

A) Detection of HAL-2 protein in whole worm lysates of the indicated genotype. HAL-2 expressed from the endogenous locus (36kDa, red

В

asterisks) and GFP::HAL-2 expressed from an integrated transgene (62kDa, green asterisks) were detected by Western blot analysis

Methods). HAL-2 peptides were detected only in the nuclear soluble fraction derived from worms expressing GFP::HAL-2, consistent with

Western blot analyses of fractionated extracts (SILVA et al. 2014). Twenty additional proteins exhibited this same profile, including

0

0

0

0

0

performed using rabbit anti-HAL-2 antibody ((ZHANG et al. 2012) at 1:10,000 dilution). As the level of GFP::HAL-2 is higher in the hal-2(me79) homozygous mutant background (lane 4) than in otherwise wild-type animals (lane 3) the hal-2; gfp::hal-2 strain was therefore used for our proteomics strategy. B) Schematic representation of the proteomics strategy used to identify HAL-2 partners (see Materials and

RAD-50, which has previously-demonstrated roles in meiotic recombination. Additional criteria were applied to define a set of candidate genes among the remaining 19 to test for roles in regulating meiotic prophase events. Eight genes were excluded because they are located on the X chromosome (which lacks known meiosis genes) and/or encode tubulin isoforms. Two additional genes were excluded based on

evidence that they are likely not expressed in the germ line (Wormbase). Six genes were not pursued further based on prior annotation/functional analysis, including three genes encoding proteins (PGL-1, CGH-1 and OMA-1) that are known to localize to P-granules and/or

cytoplasm and have previously-described roles in translational regulation. The remaining genes (T15H9.2, K07F5.14, R02F2.7) were prioritized as candidates for further study based on strong evidence for germline-enriched expression (REINKE et al. 2000; KIM et al. 2001; HAN

et al. 2017), yet little or no prior in-depth analysis of germline function. C) Table showing numbers of peptides detected for HAL-2, RAD-50, and the three prioritized candidate proteins (with peptides detected specifically in the nuclear soluble fraction from worms expressing GFP::HAL-2. D) Images of DAPI-stained chromosomes in diakinesis-stage oocytes, illustrating the normal diakinesis karyotypes observed following depletion of the two candidate HAL-2 interacting partners K07F5.14 and R02F2.7.