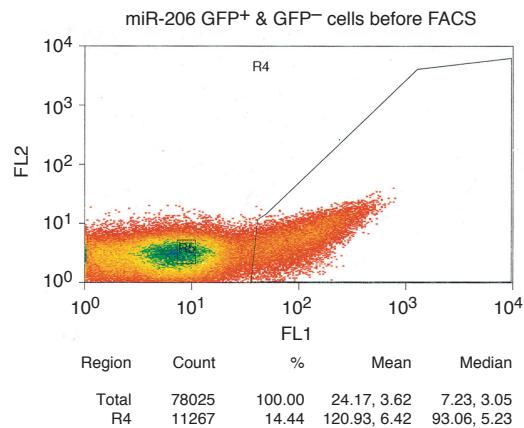
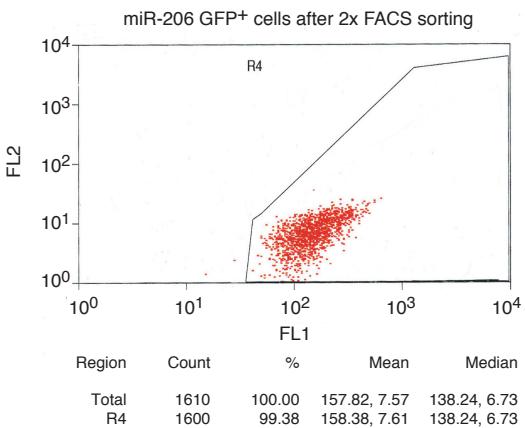


Supplementary Figure 1: Shkumatava et al.

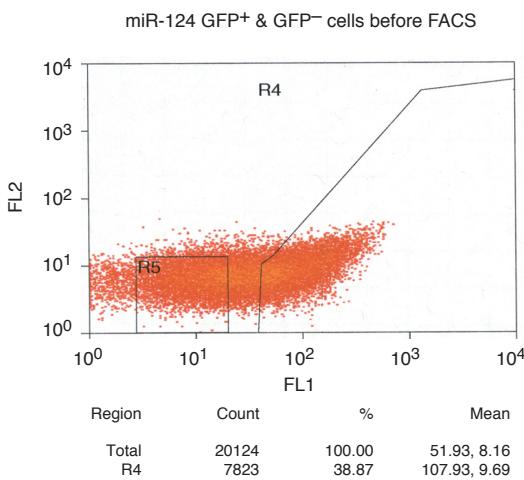
A



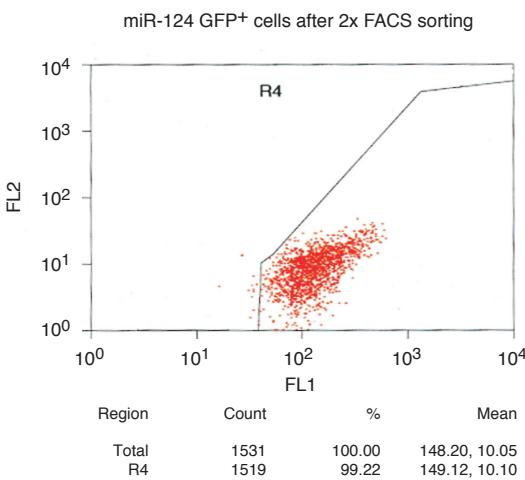
B



C



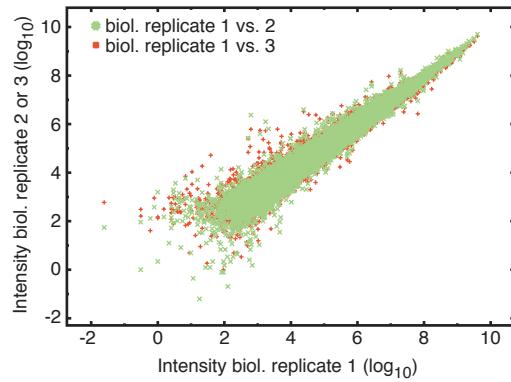
D



Supplementary Figure 2: Shkumatava et al.

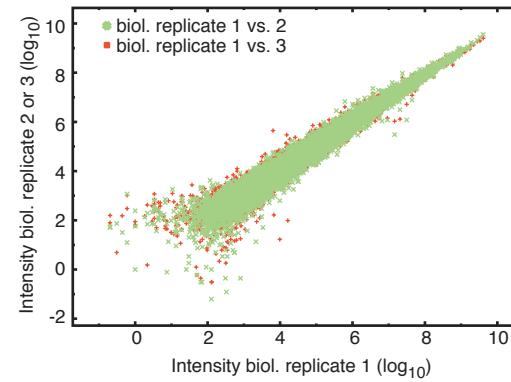
A

miR-206 GFP<sup>+</sup>

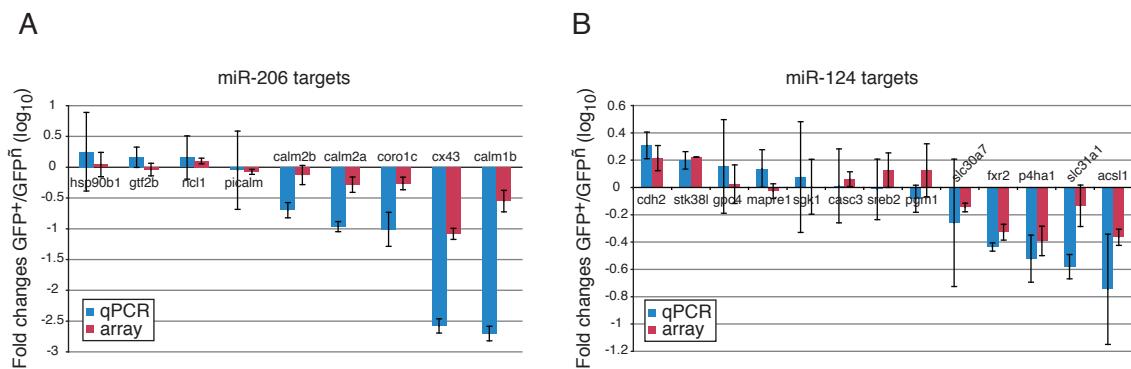


B

miR-124 GFP<sup>+</sup>

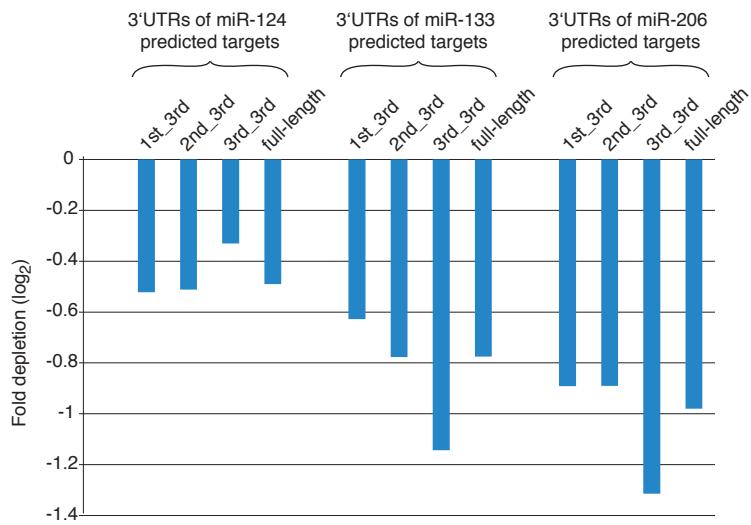


Supplementary Figure 3: Shkumatava et al.

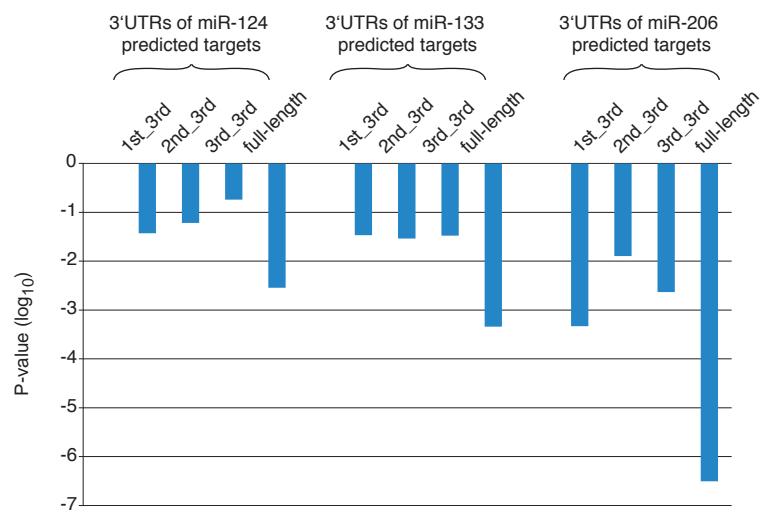


Supplementary Figure 4: Shkumatava et al.

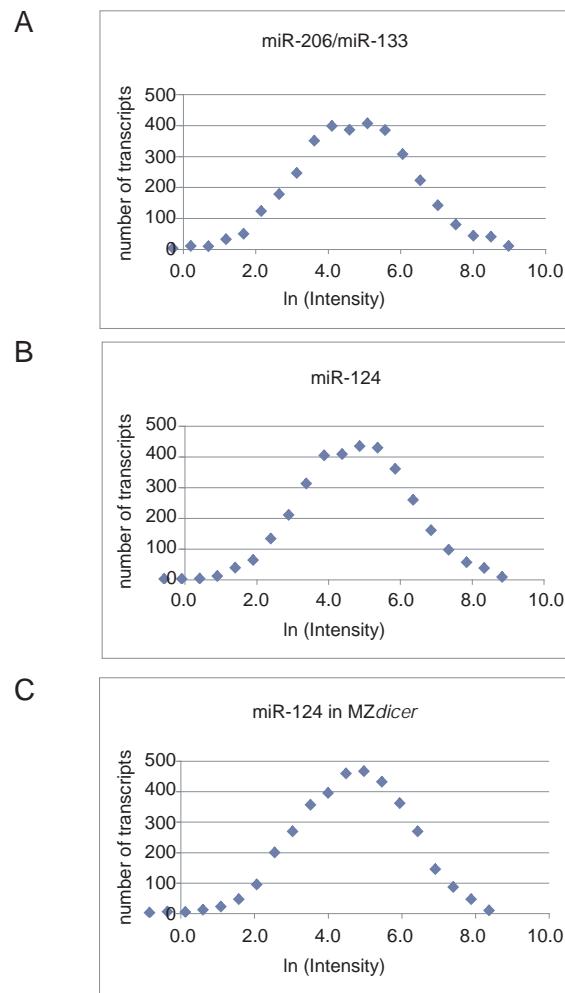
A



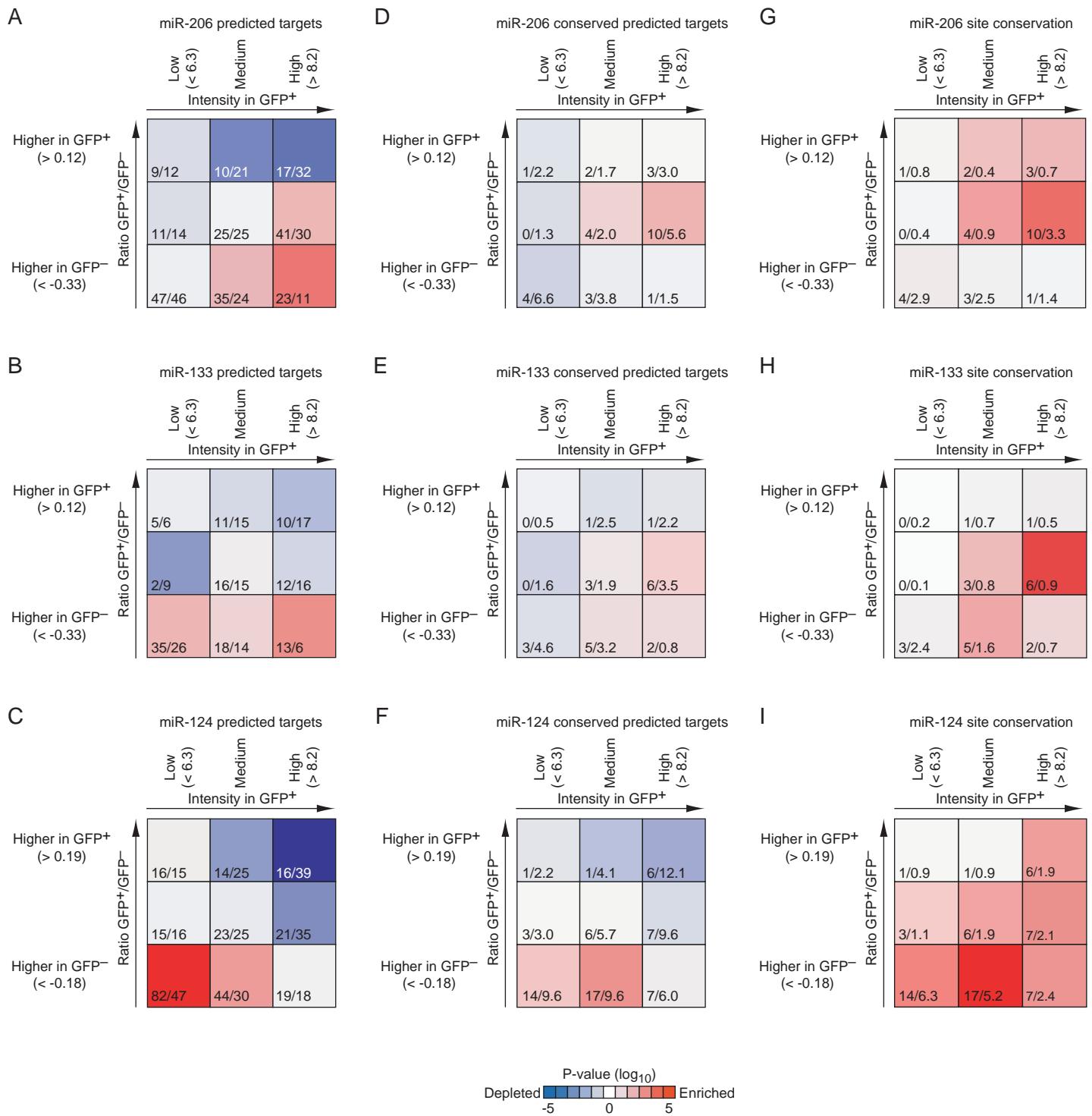
B



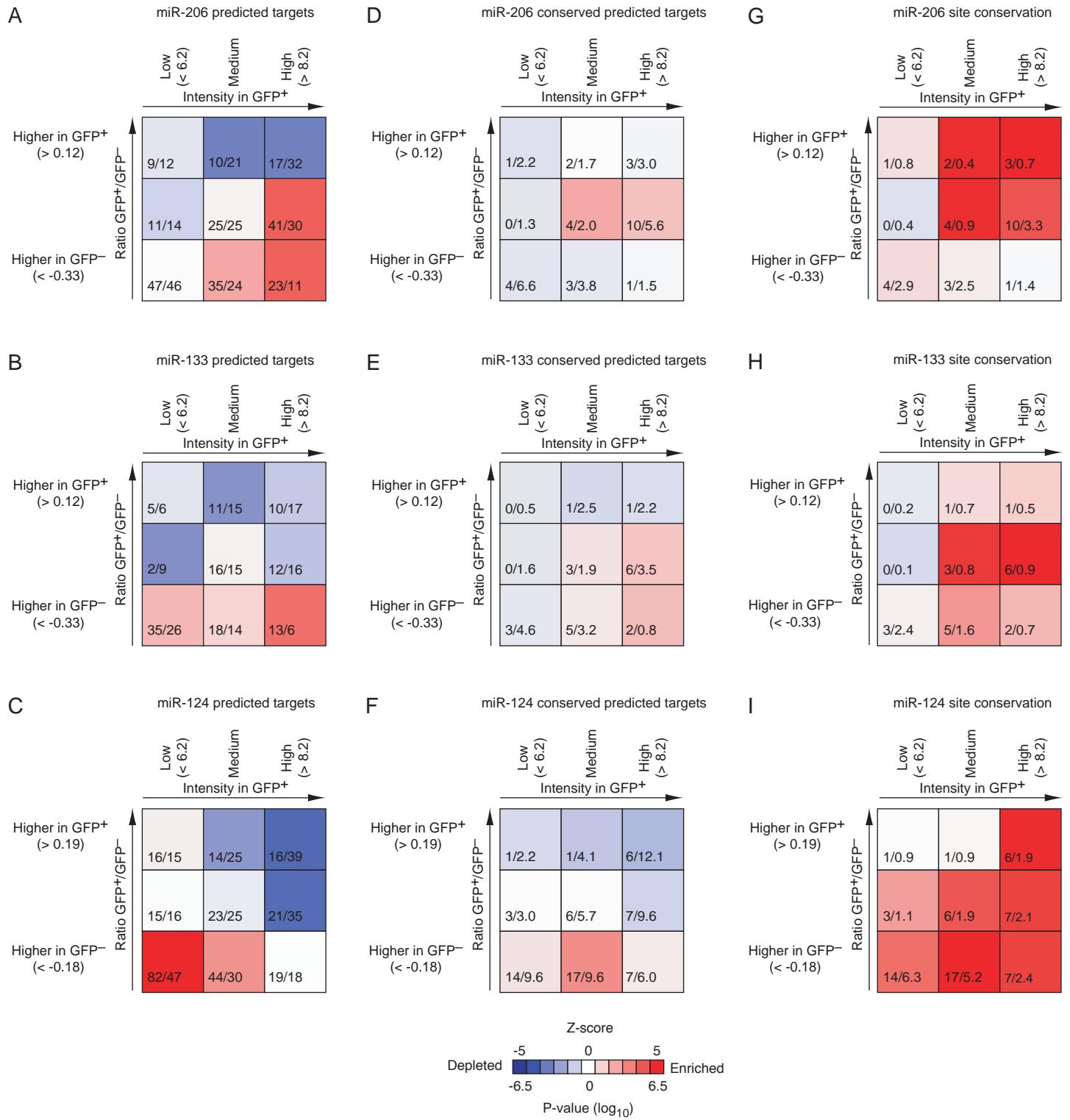
Supplementary Figure 5: Shkumatava et al.



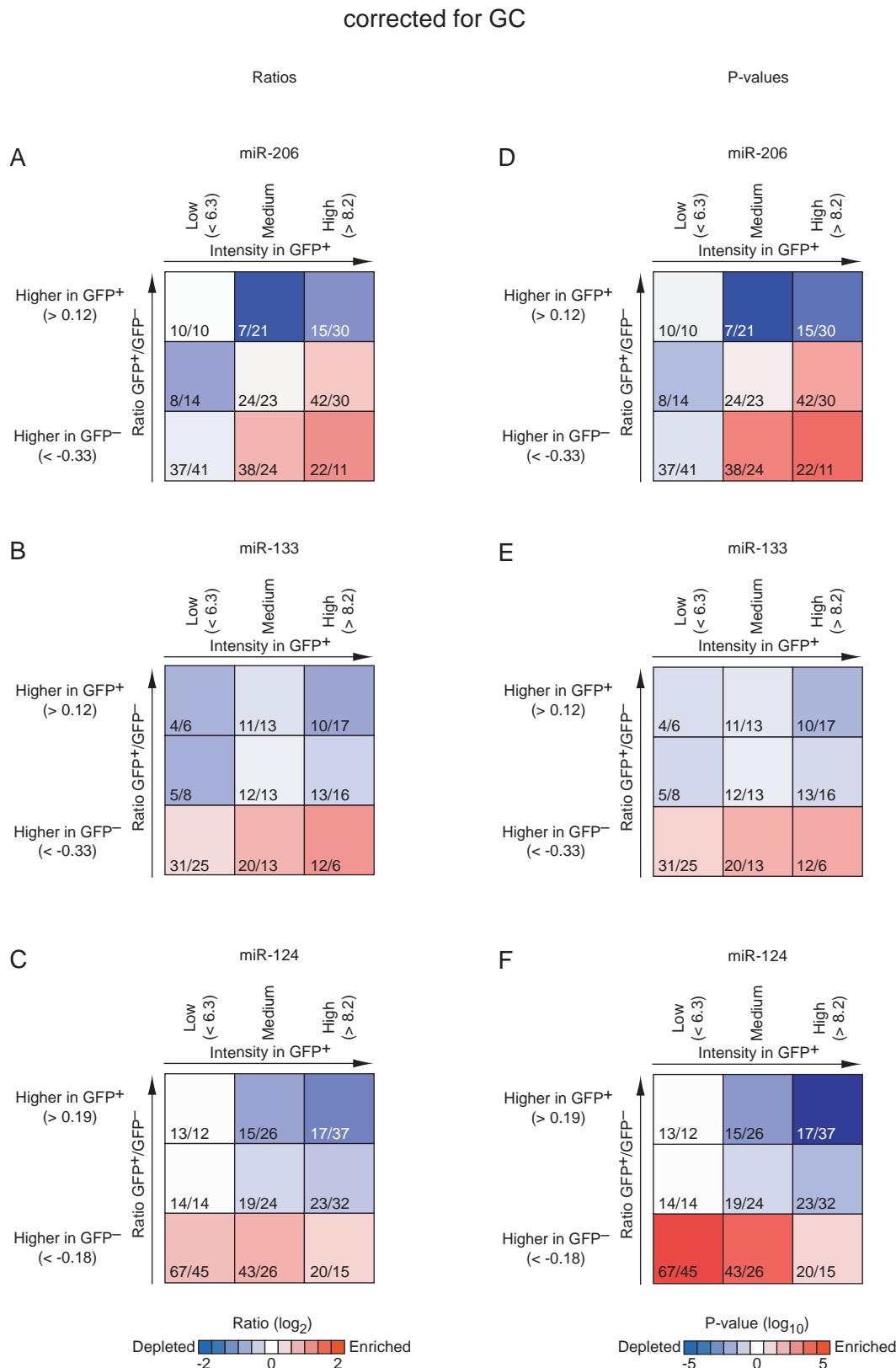
Supplementary Figure 6: Shkumatava et al.



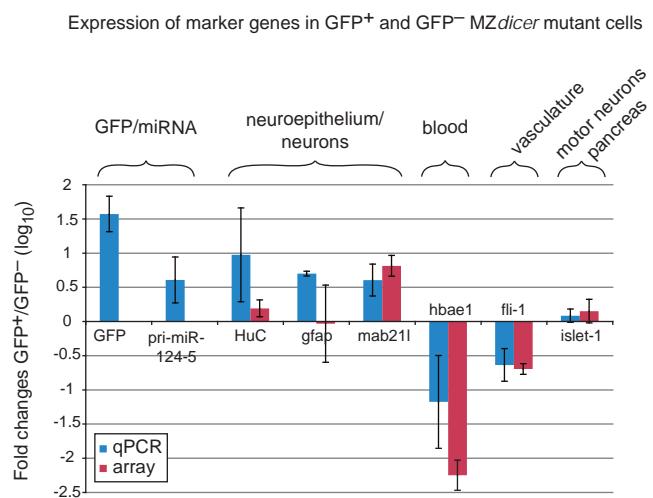
Supplementary Figure 7: Shkumatava et al.



Supplementary Figure 8: Shkumatava et al.

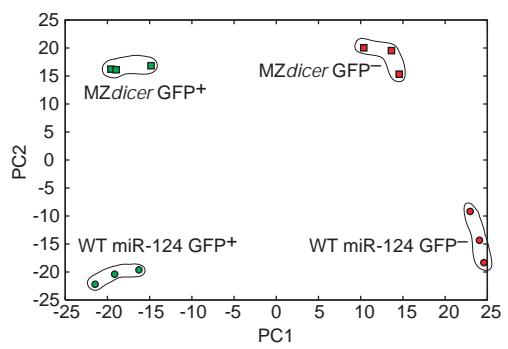


Supplementary Figure 9: Shkumatava et al.

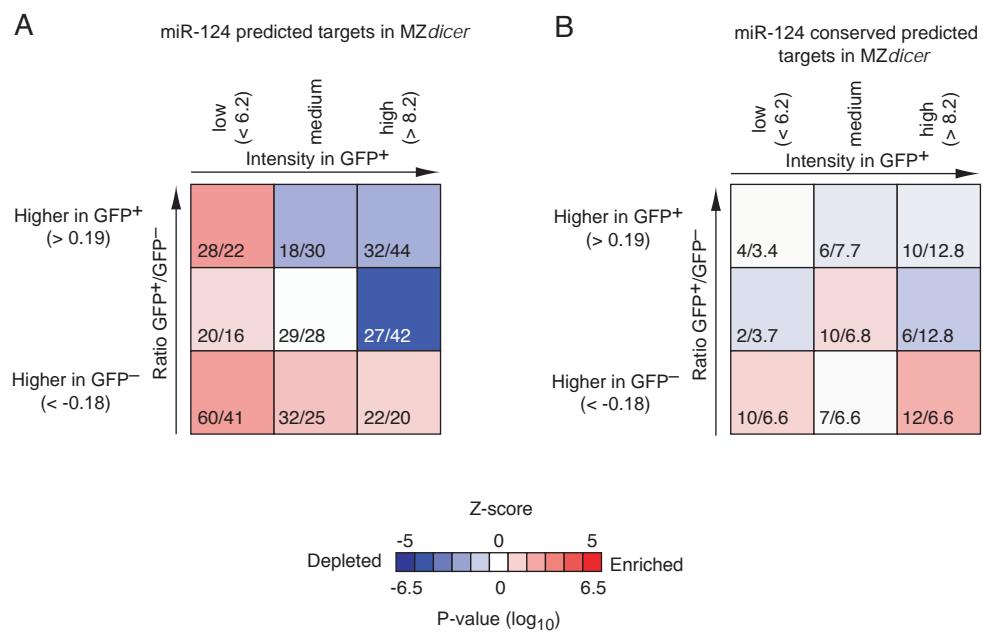


Supplementary Figure 10: Shkumatava et al.

PCA of miR-124 wild-type & miR-124 *MZdicer* microarray data



Supplementary Figure 11: Shkumatava et al.



## Supplementary Figure Legends

**Figure S1.** FACS sorting of miR-206/miR-133 GFP<sup>+</sup> and miR-124 GFP<sup>+</sup> cells. (A) GFP<sup>+</sup> and GFP<sup>-</sup> cells from miR-206/miR-133 reporter construct before FACS sorting. (B) Diagnostic sorting of miR-206/miR-133 GFP<sup>+</sup> cells after two rounds of FACS sorting, indicating cell purity of 99.4%. (C) GFP<sup>+</sup> and GFP<sup>-</sup> cells from miR-124 reporter construct before FACS sorting. (D) Diagnostic sorting of miR-124 GFP<sup>+</sup> cells after two rounds of FACS sorting, indicating cell purity of 99.2%.

**Figure S2.** Correlation between array values for biological replicates for (A) miR-206/miR-133 GFP<sup>+</sup> cells and (B) for miR-124 GFP<sup>+</sup> cells.

**Figure S3.** Correspondence between qRT-PCR and microarray data for (A) miR-206 and (B) miR-124 predicted targets. Blue bars show log<sub>10</sub> fold changes between GFP<sup>+</sup> and GFP<sup>-</sup> cells by qRT-PCR. Red bars show log<sub>10</sub> fold changes between GFP<sup>+</sup> and GFP<sup>-</sup> cells by microarrays. Error bars indicate standard deviations between three biological replicates.

**Figure S4.** Depletion of predicted miRNA targets in GFP<sup>+</sup> cells dependent on target site positions in 3'UTRs. For each transcript with a unique continuous 3'UTR we calculated enrichment/depletion of targets in GFP+ cells as in Figure 4 considering all sites (full-length) or only sites at specific locations in the 3'UTRs (1<sup>st</sup> third adjacent to the coding sequence, 2<sup>nd</sup> third, and 3<sup>rd</sup> third close to the end of the 3'UTR). We find depletion of targets independent of the location of sites, indicating that alternative poly-adenylation does not influence our conclusions. (A) Fold depletion in log<sub>2</sub> (B) P-values in log<sub>10</sub>. Note that P-values are more dependent on the number of sites, which explains the more significant P-values for full-length 3'UTRs at similar depletion levels.

**Figure S5.** Distribution of expression intensities (natural log) in (A) miR-206/miR-133 GFP<sup>+</sup> cells, (B) miR-124 GFP<sup>+</sup> cells, and (C) MZdicer GFP<sup>+</sup> cells.

**Figure S6.** Predicted miRNA target enrichment or depletion for total (left column) and conserved targets (middle column) and target site conservation rate (right column), as in Figure 5 but showing hypergeometric P-values (-log<sub>10</sub>) instead of fold-enrichment or depletion. Negative values indicate depletion.

**Figure S7.** Predicted miRNA target enrichment or depletion for total (left column) and conserved targets (middle column) and target site conservation rate (right column), as in Figure 5 but showing Z-scores instead of fold-enrichment (positive values) or depletion (negative values). Z-scores assess the significance with respect to the differences among the shuffled controls, i.e. the trends observed for the individual 7mers.

**Figure S8.** Predicted miRNA target enrichment or depletion as in Figure 5 left column, but corrected for transcript GC content. All transcripts were divided into 10 bins based on their GC content and then the matrix columns were populated equally with transcripts from each of the 10 bins according to their absolute expression levels. Log ratios (left) and P-values (right) are positive for enrichments and negative for depletions.

**Figure S9.** Expression of established marker genes in GFP<sup>+</sup> and GFP<sup>-</sup> MZ*dicer* mutant cells. Blue bars show fold differences between GFP<sup>+</sup> and GFP<sup>-</sup> cells obtained by qRT-PCR. Red bars show fold changes between GFP<sup>+</sup> and GFP<sup>-</sup> MZ*dicer* cells in expression of marker genes obtained from microarrays. Error bars indicate standard deviations between three biological replicates.

**Figure S10.** Principle component analysis (PCA) of microarray data from GFP<sup>+</sup> and GFP<sup>-</sup> MZ*dicer* and wild-type cells. The intensity vectors for GFP<sup>+</sup> cells (green) and GFP<sup>-</sup> cells (red) are projected onto the first two principal components, revealing dense clustering of biological replicates of each condition and substantial separation between conditions.

**Figure S11.** Predicted miRNA target enrichment or depletion for total (A) and conserved targets (B), as in Figure 6B, C but showing Z-scores instead of fold-enrichment or depletion. Z-scores assess the significance with respect to the differences among the shuffled controls, i.e. the trends observed for the individual 7mers.

#### Supplementary Table 1: Oligonucleotide sequences

Primer #	Primer name	Primer Sequence
#1	mir-124-5 promoter_forward	TGCTTGAGGCACGGTTATGGTCCC
#2	mir-124-5 promoter_reverse	CTCGGGCAGCGGCTTTTGGTCCTG
#3	mir-206-1/mir-133 promoter_forward	CTGCCCCATTAGACTCTTCTTGACG
#4	mir-206-1/mir-133 promoter_reverse	CCAGAAGAACCCAGACGAAACCCAC
#5	beta-actin_forward	CTCTTCCAGCCTTCCTTCCT
#6	beta-actin_reverse	CTTGCTGACCGTATGCAGAA
#7	beta-catenin_forward	TTGCTGACCGTATGCAGAAG
#8	beta-catenin_reverse	CTCAGACATGCGAACAGAA
#9	GFP_forward	CCCTCGTGACCACCCCTGACCTAC
#10	GFP_reverse	CCTTGATGCCGTTCTCTGCTTGTC
#11	miR-206-1_forward	TATTTTCGTCACCGGGAGT
#12	miR-206-1_reverse	CCGTATTCTGCTTCGTTTCG
#13	miR-124-5_forward	GCATCTGAGCTGTGGAAACA

**Supplemental Research Data**

Shkumatava et al., page 3

#14	miR-124-5_reverse	GAAGCATCACGAGAGCATCA
#15	mylz2_forward	CACACGGAGAGGAGAAGGAG
#16	mylz2_reverse	TCCCAGCGAGACAAGTCTTT
#17	tpm1_forward	CCCGTAAGCTGGTGATTGTT
#18	tpm1_reverse	GGACCTTCAGCGAACTCAG
#19	elavl3_forward	CGTCACCATGACCAACTACG
#20	elavl3_reverse	TCCCCCTGCATGTTAAAGAG
#21	gfap_forward	GAGATGATGGGGCTAACCGA
#22	gfap_reverse	CCTGATAGATGTCCCCCAGA
#23	mab21l1_forward	ACCGTCACCTTGAACCTACCG
#24	mab21l1_reverse	GCACGAAATGAGCTGCAATA
#25	hbae1_forward	AATTCAAGATTCTGTCCCACAA
#26	hbae1_reverse	GAGAGTTGGAGCACAGTGAAGT
#27	fli1a_forward	CGTCTCAGCGCTCTCTTCT
#28	fli1a_reverse	TCGTGCTGCTGTTCAATC
#29	isl1_forward	GTGCACAAGCAACCTGAGAA
#30	isl1_reverse	TCGGACTAAGACCCGTCATC
#31	hsp90b1_forward	AAGGCCCTGAAGGAACAAAT
#32	hsp90b1_reverse	CCTGTCTGGTAAGCCTGAGC
#33	gtfllb_forward	ACCATGATCAGCAAGGGAAC
#34	gtfllb_reverse	GCCATGGTGGTGATCTCTTT
#35	ncl_forward	AAACGCTGGCTAGAGGACAA
#36	ncl_reverse	GATGGGCCACAACAGTTCT
#37	picalm_forward	TTTGGACAAAGCGGGTTAC
#38	picalm_reverse	GGAGTGCATCCATCTGGTTT
#39	calm2b_forward	CTTCCCCTCTGTTGCTCTG
#40	calm2b_reverse	TGCACCTCCACATAAAACCA
#41	calm2a_forward	ACACGATCAGGTGCTTGTG
#42	calm2a_reverse	GGTCTCCACTCTGCTACGC
#43	coro1c_forward	ATGACCGTGCCAAGAAAATC
#44	coro1c_reverse	TACTTGAGGTGCGGGTTCT
#45	cx43_forward	GTCGCGTACTTGGATTGGT
#46	cx43_reverse	CACAGAGAGCCAGACCTTCC
#47	calm1b_forward	TGTGTCGTGGGGTTTTGTA
#48	calm1b_reverse	GCCAAACATGTCGACCTTT
#49	cdh2_forward	TGTGAATCGCGTGAAAAGAG
#50	cdh2_reverse	AGCGTGTGCTCTGTCCTT
#51	sgk_forward	ACAATCCAAACGGAGACGAG

**Supplemental Research Data**

Shkumatava et al., page 4

#52	sgk_reverse	CATCTTGTGGTGGTGTCA
#53	casc3_forward	ACCACCCGTTCTCTGTCAC
#54	casc3_reverse	CTGCTGCTGTATCGTGT
#55	sreb2_forward	TCATCGAGCGCCCTACTACT
#56	sreb2_reverse	AACAGCATGAAGGCAGTGTG
#57	pgm1_forward	GATCACATCCGGGAGAAAGA
#58	pgm1_reverse	TTTGCAGCATTGAGTCCAC
#59	slc30A7_forward	GCCCTTTCCATCATCAGAA
#60	slc30A7_reverse	GGCAAATTCTGCACCTCTTC
#61	fxr2_forward	ACTCTCTGCTGGAGGGTGAA
#62	fxr2_reverse	TCCATAAGGCTTGGTTCCAG
#63	p4ha1_forward	TGAAAGACACCAATGGA
#64	p4ha1_reverse	CCCAGCTCATAGCAGTCCTC
#65	slc31A1_forward	ATGTGGAGCTGCTTTGCT
#66	slc31A1_reverse	GTACGGTGCCATCAGATCCT

**Supplementary Table 2: Target Predictions (7 vertebrate alignment)**

Given are the predicted target genes (RefSeq IDs) for miR-124, miR-133, and miR-206 at confidence levels between 0 and 0.9.

**miR-124 targets (in alignments of 7 vertebrate genomes)****Confidence: 0.9**

NM\_201461, NM\_199992, NM\_212752, NM\_001098388, NM\_200753, NM\_001013562, NM\_214806, NM\_001083826

**Confidence: 0.8**

NM\_200773, NM\_200639, NM\_001098388, NM\_201301, NM\_001001409, NM\_131863, NM\_001009908, NM\_001077259, NM\_001007327, NM\_001083826, NM\_201461, NM\_001007403, NM\_212687, NM\_001007285, NM\_001020656, NM\_131872, NM\_001044911, NM\_001013562, NM\_131427, NM\_001035261, NM\_131267, NM\_205644, NM\_001045447, NM\_131856, NM\_001002471, NM\_198805, NM\_199992, NM\_001002228, NM\_205674, NM\_001003781, NM\_001099254, NM\_201308, NM\_001020595, NM\_199212, NM\_131862, NM\_001001816, NM\_213104, NM\_213478, NM\_131665, NM\_001089352, NM\_131686, NM\_213538, NM\_001030164, NM\_001044709, NM\_200753, NM\_130941, NM\_212906, NM\_001014354, NM\_214806, NM\_212637, NM\_199567, NM\_201504, NM\_199479, NM\_001001818, NM\_213459, NM\_001025526, NM\_001040326, NM\_131520, NM\_213405, NM\_001009918, NM\_001017640, NM\_001013538, NM\_001045019, NM\_212966, NM\_194396, NM\_201471, NM\_001077370, NM\_001100008, NM\_212752, NM\_212794, NM\_001013278, NM\_201480, NM\_001077535, NM\_131311, NM\_131193, NM\_205726, NM\_205566, NM\_001083812, NM\_001080590, NM\_001089498, NM\_199868, NM\_001007771, NM\_001003518, NM\_212585, NM\_130978, NM\_200441, NM\_001017819, NM\_001045143, NM\_131670, NM\_131044, NM\_131452, NM\_199744, NM\_201454, NM\_131499, NM\_200629, NM\_173259, NM\_194380, NM\_001007775, NM\_199658, NM\_001007309, NM\_001089524, NM\_001077279, NM\_200950, NM\_001045349, NM\_001044927, NM\_001007440, NM\_001002655, NM\_212565, NM\_001004597, NM\_207060, NM\_001002467, NM\_001007366, NM\_131184, NM\_001002165, NM\_205705, NM\_001045175

**Confidence: 0.7**

NM\_200773, NM\_200639, NM\_001098388, NM\_201301, NM\_001001409, NM\_131863, NM\_001005292, NM\_001009908, NM\_001077259, NM\_001007327, NM\_001083826, NM\_001002214, NM\_201461, NM\_001007403, NM\_212687, NM\_199941, NM\_001007285, NM\_001020656, NM\_131872, NM\_200211, NM\_001008616, NM\_001044911, NM\_001013562, NM\_131427, NM\_001035261, NM\_131267, NM\_205644, NM\_001045447, NM\_001002467, NM\_131856, NM\_001002471, NM\_198805, NM\_199992, NM\_001002228, NM\_205674, NM\_001003781, NM\_001099254, NM\_152978, NM\_213439, NM\_130989, NM\_201308, NM\_001005955, NM\_001020595, NM\_199212, NM\_131862, NM\_200062, NM\_001001816, NM\_213104, NM\_213638, NM\_213478, NM\_001080044, NM\_131665, NM\_001089352, NM\_131686, NM\_001039804, NM\_200971, NM\_213538, NM\_001030164, NM\_001044709, NM\_200753, NM\_130941, NM\_212906, NM\_001014354, NM\_001002227, NM\_214806, NM\_212637, NM\_199567, NM\_201504, NM\_131885, NM\_199479, NM\_131553, NM\_001001818, NM\_213459, NM\_001025526, NM\_200649, NM\_001040326, NM\_131520, NM\_213405, NM\_001009918, NM\_001077765, NM\_001017640, NM\_001013538, NM\_001045019, NM\_212966, NM\_194396, NM\_001001398, NM\_201471, NM\_001077370, NM\_001077391, NM\_213052, NM\_001100008, NM\_212752, NM\_212794, NM\_001013278, NM\_201480, NM\_001077535, NM\_131311, NM\_001045125, NM\_131193, NM\_205726, NM\_205566, NM\_001083812, NM\_001080590, NM\_201035, NM\_001089498, NM\_199868,

## Supplemental Research Data

Shkumatava et al., page 6

NM\_001007771, NM\_001003518, NM\_212585, NM\_001004529, NM\_130978, NM\_200441, NM\_001017819, NM\_001045143, NM\_131670, NM\_213395, NM\_131044, NM\_131452, NM\_199744, NM\_213640, NM\_201454, NM\_131499, NM\_131041, NM\_213111, NM\_200629, NM\_001077799, NM\_001018126, NM\_173259, NM\_194380, NM\_001007775, NM\_199658, NM\_001007309, NM\_001089524, NM\_001025503, NM\_001077279, NM\_200950, NM\_001045349, NM\_001044927, NM\_001007440, NM\_001002655, NM\_001030101, NM\_212565, NM\_001017882, NM\_001006072, NM\_001004597, NM\_001030261, NM\_207060, NM\_201151, NM\_001007366, NM\_131184, NM\_001002165, NM\_205705, NM\_001045175, NM\_001080654, NM\_001045413

### **Confidence: 0.6**

NM\_001020656, NM\_212966, NM\_152978, NM\_001089352, NM\_212687, NM\_212794, NM\_001013560, NM\_001025526, NM\_205726, NM\_194396, NM\_200971, NM\_001001409, NM\_001007327, NM\_001044792, NM\_001040326, NM\_001077535, NM\_131686, NM\_001039804, NM\_201308, NM\_001045019, NM\_001099254, NM\_131863, NM\_200062, NM\_001077259, NM\_001100008, NM\_001083812, NM\_213538, NM\_001045413, NM\_200773, NM\_001080654, NM\_001045311, NM\_001002227, NM\_213052, NM\_212637, NM\_001035261, NM\_213439, NM\_001001398, NM\_131520, NM\_131665, NM\_201471, NM\_001044911, NM\_199567, NM\_001083826, NM\_131311, NM\_198805, NM\_212752, NM\_201504, NM\_001020764, NM\_131885, NM\_001020595, NM\_130989, NM\_131427, NM\_001030164, NM\_131856, NM\_213528, NM\_213104, NM\_001002214, NM\_001008616, NM\_001098388, NM\_199479, NM\_213638, NM\_131193, NM\_212906, NM\_001007403, NM\_001002471, NM\_205566, NM\_001017640, NM\_001013538, NM\_201461, NM\_131872, NM\_131553, NM\_001045175, NM\_001002228, NM\_001080590, NM\_001009918, NM\_205644, NM\_200211, NM\_213405, NM\_001001818, NM\_130941, NM\_001007285, NM\_001017552, NM\_214806, NM\_200753, NM\_201480, NM\_001013278, NM\_001003781, NM\_001077391, NM\_001014354, NM\_200649, NM\_001013562, NM\_001044709, NM\_201301, NM\_001082901, NM\_213459, NM\_001080044, NM\_001045125, NM\_001002467, NM\_131862, NM\_001030203, NM\_001009908, NM\_001045447, NM\_199941, NM\_001045487, NM\_205674, NM\_001077370, NM\_131267, NM\_001005955, NM\_001005292, NM\_213478, NM\_001001816, NM\_001077765, NM\_199992, NM\_200639, NM\_199212, NM\_199754, NM\_001089498, NM\_213395, NM\_001077742, NM\_213111, NM\_001082873, NM\_199658, NM\_001006072, NM\_001007216, NM\_205705, NM\_200629, NM\_200950, NM\_001025503, NM\_001007366, NM\_194380, NM\_130978, NM\_001005997, NM\_001077799, NM\_001004597, NM\_199744, NM\_131670, NM\_001007447, NM\_001045143, NM\_131499, NM\_001002655, NM\_001030261, NM\_001007771, NM\_001077279, NM\_200441, NM\_213640, NM\_199868, NM\_001017882, NM\_200452, NM\_201035, NM\_212565, NM\_207060, NM\_001030101, NM\_001017819, NM\_001004529, NM\_173259, NM\_001089524, NM\_131041, NM\_001007309, NM\_001002165, NM\_131044, NM\_001007775, NM\_201454, NM\_001044927, NM\_131452, NM\_201151, NM\_001007440, NM\_200571, NM\_131667, NM\_001045349, NM\_001003518, NM\_001018126, NM\_212585, NM\_131184

### **Confidence: 0.5**

NM\_001020656, NM\_212966, NM\_152978, NM\_001089352, NM\_212687, NM\_212794, NM\_178131, NM\_001013560, NM\_001025526, NM\_205726, NM\_194396, NM\_200971, NM\_001001409, NM\_001007327, NM\_001044792, NM\_001040326, NM\_001077535, NM\_131686, NM\_001039804, NM\_201308, NM\_001045019, NM\_001099254, NM\_131863, NM\_200062, NM\_001077259, NM\_001100008, NM\_001083812, NM\_213538, NM\_001045413, NM\_200773, NM\_001080654, NM\_001045311, NM\_001002227, NM\_213052, NM\_212637, NM\_001035261, NM\_213439, NM\_001001398, NM\_131520, NM\_131665, NM\_201471, NM\_001044911, NM\_199567, NM\_001083826, NM\_131311, NM\_198805, NM\_212752, NM\_201504, NM\_001020764, NM\_131885, NM\_001020595, NM\_130989, NM\_131427, NM\_001030164, NM\_131856, NM\_213528, NM\_213104, NM\_001002214, NM\_001008616,

## Supplemental Research Data

Shkumatava et al., page 7

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### Confidence: 0.4

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## Supplemental Research Data

Shkumatava et al., page 8

### Confidence: 0.3

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### Confidence: 0.2

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## Supplemental Research Data

Shkumatava et al., page 9

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### Confidence: 0.0

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## Supplemental Research Data

Shkumatava et al., page 10

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## Supplemental Research Data

Shkumatava et al., page 11

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### miR-133 targets (in alignments of 7 vertebrate genomes)

#### Confidence: 0.9

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#### Confidence: 0.8

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#### Confidence: 0.7

NM\_131595, NM\_001002527, NM\_199715, NM\_001014369, NM\_001006079, NM\_201026, NM\_001034976, NM\_131193, NM\_001017886, NM\_199687, NM\_199515, NM\_199923, NM\_001030164, NM\_199986, NM\_200929, NM\_213293, NM\_001015041, NM\_199213, NM\_001001944, NM\_001076569, NM\_199853, NM\_131452, NM\_001045170, NM\_001030238, NM\_131037, NM\_001001950, NM\_001020751, NM\_131180, NM\_131361, NM\_200911, NM\_001002667, NM\_001012373, NM\_200626, NM\_131463, NM\_199806, NM\_130949, NM\_001100086, NM\_001098730, NM\_200391, NM\_199655, NM\_130941, NM\_200080, NM\_213122, NM\_001001940, NM\_199567, NM\_205669, NM\_194273, NM\_181495, NM\_131729, NM\_001001402, NM\_131893, NM\_199538, NM\_201285, NM\_201466, NM\_212802, NM\_182967, NM\_212950, NM\_173247, NM\_199532, NM\_200874, NM\_131686, NM\_001003570, NM\_001079951, NM\_001080047, NM\_199219, NM\_001083551, NM\_214715, NM\_194384, NM\_213045, NM\_131896

#### Confidence: 0.6

NM\_001076569, NM\_200391, NM\_001014369, NM\_131452, NM\_200080, NM\_199853, NM\_199923, NM\_131037, NM\_131463, NM\_200929, NM\_131361, NM\_199687, NM\_001002667, NM\_201026, NM\_199655, NM\_001082551, NM\_001030164, NM\_001017886, NM\_213293, NM\_001001950, NM\_200626, NM\_001044793, NM\_001100086, NM\_194273, NM\_213122, NM\_199806, NM\_131595, NM\_001020751, NM\_201501, NM\_199567, NM\_199213, NM\_130949, NM\_001012373, NM\_199715, NM\_001001940, NM\_131193, NM\_001030238, NM\_200911, NM\_199986, NM\_130941, NM\_131180, NM\_001015041,

## Supplemental Research Data

Shkumatava et al., page 12

NM\_001098730, NM\_001001944, NM\_001034976, NM\_001002527, NM\_205669, NM\_199515, NM\_001006079, NM\_001045170, NM\_199532, NM\_001080047, NM\_199538, NM\_199219, NM\_001079951, NM\_131893, NM\_131896, NM\_213045, NM\_001083551, NM\_001003570, NM\_182967, NM\_194384, NM\_214715, NM\_212802, NM\_200874, NM\_199662, NM\_201285, NM\_131729, NM\_131686, NM\_181495, NM\_212950, NM\_001001402, NM\_201466, NM\_173247

### Confidence: 0.5

NM\_001076569, NM\_200391, NM\_001014369, NM\_131452, NM\_200080, NM\_199853, NM\_199923, NM\_131037, NM\_131463, NM\_200929, NM\_131361, NM\_199687, NM\_001002667, NM\_201026, NM\_199655, NM\_001082551, NM\_001030164, NM\_001077144, NM\_001017886, NM\_213293, NM\_001001950, NM\_200626, NM\_001044793, NM\_001100086, NM\_194273, NM\_213122, NM\_199806, NM\_131595, NM\_001020751, NM\_201501, NM\_199567, NM\_199213, NM\_130949, NM\_001012373, NM\_199715, NM\_001001940, NM\_131193, NM\_213045, NM\_001030238, NM\_200911, NM\_199986, NM\_130941, NM\_131180, NM\_001015041, NM\_001098730, NM\_001001944, NM\_001034976, NM\_001002527, NM\_205669, NM\_199515, NM\_001006079, NM\_001045170, NM\_199532, NM\_001080047, NM\_199538, NM\_199219, NM\_001079951, NM\_131893, NM\_001098180, NM\_131896, NM\_001083551, NM\_199881, NM\_001003570, NM\_182967, NM\_194384, NM\_214715, NM\_212802, NM\_200874, NM\_199662, NM\_201285, NM\_131729, NM\_131686, NM\_213146, NM\_181495, NM\_212950, NM\_001001402, NM\_201466, NM\_173247

### Confidence: 0.4

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### Confidence: 0.3

NM\_001030238, NM\_199923, NM\_213122, NM\_213045, NM\_001034976, NM\_131180, NM\_199806, NM\_001044793, NM\_200080, NM\_131037, NM\_130949, NM\_131361, NM\_130931, NM\_001030164, NM\_001001940, NM\_200626, NM\_213293, NM\_001014369, NM\_001006079, NM\_001020751, NM\_194384, NM\_199655, NM\_199715, NM\_001082551, NM\_199687, NM\_200391, NM\_001100086, NM\_001002667, NM\_199853, NM\_001001944, NM\_131595, NM\_131193, NM\_001045170, NM\_001077144, NM\_199213, NM\_001098730, NM\_201501, NM\_205669, NM\_199986, NM\_131463, NM\_001012373, NM\_199567, NM\_130941, NM\_001001950, NM\_001015041, NM\_199515, NM\_001076569, NM\_001002527, NM\_200929, NM\_194273, NM\_001017886, NM\_200911, NM\_001100948, NM\_131452, NM\_001017573, NM\_201026, NM\_199219, NM\_001003570, NM\_199538, NM\_001080047, NM\_212802, NM\_200874, NM\_199532, NM\_001079951, NM\_181495, NM\_199662, NM\_173247, NM\_182967, NM\_201285, NM\_001083551, NM\_001001402, NM\_201466, NM\_001098180, NM\_213146, NM\_214715, NM\_212950, NM\_131686, NM\_131729, NM\_199881, NM\_131896, NM\_131893

### Confidence: 0.2

## Supplemental Research Data

Shkumatava et al., page 13

NM\_200929, NM\_001030238, NM\_001001940, NM\_131595, NM\_001002527, NM\_130941, NM\_199715, NM\_001100086, NM\_199213, NM\_131361, NM\_200391, NM\_201026, NM\_001002667, NM\_001017886, NM\_001014369, NM\_001001950, NM\_001012373, NM\_001044793, NM\_199986, NM\_130949, NM\_199806, NM\_194384, NM\_001045170, NM\_001098730, NM\_131452, NM\_131193, NM\_213045, NM\_199515, NM\_001020751, NM\_205669, NM\_200911, NM\_199687, NM\_001100948, NM\_001015041, NM\_200626, NM\_131037, NM\_001082551, NM\_199655, NM\_001017573, NM\_201501, NM\_199923, NM\_001076569, NM\_001001944, NM\_001030164, NM\_194273, NM\_001077144, NM\_131180, NM\_199567, NM\_213293, NM\_131463, NM\_001006079, NM\_200080, NM\_001034976, NM\_130931, NM\_213122, NM\_199853, NM\_001098180, NM\_131896, NM\_173247, NM\_001083551, NM\_001080047, NM\_199881, NM\_201466, NM\_199662, NM\_199538, NM\_131686, NM\_199532, NM\_182967, NM\_181495, NM\_001003570, NM\_001079951, NM\_001001402, NM\_131893, NM\_214715, NM\_212802, NM\_212950, NM\_200874, NM\_201285, NM\_131729, NM\_213146, NM\_199219

### Confidence: 0.1

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### Confidence: 0.0

NM\_001080644, NM\_001077549, NM\_001076569, NM\_131628, NM\_001076615, NM\_001040685, NM\_131604, NM\_001003596, NM\_001030238, NM\_001045847, NM\_001075104, NM\_001002155, NM\_001001950, NM\_001002628, NM\_001100436, NM\_152965, NM\_212861, NM\_001045472, NM\_001045076, NM\_212733, NM\_001003455, NM\_001039171, NM\_001017886, NM\_001002451, NM\_200367, NM\_001007053, NM\_001001944, NM\_213045, NM\_200816, NM\_001001833, NM\_200302, NM\_213004, NM\_199715, NM\_001077144, NM\_198805, NM\_130931, NM\_001020716, NM\_001014369, NM\_001002667, NM\_213184, NM\_200757, NM\_001018165, NM\_001045203, NM\_200441, NM\_001100948, NM\_001004553, NM\_213505, NM\_001080052, NM\_001080636, NM\_001082831, NM\_130949, NM\_131304, NM\_201584, NM\_201473, NM\_001045161, NM\_001014332, NM\_001002497, NM\_200633, NM\_001002439, NM\_173283, NM\_199515, NM\_131595, NM\_001080606, NM\_001030164, NM\_001045172, NM\_213293, NM\_213474, NM\_001039834, NM\_214816, NM\_001002624, NM\_001002718, NM\_199584, NM\_001100439, NM\_001001825, NM\_199567, NM\_001017813, NM\_001017709, NM\_001098730, NM\_131874, NM\_001076757, NM\_130940, NM\_214687, NM\_001008584, NM\_201058, NM\_001002541, NM\_199991, NM\_001017573, NM\_001002729, NM\_001001940, NM\_001001947, NM\_200614, NM\_213403, NM\_198067, NM\_131260, NM\_001079704, NM\_212583, NM\_214691, NM\_001045170, NM\_001045177, NM\_001045351, NM\_200391, NM\_001029957, NM\_001002173, NM\_001002354, NM\_001008624, NM\_178297, NM\_200229, NM\_213122, NM\_001017724, NM\_200205, NM\_178437, NM\_131872, NM\_131452, NM\_001020472, NM\_131435, NM\_130928, NM\_201295, NM\_201090, NM\_001045164, NM\_201143,

## Supplemental Research Data

Shkumatava et al., page 14

NM\_001007293, NM\_001100086, NM\_213640, NM\_001002348, NM\_200810, NM\_199687, NM\_199861, NM\_001083557, NM\_001017607, NM\_173221, NM\_001044793, NM\_001080001, NM\_131463, NM\_001020751, NM\_001020717, NM\_201067, NM\_200080, NM\_201043, NM\_201335, NM\_001012386, NM\_001002154, NM\_201026, NM\_001039924, NM\_205669, NM\_194384, NM\_194273, NM\_199814, NM\_001098260, NM\_199213, NM\_001089583, NM\_001044985, NM\_001081748, NM\_001020477, NM\_001076719, NM\_131037, NM\_001044927, NM\_001030265, NM\_001045092, NM\_001005982, NM\_201189, NM\_001003833, NM\_001012373, NM\_001002148, NM\_001015041, NM\_001002527, NM\_001006079, NM\_001002305, NM\_213003, NM\_001082551, NM\_131193, NM\_001089466, NM\_131159, NM\_131823, NM\_001014361, NM\_201461, NM\_001002596, NM\_200971, NM\_001003629, NM\_200353, NM\_207640, NM\_213417, NM\_199853, NM\_199655, NM\_213306, NM\_199749, NM\_199923, NM\_198366, NM\_001080635, NM\_153650, NM\_001077154, NM\_131180, NM\_131361, NM\_130941, NM\_131018, NM\_001024389, NM\_001034976, NM\_001014355, NM\_001025526, NM\_201059, NM\_201501, NM\_199777, NM\_207050, NM\_199538, NM\_001098387, NM\_153661, NM\_199806, NM\_001020643, NM\_130935, NM\_001040354, NM\_201150, NM\_001014308, NM\_001007061, NM\_200911, NM\_199986, NM\_200626, NM\_213234, NM\_199566, NM\_001007312, NM\_001007406, NM\_131856, NM\_001077806, NM\_212880, NM\_212950, NM\_200386, NM\_001004672, NM\_001002342, NM\_001004561, NM\_001008612, NM\_001037118, NM\_200929, NM\_199570, NM\_213228, NM\_001045447, NM\_001002735, NM\_212802, NM\_181495, NM\_212562, NM\_182865, NM\_201007, NM\_001080047, NM\_213125, NM\_199614, NM\_131298, NM\_199881, NM\_198806, NM\_200417, NM\_001077557, NM\_001005967, NM\_131594, NM\_201285, NM\_201466, NM\_001079944, NM\_001045846, NM\_131494, NM\_131325, NM\_182968, NM\_214715, NM\_205565, NM\_205705, NM\_001040321, NM\_001040304, NM\_131686, NM\_001007105, NM\_001083551, NM\_001007338, NM\_200874, NM\_200256, NM\_199662, NM\_200017, NM\_001082824, NM\_131893, NM\_199219, NM\_131362, NM\_001003570, NM\_200278, NM\_200814, NM\_001079951, NM\_199532, NM\_001045148, NM\_001098180, NM\_213437, NM\_001007428, NM\_131729, NM\_001001402, NM\_001077778, NM\_173247, NM\_001017647, NM\_213146, NM\_131896, NM\_131348, NM\_212932, NM\_182967, NM\_001007051, NM\_001003741

### miR-206 targets (in alignments of 7 vertebrate genomes)

#### Confidence: 0.9

NM\_199562, NM\_201295, NM\_201466, NM\_152974, NM\_001007159, NM\_001076652, NM\_001003839, NM\_131302, NM\_199697, NM\_001080044, NM\_131336, NM\_200104, NM\_200082, NM\_001102672, NM\_212857, NM\_213019, NM\_199679, NM\_131430, NM\_212603, NM\_131340, NM\_198978, NM\_214736, NM\_131025, NM\_001002668, NM\_131220, NM\_199212, NM\_199675, NM\_001045105, NM\_201186, NM\_199996, NM\_131626, NM\_214803, NM\_181601, NM\_001003738, NM\_001003470, NM\_213463, NM\_001077391, NM\_001098730, NM\_194374, NM\_213351, NM\_001020702, NM\_178303, NM\_001081629, NM\_001040394, NM\_001001815, NM\_131031, NM\_213144, NM\_001077804, NM\_001002181, NM\_131714, NM\_194376, NM\_001089409, NM\_001033595, NM\_131563, NM\_212933, NM\_001001592, NM\_200927, NM\_131595, NM\_198210, NM\_212991, NM\_199649

#### Confidence: 0.8

NM\_001081629, NM\_131302, NM\_152974, NM\_201295, NM\_001040394, NM\_001002389, NM\_001003839, NM\_201466, NM\_213093, NM\_001002181, NM\_001102672, NM\_131714, NM\_131336, NM\_199212, NM\_001080044, NM\_199562, NM\_001020702, NM\_001007159, NM\_001076652, NM\_199697, NM\_178303, NM\_194374, NM\_001077391, NM\_001001815, NM\_001077804, NM\_213144, NM\_181439, NM\_131037, NM\_212857, NM\_131626,

## Supplemental Research Data

Shkumatava et al., page 15

NM\_213019, NM\_199779, NM\_131340, NM\_199611, NM\_131025, NM\_001045105, NM\_194376, NM\_200104, NM\_201186, NM\_199679, NM\_001003738, NM\_178302, NM\_201322, NM\_131430, NM\_001098730, NM\_214683, NM\_200185, NM\_212603, NM\_131031, NM\_212717, NM\_001003470, NM\_199996, NM\_213463, NM\_214803, NM\_198978, NM\_214736, NM\_200082, NM\_212874, NM\_213351, NM\_001089420, NM\_001002668, NM\_199675, NM\_181601, NM\_131220, NM\_001037422, NM\_001089409, NM\_131736, NM\_001014348, NM\_213453, NM\_200096, NM\_001005582, NM\_213223, NM\_001080583, NM\_131116, NM\_001080029, NM\_201336, NM\_001080033, NM\_001001818, NM\_201101, NM\_213552, NM\_199649, NM\_131563, NM\_131433, NM\_213158, NM\_001076646, NM\_001001592, NM\_001007767, NM\_198210, NM\_001033595, NM\_001077767, NM\_001089428, NM\_212758, NM\_001044855, NM\_001100001, NM\_212880, NM\_213185, NM\_131595, NM\_001045472, NM\_200927, NM\_199833, NM\_212991, NM\_212933, NM\_213641, NM\_213402, NM\_001077253

### Confidence: 0.7

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### Confidence: 0.6

NM\_200139, NM\_199562, NM\_213093, NM\_001020702, NM\_201295, NM\_152974, NM\_001002181, NM\_001007159, NM\_001102672, NM\_001081629, NM\_199697, NM\_131302, NM\_001002389, NM\_199212, NM\_001080044, NM\_213172, NM\_001040394, NM\_201466, NM\_001003839, NM\_131714, NM\_001076652, NM\_131336, NM\_001001815, NM\_001089420, NM\_001037422, NM\_131220, NM\_213463, NM\_213144, NM\_131435, NM\_001017724, NM\_001025455, NM\_001076557, NM\_001002668, NM\_001077391, NM\_205601, NM\_181601, NM\_194376, NM\_212857, NM\_001003470, NM\_201504, NM\_200104, NM\_131025, NM\_131626, NM\_213019, NM\_199675, NM\_130981, NM\_214803, NM\_200082, NM\_214736, NM\_178302, NM\_001015068, NM\_001017819, NM\_213351, NM\_199611, NM\_131430, NM\_201322, NM\_001098730, NM\_212603, NM\_194374, NM\_131232, NM\_198978, NM\_001037405, NM\_200185, NM\_131031, NM\_201094, NM\_199569, NM\_001089409, NM\_181439, NM\_178303, NM\_199679, NM\_001003587, NM\_131037, NM\_212874, NM\_001007281, NM\_214683, NM\_001003738, NM\_001001825, NM\_001077804, NM\_001045158, NM\_201186, NM\_212893, NM\_131340, NM\_212717, NM\_001001946, NM\_199779, NM\_131882, NM\_199996, NM\_001045105, NM\_199833, NM\_200278,

## Supplemental Research Data

Shkumatava et al., page 16

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### Confidence: 0.5

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### Confidence: 0.4

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## Supplemental Research Data

Shkumatava et al., page 17

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### Confidence: 0.3

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### Confidence: 0.2

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## Supplemental Research Data

Shkumatava et al., page 18

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### **Confidence: 0.1**

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### **Confidence: 0.0**

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## Supplemental Research Data

Shkumatava et al., page 19

NM\_213351, NM\_001089435, NM\_194376, NM\_001025455, NM\_198978, NM\_201504, NM\_131886, NM\_001004569, NM\_131220, NM\_131401, NM\_213164, NM\_213637, NM\_205711, NM\_200609, NM\_213019, NM\_001045177, NM\_173238, NM\_131617, NM\_001076557, NM\_213641, NM\_194374, NM\_201094, NM\_201188, NM\_001017756, NM\_001020525, NM\_201345, NM\_001045362, NM\_212603, NM\_001039971, NM\_131481, NM\_131558, NM\_131068, NM\_001077763, NM\_200759, NM\_001077302, NM\_213145, NM\_001089420, NM\_199675, NM\_001037384, NM\_181601, NM\_001001825, NM\_198070, NM\_001045158, NM\_001002668, NM\_001013523, NM\_131340, NM\_131031, NM\_200367, NM\_001020477, NM\_194379, NM\_201186, NM\_199867, NM\_213028, NM\_200104, NM\_001040362, NM\_001025545, NM\_001002380, NM\_201507, NM\_131882, NM\_131626, NM\_131025, NM\_131404, NM\_200955, NM\_199965, NM\_213300, NM\_199569, NM\_001045260, NM\_001017724, NM\_001045156, NM\_001002642, NM\_001002205, NM\_178302, NM\_001077282, NM\_213463, NM\_001003587, NM\_001005980, NM\_001034976, NM\_212717, NM\_001002385, NM\_001037422, NM\_001003411, NM\_001037405, NM\_001080663, NM\_130912, NM\_214816, NM\_212610, NM\_212704, NM\_201026, NM\_001100962, NM\_131850, NM\_001002157, NM\_130981, NM\_131232, NM\_131500, NM\_001077391, NM\_001098193, NM\_214803, NM\_205549, NM\_200906, NM\_201322, NM\_001001815, NM\_001018156, NM\_001015068, NM\_001037118, NM\_131337, NM\_200082, NM\_001020648, NM\_199611, NM\_212919, NM\_200278, NM\_001080667, NM\_200143, NM\_213130, NM\_001098256, NM\_199713, NM\_131595, NM\_001045081, NM\_212696, NM\_001007777, NM\_001102637, NM\_001089583, NM\_199543, NM\_199811, NM\_001002165, NM\_213461, NM\_201104, NM\_213024, NM\_001089570, NM\_001082826, NM\_213007, NM\_199537, NM\_198067, NM\_131448, NM\_131716, NM\_001001592, NM\_001017580, NM\_001002152, NM\_001098738, NM\_199833, NM\_001044855, NM\_001044942, NM\_131184, NM\_131563, NM\_212758, NM\_001013317, NM\_200187, NM\_200723, NM\_200706, NM\_001045171, NM\_001002688, NM\_213453, NM\_001098485, NM\_200005, NM\_001033595, NM\_001077370, NM\_001005582, NM\_131433, NM\_001007767, NM\_214770, NM\_200588, NM\_001001818, NM\_205743, NM\_199772, NM\_200419, NM\_199731, NM\_001076632, NM\_001089428, NM\_001077767, NM\_131572, NM\_001077253, NM\_131736, NM\_001002558, NM\_001008606, NM\_001003534, NM\_001045472, NM\_001002096, NM\_131216, NM\_001002212, NM\_200096, NM\_213375, NM\_205567, NM\_213223, NM\_199649, NM\_199625, NM\_001002399, NM\_131116, NM\_131408, NM\_001017728, NM\_001014348, NM\_194380, NM\_205718, NM\_001030264, NM\_152967, NM\_001075113, NM\_212991, NM\_212880, NM\_001013533, NM\_001002147, NM\_200980, NM\_001001949, NM\_001002415, NM\_001100001, NM\_213402, NM\_001080029, NM\_131867, NM\_001040379, NM\_001099417, NM\_001002484, NM\_131715, NM\_201488, NM\_212746, NM\_001039817, NM\_201336, NM\_198210, NM\_131038, NM\_213552, NM\_201101, NM\_200927, NM\_181764, NM\_199802, NM\_001030092, NM\_001099234, NM\_001040377, NM\_212587, NM\_131609, NM\_001045310, NM\_001005939, NM\_001002511, NM\_200441, NM\_194366, NM\_213505, NM\_001089498, NM\_001002097, NM\_131509

**Supplementary Table 3: Target Predictions (5 fish alignment)**

Given are the predicted target genes (RefSeq IDs) for miR-124, miR-133, and miR-206 at confidence levels between 0 and 0.9.

**miR-124 targets (in alignments of 5 fish genomes)****Confidence: 0.9**

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**Confidence: 0.8**

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**Confidence: 0.7**

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## Supplemental Research Data

Shkumatava et al., page 21

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### Confidence: 0.6

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## Supplemental Research Data

Shkumatava et al., page 22

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### Confidence: 0.5

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### Confidence: 0.4

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## Supplemental Research Data

Shkumatava et al., page 23

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### Confidence: 0.3

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## Supplemental Research Data

Shkumatava et al., page 24

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### Confidence: 0.2

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## Supplemental Research Data

Shkumatava et al., page 25

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## Supplemental Research Data

Shkumatava et al., page 26

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## Supplemental Research Data

Shkumatava et al., page 27

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## Supplemental Research Data

Shkumatava et al., page 28

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## Supplemental Research Data

Shkumatava et al., page 29

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## Supplemental Research Data

Shkumatava et al., page 30

### Confidence: 0.4

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### Confidence: 0.3

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### Confidence: 0.2

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## Supplemental Research Data

Shkumatava et al., page 31

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## Supplemental Research Data

Shkumatava et al., page 32

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## Supplemental Research Data

Shkumatava et al., page 33

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### miR-206 targets (in alignments of 5 fish genomes)

#### Confidence: 0.9

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## Supplemental Research Data

Shkumatava et al., page 34

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### Confidence: 0.8

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### Confidence: 0.7

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## Supplemental Research Data

Shkumatava et al., page 35

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### Confidence: 0.6

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### Confidence: 0.5

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## Supplemental Research Data

Shkumatava et al., page 36

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### Confidence: 0.4

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## Supplemental Research Data

Shkumatava et al., page 37

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### Confidence: 0.1

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## Supplemental Research Data

Shkumatava et al., page 38

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## Supplemental Research Data

Shkumatava et al., page 39

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## Supplemental Research Data

Shkumatava et al., page 40

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**Supplemental Research Data**

Shkumatava et al., page 41

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