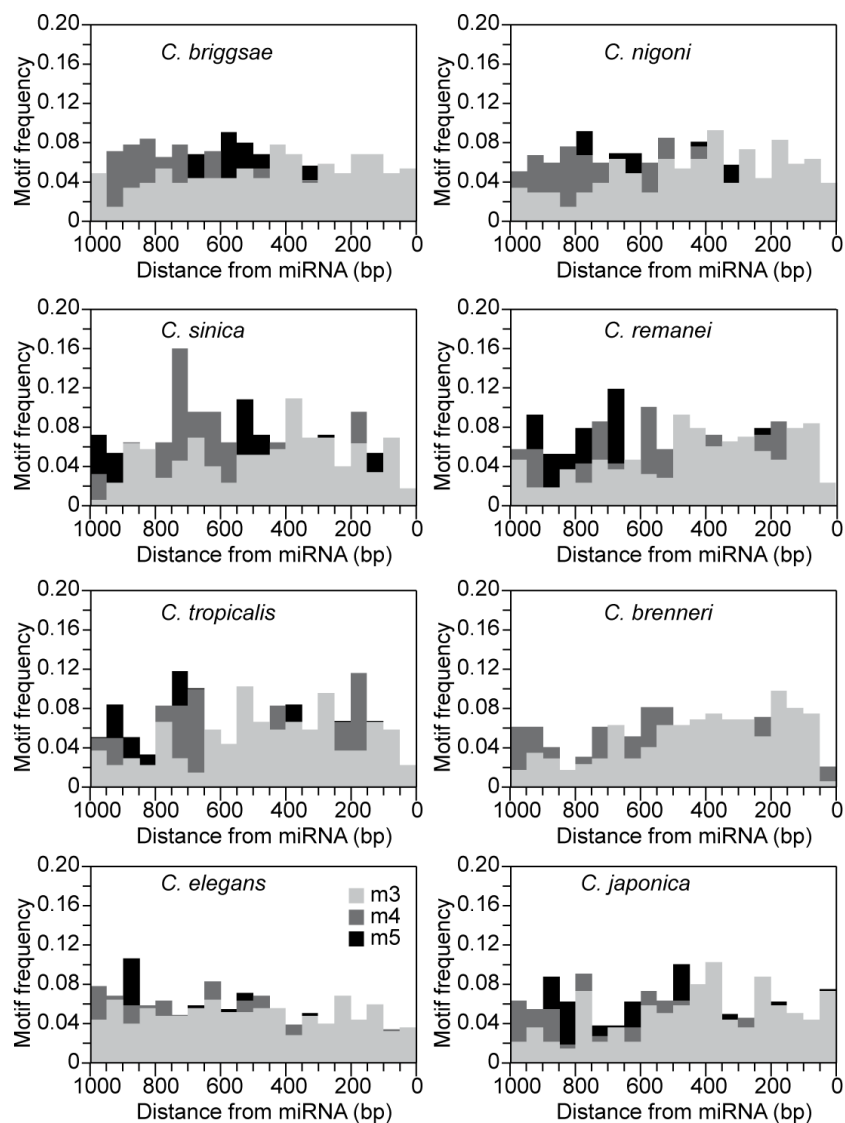


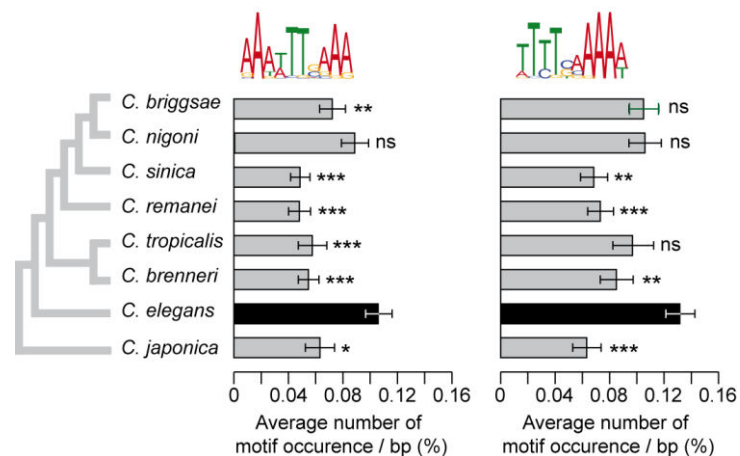
Supplementary Material for:

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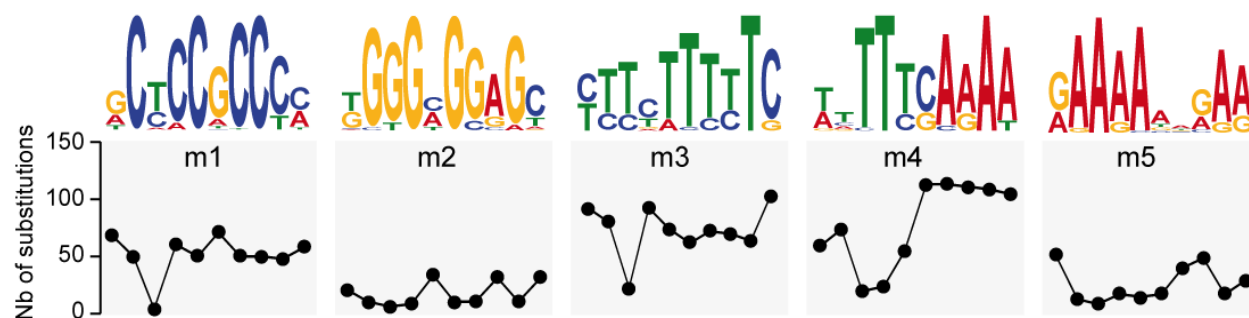
Comparative genomic analysis of upstream miRNA regulatory motifs in *Caenorhabditis*



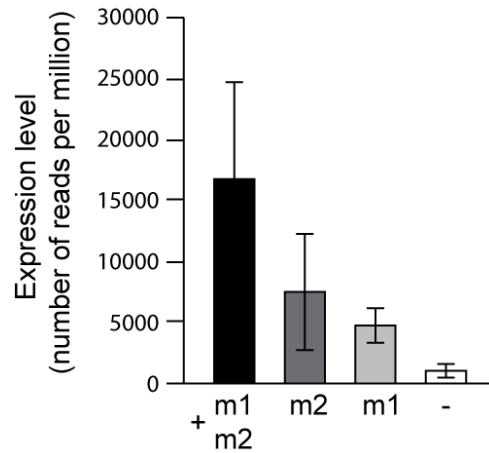
Supplementary Figure 1. The frequency distributions of motifs m3, m4 and m5 in 50 bp bins. Motifs m3-m5 are found throughout 1 Kb of miRNA upstream sequence with no preferential location.



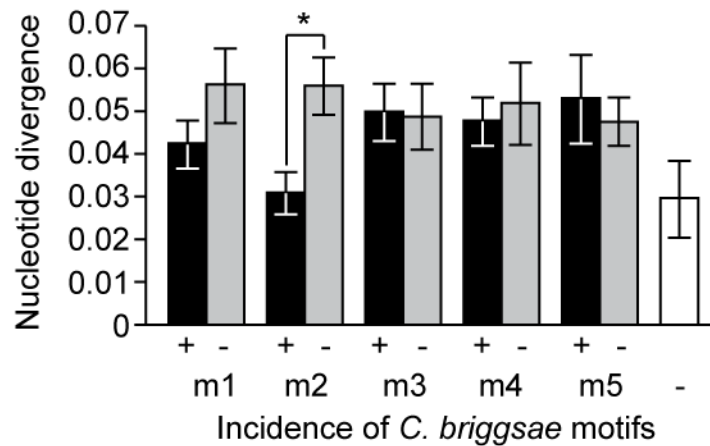
Supplementary Figure 2. Average number of motif occurrence of the *C. elegans* motifs m4 (left) and m5 (right) in miRNA upstream regions across *Caenorhabditis*. The distributions of motif occurrence are compared between *C. elegans* and each species and significance is determined by Wilcoxon sum rank tests. Means are represented ± 1 standard error. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: not significant.



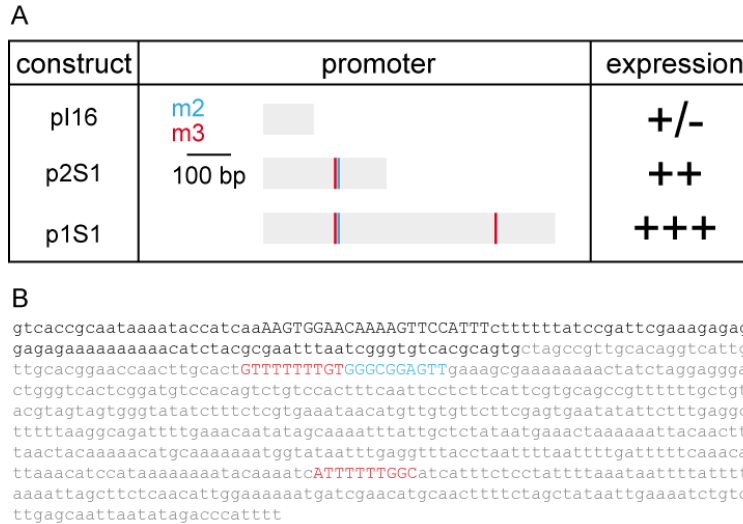
Supplementary Figure 3. Number of substitutions between *C. briggsae* and *C. nigoni* at each position of the *C. briggsae* motifs m1 to m5.



Supplementary Figure 4. Expression level differences between miRNAs having both motifs m1 and m2, only one of these motifs or none in their upstream regions. Means are represented ± 1 standard error. $F(3, 176) = 8.867$, $P < 0.0001$



Supplementary Figure 5. Nucleotide divergence between *C. briggsae* and *C. nigoni* orthologous hairpin miRNA sequences as a function of the presence (+) of absence (-) of motifs upstream of *C. briggsae* miRNAs. Nucleotide divergence of miRNAs lacking all motifs is indicated in orange. Means are represented ± 1 standard error. Wilcoxon two-sample tests: * $P < 0.05$



Supplementary Figure 6. Expression and sequence of *let-7* reporter constructs from Johnson et al (Johnson et al. 2003). A. pI16 is the minimum enhancer driving expression of *let-7* in the seam cells. This reporter is faintly expressed and has low penetrance. Importantly, reporter p2S1 which has a single instance of motif m2 and m3 is more strongly expressed and has high penetrance and reporter p1S1 which has an additional instance of motif m3 shows very bright GFP expression with high penetrance. B. The sequence of p1S1 reporter with the sequence of the minimum *let-7* enhancer pI16 in bold and motif m2 in blue and motif m3 in red.

Supplementary Table 1. Motif abundance is greater upstream the miRNA transcription start site (TSS) in the promoter than between the TSS and the miRNA start.

Motif	N ^a	U ^b	D ^c	^d Up	^e Down	<i>P</i>	^f NormUp	^g NormDown	<i>P</i>
m1	28	42	4	1.5	0.143	2.13E-05	0.0017	0.0006	0.0021
m2	11	10	1	0.909	0.091	0.0066	0.0013	0.0003	0.0505
m3	29	47	6	1.621	0.207	2.10E-05	0.0018	0.0013	0.0048
m4	27	48	2	1.778	0.074	1.23E-05	0.0020	0.0009	0.0006
m5	29	50	4	1.724	0.138	3.73E-06	0.0020	0.0013	0.0029

^aN: Number of miRNAs with TSS annotations; ^bU: Number of motifs upstream the TSS; ^cD: Number of motifs downstream the TSS; ^dUp: Mean motif abundance upstream the TSS; ^eDown: mean motif abundance downstream the TSS; ^fNormUp: Mean motif abundance per nucleotide upstream the TSS; ^gNormDown: mean motif abundance per nucleotide downstream the TSS. *P*: Wilcoxon paired tests