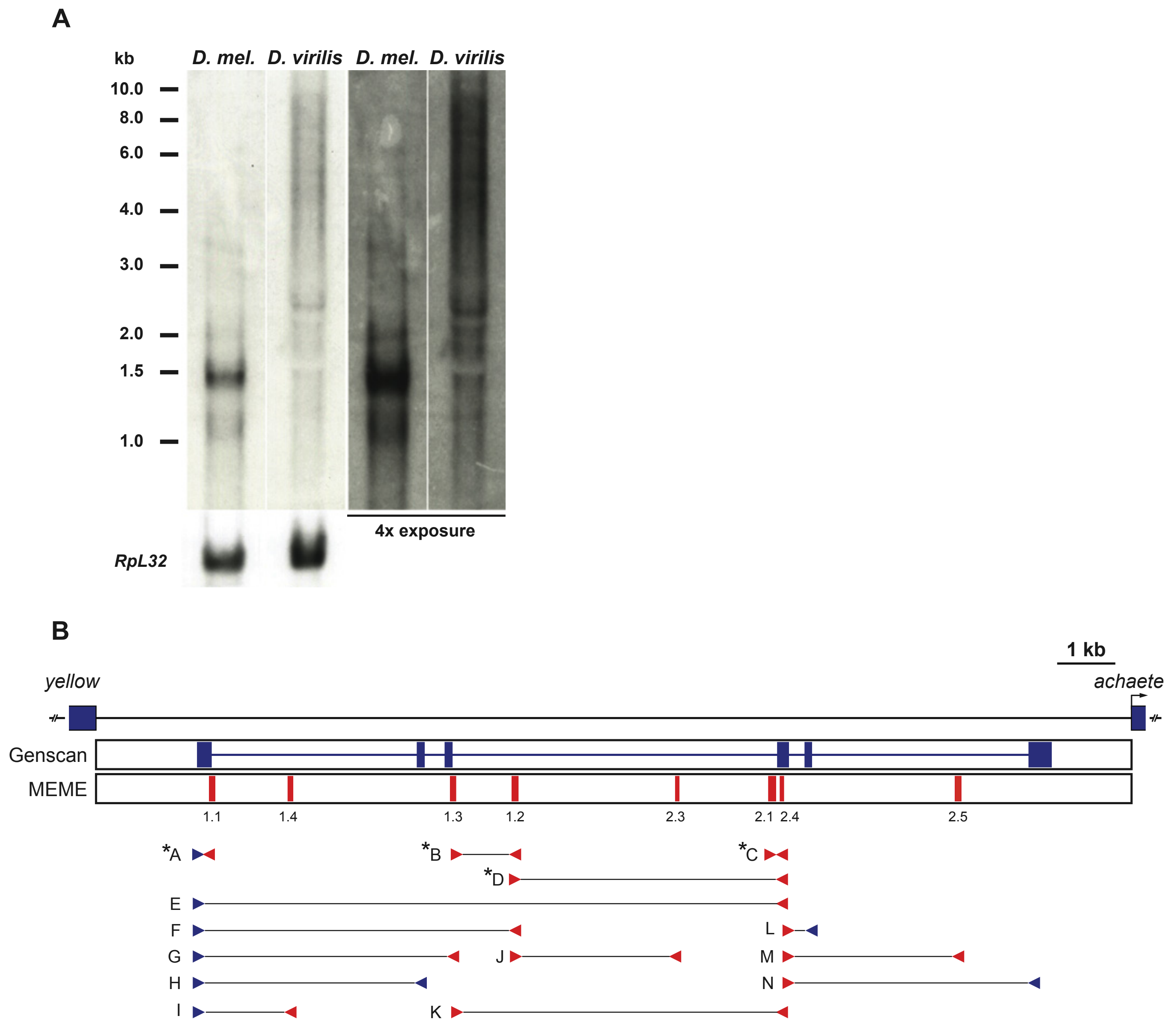

Figure S2 RNA analyses of D. virilis and D. melanogaster yar. A. Northern analysis of Poly A+ RNA from 6-12 hour D. melanogaster Canton S embryos (3.5 µg) or 16-24 hour D. virilis embryos (10 µg) hybridized with 32P-dATP- labeled DNA probes corresponding to D. melanogaster yar isoform containing exons 1, 3 and 4 (Genbank GQ329854) or D. virilis yar cDNA B. RpL32 is a constitutively expressed gene that served as a loading control. The far right panels show the same blots exposed four times longer. B: Strategy for analysis of intergenic transcription in D. virilis. Top, structure of the y-ac intergenic region in D. virilis, showing the location of the Genscan-predicted gene (blue) and MEME-identified conserved motifs (red). Bottom, schematic of the strategies used for PCR analyses of RNAs isolated from 16-24 hr D. virilis embryos using the indicated primer pairs. This developmental time was chosen, as it represents the period of maximal expression of the yar gene (Figure 2). Primers were anchored in Genscan-predicted exons (blue arrowheads) and MEME-identified motifs (red arrowheads). As a positive control, all primer pairs were tested with D. virilis genomic DNA, and each yielded products of expected size. Only primer pairs shown in A, B, C and D yielded products when embryonic cDNAs were used as a template. These are indicated by asterisks (*).