Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information: Figs S1 to S8

High-throughput transcriptomics reveals mechanisms of nanopesticides – nanoformulation, commercial, active ingredient – finding safe and sustainable-by-design (SSbD) options for the environment

Susana I. L. Gomes[*], *Estefânia V. R. Campos, Leonardo F. Fraceto, Renato Grillo, Janeck J. Scott-Fordsmand, and Mónica J. B. Amorim*[*]

*Corresponding authors' e-mails: susana.gomes@ua.pt; mjamorim@ua.pt

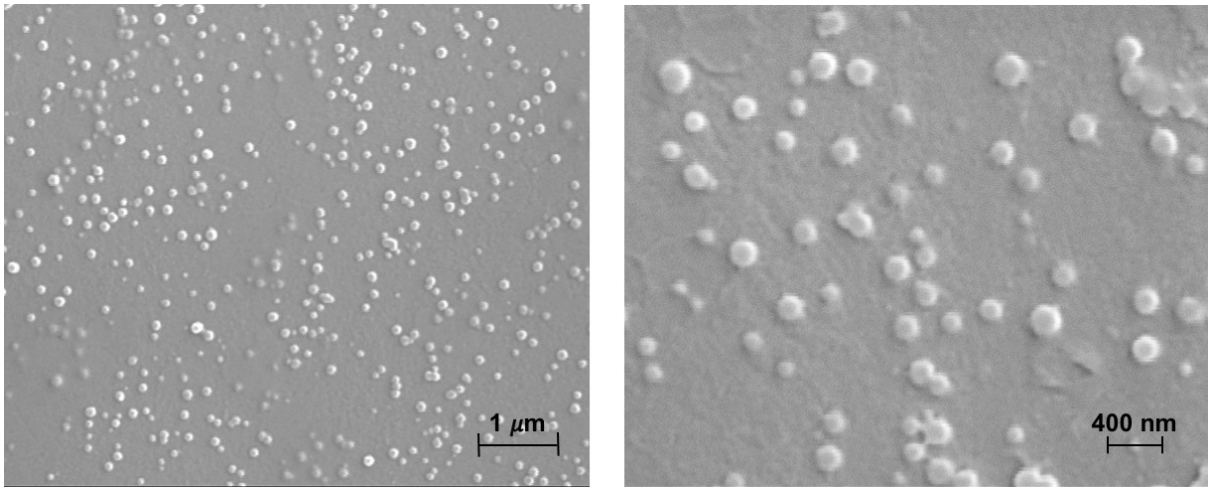


Fig. S1. Scanning electron microscopy (SEM) of the nano_ATZ formulation with 30 000x (left) and 50 000x (right) magnification, providing information about the size and the shape of the nanoformulation.

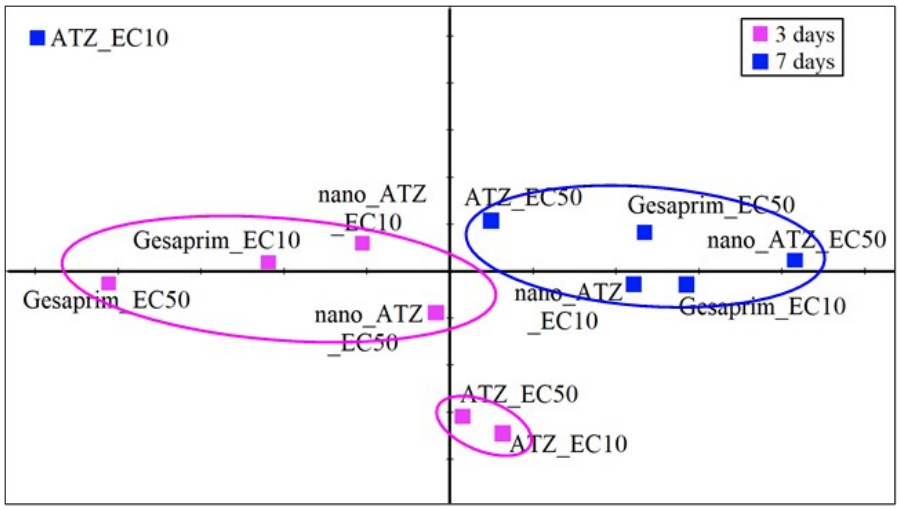
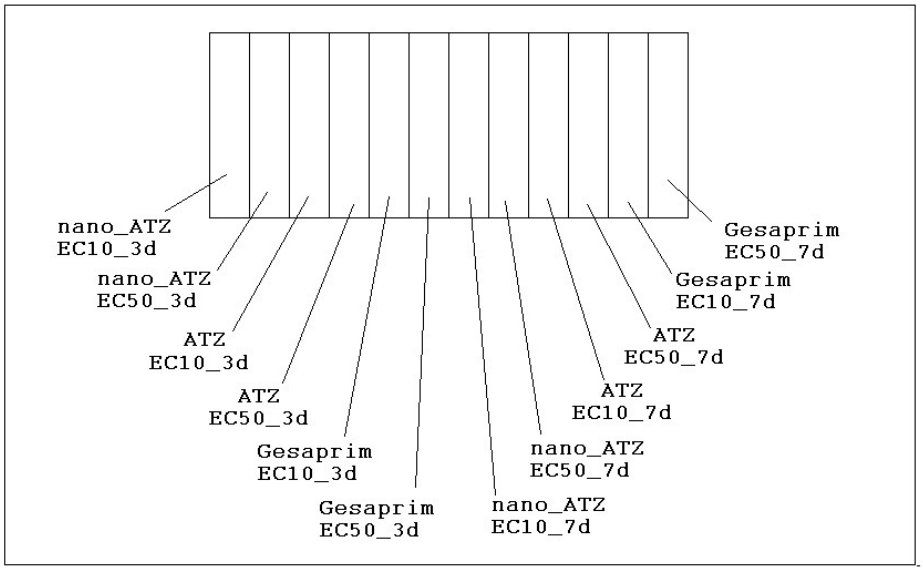


Fig. S2. Principal component analysis (PCA) of the samples (treatments) based on all the DEGs, in at least one test condition (adjusted $p<0.05$) in *Enchytraeus crypticus* when exposed to nanocapsules containing atrazine (nano_ATZ), pure atrazine, a.i. (ATZ), and Gesaprim, at reproduction effect concentrations EC10 and EC50, in LUFA 2.2 soil for 3 and 7 days. The first two components presented explain 48.9% of the data variance (PC1-x axis = 28.4%, PC2-y axis = 20.5%).

For Figs. S3 to S8, KEGG graphs – rendered by Pathview: Fold-change (treatments versus control) of genes representing components of the different KEGG pathways, as examples of pathways that are significantly affected by atrazine exposure, in *Enchytreaus crypticus*. Green and red indicate down- and up-regulation, respectively. Each rectangle (corresponding to a gene) is divided in 12, corresponding to: nano_ATZ_EC10_3d, nano_ATZ_EC50_3d, ATZ_EC10_3d, ATZ_EC50_3d, Gesaprim_EC10_3d, Gesaprim_EC50_3d, nano_ATZ_EC10_7d, nano_ATZ_EC50_7d, ATZ_EC10_7d, ATZ_EC50_7d, Gesaprim_EC10_7d, Gesaprim_EC50_7d, as depicted bellow:



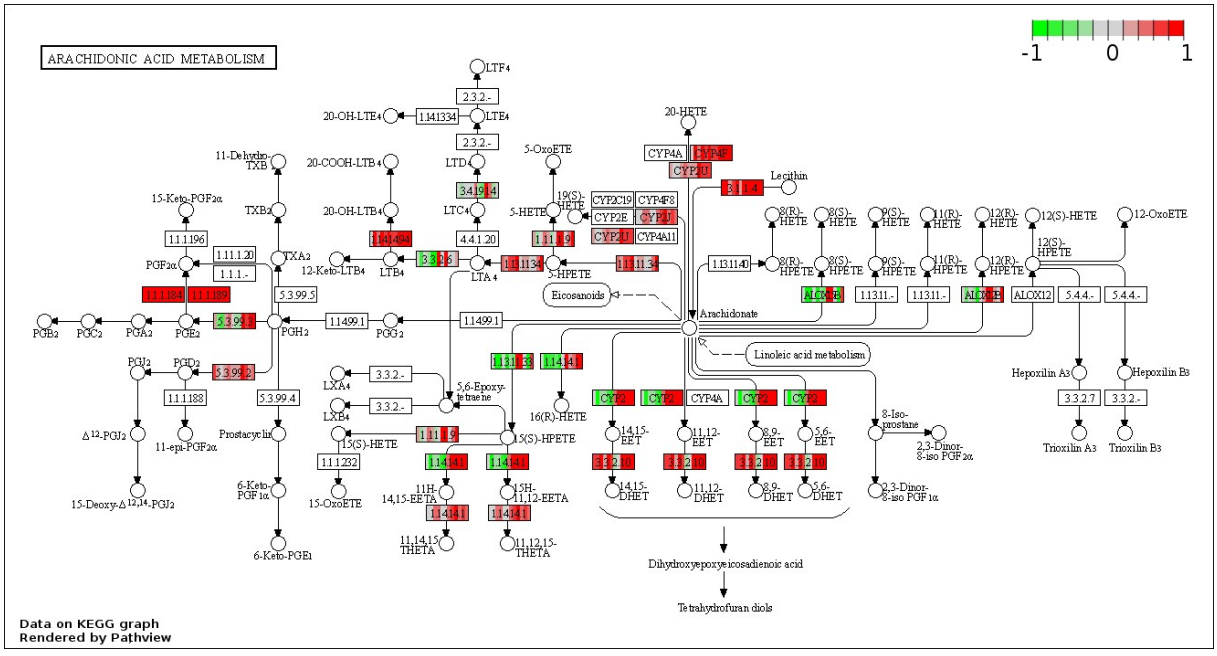


Fig. S3. Details in this pathway can be retrieved from the KEGG website: https://www.genome.jp/kegg-bin/show_pathway?ko00590.

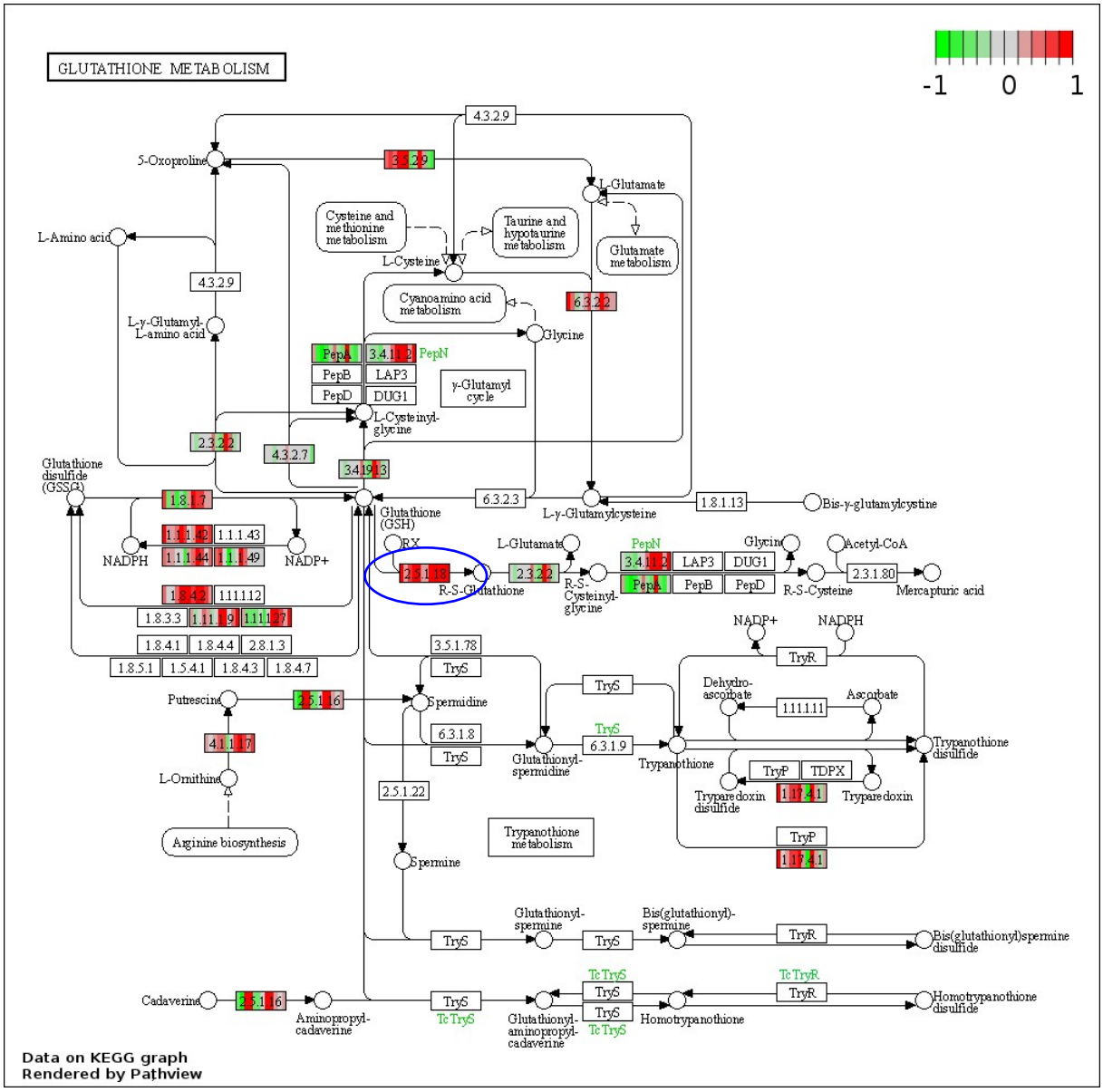


Fig. S4. Details in this pathway can be retrieved from the KEGG website: https://www.genome.jp/kegg-bin/show_pathway?ko00480. Blue circle marks glutathione S-transferase (GST).

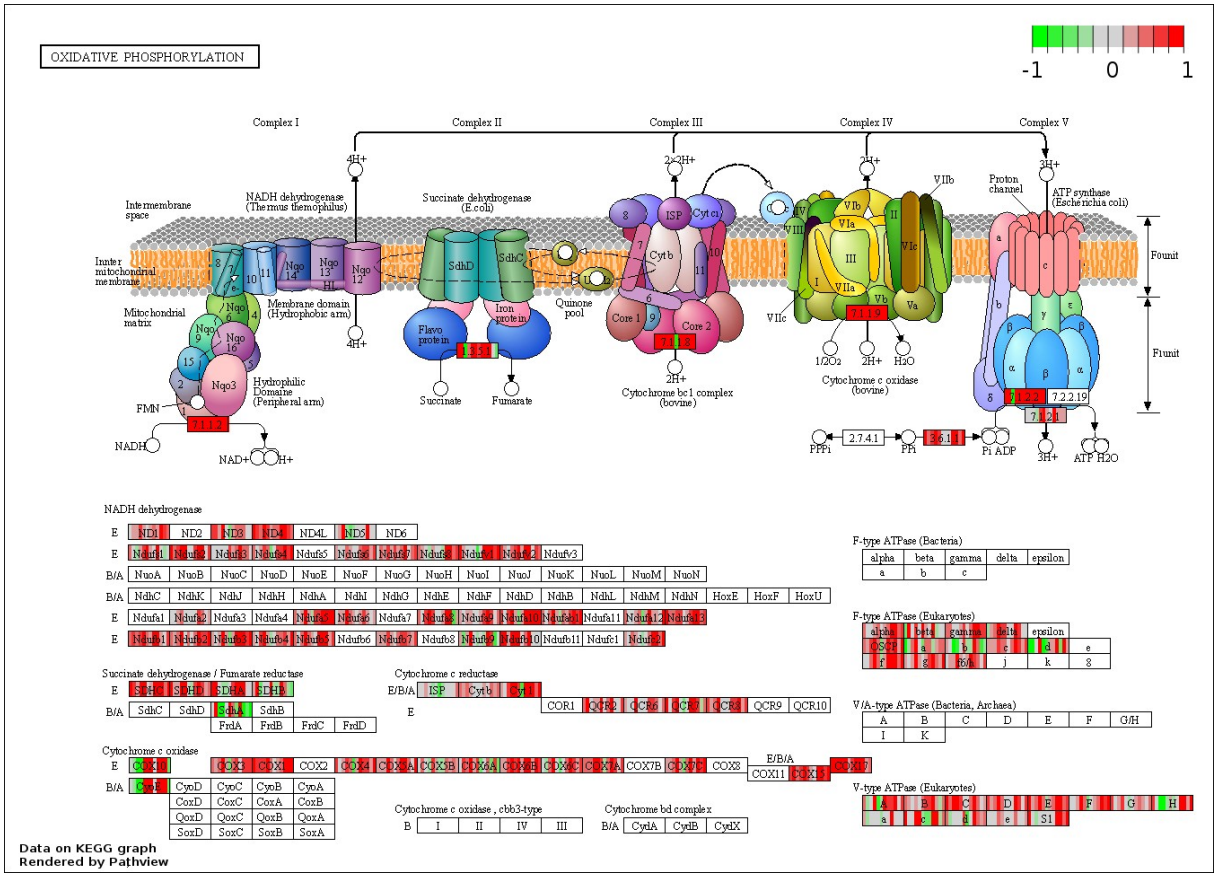


Fig. S5. Details in this pathway can be retrieved from the KEGG website: https://www.genome.jp/kegg-bin/show_pathway?ko00190.

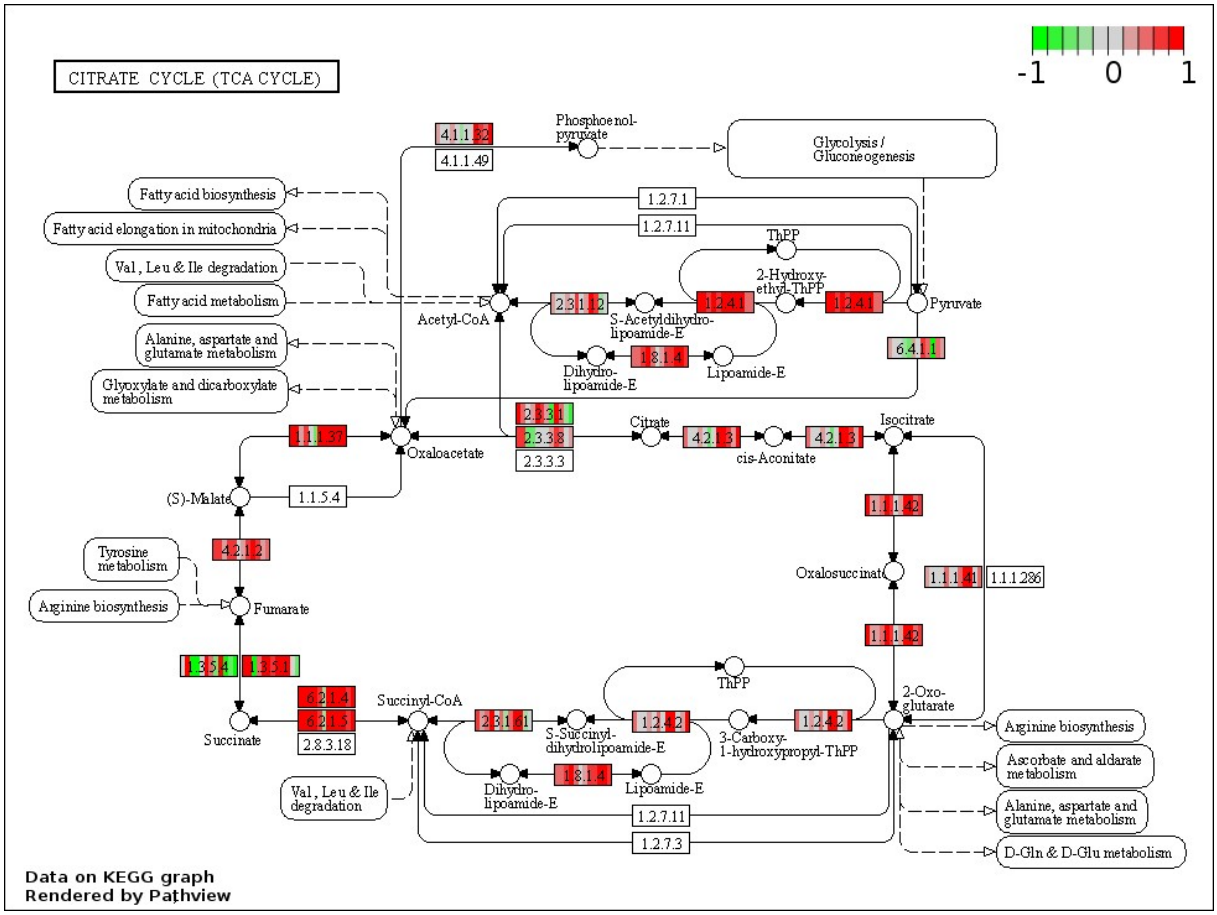


Fig. S6. Details in this pathway can be retrieved from the KEGG website: https://www.genome.jp/kegg-bin/show_pathway?ko00020.

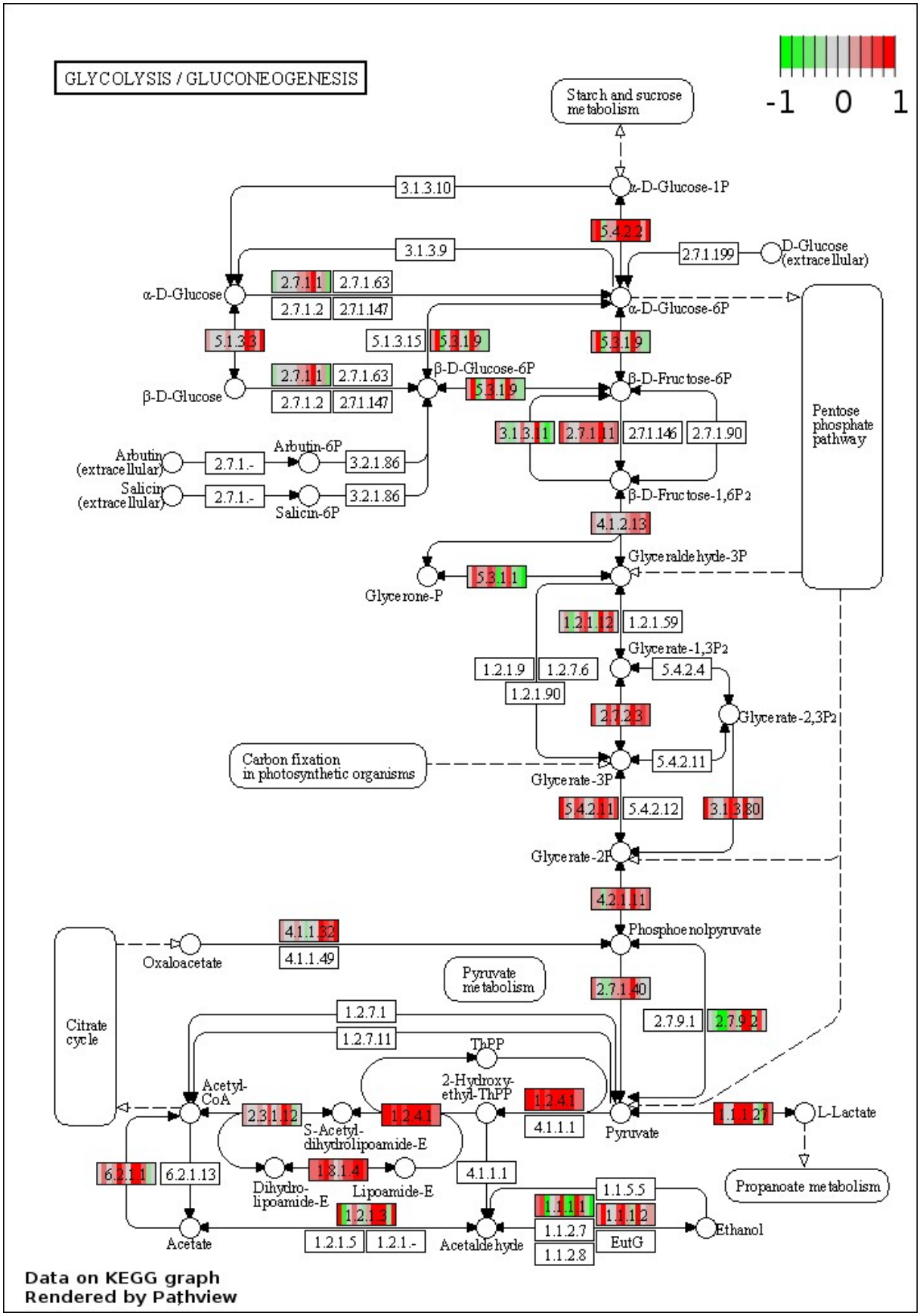


Fig. S7. Details in this pathway can be retrieved from the KEGG website: https://www.genome.jp/kegg-bin/show_pathway?ko00010.

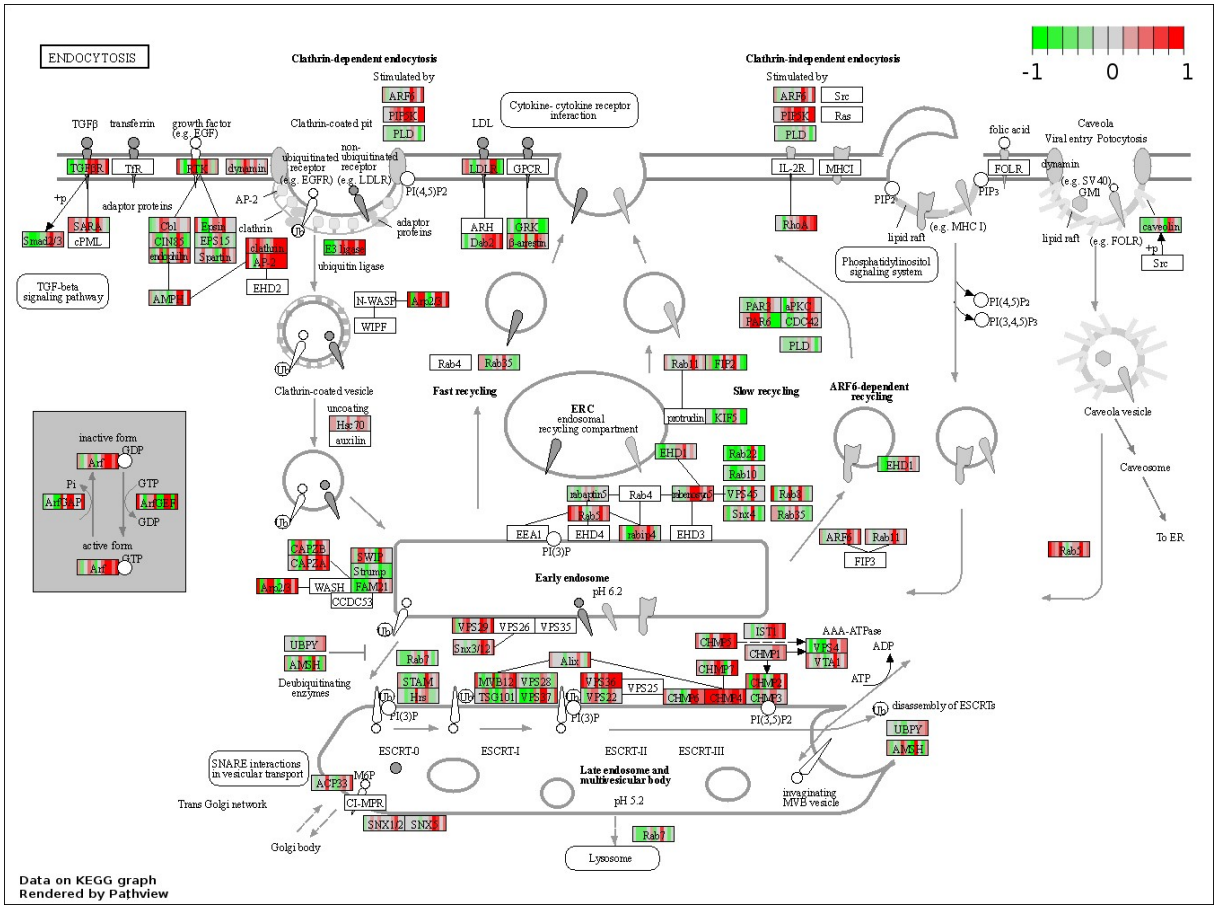


Fig. S8. Details in this pathway can be retrieved from the KEGG website: https://www.genome.jp/kegg-bin/show_pathway?ko04144.