

# Supplementary Material: Exercise Reduces the Resumption of Tumour Growth and Proteolytic Pathways in the Skeletal Muscle of Mice Following Chemotherapy

Edson Alves de Lima, Jr., Alexandre Abilio de Souza Teixeira, Luana Amorim Biondo, Tiego Aparecido Diniz, Loreana Sanches Silveira, Dario Coletti, Sílvia Busquets Rius and José Cesar Rosa Neto

**Table S1.** Sequences of the primers used in real time-PCR.

| Gene          | Left Primer                  | Right Primer                  | GenBank*       |
|---------------|------------------------------|-------------------------------|----------------|
| <i>Trim63</i> | 5'-GTGTGAGGTGCCTACTTGCTC-3'  | 5'-GCTCAGTCTTCTGTCCTTGA-3'    | NM_001039048.2 |
| <i>Fbxo32</i> | 5'-ACAAAGGAAGTACGAAGGAGCG-3' | 5'-GGCAGTCGAGAAGTCCAGTC-3'    | NM_026346.3    |
| <i>Foxo1</i>  | 5'-CCCAGGCCGGAGTTTAACC-3'    | 5'-GTTGCTCATAAAGTCGGTGCT-3'   | NM_019739.3    |
| <i>Foxo3</i>  | 5'-CTGGGGGAACCTGTCCTATG-3'   | 5'-TCATTCTGAACGCGCATGAAG-3'   | NM_019740.2    |
| <i>Il6</i>    | 5'-GTTGTGCAATGGCAATTCTG-3'   | 5'-CCAGTTTGGTAGCATCCATC-3'    | NM_001314054.1 |
| <i>Msmt</i>   | 5'-AGTGGATCTAAATGAGGGAGT-3'  | 5'-GGAGTACCTCGTGTGTTTGTCTC-3' | NM_010834.3    |
| <i>Rpl19</i>  | 5'-CAATGCCAACTCCCGTCA-3'     | 5'-GTGTTTTCCGGCAAACGAG-3'     | NM_009078.2    |

\* <https://www.ncbi.nlm.nih.gov/nucleotide/>.

**Table S2.** Effects of only doxorubicin and the combination of doxorubicin plus endurance exercise on muscle and adipose tissue weight.

| Parameters        | Experimental Groups |                      |                   |                |                   |                  |
|-------------------|---------------------|----------------------|-------------------|----------------|-------------------|------------------|
|                   | 21st day            |                      |                   | 28th day       |                   |                  |
| Tissue Weight     | LLC                 | LLC + DOX            | LLC + DOX + EXER  | LLC            | LLC + DOX         | LLC + DOX + EXER |
| Tibialis anterior | 68.79 ± 2.24        | 64.67 ± 1.99         | 69.33 ± 2.09      | 65.00 ± 3.27   | 60.30 ± 3.60      | 71.89 ± 3.74 #   |
| EDL               | 25.00 ± 3.90 aa     | 22.11 ± 2.94 a       | 22.22 ± 1.96 a    | 16.50 ± 0.56   | 14.30 ± 0.73      | 14.89 ± 0.56     |
| Soleus            | 14.50 ± 0.77        | 12.47 ± 0.50 *       | 12.08 ± 0.62 *    | 13.70 ± 0.33   | 12.00 ± 0.65      | 14.11 ± 0.70     |
| Adiposity index   | 531.79 ± 32.25 aa   | 311.07 ± 49.08 ***aa | 388.58 ± 50.41 *a | 338.00 ± 41.90 | 125.60 ± 12.23 ** | 208.89 ± 16.50   |

Values represent the mean and standard error of the data obtained (mg).  $n = 15-9$ . \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  vs. LLC group; #  $p < 0.05$  compared with the LLC + DOX group; a:  $p < 0.05$  compared with the respective group within 28 days; aa:  $p < 0.01$  compared with the respective group within 28 days. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.

**Table S3.** Levels of pro- and antiinflammatory cytokines in the gastrocnemius muscle after the chemotherapy treatment.

| Parameters    | Experimental Groups |                   |                   |
|---------------|---------------------|-------------------|-------------------|
| Cytokines     | 21st day            |                   |                   |
|               | LLC                 | LLC + DOX         | LLC + DOX + EXER  |
| TNF- $\alpha$ | 38.48 $\pm$ 23.58   | 59.5 $\pm$ 23.08  | 60.42 $\pm$ 16.09 |
| IL-1 $\beta$  | 20.97 $\pm$ 12.92   | 10.43 $\pm$ 1.40  | 12.14 $\pm$ 2.61  |
| IL-1 $\alpha$ | 28.93 $\pm$ 8.32    | 67.29 $\pm$ 25.88 | 76.82 $\pm$ 26.16 |
| IL-10         | 30.07 $\pm$ 7.56    | 38.06 $\pm$ 9.70  | 69.39 $\pm$ 32.50 |

Values expressed as pg/mg protein. Values represent the mean and standard error of mean of the data obtained.  $n = 9-7$ . LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.

**Table S4.** Serum parameters.

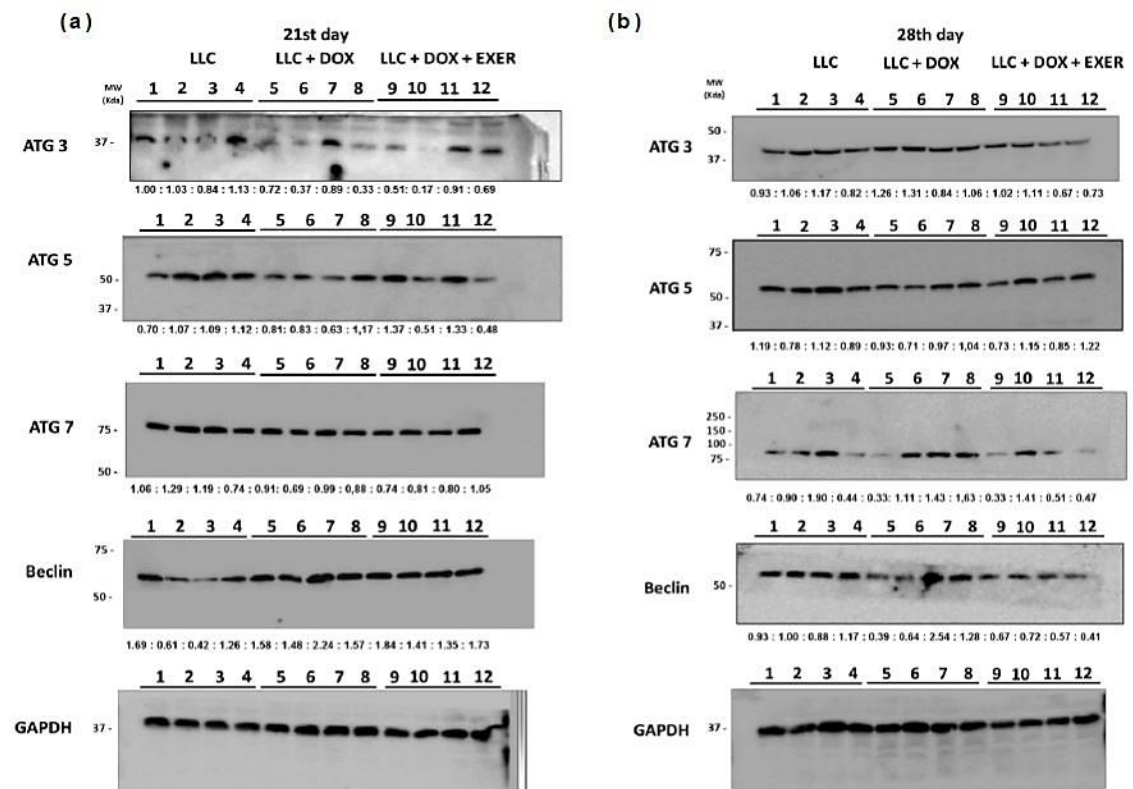
| Parameters                | Experimental Groups |                    |                     |                    |                     |                    |
|---------------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|
| Serum                     | 21st day            |                    |                     | 28th day           |                     |                    |
|                           | LLC                 | LLC + DOX          | LLC + DOX + EXER    | LLC                | LLC + DOX           | LLC + DOX + EXER   |
| Total cholesterol (mg/dL) | 174.00 $\pm$ 19.52  | 160.90 $\pm$ 21.7  | 132.50 $\pm$ 18.53  | 126.62 $\pm$ 17.54 | 122.22 $\pm$ 4.78   | 120.95 $\pm$ 4.54  |
| Triglycerides (mg/dL)     | 118.80 $\pm$ 3.51   | 110.80 $\pm$ 9.76  | 127.70 $\pm$ 15.56  | 151.86 $\pm$ 20.56 | 114.79 $\pm$ 32.69  | 89.63 $\pm$ 13.95  |
| Lactate (mg/dL)           | 47.87 $\pm$ 3.42    | 55.48 $\pm$ 7.81 a | 35.37 $\pm$ 1.56 #a | 46.16 $\pm$ 4.15   | 103.47 $\pm$ 7.18 * | 88.31 $\pm$ 4.49 * |

Values represent the mean and standard error of the mean of the data obtained.  $n = 9-6$ . \*  $p < 0.05$  vs. LLC group; #  $p < 0.05$  compared with the LLC+DOX group. a:  $p < 0.05$  in compared with the respective group on the 28th day. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.

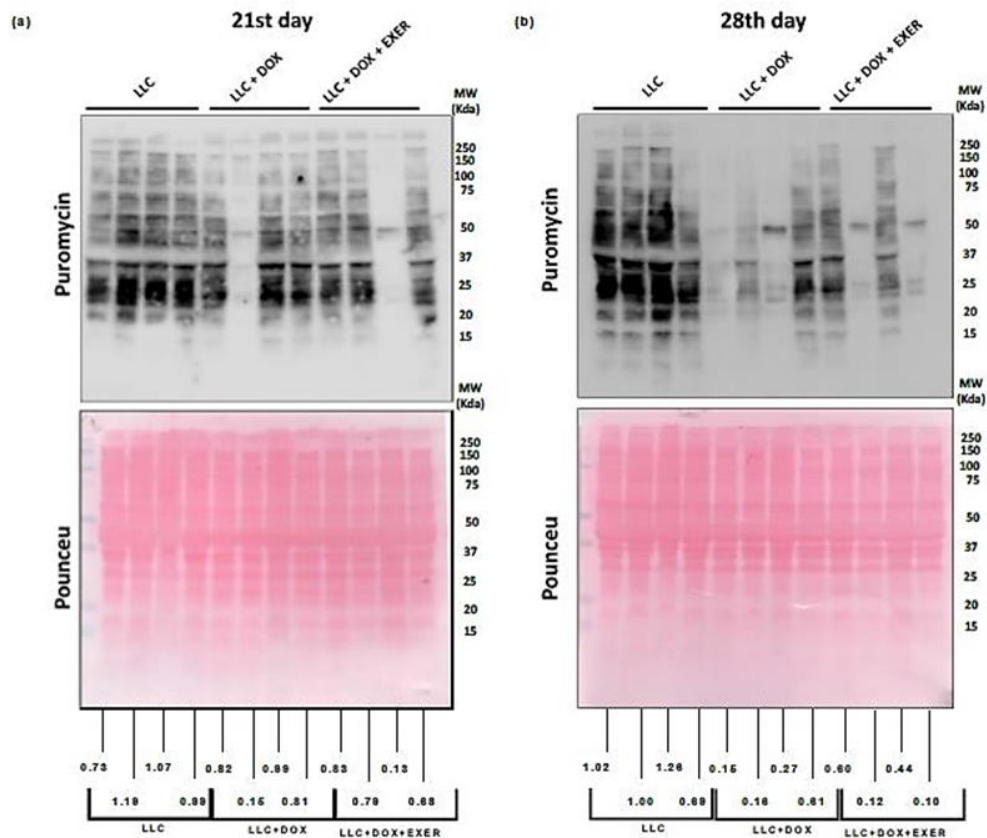
**Table S5.** Glucose metabolism in tumour-bearing mice.

| Parameters         | Experimental Groups   |                  |                      |                    |                 |                   |
|--------------------|-----------------------|------------------|----------------------|--------------------|-----------------|-------------------|
| Glucose metabolism | 21st day              |                  |                      | 28th day           |                 |                   |
|                    | LLC                   | LLC + DOX        | LLC + DOX + EXER     | LLC                | LLC + DOX       | LLC + DOX + EXER  |
| Glucose (mg/dL)    | 142.63 $\pm$ 10.20 aa | 116.7 $\pm$ 7.34 | 117.89 $\pm$ 6.55 aa | 190.26 $\pm$ 22.35 | 150 $\pm$ 10.27 | 175.40 $\pm$ 5.72 |
| Insulin (ng/mL)    | 1.14 $\pm$ 0.51       | 0.89 $\pm$ 0.10  | 0.92 $\pm$ 0.19      | 1.16 $\pm$ 0.14    | 1.00 $\pm$ 0.47 | 0.87 $\pm$ 0.16   |

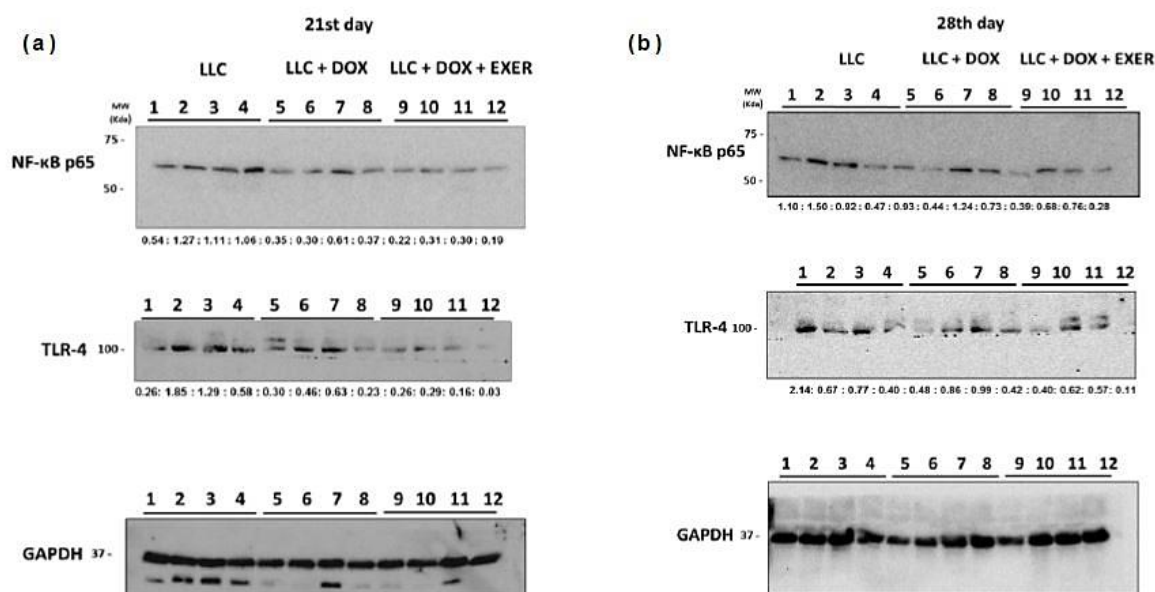
Values represent the mean and standard error of the mean of the data obtained.  $N = 8$ . aa:  $p < 0.01$  compared with the respective group on the 28th day. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.



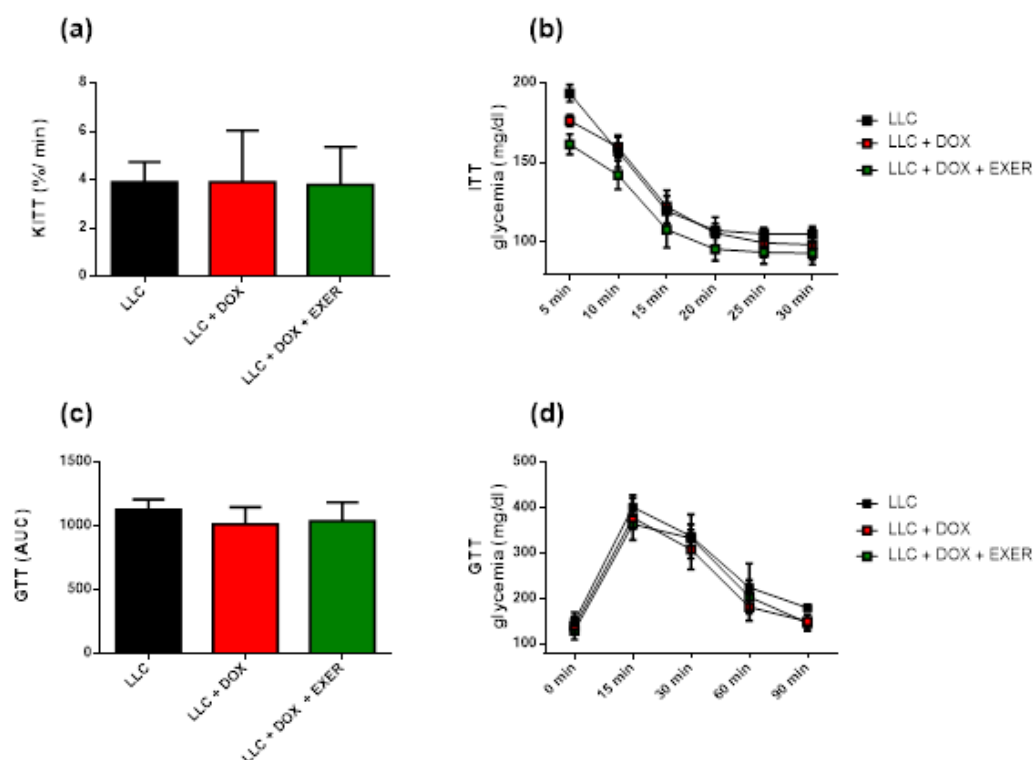
**Figure S1.** Uncropped Western Blot of Figure 4. Autophagic signaling in gastrocnemius. *n* = 4. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.



**Figure S2.** Uncropped Western Blot of Figure 5. Evaluation of total protein synthesis immediately and after chemotherapy.  $n = 4$ . LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.



**Figure S3.** Uncropped Western Blot of Figure 6. Inflammatory signalling in skeletal muscle.  $n = 4$ . LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.



**Figure S4.** Effect of treatments on glucose metabolism immediately after the interruption of chemotherapy. (a). Serum glucose disappearance rate (KiTT) during the insulin tolerance test.  $n = 14-13$ ; (b) Serum glucose levels after intraperitoneal insulin administration (0.5 IU); (c) Area under the curve of the glucose tolerance test.  $n = 5$ ; (d) Serum glucose levels after intraperitoneal glucose administration (2 g/kg body weight). Values represent the mean and standard error of the mean of

the analysis of the data obtained. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).