Supplementary Figures, Legends and Table

Melanocortin-4 receptor antagonist TCMCB07 ameliorates cancer- and chronic kidney disease-associated cachexia

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Table S1. Summary of TCMC compound evaluation

TCMC compounds	Rat models	Number of rats	TCMC treatment routes	TCMC treatment doses	Test results	Notes
B01	Tumor	24	IP	4X[A], 28 & 280 μg/rat/d	Negative	A (for all)
B02, SHU9119	LPS[B]	8	ICV	1X, 1.5 μg/rat/d	Positive	B
B02	LPS[B]	20	IP	1X, 5 mg/kg/d	Negative	B
B02	LPS[B]	20	Gavage	1X, 5 mg/kg/d	Negative	B
B03, PG932	LPS[C]	16	IP	1X, 4 mg/kg/d	Negative	C
B04	LPS[B]	5	ICV	1X, 2 μg/rat/d	Positive	B
B04	LPS[B]	20	IP	1X, 4.5 mg/kg/d	Negative	B
B05, 06, 07	LPS[C]	8	ICV	1X, 2 μg/rat/d	Positive	C
B05, 06, 07	LPS[C]	24	IP	1X, 3 mg/kg/d	Negative	C
B05, 07	LPS[C]	28	Gavage	1X, 10 mg/kg/d	B05-Negative B07-Positive	C
B07, 08, 7A	LPS[C]	8	ICV	1X, 2 μg/rat/d	Positive	C
B07, 08, 7A	LPS[C]	28	Gavage	1X, 10 mg/kg/d	B07-Positive B08, B7A-Negative	C
B07	LPS[D]	18	IP	1X, 0.6 mg/kg/d	Negative	D
B07, 7A	LPS[C]	18	IP	1X, 3 mg/kg/d	Positive	C
B07, 7A	LPS[D]	30	Gavage	1X, 12 mg/kg/d	Negative	D
B07	Tumor	20	ICV	4X, 2 & 20 μg/rat/d[E]	Positive	E
B07	Tumor	24	IP	6X, 3 mg/kg/d	Positive	/
B07	Tumor	33	SC	6X, 1.5 & 3 mg/kg/d	Positive	/
B07	Nephrectomy	28	SC	14X, 3 mg/kg/d	Positive	/
B08, 09, 10	LPS[C]	24	IP	1X, 3 mg/kg	Negative	C

A: X: Total doses of TCMC compounds. B: LPS dose: 250 μg/kg/d. C: LPS dose: 100 μg/kg/d. D: LPS dose: 150 μg/kg/d. E: Some animals died after ICV injection with high dose of 20 μg/rat/d.

Table S1

Summary of a series of TCMC compound evaluation. A total of eleven compounds (TCMCB01-B10 plus TCMCB07A) were evaluated throughout entire study with more than 20 independent experiments using three rat models of LPS and cachexia associated with cancer and CKD, respectively. The routes of administration of compounds included intracerebroventricular (ICV) injection, intraperitoneal (IP) injection, subcutaneous (SC) injection and oral gavage (intragastric gavage). The LPS dose range was 100-250 μg/kg/day, and the compound dose range was 2-20 μg/rat/day (for ICV injection) and 0.6-12 mg/kg/day (for IP, SC injection and oral gavage). Among eleven TCMC compounds, TCMCB07 was tested with over 12 independent experiments. Both central and peripheral treatment of TCMCB07 showed a high efficacy in attenuation of LPS-induced anorexia and cancer- and CKD- associated cachexia.

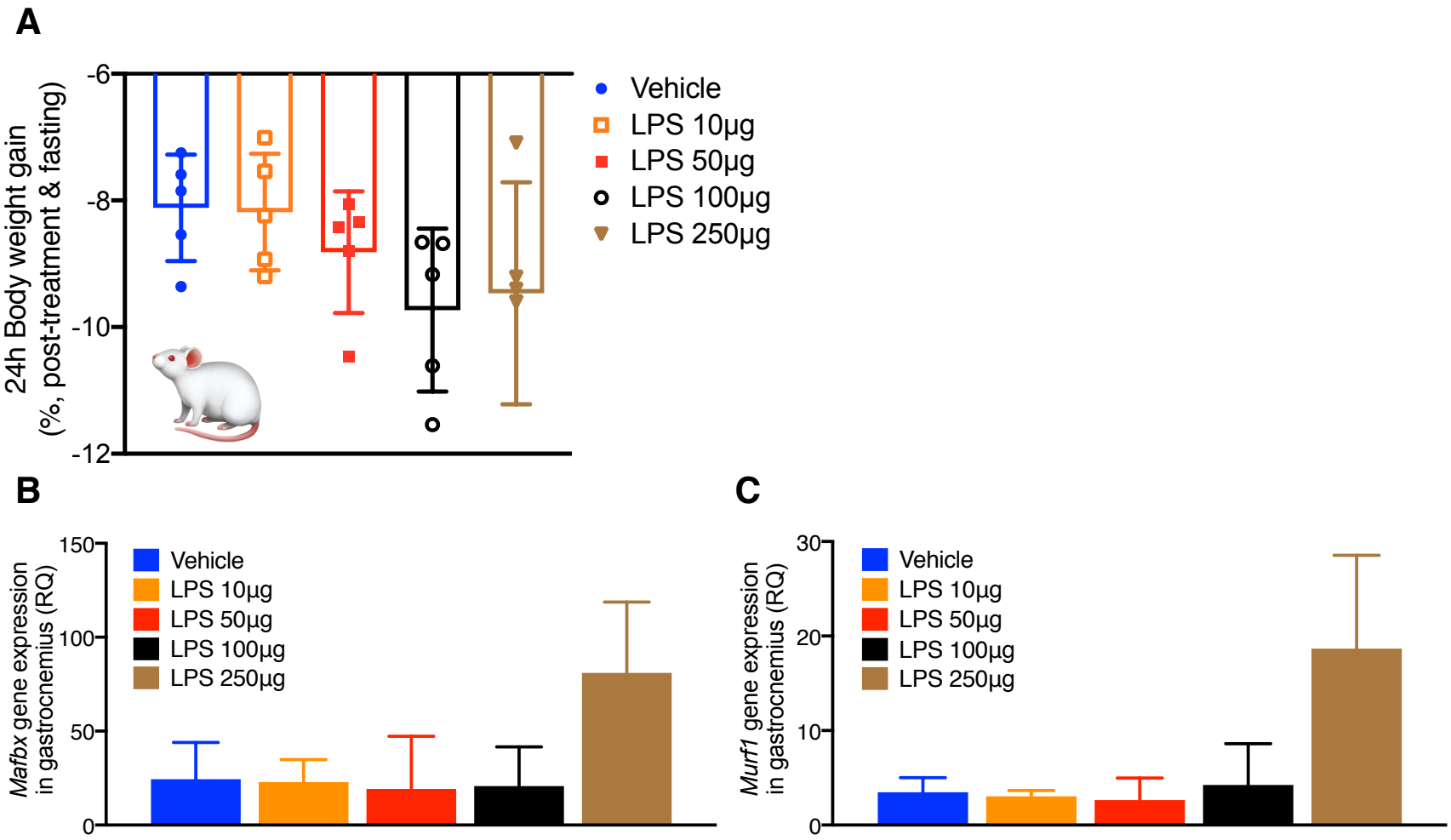

Figure S1 (related to Figure 1)

LPS dose-response study in SD rats for LPS dose selection. Rats were fasting after receiving IP injection of vehicle or LPS with 4 different doses of 10, 50, 100, 250 *μ*g/kg/day. (**A**) 24h body weight gain (%, net gain normalized to baseline) post-IP injection of vehicle (n = 5) vs. LPS (each dose n = 5). qRT-PCR analysis for *Mafbx* (**B**) and *Murf1* (**C**) gene expression in the rat gastrocnemius after IP injection of vehicle or LPS. Saline group (n = 5) vs. LPS groups (each dose n = 4-5)**. D**ata in (**A**) were expressed with each dot representing one sample, and data in (**B** and **C**) are expressed as mean ± SEM for each group.

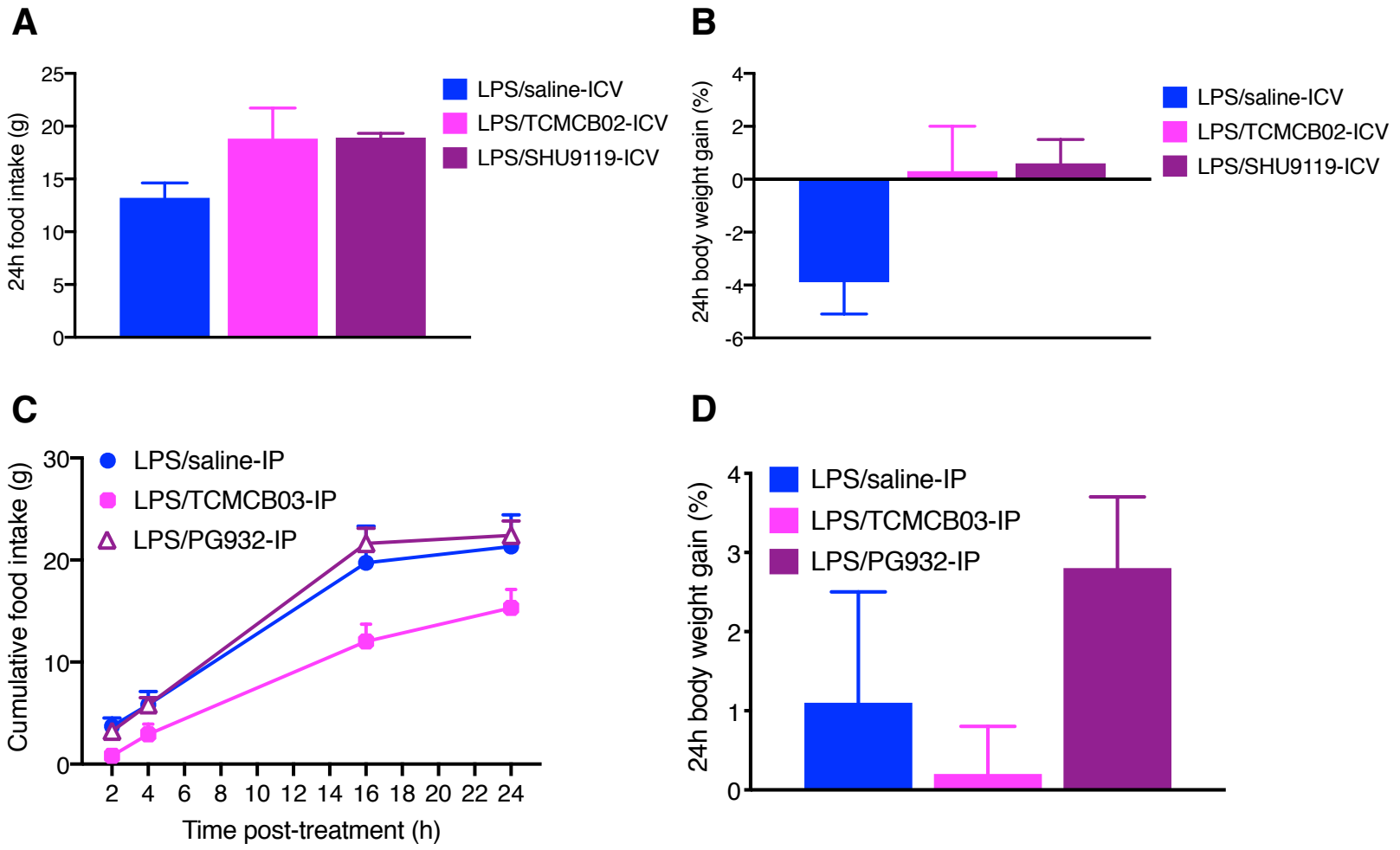

Figure S2 (related to Figure 1)

Central administration of TCMCB02 or SHU9119 but not peripheral administration of TCMCB03 or PG932 improved food intake and body weight in rats treated with LPS. (**A**) 24 h food intake and (**B**) body weight gain (%, net gain normalized to baseline) in LPS-treated rats post-ICV injection of saline *(n* = 3), TCMCB02 (1.5 nmol/rat/day, *n* = 3), or SHU9119 (1.5 nmol/rat/day, *n* = 2). (**C**) Cumulative food intake at 2 h, 4 h, 16 h and 24 h, and (**D**) 24 h body weight gain (%, net gain normalized to baseline) in LPS-treated rats post-IP injection of saline (*n* = 5), TCMCB03 (4 mg/kg/day, *n* = 5), or PG932 (4 mg/kg/day, *n* = 6). TCMCB03 treatment showed even worse response as the rats had lower food intake and body weight gain after receiving TCMCB03 treatment, compared to saline treated rats. Data are expressed as mean ± SEM for each group.

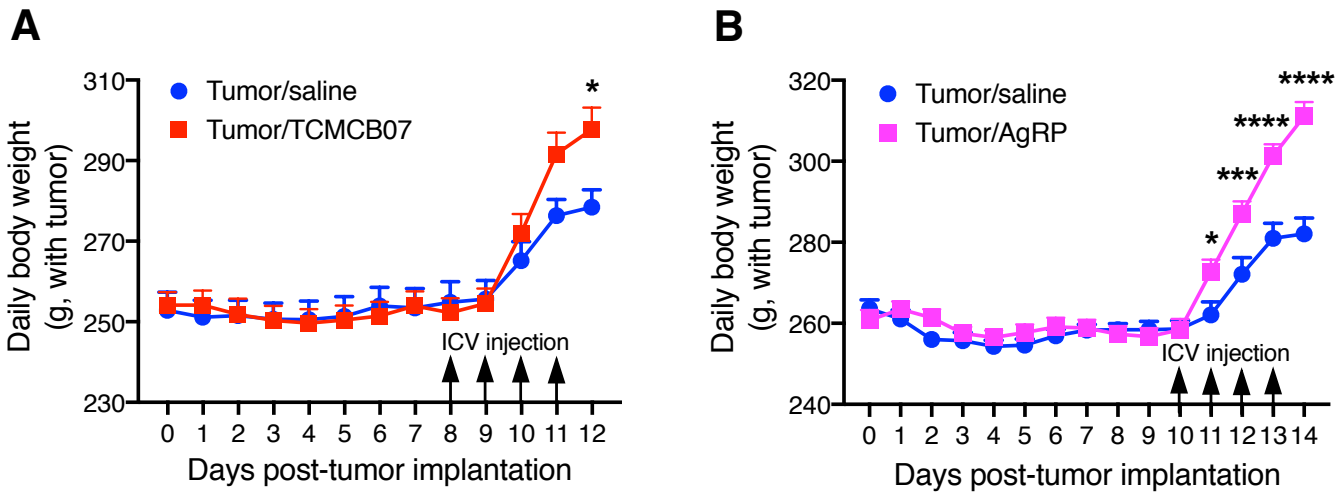

Figure S3 (related to Figure 2)

Central administration of TCMCB07 or AgRP attenuates body weight loss in rats with cancer cachexia that is similar to AgRP treatment. Daily body weight change post-tumor implantation and post-ICV administration of saline, TCMCB07 or AgRP. **(A)** Tumor-bearing rats received ICV injection once daily with saline *(n* = 6) or TCMCB07 (1.5 nmol/rat/day, *n* = 11) between day 8 and 11 post-tumor implantation**. (B)** In a separate experiment, tumor-bearing rats received ICV injection once daily with saline (*n* = 8) or AgRP (1 nmol/rat/day, *n* = 8) between day 10 and 13 post-tumor implantation. Data are expressed as mean ± SEM for each group. Two-way ANOVA (**A** and **B**). * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

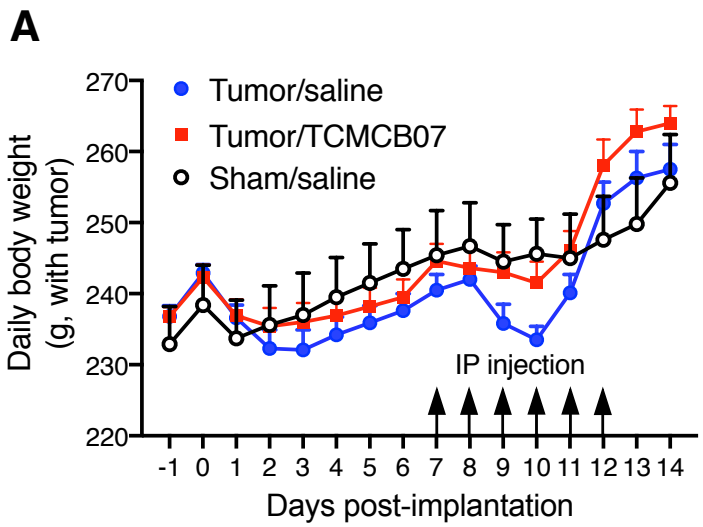

Figure S4 (related to Figure 3)
Effects of peritoneal administration of TCMCB07 on body weight loss in rats with cancer cachexia. (**A**) Daily body weight change post-implantation in tumor/saline group (n = 9), tumor/TCMCB07 group (n = 8), and sham/saline group (n = 6). Rats received IP injection once daily with saline or TCMCB07 (3 mg/kg/day) between day 7 and 12 post-implantation. Data are expressed as mean ± SEM for each group.

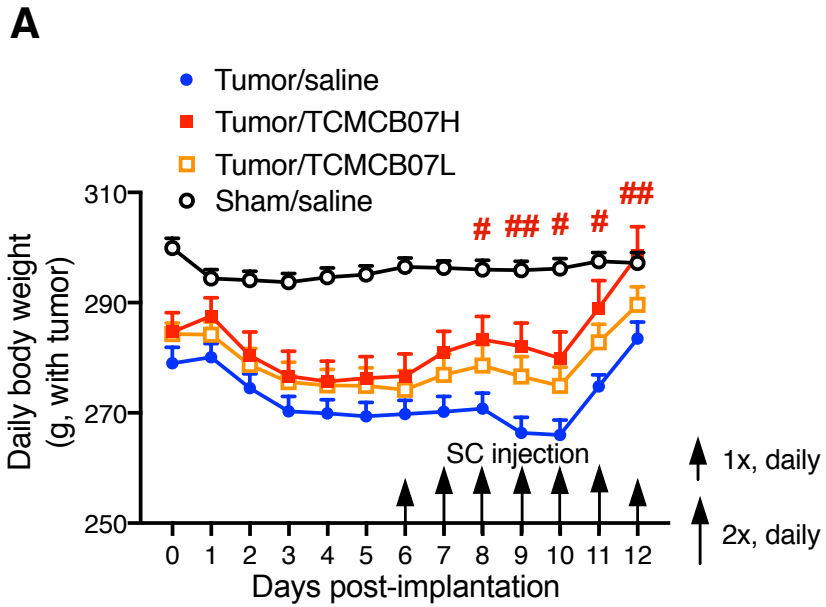

Figure S5 (related to Figure 4)
Effects of subcutaneous administration of TCMCB07 on body weight loss in rats with cancer cachexia. (**A**) Daily body weight change post-implantation in tumor/saline group (n = 8), tumor/TCMCB07L group (low dose, n = 8), tumor/TCMCB07H group (high dose, n = 8), and sham/saline group (n = 9). Rats received SC injection once (1x) or twice (2x) daily with saline or TCMCB07L (1.5 mg/kg/day) or TCMCB07H (3 mg/kg/day) between day 6 and 12 post-implantation. Data are expressed as mean ± SEM for each group. Two-way ANOVA, # P < 0.05, ## P < 0.01, Tumor/TCMCB07H group versus Tumor/saline group. Sham/saline group was excluded for Two-way ANOVA analysis in order to clearly show a treatment comparison between three tumor groups (tumor/saline, tumor/TCMCB07L, tumor/TCMCB07H).