

Fig S1

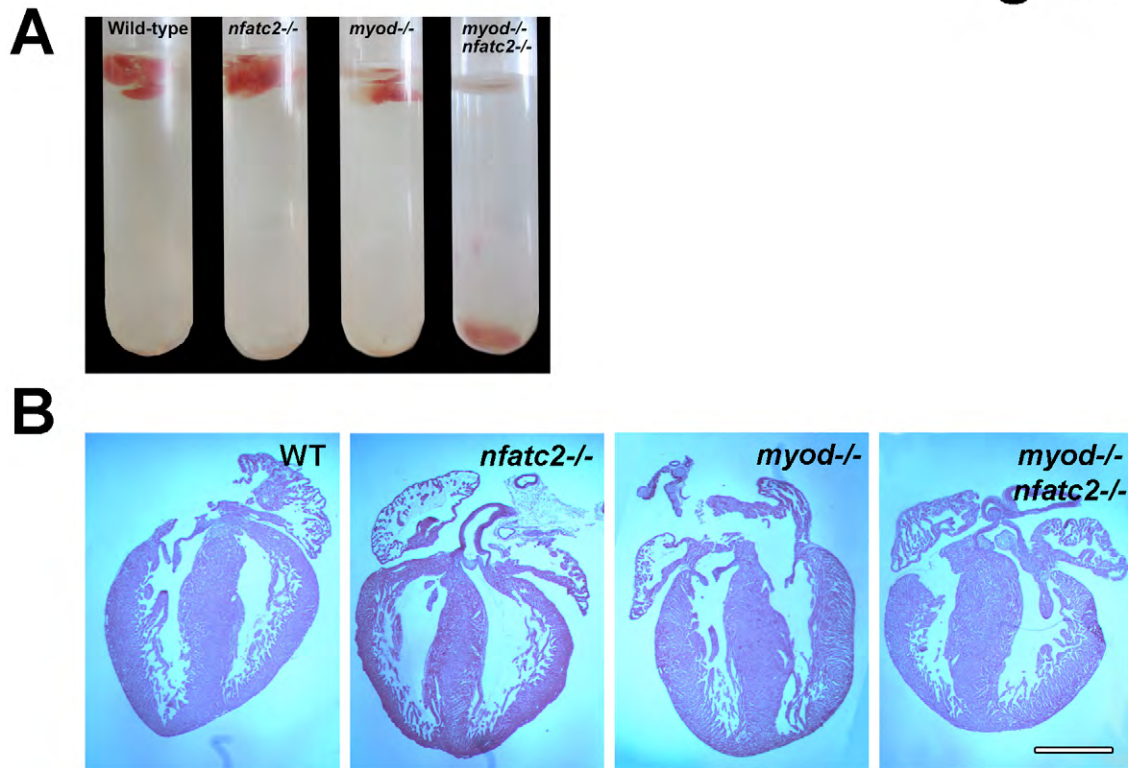


Fig. S1: *myod/nfatc2* double null mice die at birth with respiratory defects. (A) Lungs were dissected out from newborn mice and dropped in water. All lungs float except the *myod*^{-/-}: *nfatc2*^{-/-} lungs that sink to the bottom, indicating that they were not inflated with air. (B) Representative H&E stained four-chamber view of hearts dissected from newborn mice of indicated genotypes. No obvious cardiac phenotype was observed in any transgenic mice. Bar=750 μ m.

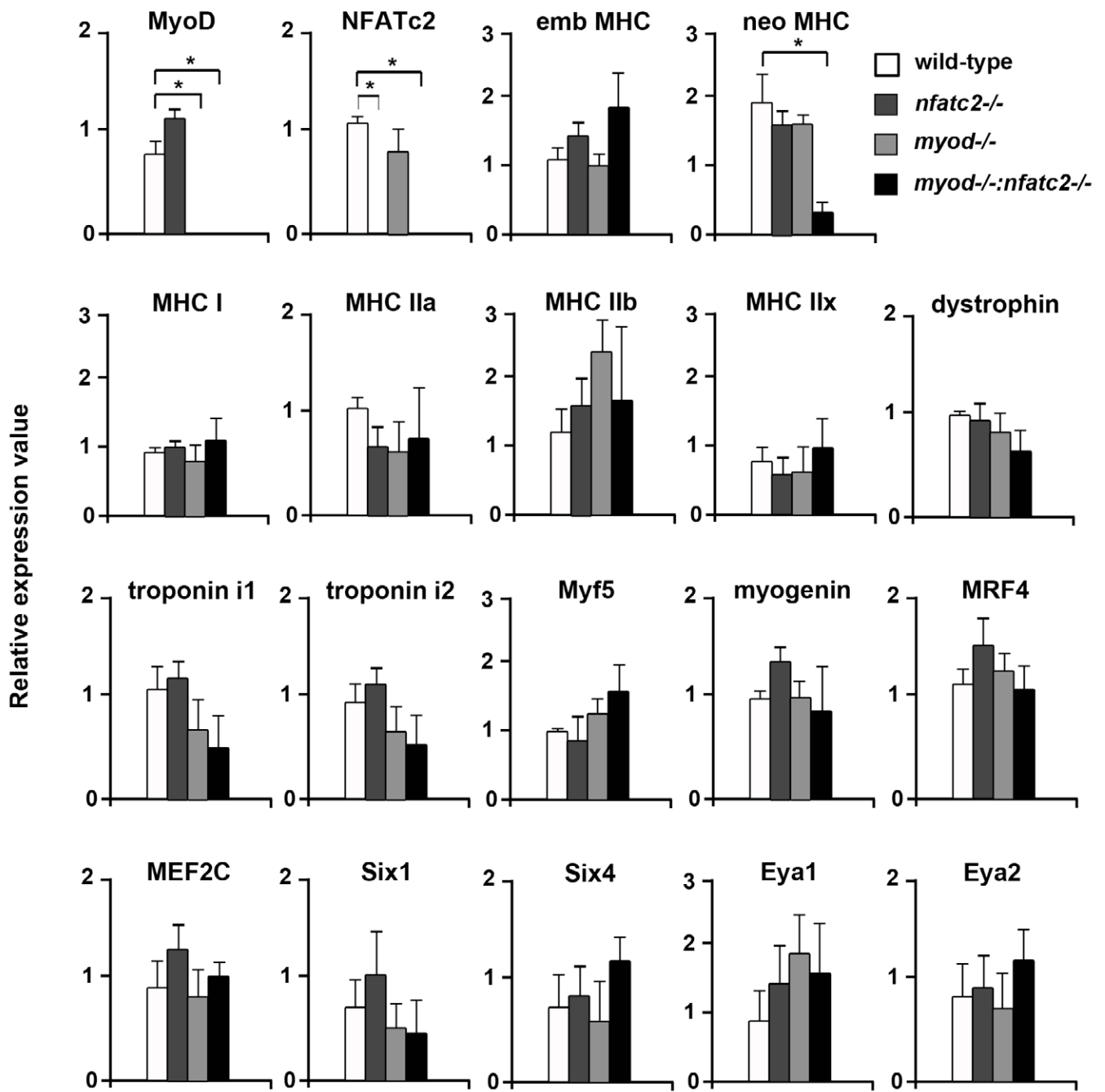


Fig. S2: The expression of the indicated muscle specific genes was analyzed by RT-qPCR from E18.5 limb muscles of WT, *nfatc2*^{-/-}, *myod*^{-/-}, *myod*^{-/-}:*nfatc2*^{-/-} mutant mice. Data are means±s.e.m. of three different experiments. Five to seven embryos of each genotype were analyzed. * indicates $P < 0.01$ vs WT.

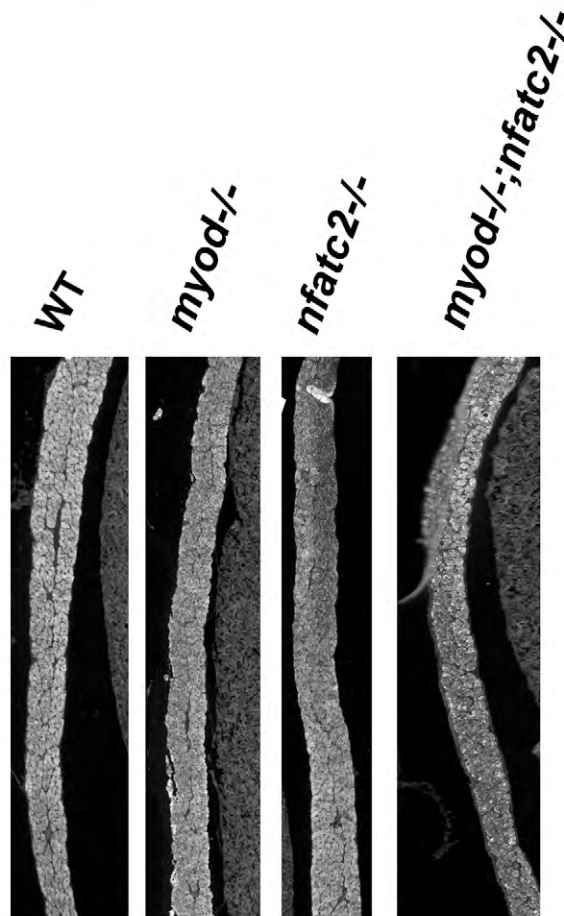
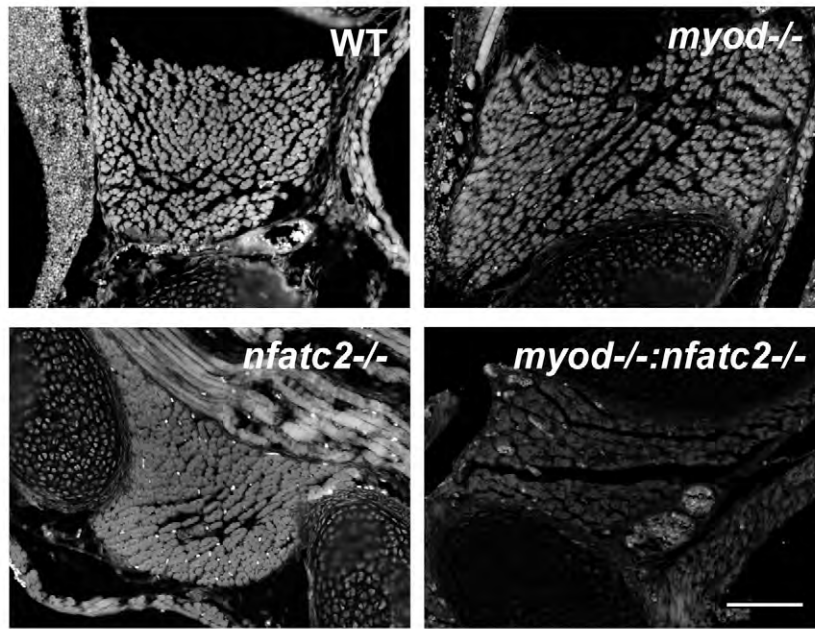


Fig. S3: MyoD and NFATc2 are required for neo-MHC expression in intercostal muscles at E18.5. Transverse cryosections of intercostal muscles (upper panels) and diaphragm (lower panel) were immunostained with neo-MHC antibodies, demonstrating that *myod/nfatc2* double null intercostal muscles but not diaphragm are deficient in neo-MHC proteins. Bar=40 μ m.

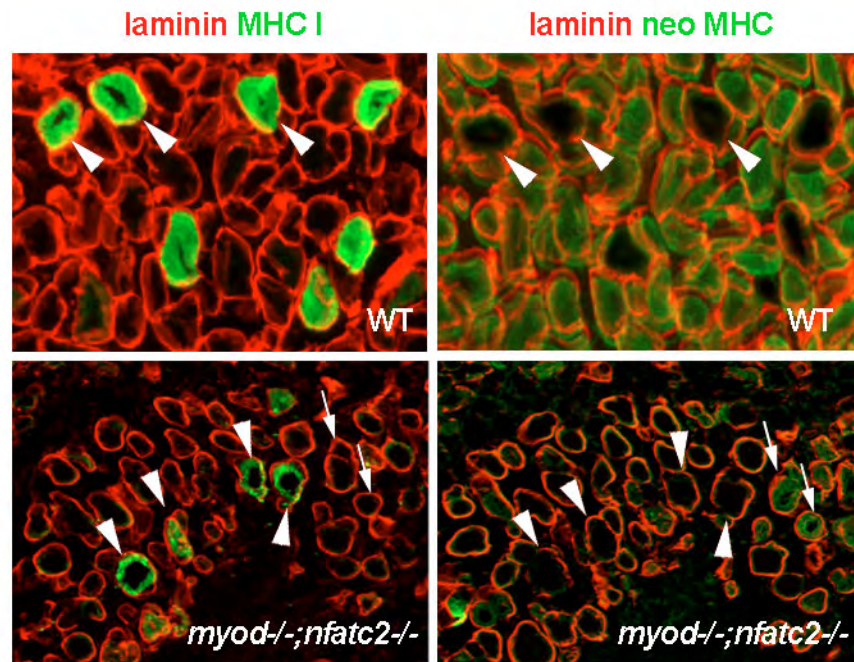


Fig. S4: Immunostaining of serial sections of E18.5 WT and *myod*^{-/-};*nfatc2*^{-/-} limb muscles using anti-MHC I or anti-neo-MHC antibodies with the specific anti-laminin antibody. Primary myofibers are indicated by arrowheads. They express MHC I but not neo-MHC at this stage (Condon et al., 1990b). Secondary WT myofibers always express neo-MHC. In *myod*/*nfatc2* double null muscles, few secondary myofibers (pointed by arrows) express faint amount of neo-MHC.

Table S1. Primers

		Sequence (5'-3') Forward primer	Sequence (5'-3') Reverse primer	
Chromatin IP	Amplicon a	AATGAGGGGATGGAAGATGA	CCAGACTTTGAGGGTGCATT	
	Amplicon b	TACGACATCCCTGCCTTCAT	CGGAAAGAAAAGAGAAAATGC	
	Amplicon c	ATCCCCATGTATCTCTCC	CTCATCCTTGAGCCTGACT	
	Amplicon d	AGTCAGGCTCCAAGGATGAG	CTGTGCTGCCAAAGATCAGA	
	Amplicon e	TCTGATCTTTGGCAGCACAG	GCGGAGATAGGGTGTGAGG	
	Amplicon f	GCTTGAGGGTAGGGGAAGCTT	CCAGGCTTCAGTGAGGGTAA	
	Amplicon g	TTTTTGAGGGACGGCTTGTA	GGAGGCTTGGCATGAAATAG	
	Amplicon h	GAACCAGGGCAGTGCTAAGT	CCATTGTTGCTCAAACCAA	
	Amplicon i	CAAAGCCATGTTTCCTTCT	CTCGCCAGAATCTTGACTCC	
	Amplicon a'	TGGACCAGGAAAGAGAGGAA	AGCAGGACCAACAAGCAAGT	
	Amplicon b'	TGATTTCAAGGGTGGGATGG	CGTATTTTTCGCCGTTGCC	
	Amplicon c'	TCAAGGCGTTCTCAAGGAAT	GTCCCTGTTCATTCTGTGG	
	Amplicon d'	CCATACGTCCACAAGTCTG	AAGCCTGCATTCTCCGTTA	
	Amplicon e'	GTTTGCTCTACCCGTGC	GCAATGAATGGAAGCCTCTC	
	Amplicon f'	GTGCTGAACCTTGTGTGAA	TCCATCTCGGTGTCGCTACT	
	Control amplicon	TCCTGGATTACTGTCAAGC	AACGAGTCTATGAGGGTAAAG	
qRT-PCR	NFATc2.2	ATCTACCCAGATCAGTATGG	TCAGGAGTATACCATTCTCTC	
	MyoD	GGCTCTCTCTGCTCCTTTGA	AGTAGGGAAGTGTGCGTGTCT	
	Emb MHC	GCAAAGACCCGTGACTTCACCTCTAG	GCATGTGGAAAAGTGATACGTGG	
	neo MHC	GTCACGCAATGCAGAAGAGA	CAGGTCCTTCACCGTCTGTT	
	MHC I	AGGGCGACCTCAACGAGAT	CAGCAGACTCTGGAGGCTCTT	
	MHC IIa	CCAAGAAAGGTGCCAAGAAG	CGGGAGTCTTGTTTCATTG	
	MHC IIb	GCTTGAAAACGAGGTGGA	CCTCCTCAGCCTGTCTCTTG	
	MHC IIx	CGGTGTTGGAAGAAAGG	CAGGAGTCTTGTTTCATT	
	myogenin	TGACCCTACAGACGCCACAATC	CACACCCAGCCTGACAGACAATC	
	MRF4	TGCTAAGGAAGGAGGAGCAA	CCTGCTGGGTGAAGAATGTT	
	Myf5	AGGAAAAGAAGCCCTGAAGC	GCAAAAAGAACAGGCAGAGG	
	Six1	CTTTAAGGAGAAGTCTCGGG	TTCCAGAGGAGAGAGTTGAT	
	Six4	TCACTCCACATCCCTCTTC	CATCTGTTTCAAAGCGAGCA	
	Eya1	ACCTCCCGACTTCTCATT	AGAAGTGTGCTGACCCAGT	
	Eya2	ACCCGTTACTCCATTACCC	CCCCCTCTCTACAACACAA	
	Troponin i1	ATGCCGGAAGTTGAGAGGAAA	TCCGAGAGGTAACGCACCTT	
	Troponin i2	AGAGTGTGATGCTCCAGATAGC	AGCCACGTGATCTTCGCA	
	Dystrophin	TCTCCTTGCATTGGTTCC	GAGTTTGAGACGCTTTTGC	
	MEF2C	TGCTGGTCTCACCTGGTAAAC	ATCCTTTGATTCACTGATGGCAT	
	26S	AGGAGAAAACAAGGTCGTGCCAAAA	GCGCAAGCAGGTCTGAATCGTG	
	Mutagenesis	N2 neo	GGCACTGGTCCAATGGTCTCGAGTCAGCATAAAGGTTCTTCTCG	
		N3 neo	CCTGTTGGAAACAAATACTCGAGGTGAGTTGCTGGGCATCTG	
E8 neo		GAATTTTGTCAAGTATGAGTACCTAACTTAGTGGCAACC		
EMSA	N2 neo	gatcCCAATGGTGGAGACTCAGCATA		
	N3 neo	gatcACAAATAAGGAAAGTGAGTTGC		
	N3 emb	gatcGGATTATATCCAGTGCTGAA		
	E8 neo	gatcGAGTTATCAGCTGTAACCTTAGT		

Table S2. Antibodies

	Antibody		
Chromatin IP	NFATc3, monoclonal	Santa Cruz (sc-8405X)	5µg
	NFATc2, polyclonal	Santa Cruz (sc-13034X)	5 µg
	NFATc2, monoclonal	Santa Cruz (sc-7296X)	5 µg
	NFATc1, polyclonal	Santa Cruz (sc-1789)	5µg
	MyoD, monoclonal	Santa Cruz (sc-760)	5µg
	MyoD, polyclonal	Santa Cruz (sc-304X)	5µg
	NFATc2, polyclonal	Gift from JM Redondo, Madrid, Spain	5µg
	Laminin, polyclonal	Sigma	5µg
Immuno-detection	Sarcomeric MHC, monoclonal	DSHB (Iowa University) (MF20)	IHC: 1/100 WB: 1/20
	Embryonic MHC, monoclonal	DSHB (Iowa University) (F1.652)	IHC: 1/20 WB: 1/200
	Neonatal MHC, monoclonal	DSHB (Iowa University) (N3.36)	IHC: 1/20 WB: 1/200
	V5, monoclonal	Invitrogen	WB: 1/1000
	Gal4, monoclonal	Santa Cruz	WB: 1/2500
	Cleaved caspase-3, polyclonal	Millipore	IHC: 1/20
	GAPDH, monoclonal	Millipore	WB: 1/5000
	MHC I, monoclonal	Leica (NCL-MHCS)	IHC: 1/20 WB: 1/200
	Desmin, monoclonal	Dako	IHC: 1/200
	GFP, polyclonal	Abcam	IHC: 1/250
	Laminin, polyclonal	Sigma	IHC: 1/200
	Bis-benzimide	Sigma	IHC: 1/30 000
	EMSA	Gal4, monoclonal	Santa Cruz
NFATc2, monoclonal		Santa Cruz (sc-7296X)	0.8 µg
MyoD, polyclonal		Santa Cruz (sc-759X)	0.8µg
IP	NFATc2, polyclonal	Santa Cruz (sc-13034)	2 µg