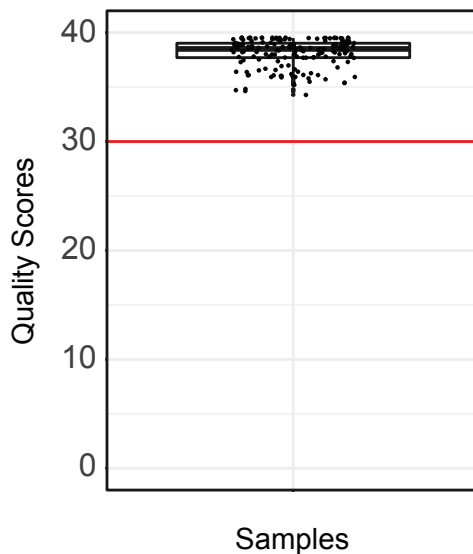
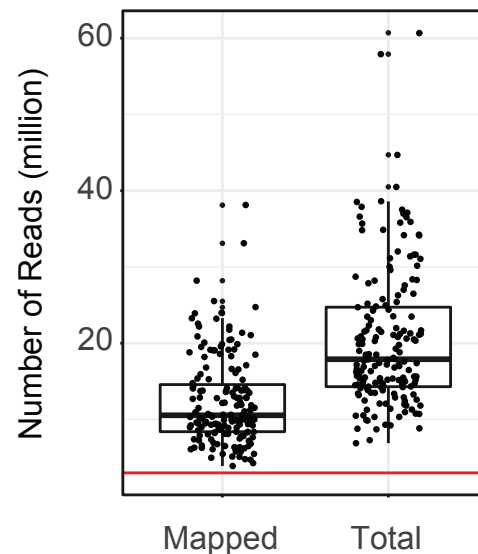
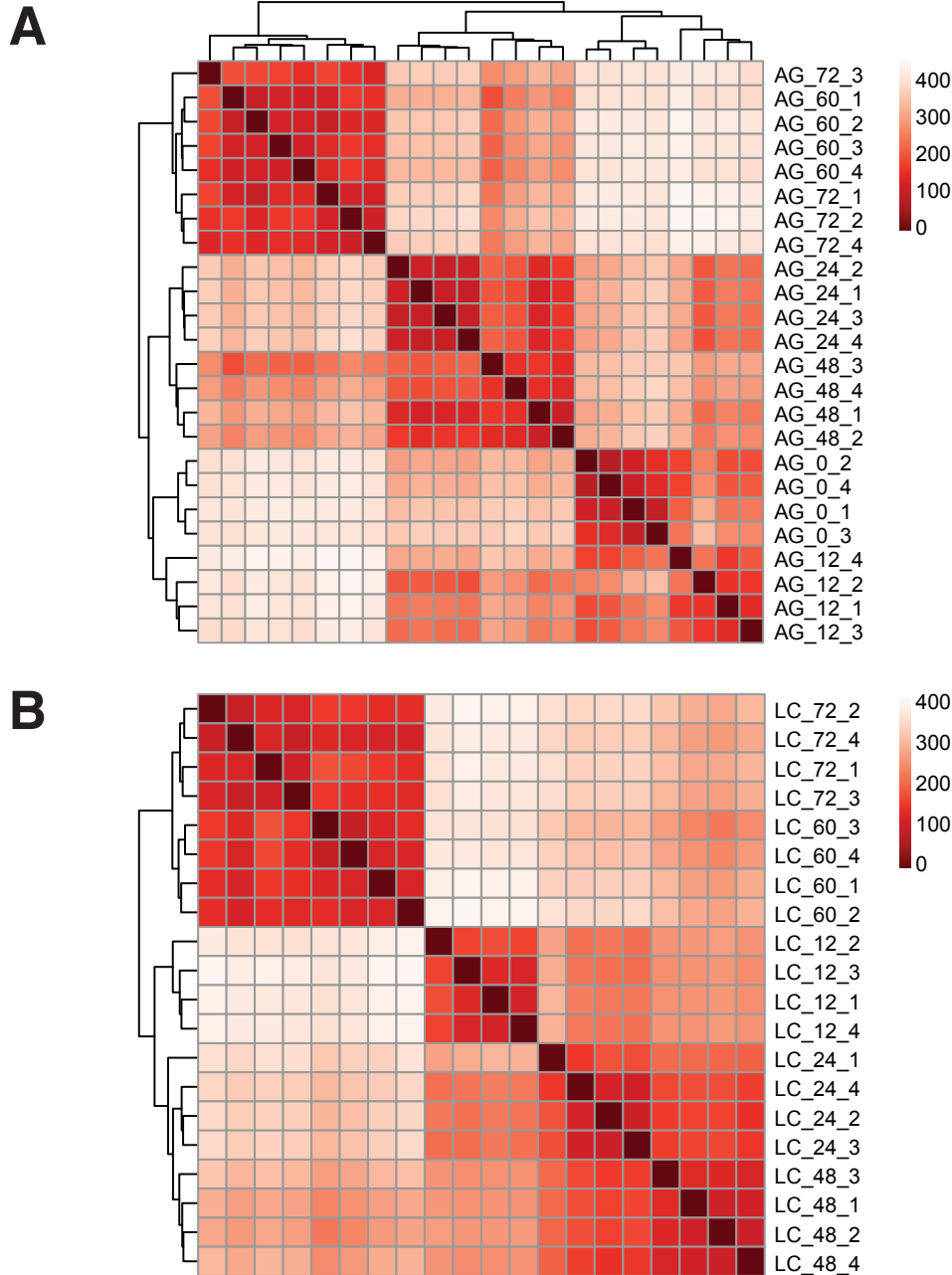


A

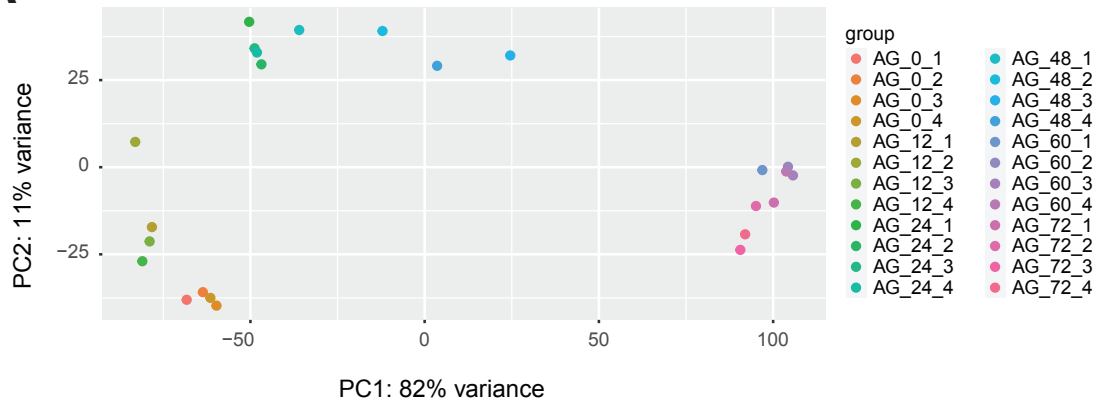
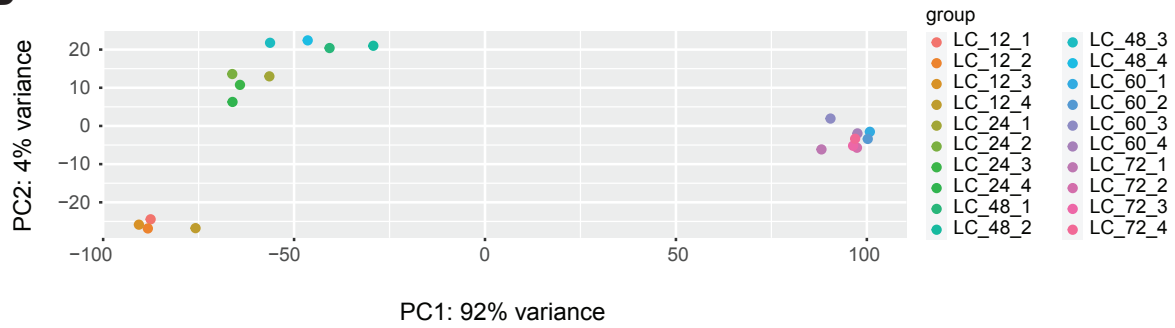
<i>Environment</i>	<i>Mutants</i>	<i>Genetic Background</i>
liquid culture (St) agar (Eu)	<i>Ppa-eud-1</i> (St) <i>Ppa-Ex[eud-1]</i> (Eu) <i>Ppa-nhr-40 g.o.f.</i> (Eu) <i>Ppa-mbd-2</i> (St) <i>Ppa-lsy-12</i> (St) <i>Ppa-sult-1</i> (Eu) <i>Ppa-nag-1/nag-2</i> (Eu)	RSC017 (St) RS5410 (St) RSA100 (Eu) RS5427 (Eu) <i>P. expectatus</i> (St)
6 timepoints/condition 4 replicates/timepoint	6 timepoints/mutant 2 replicates/timepoint	4 timepoints/strain 6 timepoints/ <i>P. expectatus</i> 2 replicates/timepoint

B**C**

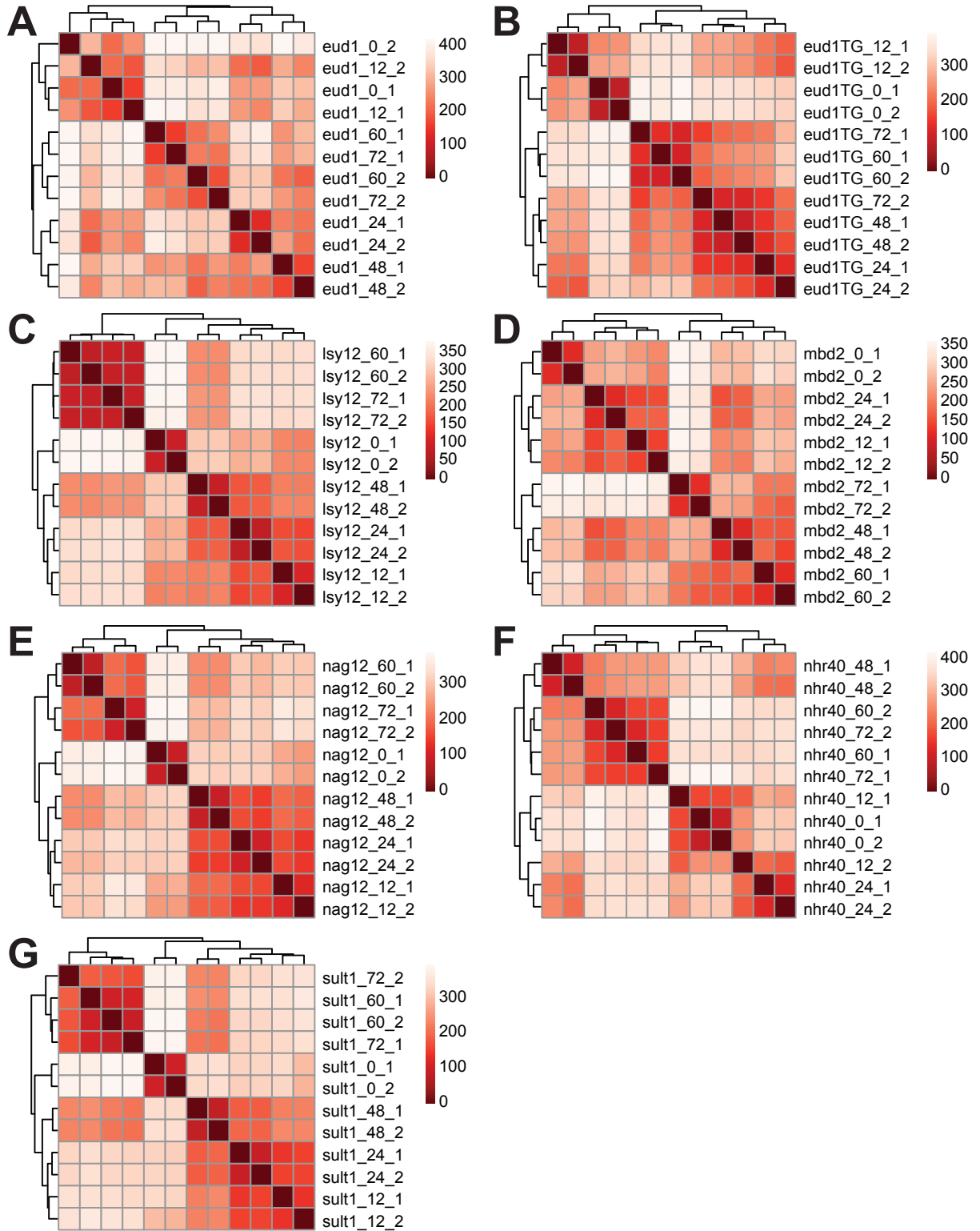
Supplemental Figure S1. Experimental design and RNA Sequencing quality scores
(A) Table of conditions and worm lines sampled for RNA-seq along with time points and number of replicates. **(B)** Quality scores of RNA-seq data. Red line represents threshold of 30. **(C)** Total number of RNA-seq reads and number of reads that mapped to transcriptome. Red line represents threshold for sample inclusion of 3 million reads.



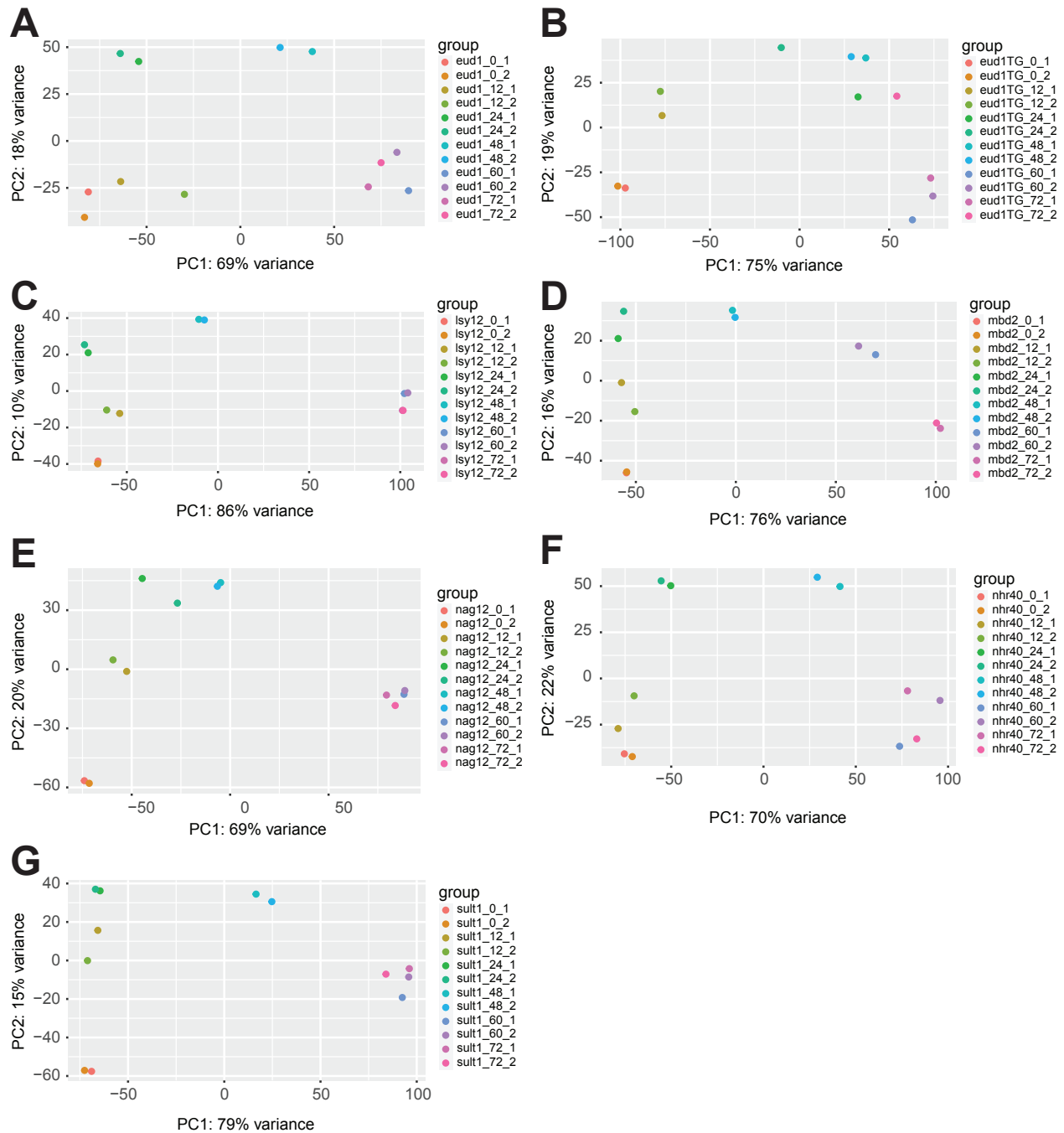
Supplemental Figure S2. Heatmaps of normalized gene expression from agar and liquid cultures. (A) Data from NGM agar include six developmental time points. The 0 h time point represents eggs which were collected from agar plates. To generate all subsequent time points, bleached eggs from agar plates were aliquoted between agar and liquid cultures (see Methods). Thus the AG_0 time points are representative of gene expression at 0 h for both agar and liquid culture conditions. **(B)** Data from S-medium liquid cultures include five developmental time points.

A**B**

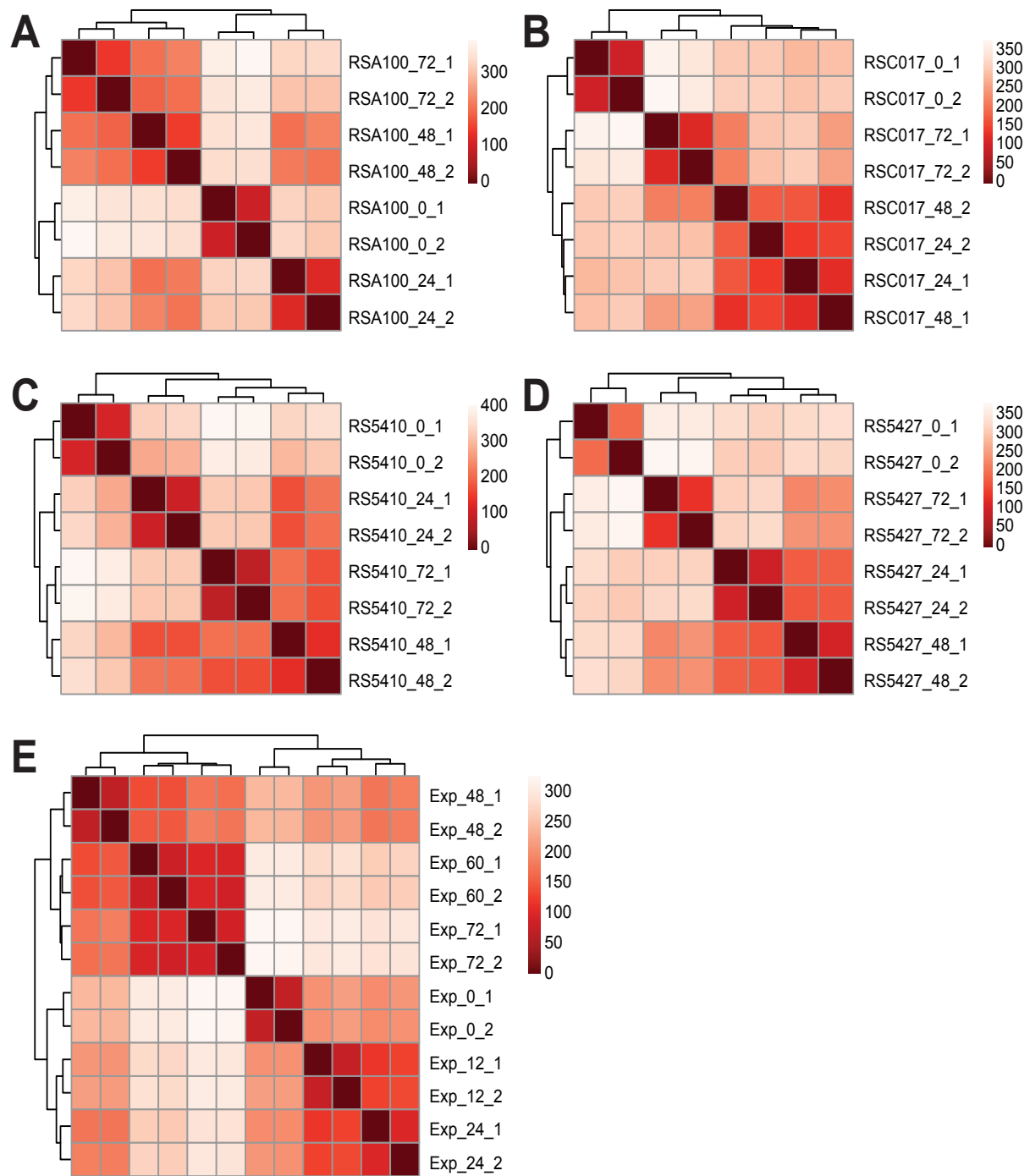
Supplemental Figure S3. Principal Component Analysis (PCA) of normalized gene expression from agar and liquid culture conditions. Plots show data from **(A)** eggs and worms grown on NGM agar plates and **(B)** worms grown in S-medium liquid culture.



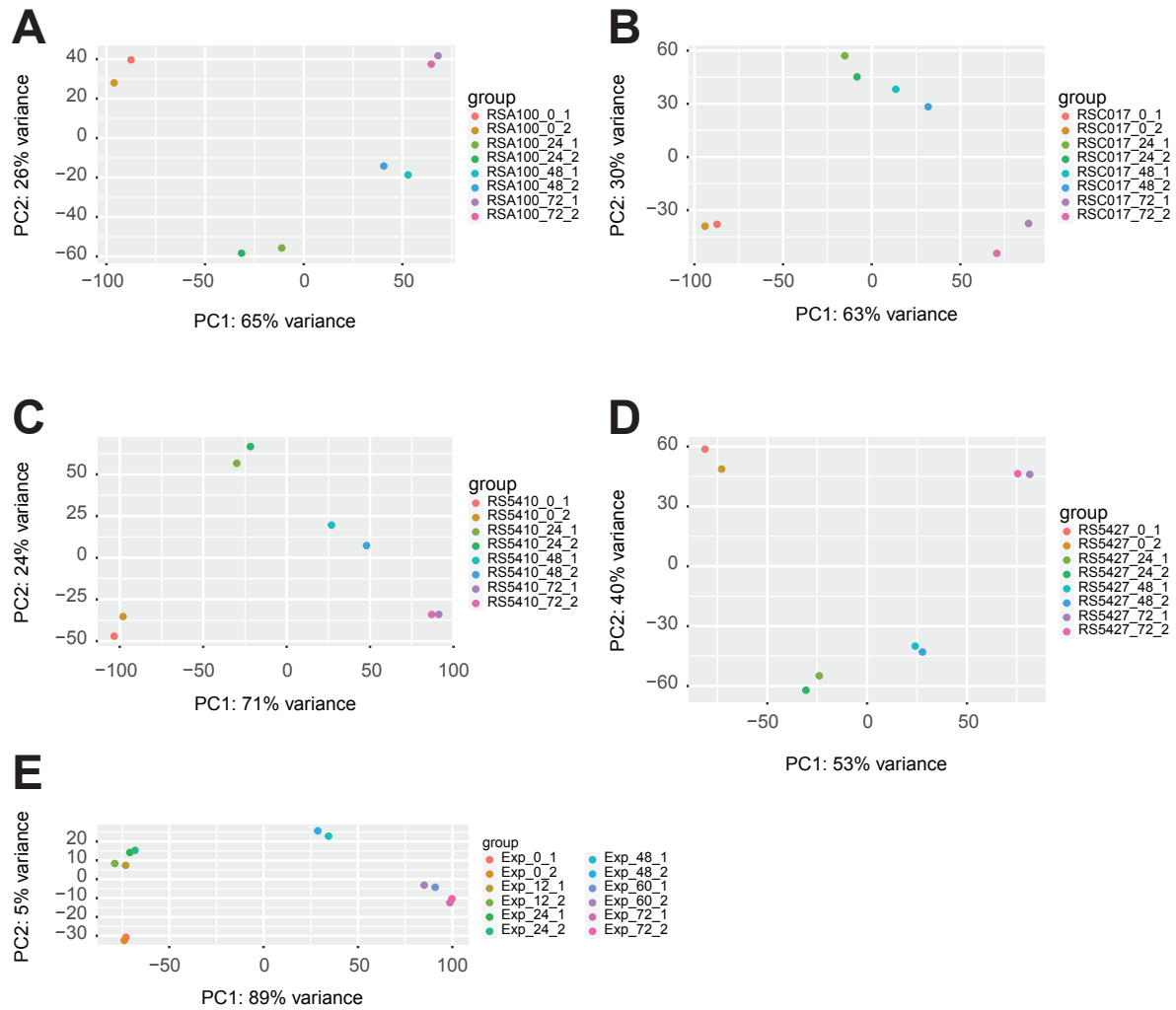
Supplemental Figure S4. Heatmaps of gene expression from seven mutant strains of *P. pacificus*. Plots represent data from eggs and five developmental time points in (A) *Ppa-eud-1*, (B) *Ppa-Ex[eud-1]*, (C) *Ppa-lsy-12*, (D) *Ppa-mbd-2*, (E) *Ppa-nag-1/nag-2*, (F) *Ppa-nhr-40 g.o.f.*, and (G) *Ppa-sult-1*.



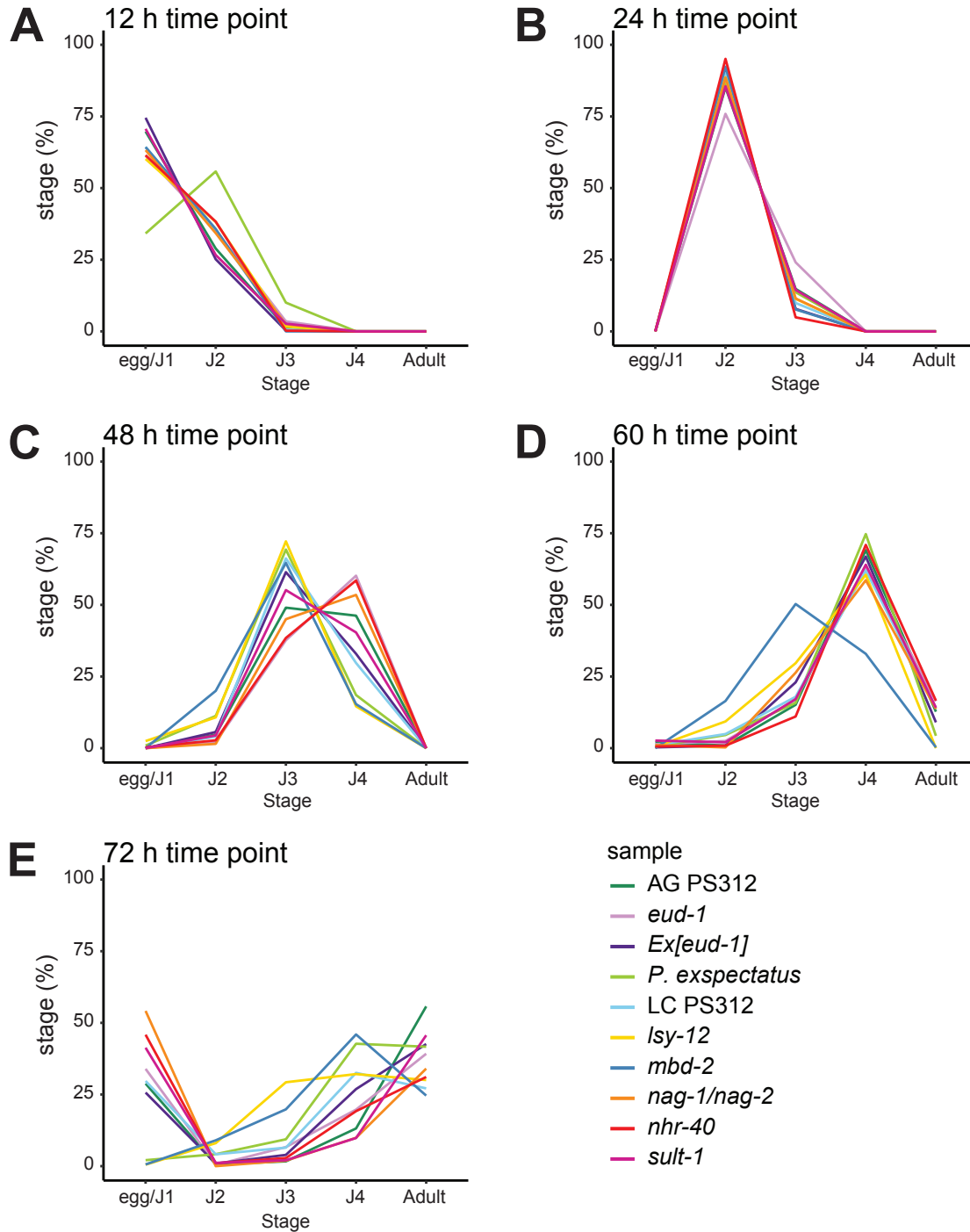
Supplemental Figure S5. Principal Component Analysis (PCA) of normalized gene expression from seven mutant strains. Plots show data from **(A)** *Ppa-eud-1*, **(B)** *Ppa-Ex[eud-1]*, **(C)** *Ppa-lsy-12*, **(D)** *Ppa-mbd-2*, **(E)** *Ppa-nag-1/nag-2*, **(F)** *Ppa-nhr-40* g.o.f., and **(G)** *Ppa-sult-1*.



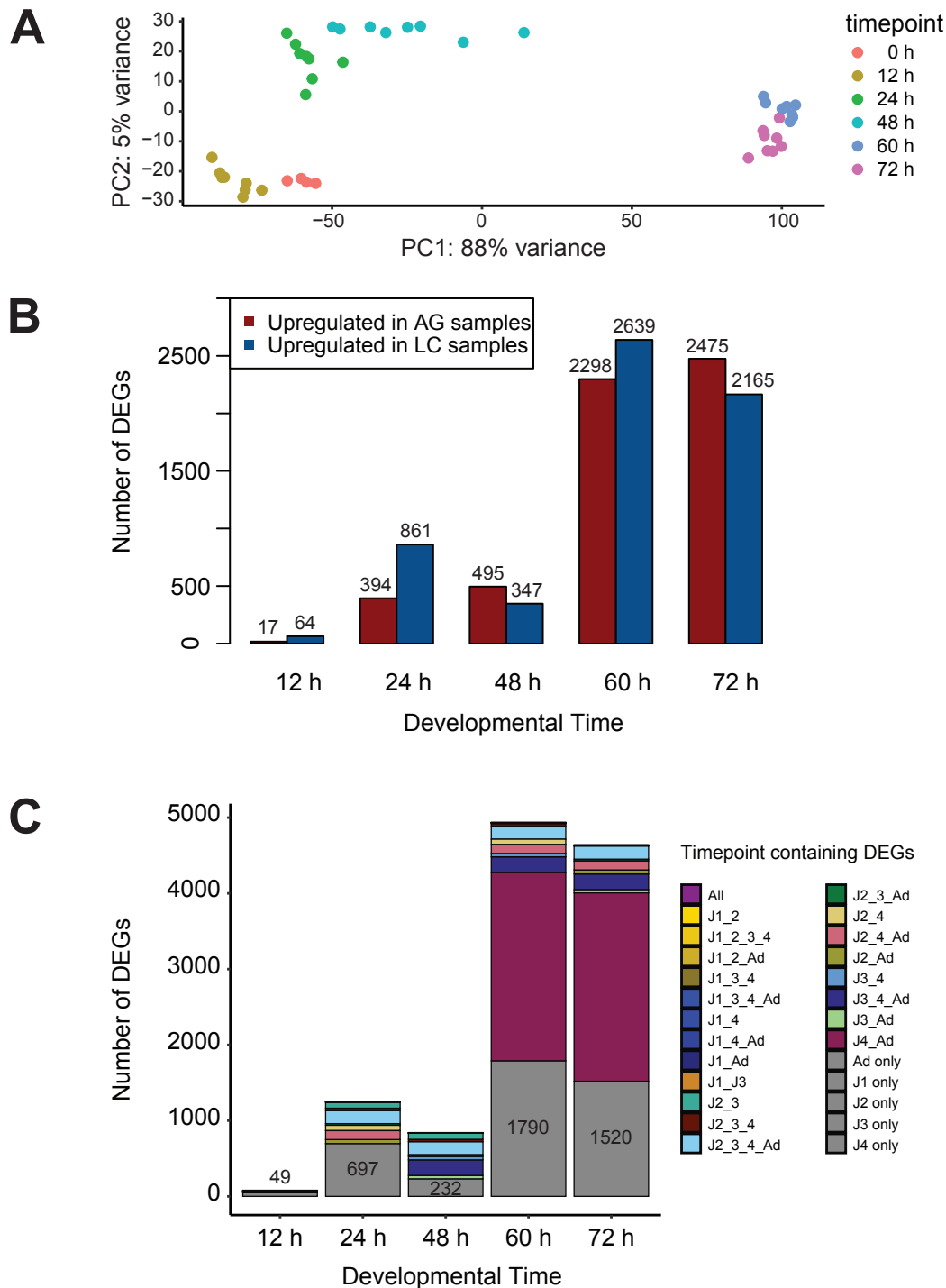
Supplemental Figure S6. Heatmaps of normalized gene expression from four *P. pacificus* strains and the sister species, *P. expectatus*. Plots show data from (A) RSA100, (B) RSC017, (C) RS5410, (D) RS5427, and (E) *P. expectatus*.



Supplemental Figure S7. Principal Component Analysis (PCA) of normalized gene expression from four *P. pacificus* strains and *P. exspectatus*. Plots show data from (A) RSA100, (B) RSC017, (C) RS5410, (D) RS5427, and (E) *P. exspectatus*.

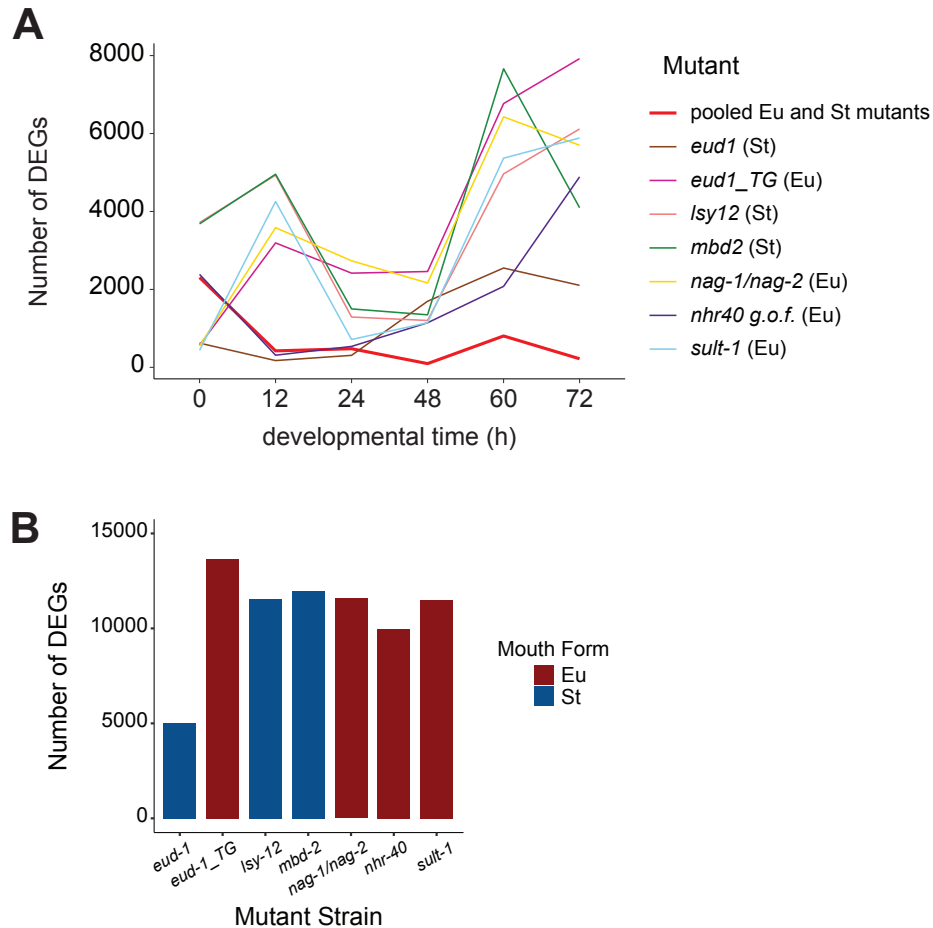


Supplemental Figure S8. Developmental stage of worms at from agar, liquid culture, mutant, and *P. expectatus* samples. Plots show stages present at **(A)** 12 h, **(B)** 24 h, **(C)** 48 h, **(D)** 60 h, and **(E)** 72 h time points.

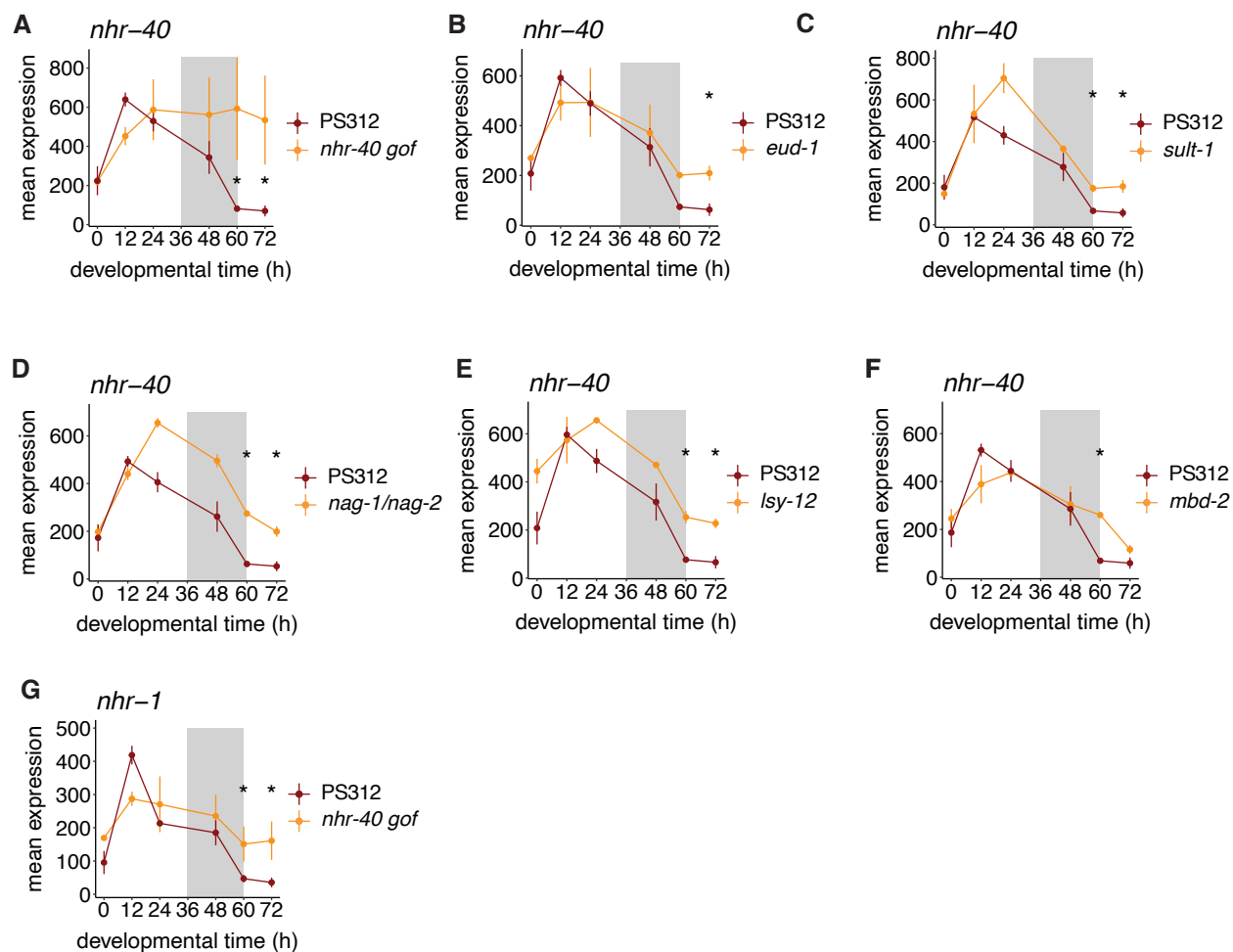


Supplemental Figure S9. Additional analyses from the environmental comparison.

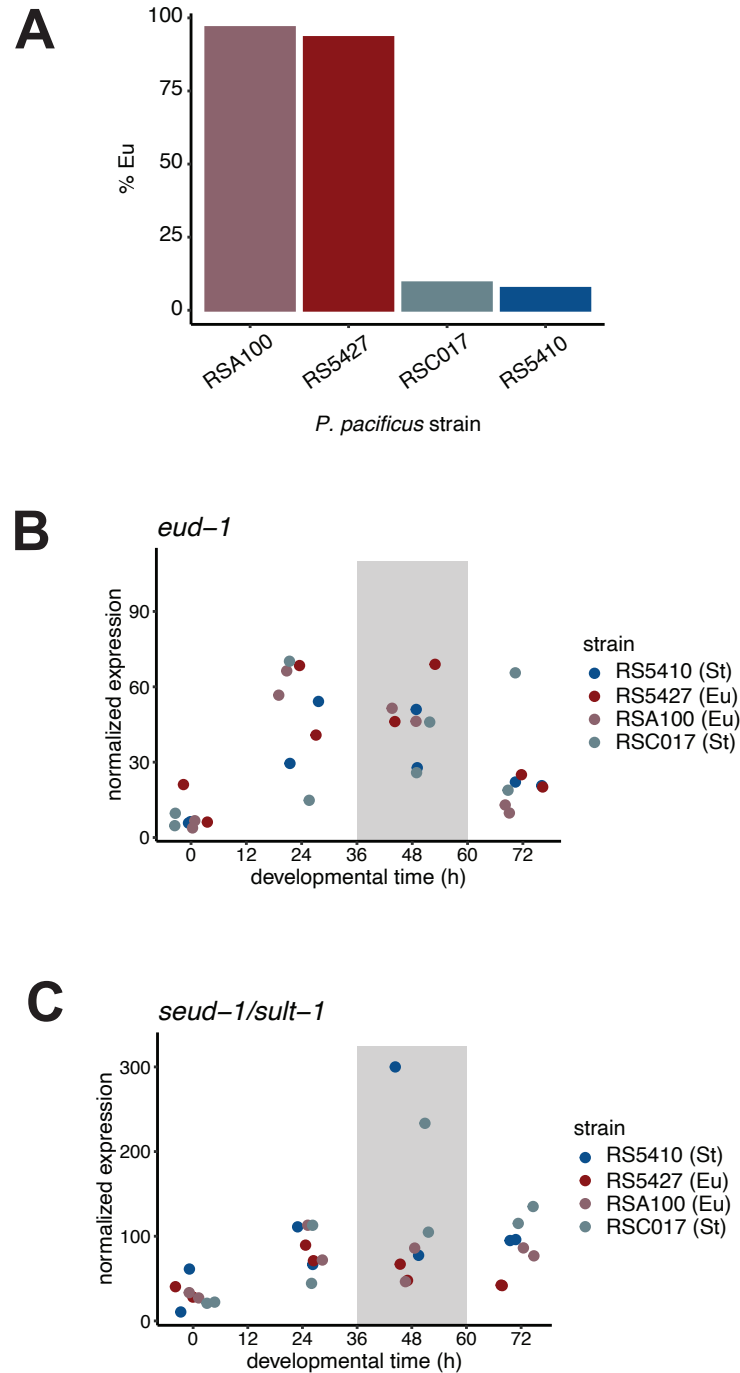
(A) Principal Component Analysis of normalized gene expression results from PS312 grown on NGM agar plates and S-medium liquid cultures with samples colored by time point. **(B)** Number of differentially expressed genes (DEGs) at each sampled time point upregulated in agar (AG, red) or liquid culture (LC, blue). **(C)** Number of differentially expressed genes (DEGs) at each sampled time point with DEGs shared between time points colored the same. The exception is the gray blocks, which represent the number of DEGs that were unique to each time point.



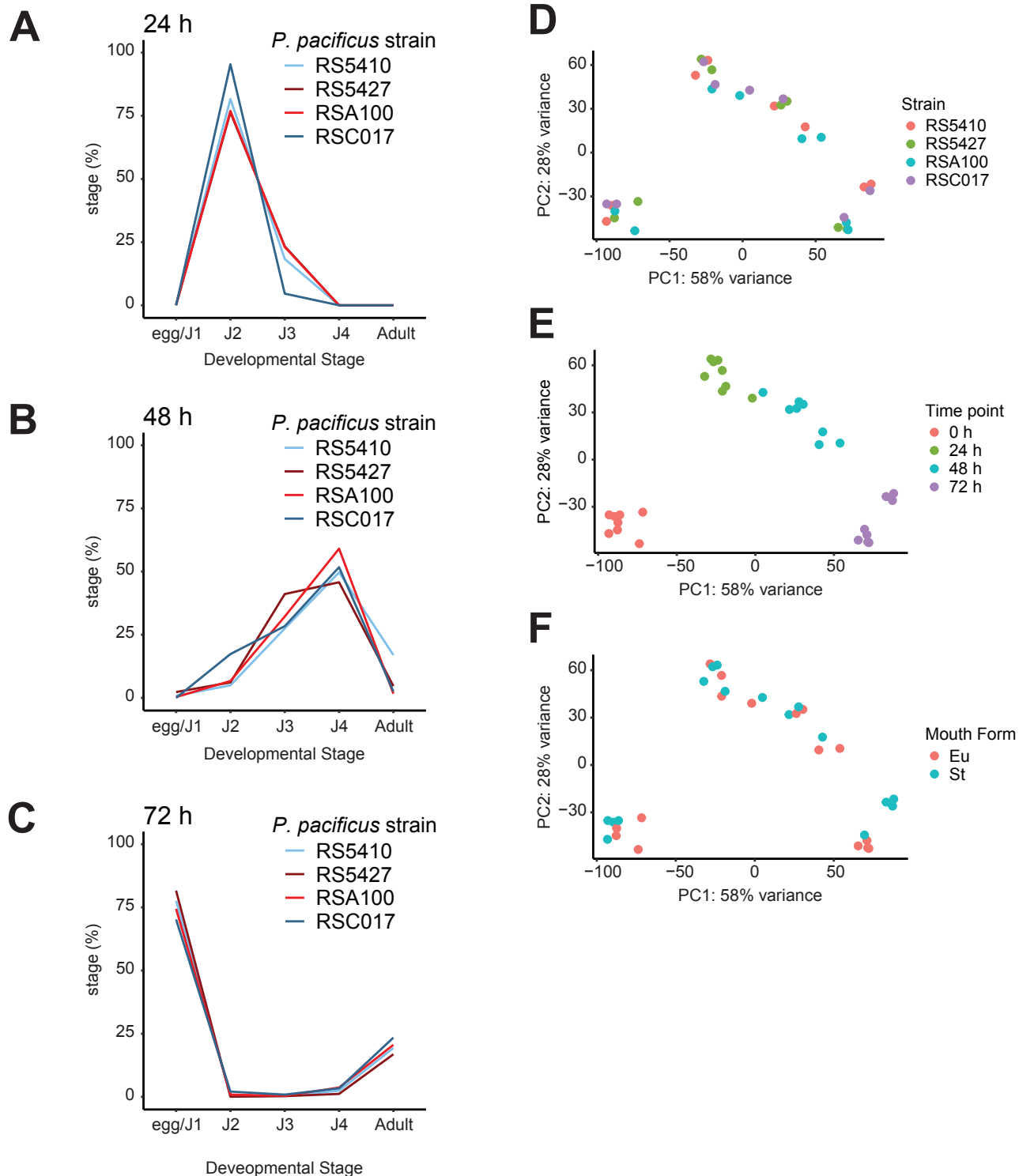
Supplemental Figure S10. Transcriptomic comparisons between mouth-form mutants and PS312 grown on agar plates. (A) Number of differentially expressed genes (DEGs) per time point between each mutant strain and PS312. Red line shows the number of DEGs between pooled Eu and St mutants. **(B)** Total number of unique DEGs across all time points for each mutant line compared to wildtype PS312 (adjusted P -value < 0.05). Bars are colored by mouth-form of mutant strain. *eud1_TG* is the overexpression line *Ppa-Ex[eud-1]*.



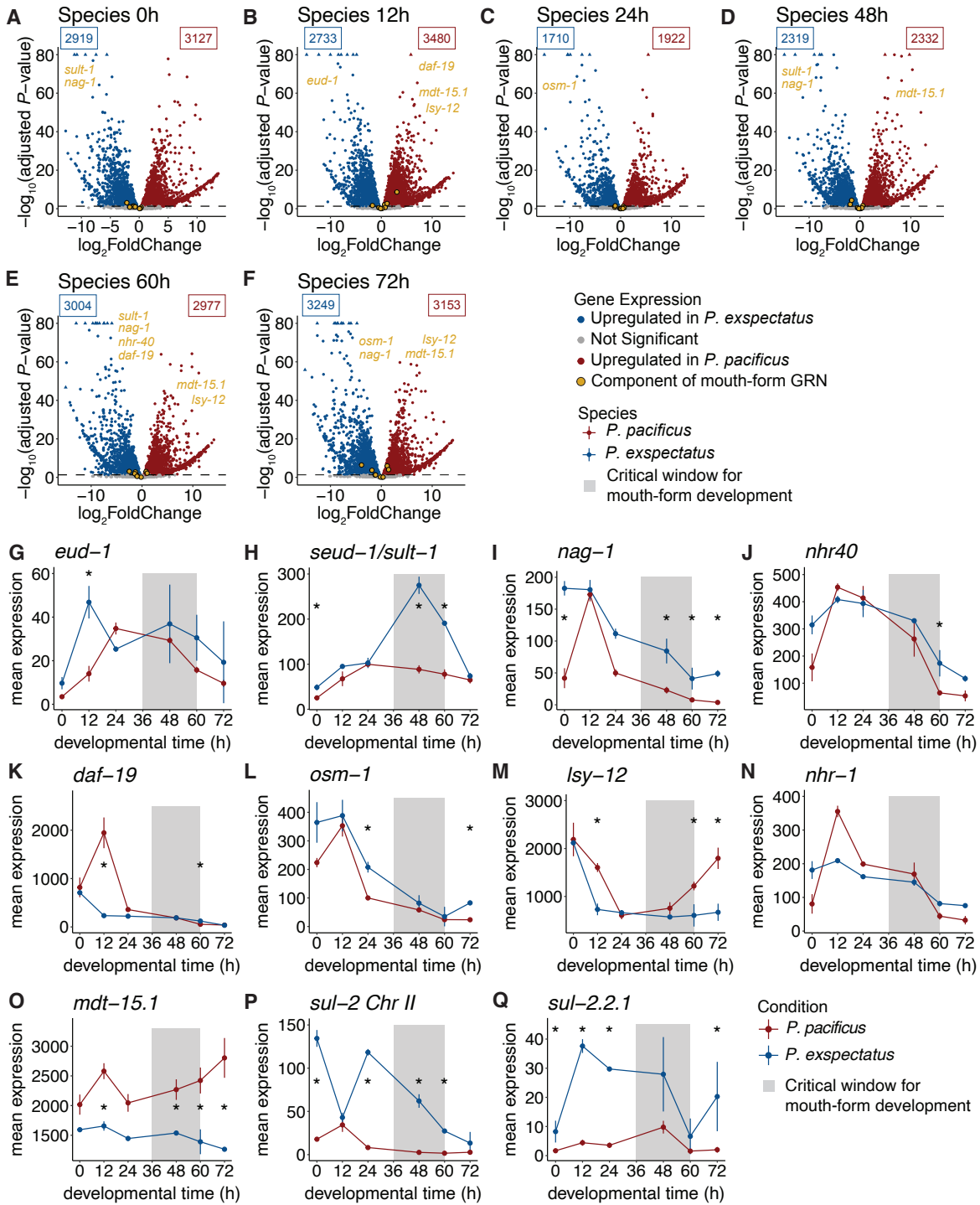
Supplemental Figure S11. Expression of nuclear hormone receptors is affected by mutating mouth-form genes. Plots show mean expression of normalized counts from DESeq2 for **(A-F)** *nhr-40* in six different mutants lines relative to PS312 and **(G)** *nhr-1* in the *Ppa-nhr-40 g.o.f.* mutant line.



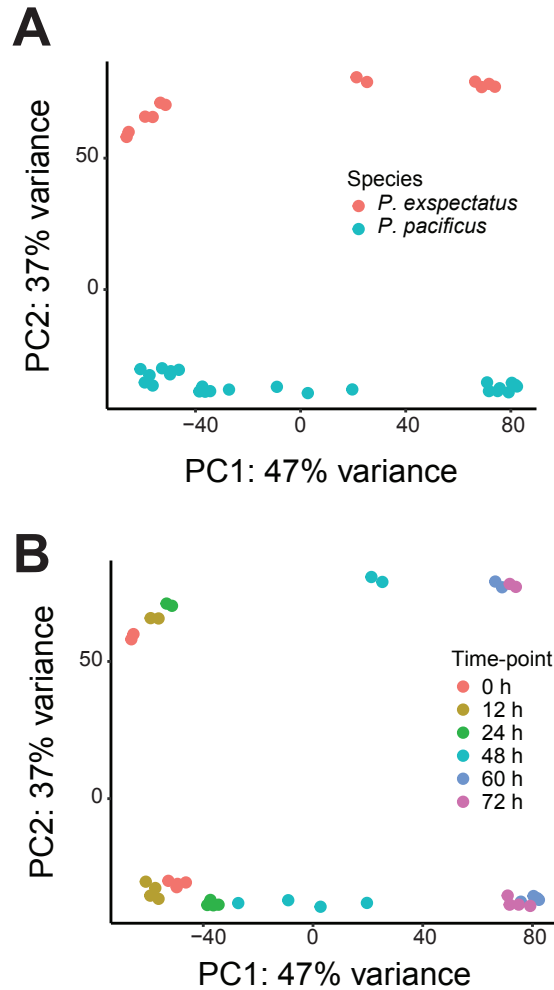
Supplemental Figure S12. Mouth-form ratios and switch gene expression in natural isolates of *P. pacificus*. (A) Mouth-form ratios (% Eurystomatous) for each of the natural isolates used in this study. Data for RS5410 are adapted from Ragsdale et al. 2013. Mouth-form ratios for other strains were empirically determined by authors of this manuscript. Expression of mouth-form switch genes (B) *eud-1* and (C) *seud-1/sult-1* in four different natural isolates of *P. pacificus*. Mouth-form bias of strain is given in parentheses.



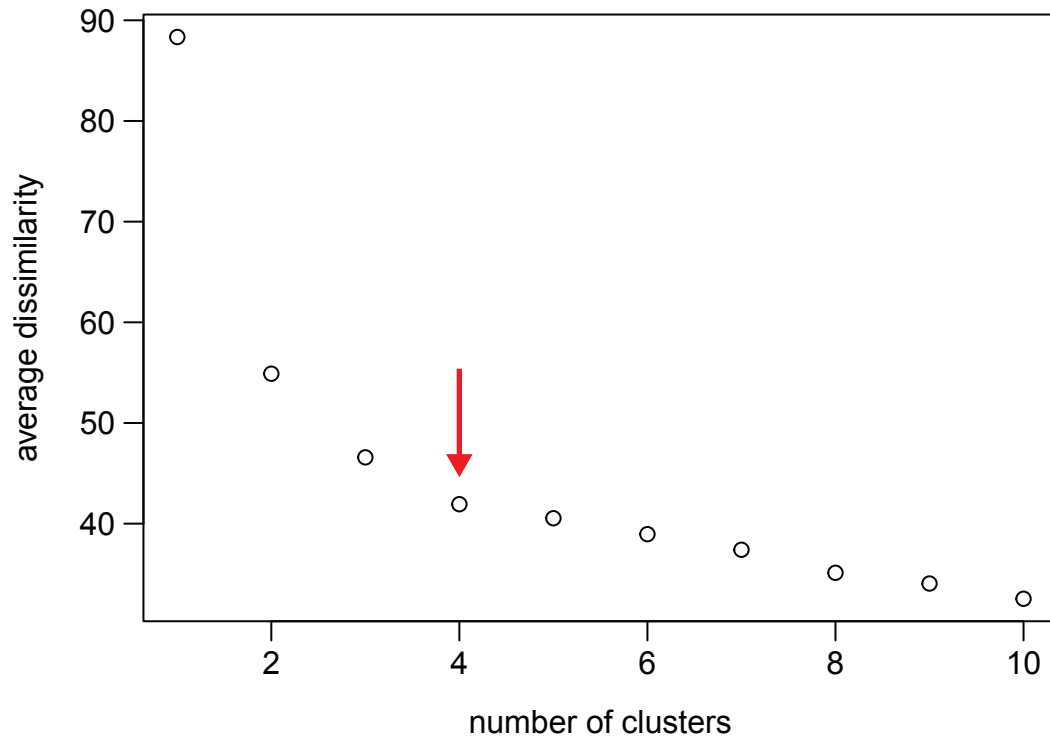
Supplemental Figure S13. Developmental staging and Principal Component Analysis (PCA) of Eu- and St-biased *P. pacificus* strains. Stages present at (A) 24 h, (B) 48 h, and (C) 72 h time points. PCA of normalized gene expression with samples colored by (D) strain ID, (E) sampling time point, and (F) mouth-form bias.



Supplemental Figure S14. *Pristionchus* species exhibit large transcriptomic differences. (A-F) Volcano plots of differentially expressed genes (DEGs) between 1:1 orthologs of *P. pacificus* (Eu-biased) and *P. expectatus* (St-biased) at six developmental time points. Significant DEGs (adjusted P -values < 0.05) are colored by whether they are upregulated in *P. expectatus* (blue) or *P. pacificus* (red). Mouth-form genes are colored in gold and labeled on plot when significant. (G-Q) Mean expression of normalized counts from DESeq2 for mouth-form genes in two species at six developmental time points. * time points with significant differences in expression (adjusted P -value < 0.05).

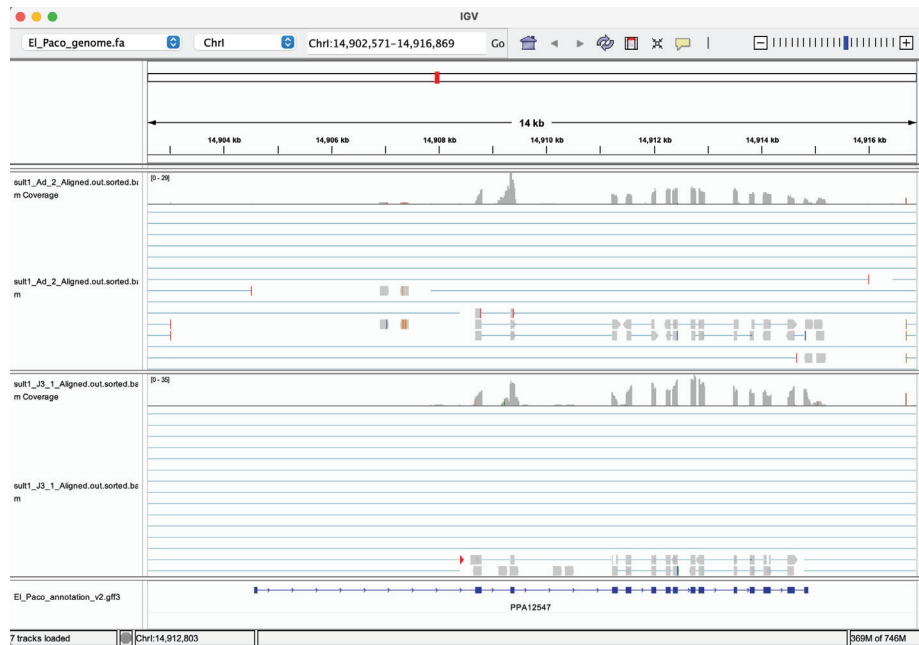


Supplemental Figure S15. Species differences explain a substantial amount of variance in gene expression between *P. pacificus* and *P. expectatus*. Principal component analysis of normalized differential gene expression between species using reads from 1:1 orthologs between the species and each species' transcriptome. Each dot represents a different sample, and samples are colored by **(A)** species and **(B)** sampling time point.



Supplemental Figure S16. Dissimilarity analysis used to determine number of clusters for *k*-medoid clustering. Dissimilarity plot showing the average dissimilarity within a cluster when samples were divided into a given number of clusters. We decided on 4 clusters (indicated by red arrow) based on the declining reduction of dissimilarity when samples were divided into 4 or 5 clusters.

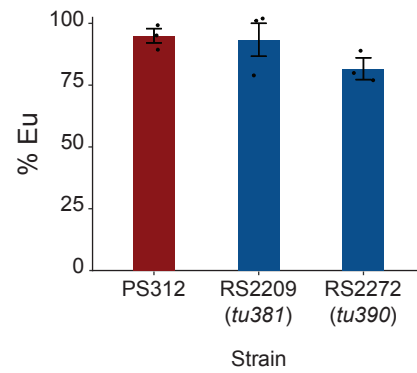
A



B



Supplemental Figure S17. Mapping reads from *Ppa-sult-1* samples to genome to check location of the mutation. (A) Screenshot of window from IGV showing the entire *seud-1/sult-1* locus and the counts of reads in two different *Ppa-sult-1* samples aligned across the locus. **(B)** Zoomed in screenshot of window from IGV showing the 9 bp deletion in the 9th exon of *seud-1/sult-1*.



Supplemental Figure S18. Mouth-form phenotype of two different *daf-12* mutants and wildtype PS312 grown on NGM agar plates. Error bars represent S.E.M. for $n=3$ independent biological replicates, with >20 worms phenotypes per replicate.