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Article

Angiotensin-(1-7) Prevents Lipopolysaccharide-Induced Autophagy via the Mas Receptor in Skeletal Muscle

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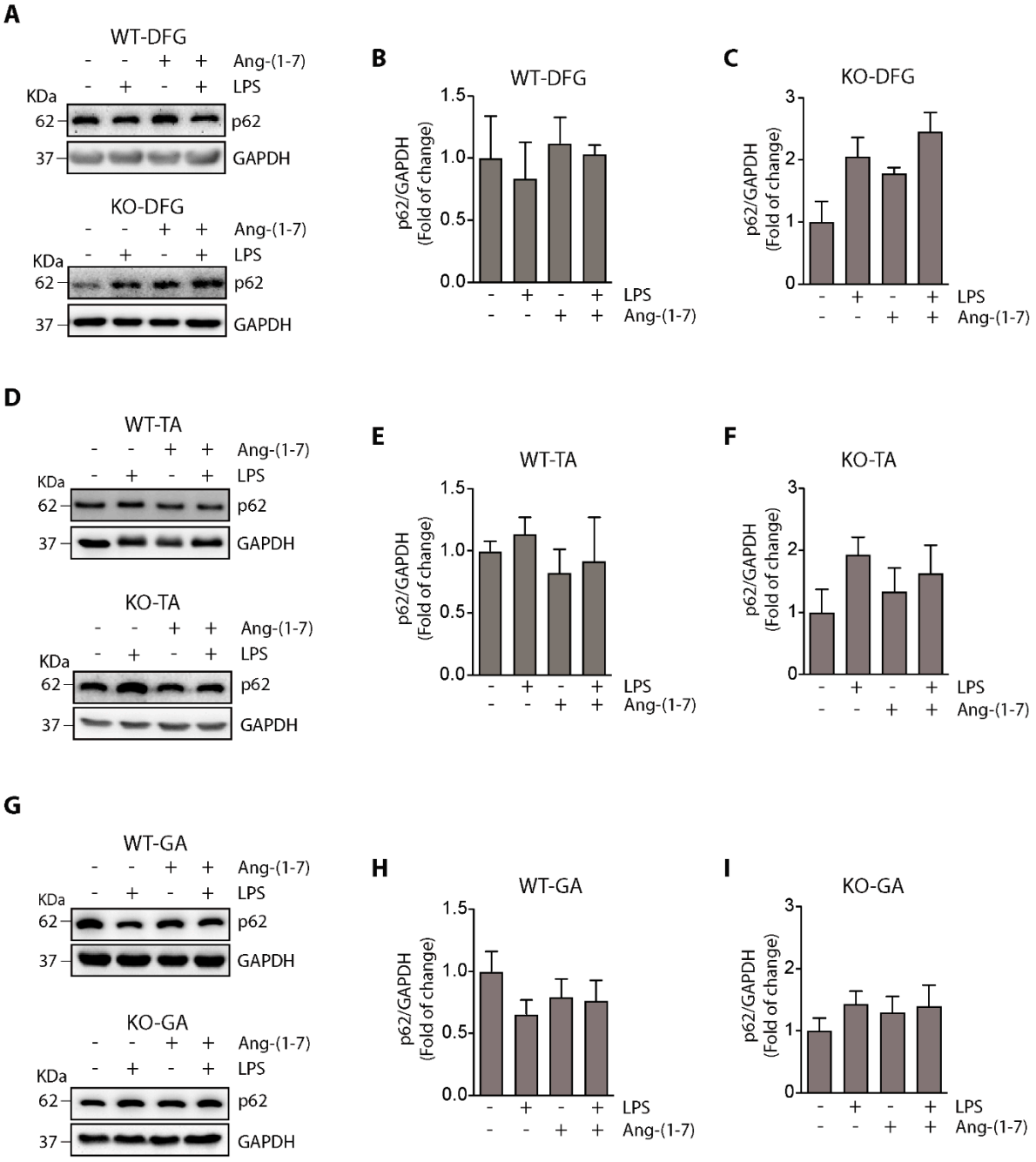

Figure S1. Treatment with LPS and/or Ang-(1-7) does not change the p62/SQSTM1 protein levels in muscles from mice. C57BL/6J wild-type or Mas KO male mice were treated with the vehicle, LPS, Ang-(1-7) or LPS+Ang-(1-7) for 18 h. When the experiments were finished, the mice were sacrificed, and the muscle was excised and homogenised to evaluate p62/SQSTM1 protein levels through Western blot analysis in DFG **(A)**, TA **(D)** and GA **(G)**. GAPDH levels are shown as the loading control (The images for GAPDH are the same as those for Figure 2). Molecular weight markers are depicted in kDa. The quantitative analysis of the experiments is shown for DFG **(B, C)**, TA **(E, F)** and GA **(H, I)**. The results were expressed as the mean ± S.E. (the fold of change relative to the vehicle group). Nine animals per group were used for these experiments (*, $P < 0.05$ vs. vehicle. #, $P < 0.05$ vs. LPS).

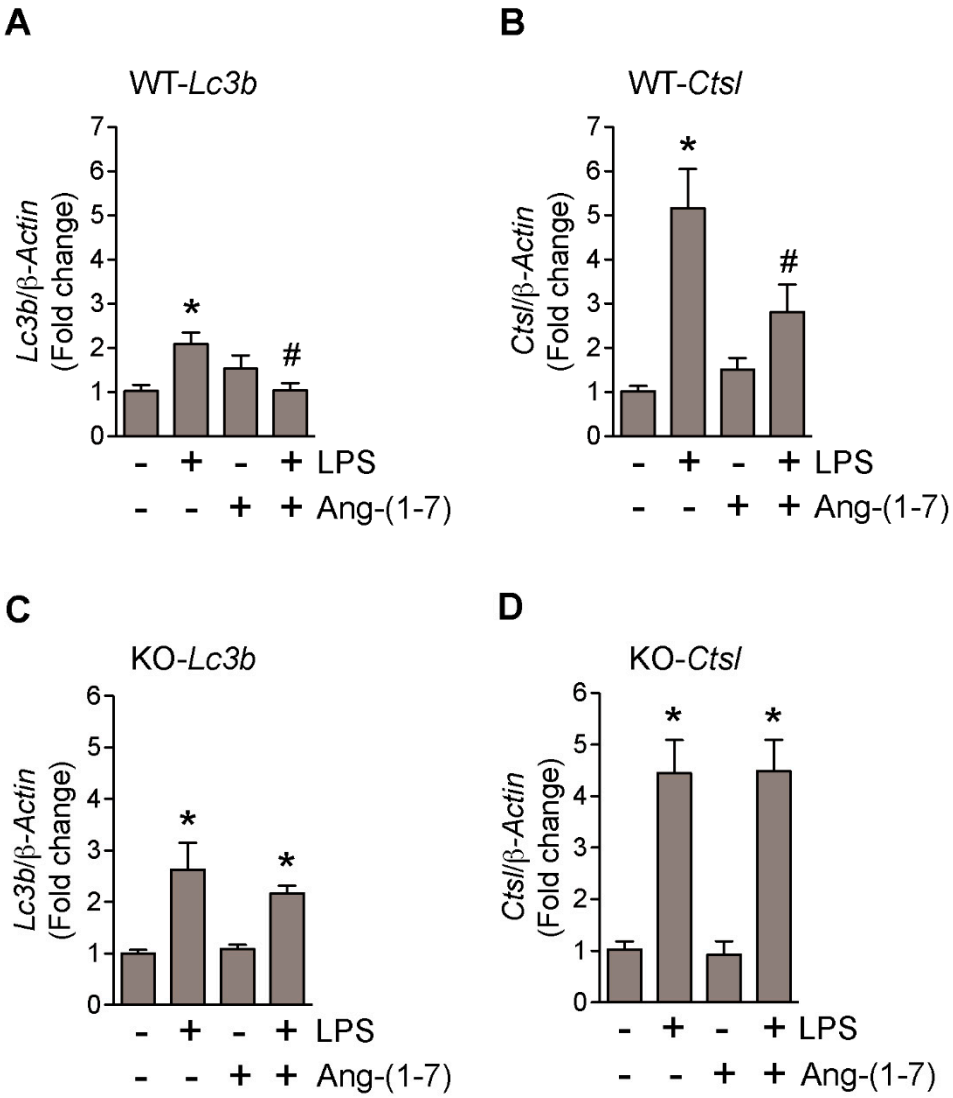

Figure S2. Ang-(1-7) reduced LPS-induced *Lc3b* and *Ctsl* gene expressions via the Mas receptor in muscles from mice. *Lc3b* and *Ctsl* gene expressions were evaluated by RT-qPCR in DFG from WT **(A, B)** and KO Mas **(C, D)** mice treated with the vehicle, LPS, Ang-(1-7) or LPS+Ang-(1-7) for 18 h. The graph shows the mRNA levels of *Lc3b* **(A, C)** and *Ctsl* **(B, D)** normalised to the β-actin levels used as the loading control. The levels are represented as the mean ± S.E. (the fold of change relative to the vehicle). Nine to twelve animals per group were used in these experiments (*, $P < 0.05$ vs. vehicle; #, $P < 0.05$ vs. LPS).

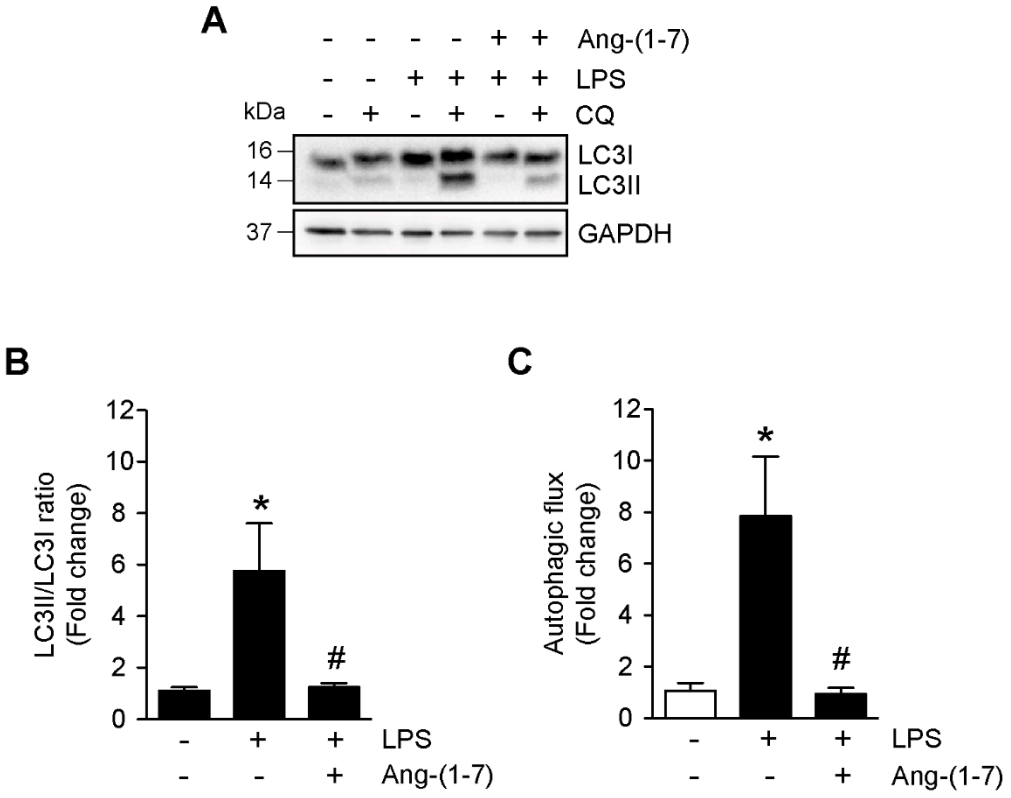

Figure S3. Ang-(1-7) reduced LPS-induced autophagy in C2C12 myoblasts. C2C12 cells were pre-incubated without or with of Ang-(1-7) (100 nM) for 30 min. Then, the cells were incubated with or without CQ (50 µM) for 5 min. Finally, the cells were incubated without or with LPS (500 ng/mL) for 8 h. **(A)** LC3I and LC3II protein levels were detected by Western blot analysis. The levels of GAPDH are shown as the loading control. Molecular weights are shown in kDa. **(B)** Quantitative analysis of three independent experiments represented in **(A)** of treatments with CQ. The results were described as the LC3II/LC3I ratio and expressed as the mean ± S.E. (the fold of change relative to the control group. *, $P < 0.05$ vs. vehicle. #, $P < 0.05$ vs. LPS). **(C)** Autophagic flux derived from **(A)**. The value of LC3II was normalised to GAPDH and expressed as the mean ± S.E. (the fold of change relative to the control group. *, $P < 0.05$ vs. vehicle. #, $P < 0.05$ vs. LPS).

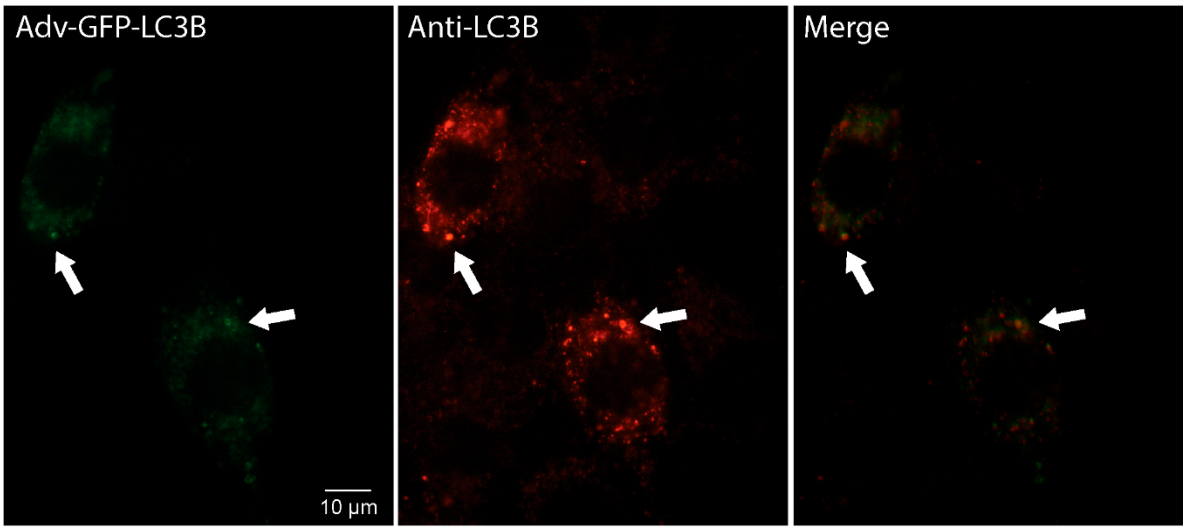

Figure S4. Colocalization of overexpressed GFP-LC3B with endogenous LC3B in LPS-induced autophagosomes *in vitro*. C2C12 cells were transduced with Adv-GFP-LC3. The cells were incubated with CQ (50 µM) for 5 min and further incubated with LPS (500 ng/mL) for 8 h. At the end of the experiment, fluorescent detection of GFP (left) and LC3B was performed using indirect

immunofluorescence with anti-LC3B (centre). The colocalisation of overexpressed GFP with endogenous LC3B is shown (right). The arrows indicate examples of autophagosomes (puncta).