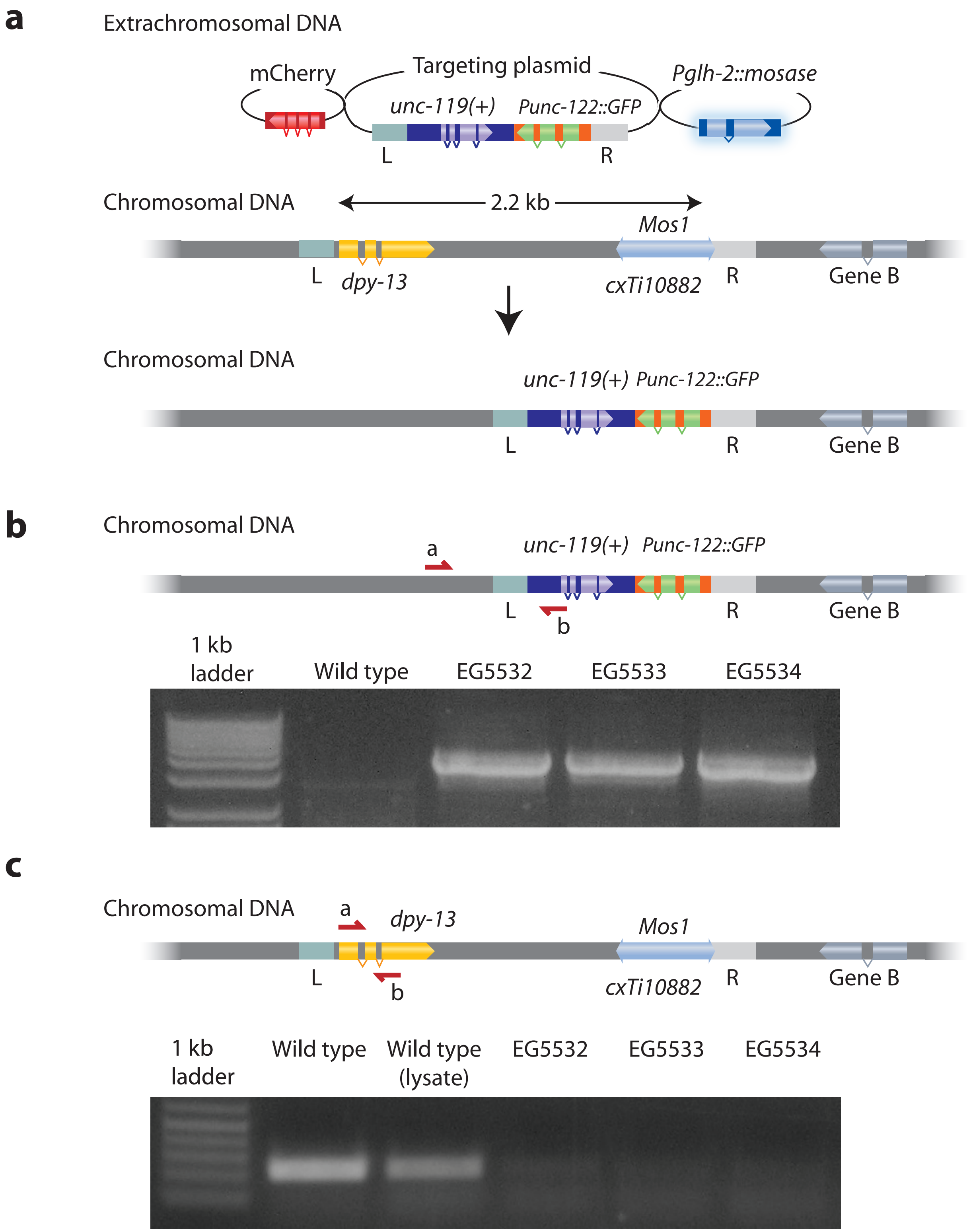
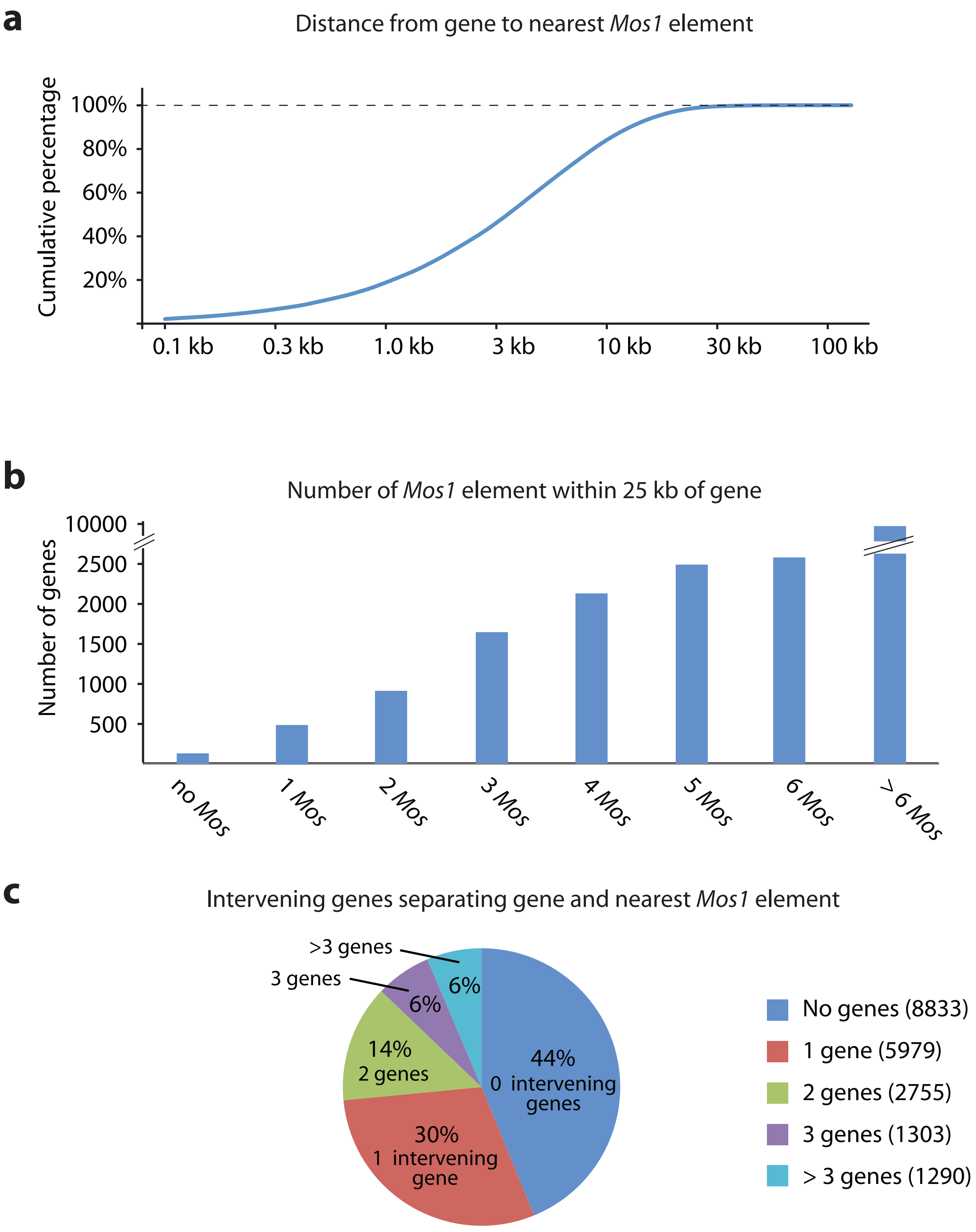
Supplementary Figure 1. PCR verification of *dpy-13* deletion



Supplementary Figure 1: PCR verification of *dpy-13* deletion.
(**a**) Schematic of targeting construct to delete a 2.2 kb genomic fragment adjacent to *cxTi10882* including *dpy-13*. The *Mos1* allele was crossed into the *unc-119(ed3)* background (not shown). Three independent deletion strains were generated: EG5532, EG5533 and EG5534. (**b**) PCR verification of deletions. Oligos annealing outside the displaced targeting homology and inside the *cb-unc-119(+)* were used to amplify across the junction. PCR products were subsequently sequenced to verify correct deletion junctions. (**c**) PCR verification of deletions. Oligos were used to amplify a 245 bp fragment from *dpy-13*. The fragment could be detected from high quality wild type genomic DNA ("wild-type") and single worm lysates from wild type ("wild type lysate") but not from any of the deletion mutants.

Supplementary Figure 2. *Mos1* distribution in the genome



Supplementary Figure 2: *Mos1* distribution in the genome.
We analyzed the distribution of *Mos1* elements in the *C.elegans* genome (WS205) with a total of 14,305 *Mos1* elements and 20,160 coding genes. See Supp. Table 2 for a comprehensive list of the data. (**a**) Distance from start codon to closest *Mos1* element. Shown is a cumulative plot of the percentage of coding genes within a given distance from the nearest Mos1 element. 99.4 % of genes are within 25 kb. (**b**) Number of *Mos1* elements within 25 kb. Shown is a histogram of the number of *Mos1* elements within 25 kb of the start codon. The *Mos1* strain collection was frozen as pools of animals and therefore not all inserts can be recovered. The presence of more than one *Mos1* element close to a given gene should minimize the chances of not recovering a *Mos1* allele within 25 kb. (**c**) Intervening genes. Shown is a pie diagram of the number of genes between the closest *Mos1* element and the start codon. Shown in parenthesis to the right are the number of genes for each category. For example, 8833 genes can be deleted without perturbing coding sequence from other genes.

Supplementary Table 1. Deletion frequency

Deletion size	Insert	Displaced ('left') homology size	Worms injected	Stable unc-119(+) inserts	Insertions per injected animal	Complete deletions	Successful deletions per *unc-119(+)* insertion	Deletions per injected animal
***cxTi10882* locus (*dpy-13*)**								
2.2 kb	*Cb-unc-119(+)* and *Punc-122::GFP*	1.5 kb	83	3	3.6 % (3/83)	3	100% (3/3)	**3.6 %** (3/83)
5.5 kb	*Cb-unc-119(+)* and *Punc-122::GFP*	2.8 kb	66	2	3.0 % (2/66)	2	100 % (2/2)	**3.0 %** (2/66)
10 kb	*Cb-unc-119(+)* and *Punc-122::GFP*	2.5 kb	108	4	3.7 % (4/108)	3	75 % (3/4)	**2.8 %** (3/108)
15 kb	*Cb-unc-119(+)*	3.1 kb	45	2	4.4 % (2/45)	1	50 % (1/2)	**2.2 %** (1/45)
25 kb	*Cb-unc-119(+)*	3.2 kb	81	7	8.6 % (7/81)	4	57 % (4/7)	**4.9 %** (4/81)
35 kb	*Cb-unc-119(+)*	3.0 kb	no data	8	no data	0	0 % (0/8)	**0%**
50 kb	*Cb-unc-119(+)*	3.3 kb	162	5	3.1 % (5/162)	0	0 % (0/5)	**0%**
***ttTi14024* locus (*dyn-1*)**								
6.0	*Cb-unc-119(+)*	2.4 kb	100	17	17 % (17/100)	4 of 5 tested	80 % (4/5)	**8-9 %**$^{\$}$ (8-9/100)

Supplementary Table 1. **Deletion frequency**. Please note that the frequency of deletions from the different injections are not directly comparable. In some cases (2.2 kb, 5.5 kb and 10 kb) a larger fragment was inserted. In other cases, two different experimenters performed injections. In our hands, approximately 70 % of all injected worms give rescued transgenic progeny. All deletion strains were isolated from plates that had a mixed population of fluorescent animals rescued by extra-chromosomal arrays and non-fluorescent animals rescued by stable insertion of *unc-119*(+). $^{\$}$ 11 of 17 strains (65 %) could not be homozygosed for the *unc-119(+)* marker as expected from a lethal mutation balanced by the insertion of *unc-119(+)*. 4 of 5 tested strains (80 %) had a full *dyn-1* deletion. We therefore estimate that 8 or 9 of the 11 strains (80 % * 11 strains = 8.8 strains) were full deletions.

Supplementary Table 3 - Strains

	Strain	Genotype
Mos1 strains		
	EG5003	*unc-119(ed3)* III; *cxTi10882* IV
	EG5817	*unc-119(ed3)* III ; *ttTi14024* X
	EG5618	*unc-119(ed3)* III ; *ttTi44501* X
Deletion strains		
		cxTi10882 mos1 allele
2.2 kb	EG5532	*unc-119(ed3)* III ; *dpy-13(ox448::cb-unc-119(+) Punc-122::GFP)* IV
	EG5533	*unc-119(ed3)* III ; *dpy-13(ox449::cb-unc-119(+) Punc-122::GFP)* IV
	EG5534	*unc-119(ed3)* III ; *dpy-13(ox450::cb-unc-119(+) Punc-122::GFP)* IV
5.0 kb	EG5637	*unc-119(ed3)* III ; *dpy-13(ox434::cb-unc-119(+) Punc-122::GFP)* IV
	EG5638	*unc-119(ed3)* III ; *dpy-13(ox435[cb-unc-119(+) Punc-122::GFP])* IV
10 kb	EG5620	*unc-119(ed3)* III ; *oxDf19* IV. EG5621: *unc-119(ed3)* III ; *oxDf20* IV
	EG5862	*unc-119(ed3)* III ; *oxIs562[Punc-122::GFP cb-unc-119(+)]* IV
	EG5863	*unc-119(ed3)* III ; *oxDf21* IV
15 kb	EG5864	*unc-119(ed3)* III ; *dpy-13(ox451::cb-unc-119(+))*
	EG5865	*unc-119(ed3)* III ; *oxDf22* IV
25 kb	EG5810	*unc-119(ed3)* III ; *oxDf13* IV.
	EG5866	unc-119(ed3) III ; oxDf23 IV
	EG5867	*unc-119(ed3)* III ; *oxIs563[cb-unc-119(+)]* IV
	EG5858	*unc-119(ed3)* III ; *oxIs564[cb-unc-119(+)]* IV
	EG5811	*unc-119(ed3)* III ; *oxDf14* IV
	EG5812	*unc-119(ed3)* III ; *oxDf15* IV
	EG5869	*unc-119(ed3)* III ; *dpy-13(ox452::cb-unc-119(+))* IV
		ttTi14024 mos1 allele
dyn-1 deletions	EG5815	*unc-119(ed3)* III ; *dyn-1(ox447::cb-unc-119(+))* X
	EG5878	*unc-119(ed3)* III ; *dyn-1(ox456::cb-unc-119(+))* X
	EG5879	*unc-119(ed3)* III ; *dyn-1(ox457::cb-unc-119(+))* X
	EG5880	*unc-119(ed3)* III ; *dyn-1(ox458::cb-unc-119(+))* X
	EG5881	*unc-119(ed3)* III ; *dyn-1(ox459::cb-unc-119(+))* X
dyn-1 rescue strain	EG6028	*unc-119(ed3)* III ; *dyn-1(ox447::cb-unc-119(+)*) X ; *oxEx1473[*pMPD13(*dyn-1(+) unc-119(+)) Punc-122::GFP litmus38i]*
		ttTi44501 mos1 allele
cst-1/cst-2 deletions	MJB1110	*unc-119(ed3)* III ; *basIs10[cb-unc-119(+) Punc-122::GFP]* X
	MJB1111	*unc-119(ed3)* III ; *basDf1* X

Supplementary Table 3 - Oligos

Uppercase sequences are gene specific; lowercase sequences are Gateway linkers.

Oligo	**Sequence (5'->3')**
oBN1	ggggacagctttcttgtacaaagtggATAGAATCAAGCATGCTCCG
oBN2	ggggacaactttgtataataaagttgGGCCCATTAGGTCAGACAAA
oCF125	GGGTGCCAAATAACCAGCTA
oCF168	CAGGAGAGCAAGGACCAAAG
oCF250	GTTGTCCTGAAAGATGTGGTGA
oCF400	CCGAATTCACGAAACAACTG
oCF570	ggggacaactttgtatagaaaagttgGGCGAGGAGGTACCAAAGAT
oCF571	ggggactgctttttgtacaaacttgCTTTCGTCCGTTGACAAGGT
oCF607	ggggacaactttgtatagaaaagttgGTGAATTGTAATACGACTCACTATAGGG
oCF608	ggggactgctttttgtacaaacttgCTGCCAAAGTTGAGCGTTTATTCTGA
oCF673	ggggacaactttgtatagaaaagttgCACGGATTGACGGACAATAA
oCF674	ggggactgctttttgtacaaacttgGCACATCCCTTTGCTGTAAAA
oCF677	ggggacagctttcttgtacaaagtggTGCACCAGAGAAACACAAAGA
oCF678	ggggacaactttgtataataaagttgCCGTAAACAAATAAAATTGTGTGG
oCF681	ggggacaagtttgtacaaaaaagcaggctTGAGCCAATTTATCCAAGTCC
oCF682	ggggaccactttgtacaagaaagctgggtCAGTTGAAATTGAAAATGAGTTAAAG
oCF692	ATAGACGGGGCCAAATTTTC
oCF693	TGTCAACCATTGGTGCTTGT
oCF711	GGAAGACCTTAAGCCGGTTC
oCF712	AGCGAGAGCCTGAAGAAGTG
oCF713	GAACAGGTAGCCCATTGGAA
oCF714	ATTGGCACGTTTCATTAGCC
oCF715	GTTTTCTTGGTGCTGGCCTA
oCF716	AAAACGGGGGAAAACTGACT
oCF717	TTTGGAGCCCATAAAATTGC
oCF718	AATGCAAAACTGCTGTCACG
oCF719	ATGTTGGCGGCTATTCAAAC
oCF720	GTGCAGAAATTTGGGGCTTA
oCF721	GTAGGCGGAGCATACATCGT
oCF722	TAGAACGCCCTTAACCATGC
oCF723	GGCGATTTGTCGGTACTTTG
oCF724	CTTTCACGGGAATGTCTGGT
oCF725	GCGGATTGGAAGGTCATAAG
oCF726	GGAAACCTTGCTCAAATCCA
oCF727	CAGGCAAGGGAGTGCTTTTA
oCF728	GCGTGGAATCTGTCCAAAAT
oCF729	TCCCCATTTCACCAGAGAAC
oCF730	GCCTACTGGGTCAACGACAC
oCF731	TGGGCAGTAGTATGGCAAAA
oCF732	TCAAATTTTCCCGTAGACGA
oCF733	CGCCATAATTGCTTGGCTAC
oCF734	GCCACTTCAAGGGAATTCAG
oCF735	AAGGATGCTGCTTACCTGCT
oCF736	AAGCGGATCGCCTTTAATTT
oCF737	ACAGATTCCACGCTCTCCTG
oCF738	ACGGAACCCCAGGTAATCAT
oCF739	GATCTCCTCGCAAGGCTTCT
oCF740	CTGGAAACGGACCAAACTTC

oCF741	CAGAGATTCCGTGTGCAAGA
oCF742	GGTTTCGGGGGAAAAGAATA
oCF743	TTCACACATAGGCGCTGAAG
oCF744	GTCCCTCCCTCCCTAAACTG
oCF745	TTATTGCCGCACACACCTAA
oCF746	ATTACCGAAACATCGCCAAG
oCF747	GGTGGGAGGCAATTGTTAAA
oCF748	ATTCCAGATTGCCGAGTTTG
oCF749	AGTGGTCGATGGAGATTTCG
oCF750	AATTTCCGGCTTTTTGAGGT
oCF751	TGGGGAGGTTGGAGTAGTTG
oCF752	TTCTCACAGCAGTCCAGGTG
oCF753	TTGCTTGGTGCTTGGTGTTA
oCF754	GCGGCCTTATTTTTGAAATG
oCF755	TGCCTTCCCTGCTTTTCTAA
oCF756	ATATGAACGTTGGCGAGCTT
oCF786	ggggacaactttgtatagaaaagttgGGCTTCTCTGTCGTTCTTCG
oCF787	ggggactgctttttgtacaaacttgCCATTAAACCTGAAAAGACTCTGA
oRL1	ggggacaactttgtatagaaaagttgTCCACTACCCAAATGTGCCATC
oRL2	ggggactgctttttgtacaaacttgGCTGGCGATGCTGGTTATAGTT
oRL4	ggggacaactttgtatagaaaagttgGATAAATGTTCCAGCGGCAAGGG
oRL5	ggggactgctttttgtacaaacttgAACAGTTCAGTCCCTGCGTG
oRL12	ggggacaactttgtatagaaaagttgGGGCTGAAACCATTTCAAGA
oRL13	ggggactgctttttgtacaaacttgGGGATTGTCCGTGCTACACT
oRL14	ggggacaactttgtatagaaaagttgGAGGTGGTGGCTTGGAATTA
oRL15	ggggactgctttttgtacaaacttgAAGTGCCGAAAACCACTTTG
oRL16	ggggacaactttgtatagaaaagttgCTGCAATGTTTTGCGGACTA
oRL17	ggggactgctttttgtacaaacttgGGAAAAATGCCCAGAGTTCA
oGH128	ggggacagctttcttgtacaaagtggTTTGTTTGTGAGTTTCAGGAAACTTG
oGH129	ggggacaactttgtataataaagttgGGGGATGAAGTTTAAAGGTTGAGTAGC
oGH130	ggggacaactttgtatagaaaagttgCAGTCCAGCTATCCGTCCGTC
oGH131	ggggactgctttttgtacaaacttgGTCGATTAATGATATGAGGAAGAGCATTG
oGH132	ggggacaactttgtatagaaaagttgCTGCTCCTGGTATCCTTAACCAG
oGH133	ggggactgctttttgtacaaacttgATTTCGCAAAGTTATGTTTTTTTTCTTC
oGH134	ggggacaagtttgtacaaaaaagcaggctAAAATCACAATTTTTTTCCTTTCCAG
oGH135	ggggaccactttgtacaagaaagctgggtGGTTTTTCTGTCTTACAAGTCATTAAAGAAG
oGH136	ggggacagctttcttgtacaaagtggTTTGTGTTGTCGTCCTTGTGATC
oGH137	ggggacaactttgtataataaagttgCCGTTTTAACTTTATTCCACTTTCACG
oGH154	GTGTTCTAGAACTCAATGGCACAG
oGH155	CTATGGTTTTGGAACACTGGCTAG
oGH156	AAACGACATTTCATACTTGTACACCTG

Supplementary Table 3 - PCR verification

	Annealing	Forward Oligo	Reverse Oligo	PCR product size
cxTi10882 locus	39 kb upstream	oCF713	oCF714	451 bp
(Figure 1c)	29 kb upstream	oCF715	oCF716	382 bp
	19 kb upstream	oCF717	oCF718	409 bp
	14 kb upstream	oCF719	oCF720	385 bp
	9 kb upstream	oCF721	oCF722	500 bp
	4 kb upstream	oCF723	oCF724	483 bp
	1.3 kb upstream	oCF168	oCF250	245 bp
	0.5 kb downstream (control)	oCF725	oCF726	447 bp
	5.7 kb downstream (control)	oCF727	oCF728	312 bp

ttTi44501 (cst-1/cst-2)	27.5 kb upstream	oCF741	oCF742	263 bp
(Figure 2d)	24 kb upstream	oCF743	oCF744	253 bp
	18 kb upstream	oCF745	oCF746	268 bp
	13.0 and 5.5 kb upstream (inverted repeat)	oCF692	oCF693	327 bp
	9.4 kb upstream	oCF747	oCF748	105 bp
	4.2 kb upstream	oCF749	oCF750	232 bp
	2.2 kb upstream	oCF751	oCF752	245 bp
	1.7 kb downstream(control)	oCF753	oCF754	272 bp
	5 kb downstream	oCF755	oCF756	290 bp

Supplementary Table 3 - Plasmids

Entry vectors

Positive selection vectors

Name	Forward Oligo	Reverse Oligo	Template	DONR vector	Description
pCFJ66					[1-2] Entry vector with *C.briggsae-unc-119(+)* and *Punc-122::GFP*. Described in Frøkjær-Jensen *et al.*, (2008
pRL8	oCF681	oCF682	pCFJ66	pDONR221	[1-2] Entry vector with *C.briggsae-unc-119(+)*

cxTi10882 locus

pBN03	oBN1	oBN2	N2 DNA	pDONRP2-P3	Right homology vectors
pCFJ215	oCF570	oCF571	N2 DNA	pDONRP4-P1R	2.2 kb deletion
pRL3	oRL4	oRL5	N2 DNA	pDONRP4-P1R	5.0 kb deletion
pRL4	oRL1	oRL2	N2 DNA	pDONRP4-P1R	10 kb deletion
pRL11	oRL12	oRL13	N2 DNA	pDONRP4-P1R	15 kb deletion
pRL19	oRL14	oRL15	N2 DNA	pDONRP4-P1R	25 kb deletion
pCFJ281	oCF786	oCF787	N2 DNA	pDONRP4-P1R	35 kb deletion
pRL12	oRL16	oRL17	N2 DNA	pDONRP4-P1R	50 kb deletion

ttTi44501 locus (cst-1/cst-2)

pCFJ251	oCF677	oCF678	N2 DNA	pDONRP2-P3	Right homology region
pCFJ249	oCF673	oCF674	N2 DNA	pDONRP4-P1R	Left homology region

ttTi14024 locus (*dyn-1*)

pMPD6	oGH128	oGH129	N2 DNA	pDONRP2-P3	Right homology region
pMPD8	oGH130	oGH131	N2 DNA	pDONRP4-P1R	Left homology region
[4-1] dyn-1(+)	oGH132	oGH133	N2 DNA	pDONRP4-P1R	Part rescue fragment dyn-1
[1-2] dyn-1(+)	oGH134	oGH135	N2 DNA	pDONR221	Part rescue fragment dyn-1
[2-3] dyn-1(+)	oGH136	oGH137	N2 DNA	pDONRP2-P3	Part rescue fragment dyn-1

Expression vectors

cxTi10882 targeting plasmids

Name	[4-1]	[1-2]	[2-3]	pDEST	Description
pCFJ216	pCFJ215	pCFJ66	pBN03	pDESTR4-R3	2.2 kb deletion
pRL5	pRL3	pCFJ66	pBN03	pDESTR4-R3	5.0 kb deletion
pRL6	pRL4	pCFJ66	pBN03	pDESTR4-R3	10 kb deletion
pRL13	pRL11	pRL8	pBN03	pDESTR4-R3	15 kb deletion
pCFJ260	pRL19	pRL8	pBN03	pDESTR4-R3	25 kb deletion
pCFJ287	pCFJ281	pRL8	pBN03	pDESTR4-R3	35 kb deletion
pRL14	pRL12	pRL8	pBN03	pDESTR4-R3	50 kb deletion

ttTi44501 (*cst-1/cst-2*) targeting plasmids

pCFJ255	pCFJ249	pCFJ66	pCFJ251	pDESTR4-R3	cst-1/cst-2 deletion

ttTi14024 (*dyn-1*) targeting plasmids

pGH238	pMPD6	pRL8	pMPD8	pDESTR4-R3	dyn-1 deletion

pMPD13	[4-1] dyn-1(+)	[1-2] dyn-1(+)	[2-3] dyn-1(	pCFJ150	dyn-1 rescue plasmid