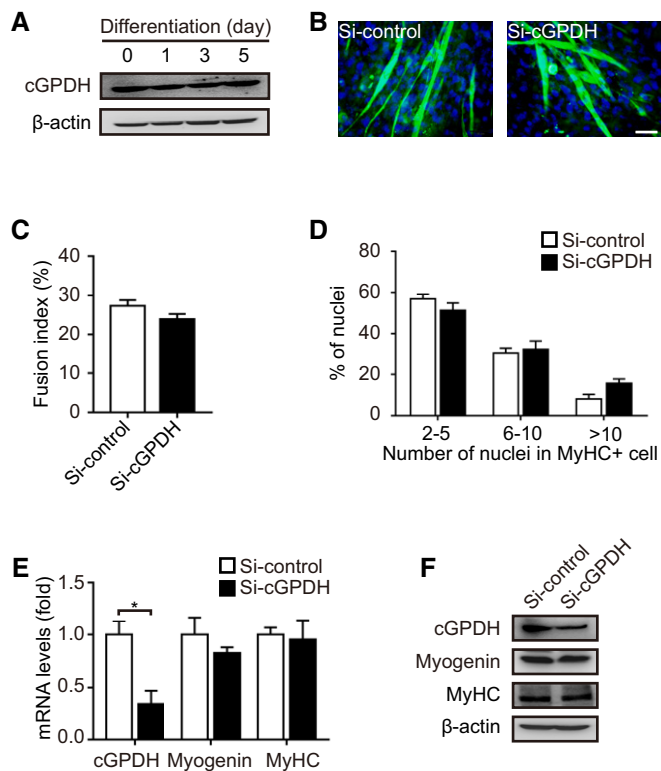


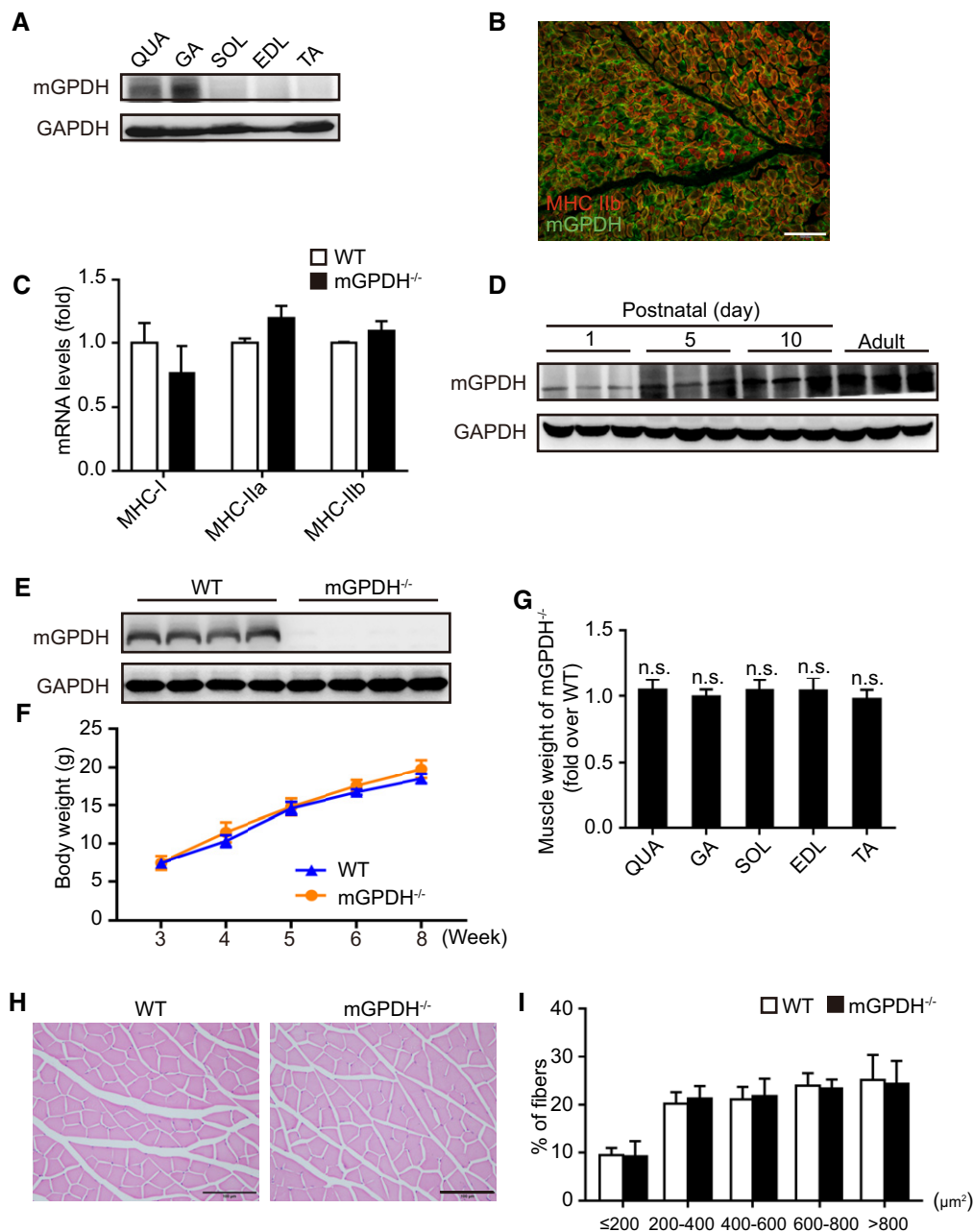
## Expanded View Figures



**Figure EV1. Effect of cGPDH on myoblast differentiation.**

A cGPDH expression during C2C12 myocyte differentiation.  
 B–D Representative images of MyHC immunofluorescence (B) of C2C12 myocytes transfected with siRNA targeting cGPDH; the fusion index (C) and the distribution of nuclei per myotube (D) were calculated.  
 E, F qRT-PCR (E) and Western blot analysis (F) of myogenin and MyHC in C2C12 myocytes transfected with siRNA targeting cGPDH.

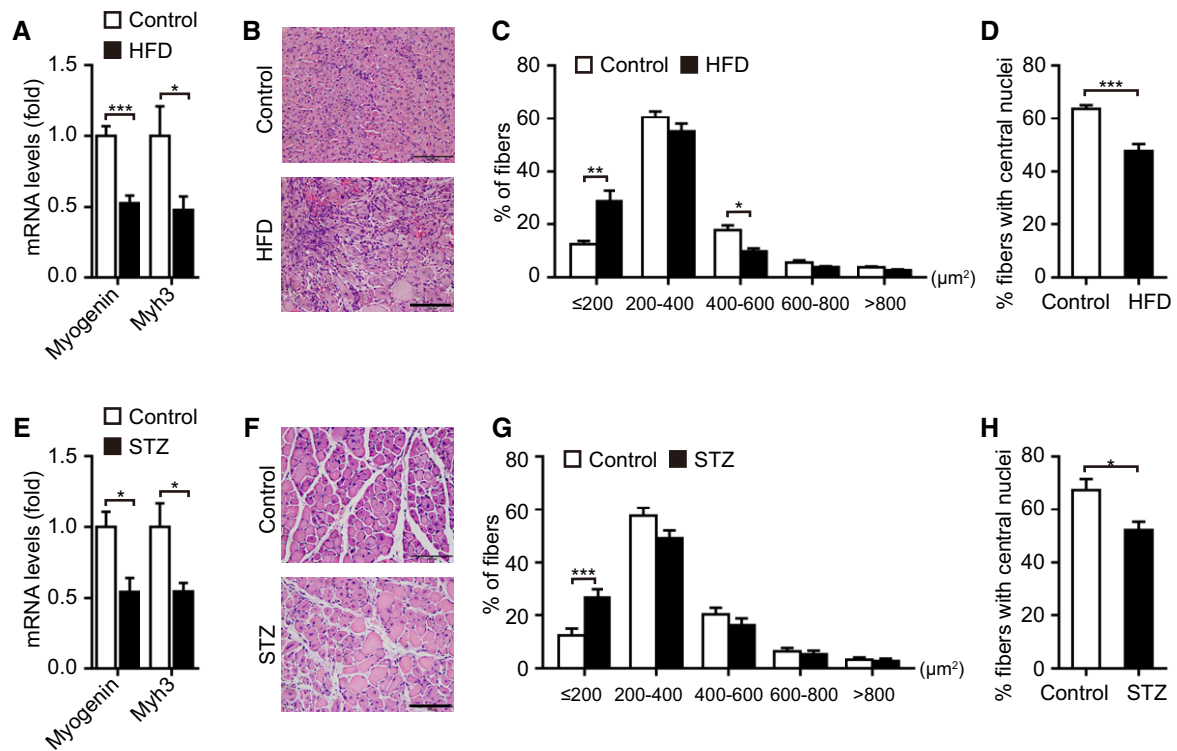
Data information: Data are presented as the mean ± s.e.m. Scale bars represent 50 μm in panel (B). In panels (A–F),  $n = 3$ . \* $P < 0.05$ . Unpaired  $t$ -test was used for all analyses except in panel (D), where the Kolmogorov–Smirnov test was used.



**Figure EV2. mGPDH is not essential to muscle development.**

- A** Immunoblot of mGPDH in the quadriceps (QUA), gastrocnemius (GA), soleus (SOL), extensor digitorum longus (EDL), and tibialis anterior (TA) muscles of 8-week-old C57BL/6J mice.
- B** Immunofluorescence showing localization of mGPDH with fiber type marker MHC IIb on cryosections from uninjured GA muscle of 8-week-old C57BL/6J mice.
- C** qRT-PCR analyses of the indicated fiber type markers (MHC I, IIa, and IIb) in the uninjured GA muscles of 8-week-old WT and mGPDH<sup>-/-</sup> mice.
- D** Immunoblot of mGPDH in C57BL/6J mouse skeletal muscle at postnatal days 1, 5, and 10 and 8 weeks.
- E** Immunoblot of mGPDH in the GA muscle of 8-week WT and mGPDH<sup>-/-</sup> mice.
- F** Body weight of WT and mGPDH<sup>-/-</sup> mice at the indicated week of age.
- G** Muscle weight of the indicated 8-week-old mGPDH<sup>-/-</sup> mice normalized to WT.
- H, I** Hematoxylin–eosin (H&E) staining (H) and average myofiber cross-sectional area (CSA) (I) in the GA muscle of 8-week-old WT and mGPDH<sup>-/-</sup> mice.

Data information: Data are presented as the mean  $\pm$  s.e.m. Scale bars represent 200  $\mu$ m in panel (B) and 100  $\mu$ m in panel (H). In panels (A and D),  $n = 3$  mice per group; in panels (B and C),  $n = 6$  mice per group; in panels (E–I),  $n = 4$  mice per group; in panels (H and I), three sections were obtained per mouse. n.s., not significant. Unpaired  $t$ -test was used for all analyses except in panel (I), where the Kolmogorov–Smirnov test was used.

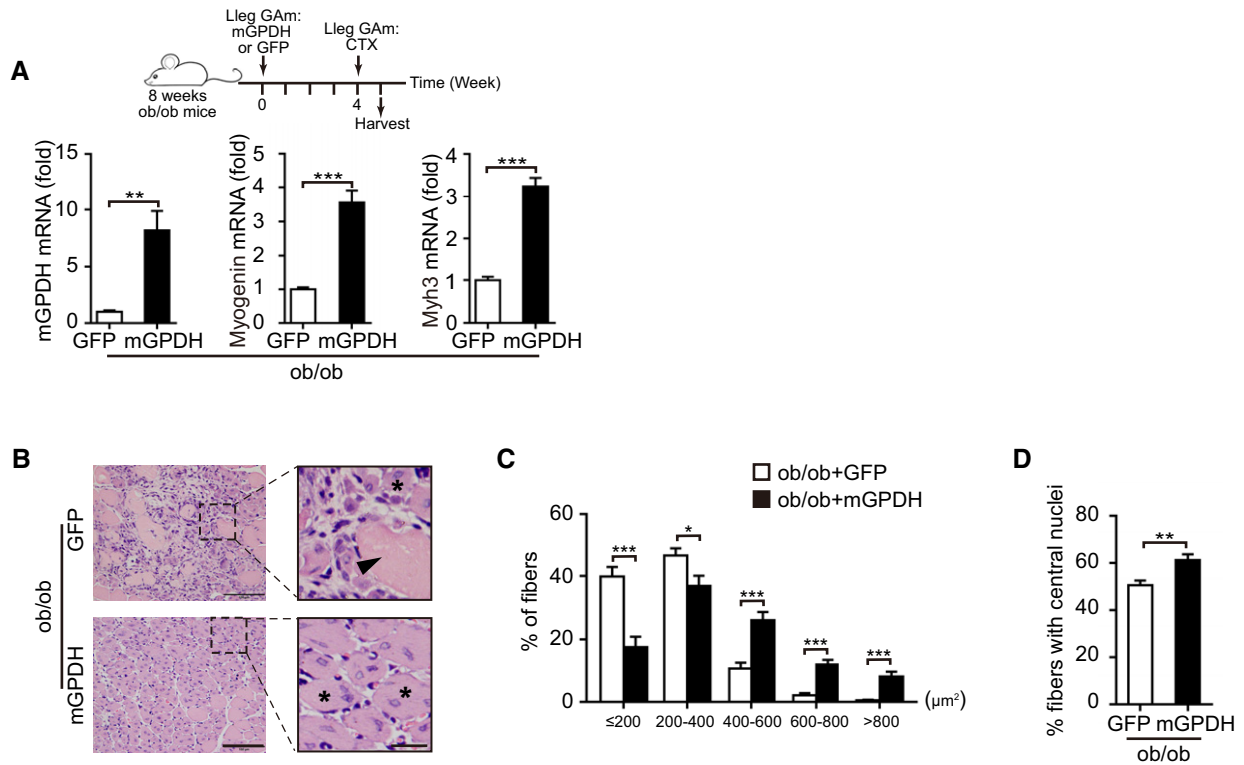


**Figure EV3. Skeletal muscle regeneration is impaired in HFD-fed mice and STZ-injected mice.**

A–D Gastrocnemius (GA) muscles were obtained from HFD-fed mice at day 7 post-CTX injury. Quantification of myogenin and myh3 by qRT–PCR (A), representative images of H&E staining (B), distribution of the CSA (C), and percentage of myofibers with central nuclei (D).

E–H GA muscles were obtained from STZ-treated mice 4 weeks after STZ injection and at day 7 post-CTX injury. Quantification of myogenin and myh3 by qRT–PCR (E), representative images of H&E staining (F), distribution of the fibers CSA (G), and percentage of myofibers with central nuclei (H).

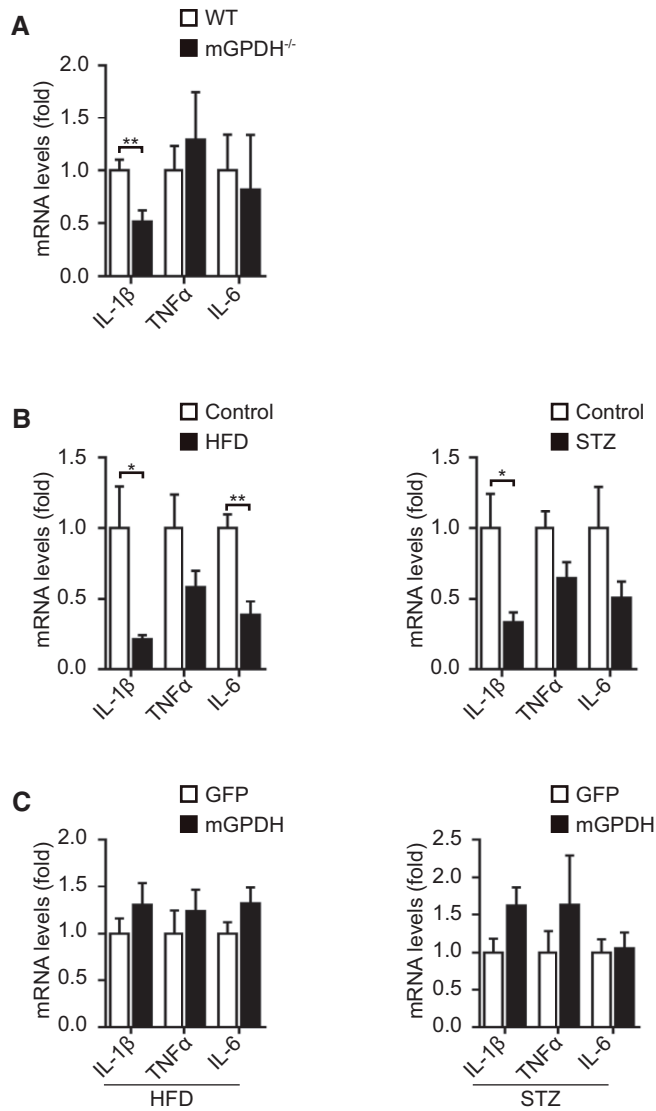
Data information: Data are presented as the mean  $\pm$  s.e.m. Scale bars represent 100  $\mu\text{m}$  in panels (B and F).  $n = 6$  mice per group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Unpaired  $t$ -test was used for all analyses except in panels (C and G), where the Kolmogorov–Smirnov test was used.



**Figure EV4. mGPDH activation improves skeletal muscle regeneration in *ob/ob* mice.**

A–D Experimental setup (A, upper panel) and qRT–PCR of mGPDH, myogenin, and myh3 gene expressions (A, bottom panel), H&E staining (arrowhead, necrotic myofibers; asterisks, regenerating fibers) (B), distribution of CSA (C), and percentage of fibers with central nuclei (D) in GA muscle from AAV–mGPDH-treated *ob/ob* mice at day 7 post-CTX.

Data information: Data are presented as the mean  $\pm$  s.e.m. Scale bars represent 100  $\mu\text{m}$  (25  $\mu\text{m}$  for magnification insets) in panel (B). In panels (A–D),  $n = 6$  mice per group; in panels (B–D), three sections were obtained per mouse. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Unpaired  $t$ -test was used for all analyses except in panel (C), where the Kolmogorov–Smirnov test was used.



**Figure EV5. Effect of mGPDH on inflammatory signaling.**

A–C Quantification of the indicated inflammatory cytokines by qRT–PCR in GA muscles of mGPDH<sup>-/-</sup> mice (A), HFD-fed and STZ-treated mice (B), and HFD-fed and STZ-treated mice intramuscularly injected with AAV-mGPDH (C) at day 7 post-CTX injury.

Data information: Data are presented as the mean  $\pm$  s.e.m.  $n = 6$  mice per group. \* $P < 0.05$ , \*\* $P < 0.01$ . Unpaired t-test was used for all panels.