The proteasome inhibitor, MG132, enhances LDL uptake in HepG2 cells through regulating LDLR and PCSK9 expression: novel roles in cholesterol homeostasis

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SUPPLEMENT FIGURE

Figure S1

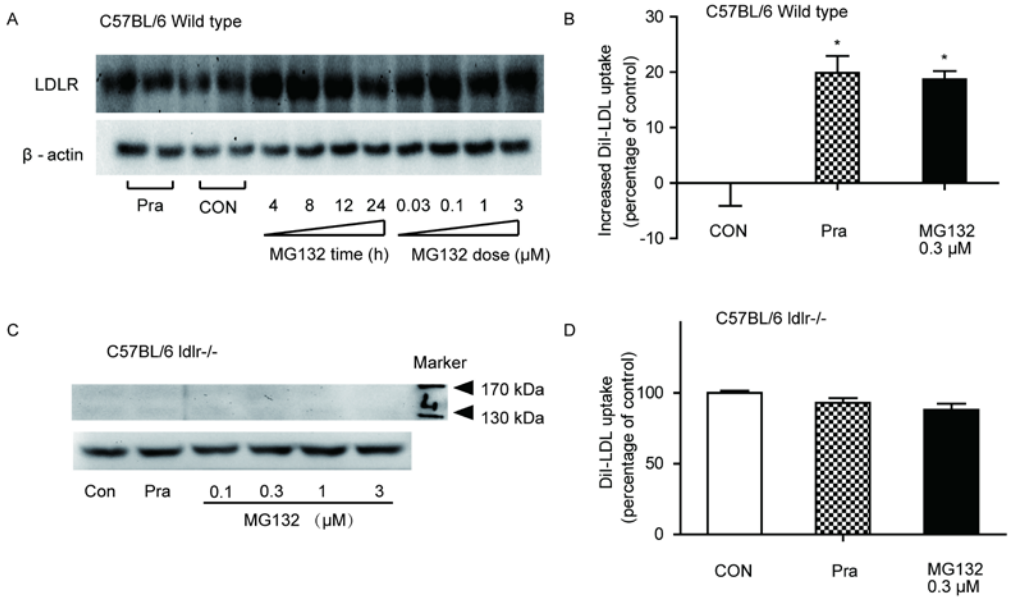

Figure S1. Effects of MG132 on LDLR expression and function in primary mouse hepatocytes derived from wild-type or ldlr$^{-/-}$ C57BL/6 mice. (A, C) Western blot analysis of LDLR expression and (B, D) DiI-LDL uptake in cells treated with MG132 for indicated times and dosages (**Pra-** pravastatin 5 μM, positive control). Data are representative of three independent experiments with similar results, and presented as means ± SEM. **P*<0.05 vs. vehicle-treated groups.

Figure S2

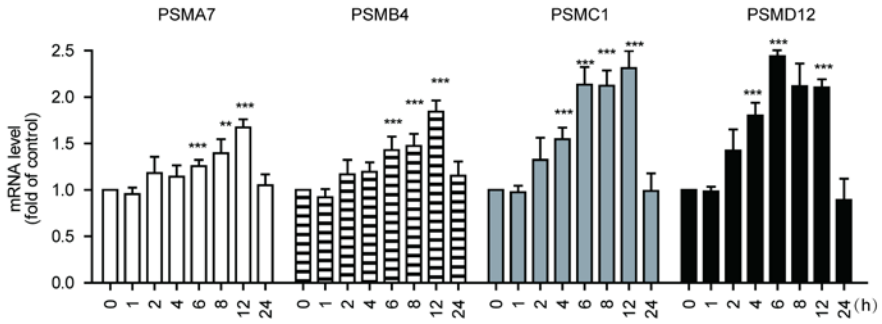

Figure S2. Time-curve of mRNA levels of specific proteasome subunits in HepG2 cells treated with MG132 (0.3 μM). Primer sequences are listed in Table S1 (**PSMA7**- Proteasome subunit alpha type-7, **PSMB4**- Proteasome subunit beta type-4, **PSMC1**- 26S protease regulatory subunit 4, **PSMD12**- 26S proteasome non-ATPase regulatory subunit 12). Data are presented as means ± SEM of three or more independent experiments. ***P*<0.01, *** *P*<0.001 vs. vehicle-treated groups.

Table S1. Sequences of primers used to detect mRNA expression of indicated proteasome subunits

gene name	accession No.	Primer (5'-3')	product length (bp)
PSMA7	NM_002792.3	CTGTGCTTTGGATGACAACG	149
		CGATGTAGCGGGTGATGTACT	
PSMB4	NM_002796.2	CTCGTTTCCGCAACATCTCT	142
		TGTCCATCTCCCAGAAGCTC	
PSMC1	XM_005267875.1	TTCCGAGTTGCTGAAGAACA	145
		ATCCATCCAACTGGTTCAGC	
PSMD12	NM_174871.2	GTGCGCGACTGACTAAAACA	158
		TAGGCAGAGCCTCATTTGCT	

Figure S3

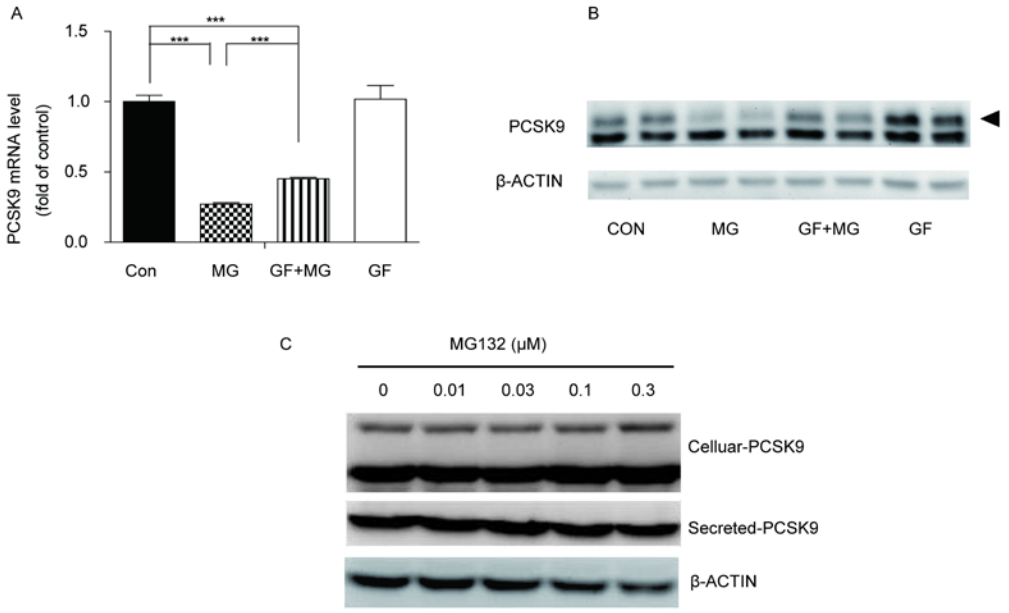

Figure S3. (A) Real-time quantification and (B) Western blot analysis of PCSK9 expression in HepG2 cells incubated with MG132 (0.3 μM), GF 109203X (5 μM), or both for 24 h (black triangle indicates the PCSK9 band). (C) HEK293T cells stably expressing PCSK9-Flag were incubated with increasing concentrations of MG132 (0 to 0.3 μM) in DMEM supplemented with 1% FBS for 24 h. PCSK9 protein levels were determined using Western blot. Data are representative of three independent experiments with similar results, and presented as means ± SEM. ****P*<0.001 vs. the vehicle treated groups.

Figure S4

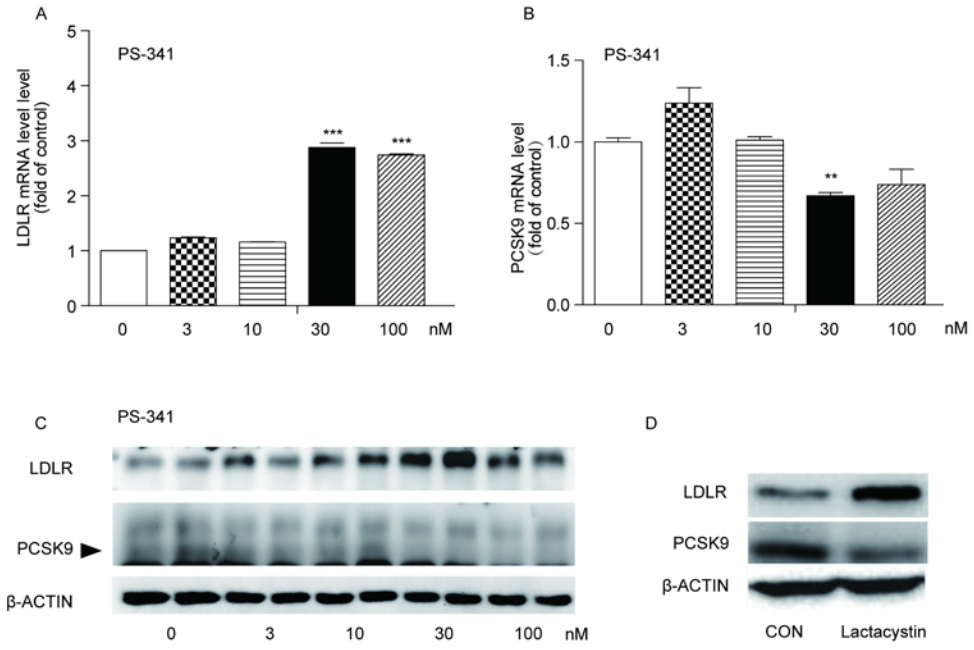

Figure S4. Two other proteasome inhibitors upregulate LDLR expression while downregulate PCSK9 expression. (A, B) Real-time PCR quantification of LDLR and PCSK9 mRNA level in HepG2 cells incubated with increasing concentrations of PS-341 for 6 h and 24 h, respectively. (C) Western blot analysis of LDLR an PCSK9 protein levels in HepG2 cells treated with increasing PS-341(black triangle indicates the PCSK9 band) or 20 nM Lactacystin (D) for 24 h. Data are representative of three independent experiments with similar results. ***P*<0.01, ****P*<0.001 vs. the vehicle treated groups.

Supplementary Methods

Primary Mouse Hepatocyte

All procedures performed on animals were approved by the Animal Care and Use committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8^{th} edition, ISBN-13: 978-0-309-15400-0). C57BL/6 mice (20-40 g, male) were anesthetized by pentobarbital (i.p., 200 mg/kg), and primary mouse hepatocytes were isolated by liver perfusion followed by enzymatic digestion and purification on a PBS-Percoll gradient as described with minor modifications.(Li et al., 2010) Hepatocytes were plated at the density of 3×10^5 cells per well in a 6-well plate or 1.5×10^5 cells per well in a 12-well plate in DMEM/F12 (Hyclone) supplemented with 10% fetal bovine serum and antibiotics. Cells were allowed to attach for 2 hours before switching to low glucose-DMEM (Hyclone) with 2% LPDS and adding all treatments.

Washing medium: HBSS (KCl 0.4, KH_2PO_4 0.06, $NaHCO_3$ 0.35, NaCl 8.0, $Na_2HPO_4 \cdot 12H_2O$ 0.121, D-glucose 1, g/L) with 0.5 mM EGTA and 25 mM HEPES

Digestion medium: low glucose-DMEM with 100 CDU/ml collagenase (Worthington, U.S.A)

REFERENCES

Li WC, Ralphs KL and Tosh D (2010) Isolation and culture of adult mouse hepatocytes. Methods in molecular biology (Clifton, NJ) 633:185-196.