Supplemental Figures



S1. Creatine Kinase concentration changes in contusion-modeled muscle. Related to figure 2. (A) The serum creatine kinase was analysis at before injured and 2h, 24h after injured. (n=4) *P <

0.05 ****P < 0.0001.



S2. Image of LIPUS treatment in mice and 2D gait patterns. Related to figure 2.

(A) The contusion-modeled mouse was placed in a container to fully expose the right contusion gastrocnemius muscle. LIPUS is then fixed with a designated scaffold to keep the ultrasound transducer parallel to the muscle, and the appropriate amount of coupling agent was applied before treatment.

(B) Representative images of 2D gait patterns of different groups. The difference in gait cycle was visualized.



S3. Histological staining images of injured muscle. Related to figure 3.

(A) Representative HE staining images of injured muscle among different groups on Day 3.

(B) Representative Masson staining images of injured muscle among different groups on Day 7.



S4. The representative data of blood perfusion in the Contusion group. (A)The injured hind of the Contusion group. (B) The healthy hind of the Contusion group. Related to figure 3.



S5. Relative protein expressions of insignificantly changed inflammatory cytokines after LIUPS treatment. Related to figure 4.



S6. Histological staining images of injured muscle with macrophage depletion. Related to figure 5.

(A) Representative HE staining images of injured muscle with macrophage depletion among different groups on Day 7.

(B) Representative Masson staining images of injured muscle with macrophage depletion among different groups on Day 14.



S7. Image of LIPUS treatment on cells and the cellular viability at different LIPUS intensities treatment. Related to figure 6.

(A) We fixed LIPUS on a flat table with the ultrasound treatment head up and placed six-well plates on it to keep them stable and parallel between them. The cells were then treated.

(B) Cell Counting Kit-8 (CCK-8) is tested at different LIPUS intensities treatment for determining range of treatment intensity (n=4) *P < 0.05. **P < 0.01.

(C) Cell Counting Kit-8 (CCK-8) is tested at different LPS concentration stimulating RAW 264.7 (n=4) P < 0.05.

(D-E) Masson staining is conducted for muscle fibrosis for different LIPUS intensities (10mW/cm², 20mW/cm², 30mW/cm², 60mW/cm², and 90mW/cm²) treatment for determining range of treatment intensity (n=4) *P < 0.05. The scale bar from top to bottom is 250µm, 100µm, and 50µm. Muscle fibers on Day 14 were calculated in different groups



S8. Morphology of LPS-induced M1 macrophages. Related to figure 6.

(A) Negative controlled RAW264.7 cell morphology under microscope. Scale bar=200, 100, or 50µm.

(B) LPS-induced RAW264.7 cell morphology under microscope. Scale bar=200, 100, or 50µm.

(C) LIPUS-treated and LPS-induced RAW264.7 cell morphology under microscope. Scale bar=200, 100, or 50µm.



S9. LIPUS regulated the expression of FZD5 *in vitro*. Related to figure 7.

(A-B) Relative protein expression of FZD5 and FZD1 in RAW 264.7 after three different condition treatment was calculated after normalized with GAPDH. (n=4). *P < 0.05.



S10. Drug titration (XAV-939 and QS11) curve with cell viability(RAW264.7).

(A-B) Cell Counting Kit-8 (CCK-8) is tested at different concentration of XAV-939 and QS11 for cell viability of RAW264.7. (n=4) P < 0.05. P < 0.01.

Supplemental Movies

- Sup M1. Ultrasound therapy in mice
- Sup M2. Water maze test
- Sup M3. Catwalk
- Sup M4. Rotarod test
- Sup M5. Treadmill test
- Sup M6. Ultrasound treatment in vitro

Original Western Blot Date

Related to Fig.4 (C) : Day 3 In vivo

CD206(166kDa)

- Beta-tubulin(55kDa) 5
 - ARG1(38kDa)













iNOS(130kDa) 11 7

Related to Fig.4 (c):

Day 7 In vivo

Beta-tubulin(55kDa)

CD86(38kDa)







Related to Fig.6 (E): In vitro

kDa 200 140 Contusion+ Contusion+ Control Ultrasound^H Ultrasound^L iNOS(130kDa) 110 Contusion+ Contusion+ 75 $Ultrasound^{\rm H}$ Control Ultrasound^L Beta-tubulin(55kDa) 55 42 CD86(38kDa) 30 22 18





Related to Fig.7 (A): In vitro







Related to Fig.7 (A): In vivo



:

FZD5(65kDa)

GAPDH(38kDa)



Supplemental Movies Sup M1. Ultrasound therapy in mice Sup M2. Water maze test Sup M3. Catwalk Sup M4. Rotarod test Sup M5. Treadmill test Sup M6. Ultrasound treatment in vitro