

Supplementary Data

Melatonin improves mitochondrial function by promoting MT1/SIRT1/PGC-1 alpha-dependent mitochondrial biogenesis in cadmium-induced hepatotoxicity in vitro

Authors:

Pan Guo^{1*}, Huifeng Pi^{1*}, Shangcheng Xu^{1*}, Lei Zhang¹, Yuming Li², Min Li¹, Zhengwang Cao¹, Li Tian¹, Jia Xie¹, Renyan Li¹, Mindi He¹, Yonghui Lu¹, Chuan Liu¹, Weixia Duan¹, Zhengping Yu^{1†}, Zhou Zhou^{1†}

Authors' Affiliations:

¹Department of occupational health, Third Military Medical University, Chongqing 400038, People's Republic of China

²Institute of Hepatobiliary Surgery, XinQiao Hospital, Third Military Medical University, Chongqing 400037, People's Republic of China

Correspondence to: Zhengping Yu, Ph.D., Zhou Zhou, Ph.D., Department of Occupational Health, Third Military Medical University, Chongqing 400038, China, Tel: +86-23-68752290, Fax: +86-23-68752290, Email: yuzping_tmmu@126.com (ZP. Yu); 510786341@qq.com (Z. Zhou)

*contributed equally to this study

†corresponding author

Table S1**The treatment groups in our study**

	Step 1	Step 2	Step 3		Step 4
			i	ii	
	Control	Control	Control	Control-siRNA+ Cd	Control
	Cd (2.5 μ M)	Melatonin	Melatonin+ Cd	Control-siRNA+	Cd
The group in each step	Cd (5 μM)	Cd	Sirtinol +melatonin + Cd	SIRT1-siRNA+ melatonin + Cd	Melatonin+ Cd
	Cd (10 μ M)	Melatonin+ Cd	Sirtinol +Cd	SIRT1-siRNA+ Cd	luzindole(10 μ M) + Cd

Note: In step 2, 3 and 4, the doses of Cd, melatonin and sirtinol were 5 μ M, 0.5 μ M and 30 μ M, respectively.

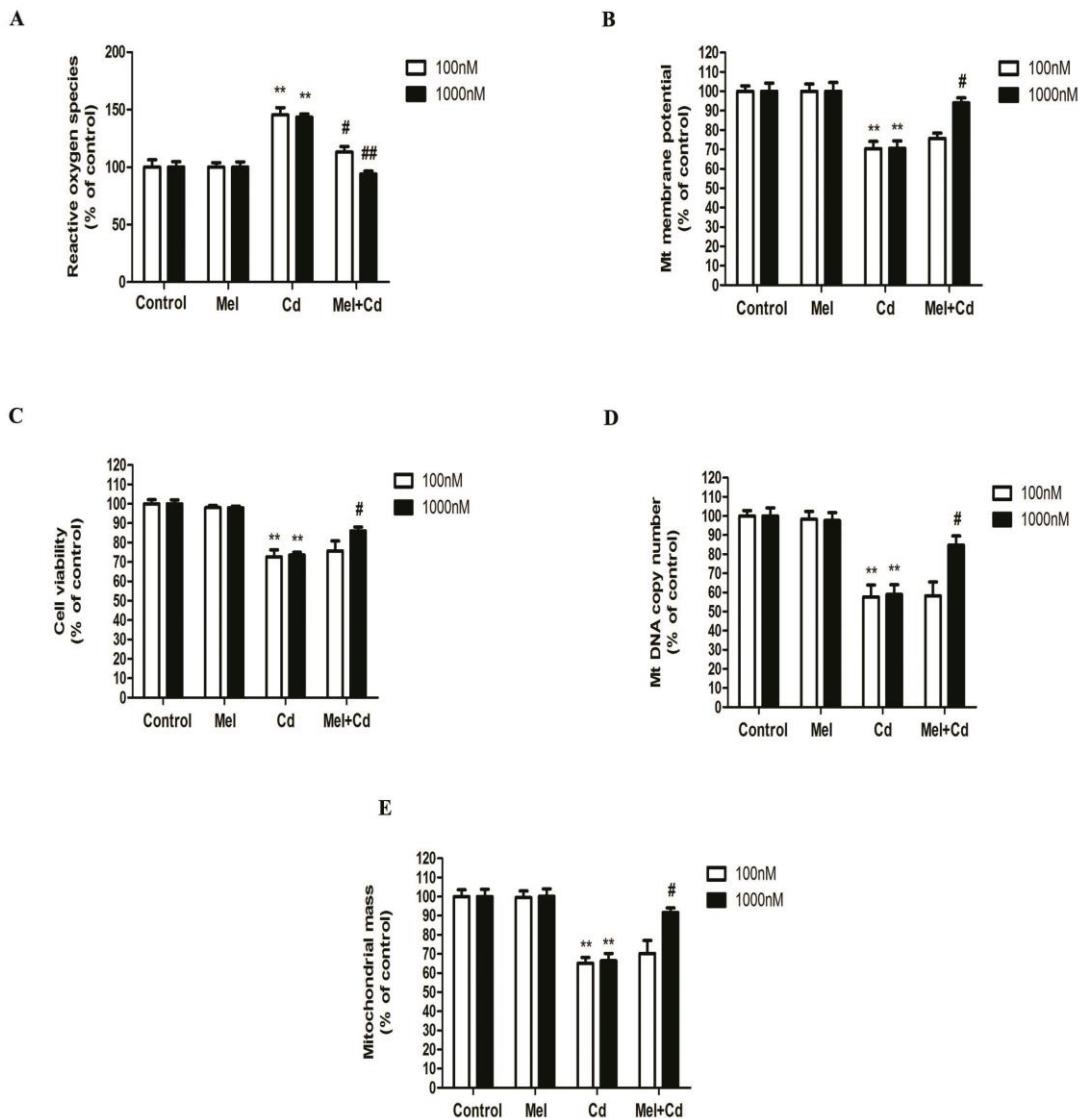


Fig. S1. Melatonin protects against mitochondrial dysfunction and mitochondrial biogenesis

disruption after exposure to Cd *in vitro*. HepG2 cells were pretreated with 0.1 μ M or 1 μ M melatonin for 2 h prior to 5 μ M Cd treatment. (A) ROS production, (B) $\Delta\Psi_m$ level, (C) cell viability, (D) mtDNA copy number, (E) mitochondrial mass. The results are expressed as a percentage of the control, which was set at 100%. The values are presented as the mean \pm SEM,

** $p < 0.01$ versus control group, # $p < 0.05$, ## $p < 0.01$ versus the Cd (5 μ M) group (n=6).

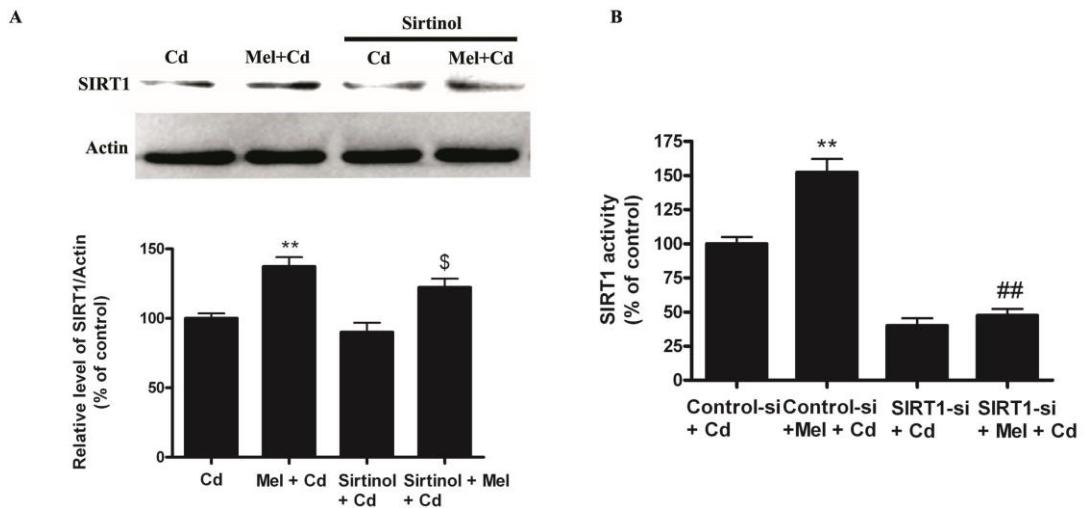


Fig. S2. (A) The effects of melatonin and sirtinol pretreatment on SIRT1 expression, ** $p < 0.01$ versus the Cd (5 μ M) group, and $^{\$}p < 0.05$ versus the sirtionl + Cd (5 μ M) group (n=6). (B) The effects of melatonin and SIRT1 siRNA pretreatment on SIRT1 activity. ** $p < 0.01$ versus the Control siRNA + Cd (5 μ M) group, $^{##}p < 0.01$ versus the Control siRNA + Cd (5 μ M) + melatonin group (n=6). The results are expressed as a percentage of the control (“Cd” group taken as a control), which is set at 100%. The values are presented as the mean \pm SEM.