

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

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**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>Msnt</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 |                   |                   |
|---------------|---------------------|-------------------|-------------------|
|               | 21st day            |                   |                   |
| Cytokines     | 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-1ra        | 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.

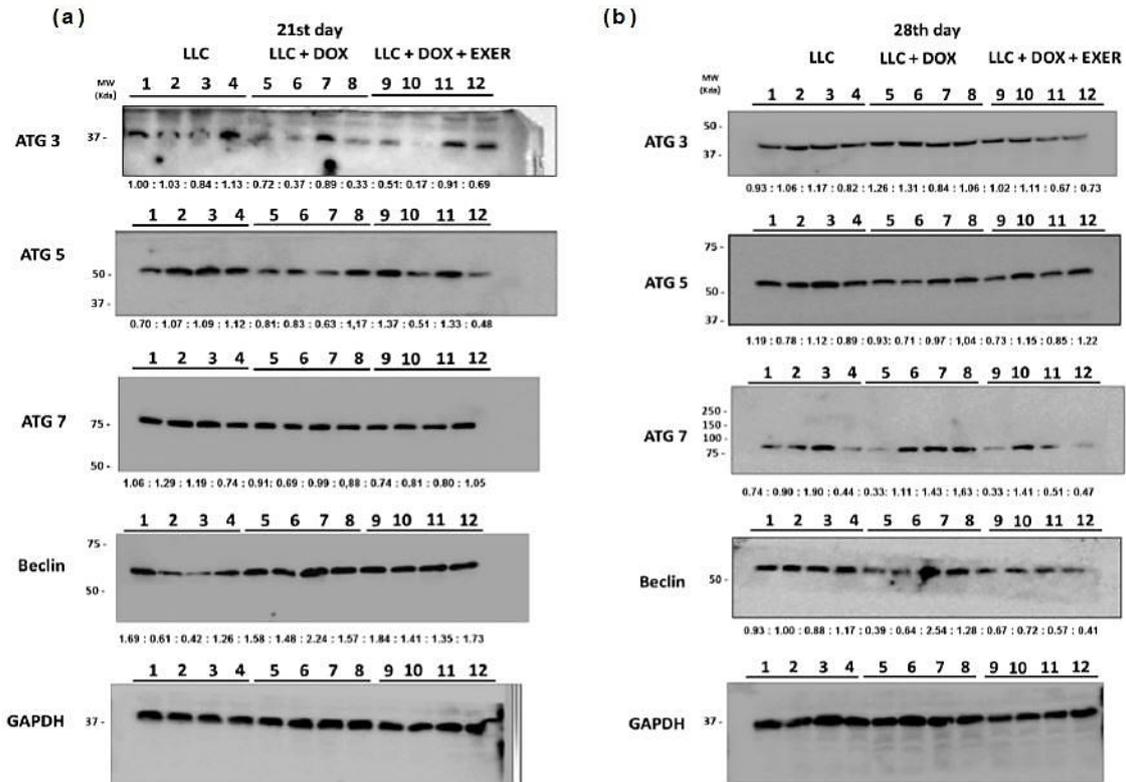
| Parameters                | Experimental Groups |                    |                     |                    |                     |                    |
|---------------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|
|                           | 21st day            |                    |                     | 28th day           |                     |                    |
| Serum                     | 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.

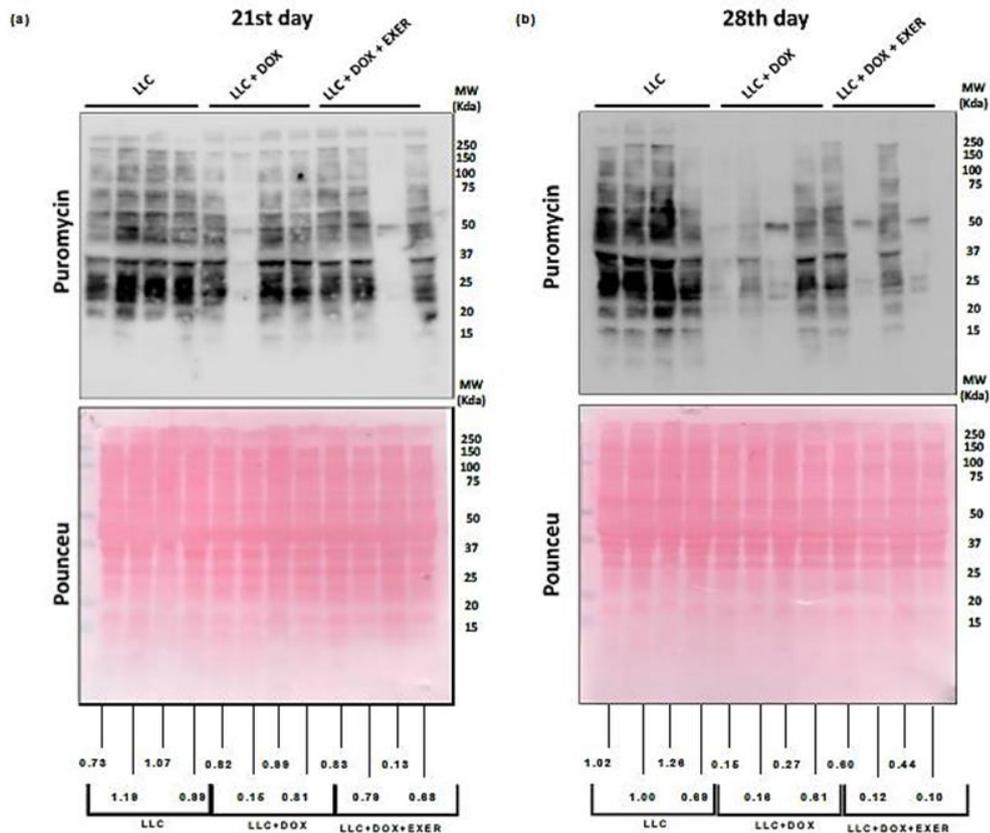
**Table S5.** Glucose metabolism in tumour-bearing mice.

| Parameters         | Experimental Groups   |                  |                      |                    |                 |                   |
|--------------------|-----------------------|------------------|----------------------|--------------------|-----------------|-------------------|
|                    | 21st day              |                  |                      | 28th day           |                 |                   |
| Glucose metabolism | 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   |

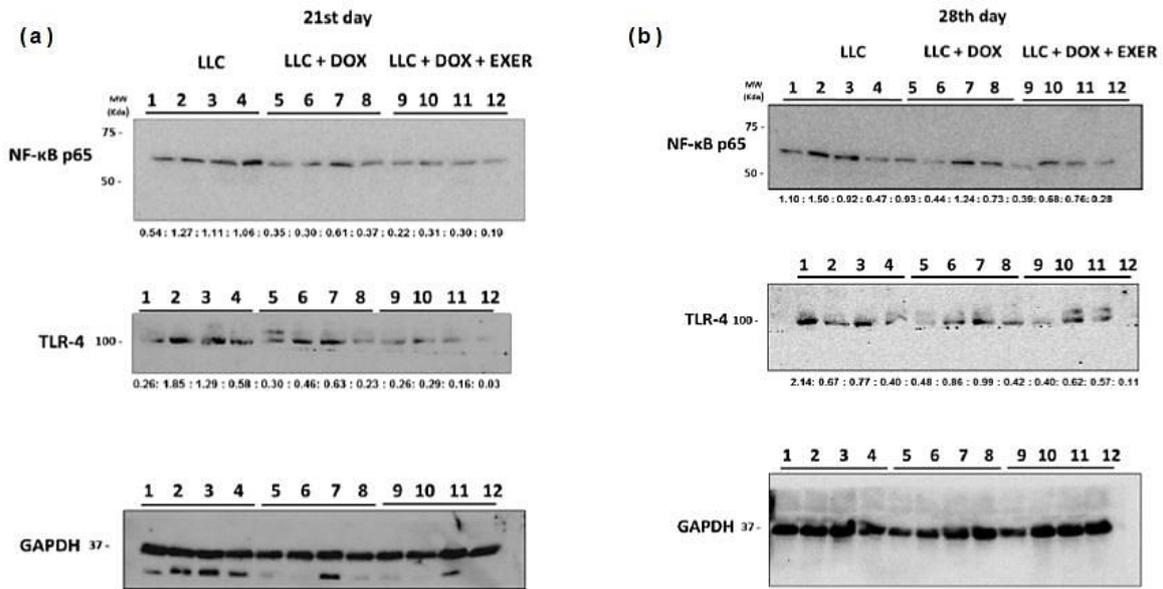
Values represent the mean and standard error of the mean of the data obtained.  $N = 8$ . aa:  $p < 0.01$  compared with the respective group on the 28th day. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.



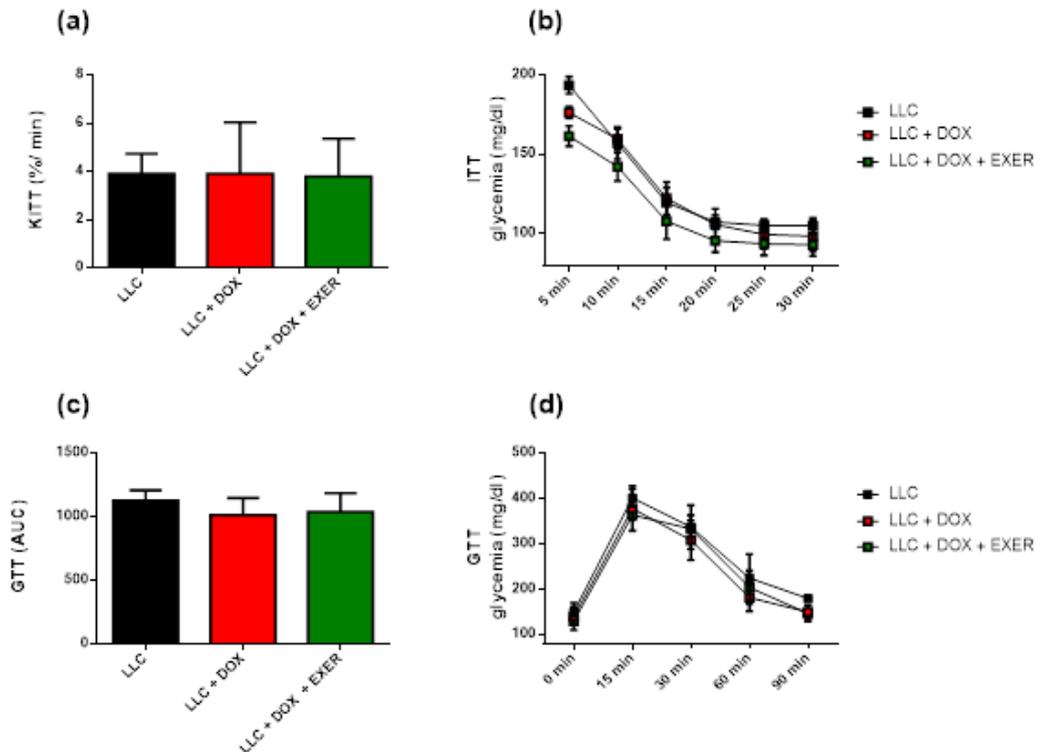
**Figure S1.** Uncropped Western Blot of Figure 4. Autophagic signaling in gastrocnemius. *n* = 4. LLC = Lewis lung carcinoma; DOX = doxorubicin; EXER = endurance exercise.



**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.

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