

Supplementary figures

Figure S1: The following plot shows that DNA methylation is found all 16 of the largest contigs (putative chromosomes), and that sites showing caste-specific methylation are widely distributed across the methylome. The solid black line shows the density of sites with $>50\%$ DNA methylation, which closely correlates with the density of genes (shown by the dashed black line). The purple line shows the density of sites that we identified as having significant caste-specific methylation ($p < 0.0001$), which also follows the overall distribution of genes closely. The rug plot at the bottom marks methylated sites (grey) and caste-differentially methylated sites (red: QDL-biased, and blue: WDL-biased).

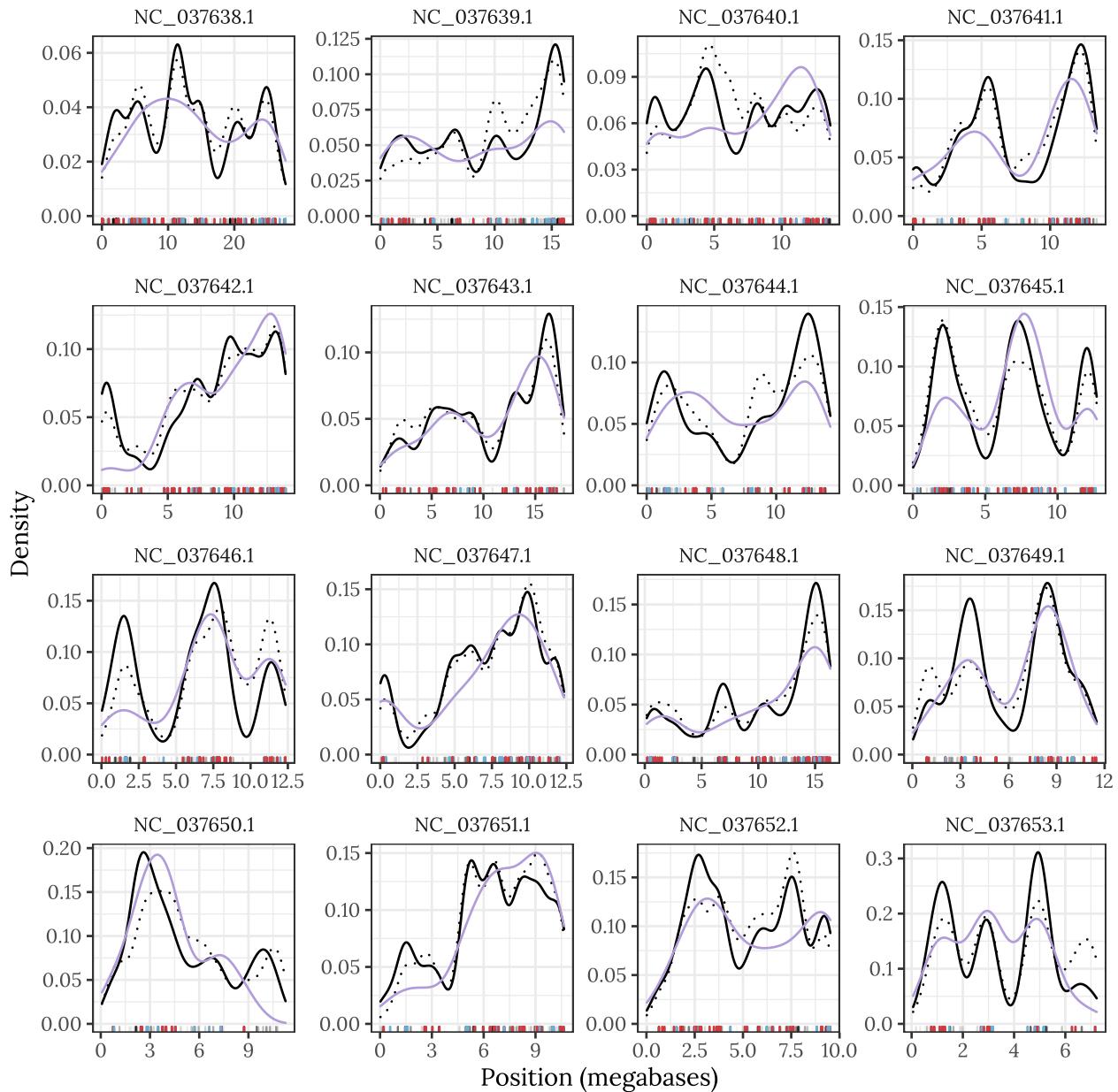


Figure S2: Similar heatmap to Figure 1C, except showing all CpG sites with at least 2.7% methylated sequencing reads (with at least 4x coverage in all 36 samples, n = 110,626 sites). The samples cluster by caste to an extent, though not as strongly as in Figure 1C (which focuses on sites with $\geq 75\%$ methylation), suggesting that is the more-methylated sites that vary the most between castes.

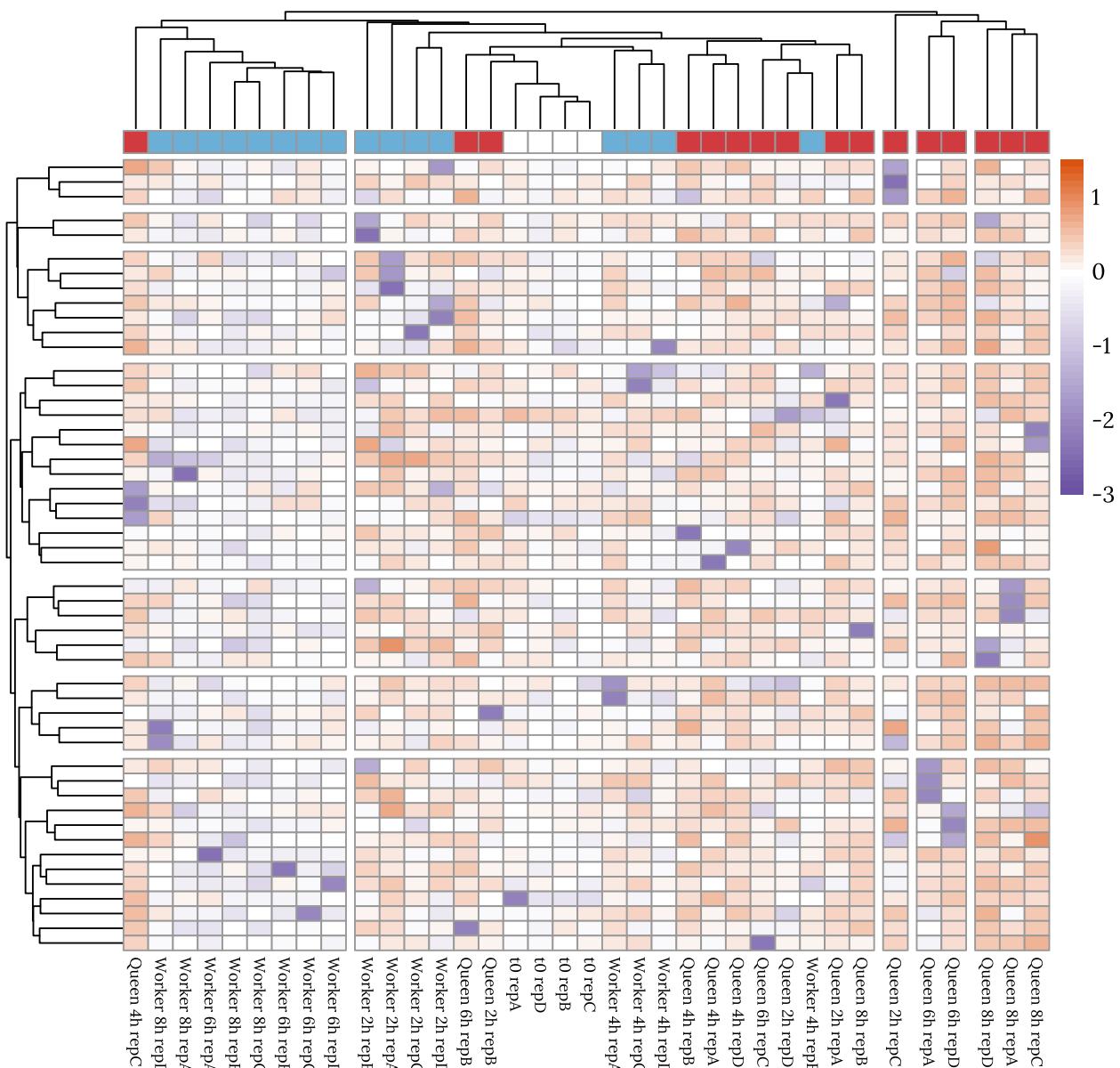


Figure S3: Difference in mean % methylated CpG sites (mCpGs) between time points in QDL (panel A) and WDL (panel B), $\pm 95\%$ confidence intervals (estimated using a linear model); positive values mean higher % mCpGs at the later time point.

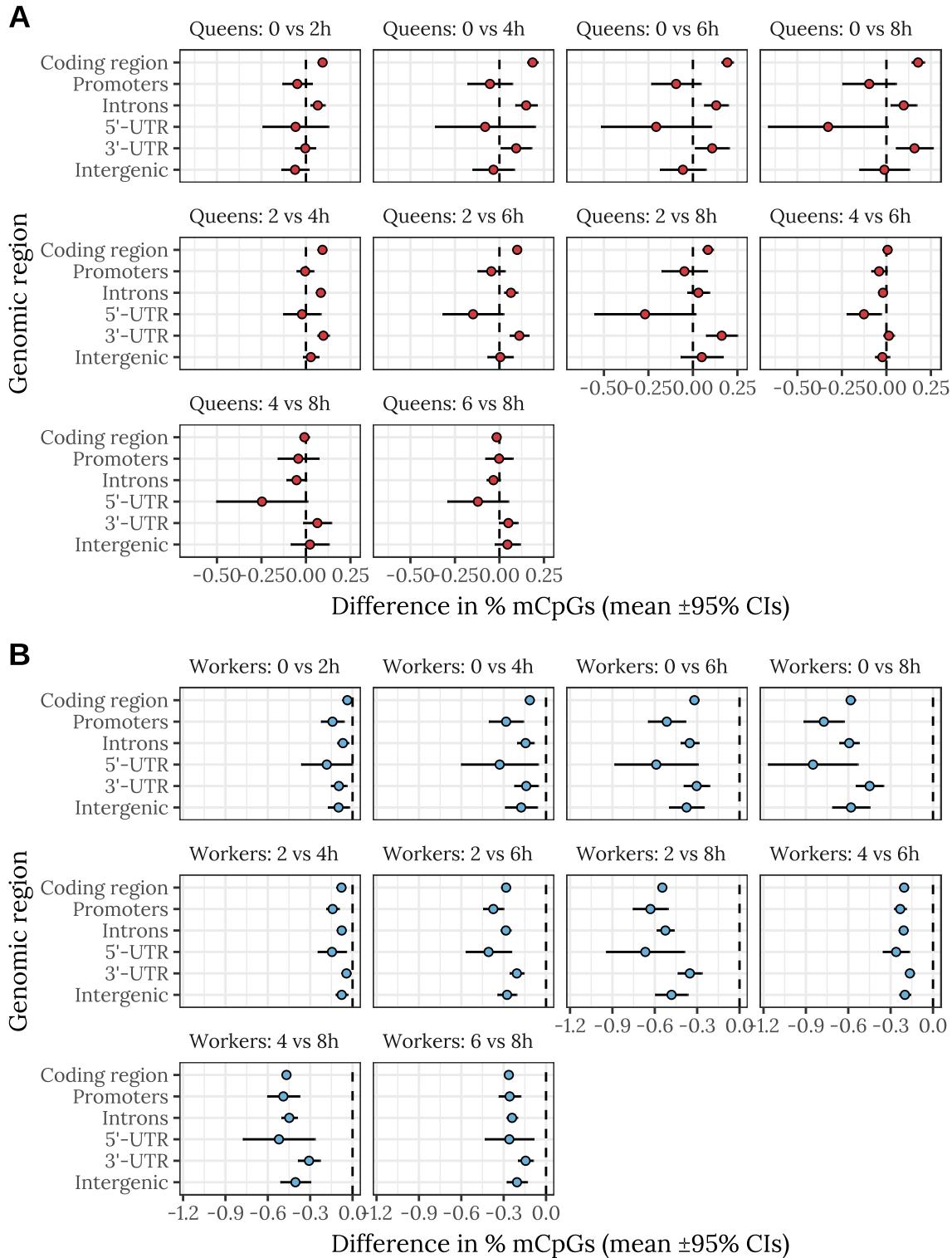


Figure S4: GO terms (and one KEGG term) that were enriched among genes showing QDL- or WDL-biased methylation (as measured by the difference in mean % CpG methylation for all sites in the introns, exons, and promoters of the gene; red: QDL-biased, blue: WDL-biased). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO annotations. All terms passed the cutoff $p < 0.01$ for one or more time points, and asterisks indicate results that remained significant after FDR correction.

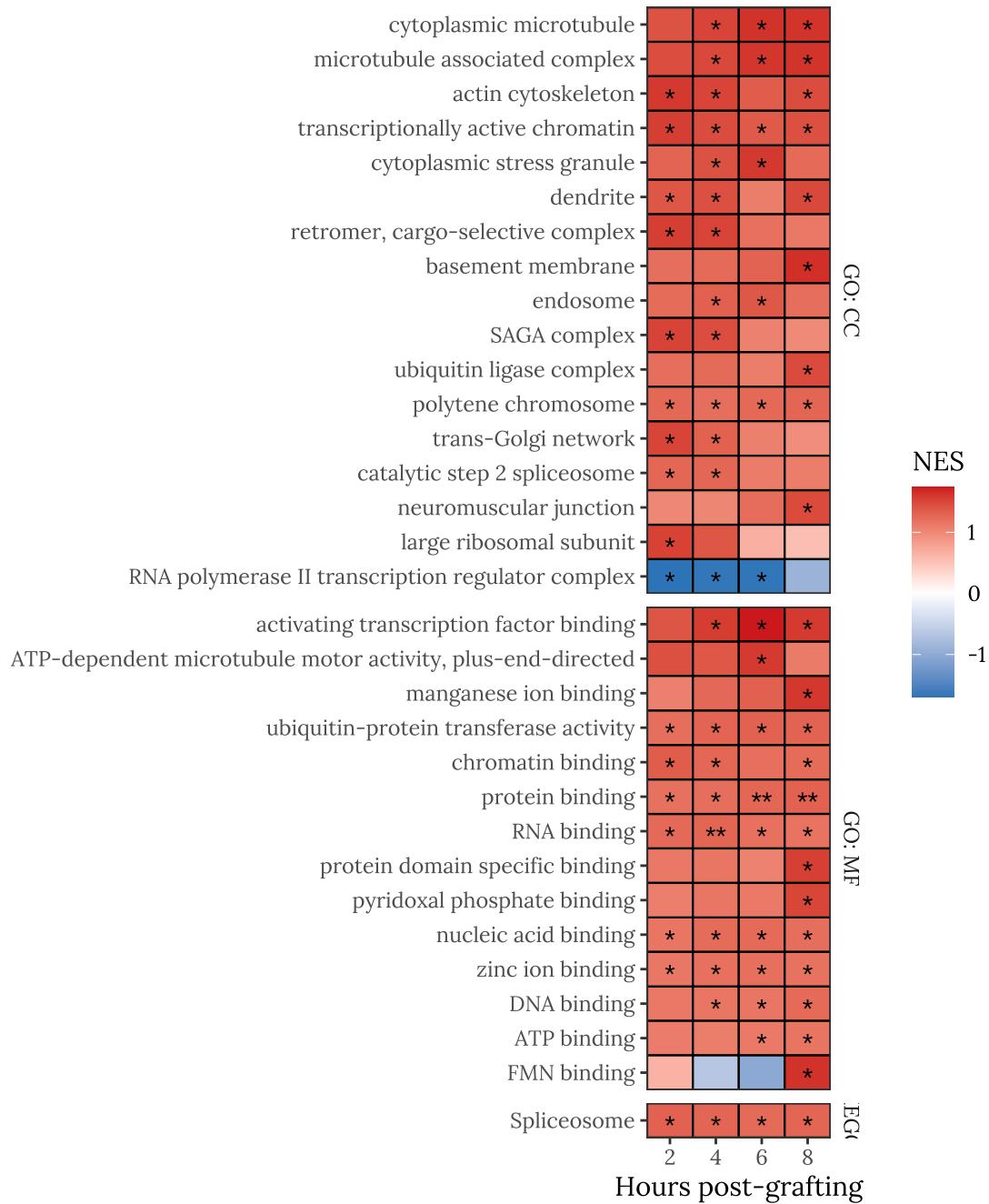


Figure S5: GO and KEGG terms that are enriched among genes showing temporally variable methylation within each treatment group (as measured by the difference in mean % CpG methylation between the 0h and 8h QDL or WDL samples for all sites in the introns, exons, and promoters of the gene). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations. Orange indicates functional categories for which the relevant genes show increasing methylation over time, purple indicates decreasing methylation over time. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.

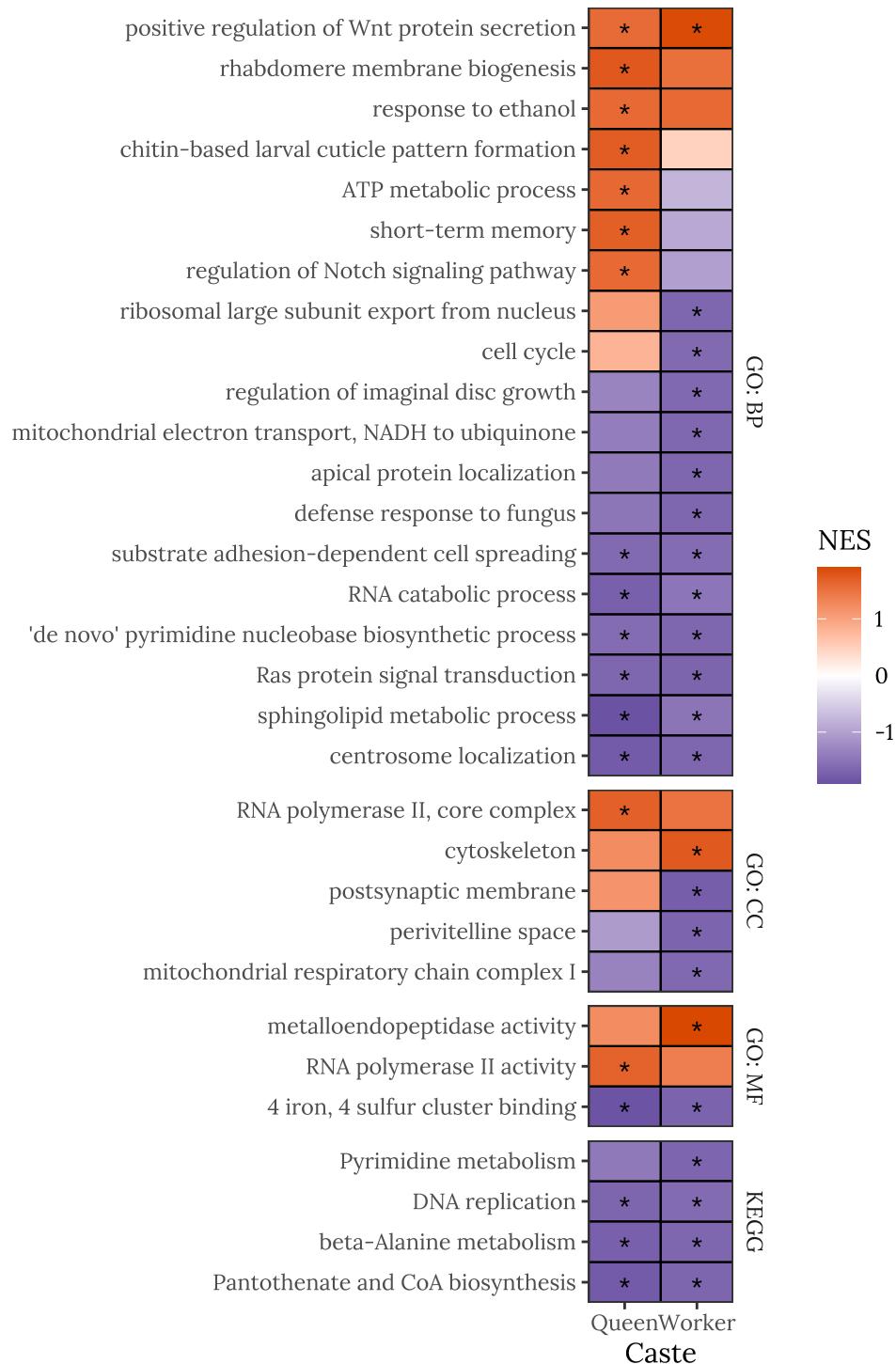


Figure S6: Analysis of the transcriptome data using non-metric multidimensional scaling, which reduces the distance matrix between each sample's transcriptome to three dimensions (variance explained: 96.6%). The plot suggests that queen- and worker-destined larvae have different gene expression profiles and that the transcriptome changes over time, and there is some indication that the caste difference is larger at later times post-grafting into a queen or worker cell.

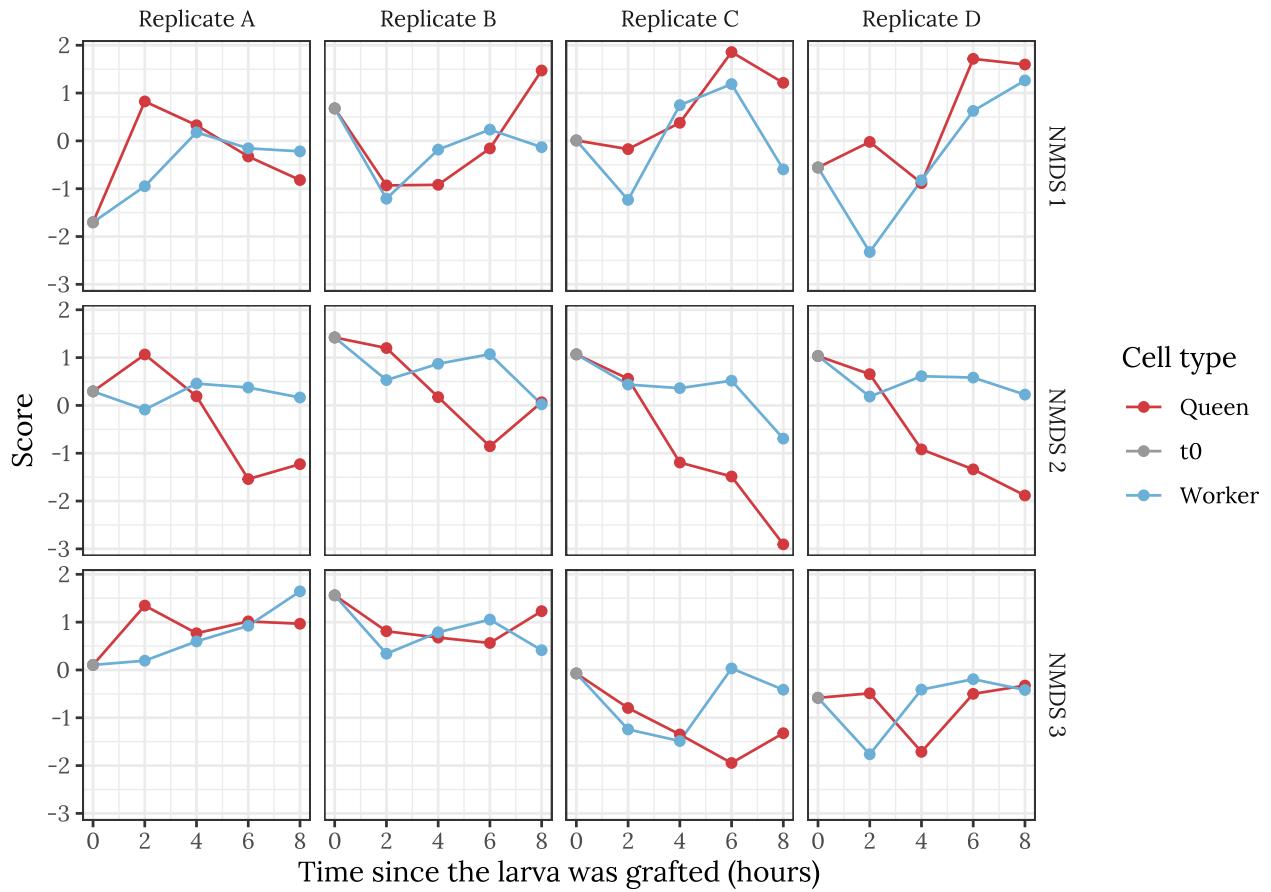


Figure S7: The remaining GO (Biological Process) terms that were enriched among genes showing a caste difference in expression (excluding the 50 terms shown in Figure 3B). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; red: QDL-biased, blue: QDL-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction. The figure is split over two columns to fit onto the page.

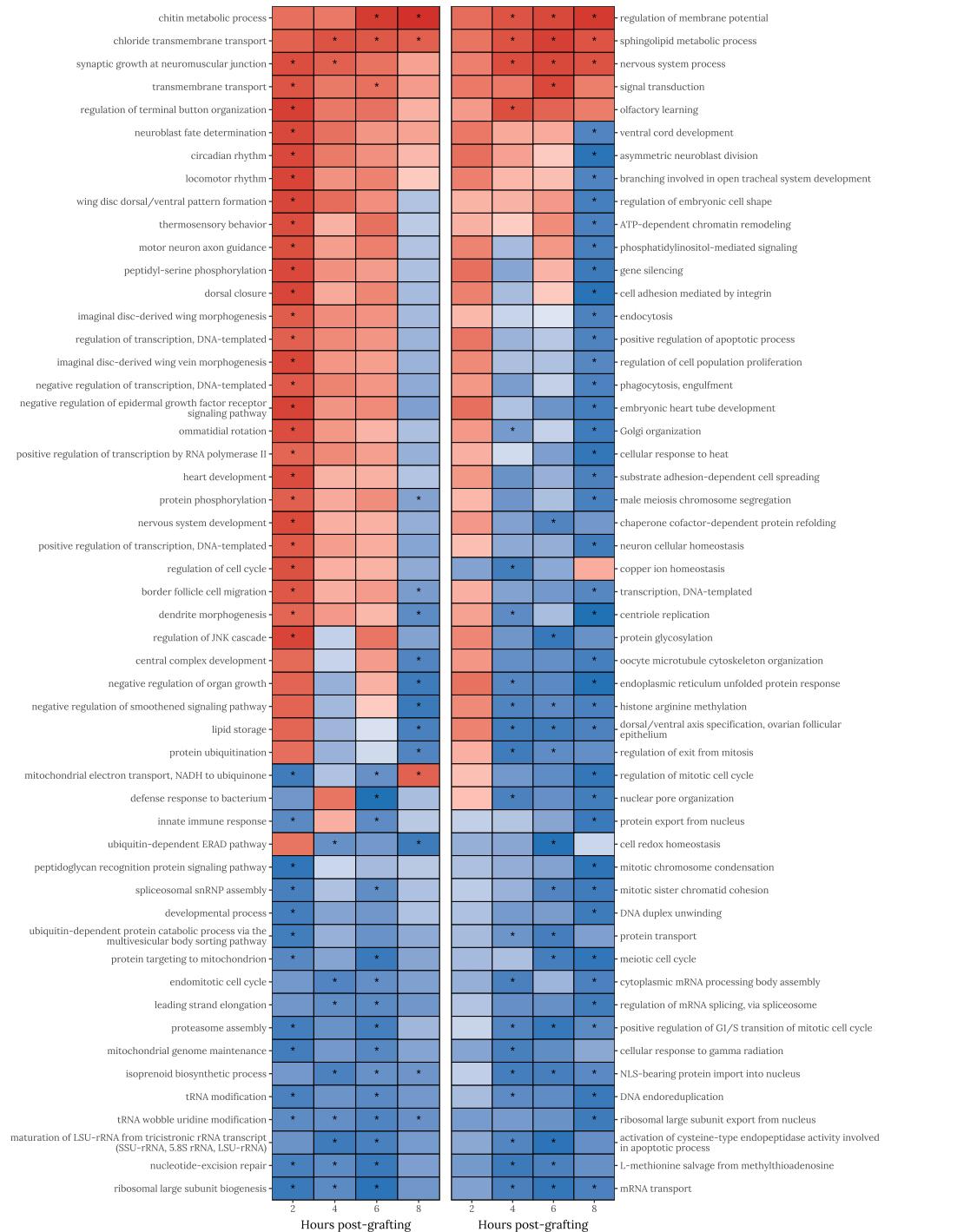


Figure S8: GO (Molecular Function) terms that were enriched among genes showing a caste difference in expression. Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; red: QDL-biased, blue: QDL-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction. The figure is split into two columns to fit onto the page.

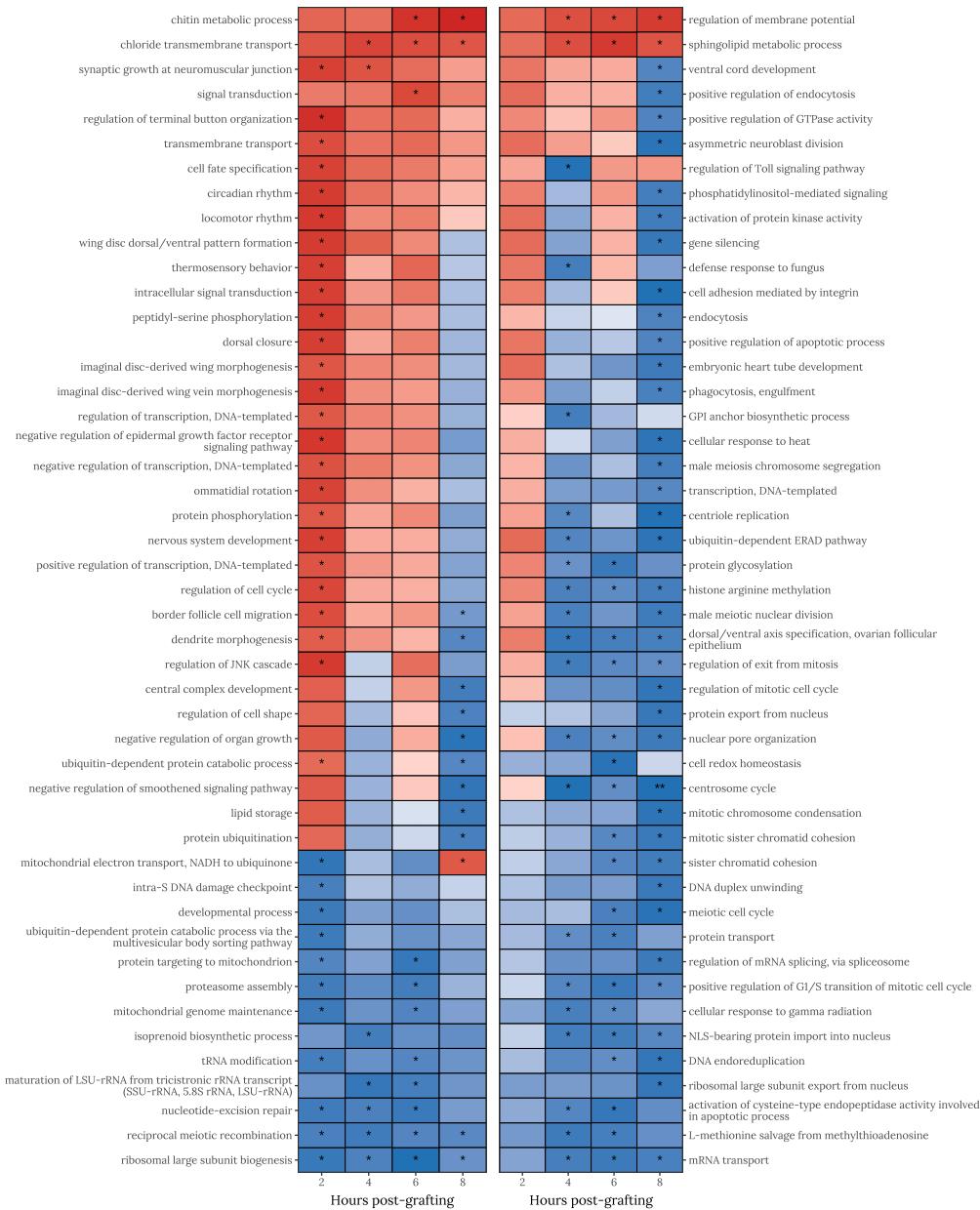


Figure S9: GO (Cellular Component) terms that were enriched among genes showing a caste difference in expression. Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; red: QDL-biased, blue: QDL-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.

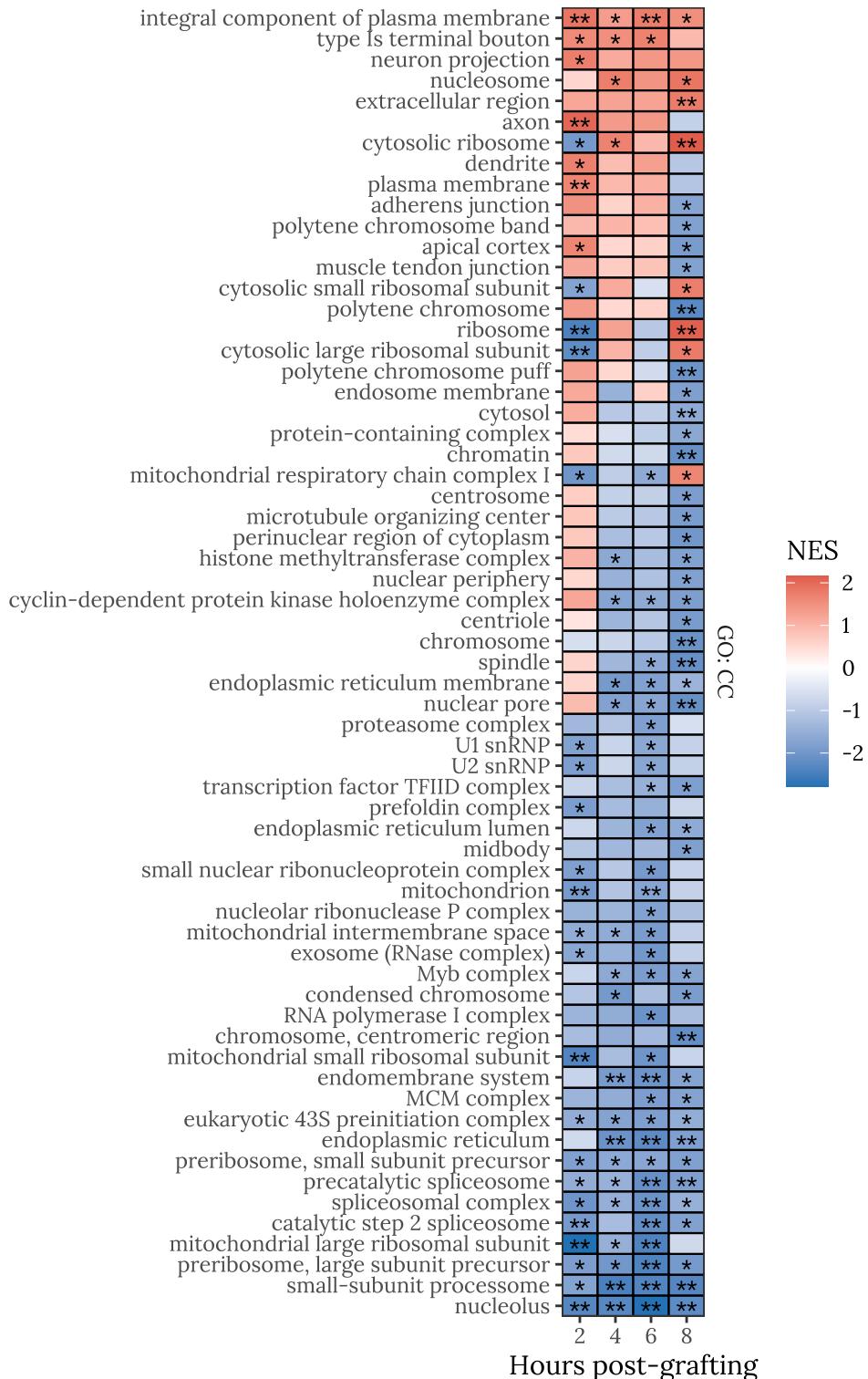


Figure S10: KEGG terms that were enriched among genes showing a caste difference in expression. Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; red: QDL-biased, blue: QDL-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.

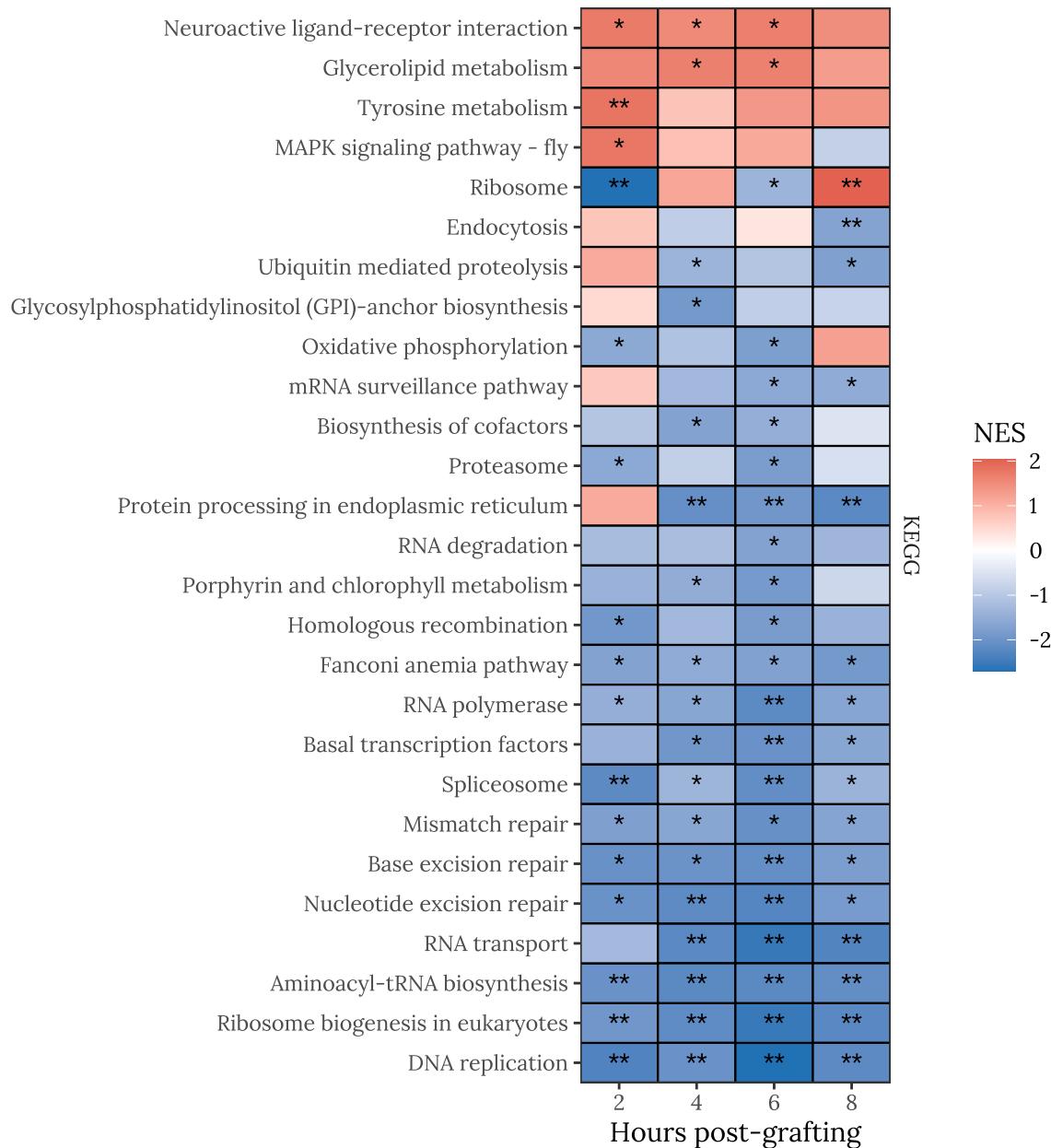


Figure S11: GO (Biological Process) terms that were enriched among genes showing a difference in expression between the 0h and 8h time points in QDL (left) and WDL (right). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; orange: 8h-biased, purple: 0h-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.

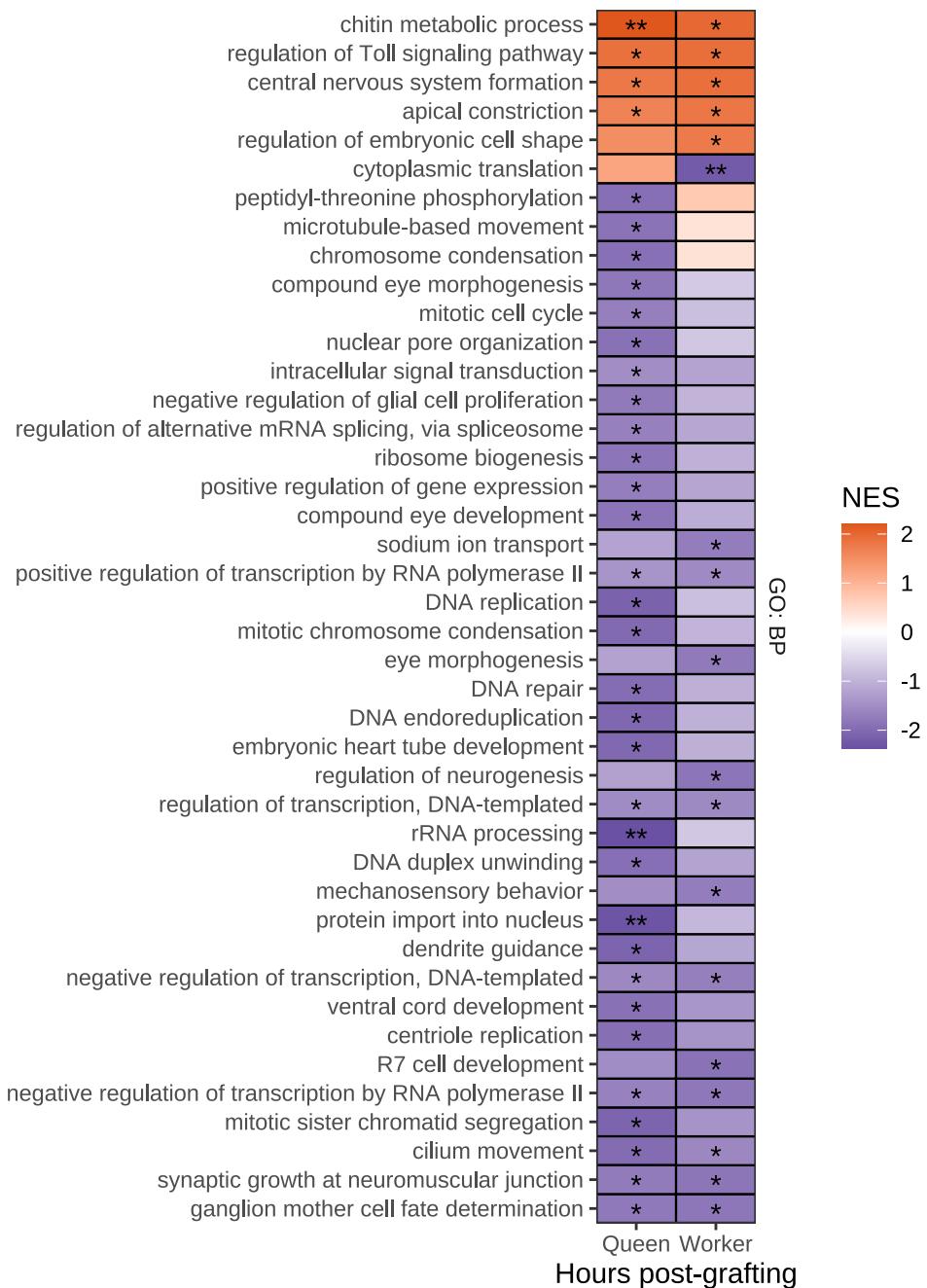


Figure S12: GO (Molecular Function) terms that were enriched among genes showing a difference in expression between the 0h and 8h time points in QDL (left) and WDL (right). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; orange: 8h-biased, purple: 0h-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.

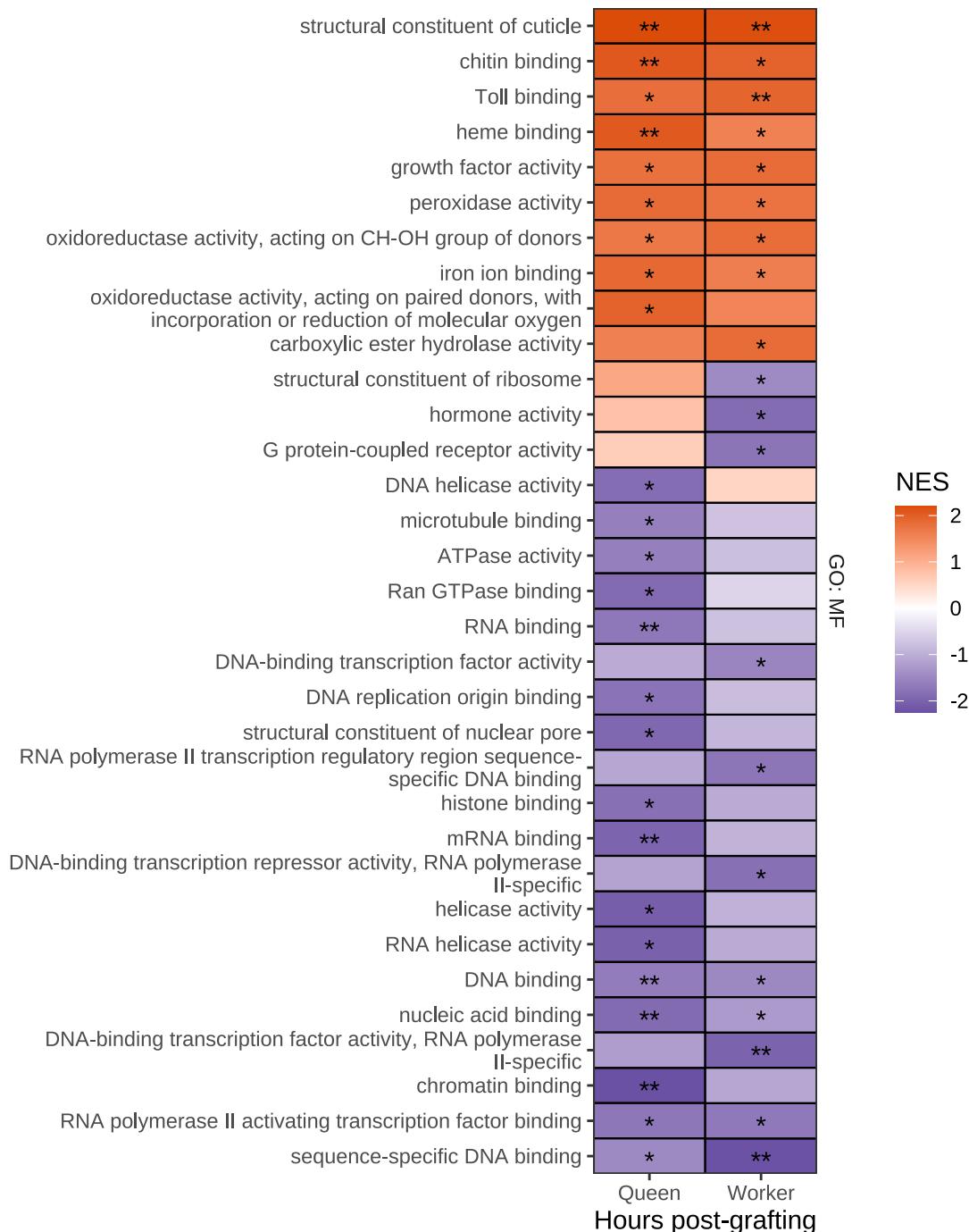


Figure S13: GO (Cellular Component) terms that were enriched among genes showing a difference in expression between the 0h and 8h time points in QDL (left) and WDL (right). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; orange: 8h-biased, purple: 0h-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.

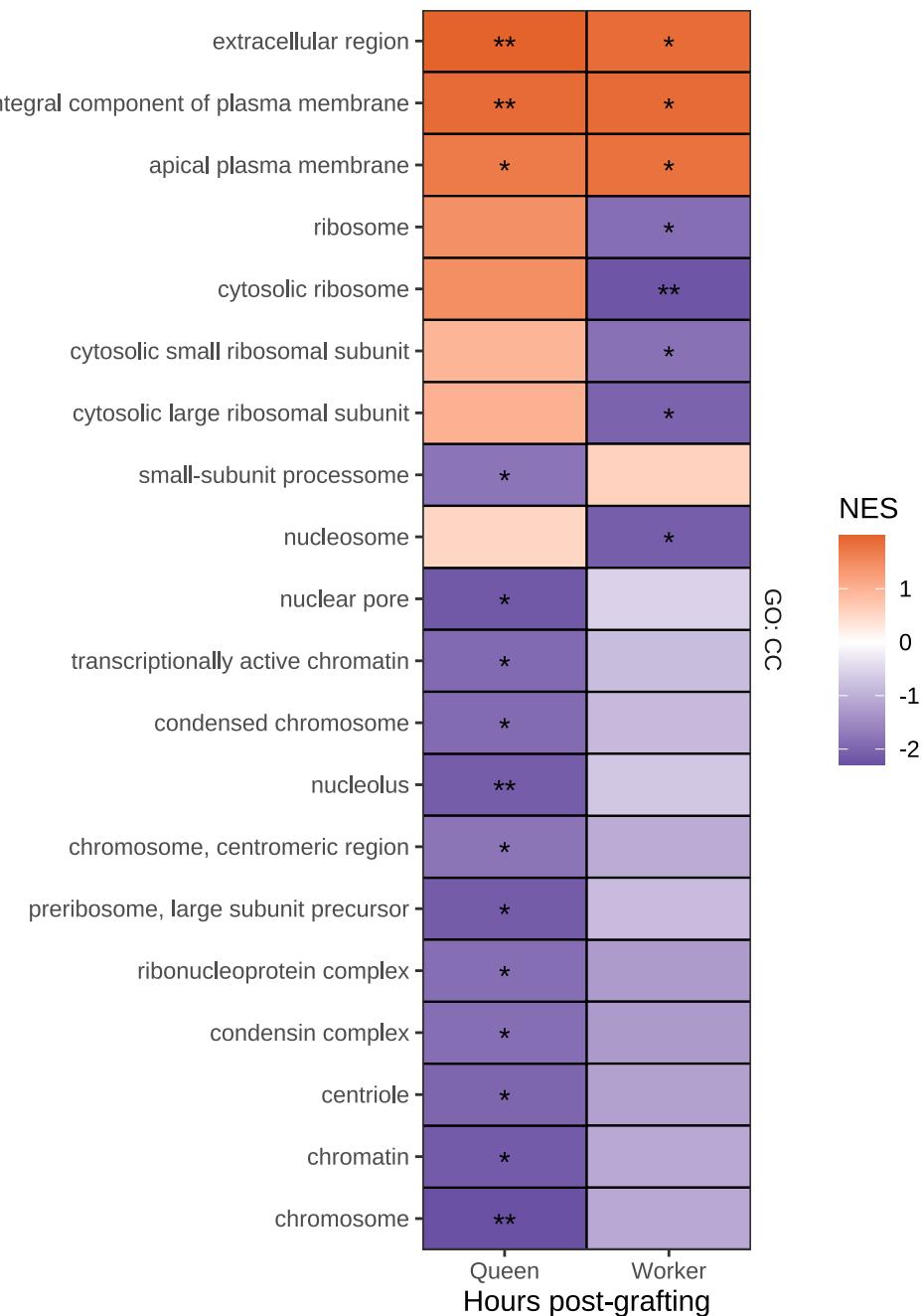
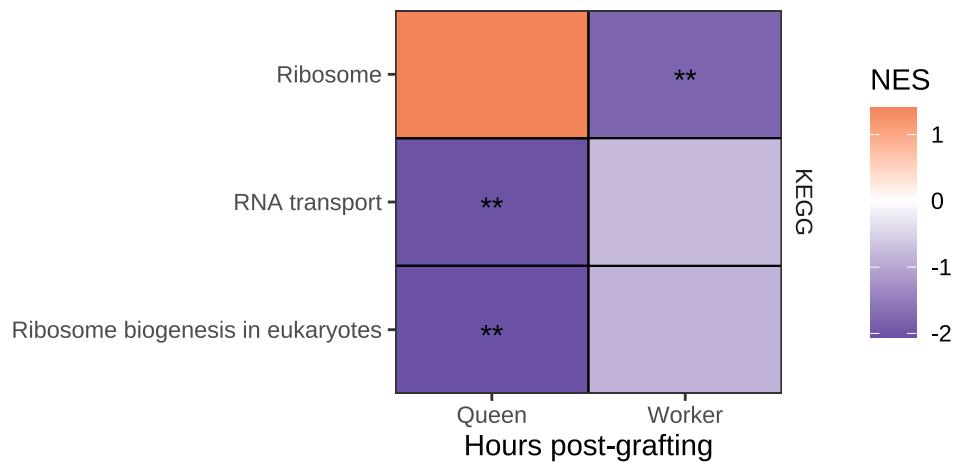


Figure S14: KEGG terms that were enriched among genes showing a difference in expression between the 0h and 8h time points in QDL (left) and WDL (right). Results are from Kolmogorov-Smirnov enrichment tests using our custom GO and KEGG annotations; orange: 8h-biased, purple: 0h-biased. All terms passed the cutoff $p < 0.01$, and asterisks indicate results that remained significant after FDR correction.



Supplementary tables

Table S1: This table is provided separately as an Excel spreadsheet. For each sample, there are columns listing the number of sequencing reads and their mean length (post-trimming), the number and percentage of reads that were mapped, the genome coverage, the bisulphite conversion failure rate (estimated from phage Lambda DNA), the number of reads required to label a site as ‘sufficiently covered’ (defined by the BWASP pipeline), the total number of methylated and unmethylated CpG sites identified (note: this includes only the ‘sufficiently covered’ sites, and sites were designated as methylated if at least one mapped read contained a C), and lastly the estimated % methylated CpG sites (calculated by BWASP).

Table S2: Source, description, and guide to interpretation for each of the gene-level variables in Figure 6.

Variable	Source	Description	Interpretation
% mCpGs in gene body	Present study	Average % mCpGs inside the gene body (averaged over all eight 8h samples). Output by BWASP.	Larger values mean higher average % methylation per CpG site inside the gene body
% mCpGs: QDL vs WDL	Present study	The absolute value of the mean % mCpGs for 8h queen-destined larvae, minus the mean for 8h worker-destined larvae (averaged over all sites within the gene, then averaged over the four replicate samples).	Higher values mean a larger caste difference in % methylation.
Brain / CNS expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila brain and CNS, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Connectivity in the methylome	Present study	'Connectedness' of each gene in the co-methylation network, i.e. the sum of all the correlations between the focal gene and all the other genes. Output by the WGCNA package (which calls this parameter kTotal).	High values mean the gene is strongly co-methylated with many other genes, lower values mean less co-methylation.
Connectivity in the transcriptome	Present study	'Connectedness' of each gene in the co-expression network, i.e. the sum of all the correlations between the focal gene and all the other genes. Output by the WGCNA package (which calls this parameter kTotal).	High values mean the gene is strongly co-expressed with many other genes, lower values mean less co-expression.
Crop expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila crop, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Expression in L2 larvae: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in expression between queen- and worker-destined L2 larvae (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in L3 larvae: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in expression between queen- and worker-destined L3 larvae (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in L4 larvae: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in expression between queen- and worker-destined L4 larvae (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in L5 larvae: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in expression between queen- and worker-destined L5 larvae (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in Pupae: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in expression between queen- and worker-destined pupae (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in the gaster: Male vs Queen	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in gaster tissue from adult males versus adult, mated queens (absolute log fold difference)	Higher values indicate a higher sex bias in gene expression.

Expression in the gaster: Nurse vs Forager	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in gaster tissue from adult nurse workers versus adult, forager workers (absolute log fold difference)	Higher values indicate a higher worker type bias in gene expression.
Expression in the gaster: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in gaster tissue from adult queens versus adult workers (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in the head: Male vs Queen	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in head tissue from mated, adult queens and adult males (absolute log fold difference)	Higher values indicate a higher sex bias in gene expression.
Expression in the head: Nurse vs Forager	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in head tissue from adult nurse workers versus adult, forager workers (absolute log fold difference)	Higher values indicate a higher worker type bias in gene expression.
Expression in the head: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in head tissue from adult queens versus adult workers (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression in the mesosoma: Male vs Queen	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in mesosoma tissue from mated, adult queens and adult males (absolute log fold difference)	Higher values indicate a higher sex bias in gene expression.
Expression in the mesosoma: Nurse vs Forager	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in mesosoma tissue from adult nurse workers versus adult, forager workers (absolute log fold difference)	Higher values indicate a higher worker type bias in gene expression.
Expression in the mesosoma: Queen vs Worker	Warner et al. (2018) Nature Comms 10.1038/s41467-019-10546-w	Difference in gene expression in mesosoma tissue from adult queens versus adult workers (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Expression: QDL vs WDL	Present study	Difference in expression between queen- and worker-destined larva at 8h post-grafting (absolute log fold difference)	Higher values indicate a higher caste bias in gene expression.
Eye expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila eye, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Fat body expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila fat body, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Head expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila head, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Hindgut expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila hindgut, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.

Larval histone modification (H3K27ac): QDL vs WDL	Wojciechowski et al. 2018 Genome Biology, 10.1101/gr.236497.118	The authors collected chromatin from the head tissues of queen and worker larvae, 96h post-hatching, then measured histone modifications using Chip-Seq. We re-calculated the absolute caste difference in the Chip-Seq signal for each gene from the raw data (see wojciechowski_histone_analysis.R).	Higher values indicate a higher caste bias in H3K27ac histone modification.
Larval histone modification (H3K36me3): QDL vs WDL	Wojciechowski et al. 2018 Genome Biology, 10.1101/gr.236497.118	The authors collected chromatin from the head tissues of queen and worker larvae, 96h post-hatching, then measured histone modifications using Chip-Seq. We re-calculated the absolute caste difference in the Chip-Seq signal for each gene from the raw data (see wojciechowski_histone_analysis.R).	Higher values indicate a higher caste bias in H3K36me3 histone modification.
Larval histone modification (H3K4me3): QDL vs WDL	Wojciechowski et al. 2018 Genome Biology, 10.1101/gr.236497.118	The authors collected chromatin from the head tissues of queen and worker larvae, 96h post-hatching, then measured histone modifications using Chip-Seq. We re-calculated the absolute caste difference in the Chip-Seq signal for each gene from the raw data (see wojciechowski_histone_analysis.R).	Higher values indicate a higher caste bias in H3K4me3 histone modification.
Malpighian tubules expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila MTs, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Mated spermatheca expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the mated female Drosophila spermatheca, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Midgut expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila midgut, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Ovary expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the Drosophila ovary, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Positive selection (gamma)	Harpur et al. (2014) PNAS 10.1073/pnas.1315506111	The population size-scaled selection coefficient ‘gamma’ on nonsynonymous mutations, calculated from a Bayesian McDonald-Kreitman test.	Negative gamma denotes purifying selection and positive gamma denotes positive selection

Rectal pad expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila rectal pad, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Salivary gland expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila salivary gland, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Thoraciocoabdominal ganglion expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the female Drosophila TAG, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Virgin spermatheca expression	Leader et al. (2018) Nucl acids Res 10.1093/nar/gkx976	Expression level (FPKM) of the Drosophila 1-to-1 ortholog of the focal gene in the virgin female Drosophila spermatheca, as a proportion of the highest FPKM for the focal gene among the tissues listed here.	Higher values mean the expression level is relatively high in the focal tissue.
Worker expression: Brood pheromone vs control	Ma et al. (2019) BMC Genomics 10.1186/s12864-019-5923-7	Difference in gene expression in worker bees exposed to 'brood pheromone' (a blend of 10 chemicals) versus a control (absolute logFC)	Larger values indicate a greater difference in gene expression between the brood pheromone treatment and the control.
Worker expression: doublesex RNAi vs control	Velasque et al (2018) bioRxiv. https://doi.org/10.1101/314492	Change in gene expression in the dsx RNAi treatment group relative to the control, expressed as absolute Log fold change. Calculated from the raw data using EBSeq; see <code>create_merged_data.R</code>	Larger values indicate a greater difference in gene expression between the doublesex RNAi treatment and the control.
Worker expression: EBO pheromone vs control	Ma et al. (2019) BMC Genomics 10.1186/s12864-019-5923-7	Difference in gene expression in workers exposed to the pheromone EBO ((E)-beta-ocimene) versus a control (absolute logFC)	Larger values indicate a greater difference in gene expression between the EBO treatment and the control.
Worker expression: Pollen foragers vs Nectar foragers	Ma et al. (2019) BMC Genomics 10.1186/s12864-019-5923-7	Difference in gene expression in brain tissue from adult pollen-foraging workers versus nectar-forager workers (absolute log fold difference)	Higher values indicate a higher worker type bias in gene expression.
Worker expression: Queen pheromone vs control	Holman et al. (2019) Nature Comms 10.1038/s41467-019-09567-2	Difference in gene expression in worker bees exposed to 'queen pheromone' (a blend of 5 chemicals) versus a control (absolute logFC)	Larger values indicate a greater difference in gene expression between the queen pheromone treatment and the control.