

Supplementary Materials and Methods:

Genotyping:

Genotyping strategies for MRTF-A wild-type and null alleles, and for MRTF-B wild-type, null and floxed alleles have been described previously (Li et al., 2006; Mokalled et al., 2010). The following primers were used for genotyping:

MRTF-A:

GT5: GTT GCT CAG TCA TGT GAC ACC TGT ACA G

GT6: GGC TTC AGT ACC TTC CTA AGC TCT GCA G

LacZ3-QB: CAT GGT GGA TCC TGA GAC TGG CGA ATT C

MRTF-B:

QC SA F: CAT GGC GAC TTC CTT CTC CTC TTC TCA AGG CTG

QC EX R: GGC TTA GAC AAG ATG GTT GGT CTG GCA CTG C

QC LA R: CCA GTG GTG TCC AGT CTT ACT GAA CAG CTC ACT CAG

Quantitative Realtime PCR Analysis:

Total RNA was extracted from tissue with TRIZOL reagent (Invitrogen) according to manufacturer instructions. cDNA was synthesized using Superscript III reverse transcriptase with random hexamer primers (Invitrogen), as per manufacturer instructions. Gene expression was analyzed by qPCR using KAPA SYBR FAST (Kapa Biosystems) or KAPA PROBE FAST (Kapa Biosystems) on a 7900HT Fast Real-Time PCR machine (Life Technologies). See Supplementary Table 1 for Taqman probe IDs. The following primer sets were used for LMOD3 and KLHL40:

Lmod3-F: CCGCTGGTGGAAATCACTCCC

Lmod3-R: ACTCCAGCTCCTTTGGCAGTTGC

Klh40-F: CCCAAGAACCATGTCAGTCTGGTGAC

Klh40-R: TCAGAGTCCAAGTGGTCAAACCTGCAG

Bioinformatics:

Differential Gene Expression Analysis

Quality assessment of the RNA-Seq data was done using NGS-QC-Toolkit (Patel and Jain, 2012) with default setting. Quality filtered reads generated by the tool were then aligned to the mouse reference genome GRCm38 (mm10) using the Tophat2 (v 2.0.0) aligner (Kim et al., 2013) using default setting. Differential gene expression analysis was done using the R package DESeq2 (v 1.6.3) (Anders and Huber, 2010). Read counts were normalized by taking the median of each gene count across samples and dividing each sample gene count by the relative ratio of library sizes between the calculated median and sample size. The averaged normalized expressions values of the samples were used to calculate fold change and p-values. Cutoff values of fold change greater than 2 and p-value less than 0.05 were then used to select for differentially expressed genes between sample group comparisons.

Pathway Enrichment Analysis

Significant pathway enrichment analysis was performed using Ingenuity Pathways Analysis (Ingenuity® Systems). Differentially expressed genes from the RNA expression data are associated with a biological function supported by at least one publication in the Ingenuity Pathways Knowledge Base. Fisher's exact test is then used to calculate the p-value and determine the probability that each biological function is enriched in the dataset due to chance alone. Statistically significant biological pathways were then identified by selection for pathways with p-values less than 0.05. DAVID gene functional annotation and classification tool (Huang et al., 2007) was used to annotate the list of differentially expressed genes with respective Gene Ontology terms and perform GO enrichment analysis for molecular and biological functional categories. Functional Gene Ontology groups were selected for significance by using a p-value cutoff of 1%.

Supplemental tables:**Supplemental Table 1. Taqman probes used in quantitative real-time PCR analysis.**

Mm00808218_g1	Acta1	actin, alpha 1, skeletal muscle
Mm00725412_s1	Acta2	actin, alpha 2, smooth muscle
Mm00432556_m1	Ckm	creatine kinase, muscle
Mm00442776_m1	Crabp1	cellular retinoic acid binding protein I
Mm00458042_m1	Dysf	dysferlin
Mm00517621_m1	Jph2	junctionophilin 2
Mm00461840_m1	Mkl1	MKL (megakaryoblastic leukemia)/myocardin-like 1
Mm00461844_m1	Mkl1	MKL (megakaryoblastic leukemia)/myocardin-like 1
Mm00463877_m1	Mkl2	MKL/myocardin-like 2
Mm00803032_m1	Myl3	myosin, light polypeptide 3
Mm00447745_m1	Vcl	vinculin
Mm00449201_m1	Vim	vimentin

Supplemental Table 2. List of differentially expressed genes in whole transcriptome analysis of MRTF HdKO skeletal muscle.

Gene ID	baseMean	log2FoldChange	Fold Change	pvalue	padj
Hmgn2	840.943	3.531	11.560	0.000	0.000
Rps18	550.720	3.323	10.005	0.000	0.000
Hspa8	2031.694	2.455	5.482	0.000	0.000
Hoxb8	217.141	2.440	5.425	0.000	0.000
AA388235	41.192	2.312	4.967	0.000	0.000
Fbxo44	62.109	2.292	4.899	0.000	0.000
Mybpc3	77.293	2.177	4.522	0.000	0.000
C920006O11Rik	42.362	2.100	4.288	0.000	0.000
B3gat2	58.160	2.066	4.188	0.000	0.000
Ret	224.718	2.027	4.077	0.000	0.000
Pcdhgb8	35.567	2.011	4.031	0.000	0.000
Acp1	188.111	1.987	3.963	0.000	0.000
H2-T23	55.890	1.930	3.811	0.000	0.000
Ankrd45	37.620	1.924	3.795	0.000	0.000
Nell2	225.997	1.837	3.573	0.000	0.000
St8sia1	117.456	1.800	3.483	0.000	0.000

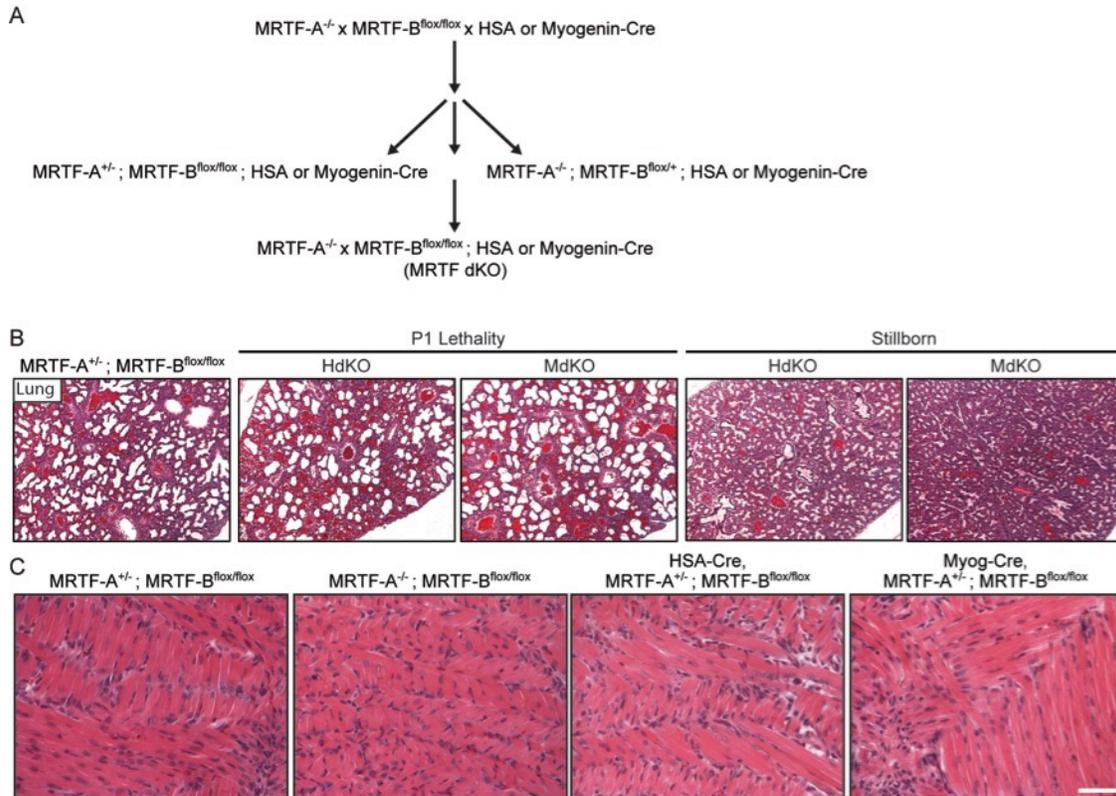
Xaf1	101.835	1.796	3.471	0.000	0.000
Mettl21c	150.126	1.791	3.460	0.000	0.000
Hspe1	520.723	1.778	3.430	0.000	0.000
Slc13a3	34.920	1.766	3.402	0.000	0.000
Gm3500	17.993	1.760	3.387	0.000	0.000
Plekhd1	36.014	1.744	3.350	0.000	0.000
Shank2	84.600	1.721	3.296	0.000	0.000
Pla2g7	221.457	1.715	3.282	0.000	0.000
Gm10406	29.100	1.685	3.215	0.000	0.000
Pcp4	81.244	1.661	3.163	0.000	0.000
Srp9	403.177	1.647	3.132	0.000	0.000
Kcnd3	122.069	1.631	3.097	0.000	0.000
Scn8a	148.500	1.585	3.001	0.000	0.000
Dclk3	39.834	1.585	2.999	0.000	0.000
Megf11	35.524	1.576	2.982	0.000	0.000
Myo5c	89.254	1.576	2.982	0.000	0.000
Slc30a10	138.319	1.575	2.979	0.000	0.000
Syn3	156.519	1.566	2.961	0.000	0.000
Ppm1h	167.288	1.549	2.925	0.000	0.000
Hoxb9	265.494	1.542	2.912	0.000	0.000
Kcnt1	108.983	1.540	2.907	0.000	0.000
Cldn11	127.726	1.491	2.810	0.000	0.001
BC068157	72.442	1.469	2.768	0.000	0.001
Pcdhb6	32.861	1.467	2.764	0.000	0.001
Trank1	199.436	1.467	2.764	0.000	0.001
Dmtn	177.805	1.444	2.721	0.000	0.001
Aqp4	90.546	1.436	2.706	0.000	0.001
Ptpro	149.162	1.433	2.701	0.000	0.001
Fut9	179.221	1.431	2.697	0.000	0.001
Susd4	87.785	1.426	2.687	0.000	0.001
Slit1	187.423	1.419	2.674	0.000	0.002
Esrrg	177.264	1.409	2.655	0.000	0.000
Rap1gap	195.314	1.399	2.637	0.000	0.000
Stxbp5l	52.744	1.396	2.632	0.000	0.002
Slc15a2	200.424	1.393	2.625	0.000	0.002
Cux2	91.935	1.391	2.623	0.000	0.001
Grik4	30.691	1.389	2.620	0.000	0.002
Asb15	35.587	1.388	2.617	0.000	0.001
Galnt14	53.684	1.384	2.610	0.000	0.002
Gpm6a	585.057	1.375	2.594	0.000	0.002

Hap1	381.079	1.370	2.584	0.000	0.002
Hdac1	374.790	1.368	2.582	0.000	0.000
Rab11fip4	106.011	1.365	2.576	0.000	0.002
Gm5111	28.702	1.362	2.571	0.000	0.003
Rpl21	112.623	1.362	2.570	0.000	0.001
Shd	102.491	1.360	2.568	0.000	0.001
Cx3cl1	147.641	1.359	2.564	0.000	0.002
Pcp4l1	39.250	1.357	2.561	0.000	0.003
Rpl14	644.163	1.355	2.558	0.000	0.000
Cacnb4	82.270	1.352	2.552	0.000	0.002
4632415L05Rik	193.820	1.338	2.527	0.000	0.000
Hist3h2ba	49.615	1.330	2.513	0.000	0.001
Bzap1	146.414	1.311	2.481	0.000	0.004
Lhx1	66.904	1.302	2.466	0.000	0.003
Rundc3b	76.470	1.286	2.438	0.000	0.006
Fam169a	70.366	1.283	2.433	0.000	0.004
6330403A02Rik	321.708	1.283	2.433	0.000	0.005
Rprm	147.151	1.281	2.430	0.000	0.000
Gpd1	437.438	1.276	2.421	0.000	0.006
9330159F19Rik	215.867	1.274	2.418	0.000	0.007
Hfm1	25.515	1.272	2.414	0.000	0.007
Amy1	41.040	1.271	2.414	0.000	0.007
Gria4	179.283	1.271	2.413	0.000	0.006
Inpp5j	24.472	1.256	2.389	0.000	0.006
Slc2a5	58.674	1.246	2.373	0.000	0.002
Adora1	117.349	1.243	2.366	0.000	0.006
Ano4	36.945	1.242	2.365	0.000	0.006
Adam11	163.195	1.238	2.358	0.000	0.007
A730017C20Rik	97.126	1.232	2.349	0.000	0.010
Map7d2	83.541	1.227	2.341	0.000	0.008
Hoxd10	155.391	1.215	2.321	0.000	0.010
Rims2	67.040	1.211	2.316	0.000	0.009
Mtus2	183.732	1.209	2.312	0.000	0.005
Sall3	68.989	1.205	2.306	0.000	0.008
Rpl35a	97.618	1.200	2.297	0.000	0.002
Gm10516	46.519	1.198	2.294	0.000	0.003
Zcchc18	219.925	1.183	2.271	0.000	0.010
Nrn1	191.918	1.179	2.264	0.000	0.003
BC005764	382.326	1.174	2.257	0.000	0.008
Plcb1	208.608	1.173	2.255	0.000	0.002

A330021E22Rik	46.436	1.172	2.253	0.000	0.008
Vcp	1135.394	1.167	2.246	0.000	0.000
Prkcz	116.095	1.124	2.180	0.000	0.010
Rpsa	2901.178	1.120	2.174	0.000	0.000
Slc25a23	455.569	1.117	2.169	0.000	0.000
Prr33	43.890	1.114	2.164	0.000	0.007
Ehd3	232.116	1.100	2.144	0.000	0.000
Ppp1cc	243.674	1.100	2.143	0.000	0.000
Mrps10	211.070	1.088	2.126	0.000	0.000
Cystm1	202.278	1.062	2.088	0.000	0.008
Socs2	179.265	1.061	2.086	0.000	0.000
Paqr8	135.745	1.031	2.044	0.000	0.008
Robo2	546.658	1.031	2.044	0.000	0.009
Ccl27a	67.957	1.029	2.040	0.000	0.007
Acaa1a	587.268	1.023	2.033	0.000	0.000
Lyve1	669.905	-1.038	0.487	0.000	0.010
Prkab2	615.096	-1.047	0.484	0.000	0.000
Slfn9	361.803	-1.081	0.473	0.000	0.000
Pdlim7	1267.551	-1.107	0.464	0.000	0.000
Eno1b	1833.057	-1.135	0.455	0.000	0.000
Clec12b	47.503	-1.138	0.454	0.000	0.008
Tgfb1i1	567.423	-1.177	0.442	0.000	0.000
Abrac1	519.251	-1.204	0.434	0.000	0.000
Siglec1	92.477	-1.222	0.429	0.000	0.003
Gm9054	21.630	-1.231	0.426	0.000	0.009
Ifi202b	81.393	-1.247	0.421	0.000	0.003
Ulbp1	117.541	-1.280	0.412	0.000	0.000
Adh7	133.302	-1.297	0.407	0.000	0.004
4833412C05Rik	22.543	-1.303	0.405	0.000	0.005
Srf	1463.674	-1.309	0.404	0.000	0.000
H2-T10	20.096	-1.325	0.399	0.000	0.004
Lym7	63.741	-1.330	0.398	0.000	0.000
Rpl23	1024.645	-1.348	0.393	0.000	0.000
Hmga2-ps1	47.309	-1.370	0.387	0.000	0.001
Tbc1d1	322.088	-1.375	0.386	0.000	0.000
C130080G10Rik	212.339	-1.389	0.382	0.000	0.000
C130046K22Rik	51.107	-1.412	0.376	0.000	0.001
Acta1	40420.212	-1.423	0.373	0.000	0.000
Rbm46	87.645	-1.428	0.372	0.000	0.000
Car3	5270.338	-1.429	0.371	0.000	0.001

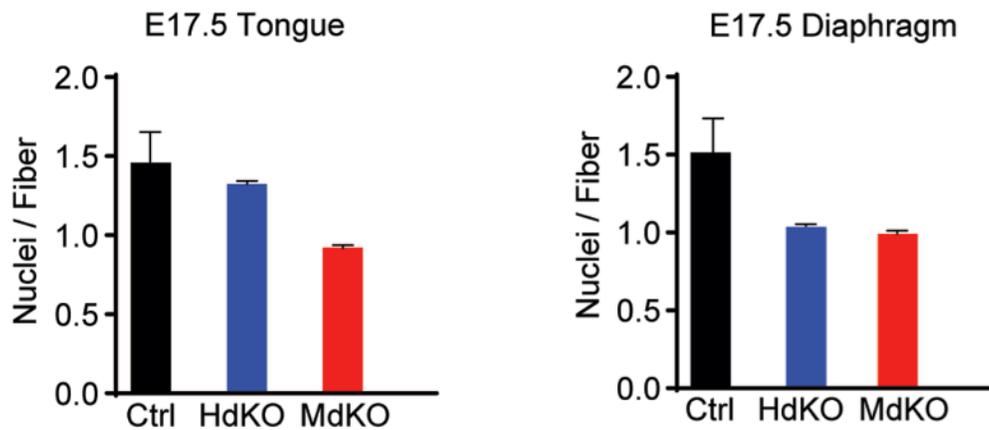
Tagap1	171.453	-1.436	0.370	0.000	0.000
2610305D13Rik	100.616	-1.445	0.367	0.000	0.000
Rplp0	5210.944	-1.453	0.365	0.000	0.000
Gm13212	52.648	-1.627	0.324	0.000	0.000
Gm1943	89.663	-1.681	0.312	0.000	0.000
Gm8801	77.195	-1.735	0.300	0.000	0.000
Gm14403	113.466	-1.870	0.274	0.000	0.000
Mkl1	447.413	-1.981	0.253	0.000	0.000
Rhoj	488.347	-2.003	0.249	0.000	0.000
Fbxl22	441.277	-2.113	0.231	0.000	0.000
Tmem181c-ps	54.023	-2.977	0.127	0.000	0.000
Al506816	225.186	-3.967	0.064	0.000	0.000

Supplemental Figures

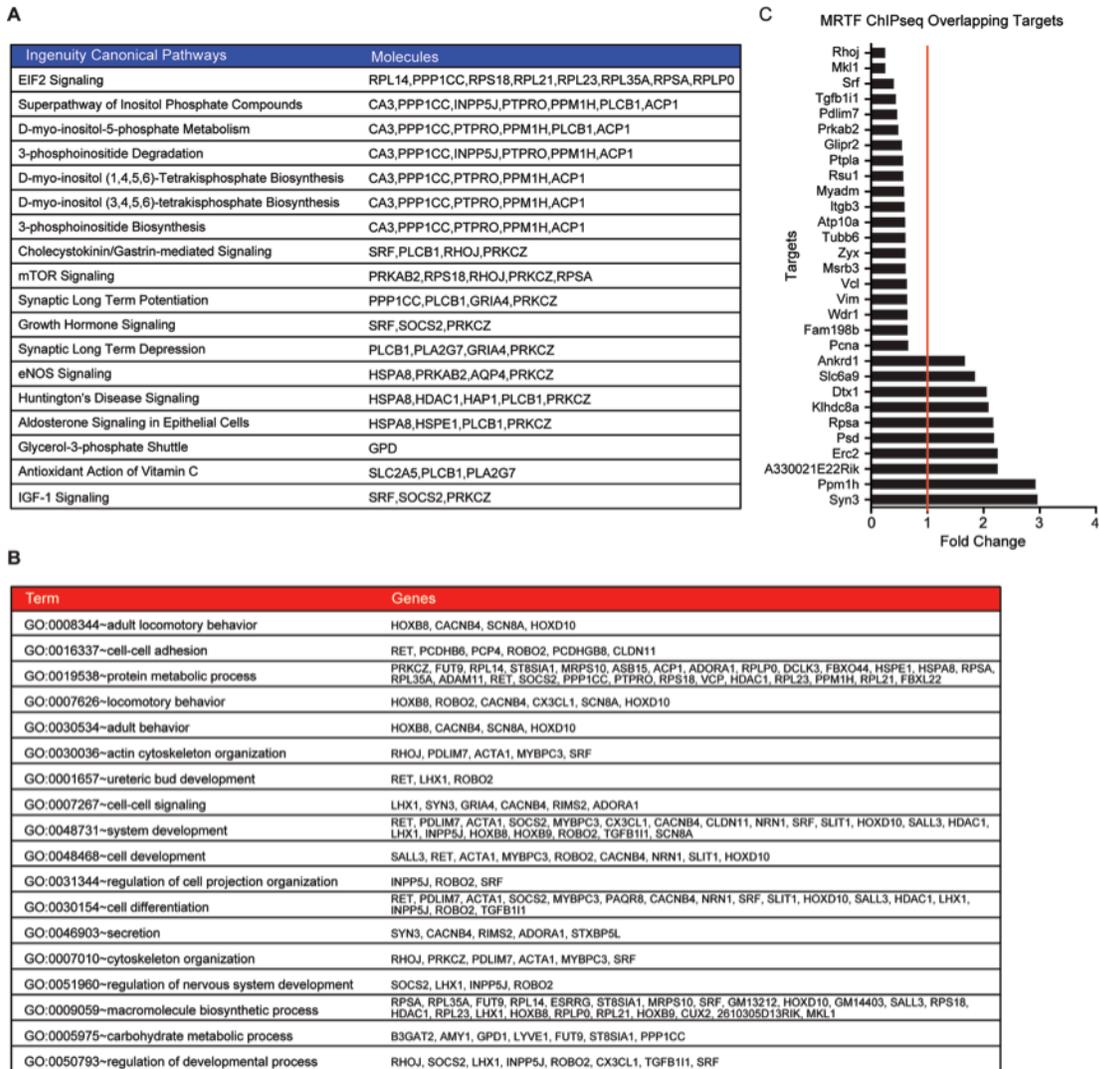


Supplemental Figure 1. Breeding strategy and histological analysis of control and *dKO* animals. (A) Breeding strategy: muscle-specific Cre-bearing mice were crossed with MRTF-A^{-/-}, MRTF-B^{flox/flox} animals in order to generate MRTF *dKO* mice. (B) Transverse sections from P1 lung tissue of MRTF-A^{+/-}, MRTF-B^{flox/flox}; *HdKO* and *MdKO* animals were stained with H&E to assess patency of alveoli: *HdKO* and *MdKO* mice that died perinatally demonstrated patent alveoli whereas animals that died *in utero* had non-expanded alveoli. (C) Coronal sections of P1 tongue tissue from Cre-negative, MRTF-A^{+/-}, MRTF-B^{flox/flox}; Cre-negative, MRTF-A^{-/-}, MRTF-B^{flox/flox}; and Cre-positive, MRTF-A^{+/-}, MRTF-B^{flox/flox} animals were analyzed by H&E staining for dosage effects. Scale bars: 40 μ m.

A



Supplemental Figure 2. Nuclei number quantification of MRTF *dKO* myofibers. (A) Transverse sections from E17.5 tongue and diaphragm were stained with WGA and DAPI. DAPI+ nuclei were counted and divided by total number of myofibers, which was determined by WGA staining. Images were acquired from at least 3 mice per genotype. All data are shown as mean \pm SEM. The non-parametric Kruskal-Wallis test for multiple comparisons was conducted in order to compare columns. p = not significant.



Supplemental Figure 3. (A) Ingenuity pathway analysis and (B) Gene ontology analysis (red) of RNA-seq targets. (C) Overlay of published MRTF-A ChIP-seq data with the *HdKO* RNA-seq data.

References

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