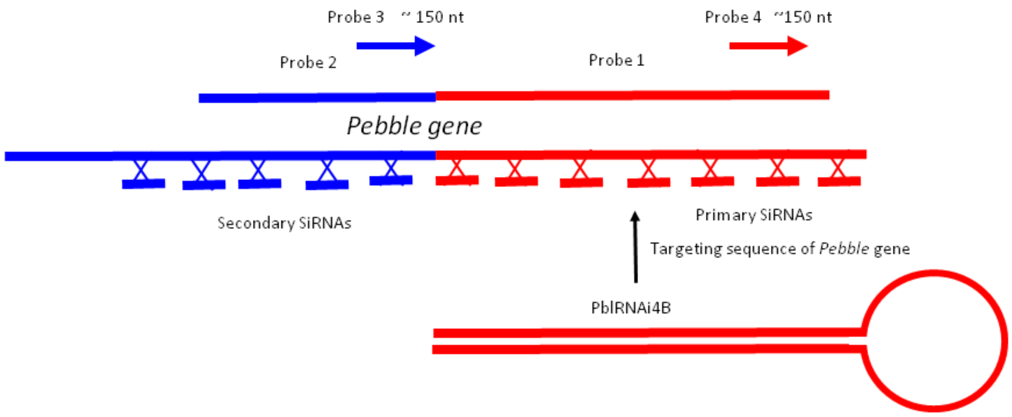
Supplementary Figures S1-13

S1



S2

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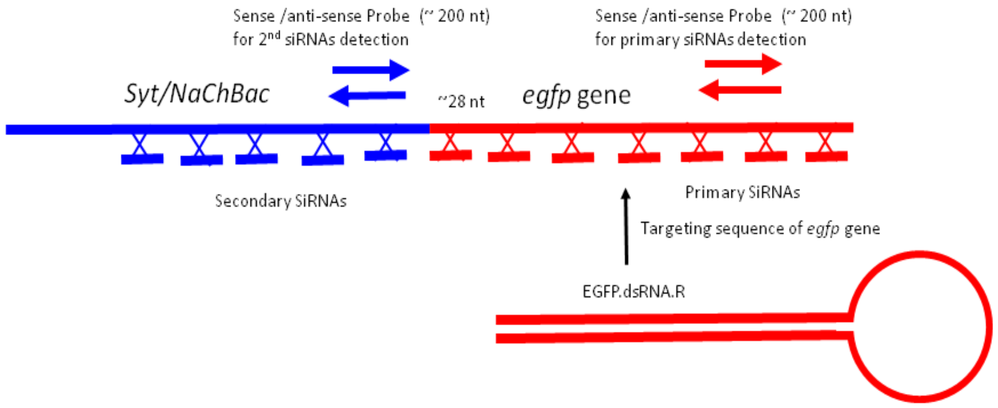
S3



S4

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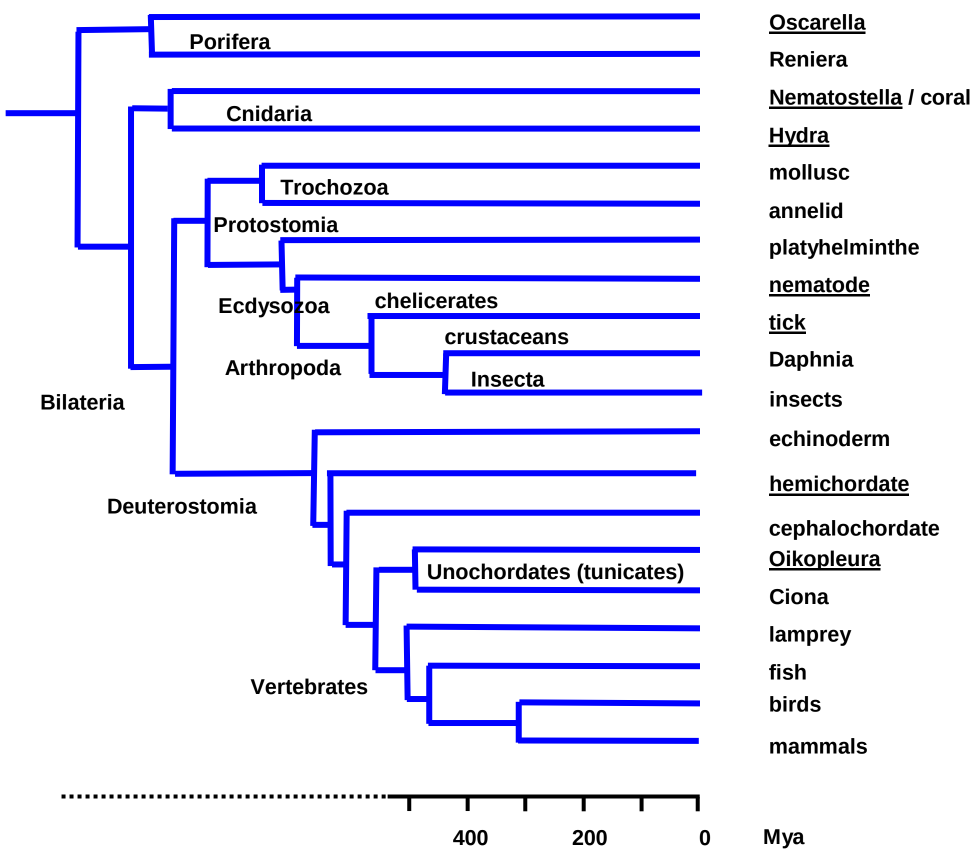
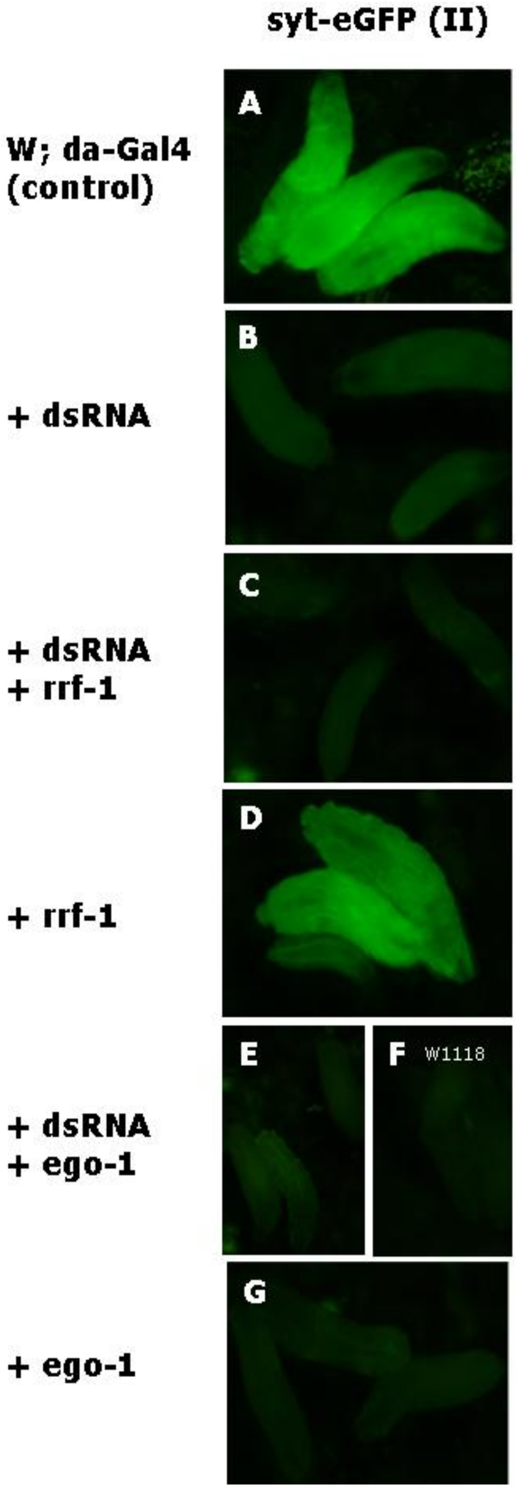
S5



S6


syt-eGFP (II)
W; da-Gal4 (control)
A
+ dsRNA
B
+ dsRNA
+ rrf-1
C
+ rrf-1
D
+ dsRNA
+ ego-1
E
F
W1118
+ ego-1
G

S7

w; da-Gal4; UAST-ego-1 x w; +; UAS-syt.eGFP

↓

♂ w; da-Gal4/+; UAST-ego-1/ UAS-syt.eGFP + x ♀ w; If/CyO; MKRS/TM6B

↓

¼ of the progeny should be: w; da-Gal4 /CyO; UAS-syt.eGFP /TM6B

i.e. da-Gal4>> UAS-syt.eGFP, up to 100 1st or 2nd instar larvae were checked, however none of them were GFP fluorescent, indicating that silencing by EGO-1 is irreversible.

S8

Three parallel crosses were set up as follow to test if RRF-1 and EGO-1 can independently silence hairpin *pblRNAi* $^{4B(3.1)}$:

A: w; da-Gal4; + x w; *pblRNAi* $^{4B(3.1)}$; +

All progeny were killed at the pupae stage

B: w; da-Gal4; UAST-rrf-1 x w; *pblRNAi* $^{4B(3.1)}$; +

All progeny were killed at the pupae stage

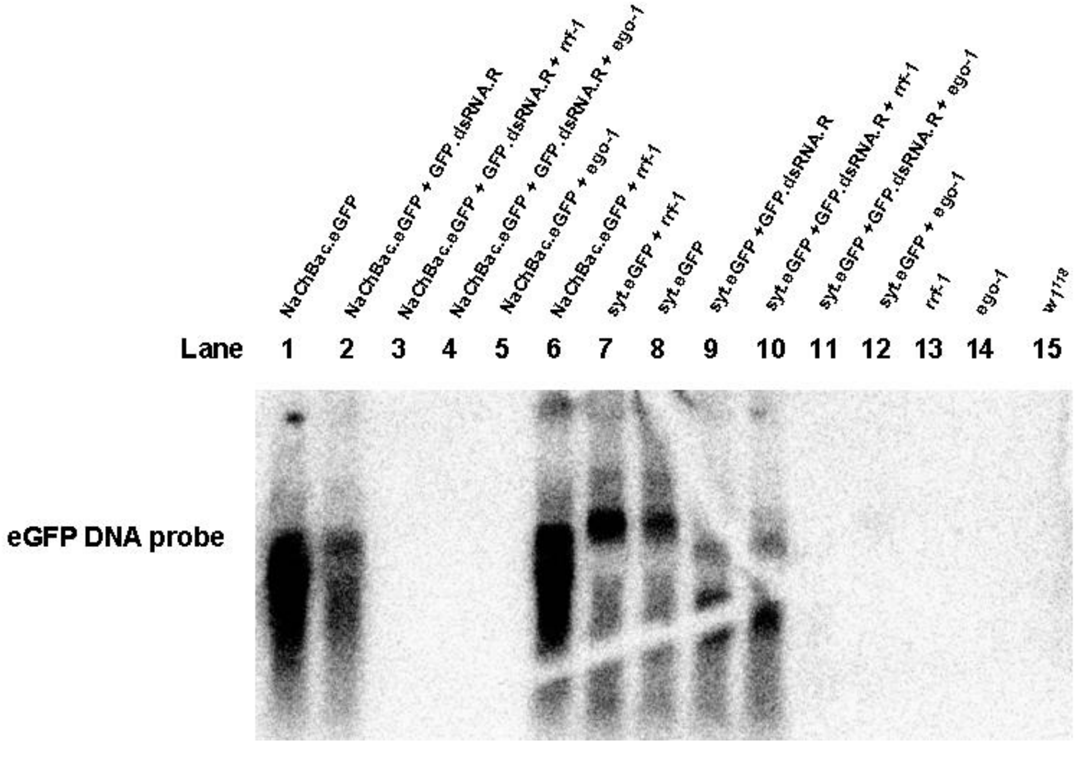
C: w; da-Gal4; UAST-ego-1 x w; *pblRNAi* $^{4B(3.1)}$; +

All progeny developed to the adult stage, indicating that *ego-1* rescued flies by directly silencing hairpin *pblRNAi* $^{4B(3.1)}$

S9

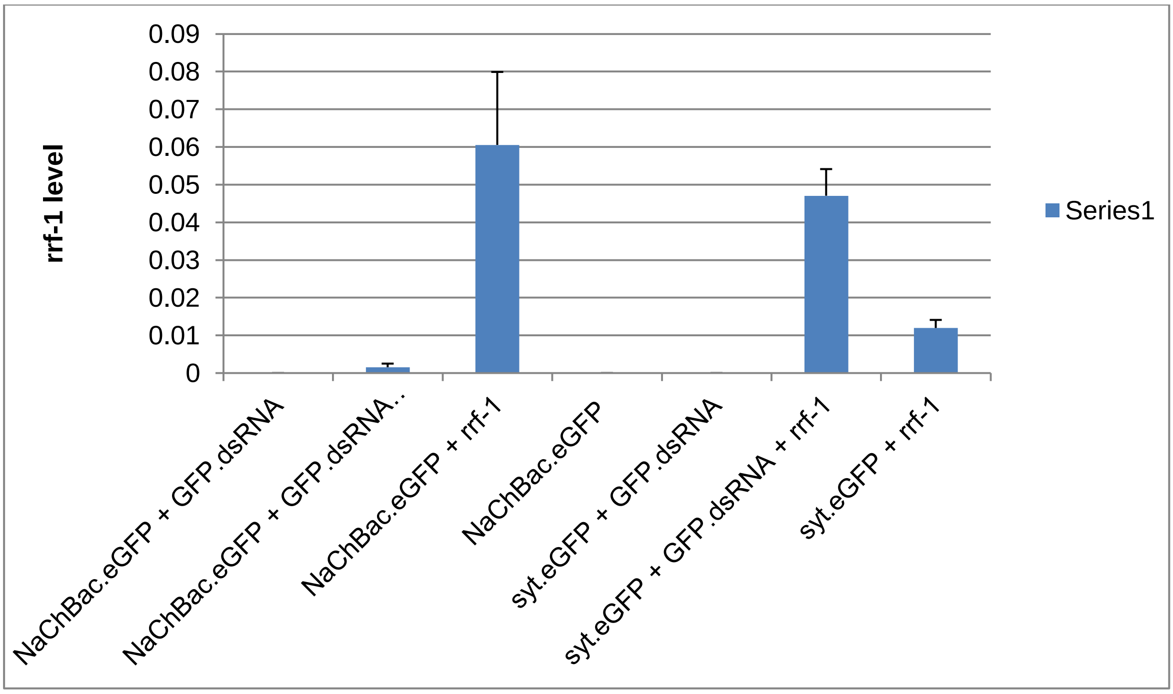
Cross	Cross in Genotype	Number of Progenies with or without *CyO* phenotype		Number of progenies with *pebble* gene silenced on the posterior compartment of the wing disc
A	*w; en-Gal4/CyO; UAST-rrf-1 x w; pblRNAi* $^{4B(3.1)}$*; +*	*CyO*	102	0
		en-Gal4>> pblRNAi $^{4B(3.1)}$ *+ UAST-rrf-1*	10	10
B	*w; en-Gal4/CyO; UASP-ego-1 x w; pblRNAi* $^{4B(3.1)}$*; +*	*CyO*	76	0
		en-Gal4>> pblRNAi $^{4B(3.1)}$ *+ UASP-ego-1*	8	8
C*	*w; en-Gal4/CyO; UAST-ego-1 x w; pblRNAi* $^{4B(3.1)}$*; +*	*CyO*	147	0
		en-Gal4>> pblRNAi $^{4B(3.1)}$ *+ UAST-ego-1*	200	0

S10

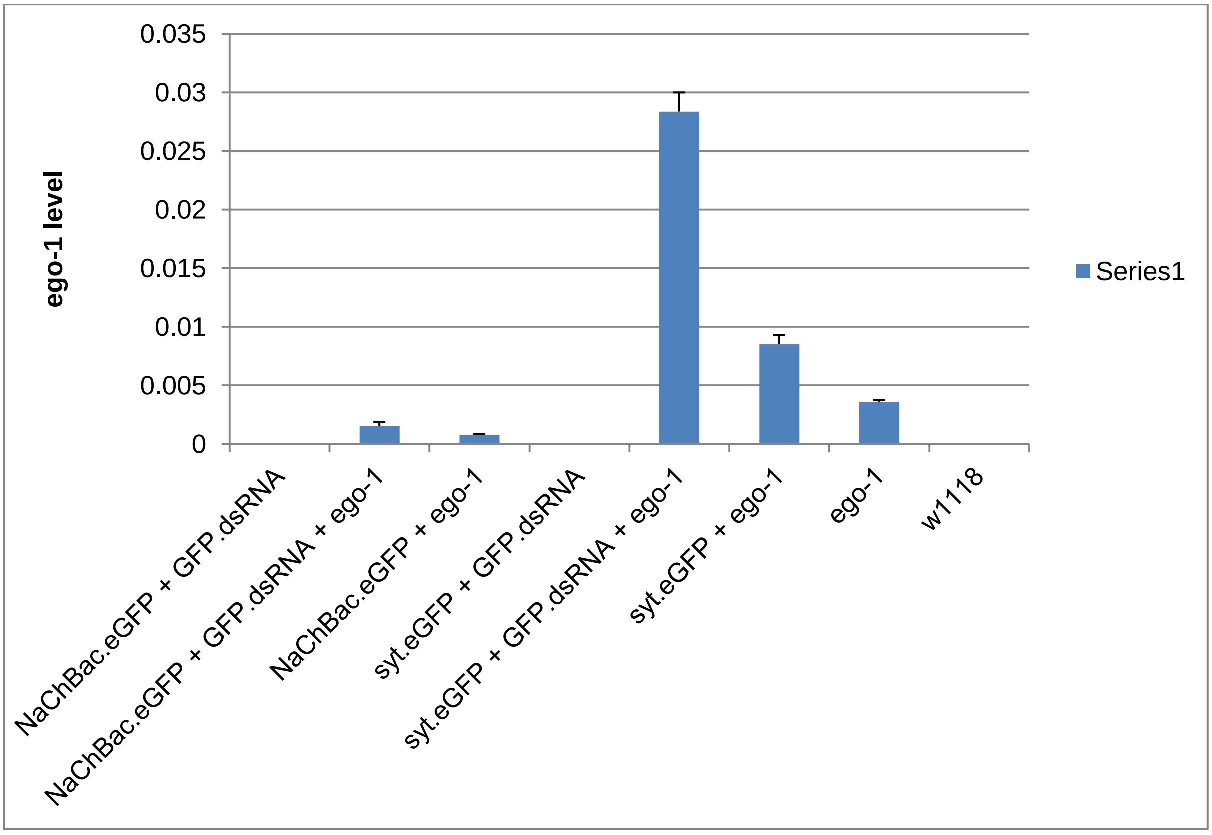


S11

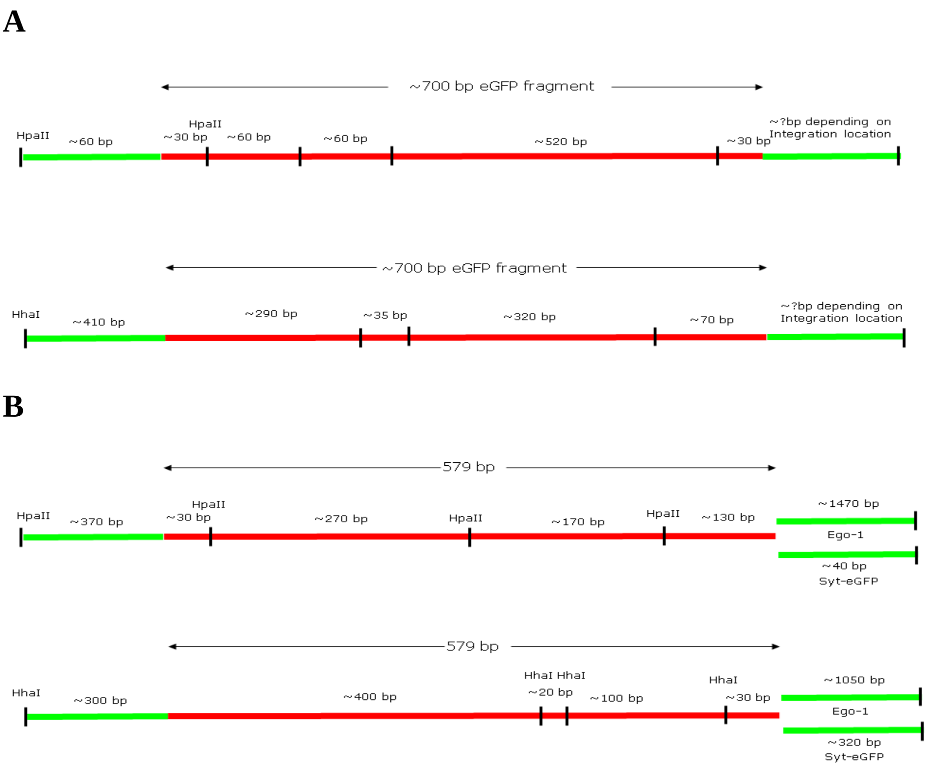
A


rrf-1 level
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
NaChBac.eGFP + GFP dsRNA
NaChBac.eGFP + GFP dsRNA…
NaChBac.eGFP + rrf-1
NaChBac.eGFP
syt.eGFP + GFP dsRNA
syt.eGFP + GFP dsRNA + rrf-1
syt.eGFP + rrf-1
■ Series1

B


ego-1 level
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
NaChBac.eGFP + GFP dsRNA
NaChBac.eGFP + GFP dsRNA + ego-1
NaChBac.eGFP + ego-1
syt.eGFP + GFP dsRNA
syt.eGFP + GFP dsRNA + ego-1
syt.eGFP + ego-1
ego-1
w1118
■ Series1

S12



S13

```
450

UAST promoter region    (387)
CTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAG

                seq29    (212)
TTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAG

            Consensus    (401)
TCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGAGCGGAG


500

UAST promoter region    (437)
TACTGTCCTCCGAGCGGAGACTCTAGCGAGCGCCGGAGTATAAATAGAGG

                seq29    (262)
TACTGTCCTCCGAGCGGAGACTCTAGTGAGCGCCGGAGTACAAATAGAGG
```

Consensus (451)

TACTGTCCTCCGAGCGGAGACTCTAG GAGCGCCGGAGTA AAATAGAGG

550

UAST promoter region (487)
CGCTTCGTCTACGGAGCGACAATTCAATTCAAACAAGCAAAGTGAACACG

seq29 (312)
CGCTTCGTCTACGGAGCGACAATTCAATTCAAACAAGCAAAGTGAACACG

Consensus (501)
CGCTTCGTCTACGGAGCGACAATTCAATTCAAACAAGCAAAGTGAACACG

600

UAST promoter region (537)
TCGCTAAGCGAAAGCTAAGCAAATAAACAAGCGCAGCTGAACAAGCTAAA

seq29 (362)
TCGCTAAGCGAAAGTTAAGTAAATAAATAAGTGTAGTTGAATAAGTTAAA

Consensus (551)

TCGCTAAGCGAAAG TAAG AAATAAA AAG G AG TGAA AAG TAAA

650

UAST promoter region (587)
CAATCTGCAGTAAAGTGCAAGTTAAAGTGAATCAATTAAAAGTAACCAGC

seq29 (412)
TAATTTGTAGTAAAGTGTAAGTTAAAGTGAATTAATTAAAAGTAATTAGT

Consensus (601)

AAT TG AGTAAAGTG AAGTTAAAGTGAAT AATTAAAAGTAA AG

700

UAST promoter region (637)
AACCAAGTAAATCAACTGCAACTACTGAAATCTGCCAAGAAGTAATTATT

seq29 (462)
AATTAAGTAAATTAATTGTAATTATTGAAATTTGTTAAGAAGTAATTAT-

Consensus (651)

AA AAGTAAAT AA TG AA TA TGAAAT TG AAGAAGTAATTAT