SUPPLEMENTARY DATA

Chronic Swimming Routine Promotes Gut Microbiota Remodeling and Improvements in Physical Resilience, Episodic-Like Memory, and Inflammatory Status in Late Middle-Aged Mice

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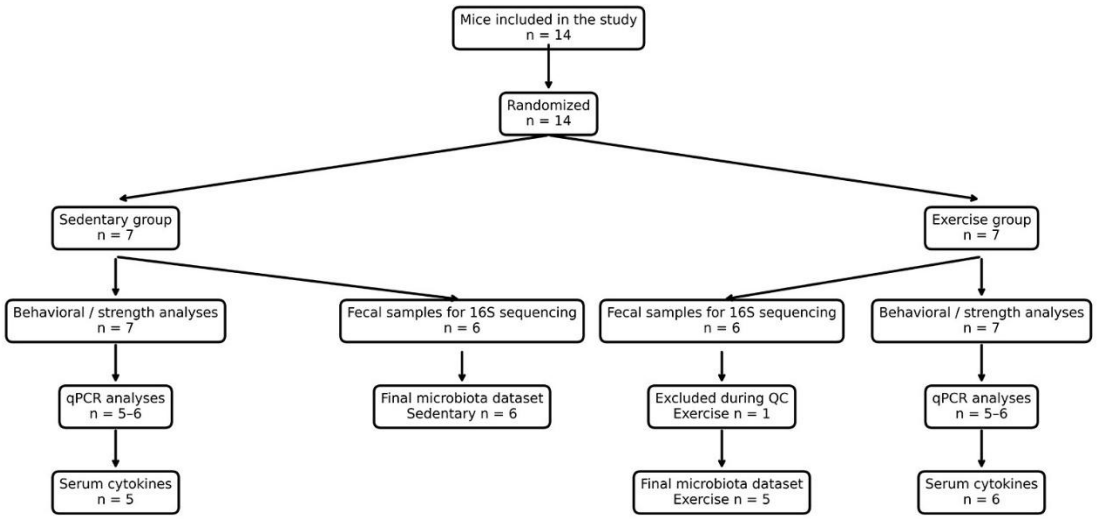
Supplementary Table 1. Primer sequences and thermocycling parameters used for qPCR analyses.

Gene	Primers	Thermocycling conditions
β-actina	Forward 5'- TACGACCAGAGGCATACAG -3' Reverse 5'- GCCAACCGTGAAAAGATGAC -3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 58°C for 15 seconds and 72°C for 20 seconds
Il-1β	Forward 5'-GCAACTGTTCCTGAACTCAACT-3' Reverse 5'-TCTTTTGGGGTCCGTCAACT-3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 15 seconds and 72°C for 15 seconds
Tnf-α	Forward 5'-CTGAACTTCGGGGTGATCGG-3' Reverse 5'-GGCTTGTCACTCGAATTTTGAGA-3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 58°C for 15 seconds and 72°C for 20 seconds
Bdnf	Forward 5'-CCATAAGGACGCGGACTTGTAC-3' Reverse 5'-GAGGAGGCTCCAAAGGCACTT-3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 58°C for 15 seconds and 72°C for 15 seconds
Il-6	Forward 5'-CCAATTTCCAATGCTCTCCT-3' Reverse 5'-ACCACAGTGAGGAATGTCCA-3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 51°C for 16 seconds and 72°C for 20 seconds
Emr1	Forward 5'- AATCGCTGCTGGTTGAATACAG -3' Reverse 5'- CCAGGCAAGGAGGACAGAGTT -3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 54°C for 15 seconds and 72°C for 15 seconds
Gfap	Forward 5'- AACGACTATCGCCGCCAACTG -3' Reverse 5'- CTCTTCCTGTTCGCGCATTTG -3'	95°C for 10 minutes, followed by 40 cycles of 95°C for 30 seconds, 55°C for 60 seconds and 72°C for 20 seconds



Supplementary Figure 1. Animal and sample inclusion flow diagram. A total of 14 mice were included in the study and randomized into sedentary and exercise groups (n = 7 per group). All animals were included in behavioral and strength analyses. Molecular analyses were performed on subsets of animals depending on sample availability and assay requirements. qPCR analyses were conducted in 5–6 animals per group, and serum cytokine measurements were obtained from 5 sedentary and 6 exercised mice. For gut microbiota profiling, fecal samples from 6 animals per group were submitted for 16S rRNA gene sequencing. One sample from the exercise group did not pass sequencing quality control and was excluded prior to downstream analysis, resulting in a final microbiota dataset of 6 sedentary and 5 exercised samples.

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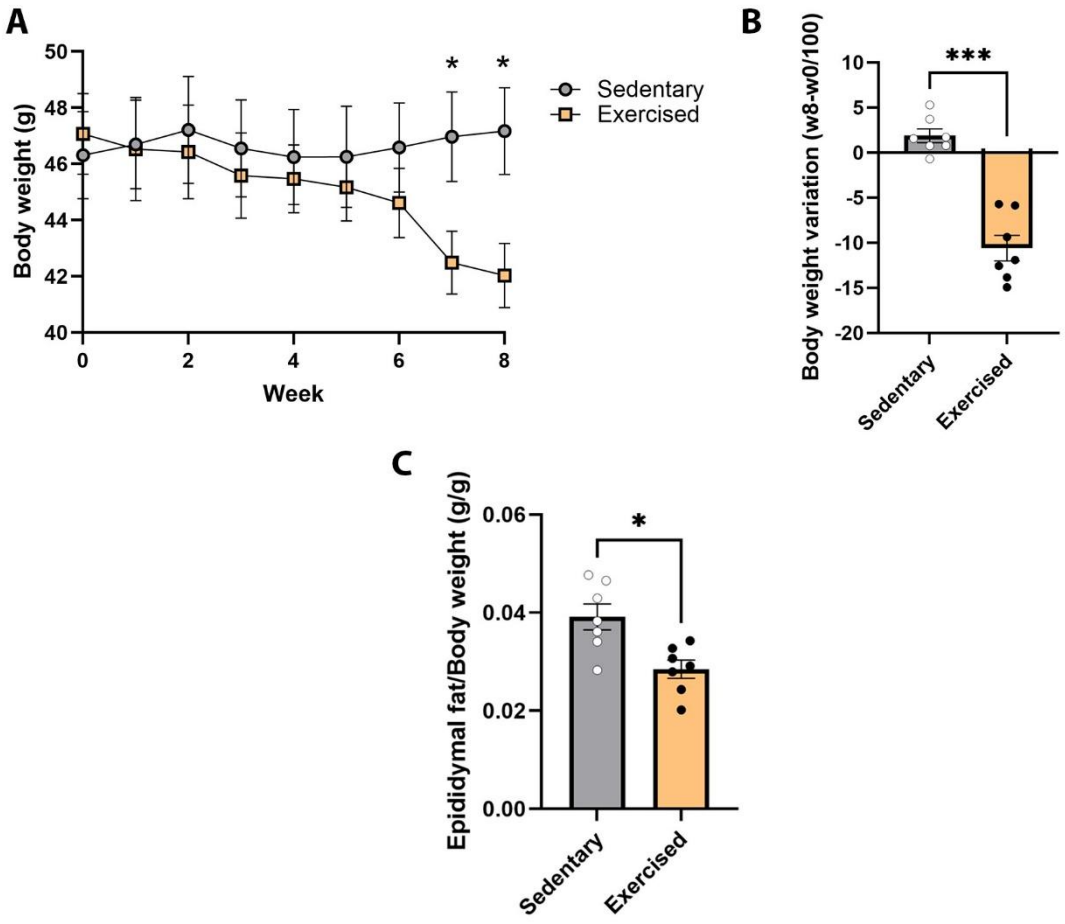

Supplementary Figure 2. Chronic swimming exercise reduces body weight and adiposity in middle-aged mice. Body weight was monitored weekly throughout the 8-week swimming training protocol. Chronic swimming exercise induced a progressive reduction in body weight compared with sedentary controls. (**A**) Weekly body weight evolution in sedentary and swimming-exercised mice. (**B**) Body weight variation from week 0 to week 8 (w8–w0), showing a significant decrease in exercised mice compared with sedentary controls. (**C**) Epididymal fat-to-body weight ratio at the end of the protocol, showing reduced adiposity in exercised mice. Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean ± SEM (n = 7 mice per group). Each dot represents an individual mouse. Statistical significance was determined using the Mann–Whitney U test. *$p < 0.05$, ***$p < 0.001$ versus sedentary mice.

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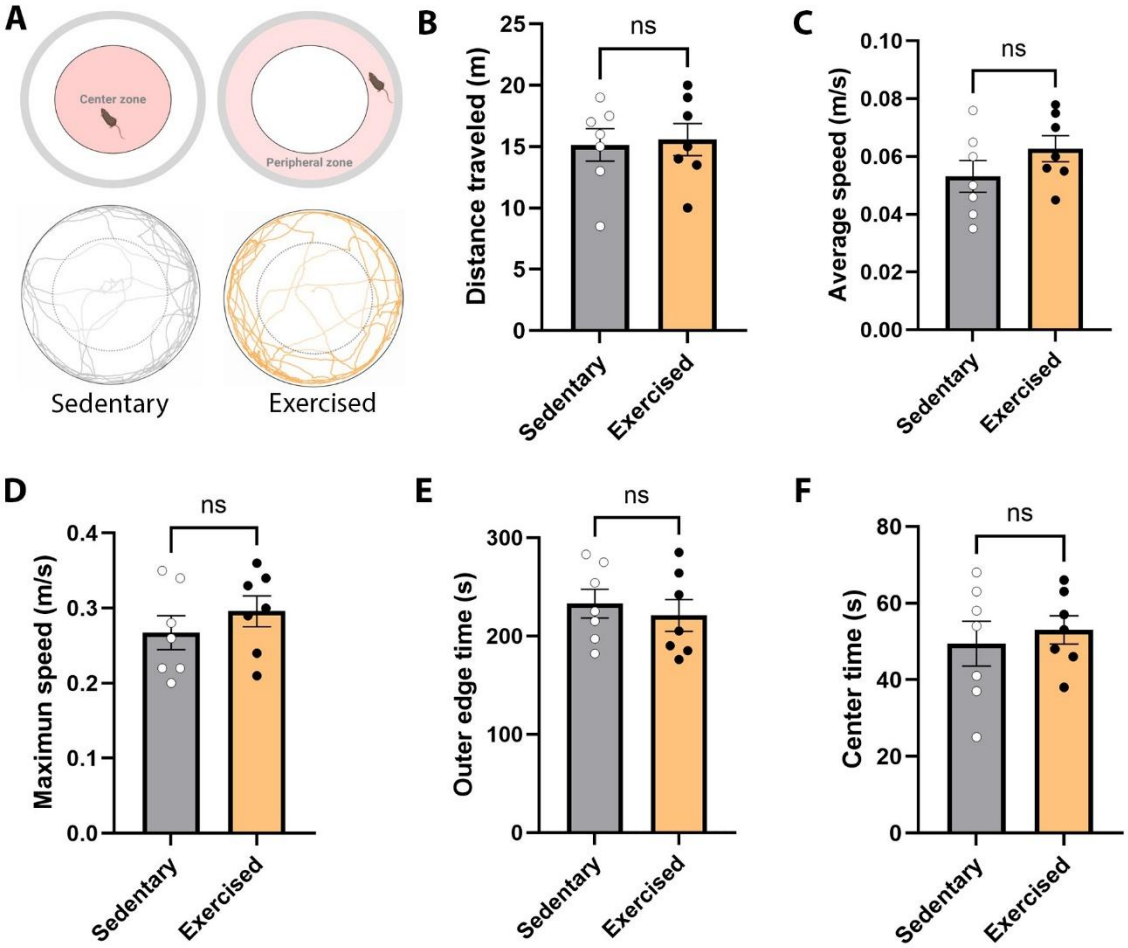

Supplementary Figure 3. Chronic swimming exercise does not alter locomotor activity or anxiety-like behavior in the open field test in middle-aged mice. Mice were subjected to the open field test to evaluate locomotor activity and anxiety-like behavior. (**A**) Schematic representation of the arena and representative movement trajectory maps of sedentary and swimming-exercised mice. (**B**) Total distance traveled. (**C**) Average speed. (**D**) Maximum speed. (**E**) Time spent in the outer edge zone. (**F**) Time spent in the center zone. No significant differences were observed between sedentary and exercised mice across any of the analyzed parameters. Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean ± SEM (n = 7 mice per group). Each dot represents an individual mouse. Statistical significance was determined using the Mann–Whitney U test. ns: not significant versus sedentary mice.

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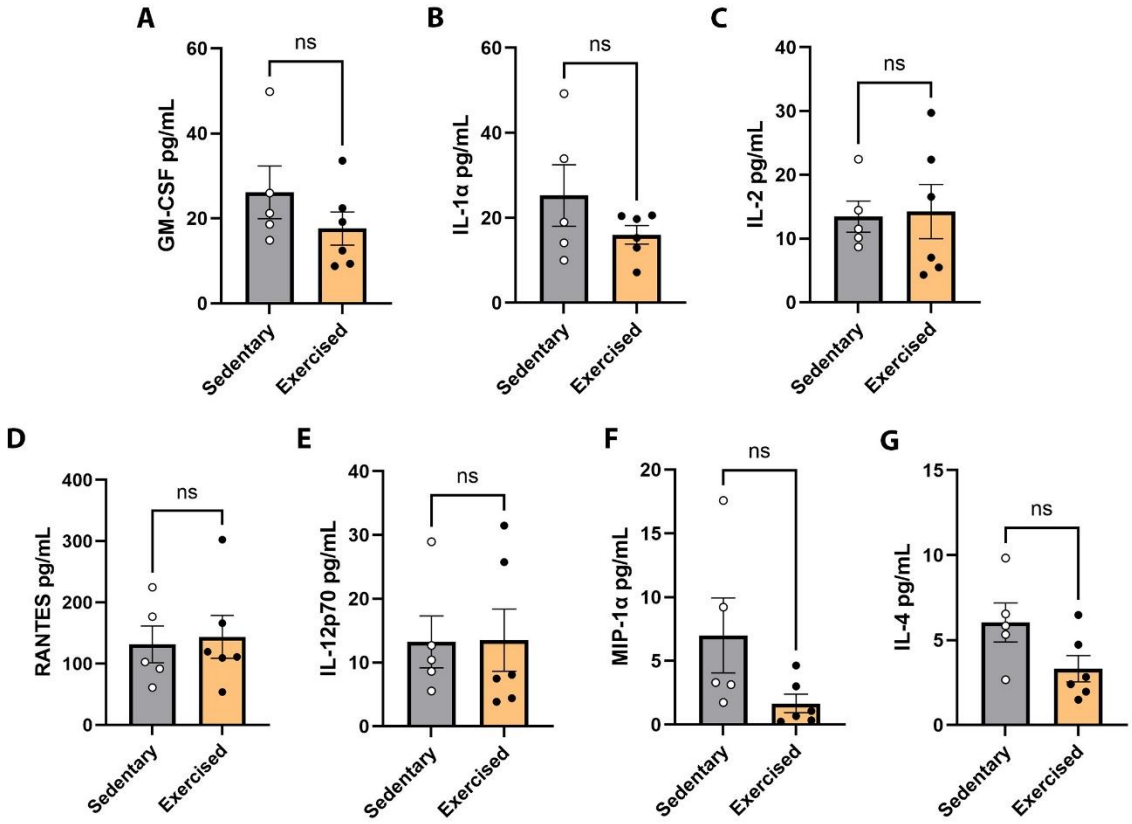

Supplementary Figure 4. Chronic swimming exercise does not affect a subset of circulating immune mediators in middle-aged mice. Circulating cytokines and chemokines were quantified in serum from swimming-exercised and sedentary middle-aged mice using multiplex assays. Comparisons between groups were performed using the Mann–Whitney U test with Benjamini–Hochberg false discovery rate (FDR) correction applied across the cytokine panel. (**A**) Serum levels of granulocyte–macrophage colony-stimulating factor (GM-CSF). (**B**) Serum levels of interleukin-1α (IL-1α). (**C**) Serum levels of interleukin-2 (IL-2). (**D**) Serum levels of regulated upon activation, normal T cell expressed and secreted (RANTES). (**E**) Serum levels of interleukin-12p70 (IL-12p70). (**F**) Serum levels of macrophage inflammatory protein-1α (MIP-1α). (**G**) Serum levels of interleukin-4 (IL-4). No significant differences were detected for these analytes after FDR correction. Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean ± SEM (n = 5–6 mice per group). Each dot represents an individual mouse. ns, not significant after FDR correction.

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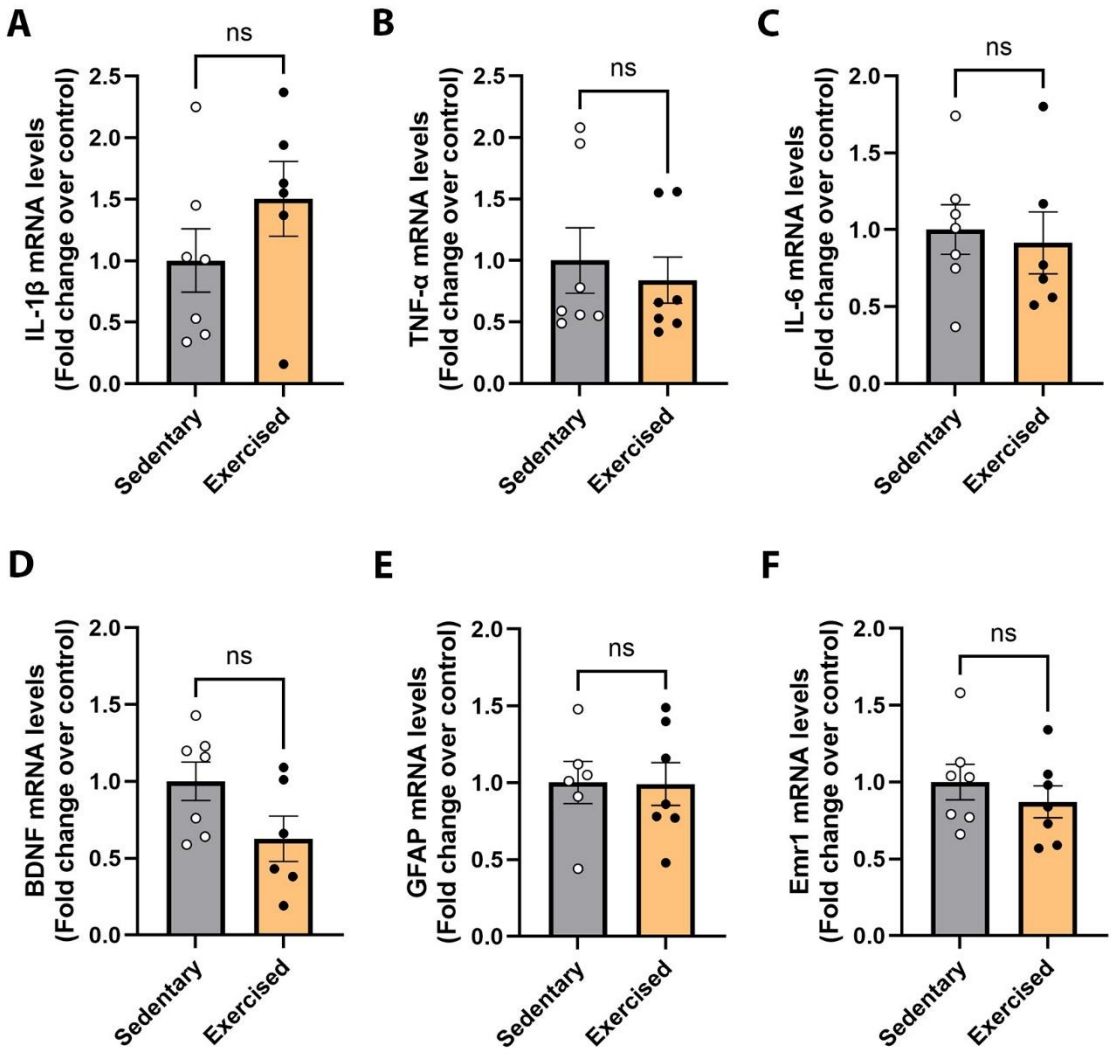

Supplementary Figure 5. Chronic swimming exercise does not modify inflammatory, glial, or neurotrophic marker expression in the medial prefrontal cortex of middle-aged mice. mRNA expression of inflammatory, glial, and neurotrophic markers was analyzed in medial prefrontal cortex (mPFC) tissue by RT-qPCR. No significant differences were observed between sedentary and swimming-exercised mice across any of the analyzed transcripts. (**A**) Relative expression of interleukin-1β (IL-1β). (**B**) Relative expression of tumor necrosis factor-α (TNF-α). (**C**) Relative expression of interleukin-6 (IL-6). (**D**) Relative expression of brain-derived neurotrophic factor (BDNF). (**E**) Relative expression of glial fibrillary acidic protein (GFAP). (**F**) Relative expression of adhesion G protein-coupled receptor E1 (Emr1-F4/80). Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean ± SEM and expressed as fold change relative to sedentary controls (n=6-7 mice per group). Each dot represents an individual mouse. Statistical significance was determined using the Mann–Whitney U test. ns: not significant versus sedentary mice.

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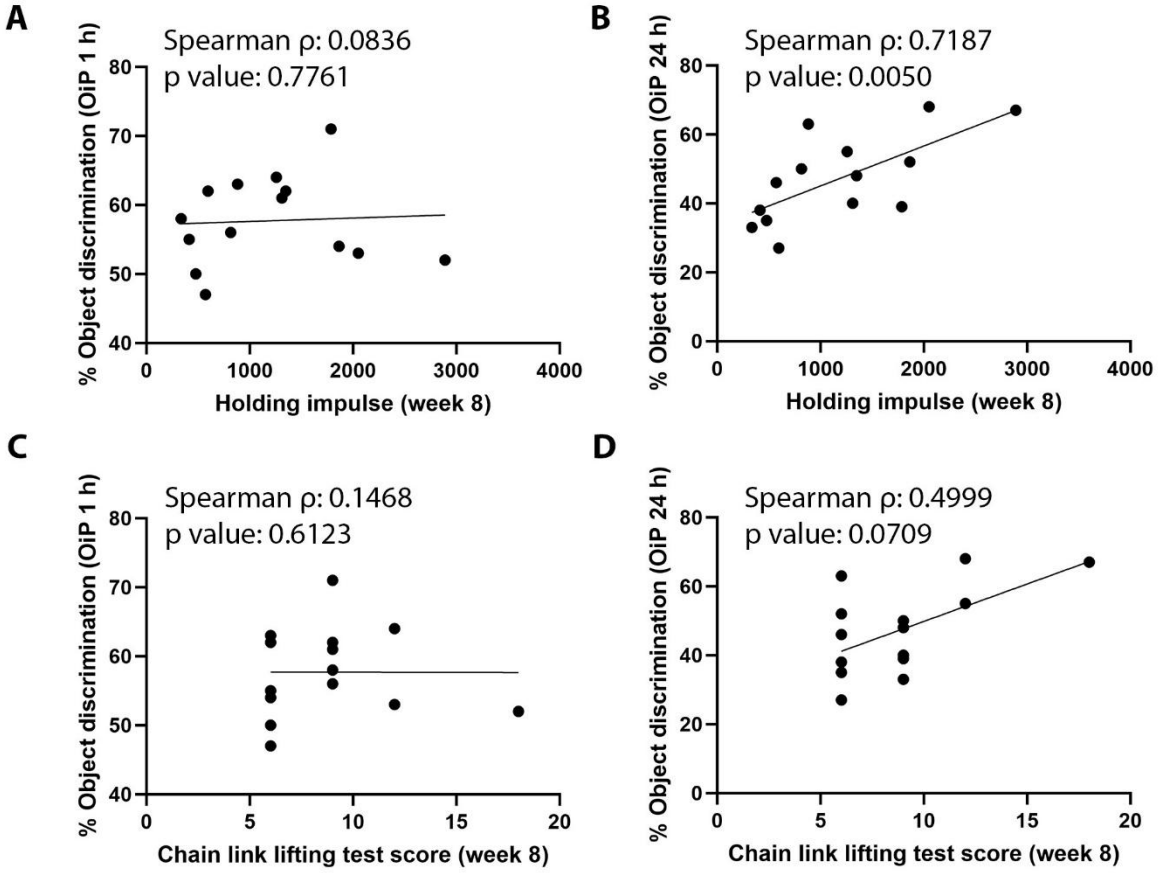

Supplementary Figure 6. Muscle performance correlates with associative recognition memory assessed by the object-in-place test in middle-aged mice. Correlation analyses were performed between muscle function assessed at week 8 of the training protocol and discrimination performance in the object-in-place (OiP) memory test. Overall, muscle performance showed a positive association with long-term associative memory performance. (**A**) Correlation between holding impulse and the OiP discrimination index at the 1 h retention interval. (**B**) Correlation between holding impulse and the OiP discrimination index at the 24 h retention interval. (**C**) Correlation between chain link lifting test score and the OiP discrimination index at the 1 h retention interval. (**D**) Correlation between chain link lifting test score and the OiP discrimination index at the 24 h retention interval. Correlations were assessed using Spearman's rank correlation coefficient (ρ), and the corresponding ρ and p values are indicated in each panel. Each dot represents an individual mouse.

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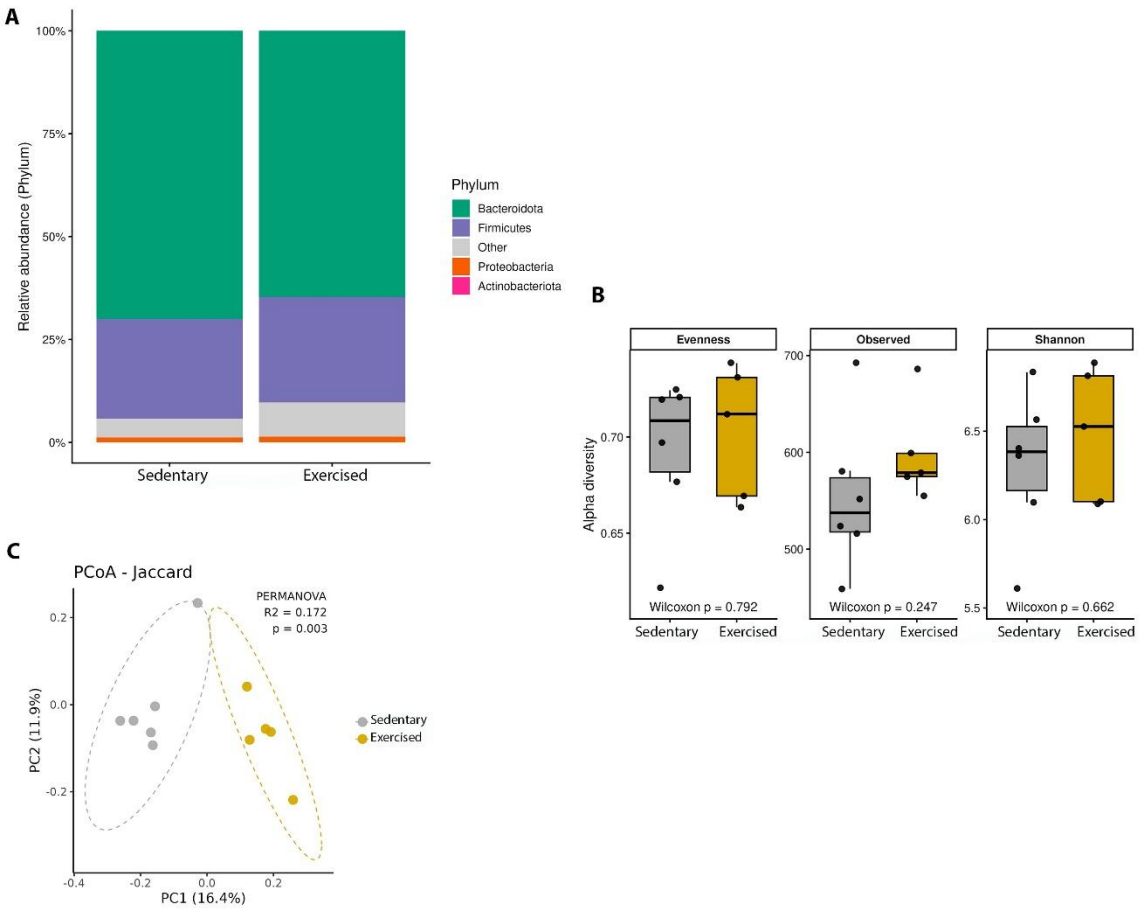

Supplementary Figure 7. Exercise-induced modulation of gut microbiota diversity and presence–absence structure in late middle-aged mice. (**A**) Alpha-diversity metrics, including observed richness, Shannon diversity, and Pielou's evenness, did not differ between sedentary and exercised mice, indicating that chronic exercise did not alter global within-sample diversity. (**B**) Relative abundance at the phylum level revealed comparable overall phylum distribution between groups, with no significant differences in dominant phyla. (**C**) Presence–absence–based beta-diversity analysis using Jaccard distance demonstrated a significant separation between sedentary and exercised groups, indicating that exercise influenced community composition independently of abundance weighting. Principal coordinates analysis (PCoA) is shown, and statistical significance was assessed by PERMANOVA. n = 5–6 mice per group.