

1 **Supplementary Information**

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3 **OXIDATIVE STRESS, INFLAMMATION AND ACTIVATORS OF MITOCHONDRIAL**  
4 **BIOGENESIS: TEMPOL TARGETS IN THE DIAPHRAGM MUSCLE OF EXERCISE**  
5 **TRAINED-MDX MICE**

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21 **Supplementary Figure 1S. Tempol effects on forelimb muscle strength**

22 Representative forelimb muscle strength, assessed by taking measurements of force at time points  
23 Start and End, in the 7 animals from each experimental group: (A) sedentary *mdx* mice (*mdxSed*), (B)  
24 exercise trained *mdx* mice (*mdxEx*) and (C) exercise trained *mdx* mice treated with Tempol  
25 (*mdxEx+T*).

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27 **Supplementary Figure 2S. Tempol effects on regenerative muscular process (fibers which  
28 central nuclei) and on inflammatory response morphology (inflammatory area)**

29 Representative diaphragm (DIA) cross-sections showing fibers which central nuclei (black  
30 arrowheads) and the inflammatory area (outline indicates the representative area) in the 7 animals  
31 from each experimental group: sedentary *mdx* mice (*mdxSed*), exercise trained *mdx* mice (*mdxEx*)  
32 and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*).

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34 **Supplementary Figure 3S. Tempol effects on inflammatory response morphology (macrophage  
35 infiltration)**

36 Representative diaphragm (DIA) cross-sections showing the macrophage infiltration (white  
37 arrowheads) in the 7 animals from each experimental group: sedentary *mdx* mice (*mdxSed*), exercise  
38 trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*).

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40 **Supplementary Figure 4S. Tempol effects on oxidative stress markers (DHE)**

41 Representative diaphragm (DIA) cross-sections showing the dihydroethidium (DHE) fluorescence in  
42 the 7 animals from each experimental group: sedentary *mdx* mice (*mdxSed*), exercise trained *mdx*  
43 mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*).

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45 **Supplementary Figure 5S. Tempol effects on oxidative stress markers (Lipofuscin granules)**

46 Representative diaphragm (DIA) cross-sections showing the autofluorescent lipofuscin granules  
47 (white arrowheads) in the 7 animals from each experimental group: sedentary *mdx* mice (*mdxSed*),  
48 exercise trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*).

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50 **Supplementary Figure 6S. Tempol effects on inflammatory markers (TNF- $\alpha$  and NF- $\kappa$ B)**

51 (A) Representative membranes stained with Ponceau of the different bands shown in this paper. (B)  
52 Representative bands analyzed for the tumor necrosis factor-alpha (TNF- $\alpha$ ), nuclear factor kappa B  
53 (NF- $\kappa$ B) and  $\beta$ -actin quantified by western blotting from the different experimental groups: sedentary  
54 *mdx* mice (*mdxSed*), exercise trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with  
55 Tempol (*mdxEx+T*). The images in Figure 4B represent one animal per group in sequence (n=7  
56 animals per group).

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58 **Supplementary Figure 7S. Tempol effects on oxidative stress markers (4-HNE)**

59 (A) Representative membranes stained with Ponceau of the different bands shown in this paper. (B)  
60 Representative bands analyzed for the 4-hydroxynonenal (4-HNE)-protein adducts and  $\beta$ -actin  
61 quantified by western blotting from the different experimental groups: sedentary *mdx* mice (*mdxSed*),  
62 exercise trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*).  
63 The images in Figure 7B represent one animal per group in sequence (n=7 animals per group).

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65 **Supplementary Figure 8S. Tempol effects on antioxidant system levels (CAT, SOD2, GPx1 and**  
66 **GR)**

67 (A) Representative membranes stained with Ponceau of the different bands shown in this paper. (B)  
68 Representative bands analyzed for the catalase (CAT), manganese-superoxide dismutase (SOD2),  
69 glutathione peroxidase (GPx1), glutathione reductase (GR) and  $\beta$ -actin quantified by western blotting  
70 from the different experimental groups: sedentary *mdx* mice (*mdxSed*), exercise trained *mdx* mice  
71 (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*). The images in Figure 8B  
72 represent one animal per group in sequence (n=7 animals per group).

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74 **Supplementary Figure 9S. Tempol effects on VEGF factor**

75 (A) Representative membranes stained with Ponceau of the different bands shown in this paper. (B)  
76 Representative bands analyzed for vascular endothelial growth factor (VEGF) and  $\beta$ -actin quantified  
77 by western blotting from the different experimental groups: sedentary *mdx* mice (*mdxSed*), exercise  
78 trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*). The  
79 images in Figure 9B represent one animal per group in sequence (n=7 animals per group).

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81 **Supplementary Figure 10S. Tempol effects on activators of mitochondrial biogenesis and**  
82 **oxidative phosphorylation**

83 (A) Representative membranes stained with Ponceau of the different bands shown in this paper. (B)  
84 Representative bands analyzed for peroxisome proliferator-activated receptor  $\delta$  (PPAR $\delta$ ) and  
85 peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) and  $\beta$ -actin quantified by  
86 western blotting from the different experimental groups: sedentary *mdx* mice (*mdxSed*), exercise

## Tempol effects on exercise-trained-mdx mice

87 trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*). The  
88 images in Figure 10B represent one animal per group in sequence (n=7 animals per group).

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90 **Supplementary Figure 11S. Tempol effects on activators of mitochondrial biogenesis and**  
91 **oxidative phosphorylation**

92 (A) Representative membranes stained with Ponceau of the different bands shown in this paper. (B)  
93 Representative bands analyzed for OXPHOS complexes (complex V, III, IV, II and I) and  $\beta$ -actin  
94 quantified by western blotting from the different experimental groups: sedentary *mdx* mice (*mdxSed*),  
95 exercise trained *mdx* mice (*mdxEx*) and exercise trained *mdx* mice treated with Tempol (*mdxEx+T*).  
96 The images in Figure 10B represent one animal per group in sequence (n=7 animals per group).