Supplementary data

Loss of CD36 protects against diet-induced obesity but results in impaired muscle stem cell function, delayed muscle regeneration and hepatic steatosis

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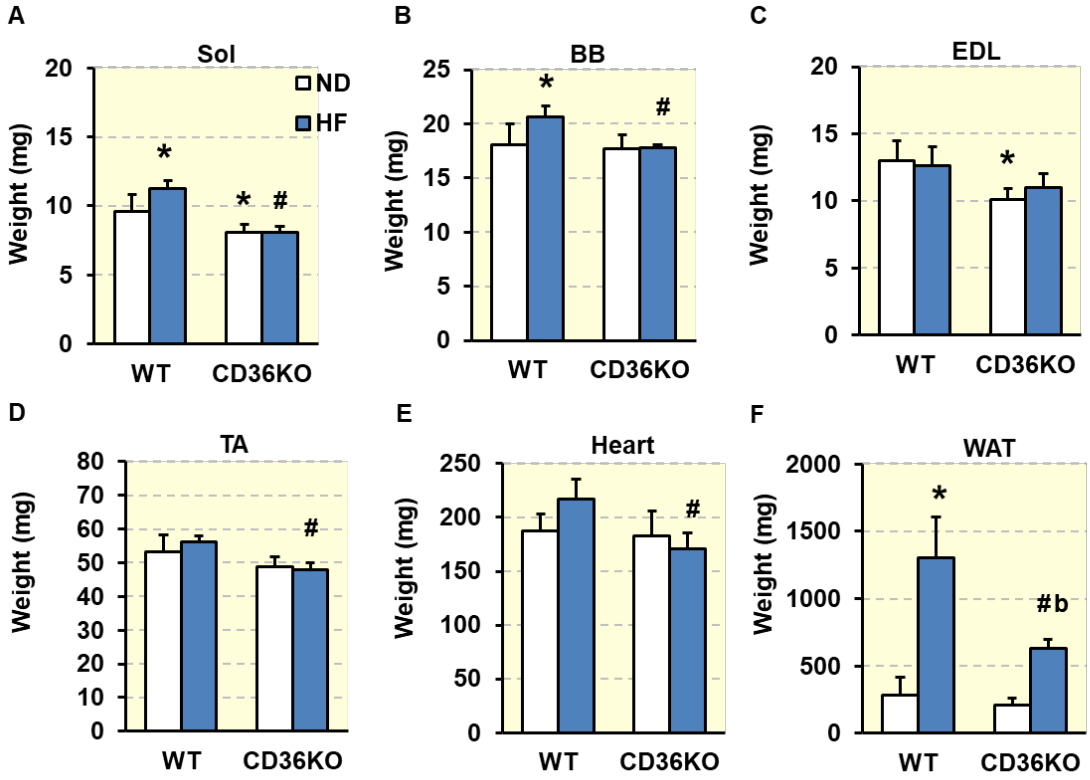
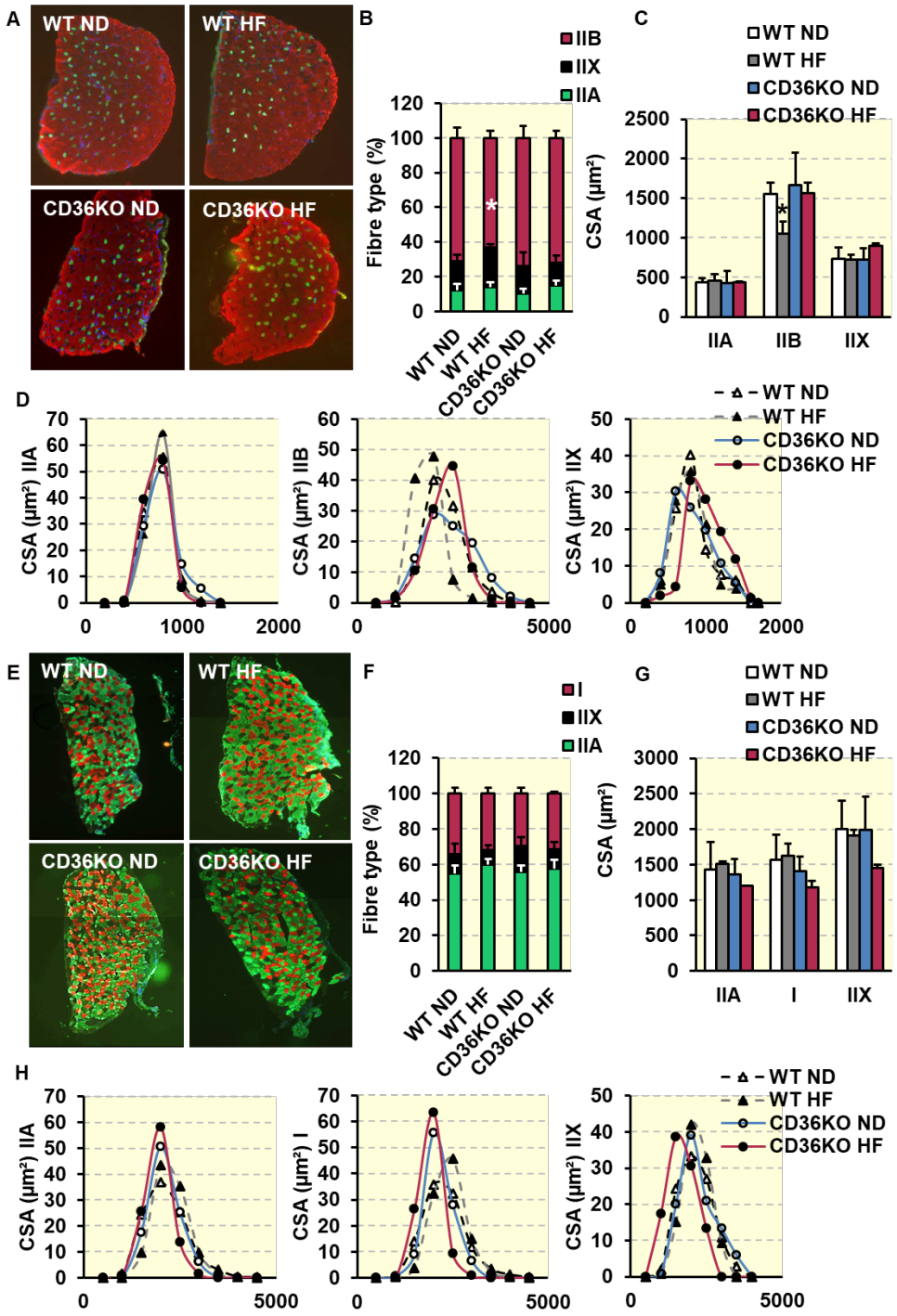
Supplementary Table 1. Overview of the effect of CD36 deficiency on blood lipids and glucose

	CD36 KO ND vs. WT ND	**CD36 KO HF vs. WT HF**
Total Cholesterol	↑**0- 1.3fold** (Febbraio, M. et al. 1999 PMID: 10383407 Goudriaan et al . 2003 PMID12923231, Brundert et al. 2011 PMID3284166)	No difference (Berger et al. 2019 PMID31289200)
VLDL/IDL	↑**1.4 fold** (Masuda, D. et al. 2008 PMID: 2666186,	↑**3fold** (Masuda, D. et al. 2008 PMID: 2666186)
LDL	No difference (Masuda, D. et al. 2008 PMID: 2666186	↑**2-fold** (Masuda, D. et al. 2008 PMID: 2666186)
TGs	↑**1.3fold- 2.4fold** (Jeltje R. Goudriaan et al. 2005 PMID: 16024917, Febbraio, M. et al. 1999 PMID: 10383407, Coburn et al. 2000 PMID: 10913136, Masuda, D. et al. 2008 PMID: 2666186, Goudriaan et al . 2003 PMID12923231, Hajri et al 2002 PMID150975)	↑**1.3fold** (Jeltje R. Goudriaan et al. 2005 PMID: 16024917, Hajri et al 2002 PMID150975)
FFA	↑**1.4- 1.9 fold** (Febbraio, M. et al. 1999 PMID: 10383407, (Masuda, D. et al. 2008 PMID: 2666186, Jeltje R. Goudriaan et al. 2005 PMID: 16024917, Goudriaan et al . 2003 PMID12923231, Hajri et al 2002 PMID150975	↑**1.4-2fold** (Masuda, D. et al. 2008 PMID: 2666186, Koonen et al. 2010 PMID2874697, Hajri et al 2002 PMID150975
Glucose	↓**0.5- 0.7fold** Goudriaan et al . 2003 PMID12923231, Hajri et al 2002 PMID150975	↓**1.8- 1.9 fold** Koonen et al. 2010 PMID2874697, Hajri et al 2002 PMID150975

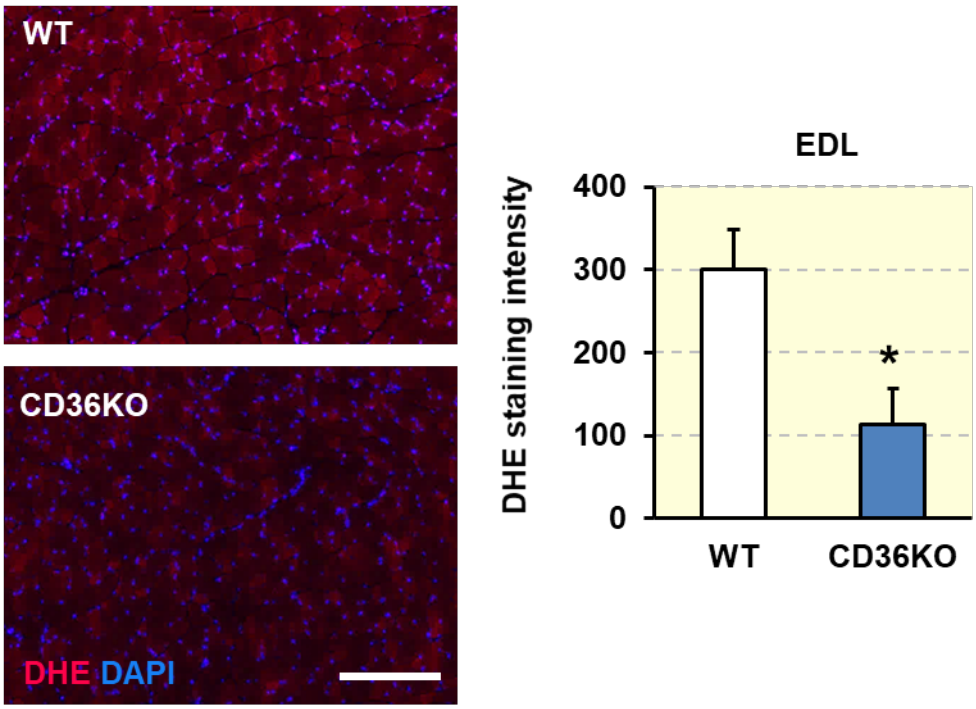
Supplementary figures



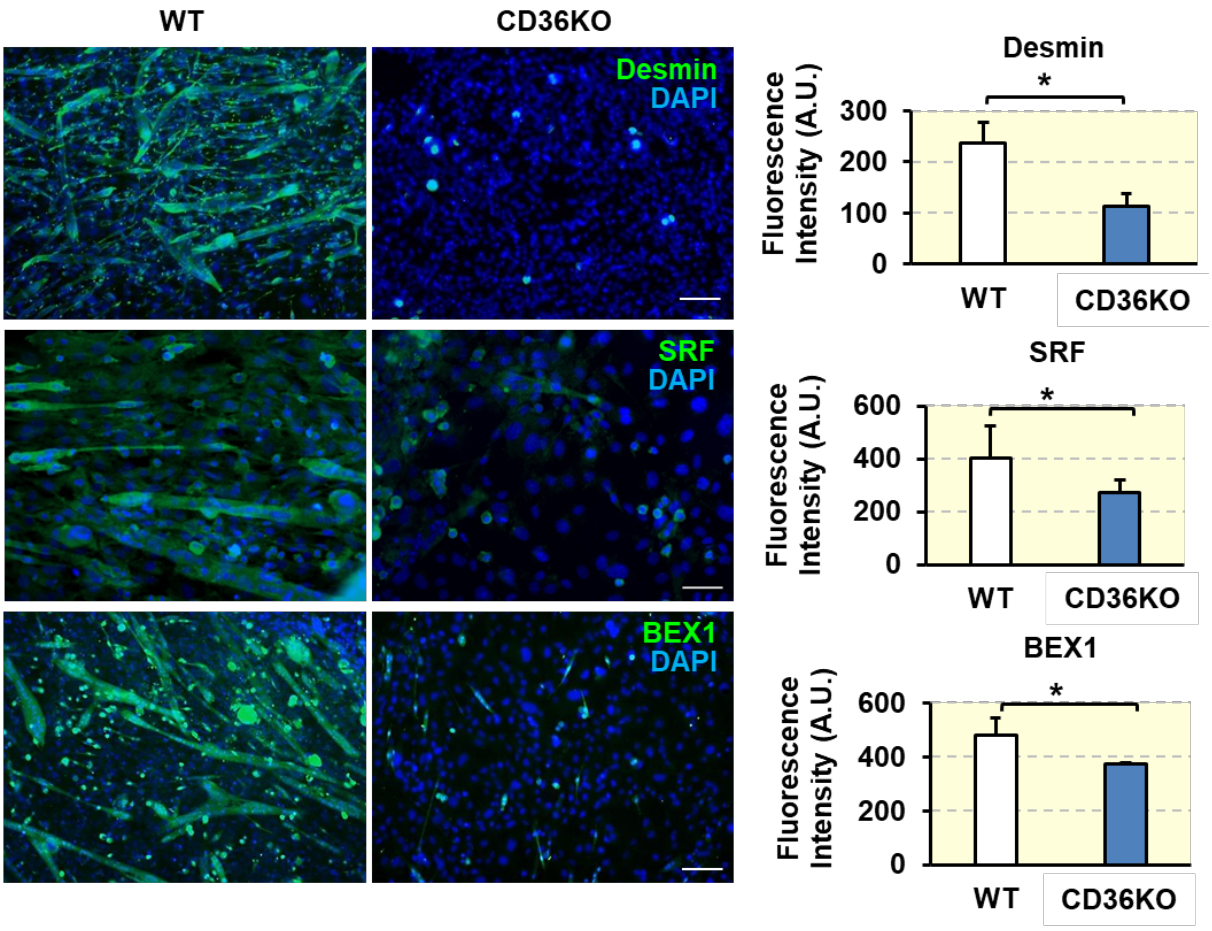
Supplementary Figure 1. Tissue weight differences in Wild-type and CD36 deficient mice. **(A)** Soleus (Sol), **(B)** Biceps Brachii (BB), **(C)** Extensor digitorum longus (EDL), **(D)** Tibialis Anterior (TA), **(E)** heart and **(F)** White-Adipose-Tissue (WAT) from WT and CD36 KO mice fed a ND or a HF diet. Data are represented as mean±SD (N=8 per group). Statistical analysis was performed using two-way ANOVA, *p<0.05 *vs.* WT ND, #p <0.05 *vs.* WT HF, ^{b}p<0.05 *vs.* CD36 KO ND.



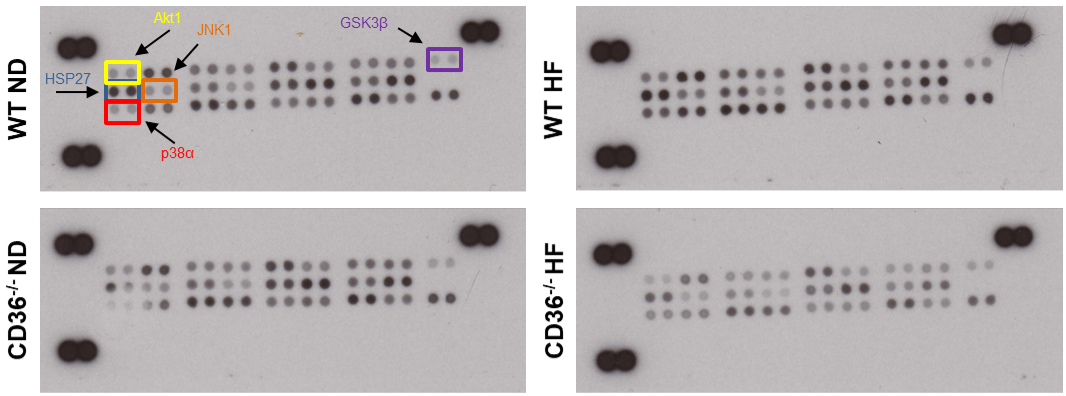
Supplementary Figure 2. CD36 deficiency alters diet induced muscle specific adaptations/morphometric changes. **(A, E)** Representative images of cross-sections from WT and CD36 KO mice fed a ND or HF diet from EDL and Sol muscle stained for myosin heavy chain (Mhc) isoforms. EDL and Sol muscle were examined for fibre type composition **(B, F)**, and cross-sectional area (CSA) **(C-D, G-H)**, respectively. EDL fibre type expression profile (IIA IIB IIX) and Soleus fibre type expression profile (IIA I IIX) shown in percentage [%]. Data are represented as mean±SD (N=8) per group). Statistical analysis was performed using two-way ANOVA or chi square analysis as appropriate, *p<0.05 vs WT ND.



Supplementary Figure 3. Attenuated DHE staining in the EDL muscle of CD36 KO mice. Representative images of DHE staining to assess ROS production in EDL transverse sections from WT and CD36 KO mice. Quantification of DHE staining intensity. Data are represented as mean±SD (N=6) per group. Statistical analysis was performed using Student's *t*-Test, *p<0.05.

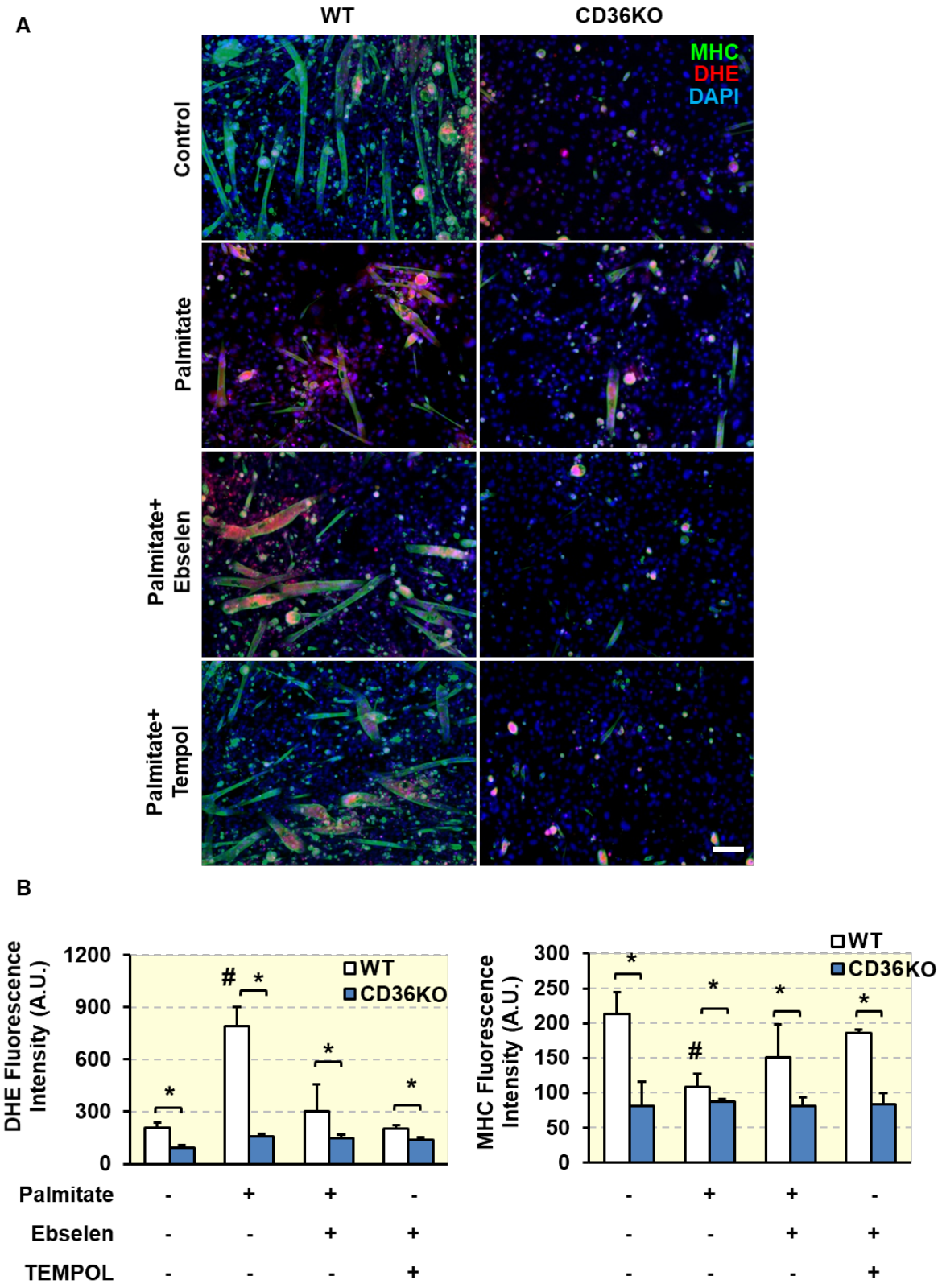


Supplementary Figure 4. Compromised myogenic differentiation in CD36 KO skeletal myoblasts versus WT. Immunohistochemistry was performed on isolated satellite cells derived from WT or CD36 KO mice. Following expansion in growth medium, differentiation was quantified after 4 days in differentiation medium (x10 magnification, scale bar 100μm). Satellite cell differentiation and myotube formation was measured by Desmin, SRF and BEX1 and nuclei were counterstained with DAPI. Statistical analysis was performed by Student's *t*-test. Differences are *p<0.05.



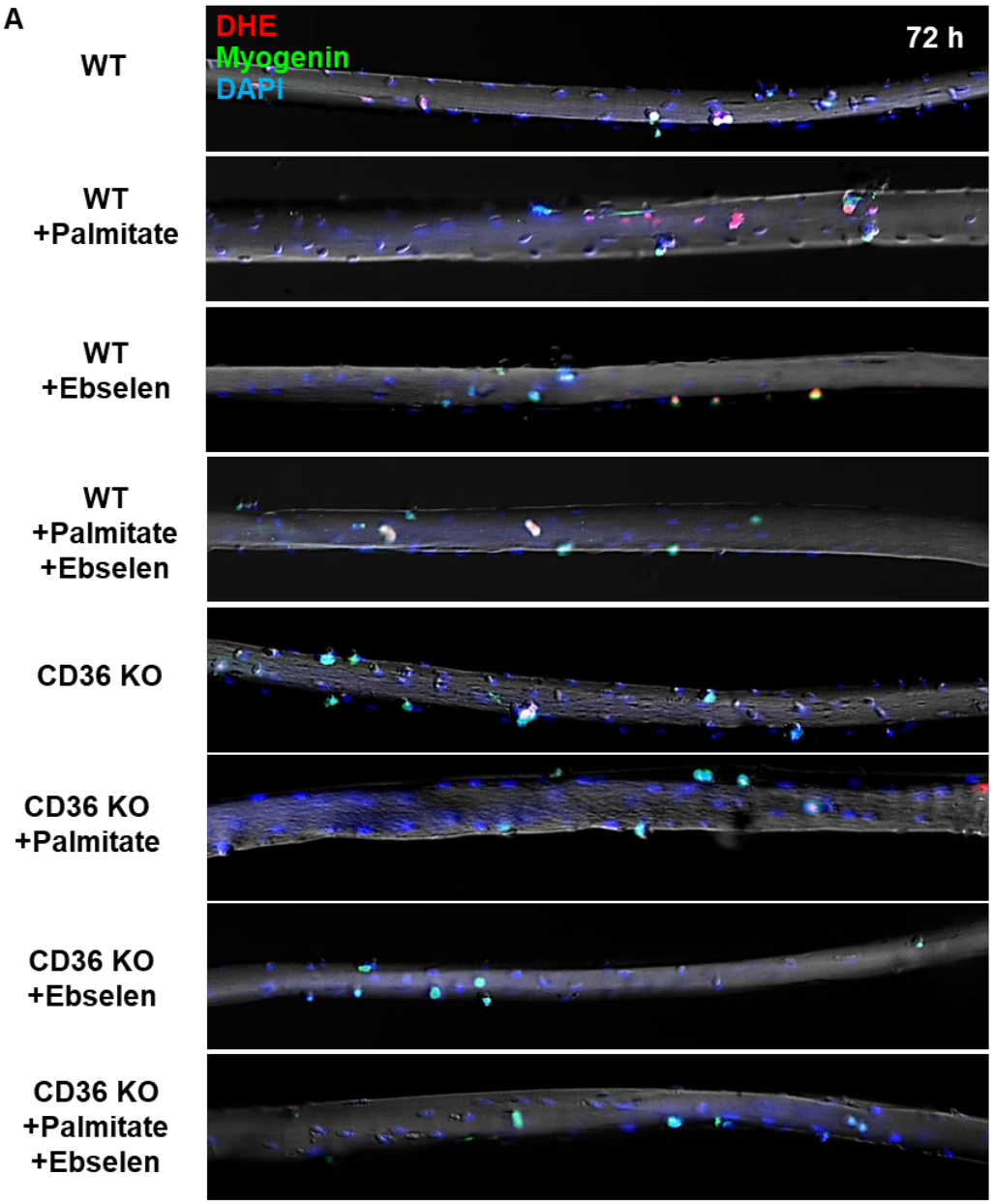
MAPKs	Fold Changes vs. WT ND			vs. CD36$^{-/-}$ ND
	CD36$^{-/-}$ ND	WT HF	CD36$^{-/-}$ HF	CD36$^{-/-}$ HF
Akt1^{S473}	1.00	**1.52**	**0.53**	**0.52**
Akt2^{S474}	0.98	1.21	**0.67**	**0.68**
Akt3^{S472}	0.81	1.15	**0.53**	**0.65**
Akt Pan^{S473,S474,S472}	0.97	1.24	**0.62**	**0.64**
CREB^{S133}	1.07	1.15	**0.84**	**0.79**
ERK1$^{T202/Y204}$	0.93	0.90	**0.56**	**0.60**
ERK2$^{T185/Y187}$	1.07	1.12	**0.46**	**0.43**
GSK3α/β$^{S21/S9}$	1.01	1.10	**0.55**	**0.55**
GSK3β^{S9}	1.08	**1.65**	1.01	0.94
HSP27$^{S78/S82}$	**0.54**	1.19	**0.67**	1.23
JNK1$^{T183/Y185}$	0.95	**1.63**	**0.63**	**0.66**
JNK2$^{T183/Y185}$	1.02	1.28	**0.60**	**0.59**
JNK3$^{T221/Y223}$	0.90	1.17	**0.47**	**0.53**
JNK pan$^{T183/Y185,T221/Y223}$	1.01	1.05	**0.68**	**0.67**
MKK3$^{S218/T222}$	1.08	1.03	**0.81**	**0.75**
MKK6$^{S207/T211}$	0.93	0.87	**0.54**	**0.58**
MSK2^{S360}	0.95	0.91	**0.56**	**0.59**
p38α$^{T180/Y182}$	**0.19**	**2.29**	1.18	**6.19**
p38β$^{T180/Y182}$	**0.69**	1.38	**0.65**	0.94
p38δ$^{T180/Y182}$	0.98	1.13	**0.74**	**0.76**
p38γ$^{T183/Y185}$	1.11	1.29	**0.77**	**0.69**
p53^{S46}	1.05	1.16	**0.70**	**0.66**
p70S6K$^{T421/S424}$	0.98	0.94	**0.56**	**0.57**
RSK1^{S380}	1.00	0.95	**0.71**	**0.71**
RSK2^{S386}	0.92	0.91	**0.54**	**0.58**
TOR	0.96	1.15	**0.75**	**0.79**

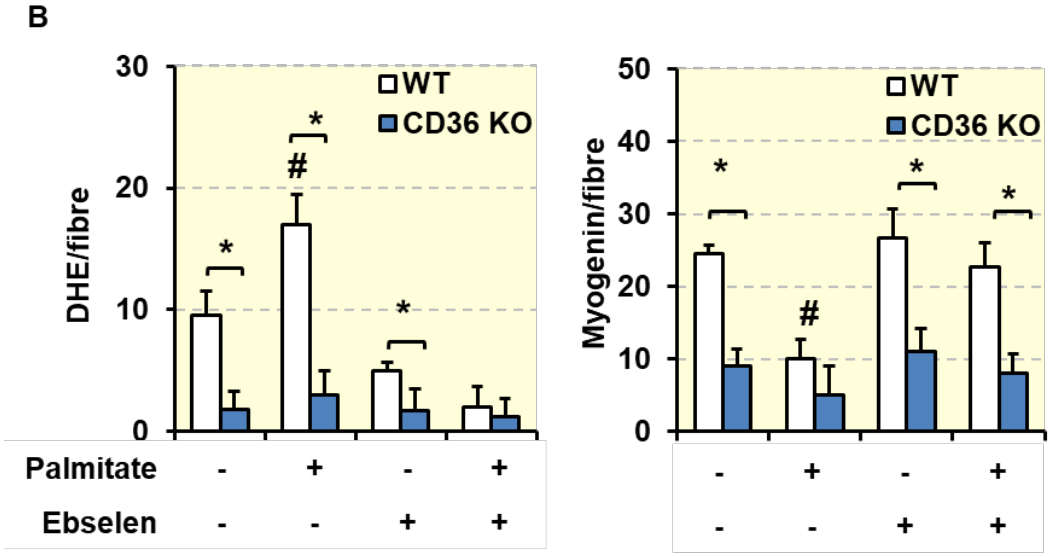
Supplementary Fig. 5. The effect of high-fat diet and CD36 deficiency on MAPKs in skeletal muscle. Phosphorylation levels of various MAPKs were assessed in skeletal muscle of WT and CD36 KO mice fed a standard chow diet (normal diet, ND) or High-fat (HF) diet for 13 weeks. Results of Phospho-MAPK antibody array (R&D). The densitometric analysis was presented as fold change. Fold change was calculated as: the ratio of each of the 3 groups to WT ND group (n=6/group).



Supplementary Figure 6. Palmitate increases DHE levels and compromises muscle stem cell differentiation that can be reversed by antioxidants in WT mice, but not in CD36 KO. (A) Immunohistochemistry was performed on isolated satellite cells derived from WT or CD36 KO mice. Following expansion in growth medium (GM), differentiation was

quantified after 4 days in differentiation medium (DM) with DMSO (control), Palmitate, Palmitate + Ebselen (20µM), or Palmitate + TEMPOL (1mM). Satellite cells were immunostained for MHC, DHE and nuclei counterstained using DAPI (Magnification x20, scale bar 50µm). **(B)** Quantification of DHE and MHC staining intensity (A.U.). Data are represented as mean±SD (N=6 technical replicates, 2 independent experiments) per group. Statistical analysis was performed using two-way ANOVA, *p<0.05 *vs* WT, #p<0.05 *vs* every other WT group.


A
WT
WT
+Palmitate
WT
+Ebselen
WT
+Palmitate
+Ebselen
CD36 KO
CD36 KO
+Palmitate
CD36 KO
+Ebselen
CD36 KO
+Palmitate
+Ebselen
DHE
Myogenin
DAPI
72 h


B
DHE/fibre
30
20
10
0
WT
CD36 KO
*
#
*
*
Palmitate - + - +
Ebselen - - + +
Myogenin/fibre
50
40
30
20
10
0
WT
CD36 KO
*
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- + - +
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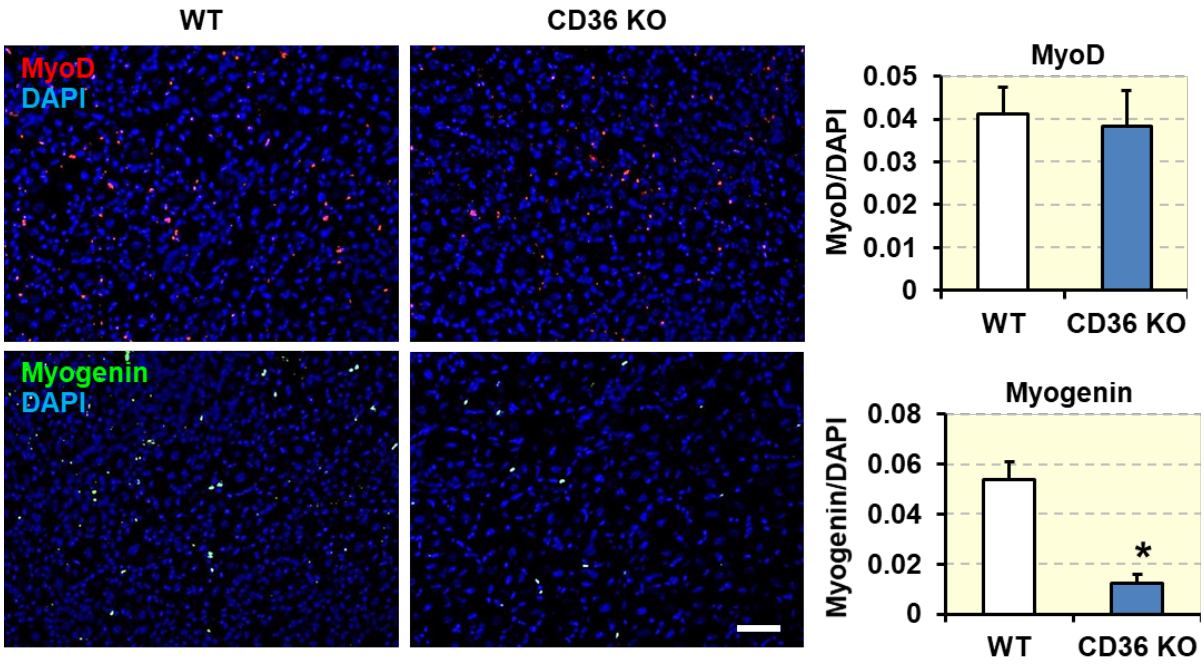
Supplementary Figure 7. Palmitate increases DHE levels and compromises muscle stem cell differentiation that is reversible by the antioxidant agent ebselen in WT, but not in CD36 KO single fibre cultures. (A) Representative images show WT and CD36 KO EDL satellite cell staining for DHE and Myogenin counterstained with DAPI on single myofibres after 72 h (72 hours) treated with Palmitate (0.3mM) and/or Ebselen (20μM). **(B)** Quantification of DHE levels and Myogenin. Data are represented as mean±SD (N=5 mice per condition, 40 fibres per group). Statistical analysis was performed using two-way ANOVA, *$p<0.05$ *vs.* WT ND, $^{\#}p<0.05$ *vs.* every other WT group.



Supplementary Figure 8. The effect of CD36 deficiency on MyoD and Myogenin expression in progenitor cells of injured muscle. Acute injury was induced by Cardiotoxin injection into the tibialis anterior muscle of WT and CD36 KO mice and specimens were studied 5 days post-injury. Representative images and quantitative data for MyoD and Myogenin immunohistochemical staining on transverse sections of injured muscles, co-stained with DAPI to visualise nuclei. Representative images (x20 magnification, scale bar 50μm). Statistical analysis was performed by Student's *t*-test. n=5 animals per group. Differences are *p<0.05.