

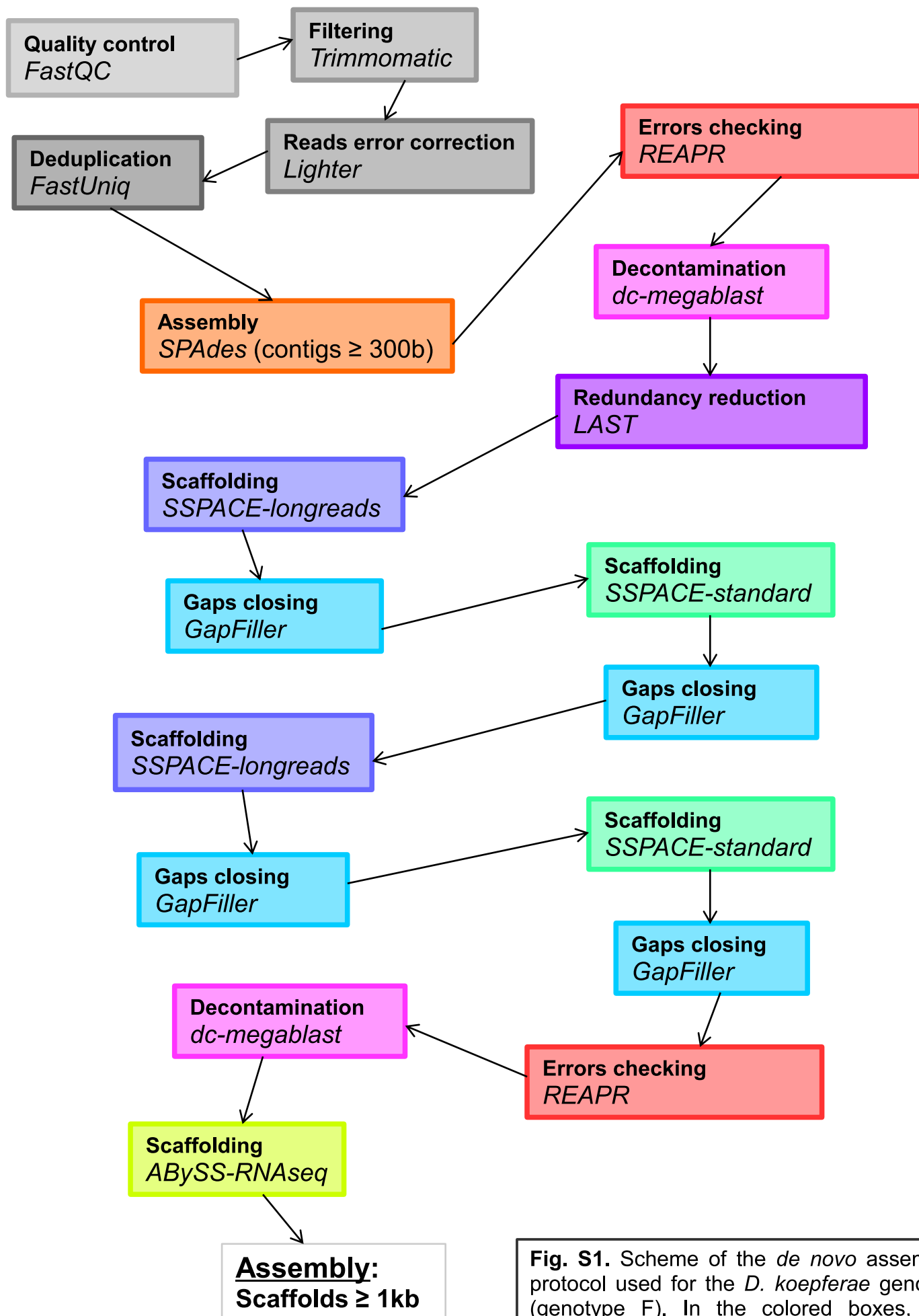
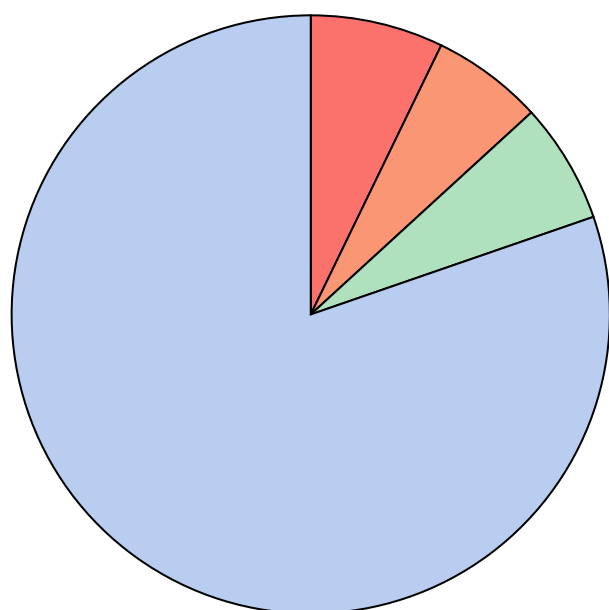


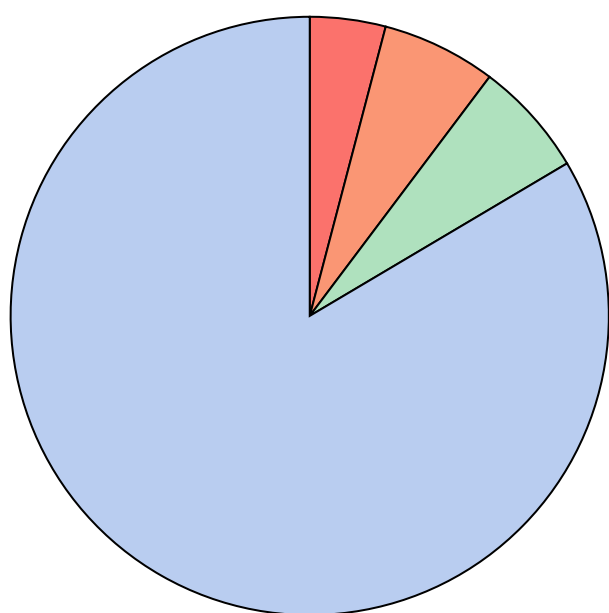
Fig. S1. Scheme of the *de novo* assembly protocol used for the *D. koepferae* genome (genotype F). In the colored boxes, the programs used are named in italics.



***D. buzzatii* (reference)**

Total genes: 13567

- Annotated: 10983 (80%)
- With GO terms: 883 (7%)
- Blast only: 816 (6%)
- No Blast: 975 (7%)



***D. koepferae* (genotype F)**

Total genes: 14134

- Annotated: 11804 (84%)
- With GO terms: 578 (4%)
- Blast only: 873 (6%)
- No Blast: 879 (6%)

Fig. S2. Data distribution chart for the functional annotation pipeline with Blast2GO. "Annotated" depicts the amount of genes finally annotated at the end of the pipeline; "With GO terms" depicts the amount of genes with GO terms association that did not exceed the threshold of the default parameters to be tagged as annotated, "Blast Only" depicts the amount of sequences with a positive Blast that did not mapped to GO terms; "No Blast" depicts the amount of genes without a positive blast for the default parameters.

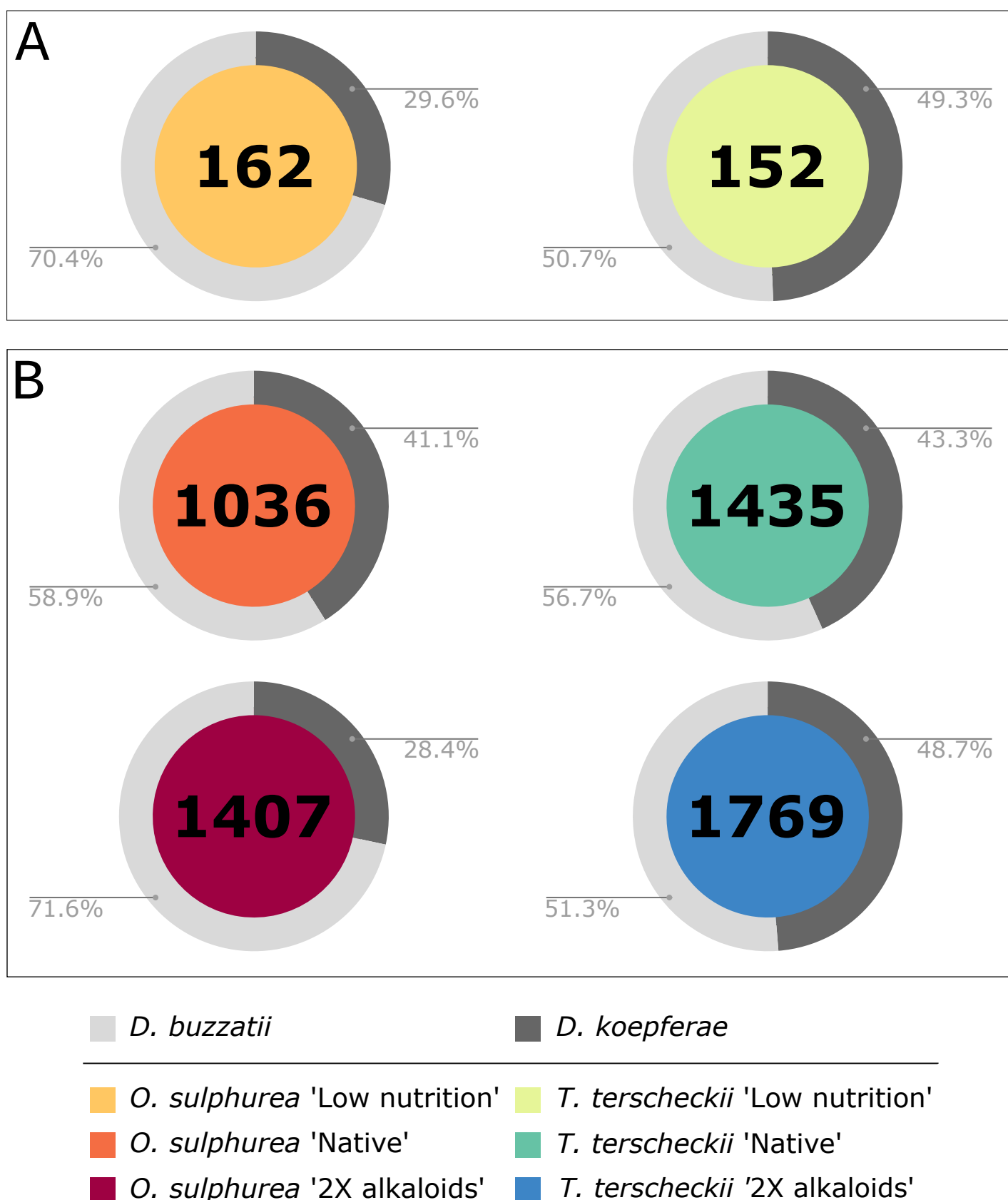


Fig. S3. Doughnuts plots representing the proportion of the DE genes comparatively overexpressed in each species during exploratory INTER-specific comparisons involving treatments with two (A) and three (B) biological replicates. The number in the centre corresponds to total DE genes, the portion and percentage corresponds to overexpressed genes in *D. buzzatii* or *D. koepferae*.

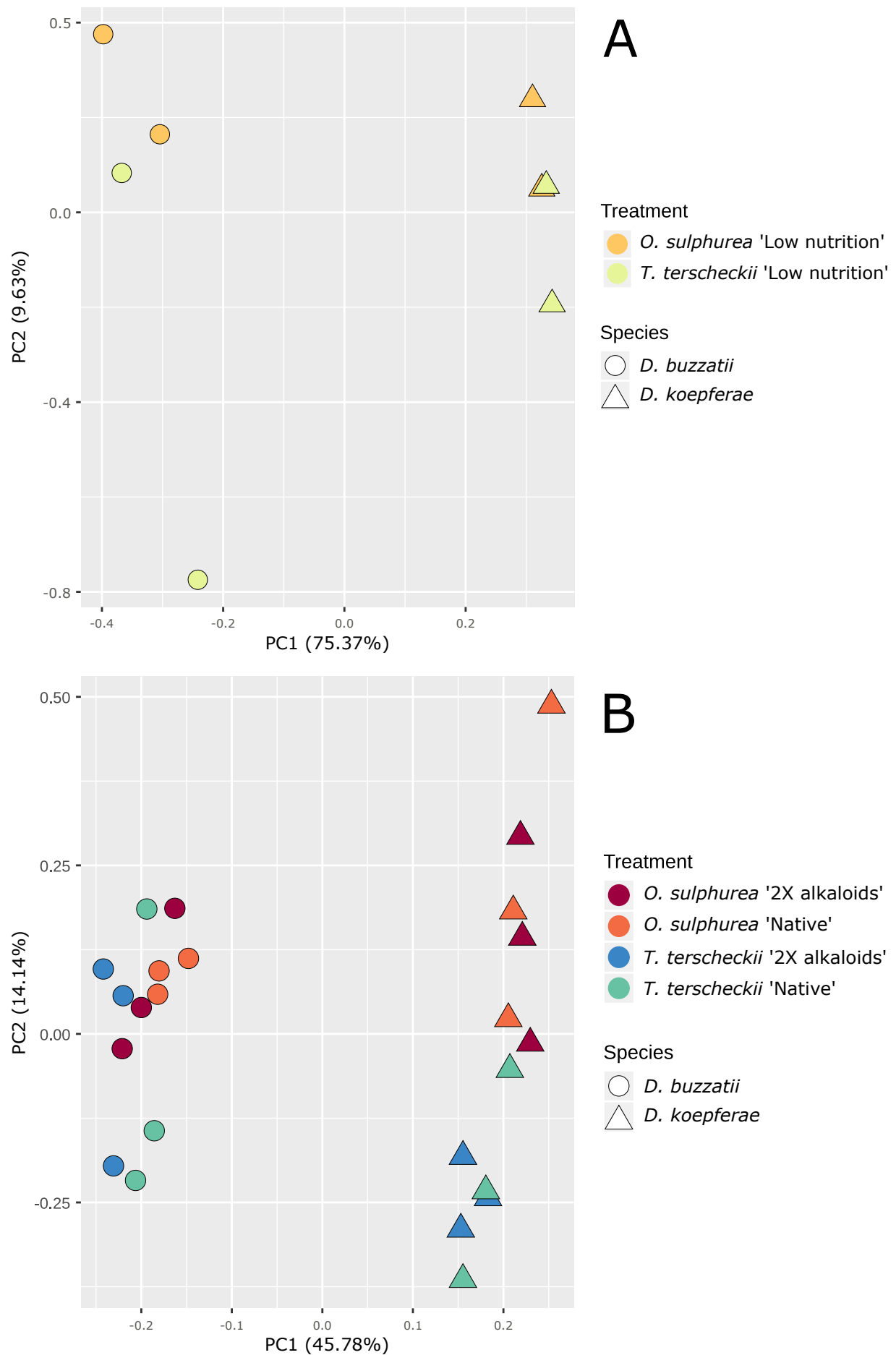
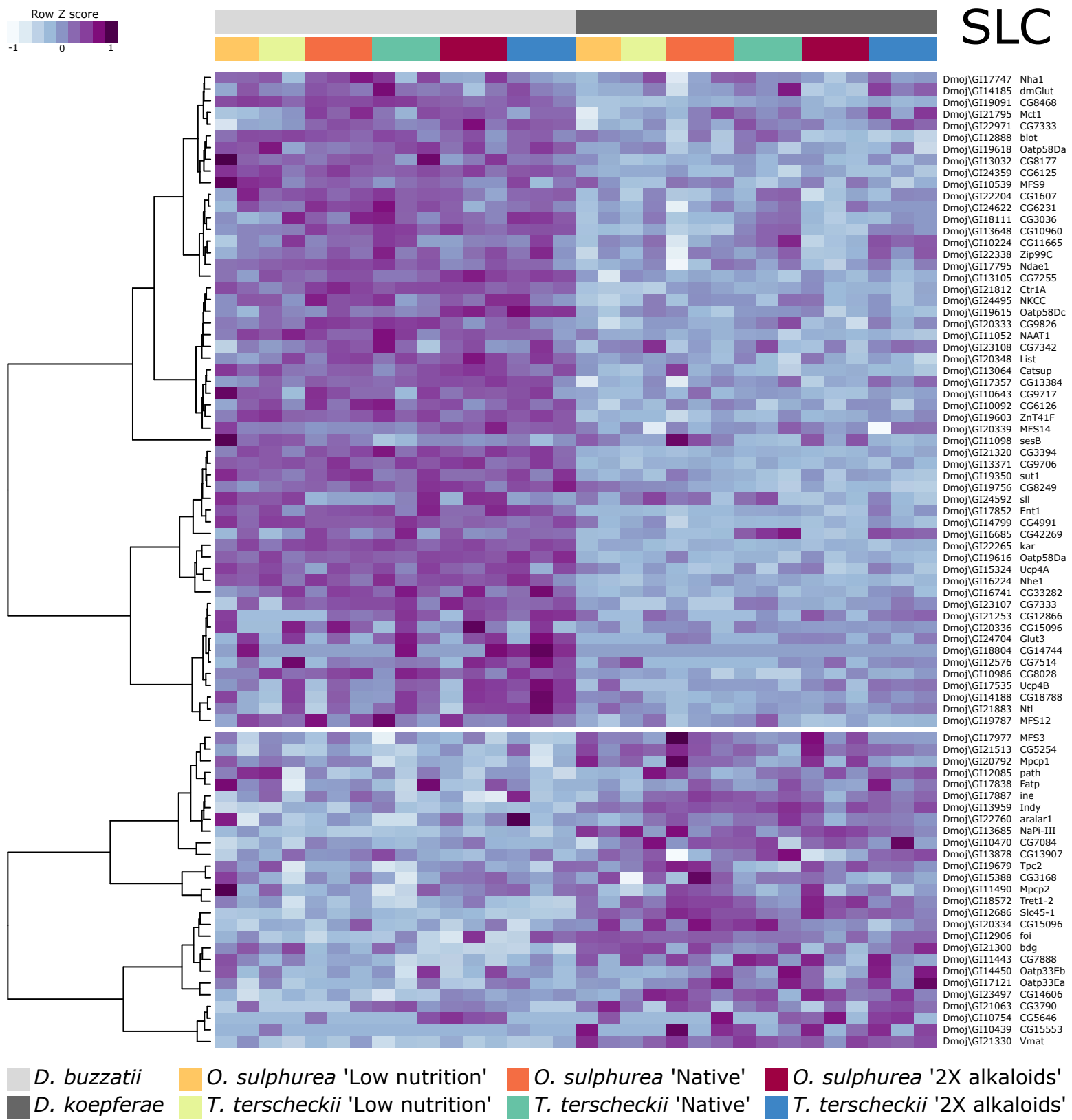


Fig. S4. Principal component analysis to visualize patterns of gene expression in exploratory INTER-specific analysis. **A.** Treatments without nutritional supplementation (three-replicate treatments). Variance explained by the axis: 85%. **B.** Nutritionally-supplemented treatments (two-replicate treatments). Variance explained by the axis: ~60%.

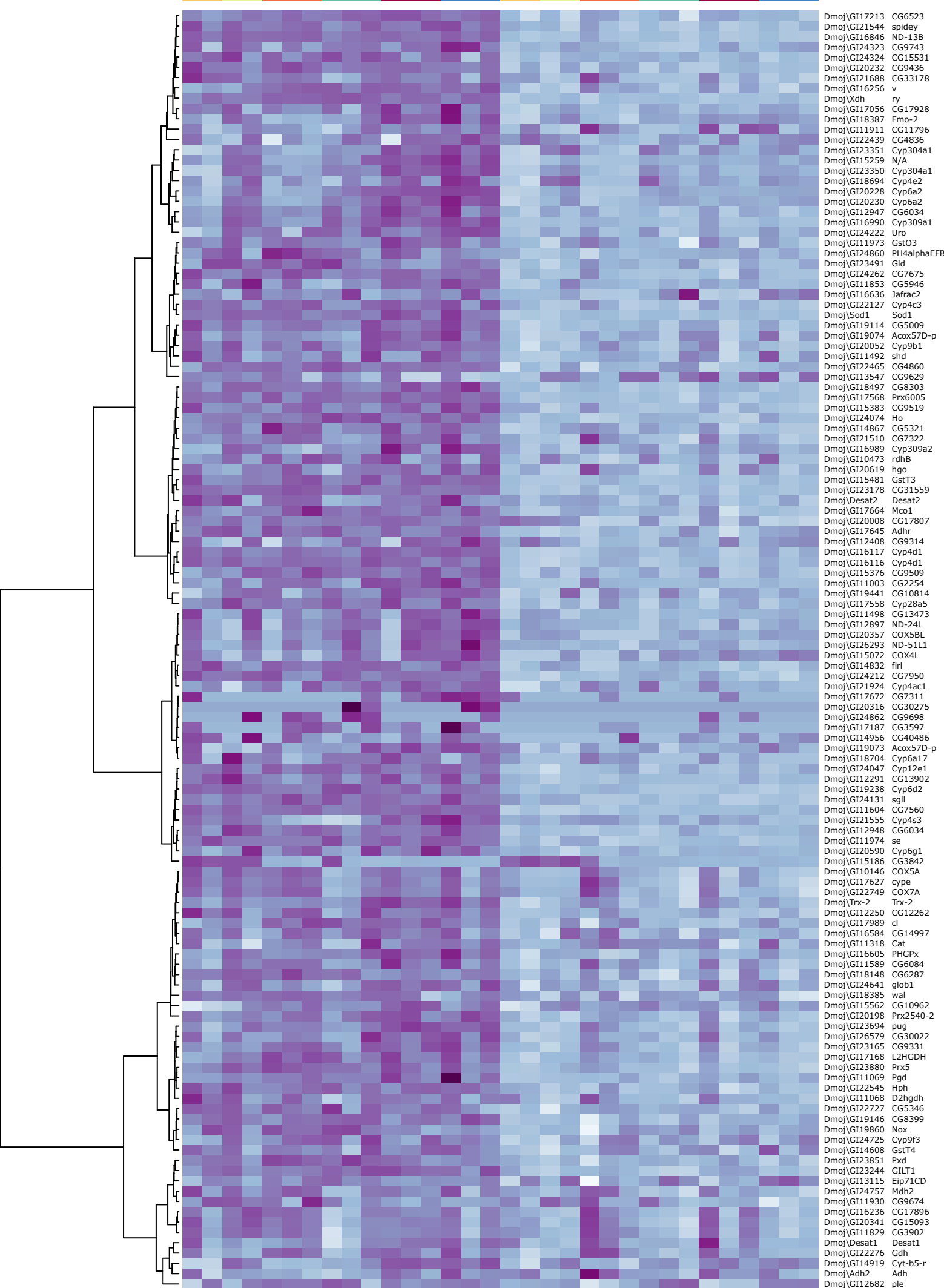
Fig. S5. Differentially expressed genes in exploratory INTER-specific analysis across treatments involved in the four phases of general xenobiotic transport and metabolism, corresponding mainly to six enzymatic groups: Solute carriers (SLC) transporters involved in Phase 0; Carboxylesterases (CE) and Oxidoreductases (OXR) in Phase I; Glutathione S-transferases (GST) and Glycosyltransferases (GT) in Phase II; and ATP-binding cassette (ABC) transporters in Phase III. Genes are identified by the respective *D. melanogaster* homolog. Row dendrograms are not shown. Heatmaps are split by species general overexpression for better visualization.

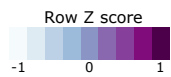


Row Z score

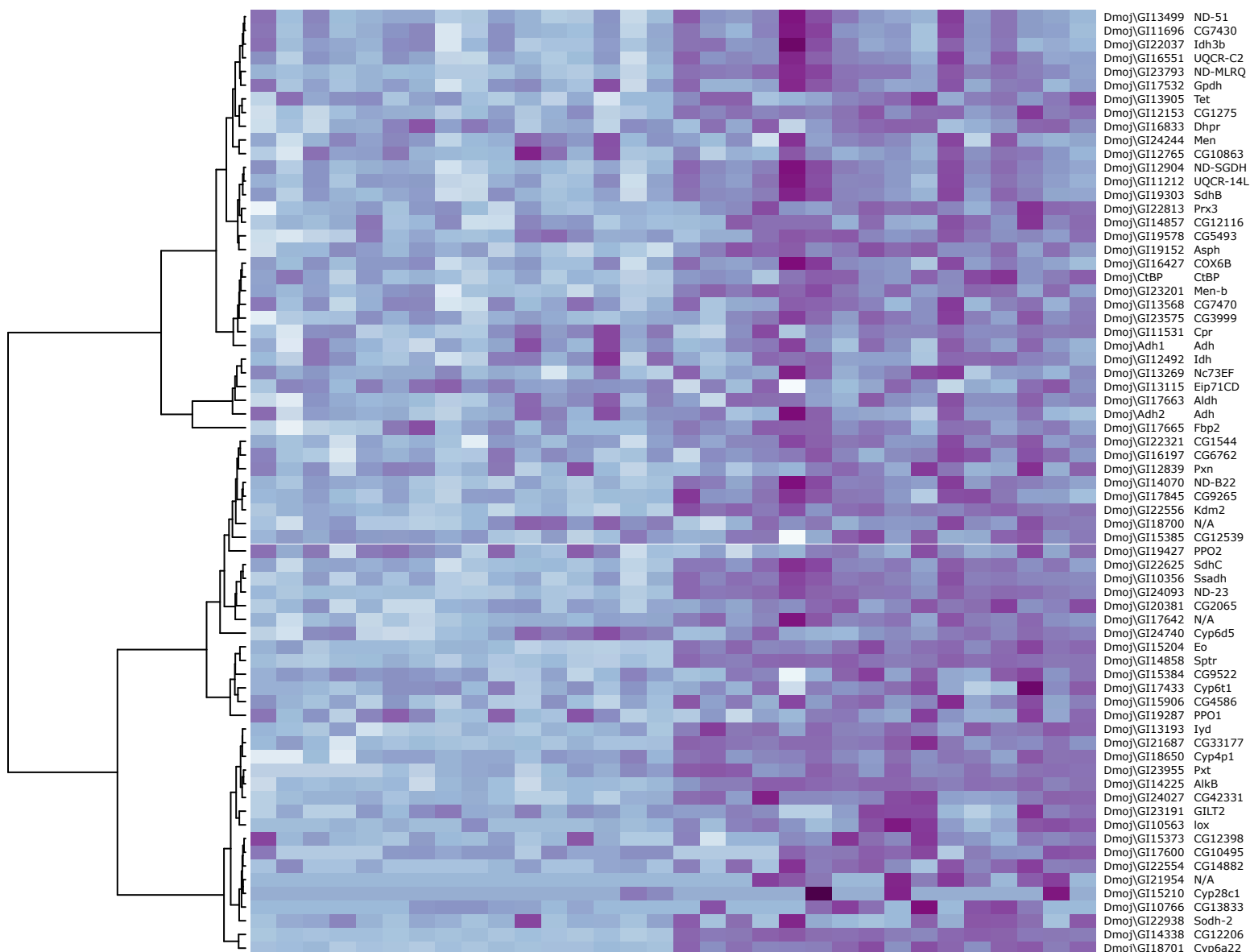


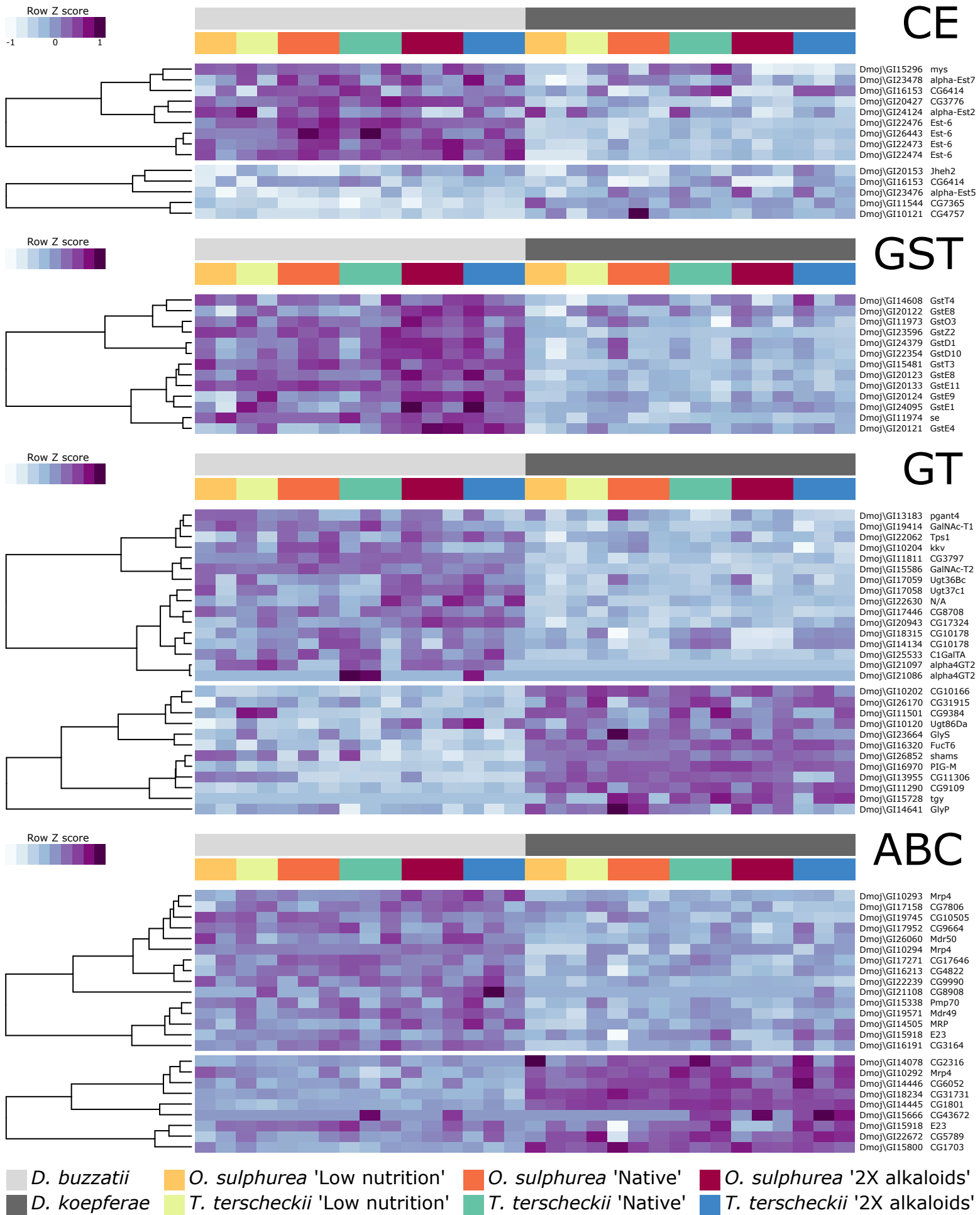
OXR



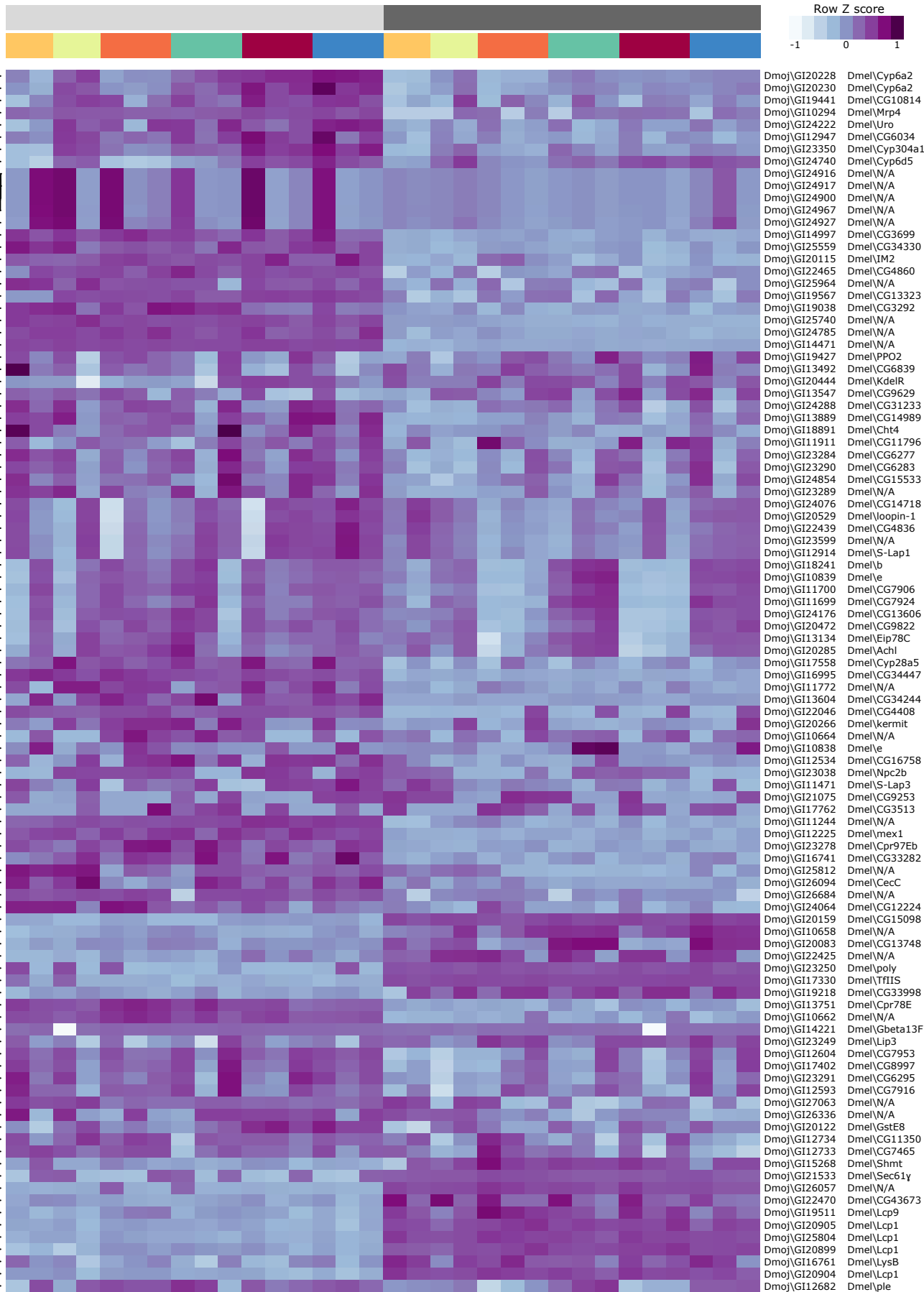


OXR





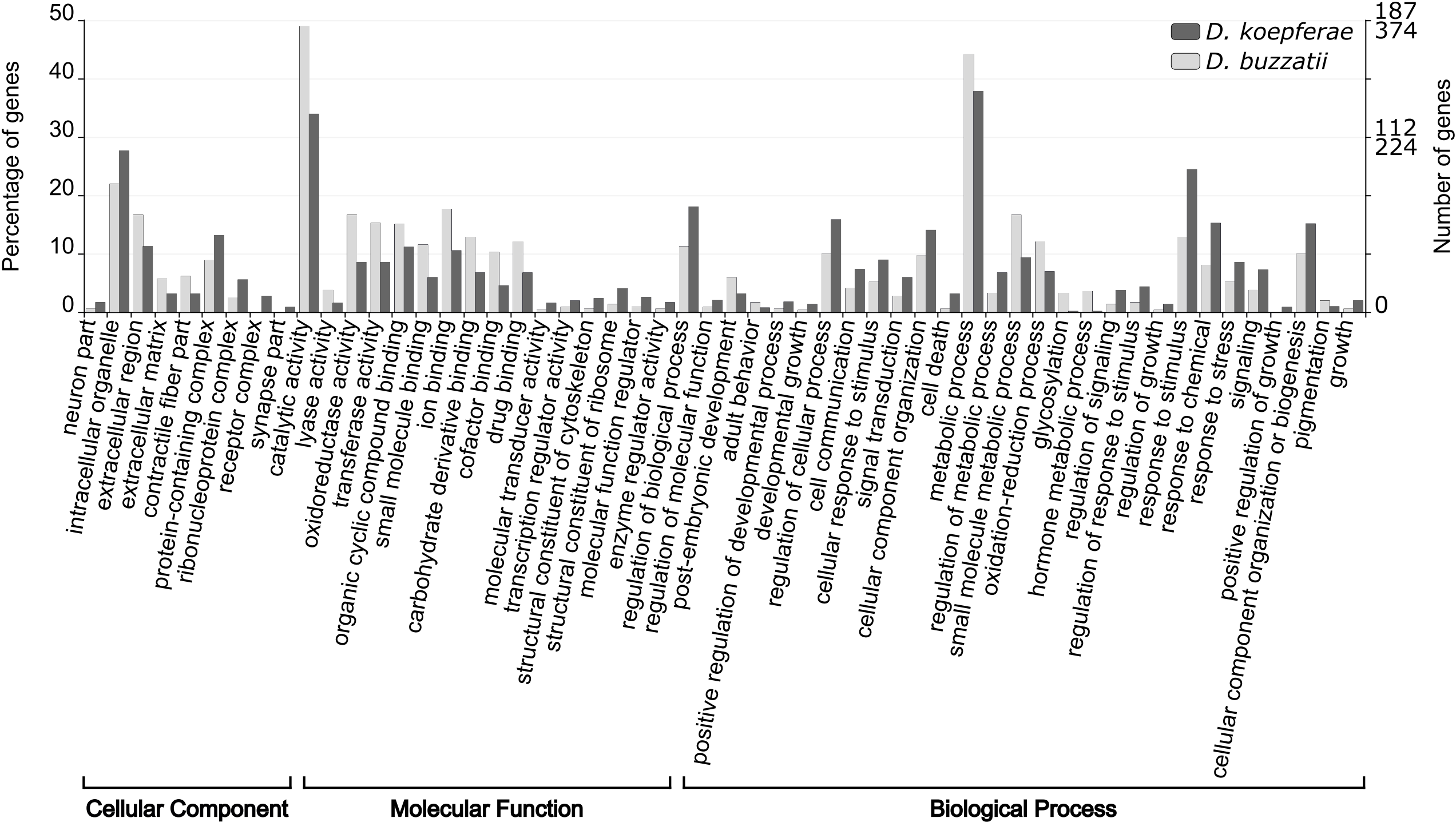
Top100

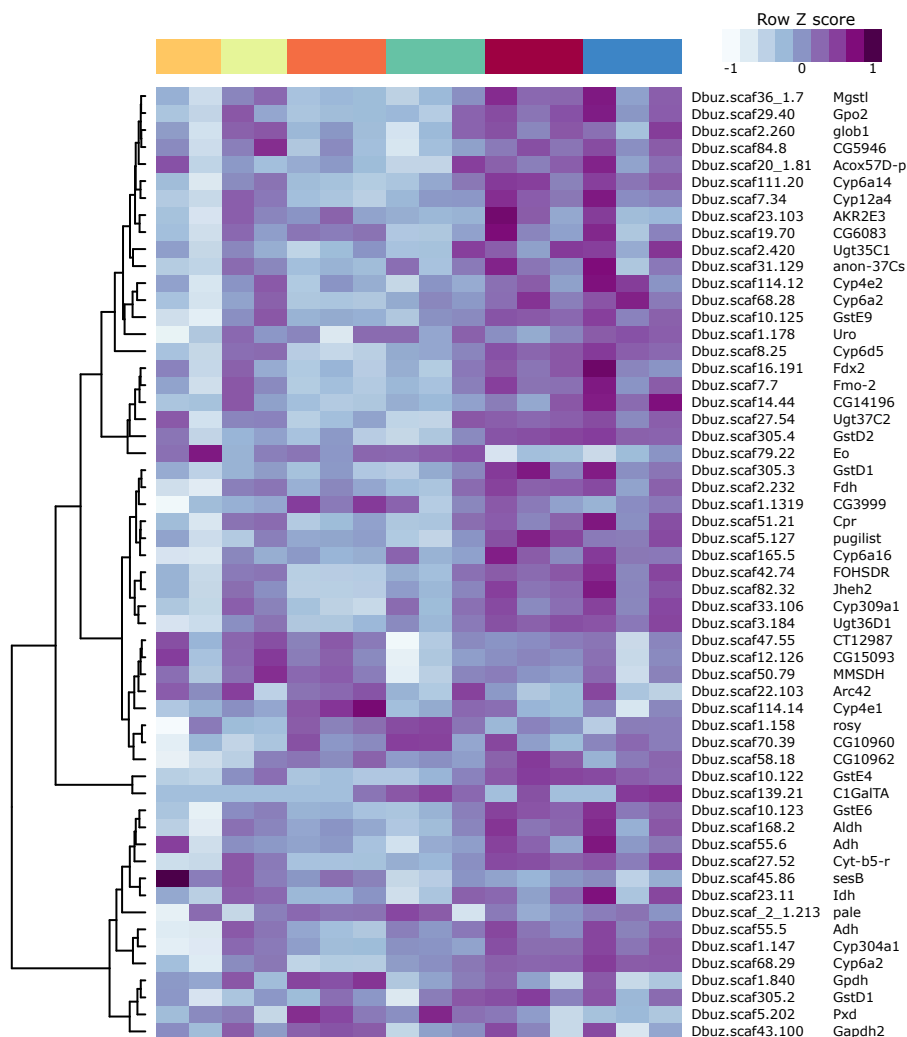


D. buzzatii
 O. sulphurea 'Low nutrition'
 O. sulphurea 'Native'
 O. sulphurea '2X alkaloids'
 D. koepferae
 T. terscheckii 'Low nutrition'
 T. terscheckii 'Native'
 T. terscheckii '2X alkaloids'

Fig. S6. Top 100 most variable differentially expressed genes in exploratory INTER-specific analyzes across treatments. Genes are identified by the respective *D. melanogaster* homolog. Row dendrograms are not shown.

Fig. S7. Main ontology terms taking into account all differentially expressed genes in INTRA-specific analyzes.





■ *O. sulphurea* 'Low nutrition'
 ■ *O. sulphurea* 'Native'
 ■ *O. sulphurea* '2X alkaloids'
■ *T. terscheckii* 'Low nutrition'
 ■ *T. terscheckii* 'Native'
 ■ *T. terscheckii* '2X alkaloids'

Fig. S8. *D. buzzatii* differentially expressed genes in INTRA-specific analyzes across treatments involved in the four phases of general xenobiotic transport and metabolism. Genes are identified by the respective *D. melanogaster* homolog. Row dendrograms are not shown.

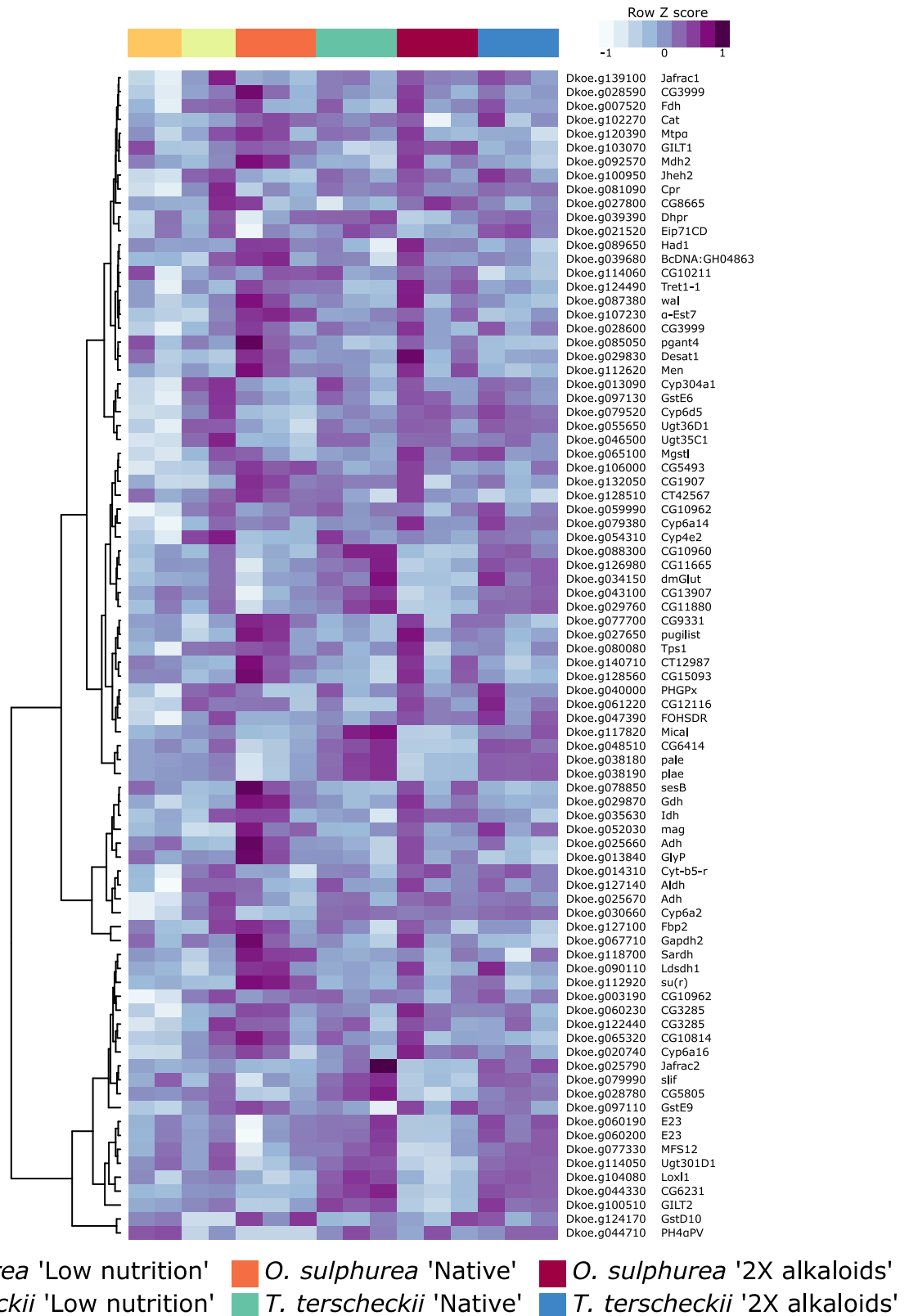


Fig. S9. *D. koepferae* differentially expressed genes in INTRA-specific analyzes across treatments involved in the four phases of general xenobiotic transport and metabolism. Genes are identified by the respective *D. melanogaster* homolog. Row dendrograms are not shown.

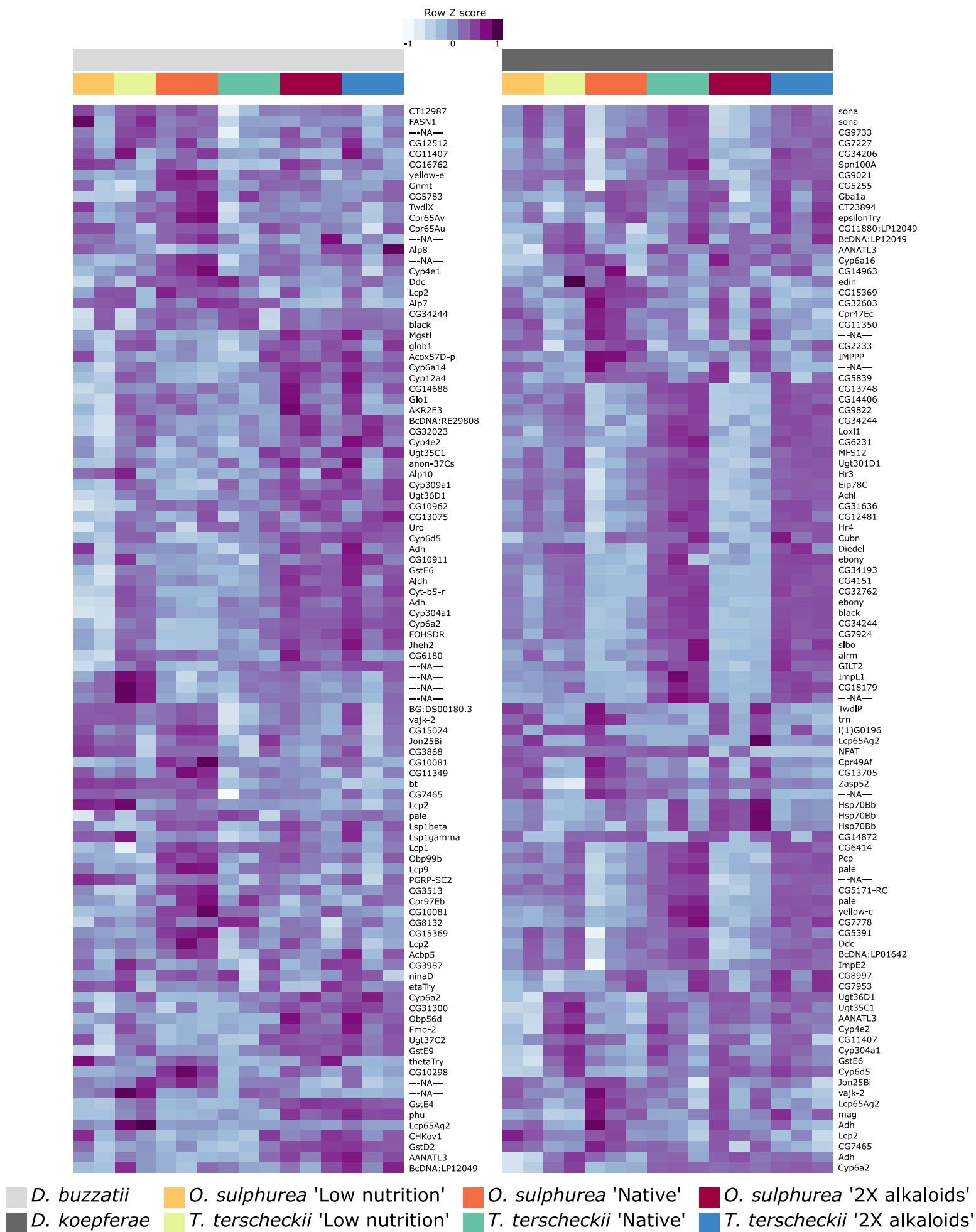


Fig. S10. Top 100 most variable differentially expressed genes in INTRA-specific analyzes in each species across treatments. Genes are identified by the respective *D. melanogaster* homolog. Row dendrograms are not shown.