

Clonostachys rosea* ‘omics profiling: identification of putative metabolite-gene associations mediating mycoparasitic properties against *Fusarium graminearum

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Supplementary Figures S1 – S10

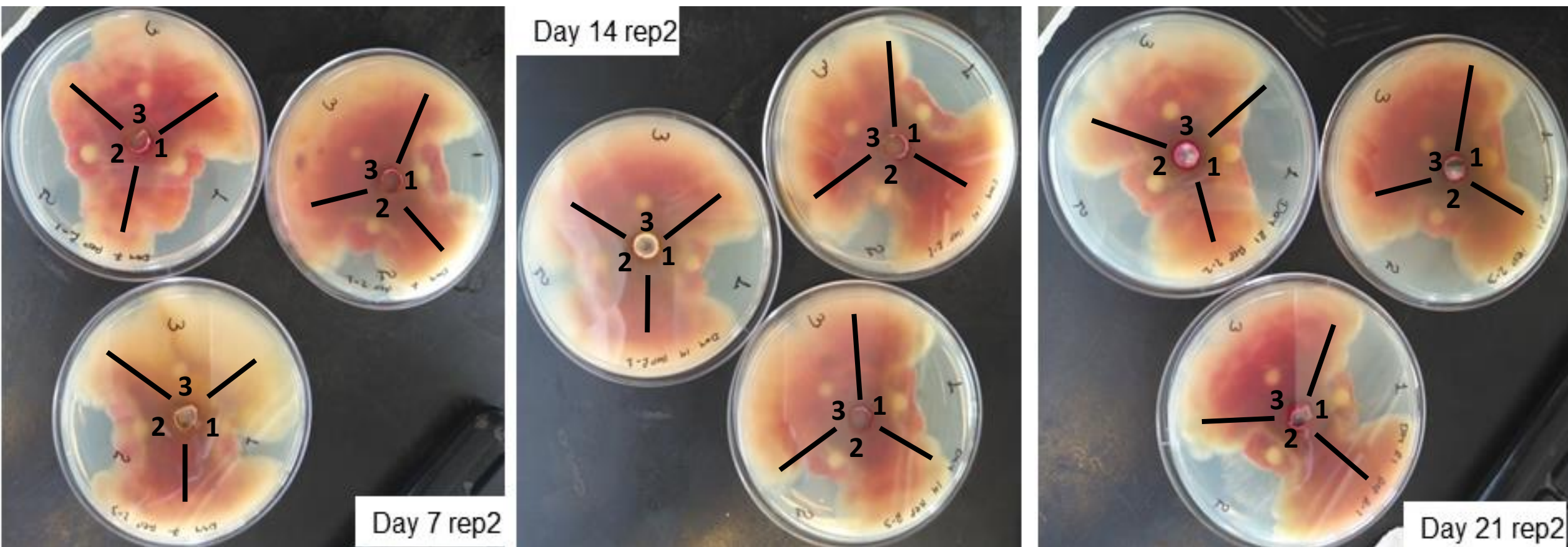


Fig. S1. Representative images that were used to quantify the growth inhibitory activity of exometabolites derived from *C. rosea* spent media after 7, 14 and 21 days of culturing. Growth inhibition of *F. graminearum* strain GZ3639 by exometabolites of *C. rosea* strain ACM941 (1) and 88-710 (2) fermented in Czapek Dox media as indicated. These were compared to methanol-only negative control (3). Zones of inhibition were quantified using ImageJ. Sample plates for n = 3.

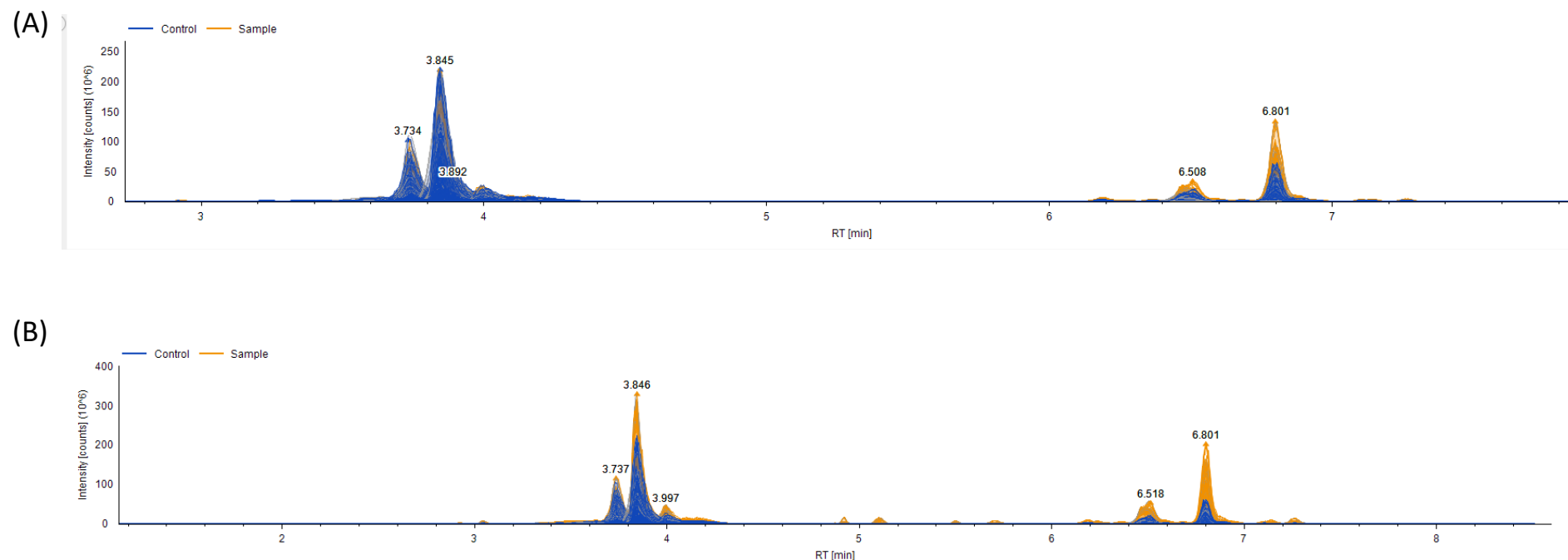


Fig. S2. The differential exometabolite profile of strain ACM-941 over 21 days. **(A)** Day 14 strain ACM-941 extracts (sample-orange) vs D7 extracts (control-blue) shows increase metabolite production for compounds eluting at 6-7.5 min, as opposed to compounds eluting earlier in the range 3.5-4.5 min. **(B)** Day strain 21 ACM-941 extracts (sample-orange) vs D7 extracts (control-blue) shows additional increase metabolite production for compounds at 6-7.5 min range.

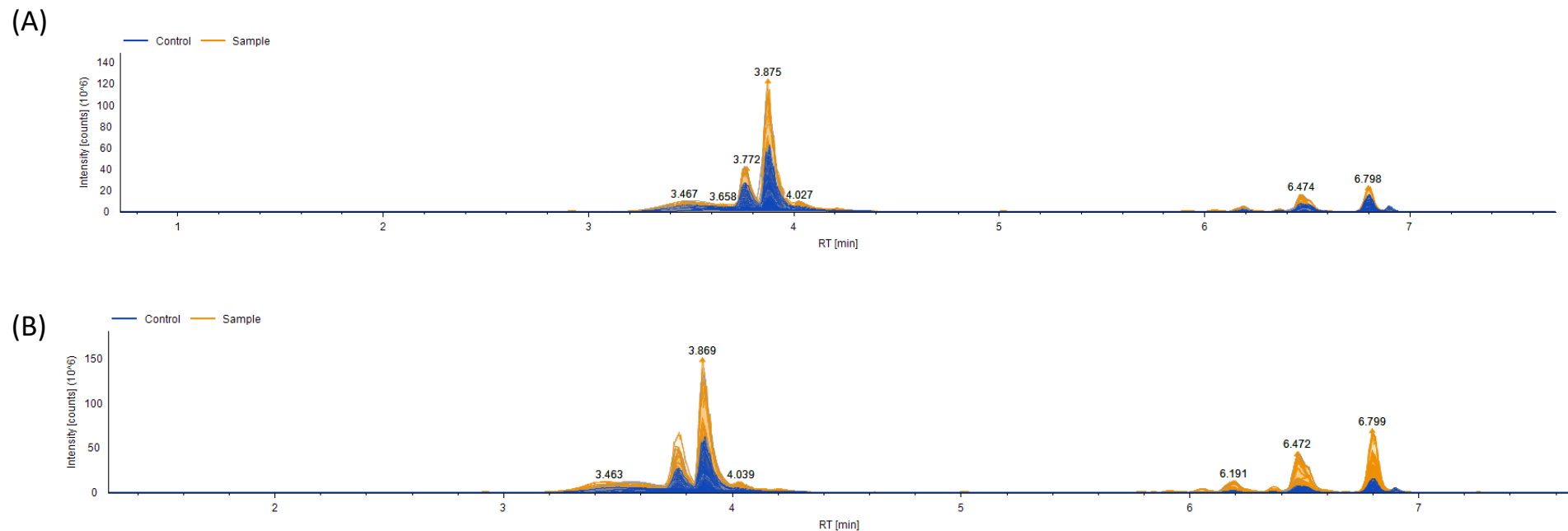


Fig. S3. The differential exometabolite profile of strain 88-710 over 21 days. **(A)** Day 14 strain 88-710 extracts (sample-orange) vs D7 extracts (control-blue) shows muted increase in the of production for compounds eluting at 6-7.5 min range. **(B)** Day 21 strain 88-710 extracts (sample-orange) vs D7 extracts (control-blue) shows clear increase in metabolite production for compounds at 6-7.5 min range.

Fig. S4. Validation of RNAseq results by qPCR. Results arising for a number of selected unchanged, upregulated and downregulated transcripts are shown. Gene ID description scf: 271.g103: , 291.g163: , c_3.g97: , c_291.g117: , 298.g180: , 027.g180: , 050.g111: , 014.g324: , 003.g301: , 289.g159: , 092.g104: , 092.g101: , 068.g206: , 049.g267: , 015.g172: , 027.g161: , 032.g327: , 001.g913: and 097.g246: . RNAseq outcomes are represented by black circles, qPCR outcomes are represented by circles with a central cross.

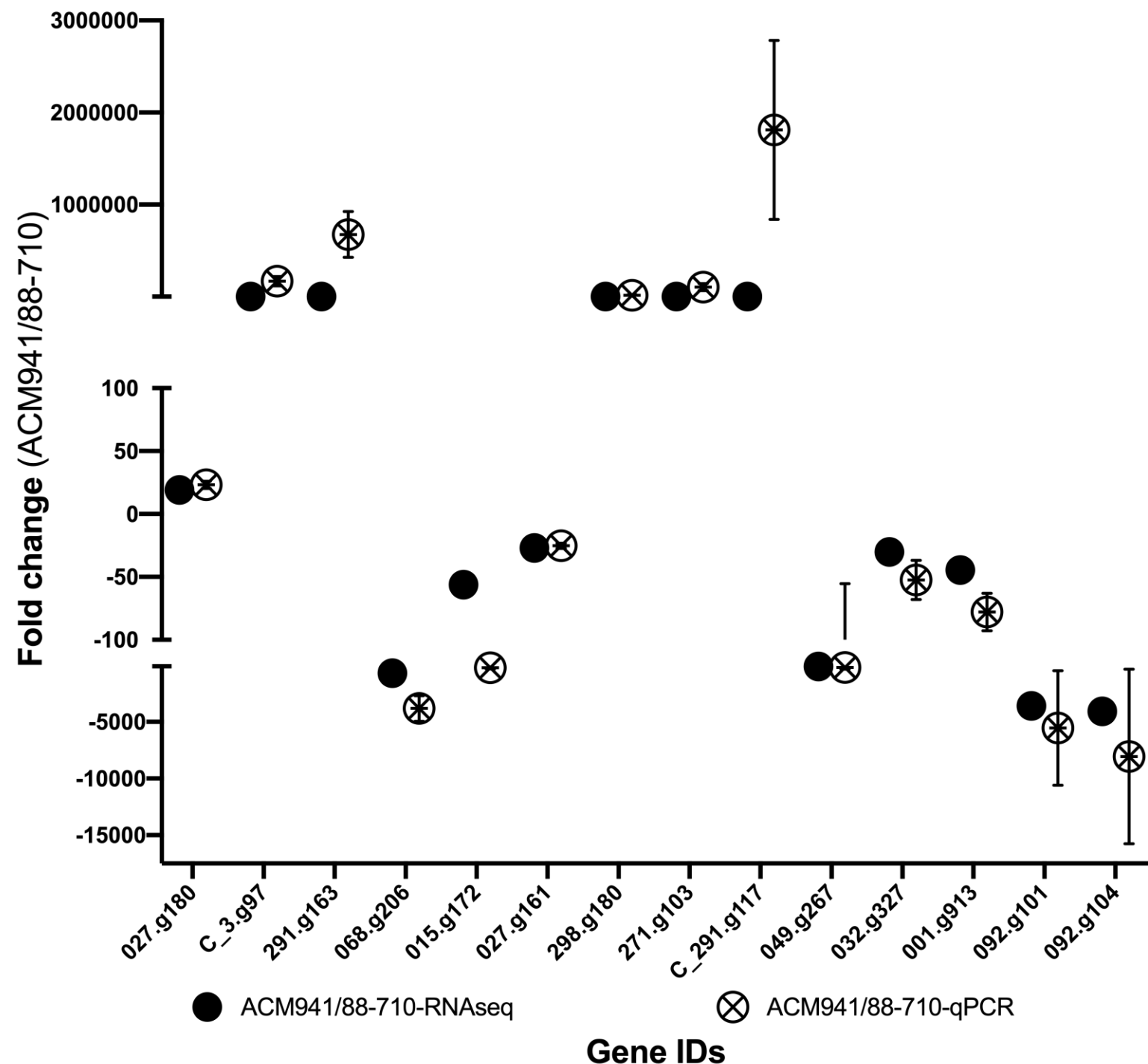
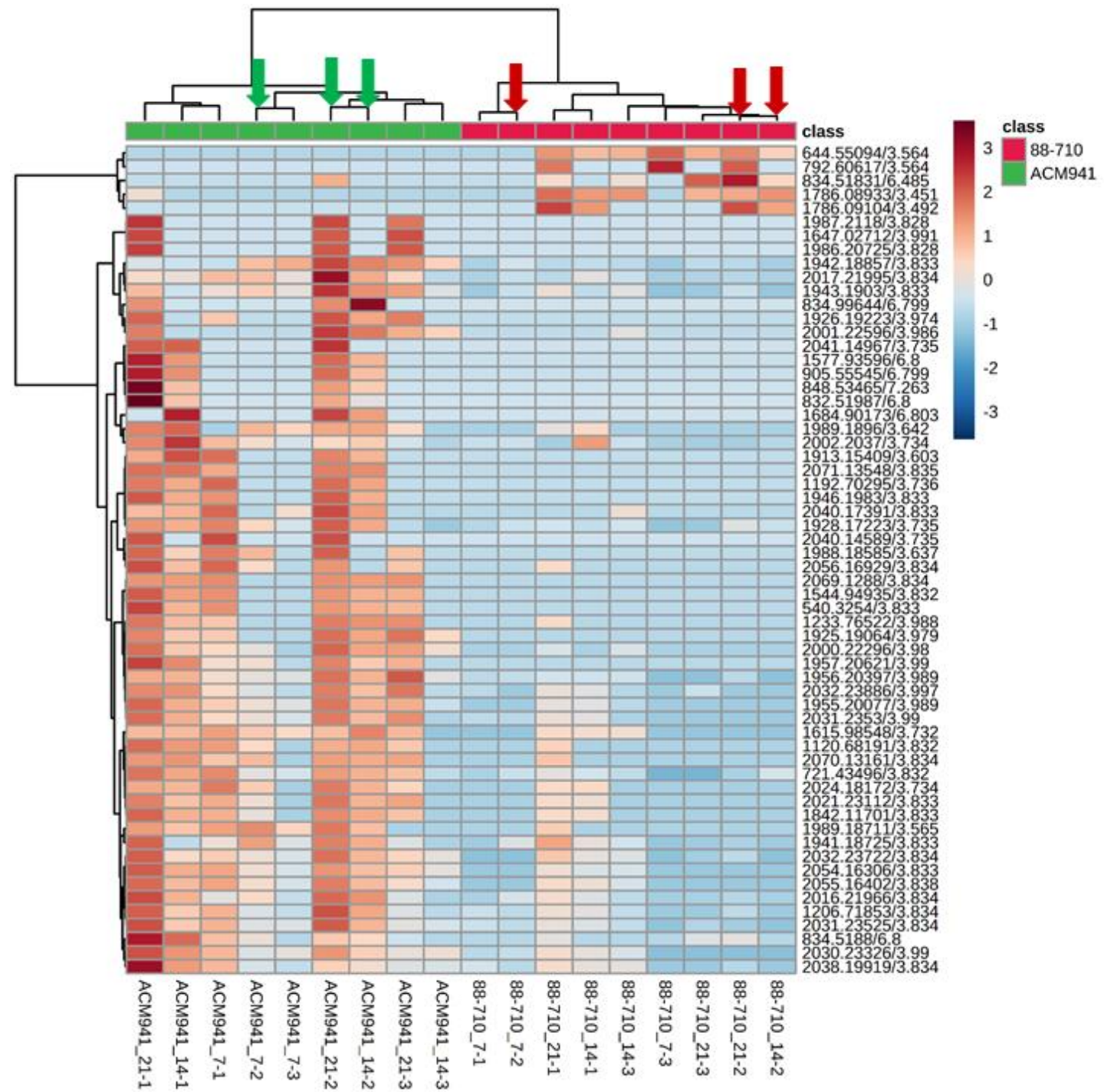


Fig. S5. Heatmap representation of the top 75 highly accumulated metabolites by *C. rosea* strains ACM941 and 88-710 after 7-, 14- and 21-days fermentation in Czapek Dox media. Metabolites are labelled based on mass/charge ratio and retention time (m/z/RT). For sample names, numbers after the hyphens represent replicate number.



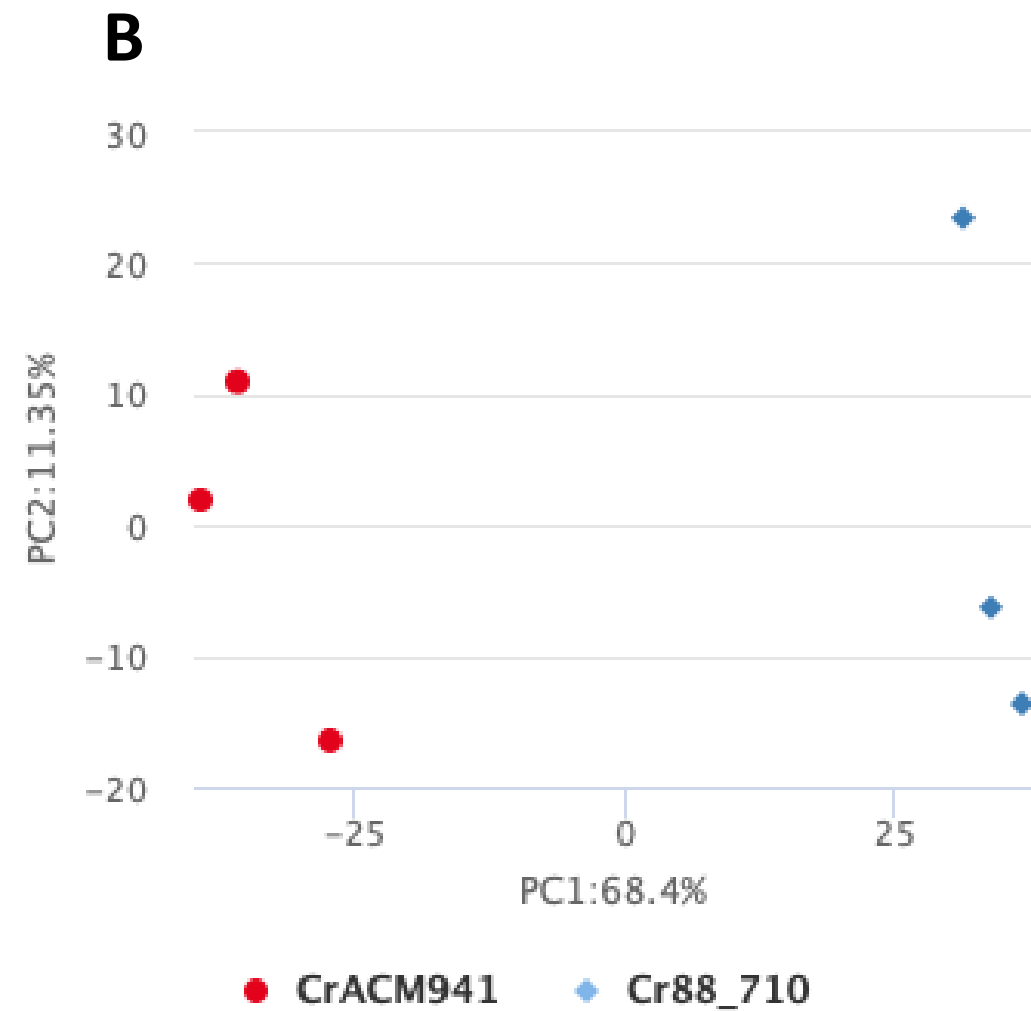
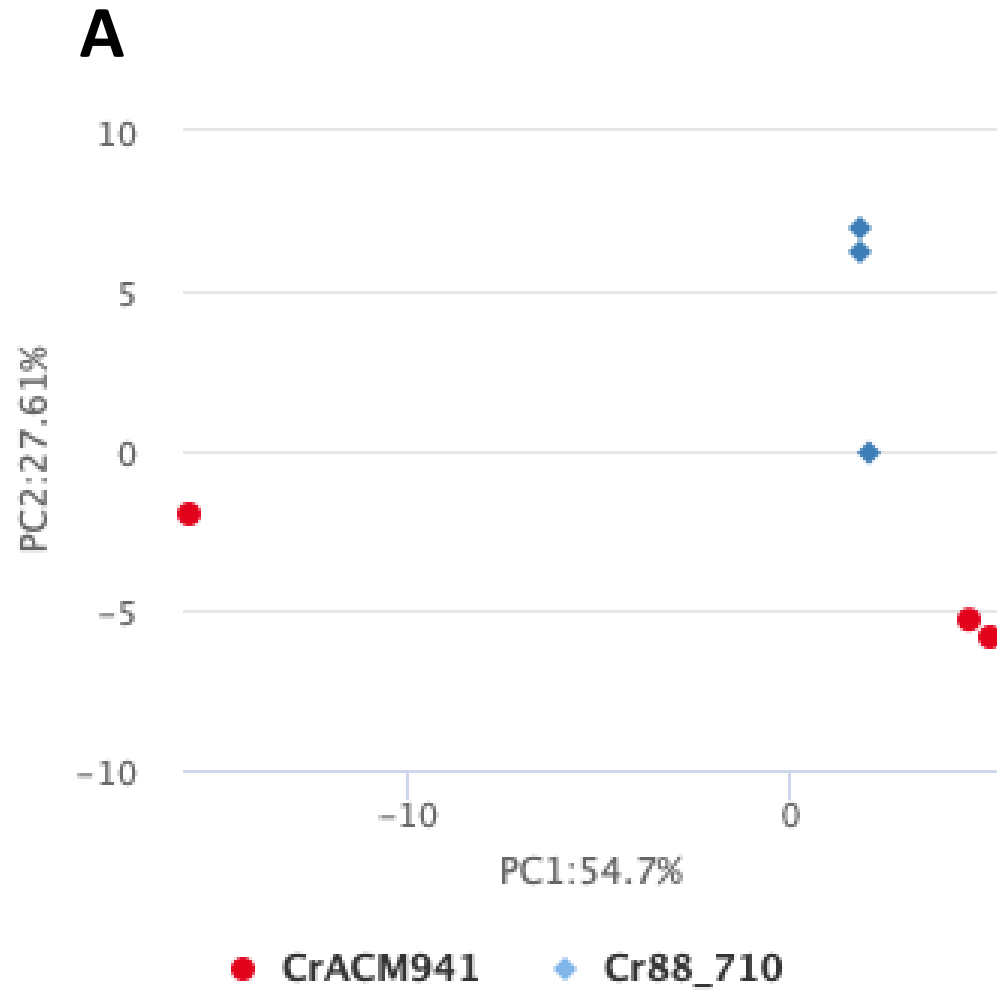


Fig. S6. Principal component analysis of metabolomics (**A**) and gene expression (**B**) data. Red coloured dots represent ACM941 and blue represent 88-710. Sampling was performed after 11 days of fermentation for transcriptomic and exometabolomics analysis.

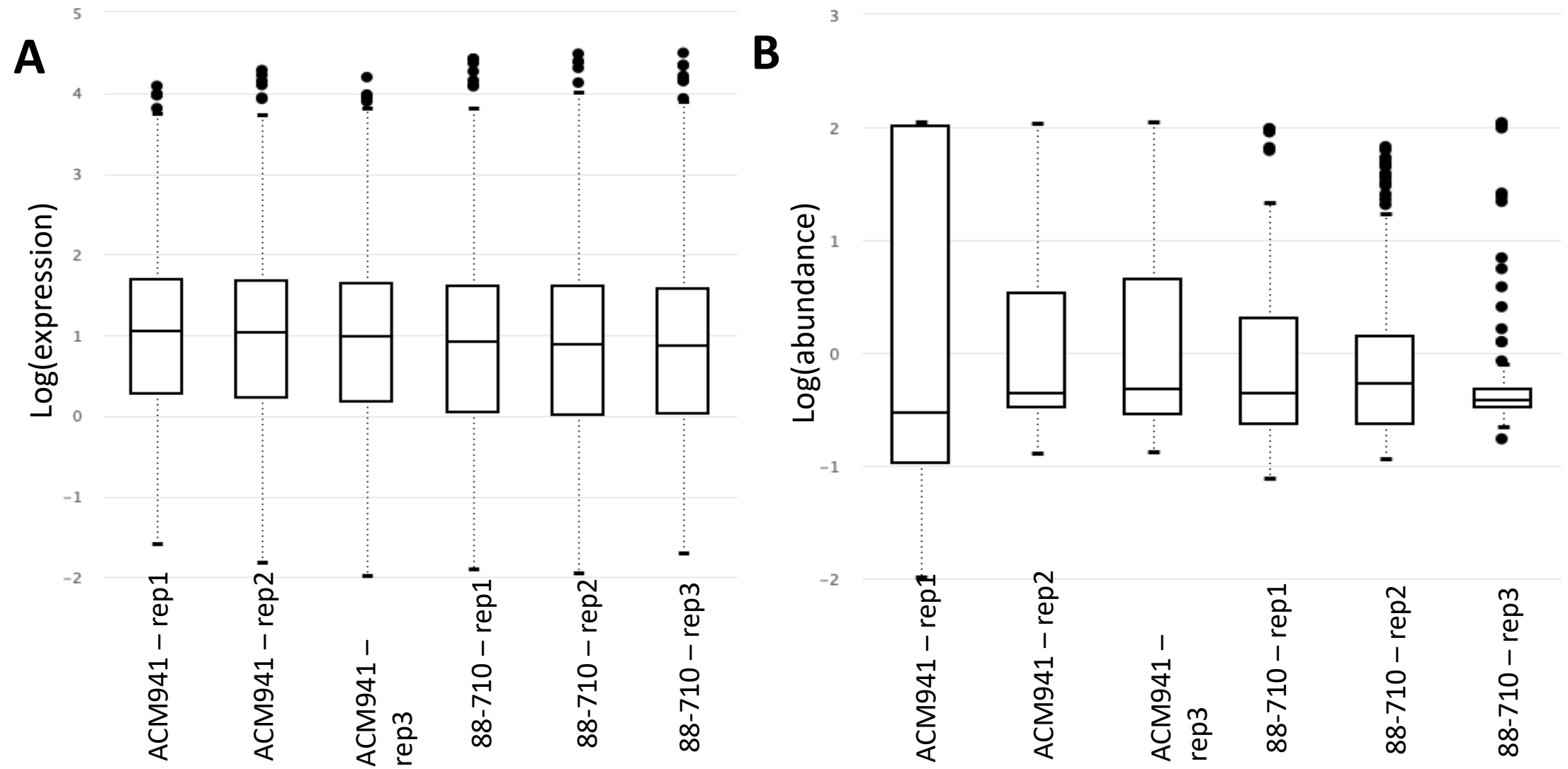


Fig. S7. Box plot representation of distribution of normalized and log2 transformed gene expression **(A)** and metabolomics **(B)** data.

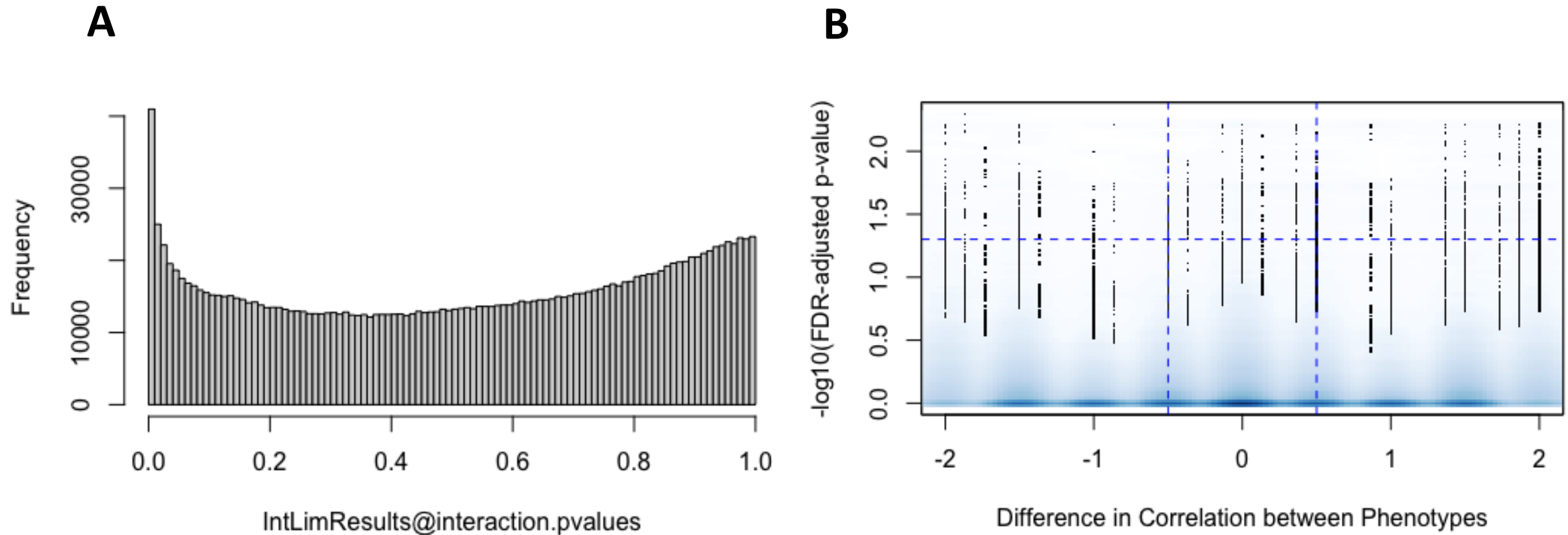


Fig. S8. IntLIM linear modeling gene-metabolite pairing. **A)** Histogram of Interaction p-values. All possible gene-metabolite pairs and extract FDR-adjusted interaction p-values < 0.05 . **B)** A volcano plot depicting the absolute difference in correlation vs. $-\log_{10}(\text{FDR-adjusted p-values} < 0.01)$.

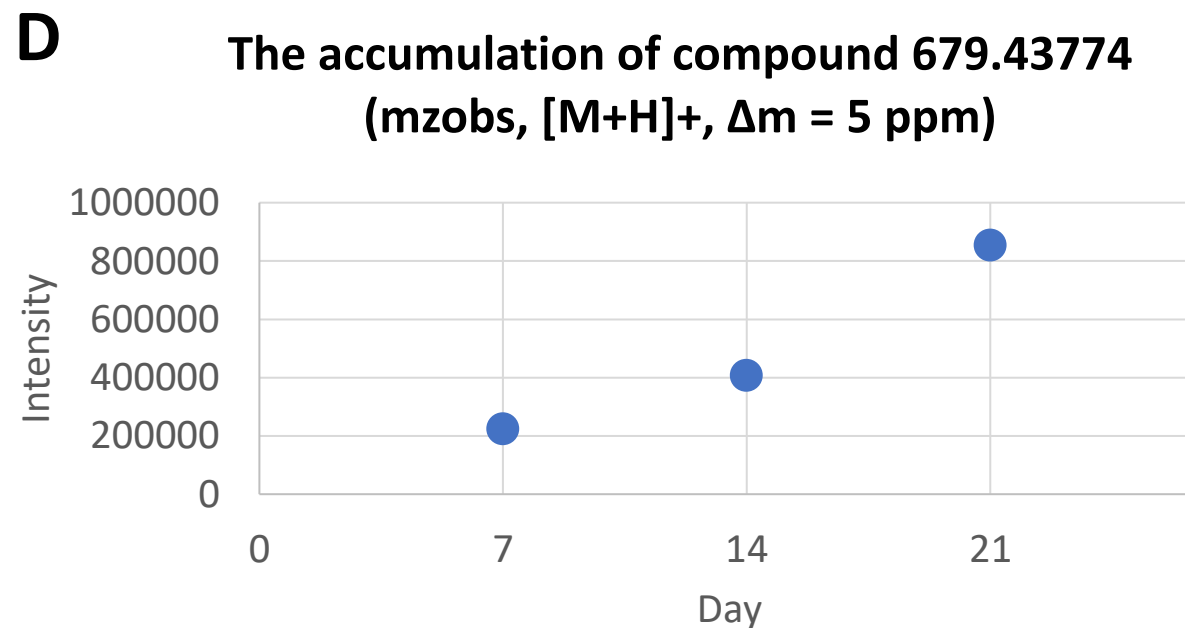
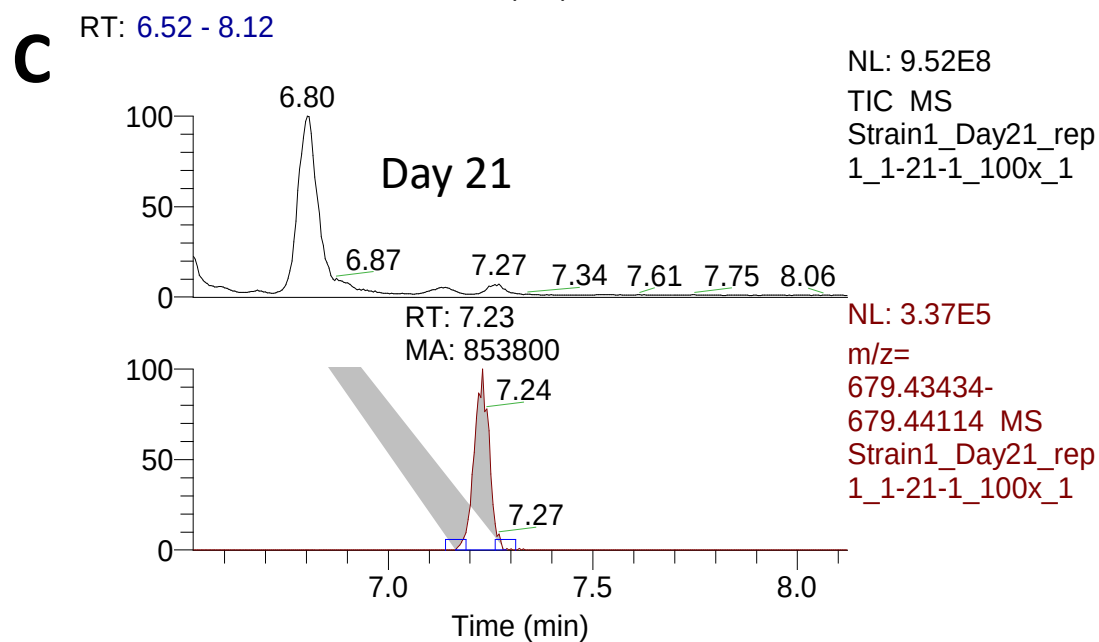
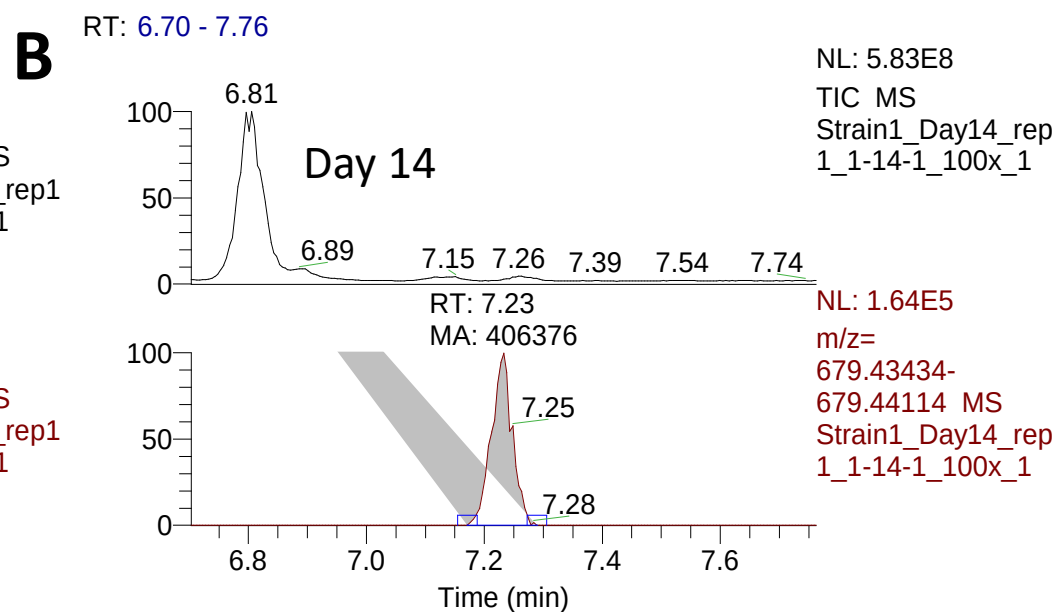
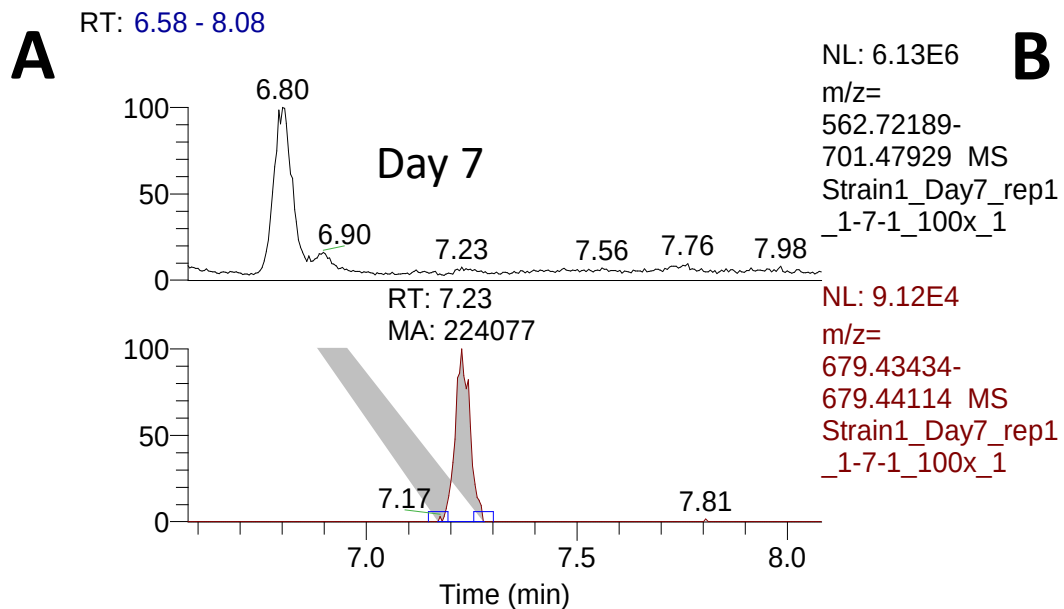


Fig. S9. Compound with mz_{obs} 679.43774 elutes at 7.23 minutes is consistent with observed. Extracted ion chromatogram at **A)** Day 7, **B)** Day 14 and **C)** Day 21 for mz_{obs} 679.43774 ($[M+H]^+$). **D)** The intensity of this metabolite was found to double every 7 days.

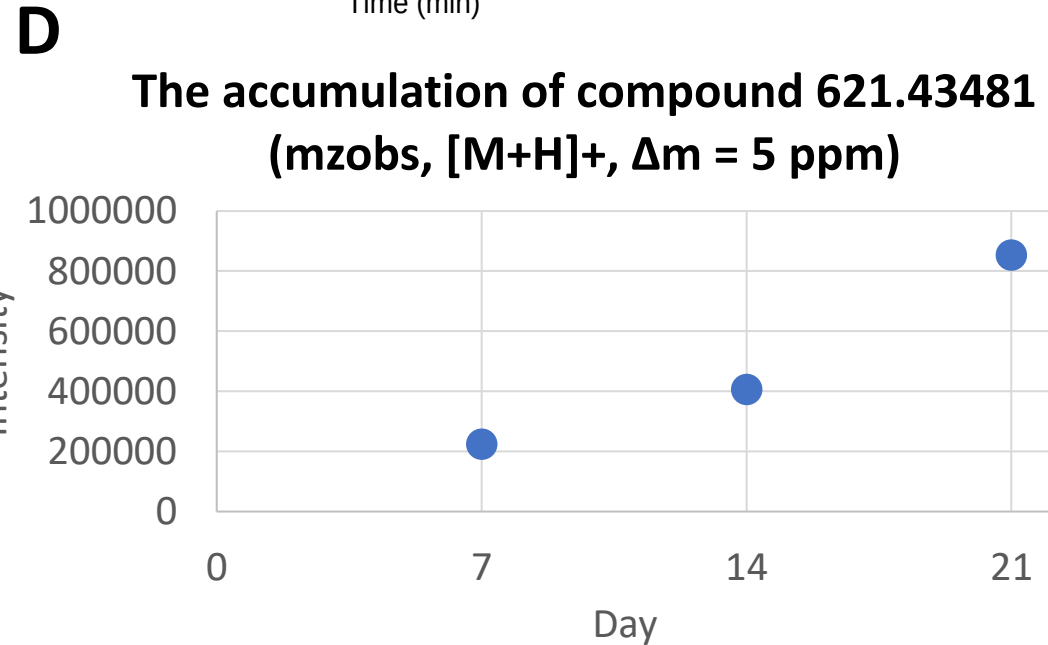
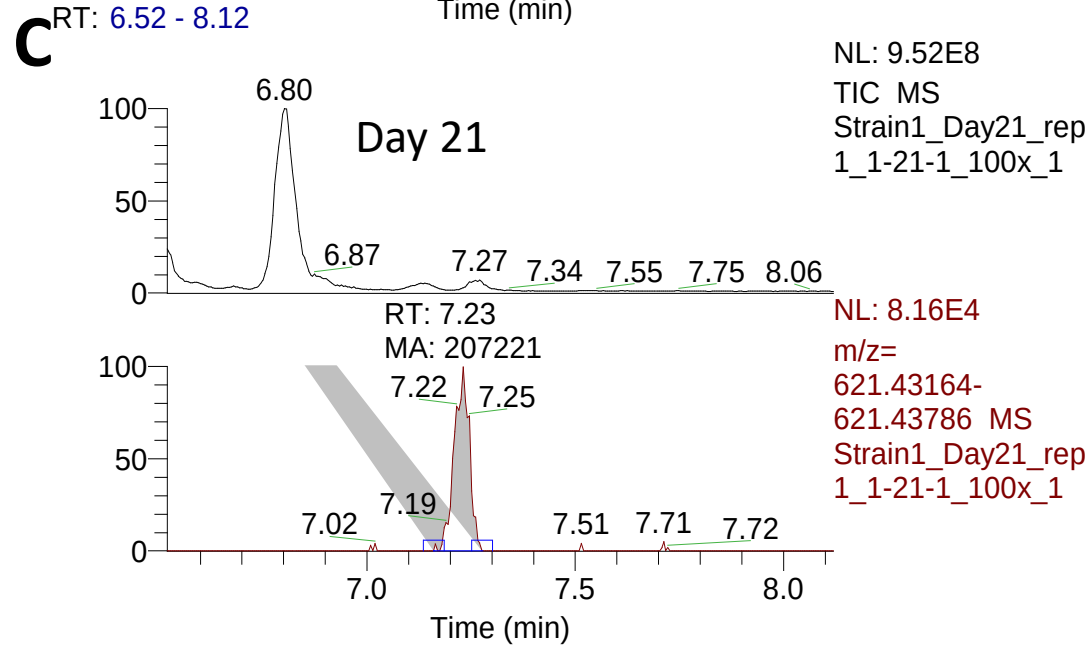
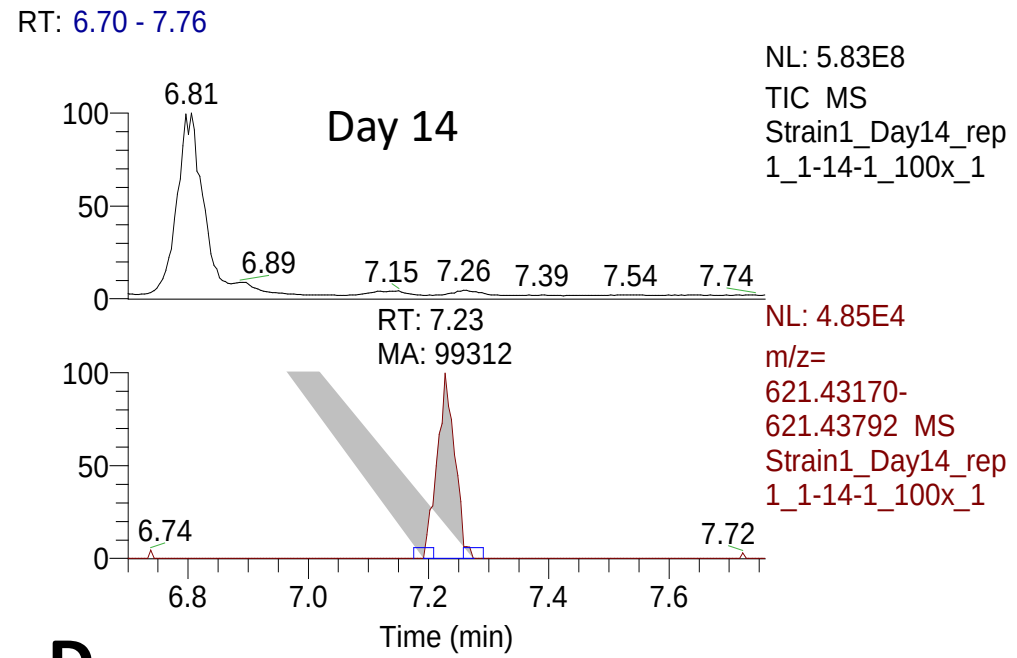
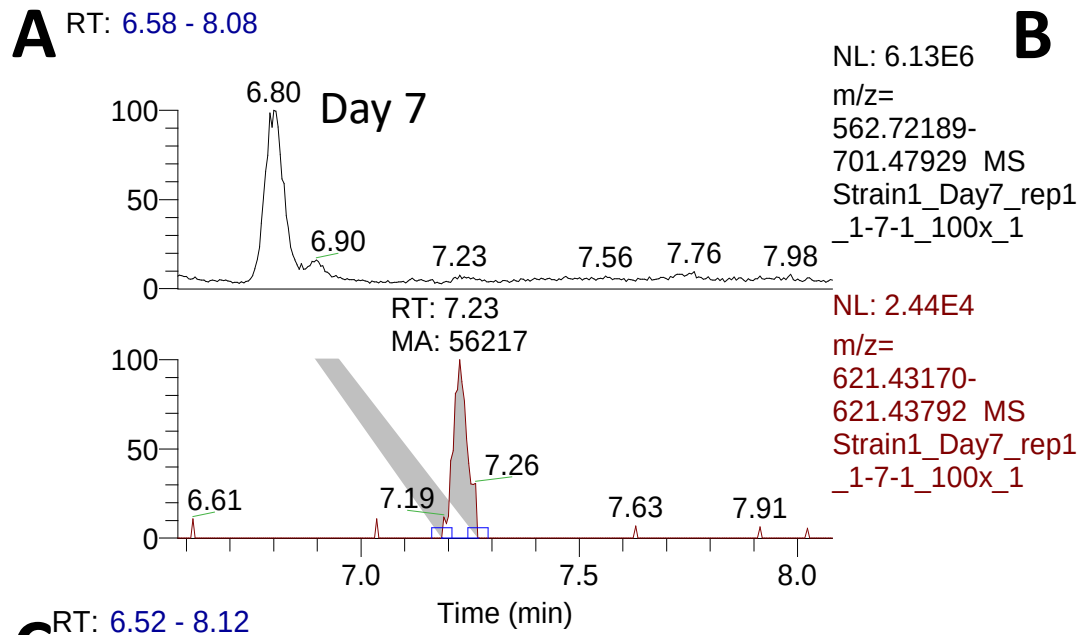


Fig. S10. Compound with mz_{obs} 621.43481 also elutes at 7.23 minutes. Extracted Ion chromatogram at **A)** Day 7, **B)** Day 14 and **C)** Day 21 for mz_{obs} 621.43481 ($[M+H]^+$). **D)** The intensity of this metabolite was also found to double every 7 days.