Expanded View Figures

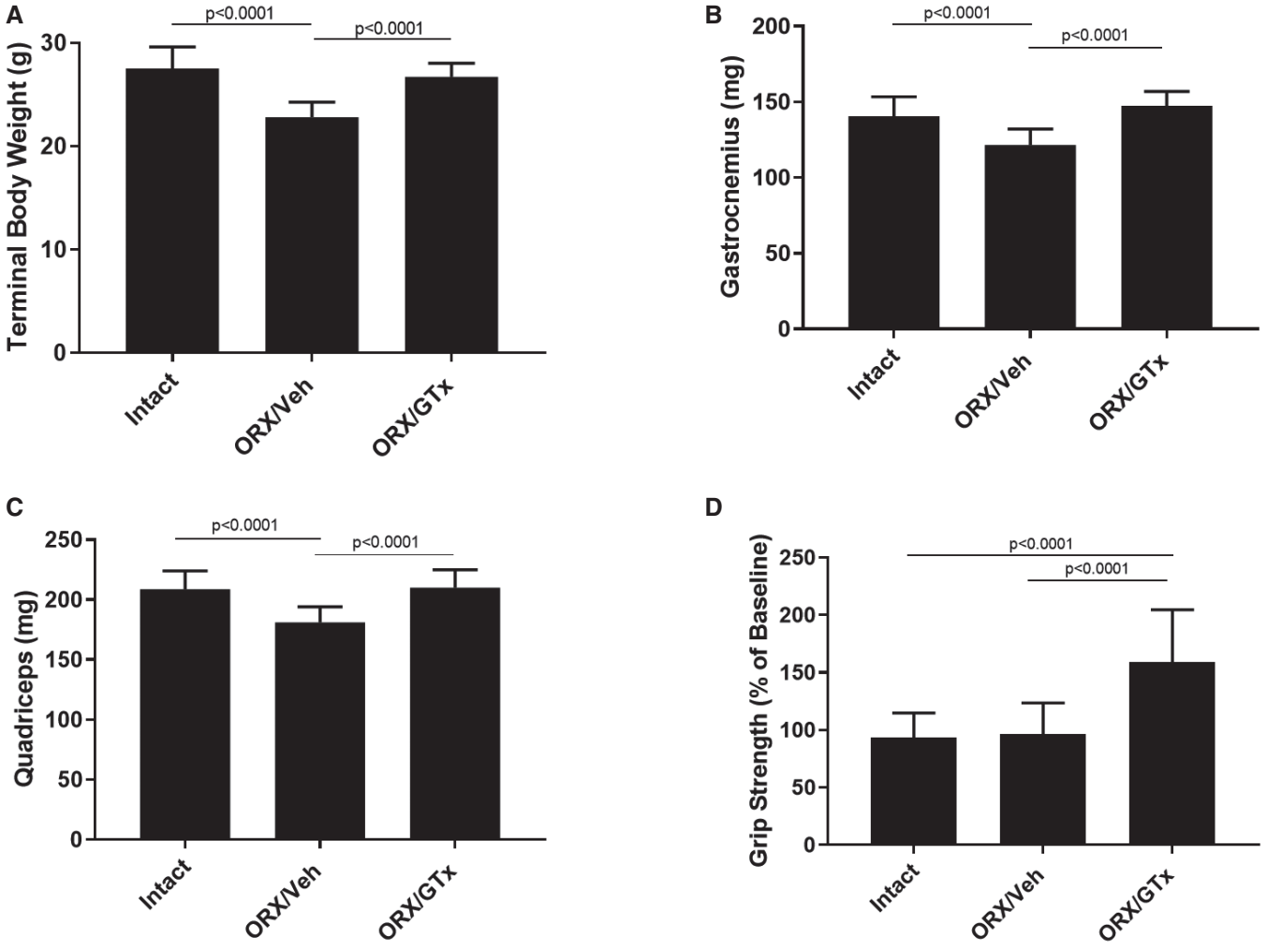

Figure EV1. Confirmation of the anabolic activity of GTx-024.

Orchiectomized (ORX) male CD2F1 mice were treated with GTx-024 (GTx; 15 mg/kg; $n = 12$) or vehicle (Veh; $n = 13$) once daily by oral gavage for 28 days. Sham-orchiectomized (intact) mice ($n = 14$) were treated identically with vehicle.

A Terminal body weights.
B Terminal gastrocnemius weights.
C Terminal quadriceps weights.
D Forelimb grip strength expressed as % baseline defined as (end of study grip strength) × 100/(pre-treatment grip strength).

Data information: *P*-values are provided in Appendix Table S9. One-way ANOVA followed by Tukey's multiple comparisons procedure. Data are presented as means ± SD.

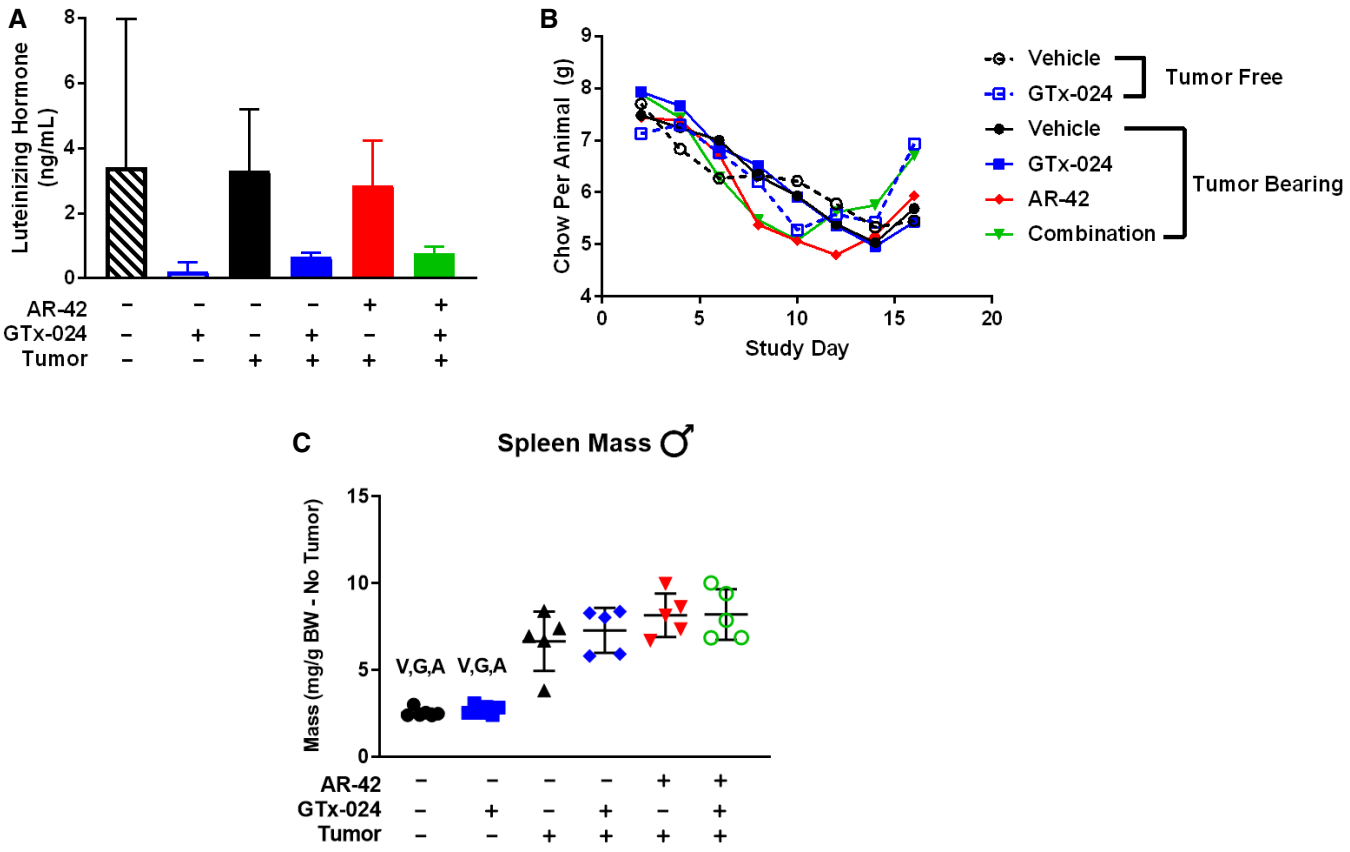

Figure EV2. Anti-cachectic effects of combination therapy in male C-26 tumor-bearing mice.

Study 1, tumor-bearing male mice receiving GTx-024 (15 mg/kg; $n = 5$), AR-42 (10 mg/kg; $n = 5$), combination (15 mg/kg GTx-024 and 10 mg/kg AR-42; $n = 5$), or vehicle ($n = 5$) and tumor-free mice receiving GTx-024 (15 mg/kg; $n = 6$) or vehicle ($n = 6$) were treated daily by oral gavage for 13 days starting 6 days post-injection of C-26 cells.

A Serum-luteinizing hormone levels measured on day 18.
B Per animal food consumption measured every 2 days.
C Spleen weights normalized to tumor mass-corrected terminal body weight.

Data information: V, G, A indicate statically significant differences versus tumor-bearing vehicle-treated, tumor-bearing GTx-024-treated, and tumor-bearing AR-42-treated groups, respectively. *P*-values are provided in Appendix Table S10, one-way ANOVA followed by Tukey's multiple comparison test. Means ± SD, except for (B) in which error bars are not shown to improve clarity of presentation. BW, body weight.

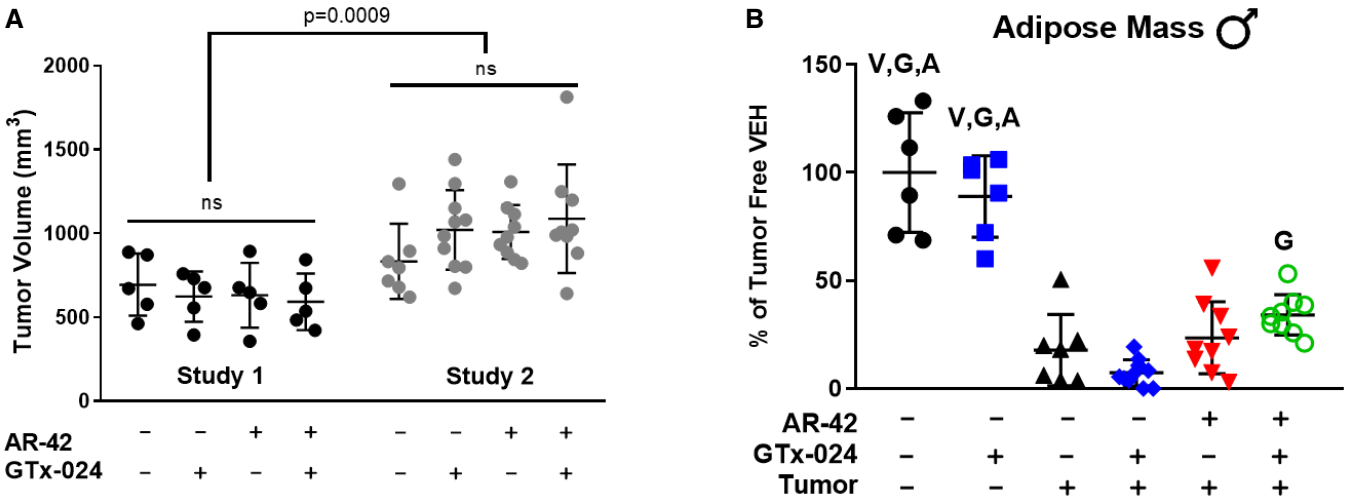

Figure EV3. Terminal tumor volume and fat mass in Study 2.

Study 2, tumor-bearing male mice receiving GTx-024 (15 mg/kg; $n = 10$), AR-42 (10 mg/kg; $n = 9$), combination (15 mg/kg GTx-024 and 10 mg/kg AR-42; $n = 9$) or vehicle ($n = 7$) and tumor-free male mice receiving GTx-024 (15 mg/kg; $n = 6$) or vehicle ($n = 6$) were treated daily by oral gavage for 12 days starting 6 days post-injection of C-26 cells.

A Terminal tumor volume comparisons between the initial (Study 1, day 18) and confirmatory (Study 2, day 17) combination studies. *Statistics:* $P = 0.0009$, Student's *t*-test of combined tumor volumes (Study 1 versus Study 2). ns, no significant differences among treatment groups within each study, one-way ANOVA followed by Tukey's multiple comparison test.

B Terminal epididymal fat pad mass.

Data information: V, G, A indicate statistically significant differences versus tumor-bearing vehicle-treated, tumor-bearing GTx-024-treated, and tumor-bearing AR-42-treated groups, respectively. *P*-values are provided in Appendix Table S11, one-way ANOVA followed by Tukey's multiple comparison test. Data are presented as means ± SD.

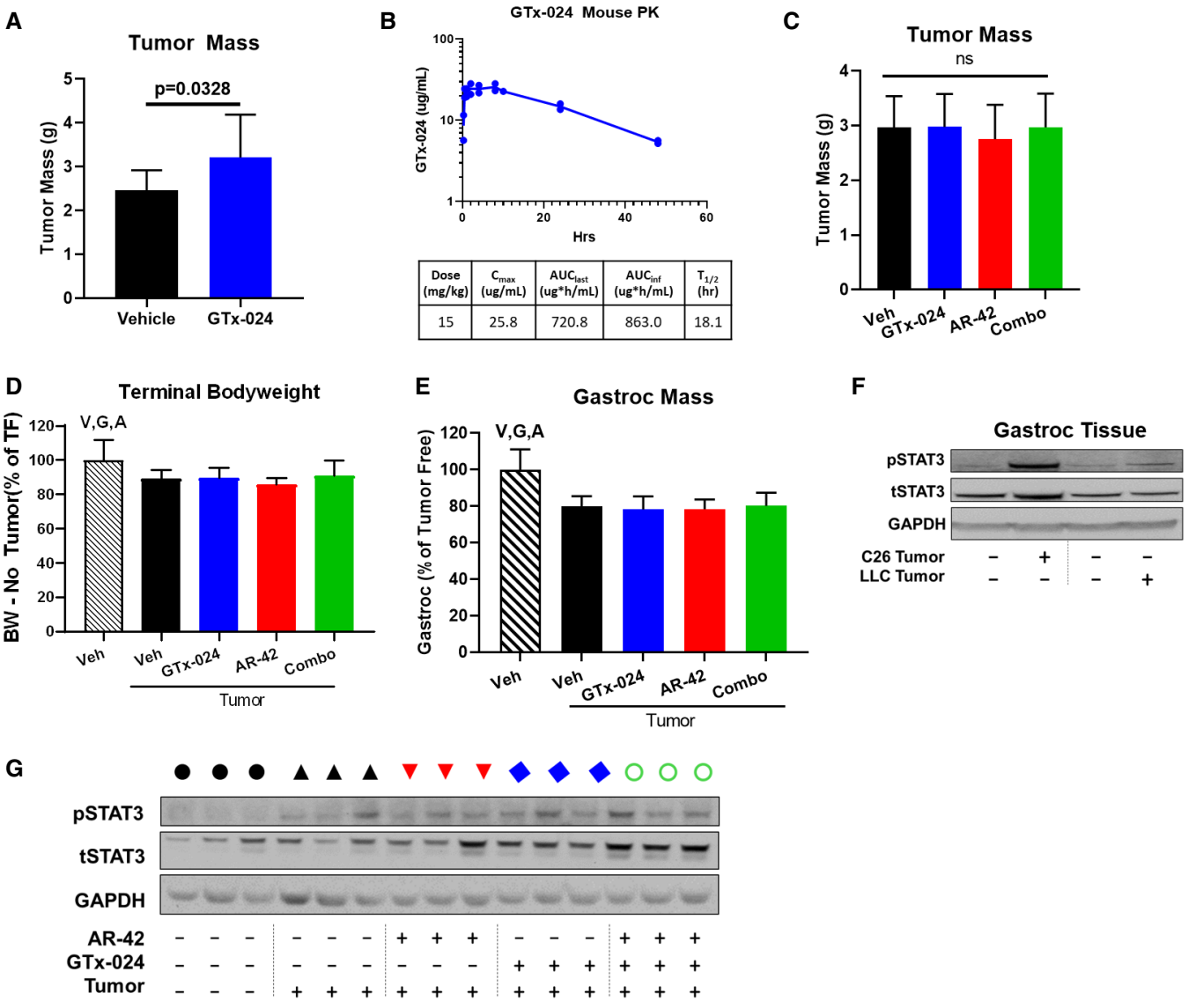

Figure EV4. Anabolic resistance in the LLC model.

Study 5, tumor-bearing male mice receiving GTx-024 (15 mg/kg; $n = 12$), AR-42 (10 mg/kg; $n = 12$), combination (15 mg/kg GTx-024 and 10 mg/kg AR-42; $n = 12$), or vehicle ($n = 12$) and tumor-free mice receiving vehicle ($n = 6$) were treated daily by oral gavage for 14 days starting 6 days post-injection of LLC cells.

A LLC tumor-bearing mice receiving 15 mg/kg GTx-024 monotherapy exhibited larger terminal tumor mass than tumor-bearing vehicle-treated controls.

B Plasma pharmacokinetics of GTx-024 following a single 15 mg/kg oral dose.

C–E Repeat of Study 5 with lower dose of GTx-024. Tumor-bearing male mice receiving GTx-024 (0.5 mg/kg; $n = 12$), AR-42 (10 mg/kg; $n = 12$), combination (0.5 mg/kg GTx-024 and 10 mg/kg AR-42; $n = 12$), or vehicle ($n = 12$) and tumor-free mice receiving vehicle ($n = 6$) were treated daily by oral gavage for 13 days starting 6 days post-injection of LLC cells. (C) Terminal tumor masses (mean ± SD). ns, no significant differences among treatment groups, one-way ANOVA followed by Tukey's multiple comparison test. (D) Terminal body weights corrected for tumor mass compared to tumor-free controls (mean ± SD). (E) Terminal gastrocnemius mass (mean±SD) compared to tumor-free controls.

F Western blot of pSTAT3 (Y705) and total (t)STAT3 in pooled gastrocnemius muscle from C-26 and LLC tumor-bearing animals and associated tumor-free controls.

G Western blot of pSTAT3/tSTAT3 levels in gastrocnemius tissue from representative individual animals from Study 5. Black circle—tumor-free, blue square—tumor-free/GTx-024, black triangle—tumor-bearing, blue diamond—tumor/GTx-024, red triangle—tumor/AR-42, green circle—tumor/combo.

Data information: (A) $P = 0.0328$ versus tumor-bearing vehicle-treated controls. One-way ANOVA followed by Dunnett's multiple comparison test. (C, D) V, G, A indicate statistically significant differences for tumor-free versus tumor-bearing vehicle-treated ($P = 0.0294$), tumor-bearing GTx-024-treated ($P = 0.0327$), and tumor-bearing AR-42-treated groups ($P = 0.0015$), respectively. *P*-values are provided in Appendix Table S12. One-way ANOVA followed by Tukey's multiple comparison test. (E) V, G, A indicate statistically significant differences for tumor-free versus tumor-bearing vehicle-treated ($P < 0.0001$), tumor-bearing GTx-024-treated ($P < 0.0001$), and tumor-bearing AR-42-treated ($P < 0.0001$) groups, respectively. *P*-values are provided in Appendix Table S12. One-way ANOVA followed by Tukey's multiple comparison test.

Source data are available online for this figure.