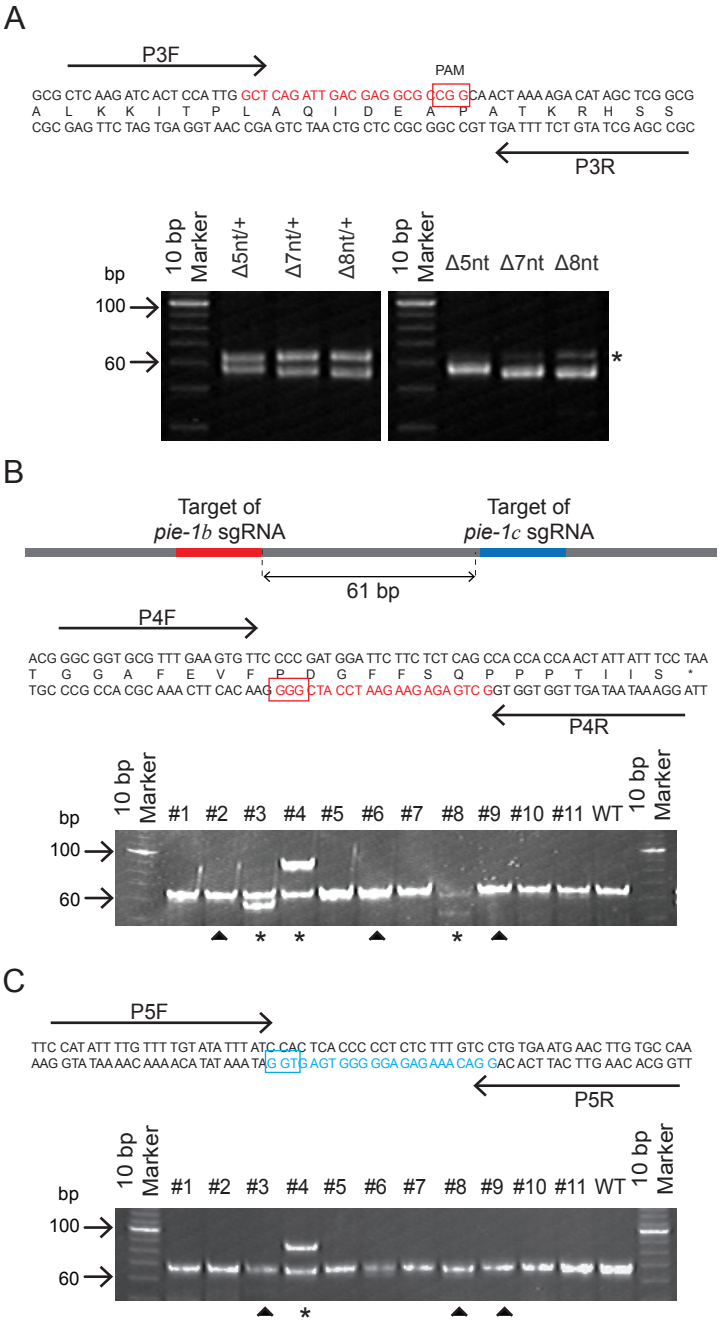

Figure S1. Detecting small indels on 15% polyacrylamide gels. (A) The indicated primers (arrows) were used to amplify sequences immediately surrounding the CRISPR-Cas9 target site (red). The indels in this experiment were from an HR experiment, so an initial PCR was performed using primers outside of 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.