Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Yonatan Feuermann, Keunsoo Kang, Oksana Gavrilova, Nadine Haetscher, Seung Jin Jang1, Kyun Hyun Yoo, Jiang Changtao, Frank Gonzalez, Gertraud W Robinson, and Lothar Hennighausen

MiR-193b and miR-365–1 are not required for the development and function of brown fat in the mouse

2013; 10(12)
http://dx.doi.org/10.4161/rna.27239

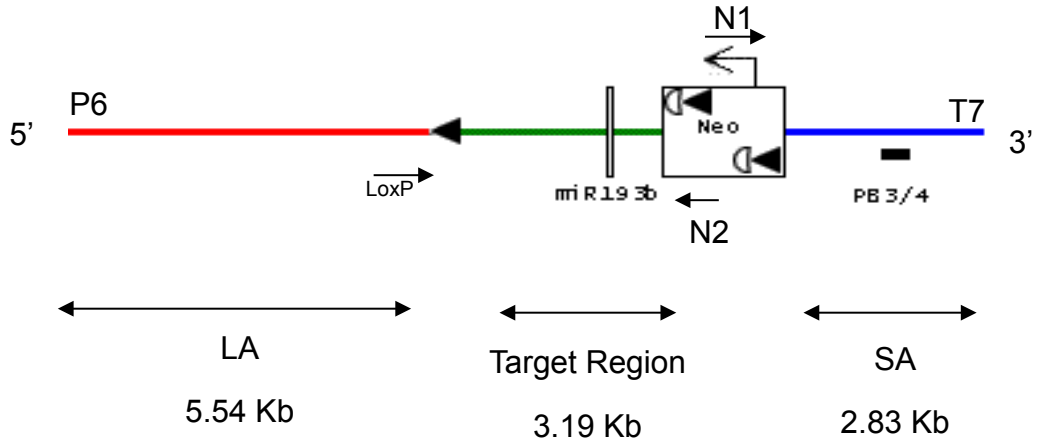
www.landesbioscience.com/journals/rnabiology/article/27239/

www.landesbioscience.com/journals/rnabiology/article/27239/ 2013RNABIOL0224R_ST1

www.landesbioscience.com/journals/rnabiology/article/27239/ 2013RNABIOL0224R_ST2

Vector Design Outline

AN 11.57 Kb region used to construct the targeting vector was first sub-cloned from a positively identified C57BL/6 BAC clone (RP23: 239H18). The region was designed such that the long homology arm (LA) extends 5.54 Kb 5' to miR193b. The short homology arm (SA) ends 3' to miR193b, and is 2.83 Kb. The LoxP/FRT flanked Neo cassette is inserted 603 bp downstream of miR193b. The single loxP site, containing engineered KpnI, EcoRV, EcoRI, PstI and SpeI sites for southern blot analysis, is inserted 179 bp 5' of miR193b. The target region is 3.19 Kb, and includes miR193b.The targeting vector is confirmed by restriction analysis after each modification step. P6 and T7 primers anneal to the backbone vector sequence and read into the 5' and 3' ends of the BAC sub-clone. N1 and N2 primers anneal to the 5' and 3' ends of the LoxP/FRT Neo cassette and sequence the SA and LA, respectively.



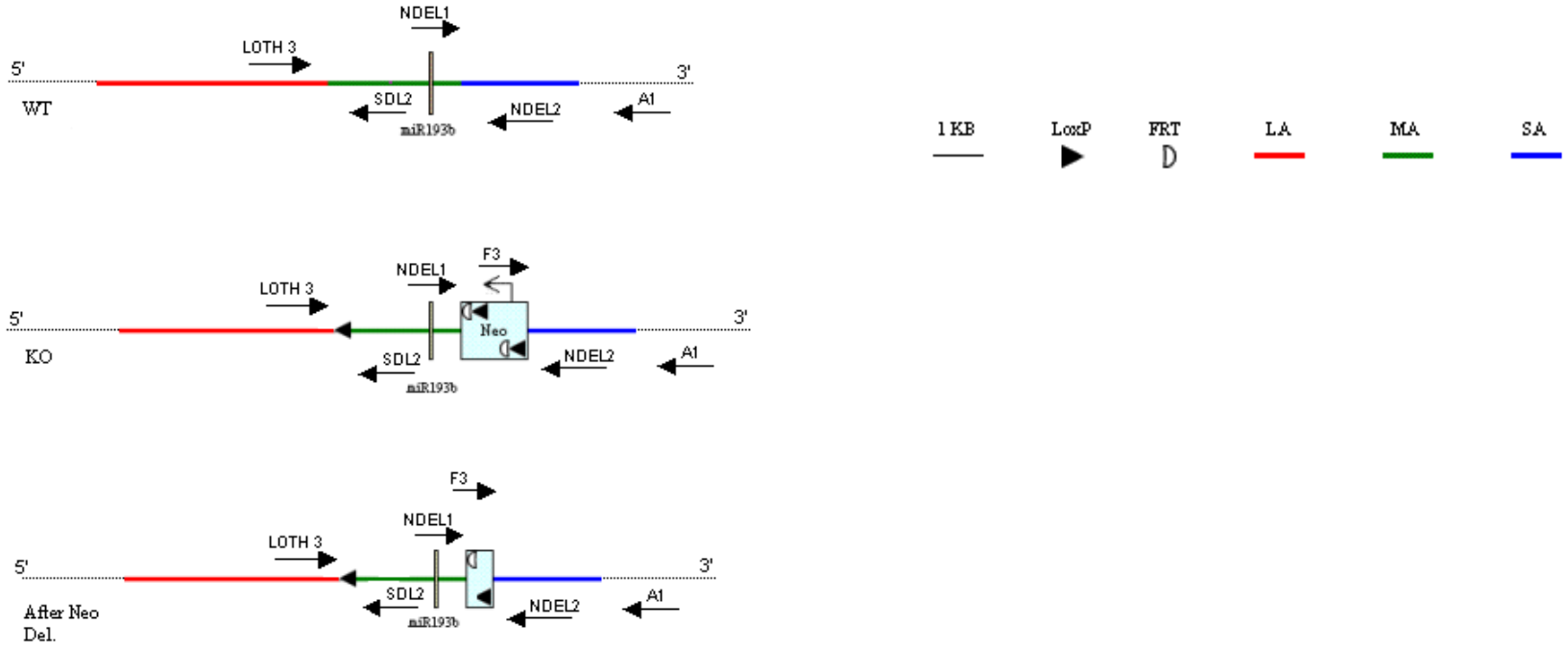
PCR primers used for sequencing:

Primer P6: 5'-GAG TGC ACC ATA TGG ACA TAT TGT C-3'
Primer T7: 5'-CGA TAA GCC AGG TTA ACC TGC ATT A-3'
Primer N1: 5'-TGC GAG GCC AGA GGC CAC TTG TGT AGC-3'
Primer N2: 5'-TTC CTC GTG CTT TAC GGT ATC G-3'
Primer loxP: 5'-AGA AGA GTG ATG GCA TCT CTG ACG-3'

Supplementary Figure 1. Conditional gene targeting scheme for generating miR-193b flox mice.
(A) Vector Design Outline. (B) Schematic and Information

B. Schematic and Information

Targeted iTL BA1 (C57BL/6N x 129/SvEv) hybrid embryonic stem cells were microinjected into C57BL/6 blastocysts. Resulting chimeras with a high percentage agouti coat color were mated to C57BL/6 FLP mice to remove the Neo cassette. Tail DNA was analyzed as described below from pups with agouti or black coat color.

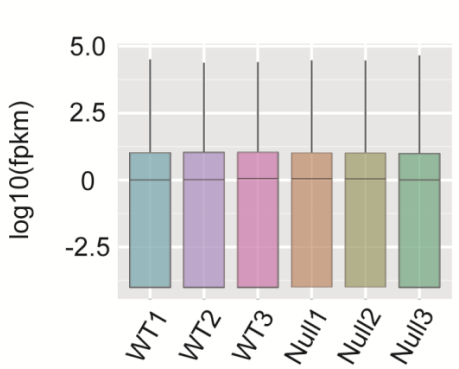


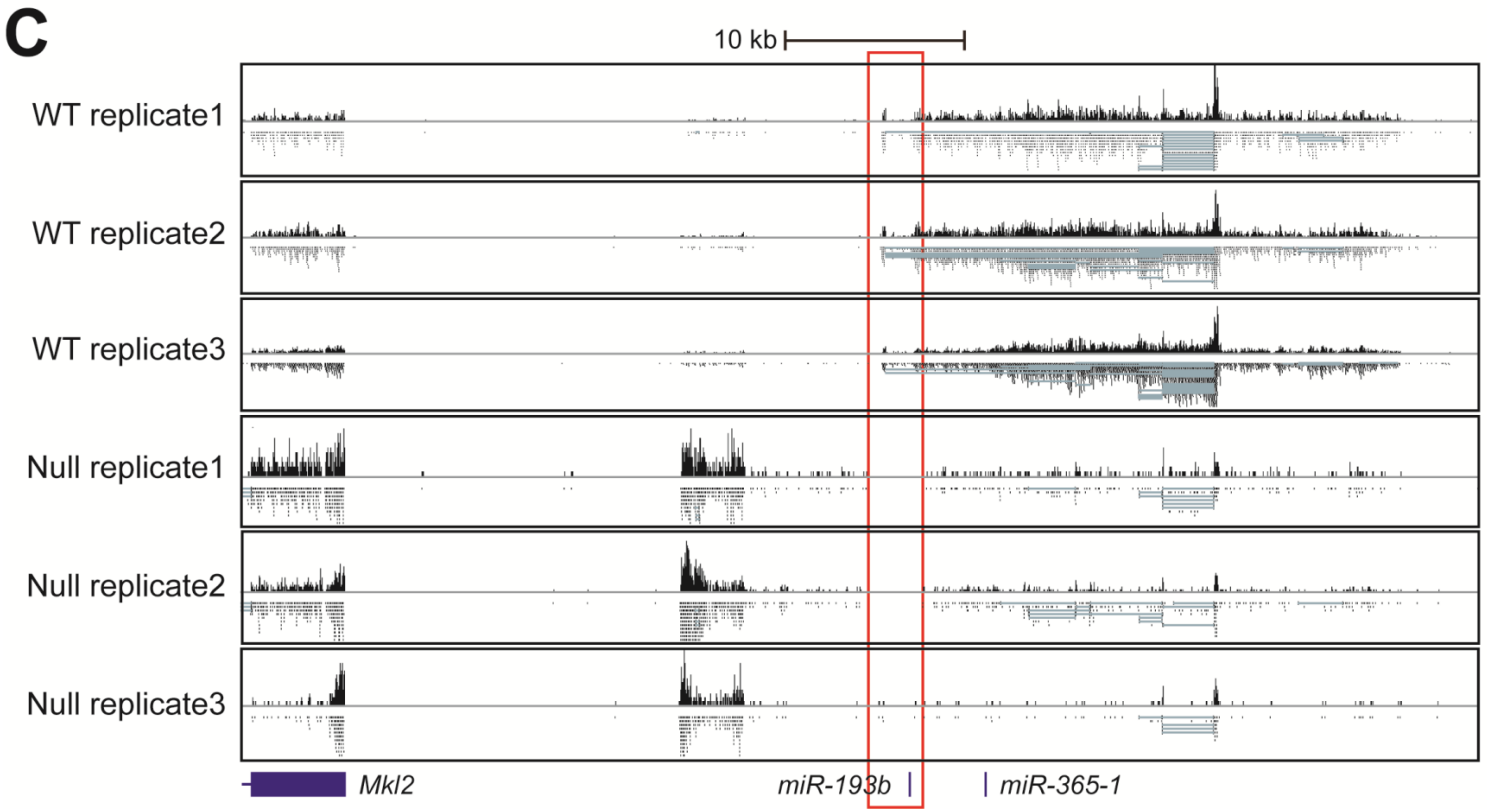
Primers for PCR Screening

NDEL1: 5'- GCG AAT GAG ACC AGA AGG AAA CTG -3'
NDEL2: 5'- GGG CTC ATT GTG TCG CGT AAG G -3'
FLP1: 5'- CAC TGA TAT TGT AAG TAG TTT GC -3'
FLP2: 5'- CTA GTG CGA AGT AGT GAT CAG G -3'
LOTH 3: 5'- AGA AGA GTG ATG GCA TCT CTG ACG -3'
SDL2: 5'- TCC GCC CAT TCC TTG TCT GTC TTA -3'
F3: 5'- GCA TAA GCT TGG ATC CGT TCT TCG GAC -3'
A1: 5'- CCT TGG CAG TTT CTC CAT GAA GG -3'

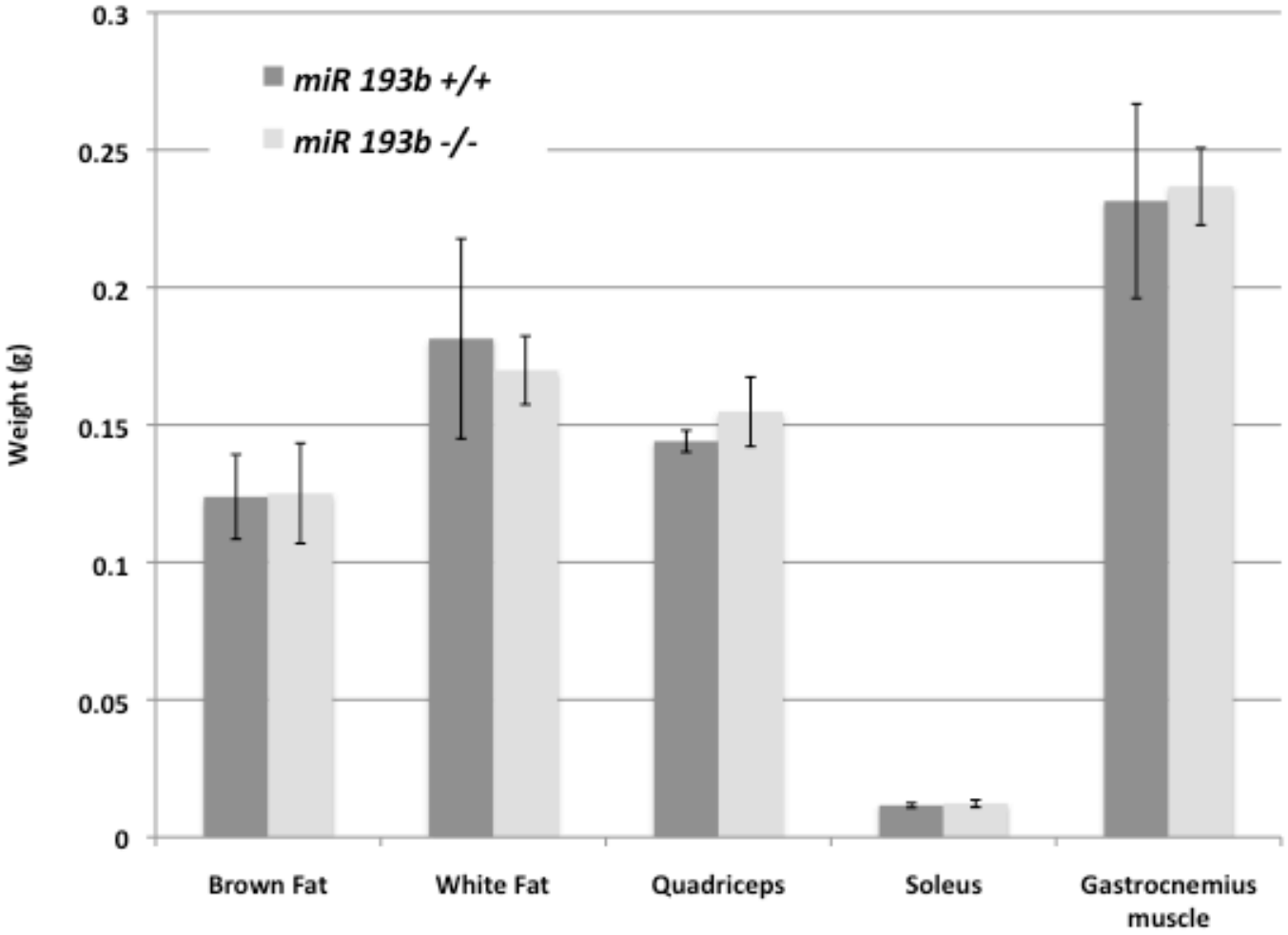
A

BAT sample	Total mapped reads
WT replicate1	29,148,888
WT replicate2	32,073,088
WT replicate3	49,551,151
Null replicate1	26,488,582
Null replicate2	35,655,594
Null replicate3	27,933,153





Supplementary Figure 2. RNA-seq quality check and confirmation of the miR-193b deletion. (A) Total number of mapped reads is shown. (B) Box plots show distributions of whole gene expression levels for individual conditions. (C) Examination of mapped reads over the miR-193b locus confirmed the deletion of the miR-193b locus in all BAT-Null replicates (red box).



Supplementary Figure 3. Equivalent weights of fat and muscle tissues in *miR-193b*$^{-/-}$ and *miR-193b*$^{+/+}$ control mice. Three mice from each genotype were selected and their brown fat, white fat, quadriceps, soleus, and gastrocnemius muscle were extracted and weighed. The graphs show average weights of fats and muscles in wild-type and null groups.