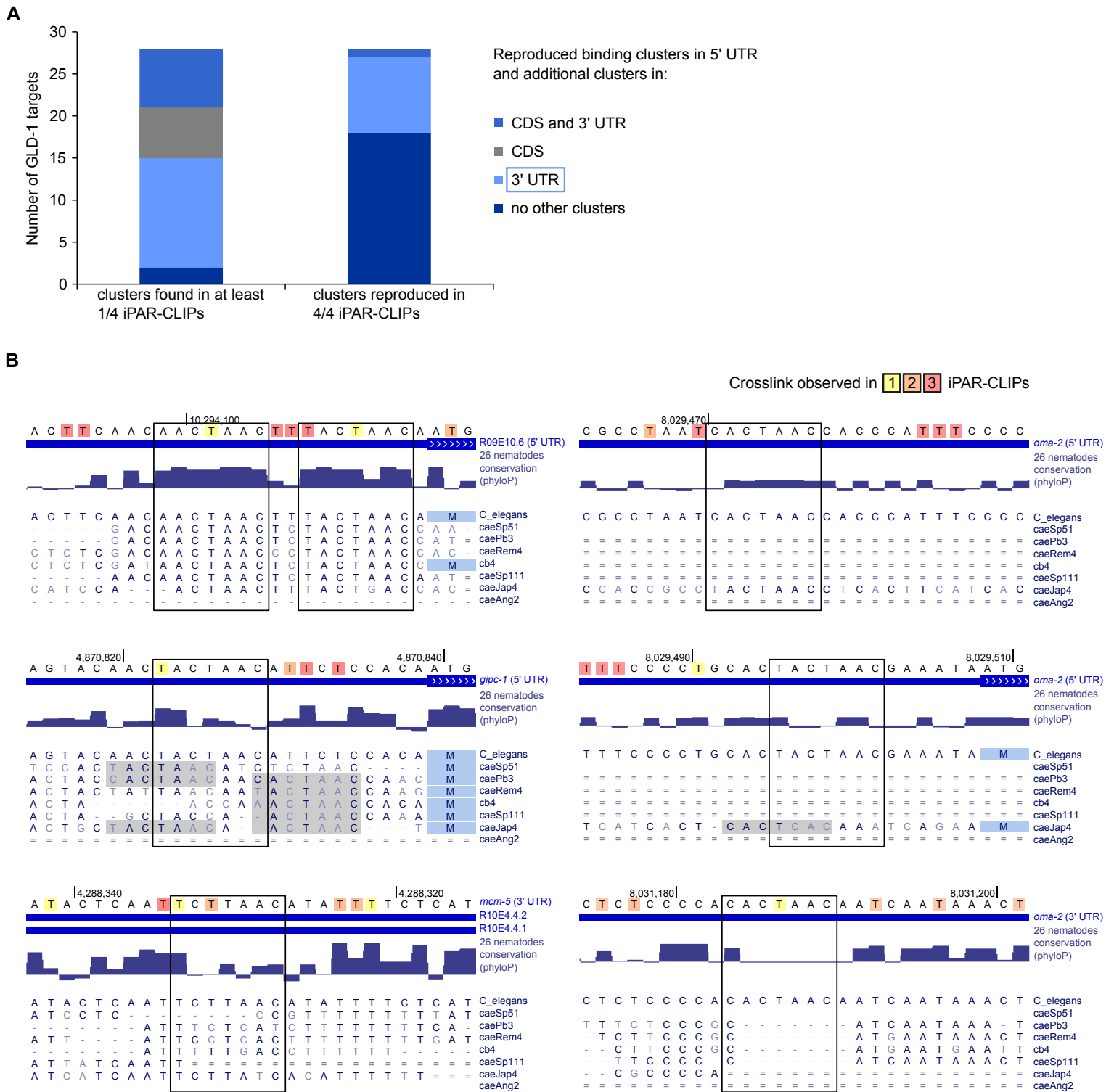


Cell Reports, Volume 22

**Supplemental Information**

**Post-transcriptional Regulation by 3' UTRs Can  
Be Masked by Regulatory Elements in 5' UTRs**

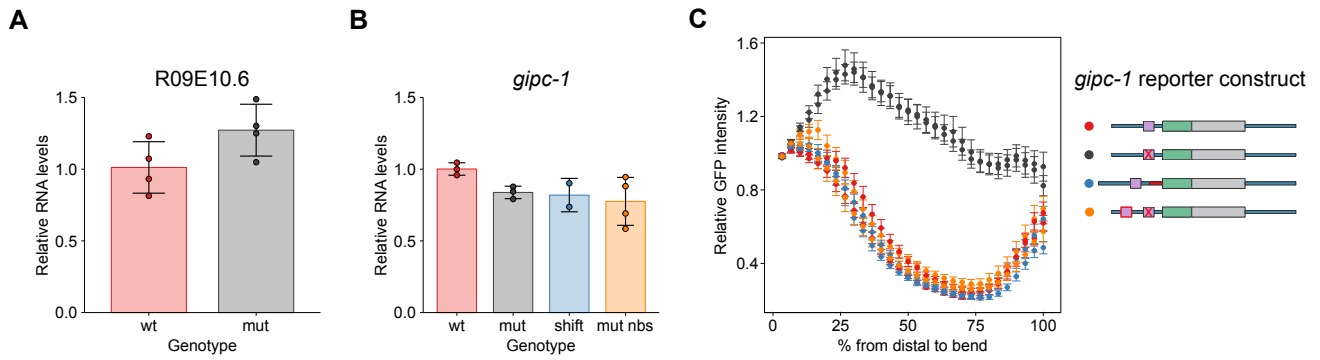
**Kathrin Theil, Margareta Herzog, and Nikolaus Rajewsky**



**Figure S1. GLD-1 binds to conserved and non-conserved sites within *C. elegans* transcript UTRs. Related to Figure 1.**

(A) Number of GLD-1 targets that have reproduced clusters in 5' UTRs (4/4 iPAR-CLIP experiments) and additional reproduced or non-reproduced clusters in other transcript regions (Jungkamp *et al.*, 2011).

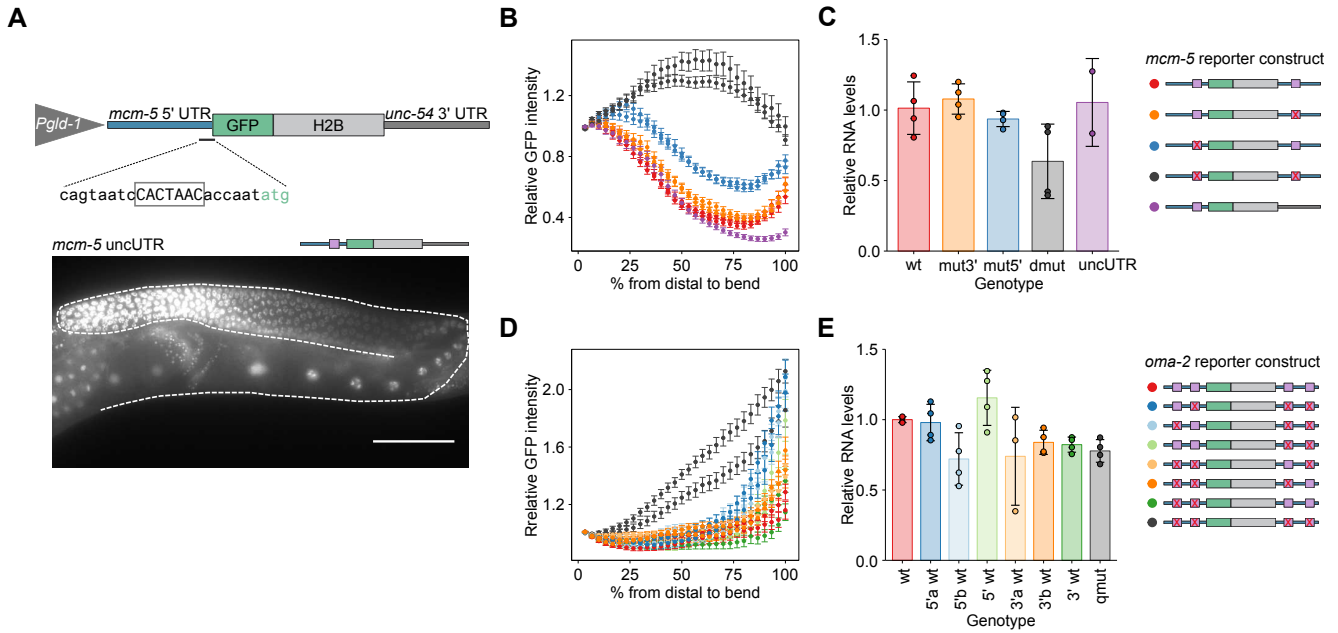
(B) GLD-1 binding sites investigated in this study (see also **Table 1** and **Figure 1B**). Crosslinking sites from iPAR-CLIP experiments, indicating direct protein-RNA contacts, were found in immediate vicinity of GLD-1 binding motifs (Jungkamp *et al.*, 2011). Genome browser screen shots adapted from <http://genome.ucsc.edu> (Kent *et al.*, 2002). Boxes indicate GLD-1 binding motifs; shifted motifs are highlighted in grey.



**Figure S2. Reporters with single 5' UTR binding sites show comparable RNA levels and reproducible GFP expression along the germline. Related to Figure 2.**

(A and B) Reporter RNA levels, as determined by RT-qPCR, for the R09E10.6 (A) and *gipc-1* (B) constructs.  $C_t$  values were normalized to three reference genes (*pmp-3*, *tba-1*, Y45F10D.4; Zhang *et al.*, 2012), and are plotted as ratio to wild-type reporter levels. Individual data points correspond to biological replicates of the same or independent transgenic lines. Bars depict mean  $\pm$  SD.

(C) Quantification of *gipc-1* reporter expression (analogous to Figure 2E) along the germline resulted in highly reproducible patterns for independent lines of the same constructs (plotted in same color). Measured GFP pixel intensities were normalized to the mean of the first 5 % of each gonad (where GLD-1 is not expressed), and grouped into 30 bins. Displayed are mean  $\pm$  SEM for each bin. Per reporter construct, two independent transgenic lines were analyzed (number of gonads per line: wt, n = 23; mut, n = 28; mut nbs, n = 22; shift, n = 25).

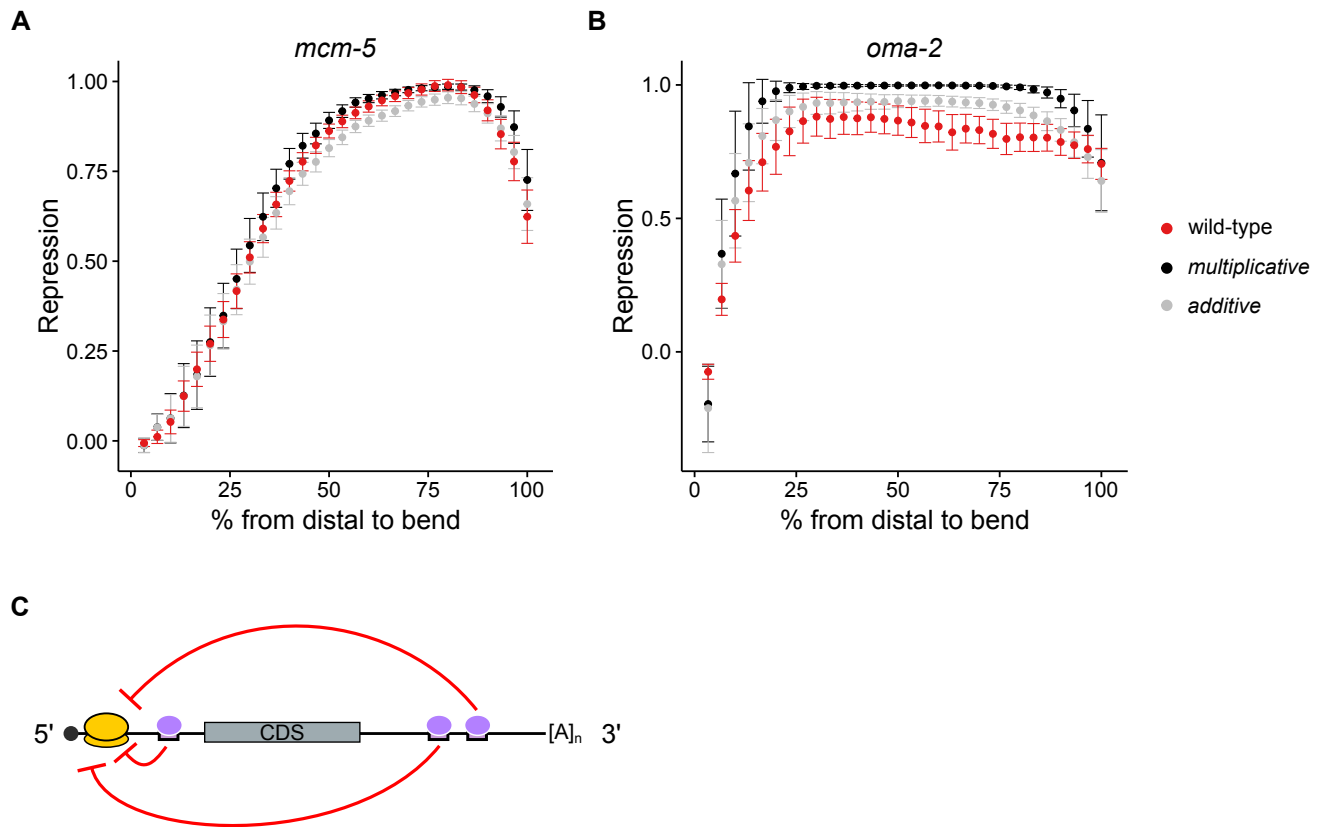


**Figure S3. The *mcm-5* 5' UTR is sufficient for regulation, and independent lines of 5' and 3' UTR reporter constructs show reproducible expression. Related to Figure 3 and Figure 4.**

**(A)** Replacement of the endogenous *mcm-5* 3' UTR by a non-regulated 3' UTR retains regulation. Transgene design with blow-up of GLD-1 binding site. *Pgld-1*, *gld-1* promoter; capital letters indicate GLD-1 binding motif; box indicates intact site. Dashed line encircles gonad. Scale bar: 50  $\mu$ m.

**(B and D)** Quantifications of reporter expression (analogous to **Figure 2E**) along the germline for independent lines (plotted in same color) of the *mcm-5* (**B**) and *oma-2* (**D**) constructs show reproducible patterns. Displayed are mean  $\pm$  SEM for each bin. Per reporter construct, two independent transgenic lines were analyzed, except for *mcm-5* uncUTR where only one line was analyzed (number of gonads per line of *mcm-5* reporters: wt, n = 28; dmut, n = 32; mut3', n = 27; mut5', n = 24; uncUTR, n = 31; number of gonads analyzed per line of *oma-2* reporters: wt, n = 27; qmut, n = 32; 5'a wt, n = 28; 5'b wt, n = 29; 3'a wt, n = 24; 3'b wt, n = 28; 3' wt, n = 28; 5' wt, n = 20). Colors as in **C** and **E**.

**(C and E)** Reporter RNA levels, as determined by RT-qPCR, for the *mcm-5* (**C**) and *oma-2* (**E**) constructs.  $C_t$  values were normalized to three reference genes (*pmp-3*, *tba-1*, Y45F10D.4; Zhang *et al.*, 2012), and are plotted as ratio to wild-type reporter levels. Individual data points correspond to biological replicates of the same or independent transgenic lines. Bars depict mean  $\pm$  SD.



**Figure S4. Modeling of repression suggests that sites act independently. Related to Figure 3 and Figure 4.**

(A and B) Repression was calculated by assuming (1) complete repression at minimal GFP signal for each wild-type reporter, and (2) GLD-1-independent expression for reporters without GLD-1 sites. Wild-type: calculated experimental repression, *multiplicative*: theoretical wild-type reporter repression if repression was conferred independently by both sites, *additive*: theoretical wild-type reporter repression if binding of additional sites did not result in increased repression. For detailed explanation of calculations, see the **Supplemental Experimental Procedures**. Displayed are mean  $\pm$  SEM.

(C) Sites in both 5' and 3' UTRs can potentially repress translation. CDS: coding sequence.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Raw data, scripts, reporter constructs and worm strains are available upon request.

### Estimation of repression

To estimate the degree of repression, we made the following assumptions: (1) RNA levels are the same along the germline for different reporter constructs of the same target (see **Figures S3C** and **S3E**). (2) repression reaches its maximum in the medial gonad of wild-type reporters, and remaining signal is due to autofluorescence (baseline ( $b$ ) = complete repression, for *oma-2*:  $b = 0.9$ , for *mcm-5*:  $b = 0.35$ ), and (3) the *mcm-5* double mutant and the *oma-2* quadruple mutant (“no site” mutants) are expressed independently of GLD-1. Normalization of relative GFP intensities of reporters with intact binding sites to the respective “no site” mutant yields GLD-1-dependent repression. Repression fold changes ( $f$ ) along the germline were calculated for each reporter with intact GLD-1 site(s) as follows ( $E$ : relative GFP expression of respective reporter):

$$f = (E[\text{"no site"}] - b) / (E[\text{"intact site(s)"}] - b)$$

Based on repression fold changes of “single site” reporters, theoretical wild-type repression along the germline was calculated, assuming either multiplicative (independent repression by multiple sites) or additive repression (binding of additional sites within a transcript already bound does not lead to increased repression).

Multiplicative repression:

*mcm-5*:

$$f[\text{"wild-type"}] = f[\text{"intact 5' site"}] \times f[\text{"intact 3' site"}]$$

*oma-2* :

$$f[\text{"wild-type"}] = f[\text{"intact 5'a site"}] \times f[\text{"intact 5'b site"}] \times f[\text{"intact 3'a site"}] \times f[\text{"intact 3'b site"}]$$

Additive repression:

*mcm-5*:

$$f[\text{"wild-type"}] = f[\text{"intact 5' site"}] + f[\text{"intact 3' site"}] - 1$$

*oma-2* :

$$f[\text{"wild-type"}] = f[\text{"intact 5'a site"}] + f[\text{"intact 5'b site"}] + f[\text{"intact 3'a site"}] + f[\text{"intact 3'b site"}] - 3$$

### Oligonucleotides for amplification, mutagenesis and sequencing

Restriction sites and introduced mutations are in capital letters.

#### Promoter:

Gene	Description	Sequence
<i>gld-1</i>	fwd	actACTAGTattgagatacacaagtgttttta
	rev	actAGATCTtcttcgatggtaaacctgtaag
	rev2 <sup>a</sup>	actAGATCTctgtaagaaacttagtaataatattttc

<sup>a</sup>used instead of “rev” for *oma-2* reporters and for *mcm-5* uncUTR reporter; generates a 15 nt-shortened *gld-1* promoter sequence (→ *gld-1* promoter without annotated *gld-1* 5' UTR)

**3' UTRs:**

Gene	Description	Sequence
R09E10.6	fwd	actCCTAGGcgctctttgcgttgctagac
	rev	actCTTAAGtggtccatttcacactcc
<i>gipc-1</i>	fwd	actCCTAGGcttttactgtggaataaatttttaac
	rev	actCTTAAGaactgggaatcgaatgaatgc
<i>mcm-5</i>	fwd	actCCTAGGattctcaaaattttgtctttttatctg
	rev	actCTTAAGaaactagaatttgattattttcaagg
<i>unc-54</i>	fwd	actCCTAGGgtccaattactctcaacatcc
	rev	actCTTAAGaaacagttatgtttggtatattggg
<i>oma-2</i>	fwd	actCCTAGGataaccacctctcccca
	rev	actCTTAAGccctgtcgcgaaactaattg

**5' UTR::GFP::H2B fusions and mutagenesis (Hobert, 2002):**

Gene	Description	Sequence
GFP::H2B	primer D	ttgtccggcagtgcactgatgataa
	primer D*	actCCTAGGttacttctgctggaagtgtacttggtgacg
	primer C	atgagtaaaggagaagaactttc
R09E10.6	primer A	cgtgaaagcctattcaagttc
	primer A*	actAGATCTcagtactagaaaattttctgacttc
	primer B	gaaaagttcttctcttactcattgtagtaagttagttggaag
	primer B with mutation	gaaaagttcttctcttactcattgCtCgtaaagctcgttggaa
<i>gipc-1</i>	primer A	cagcgtggcactctaattt
	primer A*	actAGATCTtgattcattcatcgatttaacacc
	primer B	gaaaagttcttctcttactcattgtggagaatgtagtagttg
	primer B with mutation	gaaaagttcttctcttactcattgtggagaatgCtCgtagttg
<i>mcm-5</i>	primer A	ccgattctgtttatttcca
	primer A*	actAGATCTatggtttaaaatttcagtaatccac
	primer B	gaaaagttcttctcttactcatattggtgtagtggacttg
	primer B with mutation	gaaaagttcttctcttactcatattggtgCtCgtggacttg
<i>oma-2</i>	primer A	ttataccgtttggcgcat
	primer A*	actAGATCTgggaatcaattaaccgagcac
	primer B	gaaaagttcttctcttactcattatttcgtagtagtcagg

**Site-directed mutagenesis (3' UTR):**

Gene	Description	Sequence
<i>mcm-5</i>	fwd	ctttctactcaattctGAGcatatttctcattcaattg
	rev	caaattgaatgagaaaatagCtCagaattgagtatgagaag
<i>oma-2</i> (3'a site)	fwd	ccatacgttttatgtcccatacGAGctataataacgcgcgaattt
	rev	aaattgcgcgcgttattatagCtCgtatggacataaaaacgtatgg
<i>oma-2</i> (3'b site)	fwd	cgtatctctccccacacGAGcaatcaataaactttatcgaatgca
	rev	tgacattcgataaagtattgattgCtCgtgtggggagaggatacg

**Site-directed mutagenesis (5' UTR):**

Gene	Description	Sequence
<i>gipc-1</i> (mut nbs)	fwd	tcttgattcattcatcgTACtaacaccaactcacttc
	rev	gaagtgagttgggttaGTAcgatgaatgaatcaaga
<i>gipc-1</i> (shift)	fwd	acaactactaacattctccacaATCGATTTAACAAatgagtaaaggagaagaactttc
	rev	gaaaagttcttctcttactcatTGTTAAATCGATtgtggagaatgtagtagttg
<i>oma-2</i> (5'a site)	fwd	cacccacgcctaatacGAGccaccattcccctgca
	rev	tgcaggggaaatgggtggCtCgtgattagcgtgggggtg
<i>oma-2</i> (5'b site)	fwd	caccatttcccctgcactacGAGcgaataatgagtaaaggagaagaac
	rev	gttcttctcttactcattatttcGtCgtagtcaggggaaatgggtg

**Verification of integration on chromosome II (Frøkjær-Jensen *et al.*, 2008):**

Region	Name	Sequence
Insertion site	oCF418	tctggctctgcttctcggt
Transgene	oCF419	caattcatcccggttctgt

**Sequencing:**

Region	Description	Sequence
5' UTR, CDS, 3' UTR	fwd	gcagagtttaagcttatgtcg
	rev	cgctgtcctgtcacactc
<i>gld-1</i> promoter	fwd	tacagaagaccgttacgaaac
	rev	gaattgggacaactccagtg

**qPCR:**

Gene	Description	Sequence
<i>gfp</i> (1)	fwd	ggccctgtcctttaccagac
	rev	tcgtccatgccatgtgtaatc
<i>gfp</i> (2)	fwd	tggaaacattcttggacacaa
	rev	tcgccaattggagtattttg
<i>pmp-3</i> (control)	fwd	gttcccgtgttcactcactcat
	rev	acaccgtcgagaagctgtaga
<i>tba-1</i> (control)	fwd	gtacactccactgatctctgctgacaag
	rev	ctctgtacaagaggcaaacagccatg
Y45F10D.4 (control)	fwd	gtcgttcaaatcagttcagc
	rev	gttcttgcaagtgatccgaca