

Maximal isometric tetanus
assessed and SSC protocol
administered to left dorsiflexors
of young and old rats.

$N = 30$ per age group



Maximal isometric tetanus assessed for
left dorsiflexors at either 6, 24, 48, 72,
or 120 hours post SSC exposure. At
each time point, right (non-exposed)
and left (SSC-exposed) tibialis anterior
muscles removed for analysis.

$N = 6$ per age group at each time point

Figure S1. Study design

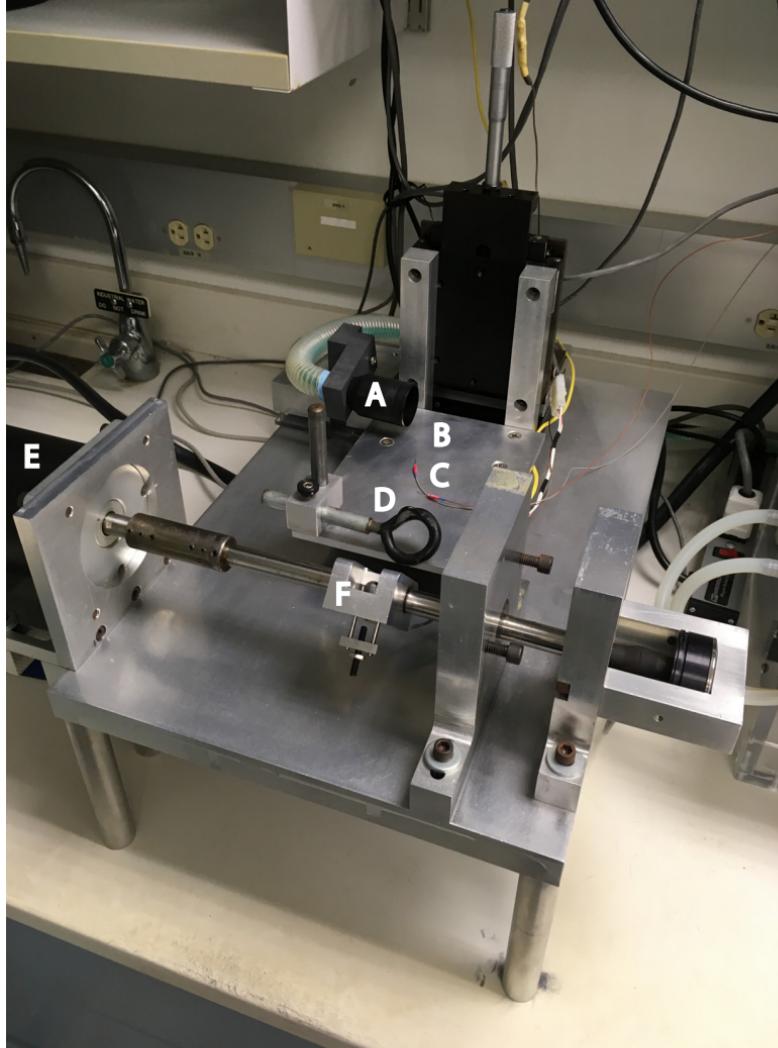


Figure S2. Apparatus for SSC exposure. (A) Anesthesia nose cone, (B) heated table, (C) platinum needle electrodes, (D) knee holder, (E) servomotor and (F) fixture with load cell to secure foot are noted.

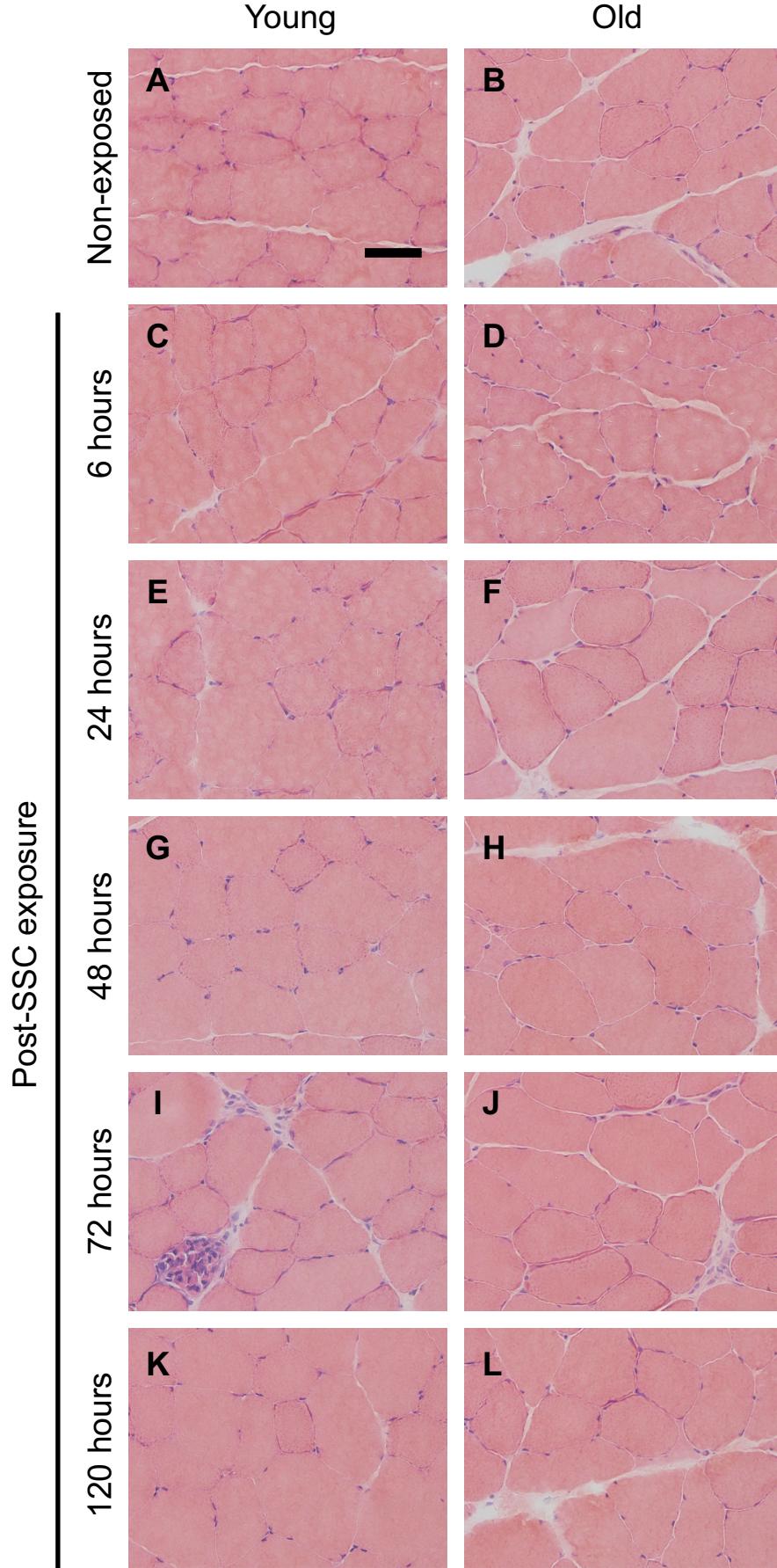


Figure S3. Hematoxylin and eosin stained muscle sections from non-exposed (A,B) and SSC-exposed muscles (C-L) of young and old rats. 40x magnification. Scale bar = 50 μ m.

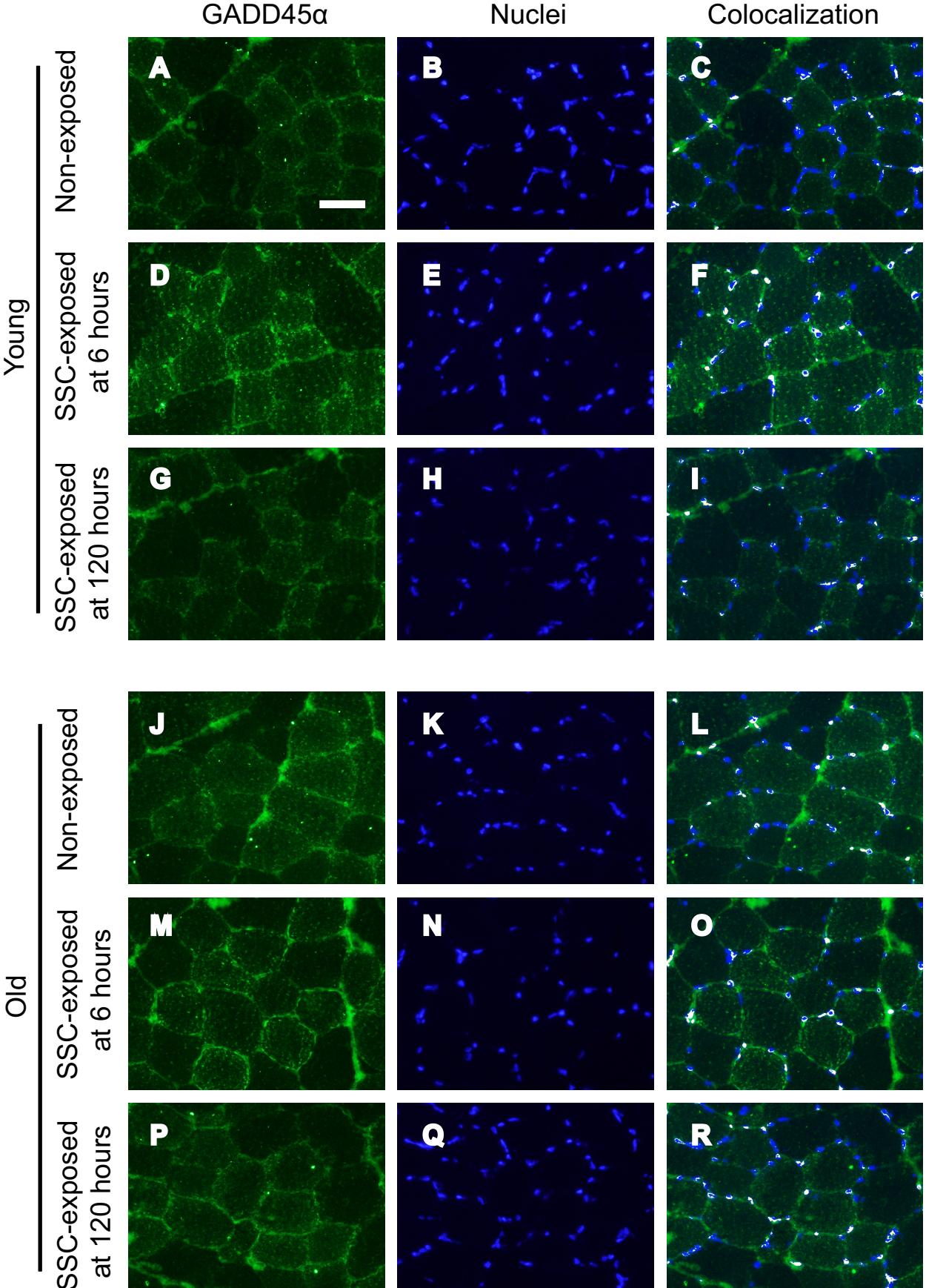


Figure S4. GADD45 α (green) and nuclei (blue) were identified by immunofluorescence. Colocalization (light yellow) was determined utilizing ImageJ. Images depicted are for non-exposed and SSC-exposed muscles of young (A-I) and old (J-R) rats. 40x magnification. Scale bar = 50 μ m.

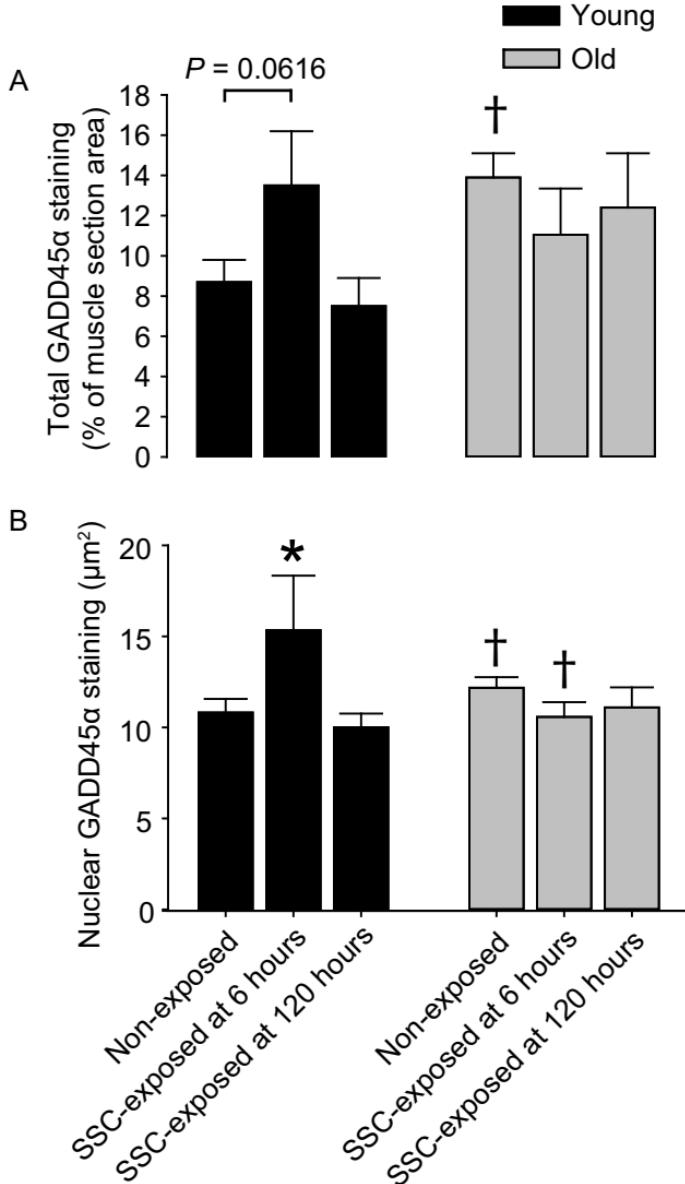


Figure S5. Increased area of nuclear GADD45 α immunostaining at 6 hours post SSC exposure exclusively at young age. (A) Total GADD45 α staining was determined by assessing the GADD45 α distribution over the entire muscle section area evaluated. (B) Nuclear GADD45 α staining represents the typical particle size (in terms of area) of colocalized GADD45 α and DAPI staining. Sample sizes were N = 5 to 11 per group. *Value for SSC-exposed muscles distinct from non-exposed value, †Value for old different from value from young, P < 0.05.

	Young		Old		Fold change	
	Mean	SE	Mean	SE	Mean	SE
<i>Bax</i>	0.33%	0.25%	0.81%	0.21%	2.47	0.63
<i>Bcl2l1</i>	0.88%	0.32%	2.04%	0.55%	2.31	0.62
<i>Bcl2l11</i>	0.18%	0.05%	0.23%	0.07%	1.28	0.40
<i>Bclaf1</i>	1.51%	0.84%	1.23%	0.28%	0.82	0.19
<i>Bid</i>	2.98%	0.69%	2.58%	0.25%	0.87	0.08
<i>Bik</i>	0.11%	0.07%	0.15%	0.08%	1.37	0.75
<i>Birc2</i>	0.68%	0.26%	1.06%	0.26%	1.55	0.37
<i>Bnip3l</i>	1.59%	0.43%	1.49%	0.35%	0.94	0.22
<i>Casp3</i>	1.08%	0.43%	1.01%	0.20%	0.94	0.18
<i>Casp9</i>	2.66%	0.70%	2.37%	0.40%	0.89	0.15
<i>Cideb</i>	1.08%	0.48%	1.07%	0.25%	1.00	0.23
<i>Cradd</i>	0.20%	0.07%	0.17%	0.04%	0.85	0.23
<i>Dapk1</i>	0.12%	0.08%	0.27%	0.11%	2.38	0.94
<i>Dffa</i>	13.33%	2.10%	11.64%	1.28%	0.87	0.10
<i>Fadd</i>	1.85%	0.91%	1.88%	0.53%	1.01	0.29
<i>Gadd45a</i>	2.53%	0.91%	2.36%	0.37%	0.93	0.15
<i>Hrk</i>	0.86%	0.16%	2.87%	1.03%	3.34	1.20
<i>Tnfrsf10b</i>	0.38%	0.10%	0.34%	0.12%	0.89	0.32
<i>Tnfrs21</i>	0.05%	0.02%	0.04%	0.01%	0.79	0.19
<i>Tp53</i>	0.11%	0.06%	0.09%	0.08%	0.76	0.67

Table S3. Percent methylation of genes relevant to stress response for non-exposed muscles of young and old rats. Fold change calculated as old value divided by young value. Sample sizes of $N = 9$ to 12 per value.

	6 hours						120 hours					
	Non-exposed		Exposed		Fold change		Non-exposed		Exposed		Fold change	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Bax</i>	0.03%	0.01%	0.57%	0.18%	19.81	6.19	0.63%	0.50%	0.45%	0.21%	0.72	0.34
<i>Bcl2l1</i>	1.17%	0.59%	2.31%	0.99%	1.97	0.84	0.59%	0.24%	0.34%	0.12%	0.57	0.20
<i>Bcl2l11</i>	0.14%	0.05%	0.14%	0.05%	0.97	0.32	0.22%	0.08%	0.22%	0.05%	1.00	0.24
<i>Bclaf1</i>	1.70%	1.61%	2.24%	1.32%	1.32	0.77	1.32%	0.74%	0.20%	0.14%	0.15	0.11
<i>Bid</i>	3.14%	0.89%	3.51%	1.10%	1.12	0.35	2.83%	1.16%	2.51%	0.75%	0.89	0.26
<i>Bik</i>	0.19%	0.14%	0.73%	0.69%	3.79	3.61	0.02%	0.01%	0.03%	0.02%	1.32	0.71
<i>Birc2</i>	0.75%	0.47%	1.08%	0.61%	1.44	0.82	0.61%	0.30%	0.38%	0.15%	0.61	0.24
<i>Bnip3l</i>	1.45%	0.49%	2.56%	1.12%	1.77	0.78	1.74%	0.75%	0.34%	0.09%	0.20	0.05
<i>Casp3</i>	1.15%	0.77%	1.50%	0.59%	1.31	0.51	1.01%	0.49%	0.76%	0.28%	0.76	0.28
<i>Casp9</i>	2.97%	1.38%	3.28%	1.28%	1.11	0.43	2.35%	0.49%	1.57%	0.52%	0.67	0.22
<i>Cideb</i>	1.01%	0.85%	1.74%	0.97%	1.72	0.97	1.16%	0.44%	0.59%	0.30%	0.51	0.26
<i>Cradd</i>	0.10%	0.04%	0.21%	0.10%	2.08	0.98	0.29%	0.12%	0.08%	0.04%	0.28	0.13
<i>Dapk1</i>	0.04%	0.03%	0.08%	0.06%	1.83	1.31	0.19%	0.16%	0.21%	0.07%	1.15	0.38
<i>Dffa</i>	13.29%	3.42%	13.72%	2.49%	1.03	0.19	13.37%	2.84%	10.97%	1.16%	0.82	0.09
<i>Fadd</i>	2.10%	1.74%	2.81%	1.30%	1.34	0.62	1.60%	0.81%	0.61%	0.16%	0.38	0.10
<i>Gadd45a</i>	2.52%	1.78%	3.04%	1.15%	1.21	0.46	2.53%	0.75%	1.00%	0.33%	0.39	0.13
<i>Hrk</i>	0.74%	0.19%	1.57%	0.94%	2.13	1.28	1.01%	0.32%	1.10%	0.19%	1.09	0.19
<i>Tnfrsf10b</i>	0.38%	0.18%	0.75%	0.43%	1.98	1.13	0.39%	0.12%	0.13%	0.11%	0.32	0.27
<i>Tnfrsf21</i>	0.06%	0.04%	0.08%	0.04%	1.35	0.66	0.04%	0.01%	0.03%	0.01%	0.77	0.16
<i>Tp53</i>	0.10%	0.05%	0.21%	0.20%	2.04	1.95	0.15%	0.14%	0.01%	0.00%	0.09	0.01

Table S4. Percent methylation of genes relevant to stress response for SSC-exposed and non-exposed muscles of young rats. Fold change calculated as exposed value divided by non-exposed value. Sample sizes of $N = 4$ to 6 per value.

	6 hours						120 hours					
	Non-exposed		Exposed		Fold change		Non-exposed		Exposed		Fold change	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Bax</i>	0.51%	0.22%	0.51%	0.15%	0.99	0.29	1.10%	0.32%	1.58%	0.54%	1.43	0.49
<i>Bcl2l1</i>	1.93%	0.44%	2.00%	0.85%	1.04	0.44	2.14%	1.06%	1.36%	0.96%	0.64	0.45
<i>Bcl2l11</i>	0.21%	0.14%	0.18%	0.09%	0.89	0.41	0.26%	0.07%	0.16%	0.03%	0.61	0.12
<i>Bclaf1</i>	1.74%	0.22%	2.42%	1.24%	1.39	0.71	0.73%	0.44%	1.03%	0.52%	1.42	0.71
<i>Bid</i>	2.43%	0.33%	3.08%	0.80%	1.27	0.33	2.74%	0.39%	3.91%	0.83%	1.43	0.30
<i>Bik</i>	0.14%	0.13%	0.92%	0.89%	6.38	6.13	0.15%	0.11%	0.07%	0.03%	0.46	0.22
<i>Birc2</i>	0.88%	0.31%	0.63%	0.20%	0.72	0.23	1.24%	0.42%	0.98%	0.36%	0.79	0.29
<i>Bnip3l</i>	1.57%	0.40%	2.85%	0.91%	1.81	0.58	1.42%	0.61%	1.23%	0.42%	0.87	0.30
<i>Casp3</i>	0.74%	0.22%	1.33%	0.46%	1.80	0.62	1.29%	0.30%	1.27%	0.42%	0.98	0.32
<i>Casp9</i>	2.31%	0.53%	2.98%	0.64%	1.29	0.28	2.43%	0.66%	2.11%	0.45%	0.87	0.19
<i>Cideb</i>	0.83%	0.20%	2.24%	0.76%	2.71	0.91	1.32%	0.46%	1.09%	0.45%	0.83	0.34
<i>Cradd</i>	0.11%	0.07%	0.19%	0.11%	1.63	0.97	0.22%	0.05%	0.24%	0.11%	1.11	0.49
<i>Dapk1</i>	0.14%	0.11%	0.21%	0.13%	1.45	0.90	0.41%	0.18%	0.49%	0.20%	1.20	0.50
<i>Dffa</i>	10.23%	1.32%	11.03%	1.58%	1.08	0.15	13.05%	2.15%	9.95%	1.91%	0.76	0.15
<i>Fadd</i>	1.68%	0.23%	2.77%	1.31%	1.65	0.78	2.07%	1.08%	1.87%	0.48%	0.90	0.23
<i>Gadd45a</i>	2.20%	0.35%	2.81%	0.71%	1.28	0.32	2.51%	0.68%	2.31%	0.73%	0.92	0.29
<i>Hrk</i>	2.58%	1.88%	0.46%	0.13%	0.15	0.04	2.73%	0.73%	1.75%	0.28%	0.64	0.10
<i>Tnfrsf10b</i>	0.34%	0.10%	0.77%	0.44%	2.26	1.30	0.34%	0.23%	0.69%	0.29%	2.01	0.86
<i>Tnfrsf21</i>	0.02%	0.01%	0.02%	0.00%	0.71	0.18	0.06%	0.02%	0.06%	0.01%	0.98	0.22
<i>Tp53</i>	0.01%	0.00%	0.47%	0.31%	57.09	37.50	0.16%	0.15%	0.01%	0.00%	0.07	0.01

Table S5. Percent methylation of genes relevant to stress response for SSC-exposed and non-exposed muscles of old rats. Fold change calculated as exposed value divided by non-exposed value. Sample sizes of $N = 6$ per value.