



**Figure S8. TS strains with mapping profiles that identify additional weak alleles of low fitness.** The TS locus within VC50178 (A) was identified on the left arm of LGIII by following competitive fitness mapping over multiple generations. The predicted 3.6 Mbp interval (B) has only two relevant coding changes with the likely candidate as *let-805* based on phenotyping of the VC50178 strain. The TS locus on LGX in VC50352 (C) was resolved with the liquid bulk segregant mapping assay. Within this interval (D) are 7 relevant candidates for which 5 are listed. The primary candidate is a *pak-1* splice-site mutation based on the observed VC50352 TS phenotype and reported phenotypes and expression data for *pak-1*. Additional fitness-defective loci present in later generations when the likely TS locus has been nearly removed from the population. Single arrows indicate positions of genes while double-arrows identify the interval spanned by a list of genes.