Melanocortin-4 receptor antagonist TCMCB07 alleviates chemotherapy-induced anorexia and weight loss in rats

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Cancer patients undergoing chemotherapy often experience anorexia and weight loss that substantially deteriorates overall health, reduces treatment tolerance and quality of life, and worsens oncologic outcomes. There are currently few effective therapeutic options to mitigate these side effects. The central melanocortin system, which plays a pivotal role in regulating appetite and energy homeostasis, presents a logical target for treating anorexia and weight loss. In this preclinical study, we evaluated the efficacy of TCMCB07, a synthetic antagonist of the melanocortin-4 receptor, in mitigating anorexia and weight loss in several rat models of chemotherapy: cisplatin, 5-fluorouracil, cyclophosphamide, vincristine, doxorubicin, and a combination of irinotecan and 5-fluorouracil. Our results indicate that peripheral administration of TCMCB07 improved appetite, stabilized body weight, preserved fat and heart mass, and slightly protected lean mass after multiple cycles of chemotherapy. Furthermore, combining TCMCB07 with a growth differentiation factor 15 antibody enhanced treatment effectiveness. Similar effects from TCMCB07 treatment were observed in a rat tumor model following combination chemotherapy. No notable adverse effects nor increased chemotherapy-related toxicities were observed with TCMCB07 treatment. These findings suggest that peripheral administration of TCMCB07 holds promise as a therapeutic approach for alleviating chemotherapy-induced anorexia and weight loss, potentially benefiting numerous patients undergoing chemotherapy.

Introduction

Chemotherapy remains a cornerstone of both curative and palliative cancer therapy. It is the primary approach for treating advanced malignancies in situations where surgical resection or radiation therapy is not viable (1, 2). Chemotherapy delivery has been improved by refining dosing strategies, incorporating neoadjuvant or adjuvant administration, and integration with more advanced supportive care (1). However, because cancer cells are so similar to healthy cells, delivering adequate cytotoxic doses often results in pronounced adverse effects such as nausea, vomiting, anorexia, and weight loss. These side effects can profoundly impact patients' quality of life and limit treatment adherence (3–5). Therefore, effective management of chemotherapy-induced side effects is imperative for both patients and clinicians.

In recent decades, there have been substantial improvements in management of chemotherapy-induced nausea and vomiting through the utilization of standard-of-care agents, such as 5-hydroxytryptamine 3 receptor (5-HT3) antagonists, neurokinin-1 receptor (NK-1) antagonists, dexamethasone, and olanzapine (6–8). However, many individuals undergoing chemotherapy experience decreased appetite and weight loss, which not only deteriorates their overall physical condition, but also reduces treatment tolerance and exacerbates the underlying disease. Recent research revealed circulating levels of GDF15 are elevated by chemotherapies (9). GDF15 triggers nausea, vomiting, anorexia, and weight loss via activating glial cell–derived neurotrophic factor receptor α-like (GFRAL) in the area postrema and nucleus of the solitary tract in the brainstem and other GDF15 receptors or pathways (10–14). A recent study reported that treatment with a GDF15-neutralizing antibody mitigated cisplatin-induced vomiting, anorexia, and weight loss, indicating the potential of this therapeutic approach for alleviating side effects induced by platinum-based chemotherapy (15).

The central melanocortin system plays a pivotal role in the regulation of appetite, body weight, and energy homeostasis. This system encompasses first-order orexigenic agouti-related peptide (AgRP) neurons and anorexigenic proopiomelanocortin (POMC) neurons in the arcuate nucleus of hypothalamus, which sense hormonal (ghrelin, leptin, and insulin) and neuronal (excitatory and inhibitory inputs) signals of energy balance and activate downstream neurons (16–18). These neurons synapse on second-order neurons in the paraventricular nucleus that express the melanocortin 3 receptor (MC3R) and melanocortin 4 receptor (MC4R),

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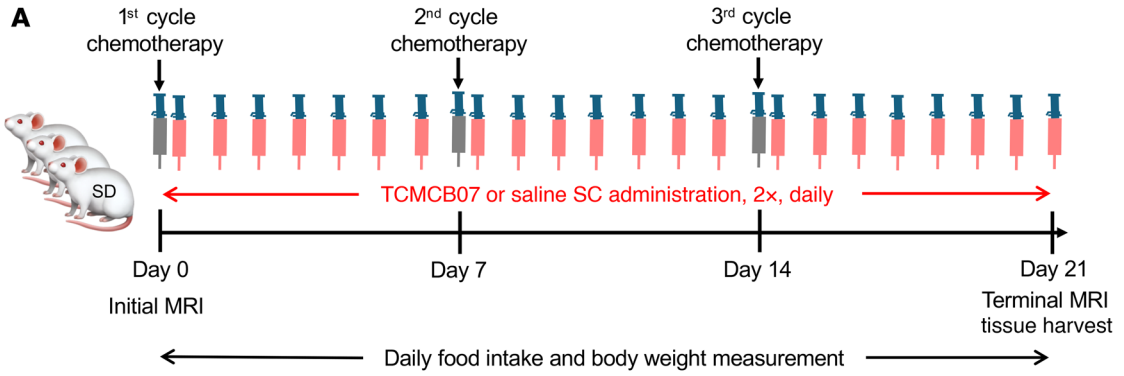

Figure 1. Schematic of TCMCB07/chemotherapy study design. (**A**) Schematic of TCMCB07/chemotherapy study design. SD male rats were treated with 3 cycles of chemotherapy via i.p. injection within 3 weeks at the following doses: cisplatin, 2.5 mg/kg; 5-FU, 70 mg/kg; CP, 65 mg/kg; vincristine, 0.27 mg/kg;, and doxorubicin 2 mg/kg. Control animals received an equivalent volume of saline i.p. injections. All rats received s.c. injections twice (2×) daily with either saline or TCMCB07 (3 mg/kg/day) from day 0 to 21. Initial and terminal body composition was measured using MRI prior to and after treatments. Food intake and body weight were monitored daily throughout entire experimental period (days 0–21). At the end of the experiment, tissues were harvested following euthanasia.

neuropeptide Y receptor 1, and $GABA_A$ receptors and are therefore capable of integrating inputs from AgRP and POMC neurons (19). These neurons transduce both anorexigenic agonists (e.g., α-melanocyte-stimulating hormone [α-MSH]) and orexigenic antagonists/inverse agonists (e.g., AgRP) of MC3R and MC4R. While MC3R neurons likely contribute to behavioral adaptation to fasting and nutrient partitioning, MC4R neurons are involved in feeding behavior, adaptive thermogenesis, and glucose homeostasis (20, 21). Therefore, this system represents a rational therapeutic target for treating anorexia, cachexia, obesity, and diabetes (19, 22–24).

Over the last decade, the development of melanocortin receptor–based therapeutics brought excitement and promise in treating obesity and anorexia-cachexia, with several melanocortin agonist drugs approved by the FDA for certain forms of obesity and other neuroendocrine disorders (19). However, despite investigations into melanocortin antagonists for therapeutic interventions to treat wasting syndromes and anorexia, no drugs in this class are yet approved for clinical use (25–30), highlighting the need to develop novel drugs with maximum safety, high efficacy, and peripheral therapeutic feasibility (e.g., effectively crossing the blood-brain barrier [BBB] to act on the central melanocortin system). Our previous work demonstrated the efficacy of TCMCB07, a synthetic MC4R antagonist, in ameliorating cachexia associated with cancer, chronic kidney disease, and other advanced illnesses (31–34). TCMCB07 recently completed a first-in-human phase 1 clinical trial, with preliminary findings supporting both safety and efficacy (35).

In this preclinical study, we aimed to investigate the potential of TCMCB07 in alleviating chemotherapy-induced anorexia and weight loss. We replicated chemotherapy-associated anorexia and weight loss in rats by administering 6 commonly used cytotoxic agents, either individually or in combination: cisplatin, 5-fluorouracil (5-FU), cyclophosphamide (CP), vincristine, doxorubicin, and irinotecan. We also utilized the rat Ward colorectal carcinoma model in combination with chemotherapy to model chemotherapy treatment of a tumor in situ (36–40). A comprehensive evaluation of TCMCB07's efficacy in stimulating appetite and maintaining body mass was performed across these rat models. Additionally, we explored the possibility of enhancing the therapeutic effectiveness by combining TCMCB07 with an anti-GDF15 antibody to combat chemotherapy-induced anorexia and weight loss.

Results

Dosing regimen selection for TCMCB07 administration and chemotherapy. We selected the dose of TCMCB07 based on our prior work demonstrating that 3 mg/kg/d effectively ameliorates cachexia associated with cancer, renal failure, or other advanced conditions (33, 34). To assess the broad effectiveness of TCMCB07 treatment in chemotherapy-induced anorexia and weight loss, we generated 6 rat models of chemotherapy, representing commonly used classes of cancer chemotherapeutics: (a) cisplatin (platinum compound), (b) 5-FU (antimetabolite), (c) CP (alkylating agent), (d) vincristine (vinca alkaloid), (e) doxorubicin (anthracycline), and (f) a combination of irinotecan (DNA topoisomerase I inhibitor) and 5-FU. A literature review of chemotherapy-induced anorexia and weight loss revealed a wide range of chemotherapy doses (41–47). Therefore, we designed and conducted a series of dose-response experiments in rats to determine doses that consistently mimicked the commonly observed clinical sickness responses, without undue toxicity. Initially, 2 doses of cisplatin and 5-FU were tested: cisplatin at 2.5 and 5 mg/kg, and 5-FU at 62.5 and 125 mg/kg (Supplemental Figure 1, A–C; supplemental material available online with this article; https://doi.org/10.1172/JCI181305DS1). For CP and vincristine, 4 doses of each agent were examined: CP at 50, 70, 90, and 110 mg/kg (Supplemental Figure 1, D and E) and vincristine at 0.18, 0.25, 0.30, and 0.40 mg/kg (Supplemental Figure 1, F and G). For doxorubicin, a dosage of 2 mg/kg was chosen based on previous reports (48, 49). For the combination of irinotecan and 5-FU, a dosage of 50 mg/kg for each agent was selected following previous studies (36–40). All chemotherapy agents were administered via i.p. injections once per week for 3 cycles in total. Control animals received an equivalent volume of saline i.p. injections. Through these dose-response experiments, we observed not only dose-dependent general behavioral responses to chemotherapy, such as reduced activity indicating fatigue, but also a gradual decline in food intake

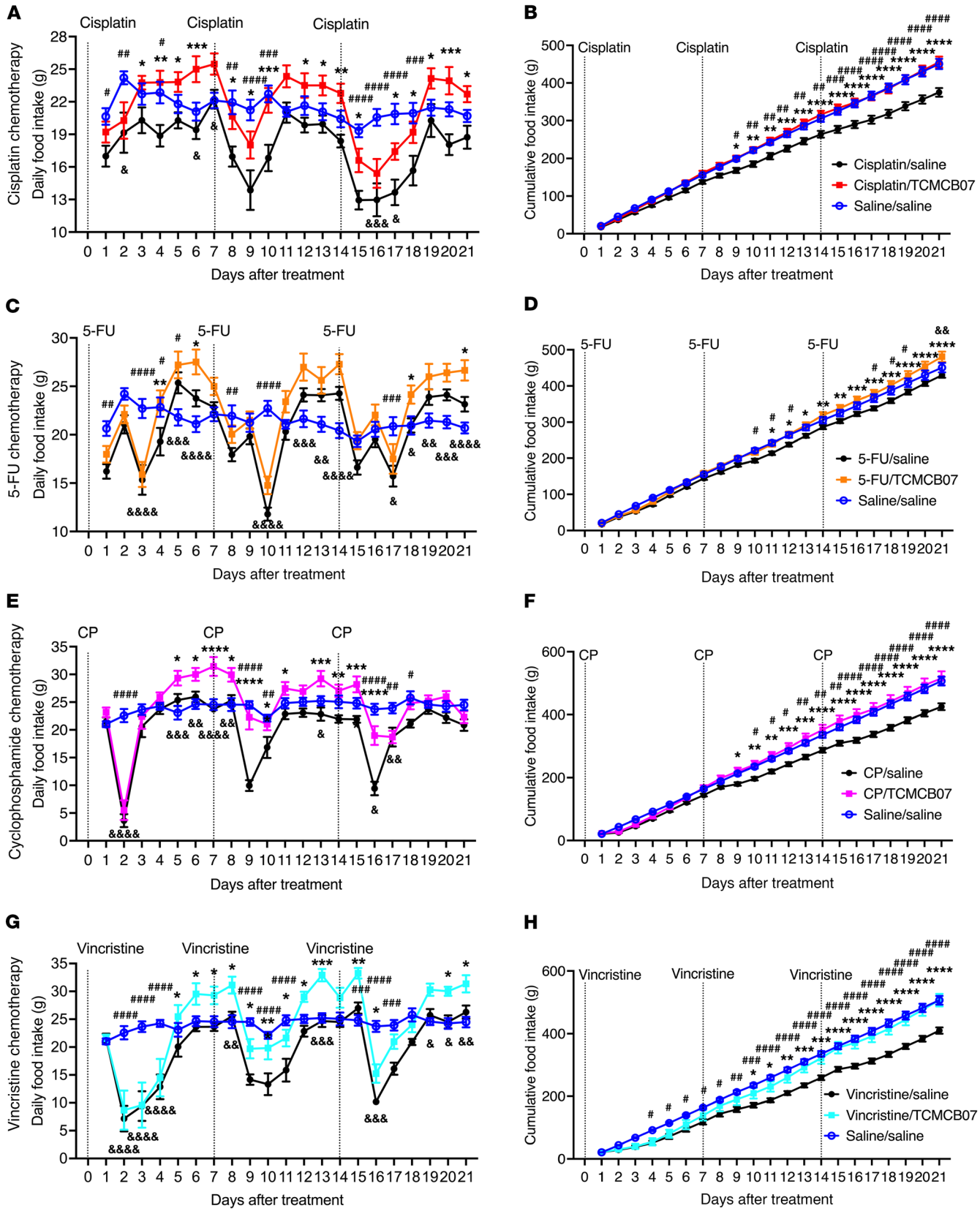

Figure 2. TCMCB07 treatment increases daily food intake throughout multiple cycles of various chemotherapy. (**A**–**H**) Daily and cumulative food intake after chemotherapy and TCMCB07 treatment throughout entire experimental period (days 0–21). (**A** and **B**) Cisplatin chemotherapy. (**C** and **D**) 5-FU chemotherapy. (**E** and **F**) CP chemotherapy. (**G** and **H**) Vincristine chemotherapy. All data in **A**–**H** were expressed as mean ± SEM for each group. *Chemotherapy/saline group versus chemotherapy/TCMCB07 group; #Chemotherapy/saline group versus saline/saline group; &Chemotherapy/TCMCB07 group versus saline/saline group. n = 10–12. $^{*,\#,\&}P < 0.05$; $^{**,\#\#,\&\&}P < 0.01$; $^{***,\#\#\#,\&\&\&}P < 0.001$; $^{****,\#\#\#\#,\&\&\&\&}P < 0.0001$. All data in **A**–**H** were analyzed by 2-way ANOVA.

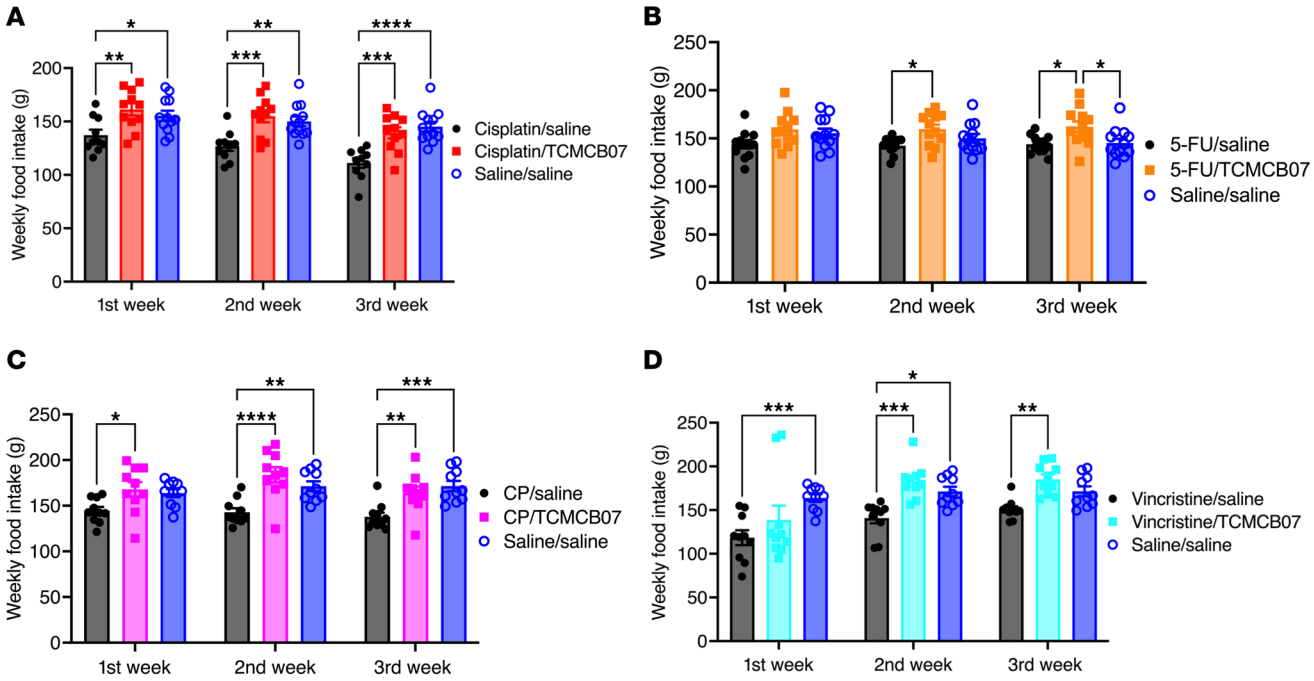

Figure 3. TCMCB07 treatment increases weekly food intake after each cycle of various chemotherapy. (**A**–**D**) Weekly food intake after chemotherapy and TCMCB07 treatment throughout entire experimental period (days 0–21). (**A**) Cisplatin chemotherapy. (**B**) 5-FU chemotherapy. (**C**) CP chemotherapy. (**D**) Vincristine chemotherapy. All data in **A**–**D** were expressed with each dot representing 1 sample. n = 10–12. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. All data in **A**–**D** were analyzed by 2-way ANOVA.

and body weight over multiple cycles of chemotherapy, consistent with common clinical side effects induced by these drugs. In addition, we noted severe morbidity and even mortality among animals receiving high doses of each chemotherapy agent. Based on these results and considering the maximum tolerance of animals to 3 consecutive cycles of chemotherapy plus daily TCMCB07 administration, we ultimately selected the optimal dose for each chemotherapy agent to induce a 10% to 30% weight loss compared with rats not receiving chemotherapy (15). Rats were treated for a total of 3 consecutive cycles (administered once per week, via i.p. injection) at the following doses: cisplatin, 2.5, 3.0, or 5.0 mg/kg (with the dose of 5.0 mg/kg administered for the first cycle only); 5-FU, 70 mg/kg; CP, 65 mg/kg; vincristine, 0.27 mg/kg; doxorubicin, 2.0 mg/kg; and irinotecan, 50 mg/kg and 5-FU, 50 mg/kg for 2 cycles of combination chemotherapy.

TCMCB07 treatment restores appetite during multiple cycles of chemotherapy. We conducted independent studies to evaluate TCMCB07's efficacy across the 5 rat models of single-agent chemotherapy (Figure 1A): (a) cisplatin, (b) 5-FU, (c) CP, (d) vincristine, and 5) doxorubicin. In the cisplatin/TCMCB07 study, during 3 cycles of chemotherapy, we observed a significant attenuation of chemotherapy-induced anorexia and a faster rebound in daily food intake among rats treated with TCMCB07 compared with those receiving saline treatment (Figure 2A). Cumulative food intake over the 21-day study period was identical between cisplatin/TCMCB07 and saline/saline groups, indicating that TCMCB07 treatment completely reversed the anorexia induced by multiple cycles of cisplatin chemotherapy (Figure 2B). Similarly, in the 5-FU/TCMCB07 study, TCMCB07 treatment led to increased cumulative food intake compared with both saline/saline and 5-FU/saline groups by day 21 (Figure 2, C and D). In the CP/TCMCB07 and vincristine/TCMCB07 studies, although TCMCB07 treatment did not elevate food intake during the initial 4 days following the first chemotherapy treatment, it increased daily food intake for the remainder of the study period (Figure 2, E and G). TCMCB07 treatment reversed any chemotherapy-induced reduction in cumulative food intake in both the CP- and vincristine-treated groups (Figure 2, F and H). Following each cycle of cisplatin or CP chemotherapy, weekly food intake was significantly greater in rats receiving TCMCB07 compared with those receiving saline (Figure 3, A and C). During the second and third cycle of 5-FU or vincristine chemotherapy, weekly food intake was higher in rats receiving TCMCB07 compared with those receiving saline (Figure 3, B and D). Notably, no significant reduction in weekly food intake was observed in any of the chemotherapy/TCMCB07 groups compared with saline/saline groups (Figure 3, A–D). Food intake was not measured in the doxorubicin/TCMCB07 study. Taken together, peripheral administration of TCMCB07 completely reversed anorexia during 3 cycles of cisplatin, 5-FU, CP, or vincristine chemotherapy, suggesting TCMCBC's efficacy in restoring appetite suppressed by these commonly used chemotherapy agents.

TCMCB07 treatment maintains body weight throughout multiple cycles of chemotherapy. In the same experiments outlined above, we monitored the daily body weight in the same groups of rats. Following each cycle of cisplatin chemotherapy, rats experienced weight loss compared with those not receiving chemotherapy, with the loss becoming more pronounced after the second and

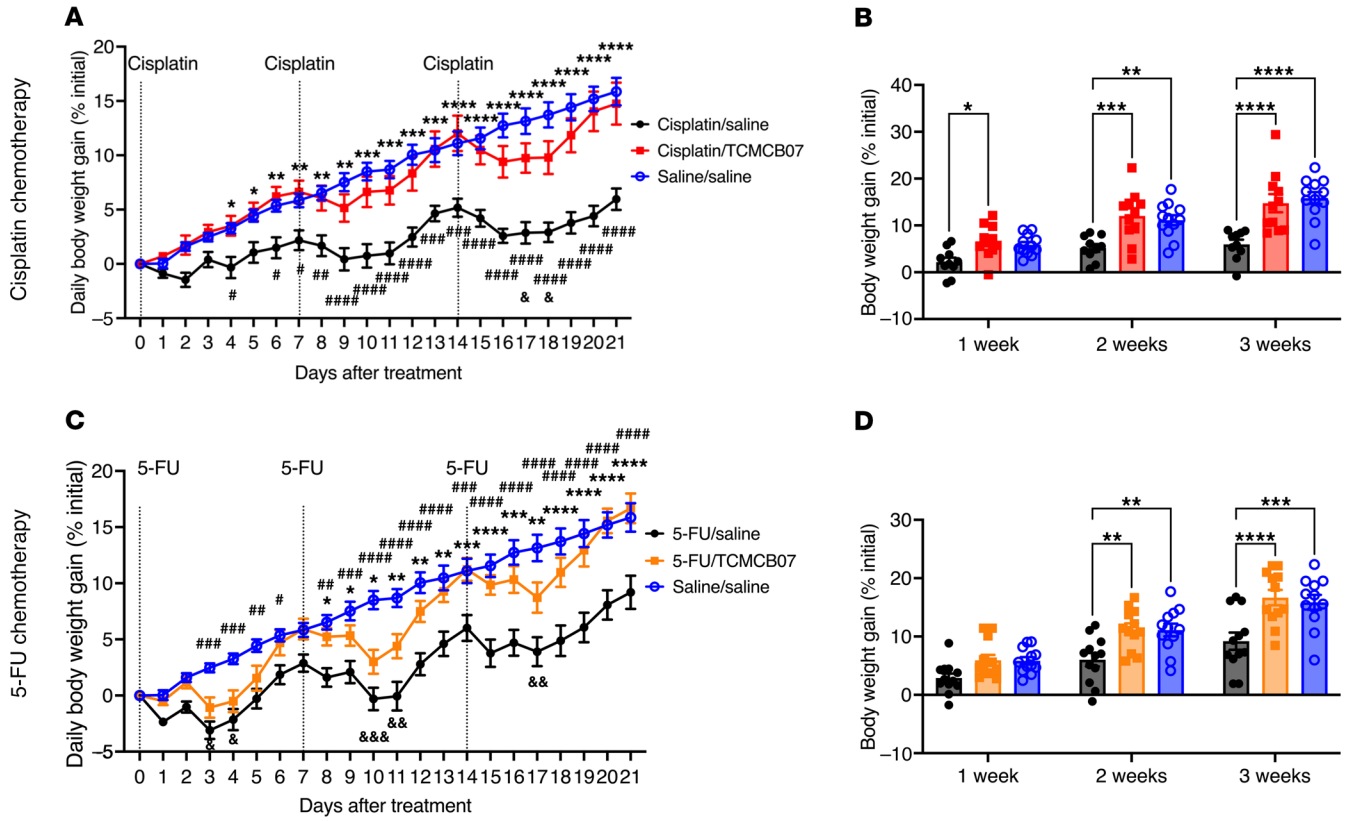

Figure 4. TCMCB07 treatment maintains body weight throughout multiple cycles of cisplatin or 5-FU chemotherapy. (**A**–**D**) Daily and weekly body weight gain (% initial body weight) after chemotherapy and TCMCB07 treatment. (**A** and **B**) Cisplatin chemotherapy. (**C** and **D**) 5-FU chemotherapy. All data in **A** and **C** were expressed as mean ± SEM for each group, and all data in **B** and **D** were expressed with each dot representing 1 sample. n = 10–12. (**A** and **C**) *Chemotherapy/saline versus chemotherapy/TCMCB07; #Chemotherapy/saline versus saline/saline; &Chemotherapy/TCMCB07 versus saline/saline. $^{*,\#,\&}P < 0.05$; $^{**,\#\#,\&\&}P < 0.01$; $^{***,\#\#\#,\&\&\&}P < 0.001$; $^{****,\#\#\#\#}P < 0.0001$. All data in **A**–**D** were analyzed by 2-way ANOVA.

third cycles of chemotherapy (Figure 4A and Supplemental Figure 2A). TCMCB07 treatment completely reversed this weight loss compared with saline treatment, as there was no difference in body weight between the cisplatin/TCMCB07 and saline/saline groups at the end of each cycle of cisplatin chemotherapy (Figure 4B). Similarly, while rats receiving 5-FU experienced weight loss compared with those receiving saline (Figure 4C and Supplemental Figure 2B), TCMCB07 treatment fully mitigated this weight loss during the 3 cycles of chemotherapy (Figure 4D). Although TCMCB07 treatment did not completely reverse the body weight loss induced by CP or vincristine chemotherapy, it significantly attenuated the reduction in body weight, particularly during the second and third cycles of chemotherapy (Figure 5, A–D, and Supplemental Figure 3, A and B). With doxorubicin chemotherapy, TCMCB07 treatment alleviated body weight loss compared with saline treatment during the 3 cycles of chemotherapy (Figure 5, E and F, and Supplemental Figure 3C). Collectively, TCMCB07 treatment attenuated body weight loss induced by 3 cycles of cisplatin, 5-FU, CP, vincristine, or doxorubicin chemotherapy, suggesting the efficacy of TCMCB07 in preserving body mass during multiple cycles of chemotherapy.

TCMCB07 treatment attenuates chemotherapy-induced tissue wasting. To assess whether TCMCB07 treatment affects whole body fat and lean mass during multiple cycles of chemotherapy, we measured body composition before and after TCMCB07 treatment. While fat mass decreased significantly in rats after 3 cycles of cisplatin, 5-FU, CP, or vincristine chemotherapy, rats treated with TCMCB07 fully retained their fat mass (Figure 6, A–D, and Supplemental Figure 4, A–D). In contrast, TCMCB07 treatment did not significantly mitigate the loss of lean mass (Figure 6, E–H, and Supplemental Figure 4, E–H). Moreover, we observed a significant normalization of heart mass in rats receiving cisplatin/TCMCB07 or 5-FU/TCMCB07 treatment compared with those receiving cisplatin/saline or 5-FU/saline treatment (Figure 7, A–D, and Supplemental Figure 5, A–D). However, there was no significant increase in gastrocnemius mass in rats treated with chemotherapy/TCMCB07 compared with those treated with chemotherapy/saline (Figure 7, E–H, and Supplemental Figure 5, E–H).

TCMCB07+GDF15 antibody combination therapy enhances effectiveness in reversing chemotherapy-induced anorexia and weight loss. Certain chemotherapy agents, particularly cisplatin, raise circulating GDF15 levels (9, 15, 50), which, in turn, suppresses appetite and promotes fat loss and muscle atrophy by activating GFRAL in the brainstem and other GDF15 receptors or pathways (9, 11, 12, 14). We sought to simultaneously target both critical central regulation systems of feeding and metabolism, the hypothalamic melanocortin and GDF15-GFRAL, using a combination therapy involving TCMCB07 and a GDF15 antibody. To test for possible

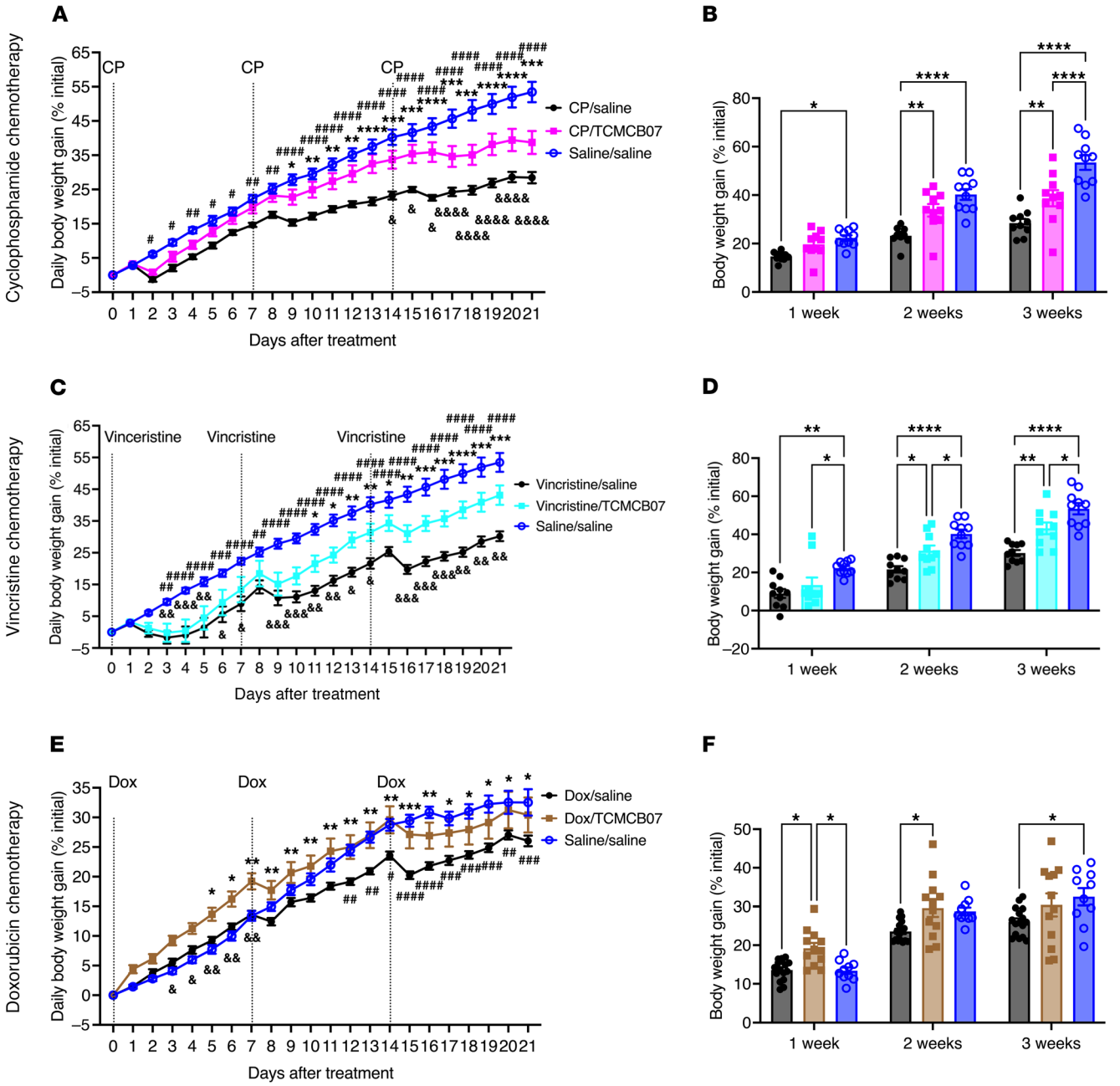

Figure 5. TCMCB07 treatment maintains body weight throughout multiple cycles of CP, vincristine, or doxorubicin chemotherapy. (**A**–**F**) Daily and weekly body weight gain (% initial body weight) after chemotherapy and TCMCB07 treatment. (**A** and **B**) CP chemotherapy. (**C** and **D**) Vincristine chemotherapy. (**E** and **F**) Doxorubicin chemotherapy. All data in **A**, **C**, and **E** were expressed as mean ± SEM for each group, and all data in **B**, **D**, and **F** were expressed with each dot representing 1 sample. n = 10–12. (**A**, **C**, and **E**), *Chemotherapy/saline versus chemotherapy/TCMCB07; #Chemotherapy/saline versus saline/saline; &Chemotherapy/TCMCB07 versus saline/saline. $^{*,\#,\&}P < 0.05$; $^{**,\#\#,\&\&}P < 0.01$; $^{***,\#\#\#,\&\&\&}P < 0.001$; $^{****,\#\#\#\#,\&\&\&\&}P < 0.0001$. All data in **A**–**H** were analyzed by 2-way ANOVA.

synergistic effects of this combination therapy in reversing anorexia and weight loss following higher doses and multiple cycles of chemotherapy, we first measured GDF15 levels in serum samples collected from TCMCB07/chemotherapy studies. Serum GDF15 levels were elevated in rats receiving chemotherapy with cisplatin, 5-FU, CP, vincristine, or doxorubicin, while no alterations in GDF15 levels were observed with TCMCB07 treatment (Figure 8, A–E). We then challenged rats with a higher dose of cisplatin chemotherapy, followed by administration of the combination therapy of TCMCB07 and GDF15 antibody (Figure 8F). Consistent with previous reports (15), the effectiveness of the GDF15 antibody was validated in mitigating cisplatin-induced anorexia and weight loss. In the present combined treatment study, all 3 groups of rats received an initial cycle of high-dose (5.0 mg/kg) cisplatin chemotherapy. However, due to mortality observed after the first cycle of chemotherapy, we reduced the dose to 3.0 mg/kg for the second and third cycles (Figure 9A). Of note, either the 5.0 mg/kg or 3.0 mg/kg dosage surpassed the 2.5 mg/kg dose employed in

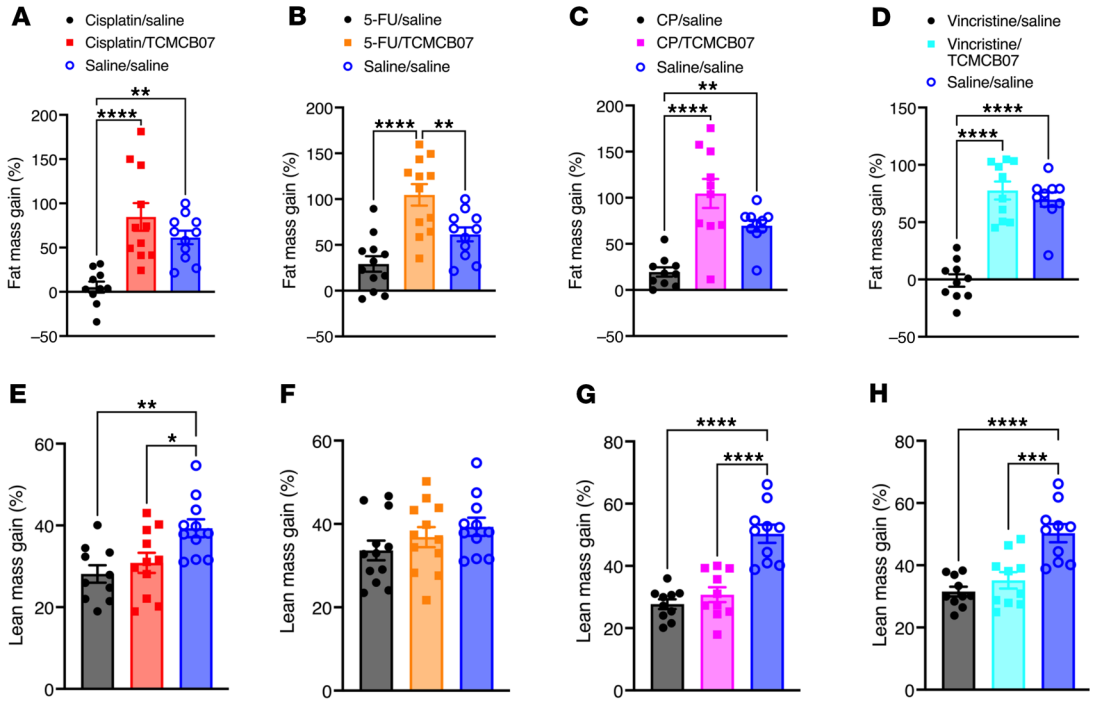

Figure 6. TCMCB07 treatment attenuates chemotherapy-induced fat and lean mass loss. (**A**–**D**) Fat mass gain (% initial) and (**E**–**H**) lean mass gain (% initial) after 3 cycles of chemotherapy and 21-day TCMCB07 treatment. (**A** and **E**) Cisplatin chemotherapy. (**B** and **F**) 5-FU chemotherapy. (**C** and **G**) CP chemotherapy. (**D** and **H**) Vincristine chemotherapy. All data in **A**–**H** were expressed with each dot representing 1 sample. n = 10–12. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. All data in **A**–**H** were analyzed by 1-way ANOVA.

the earlier monotherapy study. Over 3 cycles of higher-dose cisplatin chemotherapy, rats receiving combination therapy of TCMCB07+GDF15 antibody exhibited increased daily food intake (Figure 9A), and significant improvement in cumulative (Figure 9B), weekly (Figure 9C), and total food intake (Figure 9D), compared with those receiving IgG or GDF15 antibody monotherapy. Correspondingly, the daily body weight (Supplemental Figure 6A), body weight gain (Figure 10A), and weekly body weight gain (Figure 10B) were significantly higher in rats receiving cisplatin/TCMCB07+GDF15 antibody treatment compared with those receiving cisplatin/saline+IgG or cisplatin/saline+GDF15 treatment. Furthermore, TCMCB07+GDF15 treatment normalized fat mass (Figure 10C and Supplemental Figure 6B), and slightly increased lean mass (Figure 10D and Supplemental Figure 6C), heart mass (Figure 10E and Supplemental Figure 6D), and gastrocnemius mass (Figure 10F and Supplemental Figure 6E).

TCMCB07 treatment mitigates anorexia and weight loss in rats with Ward colorectal tumor following combination chemotherapy. To enhance the clinical relevance of this preclinical drug trial, we evaluated TCMCB07's efficacy in the rat Ward colorectal carcinoma model with combination irinotecan and 5-FU chemotherapy. We first generated the subcutaneous Ward tumor model in Fischer 344 (F344) female rats according to previous studies (36–40), monitoring the tumor growth and tumors' response to chemotherapy (Figure 11A). We then adapted the doses (50 mg/kg for each agent) and regimen (i.p. injection, once per week, 5-FU administered 24 hours after irinotecan administration) for a total of 2 cycles of the combination chemotherapy (Figure 11A), previously shown to inhibit the Ward tumor growth and also induce anorexia and weight loss (36–40). Tumor volume was reduced following the first cycle of chemotherapy, with a nadir on day 4 after chemotherapy, and then gradually increased until the second chemotherapy treatment (Figure 11B). The second cycle of chemotherapy further suppressed tumor growth (Figure 11B). There was no difference in tumor volume between chemotherapy/TCMCB07 and chemotherapy/saline groups. Chemotherapy induced anorexia and weight loss compared with both baseline levels and the nontumor (sham-operated) control group. TCMCB07 treatment mitigated anorexia in tumor/chemotherapy rats compared with saline-treated tumor/chemotherapy rats (Figure 11, C–E). In line with the increased food intake, body weight gain was significantly higher in the tumor/chemotherapy/TCMCB07 group compared with the tumor/chemotherapy/saline group (Figure 12, A and B, and Supplemental Figure 7A). This increase in body weight was attributable to a remarkable retention of fat mass and a slight preservation of lean mass (Figure 12, C and D, and Supplemental Figure 7, B and C). TCMCB07 treatment also protected cardiac muscle but had no effect on gastrocnemius mass in tumor-bearing rats following the combination chemotherapy (Figure 12, E and F, and Supplemental Figure 7, D and E). We did not observe an effect of TCMCB07 treatment on Ward tumor growth, which is consistent with our previous cancer-cachexia studies (31, 34)

TCMCB07 is detectable in the circulation without causing adverse effects. To ascertain the detectability of administered TCMCB07 in the circulation and assess its correlation with chemotherapy, we quantified TCMCB07 concentrations in rat serum using liquid chromatography/tandem mass spectrometry (LC-MS/MS) and

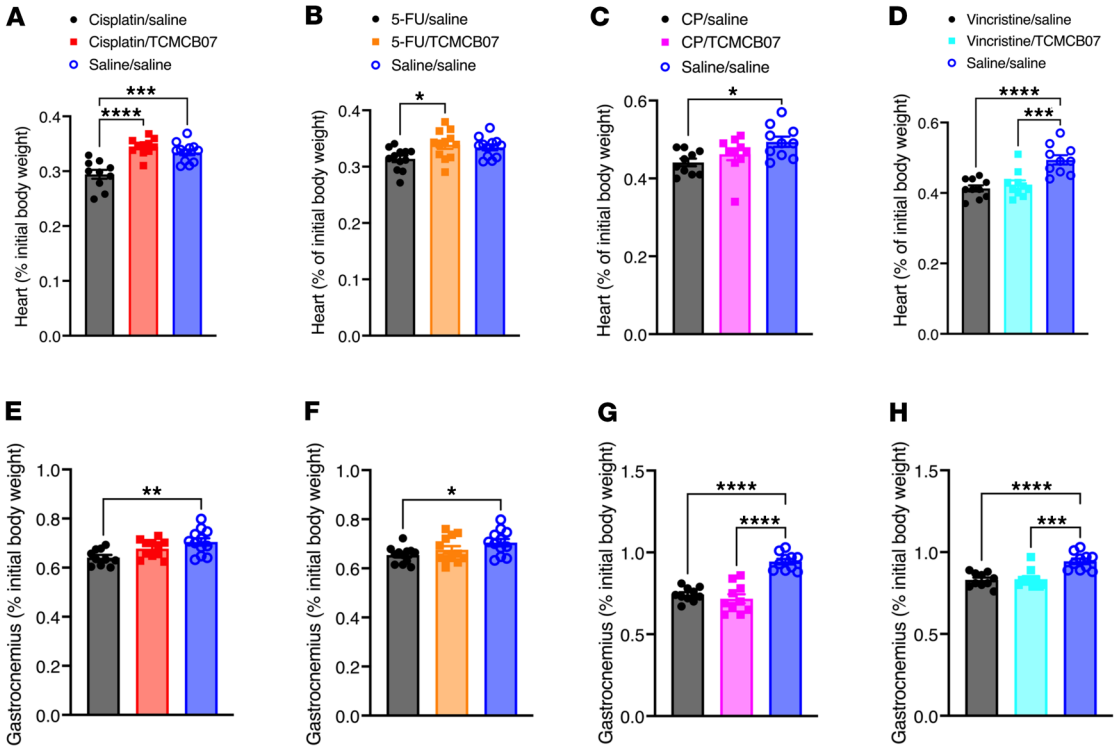

Figure 7. TCMCB07 treatment protects heart tissue during multiple cycles of chemotherapy. (**A**–**D**) Heart mass (% initial body weight) and (**E**–**H**) gastrocnemius mass (% initial body weight) after 3 cycles of chemotherapy and 21-day TCMCB07 treatment. (**A** and **E**) Cisplatin chemotherapy. (**B** and **F**) 5-FU chemotherapy. (**C** and **G**) CP chemotherapy. (**D** and **H**) Vincristine chemotherapy. All data in **A**–**H** were expressed with each dot representing 1 sample. n = 10–12. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. All data in **A**–**H** were analyzed by 1-way ANOVA.

LC–multiple reaction monitoring (LC-MRM) at the end of both the 21-day cisplatin/TCMCB07 study and a 21-day TCMCB07 test. TCMCB07 was detectable within 0.5–2.5 hours after the last TCMCB07 s.c. injection (Table 1). Serum TCMCB07 concentrations were higher in rats undergoing chemotherapy (Table 1), suggesting either reduced drug metabolism or reduced clearance due to chemotherapy-induced organ damage. Additionally, hematological parameters in blood samples were analyzed at the end of each study. We observed expected reductions in total leukocyte counts and lymphocyte counts in rats treated with chemotherapy, whereas no significant changes were noted from TCMCB07 treatment (Supplemental Figure 8, A–F). Importantly, in our daily observation of both behavioral responses and overall health status across all groups receiving TCMCB07 treatment, no significant adverse effects or morbidity was attributed to TCMCB07 administration in conjunction with 6 different chemotherapies and the GDF15 antibody.

Discussion

In this investigation, to evaluate the broad effectiveness of TCMCB07 in alleviating chemotherapy-induced anorexia and weight loss, we established rat models using 6 classical agents that represent distinct classes of chemotherapy agents frequently prescribed for various cancer types. While these 6 chemotherapy agents are commonly employed for cancer treatment, they cause a variety of common side effects and can lead to major organ damage. Substantial progress has been made in developing interventions to mitigate these side effects and address organ toxicity (51). However, only one FDA-approved drug, olanzapine, has been shown to improve weight loss in the context of chemotherapy in a randomized trial, and the contexts in which it is effective remain poorly defined (52).

A wide range of chemotherapy dosages and regimens are documented in both literature and clinical practice (42, 53). In preclinical research, the strategies for utilizing chemotherapy in experimental animals predominately depend on the specific purposes of the studies (41, 45–49, 54). However, due to the potency and toxicity, numerous aspects related to animal models of chemotherapy require careful consideration to strike a balance between effectiveness and toxicity. While clinical studies often allow for further dose escalation with extensive supportive care measures such as intravenous hydration, antiemetics, antihistamines, and corticosteroids, these measures are typically absent in animal studies (42). Moreover, animal species, strains (55), sex, age, body weight, and growth period can markedly influence responses, effectiveness, and tolerability, thereby impacting investigation outcomes. Additionally, the effects of chemotherapy are not solely determined by the dosage administered in a study but can also be affected by the quality or even the manufacturer of the agents used. For example, we observed variable degrees of sickness responses induced by different suppliers of cisplatin. Furthermore, the dosing regimen is crucial, particularly in multiple cycles of treatment (42). During the initial design of our study, we integrated previous reports and then conducted a series of dose-response experiments in both mouse

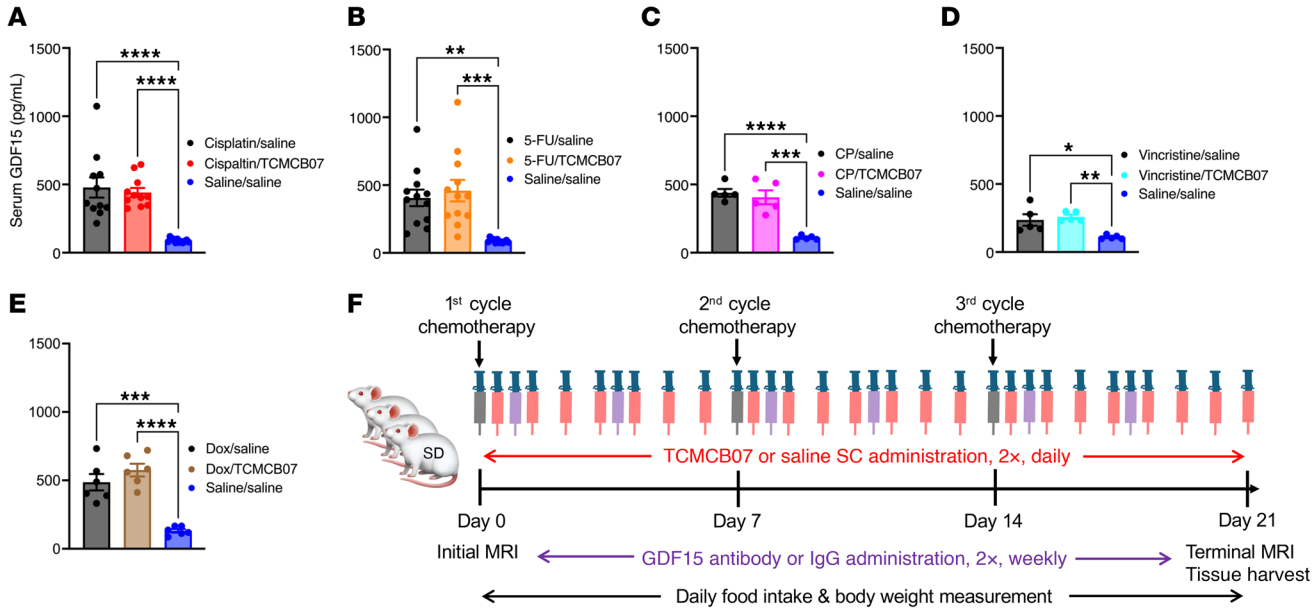

Figure 8. Chemotherapy increases circulating GDF15 levels and study design for combination therapy of TCMCB07+GDF15 antibody. (**A**–**E**) SD male rat serum GDF15 concentrations measured from experiments: cisplatin/TCMCB07 (**A**), 5-FU /TCMCB07 (**B**), CP/TCMCB07 (**C**), vincristine/TCMCB07 (**D**), and doxorubicin/TCMCB07 (**E**). (**F**) Schematic of study design. All SD male rats were treated with cisplatin chemotherapy via i.p. injection at a dose of 5.0 mg/kg (1st cycle) or 3.0 mg/kg (2nd and 3rd cycles) once per week for 3 cycles. All the rats received s.c. injections twice (2×) daily with either saline or TCMCB07 (3 mg/kg/day) from day 0 to 21. Additionally, all the rats received s.c. injections twice (2×) weekly with either IgG or GDF15 antibody from day 0 to 21. The dose of TCMCB07 was 3 mg/kg/d, and the dose of GDF15 antibody and IgG control was 10 mg/kg. Initial and terminal body composition were measured using MRI before and after treatments. Food intake and body weight were monitored daily throughout entire experimental period (days 0–21). At the end of the experiment, tissues were harvested following euthanasia. All data in **A**–**E** were expressed with each dot representing 1 sample.(**A** and **B**) n = 11–12. (**C**–**E**) n = 5–6, as blood samples were collected from half of the animals in these experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. All data in **A**–**E** were analyzed by 1-way ANOVA.

and rat species. Consistent with existing literature (42), we noted a higher tolerance to the agents we used in this study in mice compared with rats. This difference is likely due to species variations, but more importantly, the total amount of agents administered to rats is at least 10-fold higher compared with mice, as the total amount of drug in preclinical studies is generally calculated based on body weight rather than body surface area. We observed differences even within the same strain of rats: heavier rats exhibited a more pronounced response than lighter ones when given the same dose (in mg/kg). We conclude that precise dosing is critical for the analysis of sickness responses to chemotherapy, and reinforce the importance of dose-finding studies, consistent agent quality, and accurate administration volume to achieve this goal. Notably, the doses we selected for our rat model are similar to those used in patients. The clinical doses, according to a prior comparative medicine study (42), converted from mg/m^2 to mg/kg with repeated cycles, are as follows: cisplatin, 2.5 mg/kg; 5-FU, 71 mg/kg; CP, 60 mg/kg; doxorubicin, 1.9 mg/kg; and irinotecan, 8.9 mg/kg. In our rat model with multiple cycles, we used the following doses: cisplatin, 2.5, 3.0, 5.0 mg/kg; 5-FU, 70 mg/kg; CP, 65 mg/kg; doxorubicin, 2.0 mg/kg; and irinotecan, 50 mg/kg.

Our assessment of the effectiveness of TCMCB07 in reversing chemotherapy-induced adverse effects, including anorexia and weight loss, was based on our understanding of underlying mechanisms and previous studies (19, 25, 28, 30, 34, 56). Encouragingly, our data demonstrated the promising potential of TCMCB07 treatment to provide beneficial effects on both food intake and body weight in rats treated with a variety of chemotherapeutics for multiple cycles. In studies involving cisplatin or 5-FU chemotherapy, TCMCB07 treatment fully restored the loss of appetite and body weight following each chemotherapy cycle, highlighting its potential to prevent chemotherapy dose reductions in a clinical setting. In contrast to the cisplatin and 5-FU studies, TCMCB07 treatment did not fully reverse appetite and weight loss in rats undergoing CP, vincristine, or doxorubicin chemotherapy. This could be attributed to the higher toxicity of these chemotherapy agents at the experimental dosage levels and highlight the need for multiple orexigenic agents to address this heterogeneous toxicity.

Consistent with previous reports (11, 57), tissue wasting was observed following 3 cycles of chemotherapy of cisplatin, 5-FU, CP, or vincristine. Body composition analyses revealed substantially lower fat and lean mass among rats undergoing chemotherapy, including a dramatic depletion in fat mass. Furthermore, we observed cardiac and skeletal muscle loss following chemotherapy treatment. TCMCB07 treatment protected heart mass following cisplatin or 5-FU and slightly improved lean mass across all chemotherapy-treated models. This predicts a clinical benefit of melanocortin antagonism in patients receiving these agents and provides a rationale for clinical investigation of this therapeutic modality. In contrast, we did not observe a marked protective effect of TCMCB07 treatment on gastrocnemius mass in all individual studies, consistent with others' observations (15). In our previous cachexia studies with both rat models and dogs (31, 34), TCMCB07 showed muscle improvement, but this was not observed

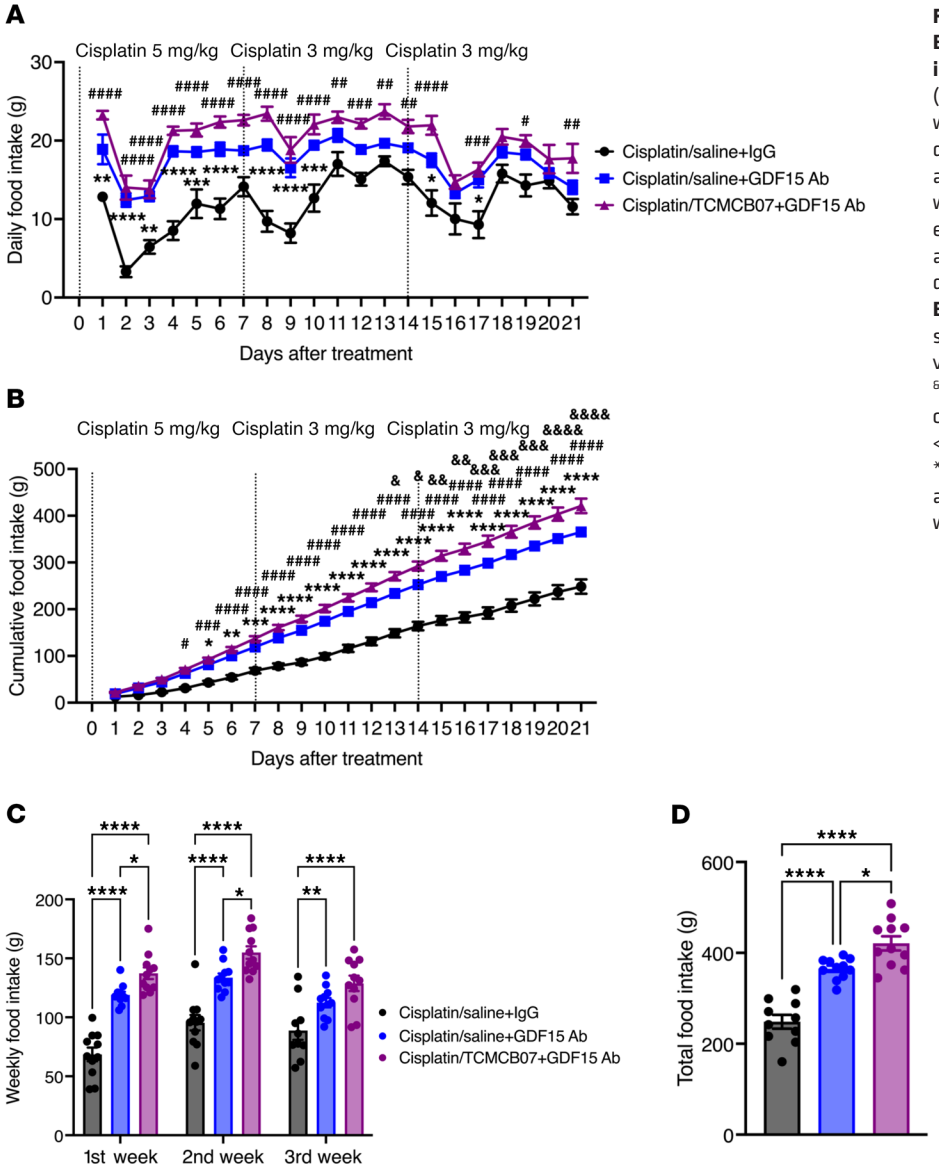

Figure 9. Combination therapy of TCMCB07+GDF15 antibody enhances effectiveness in reversing chemotherapy-induced anorexia. (**A**) Daily and (**B**) cumulative food intake, (**C**) weekly and (**D**) total food intake after a higher dose (5 or 3 mg/kg) of cisplatin chemotherapy and treatment of TCMCB07 in combination with GDF15 antibody. All data in **A** and **B** were expressed as mean ± SEM for each group, and all data in **C** and **D** were expressed with each dot representing 1 sample. n = 11–12. (**A** and **B**), *Cisplatin/saline+IgG versus cisplatin/saline+GDF15 antibody; #Cisplatin/saline+IgG versus cisplatin/TCMCB07+GDF15 antibody; &Cisplatin/saline+GDF15 antibody versus cisplatin/TCMCB07+GDF15 antibody. $^{*,\#,\&}P < 0.05$; $^{**,\#\#,\&\&}P < 0.01$; $^{***,\#\#\#,\&\&\&}P < 0.001$; $^{****,\#\#\#\#,\&\&\&\&}P < 0.0001$. All data in **A**–**C** were analyzed by 2-way ANOVA, and all data in **D** were analyzed by 1-way ANOVA.

in the chemotherapy study. We attribute this to several factors: (a) TCMCB07 does not directly stimulate protein synthesis or promote muscle proliferation and differentiation. Instead, it indirectly aids in muscle preservation by enhancing nutrient availability and general anabolism. (b) Chemotherapy-induced muscle loss occurs due to a reduction in food intake, toxicity-related tissue damage (58, 59), and inhibition of protein synthesis in skeletal muscle (60). (c) The molecular mechanisms of muscle loss induced by chemotherapy may differ from those involved in cancer cachexia-associated muscle wasting (61–64). Therefore, despite adequate nutritional availability, the anabolic potential of chemotherapy-treated skeletal muscle may be impaired.

To enhance the effectiveness and outcomes of interventions targeting the reversal of chemotherapy-induced side effects, it will be inevitable to develop novel treatment strategies that integrate various therapeutic approaches aimed at achieving synergistic effects. Accumulating evidence demonstrates that GDF15 is a key negative mediator of appetite and body weight via its action on the GFRAL receptor and potentially other pathways and receptors. Moreover, circulating GDF15 levels are elevated following chemotherapy with specific agents, such as cisplatin, and beneficial effects were observed by neutralizing the increased GDF15 (15). In our study, chemotherapy with 5 agents at the administered doses and regimens resulted in a similar elevation of circulating GDF15. It is reasonable to propose that combining both TCMCB07 and a GDF15 antibody would enhance therapeutic effectiveness. While TCMCB07 stimulates feeding and anabolism via the central melanocortin system in the forebrain, the GDF15 antibody attenuates GDF15-GFRAL–mediated feeding suppression in the hindbrain. Using a specific GDF15 antibody, we observed an augmented effectiveness from the combination therapy in rats treated with a higher dose of cisplatin, as evidenced by improved food intake and body weight gain. Additionally, the combined treatment exhibited a more pronounced preservation in fat mass and a positive trend toward an increase in lean mass and heart mass. To our knowledge, this is the first instance of this combinatorial approach being

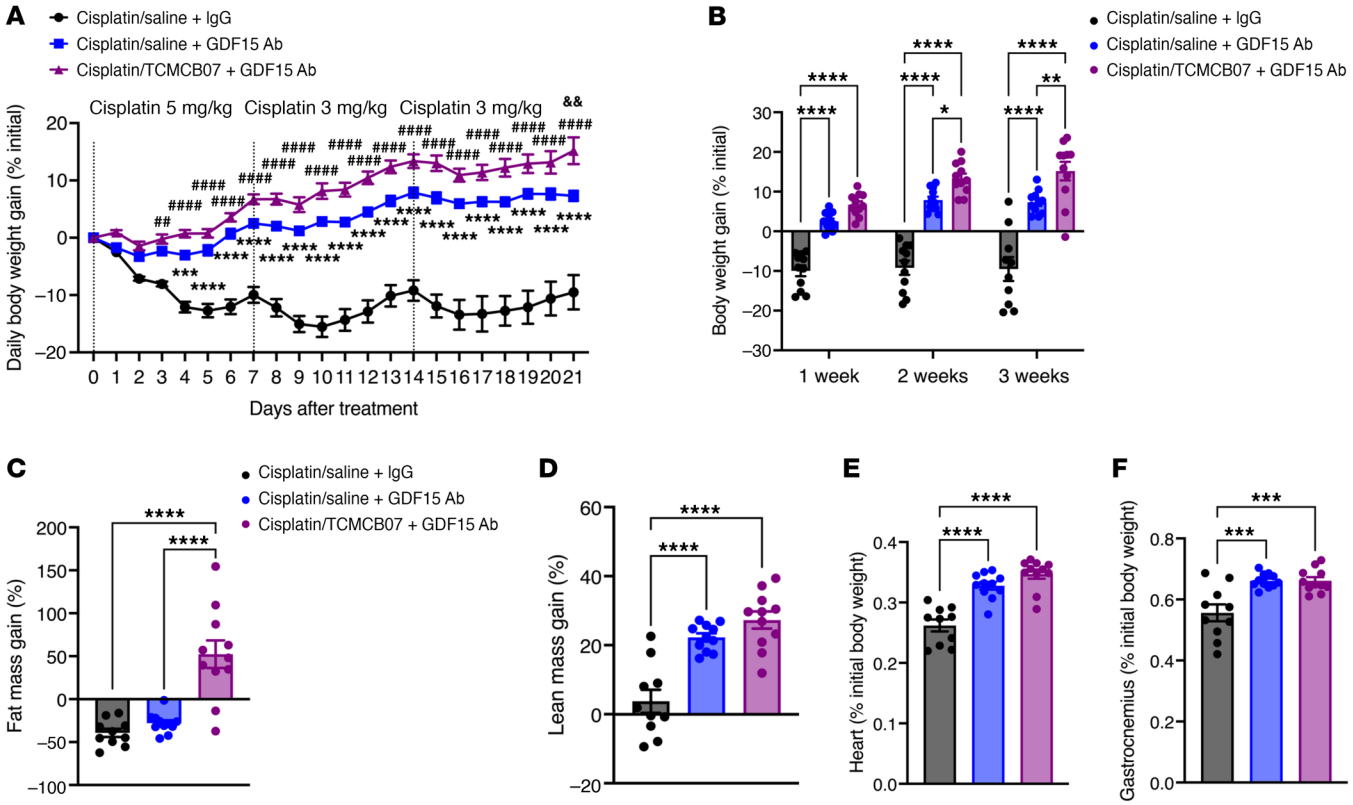

Figure 10. Combination therapy of TCMCB07+GDF15 antibody improves effectiveness in maintaining body and tissue mass during chemotherapy. (**A**) Daily and (**B**) weekly body weight gain (% initial), (**C**) fat and (**D**) lean mass gain (% initial), (**E**) heart and (**F**) gastrocnemius mass (% initial body weight), after treatment of cisplatin+saline+IgG, cisplatin+saline+GDF15 antibody (Ab), or cisplatin+TCMCB07+GDF15 Ab. All data in **A** were expressed as mean ± SEM for each group, and all data in **B**–**F** were expressed with each dot representing 1 sample. n = 10–11. (**A**), *Cisplatin/saline+IgG versus cisplatin/saline+GDF15 antibody; #Cisplatin/saline+IgG versus cisplatin/TCMCB07+GDF15 antibody; &Cisplatin/saline+GDF15 antibody versus cisplatin/TCMCB07+GDF15 antibody. $^{*}P < 0.05$; $^{**,\#\#,\&\&}P < 0.01$; $^{***}P < 0.001$; $^{****,\#\#\#\#}P < 0.0001$. All data in **A** and **B** were analyzed by 2-way ANOVA, and all data in **C**–**F** were analyzed by 1-way ANOVA.

applied to alleviating anorexia and weight loss induced by chemotherapy, supporting the potential for TCMCB07 treatment to be paired with other therapies.

It is essential to assess TCMCB07'S efficacy both with chemotherapy alone and in combination with cancer and chemotherapy. The former context reflects a common use of chemotherapy in the adjuvant setting, when there is no evidence of active disease. Furthermore, testing the effects alongside a single chemotherapy agent in healthy animals is important to identify the specific impact without potential interference from other variables. To better understand the clinical potential of TCMCB07, it is equally important to validate whether these effects persist or change in the presence of cancer and combined chemotherapy regimens (62). In clinical practice, multiple chemotherapy agents are commonly used to treat patients with tumors, both in the palliative and neoadjuvant settings. To mimic this, we utilized the Ward colorectal tumor model in female F344 rats and employed a combination chemotherapy regimen of irinotecan and 5-FU. Notably, female F344 rats exhibit absolute weight loss following chemotherapy due to their slower growth and weight gain compared with Sprague-Dawley (SD) rats (65–67). As previously reported, the combination chemotherapy of irinotecan and 5-FU effectively reduced tumor size in this model. This combination, previously used in multiple rat studies, closely resembles FOLFIRI, a regimen commonly used to treat colorectal cancer (36–39). TCMCB07 administration alleviated anorexia and weight loss in the chemotherapy-treated tumor-bearing rats. Since TCMCB07 has previously shown efficacy in ameliorating cancer cachexia in both rats and dogs (31, 34), it is rational to speculate that TCMCB07 treatment will be beneficial for cancer patients by mitigating both cancer- and chemotherapy-induced anorexia and weight loss.

Given that MC4R antagonists target the central melanocortin system, the ability of a drug to penetrate the BBB is essential for its efficacy. To develop effective drugs in this class, one of the primary challenges is that many peptides exhibit positive effects only with direct central delivery, such as AgRP and SHU9119 (melanocortin antagonists), or melanocortin-II (melanocortin agonist), but have no effects when administered peripherally (68–70). While TCMCB07 levels in the central nervous system were not measured in this study, its consistent ability to stimulate feeding and attenuate weight loss through peripheral treatment strongly implies direct activity within the brain. We found that serum TCMCB07 was detectable within 0.5–2.5 hours after final dosing and that serum concentration correlated with the duration of exposure. Notably, we observed significantly higher concentrations of TCMCB07 in rats receiving chemotherapy, indicating decreased drug metabolism or clearance (41, 45).

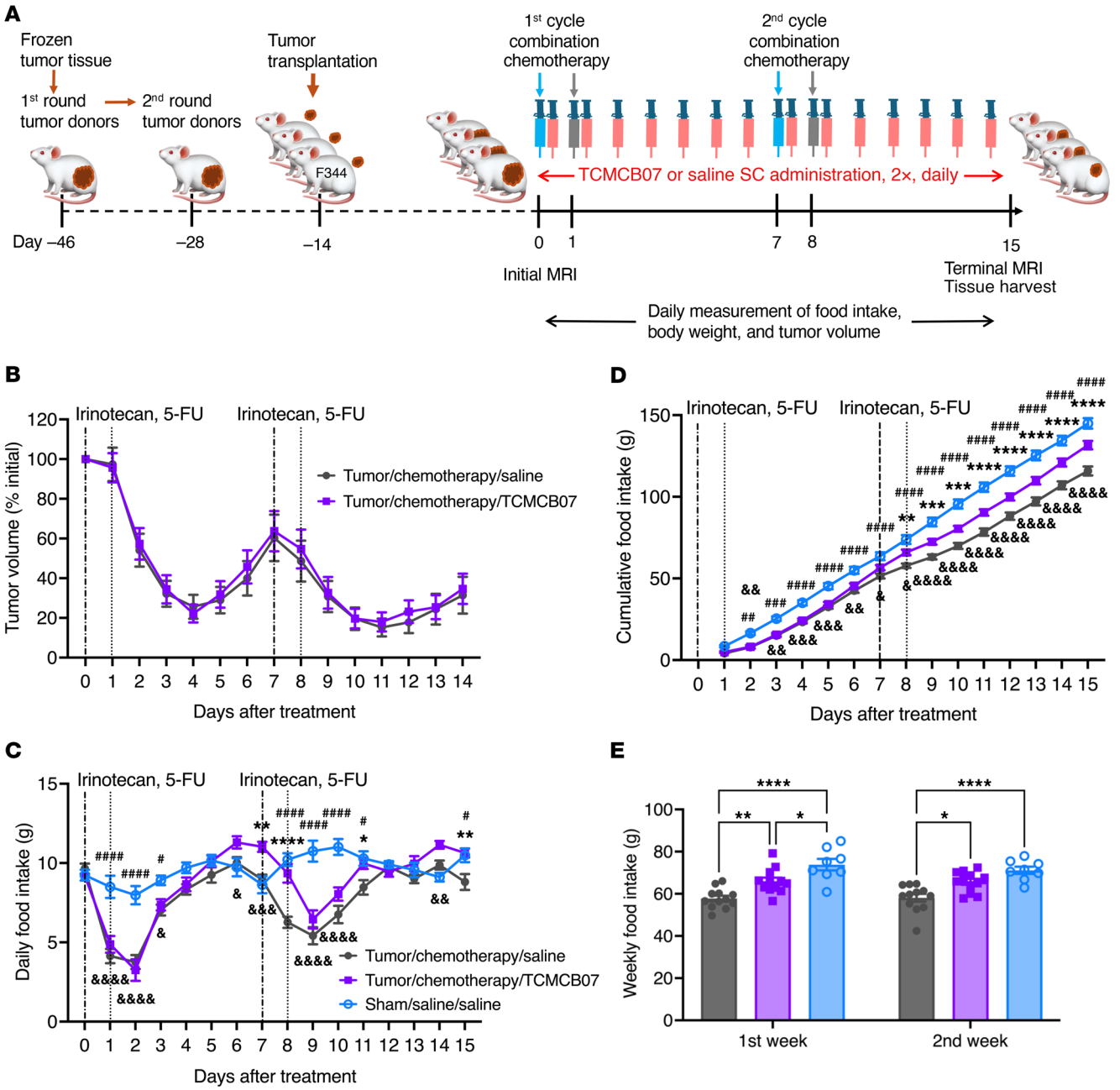

Figure 11. TCMCB07 treatment mitigates anorexia in rats with Ward colorectal tumor following combination chemotherapy. (**A**) Study design schematic. After passing the frozen tumor tissue through 2 rounds of donors, the fresh tumor tissue was s.c. implanted into Fischer (F344) female rats. Two weeks later, the tumor rats received combination chemotherapy via i.p. injection once per week for 2 cycles: irinotecan (50 mg/kg) on day 0 and day 7, and 5-FU (50 mg/kg) on day 1 and day 8. The sham control rats received s.c. sham implantation and i.p. saline injections. Additionally, all rats received s.c. injections twice (2×) daily with either saline or TCMCB07 at a dose of 3 mg/kg from day 0 to day 14. Initial and terminal body composition was measured using MRI prior to and after treatments. Food intake, body weight, and tumor volume were monitored daily from day 0 to day 15. At the end of the experiment, tissues were harvested after euthanasia. After either chemotherapy or saline and either TCMCB07 or saline treatment, (**B**) tumor volume change, (**C**) daily food intake, (**D**) cumulative food intake, and (**E**) weekly food intake were measured. All data in **B**–**D** were expressed as mean ± SEM for each group, and all data in **E** were expressed with each dot representing 1 sample. n = 8–12. (**C** and **D**), *Tumor/chemotherapy/saline versus tumor/chemotherapy/TCMCB07; #Tumor/chemotherapy/saline versus sham/saline/saline; &Tumor/chemotherapy/TCMCB07 versus sham/saline/saline. $^{*,\#,\&}P < 0.05$; $^{**,\#\#,\&\&}P < 0.01$; $^{***,\#\#\#,\&\&\&}P < 0.001$; $^{****,\#\#\#\#,\&\&\&\&}P < 0.0001$. All data in **B**–**E** were analyzed by 2-way ANOVA.

Apart from chemotherapy-induced toxicity, no additional adverse effects were observed during TCMCB07 monotherapy or combination therapy with a GDF15 antibody in rats undergoing chemotherapy. While we previously conducted a series of standard evaluations for TCMCB07's safety in various species, including rats, dogs, and humans (31, 32, 34, 35), this study marks the first time we have tested the drug candidate in combination with chemotherapy agents. Encouragingly, we observed no increased toxicity when TCMCB07 was combined with other chemotherapy agents, even during a prolonged administration period of 21 days.

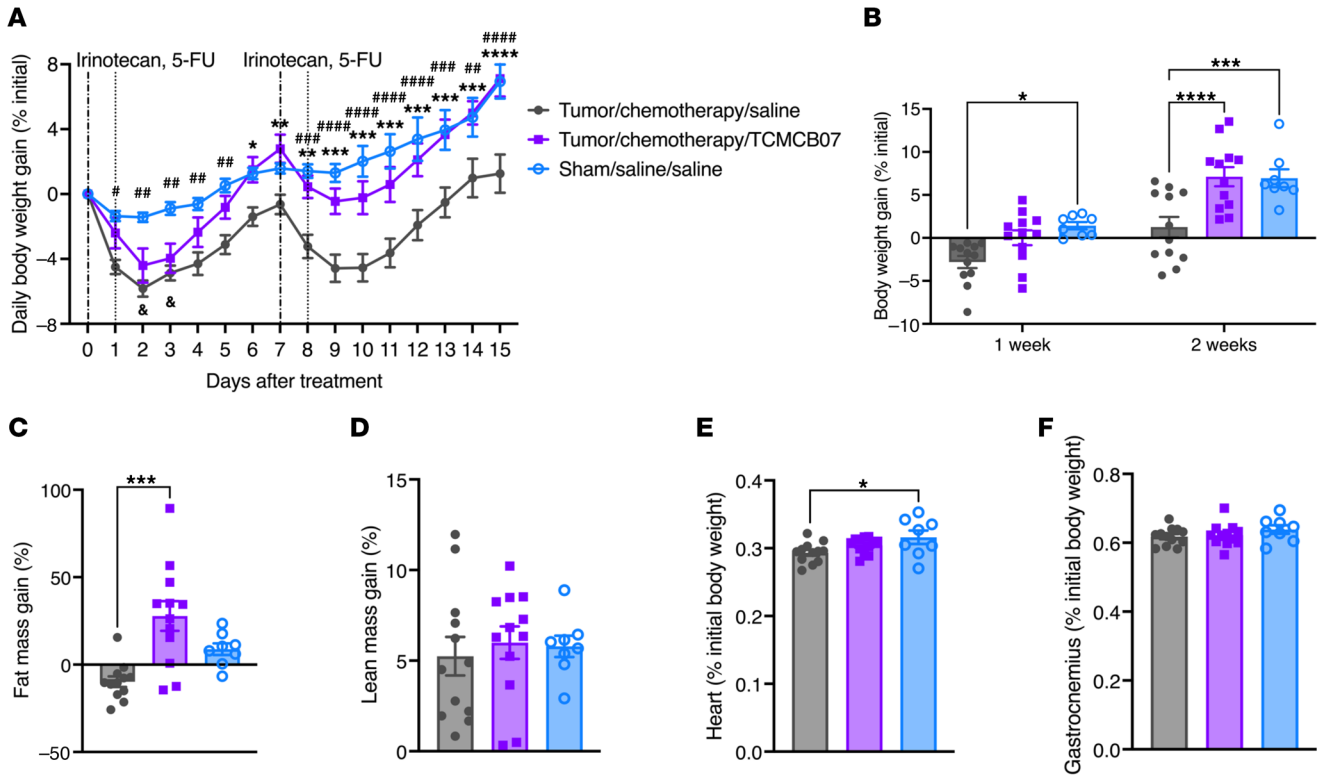

Figure 12. TCMCB07 treatment alleviates weight loss and tissue wasting in rats with Ward colorectal tumor following combination chemotherapy. (**A**) Daily and (**B**) weekly body weight gain (% initial), (**C**) fat and (**D**) lean mass gain (% initial), (**E**) heart and (**F**) gastrocnemius mass (% initial body weight). All data in **A** were expressed as mean ± SEM for each group, and all data in **B**–**F** were expressed with each dot representing 1 sample. n = 8–12. (**A**), *Tumor/chemotherapy/saline versus tumor/chemotherapy/TCMCB07; #Tumor/chemotherapy/saline versus sham/saline/saline; &Tumor/chemotherapy/TCMCB07 versus sham/saline/saline. $^{*,\#,\&}P < 0.05$; $^{**,\#\#}P < 0.01$; $^{***,\#\#\#}P < 0.001$; $^{****,\#\#\#\#}P < 0.0001$. All data in **A** and **B** were analyzed by 2-way ANOVA, and all data in **C**–**F** were analyzed by 1-way ANOVA.

This study had several key limitations. First, we predominantly used SD rats in our experiments, chosen for their widespread use in various research fields, easy availability, and cost effectiveness compared with inbred strains (65). These characteristics made SD rats a suitable choice for our studies, which were conducted across multiple locations in both the USA and China. However, like other common outbred strains such as Wistar, SD rats naturally exhibit continuous weight gain during the ages used in our studies (65–67). Male SD rats, even at 15 weeks old and weighing over 500 grams, continue to gain weight rapidly. The rapid and continuous weight gain in SD rats presented a challenge, as chemotherapy inhibited weight gain instead of inducing absolute weight loss. Although substantial relative weight loss was observed compared with nonchemotherapy-treated animals in all experiments, this limitation introduces some confusion regarding translational relevance and clinical implications. To address this, we used an inbred strain — female F344 rats — and further validated the effects of TCMCB07 following tumor growth and combination chemotherapy. Due to their slower growth rate, F344 rats allowed us to model absolute weight loss relative to baseline following chemotherapy and confirmed the ability of TCMCB07 to reverse this weight loss. Second, although i.p. injection is the most commonly used method in rodent chemotherapy models, the i.v. route better reflects clinical practice. However, we chose i.p. injection due to the considerable challenges associated with i.v. administration in rats. Unlike in clinical settings, i.v. injection in rats is technically challenging and can cause considerable stress-induced changes in feeding behavior. While our models effectively replicated chemotherapy-induced side effects such as anorexia and weight loss, we recognize that i.p. injection may result in different pharmacokinetics compared with i.v. injection (62), potentially affecting the animals' responses and the interactions between chemotherapy and TCMCB07 treatment. Third, selecting doses of the 6 chemotherapeutic agents that reflect clinical doses posed a substantive challenge. While it is important to consider clinical doses (converted from mg/m^2 to mg/kg for animals)

Table 1. TCMCB07 doses and serum concentrations in rats treated with cisplatin chemotherapy

Group (n = 10–11)	TCMCB07 doses (mg/kg/day, s.c. injection)	TCMCB07 serum concentrations (μg/mL)
Saline/saline	0.0	0.00
Saline/TCMCB07	3.0	1.48 ± 0.16
Cisplatin/saline	0.0	0.00
Cisplatin/TCMCB07	3.0	3.34 ± 0.37[A]

At the end of 21-day experiments, serum was collected after rats were euthanized. Serum TCMCB07 concentrations were quantitated. Data for the serum concentrations are expressed as mean ± SEM for each group. [A]$P < 0.001$ versus saline/TCMCB07 group.

to enhance translational relevance and clinical implications, the biological differences between humans and rats are equally critical. Rats are not simply miniature versions of humans (66). Although the doses of each single agent used in this study were similar to the clinical doses (42), confirming whether these doses are equivalent or substantially different from those used in clinical settings is difficult due to differences in tolerance and metabolism between species. We instead relied upon titrating dose to the desired adverse effects — anorexia and weight loss — which we feel provides a more relevant translational model. Further studies in humans are required to ascertain whether the beneficial effects we observed in these preclinical studies will translate to patients.

Conclusion. In conclusion, this preclinical study demonstrates that peripheral administration of TCMCB07 increases food intake, mitigates weight loss, and reduces tissue wasting over multiple cycles of various chemotherapy regimens, including combination chemotherapy in a tumor model that mirrors clinical scenarios. Moreover, the combination of TCMCB07 with a GDF15 antibody enhances treatment outcomes. These findings highlight TCMCB07 as a promising drug candidate with strong potential to alleviate chemotherapy-induced anorexia and weight loss, aligning with previous studies on its effectiveness in treating cachexia. Additionally, this study provides preliminary evidence supporting the potential of TCMCB07, in combination with other drugs, to effectively combat severe anorexia and weight loss induced by chemotherapy. TCMCB07 is expected to benefit many cancer patients undergoing chemotherapy.

Methods

Sex as a biological variable. Our study included both male and female animal models, with similar findings observed across sexes. Since the primary purpose was to evaluate the efficacy of TCMCB07 on behavioral outcomes such as food intake and body weight, male rats were initially chosen for the chemotherapy-alone experiments due to their lower variability in behavioral phenotypes. In the tumor-plus-chemotherapy experiments, female rats were selected to ensure that the findings from males could be generalized to females.

Rats. SD male rats at age of 8 weeks weighing 200–225 g and F344 female rats at age of 12 weeks weighing 130–140 g were obtained from Charles River Laboratories or Beijing Vital River Laboratory Technology and housed in animal facilities with controlled conditions, including a temperature of 20–22^{0}C and a 12-hour light/12-hour dark cycle. The rats had ad libitum access to water and food (Purina Rodent Diet 5001; Purina Mills). After being individually housed for at least 7 days for acclimation, before experiments, rats were assigned into treatment and control groups using body weight (day 0) to counterbalance groups. Rats receiving chemotherapy were monitored closely and euthanized when reaching the endpoints set by the chemotherapy study policy. Our study examined both male and female rats to test whether there was sexual dimorphism in response to chemotherapies and TCMCB07 treatment.

TCMCB07 compound and administration. TCMCB07 was designed and provided by Endevica Bio. According to previous observations, an effective dose of TCMCB07 at 3 mg/kg/d was chosen, and administration route was s.c. injection, to avoid first pass metabolism. To maintain the circulating concentration, 1 dose of TCMCB07 was split into 2 s.c. injections (1.5 mg/kg × 2 injections) performed in the morning (9–10 am) and the evening (5–6 pm). Control animals received an equivalent volume of saline via s.c. injections.

Chemotherapy agents and regimen. The following 6 medical grade chemotherapy agents were used: cisplatin (Teva), 5-FU (Xiromed, LLC), CP (Jiangsu Hengrui Pharmaceuticals), vincristine (Shenzhen Main Luck Pharmaceuticals, doxorubicin (ShanXi PUDE Pharmaceuticals, China), and irinotecan (Pfizer, PGS Pearl River). The concentrations of the agents were as follows: cisplatin, 1 mg/mL; 5-FU, 50 mg/mL; and irinotecan, 20 mg/mL. Upon reconstitution, the concentrations of the agents were as follows: CP, 20 mg/mL (in saline); vincristine, 0.2 mg/mL (in PBS); and doxorubicin, 2 mg/mL (in saline). Atropine (1 mg/kg, s.c.) was administered immediately prior to each irinotecan injection to alleviate early onset cholinergic symptoms (71). The doses of chemotherapy agents were selected through a series of dose-response experiments aiming to induce 10% to 30% weight loss compared with rats not receiving chemotherapy (15). In addition, these selected doses did not result in severe morbidity or mortality based on the observations from our doses-response experiments and the literature. The selected doses were as follows: cisplatin, 2.5, 3.0, or 5.0 mg/kg (with the 5.0 mg/kg dose given only during the first cycle); 5-FU, 70 or 50 mg/kg (with the dose of 50 mg/kg administered in the combination with irinotecan); CP, 65 mg/kg; vincristine, 0.27 mg/kg; doxorubicin, 2.0 mg/kg; and irinotecan 50 mg/kg in combination with 5-FU. All chemotherapy agents were accurately administered via i.p. injection using insulin syringes, once per week for a total of 3 cycles of a single chemotherapy or 2 cycles of combination chemotherapy. For the combination chemotherapy, irinotecan was administered 24 hours before 5-FU administration (38). Control animals received an equivalent volume of saline via i.p. injections. Data collected from animals reaching euthanasia criteria before the designed experimental endpoint were excluded from statistical analysis.

GDF15 antibody and administration. Monoclonal anti-GDF15 antibody (mAB1, catalog CHB034) was manufactured by Sanyou Biopharmaceuticals Co. Ltd., based on the published protein sequence of the mAB1 GDF15 antibody, (GDF15-001, Ponsegromab, Pfizer). The control antibody (rat IgG2a, catalog CHB001-3) was also produced by Sanyou Bio. The concentrations of both anti-GDF15 antibody and IgG2a control were verified by the manufacturer. The anti-GDF15 antibody or IgG2a control was administered at a dose of 10 mg/kg via s.c. injection, twice per week for a total of 3 weeks. The dose was determined based on previous reports (15).

Ward tumor model. Rat Ward colorectal carcinoma tissue was provided by Vickie E. Baracos, and the rat tumor model was generated as described in previous studies (36–40). Briefly, the frozen Ward tumor tissue was s.c. implanted into the first round of F344 rat donors. Eighteen days later, the fresh tumor tissue from the donors was transplanted into the second round of donors to grow tumors for 14 days. Fresh tumor fragments (0.05 g) were s.c. implanted into the right flank of experimental F344 rats via a trocar under slight isoflurane anesthesia. s.c. implantation was chosen to facilitate continuous evaluation of tumor growth and response to the chemotherapy. Rats in the sham control group received s.c. PBS injection. Tumor volume was monitored every other day prior to chemotherapy and daily after chemotherapy. Tumors were measured in 3 dimensions with a caliper: the length (L), the width (W), the height (H). The tumor volume was calculated as follows: tumor volume (cm^3) = 0.5 × L (cm) × W (cm) × H (cm) (37, 72). After approximately 2 weeks of tumor growth, when tumor volume reached approximately 2 cm^3, irinotecan and 5-FU combination chemotherapy and TCMCB07 treatment were initiated. During 2 cycles of the combination chemotherapy, tumor volume change (%) in each tumor rat was compared with the baseline volume (day 0) (37).

Food intake and body weight measurement. We measured food intake and body weight daily at the same time of day (3 hours after lights on) from the day of starting treatment (day 0) until the day of termination. Because chemotherapy-related side effects distress animals, some sick rats produced considerable food spillage (orts or crumbs) as the experiments progressed. To ensure accurate measurement of food intake, we accounted for uningested food loss by screening cage bedding and subtracting orts from total food amount reduction. We also monitored the overall health condition of animals daily to ensure no animals were moribund.

Body composition analysis. Body composition (fat mass and lean mass) was analyzed twice on the day of treatment and the end of study prior to tissue collection via EchoMRI (4-in-1, Live Animal Composition Analyzer; Echo Medical System).

Blood and tissue collection. When experimental animals reached terminal time points, we conducted blood and tissue collection immediately after the terminal MRI scan. Animals were deeply anesthetized with isoflurane, and blood was collected via cardiac puncture. Approximately 200 μL of blood was placed into a 1 mL EDTA blood collection tube for hematology assay, and the remaining blood was placed in a 10 mL serum collection tube. Serum was isolated, aliquoted, and stored at –80°C until analysis. Additionally, we dissected and weighed the heart and gastrocnemii from bilateral hind limbs. The average of both gastrocnemius masses was used in the final analysis.

Hematology assay. Whole blood was subjected to analysis using a veterinary hematology analyzer (HemaVet, 950FS, Drew Scientific) to measure various hematological parameters, including total leukocyte counts, leukocyte differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), erythrocytes, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and thrombocytes.

ELISA. Concentrations of GDF-15 in rat serum were determined using ELISA kits following the manufacturer's instructions (R&D Systems, catalog DY957).

Quantitation of serum TCMCB07. At the end of the 21-day studies, rats were euthanized and blood was collected within 0.5–2.5 hours after the last TCMCB07 or saline injection. The serum samples were submitted to the Charles W. Gehrke Proteomics Center at University of Missouri-Columbia for analysis of TCMCB07 concentration via LC-MS/MS and LC-MRM. A standard curve was established using the same batch of TCMCB07 used in the animal experiments. Nontreated rat serum samples served as the blank control. A client-specific method was developed for TCMCB07 quantitation. The serum TCMCB07 concentrations (μg/mL) were reported.

Statistics. Statistical analyses were conducted using GraphPad Prism 10.0 software. Quantitative data are reported as mean ± SEM. To compare 2 groups, a 2-tailed unpaired Student's *t* test was used. When comparing more than 2 groups, 1-way ANOVA was utilized. For comparing multiple time points and treatment groups, unless otherwise specified in the figure legends, 2-way ANOVA with Tukey's multiple-comparisons test was used. Statistical significance was considered at $P < 0.05$ for all data analyses. All measurements were taken from distinct samples, ensuring that no duplications occurred from the same samples.

Study approval. Animal studies were approved by the IACUC of the Oregon Health & Science University and conducted according to the NIH *Guide for the Care and Use of Laboratory Animals* (National Academies Press, 2011). All experiments were approved by WuXi IACUC standard animal procedures (IACUC protocol number GP02-QD009-2022v1.0.). Euthanasia criteria were adhered to as per the IACUC study protocol.

Data availability. All data associated with this study are available in the main text, main figures, and supplemental materials, or Supporting Data Values file. There are no restrictions on data availability.

Author contributions

XZ, DLM, and RP conceived and designed the study. KAG designed TCMCB07. XZ, SDN, MAN, PRL, PB, XC, and QG conducted experiments. EZ organized and oversaw some of the experiments conducted in WuXi, China. XZ, PB, and DLM analyzed the data. AJG contributed to discussion and data interpretation. XZ wrote the manuscript with input from the other authors. DLM and AJG reviewed and edited the manuscript. All authors approved the final version of the manuscript.

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1. [No authors listed]. Advancing cancer therapy. *Nat Cancer*. 2021;2(3):245–246.
2. Anand U, et al. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis*. 2023;10(4):1367–1401.
3. Kalyanaraman B. Teaching the basics of cancer metabolism: Developing antitumor strategies by exploiting the differences between normal and cancer cell metabolism. *Redox Biol*. 2017;12:833–842.
4. Kayl AE, Meyers CA. Side-effects of chemotherapy and quality of life in ovarian and breast cancer patients. *Curr Opin Obstet Gynecol*. 2006;18(1):24–28.
5. Moore A. Metabolic cycles in cancer cells? *Bioessays*. 2020;42(4):e2000048.
6. Hesketh PJ, et al. Preventing chemotherapy-induced nausea and vomiting in patients with lung cancer: efficacy of NEPA (netupitant-palonosetron), the first combination antiemetic. *Support Care Cancer*. 2018;26(4):1151–1159.
7. Raji MA. Management of chemotherapy-induced side-effects. *Lancet Oncol*. 2005;6(6):357.
8. Viale PH. Chemotherapy-induced nausea and vomiting: updates and recommendations. *J Adv Pract Oncol*. 2018;9(2):150–152.
9. Borner T, et al. GDF15 induces anorexia through nausea and emesis. *Cell Metab*. 2020;31(2):351–362.
10. Emmerson PJ, et al. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat Med*. 2017;23(10):1215–1219.
11. Hsu JY, et al. Non-homeostatic body weight regulation through a brainstem-restricted receptor for GDF15. *Nature*. 2017;550(7675):255–259.
12. Ling T, et al. Role of growth differentiation factor 15 in cancer cachexia (Review). *Oncol Lett*. 2023;26(5):462.
13. Mullican SE, et al. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat Med*.

2017;23(10):1150–1157.
14. Wischhusen J, et al. Growth/differentiation factor-15 (GDF-15): from biomarker to novel targetable immune checkpoint. *Front Immunol*. 2020;11:951.
15. Breen DM, et al. GDF-15 neutralization alleviates platinum-based chemotherapy-induced emesis, anorexia, and weight loss in mice and nonhuman primates. *Cell Metab*. 2020;32(6):938–950.
16. Barsh GS, Schwartz MW. Genetic approaches to studying energy balance: perception and integration. *Nat Rev Genet*. 2002;3(8):589–600.
17. Cone RD. Anatomy and regulation of the central melanocortin system. *Nat Neurosci*. 2005;8(5):571–578.
18. Garfield AS, et al. Role of central melanocortin pathways in energy homeostasis. *Trends Endocrinol Metab*. 2009;20(5):203–215.
19. Sweeney P, et al. Targeting the central melanocortin system for the treatment of metabolic disorders. *Nat Rev Endocrinol*. 2023;19(9):507–519.
20. Krashes MJ, et al. Melanocortin-4 receptor-regulated energy homeostasis. *Nat Neurosci*. 2016;19(2):206–219.
21. Nuzzaci D, et al. Plasticity of the melanocortin system: determinants and possible consequences on food intake. *Front Endocrinol (Lausanne)*. 2015;6:143.
22. Baldini G, Phelan KD. The melanocortin pathway and control of appetite-progress and therapeutic implications. *J Endocrinol*. 2019;241(1):R1–R33.
23. Girardet C, Butler AA. Neural melanocortin receptors in obesity and related metabolic disorders. *Biochim Biophys Acta*. 2014;1842(3):482–494.
24. Hill JW, Faulkner LD. The role of the melanocortin system in metabolic disease: new developments and advances. *Neuroendocrinology*. 2017;104(4):330–346.
25. Dallmann R, et al. The orally active melanocortin-4 receptor antagonist BL-6020/979: a promising candidate for the treatment of cancer cachexia. *J Cachexia Sarcopenia Muscle*. 2011;2(3):163–174.
26. DeBoer MD. Update on melanocortin interventions for cachexia: progress toward clinical application. *Nutrition*. 2010;26(2):146–151.
27. DeBoer MD, Marks DL. Therapy insight: Use of melanocortin antagonists in the treatment of cachexia in chronic disease. *Nat Clin Pract Endocrinol Metab*. 2006;2(8):459–466.
28. Markison S, et al. The regulation of feeding and metabolic rate and the prevention of murine cancer cachexia with a small-molecule melanocortin-4 receptor antagonist. *Endocrinology*. 2005;146(6):2766–2773.
29. Scarlett JM, Marks DL. The use of melanocortin antagonists in cachexia of chronic disease. *Expert Opin Investig Drugs*. 2005;14(10):1233–1239.
30. Sutton GM, et al. A derivative of the melanocortin receptor antagonist SHU9119 (PG932) increases food intake when administered peripherally. *Peptides*. 2008;29(1):104–111.
31. Axiak-Bechtel SM, et al. Safety of TCMCB07, a melanocortin-4 antagonist peptide, in dogs with naturally occurring cachexia. *J Vet Intern Med*. 2023;37(6):2344–2355.
32. Axiak-Bechtel SM, et al. Pharmacokinetics and safety of TCMCB07, a melanocortin-4 antagonist peptide in dogs. *Pharmacol Res Perspect*. 2021;9(3):e00777.
33. Gruber KA, et al. Development of a therapeutic peptide for cachexia suggests a platform approach for drug-like peptides. *ACS Pharmacol Transl Sci*. 2022;5(5):344–361.
34. Zhu X, et al. Melanocortin-4 receptor antagonist TCMCB07 ameliorates cancer- and chronic kidney disease-associated cachexia. *J Clin Invest*. 2020;130(9):4921–4934.
35. Qi LK, et al. Preliminary data from the phase I study of TCMCB07, a study to assess the safety, tolerability and pharmacokinetics of the melanocortin antagonist TCMCB07 in healthy subjects. *J Clin Oncol*. 2023;41(16_suppl):e15195.
36. Almasud AA, et al. Fish oil mitigates myosteatosis and improves chemotherapy efficacy in a preclinical model of colon cancer. *PLoS One*. 2017;12(8):e0183576.
37. Breuillard C, et al. Dietary citrulline does not modify rat colon tumor response to chemotherapy, but failed to improve nutritional status. *Clin Nutr*. 2021;40(7):4560–4568.
38. Cao S, Rustum YM. Synergistic antitumor activity of irinotecan in combination with 5-fluorouracil in rats bearing advanced colorectal cancer: role of drug sequence and dose. *Cancer Res*. 2000;60(14):3717–3721.
39. Xue H, et al. Single and combined supplementation of glutamine and n-3 polyunsaturated fatty acids on host tolerance and tumour response to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil chemotherapy in rats bearing Ward colon tumour. *Br J Nutr*. 2009;102(3):434–442.
40. Xue H, et al. Nutritional modulation of antitumor efficacy and diarrhea toxicity related to irinotecan chemotherapy in rats bearing the ward colon tumor. *Clin Cancer Res*. 2007;13(23):7146–7154.
41. Abd El-Rhman RH, et al. Dibenzazepine attenuates against cisplatin-induced nephrotoxicity in rats: involvement of NOTCH pathway. *Front Pharmacol*. 2020;11:567852.
42. Aston WJ, et al. A systematic investigation of the maximum tolerated dose of cytotoxic chemotherapy with and without supportive care in mice. *BMC Cancer*. 2017;17(1):684.
43. Hu C, et al. Antinociceptive effects of fucoidan in rat models of vincristine-induced neuropathic pain. *Mol Med Rep*. 2017;15(2):975–980.
44. Kamiya A, et al. Administration of cyclophosphamide to rats induces pica and potentiates 5-hydroxytryptamine synthesis in the intestine without causing severe intestinal injury. *J Pharmacol Sci*. 2021;147(3):251–259.
45. Perše M, Večerić-Haler Ž. Cisplatin-induced rodent model of kidney injury: characteristics and challenges. *Biomed Res Int*. 2018;2018:1462802.
46. VanderVeen BN, et al. The acute effects of 5 fluorouracil on skeletal muscle resident and infiltrating immune cells in mice. *Front Physiol*. 2020;11:593468.
47. Yazbeck R, et al. Combined effects of muricid extract and 5-fluorouracil on intestinal toxicity in rats. *Evid Based Complement Alternat Med*. 2015;2015:170858.
48. Podyacheva EY, et al. Analysis of models of doxorubicin-induced cardiomyopathy in rats and mice. A modern view from the perspective of the pathophysiologist and the clinician. *Front Pharmacol*. 2021;12:670479.
49. Timm KN, et al. Early detection of doxorubicin-induced cardiotoxicity in rats by its cardiac metabolic signature assessed with hyperpolarized MRI. *Commun Biol*. 2020;3(1):692.
50. Chelette B, et al. The GDF15-GFRAL axis mediates chemotherapy-induced fatigue in mice. *Brain Behav Immun*. 2023;108:45–54.
51. Nurgali K, et al. Editorial: adverse effects of cancer chemotherapy: anything new to improve tolerance and reduce sequelae? *Front Pharmacol*. 2018;9:245.
52. Sandhya L, et al. Randomized double-blind placebo-controlled study of olanzapine for chemotherapy-related anorexia in patients with locally advanced or metastatic gastric, hepatopancreaticobiliary, and lung cancer. *J Clin Oncol*. 2023;41(14):2617–2627.
53. Perše M. Cisplatin mouse models: treatment, toxicity and translatability. *Biomedicines*. 2021;9(10):1406.
54. Alotayk LI, et al. Comparative evaluation of doxorubicin, cyclophosphamide, 5-fluorouracil, and cisplatin on cognitive dysfunction in rats: Delineating the role of inflammation of hippocampal neurons and hypothyroidism. *Biomed Pharmacother*. 2023;165:115245.
55. Sears SM, et al. C57BL/6 mice require a higher dose of cisplatin to induce renal fibrosis and CCL2 correlates with cisplatin-induced kidney injury. *Am J Physiol Renal Physiol*. 2020;319(4):F674–F685.
56. Marks DL, et al. Role of the central melanocortin system in cachexia. *Cancer Res*. 2001;61(4):1432–1438.
57. Garcia JM, et al. Inhibition of cisplatin-induced lipid catabolism and weight loss by ghrelin in male mice. *Endocrinology*. 2013;154(9):3118–3129.
58. Juthani R, et al. Tumour reoxygenation after intratumoural hydrogen peroxide (KORTUC) injection: a novel approach to enhance radiosensitivity. *BJC Rep*. 2024;2(1):78.
59. Yarana C, St Clair DK. Chemotherapy-induced tissue injury: an insight into the role of extracellular vesicles-mediated oxidative stress responses. *Antioxidants (Basel)*. 2017;6(4):75.
60. Guo B, et al. Chemotherapy agents reduce protein synthesis and ribosomal capacity in myotubes independent of oxidative stress. *Am J Physiol Cell Physiol*. 2021;321(6):C1000–C1009.
61. Kadakia KC, et al. Current therapeutic targets in cancer cachexia: a pathophysiologic approach. *Am Soc Clin Oncol Educ Book*. 2023;43:e389942.
62. Klassen P, et al. Adverse effects of systemic cancer therapy on skeletal muscle: myotoxicity comes out of the closet. *Curr Opin Clin Nutr Metab Care*. 2023;26(3):210–218.
63. Neyroud D, et al. Blocking muscle wasting via deletion of the muscle-specific E3 ligase MuRF1 impedes pancreatic tumor growth. *Commun Biol*. 2023;6(1):519.
64. Sartori R, et al. Perturbed BMP signaling and denervation promote muscle wasting in cancer cachexia. *Sci Transl Med*. 2021;13(605):eaay9592.
65. Brower M, et al. Comparative analysis of growth characteristics of Sprague Dawley rats obtained from

different sources. *Lab Anim Res*. 2015;31(4):166–173.
66. Ghasemi A, et al. The laboratory rat: Age and body weight matter. *EXCLI J*. 2021;20:1431–1445.
67. Sengupta P. The laboratory rat: relating its age with human's. *Int J Prev Med*. 2013;4(6):624–630.
68. Cheung W, et al. Role of leptin and melanocortin signaling in uremia-associated cachexia. *J Clin Invest*. 2005;115(6):1659–1665.
69. Fan W, et al. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature*. 1997;385(6612):165–168.
70. Wisse BE, et al. Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. *Endocrinology*. 2001;142(8):3292–3301.
71. Blandizzi C, et al. Characterization of a novel mechanism accounting for the adverse cholinergic effects of the anticancer drug irinotecan. *Br J Pharmacol*. 2001;132(1):73–84.
72. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol*. 1989;24(3):148–154.