Improved *Mos1*-mediated transgenesis in *C. elegans*

To the Editor: The ability to add or delete genes to the genome of genetic model organisms is essential. Previously, we had developed methods based on the *Mos1* transposon[1] to make targeted transgene insertions (*Mos1*-mediated single-copy transgene insertions; MosSCI[2]) and targeted deletions (*Mos1*-mediated deletions; MosDEL[3]) in *Caenorhabditis elegans*, the latter published in *Nature Methods*. Here we present new reagents that improve the efficiency, facilitate the selection for transgenic strains and expand the set of MosSCI insertion sites (**Supplementary Table 1**).

In our system, the Mos1 transposase is expressed from a helper plasmid injected together with template DNA. Increased transposase expression would be expected to improve both insertions and targeted gene deletions. We tested several promoters driving transposase expression for their effect on MosSCI and MosDEL efficiency (**Fig. 1a** and **Supplementary Fig. 1**). Relative to the *glh-2* promoter, the most effective promoter (*eft-3;* also known as *eef-1A.1*) resulted in a more than sixfold improvement in transgene insertion efficiency (from 8% to 54% of worms) and gene deletion efficiency (from 3% (n = 66 worms)[2] to 20% (n = 30 worms); **Fig. 1b**).

An effective, inducible negative selection marker would facilitate identification of transgenic strains. We developed a negative selection marker (*Phsp-16.41::peel-1*) based on the toxin PEEL-1 (ref. 4). Worms carrying the *peel-1* plasmid were killed by a 2-h heat-shock at 34 °C with ~10% false positives (2/19 transgenic worms) (**Fig. 1c** and **Supplementary Fig. 2**). A positive selection marker is critical for identifying transgenic worms with insertions or deletions, and we have used *unc-119* selection extensively. Recently, antibiotic selection markers have been developed for nematode transgenesis[5,6]. We generated targeted *dpy-13* deletions at comparable frequencies using either the neomycin-resistance gene or *unc-119* selection (24%, 12/51 worms, **Fig. 1b**). We discuss the recommended use of selection markers in **Supplementary Methods**.

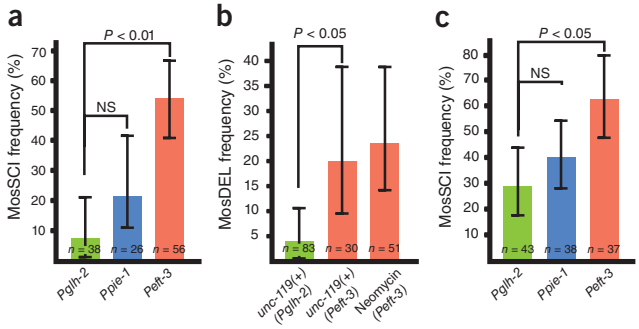

Figure 1 | Improvements to *Mos1*-based genome manipulation. (**a**) Insertion frequency with low total DNA concentration (32.5 ng μl^{-1}). A plasmid expressing Mos1 transposase from the indicated promoters was injected together with a 4.4-kb transgene into *unc-119* worms. Insertion frequency into the *ttTi5605* locus is plotted. (**b**) Frequency of a 5-kb targeted deletion of *dpy-13*. *Pglh-2* data are from ref. 2 using the indicated selection markers. (**c**) Insertion frequency with higher total DNA concentrations (~100 ng μl^{-1}) and in the presence of the negative selection marker *peel-1*. Error bars, 95% confidence intervals; significance was determined with Fischer's exact test.

Multiple insertion sites are important for generating complex genotypes. We expanded the number of MosSCI insertion sites from two to six (**Supplementary Fig. 3**) with a full set of outcrossed strains containing the *Mos1* insertion and targeting vectors (three-way Gateway-compatible or multiple cloning site–compatible) based on *unc-119* selection and for one site, *unc-18* selection (**Table 1**). All sites readily enabled generation of MosSCI inserts and expression in somatic tissue. Three of the insertion sites (*ttTi4348* I, *ttTi5605* II and *cxTi10816* IV) led to robust expression in the germline from a ubiquitous promoter (**Supplementary Fig. 4**). Because MosSCI reagents are important for expression in

Table 1 | **MosSCI site characteristics**

Selection	Locus	Genetic position[a]	Insertion strain[b]	Gateway vector[c]	Multiple cloning site vector	Germline expression[d]	Insertion frequency[e] (percentage)	Balancer strain
***unc-119*[f]**	*ttTi4348*	I:–5.32	EG6701	pCFJ210	pCFJ352	Yes	3/12 (25%)	EG6173
	ttTi4391	I:7.93	EG6702	pCFJ604	pCFJ353	No	4/14 (29%)	EG6171
	ttTi5605	II:0.77	EG6699	pCFJ150	pCFJ350	Yes	6/14 (43%)	EG6070
	cxTi10816	IV:1.41	EG6703	pCFJ212	pCFJ356	Yes	2/10 (20%)	EG6401
	cxTi10882	IV:–0.05	EG6700	pCFJ201	pCFJ351	Variable	4/14 (29%)	EG5568
	ttTi14024	X:22.84	EG6705	pCFJ606	pCFJ355	Limited	3/14 (21%)	EG6109
***unc-18*[f]**	*ttTi4348*	I:–5.32	EG6032	pCFJ448	pCFJ676	Yes	ND	EG6173

[a]Linkage group: genetic map position (cM). [b]4× outcrossed, distributed with extrachromosomal *unc-119* rescue to facilitate handling and maintenance. [c]pDESTR4-R3, three-way Gateway-compatible vector. [d]Based on germline expression of *Pdpy-30::GFP::H2B* transgene (*GFP::H2B* encodes the GFP–histone H2B fusion). [e]Insertion frequency of *Pdpy-30::GFP::H2B* transgene. ND, not determined. [f]*unc-119* is necessary for nervous system development and *unc-18* is necessary for neurotransmission. Both mutants are viable but severely uncoordinated and can be rescued by extrachromosomal arrays.

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the germline, we generated an expression vector to express GFP-histone with the inserted transgene for confirmation of expression (**Supplementary Fig. 5**). All strains are available from the *Caenorhabditis* Genetics Center, and plasmids (targeting, transposase and negative selection vectors) are available from Addgene (**Supplementary Table 1**).

Note: Supplementary information is available on the Nature Methods website.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

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Generating transgenic nematodes by bombardment and antibiotic selection

To the Editor: In an extension of methods we[1] and others[2] have previously described in *Nature Methods*, we report here single- or dual-antibiotic selection to isolate transgenic nematodes after microparticle bombardment. The protocol makes it straightforward to generate integrated transgenes in diverse *Caenorhabditis* strains and species.

Microparticle bombardment[3,4] is widely used to generate transgenic *C. elegans* but requires specialized strains, large populations of worms and a slow selection procedure. To overcome some of these shortcomings and to facilitate the generation of transgenic strains in non-model nematode species, we developed a bombardment protocol that uses antibiotic selection[1,2] (**Supplementary Fig. 1**). Selection after bombardment can be effective using single antibiotics, but 'dual selection' using a combination of puromycin and G418 is efficient, cost-effective and more robust across species (**Supplementary Figs. 2** and **3**). To facilitate dual selection, we constructed plasmid vectors that express both antibiotic-resistance genes from a single operon (**Supplementary Fig. 4**).

After bombardment, worms are plated onto a limited supply of food sufficient to allow egg laying but resulting in the arrest of progeny as starved first-stage (L1) larvae (**Supplementary Methods** and **Supplementary Fig. 1**). On the next day, worms are washed off the plates, and adults are removed by gravity sedimentation, a key step for high selection efficiency. Selection is then performed for 4 d in small volumes of liquid nematode growth medium supplemented with 0.1% (vol/vol) Triton X-100, 0.5 mg ml^{-1} puromycin and 0.5 mg ml^{-1} G418, surviving worms are plated on nematode growth medium, and transgenic worms expressing a fluorescent marker are picked after 2–3 d of growth and checked for integration by selfing.

Table 1 | Summary of bombardments in *Caenorhabditis* species and strains

Caenorhabditis species	Strain	Total bombardments	Bombardments yielding a transmitting strain	Bombardments yielding an integrated strain (percentage)
C. elegans	N2	32	24	18 (56%)
C. elegans	CB4856	2	1	1 (50%)
C. briggsae	AF16	11	9	6 (55%)
C. briggsae	HK104	1	1	1 (100%)
C. remanei	PB4641	6	4	2 (33%)
C. brenneri	PB2801	4	2	1 (25%)

Using this protocol and the standard laboratory (Bristol N2) strain of *C. elegans*, we obtained transgenic worms from >70% of bombardments, and integrated transgenes with stable expression resulted from >50% of bombardments (**Table 1**, **Supplementary Fig. 5** and **Supplementary Table 1**). We recovered both high-copy-number and single-copy transgenes, with just over half of the strains that we tested carrying fewer than ten copies of the transgene (**Supplementary Fig. 6**). Selection was also efficient in other species, with transgenic worms obtained from 83% of bombardments in *C. briggsae*, and integrated lines from 58% of bombardments (**Table 1**, **Supplementary Fig. 7** and **Supplementary Table 1**). We also obtained transgenic worms from 60% of bombardments in the gonochoristic *Caenorhabditis* species *C. remanei* and *C. brenneri*, with 30% of bombardments in these species resulting in an integrated line (**Table 1** and **Supplementary Table 1**).

Compared to other approaches for generating integrated transgenes[3,5,6], the combination of bombardment and antibiotic selection is rapid and straightforward. The protocol can be used in diverse genetic backgrounds, which should facilitate research on non-model nematode species. Details of primers, strain genotypes and vectors are available in **Supplementary Tables 2–4** and **Supplementary Figures 8** and **9**. Vectors are available from Addgene (**Supplementary Table 4**).

Note: Supplementary information is available on the Nature Methods website.

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Nature Methods

Improved *Mos1*-mediated transgenesis in *C. elegans*

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Supplementary Figure 1	Mos1 transposase optimization
Supplementary Figure 2	*peel-1* negative selection
Supplementary Figure 3	Germline expression from P*dpy-30*::GFP::H2B transgene
Supplementary Figure 4	Germline expression from operon sensor construct
Supplementary Figure 5	Overview of MosSCI insertion sites
Supplementary Table 1	Insertion sites, Vectors and Addgene Accession numbers
Supplementary Methods	Detailed protocols and discussion of selection markers

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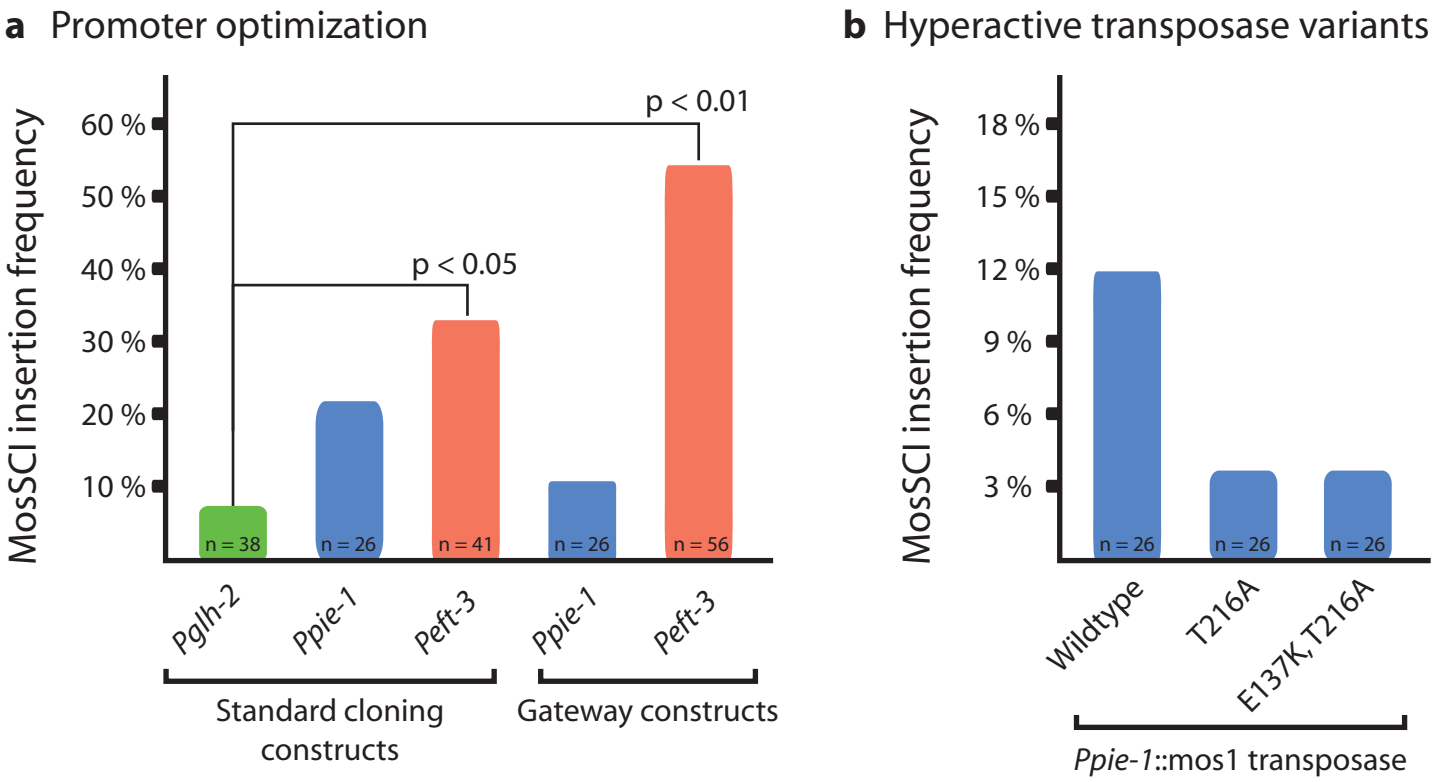

Optimizing Mos1 transposase. (a) Several different promoters were tested to enhance MosSCI insertion frequencies. All injections were done at low DNA concentration (32.5 ng/ul) to disfavor extrachromosomal array formation and to be in the dynamic range to detect differences in insertion frequencies. The *eft-3* promoter is the most efficient promoter tested both as a PCR stitched construct ("standard cloning constructs") or as a three-fragment Gateway construct. Significance was determined using Fischer's exact test compared pairwise to P*glh-2*. **(b)** Several hyperactive Mos1 transposase mutations have been published (Germon et al., 2009) that increase transposition in vitro. We tested two of the most promising mutations but could not detect enhanced transposition in vivo.
n = number of successfully injected animals.

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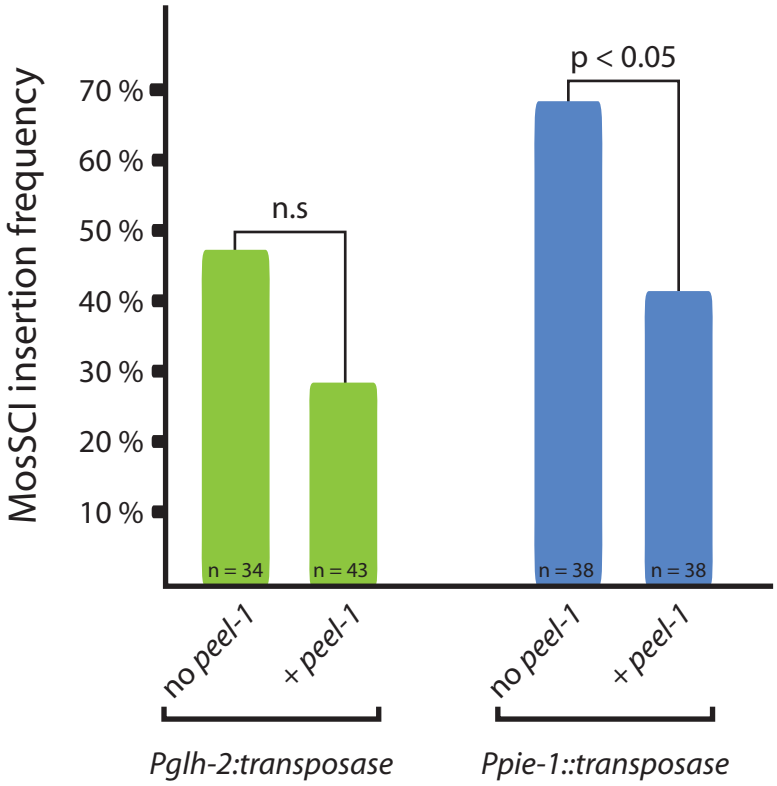
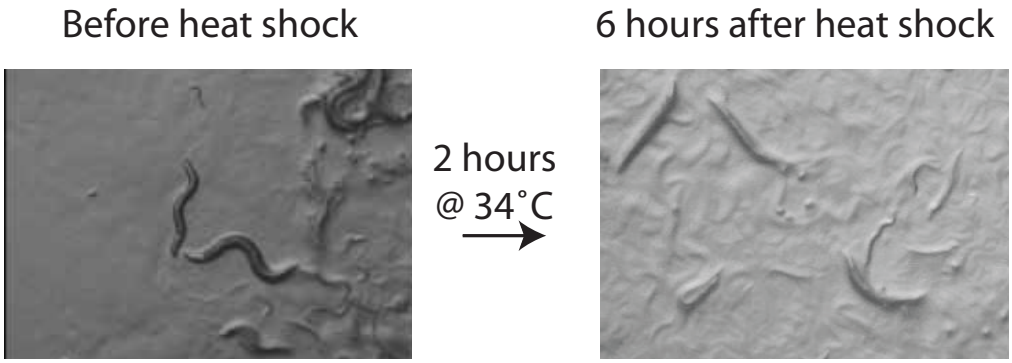
a Effect of *peel-1* on insertion frequency.

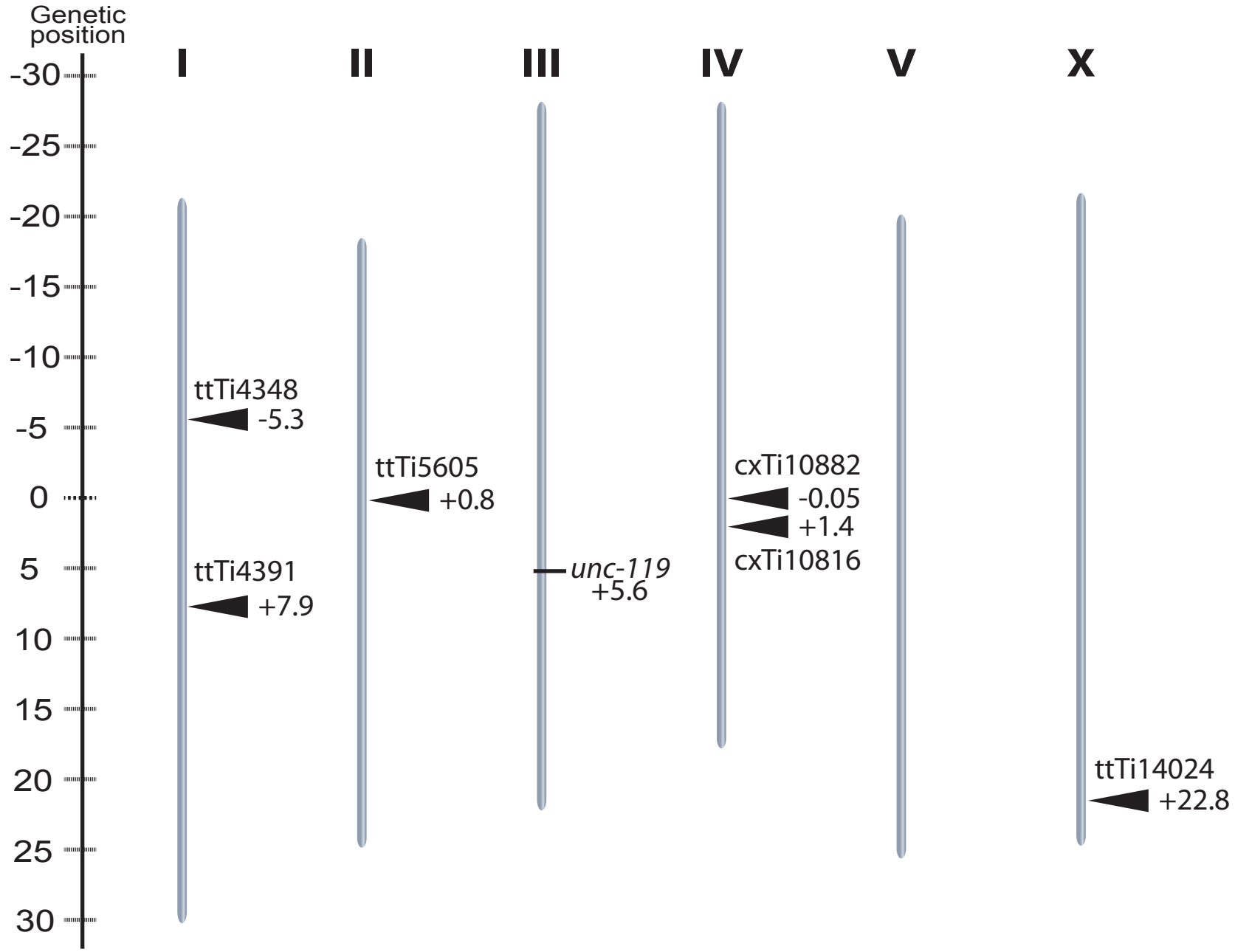


b *peel-1* induced toxicity.



Effect of peel-1 on insertion frequency. peel-1 can be used as a negative selection marker against extra-chromosomal arrays. **(a)** Bar graph of MosSCI insertions with and without the negative selection marker peel-1. peel-1 is driven by a heat-shock promoter (Phsp16.41:peel-1) and kills animals with arrays after a two-hour heat-shock at 34ºC. For both Pglh-2 and Ppie-1 driven Mos1 transposase the inclusion of peel-1 greatly enhances the speed and ability to isolate MosSCI inserts at the expense of a moderate decrease in the insertion frequency. Fischer's exact test. n = number of successfully injected animals. **(b)** Images of a plate of transgenic progeny from unc-119(-) P0s injected with 10 ng/ul Phsp::peel-1 added to an injection mix containing a unc-119(+) transgene and red co-injection markers (total concentration 100 ng/ul). Image to the left shows rescued animals before heat-shock, image to the right shows animals after a two hour heat-shock at 34ºC and four hour incubation at room temperature. Animals rescued by the peel-1 containing array are killed and essentially disintegrate. Putative MosSCI insertions were selected based on viability after heat-shock without the use of a fluorescence microscope to detect the red co-injection markers. We observed a 10% false positive rate (2 of 19 selected animals contained array markers in secondary fluorescence screen).

Supplementary Figure 3, Frøkjær-Jensen et al.



Validated MosSCI sites. Mos insertions (black arrows) were obtained from NemaGENETAG and outcrossed 4X to PS6038 (11x outcrossed *unc-119(ed3)*). Sites were identified on all chromosomes except chromosome III, which contains the selection marker *unc-119*(-), and chromosome V for which no adequate sites were identified. Each of these sites are in intergenic regions, most between annotated genes in a tail-to-tail configuration. Targeting vectors (three-way Gateway and multiple cloning site compatible) with the *unc-119*(+) selectable marker have been constructed for each insertion site. For one site (*ttTi4348* I), we also generated an insertion strain with *unc-18*(-) and a targeting vectors with an *unc-18*(+) gene for selection to facilitate making double insertion MosSCI strains.

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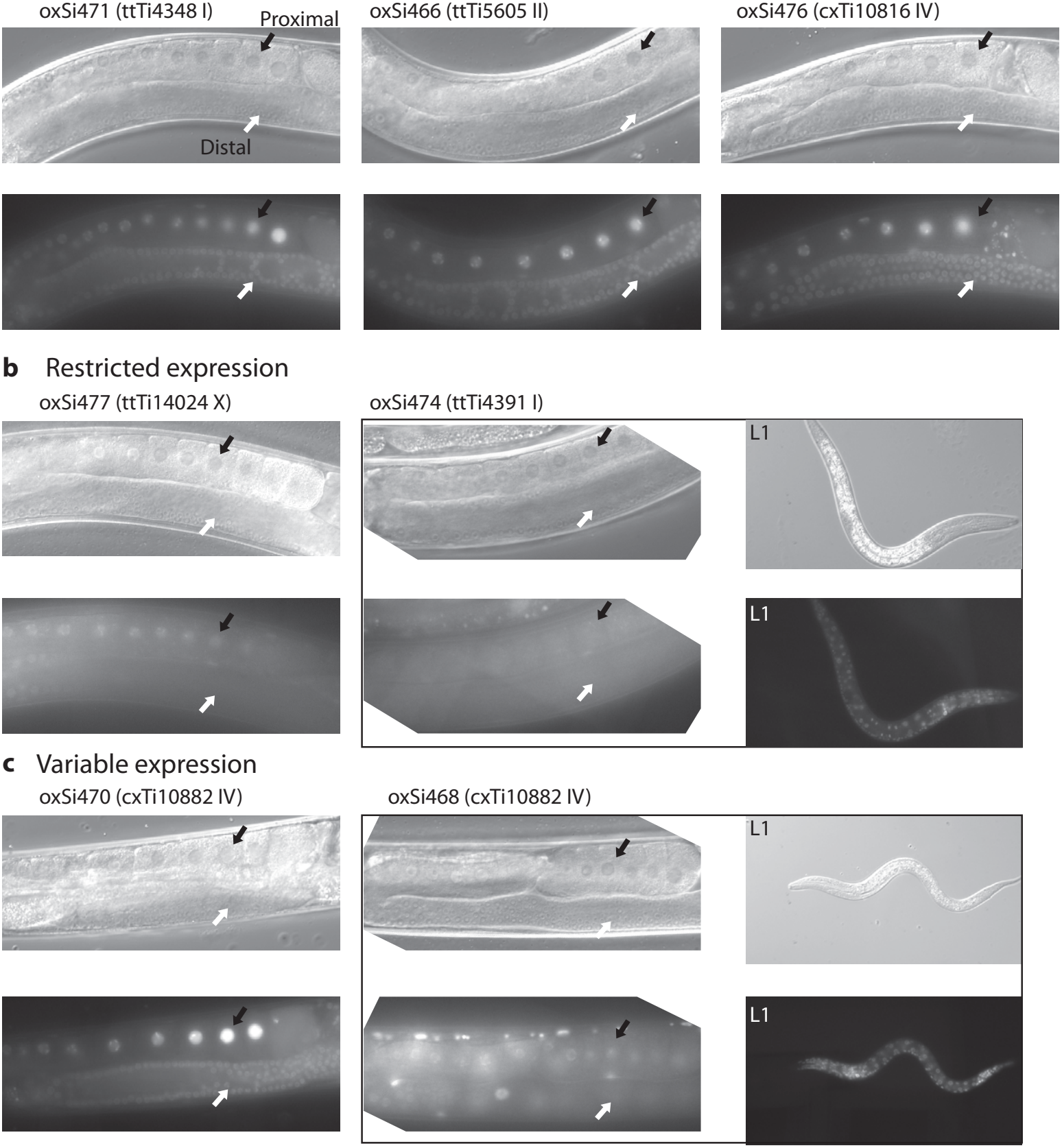

Somatic and germline expression of Pdpy-30::GFP::H2B inserts into MosSCI sites .
Black arrowhead = proximal gonad, oocytes. White arrowhead = distal gonad. (a) Consistent expression. Three MosSCI sites (ttTi4348 I, ttTi5605 II and cxTi10816 IV) exhibited consistent and bright somatic and germline expression of a Pdpy-30::GFP::H2B construct. (b) Restricted expression. Two MosSCI sites showed restricted or no germline expression, either to only the proximal arm of the gonad (ttTi14024 X) or no visible expression anywhere in the germline (ttTi4391 I). For both sites, expression in somatic tissues appeared normal. oxSi474: left image, no germline expression in adult. right image, ubiquitous somatic expression in L1 animal. (c) One MosSCI site (cxTi10882 IV) displayed variable expression in the germline. oxSi468: left image, dim expression in proximal gonad, no expression in distal gonad. right image, ubiquitous somatic expression in L1 animal.
Abbreviated figure legend. For full figure legend see **Supplementary Materials**.

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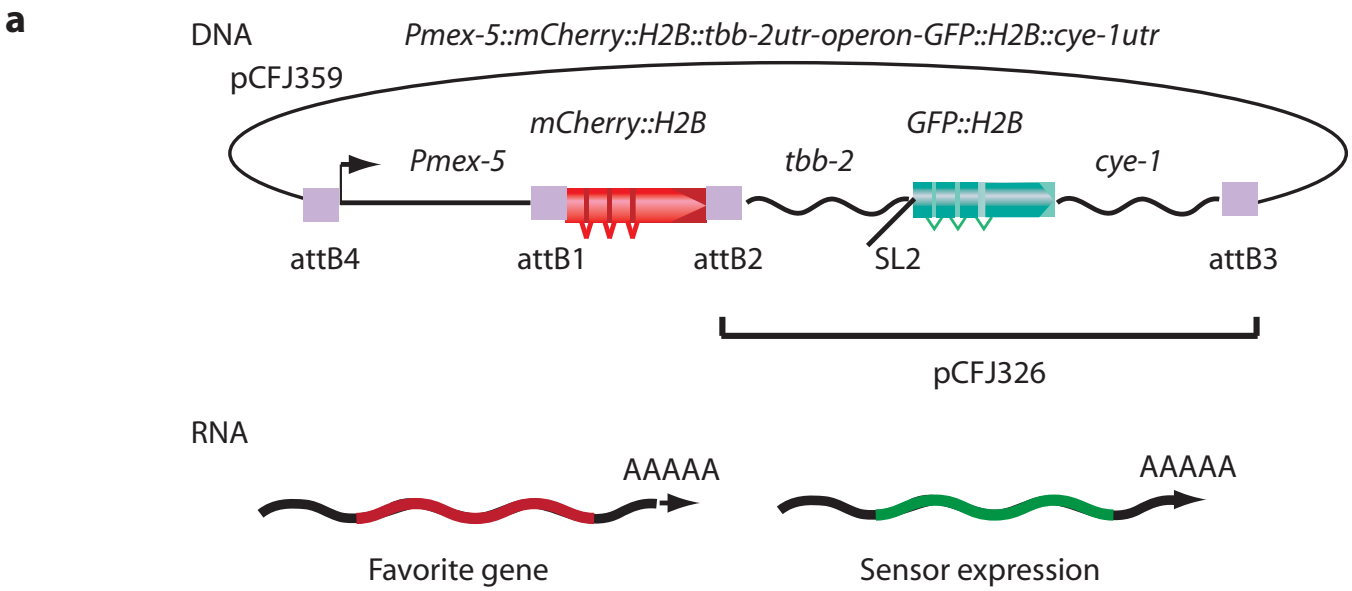

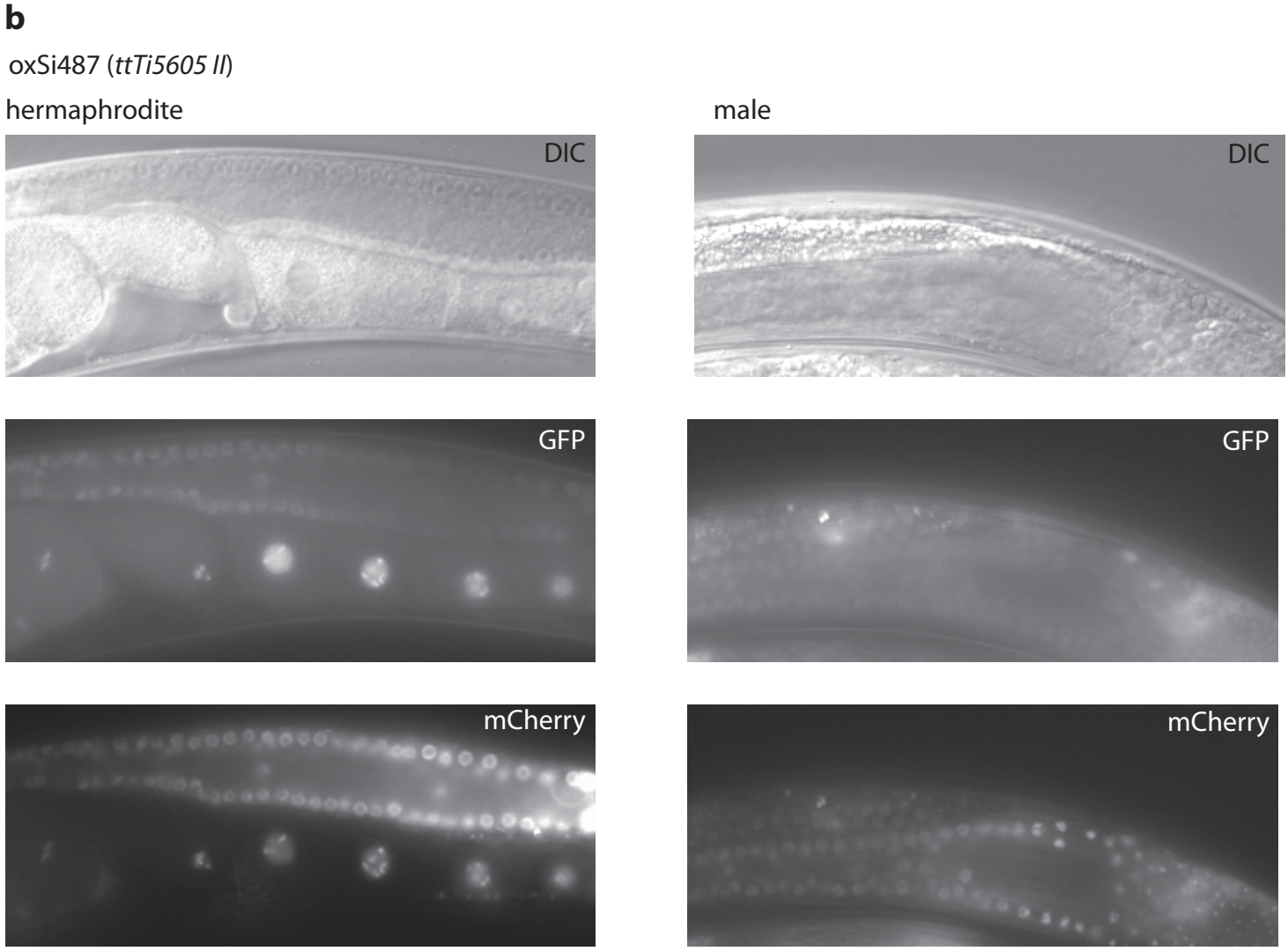

Germline fluorescence. **(a)** Schematic of the expression construct pCFJ359 which was inserted into the *ttTi5605* site to test germline expression (*unc-119* and homology arms omitted for clarity). The third gateway slot (attB3-attB2) serves as a sensor for germline expression of the second attB1-attB2 gateway slot by expressing GFP-H2B. In the schematic, the second slot (attB1-attB2) contains mCherry::H2B but any gene of interest could be cloned into a [1-2] Gateway entry vector. The entry vector pCFJ359 contains the tbb-2utr:operon:GFP::H2B:cye-1utr transgene. **(b)** An example of expression of the construct in the hermaphrodite (left) and male (right) germlines. 29% of all lines (11 of 38 independent inserts) showed germline fluorescence. In all cases where we observed GFP fluorescence we also observed mCherry fluorescence.

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Plasmids submitted to addgene

Plasmid	Name	Description	Addgene ID #
pCFJ604	pDESTR4-R3(ttTi4391, I)	ttTi4391 Gateway targeting vector	#34861
pCFJ606	pDESTR4-R3(ttTi14024, X)	ttTi14024 Gateway targeting vector	#34862
pCFJ210	pDESTR4-R3(ttTi4348, I)	ttTi4348 Gateway targeting vector	#34863
pCFJ212	pDESTR4-R3(cxTi10816, IV)	cxTi10816 Gateway targeting vector	#34864
pCFJ448	pDESTR4-R3(ttTi4348, I, unc-18)	ttTi4348 Gateway targeting vector, unc-18 selection	#34865
pCFJ350	MCS(ttTi5605, II)	ttTi5605 MCS targeting vector	#34866
pCFJ351	MCS(cxTi10882, IV)	cxTi10882 MCS targeting vector	#34867
pCFJ352	MCS(ttTi4348, I)	ttTi4348 MCS targeting vector	#34868
pCFJ353	MCS(ttTi4391, I)	ttTi4391 MCS targeting vector	#34869
pCFJ355	MCS(ttTi14024, X)	ttTi14024 MCS targeting vector	#34870
pCFJ356	MCS(cxTi10816, IV)	cxTi10816 MCS targeting vector	#34871
pCFJ676	MCS(ttTi4348, I, unc-18(+))	ttTi4348 MCS targeting vector, unc-18 selection	#34872
pMA122	peel-1 negative selection	heat-shock inducible negative selection marker	#34873
pCFJ601	Peft-3 Mos1 transposase	Improved Mos1 transposition	#34874
pCFJ326	operon for germline expression	Monitor germline expression with operon	#34875
pCFJ421	Pmyo-2::GFP::H2B (pharynx)	Co-injection marker, green pharyngeal muscle expression	#34876
pCFJ420	Peft-3::GFP::H2B (ubiquitous)	Co-injection marker, ubiquitous green expression.	#34877
pCFJ594	pENTR[1-2](NeoR)	Neomycin resistance, [1-2] gateway slot for MosDEL constructs	#34878

Please request these plasmids from the non-profit plasmid repository addgene (www.addgene.org).

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Acknowledgements

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Website

We maintain a website with up to date information on protocols, reagents and characteristics of the different MosSCI insertion sites.
Please see: https://sites.google.com/site/jorgensenmossci/

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Nematode strains

Mos1 insertion alleles were selected by visual screening in Wormbase (www.wormbase.org) for transposon insertions in intergenic regions. All insertion strains were provided by the NemaGENETAG consortium. *Mos1* insertions were homozygosed and followed in crosses by PCR. Strains were maintained on NGM plates seeded with OP50 or HB101 bacteria.

Strains used in the study (**Table 1**):
Injection strains
EG6699: *ttTi5605* II; *unc-119(ed3)* III; *oxEx1578[cb-unc-119(+); Peft-3::GFP]*
EG6700: *unc-119(ed3)* III; *cxTi10882* IV; *oxEx1579[cb-unc-119(+); Peft-3::GFP]*
EG6701: t*tTi4348 I*; *unc-119(ed3)* III; *oxEx1580[cb-unc-119(+); Peft-3::GFP]*
EG6702: *ttTi4391* I; *unc-119(ed3)* III; *oxEx1581[cb-unc-119(+); Peft-3::GFP]*
EG6703: *unc-119(ed3)* III; *cxTi10816 IV*; *oxEx1582[cb-unc-119(+); Peft-3::GFP]*
EG6705: *unc-119(ed3)* III; *ttTi14024* X; *oxEx1584[cb-unc-119(+); Peft-3::GFP]*
Strains were outcrossed and rescued with extra-chromosomal arrays to facilitate freezing, maintenance and distribution. Injections were performed using derivatives of these strains that have lost the rescuing arrays.

Outcrossing strains
EG6843: *unc-119(ed3)* III; *him-5(e1490)* V; *oxEx1605[cb-unc-119(+); Phsp-16.41::peel-1]*.
This strain is useful for outcrossing MosSCI insertions. The Him Unc-119(+) males can be crossed into the MosSCI strains with inserts. In the second or third generation from a cross, the array can be selected against by heat-shock, so the only rescue comes from the MosSCI insert.

PS6038: *unc-119(ed3)* III ; *syEx1136[Pmyo-2::GFP; unc-119(+)l]*.
11x outcross of *unc-119(ed3)* to N2 by Amir Sapir from the Sternberg Lab. This strain is useful for outcrossing MosSCI inserts. Cross males (for example, from a cross with EG6843 above) containing MosSCI insertions to animals from this strain that have lost the array rescue.

Balancer strains
EG6173: *oxSi259[Peft-3::GFP cb-unc-119(+)]* I; *unc-119(ed3)* III
EG6171: *oxSi257[Peft-3::GFP cb-unc-119(+)]* I; *unc-119(ed3)* III
EG6070: *oxSi221[Peft-3::GFP cb-unc-119(+)]* II; *unc-119(ed3)* III
EG6401: *unc-119(ed3)* III; *oxSi346[Peft-3::GFP cb-unc-119(+)]* IV
EG5568: *dpy-13(ox495::cb-unc-119(+) Pmyo-2::mCherry Punc-122::GFP)* IV
EG6109: *unc-119(ed3)* III; *oxSi230[Peft-3::GFP cb-unc-119(+)]* X
These GFP expressing chromosomes can be bounced out to homozygose the Mos bearing chromosome or a MosSCI insertion when crossing in other mutations.

Pdpy-30::GFP::H2B::tbb-2 strains
Consistent germline expression:
EG6776: *oxSi471[Pdpy-30::GFP::H2B::tbb-2; cb-unc-119(+)]* I; *unc-119(ed3)* III
EG6771: *oxSi466[Pdpy-30::GFP::H2B::tbb-2; cb-unc-119(+)]* II; *unc-119(ed3)* III
EG6781: *unc-119(ed3)* III; *oxSi476[Pdpy-30::GFP::H2B::tbb-2; cb-unc-119(+)]* IV
Restricted germline expression:
EG6782: *unc-119(ed3)* III; *oxSi477[Pdpy-30::GFP::H2B::tbb-2; cb-unc-119(+)]* X
EG6779: *oxSi474[Pdpy-30::GFP::H2B::tbb-2; cb-unc-119(+)]* I; *unc-119(ed3)* III
Variable germline expression:
EG6775: *unc-119(ed3)* III; *oxSi470[Pdpy-30::GFP::H2B::tbb-2; cb-unc-119(+)]* IV
EG6773: *oxSi468[Pdpy-30::GFP::H2B::tbb-2 cb-unc-119(+)]* IV; *unc-119(ed3)* III

Pmex-5::mCherry::H2B::tbb-2utr:operon:GFP::H2B::cye-1utr strains
EG6787: *oxSi487[Pmex-5::mCherry::H2B::tbb-2:operon:GFP::H2B::cye-1utr; unc-119(+)]* II; *unc-119(ed3)* III

dpy-13 deletion strains
EG5568: *dpy-13(ox495::cb-unc-119(+); Pmyo-2::mCherry; Punc-122::GFP)* IV

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EG5568 is a rare complex deletion/insertion. The *dpy-13* deletion is linked to an insertion of a *Pmyo-2::mCherry* co-injection marker. Presumably the template that repaired the Mos1 excision incorporated the selection marker *Pmyo-2::mCherry* from the extrachromosomal array.
EG7003: *dpy-13(ox497::neoR(+))* IV

Protocol for MosSCI insertions

We followed the protocol described in Frøkjær-Jensen et al. (2008) with a few modifications. First, all injection strains were grown on HB101 bacteria at 15ºC. The *unc-119* and *unc-18* strains are healthier on HB101 bacteria which facilitates injection and survival. The HB101 bacteria can be requested from the Caenorhabditis Genetics Center (CGC). Second, plates with a single injected worm were placed in a humidified 25ºC incubator within two hours of injection and left there until starvation. Growth at 25ºC shortens the time needed to recover insertions. Three days after injection the plates were screened for Unc-119(+) rescue; any plates with rescued progeny were kept and counted as successfully injected. After starving completely (approximately a week after injection), plates were screened for MosSCI inserts on a fluorescence dissecting microscope. MosSCI insertions can be distinguished by wild-type movement, the lack of red co-injection markers and a propensity to form dauers faster than array-rescued *unc-119* animals.
Other specific modifications are listed below.

Low DNA concentration

The data shown in **Fig. 1a** and **Supplementary Fig. 1a/b** were generated by injecting a total concentration of 32.5 ng/ul. By injecting low concentrations of DNA, F1 rescue is promoted but F2 stable array formation is inhibited (Mello *et al.*, 1991). The reduced number of stable extrachromosomal arrays facilitates screening for inserts. Also, a lower concentration of the targeting vector facilitates insertions if the targeting vector is toxic at high concentrations. A 10x MosSCI injection mix was made with 50 ng/ul pCFJ104 (*Pmyo-3:mCherry*), 50 ng/ul pGH8 (*Prab-3:mCherry*), 25 ng/ul pCFJ90 (*Pmyo-2:mCherry*) and 100ng/ul of the Mos1 transposase plasmid (for example, pCFJ601). Injection mixes were typically made up in 20 ul volumes by adding 2 ul 10x MosSCI injection mix, 10 ng/ul (final concentration) targeting vector and distilled water up to 20 ul.

High DNA concentration + *peel-1*

Although toxicity is minimized and screening for inserts is facilitated by low DNA concentration the overall frequency of insertions is reduced (see for example P*glh-2* in **Fig. 1a** vs **Supplementary Fig. 2a**). We therefore developed a method to select against animals carrying arrays by heat-shock. The *peel-1* gene encodes a cell autonomous toxin that can kill animals within hours by ectopic expression (Seidel et al., 2011). We included a P*hsp-16.41::peel-1::tbb-2utr* plasmid (pMA122) in the injection mix at 10 ng/ul to express *peel-1* after a 2 hour heat-shock at 34ºC in an air incubator. Final concentration of total injected DNA was 100 - 110 ng/ul.
P*glh-2::* Transposase
Injections were done at the original concentrations described in Frøkjær-Jensen *et al*. (2008), with the targeting vector injected at 50 ng/ul, pJL43.1(*Pglh-2::* transposase) at 50 ng/ul, pGH8 (*Prab-3:mCherry*) at 10 ng/ul, pCFJ90 (P*myo-2:mCherry*) at 2.5 ng/ul and pCFJ104 (P*myo-3:mCherry*) at 5 ng/ul. pMA122 (P*hsp-16.41*:*peel-1*) was omitted or included at 10 ng/ul.
P*pie-1::* Transposase
Injections were done with the following concentrations: Targeting vector at 10 ng/ul, *Ppie-1::* Transposase at 25 ng/ul, pGH8 (P*rab-3:mCherry*) at 10 ng/ul, pCFJ90 (*Pmyo-2:mCherry*) at 2.5 ng/ul and pCFJ104 (*Pmyo-3:mCherry*) at 5 ng/ul. pMA122 (P*hsp-16.41*:*peel-1*) was omitted or included at 10 ng/ul. In initial experiments, P*pie-1::* Transposase appeared to be toxic at 50 ng/ul (data not shown) and the concentration was therefore reduced to 25 ng/ul.
The injected animals were placed at 25ºC after injection and allowed to starve out. Starved plates were heat-shocked for 2 hours at 34ºC in an air incubator and screened for living Unc-119(+) animals either four hours later or a day later. The heat-shock kills almost all animals with extrachromosomal arrays and greatly facilitates screening. We observe an approximate 10% false positive rate if we pick Unc-119(+) adults on a regular transmitted light dissection microscope without fluorescence capabilities four hours after heatshock (**Supplementary Fig. 2**).

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Comments on the use of the negative selection marker *peel-1*

At first glance the use of the negative selection marker may seem disadvantageous. Use of the negative selection marker requires an additional step (2 hours of heat-shock at 34ºC) and reduces the insertion frequency (see **Supplementary Figure 2**). However in our hands, these disadvantages are more than compensated for by the reduction in time screening for inserts and the ability to screen without using a fluorescence microscope. Although the low DNA concentration reduces array formation there are still often array containing animals on the plates that make screening somewhat laborious. Moreover, the insertion frequency with a low DNA concentration is similar to the insertion frequency of a high DNA concentration together with the *hsp::peel-1* negative selection plasmid (54% vs 62%, respectively for *Peft-3::transposase*). In our lab, we exclusively use the protocol using a high DNA concentration with the *Peft-3* helper plasmid and the *peel-1* negative selection marker.

Protocol MosDEL injections

We followed the protocol described in Frøkjær-Jensen et al. (2010) with the same modifications described above: strains were grown on HB101 bacteria at 15ºC before injection and injected worms were placed at 25ºC after injection. Further changes to the protocol are described below.

unc-119 selection

We tested the efficiency of using P*eft-3::mosase* and the negative selection marker *peel-1* to generate a 5kb targeted deletion of *dpy-13* with the Mos1 element at *cxTi10882*. We used the plasmid pRL5 described in Frøkjær-Jensen *et al*. (2010). The injection mix consisted of pRL5 (5 kb deletion construct) at 50 ng/ul, pCFJ104 (*Pmyo-3:mCherry*) at 5 ng/ul, pGH8 (*Prab-3:mCherry*) at 10 ng/ul, pCFJ90 (*Pmyo-2:mCherry*) at 2.5 ng/ul, pCFJ631 (*Peft-3::mos1 transposase*) at 50 ng/ul and pMA122 (P*hsp-16.41::peel-1*) at 10ng/ul. *unc-119(ed3) III, cxTi10882 IV* animals were injected, singled to individual plates and placed at 25ºC. Three days later the plates were screened for *unc-119* rescue; any plates with rescued progeny were kept and counted as successfully injected. Approximately a week after injection the plates were heat-shocked for 2 hours at 34ºC and screened for rescued animals four hours later. From plates with rescued progeny, a single animal was picked and allowed to self. In the next generation, the progeny were scored for the Dpy-13 phenotype.

Neomycin selection

We generated a plasmid (pCFJ662) to delete a 5kb region adjacent to *cxTi10882* as described above, but with a neomycin selection marker in place of the *unc-119(+)* selection marker (see pCFJ594, Neomycin selection marker below). The injection mix consisted of pCFJ662 at 50 ng/ul, pCFJ104 (*Pmyo-3:mCherry*) at 5 ng/ul, pGH8 (*Prab-3:mCherry*) at 10 ng/ul, pCFJ90 (*Pmyo-2:mCherry*) at 2.5 ng/ul, pCFJ631 (*Peft-3::mos1 transposase*) at 50 ng/ul and pMA122 (*hsp-16.41::peel-1*) at 10 ng/ul. *cxTi10882 IV* animals were injected, singled to individual plates and placed at 25ºC. Three days later the plates were screened for mCherry fluorescence; any plates with transgenic progeny were kept and counted as successfully injected. Plates were flooded with 500 ul of 25 mg/ml G418 (Goldbiochem, St. Louis, MO) and allowed to dry. Approximately a week after injection the plates were heat-shocked for 2 hours at 34ºC and chunked to a new plate and flooded with 500ul of 25 mg/ml G418. Two days later, the plates were screened for Dpy-13 progeny. Dpy-13 progeny were singled and propagated for a generation to verify homozygosity of deletion. Two (see EG7003, for example) of the *dpy-13* strains were tested for G418 resistance. Growth was similar on selection and non-selection plates, whereas N2 controls did not grow on selection plates.

Comments on the use of positive selection markers *unc-119(+)* and neomycin.

unc-119 is a very strong selection marker that has been used extensively for biolistic transformation (Praitis et al., 2001), MosSCI (Froekjaer-Jensen et al., 2008) and MosDEL (Froekjaer-Jensen et al., 2010). *unc-119* is a particularly strong selection marker because *unc-119* animals are strongly Unc and cannot form dauer animals so non-rescued animals rapidly die at 25ºC (Maduro and Pilgrim, 1995). This selection makes it easy to identify inserts. Homozygous animals are easily identified based on the lack of Unc animals and *unc-119* is a strong balancer for lethal deletions (Froekjaer-Jensen et al., 2010). However, *unc-119* has a number of disadvantages: the mutant animals are sick and somewhat difficult to inject. Also, to generate Mos1 based deletions it is necessary to cross the Mos1 allele into the *unc-119* mutant background which adds an additional step.

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For these reasons, it would be highly desirable to have a selectable marker based on a drug. Two such selectable antibiotic markers have been developed for *C. elegans* based on the antibiotics G418/Neomycin (Giordano-Santini et al., 2010) and Puromycin (Semple et al., 2010). The neomycin selection had been shown to be efficient enough to generate MosSCI inserts but not strong enough to generate biolistic inserts (Giordano-Santini et al., 2010). This suggested that the neomycin selection is less efficient than *unc-119*. Because the MosDEL efficiency is lower than MosSCI and because deletions often result in sick mutant animals it was not clear if neomycin would be strong enough to select for Mos1 mediated deletions and we therefore tested the efficiency. We did not test the puromycin selection because it was not used to generate biolistic inserts or to select for MosSCI insertions (Semple et al., 2010). However, a single copy of the puromycin resistance gene inserted with *unc-119* selection is fully resistant to puromycin and this selection may therefore be strong enough as a selection marker on its own (Semple et al., 2010).

The efficiency of *dpy-13* deletions with neomycin is equal to the efficiency of *unc-119 (***Figure 1 C***)*. However, under the conditions that we used (which are a modification of the protocol used by Giordano-Santini et al.) we also observed that most of the plates without a *dpy-13* deletion had viable non-array worms on the G418 selection media. We picked "non-dead" animals from these plates but they never segregated Dpy animals suggesting that they were false positive strains that did not contain the desired deletion. In the case of *dpy-13*, the deletion generates an obvious, non-lethal phenotype which can readily be identified and selected. However for a deletion where the phenotype is not known in advance - or where the deletion is lethal - we do not expect the neomycin selection to perform as well as *unc-119* because the false positives will be difficult to distinguish from real deletions.

We therefore recommend using neomycin for deletions with a clear, non-lethal phenotype. We recommend using *unc-119* for deletions that are expected to be very severe or where the phenotype is not known from chemically induced mutations, deletions or RNA interference data.

Molecular Biology

Targeting vectors, three-way Gateway compatible

A set of targeting vectors (pDESTR4-R3) that are compatible with the three-way Gateway system (Invitrogen, Carlsbad, CA) were generated by standard cloning (pCFJ150, pCFJ201, pCFJ210, pCFJ212 and pCFJ448) or by Isothermal Assembly (Gibson et al., 2009) (pCFJ604 and pCFJ606).
The following vectors contain the *cb-unc-119* rescue marker and an attR4-ccdB-Cm-attR3 cassette flanked by homology regions to target MosSCI inserts.

Vector	**Insertion site**	**Selection Marker**
pCFJ150	*ttTi5605 II*	*unc-119(+)*
pCFJ201	*cxTi10882 IV*	*unc-119(+)*
pCFJ210	*ttTi4348 I*	*unc-119(+)*
pCFJ212	*cxTi10816 IV*	*unc-119(+)*
pCFJ604	*ttTi4391 I*	*unc-119(+)*
pCFJ606	*ttTi14024 X*	*unc-119(+)*
pCFJ448	*ttTi4348 I*	*unc-18 (+)*

Targeting vectors, Multiple Cloning Site (MCS)

A set of targeting vectors that are compatible with restriction enzyme cloning were generated by a three-way Gateway reaction that combines an [attB1-attB2] vector containing a *cb-unc-119* rescuing fragment and a multiple cloning site (MCS) with M13f and M13r sequencing sites together with two flanking constructs that contain the homology regions to target MosSCI inserts. The three entry fragments were combined with a custom-made pDESTR4-R3 vector with the M13f/M13r sites removed.

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Vector	Insertion site	Selection Marker
pCFJ350	*ttTi5605 II*	*unc-119(+)*
pCFJ351	*cxTi10882 IV*	*unc-119(+)*
pCFJ352	*ttTi4348 I*	*unc-119(+)*
pCFJ356	*cxTi10816 IV*	*unc-119(+)*
pCFJ353	*ttTi4391 I*	*unc-119(+)*
pCFJ606	*ttTi14024 X*	*unc-119(+)*
pCFJ448	*ttTi4348 I*	*unc-18 (+)*

Mos1 transposase vectors

A set of plasmids that drive *Mos1* transposase expression under different promoters (**Supplementary Table 1**) were generated either by PCR stitching or by three-fragment Gateway. In some cases, the introduction of Att sites between the promoter and the *Mos1* transposase inhibited the insertion frequency (*Ppie-1*) and in other cases the Att sites appeared to enhance the insertion frequency (*Peft-3*). In all cases the ATG start site was adjacent to the native transposase ORF and after the Att site.

Non-Gateway based

pJL43.1 (*Pglh-2::mos1 transposase::glh-2utr*) was described in (Bessereau et al., 2001). pCFJ103 (*Ppie-1(intron)::mos1 transposase::pie-1utr*) was generated by removing a *cb-unc-119* fragment from a vector containing a *Ppie-1::mos1 transposase* fragment (pWD106) (Gallo et al., 2008). pCFJ631 (*Peft-3::mos1 transposase::tbb-2utr*) was generated by PCR stitching and verified by sequencing.

Gateway based

pCFJ601 (*Peft-3::mos1 transposase::tbb-2utr*), pCFJ204 (*Pglh-2::mos1 transposase::tbb-2utr*) and pCFJ501 (*Ppie-1::mos1 transposase::pie-1utr*) were generated by a three-fragment Gateway reaction.

Mutated transposase vectors

A set of mutated transposase plasmids were generated. The mutations were chosen based on the ability of hyperactivating mutations to improve transposition in bacterial assays (Germon et al., 2009). The hyperactivating mutations were introduced by PCR and all plasmids were verified by sequencing. All mutated versions of the *Mos1* transposase were cloned into entry vectors and inserted into expression constructs by a three-fragment Gateway reaction. All wild-type and modified *Mos1* transposases were put under the control of a *pie-1* promoter and followed by a *pie-1* 3'utr.

The following expression constructs were used: pCFJ501 (*Ppie-1::mos1 transposase(wild-type)::pie-1utr*), pCFJ503(*Ppie-1::mos1 transposase(T216A): :pie-1utr) and* pCFJ504(*Ppie-1::mos1 transposase(E137K, T216A)::pie-1utr)*.

Co-injection vectors

Fluorescent markers

A set of GFP based co-injection markers (**Supplementary Table 1**) were generated to facilitate insertion of transgenes expressing red fluorophores to complement the mCherry vectors described in Frøkjær-Jensen *et al*. (2008). All vectors were generated by three-fragment Gateway reactions.

pCFJ421(*Pmyo-2::GFP::H2B::tbb-2utr*) expresses nuclear localized GFP in the pharyngeal muscles, pCFJ420(*Peft-3::GFP::H2B::tbb-2utr*) expresses nuclear-localized GFP in all somatic tissues. pCFJ90(P*myo-2::mCherry::unc-54utr*), pCFJ104(P*myo-3::mCherry::unc-54utr*) and pGH8(P*rab-3::mCherry::unc-54utr*) were described in Frøkjær-Jensen et al. (2008).

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Negative selection marker

A negative selection marker based on the *peel-1* toxic gene (Seidel et al., 2011) was developed to select against arrays following heat-shock. pMA122 (*Phsp-16.41::peel-1::tbb-2utr*) was generated by three fragment Gateway and expresses *peel-1* after heat-shock induction and kills almost all array carrying animals.

Miscellaneous vectors

Germline sensor construct

pCFJ326. An important application of MosSCI is to generate transgenic animals that express a gene of interest in the germline (see **Supplementary Fig. 4**). However, not all inserts successfully express in the germline - either because of inefficient repair or possibly because of silencing mechanisms that are not fully understood. Also, in many cases it is desirable to insert an untagged gene but still monitor its expression in the germline. We therefore developed a vector based on an operon construct described by Merritt *et al*. (2008). We modified a construct to make a [attB2-attB3] Gateway compatible vector (pCFJ326) that contains a *tbb-2utr::gpd-2:operon:GFP::H2B:cye-1utr*. We modified the construct to contain the *tbb-2* utr because it allows ubiquitous expression in the germline (Merritt *et al.*, 2008). We used a plasmid (pCFJ359) containing a 4.4 kb fragment insert consisting of *Pmex-5:mCherry::H2B::tbb-2utr::GFP::H2B::cye-1utr* to test the efficiency. 11 of 38 inserts (29%) expressed fluorescence in the germline. For all 11 fluorescent inserts we saw perfect correlation between GFP and mCherry expression, indicating that GFP::H2B expression is a good reporter for expression of the upstream gene in the operon.

Neomycin selection vector

pCFJ594. We cloned a P*rps-27::neomycin* fragment based on pRG5273-Neomycin (Giordano-Santini et al., 2010) into a [attB1-attB2] Gateway entry vector. This vector can be used to generate a targeting vector for MosDEL and select for deletions with G418/Geneticin.

Statistical Methods

All data were analyzed with the program GraphPad Prism 5 (GraphPad Software, La Jolla, CA). The proportions were analyzed in a contingency table and statistical significance was computed with Fischer's Exact Test. In cases where multiple comparisons were made with the same set of data, the significance level was adjusted for multiple comparisons by multiplying the P-value by the number of comparisons. 95% confidence intervals were calculated by the Modified Wald Method.

Figure 1

Figure 1A - Promoter optimization, low DNA concentration

Promoter	**Injected animals**	**Plates with F1 rescued animals**	**Insertions**	**Insertion frequency**	**95% confidence interval**
Pglh-2	45	38	3	0.08	0.020 - 0.22
Ppie-1	29	26	6	0.23	0.11 - 0.42
Peft-3	73	56	30	0.54	0.41 - 0.66

Fischer's exact test:
P*glh-2* vs P*pie-1*: P = 0.14, not significant
P*glh-2* vs P*eft-3*: P < 0.01, highly significant.

Figure 1B - Promoter optimization, *dpy-13* deletion

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Promoter	Selection marker	Injected animals	Plates with F1 rescued animals	Deletions	Deletion frequency	95% confidence interval
Pglh-2	unc-119(+)	N.D.	83	3	0.036	0.008 - 0.11
Peft-3	unc-119(+)	47	30	6	0.20	0.09 - 0.38
Peft-3	Neomycin	N.D.	51	12	0.24	0.14 - 0.37

Fischer's exact test:
P*glh-2(unc-119)* vs P*pie-1(unc-119)*: P = p < 0.05
P*eft-3(unc-119)* vs P*eft-3(*neomycin*)*: P = 0.78, not significant.

Figure 1C - Promoter optimization, high DNA concentration, *peel-1* negative selection

Promoter	Injected animals	Plates with F1 rescued animals	Insertions	Insertion frequency	95% confidence interval
Pglh-2	46	43	12	0.28	0.17 - 0.43
Ppie-1	48	38	15	0.39	0.26 - 0.55
Peft-3	42	37	23	0.62	0.46 - 0.76

Fischer's exact test:
P*glh-2* vs P*pie-1*: P = 0.35, not significant
P*glh-2* vs P*eft-3*: P = p < 0.05

Supplementary Figure 1

Supplementary Figure 1a - Promoter optimization

Promoter	Injected animals	Plates with F1 rescued animals	Insertions	Insertion frequency	95% confidence interval
P*glh-2 (MCS)*	45	38	3	0.08	0.020 - 0.22
P*pie-1 (MCS)*	29	26	6	0.23	0.11 - 0.42
P*eft-3 (MCS)*	59	41	12	0.32	0.19 - 0.47
P*pie-1 (Gateway)*	31	26	3	0.12	0.03 - 0.30
P*eft-3 (Gateway)*	73	56	30	0.54	0.41 - 0.66

Fischer's exact test:
P*glh-2* vs P*eft-3 (standard cloning)*: p < 0.05
P*glh-2* vs P*eft-3(gateway)*: p < 0.01

Supplementary Figure 1b - Mosase optimization

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Mosase	Injected animals	Plates with F1 rescued animals	Insertions	Insertion frequency	95% confidence interval
wildtype	31	26	3	0.12	0.03 - 0.30
T216A	37	26	1	0.04	0.00-0.20
E137K, T216A	32	26	1	0.04	0.00-0.20

The transposase variants did not improve insertion frequency.

Supplementary Figure 2

Plasmids	Injected animals	Plates with F1 rescued animals	Insertions	Insertion frequency	95% confidence interval
P*glh-2*	35	34	16	0.47	0.31 - 0.63
P*glh-2* + *peel-1*	46	43	12	0.28	0.17 - 0.43
P*pie-1*	45	38	26	0.68	0.68 - 0.52
P*pie-1* + *peel-1*	48	38	15	0.39	0.26 - 0.55

Fischer's exact test:
P*glh-2* vs P*glh-2* + *peel-1*: not significant
P*pie-1* vs P*pie-1* + *peel-1*: $p < 0.05$

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