SUPPLEMENTARY INFORMATION FOR "Pulsed glucocorticoid administration enhances dystrophic muscle performance through epigenetic and metabolic reprograming by Quattrocelli et al.

Supplemental Table 1. Biomarkers of obesity and metabolic syndrome in DMD patients receiving daily compared to weekend glucocorticoids (CG).

	daily GC regimen	**weekend GC regimen**	
MEASUREMENTS	*mean ± s.e.m*	*mean ± s.e.m*	*P value*
age (years)	10.92 ± 0.92	9.59 ± 0.82	0.199
treatment duration (months)	54.92 ± 6.94	47.17 ± 7.74	0.417
height (cm)	129.07 ± 4.33	137.09 ± 6.6	0.217
weight (kg)	36.6 ± 3.65	43.46 ± 8.63	0.377
BMI (kg/m2)	21.89 ± 1.72	21.77 ± 2.59	0.963
fat mass TBLH (%)	50.36 ± 3.07	36.28 ± 3.61	0.002
lean mass TBLH (%)	48.19 ± 3.94	64.93 ± 4.19	0.002
BMD TBLH (Z-score)	-2.92 ± 0.22	-1.2 ± 0.26	<0.001
BMD L1-L4 (Z-score)	-2.13 ± 0.28	-0.34 ± 0.25	<0.001
SERUM			
cortisol (ng/ml)	2.76 ± 0.54	15.4 ± 3.65	0.001
glucose (mg/dl)	126.67 ± 7.25	105.25 ± 3.69	0.005
insulin (ng/ml)	3.1 ± 0.79	0.64 ± 0.16	0.003
BCAA (μM)	633 ± 31.8	492 ± 31.3	0.005
free fatty acids (μM)	402.62 ± 9.03	361.86 ± 8.87	0.004
MOBILITY and MUSCLE DAMAGE			
Brooke's score (AU)	6.00 ± 1.10	5.25 ± 1.14	0.571
10m run test (sec)	*(n=5)* 7.5 ± 0.57	*(n=7)* 6.7 ± 1.07	0.785
creatine kinase (U/L)	12626 ± 3448	17770 ± 4850	0.312
HEART FUNCTION			
PR interval (msec)	113.45 ± 2.87	118.55 ± 5.32	0.339
QRS interval (msec)	83.09 ± 3.11	77.64 ± 3.01	0.202
QT interval (msec)	336.36 ± 9.20	337.09 ± 10.09	0.951
LV septum thickness (cm)	0.70 ± 0.04	0.69 ± 0.06	0.917
LV PW thickness (cm)	0.73 ± 0.04	0.70 ± 0.05	0.592
fractional shortening (%)	31.49 ± 1.35	32.05 ± 0.90	0.786

Supplemental Table 2. List of regulatory regions identified through H3K27ac ChIP-seq analysis, and targeted for ChIP-qPCR assays.

Gene	Regulatory region	H3K27ac peak region (mm10)	target element sequence
Klf15	***GRE***	chr6:90462191-90464692	TGGGACACCATGTTC
Klf15	***KRE***	chr6:90470011-90472892	TGGGGGGTGGGGTGC
Klf15	***MEF2***	chr6:90461636-90478713	ACTAAAAATAGC
Mef2c	***GRE***	chr13:83522844-83528432	TCACAGACTGTTCTG
Mef2c	***KRE***	chr13:83517868-83518938	AGACCCCGCCCCTCC
Mef2c	***MEF2***	chr13:83570825-83576273	GTTATTTTTACT
Bcat1	***GRE***	chr6:144909891-144910938	AAAAACAAAATGTTC
Bcat1	***KRE***	chr6:144909891-144910938	CAGCTCCACCCCTAA
Bcat1	***MEF2***	chr6:144909891-144910938	ACCAAAAATAGG
Bckdha	***GRE***	chr7:25651075-25652652	GAACAGACAGTGCTC
Bckdha	***KRE***	chr7:25655965-25660179	CTGGGGGTGGGGTGG
Bckdha	***MEF2***	chr7:25649671-25650527	TTTATTTTTATT
Glud1	***GRE***	chr14:34313334-34314182	CAACAGATTGTTCTG
Glud1	***KRE***	chr14:34309498-34312292	TAGCCCCGCCCCCGC
Glud1	***MEF2***	chr14:34309044-34319109	TCCTAAAATAAA
Got1	***GRE***	chr19:43521165-43529588	TTGGACACACTGTAC
Got1	***KRE***	chr19:43510300-43513519	TAGCCCCGCCCTCCC
Got1	***MEF2***	chr19:43521165-43529588	AACAGAAATAGC
Gpt2	***GRE***	chr8:85492357-85493740	GCACTGCGTGTTCCC
Gpt2	***KRE***	chr8:85519705-85525024	GTGCCCCACCCCCCT
Gpt2	***MEF2***	chr8:85511283-85517802	AATAAAAATAAC
Deptor	***GRE***	chr15:55134099-55135703	AAGAACGATGTGTTC
Deptor	***KRE***	chr15:55131703-55133628	AGCAGGGTGGGGTGT
Deptor	***MEF2***	chr15:55131703-55133628	TTTATTTTTGAT
Ldha	***GRE***	chr7:46825852-46834466	GAGTACATAATGCTC
Ldha	***KRE***	chr7:46837370-46840203	CTACCCCACCCCCGC
Ldha	***MEF2***	chr7:46844401-46847654	TTCAAAAATAGA
Pck1	***GRE***	chr2:173157052-173157552	CAGGACGGTGAGTCC
Pck1	***KRE***	chr2:173157052-173157552	CCTGGGGTGTGGCGA
Pcx	***KRE***	chr19:4509355-4511511	CGAGGGGCGGAGTCG
Pcx	***MEF2***	chr19:4535136-4535636	ACCATATATAGC
Pfkm	***GRE***	chr15:98111107-98117349	GAACACAGCGTCCCC
Pfkm	***KRE***	chr15:98108180-98110085	GAGCTACACCCAACC
Pfkm	***MEF2***	chr15:98108395-98110085	TTTATATTTAGC
Smarcd3	***GRE***	chr5:24600248-24605130	GGGAACAGACAGTTC
Smarcd3	***KRE***	chr5:24598194-24599265	GGGGGGGCGGAGTTT
Smarcd3	***MEF2***	chr5:24600248-24605130	TCTATTTCTGGG
Adipoq	***GRE***	chr16:23163366-23168343	GGGCACAGGTTGTTC
Adipoq	***KRE***	chr16:23180995-23183171	GGGAGGGTGGGGTGG
Adipoq	***MEF2***	chr16:23176392-23177054	TTTATTTTTAAC
Adipor2	***GRE***	chr6:119376615-119378483	TCACATAATGTACTG
Adipor2	***KRE***	chr6:119416203-119420293	CAGTGGGCGTGGCCT
Adipor2	***MEF2***	chr6:119416203-119420293	AATAAAAATAAA
Cd36	***GRE***	chr5:17890487-17895634	AAGAACAGTCAGTGC
Cd36	***KRE***	chr5:17884000-17889533	CAACTCCGCCCCATA
Cd36	***MEF2***	chr5:17827696-17838060	ATCCAAAATAGA
Dgat2	***GRE***	chr7:99199241-99199968	GAACAGGGTGACCTT
Dgat2	***KRE***	chr7:99182170-99183833	TATAGGGCGGGGCAA
Dgat2	***MEF2***	chr7:99201746-99203086	GTTATAAATAAC
Ech1	***GRE***	chr7:28819366-28821894	GAACAATCGGTTCTC
Ech1	***KRE***	chr7:28823813-28827057	GTGTGGGTGGGGTGG
Ech1	***MEF2***	chr7:28823813-28827057	TTTATTTTTATT
Gpd1	***GRE***	chr15:99717644-99718707	CAGGACAGTCTGTGC
Gpd1	***KRE***	chr15:99717486-99720976	AGGGGGGCGGAGTGA
Gpd2	***GRE***	chr2:57236646-57239830	AGAAACACACTGTTC
Gpd2	***KRE***	chr2:57236646-57239830	TCATGGGTGGAGTAC
Gpd2	***MEF2***	chr2:57319557-57320057	GTTATTTATATA
Pnpla2	***GRE***	chr7:141454860-141457616	CAGCACACAAAGTTC
Pnpla2	***KRE***	chr7:141454860-141457616	GAAGGGGCGGGGCCT
Pnpla2	***MEF2***	chr7:141452037-141452537	AACAAAAATAGA
Tpi1	***KRE***	chr6:124812191-124814237	GCTAGGGCGTGGCTC
Tpi1	***MEF2***	chr6:124812191-124814237	TTCTAAAATAGC

Supplemental Table 3. Regulatory sequences and transcription factor binding sites for luciferase assays in electroporated myofibers.

transcription factor binding site (position from TSS)	sequence (GRE sites in red; KRE sites in grey; MEF2 sites in blue)
Mef2C GRE-KRE (I intron; +1173bp)	AACTGTGCTTCACAGCATTTCT**CTACACATTGTTG**TATTATAGCAAATTGAAAACATTTATTTAA GCAAGGAAGCAGCTCAAAGCTAGGGACTATACATAGCAAACATATGAAACCATTTTAATAAGT AAATTCCATATTCACAAGCAACATGGGCTAATGAATGTAAAAGACACAACGGCATACATTGAT CAAGAATGCTATAAATTATTATGCATTAAAATCAATTTTCTGGGCT**GTGGGGGGTAGG**ATTGG TACTTAAGAAGAGAAAAGCTTC
Bckdha GRE-KRE (I intron; +12354bp)	TGAGCTATGGTGTCCAAGCA**GGACACACTGTCA**GGGGACCTGATGCAACCATTCAGATACCC AGGTGGACTTCACATACTGGAGCAGGCACAGACCATGTTCTCCAGTCCCCTCTTTCCAAAGG GCTGCCTTTACCCCCATGAAGTCACTGTGCTAATTCAGTGAGTTCCAAAACTGGTCAATAATG ACACTGGATGCTGGATTATAGAATGGGCAATAAAATACCTACAGAGGCTGGGCAGTGGTAGT GTACAACTTTAATCCCAGCGCTTGGGAGGCAGAGGCAGGCGGATCTCTGTGGGTTTGAGGC CAGCCTGGTATGCAAAGTGAGTTCCAGGTCAGCCAGGGTGACAGAGAAAC**CCTCCCCCACC** CAATCCTACATGTGGTCATATACCTTCTCTGTAGGA
Pfkm GRE-KRE (I intron; +26920bp)	TCGTTTTAGTAGACCCTCCTTTTCTCTCA**CCTCCCCCACC**CACCCCGTGACCCCTGACTTTGC TGTGCACCCCTCTGTTCTCTTCTCCTTTCATGTCCCCTCCCCCATCCTTCATCACCTATTCTCA TCTCTCTTGGTCTCGTCTCTAATTTTTAAGGGTTTACCCACACTTACTCCCGTTCATGCGTATA CAGGTCTACTTTACAGGGTAGGAGCTTCATATGAGAGACAGAGGAAATGTAGTTTTTGTCTTT CTGAGTTTGGGTCACTTAATA**CTACACTTTGTAG**ATCCATCCATTTTTCTAAAAAAAAAAAAAA AAGCAATAATTTAGGCTTTTTTCTTTATAGCTGAGTAAAATTCCATTCCATACATGTACCACATT TCCATTACCCA
Ech1 GRE-KRE (promoter; -8017bp)	GAGGTGACCTGGAGTGAGGGCGGTGCTTGG**CCACCCCCTCC**GGAACCACACTTCAGCGGCT CTGCCTCTGCACATTGCGCACCAACACACAGGGCACAAAACCACTAGTTCTTCCTGGAGAAC AACATGCAGCCTCCACCACGCTCCCAGAGACAGCCGCAGATTGAGCTCACTACATCAAGAAC ACCACACCGCGCTCCAAGGAACAGCTACAAGCACAGAGACAGAGGCAGACAGAGACAAAGA AAGAGACGCAACATGGCAGCAGACACTGCTTTTTTACTAAACATAAAAAAAGTCAAAAATCCA AGCAAACTTGAACCCAAGAATGTACACAGAAGTAGGAAACACAGCCCAAGTGCGCCCAGCCA GCCAGCGGTGCTCTTTTCGGAGAACGATTCGATTCATAGACTGAAGTAGATTCCATGAGCCT CCAAGACAATAGGATCCTCCATTCCTCGCCATGCAGGTTTTGTTTTTTTAAGTCCTTTGGTTTG GGGAGGGCCTTCTTTTTTTTCTTTTGTATTTTGATTTTTTTTTTTCCTGCAGTCATCGGCTGCCA ACACAGTCTGCACCAACTGGAGATCAGAAATAAAAAATTAAACCACAACAACAAAAAATCAGA AAACAGACCTAAAATCAAAACAGCTCAGAGGCACACAGTTACCTGCTGGTGGGTAACCGGGC ACGCAACCAAGGCCACAGCGCGAGCATAGTGGGGCCACTGGCACACTTCATACCAGGAGAC ACGCACATGGAGCGCTACACAGCCAGAAGCACCGCACTGCA**GCACACAGTGTGA**GGAGCTG ACCACACGGAGGGACACTACCCACCTCATACACCTTTATTT
Bckdha MEF2 (promoter; -92bp)	CTTGCGACAAAGACGCATAAATGAGTAAGGTGG**CAATTTTTAG**TCTCTAAAATTGCTCCGGTC GTCTGCTTCTAGTTGCTCCTAATTCAGGCAACTAAAAGGACAACTTAACTTGAACCTTCAGGG TTCAGGACCCGGAGCCCTGAGCAAAATGGGCCCTCTCCAAGTCCCTCCCCCTGTTCCCTGTT GTCCAATGGCTATGCCAGAATTGG
Pfkm MEF2 (promoter; -736bp)	TGGACTCTCTCCCCCAGTTAAATTAGTACCTTAAGCACCTTACTATTCTAAC**CTTTAAATAG**TA CTTAACTTCTTACACACATACACTAAGTGTGGGGTTCTGGGATATAAACACAAGAAAGCTCTG GCCTTTGCCCTTTAGTAGTTTTCAGGCCAGGCGAGGAAGCCAGATCCAGTCA
Ech1 MEF2 (promoter; -2200bp)	GGACCTGCTGAGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTATGTGTATGTGTGTGAACA CTGTTATGGGATCTGATGCCCTCTTCTGCCATGCAGGGGCACATGGAAATTAGAGTACTCAA GTTAAAAGT**CTAAAAATAA**GGTAGTTCTGATGCTGTTCTGACATGACAACTGGACCTTGGTCT GTGTACAGAGATGATTGTGCGTTGTGGTTTACTGGCCCTGAAA

Supplemental Table 4. Weekly and daily prednisone regimens exert opposing effects on nutrient disposal and insulin sensitivity in *mdx* mice (4-week treatment).

	vehicle	daily prednisone		weekly prednisone	
SERUM	*mean ± s.e.m*	*mean ± s.e.m*	*P value vs vehicle*	*mean ± s.e.m*	*P value vs vehicle*
corticosterone (ng/ml)	181 ± 1.01	127 ± 5.34	<0.0001	194 ± 5.05	0.118
insulin (ng/ml)	1.64 ± 0.09	3.09 ± 0.14	<0.0001	1.73 ± 0.07	0.820
BCAA (μM)	572 ± 25.3	657 ± 24.2	0.037	457 ± 14.1	0.005
free fatty acids (μM)	462 ± 11	546 ± 13.5	0.002	386 ± 17.3	0.005
TISSUE BCAA (nmol/mg)					
Quadriceps	105 ± 3.37	118 ± 2.74	0.019	89.6 ± 3.06	0.008
Diaphragm	51.6 ± 1.51	64 ± 3.09	0.002	43.8 ± 0.93	0.045
Heart	2.03 ± 0.08	2.38 ± 0.09	0.017	1.65 ± 0.07	0.012
omental fat	23.9 ± 1.32	28.2 ± 0.99	0.035	18.1 ± 0.94	0.005
TISSUE FREE FATTY ACIDS (nmol/mg)					
Quadriceps	2.26 ± 0.1	4.11 ± 0.11	<0.0001	1.25 ± 0.07	<0.0001
Diaphragm	1.78 ± 0.07	2.07 ± 0.09	0.021	1.11 ± 0.07	<0.0001
Heart	0.776 ± 0.06	0.86 ± 0.03	<0.0001	0.44 ± 0.02	<0.0001
omental fat	5.08 ± 0.1	6.01 ± 0.3	0.008	4.35 ± 0.07	0.038

Supplemental Table 5. Long-term weekly prednisone boosts disposal of BCAA and free fatty acids in circulation and peripheral tissues of *mdx* and *Dysf-null* mice.

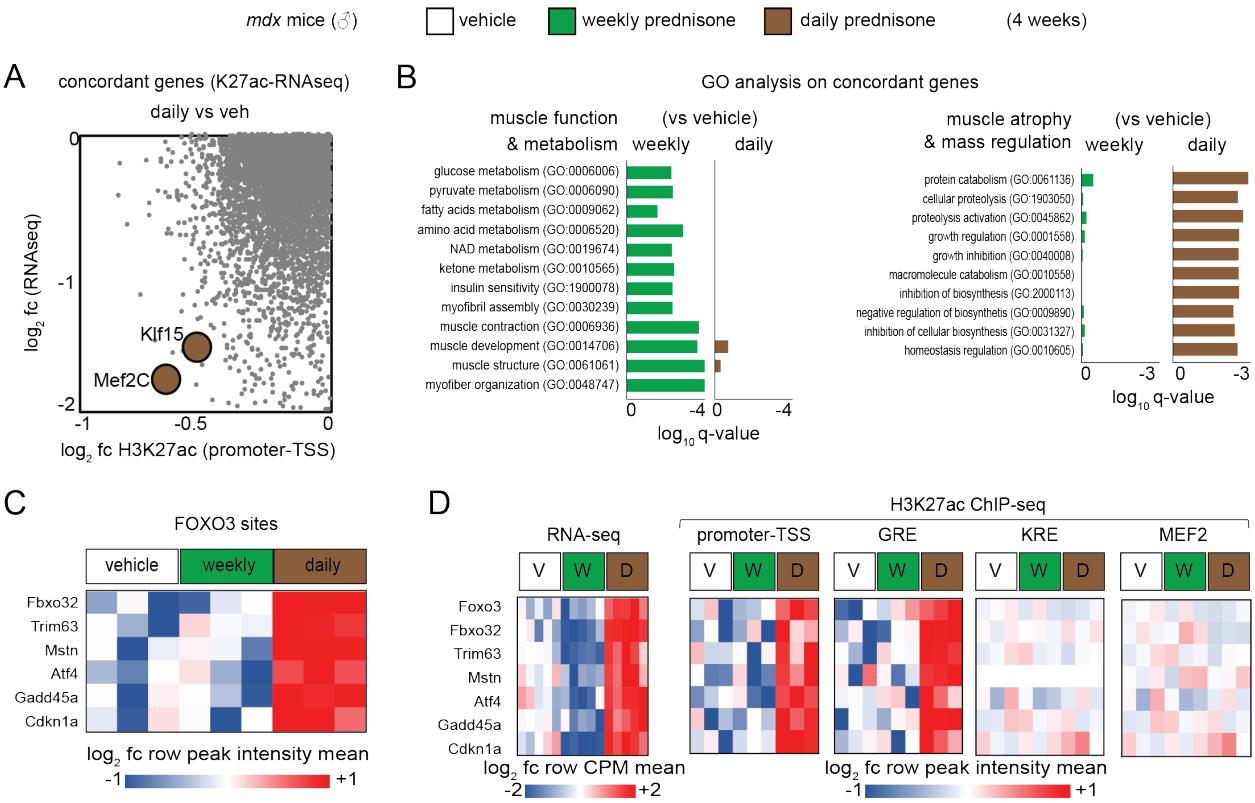
mdx mice (40 week-treatment)	**vehicle**	**weekly prednisone**	
BLOOD and SERUM	*mean ± s.e.m*	*mean ± s.e.m*	*P value*
creatine kinase (U/ml)	5.42 ± 0.4	3.1 ± 0.16	0.001
insulin (ng/ml)	1.3 ± 0.1	1.51 ± 0.14	0.219
corticosterone (ng/ml)	150 ± 10.6	133 ± 8.36	0.228
BCAA (μM)	647 ± 26	462 ± 7.32	<0.0001
free fatty acids (μM)	629 ± 13.5	547 ± 10.4	0.001
TISSUE BCAA (nmol/mg)			
quadriceps	118 ± 2.3	95.3 ± 3.21	<0.0001
diaphragm	57.5 ± 2.9	49.4 ± 2.14	0.037
heart	2.46 ± 0.05	1.74 ± 0.08	<0.0001
omental fat	31.3 ± 1.46	23.5 ± 0.43	0.001
TISSUE FREE FATTY ACIDS (nmol/mg)			
quadriceps	2.05 ± 0.08	0.752 ± 0.05	<0.0001
diaphragm	2.4 ± 0.07	1.46 ± 0.05	<0.0001
heart	0.739 ± 0.03	0.211 ± 0.01	<0.0001
omental fat	4.54 ± 0.21	3.91 ± 0.12	0.019

Dysf-null mice (32 week-treatment)	**vehicle**	**weekly prednisone**	
BLOOD and SERUM	*mean ± s.e.m*	*mean ± s.e.m*	*P value*
creatine kinase (U/ml)	2.42 ± 0.08	1.31 ± 0.04	<0.0001
fasting glycemia (mg/dl)	114 ± 3.51	109 ± 3.17	0.228
insulin (ng/ml)	1.26 ± 0.1	1.22 ± 0.11	0.815
corticosterone (ng/ml)	149 ± 6.08	143 ± 5.2	0.446
BCAA (μM)	519 ± 12.7	419 ± 9.95	<0.0001
free fatty acids (μM)	543 ± 11.7	475 ± 9.18	0.001
TISSUE BCAA (nmol/mg)			
quadriceps	107 ± 3.46	90.9 ± 2.58	0.002
diaphragm	51.3 ± 1.82	45.4 ± 1.06	0.014
heart	1.89 ± 0.04	0.91 ± 0.03	<0.0001
omental fat	35.2 ± 0.81	24.5 ± 0.8	<0.0001
TISSUE FREE FATTY ACIDS (nmol/mg)			
quadriceps	2.98 ± 0.24	1.05 ± 0.06	<0.0001
diaphragm	1.7 ± 0.26	0.753 ± 0.07	0.005
heart	0.69 ± 0.04	0.438 ± 0.05	0.001
omental fat	6.69 ± 0.31	3.49 ± 0.38	<0.0001

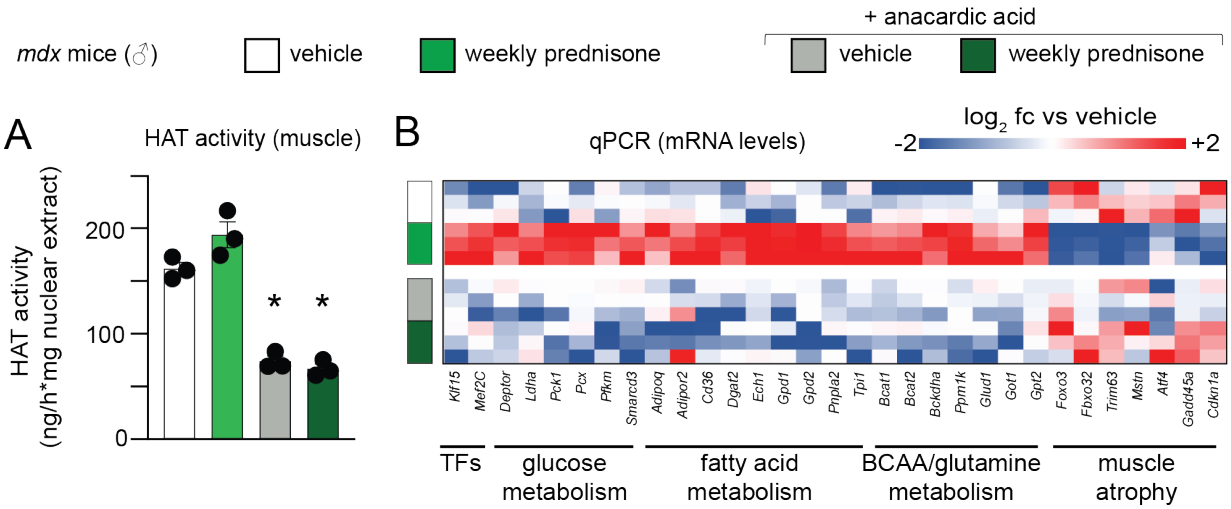
Supplemental Table 6. List of primers for ChIP-qPCR analyses.

Gene	Regulatory region	Primer F	Primer R
Klf15	*GRE*	CCTCCGTTTCTATCGGTTCA	GAACGGTCACACAAATGCAG
Klf15	*KRE*	TGTCACAGGGATGTGCCTAA	GACAAGCATCTCTGGCCTTC
Klf15	*MEF2*	ACCCTCCAGCCTATGCCTAT	GCACTCTGACTGTGCTCTGG
Mef2c	*GRE*	GATAGGGTGGAGAACGTGGA	AATCCAAACCCAGGGAAAAC
Mef2c	*KRE*	AACGCAGACCTCACAGACCT	AGTGGGTGTGCTTTGGAAAC
Mef2c	*MEF2*	GTGCCATGTGCTCTGAGAAA	GCACAGCTCAGTTCCCAAAT
Bcat1	*GRE*	CAGGCCCCAGCTATTACTGA	TCTCAGCTTTAGGGGTGGAG
Bcat1	*KRE*	ATTGCCATGATTCAACACGA	GCGGGAATGCCATAGTTTAT
Bcat1	*MEF2*	ACCCAGCTGCTCAGTCAAAT	TTTCACATGAAAGCCACAGC
Bckdha	*GRE*	AACTGAACCTGGGACCTTCA	GAACATGCGTGCTTGAAAGA
Bckdha	*KRE*	GGAAATTGGCAGCAGAGGTA	ATCCATGTCCCTGCACATCT
Bckdha	*MEF2*	TCGGTGACAATGAGCAGAAG	ATGCATGCAGACAGAACACC
Glud1	*GRE*	GTGCCAGCCTGGACTACATA	CACCCACCACAAACACAGAT
Glud1	*KRE*	AGGGGCCTGGTGACTCAT	CTTTGCAGATGGGCTTGGT
Glud1	*MEF2*	ATCGGCTCTCCCAGTGTTTA	GATTGCAGCAAGTCACCAGA
Got1	*GRE*	GTGGGGAAAGCAGAGAACAG	CTTTCTGGGGAAGCACTGAG
Got1	*KRE*	GTGATGTGGGCATGGATGTA	CATGTGCTTCTGGAACAGGA
Got1	*MEF2*	GACTGGAGGTTTTCCTGTGC	GGCCTTTGTGGTGACTTGAT
Gpt2	*GRE*	CCTTCGACGGTTCACTTGTT	CCCCTTCGTCTCACTTCAAA
Gpt2	*KRE*	GGGGCACCCTAATGTCTCTT	TGCAAAACCCTGTGTCAAAA
Gpt2	*MEF2*	GTGGGTCTCAACCTCTTTGG	GGAGCTTCAGGATTGTCTGC
Deptor	*GRE*	CAGTGGTGCAAAGTGAATGG	TGGGTTCTCACACCACTGAA
Deptor	*KRE*	GGTCTGTGGAAGAAGCCAAG	AACGAAATTGGTCAGGTTGG
Deptor	*MEF2*	CCCTTAACCAAAAGCAACCA	GGGAAGCTTGGGCTAATAGG
Ldha	*GRE*	TATGTGACCATGCCTCTGGA	TGAGCTGACAGACAGGTGCT
Ldha	*KRE*	ATCTCCAGAGCAAAGGACGA	CCCCAGTTCAGAAACCAGAA
Ldha	*MEF2*	CAGACTTGAGGTTTGCGTGA	GACAAGATCTCTGGGCAAGC
Pck1	*GRE*	CCTGGAAGAACAAGGAGTGG	CTACGGCCACCAAAGATGAT
Pck1	*KRE*	ATCATCTTTGGTGGCCGTAG	CCAGAGAAGCCATTGGTGAT
Pcx	*KRE*	AGCCAATCAGGATGAGCTTC	ACCTACCCCTCGCGTTTAAG
Pcx	*MEF2*	CGTGCCTAAGTACCCCCTTT	CAGGGAGATCTTGCCATTGT
Pfkm	*GRE*	ATTAGCAACCATGGCCACTC	CACTGAGGAAGGCTGTGTGA
Pfkm	*KRE*	GCCATCCAGACTGATTCCAT	GTGTGAAGAGCAGGCTTGG
Pfkm	*MEF2*	TGCACCTATGATGCTTCCAG	CAAGAGGCACAGGACACAAA
Smarcd3	*GRE*	CCCTCGCCACAAATAGTTTC	CCAGATTCCTTCCAGCAAAT
Smarcd3	*KRE*	AGAATGACTTGGTCGCTGCT	GCCTGTTTCTGCTTCACCTC
Smarcd3	*MEF2*	CTGTGTGCTTCCCTTCACAA	ATTTCCCCTATTGCCCTCAC
Adipoq	*GRE*	CCTGCTGGCTCTGAGACATT	CTGCAAGACACTCCCTGGAT
Adipoq	*KRE*	CAGGAGGACTGCAGACAAGA	CTTTGGTACCATCGCAACCT
Adipoq	*MEF2*	CTGCATTCCCCCAAATTAAA	GGCACATTGGTCTTGGATTC
Adipor2	*GRE*	TTTTCTTTCCTCCCTCTGGAA	ACCAAAACCCAACAAACCAA
Adipor2	*KRE*	CGGATTCTTGTGTTGTGTGG	ACACTTGAGAGGCAGGTGCT
Adipor2	*MEF2*	TTGGTTTCTCTGCCCTTTTG	GACACAGCGAATTGCTCTCA
Cd36	*GRE*	TGACATTGCTGGGAATTGAA	TGGGATTGTAGACCAGTTTGC
Cd36	*KRE*	CTACCGGGCGTTGTTCTAAA	ACCGTTCCCATAGACACTGG
Cd36	*MEF2*	TCAACCATGTGCTGATATTTGAC	CCCTCCTTCCTCGAAATGTT
Dgat2	*GRE*	GGGTCTCTTAGGACCACCAA	ACTGCAAGCAGACACACGAG
Dgat2	*KRE*	GCCACCCTAGATGAGCAGAA	CACCCCCTACAAGAACATGG
Dgat2	*MEF2*	TGAAAAAGTGGGCTGGAGAG	CCCTCATCCCAACACTGTCT
Ech1	*GRE*	GCTTGAGTGCAGCAGTGTGT	CCTGCTCTGGAGTGGAGAAC
Ech1	*KRE*	AGGGTGTCCTTTCTGCTCAC	CAGTGGGACAGTAGGGGATG
Ech1	*MEF2*	TGCAAGCCTTCAAACTCCTT	GACCTTTGGAAGAGCAGTCG
Gpd1	*GRE*	AGGGTAGGAAATGCCCTCAG	AAGGCAACAGCACTCTGCTC
Gpd1	*KRE*	CCTGTTCACCACAGGAAGGT	GGGGGAGGAACTCATAAAGC
Gpd2	*GRE*	GGTGCTCATGGGTTTCTGAT	GTCCATGCTGACTTCCCACT
Gpd2	*KRE*	GGTGCTCATGGGTTTCTGAT	GTCCATGCTGACTTCCCACT
Gpd2	*MEF2*	TTCTCCCTCCAAAATGTCTCA	CCCCAAGGAAAAGAACAACA
Pnpla2	*GRE*	GCCACACTTCTGTGCTTTGA	CCGCAGAAAACCCTAGACAA
Pnpla2	*KRE*	GGTCTCTCAGATGGCTTTGC	AGGCAGAGGAGGAGGTGTTT
Pnpla2	*MEF2*	GTTCTTGGCACCAGCATCTC	CCAGGGCTTATCCTGGTACA
Tpi1	*KRE*	GTTTGCTCGAACACGACCTT	CTGGGGCACTCAGAAAGAAG
Tpi1	*MEF2*	CCTTCCCAATGCCTCTGATA	GATCATCCATTTCCCGAAGA

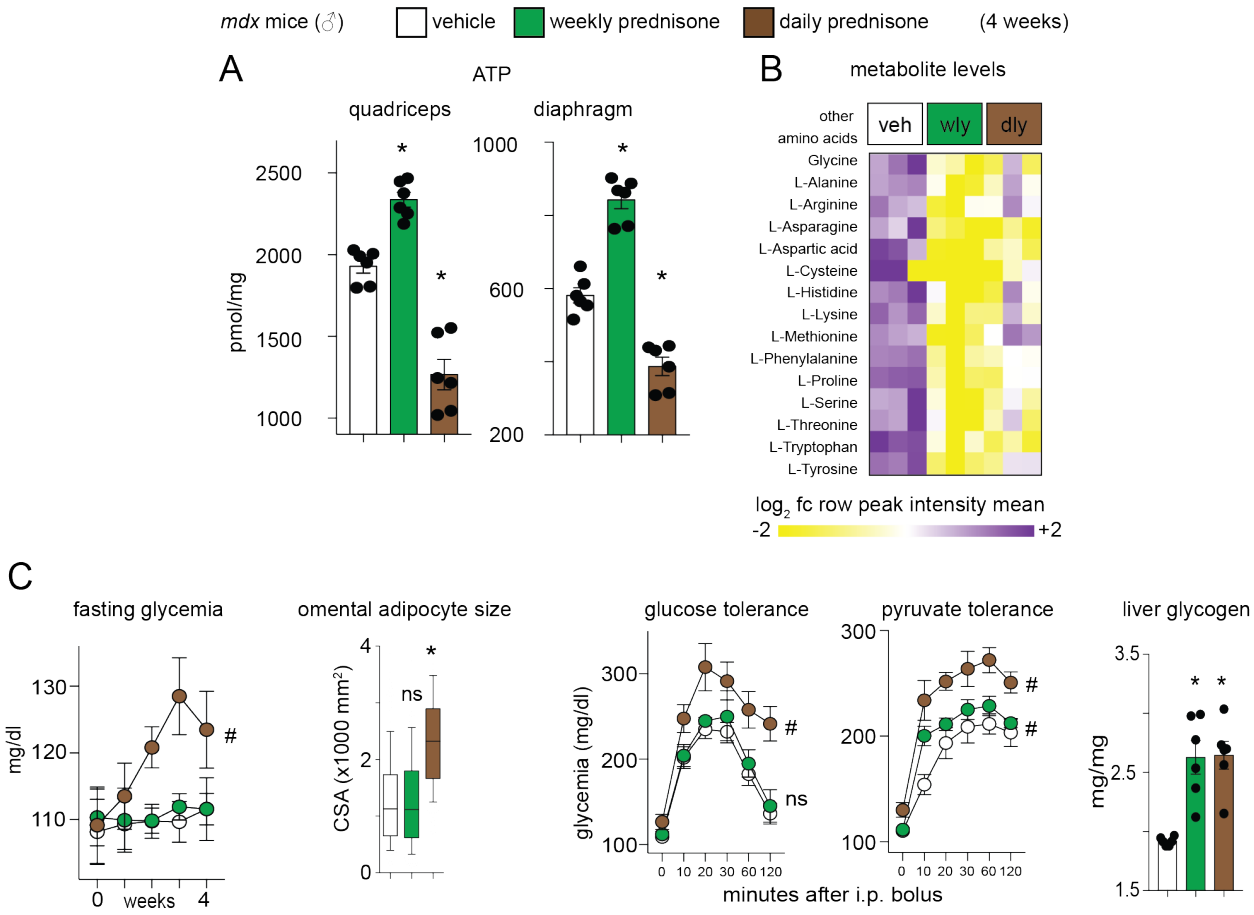
SUPPLEMENTAL FIGURES



Supplemental Figure 1. Divergent epigenomic and transcriptomic programs elicited by glucocorticoid regimens in dystrophic muscle. (A) After daily prednisone, *Klf15* and *Mef2C* showed reduced expression and H3K27 acetylation in treated *mdx* myofibers. **(B)** Genes with both increased expression and H3K27Ac marks were identified. Gene ontology (GO) analysis of these concordant genes showed that weekly prednisone enriched for nutrient metabolism and muscle function pathways, while daily prednisone exposure enriched for atrophy-related terms. **(C)** Daily prednisone drives atrophy pathways seen as enrichment for FOXO3 sites marked with increased H3K27ac marks. **(C)** Pathway-centered analysis showed that GRE but not KRE or MEF2 sites on atrophy genes were activated after daily prednisone. N=3 mice/group for K27ac ChIP-seq, n=5 mice/group for RNAseq.



Supplemental Figure 2. Anacardic acid administration reduced histone acetyl-transferase (HAT) activity in muscle and blunted transcriptional gains in nutrient metabolism pathways induced by weekly prednisone. **(A)** Muscle HAT activity was significantly reduced at endpoint of i.p. anacardic acid administration. **(B)** qPCR analyses showed loss of gene upregulation in muscles co-treated with weekly prednisone and anacardic acid. Histograms depict single values and mean±s.e.m.; n=3 mice/group *, P<0.05 vs vehicle, 1-way ANOVA test with Tukey's multiple comparison.



Supplemental Figure 3. Divergent metabolic programs elicited by pulsatile weekly versus daily prednisone in *mdx* mice. (A) ATP levels were increased in both locomotory (quadriceps) and respiratory (diaphragm) muscles compared to vehicle treated, while daily prednisone reduced ATP content in both muscles. **(B)** Weekly prednisone treatment induced a general trend in lower levels of free amino acids in muscle, as quantitated through metabolomic analysis. **(C)** Daily, but not weekly, prednisone induced hyperglycemia, adipocyte hypertrophy, and glucose intolerance. However, both regimens seemingly upregulated liver gluconeogenesis, as observed through pyruvate tolerance tests and liver glycogen levels. Curves depict mean±s.e.m.; histograms depict single values and mean±s.e.m.; box plots, Tukey distribution; n=6 mice/group **(A, C)**, n=3 mice/group **(B).** *, P<0.05 vs vehicle, 1-way ANOVA test with Tukey's multiple comparison; #, P<0.05 vs vehicle, 2-way ANOVA test.

SUPPLEMENTAL METHODS

RNA-seq. RNA-seq datasets used for analyses in this work can be accessed on the NCBI GEO databse (GSE95682). Total RNA was purified from ~30mg quadriceps muscle tissue of treated and control DBA/2J-*mdx* male 6 month-old mice with the RNeasy Protect Mini Kit (Cat #74124; Qiagen, Hilden, Germany) as per manufacturer's instructions. RNA quantity and quality were respectively analyzed with Qubit fluorometer (Cat #Q33216; Thermo Fisher Scientific, Waltham, MA) and 2100 Bioanalyzer (Cat #G2943; Agilent Technologies, Santa Clara, CA). Libraries were prepared from approximately 1mg RNA/sample with TruSeq Stranded Total RNA Library Prep Kit (Cat #RS-122-2203; Illumina, San Diego, CA). Libraries were sequenced through the NextSeq 500 System (high-throughput, paired-end 150bp fragment sequencing; #SY-415-1001; Illumina, San Diego, CA). Raw reads were aligned with TopHat v2.1.0 to the mm10 genome assembly (grcm38, version 78) (60). Transcripts were assessed and raw read counts per gene were quantified with HTseq (61). Reads Per Kilobase of transcript per Million mapped reads (RPKM) and fold-changes between groups were calculated using EdgeR from the Bioconductor package (62). Differentially expressed genes were identified by adjusted P-value <0.05. Heatmaps were visualized with GiTools (63).

ChIP-qPCR and RT-qPCR. For ChIP-qPCR assays, chromatin was immunoprecipitated following the procedures detailed for ChIP-seq, and then assayed in three replicates using 1X Sybr Green iTaq mix (Cat #1725125; Bio-Rad, Hercules, CA) and 100nM primer mix **(Supp Table 6)** at a CFX96 qPCR machine (Bio-Rad, Hercules, CA; thermal profile: 95C, 10sec; 60C, 20sec; 72C, 30sec; 60X; melting curve). Quantitation was performed as % of input, and IgG-immunoprecipitated chromatin was assayed as negative control. Primary antibodies: rabbit anti-H3K27ac (Cat #39133, Active Motif, Carlsbad, CA), rabbit anti-GR, -KLF15, -MEF2C (Cat #2164; #7194; #2585; ABclonal, Woburn, MA), rabbit IgG (Cat #ab27472, Abcam, Cambridge, MA). For qPCR assays, total mRNA was extracted using Trizol (Cat #15596026; Thermo Fisher Scientific, Waltham, MA) from cryo-pulverized quadriceps muscles, and 1μg RNA was reverse-transcribed using 1X qScript Supermix (Cat #95048; QuantaBio, Beverly, MA). 1:10-diluted cDNA was assayed with Sybr Green iTaq mix as detailed above (thermal profile: 95C, 10sec; 60C, 20sec, 72C, 30sec; 40X; melting curve). Primers were selected among validated primer sets from the MGH PrimerBank; IDs: 12963561a1, 20070856a1, 26331298a1, 33859514a1, 31982494a1, 30425290a1, 6680027a1, 6754034a1, 27805389a1, 21703930a1, 6754524a1, 7110683a1, 6679237a1, 31981185a1, 31981140a1, 31982423a1, 23271651a1, 31982474a1, 16975490a1, 7949037a1, 6753966a1, 31981769a1, 6678678a1, 26327465a1, 6678413a1, 10946948a1, 10442021a1, 21362329a1, 9789951a1, 13385848a1, 21523717a1, 6754752a1, 6753128a1, 6681149a1, 6671726a1.

Metabolic cages. VO_2 (ml/h/kg) and energy expenditure to body weight (kcal/h/kg) were assessed via indirect calorimetry using the TSE Automated Phenotyping System PhenoMaster (TSE system, Chesterfield, MO). Mice were singly housed in their home cages in an enclosed environmental chamber (part of the TSE system) with controlled temperature and light/dark cycles (12 hours each; 6AM-6PM). After a three-day period of acclimation to the metabolic chamber, data collection started at 48 hours after prednisone or vehicle injection and lasted for 5 days. Measurements of CO2 production and O2 consumption occurred using the attached gas analyzer to assess energy expenditure. In addition, physical activity in three dimensions was monitored via infrared beam breaks through frames mounted on the perimeter of the metabolic cages. Enrichment items were omitted to avoid insulation from sensors and infrared light beam path obstruction. Results are expressed as 12 hour-period values (light/dark; 10 values per mouse). Metabolic cage assays were conducted blinded to treatment groups.

Muscle function, whole-body plethysmography, echocardiography. Forelimb grip strength was monitored using a meter (Cat #1027SM; Columbus Instruments, Columbus, OH) blinded to treatment groups. Animals performed ten pulls with 5 seconds rest on a flat surface between pulls. Immediately before sacrifice, *in situ* tetanic force from *tibialis anterior* muscle was measured using a Whole Mouse Test System (Cat #1300A; Aurora Scientific, Aurora, ON, Canada) with a 1N dual-action lever arm force transducer (300C-LR, Aurora Scientific, Aurora, ON, Canada) in anesthetized animals (0.8 l/min of 1.5% isoflurane in 100% O_2). Tetanic isometric contraction was induced with following specifications: initial delay, 0.1 sec; frequency, 200Hz; pulse width, 0.5 msec; duration, 0.5 sec; using 100mA stimulation (64). Length was adjusted to a fixed baseline of 50mN resting tension for all muscles/conditions. Fatigue analysis was conducted by repeating tetanic contractions every 10 seconds until complete exhaustion of the muscle (50 cycles). Time of contraction was assessed as time to max tetanic value within the 0.0-0.5 sec range of each tetanic contraction, while time of relaxation was assessed as time to 90% min tetanic value within the 0.5-0.8 sec range of every tetanus. Unanesthetized whole-body plethysmography (WBP) was used to measure respiratory function using a Buxco Finepointe 4-site apparatus (Data Sciences International, New Brighton, MN). Individual mice were placed in a calibrated cylindrical chamber at room temperature. Each mouse was allowed to acclimate to the plethysmography chamber for 120 minutes before recording was initiated. Data was recorded for a total of 15 minutes broken into 3 consecutive 5-minute periods. All physiological studies were conducted blinded to treatment groups. Cardiac function was assessed by echocardiography, which was conducted under anesthesia (0.8L/min of 1.5% vaporized isoflurane in 100% O_2) on mice between 2 and 5 days before sacrifice. Echocardiography was performed using a Visual Sonics Vevo 2100 imaging system with an MS550D 22-55 MHz solid-state transducer (FujiFilm, Toronto, ON, Canada). Longitudinal and circumferential strain measurements were calculated using parasternal long-axis and short-axis B-mode recordings of three consecutive cardiac cycles, analyzed by the Vevo Strain software (FujiFilm, Toronto, ON, Canada). Recording and analysis were conducted blinded to treatment group.

Multi-modal imaging (FDG-PET, microCT, MRI). Mice were anesthetized in an induction chamber with 3% isoflurane in oxygen, weighed, and then transferred to a dedicated imaging bed with isoflurane delivered via nosecone at 1-2%. Mice were placed in the prone position on a plastic bed and immobilized to minimize changes in position between scans. Respiratory signals were monitored using a digital monitoring system developed by Mediso (Mediso-USA, Boston, MA). Mice were imaged with a preclinical microPET/CT imaging system (nanoScan PET/CT, Mediso-USA, Boston, MA). CT data was acquired with a 2.2x magnification, <60 µm focal spot, 2 × 2 binning, with 480 projection views over a full circle, using 50 kVp/520 µA, with a 300 ms exposure time. The projection data was reconstructed with a voxel size of 250 µm and using filtered (Butterworth filter) backprojection software from Mediso. A bone mineral density standard (GRM GmbH, Moehrendorf, Germany) with hydroxyapatite (HA) from 0 to 1200 mg HA/cm^3 was used to convert the CT images from Hounsfield units to bone mineral density. The HA standard was imaged with the same parameters. For PET imaging, a target of 10 MBq of ^{18}F-fluordeoxyglucose (FDG) was injected intravenously after mice had been fasted for four hours. PET acquisition parameters were as follows: 1:1 coincidence detection and 30-minute acquisition time. MLEM reconstruction was used with CT for attenuation correction and scattering. Pixel size was set to 0.3 × 0.3 mm. Mice were fasted overnight prior to imaging in the early morning (~8AM). After completion of PET/CT, each mouse was transferred to the MRI scanner and a reference standard consisting of one tube of canola oil and one tube of water was positioned above its back. MRI was performed on a 9.4T Bruker Biospec MRI system with a 30 cm bore, a 12 cm gradient insert, and an AutoPac laser positioned motorized bed (Bruker Biospin Inc, Billerica, MA). Respiratory signals and temperature were monitored using an MR-

compatible physiologic monitoring system (SA Instruments, Stonybrook, NY); a warm water circulating system was used to maintain body temperature. A 72mm quadrature volume coil (Bruker Biospin, Inc, Billerica, MA) was used to image each mouse's whole body in two overlapping fields of view. First, the mouse was positioned with the thorax at the magnet's isocenter and imaged using a T_1-weighted accelerated spin echo sequence (Rapid Acquisition with Relaxation Enhancement, RARE) with five pairs of interleaved axial slice stacks covering brain to mid-abdomen. TR was nominally set at 1000 ms; with respiratory gating the functional TR was approximately 1500 ms (range 1300-2000 ms). The following additional parameters were used: TE = 6.25 ms, RARE factor 4, MTX = 256 x 256, FOV 45 x 45 mm, 15 slices of 1 mm thick, 4 mm gap between slices, and 2 signal averages. Each image stack was acquired with and without fat saturation. Acquisition time was approximately 3 minutes per scan. After imaging the upper portion of the mouse, the imaging bed was moved deeper into the magnet and two more pairs of interleaved image stacks were acquired to cover the lower abdomen and legs. Parameters were the same as above, except for a 1 mm gap between slices and 3 signal averages. The reconstructed data was visualized in Amira 6.5 (FEI, Houston, TX). The interleaved MRI stacks for upper body and lower body were individually merged, then normalized to the water signal from the reference standard. Then the upper and lower body stacks were registered to each other using a combination of normalized mutual information and manual registration, and merged to create whole body fat-suppressed and non-fat-suppressed MR images. A difference (fat only) image was created by subtracting the normalized fat-suppressed image from the normalized non-fat-suppressed image and segmented by thresholding (using the water and canola oil references as a guide). A small amount of manual segmentation was necessary in regions near the testes where fat suppression pulses were less effective. CT images were registered to the MRI data using normalized mutual information. The fat region of interest (ROI) was used in both the MRI data and FDG-PET data for quantitative analysis. Additionally, each leg was segmented into its own ROI for FDG-PET analysis using the MRI images without fat saturation. A skeleton ROI was generated for each mouse by using a 750 HU threshold in the CT image. The % injected dose (%ID) of FDG in fat and muscle tissue was calculated by dividing the total PET signal found in the ROI with the total PET signal in a mouse whole-body ROI. Mass of body fat was determined by multiplying the volume of fat ROIs with the average density of adipose tissue (0.92 g/cm3) (65). The HA standard was segmented with ROIs of 0, 50, 200, 800, and 1200 mg/cm3 and used to create a linear correlation between HU and bone density with a r2 of 0.99.

CK dosing. Serum creatine kinase (CK) was analyzed in triplicate for each mouse using the EnzyChrom Creatine Kinase Assay (Cat # ECPK-100; BioAssay Systems, Hayward, CA) following manufacturer's instructions. Results were acquired with the Synergy HTX multi-mode plate reader (BioTek®, Winooski, VT) and expressed as U/ml for murine and U/l for human samples. Both HOP and CK dosing assays were conducted blinded to treatment groups.

Histology. Excised tissues (muscles, omental fat, heart) were placed in 10% formaldehyde (Cat #245-684; Fisher Scientific, Waltham, MA) for histologic processing. Seven μm sections from the center of paraffin-embedded muscles were stained with hematoxylin and eosin (H&E; cat #12013B, 1070C; Newcomer Supply, Middleton, WI) and Masson's trichrome (Cat #HT-15; Sigma-Aldrich; St. Louis, MO). Myofiber/adipocyte CSA quantitation was conducted on 400 myofibers/adipocytes per tissue per mouse. Imaging was performed using a Zeiss Axio Observer A1 microscope, using 10X and 20X (short-range) objectives. Brightfield pictures were acquired via Gryphax software (version 1.0.6.598; Jenoptik, Jena, Germany). Area quantitation was performed by means of ImageJ (59). Sample processing, imaging and CSA quantitation were conducted blinded to treatment groups.

Protein analysis. Protein lysates from ~50mg muscle tissue were obtained with homogenization at the TissueLyser II (cat #85300; Qiagen, Hilden, Germany) for two rounds of 2 minutes each with 2 minutes pause in between, using sample plates chilled at -20°C o/n and one stainless 5mm bead per sample (cat#69989; Qiagen, Hilden, Germany). Each tissue was homogenized in 250μl RIPA buffer (cat #89900, Thermo Scientific, Waltham, MA) supplemented with protease and phosphatase inhibitors (cat #04693232001 and #04906837001, Roche, Basel, Switzerland). Homogenized samples were then sonicated for 15 cycles (30 sec, high power; 30 sec pause; 200μl volume) in a water bath sonicator set at 4°C (Bioruptor 300; Diagenode, Denville, NJ) and ~10μg protein lysate was mixed with 1:1 volume of 2x Laemmli buffer (cat#161-0737; Bio-Rad, Hercules, CA) and incubated at 95°C for 15 minutes. Protein electrophoresis was performed in 4-15% gradient gels (cat#456-1086; Bio-Rad, Hercules, CA) in running buffer containing 25mM TRIS, 192mM glycine, 0.1% SDS, pH 8.3. Proteins were then blotted on 0.2μm PVDF membranes (cat#16220177; Bio-Rad, Hercules, CA), previously activated for 3 minutes in 100% methanol, in transfer buffer containing 25mM TRIS, 192mM glycine, 20% methanol at 300mA for ~3.5 hours at 4°C. Membranes were washed with TBS-T buffer containing 20mM TRIS, 150mM NaCl, 0.1% Tween-20, pH 7.6, and then blocked with StartingBlock (cat#37543, Thermo Scientific, Waltham, MA). Primary antibody incubation was performed o/n at 4°C with the following antibodies: rabbit anti-phospho BCKDHA (ser293; cat#A304-672A-T), anti-total BCKDHA (cat#A303-790A-T), rabbit anti-mTOR (cat#A301-143A-T), rabbit anti-RagC (cat# A304-299A-T), rabbit anti-S6K (cat# A300-510A-T), rabbit anti-4EBP1 (cat# A300-501A-T; Bethyl Laboratories, Montgomery, TX); rabbit anti-phopsho-S6K (Thr389; cat# AP0564), rabbit anti-phosho-4EBP1 (Ser65; cat# AP0032; ABclonal, Woburn, MA); mouse anti-myosin heavy chain (cat# MF20), mouse anti-puromycin (cat#PMY-2A4; DSHB, Iowa City, IA). Secondary antibody incubation was performed at room temperature for 1 hour with the following antibodies: donkey anti-rabbit and anti-mouse (cat#sc-2313 and #2314; Santa-Cruz Biotechnology; Dallas, TX). Blots were developed with SuperSignal Femto (cat#34096; Thermo Scientific, Waltham, MA) using the iBrightCL1000 developer system (cat #A32749; Thermo Scientific, Waltham, MA) with automatic exposure settings. Protein density was analyzed using the Gel Analysis tool in ImageJ software (59). Only bands from samples run and blotted in parallel on the same gels/membranes were analyzed for ratios. Phosphorylation levels were quantitated as ratio versus total protein; co-IP levels were quantitated as ratio versus bait protein; total protein levels were quantitated as ratio to housekeeping/structural protein control. Image acquisition and densitometric analysis were conducted blinded to treatment group.

Supplemental References

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