Supplementary Information

Chemotherapy triggers cachexia by deregulating synergetic function of histone modifying enzymes

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Supplementary information includes

1) Figure and figure legends S1 – S7
2) Supplementary movie legends – 1- 4
3) Supplementary materials and methods

Figure S1

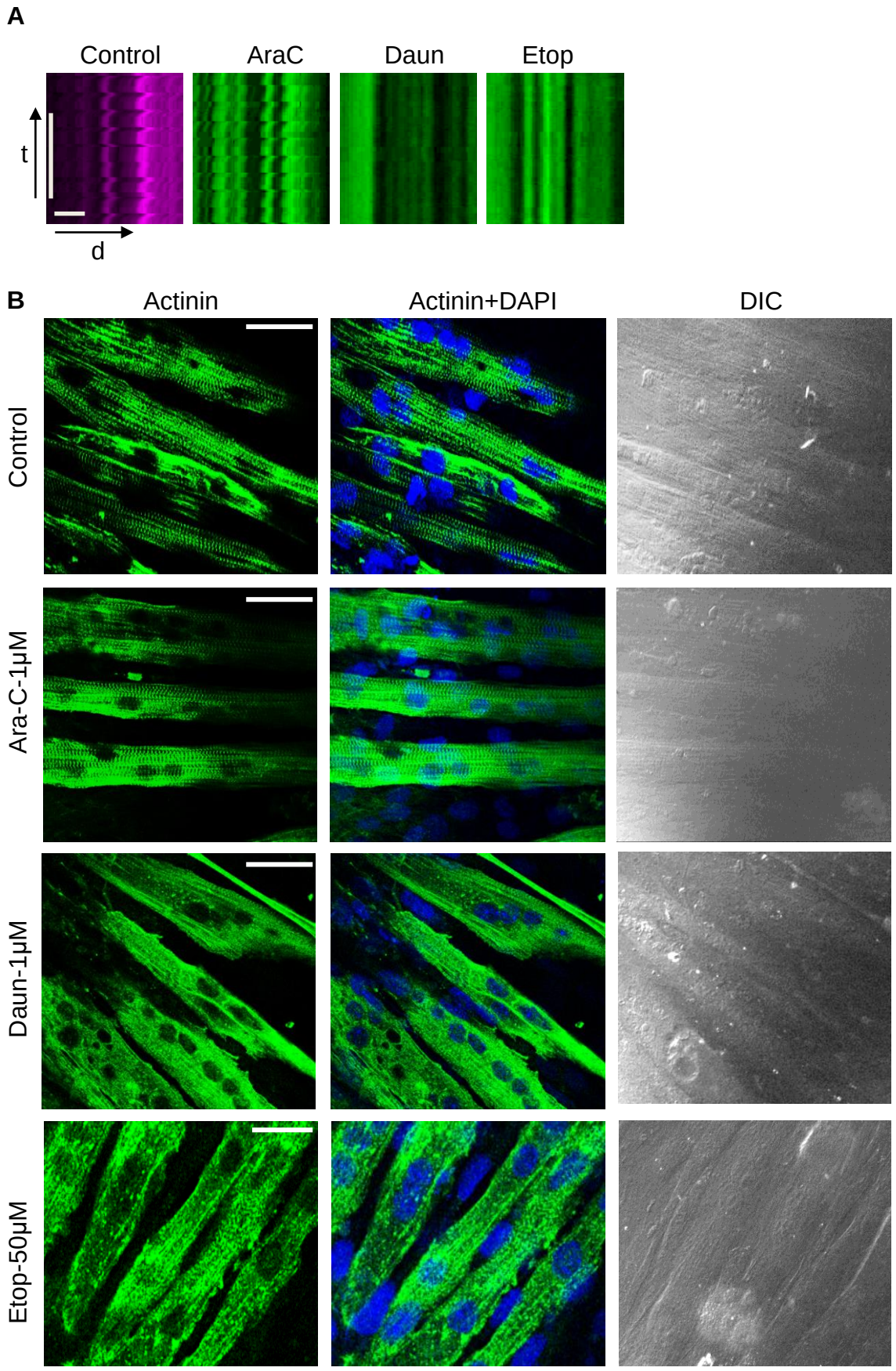
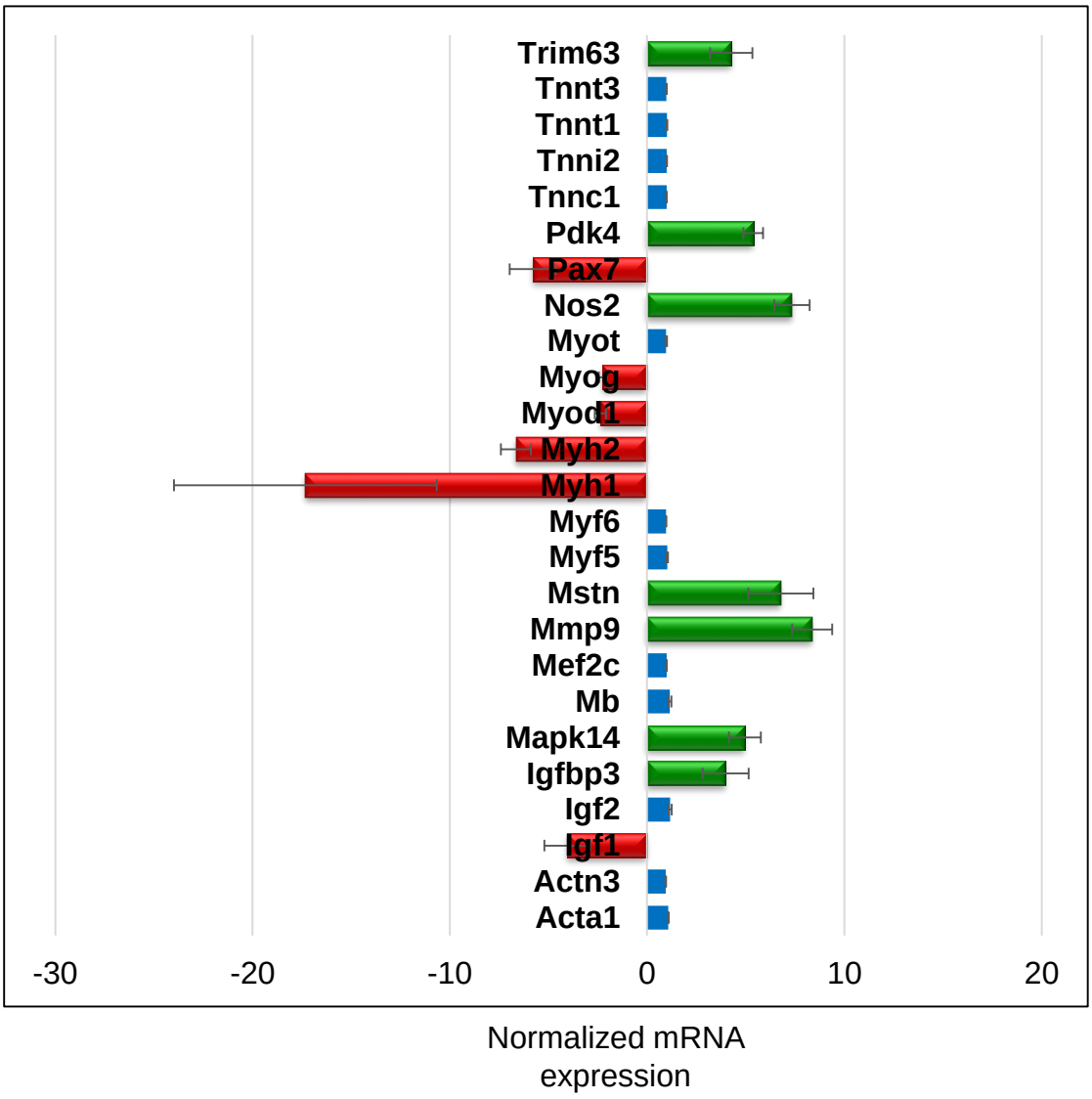

A
Control
AraC
Daun
Etop
t
d
B
Actinin
Actinin+DAPI
DIC
Control
Ara-C-1µM
Daun-1µM
Etop-50µM

Figure S1. (A) The excitation-contraction coupling of the myotubes was monitored at 1 Hz excitation frequency. The kymographs (time vs. distance plot) shows the movement of intracellular structures of the myotubes in response to the electrical stimulation. The kymographs were plotted by drawing a line across the myotube/s to monitor the changes in the intensity along the plotted line over a stack of 100 images or frames. The change in the intensity indicative of change in the position of respective organelle or structure represents the contraction. As seen for control and AraC treated cells, along the vertical axis, the change in the intensities with horizontal shifts (peaks) at regular intervals indicate contraction. For Daun and Etop however, no intensity shifts along the vertical axis was observed, thus indicating lack of contractions. (B) Same as in Figure 1A except, lower magnification confocal images shows effects of Ara-C, Daun and Etop treatment on sarcomere organization. Scale bar – 50 µm.

Figure S2

A


Trim63
Tnnt3
Tnnt1
Tnni2
Tnnc1
Pdk4
Pax7
Nos2
Myot
Myog
Myod1
Myh2
Myh1
Myf6
Myf5
Mstn
Mmp9
Mef2c
Mb
Mapk14
Igfbp3
Igf2
Igf1
Actn3
Acta1
-30
-20
-10
0
10
20
Normalized mRNA expression

B

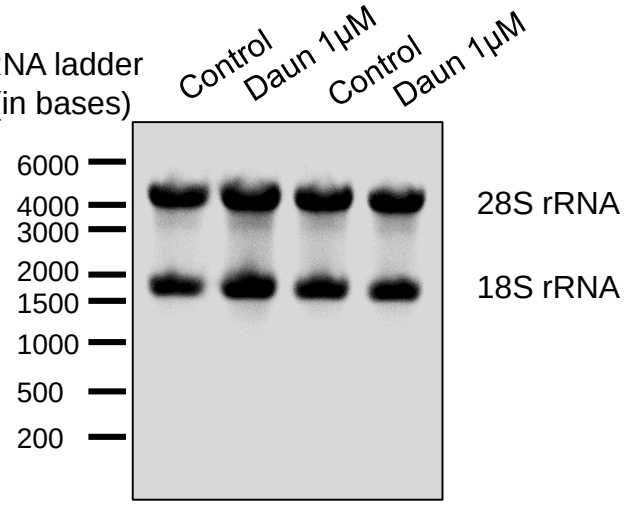

RNA ladder (in bases)
Control
Daun 1μM
Control
Daun 1μM
6000
4000
3000
2000
1500
1000
500
200
28S rRNA
18S rRNA

Figure S2.

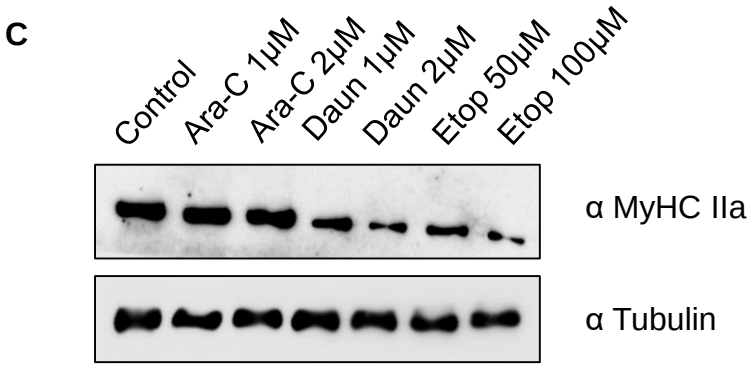


Figure S2. (A) Same as in Figure 2A except, the changes in transcript levels are shown as fold difference for the regulated genes upon Daun treatment. Green bars – upregulation, red bars – downregulation, and blue bars – for changes in the expression. The normalized data represent average (+/- SEM) of three biological experiments with technical duplicates in each experiment. Unaltered expression of other sarcomeric genes such as troponins were also shown. (B) Total RNA with indicated drug treatment (used in Figure S2A), representative from two independent experiments were loaded in agarose gel to monitor quality of RNA preparation. Two distinct bands of rRNA (28S rRNA and 18sRNA) are present, indicating high quality of total RNA with no visible degradation. (C) Same as in Figure 2F except, the examples of another independent experiment is shown.

Figure S3

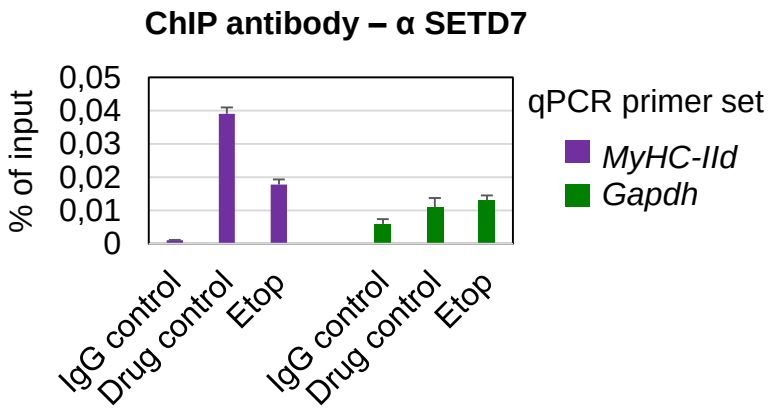


Figure S3. Same as in Figure 3B except, ChIP assays were performed with chromatin from Etop treated myotubes. Anti-SETD7 antibodies were used for ChIP.

Figure S4

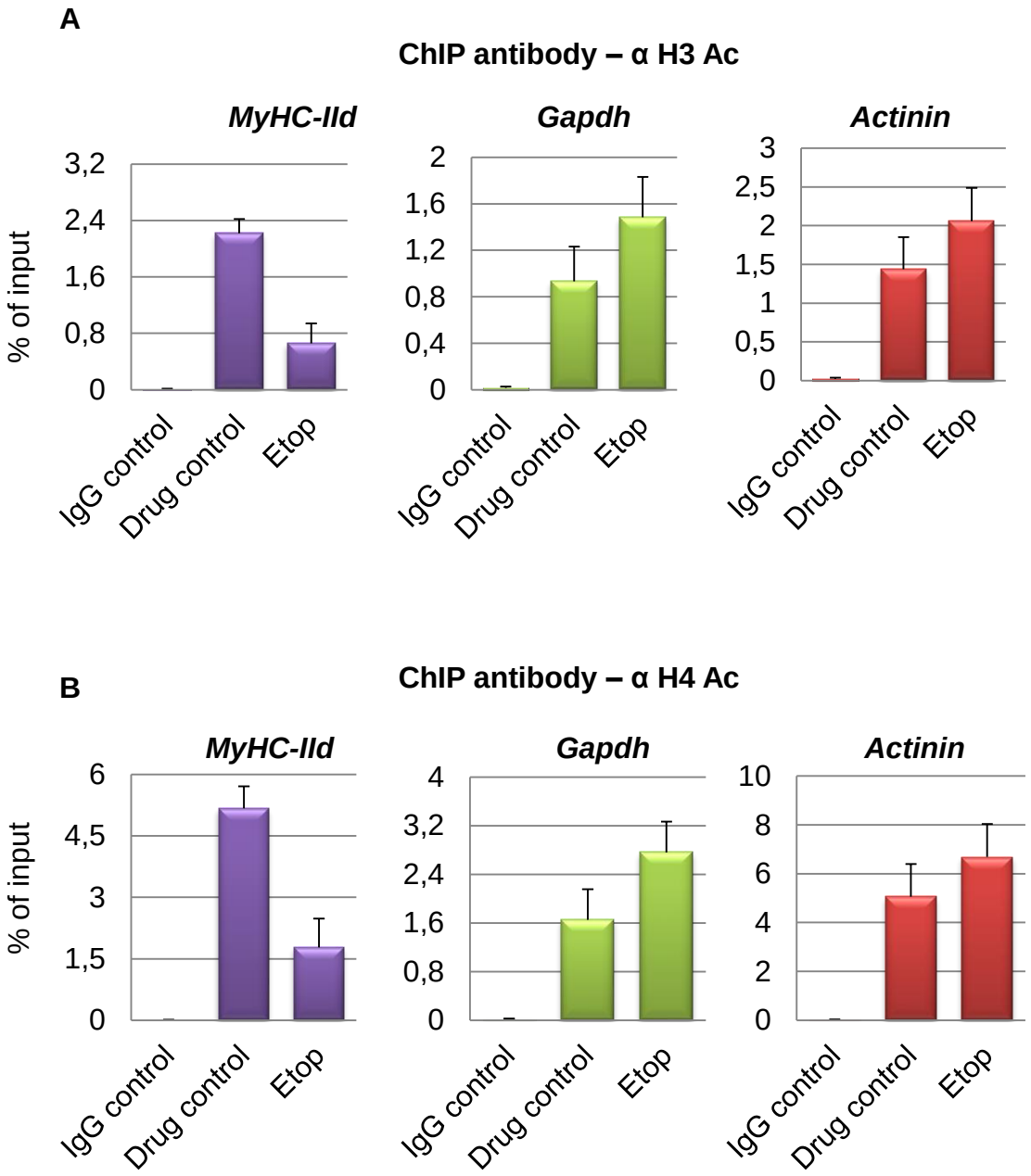


Figure S4. Similar to Figure 4B and C except, ChIP assays were performed with chromatin from Etop treated myotubes. Anti-H3 Ac and anti-H4 Ac antibodies were used for ChIP.

Figure S5

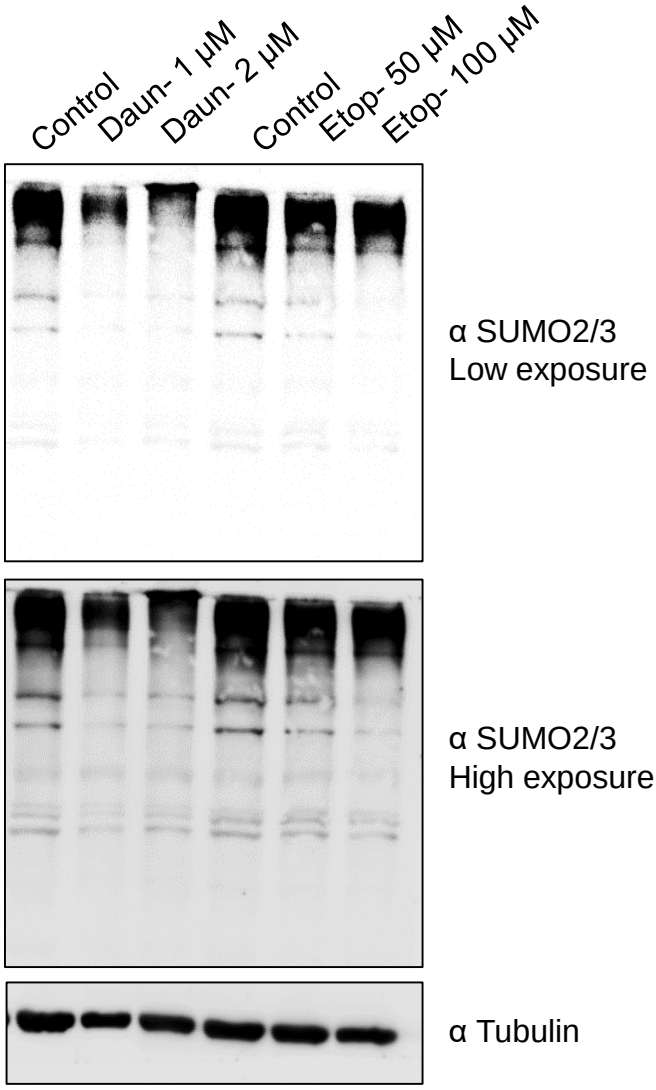


Figure S5. Western blots shows level of global SUMO2/3 conjugated proteins after Daun and Etop treatments, lysates corresponding to the Figure 6F. Different concentrations of drugs were tested.

Figure S6

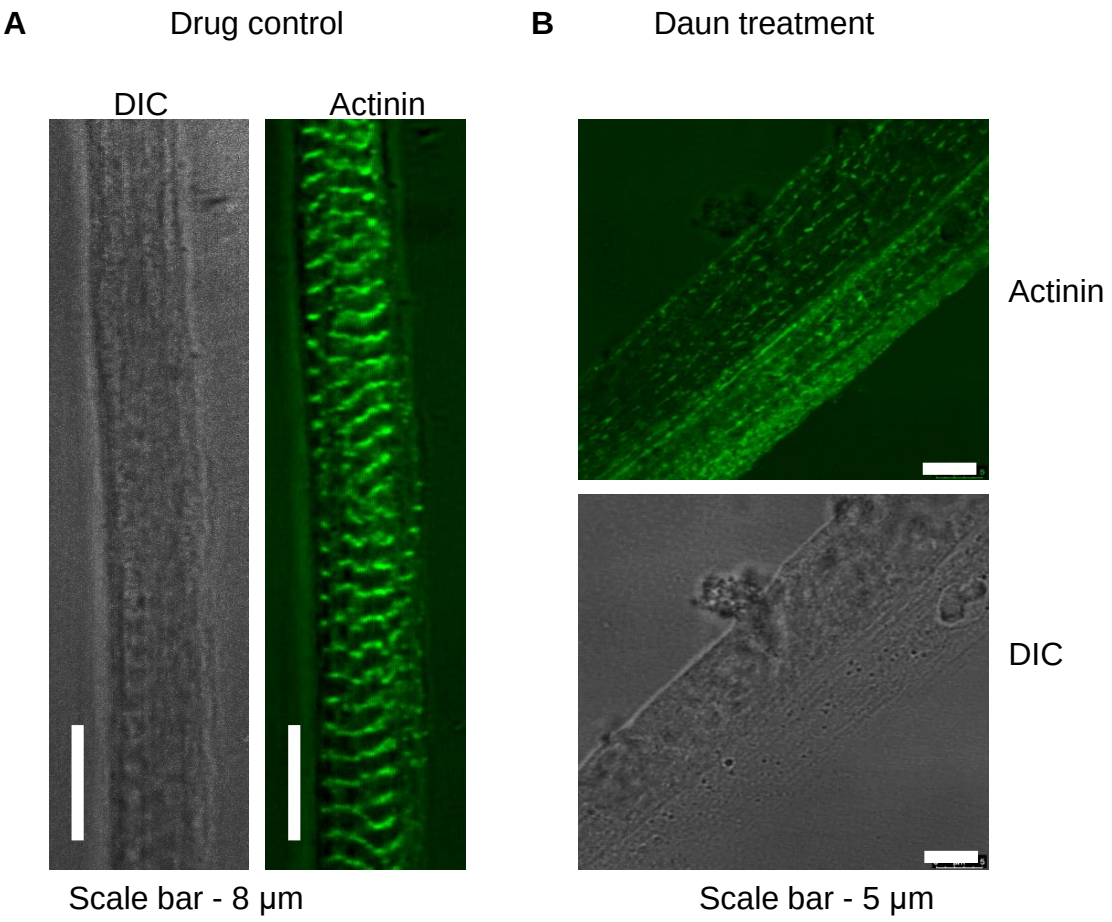


Figure S6. Similar to Figure 7A; additional examples of primary myotubes are shown.

Figure S7

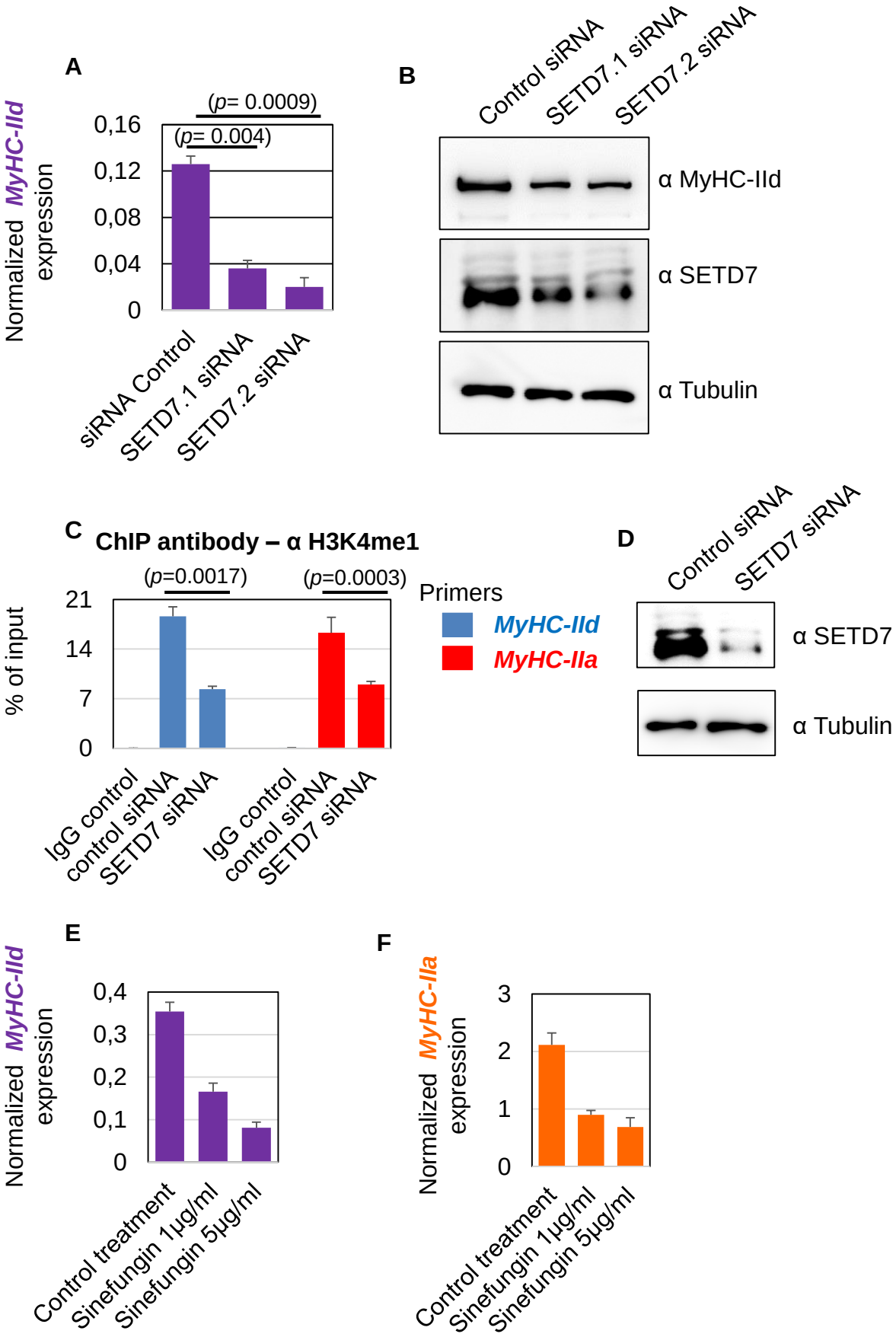

A
Normalized MyHC-IId expression
(p= 0.0009)
(p= 0.004)
0,16
0,12
0,08
0,04
0
siRNA Control
SETD7.1 siRNA
SETD7.2 siRNA
B
Control siRNA
SETD7.1 siRNA
SETD7.2 siRNA
α MyHC-IId
α SETD7
α Tubulin
C
ChIP antibody – α H3K4me1
% of input
(p=0.0017)
(p=0.0003)
21
14
7
0
IgG control
control siRNA
SETD7 siRNA
Primers
MyHC-IId
MyHC-IIa
D
Control siRNA
SETD7 siRNA
α SETD7
α Tubulin
E
Normalized MyHC-IId expression
0,4
0,3
0,2
0,1
0
Control treatment
Sinefungin 1µg/ml
Sinefungin 5µg/ml
F
Normalized MyHC-IIa expression
3
2
1
0
Control treatment
Sinefungin 1µg/ml
Sinefungin 5µg/ml

Figure S7. (A) Two days differentiated myotubes were transfected either with control or 120 pmol of SETD7 siRNA. Three days after siRNA transfection (5-days after differentiation), total RNA was isolated and RTqPCR were performed to monitor the indicated mRNA. The data represents average (+/- SEM) from two biological experiments with technical replicates in each experiment. *P*-values originated from unpaired t-test, are indicated in the figure. **(B)** siRNA efficiency of Figure S7A was checked by monitoring the level of indicated proteins. **(C)** Similar to Figure 3C except, ChIPs were performed with the control siRNA and SETD7 depleted myotubes. *P*-values originated from unpaired t-test, are indicated in the figure. **(D)** Depletion of SETD7 protein levels following SiRNA treatment (of Figure S7C) was monitored by western blot. (**E, F)** Similar to figure 7C except, indicated amount of Sinefungin (SETD7 inhibitor) was added to satellite stem cell derived primary myotubes for 20 hours before total RNA was prepared. Sinefungin treatment influenced the *Myh1* and *Myh2* expression levels.

Supplementary Movie legends:

Supplementary Movie 1: The control myotubes contract following electrical stimulation. The excitation-contraction coupling was monitored following 1HZ electric stimulation of the myotubes cultured on the coverslips. The movies were acquired at 5.8 frames/s and are played at 30 images/s. The scale bar - 10 µm.

Supplementary Movie 2: The AraC treated myotubes contract following electrical stimulation. The excitation-contraction coupling was monitored following 1HZ electric stimulation of the myotubes. The movies were acquired at 5.8 frames/s and are played at 30 images/s. The scale bar - 10 µm.

Supplementary Movie 3: Majority of the Daun treated myotubes did not contract following electrical stimulation at 1HZ frequency. The movies were acquired at 5.8 frames/s and are played at 30 images/s. The scale bar - 10 µm.

Supplementary Movie 4: A large fraction of the Etop treated myotubes did not contract following electrical stimulation at 1HZ frequency. The movies were acquired at 5.8 frames/s and are played at 30 images/s. The scale bar - 10 µm.

Supplementary material and methods

Antibodies used for western blot, immunoprecipitation, ChIP and Immunofluorescence:

Details of antibodies used for western blot, immunoprecipitation and ChIP are as follows: anti-Actinin (Sigma – A7811), anti-SENP3 (clone D20A10 cat no. - 5591, Cell signaling), anti-Tubulin (Biocat G098/AKR-009), anti-Setd7 (Santacruz Biotech, sc-390823), anti-RNA pol II CTD YSPTSPS Phos 5 (Abcam – ab5131, 17-620, Sigma-Aldrich), anti-Suv39h1 (Abcam – ab12405), anti-H3K4 monomethyl (Abcam – ab8895), anti-SUMO2/3 (MBL-M114-3), anti-myosin heavy chain (Abcam – ab91506), anti-p300 (SC-48343, Santacruz Biotech), anti-acetyl histone H4 (06-866, Millipore), anti-acetyl H3 (ab47915, abcam).

The primer pairs used for RT-qPCR are mentioned below:

GAPDH forward: 5’-AACTTTGGCATTGTGGAAGG-3’GAPDH
reverse: 5’-ACACATTGGGGGTAGGAACA-3’
MyHC-IId forward: 5’-CAAGTCATCGGTGTTTGTGG-3’
MyHC-IId reverse: 5’-TGTCGTACTTGGGAGGGTTC-3’
MyHC-IIa forward: 5’-GGCCAAAATCAAAGAGGTGA-3’
MyHC-IIa reverse: 5’-CGTGCTTCTCCTTCTCAACC-3’
SENP3 forward: 5’-AGGTGCCATCACCCTGCTG-3’
SENP3 reverse: 5’-GACTTTGGGGTCCACCTTAG-3’
Actinin forward: 5’-TGCTGCTATGGTGTCAGAGG-3’
Actinin reverse: 5’-TGGGGTCATCCTTGTTAAGC-3’

The following primer pairs were used for ChIP-qPCR to amplify ChIPed DNA.

Gapdh promoter forward: 5'- CAAAGCATCGACCAGTGCTA -3'

Gapdh promoter reverse: 5'- TGGACAGCACTGACTTCCAG -3'

MyHC-IId promoter forward: 5'-GTGAAGGCTGCCAGAAAGAG-3'

MyHC-IId promoter reverse: 5'-ACACAGAGGACAGGGGATG-3'

MyHC-IIa promoter forward: 5'-GGCCAAAATCAAAGAGGTGA-3'

MyHC-IIa promoter reverse: 5'-CGTGCTTCTCCTTCTCAACC-3'

Actinin promoter forward: 5'- GAGGAAAAGGAGGGATGAGG -3'

Actinin promoter reverse: 5'- GACAGCAACCCAGAGGAAAG -3'

RNA sample preparation and quality control –

We used Roche total RNA extraction kit. By agarose gel electrophoresis, we checked the quality/integrity of the RNA that were used for RTqPCR. The supporting information *Figure S2B* shows examples of two representative RNA quality control check. Furthermore, at the qPCR level (the gene expression array) we had six different housekeeping genes *(Actb, B2m, Gapdh, Hprt1, Rpl60 and Hgdc)* for data normalization. In addition, the array included controls to check genomic DNA contamination and general PCR performance check.