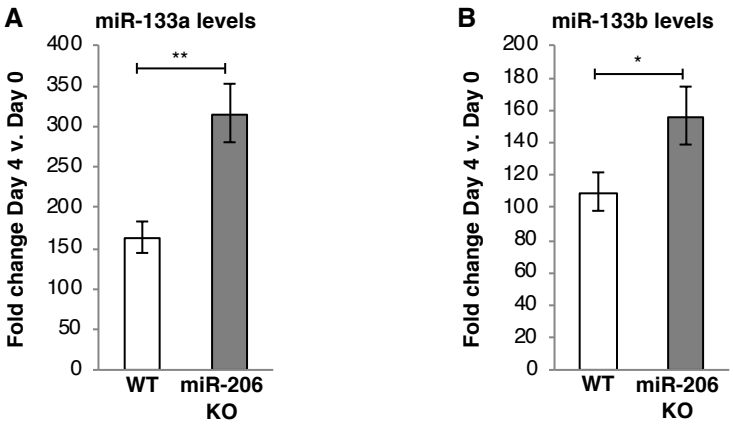
Supplementary Materials

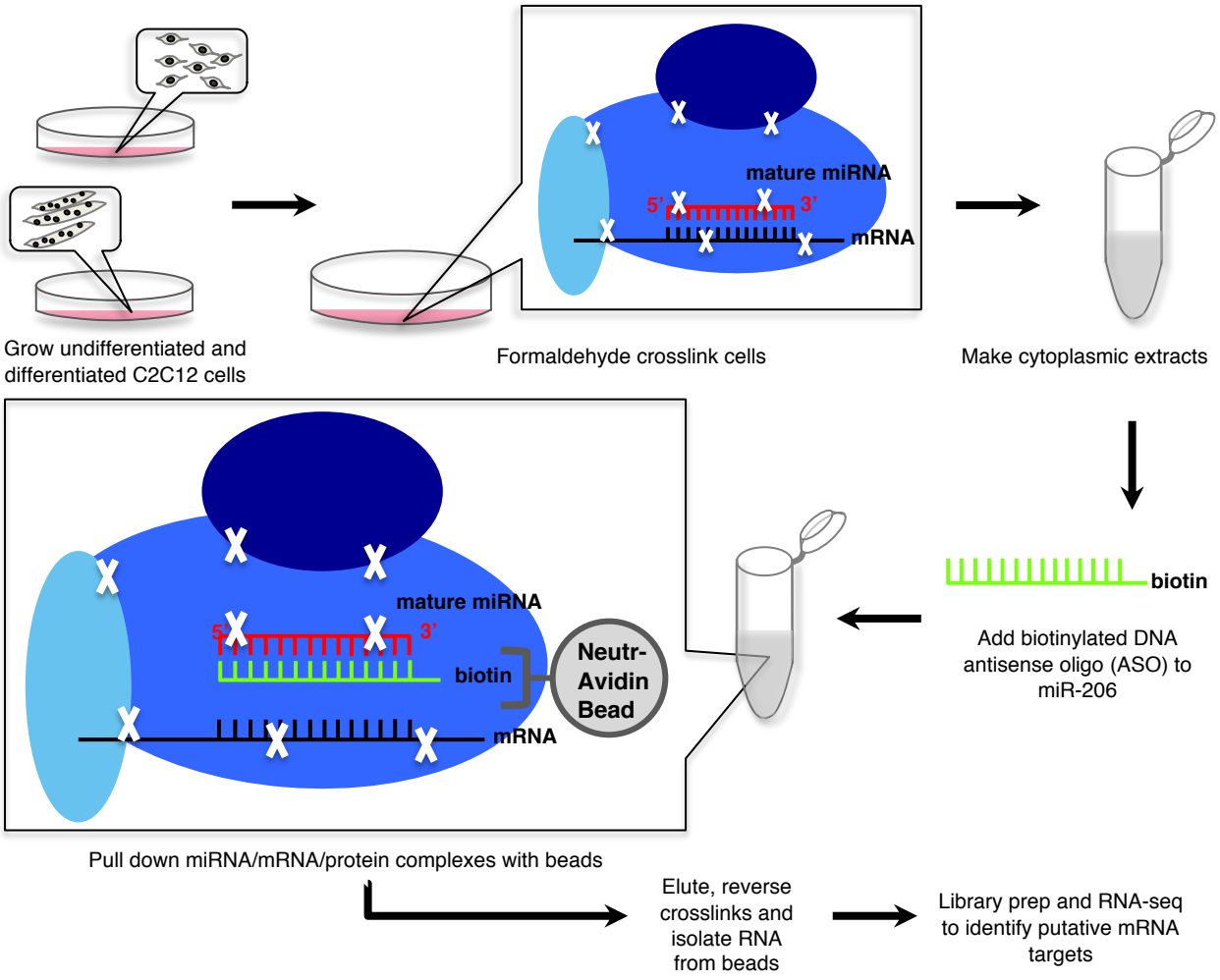
miR-206 knockout shows it is critical for myogenesis and directly regulates newly identified target mRNAs

Georgiana M. Salant, Kimngan L. Tat, James A. Goodrich, and Jennifer F. Kugel

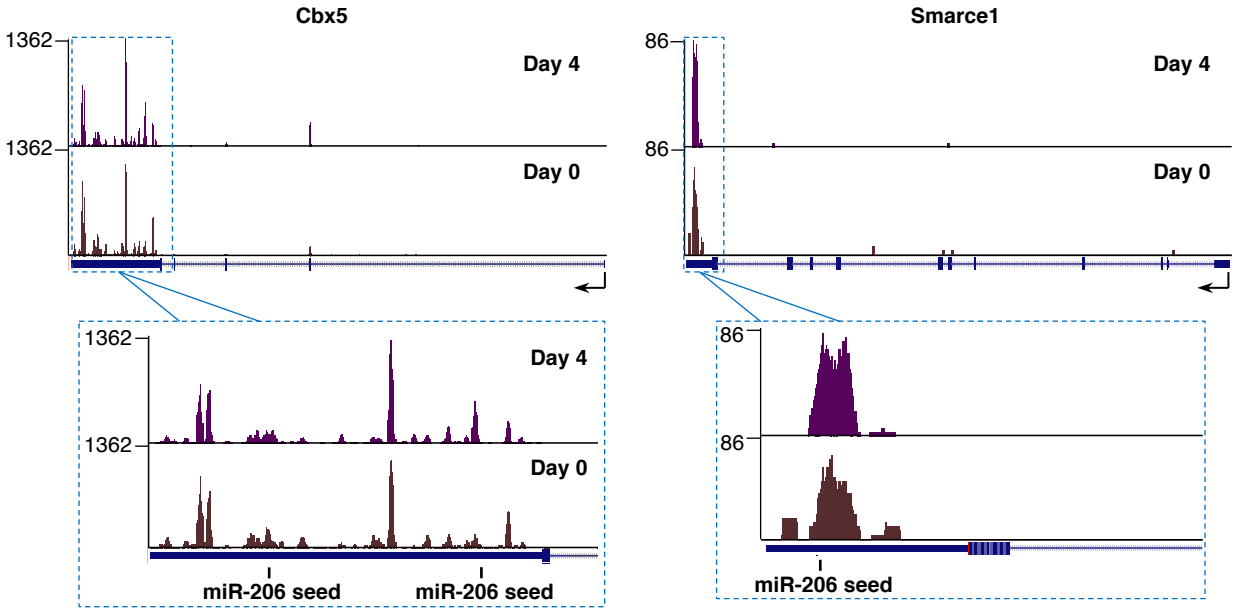
Supplemental Figures 1-3
Supplemental Tables 1-3



Supplemental Figure 1. miR-133a/b levels are upregulated after growth in differentiation media in both the WT and miR-206 KO cell lines. miR-133a (A) and miR-133b (B) expression are induced to a higher level in the miR-206 KO cells compared to WT after four days in differentiation media. Average fold change in miR-133a and miR-133b expression, normalized to control sno202 RNA levels, on Day 4 relative to Day 0 is plotted. The error bars denote the standard deviations (n=3). * indicates a p-value < 0.05 and ** indicates a p-value < 0.01, as determined from an unpaired two-tailed t-test.



Supplemental Figure 2. Schematic of xOP-seq method workflow. We developed/optimized this technique to identify the mRNA targets of a specific miRNA. xOP (crosslinking oligo-purification) is an affinity purification technology using antisense oligos to isolate individual endogenous miRNAs and their associated biomolecules from crosslinked cells (the white X's represent crosslinks). Crosslinking the cells creates a network of protein/RNA interactions that allow us to anneal an antisense oligo to an miRNA under stringent conditions and pull down complexes. Ultimately, mRNAs present in xOP-purified miRNA complexes can be identified using Illumina sequencing.



Supplemental Figure 3. xOP sequencing tracks for Cbx5 and Smarce1 show mapped reads enriched in the 3'UTRs of the genes. Reads pile up to form peaks over or near miR-206 seed sequences in the 3'UTRs of these genes. Sequencing tracks were created from the UCSC Genome Browser display of bedgraph files from replicate 1 mapped reads.

Gene name	Replicate 1 Day 4	Replicate 1 Day 0	Replicate 2 Day 4	Replicate 2 Day 0
A330074K22Rik	4.9	1.3	4.7	0.0
Abraxas2	57.5	17.8	68.6	29.6
Adam19	91.4	36.4	117.9	38.8
Adamts2	19.8	7.3	17.6	2.9
Adamtsl5	14.7	1.9	10.6	0.0
Adcy9	10.8	1.7	8.0	2.3
Adgrg6	12.3	4.6	16.8	2.4
Agap1	20.0	4.1	22.5	9.0
Apc	10.4	3.6	13.3	3.8
Arhgap23	9.8	2.0	7.5	0.0
Arhgap28	10.8	1.7	11.1	0.0
Arhgap28	10.7	1.7	11.1	0.0
Asb1	27.1	12.7	31.6	13.7
Atf5	296.1	127.6	519.8	155.3
Atf5	168.0	74.0	269.4	88.7
Atf6	90.4	25.4	113.3	30.1
AU040320	10.2	0.0	14.6	3.5
Bgn	451.8	189.3	522.1	176.0
C1qtnf3	40.3	8.7	61.3	4.6
Camk2a	109.7	1.5	123.8	2.0
Camsap1	18.2	6.3	11.6	3.5
Ccbe1	3.9	0.6	7.6	3.1
Ccl9	12.2	4.2	11.7	1.4
Cdkn1a	564.3	101.7	758.5	48.6
Cds2	40.7	15.8	36.9	15.1
Cdyl2	12.9	5.7	18.7	3.7
Chid1	5.2	0.0	8.2	2.3
Chrna1	71.7	15.3	76.3	14.1
Cln5	34.9	16.1	37.2	15.8
Cnnm4	9.2	1.2	6.6	1.6
Cnp	10.6	0.0	15.3	3.6
Cpeb4	64.6	24.7	83.1	16.6
Csdc2	10.5	0.0	17.2	0.0
Daam2	63.9	10.4	67.1	12.2
Dclk1	21.6	2.4	14.4	1.3
Dclk1	21.1	2.4	14.1	1.2
Dennd5b	19.1	5.1	11.9	4.8
Dgkd	110.3	21.9	130.9	33.7
Dip2c	20.7	9.0	29.8	4.7
Dnal1	28.3	13.2	36.6	10.0
Dnmt3a	20.2	7.8	11.5	5.1
Drp2	23.9	11.4	29.7	11.4
Emp2	116.6	56.3	125.4	48.2
Enah	40.4	12.9	48.6	14.0
Endov	12.4	3.2	9.5	3.4
Ezh1	41.1	10.0	42.1	16.8
Fam53b	10.5	1.5	10.8	0.0
Fbn1	194.1	86.5	212.9	65.1
Fbxw7	15.1	1.6	11.6	4.1
Fem1a	30.1	11.5	32.4	9.3
Foxj2	9.9	2.1	11.5	2.7
Galnt6	21.8	0.0	29.3	1.0
Ghitm	206.2	88.9	229.1	74.1
Gpd1l	48.9	18.7	40.7	16.6
Grpel2	17.2	5.5	15.8	7.1
Gstm2	28.8	0.0	27.7	9.8
Gys1	84.0	18.7	61.4	13.6
H19	261.8	96.7	320.5	74.8
Hdac4	11.5	4.0	15.8	5.2
Heyl	18.5	5.6	22.2	5.2
Hif1an	63.5	23.4	65.7	20.6
Hivep2	12.2	1.6	17.5	2.1
Igf2	272.3	26.4	341.7	12.3
Igfbp5	368.7	109.7	435.3	85.9
Jph2	133.7	32.4	151.2	18.2
Klhl31	56.3	4.3	73.2	6.4
Ksr1	23.7	9.5	29.7	9.9
Limd1	61.7	27.0	95.6	28.0
Lrig2	15.2	6.9	16.5	7.2
Lynx1	33.3	9.2	59.7	3.0
Map1a	52.8	18.0	57.7	14.9
Mapkbp1	15.7	2.5	11.6	4.9
Marc2	64.3	28.7	52.9	16.7
Mef2d	61.6	14.3	69.6	20.7
Mical3	8.8	0.8	13.8	1.1
Micu2	68.3	31.6	121.5	20.6
Mllt11	42.7	14.3	55.6	14.5
Mlst8	7.7	1.1	6.3	1.5
Mtf1	22.9	9.1	36.1	7.5
Mylk4	59.2	8.3	79.8	6.2
Ndrg1	92.0	23.5	83.7	28.5
Nrbp2	8.4	3.5	9.7	2.3
Nsmce2	18.7	7.3	20.2	9.5
Nuak1	70.0	18.7	77.1	26.7
Patl1	5.7	1.5	5.5	0.0
Pbxip1	64.0	25.0	72.5	17.1
Pcnx	8.4	3.3	9.1	1.4
Pde7a	23.7	10.9	31.8	11.3
Phf8	6.2	1.1	9.9	2.8
Pkp1	147.6	36.9	130.0	27.2
Pkp1	147.4	36.9	129.8	27.1
Pmaip1	6.9	2.4	24.2	4.7
Pnmal2	45.7	7.4	35.7	7.8
Popdc3	79.8	27.7	112.3	36.2
Prkaa2	16.7	3.2	23.8	2.6
Pvr	6.5	1.7	12.4	2.2
Qsox1	81.4	38.5	99.4	33.5
Rab5b	14.9	6.6	12.3	4.3
Rassf2	17.7	4.4	19.0	2.9
Rgma	6.7	0.0	15.4	0.0
Rgp1	23.8	11.2	24.0	10.8
Sema6a	16.0	5.8	23.0	3.3
Serpinb1a	27.5	0.0	33.1	0.0
Setbp1	7.2	2.7	8.9	2.8
Sft2d2	50.9	21.7	57.6	12.5
Sh3pxd2a	88.1	42.7	85.2	30.9
Sidt2	16.1	7.4	20.6	9.7
Slc16a2	8.6	1.1	12.3	0.0
Slc39a3	11.4	3.2	13.9	0.0
Slc7a5	89.8	19.2	55.8	18.0
Sort1	24.0	0.6	19.1	0.8
Spg20	124.1	58.2	168.5	55.6
Srl	337.2	20.8	416.7	11.8
Srl	337.4	31.3	419.1	9.1
Srr	37.5	16.0	48.7	12.0
Stard5	9.9	1.5	5.5	0.0
Stbd1	261.0	114.3	290.1	93.4
Stk25	13.0	3.0	5.5	2.0
Stom	28.1	11.1	41.0	3.6
Stx17	21.0	9.8	29.2	8.6
Susd6	133.4	47.6	143.5	30.2
Tceanc2	8.3	1.0	6.2	0.0
Tex2	15.4	7.1	19.8	4.7
Tmcc3	33.1	9.3	48.9	10.4
Tnfrsf23	11.9	0.0	7.0	1.2
Tnfrsf23	11.8	0.0	7.0	1.2
Tnfrsf23	11.7	0.0	6.9	1.2
Tom1l2	13.3	6.2	14.2	6.0
Top1	28.9	12.9	55.6	25.3
Tpm2	142.4	30.8	148.7	23.2
Trp53inp2	172.7	56.2	209.0	43.4
Ttc9	27.1	7.9	26.7	6.9
Usp20	10.7	4.4	12.3	2.9
Zfp106	94.2	41.4	113.2	31.2
Zfp322a	20.1	8.0	17.8	6.3
Zfp36l1	24.6	10.0	18.4	7.4
2010111I01Rik	8.7	1.9	7.2	1.7
2010111I01Rik	18.2	7.1	21.8	0.0

Supplemental Table 1. Putative miR-206 targets identified through xOP-seq. Shown are the normalized reads per kilobase in the 3'UTR for the 130 putative miR-206 targets identified via xOP-seq. Data for each replicate at Day 0 and Day 4 time points are shown.

TERM	GENE COUNT	%	P-value	Benjamini
intracellular signaling transduction	35	26.9	6.8E-6	1.8E-2
signal transduction	60	46.2	4.2E-5	5.5E-2
signaling	61	46.9	1.8E-4	9.0E-2
single organism signaling	61	46.9	1.5E-4	9.4E-2
regulation of response to stimulus	39	30.0	2.6E-4	9.5E-2
cell communication	61	46.9	2.4E-4	1.0E-1
regulation of cellular protein metabolic process	30	23.1	3.6E-4	1.0E-1
regulation of molecular function	29	22.3	4.1E-4	1.0E-1

Supplemental Table 2. Eight biological pathways were enriched for the list of 130 putative miR-206 targets identified by xOP-Seq. Biological pathways (category GOTERM_BP_ALL) were identified by DAVID's Functional Annotation tool. Enriched terms had Benjamini-Hochberg corrected p-values ≤ 0.1. Term refers to the enriched biological pathway. Gene count refers to the number of genes out of 130 in each term. Percent (%) refers to the percent of genes out of 130 in term. P-value refers to the uncorrected p-values for each enriched term. Benjamini refers to the Benjamini-Hochberg corrected p-values for each term.

Gene name	Forward sequence	Reverse sequence
Adam19	ACATGACCAGGATGCCACCAAACG	CACACCTCCAAGCCGACAAGTGC
Bgn	ATGACTTCAAAGGCCTCCAGCACC	AGTTTTTGCAGCTTCCGCAGAGGGC
Cbx5	TGTTGGACAGGCGCATGGTTAAGG	GACAATCCAAGTTCTTCTCAGGTTCCC
Ccnd1	GTGCAGAAGGAGATTGTGCCATCC	GCGGTCCAGGTAGTTCATGGC
Csnk2a2	GAGCTTGGGCTGCATGTTAGCG	GTTCATCTGTCCCCAGAACCTTGGC
Gja1	GTCAACGTGGAGATGCACCTGAAGC	TGCTGATGATGTAGGTTCTCAGCAGG
Hdac4	CAATGCCAATGCTGTCCACTCCATGG	CTTTTGCGCCTCAATCAGAGAGTGC
Smarce1	GAAGCCGAGCTCCTTCAGATAGAGG	GATCTCAGCCGCAATCTTCTCCATG
Spg20	CTCAACTGTCTGGCAAGGGTTGG	GTAGCTTCTCCTGCGTTGTGCC
Tmcc3	CAGGCCTATGAGCGCTCAAGG	TTCACGGCATCCGTCTGCAGG

Supplemental Table 3. qPCR Primers. Sequences are written 5' to 3'.