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Supplemental Material to:

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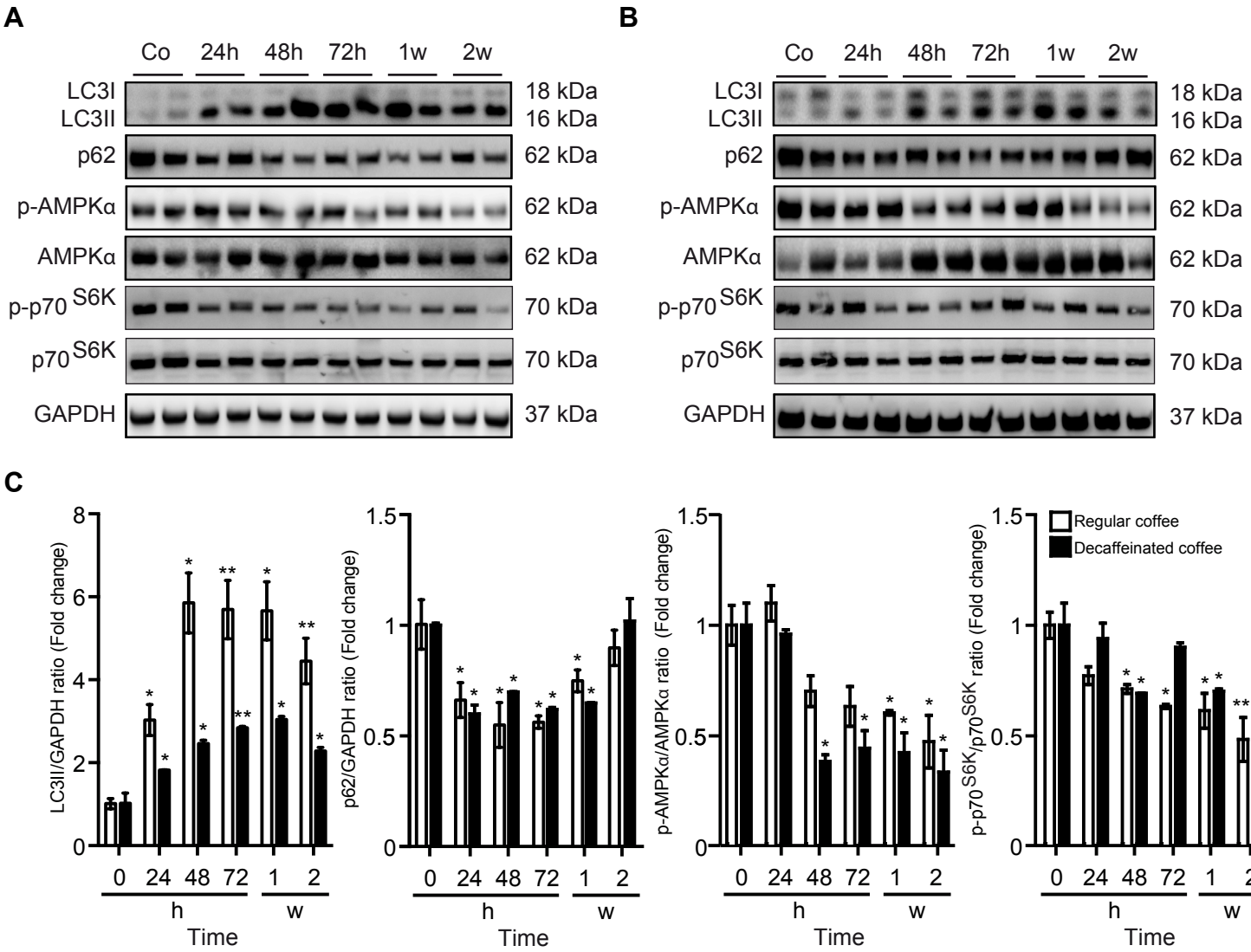
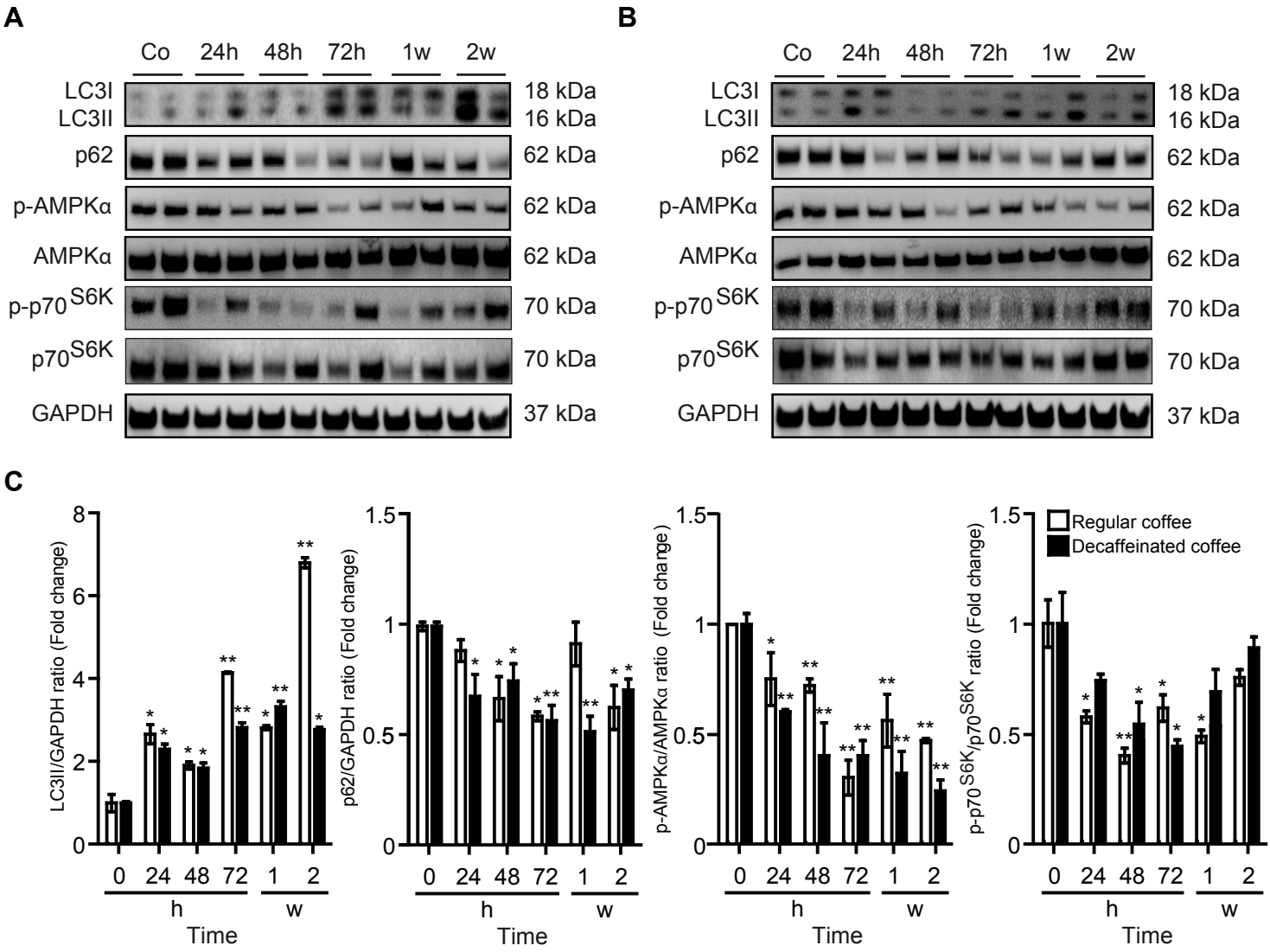
Coffee induces autophagy in vivo

Cell Cycle 2014; 13(12)
http://dx.doi.org/10.4161/cc.28929

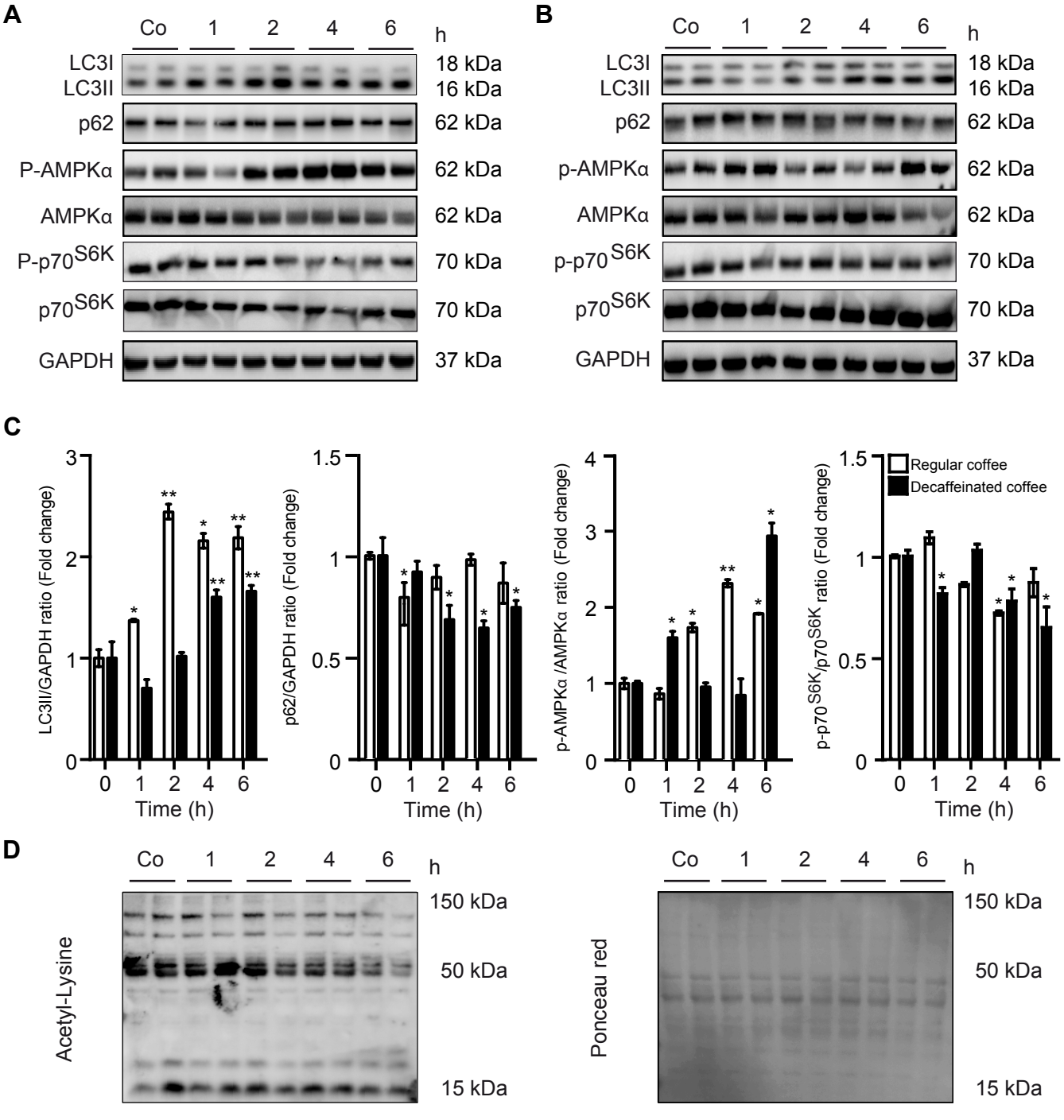
http://www.landesbioscience.com/journals/cc/article/28929



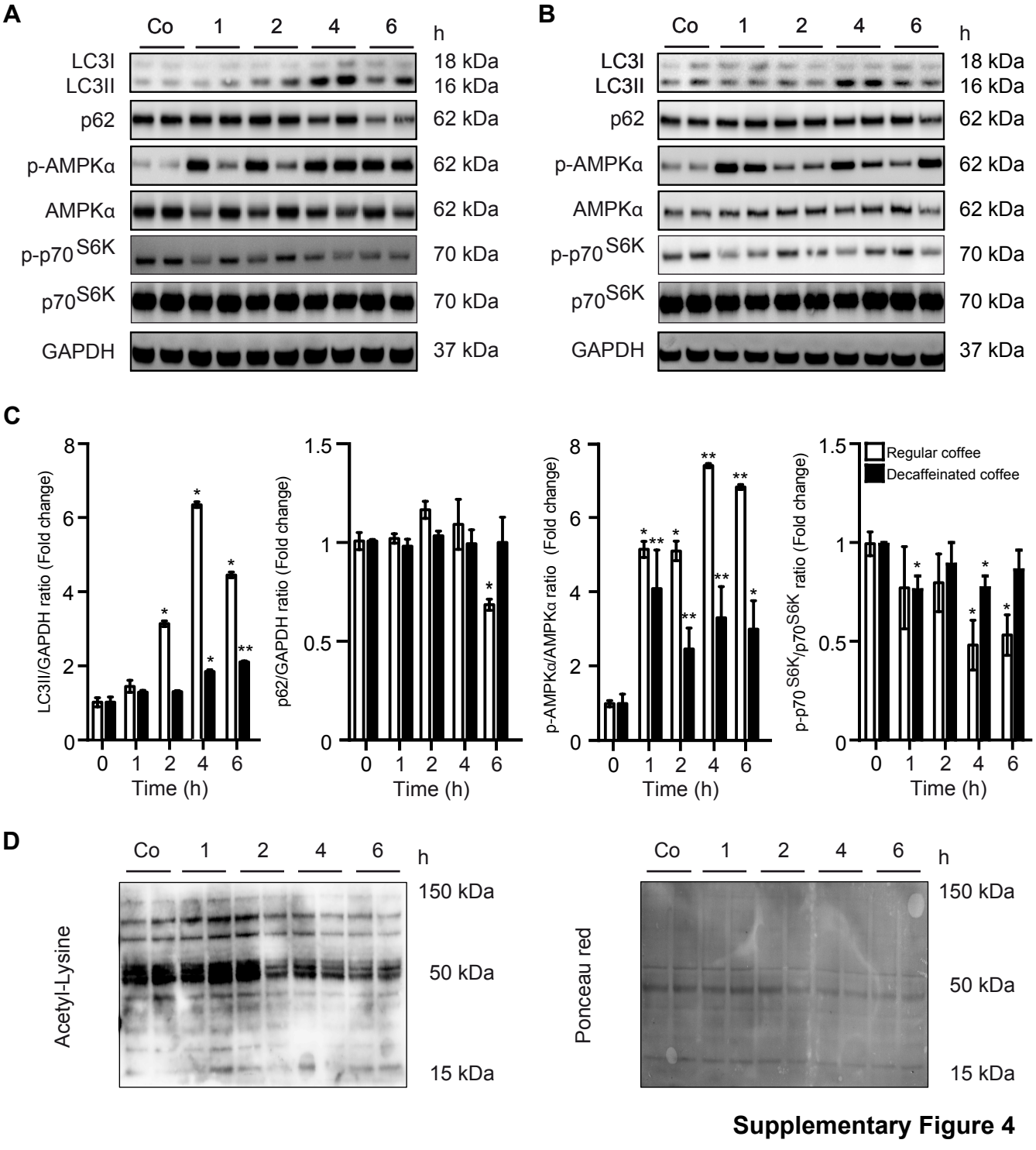
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Supplemental Figures

Fig. S1. Long-term administration of regular and decaffeinated coffee at a dose not affecting body weight induces autophagy in heart. (A-C). Immunoblot analysis of long-term coffee administration on autophagy regulation in heart. Both coffee (A) and decaffeinated coffee (B) administration for up to 2 weeks resulted in activation of autophagy, as measured by LC3 lipidation and p62 degradation.(quantified in (C)). Autophagy activation was associated to a decrease in mTORC1 activity, as measured by the phosphorylation of $p70^{s6k}$, but not to an activation of AMPK phosphorylation (quantified in (C)). Representative images are reported in (A) and (B). GAPDH levels were monitored to ensure equal loading of lanes. Results from 3 independent experiments are presented as fold change ± SEM. *p < 0.05; **p < 0.01; (unpaired, two-tailed Student's t-test), compared to untreated mice.

Fig.S2. Long-term administration of regular and decaffeinated coffee at a dose not affecting body weight induces autophagy in muscle. (A-C). Immunoblot analysis of long-term coffee administration on autophagy regulation in muscle. Both coffee (A) and decaffeinated coffee (B) administration for up to 2 weeks resulted in activation of autophagy, as measured by LC3 lipidation and p62 degradation. (quantified in (C)). Autophagy activation was associated to a decrease in mTORC1 activity, as measured by the phosphorylation of $p70^{s6k}$, but not to an activation of AMPK phosphorylation (quantified in (C)). Representative images are reported in (A) and (B). GAPDH levels were monitored to ensure equal loading of lanes. Results from 3 independent experiments are presented as fold change ± SEM. *p < 0.05; **p < 0.01; (unpaired, two-tailed Student's t-test), compared to untreated mice.

Fig. S3. **Short-term administration of regular or decaffeinated coffee induces autophagy accompanied by a reduction in global acetylation levels of proteins in the heart.**

(A-B). Immunoblot analyses of short-term coffee administration on autophagy regulation in heart. Gavage of regular (A) or decaffeinated coffee (B) resulted in an activation of autophagy, as assessed by measuring LC3 lipidation and p62 degradation (quantified in (C)). In both cases, autophagy induction was accompanied by an activation of AMPK and by a reduction in the activity of mTORC1, as measured by the phosphorylation of its substrate $p70^{s6k}$ (quantified in (C)). Representative images are depicted in (A) and (B). Results from n = 3 independent experiments are presented as fold change ± SEM. *p < 0.05; **p < 0.01; (unpaired, two-tailed Student's t-test), compared to untreated mice.

(D). Immunoblotting-assisted detection of protein acetylation in mice administered with regular coffee. Coffee administration resulted in a significant drop in the overall protein acetylation in the heart (quantified in Fig 2.E), significantly starting from 2 hours after treatment. Ponceau Red staining was used to monitor equal loading of the lanes (n = 3 independent experiments).

Fig. S4. **Short-term administration of regular or decaffeinated coffee induces autophagy accompanied by a reduction in global acetylation levels of proteins in the muscle.**

(A-B). Immunoblot analyses of short-term coffee administration on autophagy regulation in muscle. Gavage of regular (A) or decaffeinated coffee (B) resulted in an activation of autophagy, as assessed by measuring LC3 lipidation and p62 degradation (quantified in (C)). In both cases, autophagy induction was accompanied by an activation of AMPK and by a reduction in the activity of mTORC1, as measured by the phosphorylation of its substrate $p70^{s6k}$ (quantified in (C)). Representative images are depicted in (A) and (B). Results from n =

3 independent experiments are presented as fold change ± SEM. *p < 0.05; **p < 0.01; (unpaired, two-tailed Student's t-test), compared to untreated mice.

(D). Immunoblotting-assisted detection of protein acetylation in mice administered with regular coffee. Coffee administration resulted in a significant drop in the overall protein acetylation in the muscle (quantified in Fig 2.E), significantly starting from 4 hours after treatment. Ponceau Red staining was used to monitor equal loading of the lanes (n = 3 independent experiments).