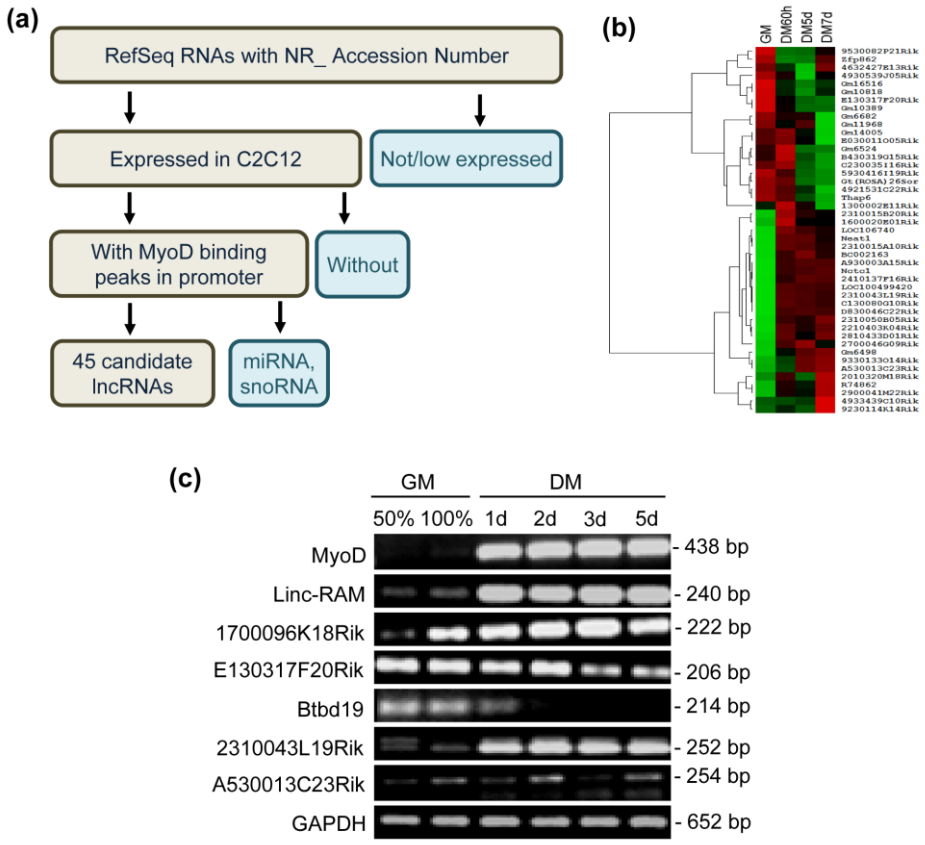
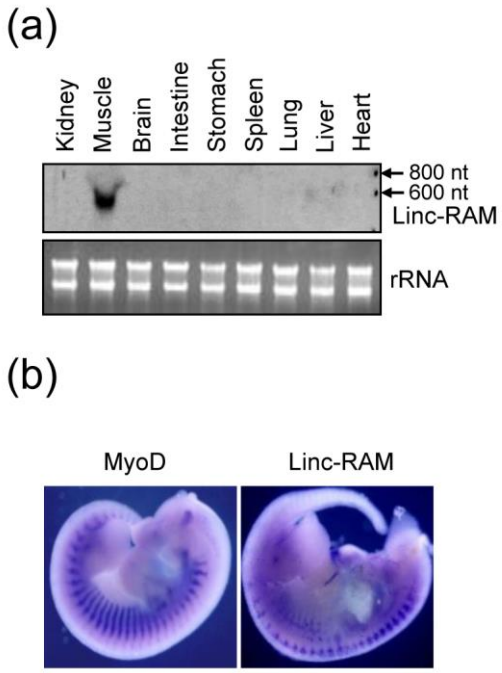
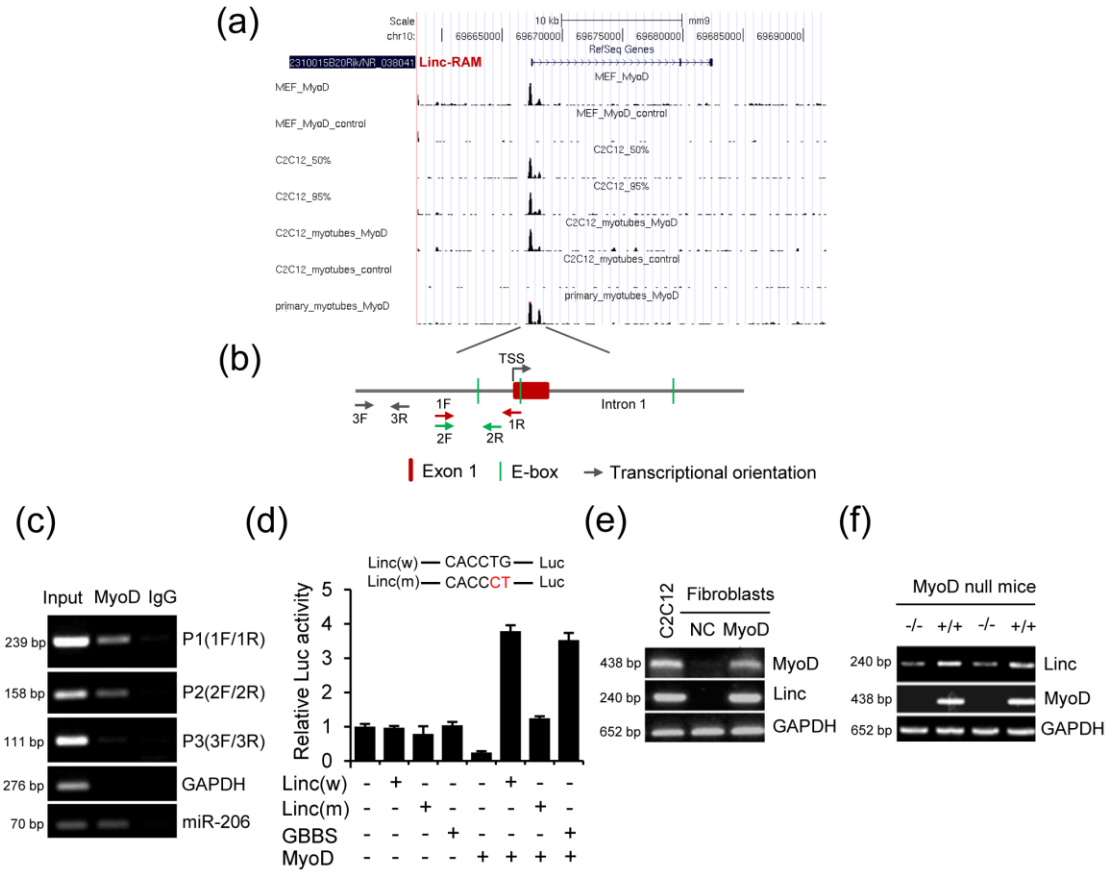


Supplementary Figure 1 | Systematic identification of muscle-expressed, MyoD-regulated lncRNAs. (a) The strategy for identifying lncRNAs that are potentially regulated by MyoD in muscle cells. **(b)** Heatmap representing hierarchical clustering of all genes that displayed a 1.5-fold difference at the transcriptional level between proliferation (GM) and differentiation (60 h, 5d, and 7d in DM). The 2310015B20Rik transcript is also known as NR_038041, termed Linc-RAM in the current study. **(c)** Six linc-RNA candidates in the top list were validated by semi-quantitative RT-PCR. Total RNA were isolated from C2C12 cells grown in either growth medium (GM) with different confluence (50% and 100%) or differentiation medium (DM) for different days (d).



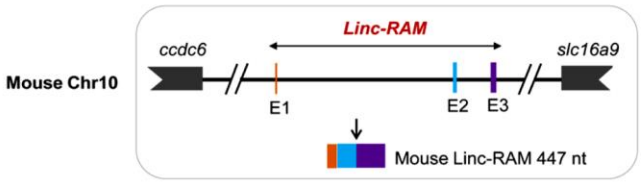
Supplementary Figure 2 | Linc-RAM is a muscle-specifically expressed lncRNA. (a) Expression of Linc-RAM in the indicated tissues was analyzed by Northern blotting. rRNA was used as a loading control. **(b)** Expression of Linc-RAM at the embryonic stage was detected by *in situ* hybridization in E11.5 mouse embryos. MyoD expression was used to demonstrate somite location.



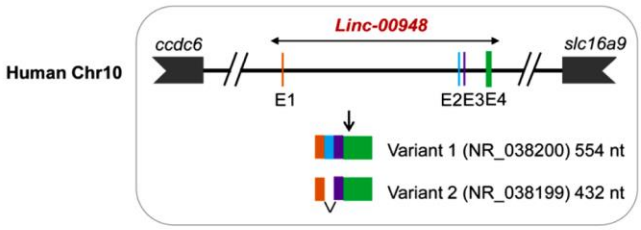
Supplementary Figure 3 | Linc-RAM is transcriptionally regulated by MyoD. (a) MyoD binding sites in the regulatory regions of the Linc-RAM gene were analyzed by using the published MyoD ChIP-Seq data (Cao et al.) **(b)** Identified E-box in the proximal promoter of Linc-RAM gene. The primers for ChIP-PCR analysis indicated as 1F/1R, 2F/2R, and 3F/3R. **(c)** ChIP assays were performed using chromatin from C2C12 cells with MyoD antibodies or control IgG. The precipitated DNA was amplified by real-time PCR with indicated primers P-1 and P-2 (covering the E-box of the *Linc-RAM* promoter) and the primers P3 (negative control covering a region in the *Linc-RAM* promoter lacking an E-box consensus sequence). *GAPDH* and *miR-206* promoter regions were also amplified as negative and positive controls, respectively. The data are representatives of three independent experiments. **(d)** Activities of *Linc-RAM* promoters containing a wild-type (Linc-W) or mutant (Linc-M) E-box were assayed by luciferase reporter assay in C3H-10T1/2 mouse embryo fibroblasts cotransfected with MyoD. The proximal promoter of the *MyoG* gene,

GBBS, a well-documented MyoD-responsive promoter, was used as positive control. The data are representatives of three independent experiments. **(e)** C3H-10T1/2 fibroblasts were transfected with MyoD to induce myogenic conversion, and Linc-RAM expression was subsequently detected by semi-quantitative RT-PCR. Myogenic C2C12 cells were used as a control for Linc-RAM expression. **(f)** Linc-RAM expression in the skeletal muscle of MyoD-knockout ($MyoD^{-/-}$) mice was examined by semi-quantitative RT-PCR. GAPDH served as a loading control.

(a)



(b)



(c)

```
Mouse_Linc-RAM          CAGTGAGCTTTTCGTCCATGGAGAGCAGATGGTGTTTTTGA-AAGGAAGGGCGGCCATTC 59
Human_Linc-RAM_v1_      -----GCCTCCTGATCCACCAAGACCATATGGTGTTTTTCATAAAAGAGAGAGATCATTC 55
                              **  *  ****   *** ** ********** * **   ** * *  *****

Mouse_Linc-RAM          CCAGGACTTTGGACTTGGCTCCGTTGCACCCCTGAACA---GAACCAAC----GTTGCTA 112
Human_Linc-RAM_v1_      CCTTGACTTTGGACTTCGCTT-ATTGAACCCCTGGACAACGGAATCTCCCTCTGTCGCCC 114
                        **  ************ ***   *** ******* ***   *** *  *    ** **

Mouse_Linc-RAM          -----GGAGAACA---------------------------------CCTGTGCCCCAGC 133
Human_Linc-RAM_v1_      AGGCTGGAGTGCAGTGGCACCATCTCGGCTTACCGCAACCTCCGCCTCCTGGGTTCAAAC 174
                             ****  **                                    **** *  * * *

Mouse_Linc-RAM          A---------CTTTGGTC------------------CAG----CAAGG--GC-AGGAGA 158
Human_Linc-RAM_v1_      AATACTCCTACCTTGGCCTCCTGAGTAGCTGGGATTACAGGAATCAAGGTTGCTAAGAGA 234
                        *         * **** *                   ***    *****  ** * ****

Mouse_Linc-RAM          ---------------------------GG--AGAGCTGGAAACTGGTAGAGCTGTGCTAC 189
Human_Linc-RAM_v1_      CCACCTGTGCTGAATACACTTTGTTCTGGCAAGAGCAGAAAACTGAGA-AGCCGTGTTAC 293
                                              **  ***** * ******  * *** *** ***

Mouse_Linc-RAM          CTGCTACCTCCTGAGGCT--CTCAGGGAGCAGGAGAAT-CTGAACATGAGTGGCAAGAGC 246
Human_Linc-RAM_v1_      CTACCACTACCTGGGATTAATTCAGGGAGCGGGATAATTCTGGATATGACTGGTAAAAAC 353
                        ** * **  **** *  *   ********* *** *** *** * **** *** ** * *

Mouse_Linc-RAM          TGGGTACTGATCTCTACTACTTCGCCCCAGAGCCTGGAAGATGAGATTCTGGGACGGCTT 306
Human_Linc-RAM_v1_      TGGATATTAATTTCTACTACTACTCCCAAAAGTCTAGAAGATGAAATTGTGGGAAGACTT 413
                        *** ** * ** ********* * *** * ** ** ******** *** ***** * ***

Mouse_Linc-RAM          CTGAAAATTCTCTTCGTTTTGTTCGTTGACTTAATGTCCATTATGTATGTCGTCATAACG 366
Human_Linc-RAM_v1_      CTAAAAATTTTGTTTGTTATCTTTGTTGACTTAATTTCTATTATATATGTTGTGATAACT 473
                        ** ****** * ** *** * ** ********** ** ***** ***** ** *****

Mouse_Linc-RAM          TCCTAGCAACCTGACTTTCTTTACTCCGTGTGTAAGTTTCAAACCAAATGCGAGCAAATA 426
Human_Linc-RAM_v1_      TCTTAGAAAGCTGACTTCCTTT---CCATGTACA--TTTCAAACTGAATTACAGTGAATA 528
                        ** *** ** ******* ****   ** ***  *  ********  ***   **  ****

Mouse_Linc-RAM          AAGATCTACATTTTAATCTGA----- 447
Human_Linc-RAM_v1_      AAAATTTGTATTTTAACTTGTAAAAA 554
                        ** ** *  *******  **
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(d)

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Mouse_Linc-RAM          CAGTGAGCTTTTCGTCCATGGAGAGCAGATGGTGTTTTTGA-AAGGAAGGGCGGCCATTC 59
Human_Linc-RAM_v2_      -----GCCTCCTGATCCACCAAGACCATATGGTGTTTTTCATAAAAGAGAGAGATCATTC 55
                              **  *  ****   *** ** ********** * **   ** * *  *****

Mouse_Linc-RAM          CCAGGACTTTGGACTTGGCTCCGTTGCACCCCTGAACAGAACCAACGTTGCTAGGAGAAC 119
Human_Linc-RAM_v2_      CCTTGACTTTGGACTTCGCTT-ATTGAACCCCTGGACAGAATCAAGGTTGCTAAGAGACC 114
                        **  ************ ***   *** ******* ****** *** ******* **** *

Mouse_Linc-RAM          ACCTGTGCCCCA-GCACTTTGGTCCAGCAAGGGCAGGAGAGGAGAGCTGGAAACTGGTAG 178
Human_Linc-RAM_v2_      ACCTGTGCTGAATACACTTTGTTCTGGCAAGAGCAG------------AAAACTGAGA- 160
                        ********   *  ******* **  ***** ****              ******  *

Mouse_Linc-RAM          AGCTGTGCTACCTGCTACCTCCTGAGGCT--CTCAGGGAGCAGGAGAAT-CTGAACATGA 235
Human_Linc-RAM_v2_      AGCCGTGTTACCTACCACTACCTGGGATTAATTCAGGGAGCGGGATAATTCTGGATATGA 220
                        *** *** ***** * **  **** *  *   ********* *** *** *** * ****

Mouse_Linc-RAM          GTGGCAAGAGCTGGGTACTGATCTCTACTACTTCGCCCCAGAGCCTGGAAGATGAGATTC 295
Human_Linc-RAM_v2_      CTGGTAAAAACTGGATATTAATTTCTACTACTACTCCCAAAAGTCTAGAAGATGAAATTG 280
                         *** ** * **** ** * ** ********* * *** * ** ** ******** ***

Mouse_Linc-RAM          TGGGACGGCTTCTGAAAATTCTCTTCGTTTTGTTCGTTGACTTAATGTCCATTATGTATG 355
Human_Linc-RAM_v2_      TGGGAAGACTTCTAAAAATTTTGTTTGTTATCTTTGTTGACTTAATTTCTATTATATATG 340
                        ***** * ***** ****** * ** *** * ** ********** ** ***** ****

Mouse_Linc-RAM          TCGTCATAACGTCCTAGCAACCTGACTTTCTTTACTCCGTGTGTAAGTTTCAAACCAAAT 415
Human_Linc-RAM_v2_      TTGTGATAACTTCTTAGAAAGCTGACTTCCTTT---CCATGTACA--TTTCAAACTGAAT 395
                        * ** ***** ** *** ** ******* ****   ** ***  *  ********  ***

Mouse_Linc-RAM          GCGAGCAAATAAAGATCTACATTTTAATCTGA----- 447
Human_Linc-RAM_v2_      TACAGTGAATAAAAATTTGTATTTTAACTTGTAAAAA 432
                           **  ****** ** *  *******  **
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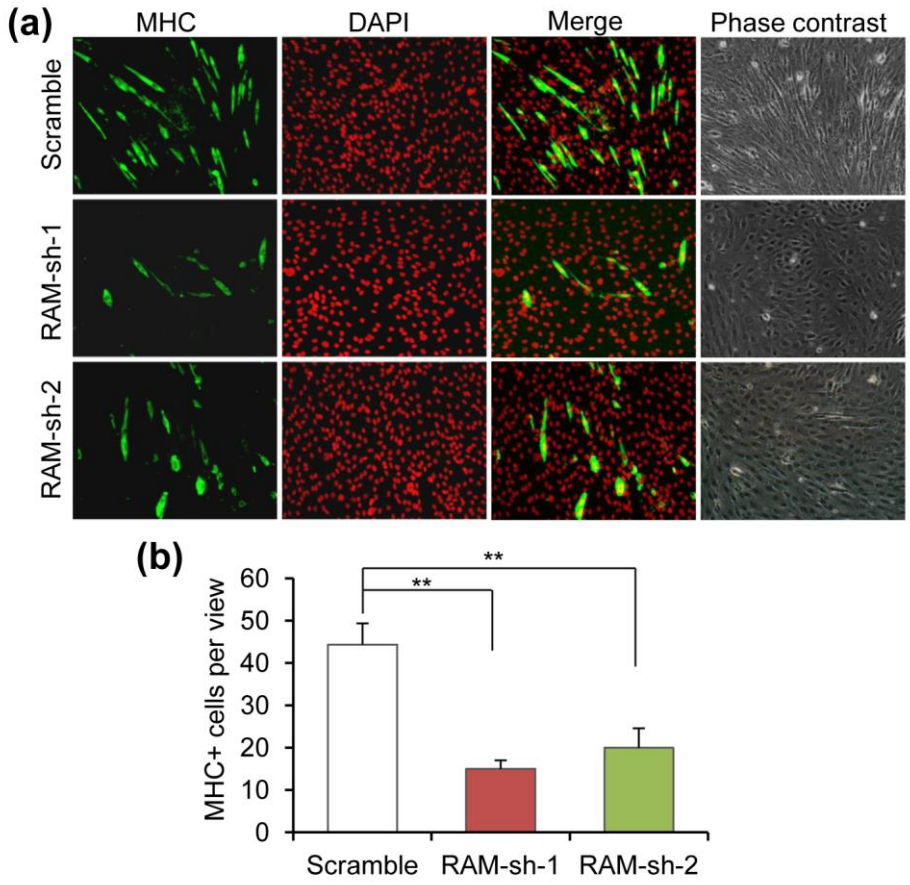
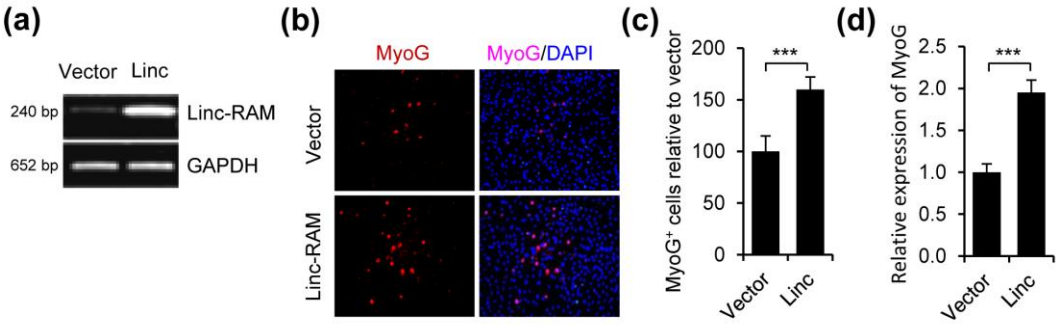
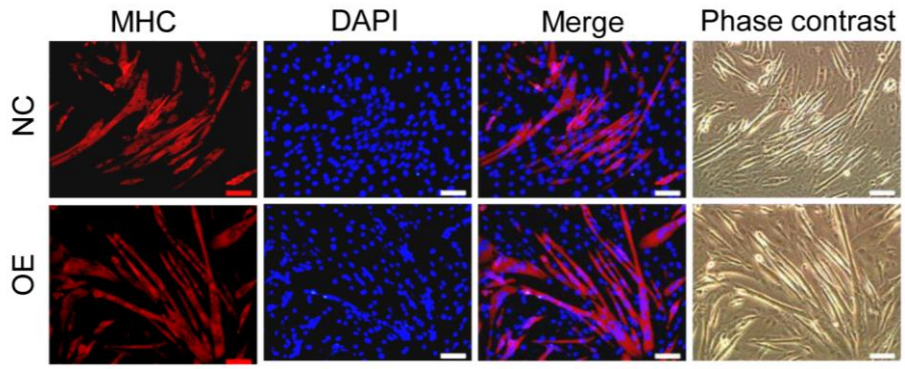
Supplementary Figure 4 | Schematic representations of the flanking genes and genomic structure of mouse Linc-RAM and its human homolog. (a) The upstream of mouse Linc-RAM is *Ccdc6* (coiled-coil domain containing 6) and the downstream gene is *Slc16a9* (solute carrier family 16, member 9). **(b)** Human Linc-00948 and mouse Linc-RAM located at the same syntenic region, suggesting Linc-00948 is a human homolog for mouse Linc-RAM. Exons of Linc-RAM and Linc-00948 are labeled E1, E2, E3, and E4. **(c, d)** Sequence similarity between human Linc-00948 and mouse Linc-RAM



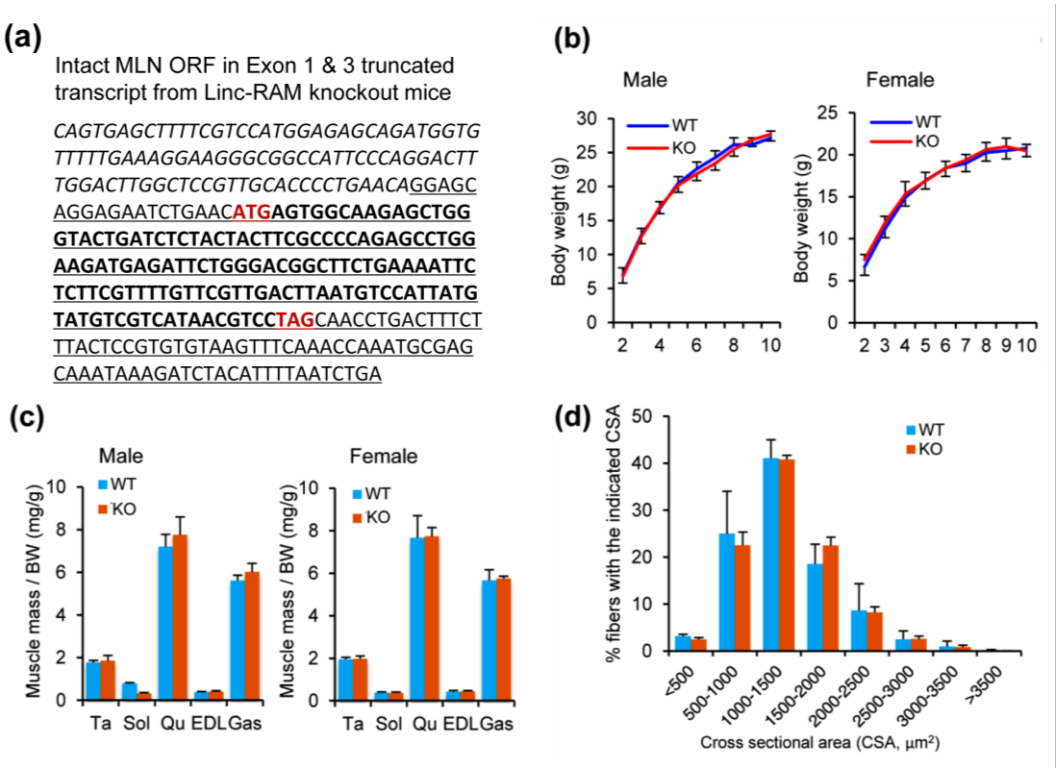
Supplementary Figure 5 | knocking down of Linc-RAM with two independent of sh-RAM significantly attenuate C2C12 cell differentiation. (A) Representative images of MHC immunostaining on C2C12 cells transiently transfected with RAM-sh-1 and RAM-sh-2 in DM for 48 hours. Scramble sequence were used as negative control. (B) MHC+ cell numbers were calculated based on MHC immunostaining in panel A. The data are presented as mean ± s.e.m from three independent experiments. The statistical significance was calculated with the t test. **P < 0.01.



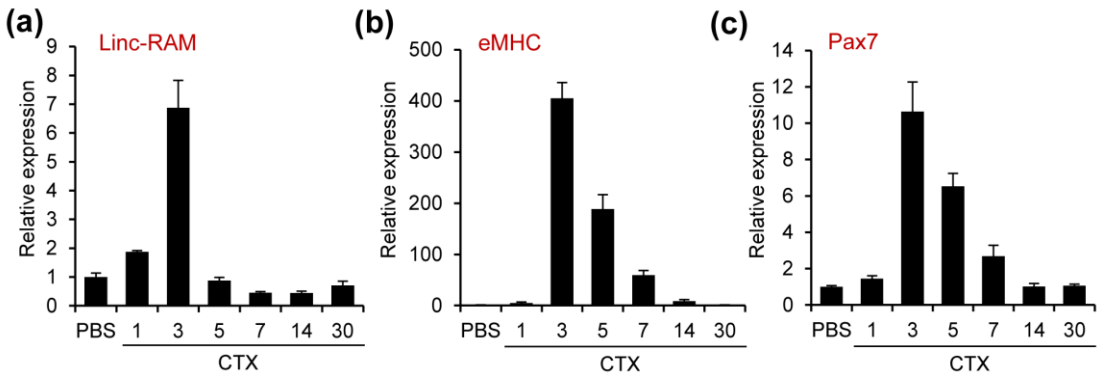
Supplementary Figure 6 | Linc-RAM promotes C2C12 cell differentiation. (**a**) C2C12 cells were transiently transfected with full-length Linc-RAM and harvested after culturing in differentiation medium (DM) for 24 hours. Overexpression of full-length Linc-RAM was examined by semi-quantitative RT-PCR. (**b**) Differentiation of C2C12 cells transfected with full-length Linc-RAM was examined by staining for MyoG after culturing in DM for 24 hours. (**c**) $MyoG^+$ cells in (**b**) were counted and presented as a percentage relative to the negative control (NC) group (defined as 100%). (**d**) MyoG mRNA expression in (**b**) was detected by RT-qPCR. All images in the figure are representatives of three independent experiments. Values are means ± s.e.m of three independent experiments. The statistical significance of the difference between two means was calculated with the *t* test. ***$P < 0.001$.



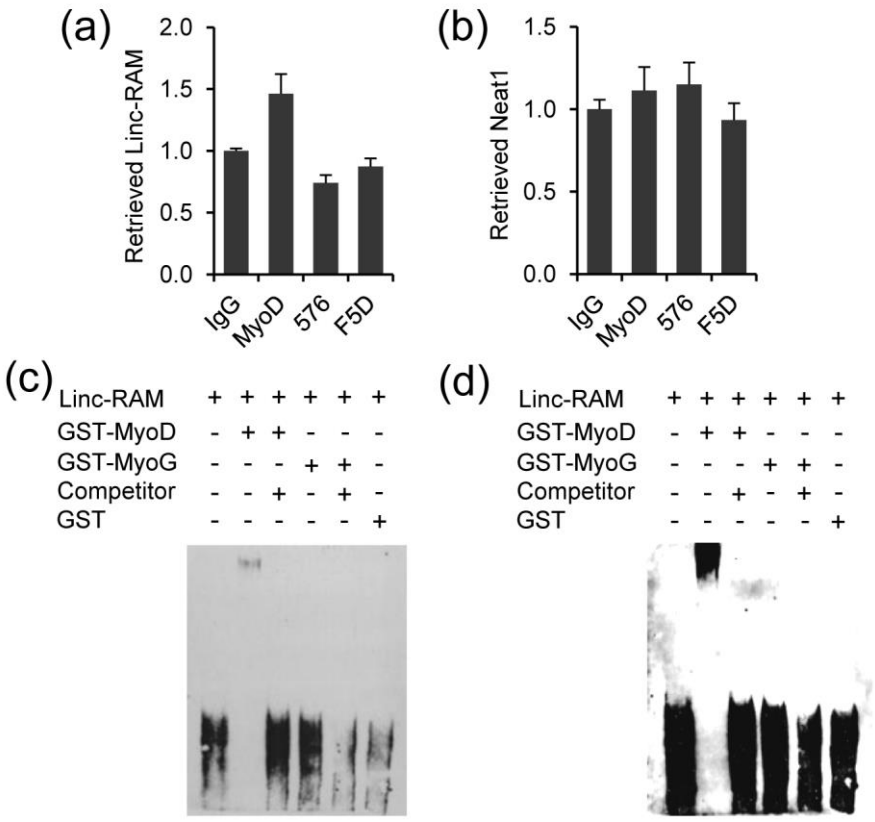
Supplementary Figure 7 | Linc-RAM enhances C2C12 cell differentiation and fusion. The C2C12 cells overexpressing Linc-RAM were differentiated for 48h in differentiation medium. MHC immunostained myotubes and the phase contrast photos were presented. Scale bar, 50μm.



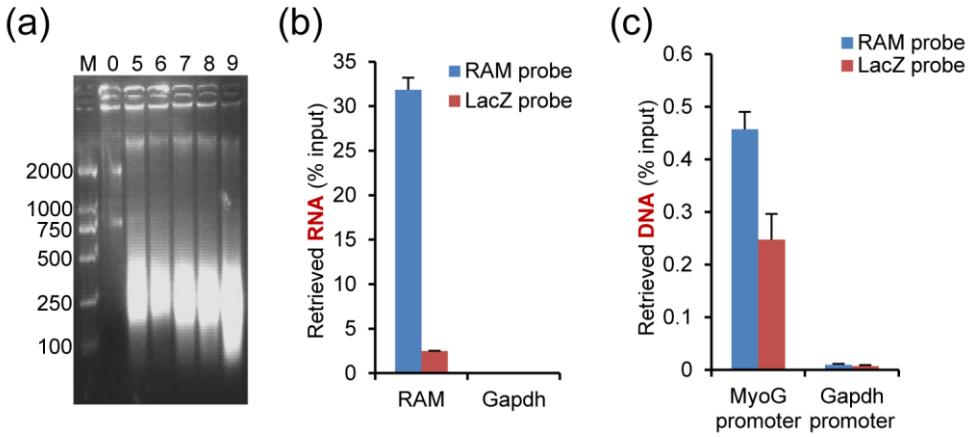
Supplementary Figure 8 | Linc-RAM knockout mice do not exhibit overt abnormalities. (**a**) Strategy for generation of Linc-RAM knockout mice. (**b**) Body weight of Linc-RAM-/- (KO) and their wild type littermates (WT). 10 mice were weighted for each group in both genders. Values are means ± s.e.m. (**c**) Muscle mass of Linc-RAM-/- (KO) and their wild type littermates (WT). Ta is *tibialis anterior*, Sol is *soleus*, EDL is *extensor digitorum longus*, Qu is *quadriceps*, and Gas is *gastrocnemius* muscle, respectively. Values are mean ± s.e.m. n = 8 mice for each group in both genders. (**d**) Cross-sectional area of myofibers in Ta muscle from the WT and KO mice. n = 7 mice of each genotype. Values are means ± s.e.m.



Supplementary Figure 9 | Expression pattern of Linc-RAM during CTX-induced muscle injury and regeneration. Muscle injury was induced by injecting CTX into the right TA muscle of C57BL/6 mice (n=5). PBS injection into the left TA muscle was the control. Damaged muscles were harvested on the indicated days after CTX injury. (**a–c**) Expression of *Linc-RAM* (**a**), *eMHC* (**b**), and *Pax7* (**c**) were determined by real-time RT-PCR. *GAPDH* was the internal control.

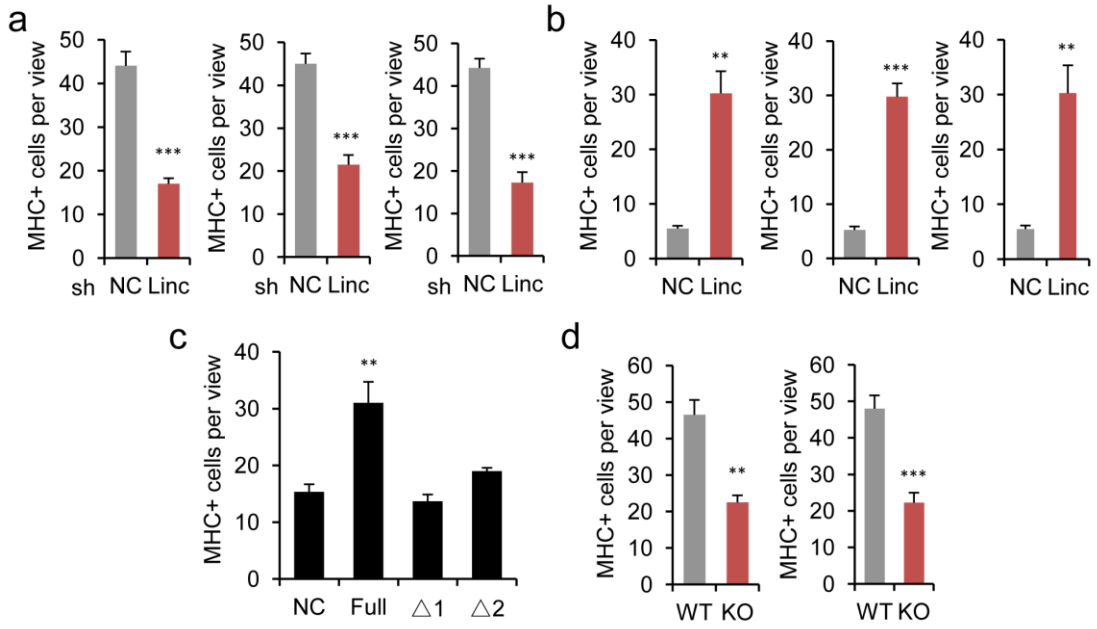


Supplementary Figure 10 | Linc-RAM does not bind MyoG. (a) C2C12 cell lysates were respectively immunoprecipitated using two anti-MyoG (sc576) and anti-MyoG (F5D). Subsequently, Linc-RAM in the immunoprecipitates was detected by real-time RT-qPCR. IgG and MyoD antibodies served as a negative and positive controls, respectively. **(b)** Neat1 as a negative control in the immunoprecipitates described in panel a was detected by real-time RT-qPCR. **(c)** The interaction between Linc-RAM and MyoG was examined by electrophoresis mobility shift assay (EMSA) with purified recombinant GST-MyoG fusion protein. GST-MyoD served as a positive control and GST only served as a negative control. Linc-RAM RNA without Dig-UTP-labeling served as competitor RNA. The presented blot was a representative of three independent experiments. **(d)** Long exposure of the blot presented in panel c of this Figure.



Supplementary Figure 11 | ChIRP analysis demonstrates Linc-RAM binds myogenin gene promoter. (a) Sonicated chromatin was separated on 1.5% agarose gel. The C2C12 cells grown in differentiation medium for 24h were crosslinked with 1% glutaraldehyde and subsequently sonicated for 9 cycles with program (30 seconds sonication, 30 seconds interval, repeat 10 times). M: molecular marker. 0: non-sonicated samples. 5~9: the samples were sonicated for 5~9 cycles, respectively. **(b)** The retrieved RNA by Linc-RAM or LacZ probe from ChIRP experiment was analyzed by real-time RT-PCR with primers against Linc-RAM RNA. Gapdh served as negative control for ChIRP analysis. **(c)** The retrieved genomic DNA by Linc-RAM or LacZ probe from ChIRP experiment was analyzed by real-time PCR with primers against myogenin gene promoter. Gapdh served as negative control.

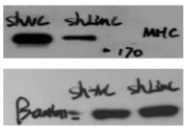
Supplementary Figure 12 | Schematic illustration of the *MyoG* gene promoter.



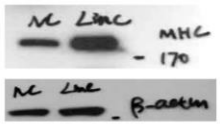
Supplementary Figure 13 | MHC positive cells calculated in three or two independent experiments. (**a**) Individual data from three independent experiments which were related to Figure 1b and 1c in main text. (**b**) Individual data from three independent experiments which were related to Figure 1f and 1g in main text. (**c**) The data were from another independent experiment which were related to Figure 1j and 1k in main text. (**d**) Individual data from additional two independent experiments which were related to Figure 2f and 2g in main text.

Original blots

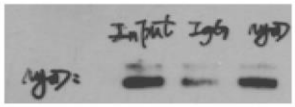
For Fig. 1d



For Fig. 1h



For Fig. 3d



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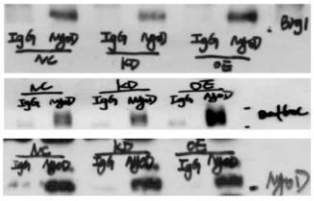
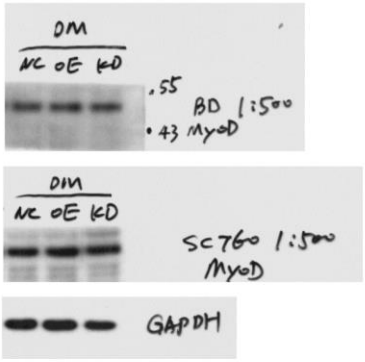
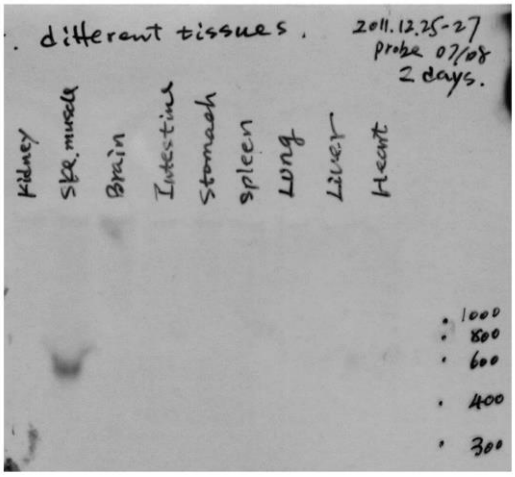


For Fig. 5h



For Supplementary Figure 2



Supplementary Figure 14 | Original blots for the Figures in main text

Supplementary Table 1 Selected genes that are coregulated by Linc-RAM and MyoD from main text Figure 4c.

Growth differentiation factor 15 (*Gdf15*), Cadherin 15 (*Cdh15*), Collagen, type IV, alpha 1 (*Col4a1*), Junction adhesion molecule 2 (*Jam2*), Junctional sarcoplasmic reticulum protein 1 (*Jsrp1*), Integrin alpha 7 (*Itga7*), transmembrane protein 8C (Tmem8c), Cholinergic receptor, nicotinic, delta polypeptide (*Chrnd*).

Function	Symbol	Fold
Differentiation		
	Myod1	2.0
	Myog	5.6
	Gdf15	4.6
Cell adhension and junction		
	Cdh15	1.8
	Col4a1	1.5
	Jam2	2.8
	Jsrp1	3.7
	Itga7	3.3
	Tmem8c	1.9
	Chrnd	2.5

Supplementary Table 2 All primers used in the study

(1) Primers used in Real-time RT-PCR experiments

Gene	**primers**
Tmem8c-FW	ATCGCTACCAAGAGGCGTT
Tmem8c-RV	CACAGCACAGACAAACCAGG
MyoG-FW	GCAATGCACTGGAGTTCGGT
MyoG-RV	GCTGTCCACGATGGACGTAAG
MyoD-FW	CAACGCCATCCGCTACAT
MyoD-RV	GGTCTGGGTTCCCTGTTCT
Col4a1-FW	CAGGACAAAAGGGTGATGCT
Col4a1-RV	CCTTTGTACCGTTGCATCCT
Linc-RAM-RW	GGCGGCCATTCCCAGGACTTTG
Linc-RAM-RV	ATCTTCCAGGCTCTGGGGCGAAG
GAPDH-FW	TGGAGAAACCTGCCAAGTATGA
GAPDH-RV	CTGTTGAAGTCGCAGGAGACA
Baf60c-FW	AGGGGAAGCTCTTGGATGAT
Baf60c-RV	CCATCTGTTTCCTGGGTTGT
Brg1-FW	CTGCGTAAGATCTGCAACCA
Brg1-RV	TTGGCAAAAGAGGAGCACTT
MHC-FW	GATGGCACCGAAGTTGCTG
MHC-RV	TACTCATTGCCGACCTTGACC
miR-133b-FW	TTTGGTCCCCTTCAACCAGCTA
miR-133-RV	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACT GGATACGACTAGCTG
miR-206-FW	TGGAATGTAAGGAAGTGTGTGG
miR-206-RV	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACT GGATACGACCCACAC
Common Reverse primer for miR	GTGCAGGGTCCGAGGT
Pax-7-FW	CCGTGTTTCTCATGGTTGTG
Pax-7-RV	GAGCACTCGGCTAATCGAAC
eMHC-FW	TCTAGCCGGATGGTGGTCC
eMHC-RV	GAATTGTCAGGAGCCACGAAA
Neat1-FW	GGGAAGGGTGACATTGAAAA
Neat1-RV	CTCCCCAGCTTCACTTCTTG

(2) Primers used in semi-quantitative RT-PCR

Gene	**primers**
GAPDG-FW	AATGTGTCCGTCGTGGATCTG
GAPDH-RV	TAGCCCAAGATGCCCTTCAGT
MyoD-FW	CAACGCCATCCGCTACAT

MyoD-RV	GGTCTGGGTTCCCTGTTCT
Neat1-FW	GGGAAGGGTGACATTGAAAA
Neat1-RV	CTCCCCAGCTTCACTTCTTG
1700096K18Rik-FW	CAGCGCACTACTTGTCGCGGA
1700096K18Rik-RV	CCCCGCCGTGGTCCCCTAAG
E130317F20Rik-FW	CGGTCGCCCTAAGCCAACCG
E130317F20Rik-RV	GACGGGTAAGTCCTGCCGCG
Btbd19-FW	CGTGGGCGCCGTTGAACAGA
Btbd19-RV	CCAACGCAGAGCACCTCGGG
2310043L19Rik-FW	GCACCTTGAGTGGTGACAGGGC
2310043L19Rik-RV	ACACAGAGGTGGGCCTCAACGT
A530013C23Rik-FW	CCCAGGGGCAACGCTTCACG
A530013C23Rik-RV	AGCTCCGCGCTGTTTGAGCAA

(3) Primers used in ChIP experiments

P1-FW (linc-RAM)	CTCCCTGCAGAAAAGGAGTG
P1-RV (linc-RAM)	CCGAGTGTTAGGCAGAAACC
P2-FW (linc-RAM)	AGGCAAGCAAACAGGCTAGA
P2-RV (linc-RAM)	GGGTGTTTAAGGAGGGCAAT
P3-FW (linc-RAM)	CTCCCTGCAGAAAAGGAGTG
P3-RV (linc-RAM)	TGCCTTAAGTCCCCATGGTA
MyoG promoter-FW	GAATCACATGTAATCCACTGGA
MyoG promoter-RV	ACGCCAACTGCTGGGTGCCA
miR-206 promoter-FW	GGAGTGATTGAGGTGGACAGA
miR-206 promoter-RV	CTCTCCCACCTGTTTGTGTCTT
GAPDH promoter-FW	AAGCCAAACTAGCAGCTAGG
GAPDH promoter-RV	GGGCTAGTCTATCATTGCAG

(4) Primers for plasmid construction

Privus-RAM-OE-FW	GGAATTCCCAGTGAGCTTTTCGTCCATGG
Privus-RAM-OE-RV	ATAAGAATGCGGCCGCTAAACTATTCAGATTAAAATG TAGATCT
Privus-Δ1-FW	GGAATTCCCAGTGAGCTTTTCGTCCATGG
Privus-Δ1-RV	ATAAGAATGCGGCCGCTAAACTATCTGAGAGCCTCAG GAGGTAG
Privus-Δ2-FW	GGAATTCCGAACCAACGTTGCTAGGAGA
Privus-Δ2-RV	ATAAGAATGCGGCCGCTAAACTATTCAGATTAAAATG TAGATCT

Lv-RAM-OE-full-FW	GGAATTCCCAGTGAGCTTTTCGTCCATGG
Lv-RAM-OE-full-RV	ATAAGAATGCGGCCGCTAAACTATTCAGATTAAAATG TAGATCT
MyoG promoter-FW	CGACGCGTCGGCTGAGCAGGAAAGAGAAGG
MyoG promoter-RV	GAAGATCTTCATAGAAGTGGGGCTCCTGGT
RAM promoyer-FW	GGGGTACCCCCTCCAGCAGGCCTGATATTC
RAM promoyer-RV	CCCAAGCTTGGGGAGCCAAGTCCAAAGTCCTG
MyoD overexpressing-FW	GGAATTCCGACAGGGAGGAGGGGTAGAG
MyoD overexpressing-RV	AAGGAAAAAAGCGGCCGCAAAAGGAAAAAGGGCTCC AGAAAGTGACAA

Supplementary Table 3 Probes for RNA-FISH and ChIRP analysis

(1) The set of probes against the Linc-RAM RNA sequence used for FISH imaging. All of the probes were labeled with a Cal 610 Fluor® Red (Cal610) flurophore at the 3’end.

1	ctgctctccatggacgaa
2	cgcccttcctttcaaaaa
3	ccaaagtcctgggaatgg
4	gggtgcaacggagccaag
5	gcaacgttggttctgttc
6	tggggcacaggtgttctc
7	ccttgctggaccaaagtg
8	agtttccagctctcctct
9	caggtagcacagctctac
10	tgagagcctcaggaggta
11	gttcagattctcctgctc
12	atcagtacccagctcttg
13	ctggggcgaagtagtaga
14	gaatctcatcttccaggc
15	attttcagaagccgtccc
16	gtcaggttgctaggacgt
17	tgctcgcatttggtttga

(2) Probes for Linc-RAM ChIRP analysis were labeled with BiotinTEG at the 3’end

#	Probe (5'-> 3')
RAM1	ccatggacgaaaagctcact
RAM2	aaaaacaccatctgctctcc
RAM3	aagtccaaagtcctgggaat
RAM4	ttctgttcaggggtgcaacg
RAM5	acaggtgttctcctagcaac
RAM6	cttgctggaccaaagtgctg
RAM7	tagcacagctctaccagttt
RAM8	cactcatgttcagattctcc
RAM9	agtagtagagatcagtaccc
RAM10	agaatctcatcttccaggct
RAM11	agaattttcagaagccgtcc
RAM12	ggacgttatgacgacataca
RAM13	ggtttgaaacttacacacgg