SUPPLEMENTAL MATERIALS AND METHODS

Strains and maintenance

Bristol N2 strain was provided by the Caenorhabditis Genetics Center (CGC), University of Minnesota. All worms were grown on nematode growth medium (NGM) agar plates seeded with the *Escherichia coli* bacterial strain OP50-1 and maintained using standard procedures (Brenner 1974).

Strains generated in this work and sequences

All strains generated in this work are listed in Table 1, and will be available for distribution through the CGC. All sequences files for SKI LODGE lines are available at https://www.themairlab.com/skilodge. All oligonucleotides, crRNAs, tracrRNA and repair templates used in this study are listed in Tables 2 and 3.

Table 1. Strains generated in this work.

Strain	Genotype	crRNA used	Recipient strain	Outcrossing
SKI LODGE strains				
WBM1119	*N2, wbmIs60[pie-1p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, III:7007600]*	*crRNA oxTi444 Chr.III*	N2	6x
WBM1126	*N2, wbmIs61[myo-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, I:2851000]*	*crRNA ttTi4348 Chr.I myo-3(8d) SKIS*	N2	6x
WBM1140	*N2, wbmIs65[eft-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, V:8645000]*	*crRNA oxTi365 Chr. V*	N2	6x
WBM1141	*N2, wbmIs66[rab-3p::3XFLAG::dpy-10 crRNA::rab-3 3'UTR, IV:5015000]*	*cxTi10816 crRNA Chr. IV*	N2	6x
WBM1179	*N2, wbmIs76[eft-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, IV:5015000]*	*cxTi10816 crRNA Chr. IV*	N2	6x
WBM1214	*N2, wbmIs88[eft-3p::3XFLAG::dpy-10 crRNA::SL2::wrmScarlet::unc-54 3'UTR, *wbmIs67]*	*3xFLAG crRNA*	WBM1143	6x
WBM1215	*N2, wbmIs89[rab-3p::3XFLAG::dpy-10 crRNA::SL2::wrmScarlet::rab-3 3'UTR, *wbmIs68]*	*3xFLAG crRNA*	WBM1144	6x
WBM1216	*N2, wbmIs96[ges-1p::3XFLAG::dpy-10 crRNA::SL2::wrmScarlet::unc-54 3'UTR, *wbmIs88]*	*SKI eft-3p 5' end crRNA + 3xFLAG crRNA #2*	WBM1214	6x

Tested SKI LODGE strains				
WBM1133	*N2, wbmIs63[myo-3p::3XFLAG:: wrmScarlet::unc-54 3'UTR, *wbmIs61]*	*dpy-10*	WBM1126	4x
WBM1143	*N2, wbmIs67[eft-3p::3XFLAG:: wrmScarlet::unc-54 3'UTR, *wbmIs65]*	*dpy-10*	WBM1140	6x
WBM1144	*N2, wbmIs68[rab-3p::3XFLAG:: wrmScarlet::rab-3 3'UTR, *wbmIs66]*	*dpy-10*	WBM1141	6x
WBM1153	*N2, wbmIs72[pie-1p::3XFLAG::GFP::unc-54 3'UTR, *wbmIs60]*	*dpy-10*	WBM1119	4x
WBM1164	*N2, wbmIs77[eft-3p::3XFLAG::GFP::unc-54 3'UTR, *wbmIs65]*	*dpy-10*	WBM1140	6x
Other strains generated in this work				
WBM1043	*N2, wbmIs53[rab-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, IV:5015000]*	*cxTi10816 crRNA Chr. IV*	N2	6x
WBM1063	*N2, wbmIs57[rab-3p::3XFLAG:: wrmScarlet::unc-54 3'UTR *wbmIs53]*	*dpy-10*	WBM1043	2x
WBM1139	*N2, wbmIs64[pie-1p::3XFLAG:: wrmScarlet::unc-54 3'UTR *wbmIs60]*	*dpy-10*	WBM1119	2x

Table 2. crRNAs and tracrRNA used in this work.

crRNA name	Target gene/region	Sequence (5'>3')
dpy-10 crRNA (Arribere *et al.* 2014)	*dpy-10*	gctaccataggcaccacgag
dpy-5 crRNA	*dpy-5*	ccaggaatgccaggaccacg
crRNA ttTi4348 Chr.I	I:2851000	ttttgtcaaaagaagagaca
myo-3(8d) SKIS	I:2851000	gaaatcgccgacttgcgagg
crRNA oxTi444 Chr.III	III:7007600	ggtatatagttaaataaacg
cxTi10816 crRNA Chr. IV	IV:5015000	acaagtgtgaaactaaactt
crRNA oxTi365 Chr. V	V:8645000	aacaagttgggacaatacgg
3xFLAG crRNA	3xFLAG	ttacaaggatgacgatgaca
3xFLAG crRNA #2	3xFLAG	atggactacaaagaccatga
SKI eft-3p 5' end crRNA	*eft-3 SKI LODGE*	taaaagaccaaaggtgccgg
tracrRNA	NA	aacagcauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuu

Identification of harbor loci

To identify safe harbor loci to knock in the SKI LODGE cassettes, we analyzed genomic regions used in the MosSCI system (Frøkjær-Jensen *et al.* 2014) (Figure 1B). SKI LODGE sites were chosen using the following basic requirements: each was in an intergenic region so as not to affect other gene expression or function, and each had a strong crRNA guide site as determined by MIT CRISPR design (http://crispr.mit.edu) and/or Benchling (https://benchling.com) platforms (Table 2). We chose the following chromosome positions to insert SKI LODGE cassettes: *myo-3p* (Chr. I:2851000), *pie-1p* (Chr. III:7007700), *rab-3p* and *eft-3p* (Chr. IV:5014900), and *eft-3p* and *ges-1p* (Chr. V:8644800).

Cas9 purification

Cas9-His-tagged was purified by overexpressing in *E. coli* BL21 Rosetta cells. Nickel-NTA beads were used to pull it down followed by HPLC purification. Briefly, we inoculated LB culture with SpCas9 containing *E. coli* Rosetta cells and agitated for 3-6 h until the culture reached an OD of 0.6-0.8. The culture was cooled down on ice water to below 20ºC, and 0.5 mM IPTG was added followed by incubation overnight in a shaker at 20ºC. The culture was spun, and the bacterial pellet was resuspended in 27.5 ml of 50 mM Tris pH 8, 150 mM NaCl, 10% glycerol and 2 mM TCEP. Cells were lysed by adding 2 ml 10X FastBreak buffer (Promega V8571) and 5 µl Benzonase, incubated for 15-20 min at RT, and spun at 38000g for 15 min at 4ºC and the supernatant collected. Ni-NTA resin was washed in an equilibration buffer (50 mM Tris pH 8, 500 mM NaCl and 10% glycerol). The supernatant was then incubated with 1 ml of this pre-washed Ni-NTA resin at 4ºC for 1 h. The sample was poured into a disposable column and washed with wash buffer (20 ml of 50 mM Tris pH 8, 500 mM NaCl and 10% glycerol, 20 mM Imidazole and 2 mM TCEP). The sample was then eluted using elution buffer containing 5 ml 50 mM Tris pH 8, 500 mM NaCl and 10% glycerol, 400 mM Imidazole and 2 mM TCEP. The eluted Cas9 protein was diluted 1-2 fold with 1X PBS and loaded onto a 5 ml heparin column equilibrated with 1X PBS. The sample was eluted in a linear salt gradient from 0.1 to 1 M NaCl in 1X PBS and 1 ml fractions were collected. Fractions were analyzed on a gel and pooled all Cas9 containing fractions followed by concentrating them down to 3 ml volume with Amicon Ultra-15 filter (30 kDa cutoff, spun 4000 g 20 min at 4 ºC). We exchanged the buffer to 1X PBS, 10% glycerol and 2 mM TCEP using a PD-10 column. The insoluble protein was spun down at 4ºC for 15 min and concentrated down to

a final volume of 500 µl. Purified Cas9 was filter sterilized (0.22 µm) and the final concentration was measured by Nanodrop and stored at -80ºC.

Microinjection and CRISPR/Cas9-triggered homologous recombination

All CRISPR edits and insertions required to generate the SKI LODGE strains were performed using the CRISPR protocol developed by (Paix *et al.* 2015). Briefly, homology repair templates were amplified by PCR, using primers that introduced a minimum stretch of 35 bp homology to the SKI LODGE cassette at both ends. (For more information please see the step-by-step guide – File S2). CRISPR injection mix reagents were added in the following order: 0.375 µl Hepes pH 7.4 (200mM), 0.25 µl KCl (1M), 2.5 µl tracrRNA (4 µg/µl), 0.6 µl *dpy-10* crRNA (2.6 µg/µl) or 0.5 µl *dpy-5* crRNA (8 µg/µl), 0.25 µl *dpy-10* ssODN (500 ng/µl) or 0.5 µl *dpy-5* ssODN (1000 ng/µl), and PCR repair template(s) up to 500 ng/µl final in the mix. Water was added to reach a final volume of 8 µl. 2 µl purified Cas9 (12 µg/µl) added at the end, mixed by pipetting, spun for 2 min at 13000 rpm and incubated at 37ºC for 10 min. Mixes were microinjected into the germ line of day 1 adult hermaphrodite worms using standard methods (Evans 2006).

SKI LODGE cassettes construction

All strains were generated by CRISPR protocol described above using at least 35 bp of homology as recombination arms (Figure S1, see Table 3 for a list of all primer sequences used for generate homologous recombination templates by PCR, Table 2 for a list of all crRNA used and Table 1 for all strains generated in this work). In order to introduce the *dpy-10* site into the SKI LODGE cassettes, we established another easily identifiable Co-CRISPR target gene, *dpy-5*. *dpy-5* gene has only one exon and a well-defined dumpy phenotype (Brenner 1974). After each edit, the modified strains were outcrossed to remove the Co-CRISPR marker mutation (*dpy-10* or *dpy-5*), as well as any additional unwanted off-site mutations. To generate the *myo-3* SKI LODGE on chromosome I:28510000 (Figure S1), 2573 bp of *myo-3* promoter was introduced, using *dpy-10* as a Co-CRISPR marker. Then, 806 bp of *3xFLAG::dpy-10 site::unc-54 3'UTR* cassette was added, using *dpy-5* as a Co-CRISPR marker. The *pie-1* SKI LODGE on chromosome III:70077000, the *rab-3* SKI LODGE on chromosome IV:50149000, *eft-3* SKI LODGE on chromosome V:86448000, and *eft-3* SKI LODGE on chromosome IV:50149000 were each made using two HR repair templates with overlapping sequence that were co-injected as part of the same

CRISPR mix, using *dpy-5* as Co-CRISPR marker (Figure S1). The templates to make or the *pie-1* cassette were 1166 bp of *pie-1* promoter and 806 bp of *3xFLAG::dpy-10 site::unc-54 3'UTR*. The templates to make the *rab-3* cassette, were 1405 bp of *rab-3p::3xFLAG::dpy-10 site* and 732 bp of *rab-3* 3' UTR. The templates to make the *eft-3* cassette on chromosome V were 682 bp of the *eft-3* promoter and 836 bp of *3xFLAG::dpy-10 site::unc-54 3'UTR*. The templates to make the *eft-3* cassette on chromosome IV were 687 bp of the *eft-3* promoter and 804 bp of *3xFLAG::dpy-10 site::unc-54 3'UTR*. *eft-3* and *rab-3* SL2 lines (Table 1) were generated introducing 402 bp of *dpy-10 site::gpd-2 SL2* between 3xFLAG and wrmScarlet in *eft-3p::3xFLAG::wrmScarlet* and *rab-3p::3xFLAG::wrmScarlet* strains, using *dpy-5* as Co-CRISPR marker. The *ges-1* SL2 line was generated by swapping out the *eft-3* promoter for 2081 bp of the *ges-1* promoter in the *eft-3* SL2 line. All final SKI LODGE strains were sequence verified and outcrossed at least six times to N2.

Screening worms with the dumpy phenotype and genotyping

Immediately after injection, individual worms were placed at 20ºC. 3-4 days after injection, all plates were screened for progeny that contained the relevant Co-CRISPR edit. ~150 F1 dumpy/roller animals were singled out, one per plate, to lay eggs. After 2 days, F1 worms were placed in 5 µl of single worm lysis buffer (30 mM Tris pH 8.0, 8 mM EDTA pH 8, 100 mM NaCl, 0.7% NP-40, 0.7% Tween-20 and 100 µg/ml proteinase K) and lysed for 1 h at 60 °C, followed by incubation at 95 °C to inactivate the proteinase K. We then screened for the CRISPR edit by PCR using Apex Taq RED Master Mix 2.0X (Genesee Scientific) as recommended by the manufacturer, using 1 µl of worm lysate as a template (see Table 3 for a list of all primers used for PCR genotyping). F2 progeny from those plates that genotyped positive for the desired edit were again genotyped and used to amplify the full cassette. PCR amplicons were then purified using the QIAquick PCR purification kit (Qiagen) as recommended by the manufacturer. Purified PCR products were then sequenced by Sanger sequencing methods (Genewiz).

Verification of SKI LODGE expression

SKI LODGE strains were tested by knock-in of 696 bp of wrmScarlet amplified from pSEM89 (Mouridi *et al.* 2017) and or by knock-in of 867 bp of eGFP amplified from pIM26. A minimum of 35 bp were used as homologous recombination arms. To generate single-strand ended wrmScarlet template we followed the protocol developed by (Dokshin *et al.* 2018). Briefly, two

PCR products were generated, one with 120 bp homology arms and the other without any homology arms (only insert sequence). PCR products were mixed 1:1 (equal concentrations), and then this mix was heat to 95 ºC and cool to 4 ºC: 95 ºC (2 min), 85 ºC (10 sec), 75 ºC (10 sec), 65 ºC (10 sec), 55 ºC (1 min), 45 ºC (30 sec), 35 ºC (10 sec), 25 ºC (10 sec), and 4 ºC (forever). Then this PCR donor cocktail was used as template in the CRISPR mix described above.

Verification of off-target events

Off-targets sites of *dpy-5* and *dpy-10* were obtained using IDT platform. To verify off-target events of *dpy-5* in the SKI LODGE strains, potential off-target sites (~900 bp) in at least three genes were amplified by PCR (Table 3) and then sequenced by Sanger sequencing methods (Genewiz). These three genes represent the closest genes to the respective SKI LODGE genomic region. It is important to note that each potential off-target sequence of the *dpy-5* crRNA has at least two mismatches to the *dpy-5* crRNA sequence. To verify off-target events for *dpy-10* in the tested SKI LODGE strains, a fragment of R12E2.15 gene (873 bp) was amplified by PCR (Table 3) and then sequenced by Sanger sequencing methods (Genewiz). All sequence files from sequencing are available on request.

Analysis of phenotypes

To study the fertility of SKI LODGE strains, seven hermaphrodites were individually selected at the L4 larval stage and transferred to new OP50-1 plates every 24 h over the course of 6 days at 20°C. Plates were scored for dead embryos and viable offspring. Embryos that did not hatch within 24 h after being laid were considered dead.

To analyze developmental timing, animals were synchronized by bleaching. After bleaching, embryos were incubated overnight at 20º C in 1X M9 buffer and gentle rocking to allow hatching. After overnight incubation, OP50-1 plates were seeded with L1 larvae. Developmental stages were evaluated every 24 hours under a dissecting scope and scored for frequency of each developmental stage present.

Lifespan experiments were performed as described previously (Burkewitz *et al.* 2015). Before the start of each lifespan experiment, gravid adult worms were bleached and eggs were seeded on HT115 bacteria (100 eggs per plate) at 20º C until they reached adulthood (72 hr), at which point 100 adult worms per treatment were transferred to fresh plates with 20 worms per plate. Worms

were transferred to fresh plates every other day until reproduction had ceased (day 10-12). Survival was scored every 1–2 days and a worm was deemed dead when unresponsive to 3 taps on the head and tail. Worms were censored due to contamination on the plate, if they crawled up onto the walls, had eggs hatching inside, or exhibited pronounced vulval protrusion.

Microscopy

DIC and fluorescence imaging of whole worms was performed using a Zeiss Discovery V8 and Apotome.2-equipped Imager M2 microscope equipped with Axiocam cameras. Worms were anesthetized in 40 mM Sodium Azide diluted in 1X M9 and mounted on 2% agarose pads on glass slides.

Statistical analysis

GraphPad Prism was used for statistical analyses. Mann-Whitney test was used to analyze fertility and embryonic lethality. The Log-rank (Mantel-Cox) analysis was used to compare survival curves: n = 85-100 worms. For all experiments p values < 0.05 were considered significant.

Ethics statement

C. elegans is not protected under most animal research legislation.

Table 3. Oligonucleotides used in this work.

Oligo name	Sequence 5' > 3'	Template	SKI LODGE line
CGSG 118	gaatctcactctgatgagcgtatctatcaagtccttgtagtgatta tagtctctgttttc	*myo-3* promoter	*myo-3*
CGSG 178	cgcaagtcggcgatttctttgaagttttgtcaaaagaagagatg gatctagtggtcgtgg	*myo-3* promoter	*myo-3*
CGSG 143	taagcttctcacacctttctctcg	genotyping	*myo-3*
CGSG 163	acctttttgcggccagacc	genotyping	*myo-3*
CGSG 124	cctcccctcatctcaattatcccg	genotyping	*myo-3*
CGSG 150	cttggacttggtacgtgatggc	genotyping	*myo-3*
CGSG 151	gattgcgcaccgtgttgttc	genotyping	*myo-3*
CGSG 120	ggcagggagccatcaaacccacgaccactagatccatatgga ctacaaagaccatgacgg	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*myo-3*
CGSG 182	caattcggcgatctctttgaagttttgtcaaaagaagagcttcca ctgagcctcaaaccc	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*myo-3*
CGSG 123	ggattctttgcttgtcaaccagc	genotyping	*myo-3*
CGSG 140	ataaagatcatgacatcgattacaaggatgacgatgacaaggt cagcaagggagaggcag	wrmScarlet	*myo-3, pie-1, eft-3*
CGSG 141	aattggacttagaagtcagaggcacgggcgcgagatgttactt gtagagctcgtccattc	wrmScarlet	*myo-3, pie-1, eft-3*
CGSG 117	aataggggtgggagcacag	genotyping	*myo-3, pie-1, eft-3*
CGSG 180	actttttgagttaaataaatgaggtatatagttaaataaaaaatcg agaaaaaatggcg	*pie-1* promoter	*pie-1*
CGSG 181	ttataatcaccgtcatggtctttgtagtccatctggaaaagaaaat ttgatttttaattg	*pie-1* promoter	*pie-1*
CGSG 179	tttcccaaacaattaaaaatcaaattttcttttccagatggactaca aagaccatgacgg	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*pie-1*
CGSG 128	atgctctccagttgaagatctgaagcccatatacctcgtcttcca ctgagcctcaaaccc	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*pie-1*
CGSG 129	cctgggaacaataagtcggtgaag	genotyping	*pie-1*
CGSG 130	cggcggcaaaaatggatttctc	genotyping	*pie-1*
CGSG 131	atgtctggcggtccaaagtg	genotyping	*pie-1*
CGSG 209	aaagatcatgacatcgattacaaggatgacgatgacaagagta	GFP	*pie-1*

	aaggagaagaactttc		
CGSG 210	taattggacttagaagtcagaggcacgggcgcgagatgctattgtatagttcatccatg	GFP	*pie-1*
CGSG 100	cacagttcatcgactagtgtgagacaagtgtgaaactaaattcagatgggagcagtggac	*rab-3p::3xFLAG::dpy-10 site*	*rab-3*
CGSG 193	attttgagtttttatagatagtataatagaacgtagaatttgctccgctaccataggcac	*rab-3p::3xFLAG::dpy-10 site*	*rab-3*
CGSG 194	acaagtaccgctcgtggtgcctatggtagcggagcaaattctacgttctattatactatc	*rab-3 3'UTR*	*rab-3*
CGSG 166	tcctcctttaagttacgattattaacaaaatgtcgcctaagacctctggaactcttcgcc	*rab-3 3'UTR*	*rab-3*
CGSG 104	gaactttgcagtttggttgtagtg	genotyping	*rab-3*
CGSG 105	ctacagtagccctattttcagatgac	genotyping	*rab-3*
CGSG 106	tcaatccgttcatttgagccc	genotyping	*rab-3*
CGSG 168	ggaaaactgagagctacgcgc	genotyping	*rab-3*
CGSG 204	tgagtttttatagatagtataatagaacgtagaatttttacttgtagagctcgtccattc	wrmScarlet	*rab-3*
oxTi365 eft-3p HR templ FOR	ctaatgtcaccgtttcgtcgcgtgtcgctcccccggcacctttggtcttttattgtcaac	*eft-3* promoter	*eft-3*
oxTi365 eft-3p HR templ REV	tgatctttataatcaccgtcatggtctttgtagtccattgagcaaagtgtttcccaactg	*eft-3* promoter	*eft-3*
oxTi365 3xFLAG dpy-10 unc-54 3'UTR HR templ FOR	attgtttttttttcagttgggaaacactttgctcaatggactacaaagaccatgacggtg	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*eft-3*
oxTi365 3xFLAG dpy-10 unc-54 3'UTR HR templ REV	ggagaaggaccgagtagaacaagttgggacaatacttccactgagcctcaaacccaaacc	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*eft-3*
eft-3p FOR RPT Chr IV	cacagttcatcgactagtgtgagacaagtgtgaaactaaagcaccttggtcttttattgtcaac	*eft-3* promoter	*eft-3* IV
CGSG 103	ctcctttaagttacgattattaacaaaatgtcgcctaagcttccactgagcctcaaaccc	*3xFLAG::dpy-10 site::unc-54 3'UTR*	*eft-3* IV
MosCRIS eft3 geno FOR	tcttgccgctctcctcattttccc	genotyping	*eft-3*
MosCRIS eft3 geno REV	ttagccaccttcttcacacagggc	genotyping	*eft-3*
eft-3p seq fwd_2	ctaccgtccgcactcttctta	genotyping	*eft-3*
CGSG 205	accttcgacctcactttccctc	genotyping	*eft-3*

CGSG 206	ccgtcctgaagtatacccagatcc	genotyping	*eft-3*
dpy-10 ssODN	cacttgaacttcaatacggcaagatgagaatgactggaaaccg taccgcatgcggtgcctatggtagcggagcttcacatggcttca gaccaacagcctat	repair template	*dpy-10 gene*
dpy-5 ssODN	gatttccgttgtttcctggctctccacgatcatcctggcattcctg ggcgtccagcgact	repair template	*dpy-5 gene*
SL2 SKIs F1	acgataagtaccgctcgtggtgcctatggtagcggagcgctgt ctcatcctactttcacc	SL2 template	*eft-3, rab-3*
SL2 scarlet R1	aacgcatgaactccttgataactgcctctcccttgctgaccatga tgcgttgaagcagtt	SL2 template	*eft-3, rab-3*
SL2 SKIs F2	ggtgattataaagatcatgacatcgattacaaggatgacgacg ataagtaccgctcgtgg	SL2 template	*eft-3, rab-3*
TIR1 R2	cgtgtccgttcatggatccctccatgtggaccttgaaacgcatg aactccttgata	SL2 template	*eft-3, rab-3*
CGSG 246	gtctcttcatcactcacactattcccaaatctagactcatctatca ctaatgtcaccgtttcgtcgcgtgtcgctcccccgaaactccga actatgatgacg	*ges-1* promoter	*ges-1*
CGSG 241	tccgctaccataggcaccacgagcggtacttgtcatcgtcatcc ttgtaatcgatgtcatgatctttataatcaccgtcatggtccttata atccatgaattcaaagataagatatgtaatag	*ges-1* promoter	*ges-1*
CGSG 167	caatacgagcgttccgaggg	genotyping	*eft-3 SL2* *rab-3 SL2*
CGSG 285	ttgagcttggcggtttggg	genotyping	*eft-3 SL2* *rab-3 SL2*
ges-1p INT SEQ	cgtctgcgtcttacagtttcaggc	genotyping	*ges-1 SL2*
CGSG 249	aggccttgtagctacgtcctg	sequencing *dpy-5* off-target (*col-8*)	*pie-1*
CGSG 250	ccgcgccattactaaagacacc	sequencing *dpy-5* off-target (*col-8*)	*pie-1*
CGSG 251	tcaagacctggctctccacag	sequencing *dpy-5* off-target (*emb-9*)	*pie-1*
CGSG 252	tccaggaattccaggctaccc	sequencing *dpy-5* off-target (*emb-9*)	*pie-1*
CGSG 253	gtcctcgaagaagactcctccg	sequencing *dpy-5* off-target (*col-92*)	*pie-1*
CGSG 254	tgcaagcactctgctcgtg	sequencing *dpy-5* off-target (*col-92*)	*pie-1*
CGSG 255	accagatggagacggtgctc	sequencing *dpy-5* off-target (*let-526*)	*myo-3*

CGSG 256	tcagcaacaagctgctgctc	sequencing *dpy-5* off-target (*let-526*)	*myo-3*
CGSG 257	caagctggacaagatggacaacc	sequencing *dpy-5* off-target (*col-51*)	*myo-3*
CGSG 258	gacgtctgtagcttccagcac	sequencing *dpy-5* off-target (*col-51*)	*myo-3*
CGSG 259	agatggacgagaagagcaaggg	sequencing *dpy-5* off-target (*col-48*)	*myo-3*
CGSG 260	ctaatatcctggagcaagacgtgc	sequencing *dpy-5* off-target (*col-48*)	*myo-3*
CGSG 261	gacagattcccttctctcctggag	sequencing *dpy-5* off-target (*col-43*)	*eft-3*
CGSG 262	gagctcagcttctgcaaggtac	sequencing *dpy-5* off-target (*col-43*)	*eft-3*
CGSG 263	tgagaagatcggagagctcaagg	sequencing *dpy-5* off-target (*col-142*)	*eft-3*
CGSG 264	cagctgaacgagctggacatg	sequencing *dpy-5* off-target (*col-142*)	*eft-3*
CGSG 281	gtccatcggatccagcatttcc	sequencing *dpy-5* off-target (*col-145*)	*eft-3*
CGSG 282	accgtcccatccctctacaac	sequencing *dpy-5* off-target (*col-145*)	*eft-3*
CGSG 267	gacgtcttcgatctaattgcctagg	sequencing *dpy-5* off-target (*col-114*)	*rab-3*
CGSG 268	cctccagttattgaagctgctgg	sequencing *dpy-5* off-target (*col-114*)	*rab-3*
CGSG 279	aagaagactcctccgtcgatagc	sequencing *dpy-5* off-target (*col-34*)	*rab-3*
CGSG 280	caatggtctacaactacgtgcacc	sequencing *dpy-5* off-target (*col-34*)	*rab-3*
CGSG 283	ttatcgccgtgctttcggtag	sequencing *dpy-5* off-target (*dpy-13*)	*rab-3*
CGSG 284	ccaggttgtcctgaaagatgtgg	sequencing *dpy-5* off-target (dpy-13)	*rab-3*
CGSG 273	gttgggtatgctcctccttgtg	sequencing *dpy-10* off-target (R12E2.15)	*pie-1, myo-3, eft-3, rab-3*
CGSG 274	agaagactacatacgacggctgg	sequencing *dpy-10* off-target (R12E2.15)	*pie-1, myo-3, eft-3, rab-3*

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