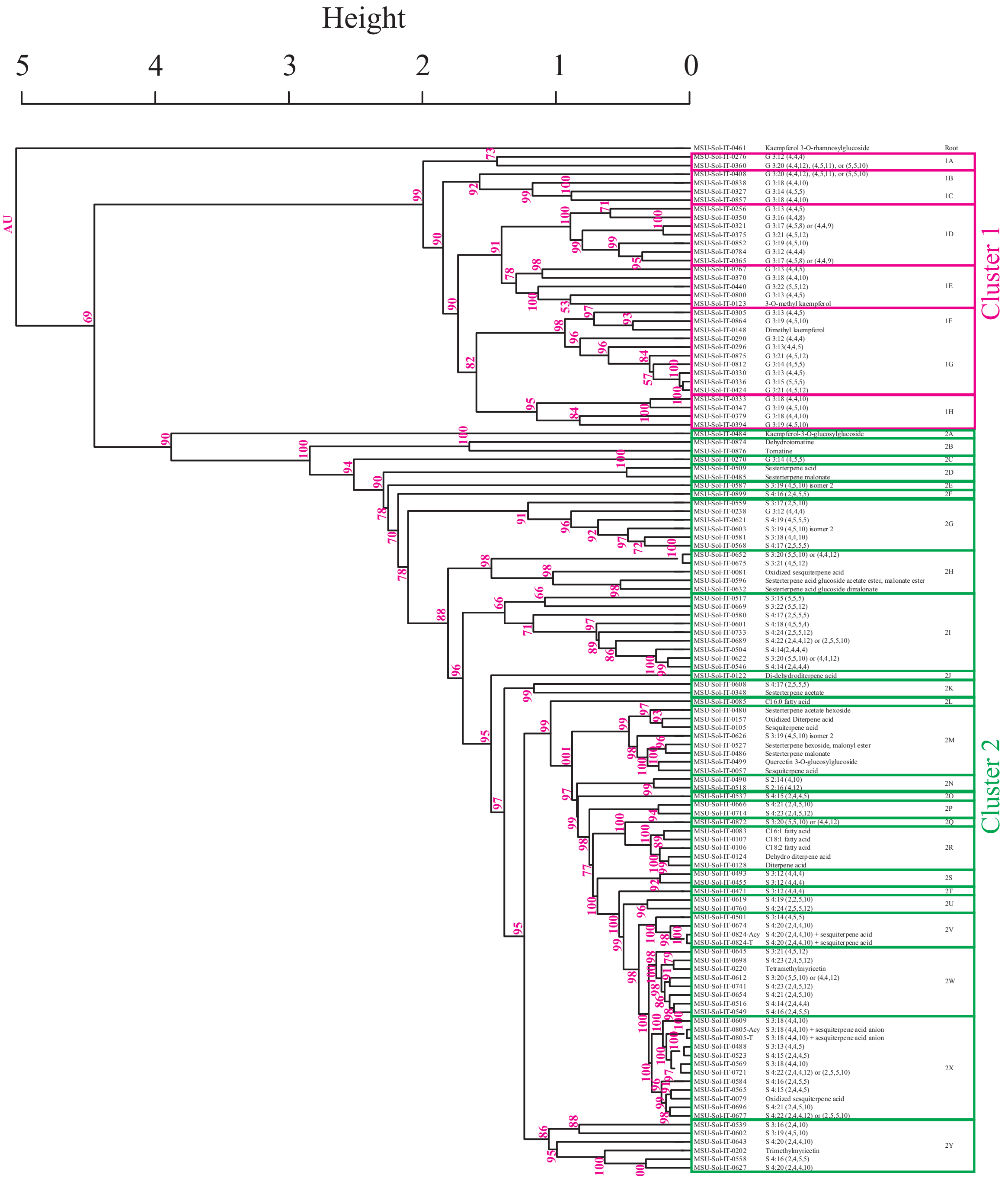
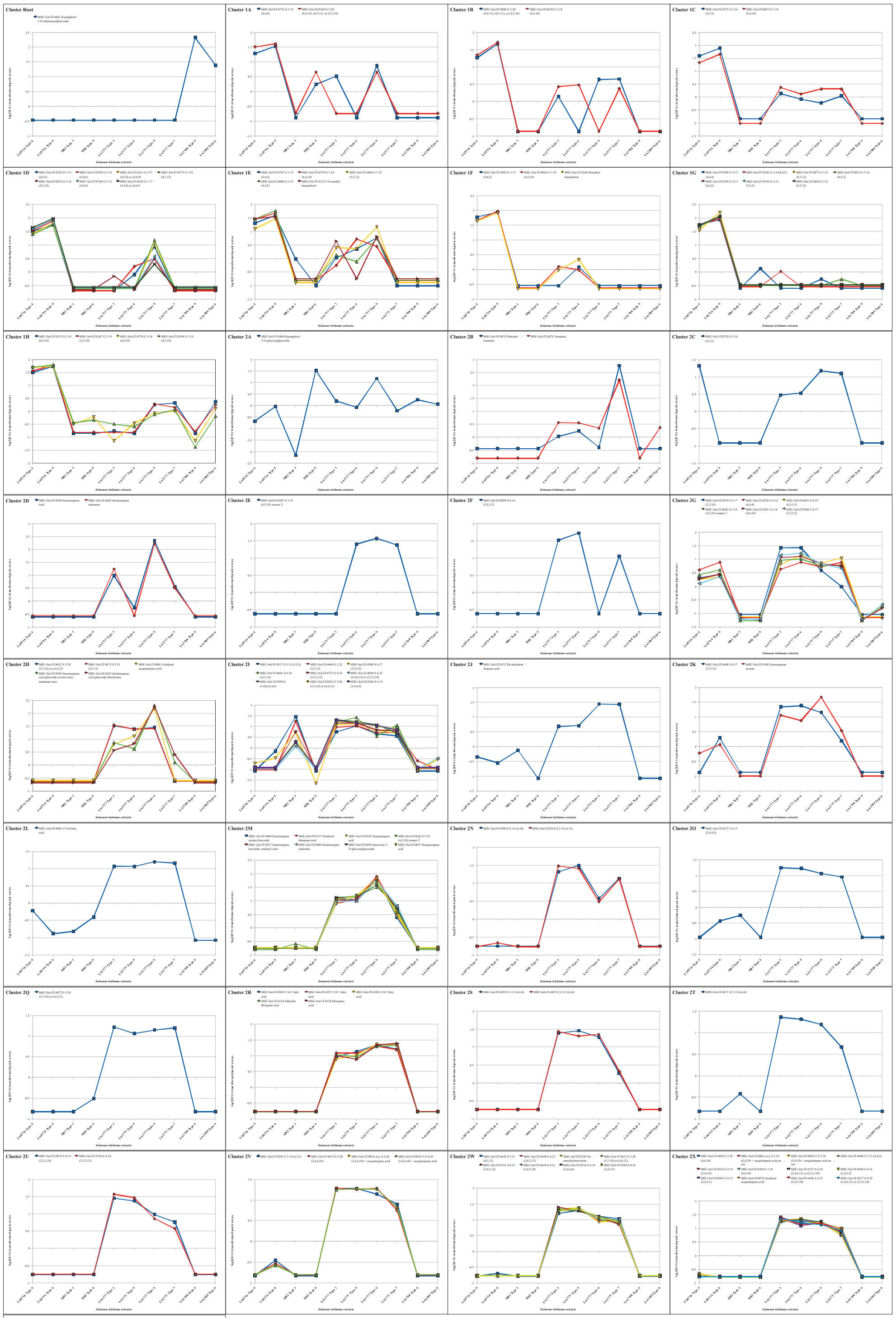


Figure S1. Two-way cluster analysis of normalized LCMS metabolite peak areas from *Solanum* glandular trichomes and their associated clusters. Dendrograms and bootstrap values (magenta numbers) calculated using R package "pvclust" (Shimodaira, 2002; Shimodaira, 2004). Clusters were identified based on having bootstrap values AU>95. Corresponding graphs of normalized metabolite levels across all extracts are displayed on Figure S2.



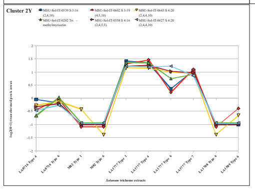


Figure S2: Clusters of normalized LCMS metabolite peak areas from *Solanum* glandular trichomes with corresponding accumulation patterns as identified by 2-way cluster analysis. Clusters are from Figure S1.

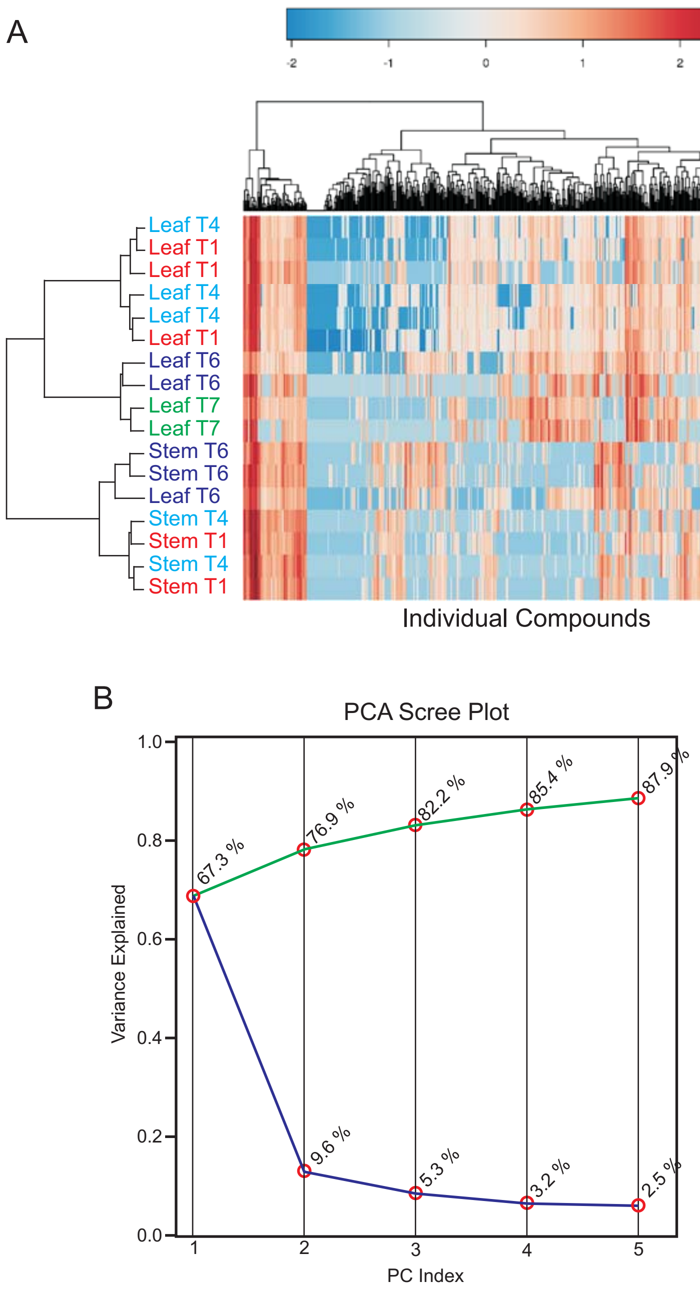


Figure S3. Multivariate analysis of metabolite data obtained for S. habrochaites, accession LA1777, the only accession that had readily isolatable trichomes for all types (Types 1, 4, 6 and 7) demonstrates that glandular trichome Types 1 and 4, which are clearly different from Types 6 and 7, are indistinguishable from each other. Principal components analysis (PCA, Figure 4A, 4D-4F), partial least squares-discriminant analysis (PLS-DA, Figure 4B and 4C) and hierarchical cluster analysis (panel A above, this case using Ward's method with Euclidean distances) all failed to separate Type 1 and 4 trichomes based on overall metabolite profiles, regardless of method used for data normalization. The normalization used for the data presented above was log2 transformed data. Panel B above shows the scree plot for data used to generate Figure 4 C - F).

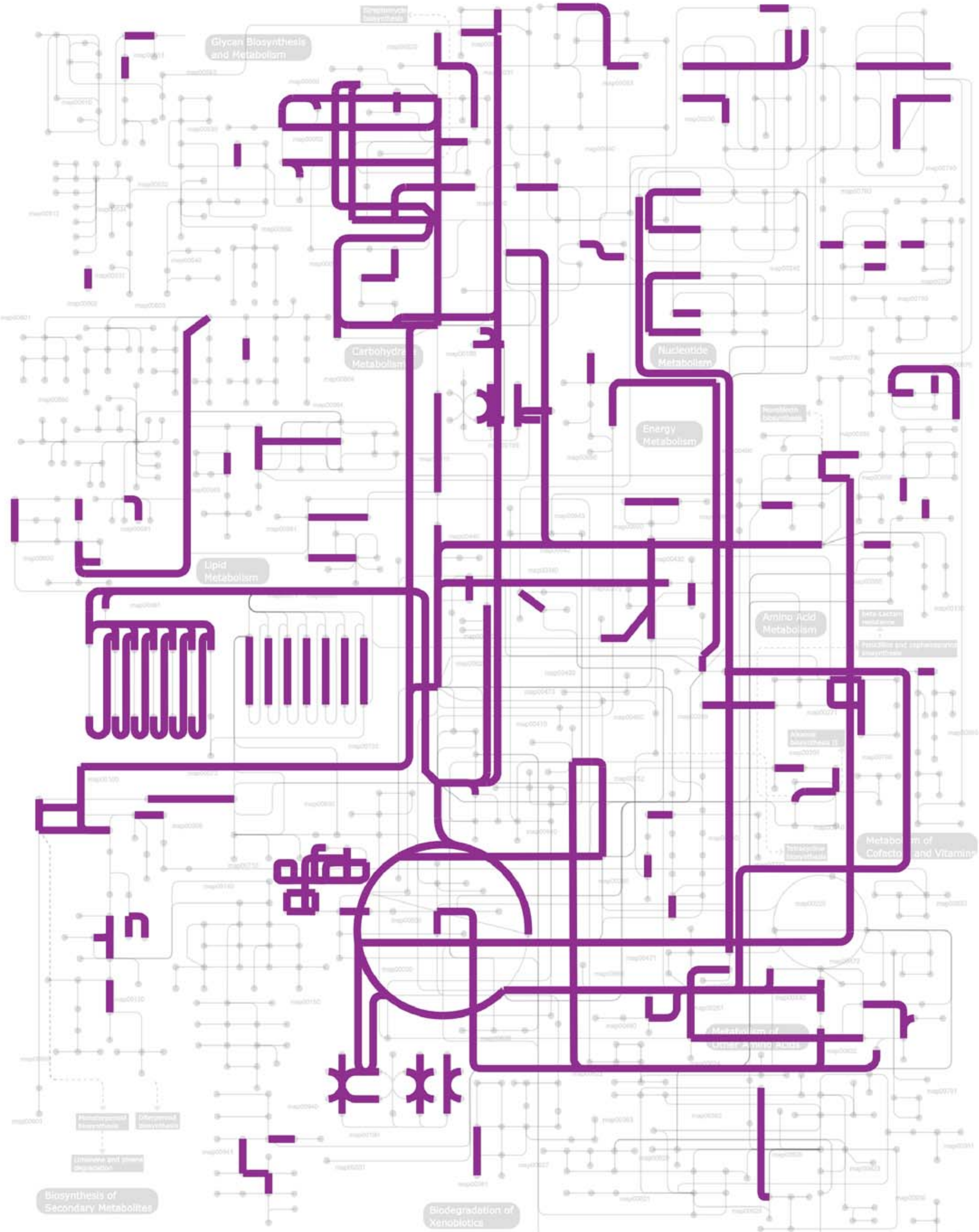


Figure S4. Biochemical pathways expressed in *S. habrochaites* type 6 glandular trichomes. Pathways highlighted in purple represent enzymes identified via EC queries on the PAVE system.

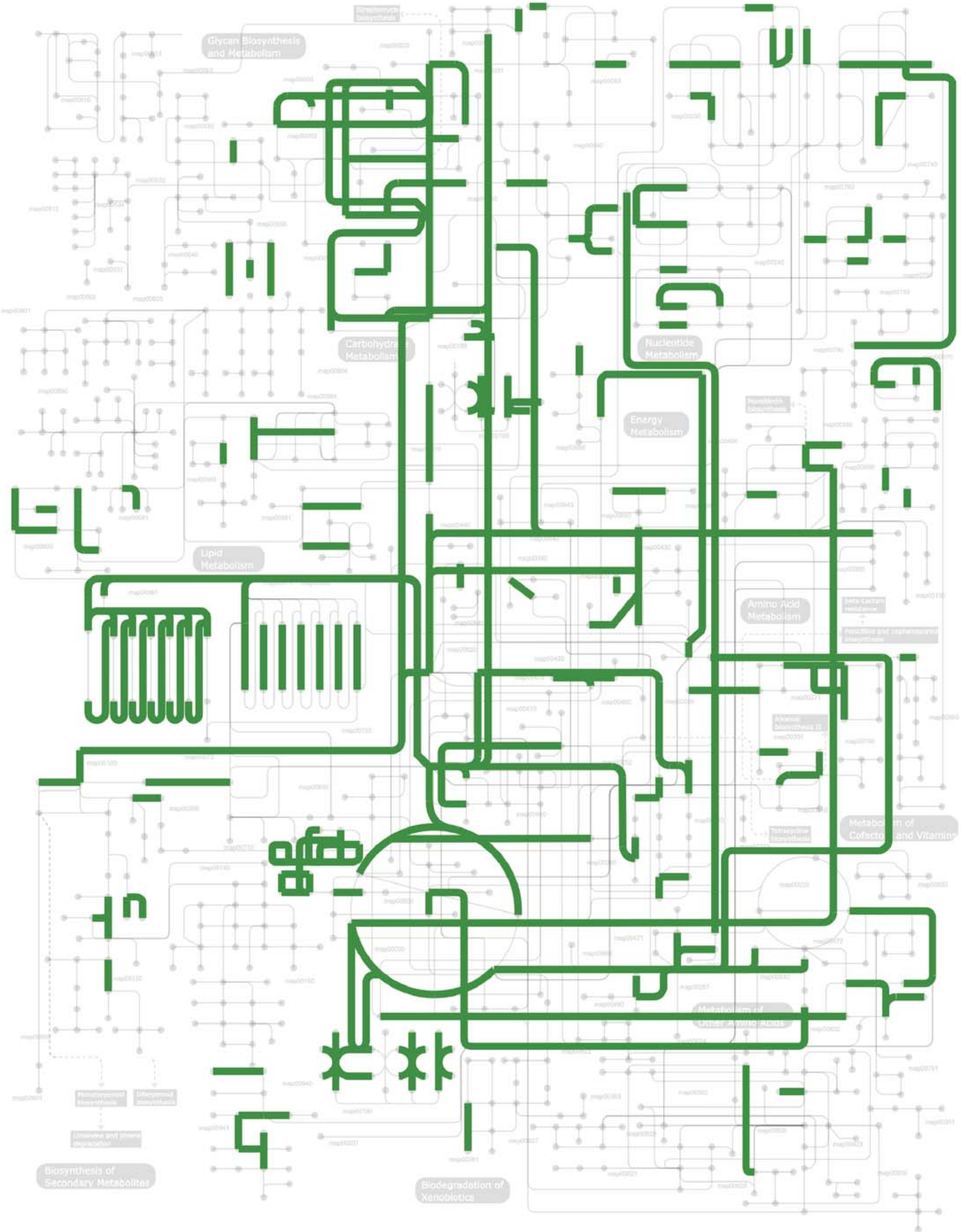


Figure S5. Biochemical pathways expressed in *S. lycopersicum* type 6 glandular trichomes. Pathways highlighted in green represent enzymes identified via EC queries on the PAVE system.

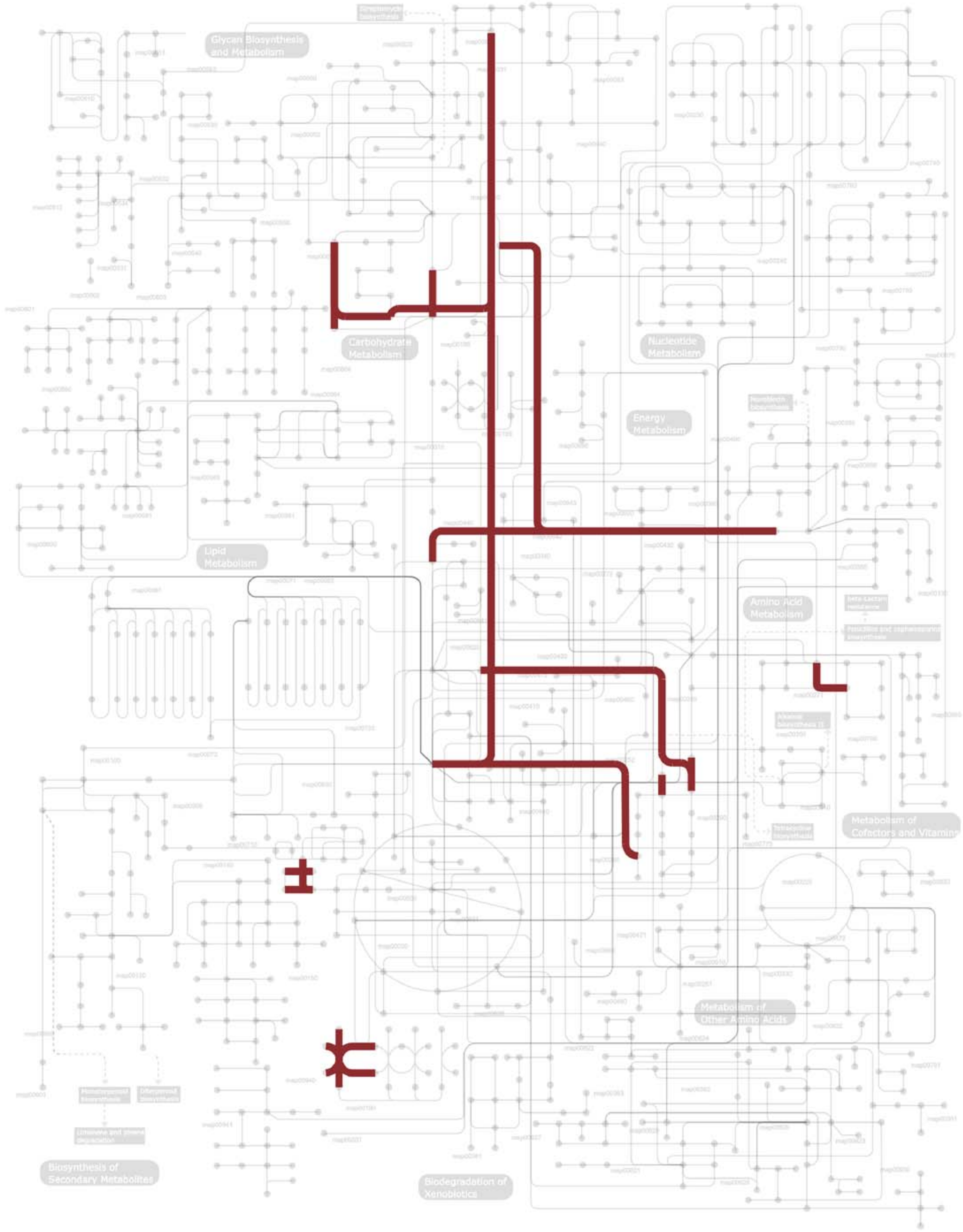


Figure S6. Biochemical pathways expressed in *S. pennellii* type 6 glandular trichomes. Pathways highlighted in maroon represent enzymes identified via blast searches with an E-vaue cutoff of 1x10E-40 against the Uniprot databases.

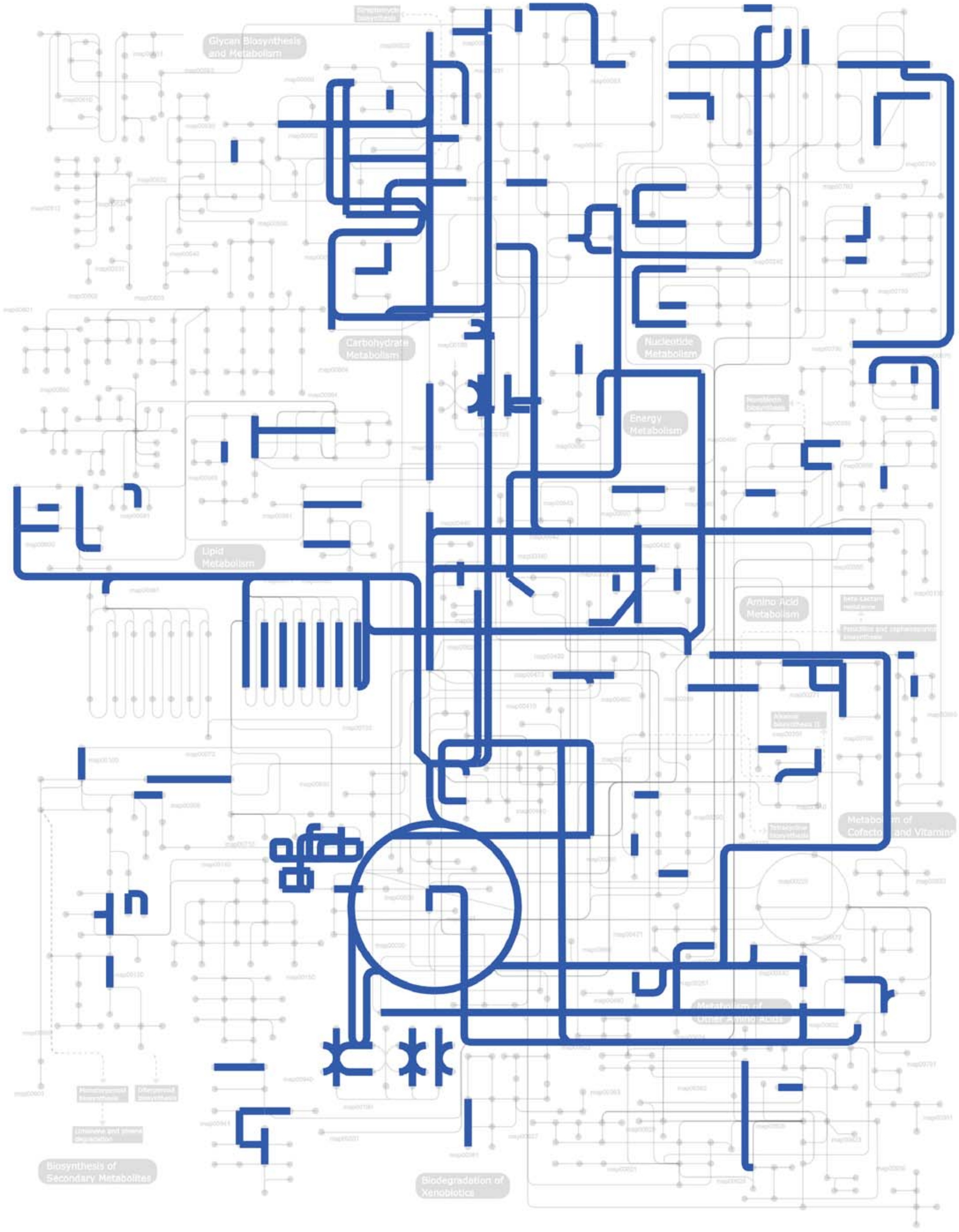


Figure S7. Biochemical pathways expressed in *S. pimpinellifolium* type 6 glandular trichomes. Pathways highlighted in blue represent enzymes identified via EC queries on the PAVE system.

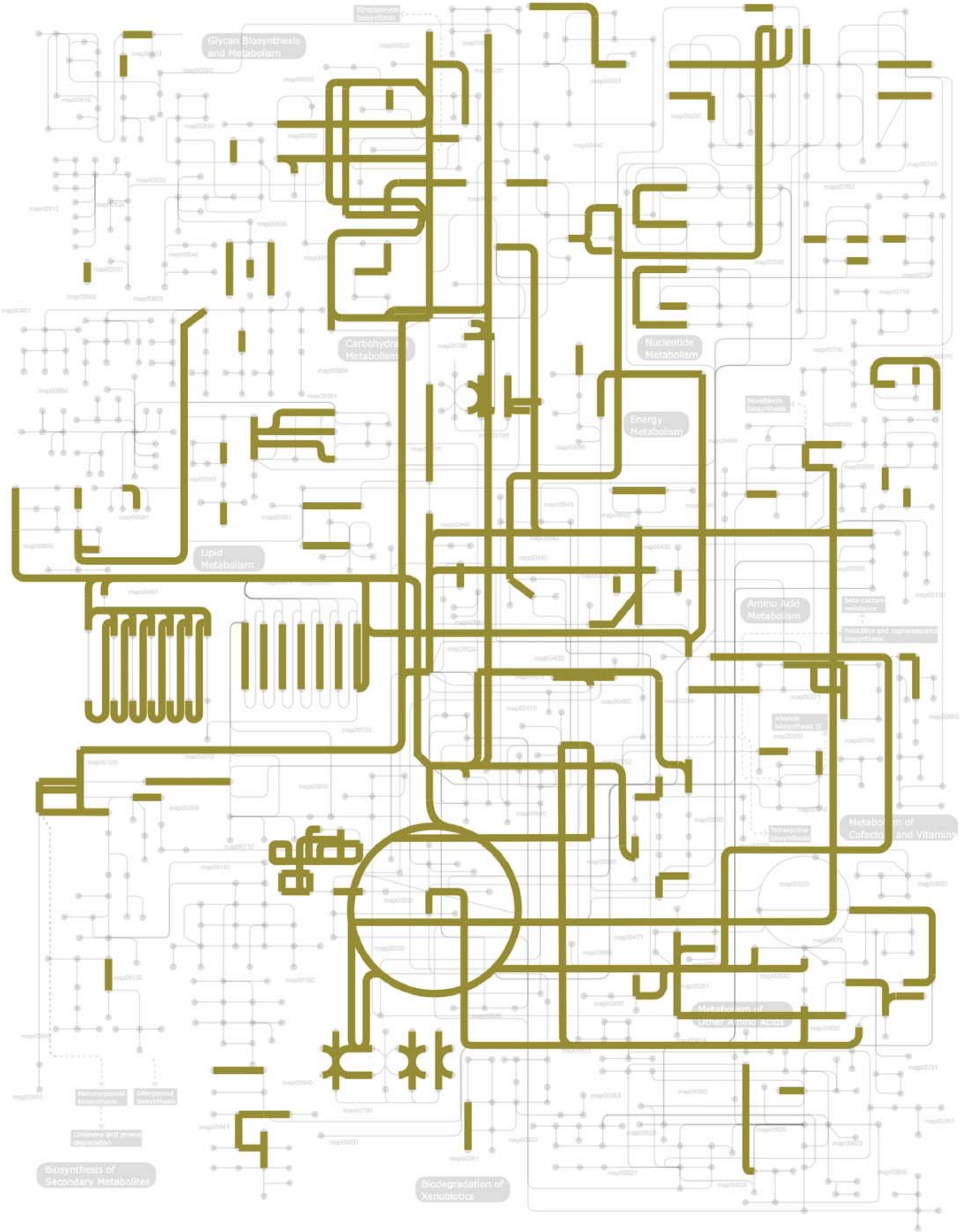


Figure S8. Biochemical pathways expressed in *S. arcanum* type 6 glandular trichomes. Pathways highlighted in olive represent enzymes identified via blast searches with an E-vaue cutoff of 1x10E-40 against the Uniprot databases.

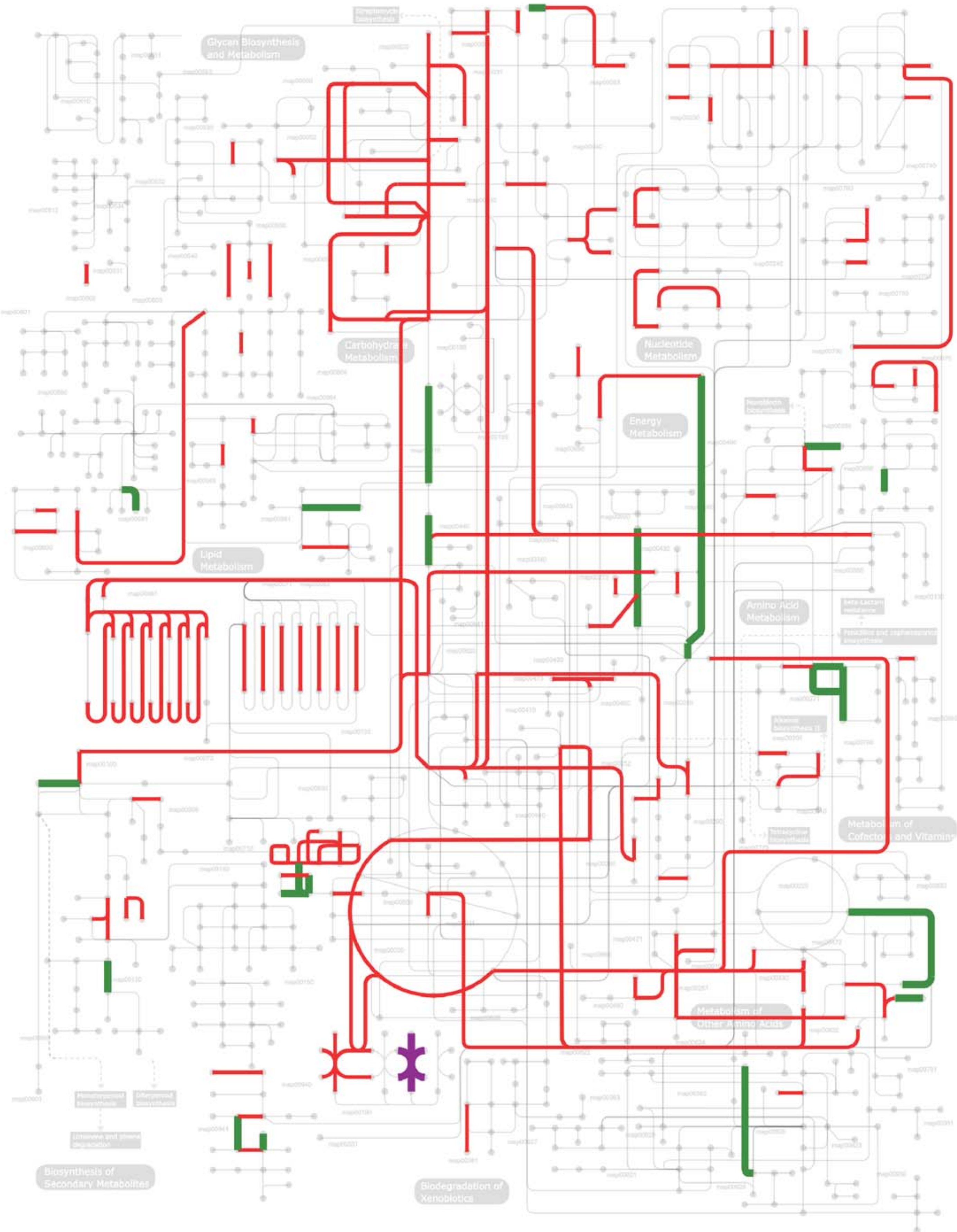


Figure S9 Biochemical pathways preferentially expressed in either *S. habrochaites* type 6 or *S. lycopersicum* type 6 glandular trichomes when queried by Uniprot ID. Pathways highlighted in red are present in both species but not at significantly different levels. Pathways highlighted in green are preferentially expressed in *S. lycopersicum* while pathways preferentially expressed in *S. habrochaites* are in purple.

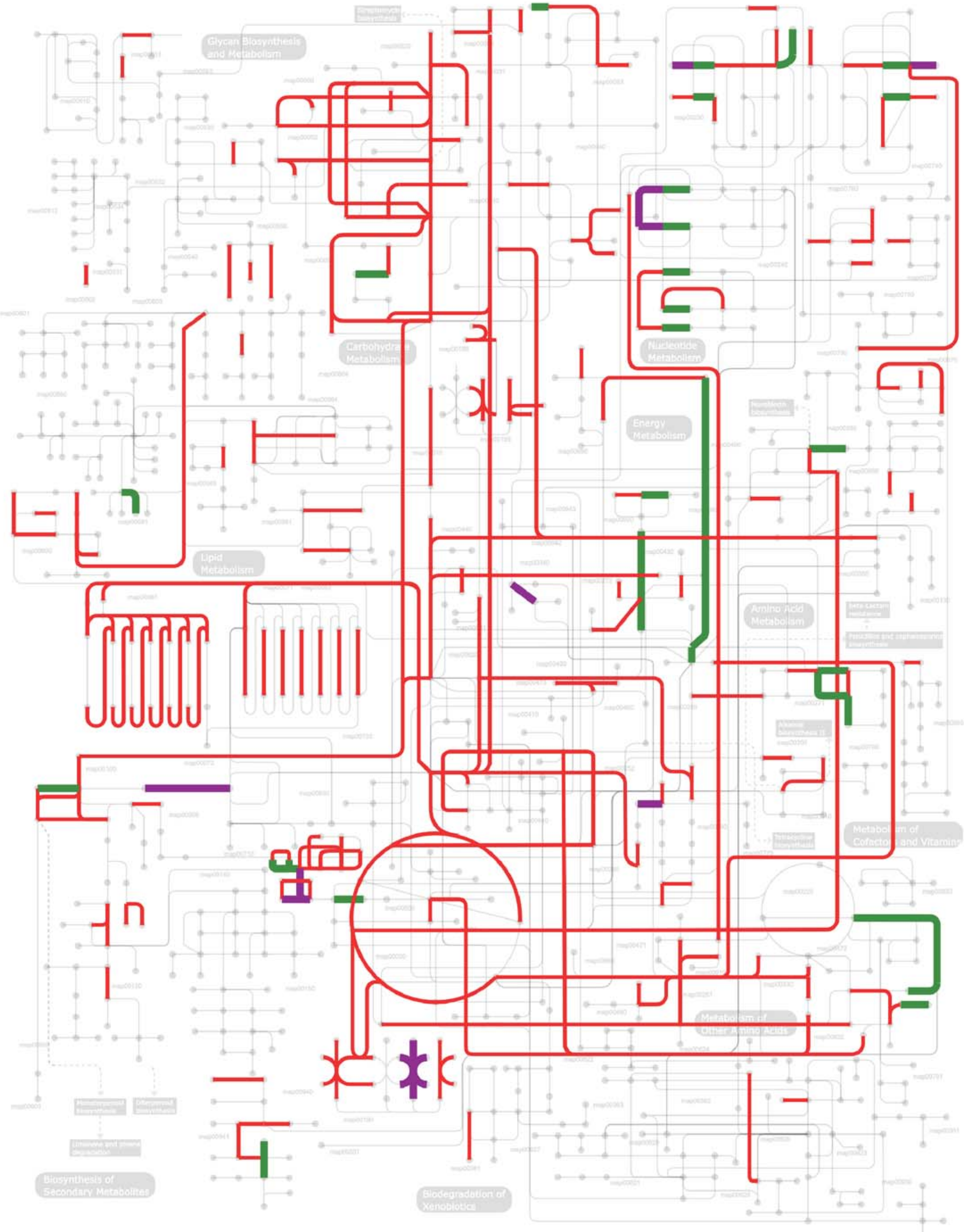


Figure S10. Biochemical pathways preferentially expressed in either *S. habrochaites* type 6 or *S. lycopersicum* type 6 glandular trichomes when queried by EC number. Pathways highlighted in red are present in both species but at not significantly different levels. Pathways highlighted in green are preferentially expressed in *S. lycopersicum* while pathways preferentially expressed in *S. habrochaites* are in purple.

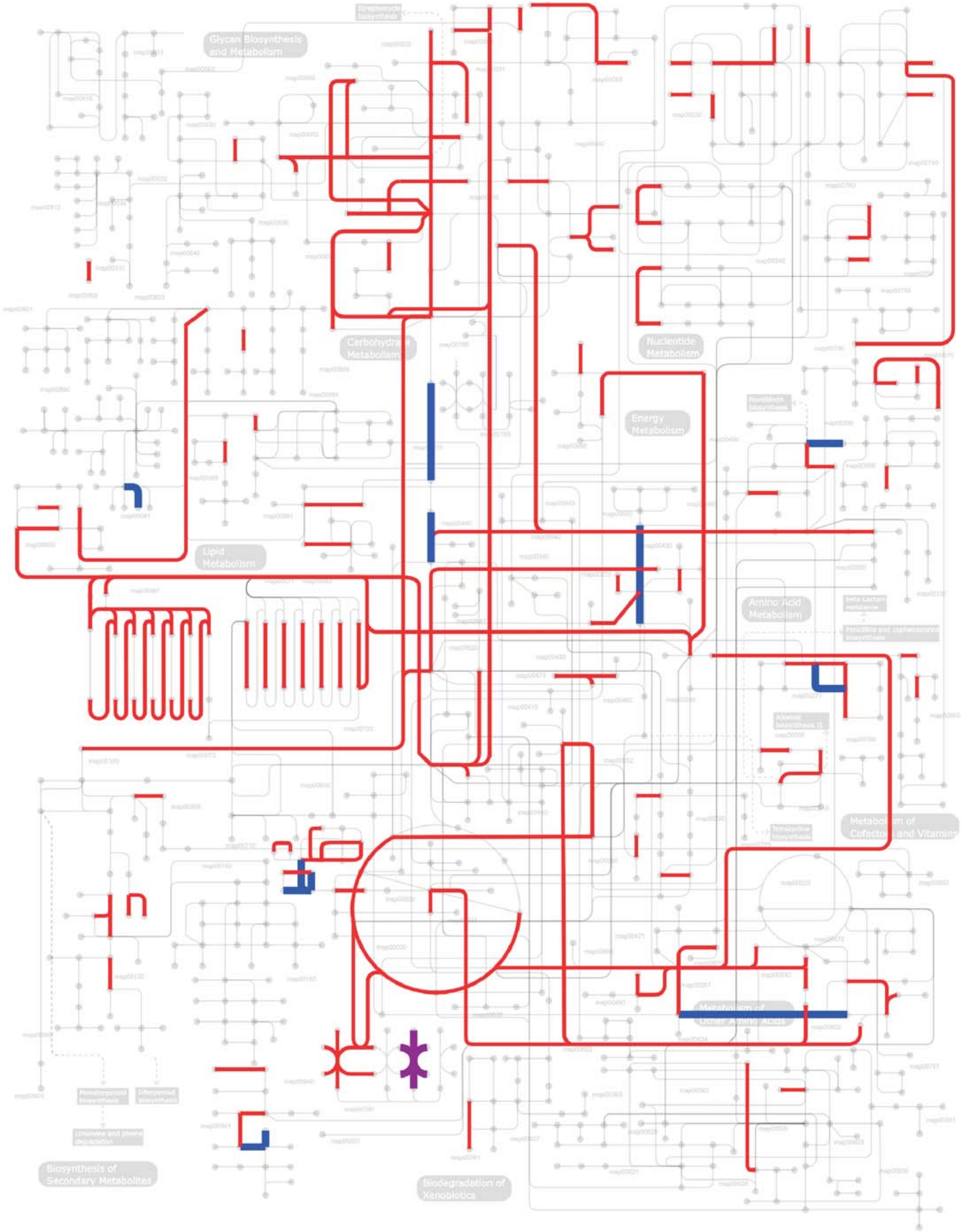


Figure S11. Biochemical pathways preferentially expressed in either *S. habrochaites* type 6 or *S. pimpinellifolium* type 6 glandular trichomes when queried by Uniprot ID. Pathways highlighted in red are present in both species but at not significantly different levels. Pathways highlighted in blue are preferentially expressed in *S. pimpinellifolium* while pathways preferentially expressed in *S. habrochaites* are in purple.

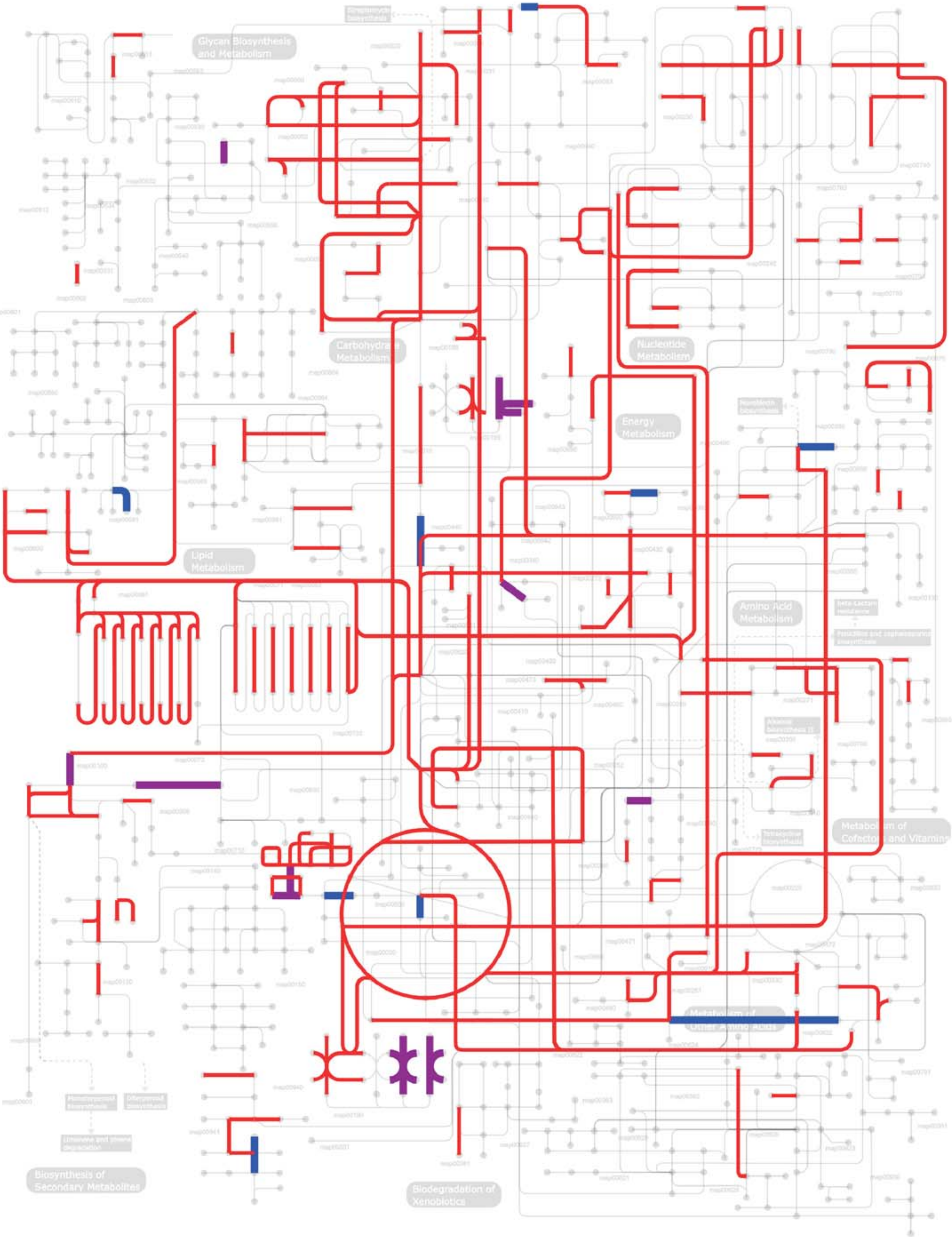


Figure S12. Biochemical pathways preferentially expressed in either *S. habrochaites* type 6 or *S. pimpinellifolium* type 6 glandular trichomes when queried by EC number. Pathways highlighted in red are present in both species but at not significantly different levels. Pathways highlighted in blue are preferentially expressed in *S. pimpinellifolium* while pathways preferentially expressed in *S. habrochaites* are in purple.

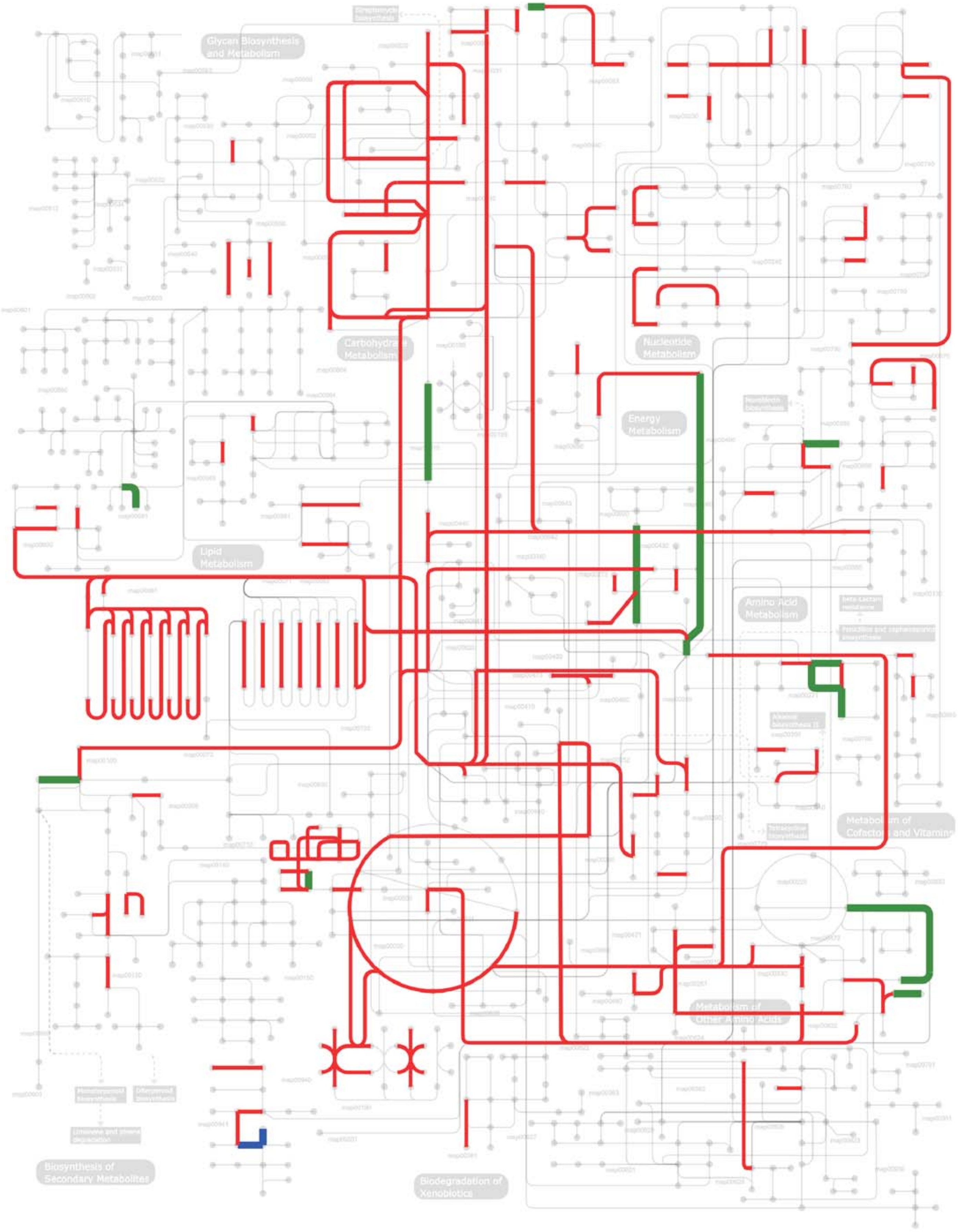


Figure S13. Biochemical pathways preferentially expressed in either *S. lycopersicum* type 6 or *S. pimpinellifolium* type 6 glandular trichomes when queried by Uniprot ID. Pathways highlighted in red are present in both species but at not significantly different levels. Pathways highlighted in green are preferentially expressed in *S. lycopersicum* while pathways preferentially expressed in *S. pimpinellifolium* are in blue.

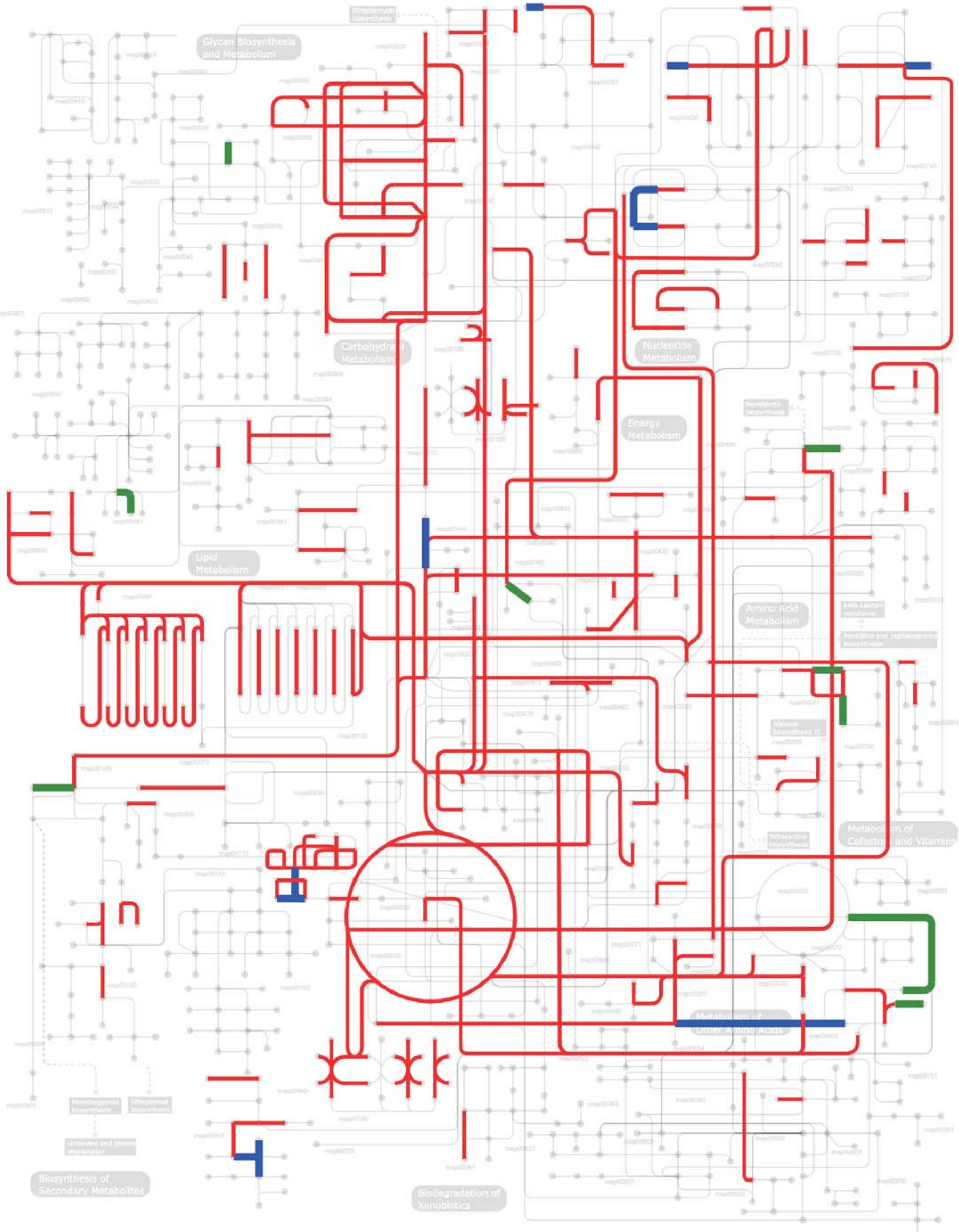


Figure S14. Biochemical pathways preferentially expressed in either *S. lycopersicum* type 6 or *S. pimpinellifolium* type 6 glandular trichomes when queried by EC number. Pathways highlighted in red are present in both species but at not significantly different levels. Pathways highlighted in green are preferentially expressed in *S. lycopersicum* while pathways preferentially expressed in *S. pimpinellifolium* are in blue.

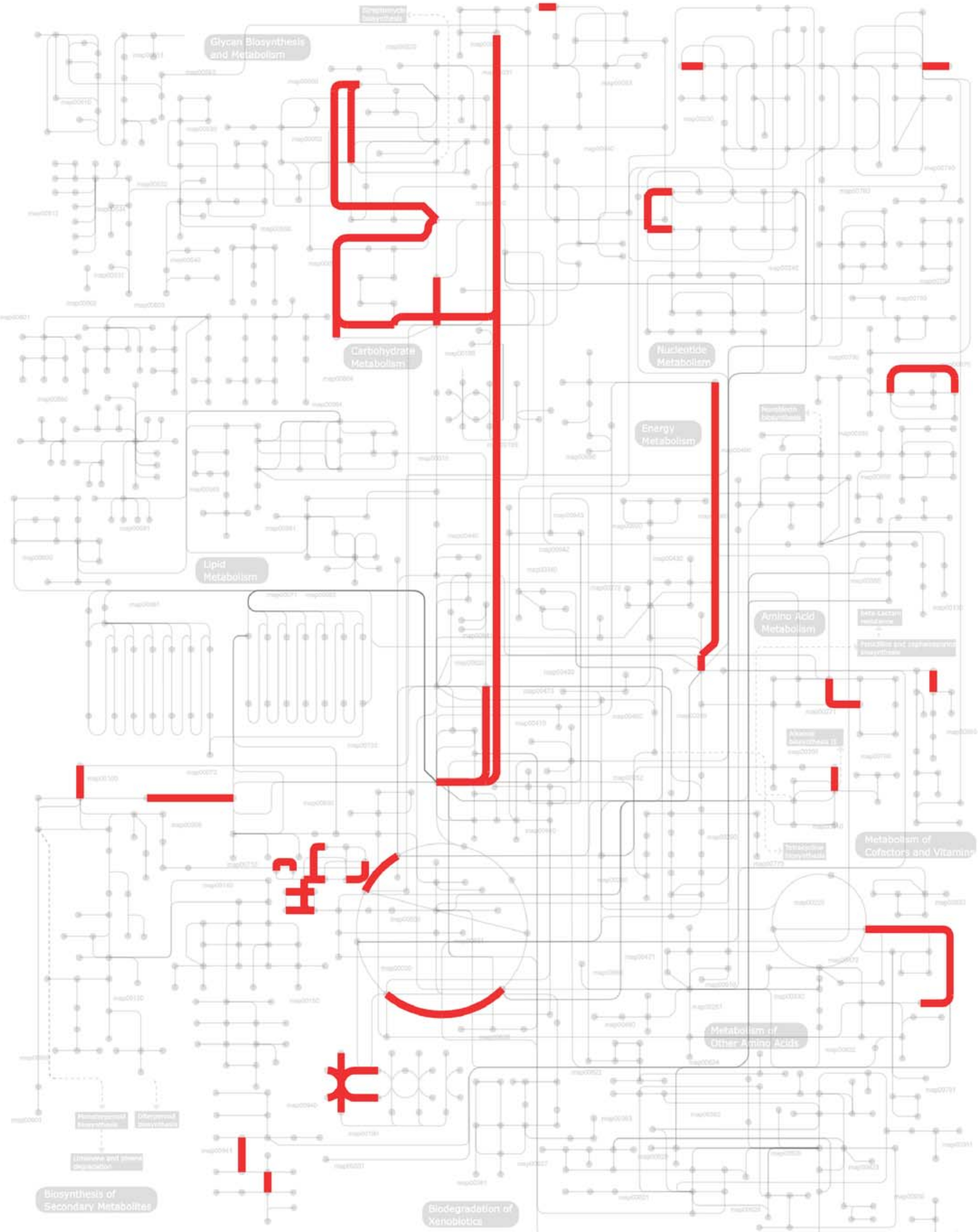


Figure S15. Biochemical pathways present in *S. habrochaites* type 1 and *S. habrochaites* type 4 glandular trichomes EST libraries when queried by EC number. Pathways highlighted in red represent biochemical pathways whose expression is present but not significantly different in either library.

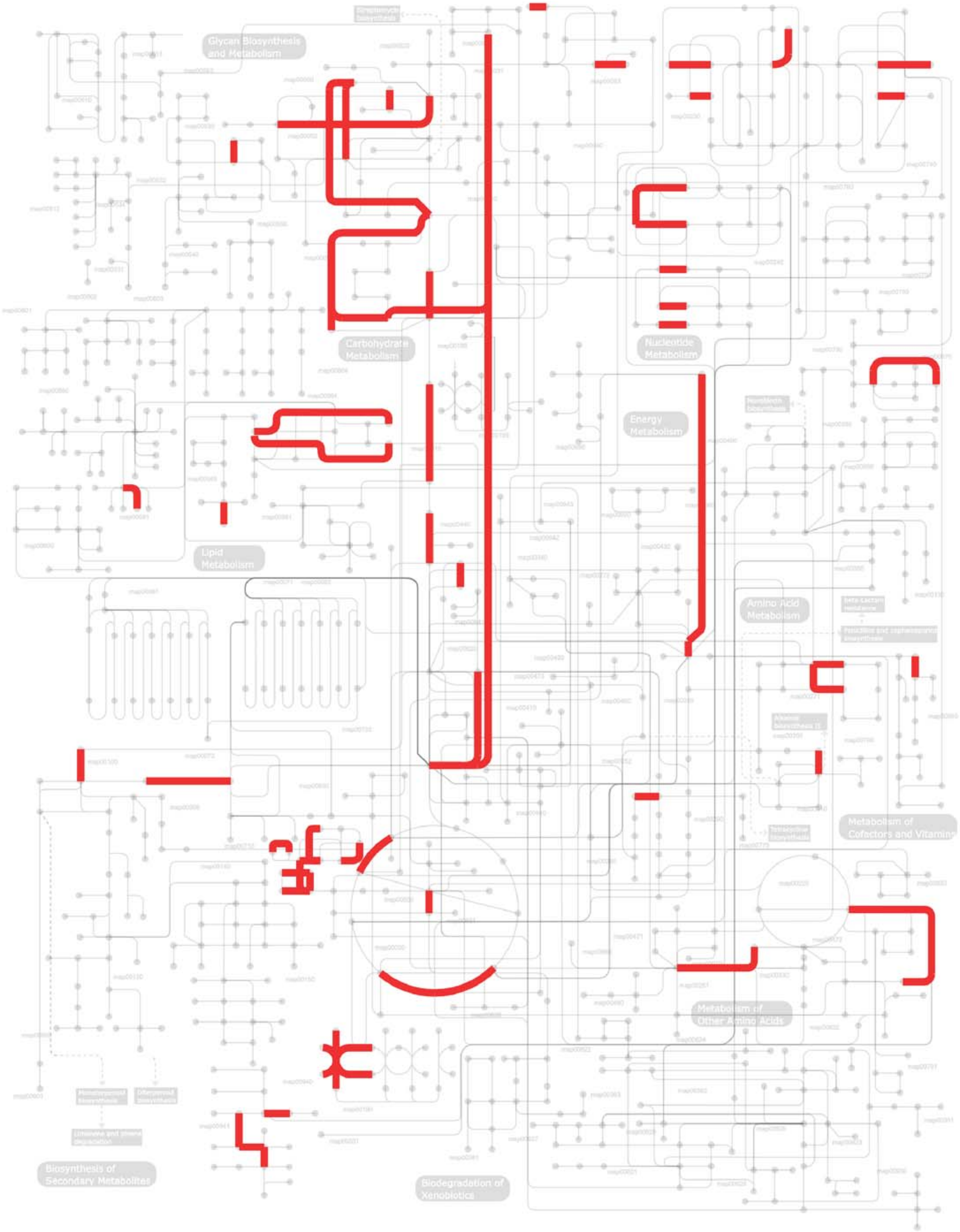


Figure S16. Biochemical pathways present in *S. habrochaites* type 1-4 composite and *S. lycopersicum* type 1 glandular trichomes EST libraries when queried by EC number. Pathways highlighted in red represent biochemical pathways whose expression is present but not significantly different in either library.

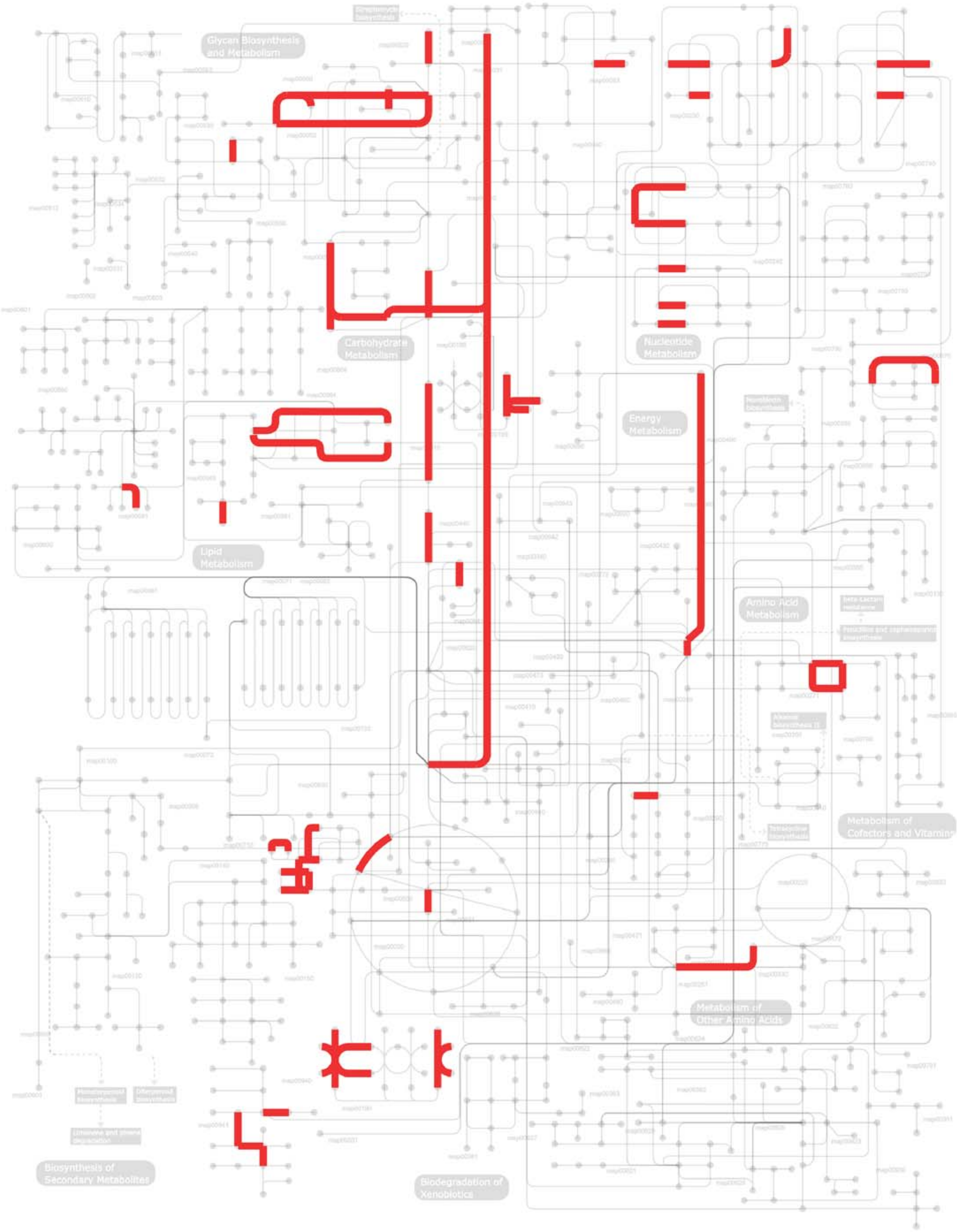


Figure S17. Biochemical pathways present in *S. lycopersicum* type 1 and *S. pennellii* type 4 glandular trichomes EST libraries when queried by EC number. Pathways highlighted in red represent biochemical pathways whose expression is present but not significantly different in either library.

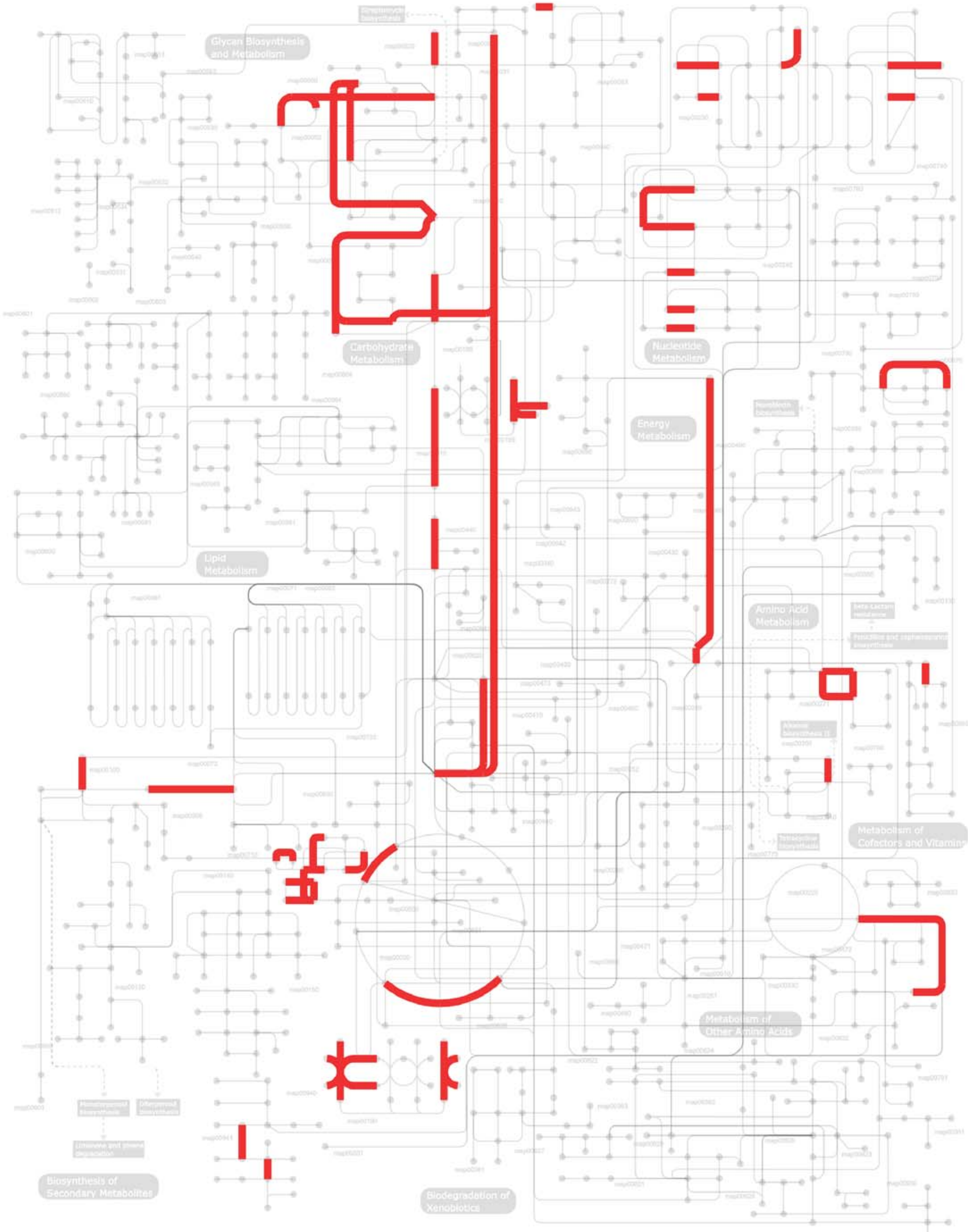


Figure S18. Biochemical pathways present in *S. habrochaites* type 1-4 composite and *S. pennellii* type 4 glandular trichomes EST libraries when queried by EC number. Pathways highlighted in red represent biochemical pathways whose expression is present but not significantly different in either library.

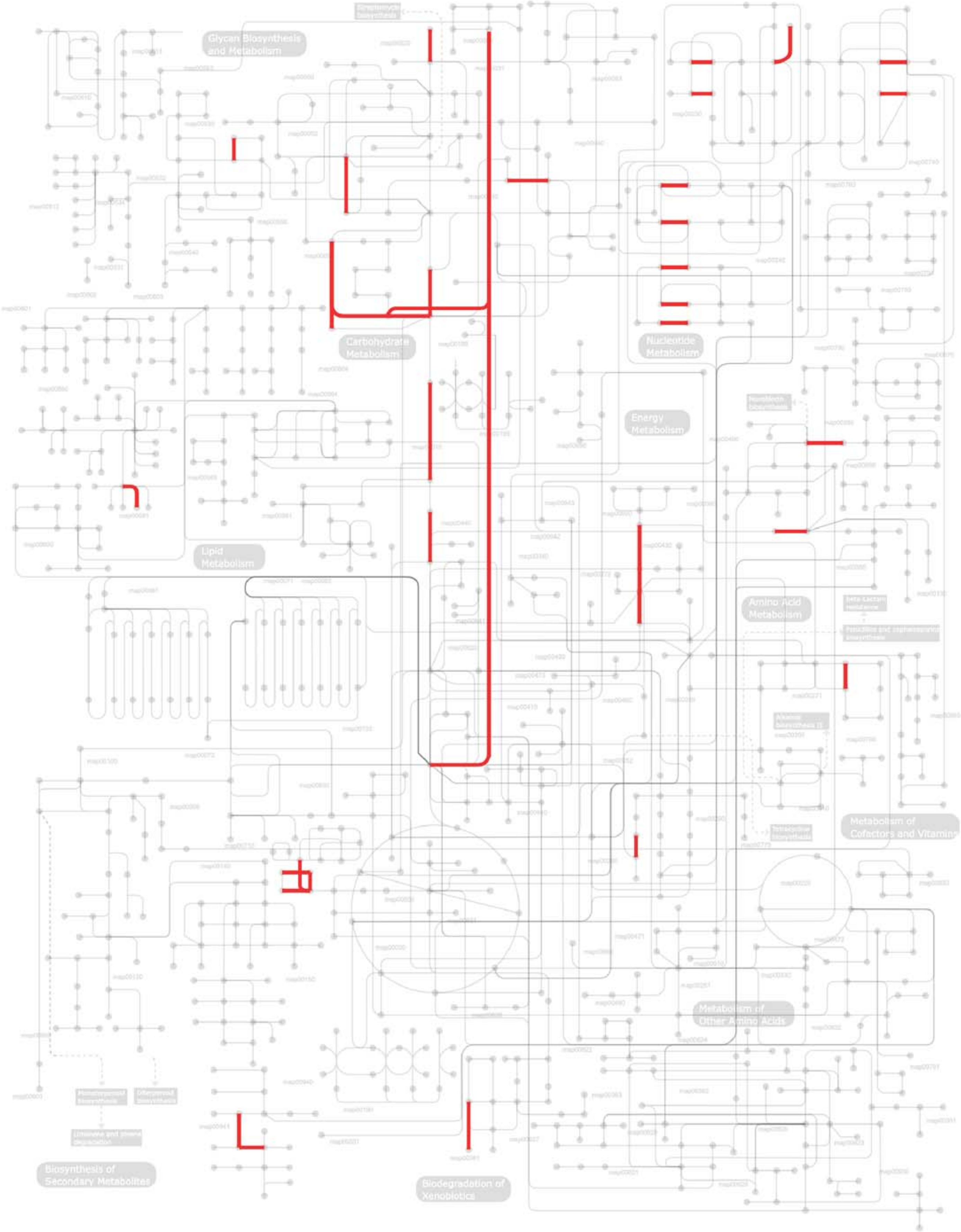


Figure S19. Biochemical pathways expressed in *S. lycopersicum* type 7 glandular trichomes when queried by EC number.

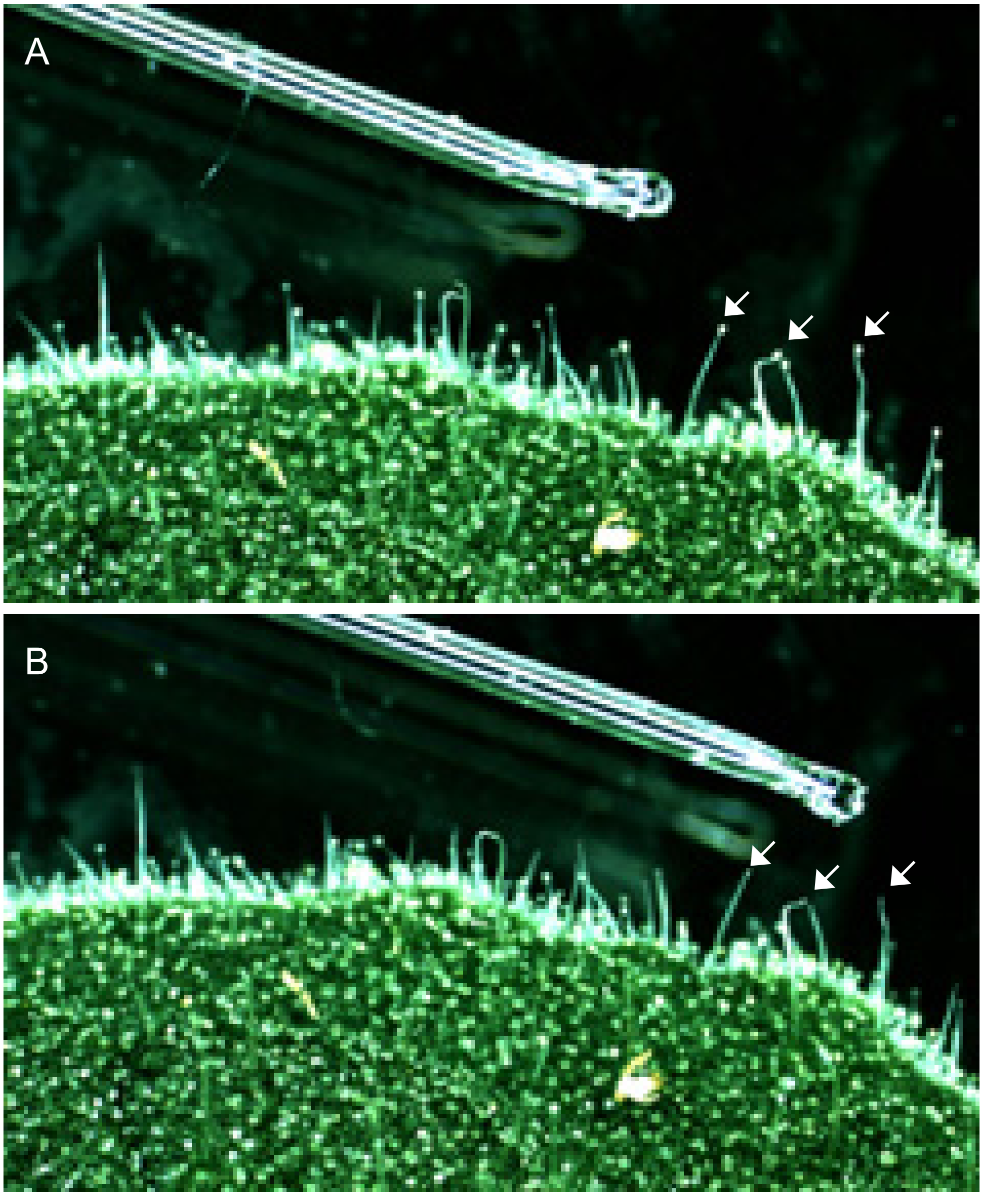


Figure S20. *Solanum habrochaites* leaf showing how gland cells are removed from trichomes without disturbing other cell types. Micro-pipettes were used to collect homogenous samples of gland cells from the different types of glandular trichome structures. **A**. Arrows indicate secreting gland cells atop type 1 trichomes. **B.** Gland cells have been removed from trichomes by “picking” with the tip of the micro-pipette shown in the background.