

Figure S1. Environmental chamber and infrared thermometer. (A) The environmental chamber used during the ES protocol. B-C) Infrared thermometer (panel B) and its use during skin temperature measurement (panel C). See Suppl. Figure 2 for the outcome of these measurements.

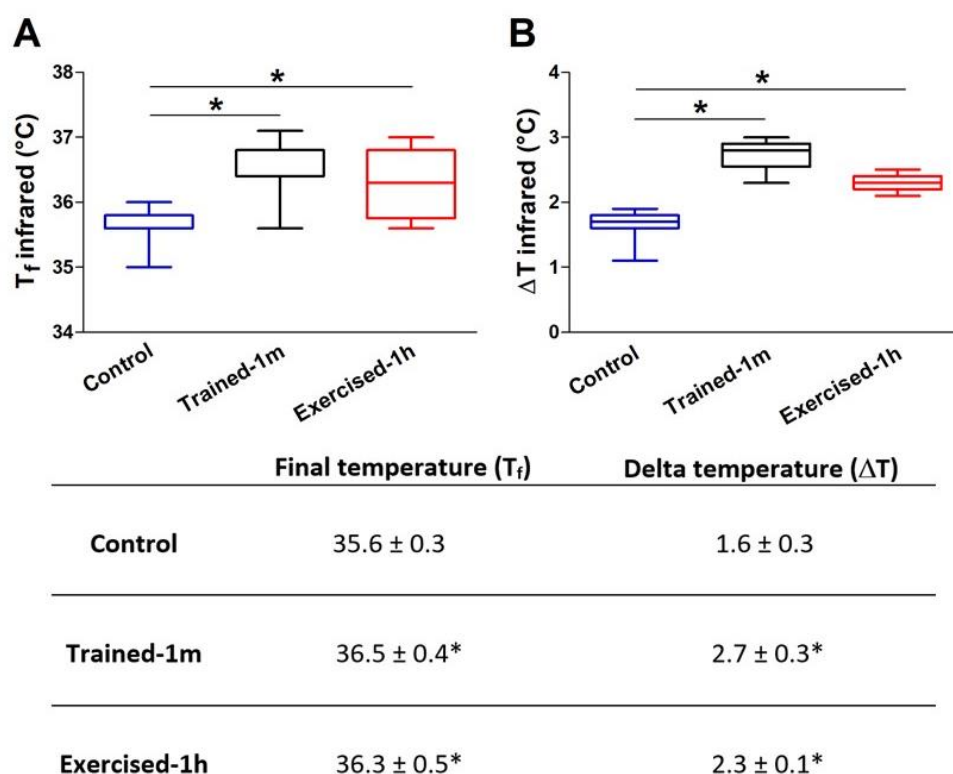


Figure S2. Average skin temperature of mice following the ES protocol recorded with the infrared thermometer (as shown in Suppl. Fig. 1 B and C). A) Average cutaneous temperature recorded at the end of the ES protocol (T_f). B) Average changes in cutaneous temperature (ΔT) during ES protocol, i.e. the difference between temperatures measured at the beginning (T_0) and at the end (T_f) of the experiment. Data are shown as follow: i) the box extends from the 25th to 75th percentiles; the line in the middle of the box is plotted at the median and the whiskers go down to the smallest value and up to the largest (graphs in panels A and B); and ii) mean \pm SEM (* $p < 0.05$), as evaluated by two-tailed unpaired Student's t-test (Table). Sample size (number of tested mice): control = 8; trained-1m = 8; exercised-1h = 9.

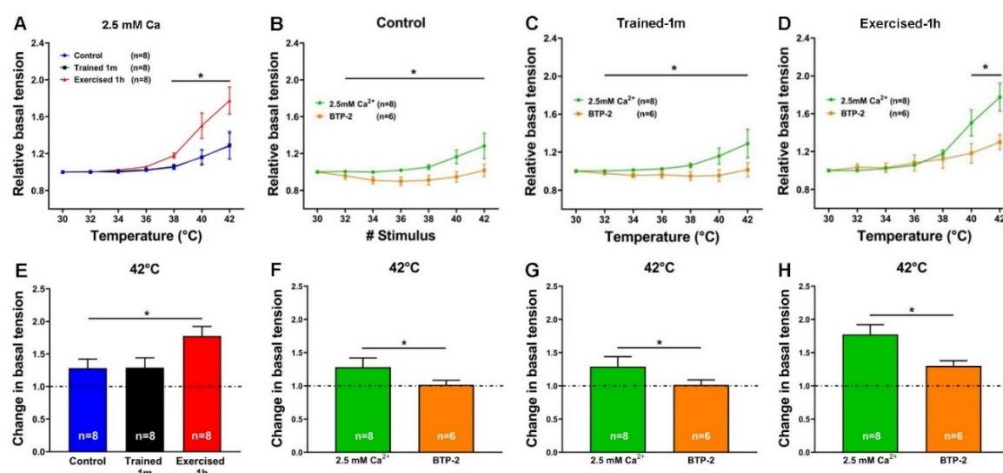


Figure S3. Relative and specific basal tension developed by isolated EDL muscles during an ex-vivo heat stress (HS) protocol. (A–D) Relative basal tension developed by EDL muscles during an ex-vivo HS protocol (i.e. 45-minute electrical stimulation with 60 single twitches, at 1 Hz, applied every 5 seconds). The chamber temperature was increased by 2°C (from 30°C to 42°C) every 5 minutes. Experiments were performed in presence of 2.5 mM of extracellular Ca^{2+} (panel A) or in a solution supplemented with 10 μM BTP-2 (panels B–D). (E–H) Specific basal tension recorded at 42°C, either in presence of 2.5 mM of extracellular Ca^{2+} (panel E) or in a solution supplemented with 10 μM BTP-2 (panels F–H). Data are shown as mean \pm SEM; in panels (A,E): * $p < 0.05$ = *indicate a difference between exercised-1h and both control and trained-

1m mice; in panels B-D and F-H: * $p < 0.05$ = difference between 2.5mM Ca^{2+} and BTP-2; as evaluated by two-tailed unpaired Student's t-test). n = number of EDL muscles analysed.

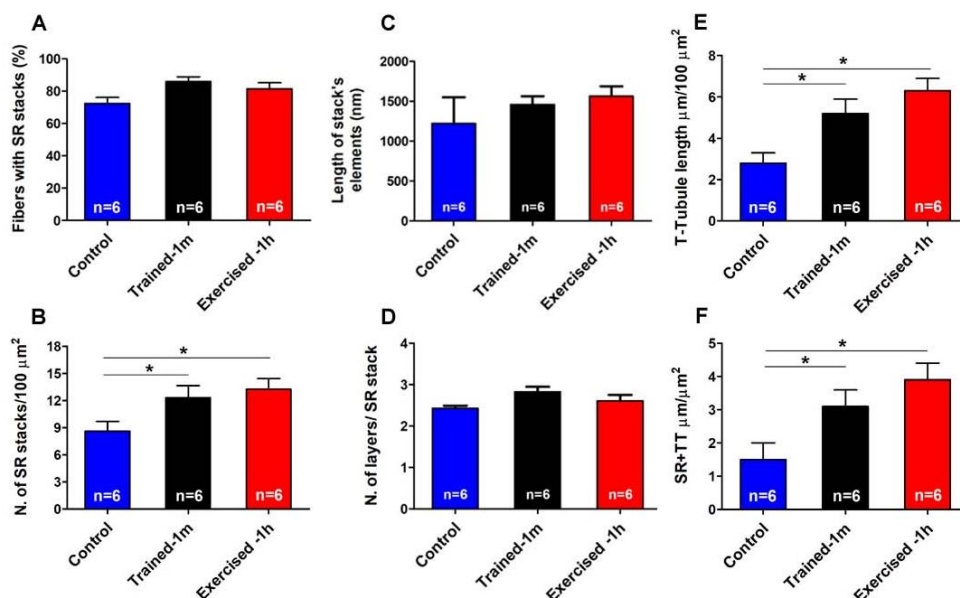


Figure S4. Quantitative EM analysis of SR-stacks and of T-tubule extension at the I band after the ES stress protocol. (A and B) Percentage of fibers containing SR-stacks and number of SR-stacks / 100 μm^2 of section. (C and D) Length of stack's elements (nm) and number of layers / SR-stack. (E and F) Extension of T-tubules at the I band in 100 μm^2 and extension of SR-TT contacts at the I band. Data are shown as mean \pm SEM (* $p < 0.05$; the two ends of the line with asterisk indicate which two groups are being compared). n = number of EDL analysed.

Table S1. Average core temperature of mice following the ES protocol.

| | Final temperature (T_f) | Delta temperature (ΔT) |
|--------------|-----------------------------|----------------------------------|
| Control | 37.9 ± 0.2 | 1.7 ± 0.1 |
| Trained-1m | $38.9 \pm 0.3^*$ | $2.7 \pm 0.2^*$ |
| Exercised-1h | $38.8 \pm 0.5^*$ | $2.3 \pm 0.1^*$ |

Sample size (number of tested mice): control, n = 8; trained-1m, n = 8; exercised-1h, n = 9. Data are shown as mean \pm SEM (* $p < 0.05$, as evaluated by two-tailed unpaired Student's t-test).

Table S2. Quantitative analysis of structural damage in longitudinal sections of EDL fibers in basal condition.

| | % of fiber with no damage | % of altered fibers |
|--------------|---------------------------|---------------------|
| Control | 98.6 ± 1.4 | 1.7 ± 1.4 |
| Trained-1m | 98.2 ± 1.8 | 2.4 ± 1.8 |
| Exercised-1h | 98.5 ± 1.5 | 2.1 ± 1.5 |

Sample size (number of tested mice): n = 3 for each sample group. Data are shown as mean \pm SEM. Statistical significance was evaluated by two-tailed unpaired Student's t-test.

Table S3. Quantitative analysis of structural damage in longitudinal sections of EDL fibers, after the ES protocol.

| | % of fiber with no damage | % of altered fibers |
|---------------------|------------------------------|---------------------|
| Control | 94.0 ± 2.3 | 6.5 ± 2.3 |
| Trained-1m | 98.5 ± 0.9 | 1.9 ± 0.9* |
| Exercised-1h | 93.1 ± 2.6 | 7.2 ± 2.6 |

Sample size (number of tested mice): n = 3 for each sample group. Data are shown as mean ± SEM (*p < 0.05 vs. control and vs. exercised-1h, as evaluated by two-tailed unpaired Student's t-test.).

Table S4. Quantitative EM analysis of mitochondria n./area and relative volume.

| | No. of mitochondria /100 μm^2 | Mitochondrial volume/total volume (%) |
|---------------------|---|--|
| Control | 26.8 ± 0.8 | 3.8 ± 0.1 |
| Trained-1m | 41.1 ± 1.2* | 7.1 ± 0.2* |
| Exercised-1h | 29.2 ± 0.9 | 4.0 ± 0.1 |

Sample size (number of tested mice): control, n = 6; trained-1m, n = 4; exercised-1h, n = 6. Data are shown as mean ± SEM (*p < 0.05 vs. control and vs. exercised-1h, as evaluated by two-tailed unpaired Student's t-test.).