Supplementary Materials

Impact of the Pd2Spm(spermine) complex on the metabolism of Triple-Negative Breast Cancer tumors of a xenograft mouse model

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Content of Supplementary Material:

Table S1. List of metabolites and corresponding spin systems visibly identified in the 500MHz ^{1}H NMR spectra of aqueous extracts of TNBC tissues from the MDA-MB-231 cell-derived xenograft (CDX) mouse model. Arrows represent the qualitative mean abundance of each compound on tumors' tissue comparatively to controls: ↑, increased levels; ↓, decreased levels. Arrows in brackets do not statistical relevance and only indicate a variation tendency. * Possible contamination obtained during the extraction procedure (material disinfection). Metabolite abbreviations: 3-AIBA, 3-aminoisobutyric acid; 3-HBA, 3-hydroxybutyrate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; DMA, dimethylamine; GPC, glycerophosphocholine; GSH, glutathione (reduced); IMP, inosine monophosphate; NAD$^+$, nicotinamide adenine dinucleotide (reduced); PC, phosphocholine; PE, phosphoethanolamine; SAH, *S*-adenosylhomocysteine; TMAO, Trimethylamine *N*-oxide; UDP-GlcA/Glc, uridine diphosphate glucuronate/ glucose; UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine; UMP, uridine monophosphate; UTP, uridine triphosphate. Multiplicity abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet.

Figure S1. Average 500 MHz ^{1}H NMR spectra of lipophilic extracts of tumors from controls group (exposure to vehicle, PBS) of MDA-MB-231 CDX mouse model. * Spectral regions assigned to water (δ 1.48 – δ 1.76), and CDCl$_3$ (and corresponding satellites) (δ 7.00 – δ 7.50), excluded from the multivariate analysis. Abbreviations: Chol., cholesterol; Ester., esterified; FA, fatty acid; PTC, phosphatidylcholine; PTE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid; SM, sphingomyelin; TG, triglycerides.

Figure S2. Heatmap illustrating the metabolic variations of aqueous extracts of tumors from MDA-MB-231 CDX mouse model relative to the pairwise comparisons cDDP / Pd2Spm *vs.* Controls (Ctr), and Pd2Spm *vs.* cDDP. The heatmap is colored according to the Effect Size (ES) in a scale from minimum (blue) to maximum (red) values. ‡ Partial integration of peak. Abbreviations: 3-letter code used for amino acids; ATP, adenosine triphosphate; Cho, choline; Cpd., compound; DMA, dimethylamine; GA, guanidine acetate; HX, hypoxanthine; NAD$^+$, nicotinamide adenine dinucleotide (reduced); PE, phosphoethanolamine; UTP, uridine triphosphate; Ui, unassigned resonance i.

Figure S3. Bar chart depicting average intensity ratios of choline compounds, obtained with the integration of signals' area for choline, PC and GPC, singlet resonances at 3.21, 3.22 and 3.23 ppm, respectively. Error bars indicate

the respective standard deviation. Asterisk indicates the significance level of 0.05 (* p-value < 5E-2). Mice groups are distinguished by the color of each bar: controls, black; cDDP, blue; Pd$_2$Spm red.

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Metabolite assignment	δ$_H$ ppm (multiplicity, assignment)	HMDB ID [92]	Pd$_2$Spm *vs.* Controls	cDDP *vs.* Controls
Amino acids and related compounds				
Alanine	1.48 (d, βCH$_3$); 3.78 (q, αCH)	HMDB0000161	(↑)	(↓)
Asparagine	2.85 (m, βCH$_2$)	HMDB0000168	↑	↑
Aspartate	2.68 (dd, βCH); 2.81 (dd, β'CH); 3.90 (dd, αCH)	HMDB0000191	(↑)	(↓)
Creatine	3.04 (s, N-CH$_3$); 3.93 (s, N-CH$_2$)	HMDB0000064	(↓)	(↑)
Glutamate	2.04 (m, βCH); 2.11 (m, β'CH); 2.36 (m, γCH$_2$); 3.76 (dd, αCH)	HMDB0000148	(↓)	(↓)
Glutamine	2.14 (m, βCH$_2$); 2.45 (m, γCH$_2$); 3.77 (t, αCH)	HMDB0000641	(↓)	(↑)
Glycine	3.55 (s, αCH$_2$)	HMDB0000123	(↓)	(↑)
GSH	2.17 (m, βCH$_2$ Glu); 2.55 (m, γCH$_2$ Glu); 2.96 (m, αCH$_2$ Cys); 3.78 (αCH Glu); 4.57 (m, βCH$_2$ Cys)	HMDB0000125	(↓)	(↓)
Histidine	3.18 (dd, βCH2); 3.99 (dd, αCH); 7.10 (s, C4H ring); 7.98 (s, C2H ring)	HMDB0000177	-	-
Isoleucine	0.94 (t, δCH3); 1.01 (d, β'CH3); 1.99 (m, βCH	HMDB0000177	-	(↓)
Leucine	0.96 (t, δCH$_3$/ δ'CH$_3$); 1.71 (m, βCH$_2$/ γCH)	HMDB0000687	-	-
Lysine	1.45 (m, γCH$_2$); 1.73 (m, δCH$_2$); 1.92 (m, βCH$_2$)	HMDB0000182	(↑)	-
Phenylalanine	7.33 (d, C2H/ C6H ring); 7.38 (m, C4H ring); 7.42 (t, C3H/ C5H ring)	HMDB0000159	(↑)	(↑)
Phosphocreatine	3.05 (s, N-CH$_3$); 3.95 (s, N-CH$_2$)	HMDB0001511	(↑)	(↑)
Proline	3.34 (m, C2H ring)	HMDB0000162		
Taurine	3.27 (t, S-CH$_2$); 3.43 (t, N-CH$_2$)	HMDB0000251	(↓)	-
Threonine	1.33 (d, γCH$_3$); 3.59 (d, αCH); 4.24 (m, βCH)	HMDB0000167		
Tyrosine	6.90 (d, C3H/ C5H ring); 7.20 (d, C2H/ C6H ring)	HMDB0000158	-	(↑)
Valine	0.99 (d, γCH$_3$); 1.05 (d, γ'CH$_3$); 2.27 (m, βCH); 3.61 (d, αCH)	HMDB0000883	(↑)	(↓)
Choline compounds				
Choline	3.21 (s, N(CH$_3$)$_3$)	HMDB0000097	↑	(↑)
GPC	3.23 (s, N(CH$_3$)$_3$); 3.92 (m, αCH$_2$); 4.33 (m, PO$_3$-αCH$_2$)	HMDB0000086	(↑)	(↓)
PC	3.22 (s, N(CH$_3$)$_3$); 4.17 (m, PO$_3$-CH$_2$)	HMDB0001565	(↑)	(↑)
Sugars				
α-Glucose	3.53 (dd, C2H); 3.83 (m, C4H); 5.23 (d, C1H)	HMDB0003345	-	(↑)
β-Glucose	3.49 (t, C3H); 3.71 (dd, C6H'); 4.65 (d, C1H)	HMDB0003345	-	(↑)

(continued)

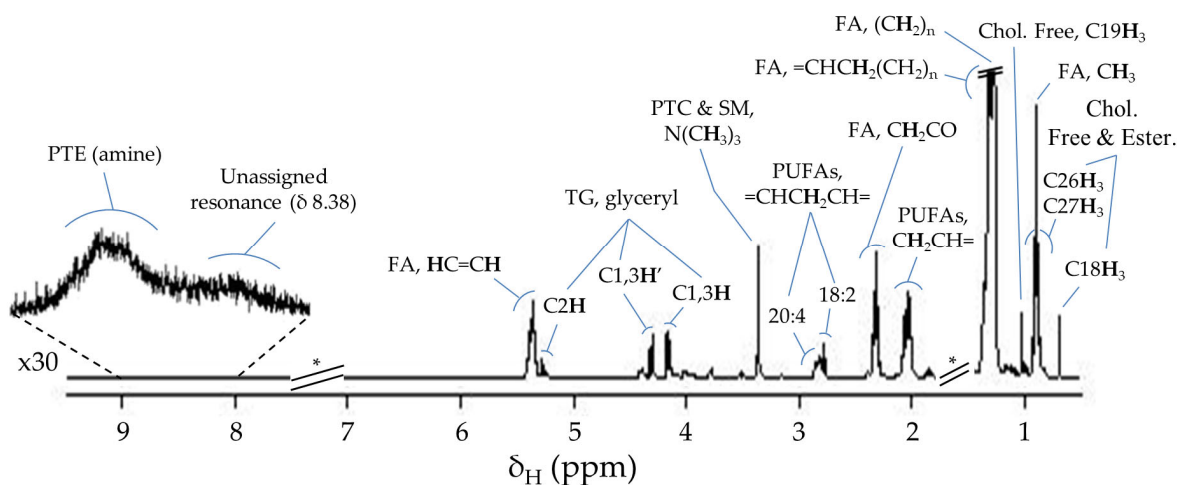| Nucleotides and related compounds				
Adenosine	4.29 (q, C4'H ribose); 4.44 (dd, C3'H ribose); 6.10 (d, C1'H ribose); 8.12 (s, C8H ring); 8.27 (s, C2H ring)	HMDB0000050	(↓)	(↓)
ADP	6.15 (d, C1'H ribose); 8.27 (s, C2H ring); 8.54 (s, C8H ring)	HMDB0001341	(↑)	(↑)
AMP	4.51 (dd, C2'H ribose); 6.14 (d, C1'H ribose); 8.27 (s, C2H ring); 8.60 (s, C8H ring)	HMDB0000045	(↓)	(↓)
ATP	6.15 (d, C1'H ribose); 8.28 (s, C2H ring); 8.54 (s C8H ring)	HMDB0000538	↑	↑
Hypoxanthine	8.18 (s, C2H); 8.20 (s, C8H)	HMDB0000157	↓	↓
IMP	8.24 (s, C8H ring); 8.58 (s, C8H ring)	HMDB0000175	(↓)	(↓)
Inosine	6.10 (d, C1'H); 8.24 (s, C8H ring); 8.35 (s, C2H ring)	HMDB0000195	(↑)	(↑)
NAD+	4.50 (m, A3'); 4.54 (m, N2'); 6.04 (d, N1'); 8.18 (s, A2); 8.19 (N5); 8.43 (s, A8); 8.83 (d, N4); 9.15 (d, N6); 9.34 (s, N2)	HMDB0000902	(↑)	-
SAH [†]	6.08 (d, N-CH-O)	HMDB0000939	(↓)	(↓)
UDP-GlcA/ Glc	5.61 (dd, C1H Glc); 7.95 (d, C2H uridine)	HMDB0000935	(↓)	(↓)
UDP-GlcNAc	5.52 (dd, C1H glucose); 5.95 (d, C5H uridine),	HMDB0000290	(↓)	-
UMP	5.99 (m, C6H ring); 8.11 (d, C5H ring)	HMDB0000288	(↓)	(↓)
Uridine	5.90 (d, C5H ring); 5.92 (d, C1'H ring); 7.88 (d, C6H ring)	HMDB0000285	(↑)	(↓)
UTP	5.97 (m, C5H ring); 8.00 (d, C6H ring)	HMDB0000285	(↑)	↑
Organic acids				
3-AIBA [†]	1.18 (d, αCH)	HMDB0003911	-	(↑)
3-HBA	1.20 (d, CH$_3$); 2.31 (m, CH$_2$)	HMDB0000357	(↓)	(↑)
Acetate	1.92 (s, CH$_3$)	HMDB0000042	(↓)	(↓)
Formate	8.46 (s, CH)	HMDB0000142	(↑)	(↑)
Fumarate	6.52 (s, CH)	HMDB0000134	↑	(↑)
GA	3.79 (s, CH$_2$)	HMDB0000128	↓	(↓)
Lactate	1.33 (d, CH$_3$); 4.10 (q, CH)	HMDB0000190	(↓)	(↓)
Succinate	2.41 (s, CH$_2$)	HMDB0000254	-	(↓)
Other compounds				
Acetone	2.24 (s, CH$_3$)	HMDB0001659	(↓)	-
DMA	2.73 (s, (CH$_3$)$_2$)	HMDB0000087	↓	↓
Ethanol *	1.19 (t, CH$_3$); 3.65 (q, CH$_2$)	HMDB0000108	(↓)	(↑)
m-Inositol	3.28 (t, C5H); 3.62 (t, C4H/ C6H); 4.06 (t, C2H)	HMDB0000211	(↓)	(↓)
PE [†]	3.99 (m, PO$_3$-CH$_2$)	HMDB0000224	↓	(↓)
TMAO	3.27 (s, CH$_3$)	HMDB0000925	(↓)	-

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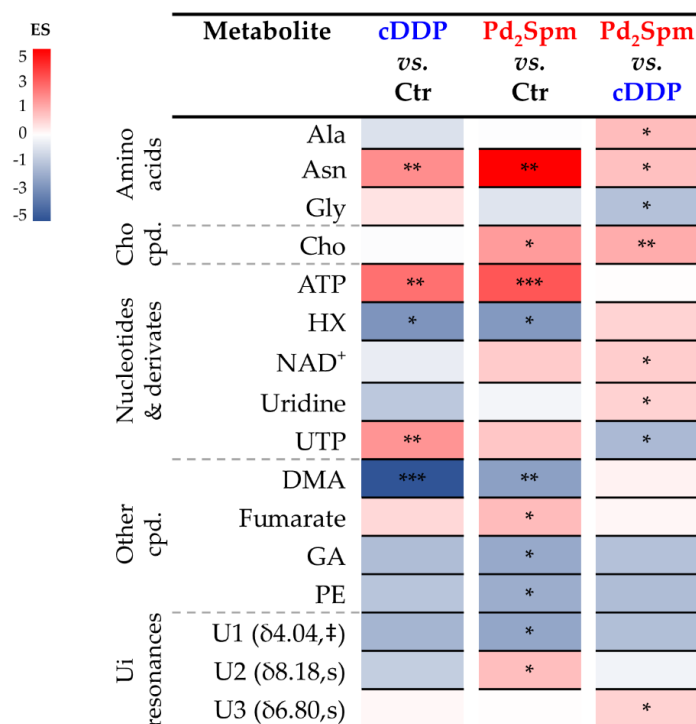
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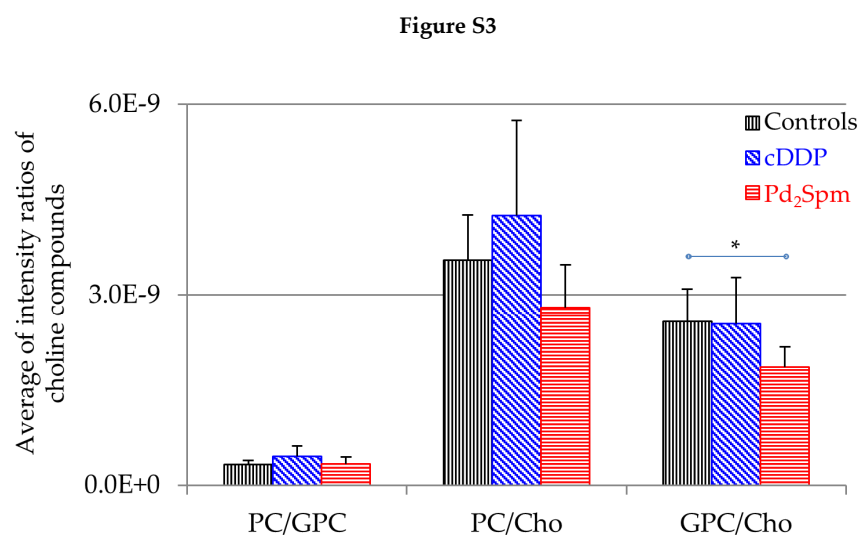
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