Supplementary Material

Acute Skeletal Muscle Contractions Orchestrate Signaling Mechanisms to Trigger Nuclear NFATc1 Shuttling and Epigenetic Histone Modifications
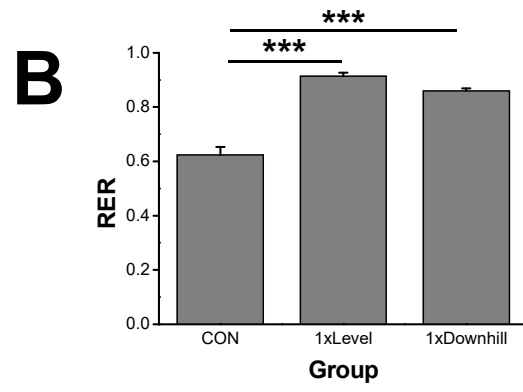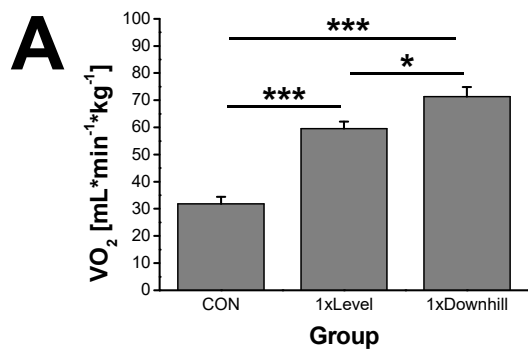
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Figure S1. **Oxygen consumption and respiratory exchange ratio (RER) with predominantly concentric and eccentric muscle contractions**. (A) VO_2 is increased in rats during 1xDownhill running compared to CON and 1xLevel running indicating increased proportions of eccentric muscle contractions due to extended recruitment of muscle fibers [26]. (B) Increased RER indicates increased metabolic demands during both 1xLevel and 1xDownhill running compared to CON. * p<0.05, *** p<0.001, bar is 1 μm.

Table S1. **Antibodies and reagents**.

Table S2. **Direct comparisons of the investigated targets between 1xLevel/1xDownhill conditions compared to CON in LAT (light gray background) and GAS (dark gray background) muscles.** '*' indicates p<0.05 compared to CON. '**' indicates p<0.01 compared to CON. '***' indicates p<0.001 compared to CON. '+' indicates a strong tendency toward significance compared to CON. '↑' indicates increased levels. '↓' indicated decreased levels. '↔' indicates no changes.

A

VO$_2$ [mL*min^{-1}*kg^{-1}]

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***

*

CON 1xLevel 1xDownhill
Group

B

RER

***

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CON 1xLevel 1xDownhill
Group

Supplements Figure S1

Antibody/Reagent	Host	Dilution	Supplier
Western blot			
pAktSer^{473}	Rb	1:400	Cell Signaling
calstabin-1	Gt	1:200	Santa Cruz
pCaMKIIThr^{286}	Rb	1:1000	Cell Signaling
GAPDH	Rb	1:8000	Cell Signaling
H3K9ac	Rb	1:1000	Cell Signaling
H3K14ac	Rb	1:1000	Cell Signaling
H3K27ac	Rb	1:1000	Cell Signaling
pH3Ser10	Rb	1:1000	Cell Signaling
total H3	Rb	1:1000	Cell Signaling
NFATc1	Mm	1:50	DSHB Iowa
PDE4D3	Gt	1:200	Epitomics
PKAc	Mm	1:700	BD Transduction Lab
PP1	Mm	1:400	BD Transduction Lab
pPTENSer^{380}	Rb	1:1000	Abcam
RyR1Ser^{2840}	Rb	1:750	Abcam
total RyR1	Rb	1:700	Abcam
anti-mouse HRP IgG	Gt	1:2000	Thermo Scientific
anti-rabbit HRP IgG	Gt	1:2000	Thermo Scientific
anti-goat HRP IgG	Rb	1:50000	Thermo Scientific
Immunohistochemistry			
alpha-actinin	Mm	1:250	Sigma Aldrich
DRAQ5		1:5000	Alexis Biochemicals
RyR1Ser^{2840}	Rb	1:100	Abcam
Alexa488 anti-mouse	Gt	1:500	Invitrogen
Alexa555 anti-rabbit	Gt	1:500	Invitrogen
biotinylated secondary antibodies		1:400	Dako Cytomation

Supplements Table S1

	LAT		GAS	
	1xLevel	**1xDh**	**1xLevel**	**1xDh**
RyR1				
pRyR1Ser^{2840} (IHC)	***↑	***↑	***↑	***↑
pRyR1Ser^{2840} (WB)	**↑	*↑	*↑	+↑
Signaling/Ca^{2+} sensors				
pCaMKIIThr^{286}	*↑	**↑	**↑	*↑
pAktSer^{473}	*↑	**↑	**↑	*↓
PI3K total	↔	↔	↔	↔
pPTENSer^{380}	*↑	**↑	**↑	*↑
PKAc (whole muscle)	*↑	*↑	*↑	*↑
PKAc (P to S)	+↑	+↑	+↑	*↑
RyR1 stabilizers				
calstabin-1 (S to P)	***↑	***↑	***↑	***↑
PDE4D3 (S to P)	**↑	***↑	**↑	↔
PP1 (S to P)	*↑	*↑	**↑	**↑
Transcription control				
H3K9ac (nuc local.)	**↑	*↑	↔	*↑
H3K14ac (nuc local.)	+↑	*↑	↔	*↑
H3K27ac (nuc local.)	*↑	*↑	*↑	*↑
pH3Ser^{10} (nuc local.)	↔	↔	+↑	+↑
NFATc1 (nuc local.)	*↑	*↑	*↑	*↑

Supplements Table S2