Supplemental Materials

Table S1. Primers.

Primers used for cDNA quantitation

Nctc1:	5'- AGATGAGCATGAAAGCCAAG
	5'- TCCATCTCCCTTGCTGTATC
Nctc1b:	5'- CTGAAGGGAAGGATCCTCTAC
	5'- CAGCTTCACTCTCAGCTCAAG
asNctc1:	5'- GTGATCTGGTGAACAAGGTCA
	5'- CCAAGTCGCTCTCTTGTAGAC
lacZ:	5'- CATCGAGCTGGGTAATAAGC
	5'- GCCATTTGACCACTACCATC
H19:	5'- TGAGTTTCTAGGGAGGGAG
	5'- ATTCCTGAGGCAGGTAGTG
Igf2:	5'- GAGCTTGTTGACACGCTTC
	5' ACGTTTGGCCTCTCTGAAC
Gapdh:	5'- CCTTCATTGACCTCAACTACAT
	5'- CAAAGTTGTCATGGATGACC
hnNctc1:	5'-CCCTATCTGCAATGTTTTCCT
	5'-GATGGTGCGTATTGTTGCTA

Primers used in strand-specific RT-PCR

1A:	5'-CAGAGTGGATGCAGAGGTG
2A:	5'-GTCTGTCATCTCACCTCTTAG
3A:	5'-GCAGCTAATGACAACCAGAG
4A:	5'-GAGCCTCGATCCTTGCTG
1B:	5'-GTTCCTCCCACATCAGGAG
2B:	5'-CTTGCCAGGAGATATGGAAC
3B:	5'-TCTGGACTAATTATTTGCTTAGG
4B:	5'-ATCAGATTCCTGACTTCGATC
1D:	5'-TTGCCCTTCTCTCTATCTAGG
2D:	5'-GGTCATGAGTGAGACACCAGTC
3D:	5'-CTATTTCTTGGGTCCCCCATGC
4D:	5'-GAATGGTGCGTATTGTTGCTA
1E:	5'-TTCCAAGCCCTTTGATCTGG
2E:	5'-GAGCCTCGCATCCTGCCTG
3E:	5'-GCTTCACAGACCTCAGGACC
4E:	5'-GATGCCCAGGTGACTCTG

Primers used in 5'-RACE

Primer for antisense priming:	5'-ATAGAGCCCAGAGAGTGCTAA
Nested primer:	5'-GTAGATGGTGAAGCAGAGTCCTT

Primers used in ChIP analysis

H19 exon1:	5'- CCAAAAGTAACCGGGATGAA
	5'- CTGGAGTCTGGCAGGAATGT
Nctc1 exon1:	5'- ACATTCAGGCAGTGACCAAT
	5'- GCTCCGACCTGAATATCTTG
Core muscle	5'- AGGAGCAGCTGTTCTCCTCATCTT
enhancer:	5'- ACAAGTGGCCATGTCCTCCTCAAA

siRNAs

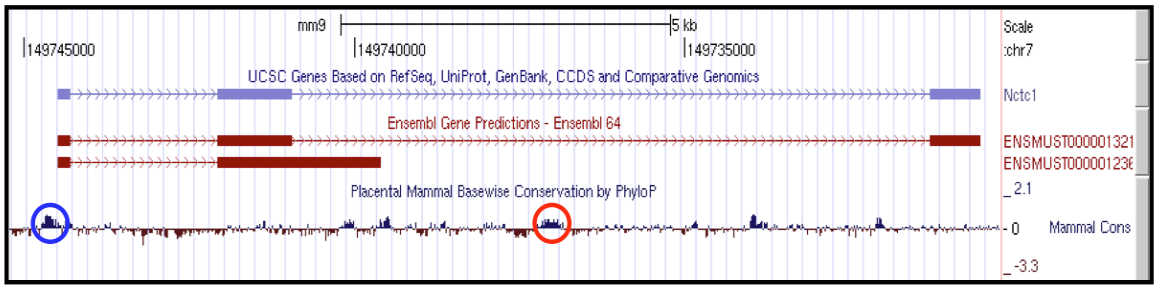
I:	GGAUGGAGAUGAGCAUGAAAGCCAA
II:	CAUCAUAGCUUGGCUGACCAGCUCA
III:	CCAGGACUAUCAAGGUCUUACUUGA

Primers used to analyze the terminator insertion

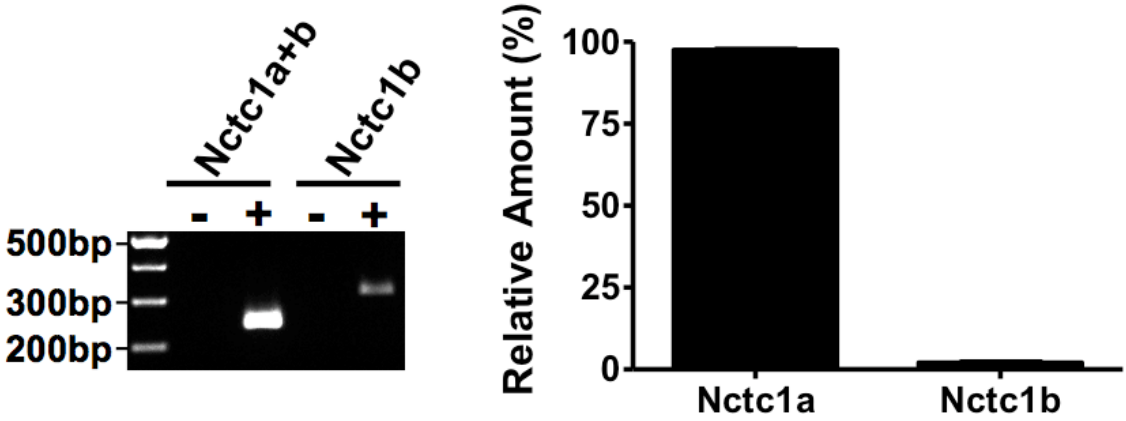
1:	5'- AGATGAGCATGAAAGCCAAG
2:	5'- GCTCCGACCTGAATATCTTG
3:	5'- ACATGAGCAGTTGAAGAAGGA
4:	5'- AGAAGGGCTCCCTAGACATGG
T1:	5'- GATCCTGAGACTTCCACACTG
T2:	5'- ATTGATGAACCTGGAGGATGT

Figure S1. Structure and transcription of the Nctc1 locus. **(A)** The *Nctc1* gene structure and conserved sequences (http://genome.ucsc.edu). The conserved promoter (blue circle) and core muscle enhancer (red circle) regions are indicated. **(B)** RT-PCR demonstrates the presence of *Nctc1a* and *Nctc1b* isoforms. Quantitative analyses indicate that the 1a isoform represents about 97% of total *Nctc1* sense transcripts (n=3). (**C**) Relative abundance of *asNctc1* transcript in stably transfected ΔME/ΔME myocytes with (construct II) and without (construct IV) the CME. N=3; mean ± standard deviation. (**D**) Relative amounts of sense and antisense transcripts were estimated by comparing crossing threshold (Ct) values in qRT-PCR analyses of 5 cDNAs prepared from RNAs purified from p4 muscle.

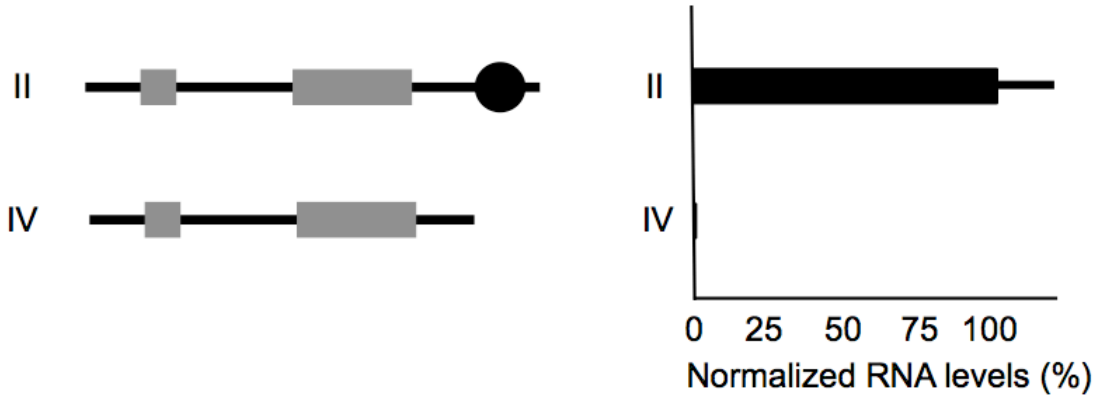
A



B



C



D ΔCt = Ct(sense transcript) – Ct(antisense transcript) = -1.1$\pm$0.6 (n = 5).

Figure S2. ***A transcriptional terminator insertion stops Nctc1 transcription through the core muscle enhancer.*** **(A)** Plasmid constructs (described in Figure 3C) with PCR primers used in transcriptional analysis. Primers 1-4 recognize *Nctc1* sequences common to both plasmids. Primers T1 and T2 recognize ends of the 2.2 kb transcriptional terminator inserted into the *NsiI* restriction site in intron 1. **(B)** To determine whether the termination signal effectively blocks transcription of *Nctc1*, Construct II (left panel) and Construct II-Stop (right panel) were separately transfected in ΔME/ΔME primary myoblasts. Since, the *Nctc1* locus is deleted from ΔME chromosomes, there is no endogenous *Nctc1* RNA to confound the PCR analysis. cDNAs were prepared without (-) or with (+) reverse transcriptase and analyzed using the primer pairs indicated. As expected, Construct II generates cDNAs recognized by primer pairs 1+2 (Nctc1 hnRNA) and 1+4 (Nctc1 mRNA). In contrast, Construct II-Stop generates cDNAs recognized by 1+2 primer pair but not by the 1+4 primer set. In addition, analysis with primers specific to the Termination insertion (T1 +3 and T2 + 4) indicates that transcription stops at the insertion. **(C)** To determine whether the termination signal effectively blocks transcription of *Nctc1* in a wild type context, construct II, construct II-Stop, or a control plasmid with no *Nctc1* insert (Ctrl) were stably transfected into primary myoblasts derived from C/C mice(Gould and Pfeifer 1998). C/C is an FVB congenic strain where the distal 7 chromosomal region is wild type but *Mus castaneus* (Cast) in origin. Plasmid constructs carry *Nctc1* sequences that are of *Mus domesticus* (Dom) in origin. Restriction length polymorphisms (RFLPs) (*) allow us to distinguish Cast and Dom *Nctc1* cDNAs. In each panel, Lane 1 (C/D) is a control that represents cDNA obtained from an untransfected wild type C/D cell line and thus demonstrates the expected band sizes for C

(Cast) and for D (Dom) cDNAs. Lanes 2-4 (II, II-Stop, Ctrl) represent cDNAs generated from C/C cells transfected with construct II, II-Stop, and control plasmids, respectively. Looking at the Dom specific PCR products, we see that Construct II transfectants generate *Nctc1* cDNAs that can be amplified by primer pairs 1+2 (left panel) and 1+4 (right panel). In contrast, Construct II-Stop generates Nctc1 cDNAs that can be amplified by primer pairs 1+2 but not by primers1+4. In each panel, M = DNA size ladder.

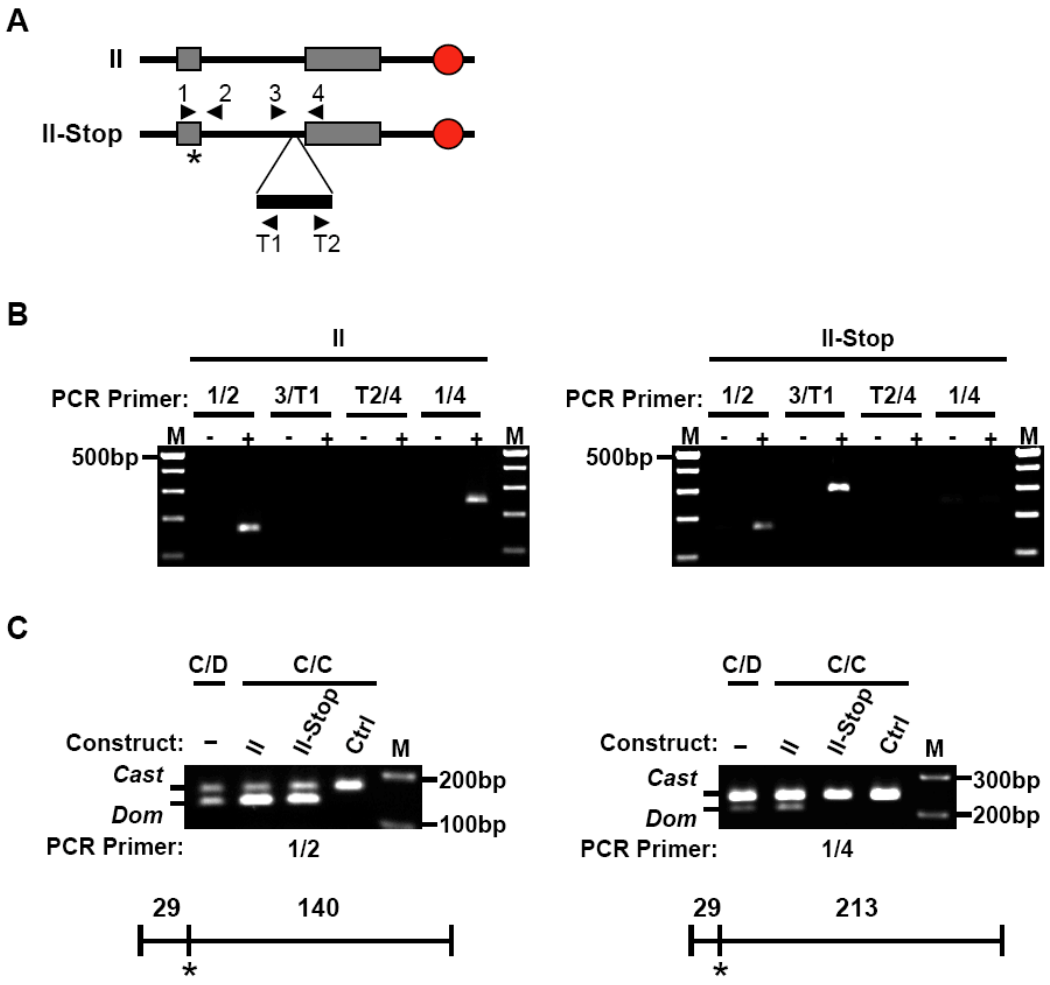


Figure S3. ***Enhancer marks at the CME are not dependent on Nctc1 transcription.*** Primary myoblasts were isolated from ΔME/ΔME pups and stably transfected with Construct II or with Construct IV (see Figure 3 for construct maps). Pooled transfectants were differentiated in vitro for 24 hours and analyzed by chromatin immunoprecipitation for Histone H3K4me1 and for Serine-5-RNA Polymerase II accumulation at the core muscle enhancer. Construct II expresses high levels of Nctc1 RNA but Construct IV carries a mutation at the *Nctc1* promoter and so expresses no antisense *Nctc1* and only minimal levels of sense *Nctc1*. (See Figure 3 for expression data). ChIP signals are normalized to input DNA and therefore account for the variable copy numbers associated with the transfections. N = 3, mean ± standard deviation.