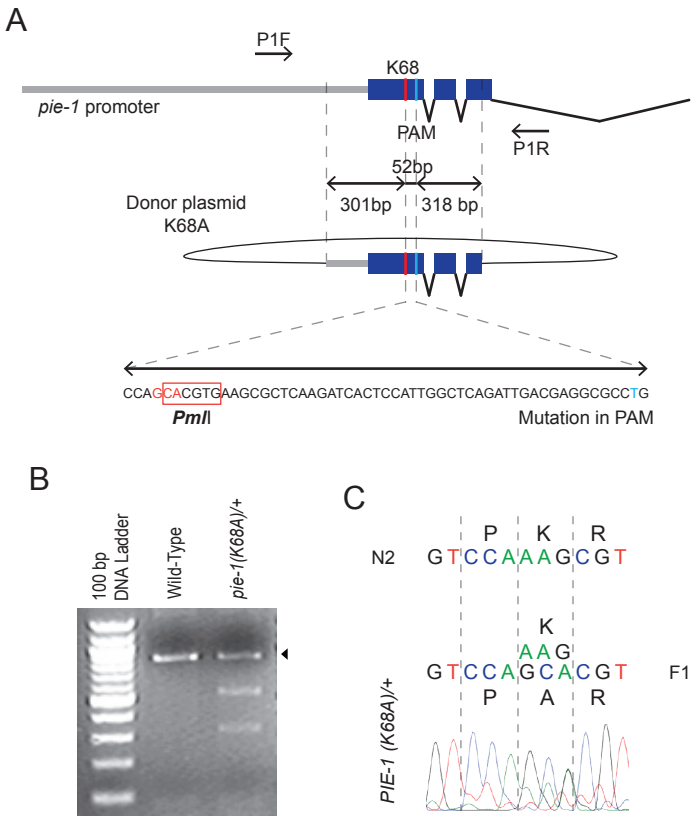

Figure S2. Site-specific mutagenesis of *pie-1* by HR. (A) Schematic of the Cas9/sgRNA target sites in *pie-1* locus and donor plasmids. The K68A donor plasmid contains ~300 bp of homology flanking the 52 bp target region between the K68 codon and PAM site and introduces a *Pml*I restriction site (red 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).