

Supporting Information for Publication

Assessing treatment response and prognosis by serum and tissue metabolomics in breast cancer patients.

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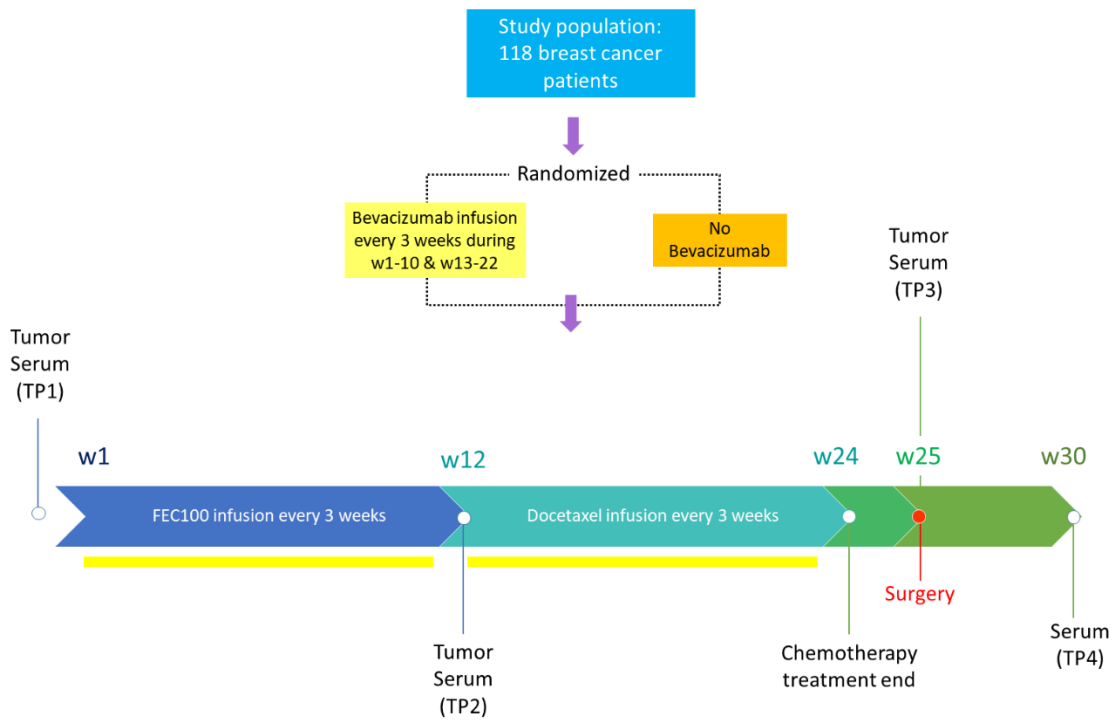


Figure S1. Diagram showing the treatment regime and experimental set up of the study.

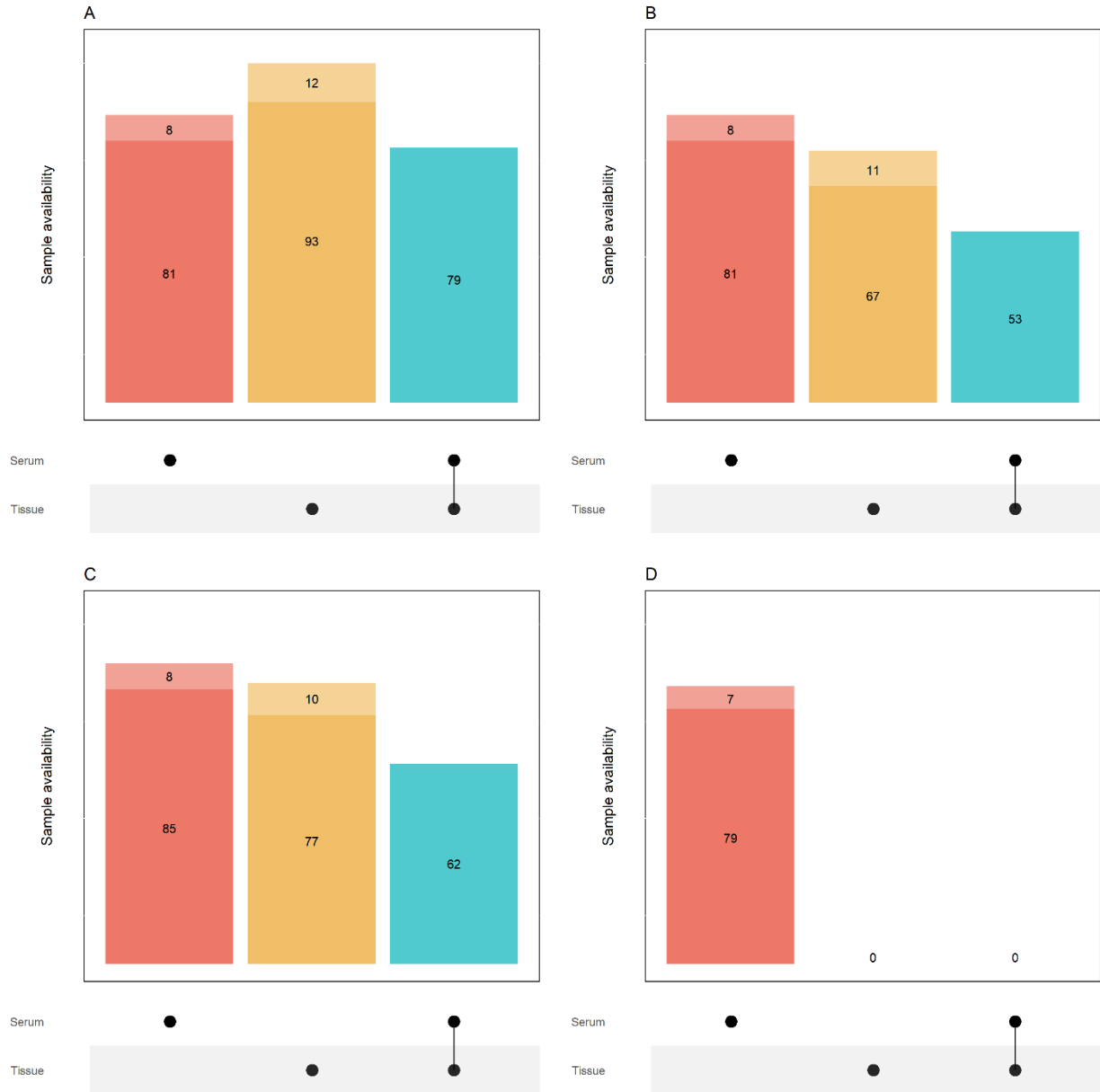


Figure S2. Sample availability of serum and tissue samples at the distinct sampling time points (TP's) in this study. Figures A, B, C, and D correspond to time points TP1, TP2, TP3, and TP4, respectively. For serum and tissue samples, a darker color denotes the number of survivors, while a lighter color denotes the number of non-survivors.

Table S1. Details on quantification of serum metabolites

	Metabolite	Chemical shift (peak multiplicity)
1	Leucine	0.95 (tr)
2	Valine	0.98 (d) 1.02 (d) 3.60 (d)
3	Isoleucine	1.00 (d)
4	2-methylglutarate	1.06 (d)
5	3-hydroxybutyrate	1.19 (d) 2.30 (q) 2.40 (q)
6	Alanine	1.47 (d)
7	Lysine	1.77 (m) 3.03 (right d)
8	Acetate	1.91 (s)
9	Acetoacetate	2.27 (s)
10	Glutamate	2.35 (d)
11	Pyruvate	2.36 (s)
12	Glutamine	2.45 (m)
13	Citrate	2.68 (right d)
14	Methionine	2.63 (right d)
15	Creatine	3.03 (s) 3.92 (s)
16	Creatinine	3.04 (s) 4.05 (s)
17	Ornithine	3.06 (d)
18	Proline betaine	3.1(s) 3.29 (s)
19	Dimethylsulfone	3.15 (s)
20	Glucose	3.25 (t) 3.42 (m) 3.36 (m) 3.49(m) 3.34 (q) 3.72 (m) 3.84 (m) 3.90 (q) 5.25 (d)
21	Methanol	3.36 (s)
22	Glycine	3.55 (s)
23	Lactate	4.12 (q)
24	Tyrosine	6.90 (d) 7.20 (d)
25	Phenylalanine	7.34 (d) 7.38 (t) 7.43 (t)
26	Histidine	7.05 (s) 7.78 (d)
27	Formate	8.46 (s)
28	Lipid 1	0.85 (br)
29	Lipid 2	1.57 (br)
*	Ethanol	1.17 (d)

Quantified metabolites and their placement on the ppm scale. ppm: parts per million; br: broad peak; s: singlet; d: doublet; dd: double doublet; t: triplet; q: quartet; m: multiplet; *: removed from analyses.

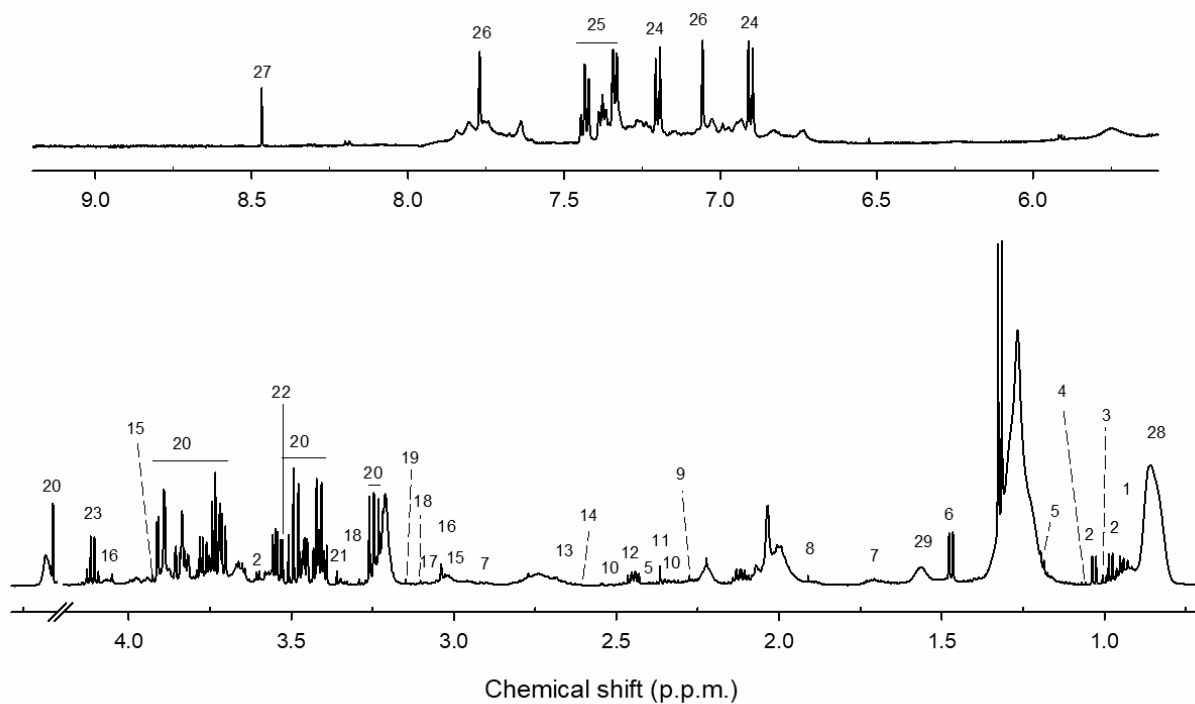


Figure S3: A representative spectrum with annotated metabolite peaks. 1: leucine; 2: valine; 3: isoleucine; 4: dimethylglutarate; 5: tri-hydroxybutyrate; 6: alanine; 7: lysine; 8: acetate; 9: acetoacetate; 10: glutamate; 11: pyruvate; 12: glutamine; 13: citrate; 14: methionine; 15: creatine; 16: creatinine; 17: ornithine; 18: proline-betaine; 19: dimethylsulfone; 20: glucose; 21: methanol; 22: glycine; 23: lactate; 24: tyrosine; 25: phenylalanine; 26: histidine; 27: formate; 28: lipid1; 29: lipid2.

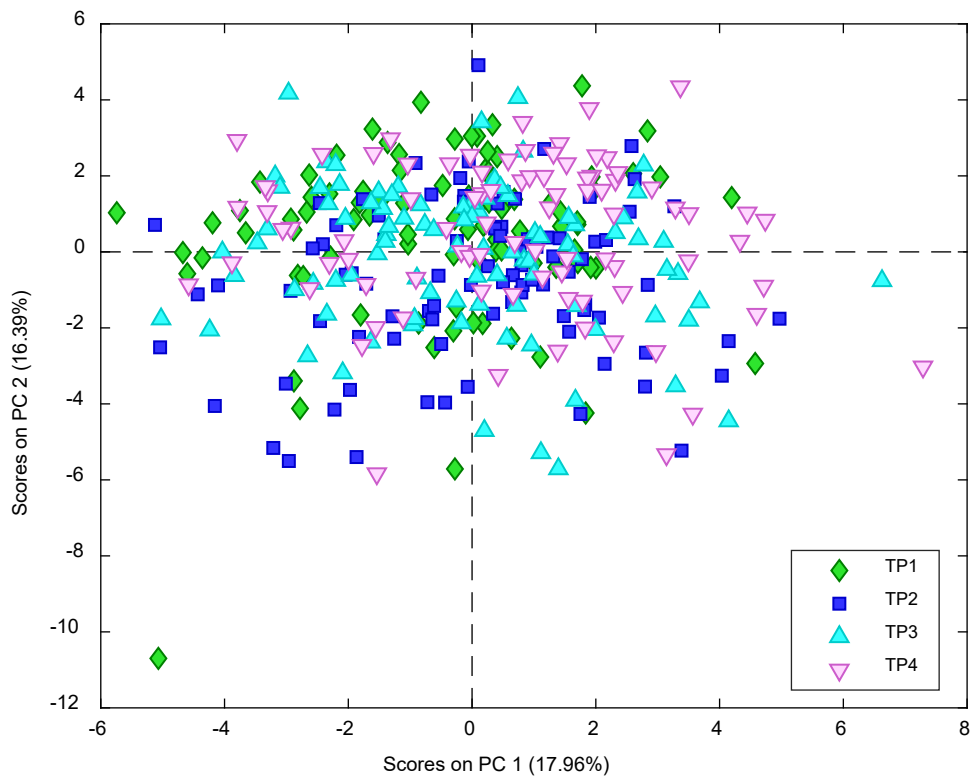


Figure S4. Scores plot for the PCA analysis of the serum metabolites. Each point corresponds to a sample colored according to the time point at which it was collected.

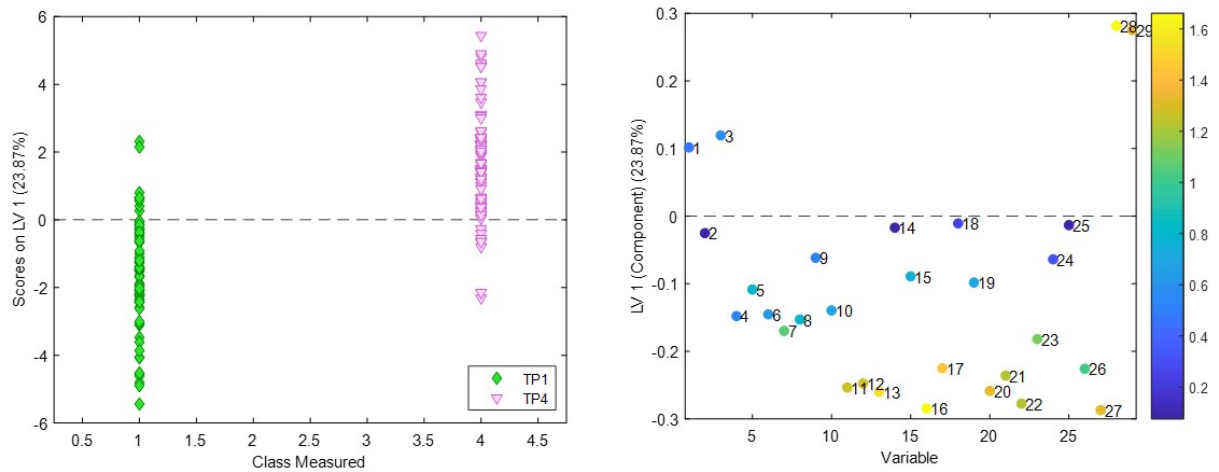


Figure S5. Scores (left) and loading plots (right) of multilevel PLS-DA analyses on serum metabolites comparing TP1 and TP4, with lipid peaks. Accuracy = 77%; p-value < 0.001. LV: latent variable. Loadings colored according to the VIP scores. 1: leucine; 2: valine; 3: isoleucine; 4: dimethylglutarate; 5: trihydroxybutyrate; 6: alanine; 7: lysine; 8: acetate; 9: acetoacetate; 10: glutamate; 11: pyruvate; 12: glutamine; 13: citrate; 14: methionine; 15: creatine; 16: creatinine; 17: ornithine; 18: proline-betaine; 19: dimethylsulfone; 20: glucose; 21: methanol; 22: glycine; 23: lactate; 24: tyrosine; 25: phenylalanine; 26: histidine; 27: formate; 28: lipid1; 29: lipid2.

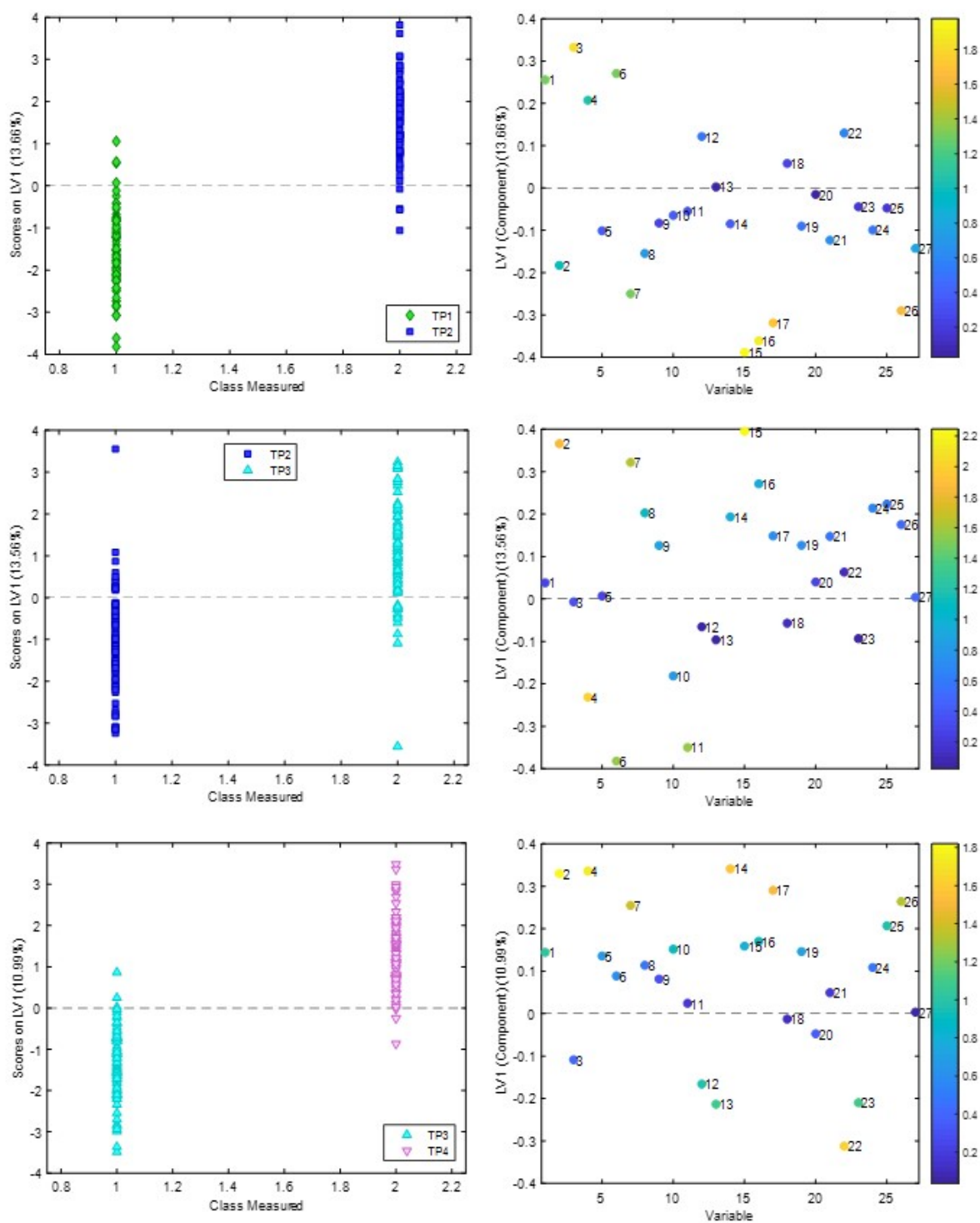


Figure S6. Scores (left) and loading plots (right) of multilevel PLS-DA analyses on serum metabolites. LV: latent variable. Top: TP2 vs TP1; Middle: TP3 vs TP2; Bottom: TP4 vs TP3. Loadings colored according to the VIP scores. 1: leucine; 2: valine; 3: isoleucine; 4: dimethylglutarate; 5: tri-hydroxybutyrate; 6: alanine; 7: lysine; 8: acetate; 9: acetoacetate; 10: glutamate; 11: pyruvate; 12: glutamine; 13: citrate; 14: methionine; 15: creatine; 16: creatinine; 17: ornithine; 18: proline-betaine; 19: dimethylsulfone; 20: glucose; 21: methanol; 22: glycine; 23: lactate; 24: tyrosine; 25: phenylalanine; 26: histidine; 27: formate