Supplemental Data File

TLR/MyD88/XBP1 signaling axis mediates skeletal muscle wasting during cancer cachexia

By

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This file contains: Figures S1-S4 and their legends

SUPPLEMENTAL FIGURES' LEGENDS

FIGURE S1. Characterization of muscle atrophy in response to tumor-derived factors. 3-month old C57BL/6J mice were injected with saline alone or 2 x 10^6 Lewis lung carcinoma (LLC) cells in the left flank. Average **(A)** body weight, and **(B)** forelimb strength of control and LLC-tumor bearing mice after 21 days of inoculation of LLC cells. N=4 in each group. C2C12 myotubes were treated with normal medium, LLC-CM, or C26-CM in 1:4 ratio for 72h. The cultures were then fixed and stained for MyHC protein using MF20 antibody. Nuclei were counterstained with DAPI. Representative images of the cultures are presented here. Scale bar: 20μm. **(D)** Average myotube diameter in control, LLC-CM, or C26-CM treated myotubes. The median and 25-75th percentiles are shown. *$p<0.05$, values significantly different from their corresponding controls.

FIGURE S2. Average muscle weight in control and LLC-tumor bearing mice. 3-month old $MyD88^{f/f}$ and $MyD88^{myoKO}$ mice were inoculated with 2 x 10^6 LLC cells in the left flank. After 21 days, the mice were euthanized and individual hind limb muscle weight was measured. Quantification of wet weight of **(A)** GA, **(B)** TA, and (C) soleus muscle of control and LLC-tumor bearing $MyD88^{f/f}$ and $MyD88^{myoKO}$ mice normalized with body weight. N=3 in each group. The median and 25-75th percentiles are shown. *$p<0.05$, values significantly different from $XBP1^{f/f}$ mice injected with saline alone. &$p<0.05$, values significantly different from LLC-tumor-bearing $MyD88^{f/f}$ mice. ns, not significant.

Figure S3. Recombinant Hsp70 protein activates UPR pathways in cultured myotubes. C2C12 myotubes were treated with Hsp70 (100 ng/ml) for 24h followed by performing biochemical analysis. **(A)** Representative immunoblots showing levels of p-eIF2α, eIF2α, tXBP1, sXBP1, and Tubulin in control and Hsp70-treated myotubes. **(B)** Densitometry quantification of bands in immunoblots for p-eIF2α, eIF2α, tXBP1, and sXBP1 in control and Hsp70-treated C2C12 myotube cultures. **(C)** Relative mRNA levels

of *ATF4, CHOP, GADD34, GRP78*, and s*XBP1* in control and Hsp70-treated C2C12 myotubes. N=3 in each group. The median and 25-75th percentiles are shown. *p<0.05, values significantly different from control myotube cultures. ns, not significant.

FIGURE S4. Average muscle weight in control and LLC-tumor bearing XBP1$^{f/f}$ and XBP1^{mKO} mice. 3-month old XBP1$^{f/f}$ and XBP1^{mKO} mice were inoculated with 2 x 10^6 LLC cells in the left flank. After 21 days, the mice were euthanized and individual hind limb muscle weight was measured. Quantification of wet weight of **(A)** GA, **(B)** TA, and **(C)** soleus muscle of control and LLC-tumor bearing XBP1$^{f/f}$ and XBP1^{mKO} mice normalized by body weight. N=3 in each group. The median and 25-75th percentiles are shown. *p<0.05, values significantly different from XBP1$^{f/f}$ mice injected with saline alone. $^{\&}$p<0.05, values significantly different from LLC-tumor-bearing XBP1$^{f/f}$ mice. ns, not significant.

FIGURE S1

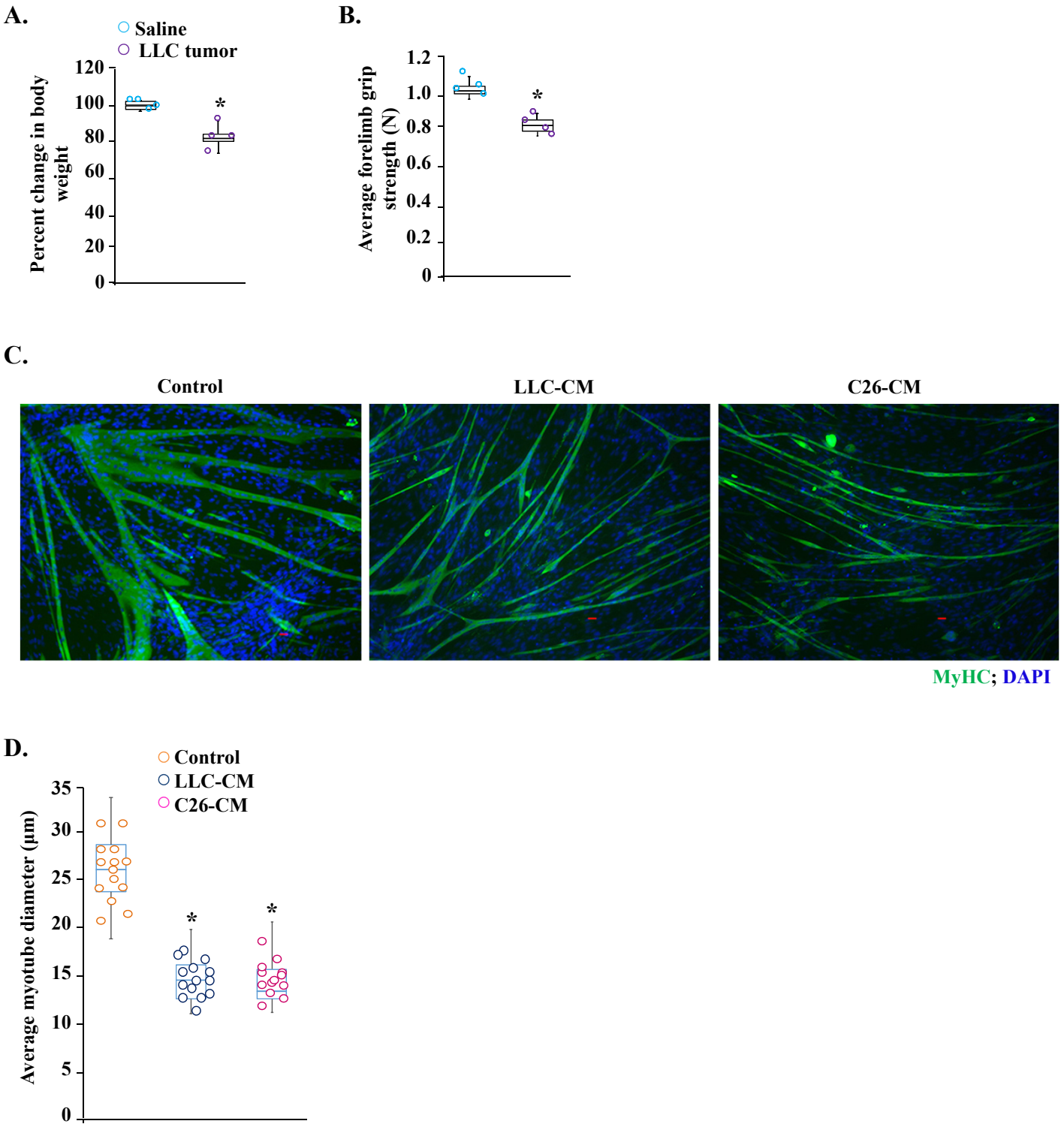

A.
Saline
LLC tumor
Percent change in body weight
120
100
80
60
40
20
0
*
B.
Average forelimb grip strength (N)
1.2
1.0
0.8
0.6
0.4
0.2
0
*
C.
Control
LLC-CM
C26-CM
MyHC; DAPI
D.
Control
LLC-CM
C26-CM
Average myotube diameter (μm)
35
30
25
20
15
10
5
0
*
*

FIGURE S2

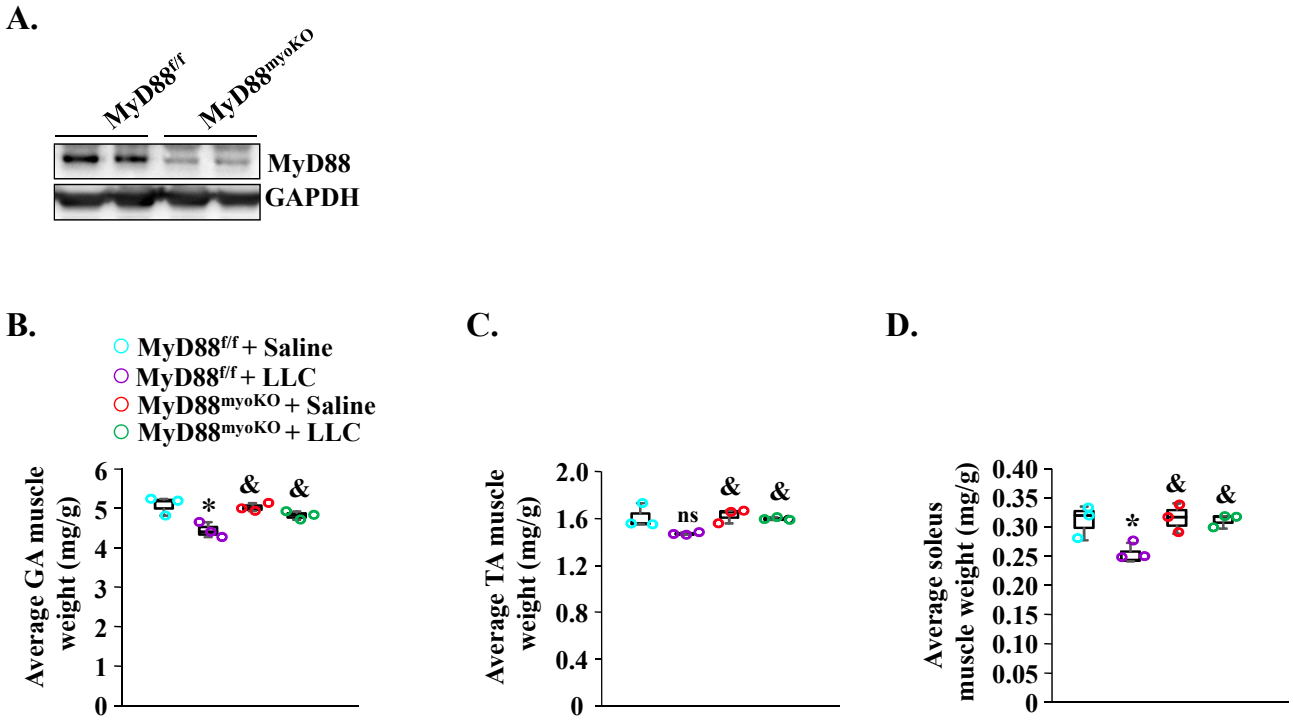

A.
MyD88f/f
MyD88myoKO
MyD88
GAPDH
B.
MyD88f/f + Saline
MyD88f/f + LLC
MyD88myoKO + Saline
MyD88myoKO + LLC
Average GA muscle weight (mg/g)
0
1
2
3
4
5
6
*
&
&
C.
Average TA muscle weight (mg/g)
0
0.4
0.8
1.2
1.6
2.0
ns
&
&
D.
Average soleus muscle weight (mg/g)
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
*
&
&

FIGURE S3

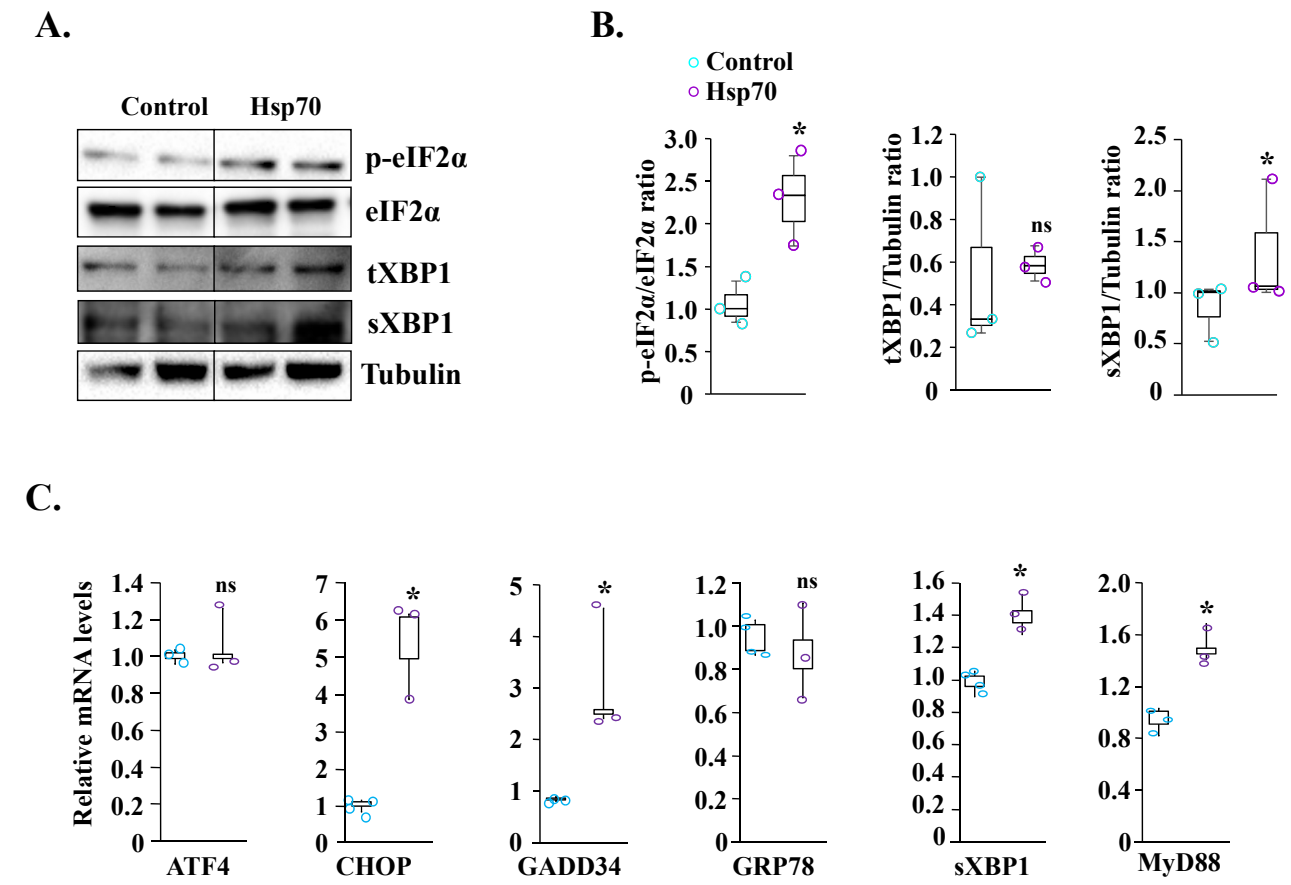
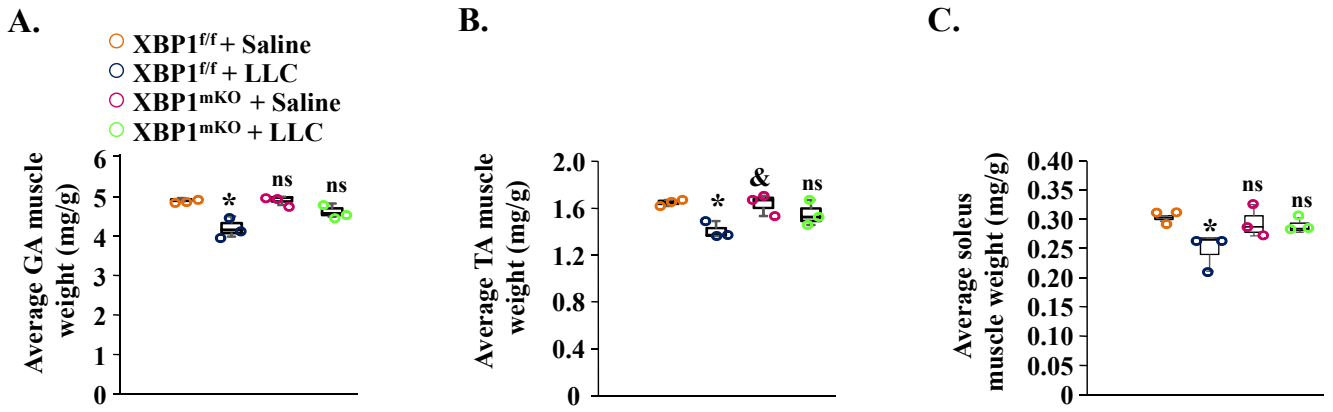

A.
Control
Hsp70
p-eIF2α
eIF2α
tXBP1
sXBP1
Tubulin
B.
Control
Hsp70
p-eIF2α/eIF2α ratio
tXBP1/Tubulin ratio
sXBP1/Tubulin ratio
ns
*
C.
Relative mRNA levels
ATF4
CHOP
GADD34
GRP78
sXBP1
MyD88
ns
*

FIGURE S4


A.
XBP1f/f + Saline
XBP1f/f + LLC
XBP1mKO + Saline
XBP1mKO + LLC
Average GA muscle weight (mg/g)
0
1
2
3
4
5
6
*
ns
ns
B.
Average TA muscle weight (mg/g)
0
0.4
0.8
1.2
1.6
2.0
*
&
ns
C.
Average soleus muscle weight (mg/g)
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
*
ns
ns