

Fig. S1. Weight of embryos during development. Embryos are collected at different time points (E12.5, E14.5, E16.5 and E18.5) from matings between *Myod*^{+/-} or *Myod*^{-/-} females and *Myod*^{+/-};*Igf2*^{+/-} males and weighed. Embryos obtained from *Myod*^{+/-} females showed a Mendelian distribution. For weight data, all litters were pooled. wt, *Myod*^{+/-} and *Myod*^{-/-} mutants have similar weight curves, showing no effect of the *Myod* mutation on the weight of the embryos before birth. By contrast, embryos carrying a deleted *Igf2* show a reduction in weight ranging from 20% at E12.5 to 40% just before birth. This reduction is identical in *Igf2*^{+/-}, *Myod*^{+/-};*Igf2*^{+/-} and in *Myod*^{-/-};*Igf2*^{+/-} embryos, showing that *Igf2* is epistatic on *Myod* for the weight phenotype.

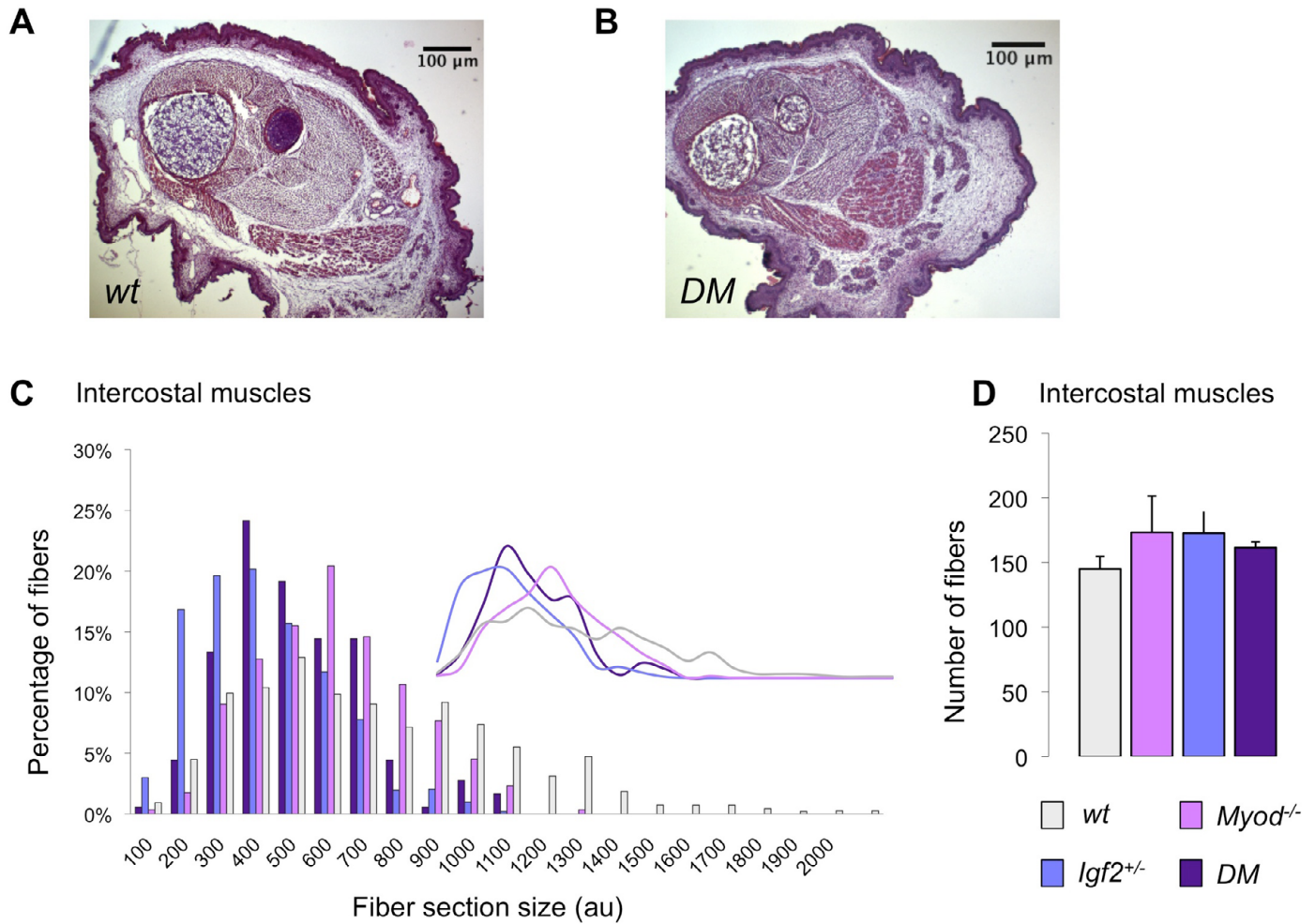


Fig. S2. Fiber repartition size in intercostal muscle. (A,B) Transverse sections of E18.5 posterior limb from *wt* (A) and *DM* (B) embryos stained with Hematoxylin and Eosin. (C) Immunostaining for laminin on E18.5 diaphragm sections from the four genotypes. Fiber cross-sectional area (as measured by MetaMorph software) is presented ($n=5$ for each genotype); au, arbitrary units. In the absence of *Igf2* protein, intercostal fibers exhibit a diminution of their cross-sectional area. (D) E18.5 intercostal muscle sections were immunostained with antibodies to laminin. Muscle fiber number was counted (using MetaMorph software) for the four genotypes.

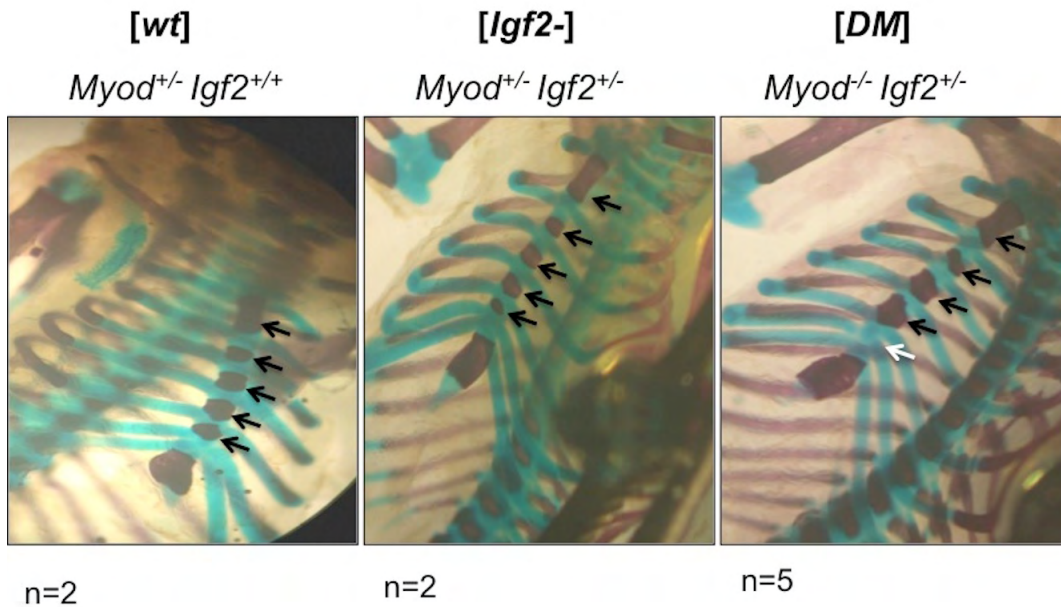
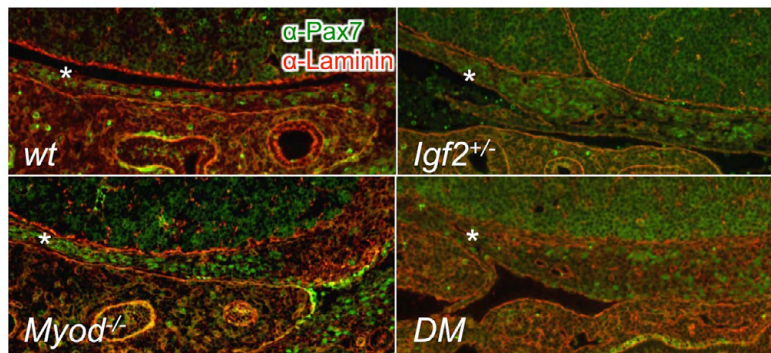


Fig. S3. Ossification of the sternum at E18.5. E18.5 embryos are stained with Alizarin Red (bone) and Alcian Blue (cartilage). Rib cages from *Myod*^{-/-}, *Myod*^{+/-};*Igf2*^{+/-} and DM embryos are shown with arrows pointing to ossification segments of the sternum. The white arrow indicates the fifth ossification segment, which is missing in the DM embryo. wt and *Myod*^{+/-}, *n*=3; *Igf2*^{+/-} and *Myod*^{+/-};*Igf2*^{+/-}, *n*=2; DM, *n*=5.

A E13.5



B E18.5

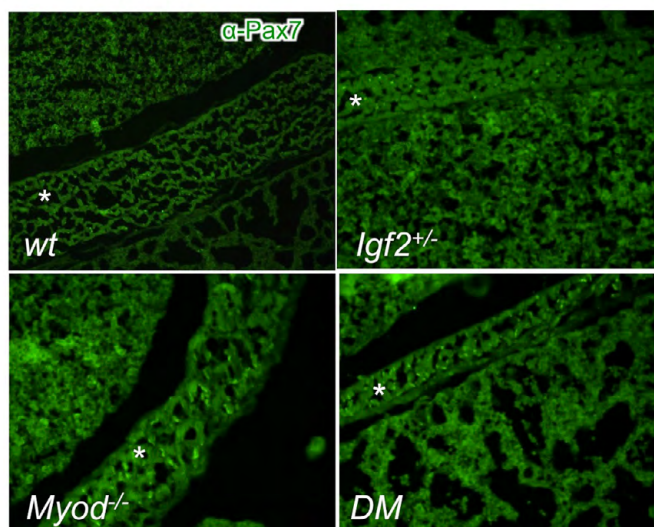


Fig. S4. Diaphragm formation during development. (A) Sagittal sections of E13.5 wt, *Igf2*^{+/-}, *Myod*^{-/-} and DM embryos were immunostained for Pax7 (α -Pax7, green) and laminin (α -Laminin, red). Stars indicate the position of the diaphragm. (B) Sagittal sections of E18.5 wt, *Igf2*^{+/-}, *Myod*^{-/-} and DM embryos were immunostained for Pax7 (green). DM diaphragms are thinner than wt, *Myod*^{-/-} and *Igf2*^{+/-} diaphragms. wt, *Myod*^{+/-}, *Igf2*^{+/-}, *Myod*^{+/-};*Igf2*^{+/-} and DM, *n*=5.

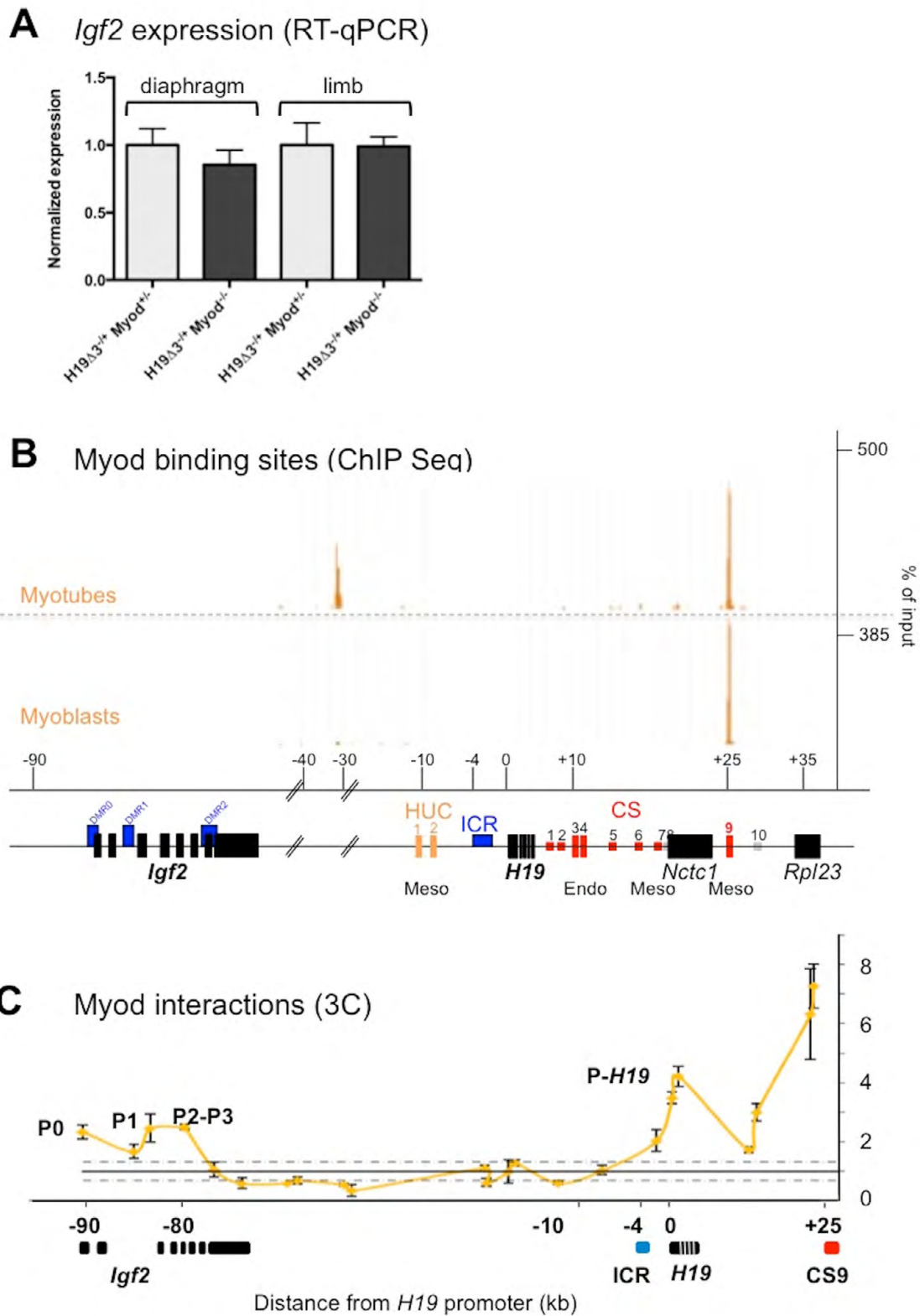


Fig. S5. Interactions of Myod with the *H19-Igf2* locus. (A) Expression level of *Igf2* mRNA as assessed by RT-qPCR in diaphragm and limb muscle samples from $H19^{\Delta 3+/+}; Myod^{+/-}$ and $H19^{\Delta 3+/-}; Myod^{+/-}$ embryos. Genotyping of $H19^{\Delta 3}$ mice is normally performed by probing for the inserted *neo* gene by PCR. However, since the *Myod* mutants also contain a *neo* insertion, the identification of the $H19^{\Delta 3}$ versus wt embryos required an RT-qPCR step to detect presence or absence of *H19* expression. In the absence of the *H19* gene, *Myod* status does not affect *Igf2* expression. (B) ChIP-Seq data showing the position of the peak of Myod binding in the *H19-Igf2* locus. The genes of the region are indicated by black boxes. Red boxes show the endodermal and mesodermal enhancers described in the literature. ICR, imprinting control region; HUC, *H19* upstream conserved region; CS, conserved sequence. (C) 3C experiment showing the interactions between the mesodermal enhancer CS9 (located at +25 kb from the start of the *H19* gene) and other regions of the locus. The -4 kb region corresponds to the localization of the ICR upstream of the *H19* gene. Interactions occur with the *H19* (p-*H19*) and *Igf2* (P0, P1 and P2-P3) promoters. Location of the ICR, *H19* and *Igf2* genes and CS9 enhancer are shown by rectangles.



Movie 1. Contraction of the diaphragm after electric stimulation of the phrenic nerve of *Igf2*^{+/-} single-mutant and DM E18.5 embryos.

Table S1. Primers for genotyping *Myod*, *Igf2* and *H19* mutants; RT-qPCR of *H19*, *Igf2*, *Myod*, *Srf*, *cAct* and *skAct*; RT- and qPCR of miR-483-5p; and the *Srf* CHIP experiment

	Gene	PCR primers	Annealing temperature
Genotyping	<i>Igf2</i>	Forward CTAGCTCAAAGCCCTGCGTTTCTTTC	58°C
		Reverse TGCCTGACAGCCGGAACAC	
	<i>Myod</i>	Forward CCCAGGGCATCTATGATTCTGCCGA	62°C
		Reverse TGTAGTAGGCGGTGTCGTAGCC	
		PGKR1 AGGGGAGGAGTAGAAGGTGGCGCGGAA	
	<i>H19^{A13}</i>	MutH19F AATGGGAAACAGAGTCACG	58°C
MutH19R GACAGTGGGAGTGCCACCTT			
wt H19 F CCATCTTCATGGCCAATTCT			
wt H19 R CTAGAGCTCGCTGATCAGCCT			
Quantitative expression	<i>Gapdh</i>	Forward ACAGTCCATGCCATCACTGCC	58°C
		Reverse GCCTGCTTCACCACCTTCCTTG	
	<i>Tbp</i>	Forward GGTATCTGCTGGCGTTTGG	60°C
		Reverse GCCCTGAGCATAAGGTGGAA	
	<i>Myod</i>	Forward GGGCCGCTGTAATCCATCATG	60°C
		Reverse CTGCCTTCTACGCACCTGGA	
	<i>Igf2</i>	Forward CGACGGTTGGCACGGCTTGA	60°C
		Reverse GGTGCTTCTCATCTCTTTGG	
	<i>H19</i>	Forward GGAGACTAGGCCAGGTCTC	60°C
		Reverse GCCCATGGTGTCAAGAAGGC	
<i>Srf</i>	Forward CACCTACCAGGTTGTCGGAAT	60°C	
	Reverse GCTGTCTGGATTGTGGAGGT		
<i>cAct</i>	Forward ACTCTCTTCCAGCCCTCCTTTCATT	60°C	
	Reverse GGAGCCAGTGCAGTGATTTCCTT		
<i>skAct</i>	Forward CGTGAAGCCTCACTTCTACC	60°C	
	Reverse AGAGCCGTTGTCACACACAA		

miR-483-5p RT stem-loop primer:

5'-GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACCTCCCTT-3'

PCR primer

F: 5'-CCGGAAGACGGGAGAAGAGA-3'

R: 5'-GTATCCAGTGCCTGTCGTGGAGT-3'

Srf ChIP primers

Myod DRR F: 5'-GCCCCGAGTAGCAAAGTAAG-3' R: 5'-GAAACCGGATCCAACACTAGCA-3'

Myod CArG F: 5'-GCCTAGCCAGACCAACATTC-3' R: 5'-CTTTGATTTCCCCCTGTCCT-3'

Il4 intron F: 5'-AGAATGAAAGGCCCCAAAGT-3' R: 5'-GGGAGGACAGATCTCTGGTG-3'

Table S2. Primers for 3C-qPCR analysis of CS9

	<i>Bam</i> HI site No	PCR primers	Annealing temperature
3C-qPCR	23	ATGACCACCAGATGTCAAGCTCG	62°C
	22	CTGCTCCGTGTGAGTTCCTTGG	64°C
	21	AGGACCCAAATCAGACAAGGG	62°C
	20	AGCCTGCGTTTCTTTCTCCAGG	62°C
	19	GGCCCTCCATCTTGTCTCTTCC	64°C
	18	GTGGCAAGGAAAGTGAAGGAGG	62°C
	17	CAAGATAAGGACTCATTAGGCCTAGG	63°C
	16	ATGGCCCCATTAGAGAGCTACTG	62°C
	15	GACACAGGCTGGGCTATGTTTTTC	62°C
	14	CTGTGACAGTGGTATGCACCAAG	62°C
	13	CTGGCCTGAGTACCTCTCCAC	64°C
	12	GTCCTCTGCCTTCTGGACTTTGG	64°C
	11	TTAGCTCTGGCTCACCCATCTG	62°C
	10	GCCTGAATACCCAAGACCTCATAAC	63°C
	9	ACACGAAGGTTGGGGAGATAGG	62°C
	8	CCAGAGCAGGATGTGAGAGGG	64°C
	7	TAGGCGGGAGACATAGAAACTGC	62°C
	6	GCAGGGTTGCCAGTAAAGACTG	62°C
	5	GCCTTGTCGTAGAAGCCGTCTG	64°C
	4	TGGAATGTGGGGAGACAAACAGC	62°C
	3	CATACCGGGCAGTAGACCTGAC	64°C
	2	CCTCCCAGGTCCTGAAGAATAC	62°C
	1	CTTTAGGTAGCCCAAGGCTCAG	62°C
		Anchor (CS9)	CCGTCCTTTGGGCATAGCTTCC