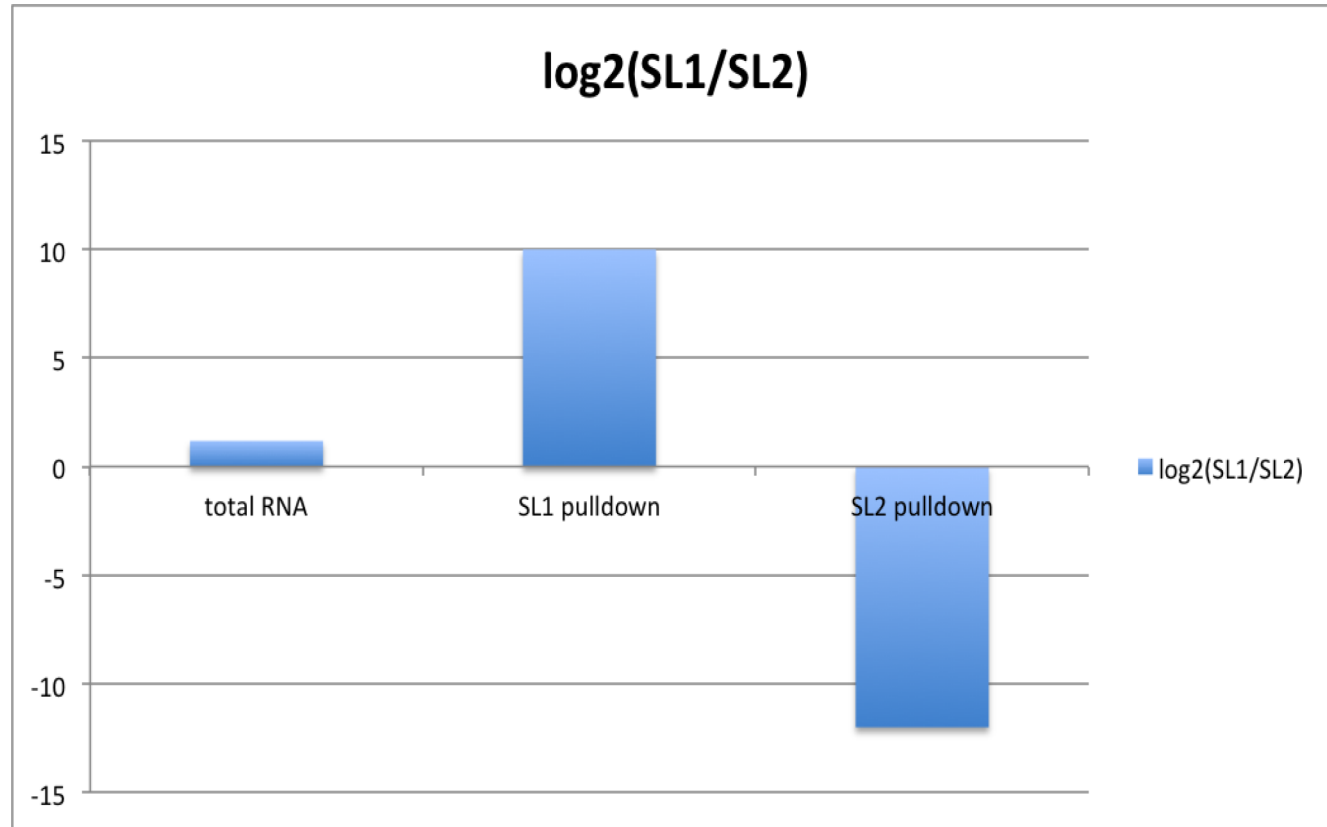
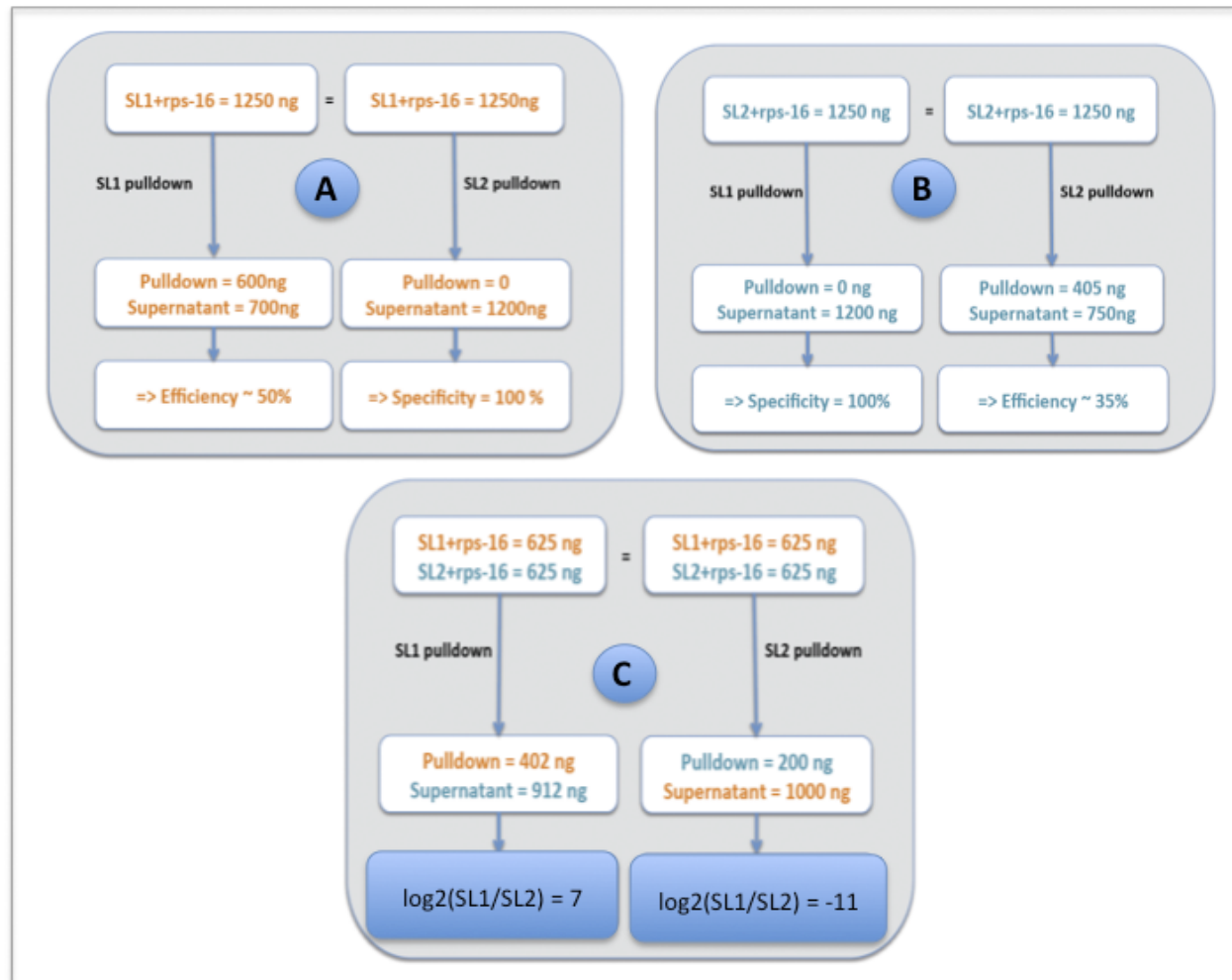


Supplementary Figure S1 : Use of splice-leader specific probes leads to very high enrichment of the corresponding trans-spliced transcripts, as verified by q-RT-PCR experiments.

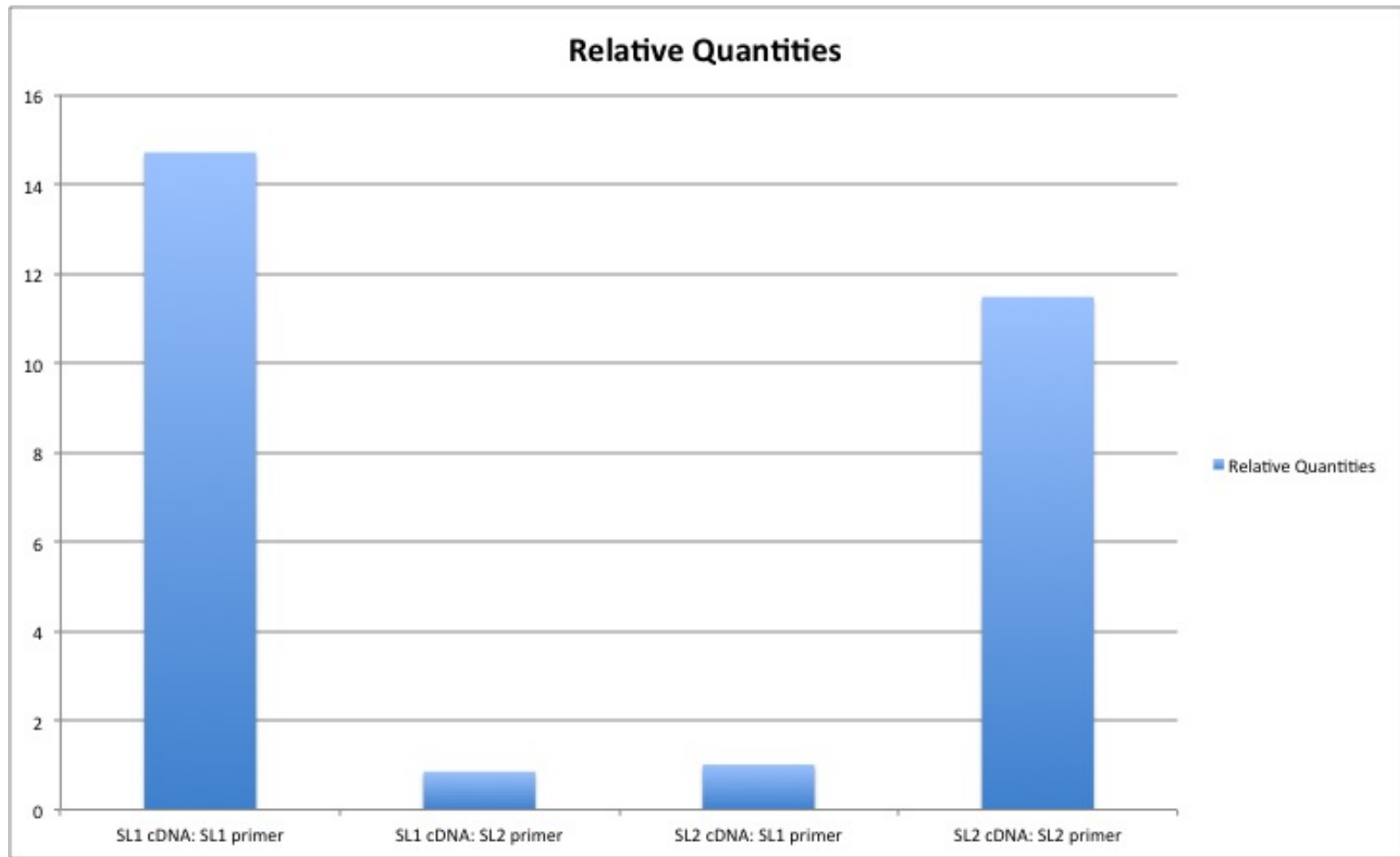


The SL1/SL2 ratio for gene Ppa-rpl-43 was calculated for the total RNA before any pull-downs, and then for mRNA pools obtained after SL1 or SL2 specific pull-downs, leading to ~1,000 fold enrichments of transcripts trans-spliced to the respective splice leaders.

Supplementary Figure S2 : High specificity and efficiency of pull-downs, as assessed on synthetic RNA. (A) pure SL1-spliced RNA (B) pure SL2- spliced RNA (C) a 1:1 mixture of pure SL1-spliced and pure SL2-spliced RNA

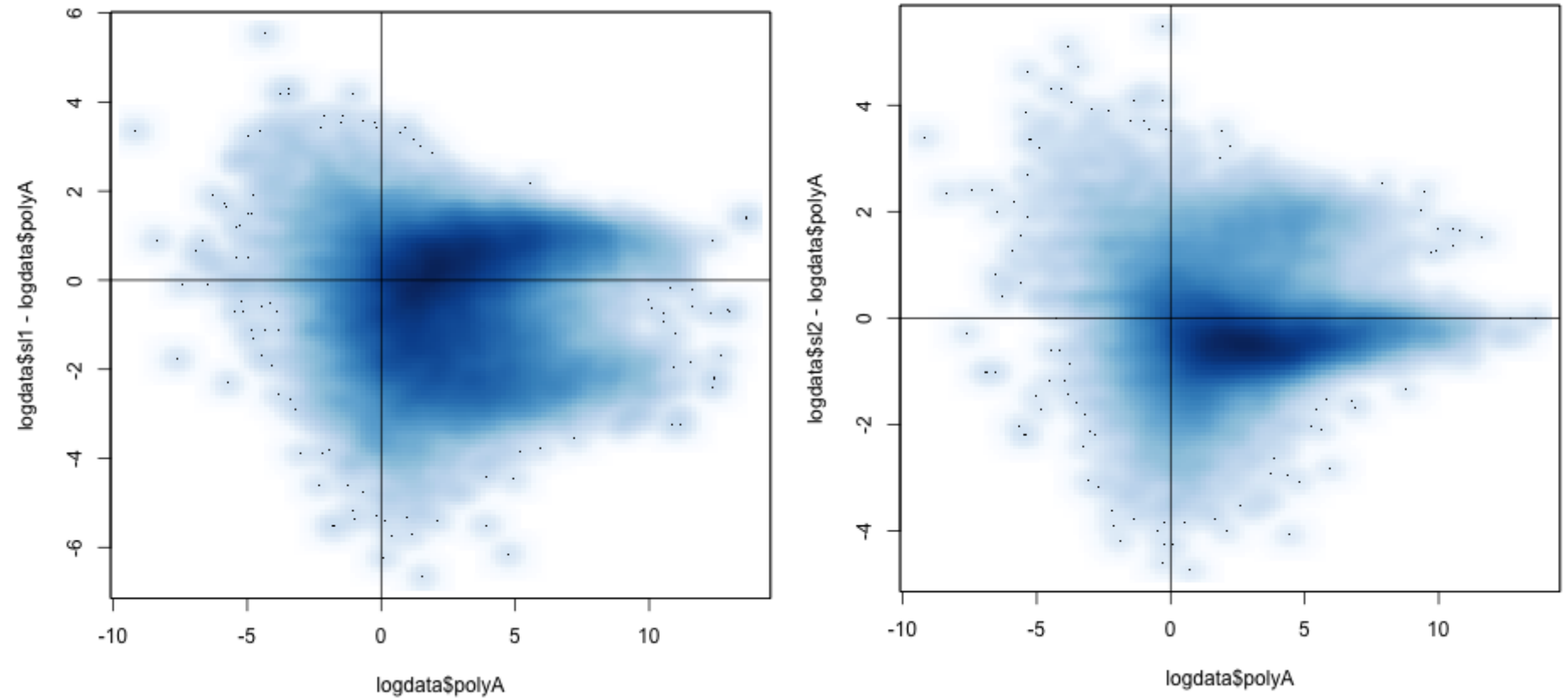


Supplementary Figure S3 : Specificity of SL1 and SL2 primers used in qPCR reactions



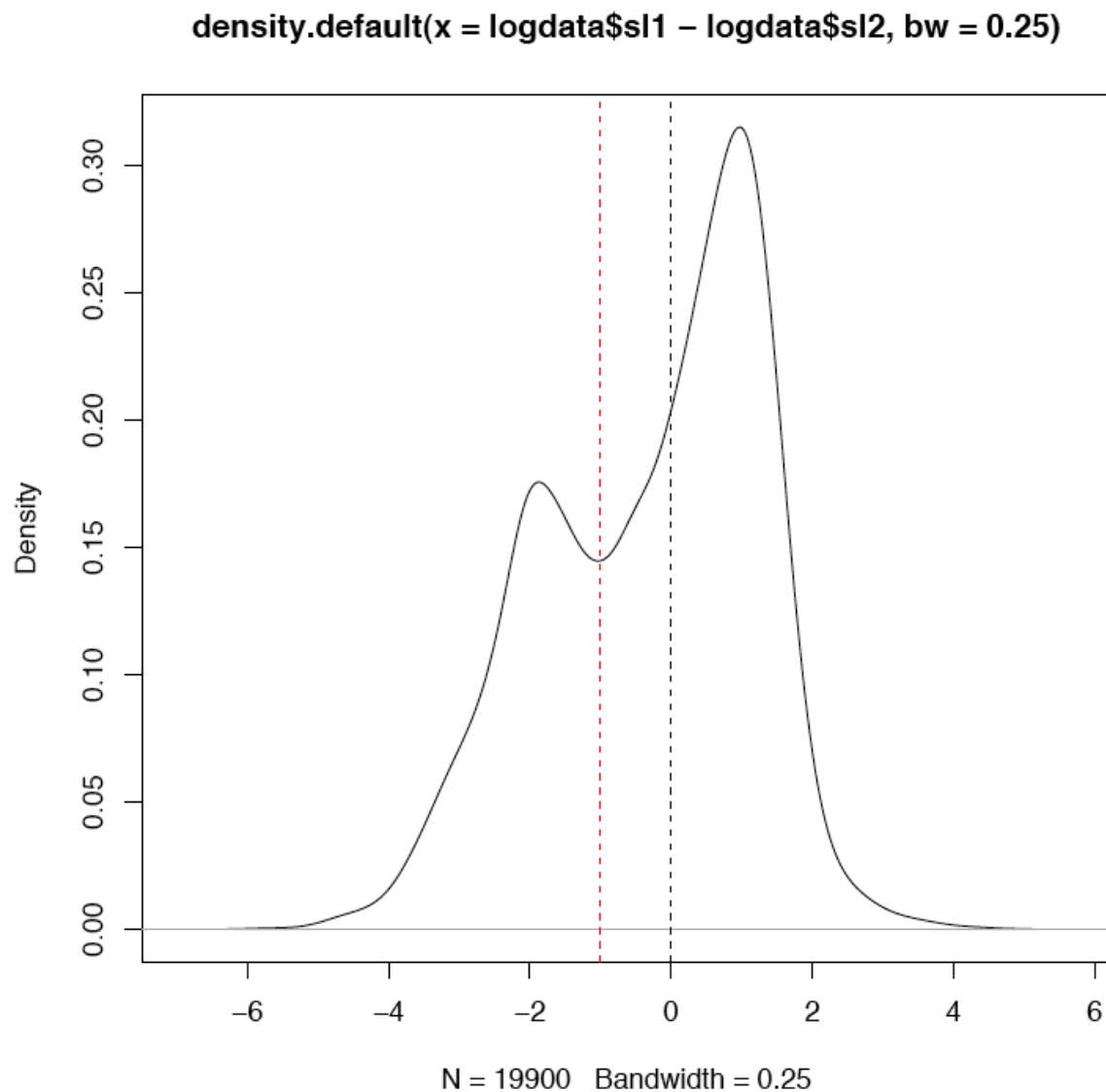
200bp fragments from *Ppa-rpl-43* cDNA that were trans-spliced to either SL1 or SL2, were first cloned into a vector. Plasmid DNA extracted from each of the two cloned fragments was then used in qPCR reactions using a gene-specific reverse primer and the forward primer corresponding to either SL1 or SL2. The relative ratios $\log_2(\text{SL1}/\text{SL2})$ calculated for each DNA:SL primer combination was used to verify that the splice-leader primers are highly specific to only their target SLs, such that negligible amplification is seen in cross-primer reactions

Supplementary Figure S4 : Extent of trans-splicing to a gene does not depend on its level of gene expression

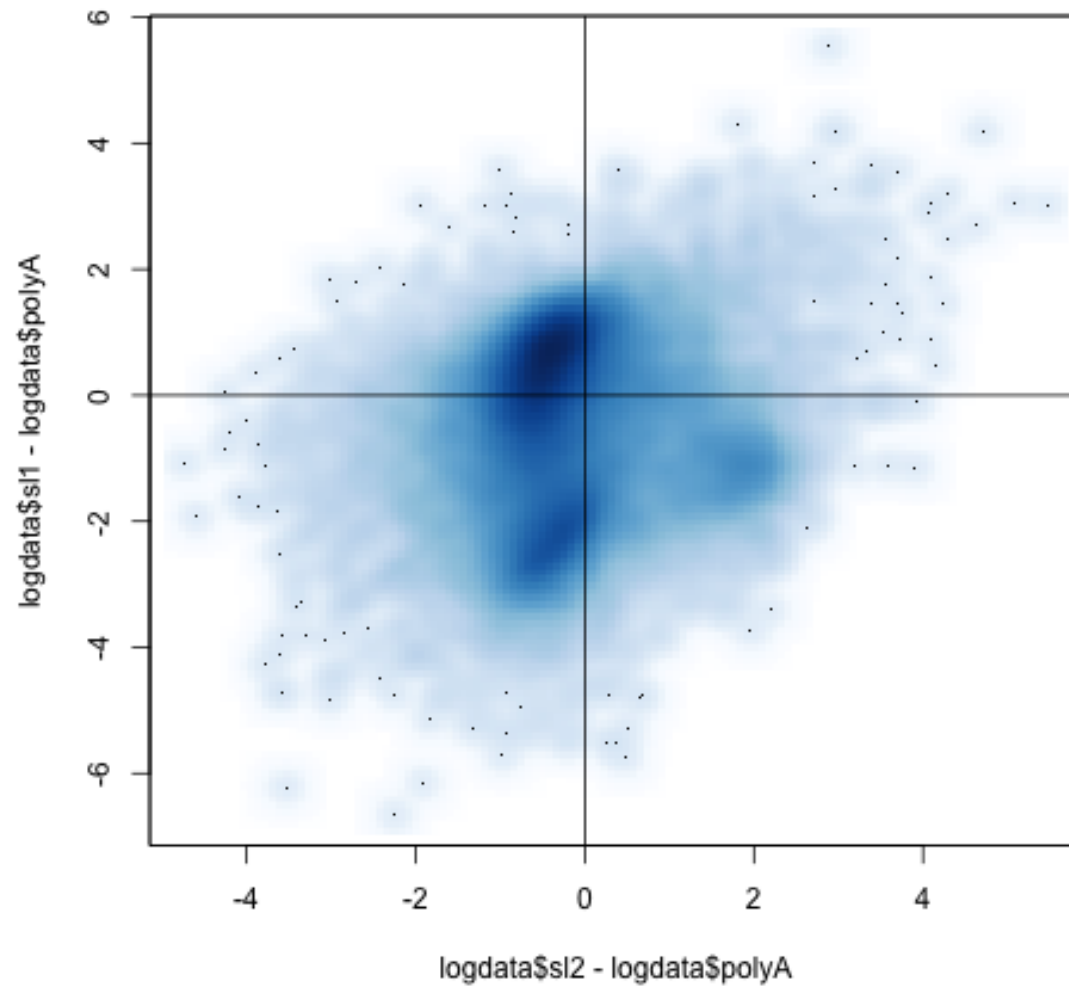


Scatter density plots of levels of trans-splicing (measured as $\log_2(\text{SL}/\text{polyA})$) against total expression levels ($\log_2(\text{polyA})$) do not show any correlations for either SL1 or SL2

Supplementary Figure S5 : Bimodal distribution of $\log_2(\text{SL1_fpkm}/\text{SL2_fpkm})$ for all genes
FPKM > 0

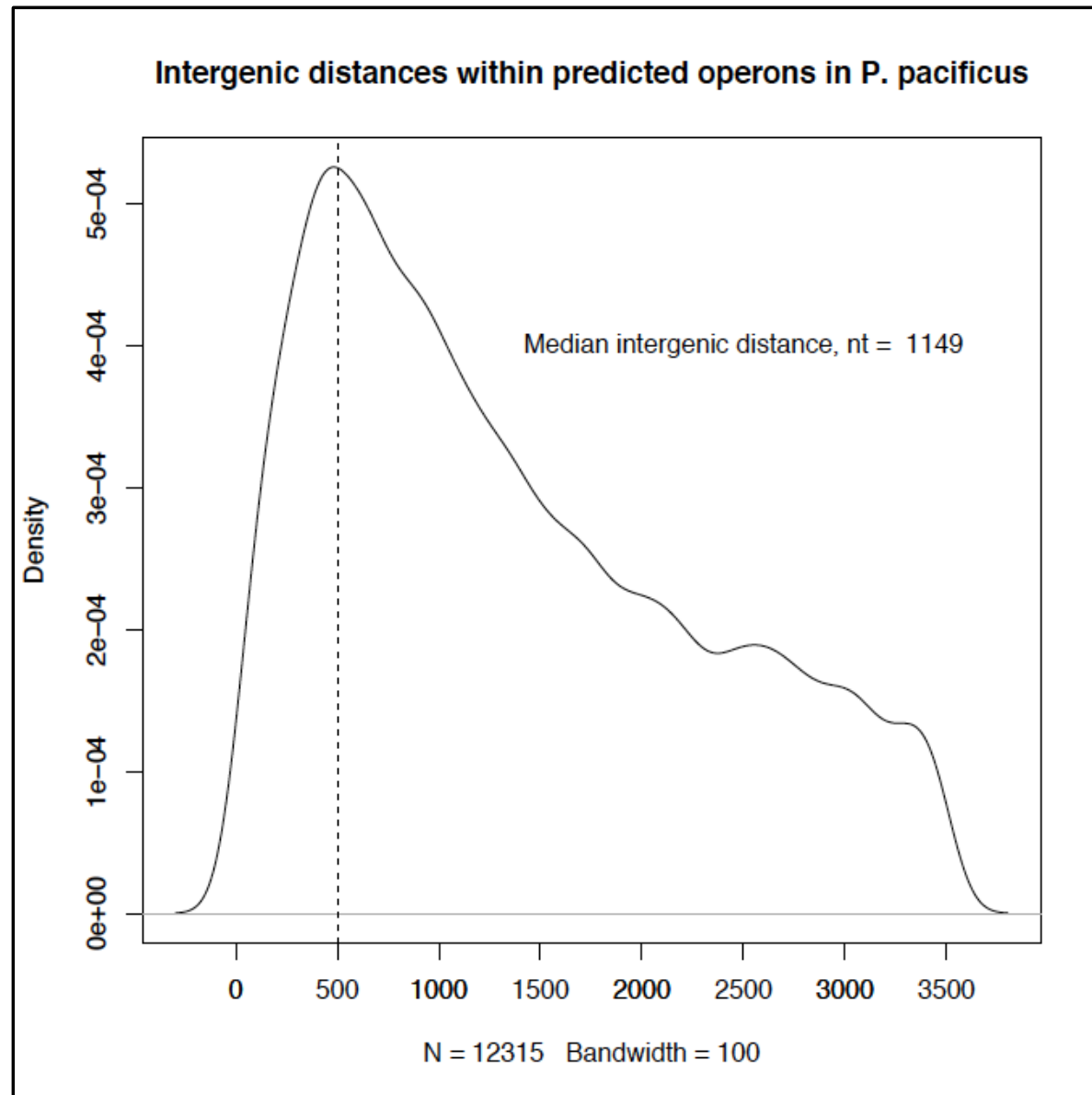


Supplementary Figure S6 : Relative proportions of SL1 versus SL2 trans-splicing are not directly correlated.

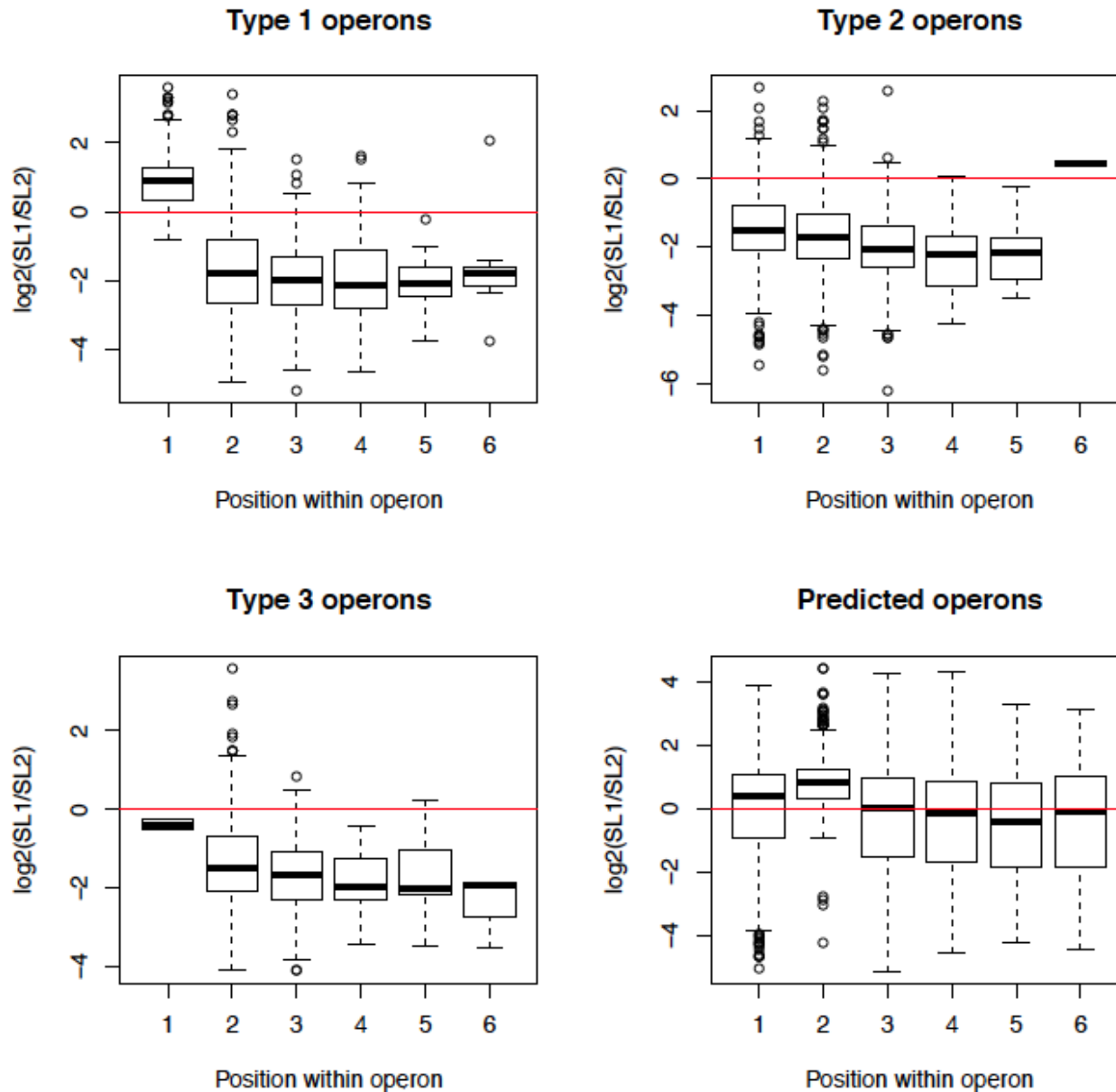


Pearson's correlation coefficient = 0.14

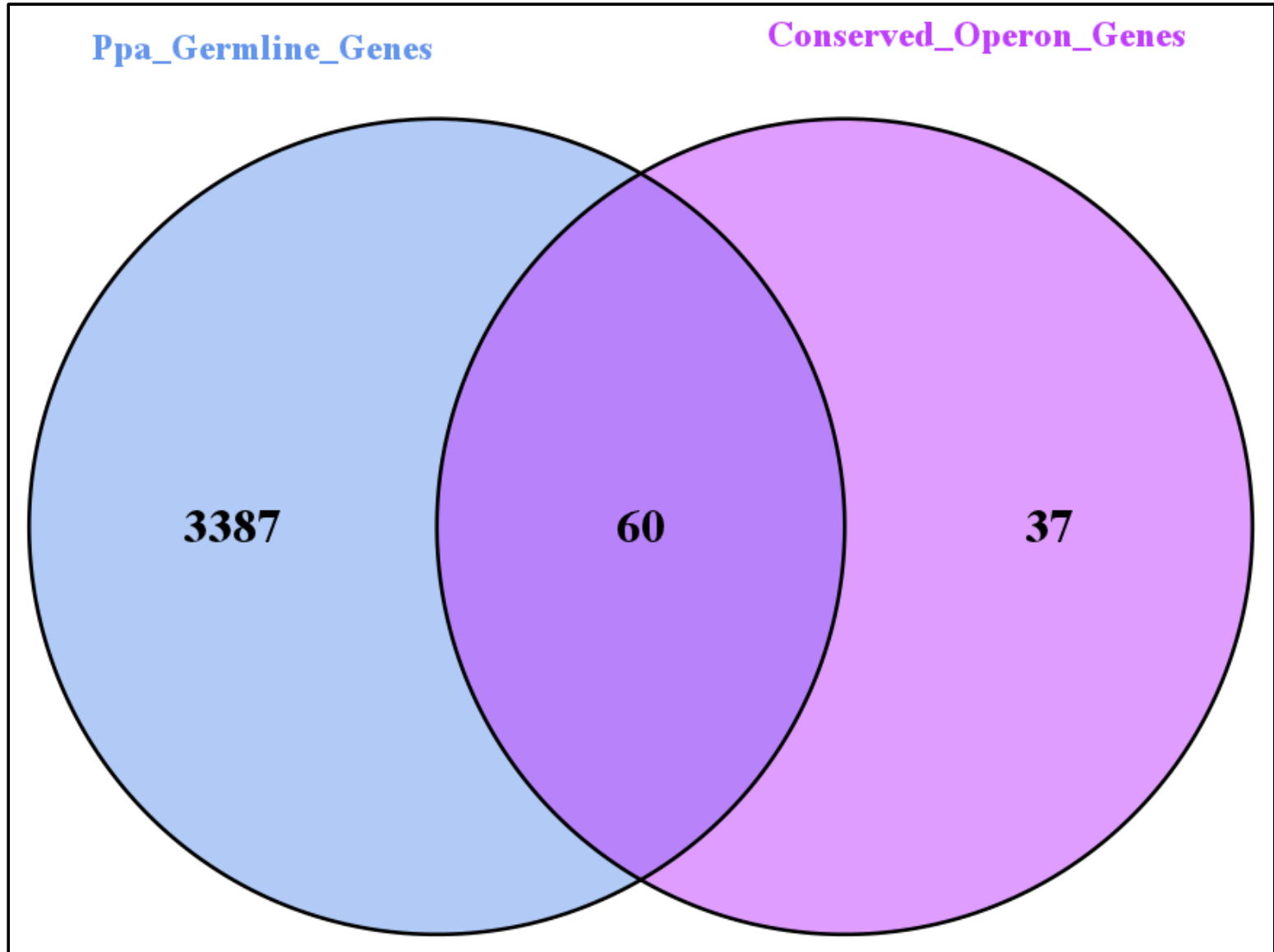
Supplementary Figure S7 : Density distribution of intergenic distances between all neighboring gene pairs in *P. pacificus* operons predicted at 3,500 nt intergenic distance threshold



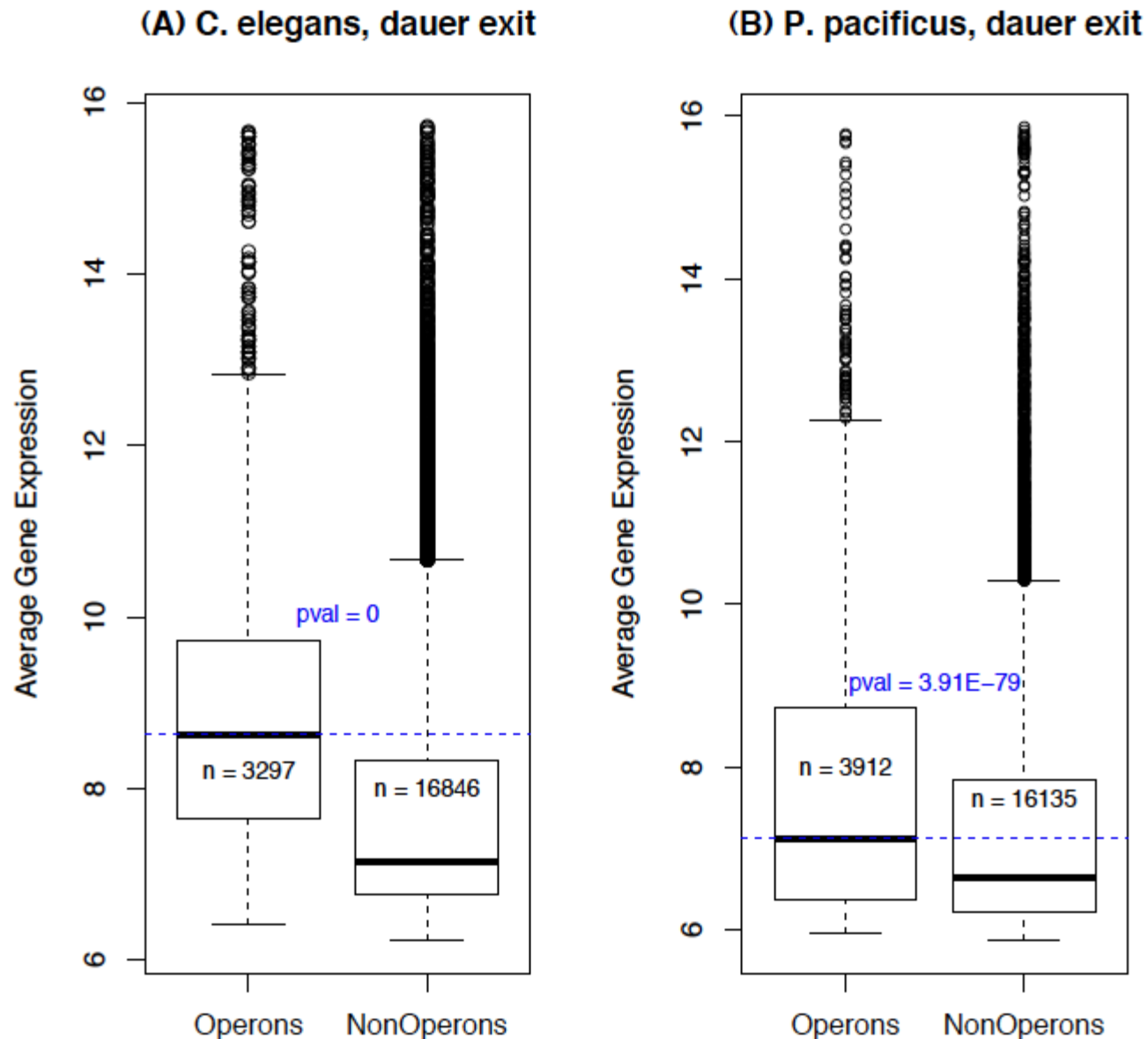
Supplementary Figure S8 : Distribution of splicing ratios($\log_2(\text{SL1}/\text{SL2})$) at different gene positions in different types of operons in *P. pacificus*



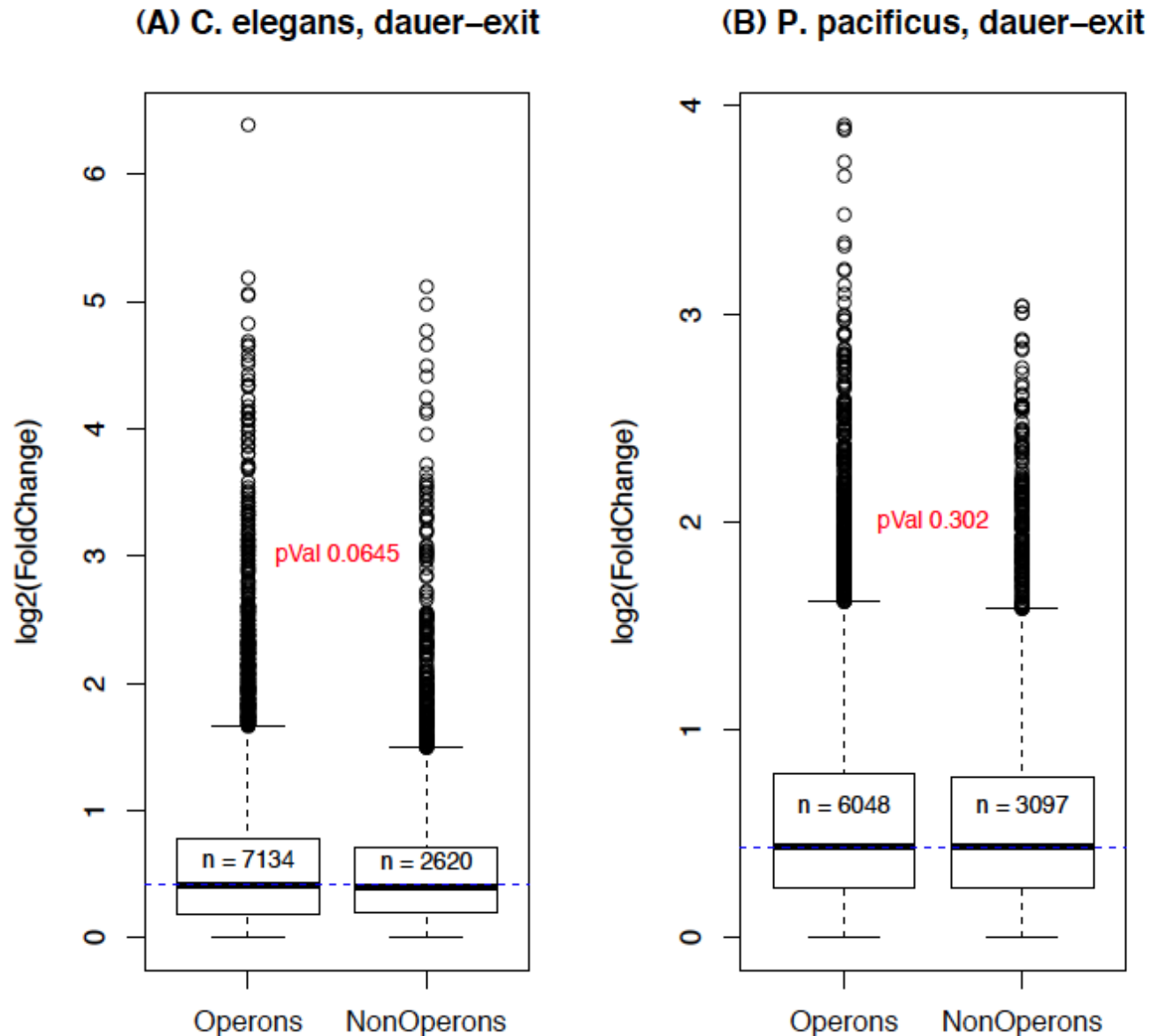
Supplementary Figure S9 : Germline expressed genes in *P. pacificus* are also significantly enriched in the annotated / validated operons conserved across *P. pacificus* and *C. elegans*
Fisher's exact test p-value = 8.56E-23



Supplementary Figure S10 : Upon dauer-exit, genes within validated operons are expressed at higher levels than genes outside these operons, in both (A) *C. elegans* and (B) *P. pacificus*



Supplementary Figure S11 : When all predicted operons are considered, the operonic genes no longer show a stronger upregulation than non-operonic genes during dauer-exit.



Supplementary Figure S12 : Multiple sequence alignment of all SL2 variants in *P. pacificus*, and the biotinylated probe used for SL2-specific pull downs.

The variant marked SL2_1 is reported to be the most abundant variant (Guiliano and Blaxter 2006). Given the high sequence similarity between all SL2 variants in *P. pacificus*, we expect the SL2-probe to also hybridize and therefore enrich for transcripts with other SL2 variants at their 5-prime ends

