



Supplementary Materials

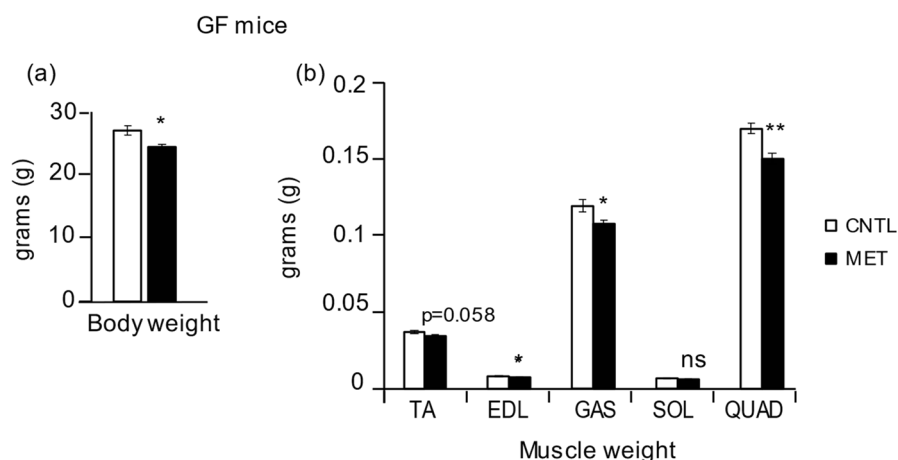
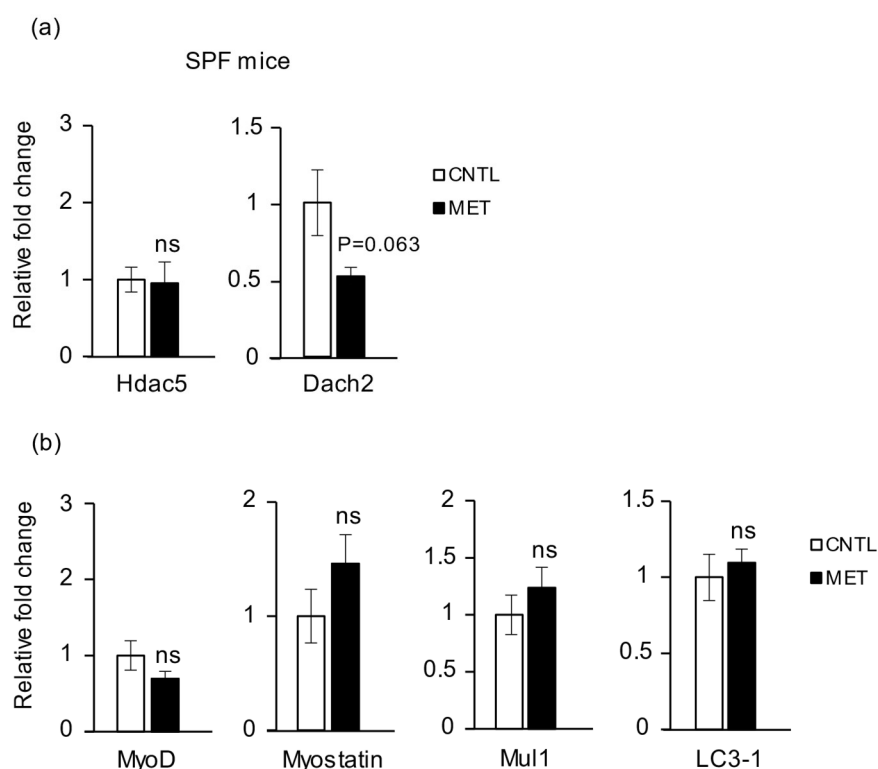


Figure S1. Metronidazole causes decreased body weight and hind limb muscle weight in germ-free mice. **(a)** Metronidazole treatment of GF mice resulted in a significant decrease in body weight compared to nontreated controls. $N = 5$ mice per group. **(b)** Absolute hind limb muscle weights of tibialis anterior (TA), extensor digitorum longus (EDL), gastrocnemius (GAS), soleus (SOL), and quadriceps (QUAD) muscles of metronidazole-treated GF mice (MET) compared to nontreated controls (CNTL). $N = 5$ mice per group. Data presented as means $\pm$ SEM. Asterisks indicate statistically significant differences (*, $p < 0.05$; **, $p < 0.01$; ns, nonsignificant with student's t test).



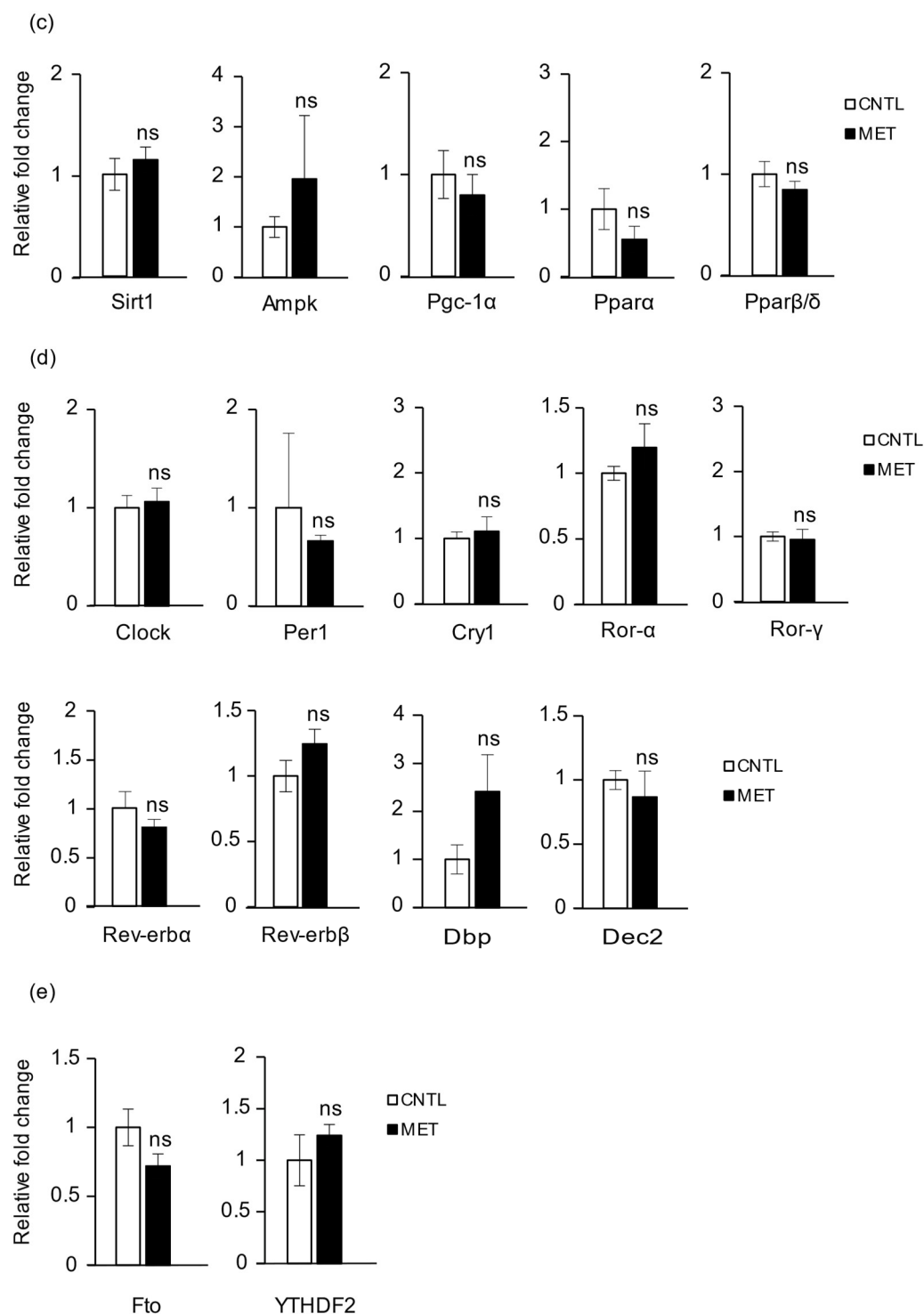


Figure S2. Metronidazole-treated SPF mice muscle mass determinants, autophagy markers, circadian metabolic regulators, and RNA epigenetics. Real-time quantitative PCR analysis of gene expression in gastrocnemius muscle of metronidazole-treated and nontreated SPF mice. (a) Neurogenic atrophy markers *Hdac5* and *Dach2*; (b) muscle mass determinants *MyoD* and *myostatin*, and autophagy markers *Mul1* and *LC3-1*; (c) circadian metabolic regulators *Sirt1*, *Ampk*, *Pgc-1 α* , *PPAR α* , and *PPAR β/δ* ; (d) clock genes *Clock*, *Per1*, *Cry1*, *Ror- α* , *Ror- γ* , *Rev-erba*, and *Rev-erb β* , and clock effector genes *Dbp* and *Dec2*; (e) mRNA demethylase *Fto* and YTH N⁶-methyladenosine RNA binding protein 2 gene *YTHDF2*. *N* = 5 mice per group. Data presented as means $\pm$ SEM (ns, nonsignificant with student's *t* test).

Table S1.

Serial No.	Primer	Species	Forward primer (5'→3')	Reverse primer (5'→3')	NCBI reference
1	Adiponectin	Mouse	TGTTGGAATGACAGGAGCTGAA	CACACTGAAGCCTGAGCGATAC	NM_009605
2	Ampk	Mouse	GCCCTTCCCGCAAGATGTTAT	TCAGCAGGGGTGGACTTTAAT	NM_01310480
3	Atrogin1	Mouse	GAGTGGCATCGCCAAAAGA	TCTCCATCCGATACACCCACA	NM_026346
4	Bmal1	Mouse	ACAGTCAGATTGAAAAGAGGCG	GCCATCCTTAGCACGGTGAG	NM_001357070
5	Clock	Mouse	ACCGTAGCAGGTTTATGGGAATG	TGGTGTCACACAATAGGCAAGA	NM_001305222
6	Cry1	Mouse	CAGACTCTCGTCAGCAAGATG	CAAACGTGTAAGTGCCTCAGT	NM_007771
7	Cry2	Mouse	GCGTCTGTTGTAGTCCGGG	TCCCAAAGGGTTCAGAGTCATA	NM_009963
8	Dach2	Mouse	TCTCCCTCGCAGATGGATCA	TGACTGGGATTTGAGCTGGT	NM_001142570
9	Dbp	Mouse	AAGAAGGCAAGGAAAGTCCA	TGTACCTCCGGCTCCAGTA	NM_016974
10	Dec2	Mouse	GCGAGACGATACCAAGGATAC	TCAGATGTTCCGGGCAGTAAA	NM_001271768
11	E4BP4	Mouse	ACGGACCAGGGAGCAGAAC	GGACTTCAGCCTCTCATCCATC	NM_017373
12	FoxO1	Mouse	ACCAGGAGAAGCTCCCAAGT	AATGTAGCCTGCTCACTAACTCTTA	NM_019739
13	FoxO3	Mouse	GCAAGCCGTGTACTGTGGA	CGGGAGCGCGATGTTATCC	NM_019740
14	Fto	Mouse	CTCCTTGAGGAGCTTGAAGACA	CCATGCTTATGCAGTGTGAGAA	NM_011936
15	Gapdh	Mouse	TGGCCTTCCGTGTTCTAC	GAGTTGCTGTTGAAGTCGCA	NM_001289726
16	Hdac4	Mouse	CTGGGGCGCCTGACG	CTCACTGGCAGCGTCTTGTA	NM_207225
17	Hdac5	Mouse	TGTTGCCACTCAAGAGCACA	CACAATGATGAAGCCCAAAGGG	NM_001077696
18	LC3-1	Mouse	GCGAGTTGGTCAAGATCATCCG	TGGACACACTCACCATGCTGTG	NM_025735
19	Mettl3	Mouse	ATCCAGGCCCCATAAGAAACAG	CTATCACTACGGAAGGTTGGG	NM_019721
20	Mettl14	Mouse	CTGAGAGTGCGGATAGCATTG	GAGCAGATGTATCATAGGAAGCC	NM_201638
21	Mul1	Mouse	AGGGCATTTCTTCAGAAAGCA	GGGGTGGAACCTTCTCGTACA	NM_026689
22	MuRF1	Mouse	GTGTGAGGTGCTACTTGCTC	GCTCAGTCTTCTGTCCTTGGA	NM_001039048
23	MyoD	Mouse	GACAGGGAGGAGGGGTAGAG	TGCTGTCTCAAAGGAGCAGA	NM_010866
24	Myogenin	Mouse	ACTCCCTTACGTCCATCGTG	CAGGACAGCCCCACTTAAAA	NM_031189
25	Myostatin	Mouse	CAGCCTGAATCCAACCTTAGG	TCGCAGTCAAGCCCAAAGTC	NM_010834
26	Pdk4	Mouse	ACCCTACGGATCCTAACACC	TCACAGGCATTTTCTGAACCAAAG	NM_013743
27	Per1	Mouse	GAATTGGAGCATATCACATCCGA	CCCGAAACACATCCCGTTTG	NM_001159367
28	Per2	Mouse	AAAGCTGACGCACAAAGAA	ACTCCTCATTAGCCTTACCT	NM_011066
29	Pgc-1α	Mouse	AAACTTGCTAGCGGTCTCTCA	TGGCTGGTGCCAGTAAGAG	NM_008904
30	Ppara	Mouse	TACTGCCGTTTTCACAAGTGC	AGGTCGTGTTTACAGGTAAGA	NM_011144
31	Pparβ/δ	Mouse	CGGCAGCCTCAACATGG	AGATCCGATCGCACTTCTCATAC	NM_011145
32	Pparγ	Mouse	TGTGGGGATAAAGCATCAGGC	CCGGCAGTTAAGATCACACCTAT	NM_011146
33	Rev-erbα	Mouse	CTTCCGTGACCTTCTCTCAGA	CAGCTCCTCCTCGGTAAGTG	NM_145434
34	Rev-erbβ	Mouse	CGCCATGGAGCTGAACG	GACAAGAGGCAGGGCTGGA	NM_011584
35	Ror-α	Mouse	ACCGTGTCATGGCAGAAC	TTTCCAGGTGGGATTTGGAT	NM_001289916
36	Ror-β	Mouse	GGCAGACCCACACCTACGA	CAGAGCCTCCCTGGACTTG	NM_001289921
37	Ror-γ	Mouse	TCTACACGGCCCTGGTTCT	ATGTTCCACTCTCTCTCTCTTG	NM_001293734
38	Sirt1	Mouse	CGGCTACCGAGGTCCATATAC	ACAATCTGCCACAGCGTCAT	NM_001159589
39	Tef	Mouse	GCCGAGCTTCGCAAGGA	ACAGGTTACAAGGGCCCGTACT	NM_153484
40	YTHDF2	Mouse	TAGCCAACCTGCGACACATTC	CACGACCTTGACGTTCTTT	NM_145393