

Supporting Information: Supplemental Figures 1-5

Running title: Metabonomics IPA of Gentamicin System Toxicity

**Ingenuity Pathways Analysis of Urine Metabonomics Phenotypes Toxicity of
Gentamicin in Multiple Organs**

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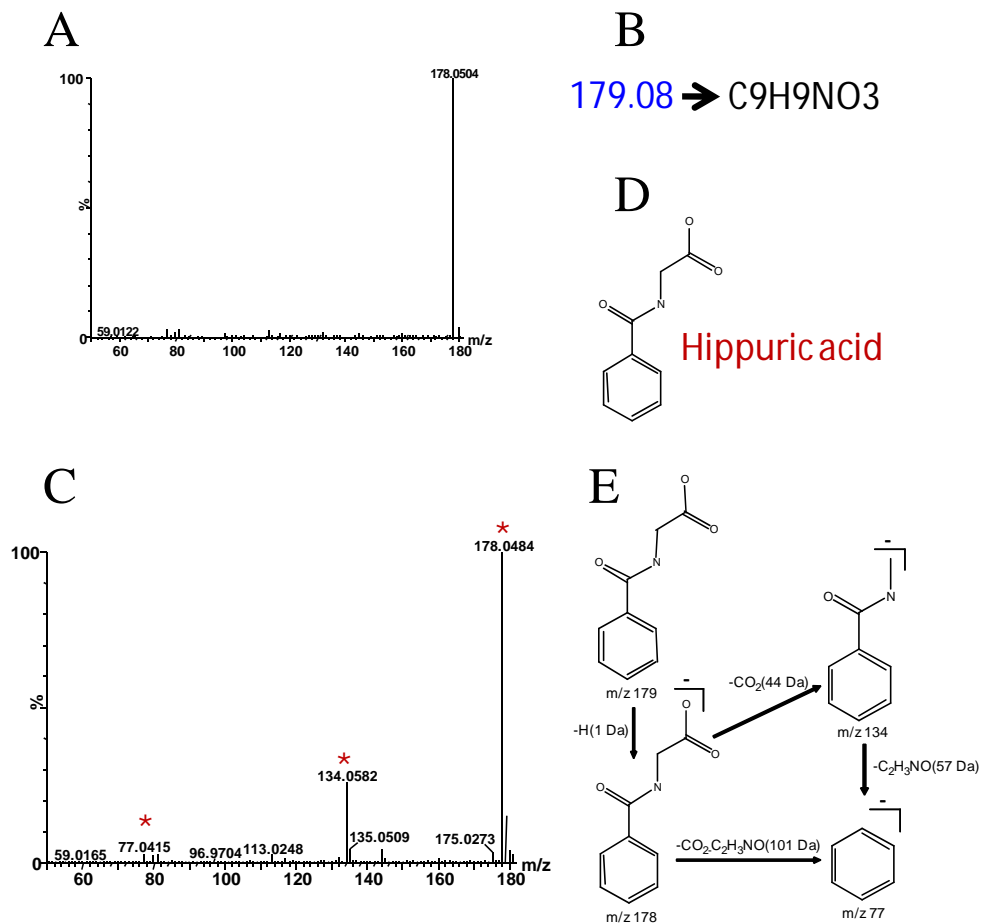
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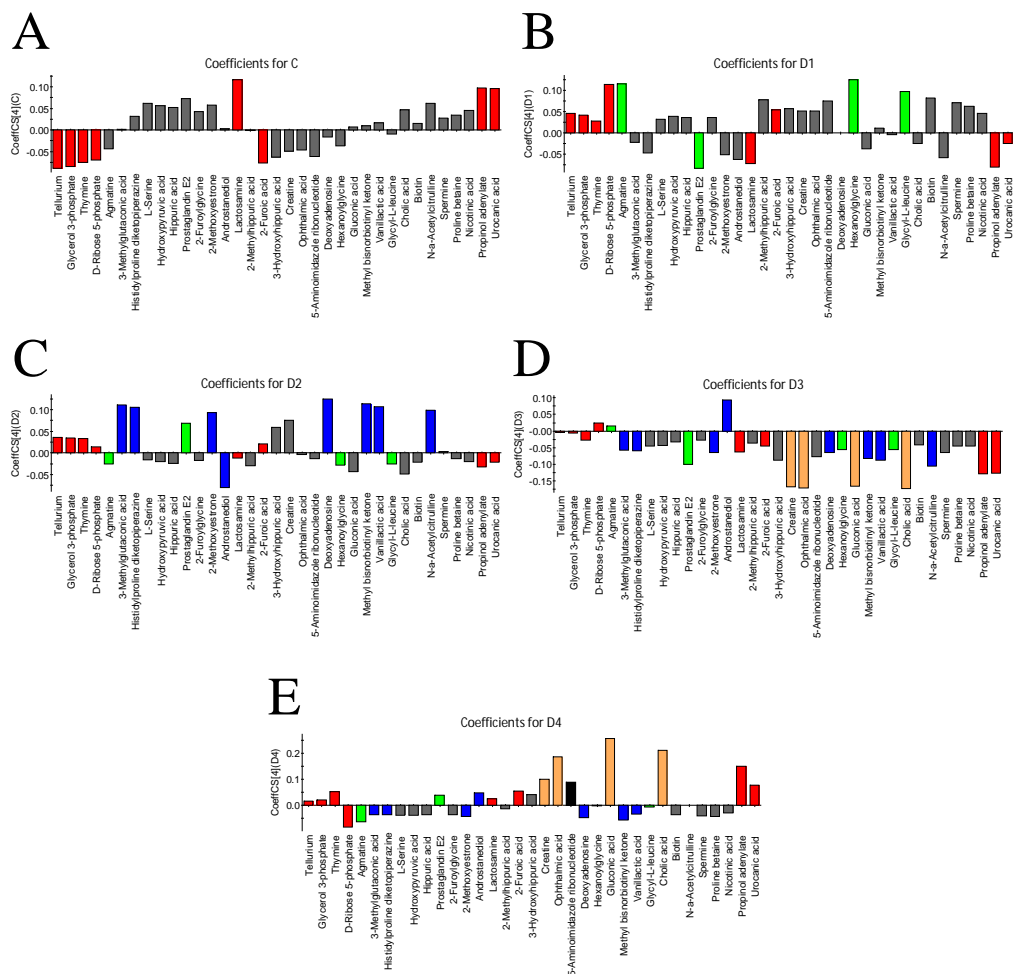
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368, New York 10461, USA.

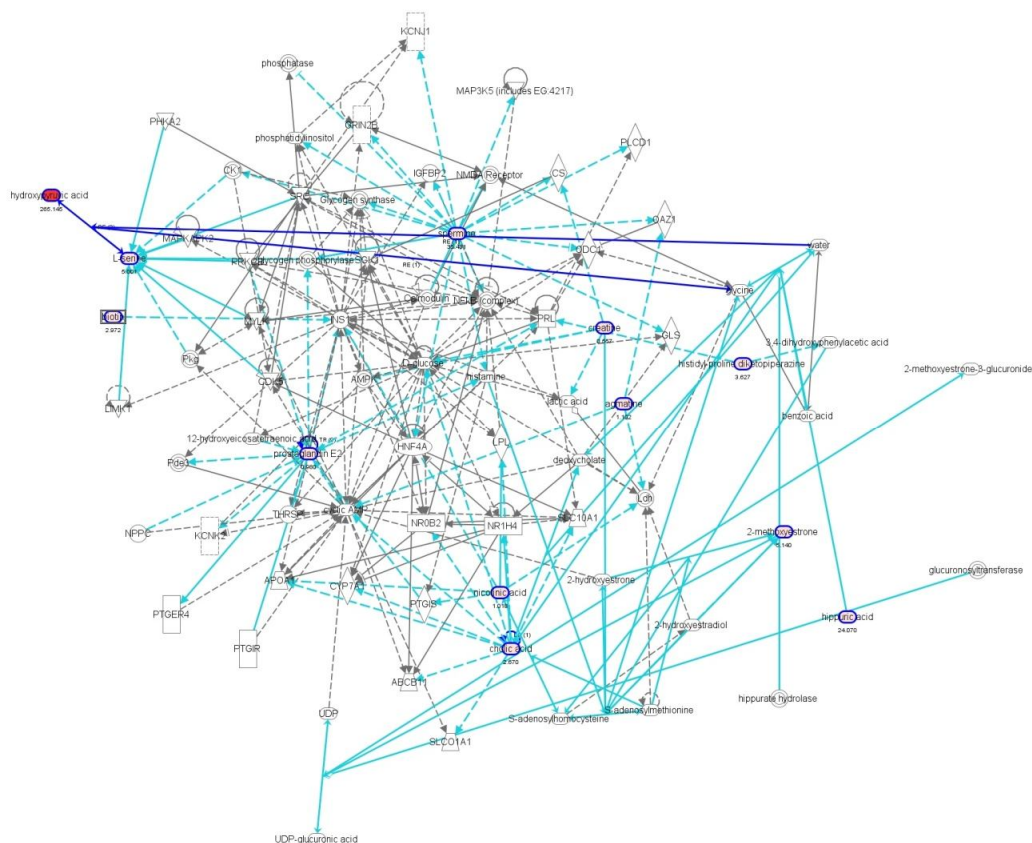


Supplemental Figure 1. The workflow of structure identification of the metabolites.

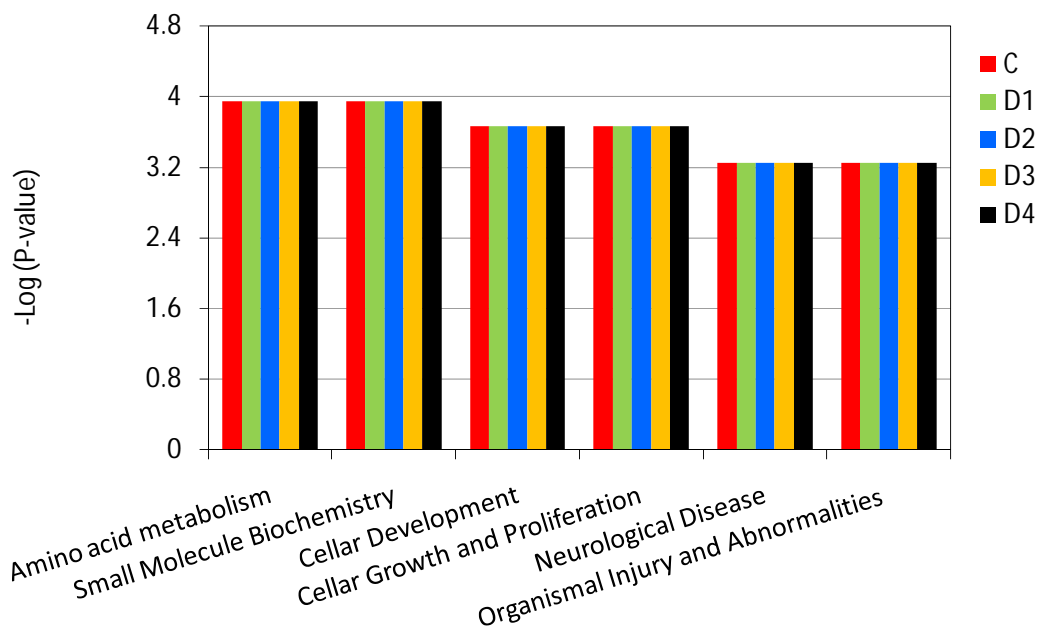
A. parent ion; B. extract mass and element composition (formula); C. daughter ion; D. the structure of hippuric acid; E. The pathway mechanism of collision induced dissection of the creatinine



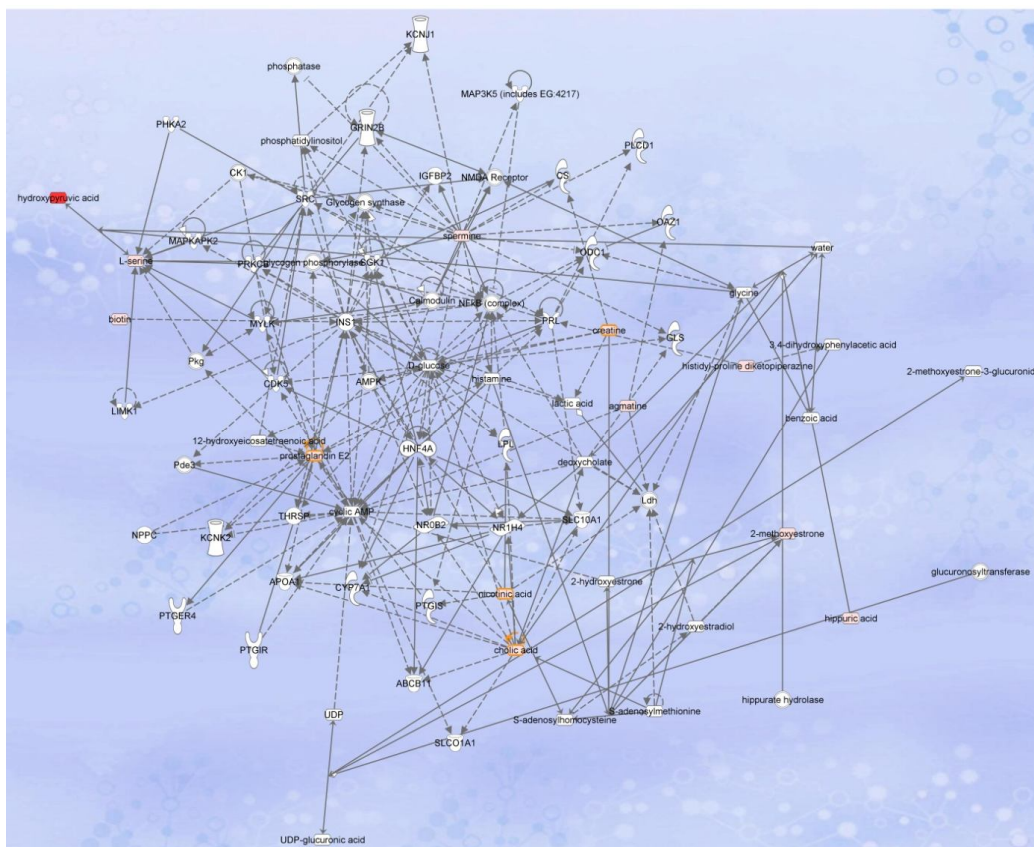
Supplemental Figure 2. The 35 metabolites with the highest regression coefficients contributing to group's discrimination observed in Fig 6C. The plots show the changes in coefficients from individual metabolites and the different sampling time-points pre- and post-dosage of gentamicin. (A) Mapping of metabolite's coefficient at C (bars in red); (B) Mapping of metabolite's coefficient at D1 (bars in green); (C) Mapping of metabolite's coefficient at D2 (bars in blue); (D) Mapping of metabolite's coefficient at D3 (bars in orange); (E) Mapping of metabolite's coefficient at D4 (bars in black); It was found that the metabolites have no absolute coefficient with specific time-point, some of them are marked coefficient with two or more time-points.



Supplemental Figure 3. Proof-of-knowledge based IPA phenotypic view of the metabolic network perturbations induced by gentamicin. The twelve main metabolites influenced by gentamicin are highlighted in blue circles. The association of the here found metabolites to proof-of-knowledge databases generated a comprehensive metabolic network map of the known connections of the metabolites, which allows to deduce the gentamicin toxicity system mechanisms.



Supplemental Figure 4. Unsupervised predictions of the biochemical network effects induced by gentamicin and generated the proof-of-knowledge based IPA analysis. IPA toxicity model generated using the 35 most relevantly changed urine metabolites predicts a drug harmful effects primarily in liver and kidney, however it is also predicted heart injury. The IPA prediction of the effects is based on the proof-of-knowledge association of changes in the phenotypic biomarkers with the most probabilistic metabolic pathways, indicating that gentamicin targeted mainly the pathways of amino acid metabolism and small molecule biochemistry. C: -24-0 h pre-dosage; D1: 0-24 h, D2: 24-48, D3: 48-72 h and D4: 72-96 h post-dosage.



Supplemental Figure 5. Proof-of-knowledge based IPA phenotypic view of the metabolic network for the IPA-defined main biomarker perturbations induced by gentamicin . The biomarkers creatine, nicotinic acid, prostaglandin E2, and cholic acid are highlighted in orange circles. The IPA-Tox evaluation showed that changes in the biomarkers significantly correlates with the toxicity of gentamicin in the liver, kidney and heart. Subsequently, the association of these four biomarkers to proof-of-knowledge databases generated a comprehensive metabolic network map of the known connections of the biomarkers, allowing to deduce the gentamicin toxicity system mechanisms.