SUPPLEMENTAL INFORMATION

NUCLEAR RESPIRATORY FACTOR 1 CONTROLS MYOCYTE ENHANCER FACTOR 2A TRANSCRIPTION TO PROVIDE A MECHANISM FOR COORDINATE EXPRESSION OF RESPIRATORY CHAIN SUBUNITS

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Running Title: *NRF1 Regulates MEF2A Expression*

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SUPPLEMENTAL TABLE

Species			MEF2 element		MEF2 element	
		consensus:	**YTAWWWWTAG**	consensus:	**YTAWWWWTAG**	
Homo sapiens	human	aggccccatt	**TTAAATATAG**	aaa....ccc	ctaagaatag	ccgccagtgc
Pan troglodytes	chimpanzee	aggccccatt	**TTAAATATAG**	aaa....ccc	ctaagaatag	ccgccagtgc
Equus caballus	horse	aggccccatt	**TTAAATATAG**	aaaa...ccc	**CTAAAAATAG**	ccacccatat
Canine familiaris	dog	aggcctcgtt	**TTAAATATAG**	aaa...cccc	**CTAAAAATAG**	ccacccatgt
Echinops telfairi	hedgehog	aggccctgtt	**TTAAATATAG**	aaccactcca	**CTAAAAATAG**	ccactcgtgg
Sorex araneus	shrew	aggtgtcatt	**TTAAATATAG**	aagg...ccc	**CTAAAAATAG**	tcgtccctct
Tupaia belangeri	tree shrew	tggtcccatt	**TTAAATATAG**	aaa...ccct	**CTAAAAATAG**	ccgcccgtgt
Cavia porcellus	guinea pig	aggcctcatt	**TTAAATATAG**	aaga...ctc	**TTAAAAATAG**	ccaccccggt
S. tridecemlineatus	squirrel	aggccccact	**TTAAATATAG**	aaga...ccc	**CTAAAAATAG**	ccacccaagt
Microcebus murinus	lemur	aggccccatt	acaaatatag	aaaa...ccc	**CTAAAAATAG**	ccactcatgt
Macaca mulatta	monkey	aggacccatt	gtaaatatag	aaa....cct	**CTAAAAATAG**	ccgctcgtgc
Bos taurus	cow	aggcctcatt	tcaaatatag	aaa...cccc	**CTAAAAATAG**	ccacccattc
Rattus norvegicus	rat	aagacccact	ttagtcagag	aaag...tcc	**CTAAAAATAG**	ccatccatgt
Mus musculus	mouse	aagacccact	ttagatagag	aaag...ccc	**CTAAAAATAG**	ccatccatgt
Oryctolagus cuniculus	rabbit	agggcccatt	ttaaagatag	aat...cgcc	**CTAAAAATAG**	gctggcacgt
	consensus	argncycryt	**TTAAATATAG**	aa..nn.ccc	**CTAAAAATAG**	ccryccrngy

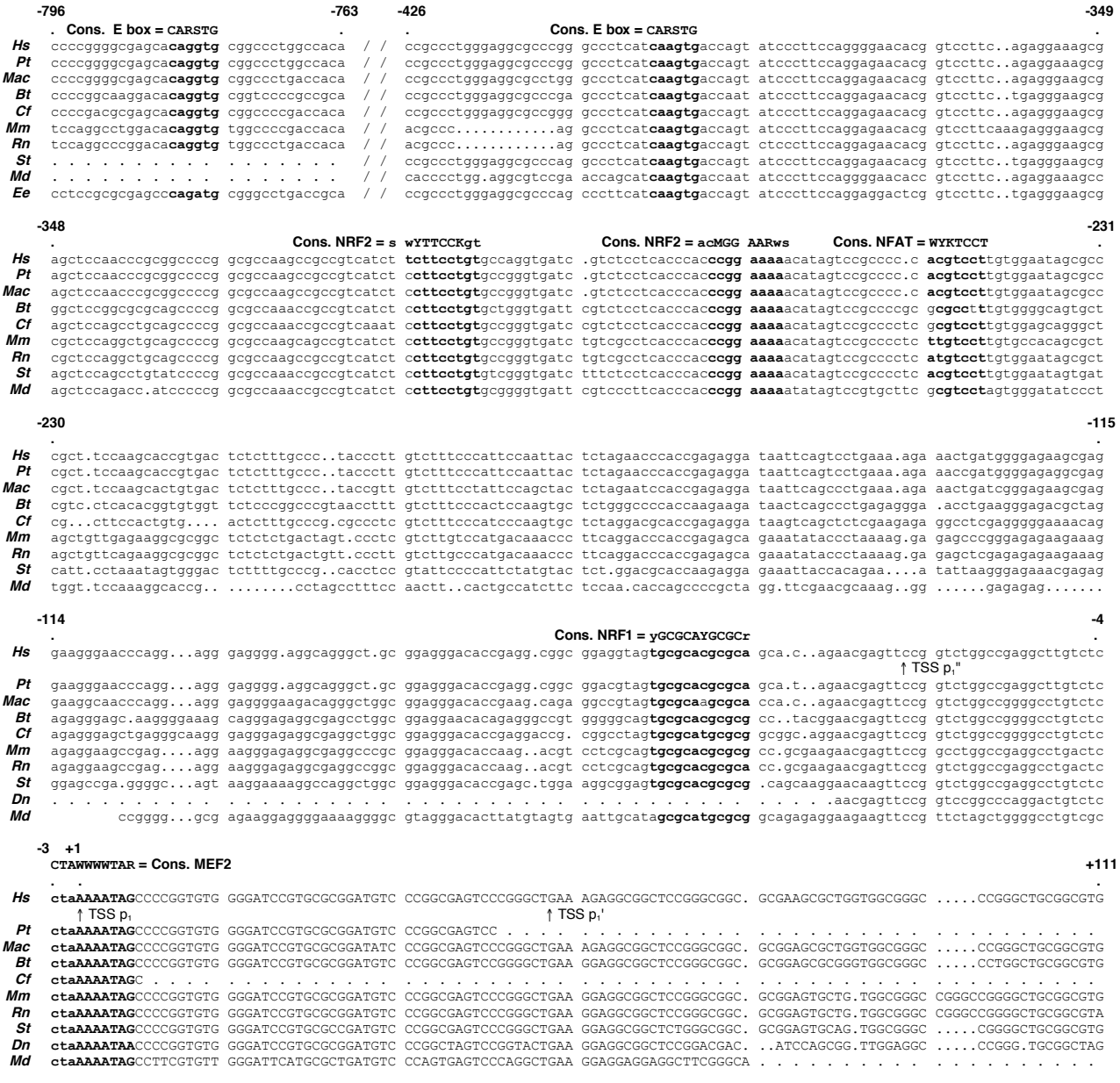
Table S1. Mammalian *COX6AH* gene promoters have one or two MEF2 elements. Alignment of proximal promoter regions of indicated mammalian *COX6AH* gene promoters. Sequences are from GenBank entries NW926306 (human), NW001225986 (chimpanzee), AAWR01032917 (horse), AAEX02025257 (dog), AAIY01181867 (hedgehog), AALT01114760 (shrew), AAPY01475073 (tree shrew), AAKN01631614 (guinea pig), AAQQ1679455 (squirrel), ABDC01254277 (lemur), AANU01233186 (rhesus monkey), AAFC03083249 (cow), NW047562 (rat), NW001030877 (mouse), AAGW01554864 (rabbit).

SUPPLEMENTAL FIGURES

Fig. S1. *MEF2A* gene promoter MEF2 and NRF elements are conserved among mammals. Alignment of mammalian *MEF2A* gene 5' transcriptional regulatory sequences showing evolutionary conservation of the NRF1 and MEF2 elements and within the ~0.8 kb expanse upstream of these sites, including E boxes and putative NRF2 and NF-AT elements. Element consensus sequences are shown above the aligned sequences, with strict consensus in bold upper case. Gene sequences that conform to consensus are in bold. Exon sequences are in upper case. Locations of the major (TSS p_1) and two minor (TSS p_1', TSS p_1") promoter 1 transcription start sites are shown (17). Numbers reference locations with respect to the human gene TSS p_1 (= +1). Sequences are from GenBank entries AC013526 (*Hs*, human), AADA01045866 (*Pt*, chimpanzee), AANU01176656 (*Mac*, rhesus monkey), AC164694 (*Bt*, cow), CE302601 (*Cf*, dog), AC120123 (*Mm*, mouse), AC134737 (*Rn*, rat), AAQQ01634896 (*St*, squirrel), AAGV01302117 (*Dn*, armadillo), and AAFR03022775 (*Md*, opossum).

Fig. S2. The *MEF2C* alternative promoter 3 has conserved NRF1 and MEF2 elements. Schematics of mammalian *MEF2C* structural gene (**A.**) and alternative splicing patterns (**B.**). Definitive alternative first exons (A_n), definitive nested 5' exons (B→D), ambiguous alternative first *vs*. nested exons (x_n), exons with coding sequences (1→9) and alternative coding sequence exons/regions (α, β, γ) (15,16) are shown. **C.** Locations of conserved regulatory elements in *MEF2C* promoter 3 (p_3). *E*, E box; *M*, MEF2; *N1*, NRF1 elements. **D.** Alignment of putative NRF1 elements from human (*Hs*), murine (*Mm*) and bovine (*Bt*) *MEF2C* p_3. Sequences are from GenBank entries AC008835 (human), AC092258 (mouse), AC172036 (cow). **E.** Mobility shift assay using $NRF1_{myc}$ and human *MEF2C* and *MEF2A* NRF1 element probes and cross competition.

Fig. S3. *Drosophila Mef2* enhancer IIE has paired functional EWG and MEF2 sites. A. Schematic of the *Drosophila Mef2* (*DMef2*) 5' regulatory region, showing location of the IIE enhancer (39) and MEF2 (*M*) (40) and putative EWG/NRF1 (*N1*) and NRF2 (*N2*) elements. **B.** Alignment of human *δALAS* (9) and *MEF2A* NRF1 elements and the putative *DMef2* IIE EWG element. **C.** Mobility shift assay using recombinant $NRF1_{myc}$ and *DMef2* and *MEF2A* NRF1 element probes and cross competition. **D.** Cells were transfected with indicated reporters, harvested for luciferase determinations after 48 hr, and analyzed and normalized as in Fig. 4. IIE-Luc contains the *DMef2* IIE enhancer upstream of the minimal E1B tata box. IIE[$m1_{EWG}$]-Luc has a mutation in the EWG/NRF1 element but is otherwise identical.


-796
-763
-426
-349
Cons. E box = CARSTG
Cons. E box = CARSTG
Hs ccccggggcgagcacaggtg cggccctggccaca / / ccgccctgggaggcgcccgg gccctcatcaagtgaccagt atcccttccaggggaacacg gtccttc..agaggaaagcg
Pt ccccggggcgagcacaggtg cggccctggccaca / / ccgccctgggaggcgcccgg gccctcatcaagtgaccagt atcccttccaggagaacacg gtccttc..agaggaaagcg
Mac ccccggggcgagcacaggtg cggccctgaccaca / / ccgccctgggaggcgcctgg gccctcatcaagtgaccagt atcccttccaggagaacacg gtccttc..agagggaagcg
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Mm tccaggcctggacacaggtg tggccccgaccaca / / acgccc.....ag gccctcatcaagtgaccagt atcccttccaggagaacacg gtccttcaaagagggaagcg
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St . . . . . / / ccgccctgggaggcgcccag gccctcatcaagtgaccagt atcccttccaggagaacacg gtccttc..tgagggaagcg
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-348
-231
Cons. NRF2 = s wYTTCCKgt
Cons. NRF2 = acMGG AARws
Cons. NFAT = WYKTCCT
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-114
-4
Cons. NRF1 = yGCGCAYGCGCr
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↑ TSS p1"
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-3 +1
CTAWWWWTAR = Cons. MEF2
+111
Hs ctaAAAATAGCCCCGGTGTG GGGATCCGTGCGCGGATGTC CCGGCGAGTCCCGGGCTGAA AGAGGCGGCTCCGGGCGGC. GCGAAGCGCTGGTGGCGGGC .....CCGGGCTGCGGCGTG
↑ TSS p1
↑ TSS p1'
Pt ctaAAAATAGCCCCGGTGTG GGGATCCGTGCGCGGATGTC CCGGCGAGTCC . . . .
Mac ctaAAAATAGCCCCGGTGTG GGGATCCGTGCGCGGATATC CCGGCGAGTCCCGGGCTGAA AGAGGCGGCTCCGGGCGGC. GCGGAGCGCTGGTGGCGGGC .....CCGGGCTGCGGCGTG
Bt ctaAAAATAGCCCCGGTGTG GGGATCCGTGCGCGGATGTC CCGGCGAGTCCGGGGCTGAA GGAGGCGGCTCCGGGCGGC. GCGGAGCGCGGGTGGCGGGC .....CCTGGCTGCGGCGTG
Cf ctaAAAATAGC . . . . .
Mm ctaAAAATAGCCCCGGTGTG GGGATCCGTGCGCGGATGTC CCGGCGAGTCCCGGGCTGAA GGAGGCGGCTCCGGGCGGC. GCGGAGTGCTG.TGGCGGGC CGGGCCGGGGCTGCGGCGTG
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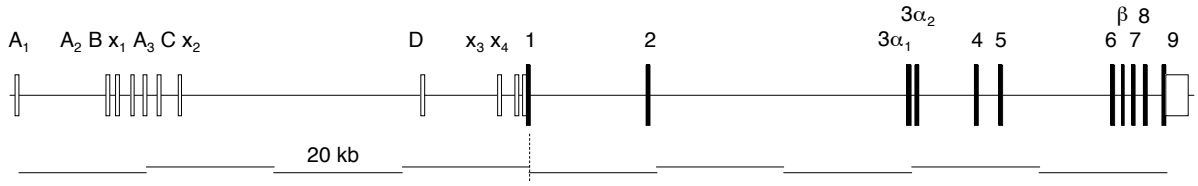
Dn ctaAAAATAACCCCGGTGTG GGGATCCGTGCGCGGATGTC CCGGCTAGTCCGGTACTGAA GGAGGCGGCTCCGGACGAC. ..ATCCAGCGG.TTGGAGGC .....CCGGG.TGCGGCTAG
Md ctaAAAATAGCCTTCGTGTT GGGATTCATGCGCTGATGTC CCAGTGAGTCCCAGGCTGAA GGAGGAGGAGGCTTCGGGCA . . . . . .

NRF1 Regulates MEF2A Expression
Figure S2.

A.



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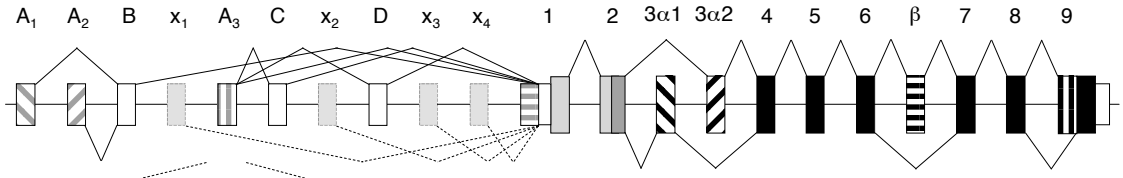

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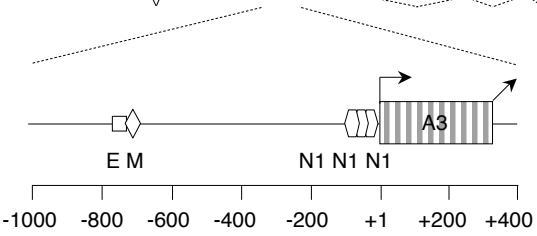


D.

Consensus NRF1	**yGCGCAYGCGCr**
Hs MEF2C p_3 NRF1$_1$	tc **TGCGC**g**CGCG**a**A** tg
Mm MEF2C p_3 NRF1$_1$	gg **TGCGC**g**CGCG**a**A** tg
Bt MEF2C p_3 NRF1$_1$	ca **CGCGC**g**CGCG**a**A** tg
Hs MEF2C p_3 NRF1$_2$	cg **CGCG**a**ATGCGCG** gc
Mm MEF2C p_3 NRF1$_2$	cg **CGCG**a**ATGCGCA** ag
Bt MEF2C p_3 NRF1$_2$	cg **CGCG**a**ATGCGCG** gc
Hs MEF2C p_3 NRF1$_3$	ct **CGCGC**g**CGC**a**CA** ca
Mm MEF2C p_3 NRF1$_3$	ct **CGCGC**g**CGC**a**CA** ca
Bt MEF2C p_3 NRF1$_3$	ct **CGCGC**g**CGCGCA** ca

Consensus MEF2	**CTAWWWWTAR**
Hs MEF2C p_3 MEF2	aaa **CTAAAATTAG** caa
Mm MEF2C p_3 MEF2	aaa **CTAAAATTAG** caa
Bt MEF2C p_3 MEF2	aaa **CTAAAATTAG** caa

E.

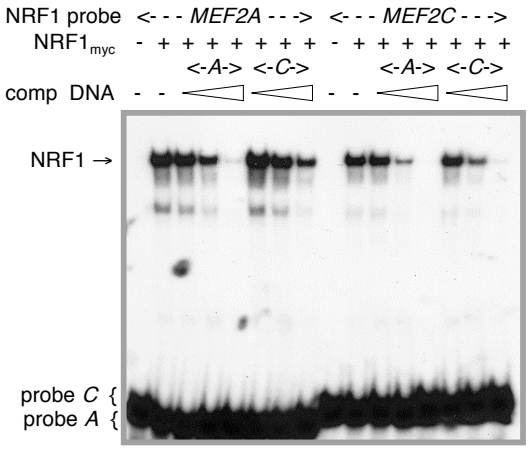
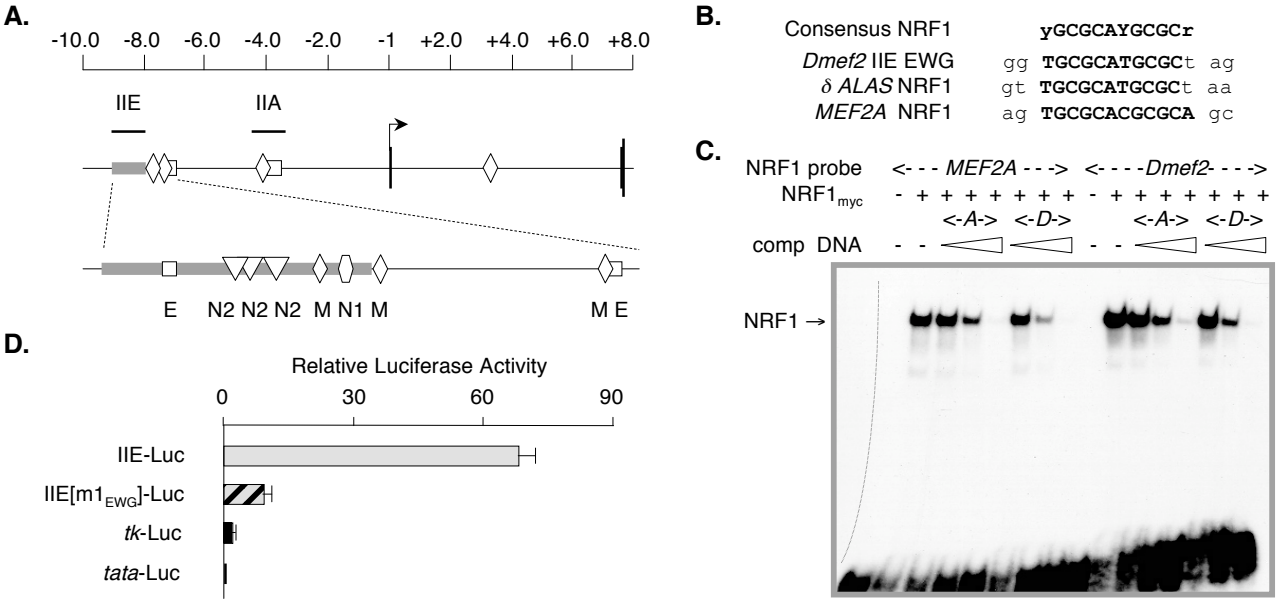


Figure S3.


A.
-10.0 -8.0 -6.0 -4.0 -2.0 -1 +2.0 +4.0 +6.0 +8.0
IIE
IIA
E N2 N2 N2 M N1 M M E
B.
Consensus NRF1 yGCGCAYGCGCr
Dmef2 IIE EWG gg TGCGCATGCGCt ag
δ ALAS NRF1 gt TGCGCATGCGCt aa
MEF2A NRF1 ag TGCGCACGCGCA gc
C.
NRF1 probe <- - - MEF2A - - -> <- - - -Dmef2- - - ->
NRF1myc - + + + + + + + - + + + + + + +
<-A-> <-D-> <-A-> <-D->
comp DNA - - - -
NRF1 →
D.
Relative Luciferase Activity
0 30 60 90
IIE-Luc
IIE[m1EWG]-Luc
tk-Luc
tata-Luc