FIGURE S2.—The HIF-1 (P621G) mutation stabilizes HIF-1 protein. A) Diagrams of minigenes that direct expression of epitope-tagged HIF-1 or HIF-1 (P621G). These transgenes are described as Phif-1::hif-1::tag and Phif-1::hif-1 (p621G)::tag. Boxes represent translated regions. The asterisk indicates sequence encoding proline 621, which was mutated to code for glycine in the P621G construct. B,C) Representative protein blots to analyze the expression of epitope-tagged HIF-1 or HIF-1 (P621G) in transgenic animals. Each strain shown carried the hif-1 (sa04) deletion allele, and HIF-1 function was restored by an integrated copy of one of the hif-1 transgenes. Blots were probed with antibodies that recognize the myc epitope. B) Regulation of epitope-tagged HIF-1 by egl-9 and vhl-1 in transgenic C. elegans. egl-9 (sa307) and vhl-1 (ok161) are strong loss-of-function alleles, and + represents the allele present in wild-type C. elegans (Bristol N2). AHA-1 protein levels are unchanged in these mutant backgrounds. C) The P621G mutation prevented vhl-1 and egl-9-mediated destabilization of HIF-1 protein. Data from at least three independent biological replicates were analyzed to determine whether loss-of-function mutations in vhl-1 or egl-9 resulted in statistically significant differences in HIF-1 or HIF-1 (P621G) protein levels. n.s.: no significant difference; *: p<0.05; **: p<0.01.