

# Supplementary information

## Supplementary figures:

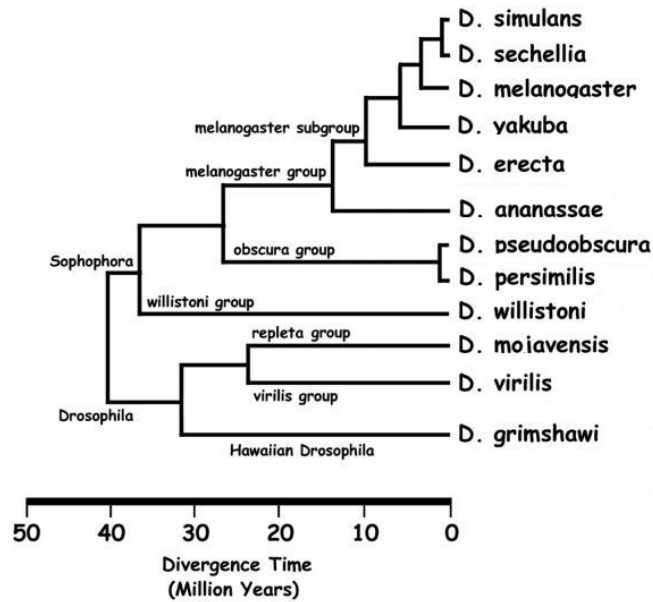


Figure S1. Phylogenetic tree of the 12 fly species involved in this work. (<http://species.flybase.net/>)

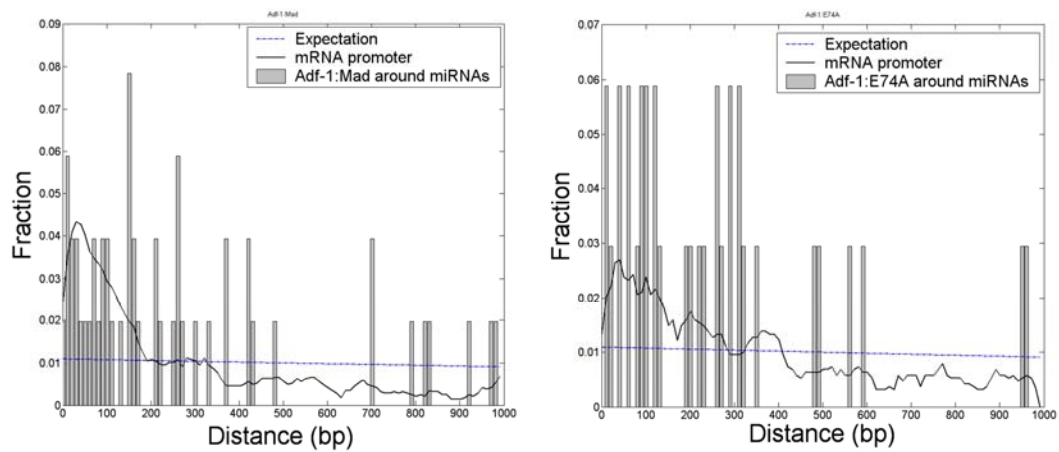


Figure S2. Putative TF-TF interactions with significant distance constraints of conserved TFBSs.





dme-mir-8	+	+	+	+	+	+	+	+	+	+	+
dme-mir-87	+	+	+	+	+	+	+	+	+	+	+
dme-mir-92a	+	+	+	+	+	+	+	+	+	+	+
dme-mir-92b	+	+	+	+	+	+	+	+	+	+	+
dme-mir-9a	+	+	+	+	+	+	+	+	+	+	+
dme-mir-9b		+	+	+	+	+	+	+	+	+	+
dme-mir-9c		+	+	+	+	+	+	+	+	+	+
dme-mir-iab-4	+	+	+	+	+	+	+	+	+	+	+

"+" represents the orthologue miRNAs can be find in that species.

The pink lines indicate that the miRNAs are not conserved across the 12 fly species.

**Table S3:** Intergenic miRNA transcription units(MIRTU).

MIRTUID	miRNAID	chromosome	strand	begin	end
mir-275_cl	mir-275,mir-305	chr2L	+	7425795	7426044
mir-87	mir-87	chr2L	-	9950418	9950516
mir-263a	mir-263a	chr2L	-	11953410	11953503
mir-124	mir-124	chr2L	+	17562299	17562398
mir-287	mir-287	chr2L	+	17570539	17570631
mir-100_cl	mir-100,let-7,mir-125	chr2L	+	18467363	18468353
mir-1	mir-1	chr2L	+	20475034	20475124
mir-133	mir-133	chr2L	+	20604210	20604307
mir-288	mir-288	chr2L	-	20605959	20606055
mir-280	mir-280	chr2R	+	3809919	3810013
mir-14	mir-14	chr2R	+	5065575	5065637
mir-184	mir-184	chr2R	-	8845309	8845408
mir-278	mir-278	chr2R	+	11171929	11172026
mir-8	mir-8	chr2R	+	12346295	12346381
mir-31a	mir-31a	chr2R	-	13298658	13298749
mir-3_cl	mir-6-3,mir-6-2,mir-6-1,mir-5,mir-4,mir-286,mir-3	chr2R	-	15175579	15176637
bantam	bantam	chr3L	+	625740	625820
mir-282	mir-282	chr3L	+	3234554	3234650
mir-276b	mir-276b	chr3L	+	10293732	10293830
mir-276a	mir-276a	chr3L	+	10339175	10339272
mir-314	mir-314	chr3L	+	11746959	11747048
mir-285	mir-285	chr3L	-	11920040	11920138
mir-219	mir-219	chr3L	+	17280265	17280364
mir-315	mir-315	chr3L	+	18826230	18826313
mir-9a	mir-9a	chr3L	+	19530390	19530467
mir-316	mir-316	chr3L	-	21602555	21602643
mir-10	mir-10	chr3R	-	2635228	2635304
mir-317	mir-317	chr3R	+	5916848	5916939
mir-277_cl	mir-277,mir-34	chr3R	+	5925744	5926756
mir-318	mir-318	chr3R	+	6234024	6234091
mir-284	mir-284	chr3R	-	8377237	8377336
mir-iab-4	mir-iab-4	chr3R	+	12681997	12682064

mir-92b	mir-92b	chr3R	+	21477120	21477219
mir-279	mir-279	chr3R	+	25041307	25041406
mir-210	mir-210	chrX	+	17962156	17962256

**Table S4. Putative CRMs identified with CCTS cutoff 8.**

MIRTU	Begin <sup>a</sup>	End <sup>a</sup>	CCTS	Locally overrepresented TFBSs
bantam	8200	9200	9	Abd-B:4 Ap:2 Kr:3
bantam	8900	9900	9	Abd-B:2 Su:2 Ap:3 En:2
mir-1	2000	3000	8	Sry-beta:2 Abd-A:2 Ap:2 Dl:2
mir-1	4300	5300	8	Abd-B:6 Cad:2
mir-1	8600	9600	9	Prd:2 Hb:2 Twi:5
mir-1	9300	10300	10	Abd-B:2 SGF-3:2 Prd:2 Hb:2 Sd:2
mir-1	11800	12800	8	Abd-B:2 Ftz:2 GCM:2 Zen-1:2
mir-1	14100	15100	9	Zeste:2 En:4 Ftz:3
mir-100_cl	3700	4700	8	Sd:2 Twi:3 Adf-1:3
mir-100_cl	8100	9100	10	Ovo-B:2 Ttk 69K:2 Elf-1:2 Adf-1:2 CF2-II:2
mir-100_cl	10000	11000	11	Twi:2 Ovo-B:2 Elf-1:3 Ap:2 Sn:2
mir-184	3600	4600	8	Prd:2 Cf1a:2 Twi:2 En:2
mir-219	2100	3100	10	Abd-B:3 SGF-3:3 Ftz:2 CF2-II:2
mir-219	5400	6400	13	Zeste:3 Abd-B:3 Prd:4 Dl:3
mir-219	6000	7000	12	Zeste:2 Prd:3 Dl:2 Ubx:3 Sn:2
mir-219	7900	8900	12	Abd-B:7 Antp:2 Cad:3
mir-219	11200	12200	9	TCF:2 Abd-B:5 Sn:2
mir-219	11900	12900	13	Abd-B:2 SGF-3:2 Ovo-B:3 byn:2 Ftz:2 Adf-1:2
mir-263a	3200	4200	19	dri:3 Abd-B:2 Abd-A:2 Prd:3 Cf1a:3 Mad:3 Bcd:3
mir-263a	6500	7500	9	Abd-B:2 HSTF:2 Prd:3 Ovo-B:2
mir-276a	200	1200	10	TCF-A:2 TCF:2 SGF-3:4 mtTFA:2
mir-276a	1800	2800	8	Cf1a:2 Sd:2 BR-C Z4:2 Zen-1:2
mir-276a	3600	4600	8	Abd-B:2 Prd:2 Twi:2 H:2
mir-276b	3400	4400	9	dri:2 Abd-B:3 Adf-1:2 Sn:2
mir-278	6300	7300	8	Abd-B:2 SGF-3:2 byn:2 Adf-1:2
mir-279	3800	4800	12	Abd-B:2 HSTF:2 Prd:5 Dl:3
mir-279	6300	7300	8	Sry-beta:2 Sd:2 byn:2 Zen-1:2
mir-279	9000	10000	11	Abd-B:2 Abd-A:3 mtTFA:2 HSTF:2 CF2-II:2
mir-280	8200	9200	8	TCF-A:2 dri:2 Abd-B:2 En:2
mir-280	9100	10100	14	TCF-A:2 TCF:2 Abd-B:2 Prd:2 Ttk 69K:2 GCM:2 BR-C Z4:2
mir-280	11900	12900	9	Abd-B:2 Kr:2 Adf-1:5
mir-287	10100	11100	8	SGF-3:2 Prd:2 Twi:2 Cad:2
mir-316	2400	3400	8	Abd-B:2 Abd-A:2 Dl:2 Cad:2
mir-9a	4900	5900	10	TCF-A:2 Abd-B:4 Cad:2 Adf-1:2
mir-9a	12200	13200	12	Cf1a:2 Ovo-B:3 CF2-II:2 Zen-1:5
mir-iab-4	3400	4400	11	Abd-B:2 HSTF:2 Dl:2 En:2 Cad:3

a: The position of the CRM relative to the extended (upstream10kb, downstream 5kb) MIRTU sequences.

**Table S5. Number of CRMs identified using different parameters**

CRM length	Minimum Conserved TFBSs	Number of CRM	Number of Shuffled CRMs	Signal to Noise ratio
600	7	13	6.7±3.2	1.93
600	8	8	4.1±2.5	1.93
600	9	3	1.9±1.6	1.55
600	10	1	1.2±1.0	0.87
600	11	1	0.5±0.6	1.96
600	12	1	0.2±0.4	4.55
800	7	31	14.0±4.9	2.21
800	8	24	9.7±3.8	2.48
800	9	13	5.2±2.7	2.50
800	10	5	3.4±2.0	1.48
800	11	3	1.9±1.4	1.60
800	12	2	1.0±1.1	1.96
1000	7	51	22.2±6.4	2.30
1000	8	36	14.6±4.4	2.47
1000	9	23	9.4±3.8	2.46
1000	10	16	6.5±2.9	2.47
1000	11	13	4.0±2.3	3.29
1000	12	8	2.7±1.9	2.93
1200	7	68	31.5±7.9	2.16
1200	8	55	23.8±6.4	2.31
1200	9	32	15.1±5.1	2.12
1200	10	24	10.7±4.1	2.25
1200	11	18	7.0±3.1	2.57
1200	12	10	4.9±2.4	2.04
1500	7	83	43.8±9.0	1.90
1500	8	77	34.9±7.8	2.21
1500	9	51	24.2±6.3	2.11
1500	10	41	17.9±5.2	2.29
1500	11	31	12.7±4.2	2.44
1500	12	23	9.4±3.6	2.44

**Table S6. Top 100 Enriched 7-mer motifs in the regulatory regions of drosophila miRNAs**

7-mer	Conserved instances	Total instances	Z_score	Transfac matrix ID
TGCAAAT	74	188	13.04473	
TTTGCAT	73	185	12.98385	
CATAAAT	72	192	12.33641	ISABDB_01
TTCAATT	75	214	11.84656	ISCF1A_Q6
AATTTGC	53	139	10.74002	
TCAATTA	51	142	9.989706	ISUBX_01,ISEN_Q6
AATGAAA	82	297	9.75088	
TGCATTT	68	230	9.553984	

AAATGAA	77	285	9.224029	
TTTATGC	45	128	9.202655	
TATGCAA	48	143	9.103192	
AATGCAA	57	187	9.025724	ISFTZ_01
CAAATTG	44	130	8.784191	
TTATGCA	40	118	8.387539	
ATTGATT	48	158	8.254528	ISCF1A_Q6
AAATCAA	59	216	8.173518	
AAAATGC	53	185	8.159513	
TTTATGA	46	150	8.15813	
TGCAATT	50	172	8.048807	ISFTZ_01
AAATTGC	50	172	8.048807	ISPRD_Q6
CAGCTGC	39	120	7.955763	
CAAATGA	41	131	7.860928	
CATAAAA	53	193	7.792803	ISHB_01,ISABDB_01,ISABDB_Q6
CATTTGC	41	133	7.74263	
TTAATTG	12	19	7.550113	ISUBX_01,ISFTZ_01,ISDRI_01
AACCGGT	48	172	7.548474	ISGRH_01
ATAAATC	37	118	7.481443	ISABDB_01
AATGCAT	38	123	7.470333	
TTTCAAT	54	206	7.46351	ISCF1A_Q6
GCAATTA	38	124	7.409614	ISUBX_01,ISFTZ_01
ATGCAAT	40	134	7.400884	ISFTZ_01
CATAATT	38	126	7.289987	
TGATTTA	37	122	7.234633	
CCATAAA	38	127	7.231056	ISABDB_01
CATGCAA	25	68	7.142698	
ATGCATA	32	100	7.098539	
GCATAAT	28	82	7.065676	
TAATTAG	32	102	6.961264	
ATGCAAC	27	79	6.944224	
CTAATTG	27	80	6.862669	
CATTTAA	44	167	6.776669	ISUBX_01,ISZEN_Q6
ATCAATT	41	151	6.768419	ISCF1A_Q6,ISCF1A_Q6
GCATATG	24	69	6.654841	
GCAACTG	23	65	6.618307	
AATGAAC	27	84	6.548876	
AGTTGCA	28	90	6.457596	
AATCAAA	45	180	6.442433	ISTCF_Q6
AAATTGA	44	177	6.326869	
AAACAAT	47	196	6.253968	ISANTP_Q6_01
TGTAATT	31	108	6.253108	
TTGCAAT	33	123	5.991187	ISFTZ_01,ISFTZ_01
AATTGCT	33	124	5.936444	ISFTZ_01

AATGCCA	32	120	5.859233	
TCTAATT	27	94	5.840019	
TGAAATT	46	201	5.824354	
ATTAATG	30	110	5.818337	I\$SUBX_01,I\$DRI_01
TAAATGC	31	116	5.78106	
TAATGAA	35	138	5.780607	I\$CF1A_Q6
TGACATT	23	75	5.768669	
AAAATCA	46	203	5.74786	I\$BYN_Q6
TCAATTG	29	106	5.740539	
TTGAACT	21	66	5.718418	
GCTAATT	25	86	5.691366	
ATTACAA	26	92	5.632	
TTCAATG	28	104	5.540441	
AGGTCAA	11	25	5.517812	
TTGACTT	25	89	5.486491	I\$TLL_Q6
ATTTTCA	43	192	5.469822	
AACAATT	38	162	5.467398	I\$ANTP_Q6_01
ATTCAAT	34	139	5.452652	
GCATTTG	35	145	5.44168	
TTGATTG	26	95	5.4377	
ATATGCA	31	123	5.399529	
TTGGCAT	28	107	5.363602	
CAATGAA	25	91	5.354574	
GCATGCA	18	56	5.347135	
TTGTTGC	39	171	5.338443	
TTTATTG	38	165	5.338051	I\$CROC_01
AAATGCC	27	102	5.336974	
GCCACGT	13	34	5.331986	
TCATTAA	32	130	5.331138	I\$ZEN_Q6
GTCACGT	9	19	5.29204	
ATTAACA	25	92	5.289942	
GTAATTG	18	57	5.254984	
CAGCTGT	22	77	5.241863	
GATGACG	14	39	5.231591	
CATTAAC	16	48	5.221089	I\$SUBX_01,I\$ZEN_Q6
AAAAGTG	39	174	5.214888	I\$BYN_Q6
TGCATTG	24	88	5.204078	
GGCACGT	8	16	5.201667	
ACCGTTA	8	16	5.201667	I\$OVO_Q6
TGTTGCA	28	110	5.192693	
GTTAATT	26	100	5.129997	I\$SUBX_01
TCAATGC	16	49	5.118962	
CTAATTT	30	123	5.1037	
CAATTTA	38	171	5.087546	

ATGCATG	20	69	5.074943
TCAAATG	27	107	5.046425
TTTACGA	17	55	4.998979
TCGTCAT	14	41	4.996187

**Table S7. Putative miRNA-TF regulatory feedback loops**

<b>Feedback loops:</b>
Trl -> dme-mir-1 -  Trl
sna -> dme-mir-1 -  sna
Su(H) -> dme-mir-1 -  Su(H)
tll -> dme-mir-124 -  tll
dl -> dme-mir-14 -  dl
Abd-B -> dme-mir-276a -  Abd-B
Abd-B -> dme-mir-276b -  Abd-B
usp -> dme-mir-277 -  usp
gcm -> dme-mir-279 -  gcm
slbo -> dme-mir-280 -  slbo
sna -> dme-mir-285 -  sna
Cf2 -> dme-mir-315 -  Cf2
Kr -> dme-mir-315 -  Kr
retn -> dme-mir-316 -  retn
Mad -> dme-mir-316 -  Mad
brk -> dme-mir-8 -  brk
hb -> dme-mir-8 -  hb
retn -> dme-mir-9a -  retn