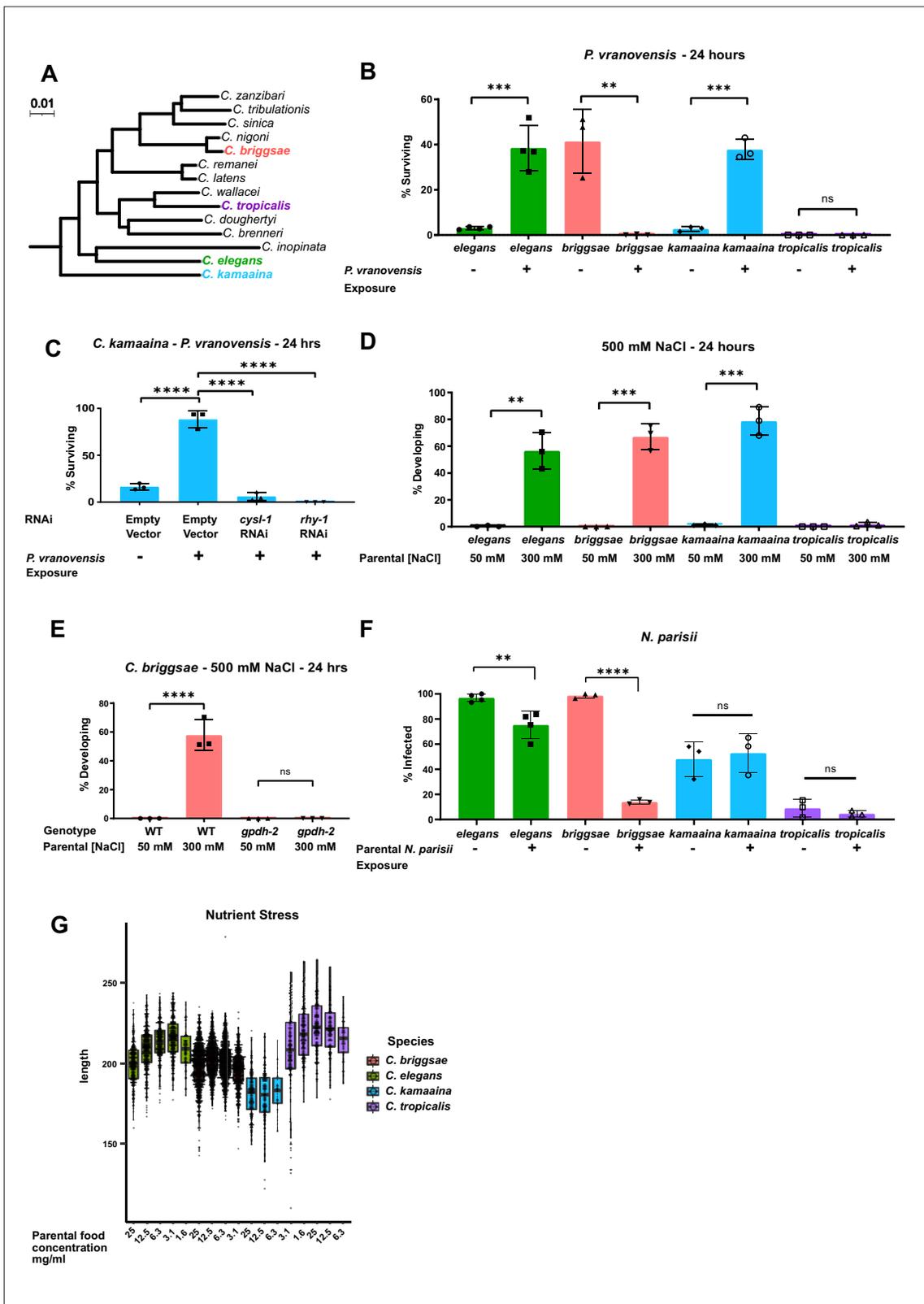# Figures and figure supplements

Intergenerational adaptations to stress are evolutionarily conserved, stress-specific, and have deleterious trade-offs

Nicholas O Burton *et al*

Figure 1. Intergenerational adaptations to multiple stresses are evolutionarily conserved in multiple species of *Caenorhabditis*. (**A**) Phylogenetic tree of the *Elegans* group of *Caenorhabditis* species adapted from **Stevens et al., 2020**. Scale represents substitutions per site. (**B**) Percent of wild-type *C. elegans* (N2), *C. kamaaina* (QG122), *C. briggsae* (AF16), and *C. tropicalis* (JU1373) animals surviving after 24 hr on plates seeded with *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3–4 experiments of >100 animals. (**C**) Percent of *C. kamaaina* wild-type (QG122) animals surviving

Figure 1 continued

after 24 hr of exposure to *P. vranovensis*. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**D**) Percent of wild-type animals mobile and developing at 500 mM NaCl after 24 hr. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**E**) Percent of wild-type and *Cbr-gpdh-2*(*syb2973*) mutant *C. briggsae* (AF16) mobile and developing after 24 hr at 500 mM NaCl. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**F**) Percent of animals exhibiting detectable infection by *N. parisii* as determined by DY96 staining after 72 hr for *C. elegans* and *C. briggsae*, or 96 hr for *C. kamaaina* and *C. tropicalis*. Data presented as mean values ± s.e.m. *n* = 3–4 experiments of 83–202 animals. (**G**) Boxplots for length of L1 progeny from P0 parents that were subject to the HB101 dose series. Larvae were measured using Wormsizer. Boxplots show median length with four quartiles. *n* = 3–8 experiments of 50–200 animals. **p < 0.01, ***p < 0.0001, ****p < 0.0001.
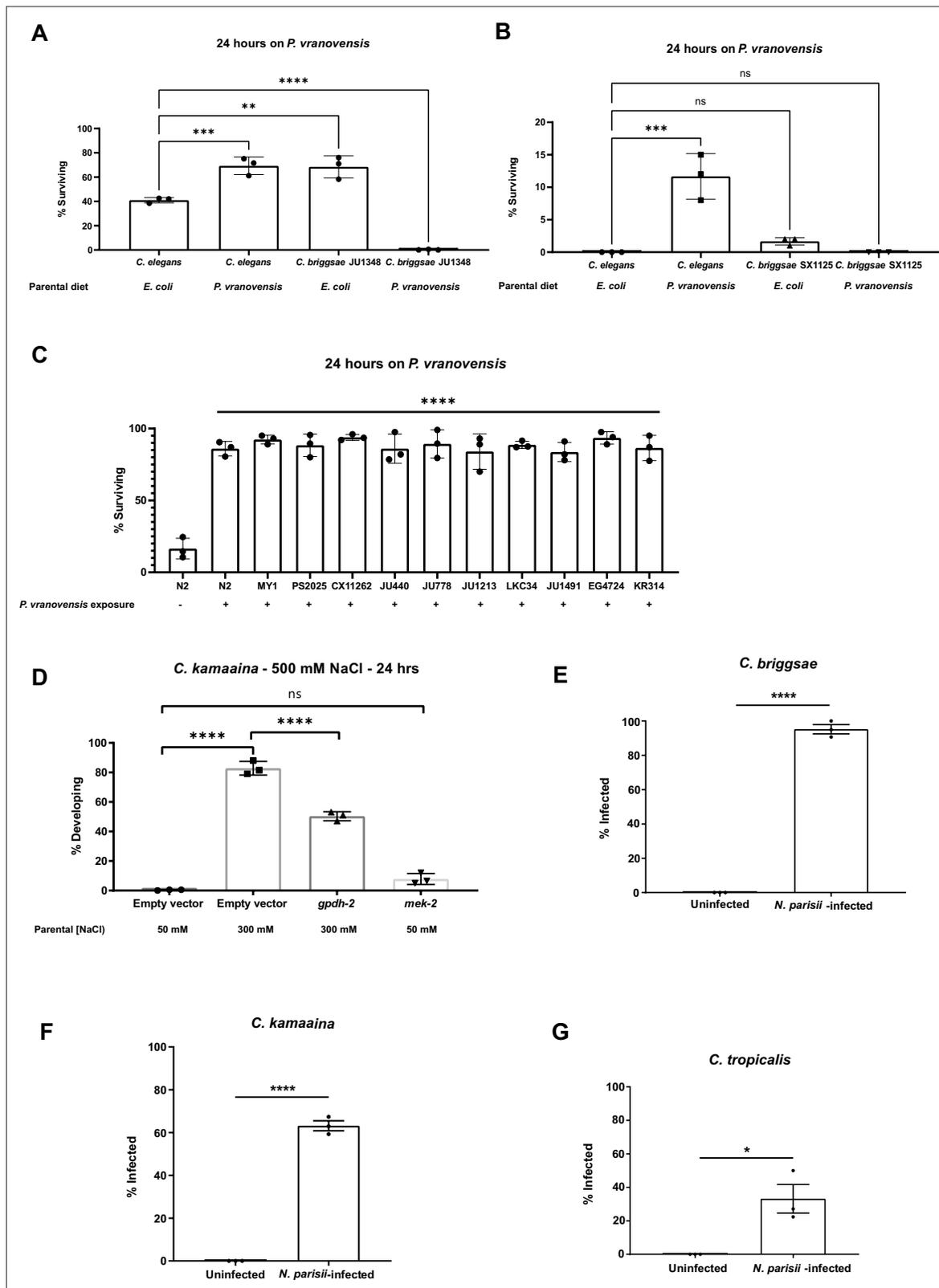
Figure 1—figure supplement 1. Intergenerational responses to environmental stress are conserved in wild isolates of *Caenorhabditis* species. (**A**) Percent of wild-type *C. elegans* (**N2**) and *C. briggsae* (JU1348) animals surviving after 24 hr on plates seeded with *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**B**) Percent of wild-type *C. elegans* (**N2**) and *C. briggsae* (SX1125) animals surviving after 24 hr on plates seeded with *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**C**) Percent of wild-

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type *C. elegans* isolates surviving after 24 hr on plates seeded with *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. n = 3 experiments of >100 animals. (**D**) Percent of wild-type *C. kamaaina* animals mobile and developing at 500 mM NaCl after 24 hr. Data presented as mean values ± s.d. n = 3 experiments of >100 animals. (**E–G**) Percent of animals exhibiting detectable infection by *N. parisii* as determined by DY96 staining after 72 hr for *C. elegans* and *C. briggsae*, or 96 hr for *C. kamaaina* and *C. tropicalis*. Data presented as mean values ± s.e.m. n = 3 experiments of 68–115 animals (E), 27–102 animals (F), and 38–104 animals (G). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.
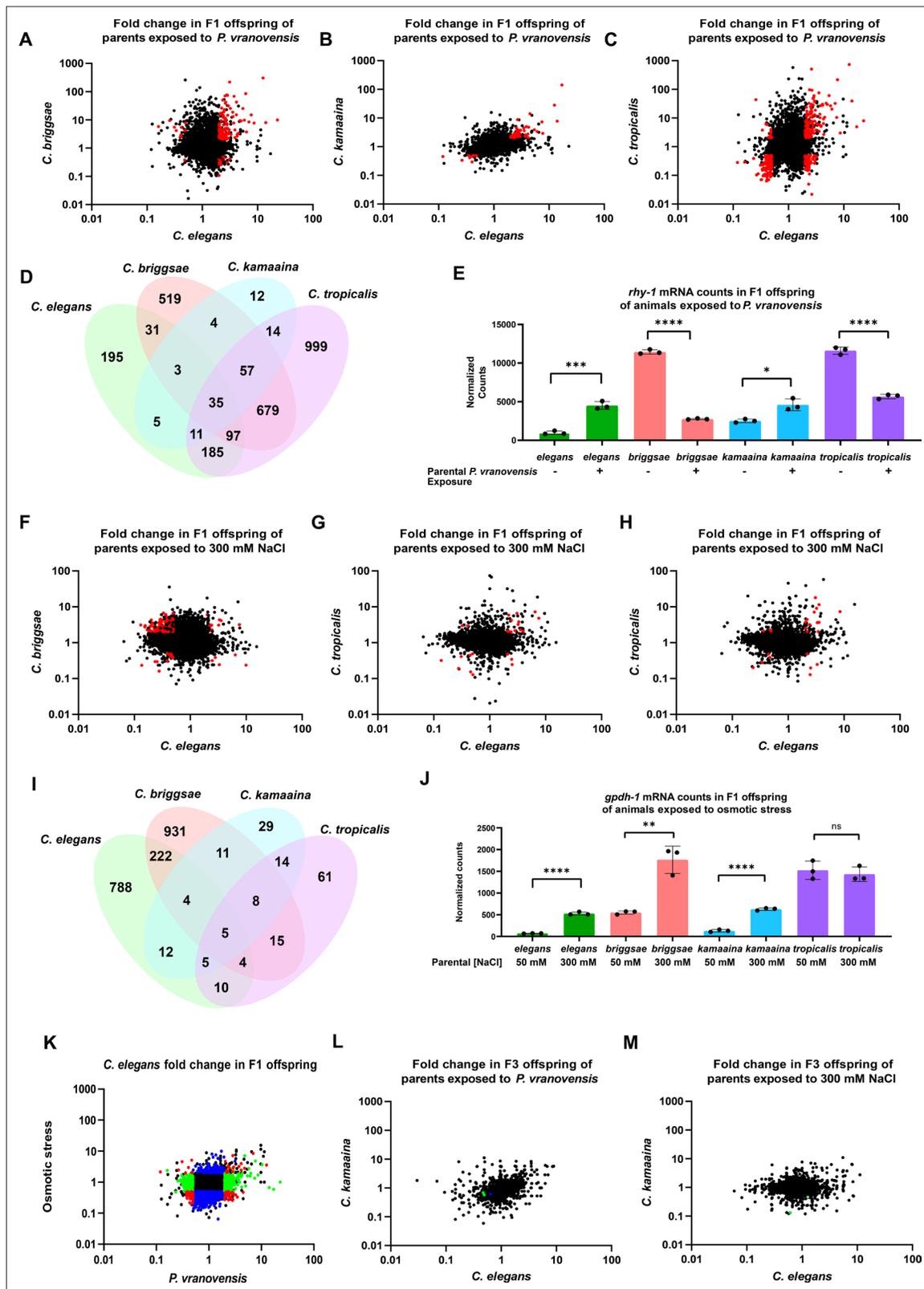
Figure 2. Parental exposure to *P. vranovensis* and osmotic stress have overlapping effects on offspring gene expression across multiple species. (**A**) Average fold change of 7587 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. briggsae* parents fed *P. vranovensis* BIGb0446 when compared to parents fed *E. coli* HB101. Average fold change from three replicates. Red dots represent genes that exhibit > twofold (*padj* <0.01) changes in expression in both species. (**B**) Average fold change of 7587 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. kamaaina*

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parents fed *P. vranovensis* BIGb0446 when compared to parents fed *E. coli* HB101. Average fold change from three replicates. Red dots represent genes that exhibit > twofold (*padj* <0.01) changes in expression in both species. (**C**) Average fold change of 7587 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. tropicalis* parents fed *P. vranovensis* BIGb0446 when compared to parents fed *E. coli* HB101. Average fold change from three replicates. Red dots represent genes that exhibit > twofold (*padj* <0.01) changes in expression in both species (**D**) Venn diagram of the number of genes that exhibit overlapping >2 fold (*padj* <0.01) changes in expression in F1 progeny of animals exposed to *P. vranovensis* BIGb0446 in each species. (**E**) Normalized counts of reads matching orthologs of *rhy-1* in the F1 offspring of parents fed either *E. coli* HB101 or *P. vranovensis* BIGb0446. Data from **Supplementary file 2**. *n* = 3 replicates. (**F**) Average fold change of 7587 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. briggsae* parents grown at 300 mM NaCl when compared to parents grown at 50 mM NaCl. Average fold change from three replicates. Red dots represent genes that exhibit > twofold (*padj* <0.01) changes in expression in both species. (**G**) Average fold change of 7587 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. kamaaina* parents grown at 300 mM NaCl when compared to parents grown at 50 mM NaCl. Average fold change from three replicates. Red dots represent genes that exhibit > twofold (*padj* <0.01) changes in expression in both species in both species. (**H**) Average fold change of 7587 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. tropicalis* parents grown at 300 mM NaCl when compared to parents grown at 50 mM NaCl. Average fold change from three replicates. Red dots represent genes exhibit > twofold (*padj* <0.01) changes in expression in both species. (**I**) Venn diagram of the number of genes that exhibit overlapping > twofold (*padj* <0.01) changes in expression in F1 progeny of animals grown at 300 mM NaCl in each species. (**J**) Normalized counts of reads matching orthologs of *gpdh-1* in the F1 progeny of parents grown at either 300 mM NaCl or 50 mM NaCl. Data from **Supplementary file 3**. *n* = 3 replicates. (**K**) Average fold change for 7587 ortholog genes in F1 progeny of *C. elegans* parents fed *P. vranovensis* or exposed to 300 mM NaCl when compared to naive parents. Average fold change from three replicates. Red dots – genes that change in expression in response to both stresses. Blue dots – genes that change in expression in response to only osmotic stress. Green dots – genes that change in expression in response to only *P. vranovensis*. (**L**) Average fold change of 7512 single-copy ortholog genes in F3 progeny of *C. elegans* and *C. kamaaina* fed *P. vranovensis* BIGb0446 when compared to those fed *E. coli* HB101. Average fold change from three replicates. Blue dots represent genes that exhibited > twofold (*padj* <0.01) changes in expression in *C. elegans*. Green dots represent genes that exhibited > twofold (*padj* <0.01) changes in expression in *C. kamaaina*. (**M**) Average fold change of 7512 single-copy ortholog genes in F1 progeny of *C. elegans* and *C. kamaaina* parents grown at 300 mM NaCl when compared to parents grown at 50 mM NaCl. Average fold change from three replicates. Green dots represent genes that exhibited > twofold (*padj* <0.01) changes in expression in *C. kamaaina*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$, ****$p < 0.0001$.
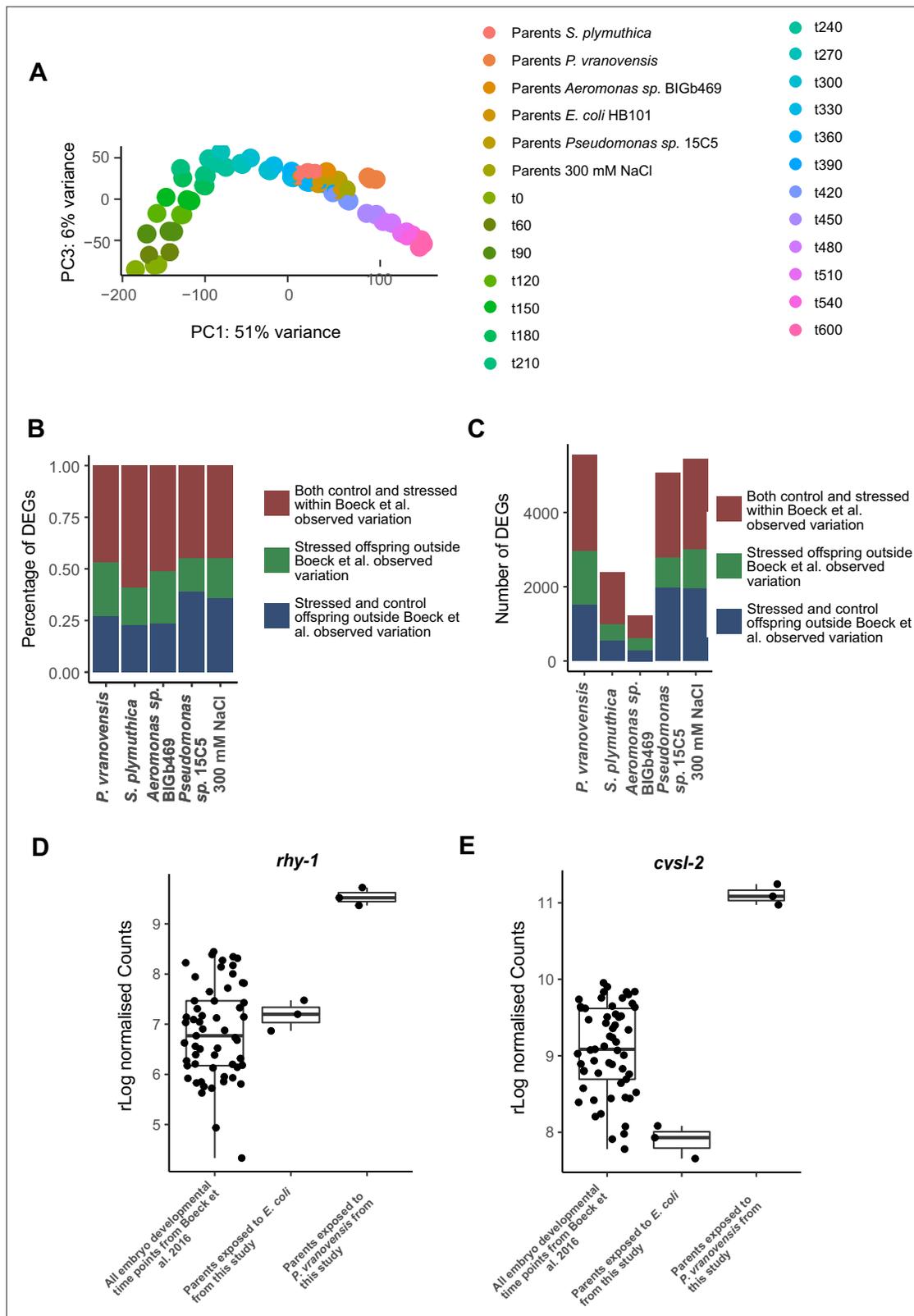
Figure 2—figure supplement 1. Differences in developmental timing are insufficient to explain a majority of the observed differences in gene expression in the offspring of stressed parents. (**A**) PCA of gene expression from *Boeck et al., 2016* compared to RNA-seq data reported in the study. Time points of development are in minutes, *t60* = 60 min postfertilization. (**B**) Percentage of genes differentially expressed in the offspring of parents exposed to different stresses that exhibit DESeq2 normalized counts that fall within or outside one standard deviation of the average normalized

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counts observed throughout all developmental time points from *Boeck et al., 2016*. (**C**) Total number of genes differentially expressed in the offspring of parents exposed to different stresses that exhibit DESeq2 normalized counts that fall within or outside one standard deviation of the average normalized counts observed throughout all developmental time points from *Boeck et al., 2016*. (**D**) *rhy-1* normalized counts from all time points during development from *Boeck et al., 2016*, the offspring of parents exposed to *E. coli* HB101 (this study), or the offspring of parents exposed to *P. vranovensis* (this study). (**E**) *cysl-2* normalized counts from all time points during development from *Boeck et al., 2016*, the offspring of parents exposed to *E. coli* HB101 (this study), or the offspring of parents exposed to *P. vranovensis* (this study).
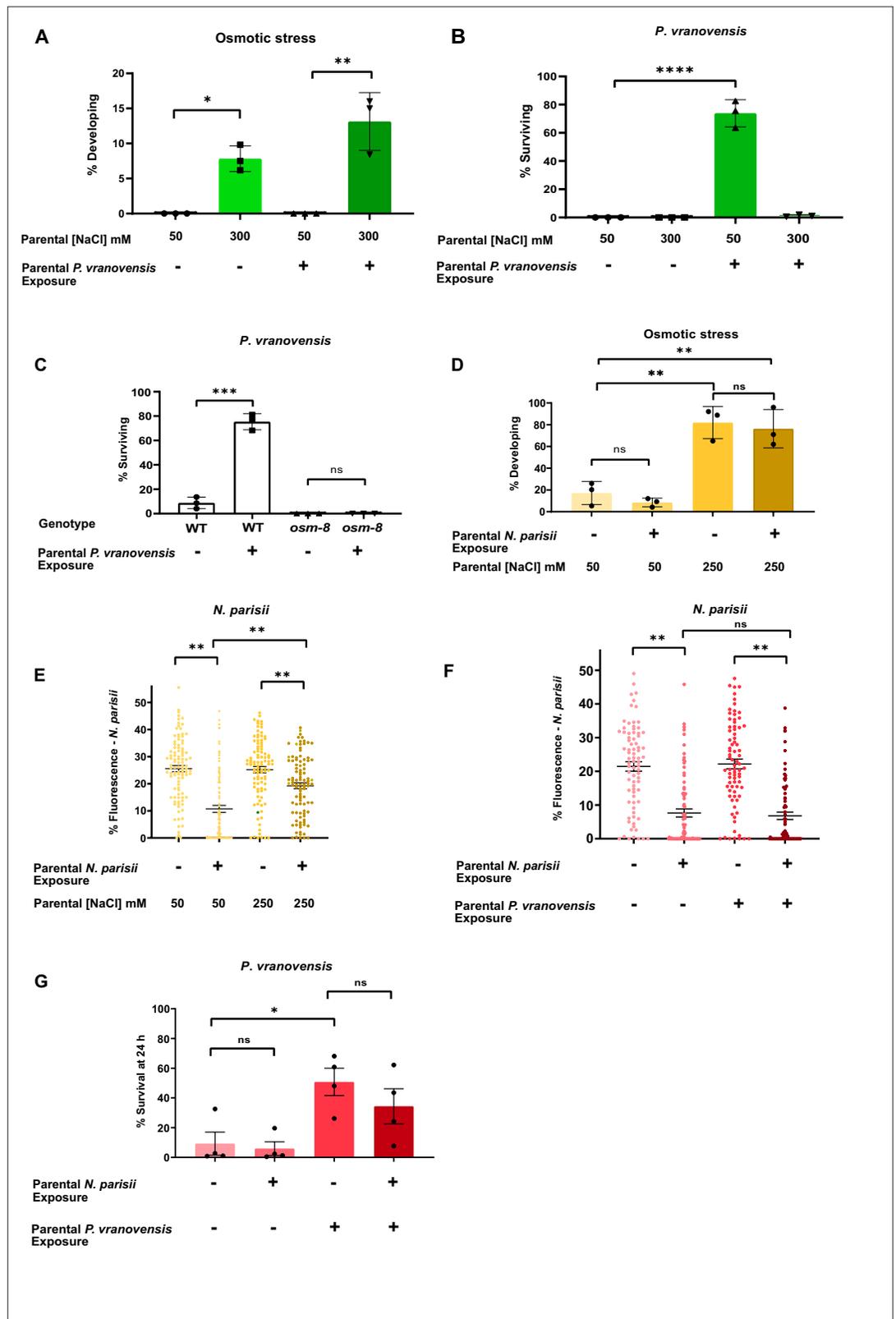
Figure 3. Intergenerational adaptations to stress are stress-specific and have deleterious tradeoffs. (**A**) Percent of wild-type *C. elegans* mobile and developing at 500 mM NaCl after 24 hr. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**B**) Percent of wild-type *C. elegans* surviving after 24 hr of exposure to *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**C**) Percent

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of wild-type and *osm-8(n1518) C. elegans* surviving after 24 hr of exposure to *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**D**) Percent of wild-type *C. elegans* mobile and developing at 420 mM NaCl after 48 hr. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**E**) *N. parisii* parasite burden of individual *C. elegans* after 72 hr (as determined by percentage fluorescence from DY96-stained spores after 72 hr). Data presented as mean values ± s.e.m. *n* = 4 experiments of 25 animals (**F**) *N. parisii* parasite burden of individual *C. elegans* after 72 hr (as determined by percentage fluorescence from DY96-stained spores after 72 hr). Data presented as mean values ± s.e.m. *n* = 3 experiments of 25 animals. (**G**) Percent of wild-type *C. elegans* surviving after 24 hr of exposure to *P. vranovensis* BIGb0446. Data presented as mean values ± s.e.m. *n* = 3 experiments of >100 animals. *p < 0.05, **p < 0.01, ***p < 0.0001, ****p < 0.0001.
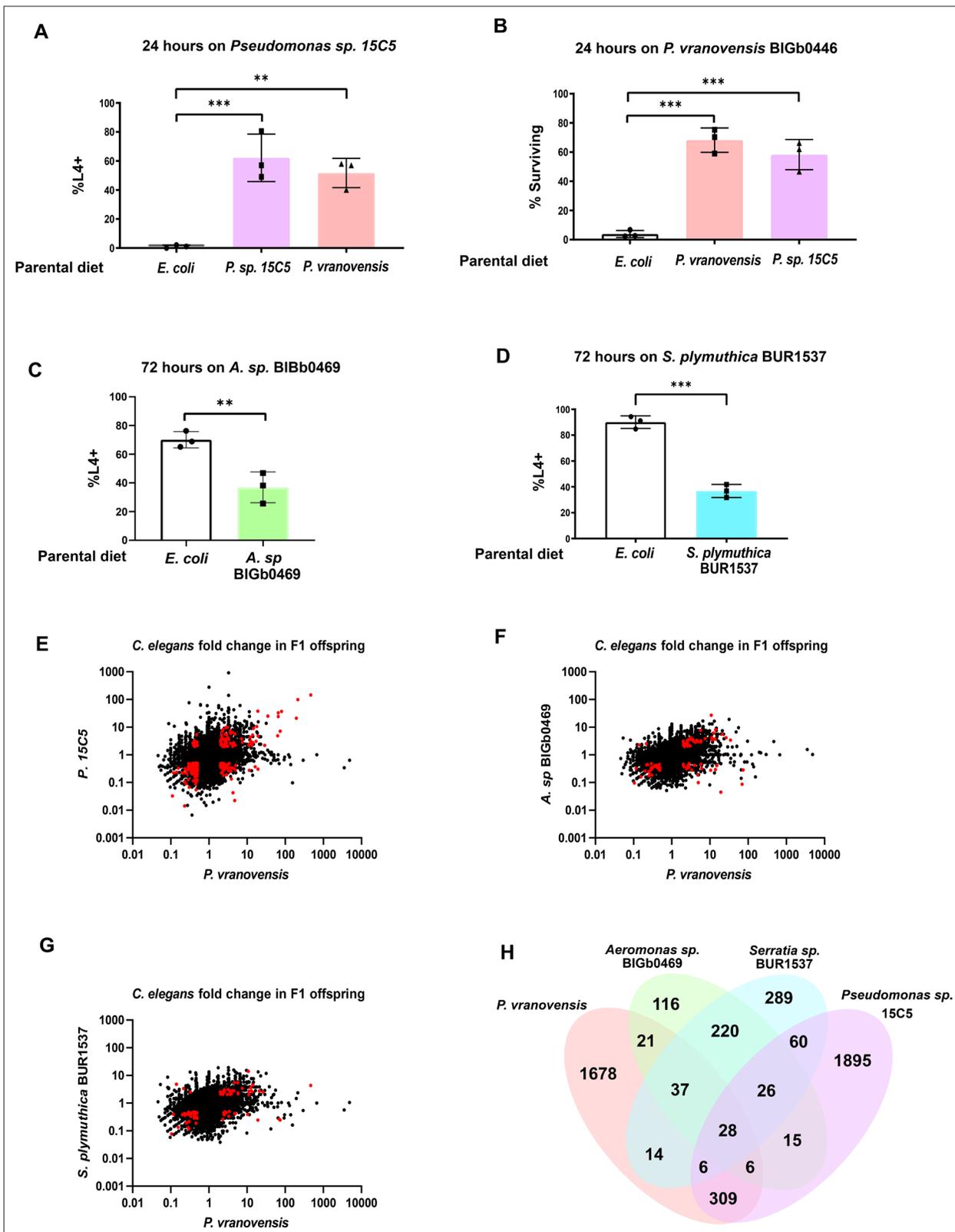
Figure 4. Many of the intergenerational effects of parental exposure to bacterial pathogens on offspring gene expression are pathogen specific. (**A**) Percent of wild-type *C. elegans* that developed to the L4 larval stage after 48 hr of feeding on *Pseudomonas* sp. 15C5. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**B**) Percent of wild-type *C. elegans* surviving after 24 hr of exposure to *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**C**) Percent of wild-type *C. elegans* that developed to the L4 larval stage after 48 hr

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of feeding on *Aeromonas* sp. BIGb0469. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**D**) Percent of wild-type *C. elegans* that developed to the L4 larval stage after 48 hr of feeding on *Serratia plymuthica* BUR1537. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. (**E**) Average fold change of genes in F1 progeny of *C. elegans* fed either *Pseudomonas* sp. 15C5 or *P. vranovensis* BIGb0446 when compared to parents fed *E. coli* HB101. Average fold change from three replicates. Red dots represent genes that exhibit statistically significant (*padj* <0.01) changes in the F1 offspring of parents fed both *Pseudomonas* sp. 15C5 and *P. vranovensis* BIGb0446. (**F**) Average fold change of genes in F1 progeny of *C. elegans* fed either *Aeromonas* sp. BIGb0469 or *P. vranovensis* BIGb0446 when compared to parents fed *E. coli* HB101. Average fold change from three replicates. Red dots represent genes that exhibit statistically significant (*padj* <0.01) changes in the F1 offspring of parents fed both *Aeromonas* sp. BIGb0469 and *P. vranovensis* BIGb0446. (**G**) Average fold change of genes in F1 progeny of *C. elegans* fed either *S. plymuthica* BUR1537 or *P. vranovensis* BIGb0446 when compared to parents fed *E. coli* HB101. Average fold change from three replicates. Red dots represent genes that exhibit statistically significant (*padj* <0.01) changes in the F1 offspring of parents fed both *S. plymuthica* BUR1537 and *P. vranovensis* BIGb0446. (**H**) Venn diagram of the number of genes that exhibit overlapping statistically significant (*padj* <0.01) changes in expression in F1 progeny of *C. elegans* parents fed each different bacterial species. **p < 0.01, ***p < 0.0001.
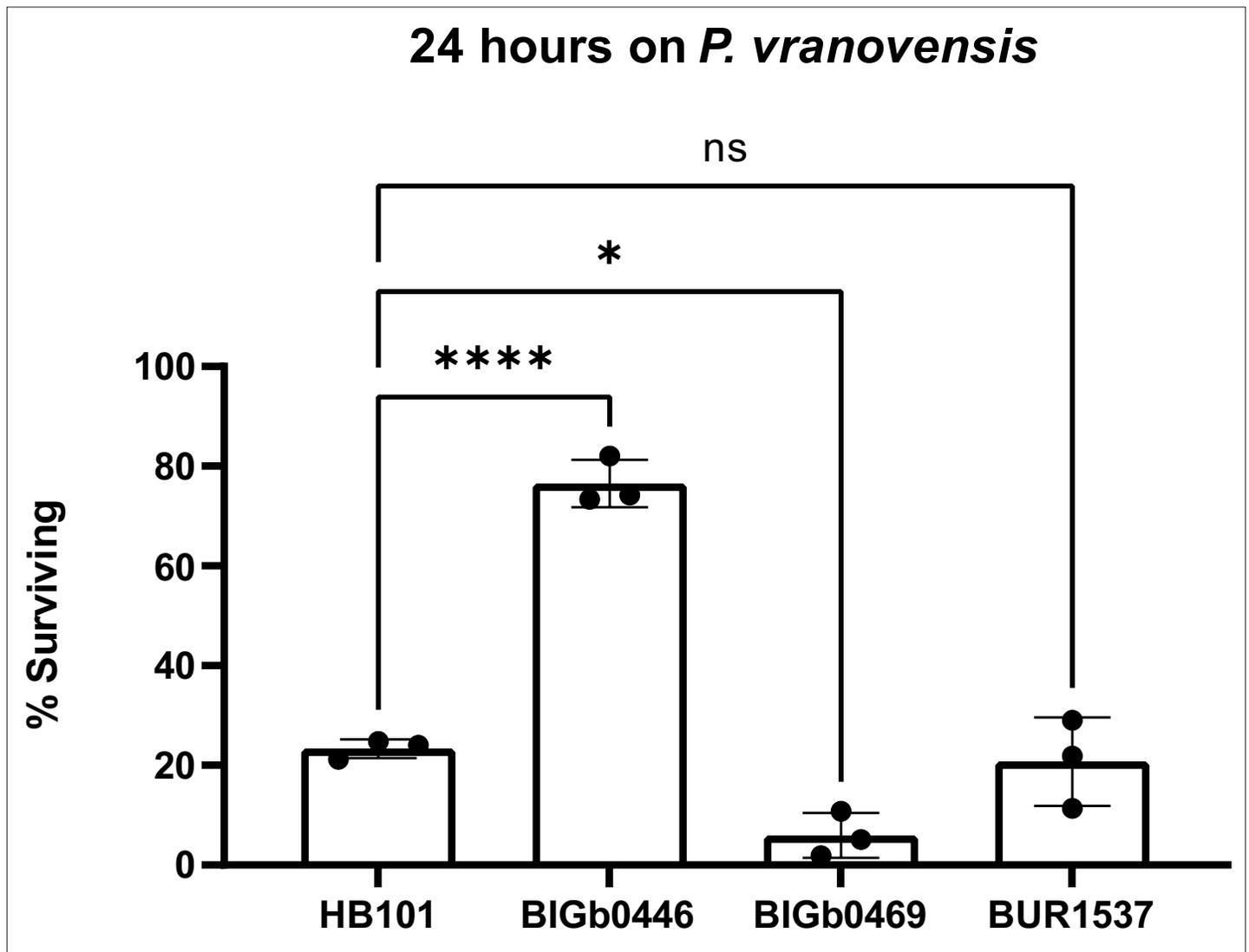
Figure 4—figure supplement 1. Parental exposure to *Aeromonas* sp. BIGb0469 and *S. plymuthica* BUR1537 does not protect offspring from *P. vranovensis*. Percent of wild-type *C. elegans* (N2) animals surviving after 24 hr on plates seeded with *P. vranovensis* BIGb0446. Data presented as mean values ± s.d. *n* = 3 experiments of >100 animals. * p < 0.05, **** p < 0.0001.