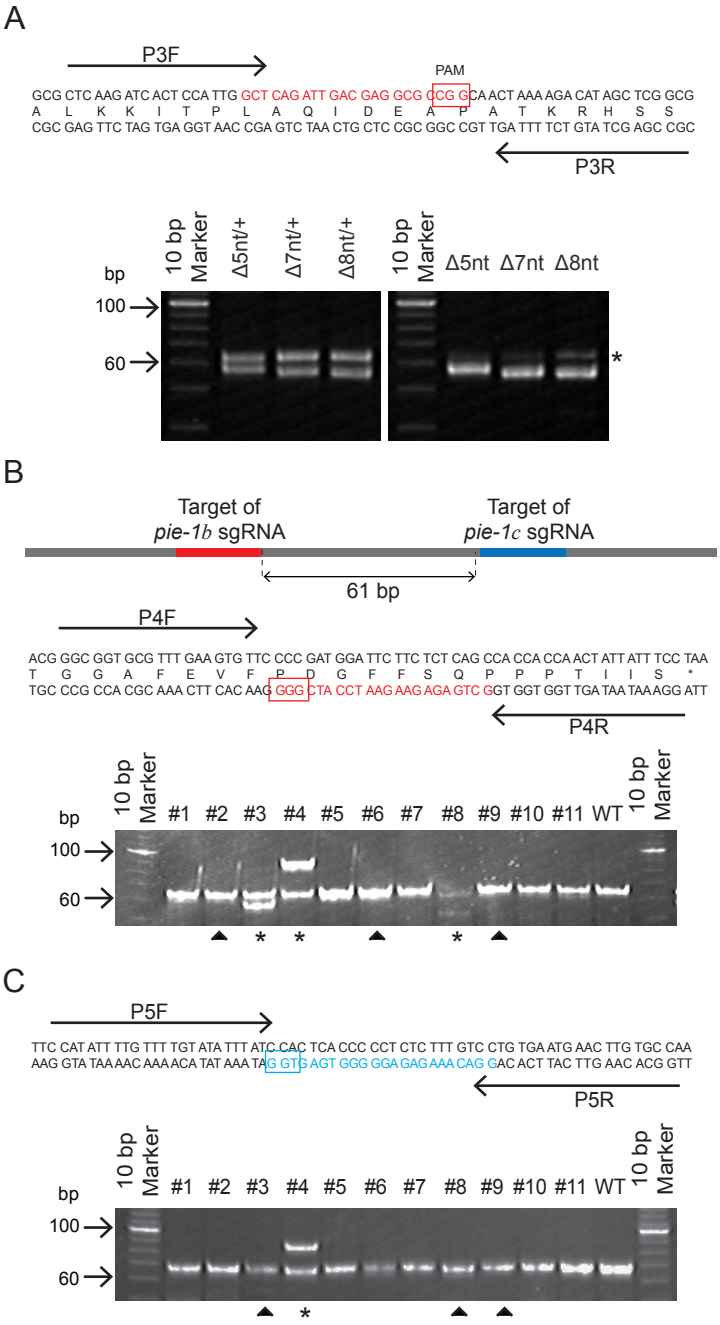

Figure S1. Detecting small indels on 15% polyacrylamide gels. (A) The indicated primers (arrows) were used to amplify sequences immediately surrounding the CRISPR-Cas9 target site (red). The indels in this experiment were from an HR experiment, so an initial PCR was performed using primers outside of the homology arms of the donor template (Figure S2A). The initial PCR was used as a template to amplify the target site using the indicated primers. PCR products from F1 heterozygotes (left) and F2 homozygotes (right) were separated on a non-denaturing 15% polyacrylamide gel and stained with ethidium bromide. The asterisk indicates the PCR product amplified from residual donor plasmids in the single worm lysate (B) and (C) Test of two uncharacterized *pie-1* sgRNAs using the Co-CRISPR strategy and PAGE analysis. The *pie-1* sgRNA vectors were combined and co-injected with the *unc-22* sgRNA, Cas9, and *rol-6* plasmids. The *pie-1* sgRNA target sites (shown in red and blue) are separated by 61 bp. As this experiment did not include an HR donor, only a single round of PCR was performed with the indicated primers (arrows). We lysed 11 F1 animals with the twitching phenotype (#3, #8, and #9-11) or that produced twitching progeny (#1-2 and #4-7). WT, wild type N2 genomic DNA was used as a template. Asterisks indicate lanes in which small indels were detected. The filled triangles indicate lanes in which the primer pair could not detect the indels.

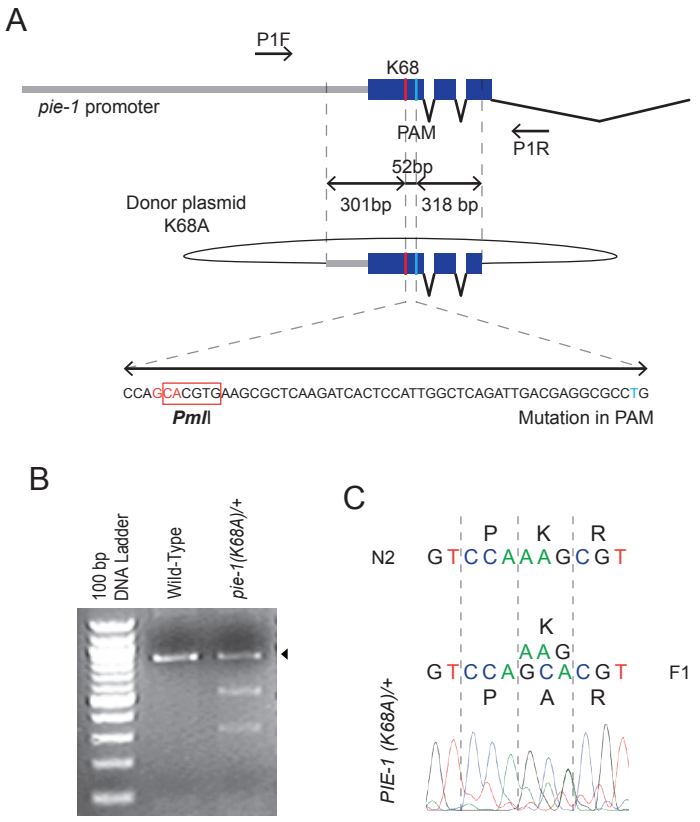

Figure S2. Site-specific mutagenesis of *pie-1* by HR. (A) Schematic of the Cas9/sgRNA target sites in *pie-1* locus and donor plasmids. The K68A donor plasmid contains ~300 bp of homology flanking the 52 bp target region between the K68 codon and PAM site and introduces a *Pml*I restriction site (red box). The PAM site of each donor was disrupted by silent mutations so that it will not be targeted by CRISPR-Cas9. The blue bar indicates the PAM site, and the red bar indicates the position of K68. (B) PCR and restriction enzyme analysis of wild type control worms and F1 rollers from K68A CRISPR-Cas9-mediated HR experiments. PCR primers outside of the donor homology arms (P1F and P1R for K68A) are indicated in (A). Restriction analysis following PCR shows the RFLP in *pie-1(K68A)/+*. The wild type product is indicated by the filled triangle. (C) DNA sequence analyses to confirm the desired point mutations. Note that the PCR products for sequencing were amplified using the primers outside of donor plasmid, as indicated in (A).

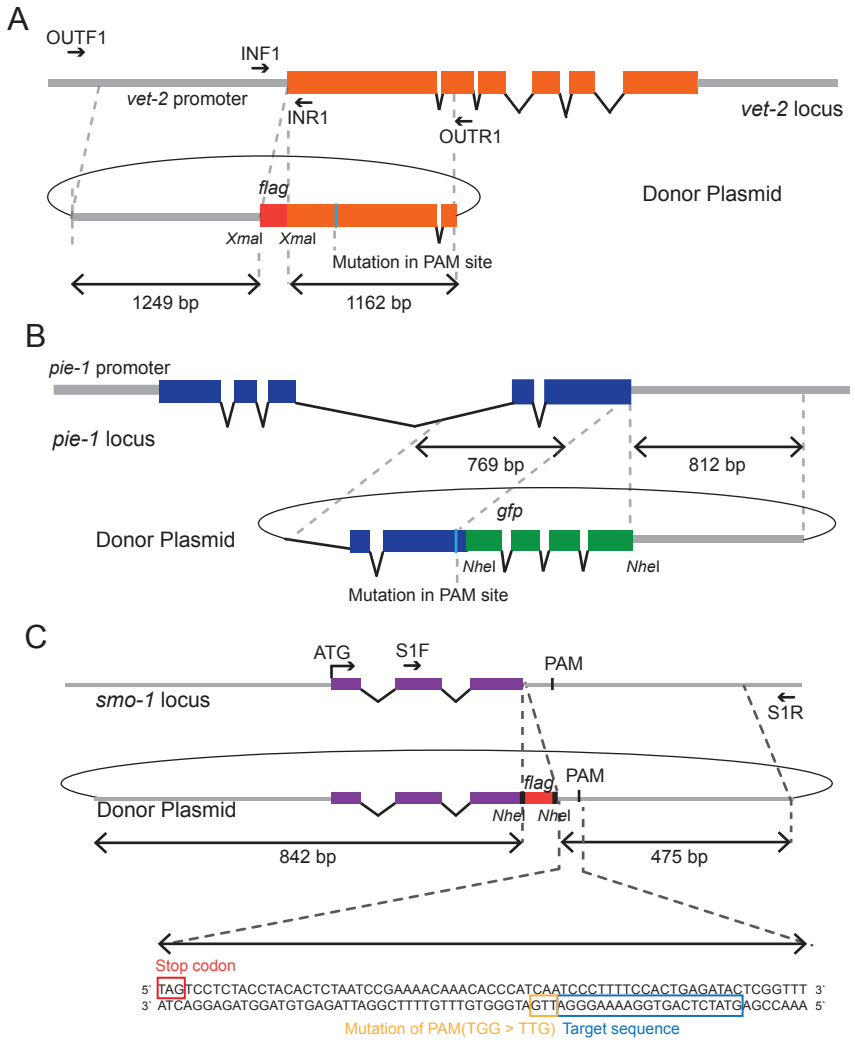

Figure S3. HR donor plasmids used in Co-CRISPR experiments. (A) Schematic of the *flag::vet-2* donor plasmid. The *flag* coding sequence was inserted immediately after the *vet-2* start codon and flanked by ~1200 bp homology arms. (B) Schematic of the *pie-1::gfp* donor plasmid. (C) Schematic of the *smo-1::flag* donor plasmid. The donor plasmid includes *flag* coding sequence immediately before the *smo-1* stop codon and asymmetrical homology arms (~800 bp and ~500 bp) flanking the target site, and the Cas9/sgRNA target sequence is located in the 3'UTR of *smo-1*. The PAM sites mutated in each donor indicate the locations of the Cas9/sgRNA target sites.

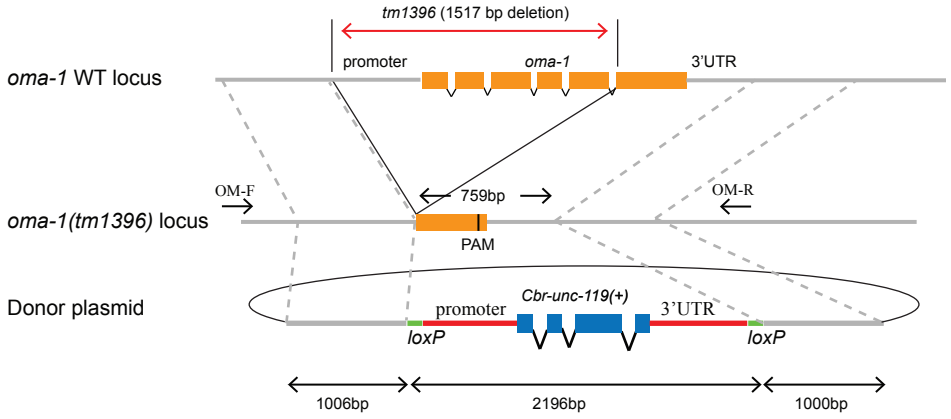

Figure S4. Selection/counterselection experiment to delete the entire *oma-1(tm1396)* locus. Schematic of the Cas9/sgRNA target site and the donor plasmid containing *Cbr-unc-119(+)* flanked by *loxP* sites and 1 kb homology arms. The indicated primers OM-F and OM-R were used for PCR analysis.

Table S1. Summary of sgRNAs sequences and their efficiency

Name	Sequence	S/AS	% efficiency
avr-14 no.1	GAATATTGAAAGACTATGAT(TGG)	S	10
avr-14 no.2	GATTGGAGAGTTAGACCACG(TGG)	S	20
avr-15 no.9	GCAGAAAATGAATGTCATAC(AGG)	AS	HIGH
avr-15 no.10	GTTTGCAATATAAGTCACCC(AGG)	AS	HIGH
unc-22 no.2	GAACCCGTTGCCGAATACAC(AGG)	S	5
unc-22 no.9	GCCTTTGCTTCGATTTTCTT(TGG)	AS	0
unc-4 no.1	GTTATCGTCATCCGGTGACG(TGG)	AS	10
rde-3 no.3	GAATTTGAGCTTGAACGAGC(TGG)	AS	LOW
rde-3 no.4	GTCGATACTTCAAAATTAAT(TGG)	AS	LOW
lon-2 no.1	GGGAAACTATACCCTCACTG(TGG)	S	30
dpy-11 no.2	GCAAGGATCTTCAAAAAGCA(CGG)	S	0.4
dpy-11 no.4	GATGCTTGTAGTCTGGAACT(GGG)	AS	
unc-32 no.1	GATAGGAAGCATCAGATTGA(AGG)	AS	0
unc-32 no.2	GTTGCTGAACTGGGAGAGCT(CGG)	S	
bli-2 no.1	GGATTTGCTGCTACTGAATC(CGG)	AS	0
bli-2 no.2	GATGGACGGGATGGTAGAGA(TGG)	S	
dpy-5 no.2	GTCGGATTCGGCGCTGCATG(CGG)	S	0
dpy-5 no.3	GGTTTCCTGGAGCTCCGGCT(GGG)	AS	
ben-1 no.3	GGATATCACTTCCCAGAACT(TGG)	AS	0
ben-1 no.5	GGGAGAAAGTGATTTGCAGT(TGG)	S	
pie-1 a	GGCTCAGATTGACGAGGCGC(CGG)	S	24
pie-1 b	GCTGAGAGAAGAATCCATCG(GGG)	AS	15
pie-1 c	GGACAAAGAGAGGGGGTGAG(TGG)	AS	7.5
pie-1 d	GTTGAGTGCAGCCATTTGCT(CGG)	AS	5
smo-1 a	GCCGATGATGCAGCTCAAGC(AGG)	S	LOW
smo-1 b	GTGCACTTCCGTGTAAAGTA(TGG)	S	HIGH
smo-1 c	GTCTACCAAGAGCAGCTGGG(CGG)	S	HIGH
smo-1 d	GTATCTCAGTGGAAAAGGGA(TGG)	S	HIGH
vet-2	GTTGGATCATAGGATACCGG(TGG)	AS	38
C35E7.6	GGGCACCATACCGAGTGATG(GGG)	AS	100
oma-1	GATCCAATGATGTCATGTAA(CGG)	S	LOW

Table S2. Summary of primer sequences for sgRNA plasmid generation

Name	Sequence
CMo16428	TGAATTCCTCCAAGAACTCG
CMo16429	AAGCTTCACAGCCGACTATG
sgRNA_F	G(N)19GTTTTAGAGCTAGAAATAGC
avr-14 sgRNA_F	GATTGGAGAGTTAGACCACGGTTTTAGAGCTAGAAATAGC
avr-15 sgRNA_F	GTTTGCAATATAAGTCACCCGTTTTAGAGCTAGAAATAGC
unc-22 sgRNA_F	GAACCCGTTGCCGAATACACGTTTTAGAGCTAGAAATAGC
pie-1 a sgRNA_F	GGCTCAGATTGACGAGGCGCGTTTTAGAGCTAGAAATAGC
pie-1 b sgRNA_F	GCTGAGAGAAGAATCCATCGGTTTTAGAGCTAGAAATAGC
pie-1 c sgRNA_F	GGACAAAGAGAGGGGGTGAGGTTTTAGAGCTAGAAATAGC
smo-1 sgRNA_F	GTATCTCAGTGGAAAAGGGAGTTTTAGAGCTAGAAATAGC
vet-2 sgRNA_F	GTTGGATCATAGGATACCGGGTTTTAGAGCTAGAAATAGC
oma-1 sgRNA F	GATCCAATGATGTCATGTAAGTTTTAGAGCTAGAAATAGC
sgRNA_R	(N)19CAAACATTTAGATTTGCAATTC
avr-14 sgRNA_R	CGTGGTCTAACTCTCCAATCAAACATTTAGATTTGCAATTC
avr-15 sgRNA_R	GGGTGACTTATATTGCAAACAAACATTTAGATTTGCAATTC
unc-22 sgRNA_R	GTGTATTCGGCAACGGGTTCAAACATTTAGATTTGCAATTC
pie-1 a sgRNA_R	GCGCCTCGTCAATCTGAGCCAAACATTTAGATTTGCAATTC
pie-1 b sgRNA_R	CGATGGATTCTTCTCTCAGCAAACATTTAGATTTGCAATTC
pie-1 c sgRNA_R	CTCACCCCCTCTCTTTGTCCAAACATTTAGATTTGCAATTC
smo-1 sgRNA_R	TCCCTTTTCCACTGAGATACAAACATTTAGATTTGCAATTC
vet-2 sgRNA_R	CCGGTATCCTATGATCCAACAAACATTTAGATTTGCAATTC
oma-1 sgRNA R	TTACATGACATCATTGGATCCAAACATTTAGATTTGCAATTC

Table S3. Summary of primer sequences for repair template and PCR screening

Name	Sequence
C_PIE-1 PF	ATAGCCCGATTTTGGAGGTG
C_PIE-1 PR	CCTCGAATTTTGGCAATTTTTC
C_PIE-1 301L	ATGGATTTCTCGCCGTTTTTTC
C_PIE-1 318R	GTTGTATCCACGTCGTCTCG
C_PIE-1(K68A)_F	GGAAAATGGCTTCGTCCAGCACGTGAAGCG
C_PIE-1(K68A)_R	CTTGAGCGCTTCACGTGCTGGACGAAGCC
C_PIE-1(K68R)_F	GGAAAATGGCTTCGTCCTAGGCGTGAAGCG
C_PIE-1(K68R)_R	CTTGAGCGCTTCACGCCTAGGACGAAGCC
C_PIE-1 a MF	GCTATGTCTTTTAGTTGCAGGCGCCTC
C_PIE-1 a MR	CAGATTGACGAGGCGCCTGCAACTAA
SMO-1 PF	CGATTTTTCGGCTCATTTCG
SMO-1 PR	CCTCGTCAAATCCGAAATCG
SMO MF	CACCCATCAATCCCTTTTC
SMO MR	GAAAAGGGATTGATGGGTG
P1F	GTTTTTGCCCCCAAATTC
P1R	TGATGCTTCGATGCTGAAGA
P2F	GGCGTCAAAAGACATATGTAAAAG
P2R	CGCAATGGATGATTTTTGTC
P3F	GCCGAGCTATGTCTTTTAG
P3R	CTCAAGATCACTCCATTGGC
P4F	GGCGGTGCGTTTGAAGTGT
P4R	GGAAATAATAGTTGGTGGTGGC
P5F	CCATATTTTGTTTTGTATATTTATC
P5R	GGCACAAGTTCATTCACAGG
P6F	GCGCAGCGAATTTTTGGGGT
P6R	TATCACAATTCTCTCCGTGC
P7R	CGGAGAACTTGCCAAAATGAAG
S1F	GAAGTGCACTTCCGTGTAAAGTATGGAACC
S1R	CCGGCTGCTATTTCATTGAT
MC. OUT F1 (vet-2)	GCTCAAGAAAGCCAATGGAG
MC. OUT R1 (vet-2)	TTCTGAACCAGTCGATGCAG
MC.IN F1 (vet-2)	ATGGAGGGATCTGTCAATGG
MC.IN R1 (vet-2)	TGGCAGTCGAGACACTTCAG
FL. IN F1 (vet-2)	CACAAAACCGGCCAAAAA
FL. IN R1 (vet-2)	TCGGTCTTGCAGAAACCAC
OM-F	CAACGTTTGCGTGTACAGAAG
OM-R	GGCTCACGTACGCAGCACTAC