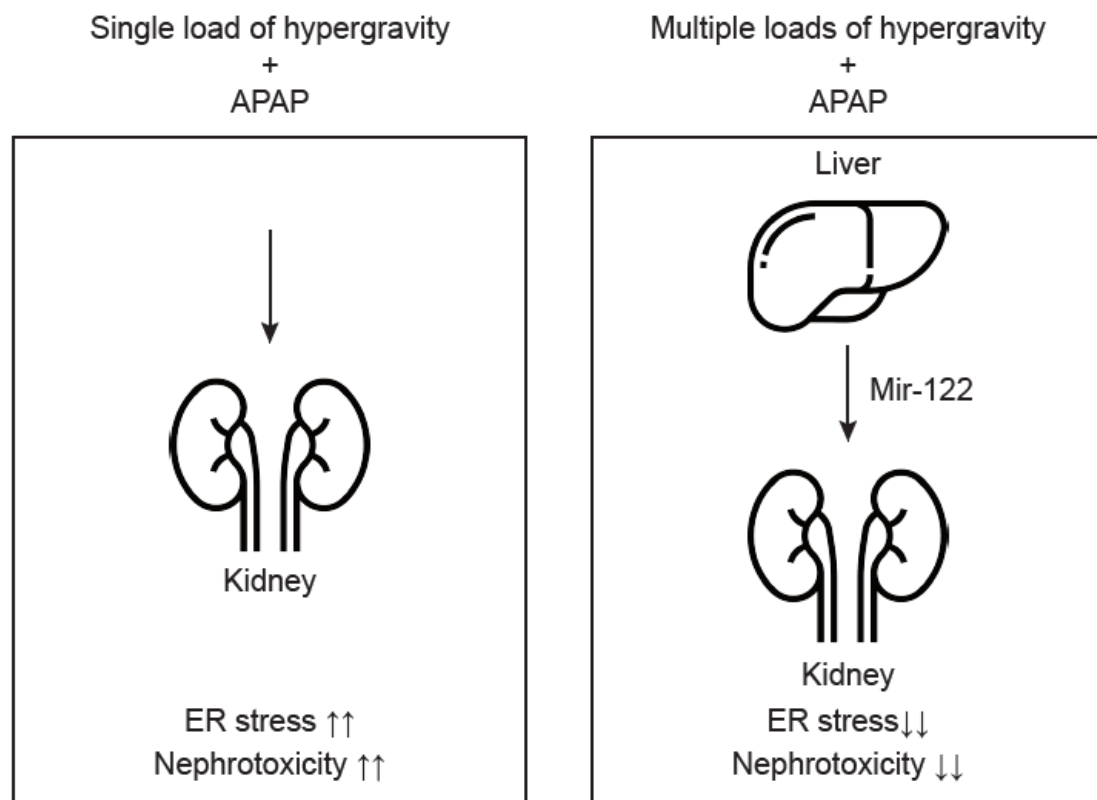
**Supplementary Figure 2. Representative miRNA levels in kidney and muscle of mice exposed to a single or multiple hypergravity loads.**

(A) Schematic of the single or multiple hypergravity load experiments. (B) qRT-PCR assays for miR-1192, miR-3473c, and miR-1933 in the renal cortex and tibialis anterior muscle of mice subjected to a single or multiple +9 Gx hypergravity loads for 1 h per day ( $n=6$ ). The data represent the mean  $\pm$  SEM (statistical differences were determined via the one-way ANOVA; \* $P<0.05$ , \*\* $P<0.01$ , n.s., not significant).



**Supplementary Figure 3. Immunoblottings for p-AMPK, p-STAT3, CREB, Sirt1 and PGC-1α in kidney of mice exposed to a single or multiple hypergravity loads.**

The samples were obtained using the same mice as in Supplementary Figure 2. Immunoblots were assessed from two samples per condition (randomly selected from 6 samples). The values represent fold changes relative to the control (β-actin).



**Supplementary Figure 4. Schematic showing the mechanistic basis of kidney protection by multiple hypergravity loads against APAP treatment.**

**Supporting Table 1. Information on antibodies or other reagents used**

Item	Supplier	Catalog no.
pCREB	Cell Signaling	9198
CREB	Cusabio	CSB-PA005947HA01HU
pAKT	Cell Signaling	9275
AKT	Cell Signaling	4685S
Grp78	Abcam	ab21685
p-PERK	Santa Cruz	sc-32577
IRE1a	Santa Cruz	sc-20790
p-AMPK	Cell Signaling	2535S
AMPK	Cell Signaling	2532
$\beta$ -actin	Sigma	A5441

**Supporting Table 2. The sequences of primers**

	Gene symbols	Forward primers	Reverse primers
Mouse mRNAs	<i>Kim-1</i>	TTCTCCCAGGCACTGTGGAT	CAGGAATCTCCACTCGACAA
	<i>Bax</i>	AGACAGGGGCCTTTTGCTAC	AATTCGCCGGAGACACTCG
	<i>Bcl2</i>	GAGAGCGTCAACAGGGAGATG	CCAGCCTCCGTTATCCTGGA
	<i>Actin</i>	CTGAGAGGGAAATCGTGC	TGTTGGCATAGAGGTCTT
microRNAs	miR-122	TGGAGTGTGACAATGGTGTTTG	Universal reverse primer (Qiagen proprietary information)
	miR-1192	AAACAAACAAACAGACCAAATT	
	miR-3473c	TCTCTCCAGCCCCATAATAAG	
	miR-1933	AGTCATGGTGTTCGGTCTTAGTTT	