

Supplemental Information

Figure Legends:

Sup. Fig. 1. Suppression of FAK expression by its miRNAs.

(A) Western blot analysis showing FAK expression in Cos-7 cells transfected with indicated plasmids.

(B) Immunostaining analysis of endogenous FAK in C2C12 cells transfected with miRNA-FAK. C2C12 cells were transiently transfected with miRNA-FAK plasmid, which encodes GFP as well as miRNA of FAK. Transfected cells were fixed and immunostained with anti-FAK antibody (rabbit polyclonal)(red). GFP (green) indicates expression of the miRNA-FAK plasmid. Open arrows indicated FAK distribution in untransfected cells, and filled arrows indicated reduced FAK expression in miRNA-FAK transfected cells. Bar, 20 μm .

Sup. Fig. 2. H₂O₂ enhancing muscle differentiation and myogenin expression.

(A) C2C12 myoblasts were transfected with GFP and cultured in growth medium for 24 hrs. The cells were then switched into FM (fusion medium) to induce myotube formation. At ~48 hr incubation with the FM, H₂O₂ (50 μM) was added to the medium. GFP labeled myotube images at the indicated time were shown in bottom panels.

(B) Western blot analysis of myogenin expression in differentiating C2C12 cells exposed to H₂O₂ (50 μM at the indicated time).

(C) Western blot analysis of H₂O₂ induced myogenin expression in differentiating C2C12 cells expressing scramble miRNA and miRNA-FAK. C2C12 myoblasts were transfected

with indicated miRNAs. Transfected cells were then cultured with FM to induce differentiation and stimulated by H₂O₂ as illustrated in (B).

Sup. Fig. 3. Dural functions of H₂O₂ on myogenic differentiation in C2C12 cells.

(A) Summary of data and method in the literature that demonstrate an inhibitory effect on myogenic differentiation by application of H₂O₂ at sub-lethal dose (>100 μM) prior to the induction of differentiation (Escobedo et al., 2004; Langen et al., 2002; Rando et al., 1998; Zaccagnini et al., 2007)

(B) Illustration of data and method in this manuscript that suggest a stimulatory effect on myogenic differentiation by application of H₂O₂ (~50 μM) after the differentiation program is established. This is in line with the observations by (Puri et al., 2002).