Supplemental Table 1

Primers used in cDNA quantitation

H19	5'-TGAGTTTCTAGGGAGGGAG
	5'-ATTCCTGAGGCAGGTAGTG
Igf2	5'- GAGCTTGTTGACACGCTTC
	5' ACGTTTGGCCTCTCTGAAC
Nctc1mRNA	5'- CAACTCCTACCACCAAAGCA
	5'-TCCATCTCCCTTGCTGTATC
Nctc1 hnRNA	5'- CCCTATCTGCAATGTTTTCCT
	5'-GAATGGTGCGTATTGTTGCTA
GAPDH	5'- CCTTCATTGACCTCAACTACAT
	5'- CAAAGTTGTCATGGATGACC

Primers used in ChIP analysis

Nctc1 Exon1	5'- ACATTCAGGCAGTGACCAAT
	5'- GCTCCGACCTGAATATCTTG
Enhancer	5'- AGGAGCAGCTGTTCTCCTCATCTT
	5'- ACAAGTGGCCATGTCCTCCTCAAA
Negative control	5'- GGTCCGATCTAAGCCCTAGCATT
	5'- TTCCTGGCACTAGCCAGTCTCTTT

Primers used in DNA melting Analysis

Nctc1 hnRNA	5'-GGTCATGAGTGAGACACCAGTC
	5' – GAATGGTGCGTATTGTTGCTA
Enhancer	5'- CCCTATCTGCAATGTTTTCCT
	5'- GAATGGTGCGTATTGTTGCTA
Nctc1 Exon1	5'- ACATTCAGGCAGTGACCAAT
	5'- GCTCCGACCTGAATATCTTG

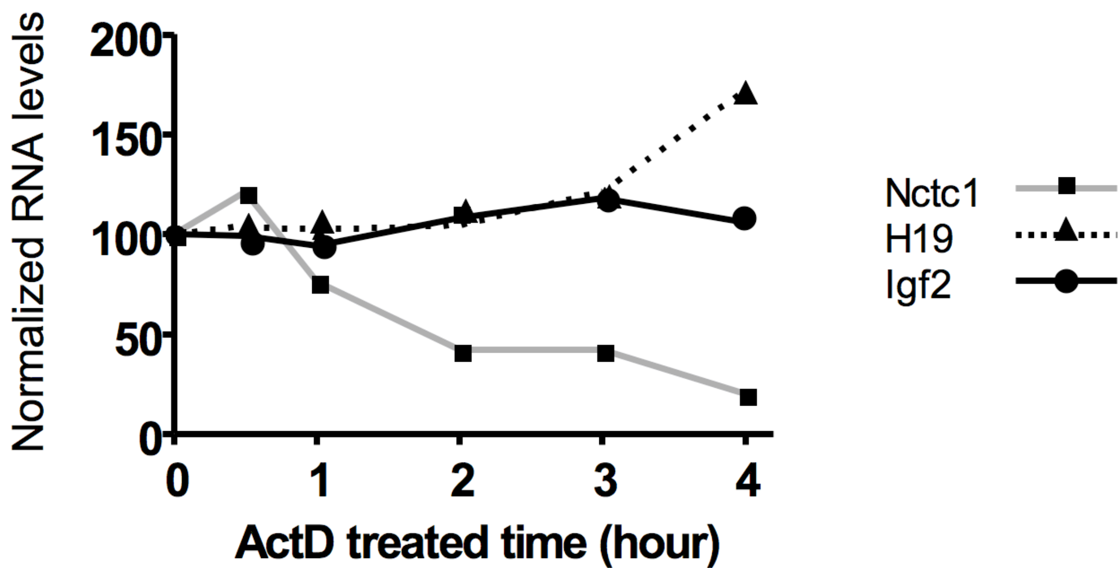
Primers used in 3C analysis (see also Fig. S1)

Primer 1	5’-CACTAAATCCTGGGTGTCTAT
Primer 2	5’-GCCATTCTCCTGGGATTAGG
Primer 3	5’-CAGGTGGAAAGAGCTCTTAGAGA
Primer 4	5’-ACAGAAGGGCAGTCATCCAG
Primer 5	5’-CTGTACCCGGAACAAGTTAGC
Primer 6	5’-GGGAATGCTGTCCTCTGAATTAATAG
Primer 7	5’-ATCTTGGGATCATGCAGAGAG
Primer 8	5’-CAGGCATGGTAGAACATGCTC

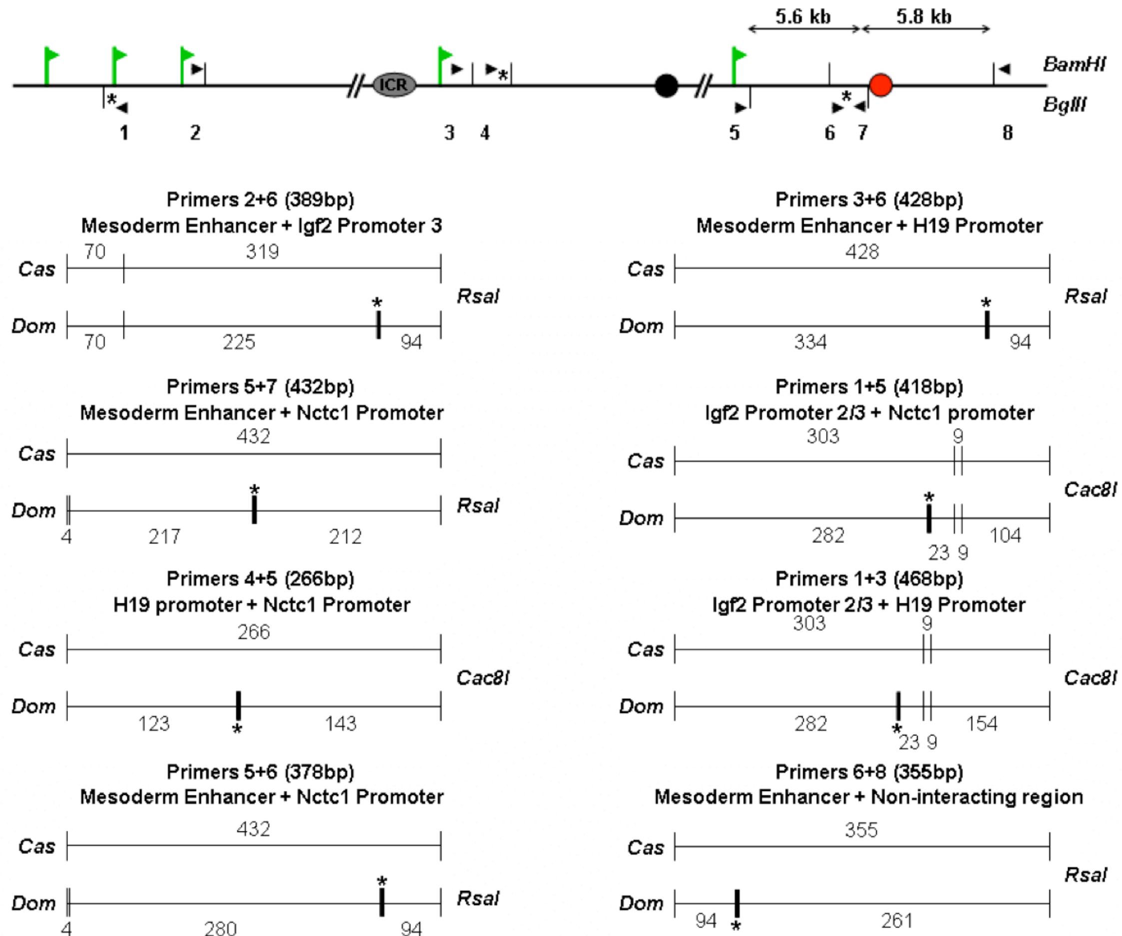
Primers used for Bisulfite Sequencing

Nctc1-Pr-Meth-For1	5’-TTG ATT TGT TTT GAG TTG GTT-3’
Nctc1-Pr-Meth-Rev1	5’-AAT TAT ATC CCT TCT TCT TAA TCC-3’
Nctc1-Pr-Meth-For2	5’-TTG TTT TGG TGG TAG GAG TTG A-3’
Nctc1-Pr-Meth-Rev2	5’-TTT CCC CAA CCT AAA CAA ACT-3’
Nctc1-En-Meth-For1	5’-AGG AGG ATA TGG TTA TTT GTG TT-3’
Nctc1-En-Meth-Rev1	5’-TAA AAA ACA TTA CCC TTC TCT CT-3’
Nctc1-En-Bis-For2	5’-GGA TGG TAG GAA TGT TTT TTA TT-3’
Nctc1-En-Meth-Rev2	5’-AAT ATC TCT TCT CAA TCC CTT T-3’

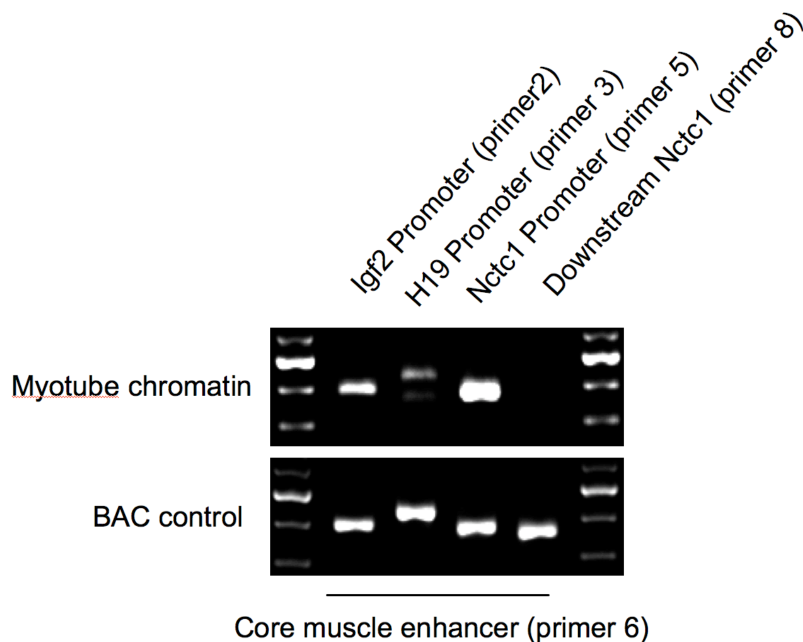
Supplemental Figure 1. RNA stability for *H19*, *Igf2* and *Nctc1*. After 24 hours in differentiation media (see Methods), primary myoblasts (C/C) were treated with 5mg/ml of actinomycin D. *Igf2*, *H19*, and *Nctc1* RNAs are quantiated relative to *GAPDH* and then normalized to the results at time 0 (no actinomycin D treatment). Normalized to GAPDH, steady state mRNA levels for H19, Igf2, and Nctc1 are 2.2$\pm$0.1, 0.6$\pm$0.1, and 0.0006$\pm$0.0001, respectively (n=4, p<0,001 for each pairwise comparison).



Supplemental Figure 2. 3C analysis of the *Igf2/H19/Nctc1* locus**.** Top line depicts the 150 kb region with key regulatory elements: promoters, green pennants; H19ICR, oval; endodermal enhancer, black circle; and core muscle enhancer, red circle. Critical BamHI and BglII sites are indicated above and below the line, respectively. PCR primers are shown with black arrowheads. Primer sequences are given in Table S1. Bottom panels indicate the PCR amplicons analyzed in the 3C study. RFLPs distinguishing castaneus (C) and domesticus (D) chromosomes are indicated (*).

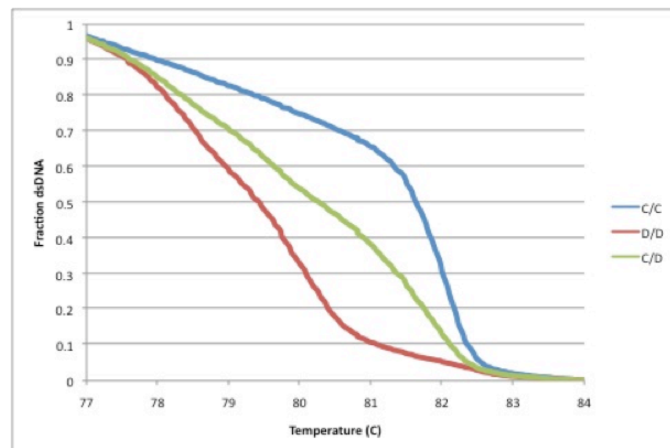


Supplemental Figure 3. Specificity of enhancer interactions detected by 3C. Top Panel*,* 3C was used to detect interactions between the core muscle enhancer (primer 6) and the *Igf2* Promoter 3 (primer 2), the *H19* Promoter (primer 3), the *Nctc1* Promoter (primer 5), or a region 5.8 kb downstream (primer 8). Primers 5 and 8 are equidistant from primer 6 but but DNA interactions are detected only using primer 5. (See Fig. S1 for primer locations and Table S1 for primer sequences.) Bottom Panel**,** To control for primer efficiency, these same primer pair combinations (Core enhancer + *Igf2* Promoter, + *H19* promoter, + *Nctc1* promoter or + *Nctc1* downstream DNA) were tested on Bacterial Artificial Chromosome (BAC) DNAs. Non-overlapping BACs 198J15 and 11301 together cover the entire locus from upstream of *Ins2* to 100 kb downstream of *Nctc1*(Gould and Pfeifer 1998). These BAC DNAs were purified and quantitated by real time PCR and equimolar quantities were digested, ligated, and analyzed by PCR as described (Palstra et al. 2003).

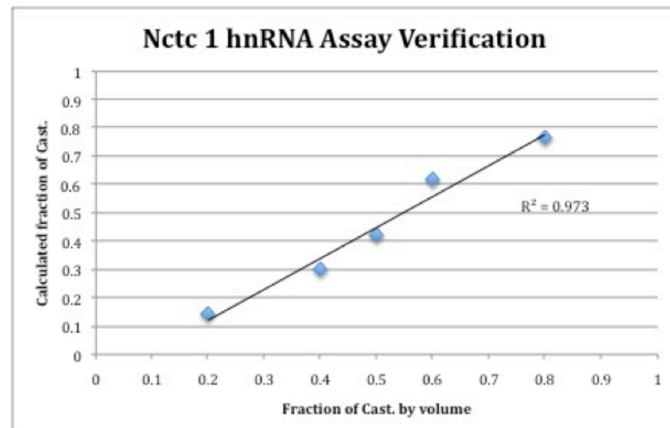


Supplemental Figure 4. Demonstration of allele-specific assays for *Nctc1* RNA. The relative fraction of *Nctc1* RNA coming from the maternal or paternal chromosome was determined using melting analyses for *Nctc1* RNA. (A) *Melting curves used to quantify allele-specific expression*. Melting curves were generated for Castaneus (blue curves) and Domesticus (red curves) alleles and for 50:50 Castaneus/Domesticus mixtures as described in Methods. (B) *Verification of allele-specific assays*. Different volume mixtures of pure Castaneus (C/C) and Domesticus (D/D) samples were made and analyzed by the allele-specific assays. The calculated fractions were compared to the known compositions to verify that the two values were well correlated.

A



B



Supplemental Figure 5. CpG methylation at the *Nctc1* locus**.** Genomic imprinting is often associated with parent-of-origin specific CpG methylation. We scanned the 13 kb Nctc1 locus plus 10 kb of additional downstream and upstream sequence for CpG rich elements and identified a single weak CpG island. The 260 bp island carries 12 CpGs and is entirely within the CME. Although not a CpG island, we also looked at the sequences just upstream of Nctc1 exon 1. This presumptive promoter has been identified by 5' RACE, is the most highly conserved part of the entire Nctc1 locus, and carries 5 CpGs within the first 250 bp. For both the CpG island/CME and the Nctc1 promoter, DNA methylation was analyzed by bisulfite sequencing using an SNP to distinguish parental origin. (A, B) The methylation status of 5 CpG dyads within the exon. Maternal (A) methylation = 3.1$\pm$0.6 residues and paternal (B) methylation = 2.9$\pm$1.2 residues (p = 0.5, student's t test). (C, D) The methylation status of 8 of 12 CpG dyads within the weak CpG island overlapping the core muscle enhancer were also analyzed. Maternal (C) methylation = 0$\pm$0 residues and paternal (D) methylation = 0.3$\pm$0.5 residues (p = 0.1).