

Electronic supplementary information (ESI) for:

## **Multiplatform metabolomics approach for comprehensive analysis of GIST xenografts with various *KIT* mutations**

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## GC-MS parameters

Table S1. GC-MS parameters of the applied method.

Parameter	Value
Injector temperature	250 °C
Detector transfer line temperature	320 °C
Filament source temperature	230 °C
Quadrupole temperature	150 °C
Electron impact voltage	70 eV
Operation mode	scan
Scan speed	2 spectra/s
Mass range	50–600 <i>m/z</i>

## LC-MS parameters

The detailed gradient programs for LC-MS analyses in RP and HILIC modes are presented in Table S2. MS parameters during LC-MS runs are presented in Table S3. The mass range for the detection of metabolic features was 61-1200 *m/z* in both RP and HILIC analyses.

Table S2. Gradient elution conditions during LC-MS analyses in RP and HILIC modes.

RP-LC-MS			HILIC-LC-MS		
Time [min]	Mobile phase A [%]	Mobile phase B [%]	Time [min]	Mobile phase A [%]	Mobile phase B [%]
0.0	18.0	82.0	0.0	5.0	95.0
30.0	4.0	96.0	22.0	45.0	55.0
38.0	4.0	96.0	23.0	45.0	55.0
38.5	0.0	100.0	23.5	5.0	95.0
40.5	0.0	100.0	29.0	5.0	95.0
42.0	18.0	82.0			
50.0	18.0	82.0			

Table S3. Mass spectrometry parameters during LC-MS analyses.

Parameter	RP	HILIC
Gas temperature	350 °C	350 °C
Gas flow	10 L/min	11 L/min
Nebulizer pressure	45 psi	45 psi
Fragmentor voltage	175 V	150 V
Skimmer voltage	65 V	65 V

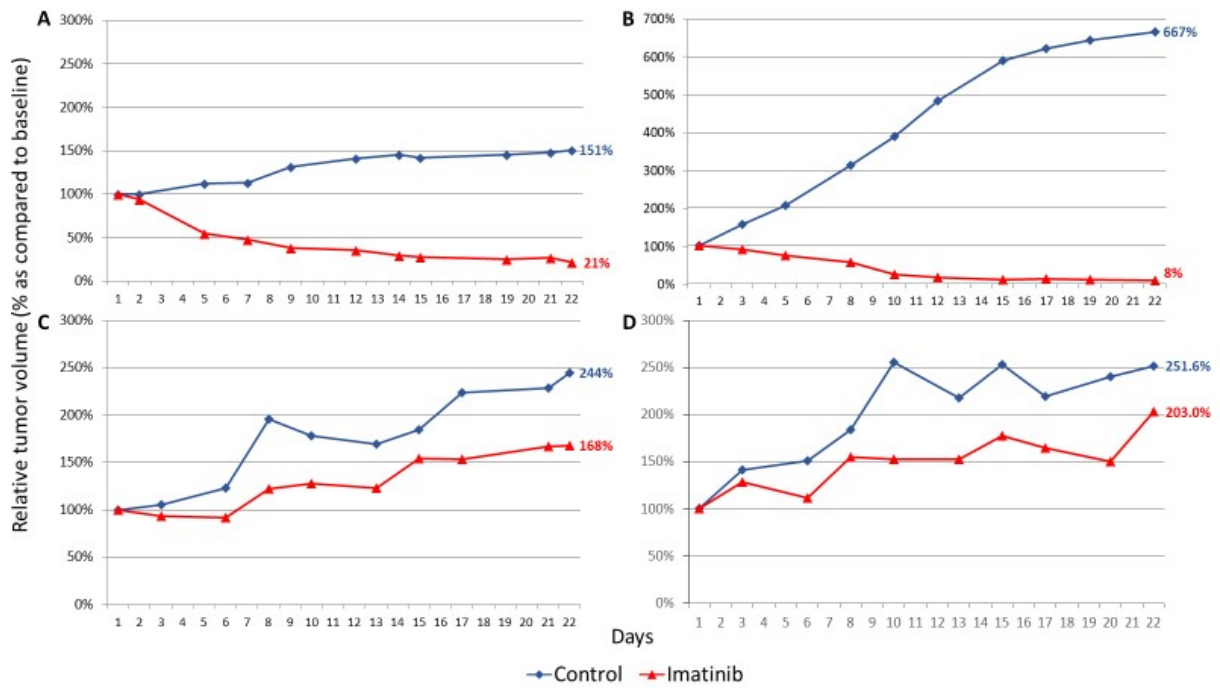


Figure S1. Relative tumour volume evolution comparing the untreated controls with imatinib twice a day orally at 50 mg/kg in UZLX-GIST1 (A)<sup>43</sup>, UZLX-GIST4 (B)<sup>44</sup>, UZLX-GIST2 (C)<sup>43</sup> and UZLX-GIST9 (D)<sup>45</sup> patient-derived xenograft models.

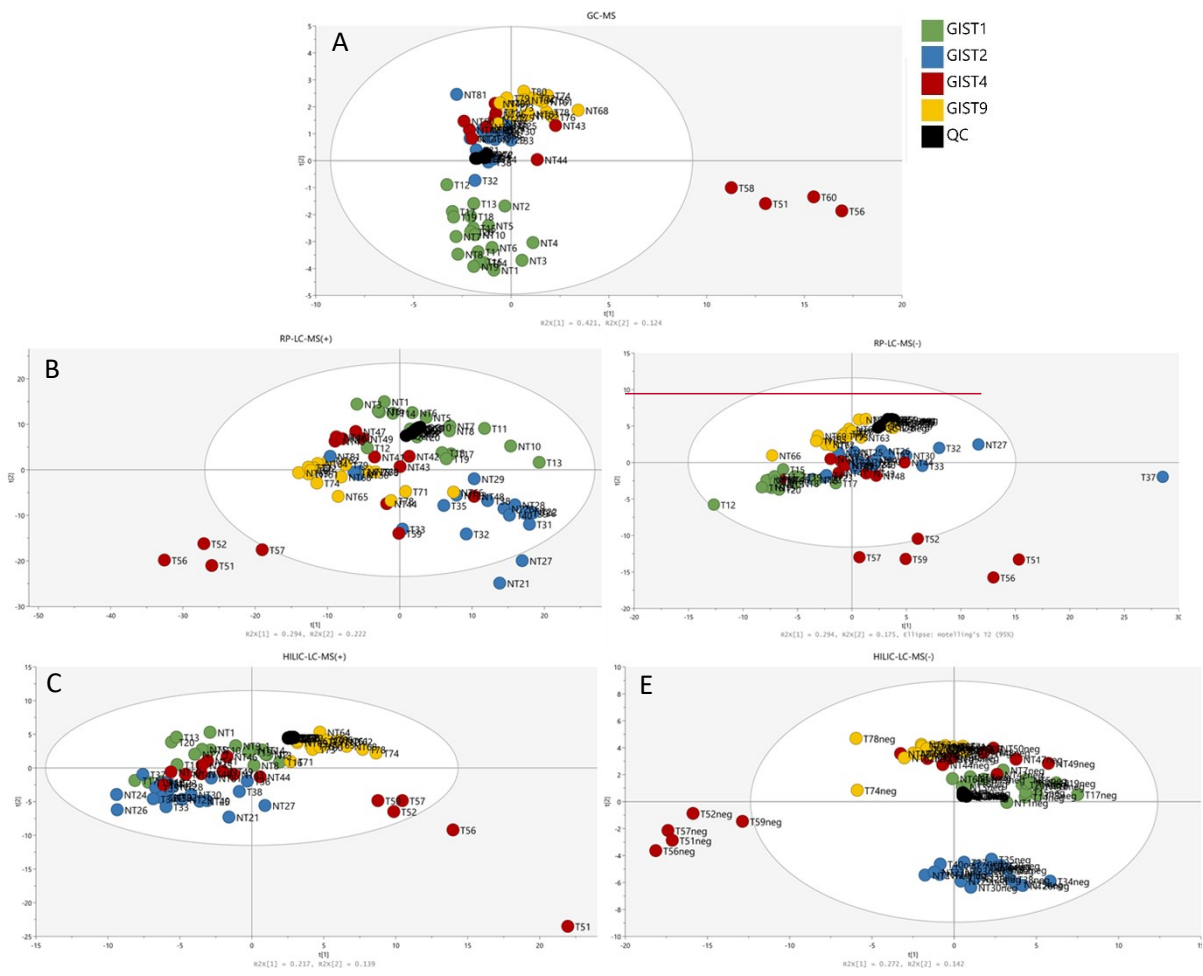


Figure S2. PCA score plots for data obtained from GIST extracts GC-MS (A) analysis . RP-LC-TOF-MS analysis in positive (B) and negative (C) ionization modes. and HILIC-LC-TOF-MS analysis in positive (D) and negative (E) ionization modes. Black colour corresponds to quality control samples.

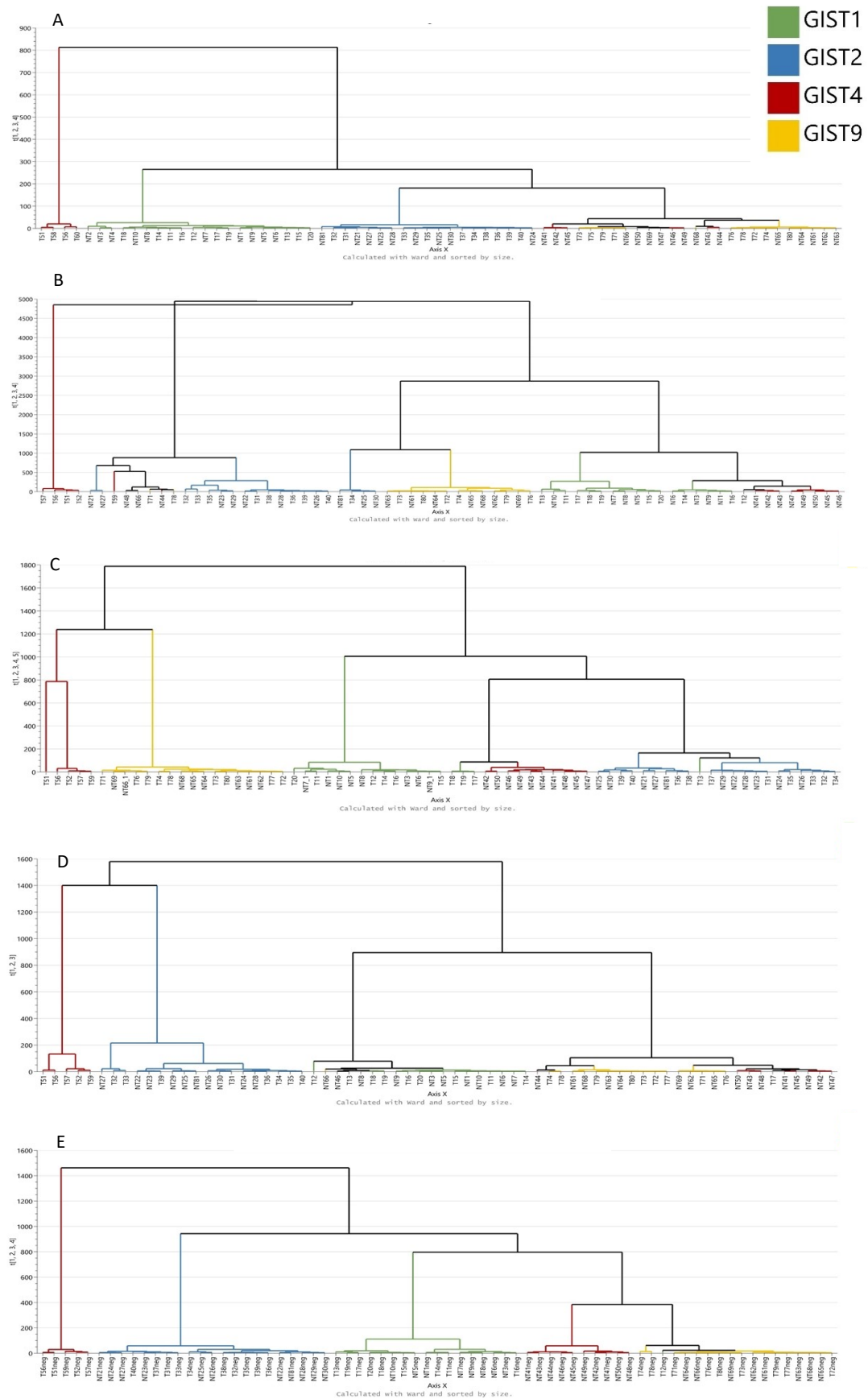


Figure S3. Representation of hierarchical cluster analysis of GIST samples analysed by GC-MS (A) analysis . RP-LC-TOF-MS analysis in positive (B) and negative (C) ionization modes. and HILIC-LC-TOF-MS analysis in positive (D) and negative (E) ionization modes. Imatinib-treated samples from GIST4 model are substantially distinct from the remaining samples.

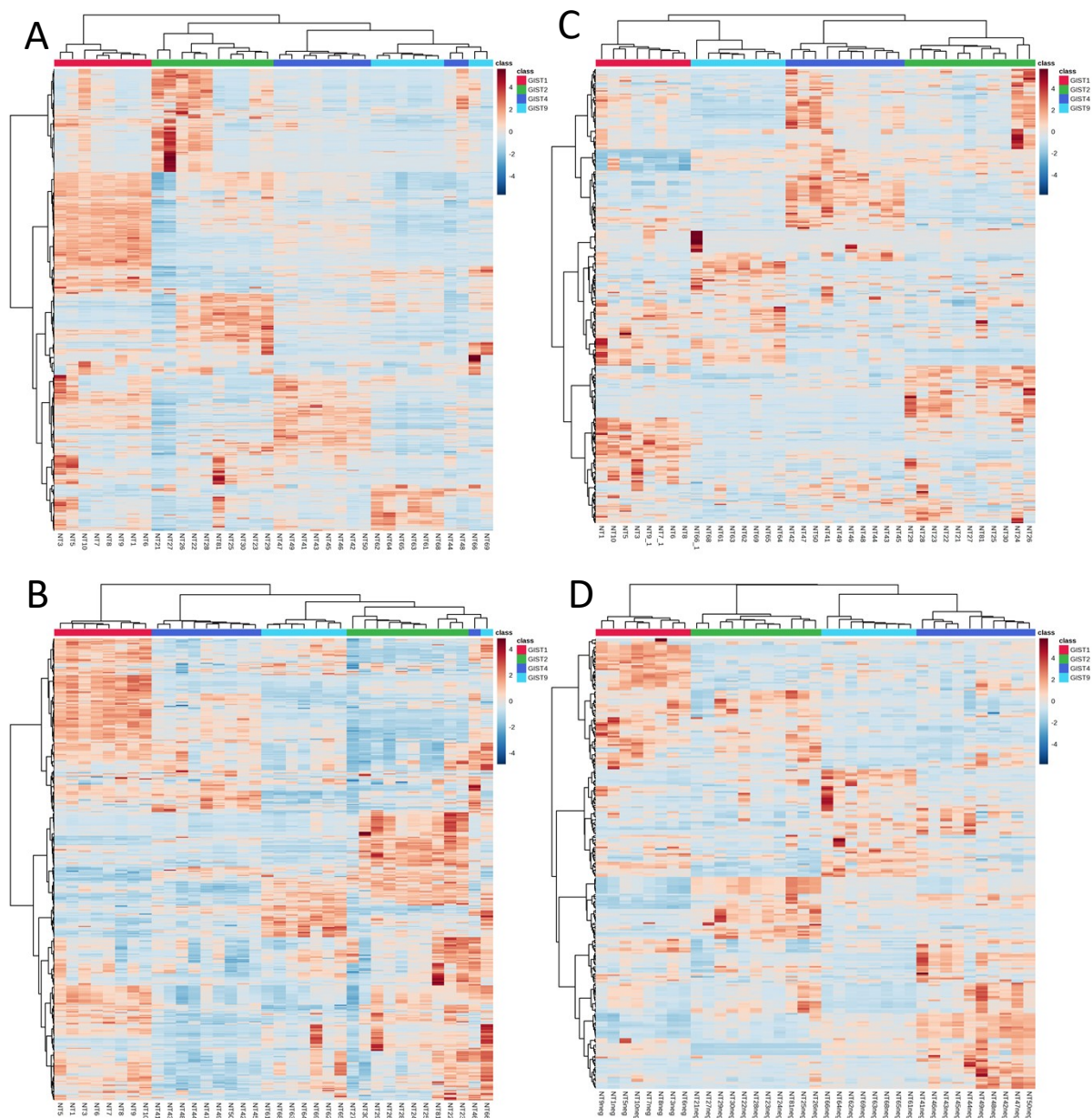


Figure S4. Heatmaps of the signal intensity of metabolic features detected by RP-LC-TOF-MS analysis in positive (A) and negative (B) ionization modes, and HILIC-LC-TOF-MS analysis in positive (C) and negative (D) ionization modes.

Table S4. Statistically significant metabolites (with 5 lowest FDR values from each analytical technique) differentiating four GIST models used in the study. *p* and FDR values result from ANOVA test. Tukey's test indicates between which groups statistical significance was confirmed.

Metabolite	<i>p</i> value	FDR	Tukey's test	Technique
<b>SM(18:0/16:1)</b>	1.40E-23	3.52E-21	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST4	RP-LC-MS(+)
<b>PE(18:0/16:1)</b>	3.10E-19	1.74E-17	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST4	RP-LC-MS(+)
<b>PC(10:0/20:0)</b>	3.24E-18	1.21E-16	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2	RP-LC-MS(+)
<b>PE(16:1/22:2)</b>	2.50E-14	3.89E-13	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST4	RP-LC-MS(+)
<b>SM(18:1/22:0)</b>	2.08E-13	2.50E-12	GIST2-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST4	RP-LC-MS(+)
<b>PE(16:0/18:2)</b>	8.69E-18	1.31E-15	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST4	RP-LC-MS(-)
<b>PE(18:0/20:5)</b>	2.01E-15	4.54E-14	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1	RP-LC-MS(-)
<b>PC(14:0/18:1)</b>	6.64E-15	1.25E-13	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2	RP-LC-MS(-)
<b>PE(18:0/22:4)</b>	4.69E-14	6.04E-13	GIST2-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2	RP-LC-MS(-)
<b>PE(16:1/22:2)</b>	6.42E-13	6.16E-12	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2	RP-LC-MS(-)
<b>2-Methylnicotinamide</b>	6.66E-20	8.90E-18	GIST9-GIST1; GIST9-GIST2; GIST9-GIST4	HILIC-LC-MS(+)
<b>PC(14:0/18:1)</b>	5.77E-17	3.31E-15	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1	HILIC-LC-MS(+)
<b>Adenosine</b>	6.23E-09	4.16E-08	GIST4-GIST1; GIST4-GIST2; GIST9-GIST2	HILIC-LC-MS(+)
<b>PC(18:1/0:0)</b>	2.72E-08	1.49E-07	GIST2-GIST1; GIST4-GIST2; GIST9-GIST2	HILIC-LC-MS(+)
<b>Hypoxanthine</b>	3.20E-08	1.67E-07	GIST4-GIST1; GIST4-GIST2; GIST9-GIST4	HILIC-LC-MS(+)
<b>Uridine/Pseudouridine</b>	1.56E-16	4.63E-15	GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2; GIST9-GIST4	HILIC-LC-MS(-)
<b>Guanosine</b>	6.94E-16	1.55E-14	GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2; GIST9-GIST4	HILIC-LC-MS(-)
<b>Taurine</b>	6.55E-09	3.44E-08	GIST2-GIST1; GIST4-GIST2; GIST9-GIST2	HILIC-LC-MS(-)
<b>Oxoglutaric acid</b>	2.64E-05	5.76E-05	GIST2-GIST1; GIST9-GIST1	HILIC-LC-MS(-)
<b>Myo-Inositol</b>	5.81E-12	2.60E-10	GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2; GIST9-GIST4	GC-MS
<b>Uridine</b>	7.97E-12	2.60E-10	GIST4-GIST1; GIST9-GIST1; GIST4-GIST2; GIST9-GIST2	GC-MS
<b>L-Glutamic acid</b>	7.31E-08	7.17E-07	GIST9-GIST1; GIST9-GIST2; GIST9-GIST4	GC-MS
<b>L-Threonine</b>	9.66E-08	8.60E-07	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1	GC-MS
<b>L-Alanine</b>	3.40E-07	2.56E-06	GIST2-GIST1; GIST4-GIST1; GIST9-GIST1	GC-MS

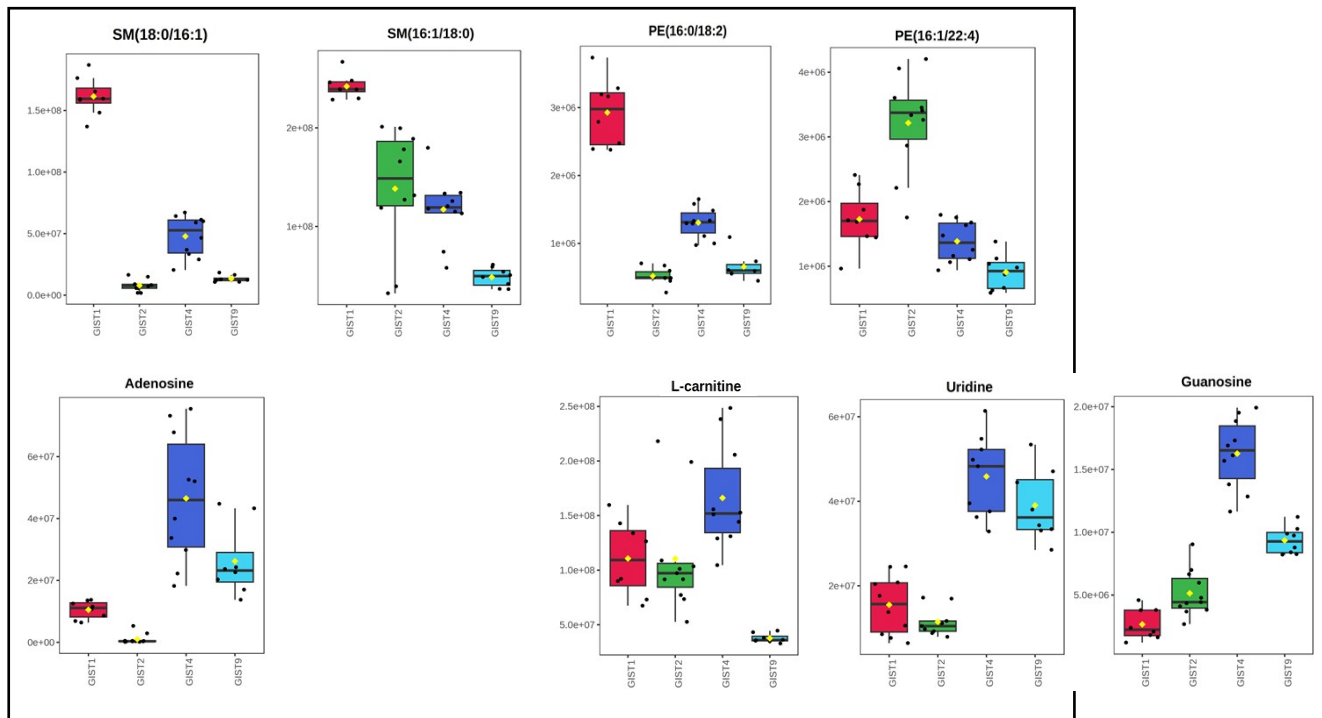


Figure S5. Box plots visualising the differences in metabolite signal intensity between four GIST models. Representative metabolites have been selected from Table S4 (generated in MetaboAnalyst 5.0).