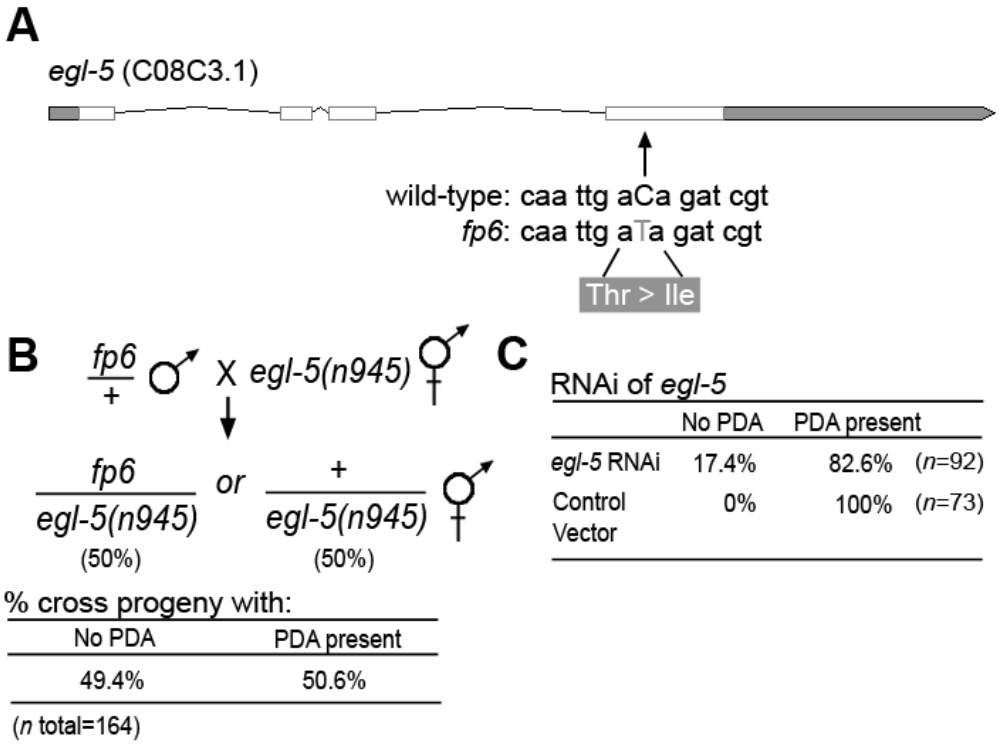

FIGURE S2.—Confirmation of *egl-5(fp6)* mutation. (A) Sanger sequencing revealed a C to T substitution in the same position of *egl-5* exon 4 as did WGS, resulting in a Threonine to Isoleucine amino acid change. The affected amino acid is a conserved residue in the highly conserved Hox domain of EGL-5. (B) *fp6* and the null allele *egl-5(n945)* did not complement for the Y-to-PDA defective phenotype confirming that *fp6* affects the *egl-5* gene and that it is the causal mutation for the "no PDA" phenotype. Both *fp6* and *egl-5(n945)* are recessive. Homozygotes for *fp6* and *egl-5(n945)* are approximately 100% penetrant for the defective Y-to-PDA phenotype. Hermaphrodite progeny from the cross between *fp6*/+ and *egl-5(n945)* were identified by the presence of *cog-1::gfp* transgene initially carried by *fp6*/+ males. Three separate crosses yielded the same result. (C) Lowering the activity of *egl-5* by RNAi results in a "no PDA" phenotype, which phenocopies the *fp6* mutants. n =164 for (B) and n = 165 for (C). Control animals were fed an empty RNAi vector. n = total number of animals scored.