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Supplemental information

Critical contribution of 3′ non-seed base pairing to the *in vivo* function of the evolutionarily conserved *let-7a* microRNA

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Table S5. Genetic alleles generated and *C. elegans* strains used in this study. Related to **Figure 2-7, S2, S5**

let-7a alleles

ALLELE	LOCATION/MUTATION	DESCRIPTION	RELATED
ma341	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma341: acactgtggatccggTGAGGTAGTATGTAATATTGTAtggaatattaccaccggTGTACAATGTTATGTTCTACCTTACcggagacagaac	*mir-84* swap	Fig.2, S2,S5
ma393	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma393: acactgtggatccgg----------TGTATCAGTTCGATATCTGAcgg----------cggagacagaac	pre-*let-7* swapped by sgINPP4A jump board. Null.	Fig.2, 3, 7, S2
ma453	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma453: acactgtggatccggTGAGGTAGGAGTTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTGCTACCTTACcggagacagaac	U9G	Fig.3, S2
ma454	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma454: acactgtggatccggTGAGGTAGTTGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTATCTACCTTACcggagacagaac	A10U	Fig.3, S2
ma431	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma431 acactgtggatccggTGAGGTAGTATGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATGTTCTACCTTACcggagacagaac	G11U	Fig.3, S2
ma448	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma448: acactgtggatccggTGAGGTAGTAGTTTGTATAGTTtggaatattaccaccggTGAACTATGCAAGTTTCTACCTTACcggagacagaac	G12U	Fig.3, S2
ma432	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma432 acactgtggatccggTGAGGTAGTAGGATGTATAGTTtggaatattaccaccggTGAACTATGCATTTTTCTACCTTACcggagacagaac	U13A	Fig.3,4,5 S2,S4,S6
ma433	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma433 acactgtggatccggTGAGGTAGTAGGTAGTATAGTTtggaatattaccaccggTGAACTATGCTATTTTCTACCTTACcggagacagaac	U14A	Fig.3, S2
ma434	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma434 acactgtggatccggTGAGGTAGTAGGTTATATAGTTtggaatattaccaccggTGAACTATGTAATTTTCTACCTTACcggagacagaac	G15A	Fig.3, S2
ma449	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma449: acactgtggatccggTGAGGTAGTAGGTTGGATAGTTtggaatattaccaccggTGAACTATTCAATTTTCTACCTTACcggagacagaac	U16G	Fig.3, S2
ma450	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma450: acactgtggatccggTGAGGTAGTAGGTTGTTTAGTTtggaatattaccaccggTGAACTAAGCAATTTTCTACCTTACcggagacagaac	A17U	Fig.3, S2
ma435	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma435: acactgtggatccggTGAGGTAGTAGGTTGTAAAGTTtggaatattaccaccggTGAACTTTGCAATTTTCTACCTTACcggagacagaac	U18A	Fig.3, S2, S3
ma436	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma436: acactgtggatccggTGAGGTAGTAGGTTGTATTGTTtggaatattaccaccggTGAACAATGCAATTTTCTACCTTACcggagacagaac	A19U	Fig.3, S2
ma452	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma452: acactgtggatccggTGAGGTAGTAGGTTGTATACTTtggaatattaccaccggTGAAGTATGCAATTTTCTACCTTACcggagacagaac	G20C	Fig.3, S2
ma456	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma456: acactgtggatccggTGAGGTAGTAGGTTGTATAGATtggaatattaccaccggTGATCTATGCAATTTTCTACCTTACcggagacagaac	U21A	Fig.3, S2
ma437	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma437 acactgtggatccggTGAGGTAGTAGGTTGTATAGTAtggaatattaccaccggTGTACTATGCAATTTTCTACCTTACcggagacagaac	U22A	Fig.3, S2
ma479	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma479: acactgtggatccggTGAGGTAGTAGGTTGGAAAGTTtggaatattaccaccggTGAACTTTTCAATTTTCTACCTTACcggagacagaac	U16G + U18A, equal to *ma449ma435*	Fig.3, S2
ma428	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma428: : acactgtggatccggTGAGGTAGTAGGATGTAAAGTTtggaatattaccaccggTGAACTTTGCATTTTTCTACCTTACcggagacagaac	U13A + U18A equal to *ma432ma435*	Fig.S2
ma455	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma455: acactgtggatccggTGAGGTAGTAGTTTGTAGAGTTtggaatattaccaccggTGAACTCTGCAATTTTCTACCTTACcggagacagaac	U18G	Fig.S2
ma451	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma451: acactgtggatccggTGAGGTAGTAGGTTGTTTAGTTtggaatattaccaccggTGAACTAAGCAATTTTCTACCTTACcggagacagaac	U18C	Fig.S2
ma476	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma476: acactgtggatccggTGAGGTAGTAGGTTGTTATCAAtggaatattaccaccggTGTTGATAGCAATTTTCTACCTTACcggagacagaac	A17U + U18A + A19U + G20C + U21A + U22A	Fig.S2
ma477	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma477: acactgtggatccggTGAGGTAGTAGGTTGTTATGTTtggaatattaccaccggTGAACATAGCAATTTTCTACCTTACcggagacagaac	A17U + U18A + A19U	Fig.S2
ma478	chr X: 14744250 <– 14744180: WT: acactgtggatccggTGAGGTAGTAGGTTGTATAGTTtggaatattaccaccggTGAACTATGCAATTTTCTACCTTACcggagacagaac ma478: acactgtggatccggTGAGGTAGTAGGTTGTATACAAtggaatattaccaccggTGTTGTATGCAATTTTCTACCTTACcggagacagaac	G20C + U21A + U22A	Fig.S2

lin-41 alleles

ALLELE	LOCATION/MUTATION	DESCRIPTION	RELATED
ma480	chr I. 9335330 <- 9335425 WT: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma480: caagtatacc**ttttatacatccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacatccattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t13 mismatch to *let-7a*, complementary to *ma432*	Fig. 4,5,6, S4,S6
ma378	chr I. 9335330 <- 9335425 WT: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma378: caagtatacc**attaatatttacagttctacactca**acgcgatgtaaatatcgcaatccct**attaatattaacattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 match *ma341*	Fig. S5
ma501	chr I. 9335330 <- 9335425 WT: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma501: caagtatacc**ttttatacaaccgttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattcta**cctctgaaccattgaaacc	LCS1 + LCS2 perfect seed pairing to *let-7a* *	Fig. 6,7
ma545	chr I. 9335330 <- 9335425 WT: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma545: caagtatacc**ttttatacatgggttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacatggattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t11-t13 mismatch to *let-7a*	Fig. 6
ma555	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma555: caagtatacc**ttttatacaacggttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaacgattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t11 mismatch to *let-7a*	Fig. 6
ma556	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma556: caagtatacc**ttttatacaagcgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaagcattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t12 mismatch to *let-7a*	Fig. 6
ma564	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma564: caagtatacc**ttttatactaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatactaccattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t14 mismatch to *let-7a*	Fig. 6
ma565	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma565: caagtatacc**ttttataaaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttataaaaccattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t15 mismatch to *let-7a*	Fig. 6
ma566	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma566: caagtatacc**ttttattcaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t16 mismatch to *let-7a*	Fig. 6
ma571	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma571: caagtatacc**ttttatacaaggttctacactcaacg**cgatgtaaatatcgcaatccct**ttttatacaaggattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t11-t12 mismatch to *let-7a*	Fig. 6
ma572	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma572: caagtatacc**ttttattgttccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttattgttccattctgcctc**tgaaccattgaaacc	LCS1 + LCS2 t13-t16 mismatch to *let-7a*	Fig. 6
ma504	chr I. 9335330 <- 9335425 WT: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma504: caagtatacc**ttttatacatccgttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttatacatccattcta**cctctgaaccattgaaacc	Identical to *ma501ma480*	Fig. 6
ma552	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma552: caagtatacc**ttttatacaacggttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttatacaacgattcta**cctctgaaccattgaaacc	Identical to *ma501ma555*	Fig. 6
ma553	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma553: caagtatacc**ttttatacaagcgttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttatacaagcattcta**cctctgaaccattgaaacc	Identical to *ma501ma456*	Fig. 6
ma511	chr I. 9335330 <- 9335425 WT: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma511: caagtatacc**ttttatacatgggttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttatacatggattcta**cctctgaaccattgaaacc	Identical to *ma501ma545*	Fig. 6
ma554	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma554: caagtatacc**ttttatacaaggttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttatacaaggattcta**cctctgaaccattgaaacc	Identical to *ma501ma571*	Fig. 6
ma525	chr I. 9335330 <- 9335425 wt: caagtatacc**ttttatacaaccgttctacactca**acgcgatgtaaatatcgcaatccct**ttttatacaaccattctgcctc**tgaaccattgaaacc ma525: caagtatacc**ttttattgttccgttctac-ctca**acgcgatgtaaatatcgcaatccct**ttttattgttccattcta**cctctgaaccattgaaacc	Identical to *ma501ma572*	Fig. 6

*This allele(*ma501)* is identical to *xe11* (Brancati and Grosshans, 2018), but was generated independently in this study.

daf-12 alleles

ALLELE	LOCATION/MUTATION	DESCRIPTION	RELATED
ma567	chr X. ma567: aaatcatctaccaaacgatgccatgccttc<CTCTTCCTCTTAATTCCTCTCTTAATT >cctacctcttaattccgtcaatactatctcagattttcattgaagaact	daf-12 Jump board	Fig. 5, S7
ma568	chr X. ma568: ACCTACTAGAAATCATCTATTTATGGTGGTGAATACCTCACATCTTGATTCTATATTGCCTCCATCCAACAAACTCAATCTAGCCAC ATTTCTTCTTTTTCACGTACCTCAACCACCCTTTCCATATTTATGGTGGTGATCTACCTCTTTAACCAATTCATCATCTTTTTATATTG TTTCTTATTTGCATTCAACTTGGAAATAGCCACTATCATATCACTATTGCGTATTTCCTTTCATTTTCTTGTCTTATTTTCTTGAGACC AGCACCAGAAGATTTTTTCGATGGAGAACTAAGCATGATAATTTGAAGTTTTCCATTTAAAAAATGCAGGTAATACGGTTAATTC AATCTGCGAGTTGATGTTCGGTCTCCGGTTTTCATGTTCTACTTCTAATGACTAGAAACCCTTTTATCTAACATCCGGTCCTCCTATC CCTAATGTACCCAGTAGATATTTTTTCCCCGAATGATTAAACCTCCCAGTCAAATATTGTATTTTGATTTATGGTGGTGACCTACCT CTTAATTCCGTCAATACTATCTC	daf-12 LCS1-3 with t11-t13 mismatch to *let-7a*	Fig. 5, S7

C. elegans strains

C.elegans: Strain N2	Caenorhabditis Genetics Center	WormBase Strain: N2
C.elegans: Strain VT1313 *(maIs105 V; mir-84(n4037) X)*	This paper	VT1313
C.elegans: Strain VT1367 (*maIs105 V*)	This paper	VT1367
C.elegans: Strain VT2692 *(maIs105 V; let-7(n2853) X)*	This paper	VT2692
C.elegans: Strain VT3439 *(maIs105 V; let-7(ma341) X)* , see Table S5	This paper	VT3439
C.elegans: Strain VT3455 *(wIs51 V)*	This paper	VT3455
C.elegans: Strain VT3460 *(wIs51 V; let-7a(ma341) X)* , see Table S5	This paper	VT3460
C.elegans: Strain VT3479 *(maIs105 V; let-7(ma341);mir-84(n4037) X)* , see Table S5	This paper	VT3479
C.elegans: Strain VT3585 *(lin-41(ma378) I; maIs105 V; let-7(ma341) X)* , see Table S5	This paper	VT3585
C.elegans: Strain VT3639 *(lin-41(ma378) I; maIs105 V)* , see Table S5	This paper	VT3639
C.elegans: Strain VT3645 *(mnDp1(umnIs25) (X;V),+ / +,maIs105 V; let-7(ma393) X)* , see Table S5	(Duan et al., 2020)	VT3645
C.elegans: Strain VT3648 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma341) X)* , see Table S5	This paper	VT3648
C.elegans: Strain VT3729 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma432ma435) X)* , see Table S5	This paper	VT3729 *ma432ma435* is also labeled as *ma428*
C.elegans: Strain VT3742 *(oxSi1091(Pmex-5::Cas-9(smu-2 introns) unc-119+) II; mnDp1(umnIs25) (X;V)/+ V; let-7(ma393) X)* , see Table S5	This paper	VT3742
C.elegans: Strain VT3793 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma431) X)* , see Table S5	This paper	VT3793
C.elegans: Strain VT3794 *(mnDp1(umnIs25) (X,* see Table S5*;V),+/+,maIs105 V; let-7(ma432) X)*	This paper	VT3794
C.elegans: Strain VT3795 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma433) X)* , see Table S5	This paper	VT3795
C.elegans: Strain VT3796 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma434) X)* , see Table S5	This paper	VT3796
C.elegans: Strain VT3797 *(maIs105 V; let-7(ma435) X)* , see Table S5	This paper	VT3797
C.elegans: Strain VT3798 *(maIs105 V; let-7(ma436) X)* , see Table S5	This paper	VT3798
C.elegans: Strain VT3799 *(maIs105 V; let-7(ma437) X)* , see Table S5	This paper	VT3799
C.elegans: Strain VT3825 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma448) X)* , see Table S5	This paper	VT3825
C.elegans: Strain VT3826 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma449) X)* , see Table S5	This paper	VT3826
C.elegans: Strain VT3827 *(maIs105 V; let-7(ma450) X)* , see Table S5	This paper	VT3827
C.elegans: Strain VT3828 *(maIs105 V; let-7(ma451) X)* , see Table S5	This paper	VT3828
C.elegans: Strain VT3829 *(maIs105 V; let-7(ma452) X)* , see Table S5	This paper	VT3829
C.elegans: Strain VT3835 *(maIs105 V; let-7(ma453) X)* , see Table S5	This paper	VT3835
C.elegans: Strain VT3836 *(maIs105 V; let-7(ma454) X)* , see Table S5	This paper	VT3836
C.elegans: Strain VT3843 *(maIs105 V; let-7(ma455) X)* , see Table S5	This paper	VT3843
C.elegans: Strain VT3844 *(maIs105 V; let-7(ma456) X)* , see Table S5	This paper	VT3844
C.elegans: Strain VT3867 *(lin-41(tn1541) I; oxSi1091 II; mnDp1(umnIs25) (X;V)/+ V; let-7(ma432) X)* , see Table S5	This paper	VT3867
C.elegans: Strain VT3868 *(lin-41(tn1541) I; mnDp1(umnIs25) (X;V)/+ V; let-7(ma432) X)* , see Table S5	This paper	VT3868
C.elegans: Strain VT3873 *(oxSi1091 II; mnDp1(umnIs25) (X;V)/+ V; let-7(ma432) X)* , see Table S5	This paper	VT3873
C.elegans: Strain VT3874 *(maIs105 V; let-7(ma476) X)* , see Table S5	This paper	VT3874
C.elegans: Strain VT3875 *(maIs105 V; let-7(ma477) X)* , see Table S5	This paper	VT3875
C.elegans: Strain VT3876 *(maIs105 V; let-7a(ma478) X)* , see Table S5	This paper	VT3876
C.elegans: Strain VT3877 *(mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma449ma435) X)* , see Table S5	This paper	VT3877 *ma449ma435* is also labeled as *ma479*
C.elegans: Strain VT3878 *(lin-41(tn1541ma480) I; let-7(ma432) X)* , see Table S5	This paper	VT3878

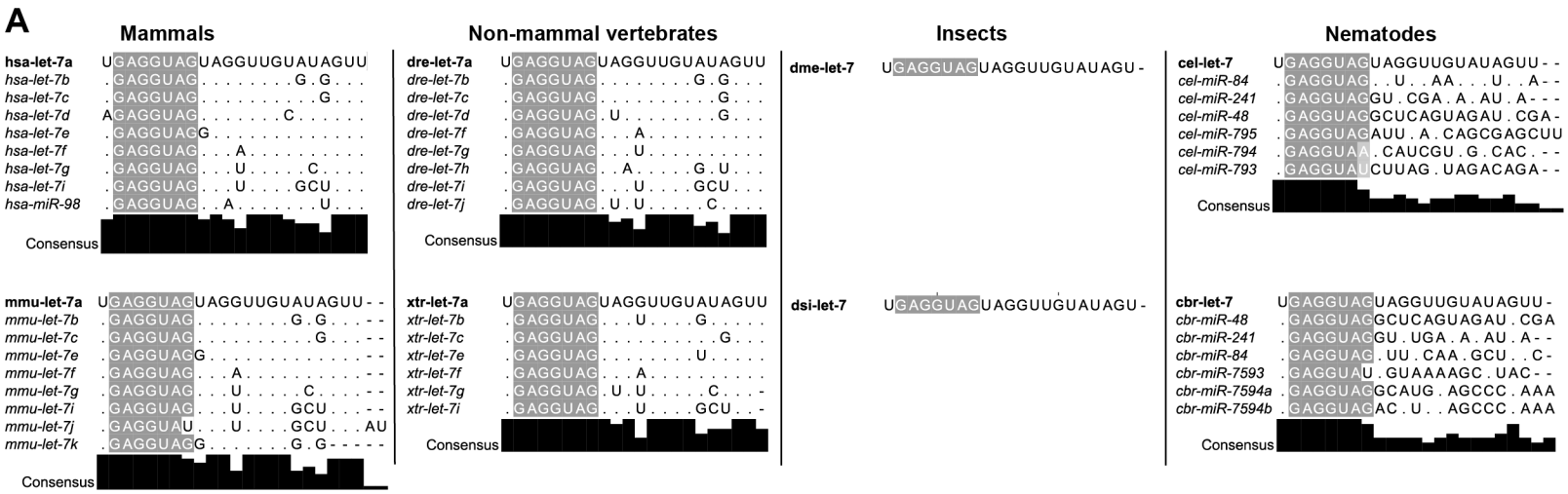
C.elegans: Strain VT3879 *(lin-41(tn1541ma480) I; maIs105 V; let-7(ma432) X)* , see Table S5	This paper	VT3879
C.elegans: Strain VT3945 *(lin-41(tn1541ma501) I; maIs105 V)* , see Table S5	This paper	VT3945
C.elegans: Strain VT3949 *(lin-41(tn1541ma480) I)* , see Table S5	This paper	VT3949
C.elegans: Strain VT3950 *(lin-41(tn1541ma480) I; maIs105 V)* , see Table S5	This paper	VT3950
C.elegans: Strain VT3954 *(lin-41(tn1541ma501) I; mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma393) X)* , see Table S5	This paper	VT3954
C.elegans: Strain VT3959 *(lin-41(tn1541) I; mnDp1(umnIs25) (X;V),+/+,maIs105 V; let-7(ma393) X)* , see Table S5	This paper	VT3959
C.elegans: Strain VT3974 *(lin-41(ma501ma545) I; maIs105 V)* , see Table S5	This paper	VT3974 *ma501ma545* is also labeled as *ma511*
C.elegans: Strain VT3975 *(lin-41(xe11) I; maIs105 V)* , see Table S5	This paper	VT3975
C.elegans: Strain VT4009 *(lin-41(ma501ma572) I; maIs105 V)* , see Table S5	This paper	VT4009 *ma501ma572* is also labeled as *ma554*
C.elegans: Strain VT4058 *(lin-41(ma501ma480) I; maIs105 V)* , see Table S5	This paper	VT4058 *ma501ma480* is also labeled as *ma504*
C.elegans: Strain VT4060 *(lin-41(ma480) I; maIs105 V)* , see Table S5	This paper	VT4060
C.elegans: Strain VT4091 *(lin-41(ma501ma555) I; maIs105 V)* , see Table S5	This paper	VT4091 *ma501ma555* is also labeled as *ma552*
C.elegans: Strain VT4092 *(lin-41(ma501ma556) I; maIs105 V)* , see Table S5	This paper	VT4092 *ma501ma556* is also labeled as *ma553*
C.elegans: Strain VT4093 *(lin-41(ma501ma571) I; maIs105 V)* , see Table S5	This paper	VT4093 *ma501ma571* is also labeled as *ma554*
C.elegans: Strain VT4094 *(lin-41(ma555) I; maIs105 V)* , see Table S5	This paper	VT4094
C.elegans: Strain VT4095 *(lin-41(ma556) I; maIs105 V)* , see Table S5	This paper	VT4095
C.elegans: Strain VT4123 *(lin-41(ma564) I; maIs105 V)* , see Table S5	This paper	VT4123
C.elegans: Strain VT4124 *(lin-41(ma565) I; maIs105 V)* , see Table S5	This paper	VT4124
C.elegans: Strain VT4125 *(lin-41(ma566) I; maIs105V)* , see Table S5	This paper	VT4125
C.elegans: Strain VT4126 *(oxSi1091 II; maIs105 V; daf-12(ma498ma567) X)* , see Table S5	This paper	VT4126
C.elegans: Strain VT4128 *(lin-41(tn1541) I; maIs105 V; daf-12(ma498ma568) X)* , see Table S5	This paper	VT4128
C .elegans: Strain VT4129 *(lin-41(tn1541) I; maIs105 V; daf-12(ma498) X)* , see Table S5	This paper	VT4129
C.elegans: Strain DG3913 *(lin-41(tn1541) I)*	Caenorhabditis Genetics Center	WormBase: DG3913

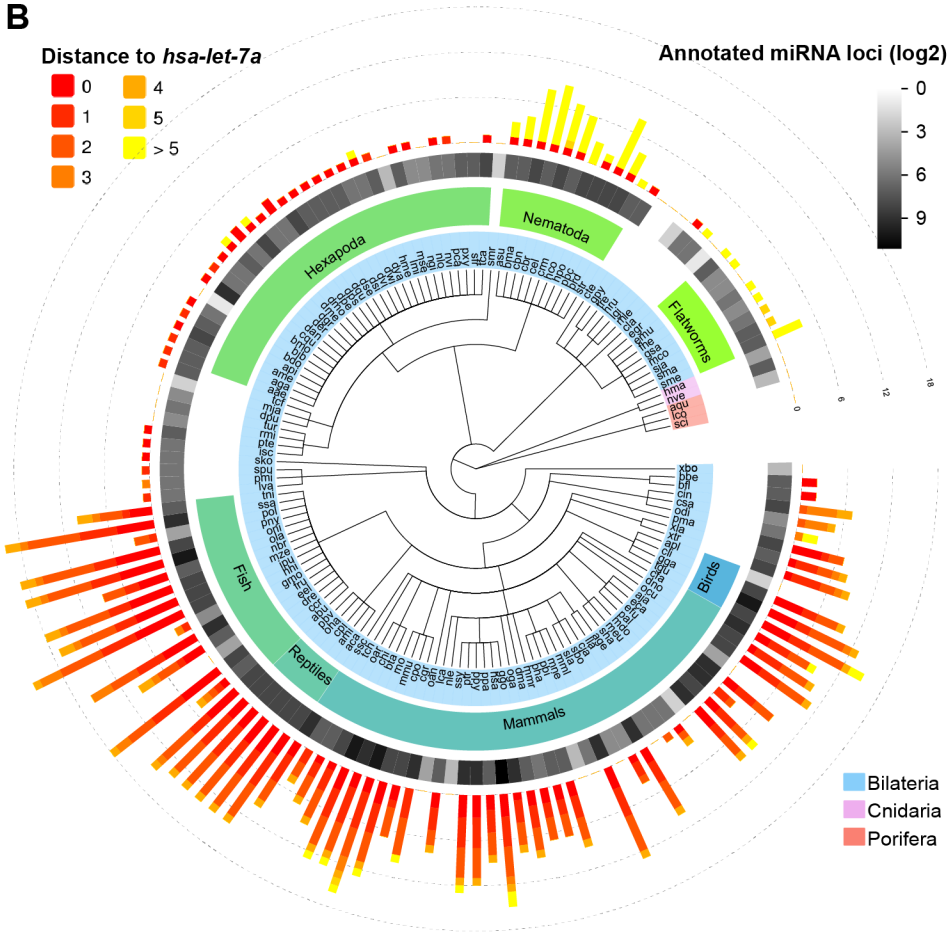
Table S6. Oligonucleotides used in this study. Related to **Figure 2-7, S2, S3, S4, S5, S7**

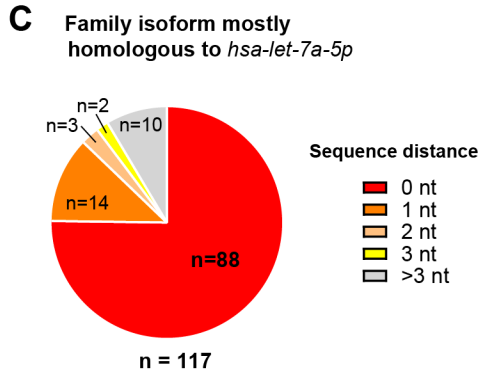
OLIGONUCLEOTIDES	SOURCE	IDENTIFIER
Alt-R *let-7a* WT crRNA: TGTGGATCCGGTGAGGTAGT + Alt-R	IDT	AltR_Cas-9 _crRNA_let-7_g3
Alt-R *let-7a* jump board crRNA_1: ATCAGTTCGATATCTGACGG + Alt-R	IDT	AltR_Cas-9 _crRNA_INPP4A_1
Alt-R *let-7a* jump board crRNA_2: TGTATCAGTTCGATATCTGA + Alt-R	IDT	AltR_Cas-9 _crRNA_INPP4A_2
Alt-R dpy-10 crRNA as co-CRISPR marker: CTACCATAGGCACCACGAG + Alt-R	IDT	AltR_Cas-9 _crRNA_dpy-10_cn64
Alt-R *lin-41* crRNA_1: CATCGCGTTGAGTGTAGAA + Alt-R	IDT	AltR_Cas-9 _crRNA_lin-41_1
Alt-R *lin-41* crRNA_2: CAATGGTTCAGAGGCAGAA + Alt-R	IDT	AltR_Cas-9 _crRNA_lin-41_2
Alt-R *daf-12* crRNA_4: AGAGAGGAATTAAGAGGAAGGTTTTAGAGCTATGCT + Alt-R	IDT	AltR_Cas-9 _crRNA_daf-12_4
Alt-R CRISPR-Cas-9 tracrRNA	IDT	Cat# 1072533
let-7a cloning primer reverse: ATTGAAAATCTGTCATTGAGCAA	this study	let-7_Donor_R4
let-7a cloning primer forward: GCTCAAGGTTTTCGATCTCTGT	this study	let-7_Donor_F4
let-7a HR donor amplifying primer forward: ATAGCACAAAATAAAGAAAAACAAAGAGGTGAAAG	this study	let-7_SEQ_F4
let-7a HR donor amplifying primer reverse: AATTTAACAACAAGTACTAATCCATTTTTCAGGCAAGC	this study	let-7_SEQ_R4
let-7a genotyping primer forward: AACGGCTCCATGGATACATTACTCAACAG	this study	let-7_SEQ_F5
let-7a genotyping primer reverse: GGTTTCTGTTCATATATGAGAAGCGCATCAG	this study	let-7_SEQ_R5
lin-41 genotyping primer forward: CATCCATTCATATGGCTCCGCCCC	this study	lin-41_SEQ_F3
lin-41 genotyping primer reverse: CACTGGGGACATTAGGCAATTGGGAC	this study	lin-41_SEQ_R3
daf-12 genotyping primer forward: CGAGGGACGTCACTCCACCGG	this study	daf-12_SEQ_F1
daf-12 genotyping primer forward: GGCGTTGGGAGTTGAAAGTCTTAAATAG	this study	daf-12_SEQ_R1
daf-12 genotyping primer forward: GATTCCAAAAGCACTGGGATTACTTAATGTAAG	this study	daf-12_SEQ_F2
daf-12 genotyping primer forward: AAACTTCAAATTATCATGCTTAGTTCTCCATCG	this study	daf-12_SEQ_R2
lin-41 qRT-PCR primer forward*:* CGGATACTCGGAATCATCGTGTTCAG	IDT	lin-41_RTPCR_F
lin-41 qRT-PCR primer reverse*:* GACTGCGAGTCGGTGATTGTTGAAG	IDT	lin-41_RTPCR_R
gpd-1 qRT-PCR primer forward*:* TGCTCCAATGTACGTGGTTGGAG	IDT	gpd-1_RTPCR_F
gpd-1 qRT-PCR primer reverse*:* CGTCATGAGTCCTTCGATGATACCG	IDT	gpd-1_RTPCR_R
lin-41 ssDNA donor for *ma480:* TCAAATTGCACCAACTCAAGTATACCTTTTATACATCCGTTCTACACTCAACGCGATGTAAATATC GCAATCCCTTTTTATACATCCATTCTGCCTCTGAACCATTGAAACCTTC (Ultramer)	IDT	N/A
lin-41 ssDNA donor for *ma504:* CCAACTCAAGTATACCTTTTATACATCCGTTCTACCTCAACGCGATGTAAATATCGCAATCCCTTTT TATACATCCATTCTACCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Ultramer)	IDT	N/A
lin-41 ssDNA donor for *ma501:* CCAACTCAAGTATACCTTTTATACAACCGTTCTACCTCAACGCGATGTAAATATCGCAATCCCTTTT TATACAACCATTCTACCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Ultramer)	IDT	N/A
lin-41 ssDNA donor for *ma511:* TTGCACCAACTCAAGTATACCTTTTATACATGGGTTCTACCTCAACGCGATGTAAATATCGCAATC CCTTTTTATACATGGATTCTACCTCTGAACCATTGAAACCTTCTCCCGTAC (Ultramer)	IDT	N/A
lin-41 ssDNA donor for *ma378:* TTCCTCAAATTGCACCAACTCAAGTATACCATTAATATTACAGTTCTACACTCAACGCGATGTAAA TATCGCAATCCCTATTAATATTACAATTCTGCCTCTGAACCATTGAAACCTTCTCCCGTAC (Ultramer)	IDT	N/A

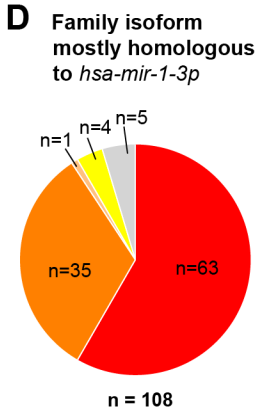
lin-41 ssDNA donor for *ma552:* TCAAATTGCACCAACTCAAGTATACCTTTTATACAACGGTTCTACCTCAACGCGATGTAAATATCG CAATCCCTTTTTATACAACGATTCTACCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Oligo-Flex)	Genewiz	N/A
lin-41 ssDNA donor for *ma553:* TCAAATTGCACCAACTCAAGTATACCTTTTATACAAGCGTTCTACCTCAACGCGATGTAAATATCG CAATCCCTTTTTATACAAGCATTCTACCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Oligo-Flex)	Genewiz	N/A
lin-41 ssDNA donor for *ma554:* TCAAATTGCACCAACTCAAGTATACCTTTTATACAAGGGTTCTACCTCAACGCGATGTAAATATCG CAATCCCTTTTTATACAAGGATTCTACCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Oligo-Flex)	Genewiz	N/A
lin-41 ssDNA donor for *ma525:* CAAATTGCACCAACTCAAGTATACCTTTTATTGTTCCGTTCTACCTCAACGCGATGTAAATATCGC AATCCCTTTTTATTGTTCCATTCTACCTCTGAACCATTGAAACCTTCTCC (Oligo-Flex)	Genewiz	N/A
lin-41 ssDNA donor for *ma555:* TCAAATTGCACCAACTCAAGTATACCTTTTATACAACGGTTCTACACTCAACGCGATGTAAATATC GCAATCCCTTTTTATACAACGATTCTGCCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Ultramer)	IDT	N/A
lin-41 ssDNA donor for *ma556:* TCAAATTGCACCAACTCAAGTATACCTTTTATACAAGCGTTCTACACTCAACGCGATGTAAATATC GCAATCCCTTTTTATACAAGCATTCTGCCTCTGAACCATTGAAACCTTCTCCCGTACTCCCA (Ultramer)	IDT	N/A
lin-41 ssDNA donor for *ma564:* CCTCTTTTCCTCAAATTGCACCAACTCAAGTATACCTTTTATACTACCGTTCTACACTCAACGCGAT GTAAATATCGCAATCCCTTTTTATACTACCATTCTGCCTCTGAACCATTGAAACCTTCTCCCGTACT CCCA (Oligo-Flex)	Genewiz	N/A
lin-41 ssDNA donor for *ma565:* CCTCTTTTCCTCAAATTGCACCAACTCAAGTATACCTTTTATAAAACCGTTCTACACTCAACGCGAT GTAAATATCGCAATCCCTTTTTATAAAACCATTCTGCCTCTGAACCATTGAAACCTTCTCCCGTACT CCCA (Oligo-Flex)	Genewiz	N/A
lin-41 ssDNA donor for *ma566:* CCTCTTTTCCTCAAATTGCACCAACTCAAGTATACCTTTTATTCAACCGTTCTACACTCAACGCGAT GTAAATATCGCAATCCCTTTTTATTCAACCATTCTGCCTCTGAACCATTGAAACCTTCTCCCGTACT CCCA (Oligo-Flex)	Genewiz	N/A
daf-12 dsDNA donor for ma568: CGAGGGACGTCACTCCACCGGAGGAATGGACGAGCTCTACAAGTAGACCTACTAGAAATCATCT ATTTATGGTGGTGAATACCTCACATCTTGATTCTATATTGCCTCCATCCAACAAACTCAATCTAGCC ACATTTCTTCTTTTTCACGTACCTCAACCACCCTTTCCATATTTATGGTGGTGATCTACCTCTTTAAC CAATTCATCATCTTTTTATATTGTTTCTTATTTGCATTCAACTTGGAAATAGCCACTATCATATCACT ATTGCGTATTTCCTTTCATTTTCTTGTCTTATTTTCTTGAGACCAGCACCAGAAGATTTTTTCGATG GAGAACTAAGCATGATAATTTGAAGTTTTCCATTTAAAAAATGCAGGTAATACGGTTAATTCAAT CTGCGAGTTGATGTTCGGTCTCCGGTTTTCATGTTCTACTTCTAATGACTAGAAACCCTTTTATCTA ACATCCGGTCCTCCTATCCCTAATGTACCCAGTAGATATTTTTTCCCCGAATGATTAAACCTCCCAG TCAAATATTGTATTTTGATTTATGGTGGTGACCTACCTCTTAATTCCGTCAATACTATCTCAGATTT TCATTGAAGAACTGGTCCGGAATTATTGAATCATCAGCTAG (fragmentGENE)	Genewiz	N/A

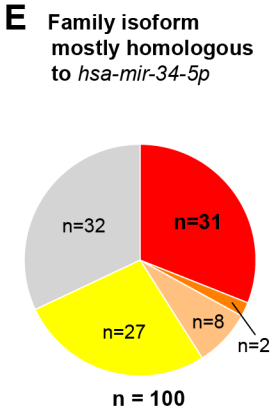
Figure S1

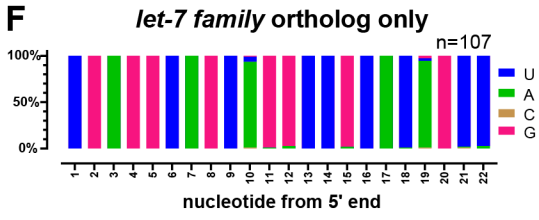

A
Mammals
hsa-let-7a
hsa-let-7b
hsa-let-7c
hsa-let-7d
hsa-let-7e
hsa-let-7f
hsa-let-7g
hsa-let-7i
hsa-miR-98
Consensus
Non-mammal vertebrates
dre-let-7a
dre-let-7b
dre-let-7c
dre-let-7d
dre-let-7f
dre-let-7g
dre-let-7h
dre-let-7i
dre-let-7j
Consensus
Insects
dme-let-7
Nematodes
cel-let-7
cel-miR-84
cel-miR-241
cel-miR-48
cel-miR-795
cel-miR-794
cel-miR-793
Consensus
mmu-let-7a
mmu-let-7b
mmu-let-7c
mmu-let-7e
mmu-let-7f
mmu-let-7g
mmu-let-7i
mmu-let-7j
mmu-let-7k
Consensus
xtr-let-7a
xtr-let-7b
xtr-let-7c
xtr-let-7e
xtr-let-7f
xtr-let-7g
xtr-let-7i
Consensus
dsi-let-7
cbr-let-7
cbr-miR-48
cbr-miR-241
cbr-miR-84
cbr-miR-7593
cbr-miR-7594a
cbr-miR-7594b
Consensus

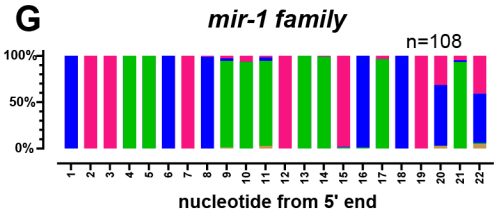

B
Distance to hsa-let-7a
0
1
2
3
4
5
> 5
Annotated miRNA loci (log2)
0
3
6
9
Hexapoda
Nematoda
Flatworms
Fish
Reptiles
Mammals
Birds
Bilateria
Cnidaria
Porifera


C
Family isoform mostly homologous to hsa-let-7a-5p
n=2
n=3
n=10
n=14
n=88
n = 117
Sequence distance
0 nt
1 nt
2 nt
3 nt
>3 nt


D
Family isoform mostly homologous to hsa-mir-1-3p
n=1
n=4
n=5
n=35
n=63
n = 108


E
Family isoform mostly homologous to hsa-mir-34-5p
n=32
n=31
n=27
n=8
n=2
n = 100


F
let-7 family ortholog only
n=107
100%
50%
0%
U
A
C
G
nucleotide from 5' end


G
mir-1 family
n=108
100%
50%
0%
nucleotide from 5' end

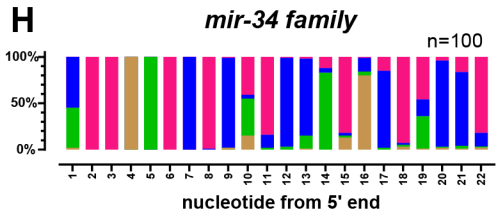

H
mir-34 family
n=100
100%
50%
0%
nucleotide from 5' end

Figure S1. *let-7a* is distinguishably conserved among bilaterian species. (Related to **Figure 1**).

A. Sequence alignments and consensus of *let-7 family* miRNAs from model organisms including *H. sapiens (hsa)*, *M. musculus (mmu), D. rerio (dre), X. tropicalis (xtr), D. melanogaster (dme), D. simulans (dsi), C. elegans (cel) and C. briggsae (cbr)*. Nucleotides identical to *hsa-let-7a-5p* are indicated by dots (.) and gaps in sequence alignment are indicated by dashes (-). *dme* and *dsi* do not have consensus scores because *let-7a* appears singly in these species. Alignments and consensus are scored by JalView (Waterhouse et al., 2009).

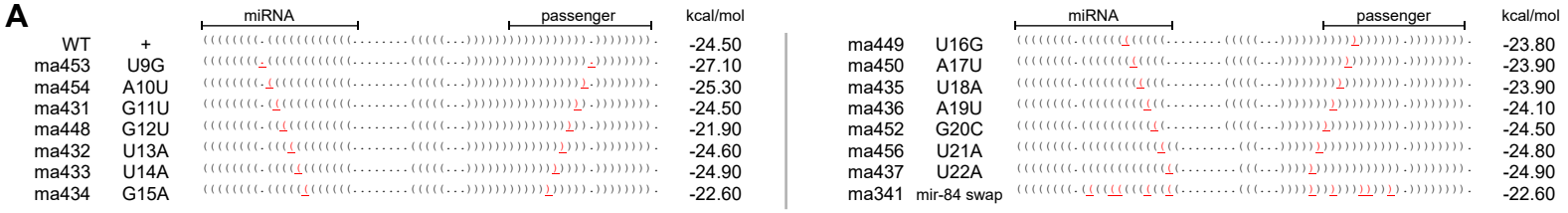
B. Summary of *let-7 family* miRNAs across animal phylogeny tree which includes all metazoans with *let-7 family* annotation in miRBase v22.1**.** In the outer circle, bar lengths indicate the number of annotated *let-7 family* isoforms, and bar colors indicate the sequence distances to *hsa-let-7a-5p*. The middle circle indicates the quality of the total miRNA annotation of each species. Note that cases of an apparent absence of *let-7 family* miRNA usually correlate with poor miRNA annotation.

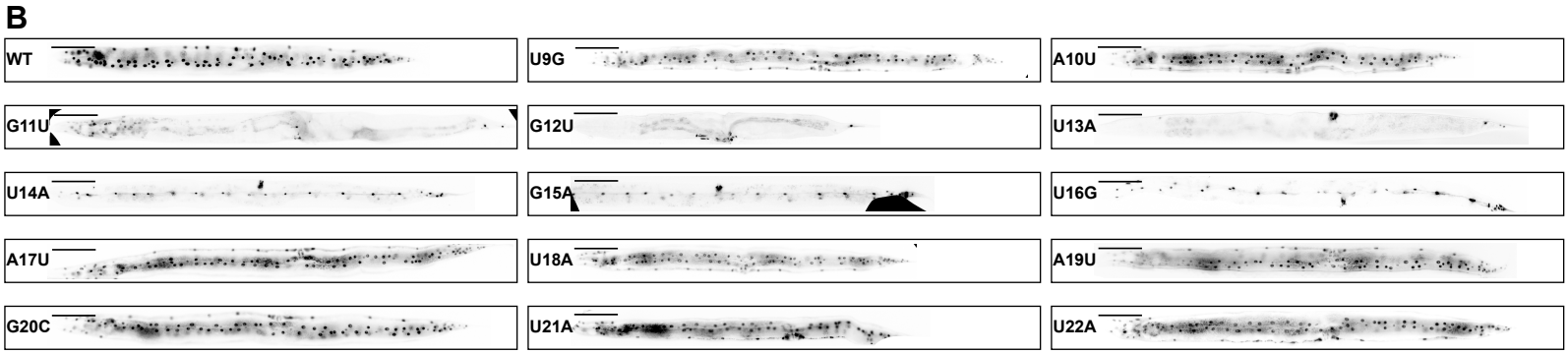
C-E. Distributions of sequence distances between *hsa-let-7a-5p* (**C**), *hsa-mir-1-3p* (**D**), *hsa-mir-34-5p* (**E**) and their closest isoforms of each species across bilaterians.

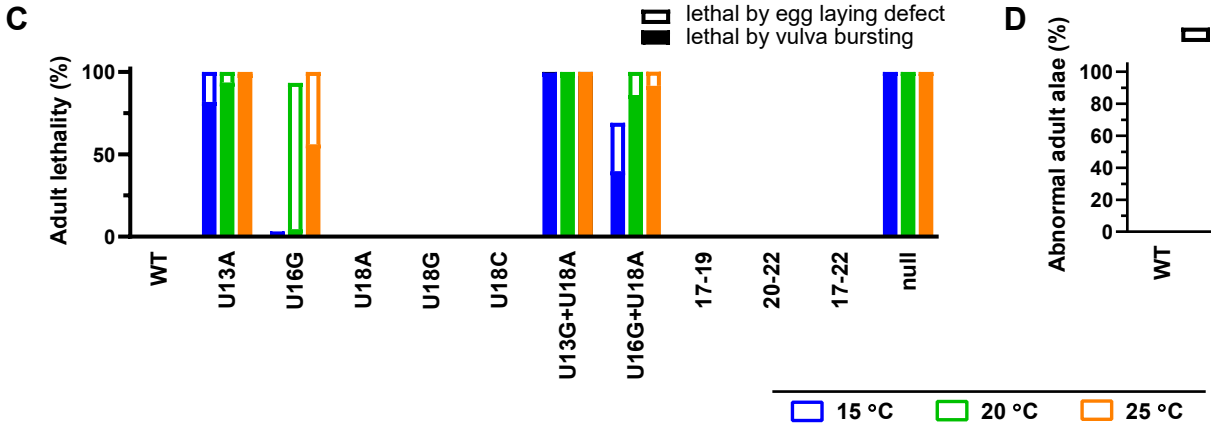
F. Nucleotide frequency of the *let-7a family* isoforms closest to *hsa-let-7a-5p*, excluding species without the *let-7a* orthologs (closest *let-7* isoform has > 3 nucleotides different from *hsa-let-7a-5p)*.

G-H. Nucleotide frequency of *mir-1 family* **(G)** and *mir-34 family* **(H)** isoforms mostly homologous to *hsa-mir-1-3p* **(G)** and *has-mir-34-5p* **(H)**.

Figure S2


A
miRNA
passenger
kcal/mol
WT +
ma453 U9G
ma454 A10U
ma431 G11U
ma448 G12U
ma432 U13A
ma433 U14A
ma434 G15A
-24.50
-27.10
-25.30
-24.50
-21.90
-24.60
-24.90
-22.60
ma449 U16G
ma450 A17U
ma435 U18A
ma436 A19U
ma452 G20C
ma456 U21A
ma437 U22A
ma341 mir-84 swap
-23.80
-23.90
-23.90
-24.10
-24.50
-24.80
-24.90
-22.60


B
WT
U9G
A10U
G11U
G12U
U13A
U14A
G15A
U16G
A17U
U18A
A19U
G20C
U21A
U22A


C
lethal by egg laying defect
lethal by vulva bursting
Adult lethality (%)
100
50
0
WT
U13A
U16G
U18A
U18G
U18C
U13G+U18A
U16G+U18A
17-19
20-22
17-22
null
15 °C
20 °C
25 °C

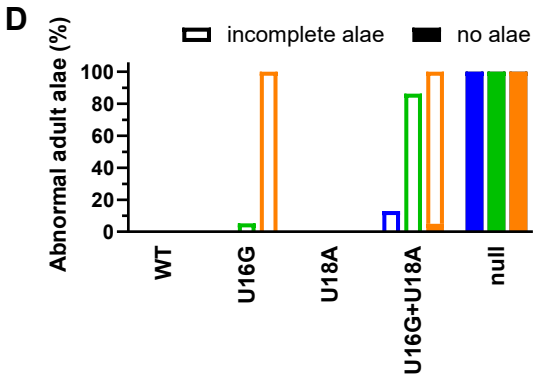

D
incomplete alae
no alae
Abnormal adult alae (%)
100
80
60
40
20
0
WT
U16G
U18A
U16G+U18A
null

Figure S2. A phenotypic mutational screen of *let-7a* 3' non-seed region. (Related to **Figure 3**).

A. Dot-bracket notations of optimal secondary structures and the minimum folding free energy of pre-miRNA for *let-7a* mutants (**Fig. 3A**). Secondary structure predictions and free energy were analyzed by RNAfold (Denman, 1993; Kerpedjiev et al., 2015). For each position, the miRNA nucleotide and the corresponding nucleotide in the passenger strand were mutated according to the following criteria (ranked by the order): (1) The resulting nucleotides are identical to the corresponding nucleotides of *let-7(ma341)*; (2) the resulting sequences cause the minimum change to pre-miRNA structure; (3) the resulting sequences cause the minimum change to pre-miRNA folding energy.

B. Representative COL-19::GFP patterns for the *let-7a* 3' non-seed mutants, scored by expression of *maIs105(col-19::gfp)*. Scale bars, 100 μm. Images are processed by ImageJ Fiji (Schindelin et al., 2012).

C-D. Synergistic effect between mutations at g17-g22 and the critical non-seed nucleotides at g11-g16. **C.** Vulva integrity phenotypes, reflected by two categories of lethality: bursting of pre-gravid adults (severe), or accumulation of hatched progeny inside the uterus due to egg-laying defects (mild). **D.** Phenotypes in heterochronic pathway based on adult alae phenotypes. The alae phenotypes are categorized as no alae (severe) or incomplete alae (mild). Simultaneously mutating g13 and g18 did not result in enhanced phenotypes compared to mutating g13 alone, likely because the phenotypes of the g13 mutation are already essentially as strong as *let-7(null)*.

Figure S3

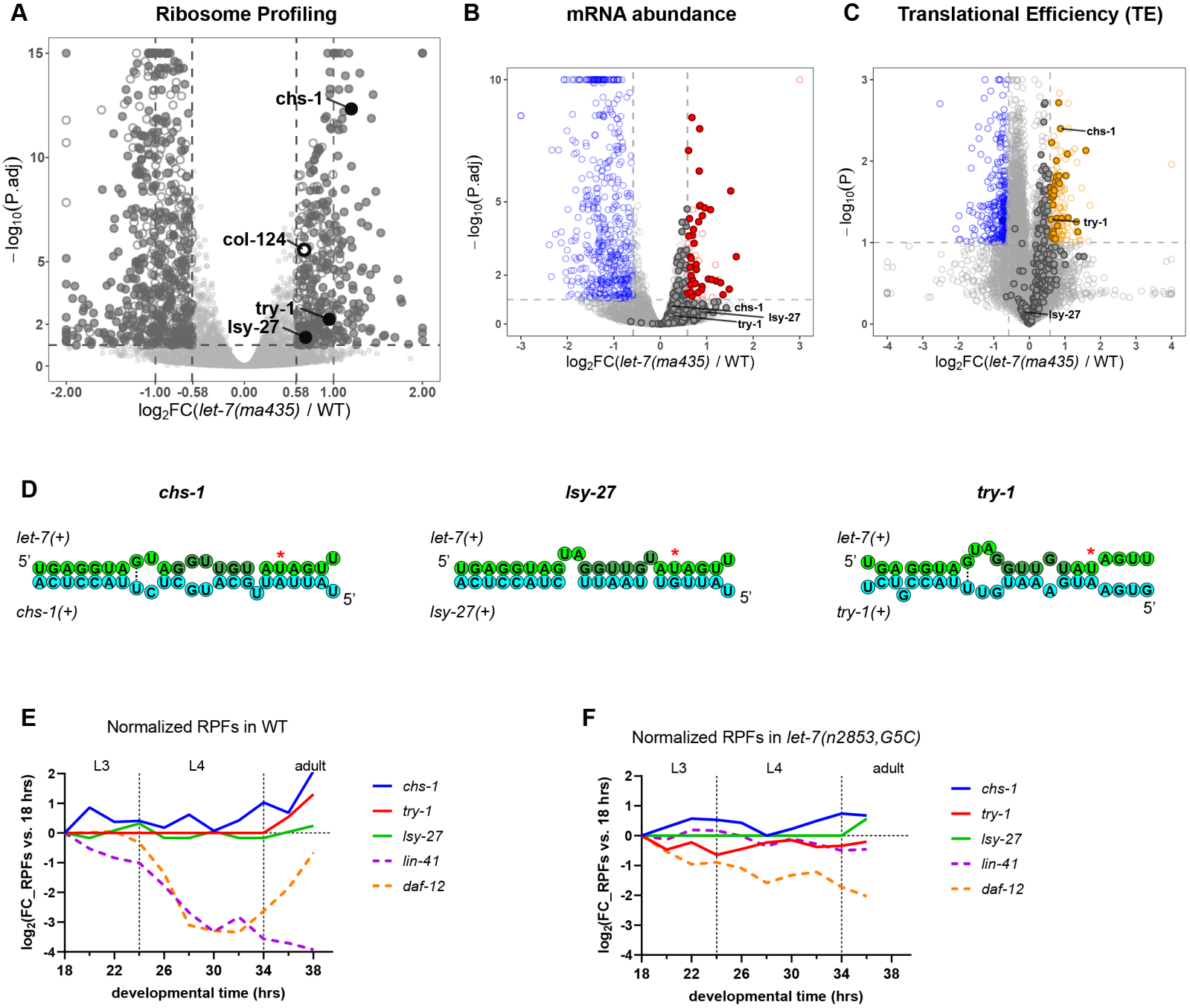

A
Ribosome Profiling
chs-1
col-124
try-1
lsy-27
$-\log_{10}$(P.adj)
$\log_2$FC(*let-7(ma435)* / WT)
B
mRNA abundance
chs-1
try-1
lsy-27
$-\log_{10}$(P.adj)
$\log_2$FC(*let-7(ma435)* / WT)
C
Translational Efficiency (TE)
chs-1
try-1
lsy-27
$-\log_{10}$(P)
$\log_2$FC(*let-7(ma435)* / WT)
D
chs-1
let-7(+)
chs-1(+)
lsy-27
let-7(+)
lsy-27(+)
try-1
let-7(+)
try-1(+)
E
Normalized RPFs in WT
L3
L4
adult
$\log_2$(FC_RPFs vs. 18 hrs)
developmental time (hrs)
chs-1
try-1
lsy-27
lin-41
daf-12
F
Normalized RPFs in *let-7(n2853,G5C)*
L3
L4
adult
$\log_2$(FC_RPFs vs. 18 hrs)
developmental time (hrs)
chs-1
try-1
lsy-27
lin-41
daf-12

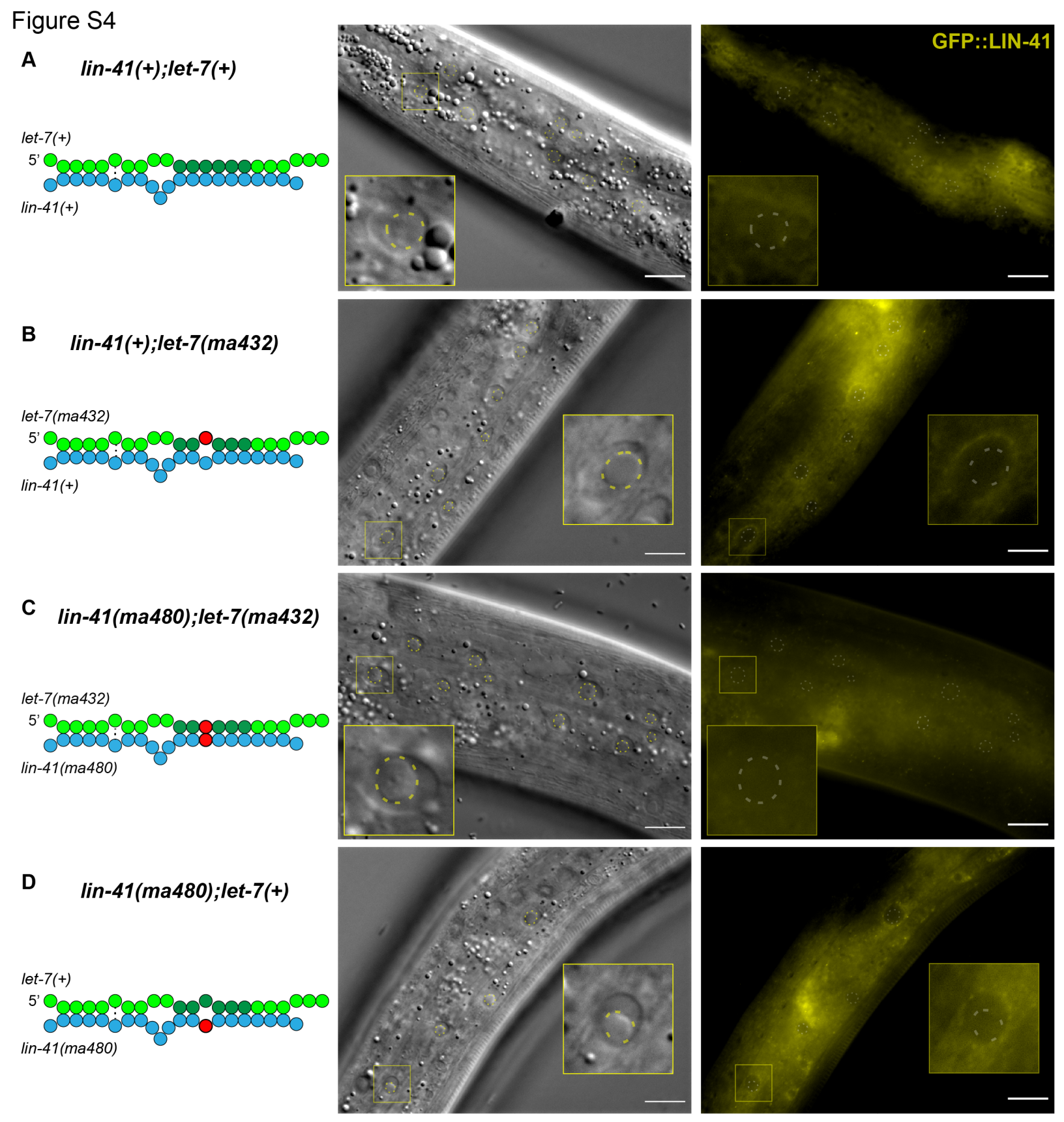
Figure S3. Molecular phenotypes of *let-7(ma435, U18A).* (Related to **Figure 3**).

A-C. Differential expression analyses of RPFs **(A)**, mRNA abundance **(B)** and TE **(C)** between *let-7(ma435);mals105* and *mals105*. Hollow points indicate developmentally dynamic genes whose perturbation in these experiments could potentially reflect imperfect synchrony between samples (See Figure 5 legend and Star Methods) and not the genotypes; solid points indicate genes likely to be perturbed specifically due to the mutation in **A**. Genes with significantly increased/decreased mRNA (FC > |1.5| and P.adj < 0.1 by DESeq2) are represented by red/blue points in **B**. Genes with significantly increased/decreased TE (FC > |1.5| and P < 0.1 by t-test) are colored orange/blue in **C**. Genes that contain predicted LCSs with g18 pairing are labeled by gene names in **A**. Solid points in **B-C** represent genes with significantly increased RPFs in ribosome profiling.

D. Predicted base-pairing configuration between *let-7a* and the predicted LCSs with g18 pairing. g18 are labeled by red asterisks (*).

E-F. Developmental profiles of the translation of the putative g18 targets in WT (**E**) and *let-7(n2853, G5C)* (**F**) at L3, L4 and young adult stages. Results were analyzed from (Aeschimann et al., 2017) and normalized by the RPFs at 18 hrs. *lin-41* and *daf-12* are used as validated *let-7a* targets which are down-regulated at L4 stages.

Figure S4


A
lin-41(+);let-7(+)
let-7(+)
5'
lin-41(+)
GFP::LIN-41
B
lin-41(+);let-7(ma432)
let-7(ma432)
5'
lin-41(+)
C
lin-41(ma480);let-7(ma432)
let-7(ma432)
5'
lin-41(ma480)
D
lin-41(ma480);let-7(+)
let-7(+)
5'
lin-41(ma480)

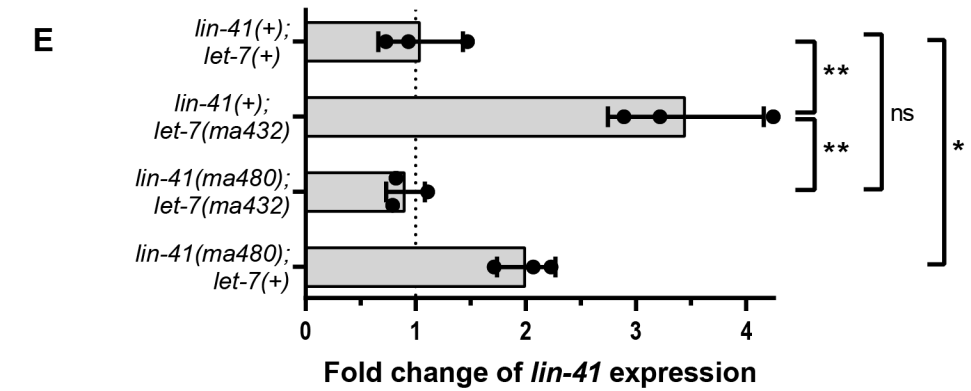

E
lin-41(+); let-7(+)
lin-41(+); let-7(ma432)
lin-41(ma480); let-7(ma432)
lin-41(ma480); let-7(+)
**
**
ns
*
0
1
2
3
4
Fold change of lin-41 expression

Figure S4. Expression of LIN-41::GFP indicates that *lin-41* is a major target of *let-7a* that requires non-seed pairing. (Related to **Figure 4**).

LIN-41 expression is visualized based on endogenous GFP-tag (*tn1541)* (Spike et al., 2014). Yellow dashed circles indicate nucleoli of Hyp7 and seam cells, which are identified using the DIC channel (middle panels) and positioned on the GFP channel image (right panels) in register with the DIC channel. The cartoons at left illustrate expected interacting configurations between *let-7a* and *lin-41* LCS1. Note that both LCSs in *lin-41* were modified using the strategy.

A. In WT, endogenously GFP tagged LIN-41 is expressed in neither Hyp7 nor in seam cells at the late L4 stage due to repression by *let-7a.*

B. In *let-7(ma432,g13*), LIN-41 is expressed abnormally in the peri-nuclear region of both Hyp7 and seam cells due to the loss of *let-7a* repression.

C. In *lin-41(ma480,t13);let-7(ma432,g13)*, restoring the interacting configurations between *let-7a* and *lin-41* rescues the *let-7a* repression, thus no abnormal expression of LIN-41 was detected.

D. In *lin-41(ma480,t13)*, disruption of non-seed paring to *let-7a* by mutations on the target causes de-repression of *lin-41*, resulting in abnormal expression of LIN-41::GFP similar to (**B**).

All strains are cultured at 25 °C. Scale bars, 10 μm. All GFP images are taken with identical exposure times and microscopy settings.

E. Normalized expression of *lin-41* at L4 stage measured by qRT-PCR. The transcription levels were calculated by the ΔΔCt relatively to *gpd-1* and were normalized by to *lin-41(+);let-7(+).* All strains are cultured at 25 °C. Statistical significance indicates t-test from 3 biological replicas.

Figure S5

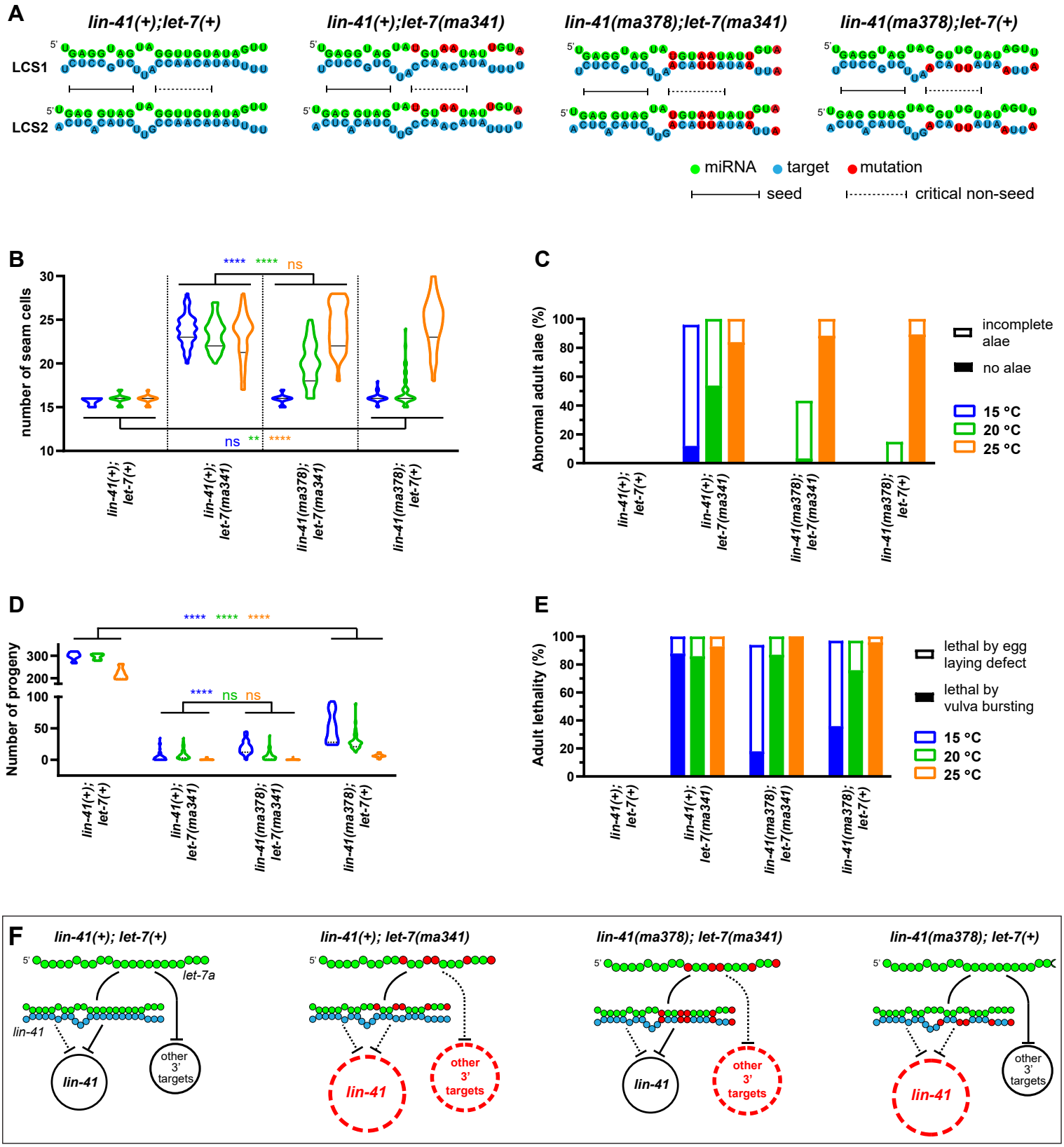

A
lin-41(+);let-7(+)
lin-41(+);let-7(ma341)
lin-41(ma378);let-7(ma341)
lin-41(ma378);let-7(+)
LCS1
LCS2
miRNA
target
mutation
seed
critical non-seed
B
number of seam cells
30
25
20
15
10
**** **** ns
ns ** ****
lin-41(+); let-7(+)
lin-41(+); let-7(ma341)
lin-41(ma378); let-7(ma341)
lin-41(ma378); let-7(+)
C
Abnormal adult alae (%)
100
80
60
40
20
0
incomplete alae
no alae
15 °C
20 °C
25 °C
lin-41(+); let-7(+)
lin-41(+); let-7(ma341)
lin-41(ma378); let-7(ma341)
lin-41(ma378); let-7(+)
D
Number of progeny
300
200
100
50
0
**** **** ****
**** ns ns
lin-41(+); let-7(+)
lin-41(+); let-7(ma341)
lin-41(ma378); let-7(ma341)
lin-41(ma378); let-7(+)
E
Adult lethality (%)
100
80
60
40
20
0
lethal by egg laying defect
lethal by vulva bursting
15 °C
20 °C
25 °C
lin-41(+); let-7(+)
lin-41(+); let-7(ma341)
lin-41(ma378); let-7(ma341)
lin-41(ma378); let-7(+)
F
lin-41(+); let-7(+)
lin-41(+); let-7(ma341)
lin-41(ma378); let-7(ma341)
lin-41(ma378); let-7(+)
5'
let-7a
lin-41
lin-41
other 3' targets
lin-41
other 3' targets
lin-41
other 3' targets
lin-41
other 3' targets

Figure S5. *let-7a* specifically targets *lin-41* and additional 3' targets compared to the family paralogs. (Related to **Figures 2, 4**).

A. Predicted pairing configurations between *let-7a* and *lin-41* LCS1/2 of WT, *let-7(ma341, mir-84 swap), lin-41(ma378);let-7(ma341)* and *lin-41(ma378)*.

B-C. The increase in seam cell numbers of young adult animals(**B**) and the lack of normal adult alae (**C**) are characteristic of *let-7a lf* phenotypes in the heterochronic pathway. Seam cell numbers of young adults were scored using *maIs105 [col-19::gfp].*

C. Proportions of animals with abnormal adult alae, categorized as no alae (severe) or incomplete alae (mild).

D-E. Penetrance of vulva integrity defects by numbers of progeny (**D**) and adult lethality (**E**)**.** The adult lethality is categorized as vulva bursting (severe) or egg-laying defective (mild).

F. Illustrative models propose that the *let-7a* 3'-sup targets additional to *lin-41* are de-repressed in *let-7a(ma341)*. The results indicate that the *lf* phenotypes of *let-7(ma341, miR-84 swap)* result from the de-repression of *lin-41* as a major effector, as well as other putative 3' targets. Thus, *let-7a* confers its functional specificity among family paralogs by regulating a multiplicity of target genes.

Figure S6

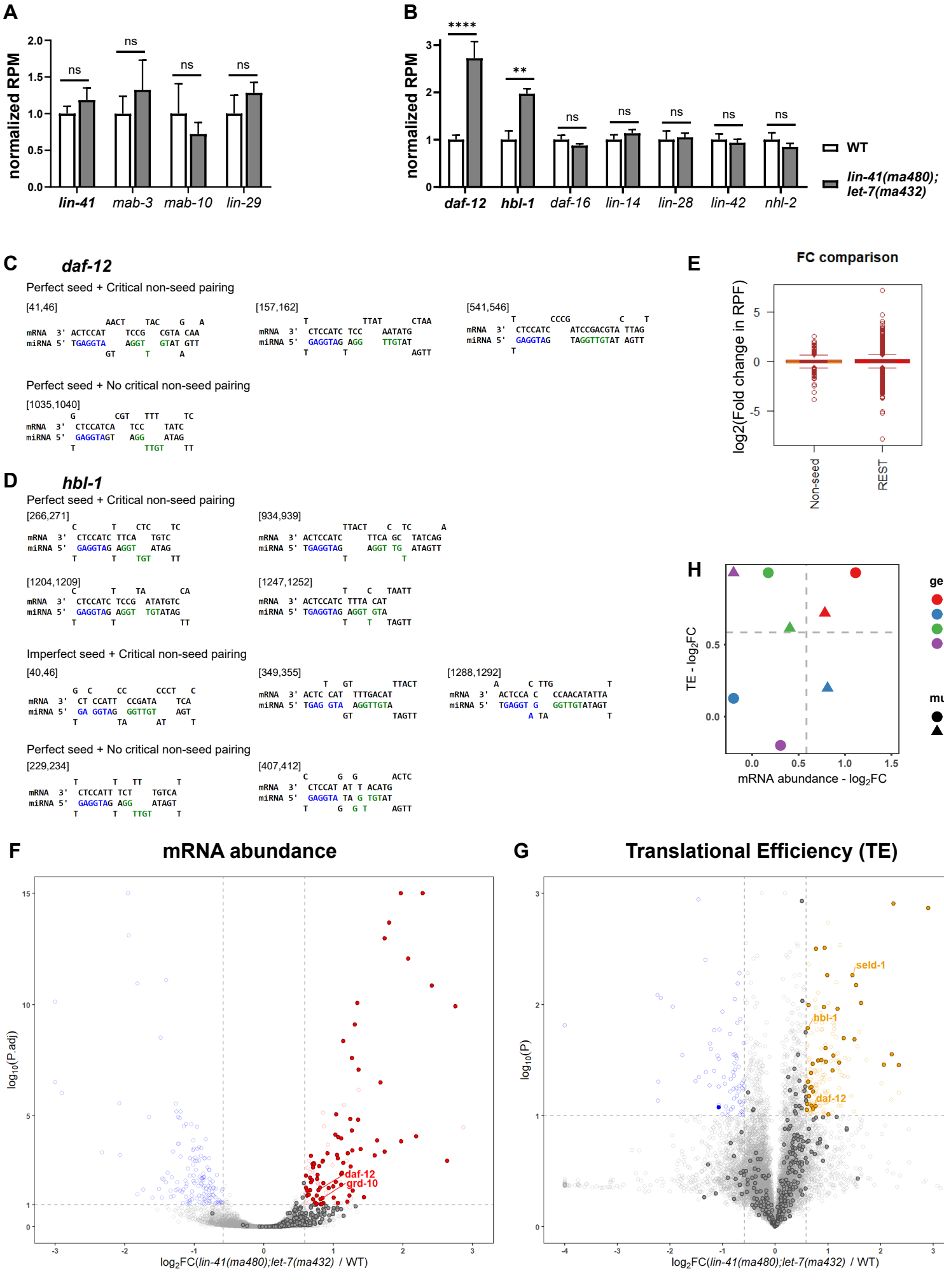

A
normalized RPM
lin-41
mab-3
mab-10
lin-29
B
daf-12
hbl-1
daf-16
lin-14
lin-28
lin-42
nhl-2
WT
lin-41(ma480); let-7(ma432)
C
daf-12
Perfect seed + Critical non-seed pairing
Perfect seed + No critical non-seed pairing
D
hbl-1
Imperfect seed + Critical non-seed pairing
Perfect seed + No critical non-seed pairing
E
FC comparison
log2(Fold change in RPF)
Non-seed
REST
H
TE - log2FC
mRNA abundance - log2FC
gene.name
daf-12
grd-10
hbl-1
seld-1
mutation
G5C
U13A
F
mRNA abundance
log10(P.adj)
log2FC(lin-41(ma480);let-7(ma432) / WT)
G
Translational Efficiency (TE)
log10(P)

Figure S6. Heterochronic genes *daf-12* and *hbl-1* are *let-7a* 3' targets that de-repressed in *let-7a* 3' non-seed mutant. (Related to **Figures 4, 5**).

A. Normalized RPFs of *lin-41* and its reported direct downstream targets in WT and *lin-41(tn1541ma480);let-7(ma432)*. (Aeschimann et al., 2017). The results further support that repression of *lin-41* has been rescued by restoring the native *let-7a:lin-41* LCSs interacting configuration in Fig. 4. Note that another reported *lin-41* target *dmd-3* is excluded for this analysis due to its low read counts. Significance is evaluated by DESeq2 (Love et al., 2014).

B. Normalized RPFs of *C.elegans* critical heterochronic genes, including *daf-12* and *hbl-1*, in WT and *lin-41(tn1541ma480);let-7(ma432)*. Significance is evaluated by DESeq2 (Love et al., 2014).

C-D. Predicted base pairing configuration between *let-7a* and the predicted LCSs of *daf-12* **(C)** and *hbl-1* **(D)** including sites with pairing in the critical non-seed region (green). The categorization of seed and non-seed types is shown in STAR-Method. Sites with "weak seed + no critical non-seed" configuration (2/3, -1) were excluded. The numbers indicate locations of the *let-7a* seed in 3' UTR. Both the interacting configurations and seed locations are further shown in Data S1 and Table S4.

E. A comparison of fold change (FC) in RPF levels between the genes that are classified as 3'-sup targets (Non-seed) and other genes (REST). Wilcoxon rank test (wilcox.test in R) confirmed that the difference between the two groups is insignificant. We reason that the lack of enrichment of 3' targets among the overexpressed genes was due to the possible scenario that these 3' targets are de-repressed in specific tissues/cells, likely where *let-7a* is highly expressed, while the RPFs in the Ribo-seq were collected from whole animals, thus signals of the significant over-expression of *let-7a* targets in *let-7a*-expressing tissues are diluted. We suggest translational profiles at single-cell resolution to be taken to further investigate the above issue.

F-G. Categorization of the repressing mechanisms of *let-7a* non-seed region, shown by volcano plots of the differential expression analysis of RNA-seq **(F)** and TE **(G)** between *lin-41(tn1541ma480);let-7(ma432)* and *lin-41(tn1541).* Significantly increased/decreased genes (FC > |1.5| and P.adj < 0.1 by DESeq2 **(F)** or P < 0.1 by t-test **(G)**) are colored red/blue. Solid points of both plots represent genes with significantly increased RPFs in ribosome profiling in Figure 5, among which genes with predicted *let-7a* non-seed sites are labeled with gene names.

H. Comparison of de-repressing models of the predicted *let-7a* 3' targets in U13A mutant (this study) and in G5C seed mutant (Aeschimann et al. 2017). RPFs and RNA-seq reads at 28-32 hrs from Aeschimann et al. 2017 were used to calculate the fold changes in TE and RNA abundance.

Figure S7

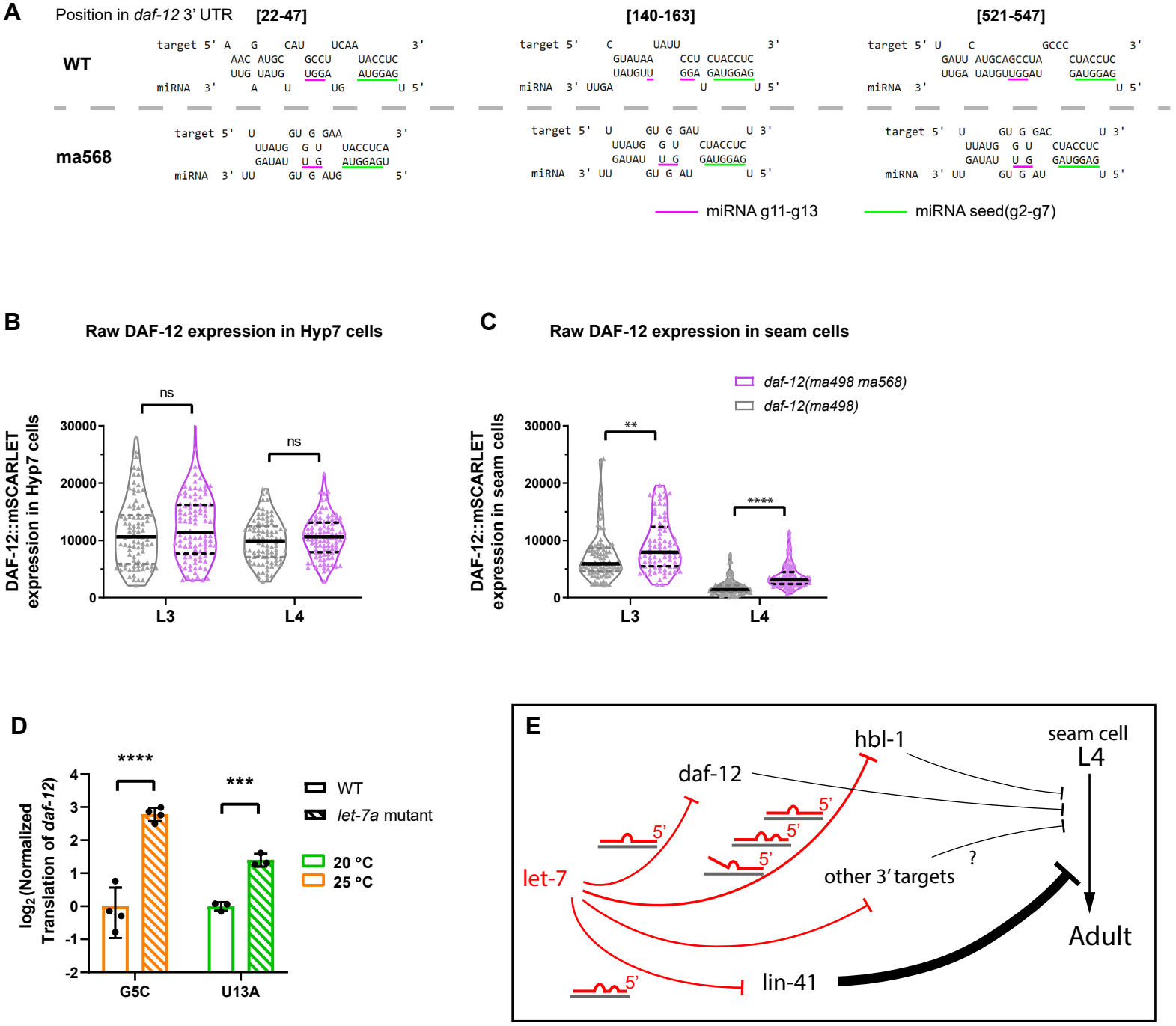

A
Position in daf-12 3' UTR
[22-47]
[140-163]
[521-547]
WT
ma568
miRNA g11-g13
miRNA seed(g2-g7)
B
Raw DAF-12 expression in Hyp7 cells
DAF-12::mSCARLET expression in Hyp7 cells
ns
L3
L4
C
Raw DAF-12 expression in seam cells
daf-12(ma498 ma568)
daf-12(ma498)
DAF-12::mSCARLET expression in seam cells
**
****
D
log2 (Normalized Translation of daf-12)
****
***
WT
let-7a mutant
20 °C
25 °C
G5C
U13A
E
hbl-1
seam cell
L4
daf-12
let-7
other 3' targets
?
lin-41
Adult
5'

Figure S7. *daf-12* is repressed by *let-7 family* miRNAs at different developmental stages with distinct pairing configuration and repressing effect. (Related to **Figure 5**).

A. Base pairing configurations between *let-7a* (miRNA) and the LCSs in *daf-12* 3' UTR which contain 3' pairing at g11-g13 in WT (target). The seed positions in 3' UTR corresponds to those in Data S1.

B-C. The DAF-12::mSCARLET fluorescent intensity at the L3/L4 stage, in Hyp7 nuclei (**B**) and seam cell nuclei (**C**). These values were used to calculate the normalized expression in Figure 5F. The fluorescent intensity per cell was quantified as the product of the area of the nucleus (determined in the register of the DIC channel) and the relative mean density; the latter was calculated by removing the average mean density of surrounding regions from the raw mean density of the nucleus region.

D. Translational expression levels of *daf-12* in *let-7(n2853, G5C)* and *lin-41(tn1541ma480);let-7(ma432, U13A)* at L4 stages. Data of *lin-41(tn1541ma480);let-7(ma432)* were obtained from Figure 5A and normalized to *lin-41(tn1541)*. Data of *let-7(n2853, G5C)* were obtained from (Aeschimann et al., 2017) at time points 26-32 hrs and normalized to N2. Note that data for the G5C mutant was obtained at 25 °C, and data of the U13A mutant was obtained at 20 °C.

E. Illustrative model for the genetic interactions of *let-7a* with heterochronic pathway genes, and the corresponding base pairing patterns. Red lines indicate the repressive effect of *let-7a*. Black lines indicate the genetic regulation to the L4-to-adult, whereas the line thickness indicates estimated regulation efficacy.