SUPPLEMENTARY INFORMATION

Mechanosensors control of skeletal muscle mass, molecular clocks, and metabolism

Mathias Vanmunster, Ana Victoria Rojo Garcia, Alexander Pacolet, Sebastiaan Dalle, Katrien Koppo, Ilse Jonkers, Rik Lories and Frank Suhr

Supplementary tables

Gene name	Sense primer (5'→3'	Antisense primer (3'→5')	Amplicon size [bp]
Bmal1	AAGTAGGTCAGGGACGGAGG	GGAAGGAAGGTGCAATGAAGG	192
Cd36	GTGGGTCTGAAGGACTGGAA	CACATTTCAGAAGGCAGCAAC	236
Clock	GCGGTGAATTTTGAGGGGTG	TCAGAACTCTTGCTGGTGATTT	249
Cry2	GGGGACTCTGTCTATTGGCATC	GGCTCTTGGGTAGGCATCTC	223
Fbxl21	TGGAAGTGAAGTCAGATGGGTG	GACTGACCTATAAAATCACTGAAGG	110
Fbxo32	TCAGAGAGGCAGATTCGCAAG	TGAGGGGAAAGTGAGACGGA	218
Fermt2	ACCAAGGGTGGCTAGATTCCTC	ATACTGCAAGGCTGCGAACA	221
G6pc3	ACCTCGTCTTCAAGTGGATTCTGT	GGAGCTGTTGCTGTAGTAGTCGGT	80
Hk2	TCAAAGAGAACAAGGGCGAGG	CGCTTGGCAAAATGGGGATGT	88
Ilk	ACGACCTCAATCAGGGGGATG	AGCATTTCAACCACCGCAGA	85
Mfn2	CCAGCTAGAAACTTCTCCTCTGTTC	GGGGAAGCATTCACTTCAGCCA	181
Mtor1	TGCGGCAGGATGAACGAGTG	GCCCGAGTTGGTGGACAGAG	131
Nrf1	GCTGCAGGTCCTGTGGGAA	GTTGGGTTTGGAGGGTGAGA	207
Opa1	GGGATCTGCTGTTGGAGGTGG	CCTAACAAGAGAAGGGCCTCAGAAA	297
P70s6kb1	CACGAACACCTGTCAGCCCA	CCCCGCTCACTGTCACATCC	147
Per1	TTGATGTGACGGCGTGTGT	CAGCCCCAATCCATCCAGTT	95
Per2	CCCGAGCTAGAGACGGTAGAA	AGCCAGAACTTCCTTGGTGAG	123
Pfkm	GTTTGGAAGCCTCTCCTCCTC	GCATTCATACCTTGGGCATCT	233
Pxn	GCCTGGAACAGAGAGTGGATGG	GGGTGAGCTGCTCCCAGTTTT	131
RevErba	CCTCAGCGTCCTACCCTTCT	ACACCACCTGTGTTGTTATTGG	234
RevErbb	GTGGACAGTACAAAGGGAGGA	TTGCTCATAGGACACACCAGA	208
Rpl41	ATGAGAGCGAAGTGGCGGAAG	CAGGGCAGAGGGACTGTTTTG	260
Slc2a4	AGGCACCCTCACTACGCTCT	GGCCAGCATAGCCCTTTTCCTTC	121
Tbc1d1	CAGCGTGGGGCGGCAAGAG	CTGAGGGCAGAATCCAGTGAGGTG	145
Tcap	GAATACCAGCTGCCGTACCA	TTGTGATCTCAGCCACGTCC	175
Tln2	CAAGGTTCTGGGCGAGTCAA	ACACATTCTCCGAAGGCAGG	78
Trim63	GGTGCCTACTTGCTCCTTGT	ACCTGGTGGCTATTCTCCTTG	201
Vcl	CGATGAGGCTGAGGTTCGTAAAA	AGTCAACTCCTGCTGTCTCTC	172
Yap	TCGGCAGGCAATACGGAATATCAA	GCATTCGGAGTCCCTCCATC	114

Table S1 Primer sequences and amplicon sizes.

Supplementary figures

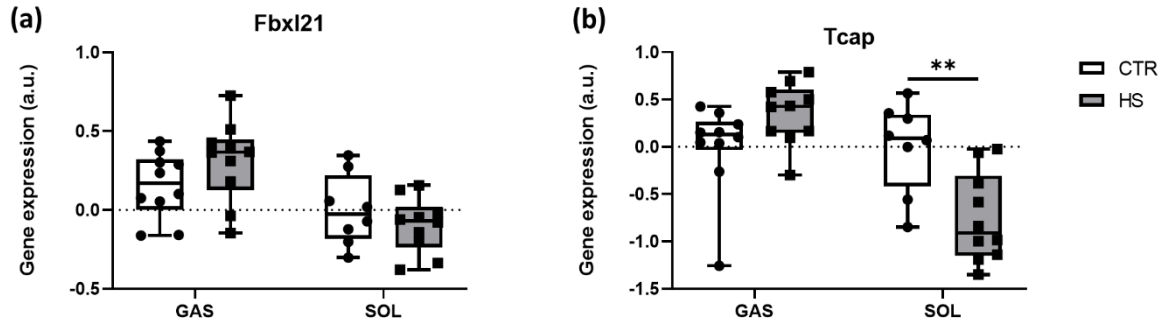

Fig. S1 Skeletal muscle unloading modifies gene expressions of circadian-related ligases, sarcomeric Z-disk control and gluconeogenesis. Relative gene expression profiles of **(a)** Fbxl21 and **(b)** Tcap between CTR and HS mice in GAS and SOL muscles. Expression is relative to the housekeeping gene Rpl41. Statistical analysis used was two-way ANOVA with Sidak's multiple comparison post hoc test on the following number of animals: GAS 10 CTR and 10 HS animals, SOL 8 CTR and 10 HS animals. **p value<0.01.

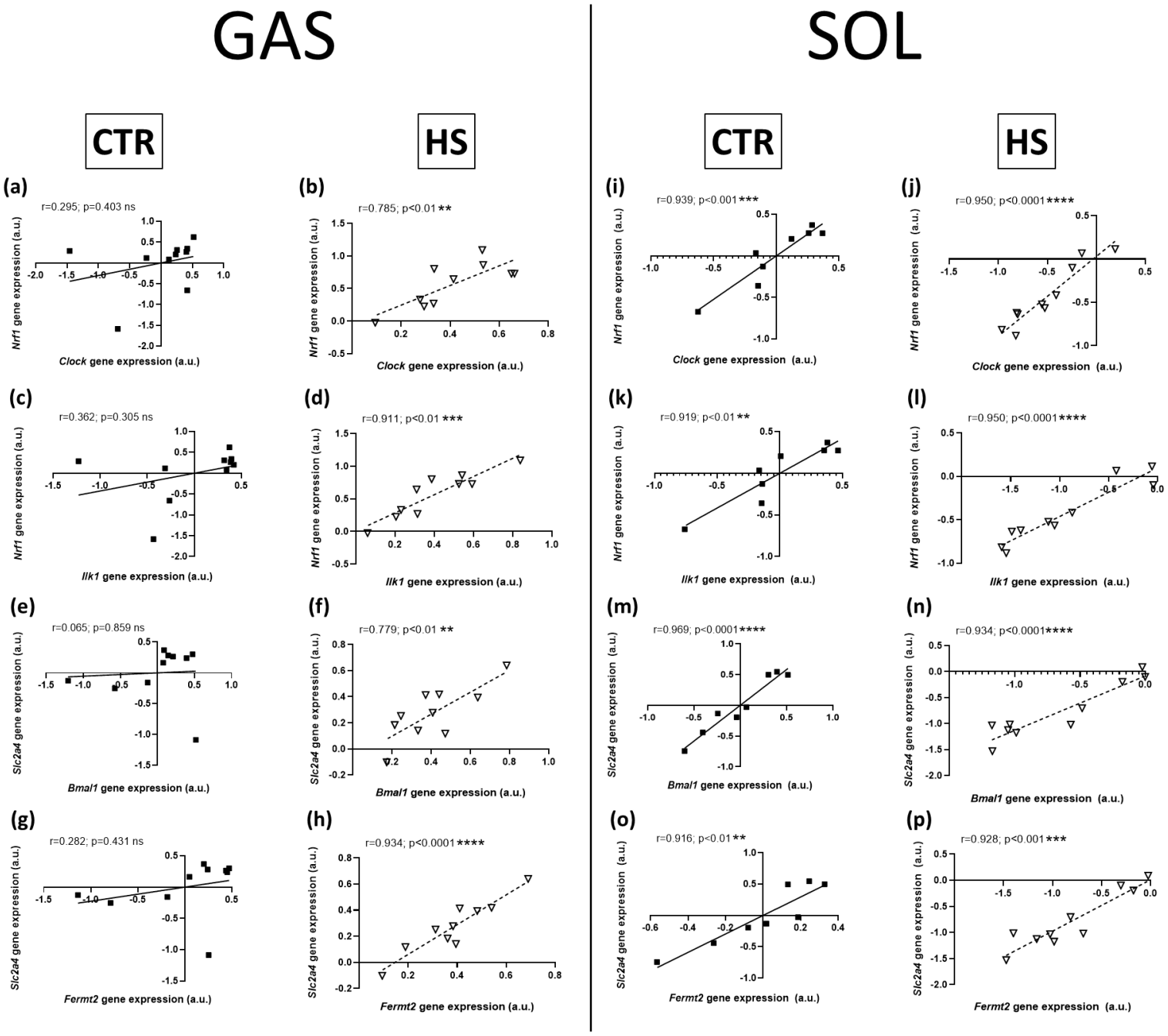

Fig. S2 Skeletal muscle unloading results in strong correlations between metabolism-related and circadian clock and mechanosensor genes. Correlations in GAS between *Nrf1* and *Clock* under CTR **(a)** and HS **(b)** conditions; between *Nrf1* and *Ilk* under CTR **(c)** and HS **(d)** conditions; between *Slc2a4* and *Bmal1* under CTR **(e)** and HS **(f)** conditions; between *Slc2a4* and *Fermt2* under CTR **(g)** and HS **(h)** conditions. Correlations in SOL between *Nrf1* and *Clock* under CTR **(i)** and HS **(j)** conditions; between *Nrf1* and *Ilk* under CTR **(k)** and HS **(l)** conditions; between *Slc2a4* and *Bmal1* under CTR **(m)** and HS **(n)** conditions; between *Slc2a4* and *Fermt2* under CTR **(o)** and HS **(p)** conditions. Correlations between mechanosensor, circadian clock and metabolism-related genes were assessed by using Pearson correlation coefficient analyses on the following number of animals: GAS 10 CTR and 10 HS animals, SOL 10 CTR and 10 HS animals. Correlation coefficients r and significance levels are provided. ** p value<0.01, *** p value<0.001, **** p value<0.0001.

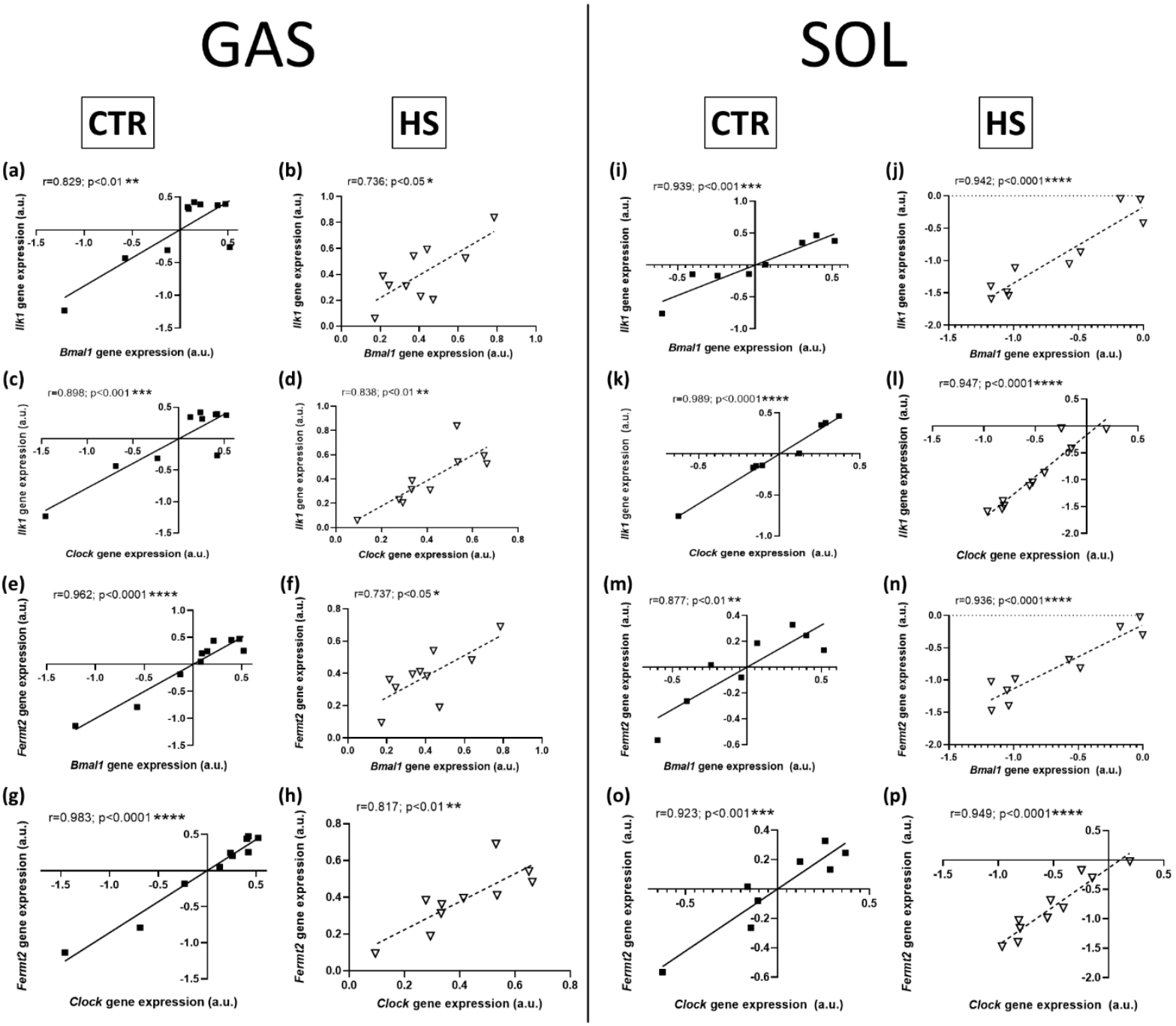

Fig. S3 Skeletal muscle unloading results in strong correlations between mechanosensor and circadian clock genes. Correlations in GAS between *Ilk* and *Bmal1* under CTR **(a)** and HS **(b)** conditions; between *Ilk* and *Clock* under CTR **(c)** and HS **(d)** conditions; between *Fermt2* and *Bmal1* under CTR **(e)** and HS **(f)** conditions; between *Fermt2* and *Clock* under CTR **(g)** and HS **(h)** conditions. Correlations in SOL between *Ilk* and *Bmal1* under CTR **(i)** and HS **(j)** conditions; between *Ilk* and *Clock* under CTR **(k)** and HS **(l)** conditions; between *Fermt2* and *Bmal1* under CTR **(m)** and HS **(n)** conditions; between *Fermt2* and *Clock* under CTR **(o)** and HS **(p)** conditions. Correlations between mechanosensor and circadian clock genes were assessed by using Pearson correlation coefficient analyses on the following number of animals: GAS 10 CTR and 10 HS animals, SOL 10 CTR and 10 HS animals. Correlation coefficients r and significance levels are provided. * p value<0.05, ** p value<0.01, *** p value<0.001, **** p value<0.0001.

In addition to data presented in figure 6, we performed supplementary analyses in *Ilk1* and *Fermt2* siRNA-treated C2C12 myoblasts on a selection of genes, involving mechanosensors, myosin heavy chains, transcription factors, reference genes and metabolic markers. Our findings are presented below, but are not further discussed in the main body of this paper.

In *Ilk1* siRNA-treated C2C12 cells, gene expression levels of the mechanosensor *Yap* tended to be lower, while we observed no change in *Pxn* relative to control siRNA (both fig. S4a). We next analyzed myosin heavy chain gene expression and confirmed previously reported findings with significant higher levels of the slow-myosin-coding *Myh7* gene and significant lower levels of fast-myosin-coding *Myh1* gene in *Ilk1* siRNA-treated C2C12 myoblast (both fig. S4b). In addition, expression levels of transcription factors *Six1* and *Six4* were significantly lower upon treatment with *Ilk1* siRNA, while gene expression levels *Nfact1* did not change relative to control siRNA (all fig. S4c). We next analyzed the expression levels of reference genes commonly used to normalize RT-qPCR data. We observed no changes in the expression of *Rpl27*, *18S* and *28S* between control- and *Ilk* siRNA-treated C2C12 myoblasts (all fig. S4d). We further analyzed gene expression levels of several metabolic markers upon treatment of C2C12 myoblast with *Ilk1* siRNA (all in fig. S4e). Expression levels of the glycolytic marker *Slc2a4* did not change upon treatment in *Ilk1* siRNA-treated C2C12 cells. Gene expression levels of *Tfam* and *Sdhb*, both markers of the oxidative metabolism, were significantly lower, while expression levels of *Ppargc1a* did not change in the *Ilk1* siRNA approach. Relative to control siRNA, gene expression levels of autophagy markers (i.e. *Foxo3*, *Bcl2* and *Bag3*) and markers of angiogenesis (*Vegfa* and *Flt1*) were significantly lower in *Ilk1* siRNA-treated C2C12 myoblasts.

In Fermt2-treated C2C12 cells, gene expression levels of the mechanosensor *Yap* tended to be lower, while expression *Pxn* did not change relative to control siRNA (both fig. S4f). We next analyzed myosin heavy chain gene expression and reported significant higher levels of the fast-myosin-coding *Myh4* gene and the slow-myosin-coding *Myh7* gene upon treatment with *Fermt2* siRNA (both fig. S4g). In addition, expression levels of transcription factor *Six1* tended to be lower, while *Nfatc1* expression was significantly lower in *Fermt2* siRNA-treated myoblasts (both fig. S4h). Expression levels of *Six4* (fig. S4h) did not change upon treatment with *Fermt2* siRNA. Expression levels of commonly used reference genes (*Rpl27*, *18S*, *28S* (all fig. S4i)) did not change between control- and *Ilk* siRNA-treated C2C12 myoblasts. We further analyzed gene expression levels of several metabolic markers upon treatment of C2C12 myoblast with *Fermt2* siRNA (all fig. S4j). Expression levels of the glycolytic marker *Slc2a4* did not change upon treatment in *Ilk1* siRNA-treated C2C12 cells. Gene expression levels of markers of the oxidative metabolism, *Tfam* and *Sdhb*, were significantly lower upon treatment with *Fermt2* siRNA, while expression levels of *Ppargc1a* tended to be lower. Relative to control siRNA, gene expression levels of autophagy markers (both *Foxo3* and *Bag3*, but not *Bcl2*) and markers of angiogenesis (*Vegfa* and *Flt1*) were significantly lower in *Ilk1* siRNA-treated C2C12 myoblasts.

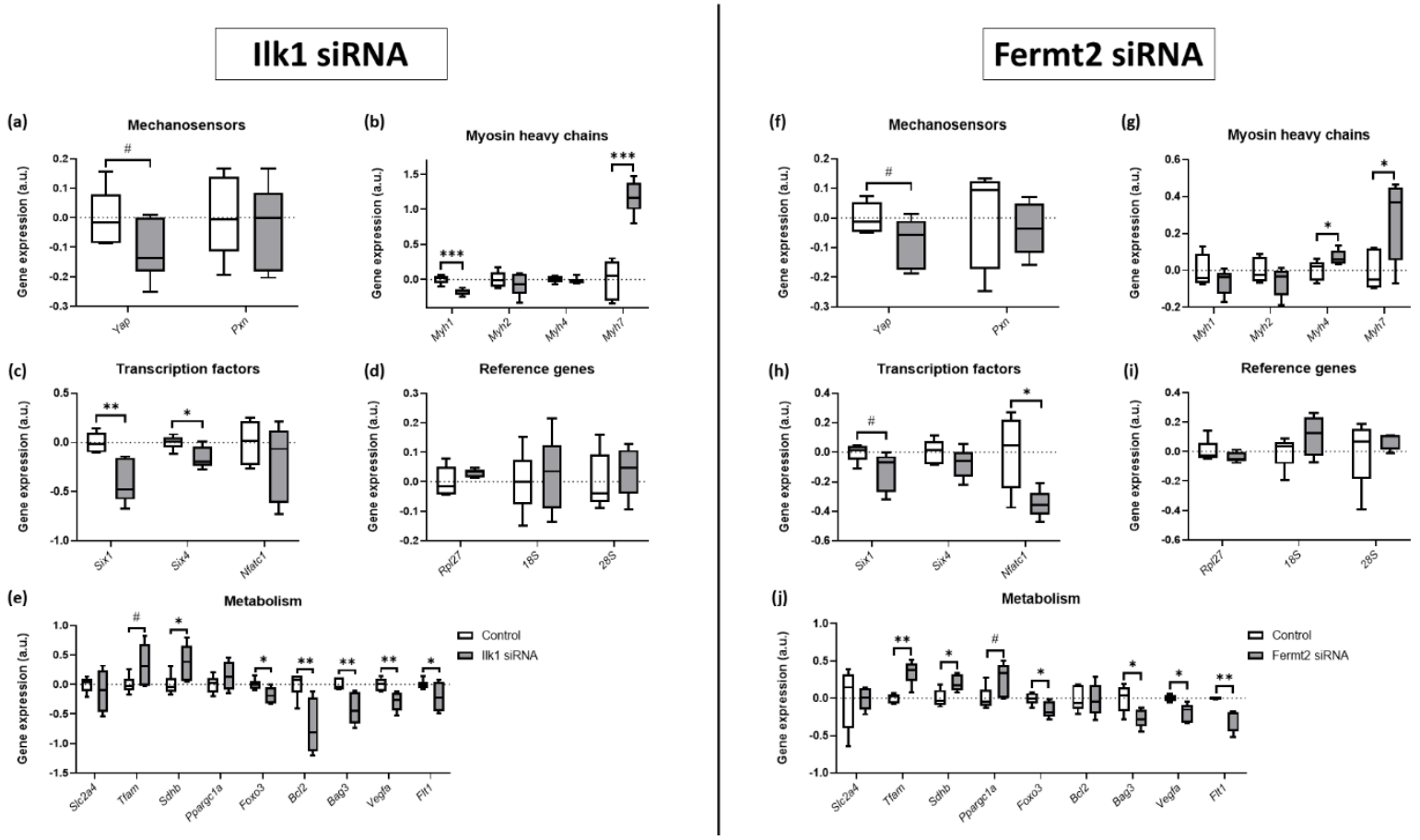

Fig. S4 Gene expression levels of mechanosensors, myosin heavy chains, transcription factors and metabolic markers upon Knockdown of either Ilk1 or Fermt2 in C2C12 myotubes. Relative gene expression profiles of **(a)** mechanosensors (*Yap and Pxn*), **(b)** myosin heavy chains (*Myh1*, *Myh2*, *Myh4* and *Myh7*), (**c**) transcription factors (*Six1*, *Six4* and *Nfatc1*), (**d**) reference genes (*Rpl27*, *18S*, *28S*), and (**e**) metabolic markers (*Slc2a4*, *Tfam*, *Sdhb*, *Ppargc1a*, *Foxo3*, *Bcl2*, *Bag3*, *Vegfa* and *Flt1*) between Ilk1 SiRNA-treated (n=6) and control SiRNA-treated (n=6) C2C12 myotubes. Relative gene expression profiles of **(f)** mechanosensors (*Yap and Pxn*), (**g**) myosin heavy chains (*Myh1*, *Myh2*, *Myh4* and *Myh7*), (**h**) transcription factors (*Six1*, *Six4* and *Nfatc1*), (**i**) reference genes (*Rpl27*, *18S*, *28S*), and (**j**) metabolic markers (*Slc2a4*, *Tfam*, *Sdhb*, *Ppargc1a*, *Foxo3*, *Bcl2*, *Bag3*, *Vegfa* and *Flt1*) between Fermt2 SiRNA-treated (n=6) and control SiRNA-treated (n=6) C2C12 myotubes. Expression is relative to the housekeeping gene Rpl41. Statistical analysis used was Student's t test. *p value<0.05; **p value<0.01; ***p value<0.001; #p value=0.05-0.1.