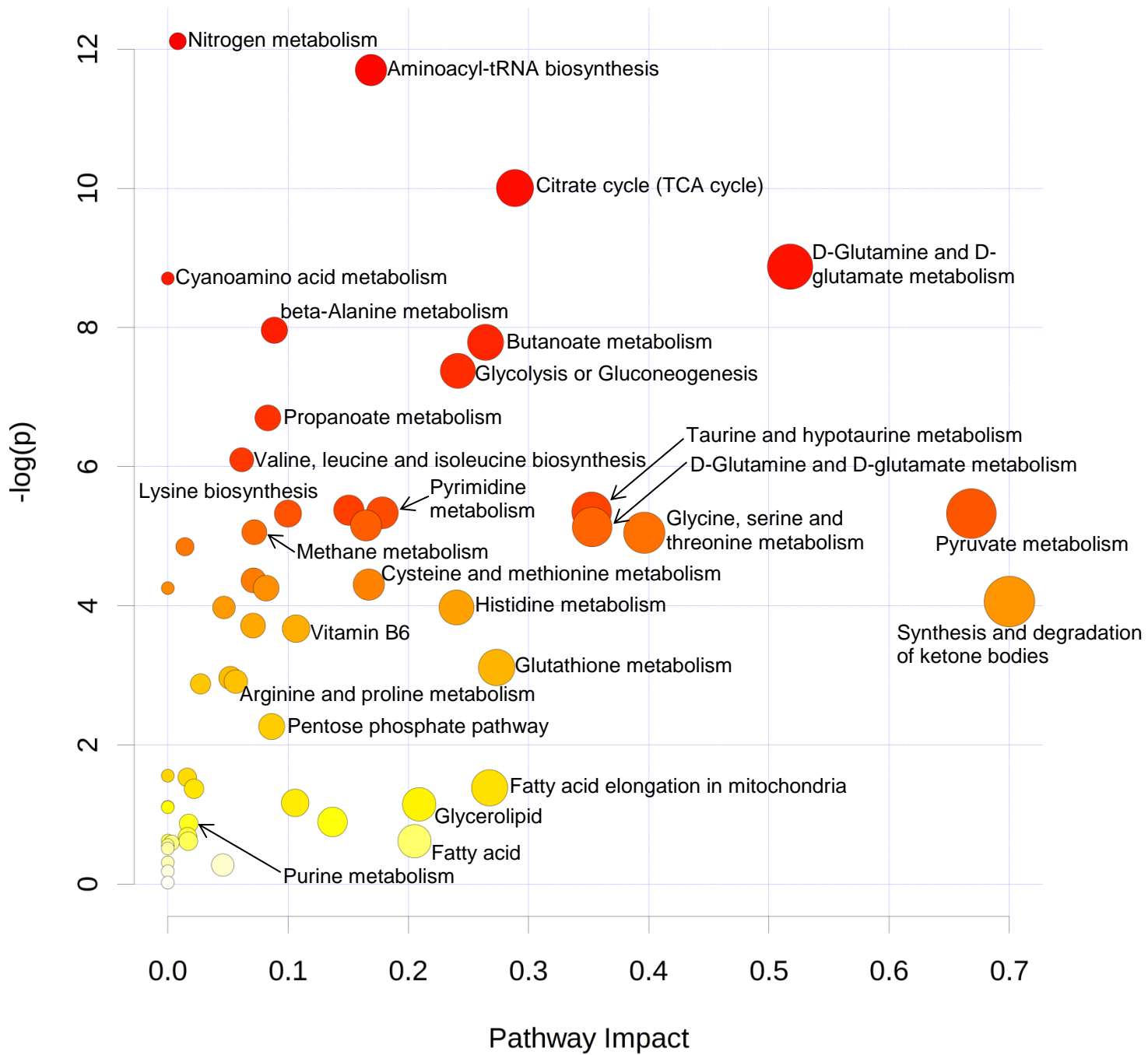


Supplementary Figure 1 OPLS-DA scores plot showing clustering of bladder cancer (BC) samples according to classification of tumors. Tumors were classified according World Health Organization (WHO)/International Society of Urological Pathology (ISUP) classification criteria. PUNLMP, papillary urothelial neoplasm of low malignant potential; Ta, noninvasive papillary tumors confined to the mucosa; T1, invasive tumors that invade the submucosa (lamina propria). Low grade and high grade are represented as LG and HG respectively.



Supplementary Figure 2 Bladder cancer metabolic pathway analysis and visualization using MetaboAnalyst. The metabolic pathways are arranged according to the scores from enrichment analysis (y axis) and from topology analysis (x axis), and displayed as circles based on their individual *P* values (intensity of color) and pathway impact values (size of circle).

Supplementary Table 1 Metabolic pathways and respective marker metabolites perturbed in bladder cancer

| Pathway | $-\log(p^a)$ | Impact ^b | Metabolites | Direction of change & biofluids utilized |
|--|--------------|---------------------|---|---|
| Alanine, aspartate and glutamate metabolism | 8.8082 | 0.51757 | Glutamine ^{1,2} Aspartate ¹ Asparagine ³ Oxoglutarate ⁴ Pyruvate ⁴ Succinate ⁴ | + in tissue & serum + in tissue + in tissue & urine + in urine + in urine + in urine |
| Aminoacyl-tRNA biosynthesis | 11.575 | 0.16902 | Asparagine ³ Histidine ^{2,3} Phenylalanine ^{1,3,5} Glutamine ^{1,2} Aspartate ¹ Glycine ⁵ Serine ³ Valine ^{3,6} Lysine ³ Tyrosine ^{1,3,5} Isoleucine/Leucine ^{3,5} Tryptophan ³ | + in tissue & urine + in tissue, serum & urine – in serum; + in tissue & urine + in tissue & serum + in tissue – in serum + in tissue & urine + in tissue, serum & urine + in tissue & urine – in serum; + in tissue & urine – in serum; + in tissue & urine + in tissue & urine |
| Amino sugar and nucleotide sugar metabolism | 0.17818 | 0 | Glucose ⁵ | + in serum |
| Arginine and proline metabolism | 2.8632 | 0.05655 | Creatine ^{1,3} S-Adenosylmethionine ³ Spermidine ³ Glutamine ^{1,2} Aspartate ¹ Pyruvate ⁴ | + in tissue & urine + in tissue & urine + in tissue & urine + in tissue & serum + in tissue + in urine |
| Ascorbate and aldarate metabolism | 1.5112 | 0.01617 | Myo-inositol ¹ Pyruvate ⁴ Oxoglutarate ⁴ | + in tissue + in urine + in urine |
| beta-Alanine metabolism | 7.8956 | 0.08863 | Histidine ^{2,3} Spermidine ³ Uracil ³ Aspartate ¹ | + in tissue, serum & urine + in tissue & urine + in tissue & urine + in tissue |

| | | | | |
|---|---------|---------|------------------------------------|---------------------------|
| | | | Pantothenic acid ³ | + in tissue & urine |
| | | | Malonate ² | + in serum |
| | | | Acetyl-CoA ⁴ | + in urine |
| Biotin metabolism | 1.0979 | 0 | Lysine ³ | + in tissue & urine |
| Butanoate metabolism | 7.714 | 0.26429 | 2-Butenedioic acid ^{6, 7} | - in urine |
| | | | Acetoacetate ⁵ | + in serum |
| | | | Oxoglutarate ⁴ | + in urine |
| | | | Succinate ⁴ | + in urine |
| | | | Pyruvate ⁴ | + in urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| Citrate cycle (TCA cycle) | 9.9362 | 0.28874 | Citrate ⁵⁻⁸ | - in urine & serum |
| | | | Acetyl-CoA ⁴ | + in urine |
| | | | Pyruvate ⁴ | + in urine |
| | | | Succinate ⁴ | + in urine |
| | | | Oxoglutarate ⁴ | + in urine |
| | | | Phosphoenolpyruvate ⁴ | + in urine |
| Cyanoamino acid metabolism | 8.6486 | 0 | Alanine ^{1, 3} | +/- in tissue; - in urine |
| | | | Serine ³ | + in tissue, urine |
| | | | Asparagine ³ | + in tissue, urine |
| | | | Aspartate ¹ | + in tissue |
| | | | Glycine ⁵ | - in serum |
| Cysteine and methionine metabolism | 4.2505 | 0.16719 | S-Adenosylmethionine ³ | + in tissue & urine |
| | | | Glutathione ¹ | + in tissue |
| | | | Homoserine ³ | + in tissue & urine |
| | | | Serine ³ | + in tissue & urine |
| | | | Aspartate ¹ | + in tissue |
| | | | Pyruvate ⁴ | + in urine |
| D-Glutamine and D-glutamate metabolism | 5.0996 | 0.35294 | Glutamine ^{1, 2} | + in tissue & serum |
| | | | Glutamate ¹ | + in tissue |
| | | | Oxoglutarate ⁴ | + in urine |
| Ether lipid metabolism | 0.55708 | 0 | Glycerophosphocholine ¹ | + in tissue |
| Fatty acid biosynthesis | 1.3481 | 0.0218 | Oleic acid ³ | - in tissue & urine |
| | | | Palmitic acid ³ | - in tissue & urine |
| | | | lauric acid ³ | - in tissue & urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| Fatty acid elongation in mitochondria | 1.3663 | 0.26765 | Palmitic acid ³ | - in tissue & urine |
| | | | Acetyl-CoA ⁴ | + in urine |

| | | | | |
|---|---------|---------|--|--|
| Fatty acid metabolism | 0.60483 | 0.20518 | Palmitic acid ³ Acetyl-CoA ⁴ | – in tissue & urine + in urine |
| Galactose metabolism | 2.8416 | 0.02724 | Fructose ⁶ Glucose ⁵ Glyceraldehyde-3-phosphate ³ Glycerol ^{6, 7} Melibiose ^{6, 7} <i>Myo</i> -inositol ¹ | – in urine + in serum – in tissue & urine – in urine + in urine + in tissue |
| Glutathione metabolism | 3.0767 | 0.27351 | Glutathione ¹ Spermidine ³ Glycine ⁵ Acetyl-CoA ⁴ | + in tissue + in tissue & urine – in serum + in urine |
| Glycerolipid metabolism | 1.1302 | 0.20907 | Dihydroxyacetone ⁷ Glycerol ^{6, 7} | – in urine – in urine |
| Glycerophospholipid metabolism | 0.87912 | 0.05556 | Phosphorylcholine ⁹ Choline/Glycerophosphocholine ¹ | – in urine + in tissue |
| Glycine, serine and threonine metabolism | 4.9927 | 0.39631 | Choline ¹ Creatine ^{1, 3} Homoserine ³ Aspartate ¹ Glycine ⁵ Serine ³ Tryptophan ³ Pyruvate ⁴ | + in tissue + in tissue & urine + in tissue & urine + in tissue – in serum + in tissue & urine + in tissue & urine + in urine |
| Glycolysis or Gluconeogenesis | 7.3129 | 0.24128 | Acetate ¹ Lactate ^{1, 2, 5, 7} Glucose ⁵ Glyceraldehyde-3-phosphate ³ Acetyl-CoA ⁴ Phosphoenolpyruvate ⁴ Pyruvate ⁴ | + in tissue +/- in serum; + in urine & tissue + in serum – in tissue & urine + in urine + in urine + in urine |
| Glyoxylate and dicarboxylate metabolism | 4.792 | 0.01426 | Citrate ⁵⁻⁸ Acetyl-CoA ⁴ Succinate ⁴ Oxoglutarate ⁴ Pyruvate ⁴ | – in urine & serum + in urine + in urine + in urine + in urine |
| Histidine metabolism | 3.9293 | 0.24013 | Urocanic acid ³ | – in tissue & urine |

| | | | | |
|---|---------|---------|---|---------------------------------|
| | | | Histidine ^{2,3} | + in tissue, serum & urine |
| | | | Histamine ³ | - in tissue & urine |
| | | | Aspartate ¹ | + in tissue |
| | | | Oxoglutarate ⁴ | + in urine |
| Inositol phosphate metabolism | 0.87912 | 0.13703 | Myo-inositol ¹ | + in tissue |
| | | | Glyceraldehyde-3-phosphate ³ | - in tissue & urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| Limonene and pinene degradation | 0.11755 | 0.00842 | Pinene ⁷ | - in urine |
| Lysine biosynthesis | 5.2748 | 0.09993 | Homoserine ³ | + in tissue & urine |
| | | | Aspartate ¹ | + in tissue |
| | | | Lysine ³ | + in tissue & urine |
| | | | Oxoglutarate ⁴ | + in urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| Lysine degradation | 5.0973 | 0.1649 | Lysine ³ | + in tissue & urine |
| | | | Pipecolic acid ³ | - in tissue & urine |
| | | | Glycine ⁵ | - in serum |
| | | | Carnitine ^{4,3} | + in tissue & urine |
| | | | Trimethyllysine ⁴ | + in urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| Methane metabolism | 5.0082 | 0.07195 | Dihydroxyacetone ⁷ | - in urine |
| | | | Glyceraldehyde-3-phosphate ³ | - in tissue & urine |
| | | | Serine ³ | + in tissue & urine |
| | | | Glycine ⁵ | - in serum |
| | | | Dimethylamine ² | + in serum |
| | | | Acetyl-CoA ⁴ | + in urine |
| Nicotinate and nicotinamide metabolism | 3.9293 | 0.0467 | Trigonelline ⁹ | - in urine |
| | | | Niacinamide ³ | + in tissue & urine |
| | | | Aspartate ¹ | + in tissue |
| | | | 2-Butenedioic acid ^{6,7} | - in urine |
| | | | Pyruvate ⁴ | + in urine |
| Nitrogen metabolism | 12.015 | 0.0083 | Anthranilic acid ⁷ | + in urine |
| | | | Asparagine ³ | + in tissue & urine |
| | | | Aspartate ¹ | + in tissue |
| | | | Glutamine ^{1,2} | + in tissue & serum |
| | | | Glycine ⁵ | - in serum |
| | | | Histidine ^{2,3} | + in tissue, serum & urine |
| | | | Phenylalanine ^{1,3,5} | - in serum; + in tissue & urine |

| | | | | |
|--|---------|---------|---|------------------------------------|
| | | | Taurine ^{1, 8, 10, 3} | +/- in urine & tissue; - in serum; |
| | | | Tyrosine ^{1, 3, 5} | + in tissue & urine |
| | | | Tryptophan ³ | + in tissue & urine |
| Pantothenate and CoA biosynthesis | 4.2135 | 0 | Aspartate ¹ | + in tissue |
| | | | Uracil ³ | + in tissue & urine |
| | | | Valine ^{3, 6} | + in tissue, serum & urine |
| | | | Pantothenic acid ³ | + in tissue & urine |
| | | | Pyruvate ⁴ | + in urine |
| Pentose and glucuronate interconversions | 0.54774 | 0 | Ribitol ^{6, 7} | - in urine |
| | | | Glyceraldehyde 3-phosphate ³ | - in tissue & urine |
| | | | Pyruvate ⁴ | + in urine |
| Pentose phosphate pathway | 2.2379 | 0.08639 | Gluconic acid ^{6, 7} | - in urine |
| | | | Glucose ⁵ | + in serum |
| | | | Glyceraldehyde-3-phosphate ³ | - in tissue & urine |
| | | | Pyruvate ⁴ | + in urine |
| Phenylalanine metabolism | 5.3158 | 0.15056 | Hippuric acid ^{3, 8-10} | +/- in urine; + in tissue |
| | | | Phenylacetylglutamine ^{9, 10} | - in urine |
| | | | Phenylalanine ^{1, 3, 5} | - in serum; + in tissue & urine |
| | | | Tyrosine ^{1, 3, 5} | - in serum; + in tissue & urine |
| | | | 4-Hydroxy phenylactic acid ³ | + in tissue & urine |
| | | | Succinate ⁴ | + in urine |
| | | | Pyruvate ⁴ | + in urine |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 4.2135 | 0.0818 | Anthranilic acid ⁷ | + in urine |
| | | | Phenylalanine ^{1, 3, 5} | - in serum; + in tissue & urine |
| | | | Tyrosine ^{1, 3, 5} | - in serum; + in tissue & urine |
| | | | Tryptophan ³ | + in tissue & urine |
| | | | Phosphoenolpyruvate ⁴ | + in urine |
| Porphyrin and chlorophyll metabolism | 0.02015 | 0 | Glycine ⁵ | - in serum |
| Primary bile acid biosynthesis | 0.66855 | 0.01644 | Taurine ^{1, 8, 10, 3} | +/- in tissue & urine |
| | | | Glycine ⁵ | - in serum |
| Propanoate metabolism | 6.6387 | 0.08322 | 2-Hydroxybutyric acid ³ | - in tissue & urine |
| | | | Acetoacetate ⁵ | + in serum |
| | | | Valine ^{3, 6} | + in tissue, serum & urine |
| | | | Lactate ^{1, 2, 5, 7} | +/- in serum; + in tissue & urine |
| | | | Succinate ⁴ | + in urine |
| | | | Acetyl-CoA ⁴ | + in urine |

| | | | | |
|---|---------|---------|---|--|
| Purine metabolism | 0.84908 | 0.01738 | Guanine ³ Hypoxanthine ³ Glutamine ^{1,2} Glycine ⁵ | + in tissue & urine + in tissue & urine + in tissue & serum – in serum |
| Pyrimidine metabolism | 5.2705 | 0.1784 | Cytidine monophosphate ³ Pseudouridine ⁷ Thymine ³ Uridine ^{6,7} Glutamine ^{1,2} Uracil ³ Malonate ² | + in tissue & urine + in urine + in tissue & urine + in urine + in tissue & serum + in tissue & urine + in serum |
| Pyruvate metabolism | 5.2748 | 0.66833 | Lactate ^{1,2,5,7} Acetate ¹ Acetyl-CoA ⁴ Phosphoenolpyruvate ⁴ Pyruvate ⁴ | +/- in serum; + in tissue & urine + in tissue + in urine + in urine + in urine |
| Riboflavin metabolism | 0.61631 | 0 | Ribitol ^{6,7} | – in urine ^{6,7} |
| Selenoamino acid metabolism | 0.58571 | 0.00321 | Acetate ¹ | + in tissue |
| Sphingolipid metabolism | 0.50502 | 0 | Serine ³ | + in tissue & urine |
| Starch and sucrose metabolism | 0.60483 | 0.01703 | Fructose ⁶ Glucose ⁵ | – in urine + in serum |
| Sulfur metabolism | 3.6839 | 0.07087 | Acetate ¹ Homoserine ³ Serine ³ | + in tissue + in tissue & urine + in tissue & urine |
| Synthesis and degradation of ketone bodies | 4.0385 | 0.7 | Acetoacetate ⁵ Acetyl-CoA ⁴ | + in serum + in urine |
| Taurine and hypotaurine metabolism | 5.308 | 0.35252 | Taurine ^{1,8,10,3} Acetate ¹ Pyruvate ⁴ Acetyl-CoA ⁴ | +/- in tissue & urine + in tissue + in urine + in urine |
| Terpenoid backbone biosynthesis | 1.0894 | 0 | Glyceraldehyde 3-phosphate ³ Pyruvate ⁴ Acetyl-CoA ⁴ | – in tissue & urine + in urine + in urine |
| Thiamine metabolism | 1.54 | 0 | Tyrosine ^{1,3,5} Glycine ⁵ | – in serum; + in tissue & urine – in serum |
| Tryptophan metabolism | 1.1423 | 0.10593 | 3-Hydroxykynurenine* ³ N-acetyl-anthranilic acid* ⁷ | + in tissue & urine + in urine |

| | | | | |
|--|--------|---------|--|---------------------------------|
| | | | Kynurenine* ³ | + in tissue & urine |
| | | | Anthranilic acid ⁷ | + in urine |
| | | | Tryptophan ³ | + in tissue & urine |
| | | | 5-Hydroxyindoleacetic acid ³ | + in tissue & urine |
| | | | Malonate ² | + in serum |
| | | | Acetyl-CoA ⁴ | + in urine |
| Tyrosine metabolism | 2.9159 | 0.05201 | <i>p</i> -Cresol* ⁷ | + in urine |
| | | | 3,4-Dihydroxyphenylpyruvate ⁷ | + in urine |
| | | | Vanillylmandelic acid ⁷ | + in urine |
| | | | Acetoacetate ⁵ | + in serum |
| | | | Tyrosine ^{1, 3, 5} | – in serum; + in tissue & urine |
| | | | Norepinephrine ³ | – in tissue & urine |
| | | | 4-Hydroxy phenylactic acid ³ | + in tissue & urine |
| | | | Pyruvate ⁴ | + in urine |
| | | | Succinate ⁴ | + in urine |
| Ubiquinone and other terpenoid-quinone biosynthesis | 0.3053 | 0 | Tyrosine ^{1, 3, 5} | – in serum; + in tissue & urine |
| Valine, leucine and isoleucine degradation | 4.3175 | 0.0713 | Isoleucine/Leucine ^{3, 5} | – in serum; |
| | | | Acetoacetate ⁵ | + in tissue & urine |
| | | | Valine ^{3, 6} | + in tissue, serum & urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| Valine, leucine and isoleucine biosynthesis | 6.0465 | 0.06148 | Valine ^{3, 6} | + in tissue, serum & urine |
| | | | Isoleucine/Leucine ^{3, 5} | – in serum; + in tissue & urine |
| | | | Acetyl-CoA ⁴ | + in urine |
| | | | Pyruvate ⁴ | + in urine |
| Vitamin B6 metabolism | 3.6329 | 0.1067 | 4-Pyridoxic acid ³ | – in tissue & urine |
| | | | Glutamate ¹ | + in tissue |
| | | | Glyceraldehyde-3-phosphate ³ | – in tissue & urine |
| | | | Oxoglutarate ⁴ | + in urine |
| | | | Pyruvate ⁴ | + in urine |

| Carnitine species* | | | | |
|--------------------|---|---|--|---------------------|
| | - | - | Carnitine ^{4,3} | + in tissue & urine |
| | | | 2,6-Dimethylheptanoyl carnitine ⁹ | - in urine |
| | | | Acetylcarnitine ⁹ | + in urine |
| | | | Carnitine C10:1 ⁹ | - in urine |
| | | | Carnitine C10:3 ¹⁰ | + in urine |
| | | | Carnitine C8:1 ^{9,10,4} | +/- in urine |
| | | | Carnitine C9:0 ¹⁰ | - in urine |
| | | | Carnitine C9:1 ^{9,10} | - in urine |
| | | | Isobutyryl carnitine ³ | + in tissue & urine |
| | | | Isovalerylcarnitine ⁴ | + in urine |
| | | | Glutarylcarnitine ⁴ | - in urine |
| | | | Decanoylcarnitine ⁴ | - in urine |

(+) refers to elevated levels in BC patients compared to benign or healthy controls

(-) refers to decreased levels in BC patients compared to benign or healthy controls

^a *p* values are calculated from pathway enrichment analysis using MetaboAnalyst

^b Impact values are calculated from pathway topology analysis using MetaboAnalyst

* Pathways or metabolites are enriched based on literatures

Supplementary Table 2 Bladder cancer marker metabolites that are not documented within KEGG database nor associated with any specific metabolic pathways

| Metabolites | Direction of change & biofluids utilized |
|---|---|
| 2,5-Furandicarboxylic acid^{6,7} | – in urine |
| 2-Aminoisobutyric acid⁷ | + in urine |
| 2-Hydroxyglutaric acid⁷ | – in urine |
| 2-Propenoic acid⁶ | – in urine |
| 3-Hydroxysebacic acid⁷ | + in urine |
| 3-Methyladipic acid⁷ | + in urine |
| 4-Methoxycinnamic acid⁷ | + in urine |
| Adipic acid⁷ | + in urine |
| Aminobutyric acid³ | + in tissue & urine |
| Aniline³ | + in tissue & urine |
| Citramalic acid³ | + in tissue & urine |
| Coumarin³ | – in tissue & urine |
| DL-3-phenyllactic acid³ | + in tissue & urine |
| D-Ribonolactone³ | + in tissue & urine |
| Erythritol⁷ | + in urine |
| Ethylmalonic acid⁷ | + in urine |
| Heptadecanoic acid⁷ | + in urine |
| Homocysteine³ | – in tissue & urine |
| Itaconic acid⁷ | + in urine |
| Leucylproline⁹ | – in urine |
| N1-Acetylspermine³ | + in tissue & urine |
| Phthalic acid Mono-2-ethylhexyl Ester³ | – in tissue & urine |
| Ribonic acid^{6,7} | – in urine |
| Sebacic acid⁷ | – in urine |
| Senecioic acid⁶ | – in urine |
| Sumiki's acid^{6,7} | – in urine |
| Triglycerides¹ | – in tissue |
| Valerate⁶ | – in urine |
| Cyclopentane-1,2-diamine⁷ | + in urine |
| Erythro-pentonic acid⁷ | + in urine |
| Ethyl tartrate⁷ | – in urine |
| Levulinic acid enol⁷ | – in urine |
| Talonic acid⁷ | + in urine |
| 2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone⁷ | + in urine |
| 2-Hydroxymalonic acid⁷ | + in urine |
| 2-Pentadecanol⁷ | + in urine |

(+) refers to elevated levels in BC patients compared to benign or healthy controls

(–) refers to decreased levels in BC patients compared to benign or healthy controls

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