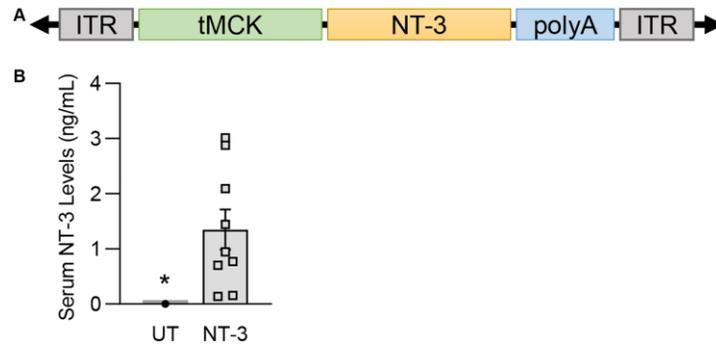
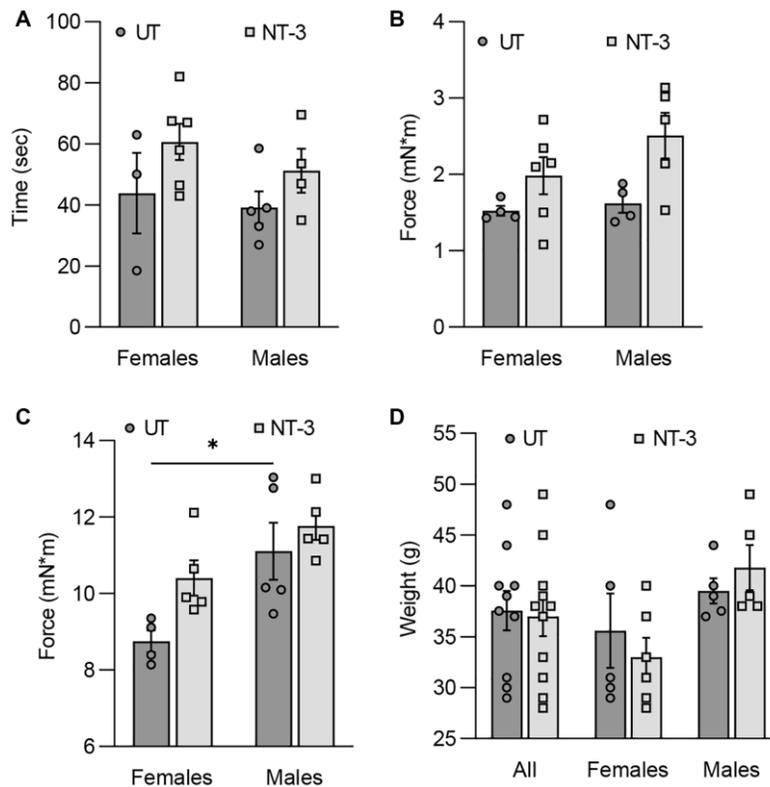


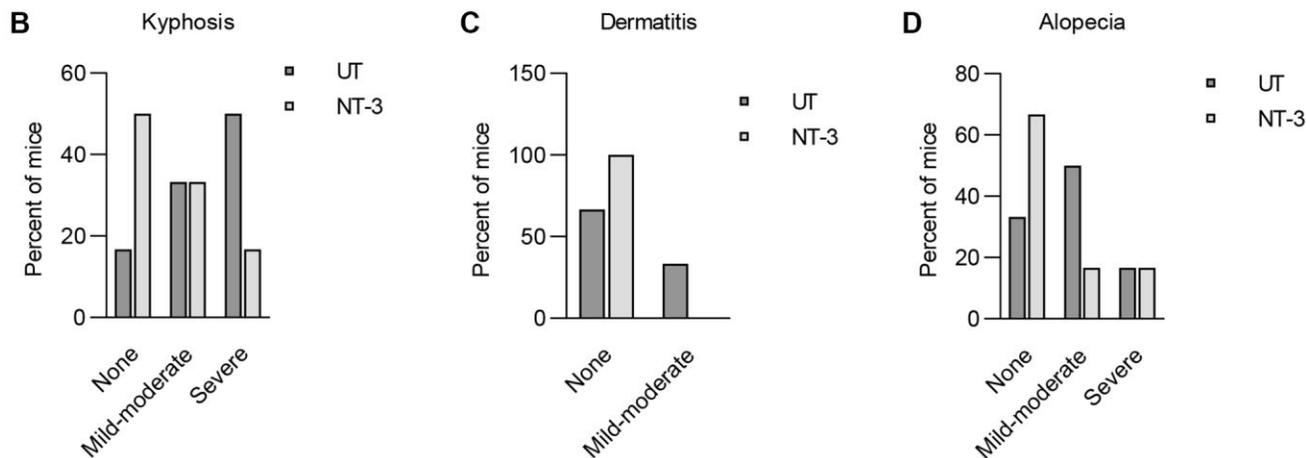
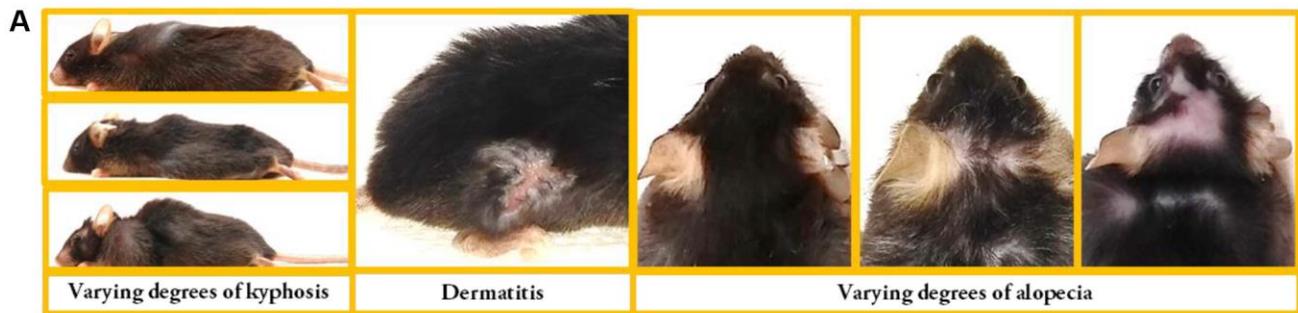
## SUPPLEMENTARY FIGURES



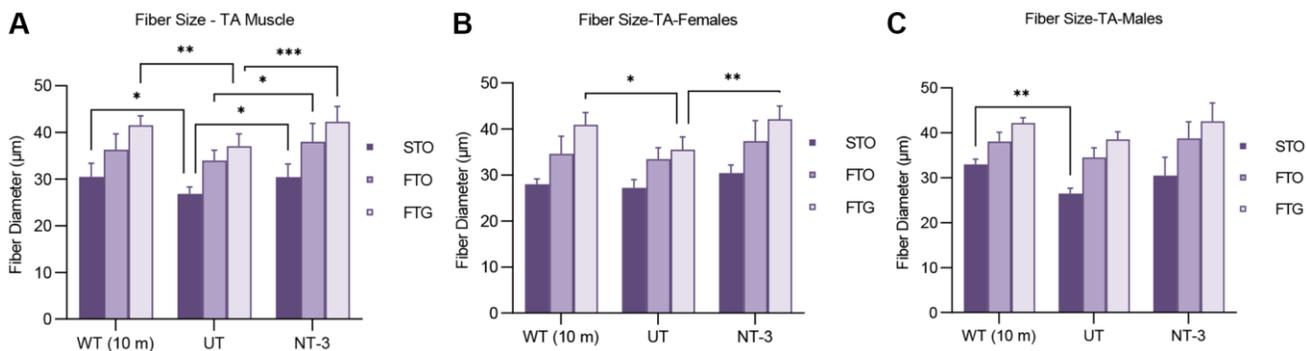
**Supplementary Figure 1. scAAV1.tMCK.NT-3 vector and serum NT-3 levels.** (A) The diagram shows the NT-3 expression cassette composed of a tMCK enhancer/promoter region (714 bp), the full-length NT-3 cDNA (774 bp) and the SV40 polyA tail (211 bp). Diagram is modified from Ozes et al., 2021 [16]. (B) 18 months old C57BL/6 mice were injected with  $1 \times 10^{11}$  vg of scAAV1.tMCK.NT-3 vector, or with Ringer's lactate as control in the right gastrocnemius muscle. At endpoint, serum samples were obtained from treated (NT-3) and untreated (UT) mice via cardiac puncture, and NT-3 levels were determined by ELISA. Error bars are  $\pm$  SEM;  $n = 9$  for treated cohort. Asterisk depicts UT mice ( $n = 8$ ) with NT-3 serum levels below detection range.



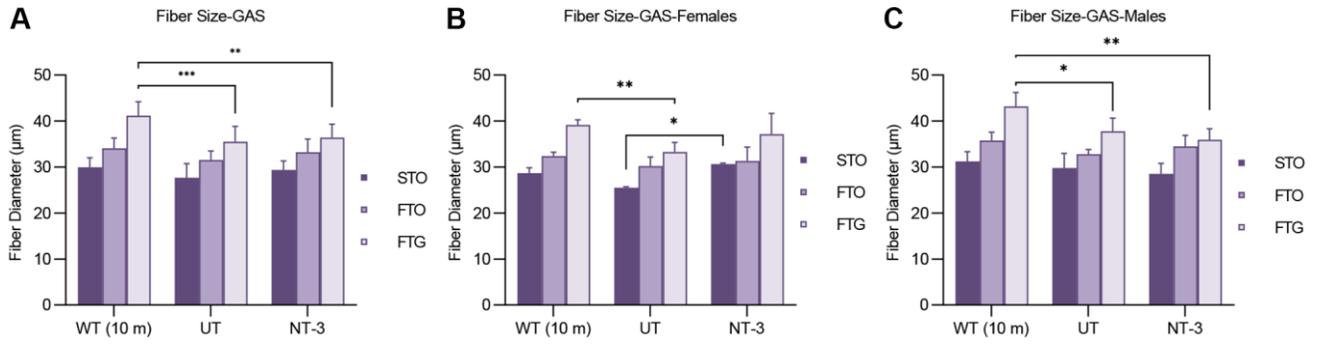
**Supplementary Figure 2. Rotarod and *in vivo* muscle physiology data from 2-year-old male and female C57BL/6 mice.** (A) The bar graphs show sex-separated rotarod data at endpoint (Females: NT-3,  $60.67 \pm 7.80$  sec,  $n = 6$ ; UT,  $43.83 \pm 15.19$  sec,  $n = 3$ ; Males: NT-3,  $51.25 \pm 8.90$  sec,  $n = 4$ ; UT,  $39.10 \pm 7.07$  sec,  $n = 5$ ). Sex-separated *in vivo* muscle contractility data for (B) maximum twitch (Females: NT-3,  $1.984 \pm 0.24$  mN\*m,  $n = 6$ ; UT,  $1.523 \pm 0.06$  mN\*m,  $n = 4$ ; Males: NT-3,  $2.509 \pm 0.30$  mN\*m,  $n = 5$ ; UT,  $1.619 \pm 0.12$  mN\*m,  $n = 4$ ) and (C) maximum tetanic responses (Females: NT-3,  $10.40 \pm 1.04$  mN\*m,  $n = 5$ ; UT,  $8.75 \pm 0.58$  mN\*m,  $n = 4$ ; Males: NT-3,  $11.77 \pm 0.82$  mN\*m,  $n = 5$ ; UT,  $11.11 \pm 1.66$  mN\*m,  $n = 5$ ). (D) The treatment did not alter the endpoint weight of the treated cohort (All: NT-3,  $37.0 \pm 1.95$  g,  $n = 11$ ; UT,  $37.6 \pm 1.94$  g,  $n = 10$ ; Females: NT-3,  $33.0 \pm 1.91$  g,  $n = 6$ ; UT,  $35.6 \pm 3.67$  g,  $n = 5$ ; Males: NT-3,  $41.8 \pm 2.22$  g,  $n = 5$ ; UT,  $39.5 \pm 1.24$  g,  $n = 5$ ). Data is represented as mean  $\pm$  SEM; Two-way ANOVA, Tukey's multiple comparisons test for (A–C) and Sidak's multiple comparisons test for (D);  $*p < 0.05$ .



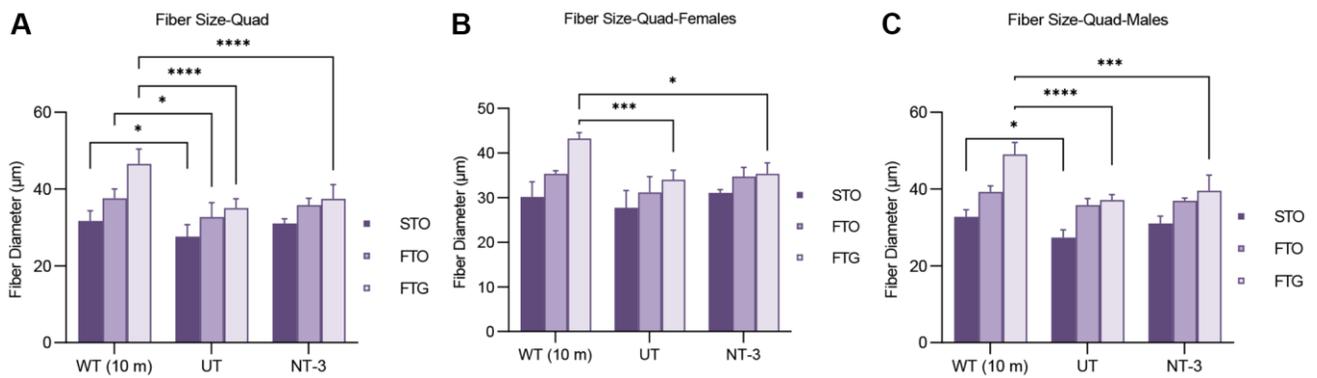
**Supplementary Figure 3. NT-3 improves age-related musculoskeletal and skin changes.** (A) Representative photographs of C57BL/6 mice with various degrees of kyphosis, dermatitis, and alopecia. (B–D) Bar graphs display the percent distribution of the musculoskeletal and skin changes calculated based on the severity scores (0: none, 0.5: mild-moderate, 1: severe). (B) Kyphosis scoring suggested that 50% of the untreated mice had severe kyphosis while this ratio decreased to 16.7% in the treated group. (C) Dermatitis was not observed in the NT-3 treated cohort however 33% of untreated mice had mild-moderate dermatitis. (D) Percent of the moderate to severe alopecia was 67% in the untreated mice whereas this ratio reduced to 33% with treatment ( $n = 6$  for each cohort).



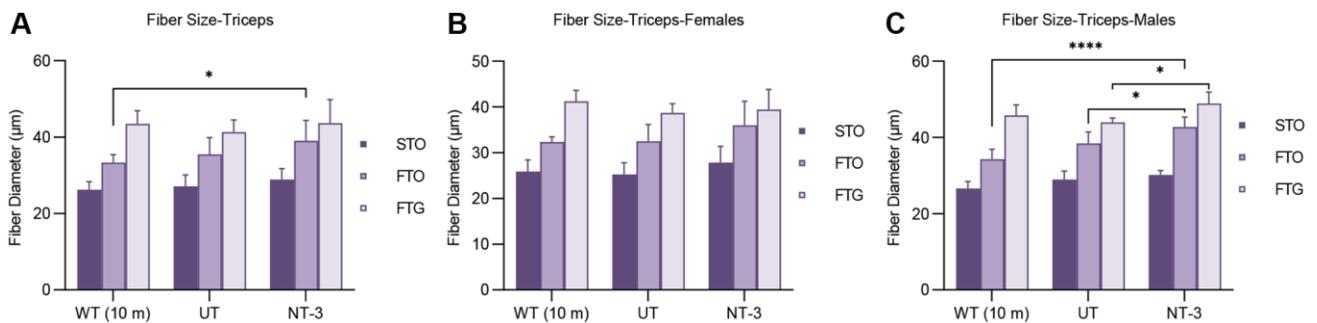
**Supplementary Figure 4. Fiber size analysis of the tibialis anterior muscle.** Fiber size measurements of the tibialis anterior muscle from 10-month-old (mo), 2-year-old untreated and 2-year-old NT-3-treated C57BL/6 mice shown as (A) combined, (B) females only, and (C) males only. Each bar represents mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , 2-way ANOVA, Tukey's multiple comparison test.



**Supplementary Figure 5. Fiber size analysis of the gastrocnemius muscle.** Fiber size measurements of the gastrocnemius muscle from 10-month-old (mo), 2-year-old untreated and 2-year-old NT-3-treated C57BL/6 mice shown as (A) combined, (B) females only, and (C) males only. Each bar represents mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , 2-way ANOVA, Tukey's multiple comparison test.



**Supplementary Figure 6. Fiber size analysis of the quadriceps muscle.** Fiber size measurements of the quadriceps muscle from 10-month-old (mo), 2-year-old untreated and 2-year-old NT-3-treated C57BL/6 mice shown as (A) combined, (B) females only, and (C) males only. Each bar represents mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , 2-way ANOVA, Tukey's multiple comparison test.



**Supplementary Figure 7. Fiber size analysis of the triceps muscle.** Fiber size measurements of the triceps muscle from 10-month-old (mo), 2-year-old untreated and 2-year-old NT-3-treated C57BL/6 mice shown as (A) combined, (B) females only, and (C) males only. Each bar represents mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , 2-way ANOVA, Tukey's multiple comparison test.