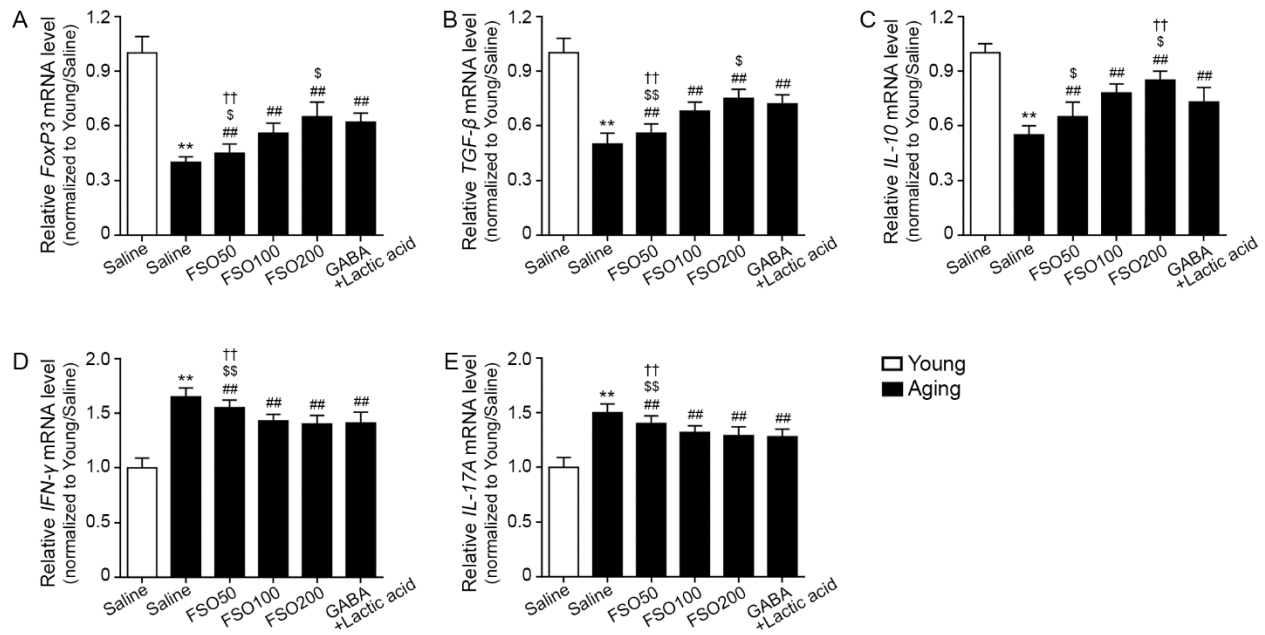# Supplementary materials

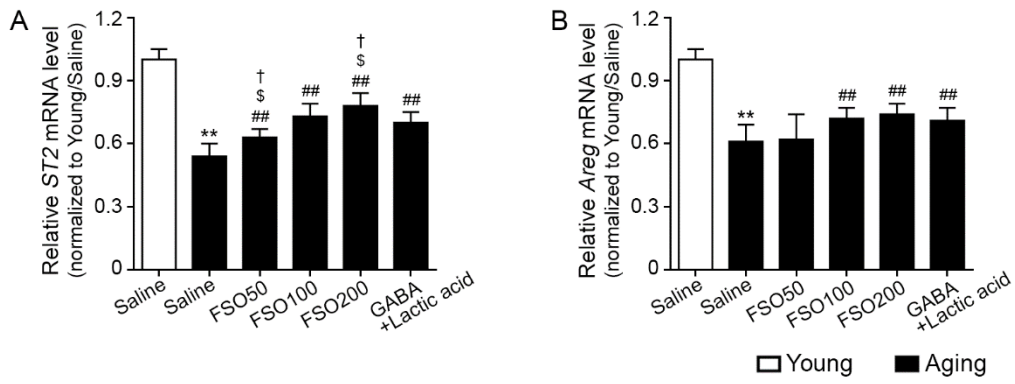

**Supplementary Fig. S1. Regulation of Treg accumulation and anti-inflammatory cytokines by FSO treatment.** (A–E) mRNA expression levels of FoxP3 (A), TGF-β (B), IL-10 (C), IFN-γ (D), and IL-17A (E) in muscle were analyzed by quantitative real-time polymerase chain reaction, normalized versus *Actb*, and expressed relative to levels in the Young/Saline group. Expression levels of FoxP3 (Treg), TGF-β and IL-10 (anti-inflammatory cytokines) are reduced in aged muscle, which are enhanced by FSO treatment. However, expression levels of IFN-γ and IL-17A (pro-inflammatory cytokines) increase in aged muscle and decrease following FSO treatment. Data are presented as the mean ± SD. $^{**}$ $p < 0.01$ second bar vs. first bar. $^{##}$ $p < 0.01$ vs. second bar. $^{\$}$ $p < 0.05$ or $^{\$\$}$ $p < 0.01$ vs. fourth bar. $^{\dagger}$ $p < 0.05$ or $^{\dagger\dagger}$ $p < 0.01$ vs. sixth bar (Mann-Whitney U test). FSO, fermented sarco oyster extract; FoxP3, forkhead box protein 3; GABA, gamma-aminobutyric acid; IFN-γ, interferon-gamma; IL, interleukin; TGF-β, transforming growth factor-beta, Treg, regulatory T cell.

**Supplementary Fig. S2. Regulation of ST2 and Areg by FSO treatment.** (A and B) mRNA expression levels of ST2 (A) and Areg (B) in muscle were analyzed by quantitative real-time polymerase chain reaction, normalized versus *Actb*, and expressed relative to levels in the Young/Saline group. Expression levels of ST2 and Areg are decreased in aged muscle, which are increased by FSO treatment. Data are presented as the mean ± SD. ** $p < 0.01$ second bar vs. first bar. ## $p < 0.01$ vs. second bar. $ $p < 0.05$ vs. fourth bar. † $p < 0.05$ vs. sixth bar (Mann-Whitney U test). Areg, amphiregulin; FSO, fermented sarco oyster extract; GABA, gamma-aminobutyric acid; ST2, interleukin 1 receptor-like 1.

22 **Supplementary Table S1.** List of antibodies for IF, WB, and IHC

| Antibody (host) | Company | Catalog no. | Dilution rate |
|---|---|---|---|
| Foxp3 (mouse) | Santa Cruz Biotechnology | sc-53876 | 1:100 (IF) |
| ATG5 (rabbit) | Novus Biologicals | NB110-53818 | 1:400 (IF) |
| GABARAP (rabbit) | Proteintech | 11010-1-AP | 1:200 (IF) |
| β-actin (rabbit) | Cell Signaling Technology | 4967s | 1:1,000 (WB) |
| Beclin1 (mouse) | Santa Cruz Biotechnology | sc-48341 | 1:500 (WB) |
| LC3 I/II (mouse) | Santa Cruz Biotechnology | sc-376404 | 1:500 (WB) |
| IL-33 (rabbit) | Bioss | BS-2208R | 1:100 (IHC) |
| CD86 (mouse) | Santa Cruz Biotechnology | sc-19617 | 1:500 (WB) |
| CD206 (mouse) | Santa Cruz Biotechnology | sc-58986 | 1:500 (WB) |
| PAX7 (mouse) | Santa Cruz Biotechnology | sc-81975 | 1:500 (WB) |

23 IF, immunofluorescence; WB, western blotting; IHC, immunohistochemistry.

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**Supplementary Table S2.** List of primer for quantitative real time polymerase chain reaction.

| Gene | Primers | |
| --- | --- | --- |
| *Actb* | Forward | 5'-CCGTAAAGACCTCTATGCCAAC-3' |
| | Reverse | 5'-GCAGTAATCTCCTTCTGCATCC-3' |
| *FoxP3* | Forward | 5'-TAC TTC AGA AAC CAC CCC GC-3' |
| | Reverse | 5'-AGT CTC ATG GTT TTG GCC CC-3' |
| *TGF-β* | Forward | 5'-GGA CTC TCC ACC TGC AAG AC-3' |
| | Reverse | 5'-CAT AGA TGG CGT TGT TGC GG-3' |
| *IL-10* | Forward | 5'-AAG GGT TAC TTG GGT TGC CA-3' |
| | Reverse | 5'-GCC TGG GGC ATC ACT TCT AC-3' |
| *IFN-γ* | Forward | 5'-TGG AGG AAC TGG CAA AAG GA-3' |
| | Reverse | 5'-TGC TGA TGG CCT GAT TGT CTT-3' |
| *IL-17A* | Forward | 5'-TCT TTA ACT CCC TTG GCG CA-3' |
| | Reverse | 5'-CCA CCA GCA TCT TCT CGA CC-3' |
| *ST2* | Forward | 5'-TCC TGC AGA GTC ATG CTT CG-3' |
| | Reverse | 5'-GGA ATG GGC AGT GCT GTA GT-3' |
| *AREG* | Forward | 5'-ACT TTG GTG AAC GGT GTG GA-3' |
| | Reverse | 5'-GTG ATA ACG ATG CCG ATG CC-3' |
| *MYF5* | Forward | 5'-TCT GGT CCC GAA AGA ACA GC-3' |
| | Reverse | 5'-GCT CGG ATG GCT CTG TAG AC-3' |
| *MyoD* | Forward | 5'-CAG CAT AGT GGA GCG CAT CT-3' |
| | Reverse | 5'-TTC CCT GTT CTG TGT CGC TT-3' |
| *Myogenin* | Forward | 5'-ATC CAG TAC ATT GAG CGC CT-3' |

| | | |
|---|---|---|
| | Reverse | 5'-CGC GAG CAA ATG ATC TCC TG-3' |
| *MuRF-1* | Forward | 5'-CAC GTG TGA GGT GCC TAC TT-3' |
| | Reverse | 5'-TCT TGA TGA GCT GCT TGG CA-3' |
| *Atrogin-1* | Forward | 5'-AGG AGC GCC ATG GAT ACT GT-3' |
| | Reverse | 5'-CCA CTC AGG GAT GTG AGC TG-3' |