Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary information

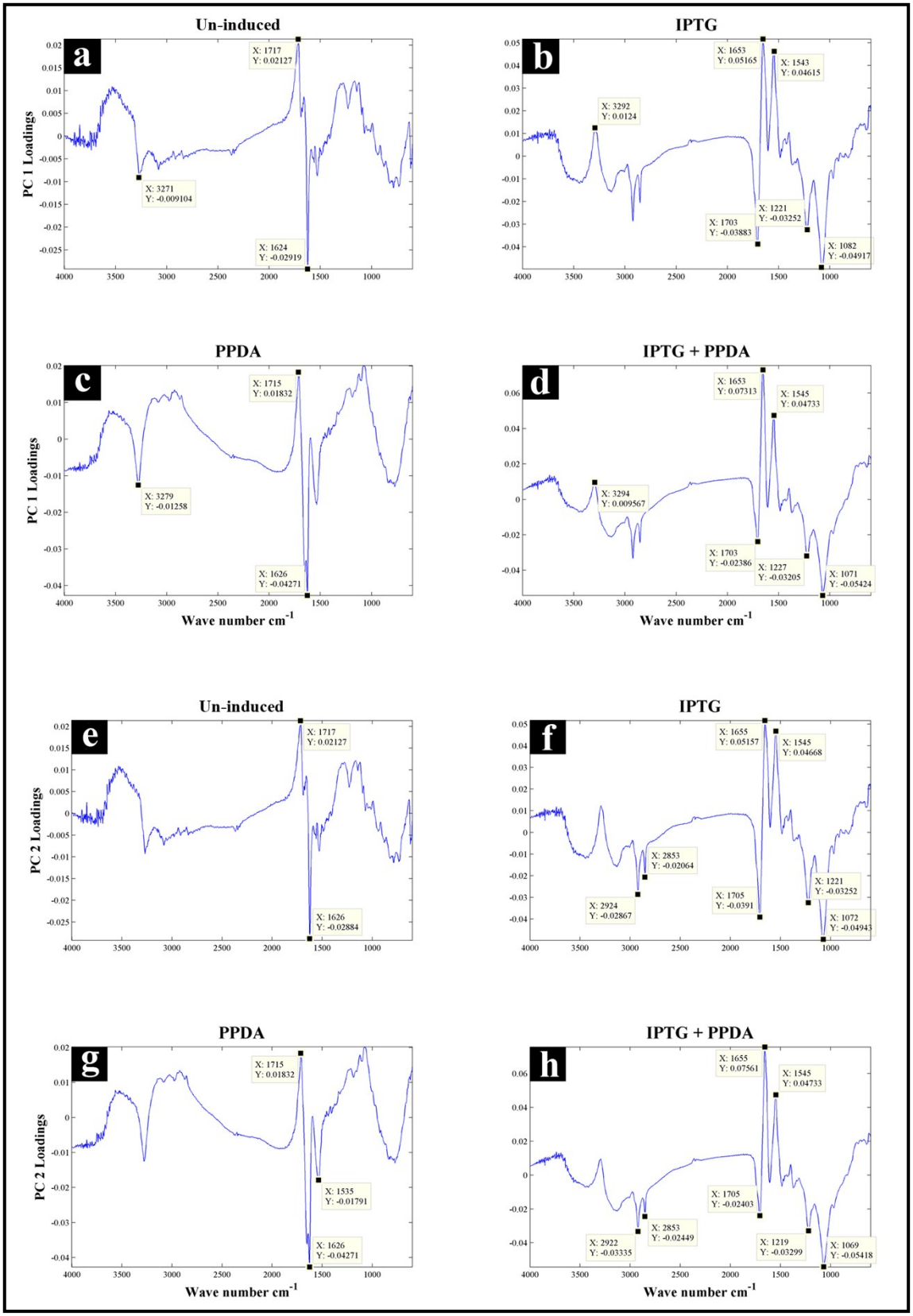

Figure S1. Loadings plots of PC1 (a-d) and PC2 (e-h) for the inducer-blocked FT-IR data. The corresponding PC scores plot is provided in Figure 4.

GC-MS multiblock PCA results

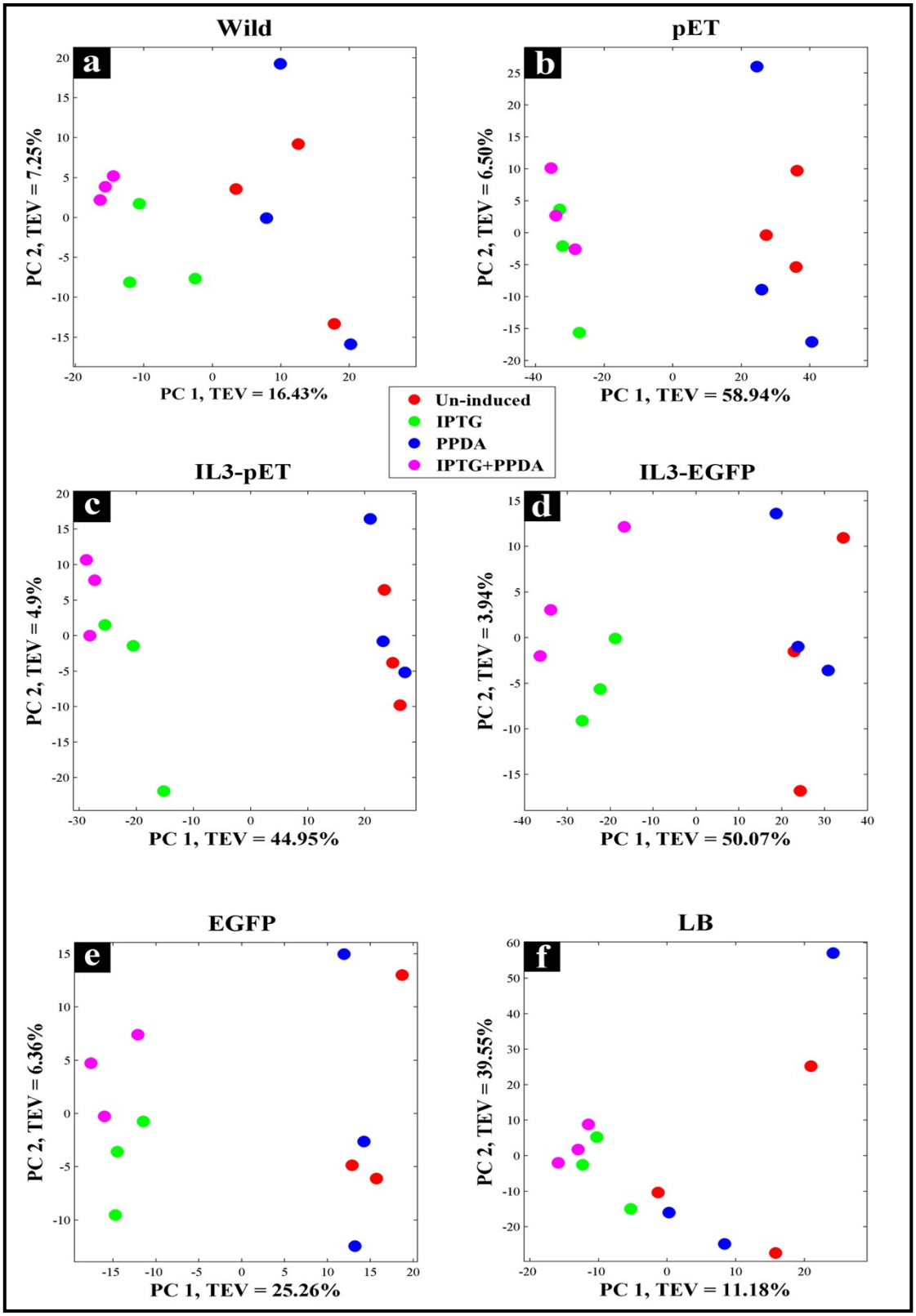

Figure S2. CPCA-W scores plots of GC-MS footprints for strain-blocked data. Different colours in the legend represent the examined inducing conditions.

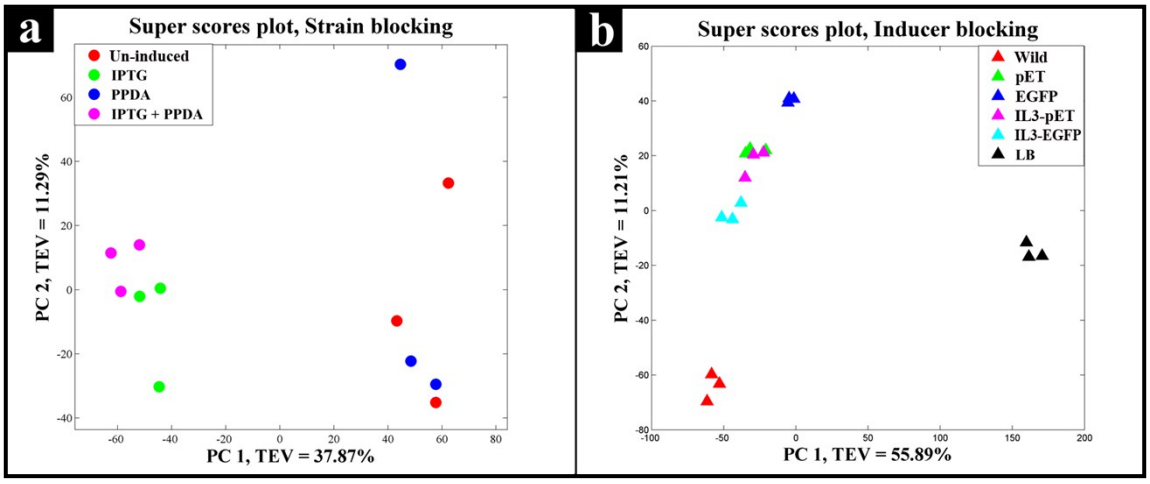

Figure S3. CPCA-W super scores plots of GC-MS footprints a) strain-blocked and b) inducer-blocked data.

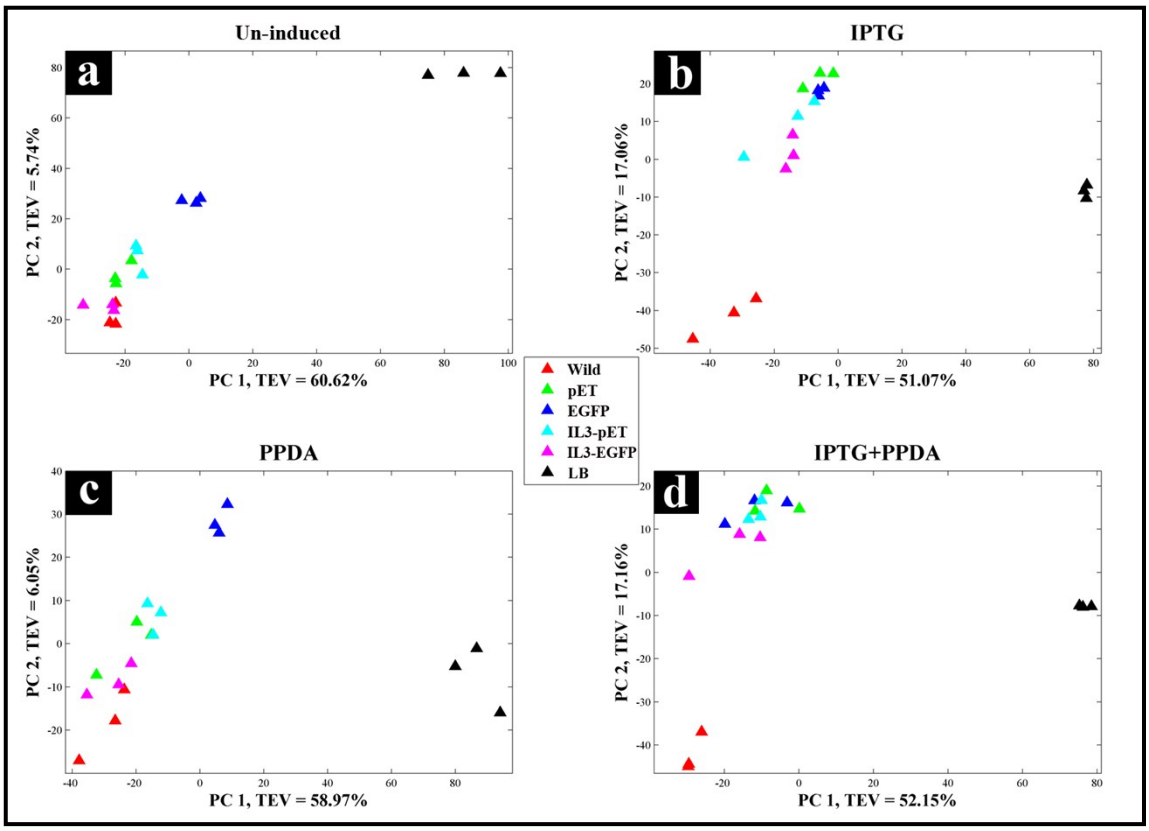

Figure S4. CPCA-W scores plots of GC-MS footprints of inducer-blocked data. Different colours in the legend represent various strains.

PPDA solutions and quantification

A 20 mM PPDA solution was prepared by dissolving 56 mg of PPDA powder in 10 mL DI water followed by 20 min sonication (maximum power) at room temperature. The solution was centrifuged for 30 s at 5000 *g* and filter sterilized twice using 0.22 µM filters. The final solution was dispensed into 2 mL aliquots in microcentrifuge tubes and stored at -20 °C. The following PPDA concentrations (n = 3) were made in LB, analysed by GC-MS and used for quantification of PPDA in media and cell extract samples (Table S1). PPDA mass fragments of 305, 177, and 138 *m/z* were used for identification of the peak in the chromatogram and the total ion count (TIC) was normalised using the internal standard (succinic acid-d_4) and used for comparison and quantification purposes.

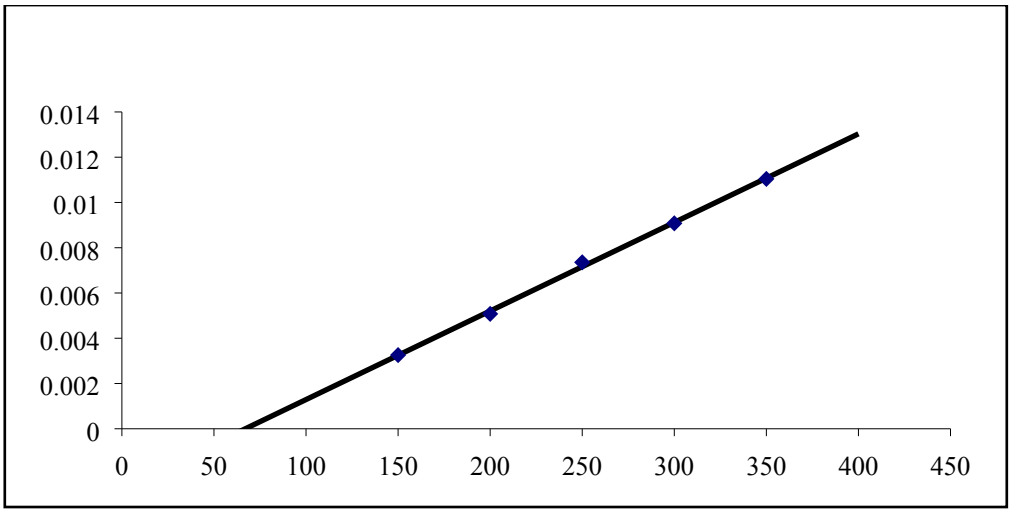

Figure S5. PPDA standard curve plotted based on normalized TICs over concentrations.

Table S1. The different concentration of PPDA used for construction of the standard curve. Calculated concentrations represent the mean of three replicates, ± mean of standard deviation.

PPDA solutions (µM)	Normalized TICs	Recalculated concentration (µM)
150	0.0033	131.40 ± 6.02
200	0.0051	176.93 ± 7.68
250	0.0074	233.88 ± 0.74
300	0.0091	276.95 ± 2.07
350	0.0110	326.02 ± 9.02

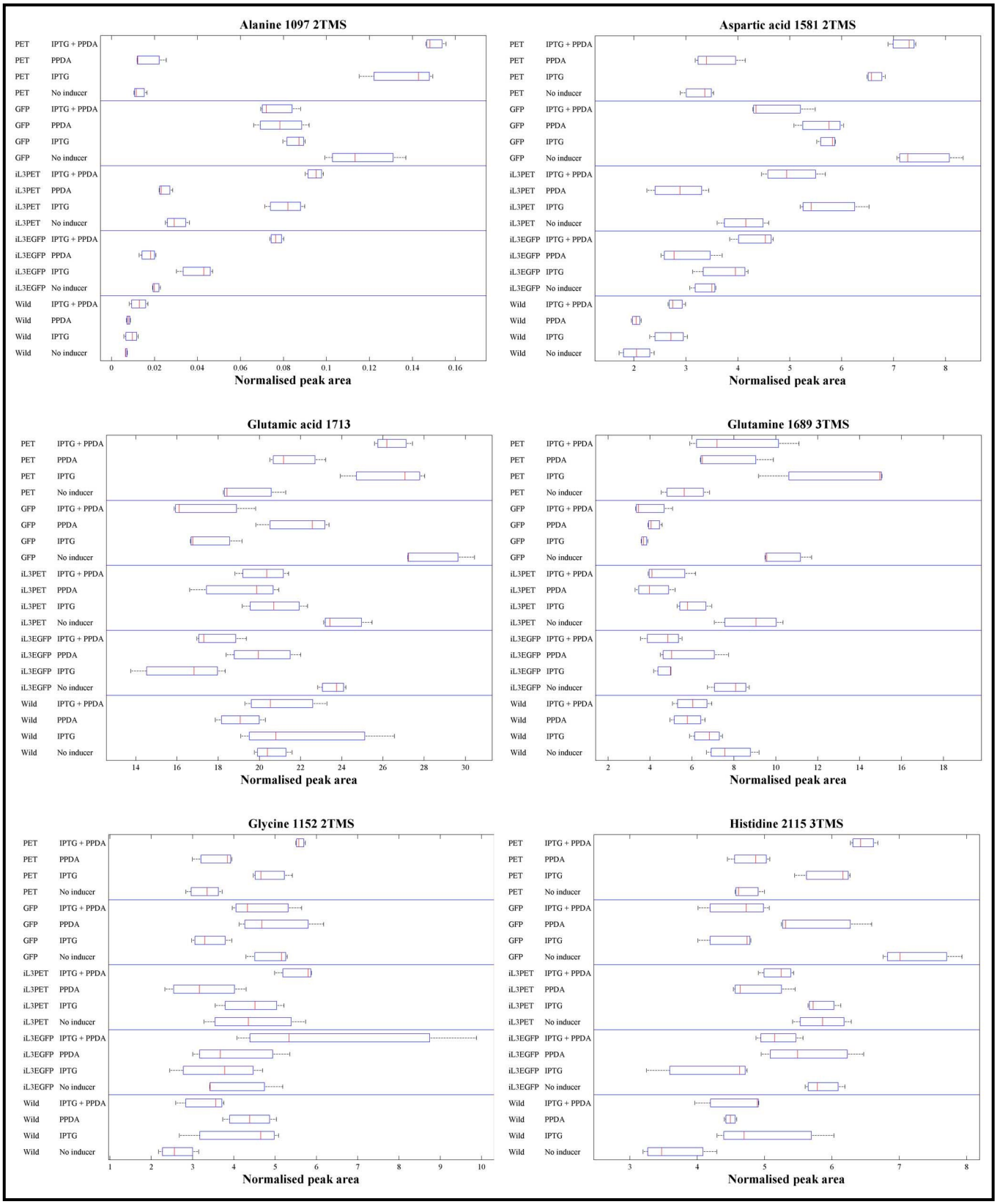

Figure S6. Relative peak intensities of different amino acids detected via GC-MS analysis in the cell extract samples under the different examined inducing conditions.

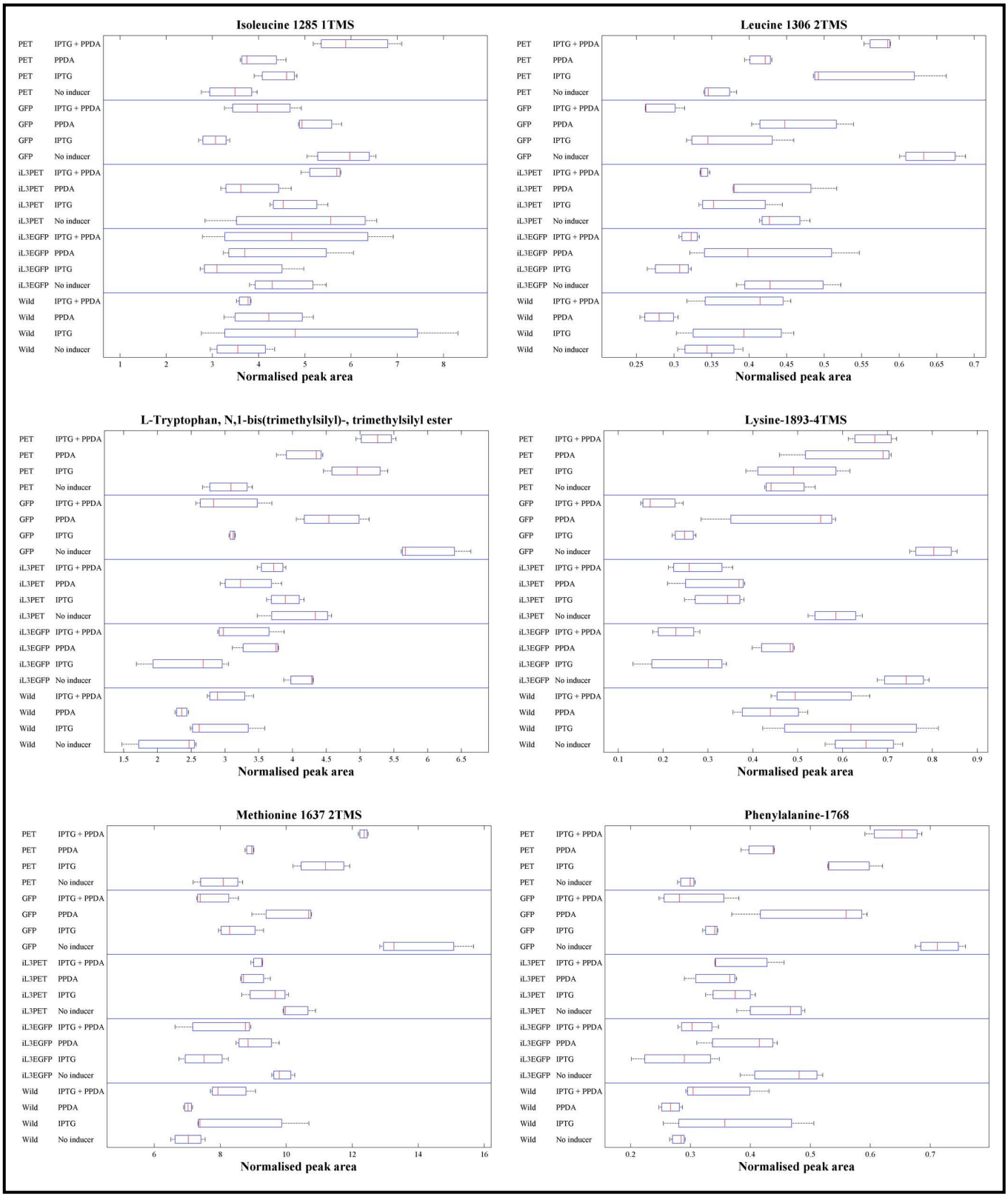

Figure S7. Relative peak intensities of different amino acids detected via GC-MS analysis of the cell extracts under the examined inducing conditions.

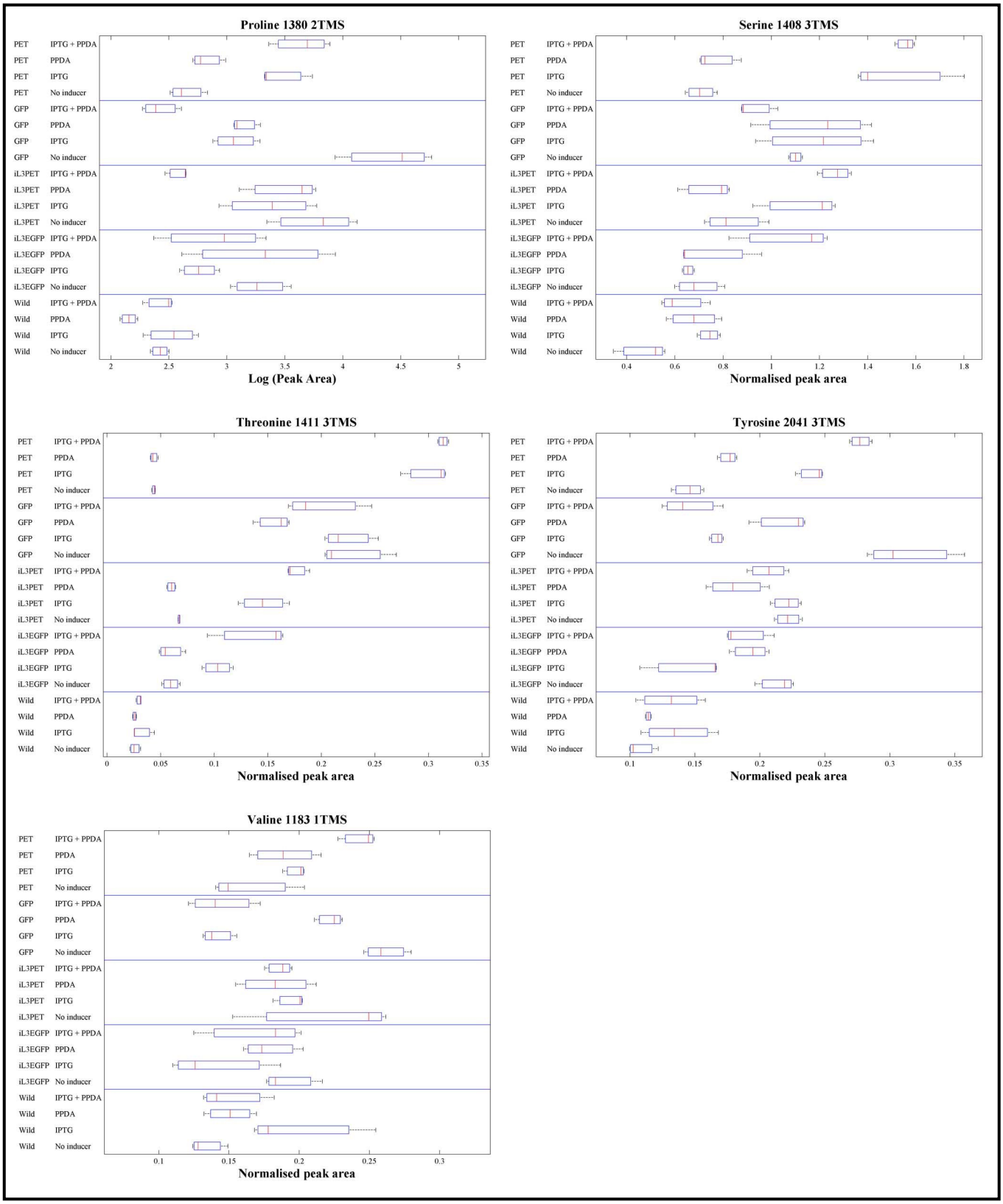

Figure S8. Relative peak intensities of different amino acids detected via GC-MS analysis in the cell extract samples under the different examined inducing conditions.

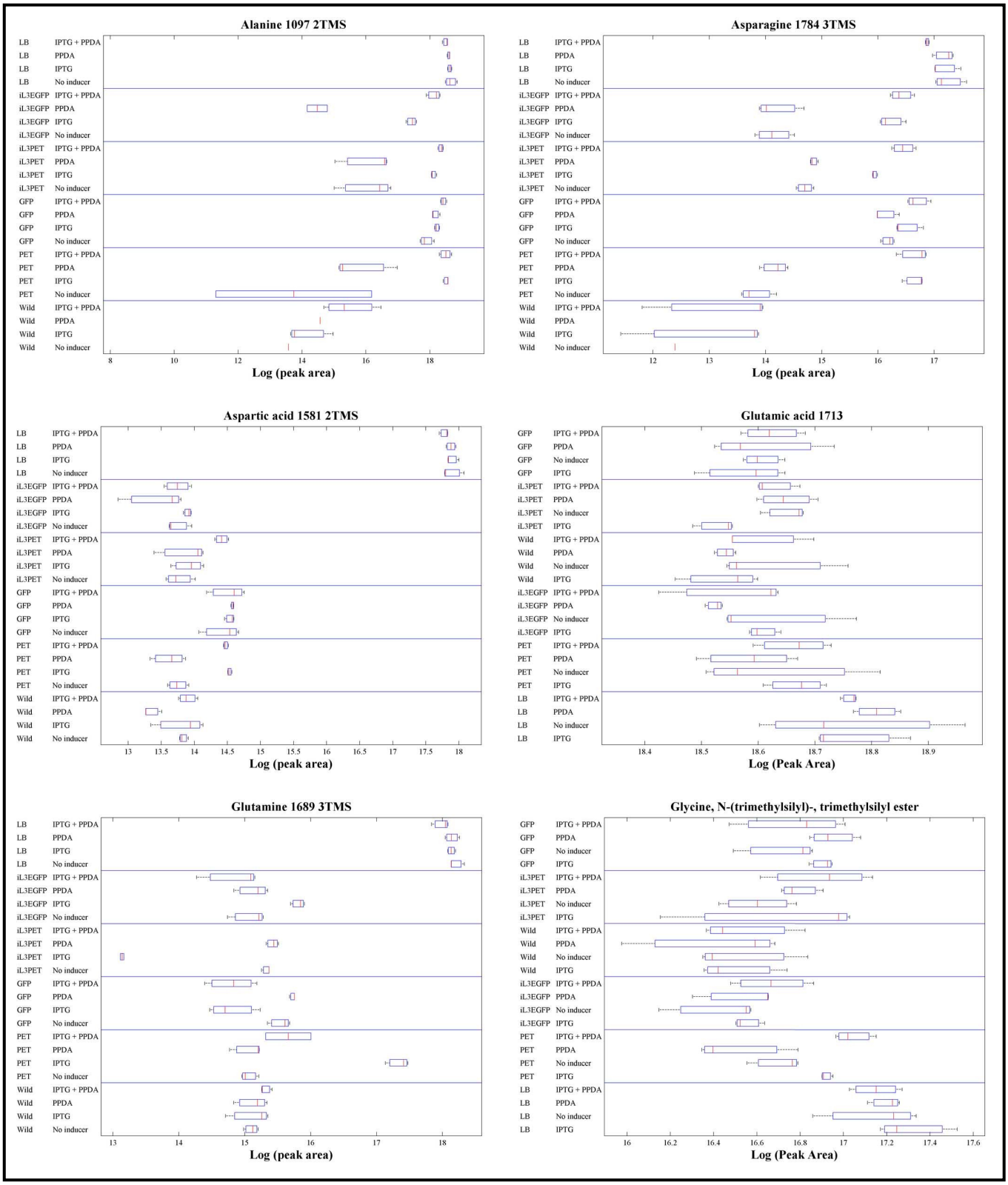

Figure S9. Relative peak intensities of different amino acids detected via GC-MS analysis in the media samples under the different examined inducing conditions.

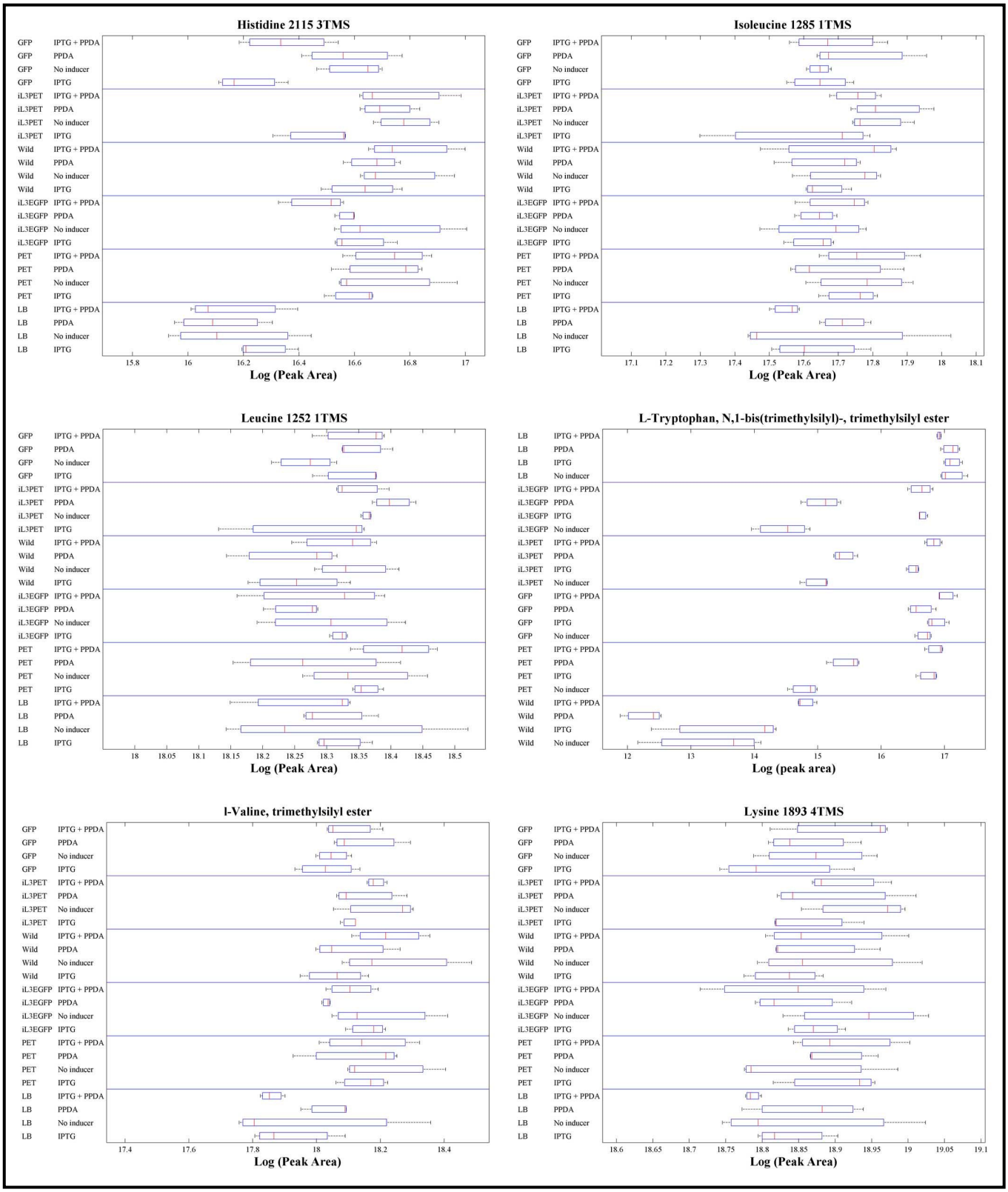

Figure S10. Relative peak intensities of different amino acids detected via GC-MS analysis of the media samples under the different examined inducing conditions.

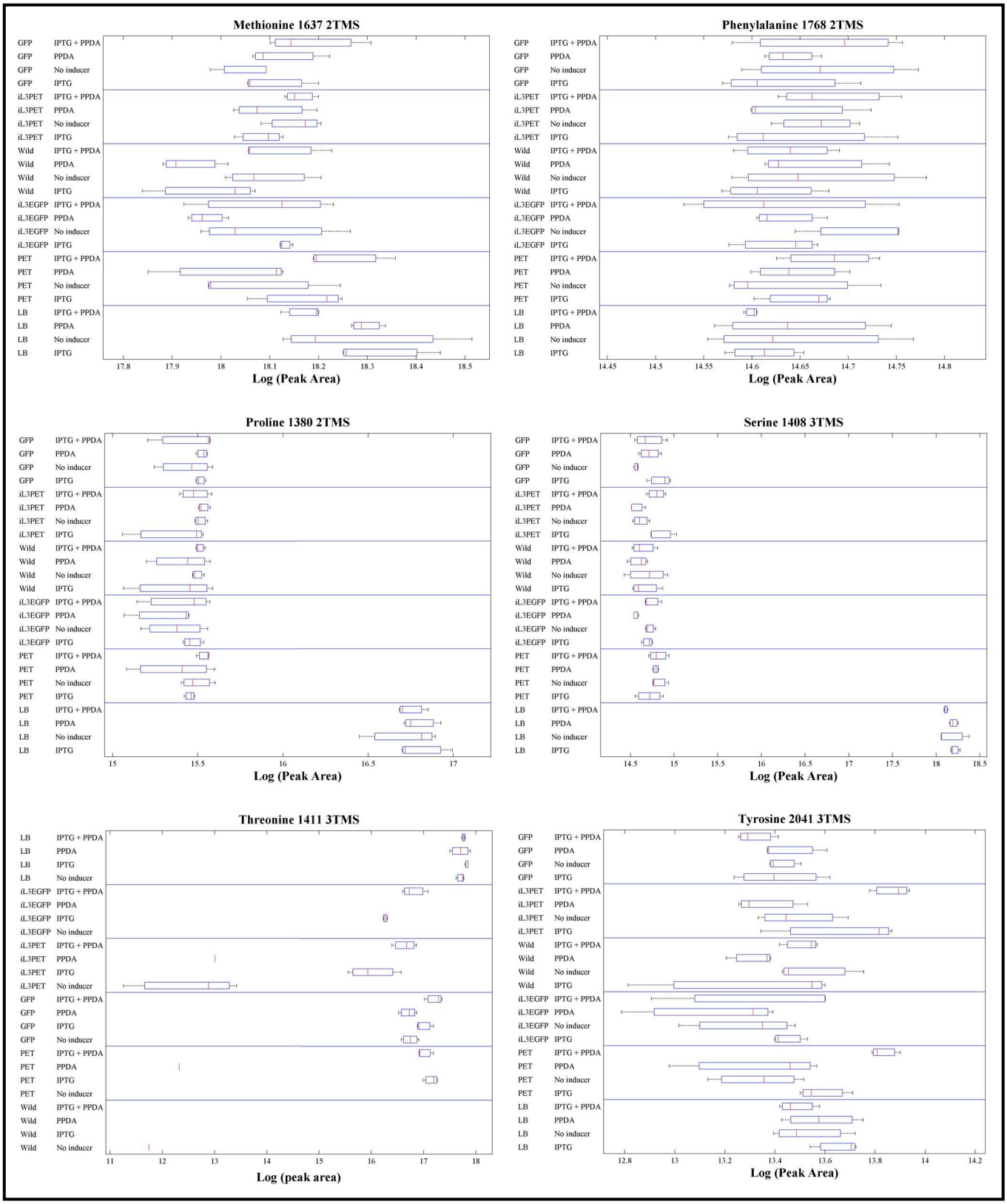

Figure S11. Relative peak intensities of different amino acids detected via GC-MS analysis of the media samples under the different examined inducing conditions.

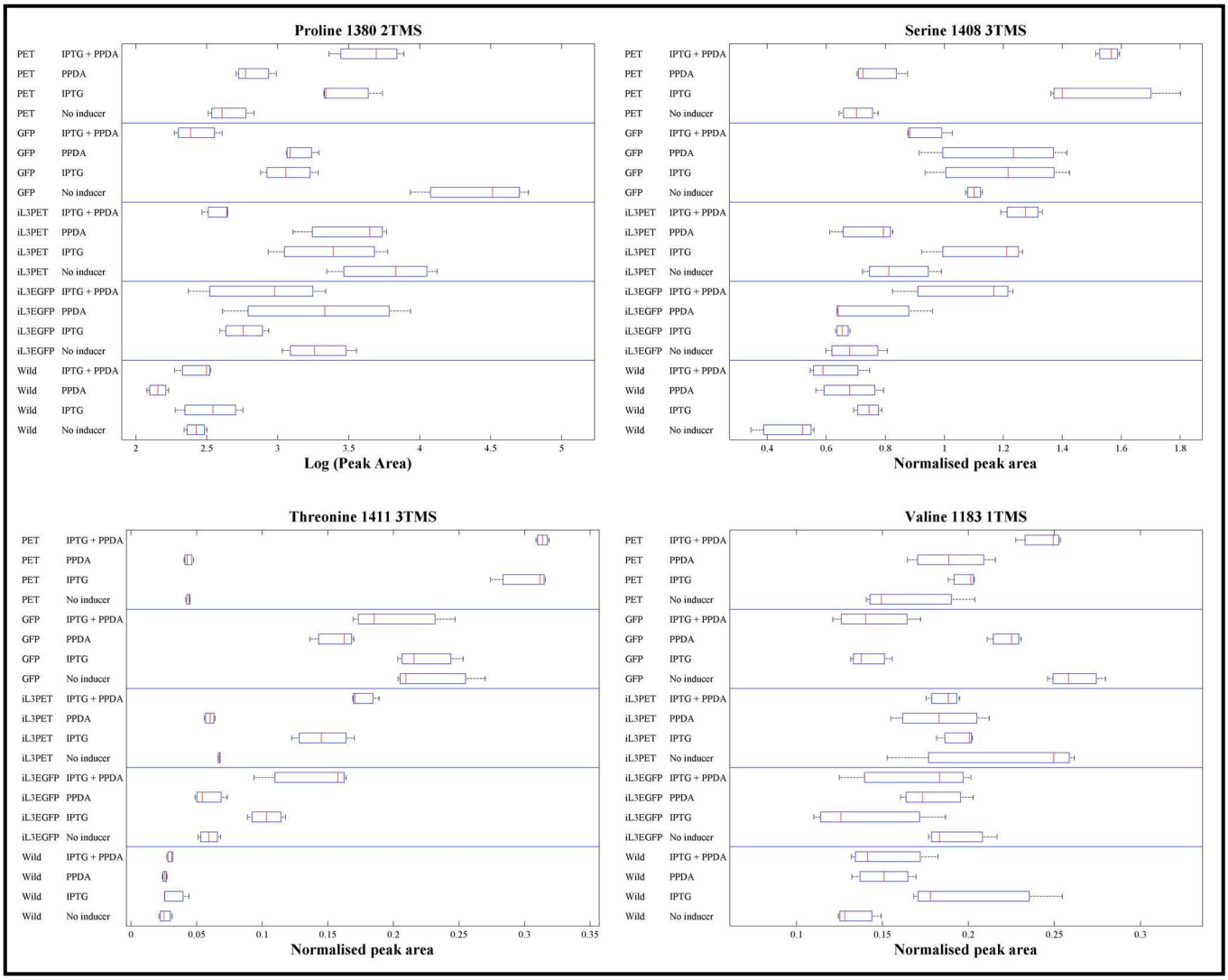

Figure S12. Box plot of the relative peak intensities of the significant metabolites in the cell extracts identified according to the CPCA-W loadings.

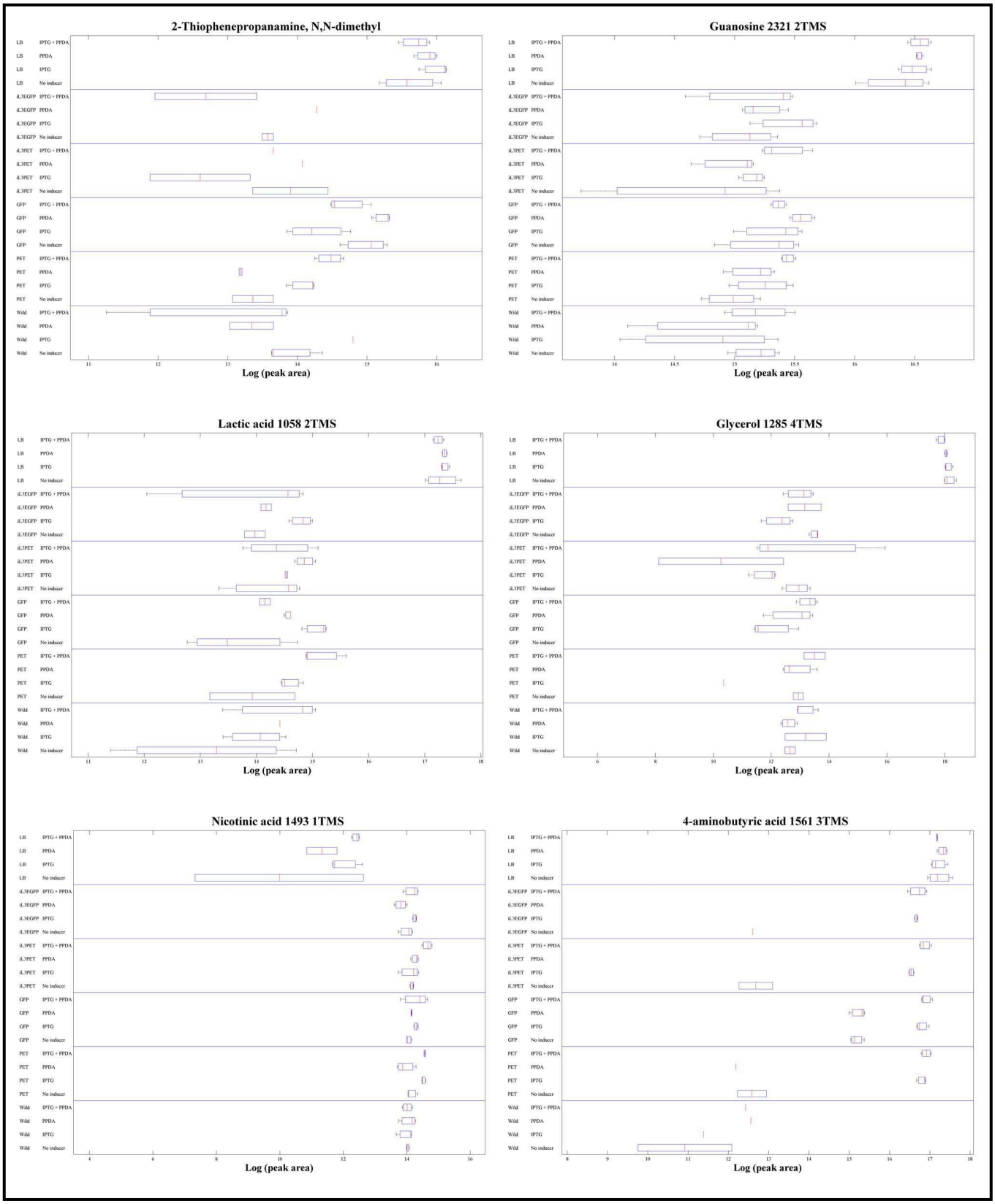

Figure S13. Box plot of the relative peak intensities of the significant metabolites in the media identified according to the CPCA-W loadings.

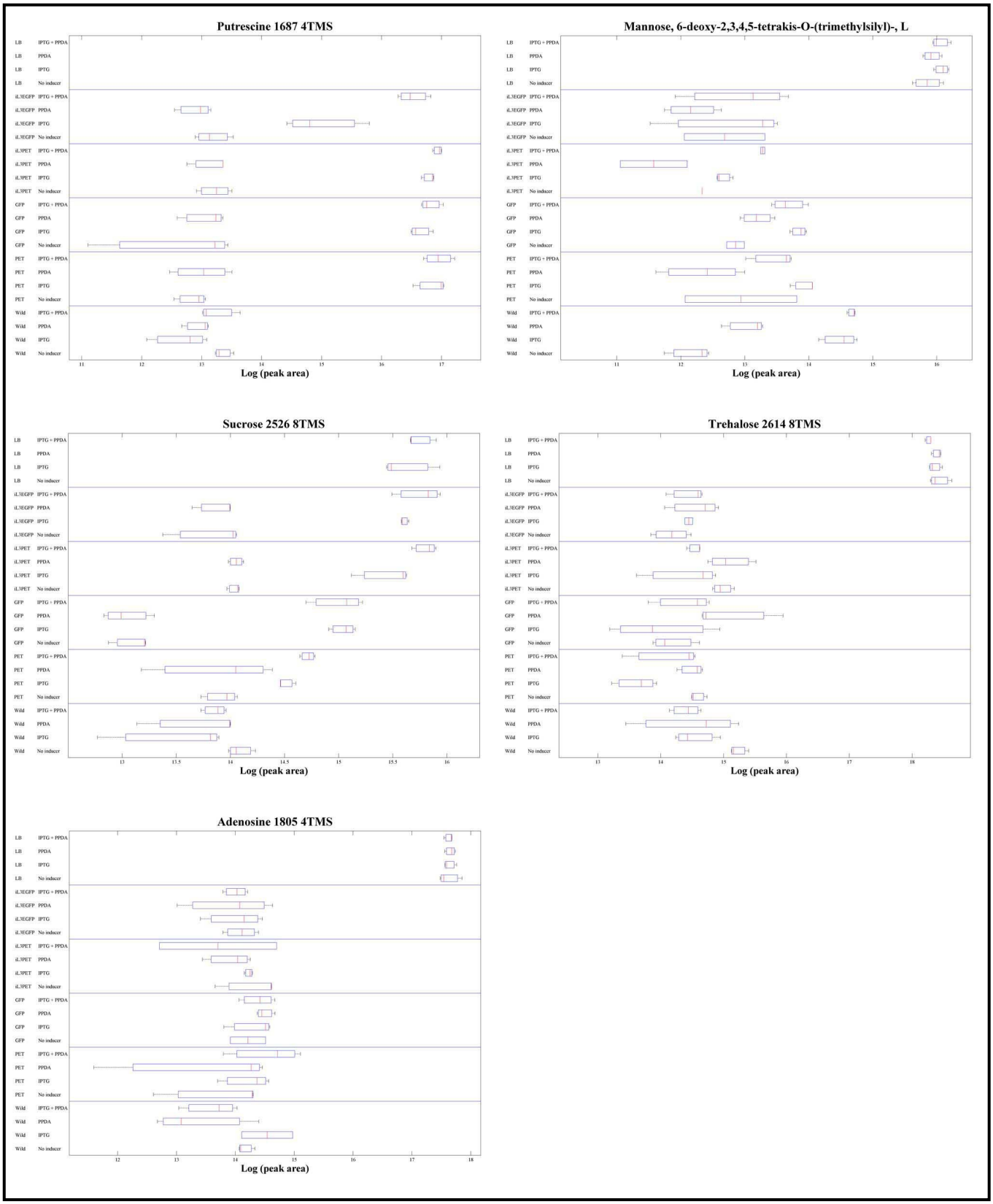

Figure S14. Box plot of the relative peak intensities of the significant metabolites in the media identified according to the CPCA-W loadings.

Table S2. List of the top 32 significant metabolites detected by GC-MS analysis of the media and cell extracts samples and identified according to the generated CPCA-W loadings plots. The level of identifications are according to the minimum metabolite reporting standards [2].

Amino acids	RT in media	RT in extract	MSI ID levels	ChEBI code
Lactic acid	352.4	NA	1	422
Alanine	374.9	373.8	1	16977
Valine	410.1	409.7	1	27266
Leucine	449.7	448.1	1	25017
Glycerol	454.843	454.328	2	17754
Isoleucine	464.7	464.1	1	17191
Glycine	488.7	488.7	2	15428
Phosphate	501.493	500.478	1	35780
Proline	505.4	504.9	1	26271
Serine	513.8	513.5	1	17822
Threonine	519.9	519.5	1	16857
Uracil	555.693	555.628	1	17568
Nicotinic acid	558.843	NA	1	NA
4-Amino butyric acid	587.293	587.078	1	16865
Aspartic acid	593.2	593.1	1	22660
Methionine	613	613	1	16811
Putrescine	628.593	628.328	1	17148
Glutamine	631.2	631.5	1	28300
Glutamic acid	644.6	643.7	1	18237
Phenylalanine	657.6	657.6	1	28044
Asparagine	665	NA	1	22653
Ornithine	670.293	669.878	1	18257
Mannose	679.393	NA	2	28563
Lysine	704.6	703.2	1	18019
Silanamine	NA	708.778	2	NA
Tyrosine	742.8	742.6	1	18186
Sucrose	756.693	NA	1	17992
Histidine	765.1	765.3	1	27570
Tryptophan	848.3	848.3	2	16828
Trehalose	885.293	NA	1	27082
Adenosine	936.443	936.428	3	16335
Guanosine	964.293	964.328	1	16750

Code: RT, retention time; MSI, Metabolomics Standards Initiative identification level; for chEBI codes see: https://www.ebi.ac.uk/chebi/

E. coli strains

Control strain (referred to as wild type); the gold-standard expression strain BL21(DE3), that expresses T7 RNA polymerase (RNAP) in an IPTG dependent manner from the lac_{uv5} P/O_{lac},. IL3-pET strain; the orthogonal riboswitch containing strain BL21(IL3), that expressed T7 RNAP upon addition of IPTG and PPDA,[1] transformed with an empty pET-plasmid. IL3-EGFP strain; same as IL3-pET transformed with a pET-eGFP plasmid. pET strain; same as wild strain transformed with an empty pET-plasmid. EGFP strain; same as strain wild strain, transformed with a pET-eGFP plasmid.

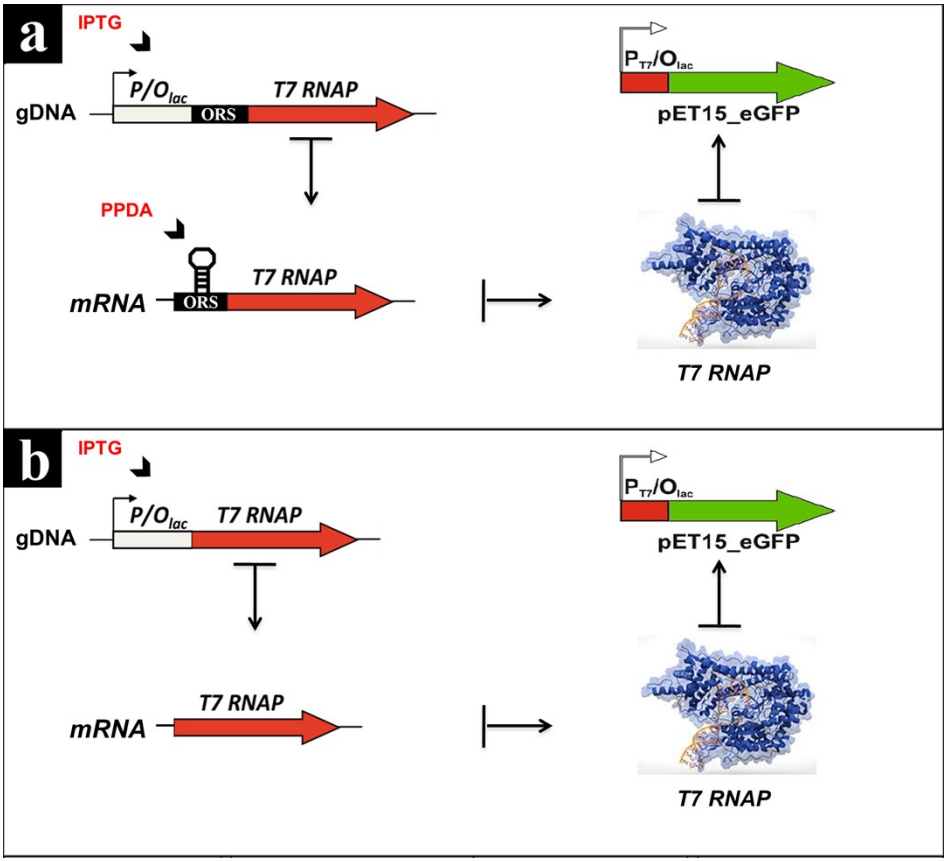

Figure S15. Strains and construct design; **a)** BL21(IL3) a chromosomal copy T7 RNAP is under the control of both the IPTG inducible lac promoter/operator and the PPDA inducible orthogonal riboswitch (ORS), transformed with pET-eGFP containing the IPTG inducible T7 promoter/ lac operator hybrid (strain engineering is described see *t*T/*t*),[1] and **b)** the standard *E. coli* BL21(DE3) expression system transformed with pET-eGFP strain (see *t*/*t*).[1]

References

1. R. Morra, J. Shankar, C. J. Robinson, S. Halliwell, L. Butler, M. Upton, S. Hay, J. Micklefield and N. Dixon, *Nucleic Acids Research*, 2015, DOI: 10.1093/nar/gkv912.
2. L. W. Sumner, A. Amberg, D. Barrett, M. H. Beale, R. Beger, C. A. Daykin, T. W. M. Fan, O. Fiehn, R. Goodacre, J. L. Griffin, T. Hankemeier, N. Hardy, J. Harnly, R. Higashi, J. Kopka, A. N. Lane, J. C. Lindon, P. Marriott, A. W. Nicholls, M. D. Reily, J. J. Thaden and M. R. Viant, *Metabolomics*, 2007, **3**, 211-221.