

## Supplementary materials

### Quantification of Cardiotonic Steroids Potentially Regulated by Paraoxonase 3 in a Rat Model of Chronic Kidney Disease using UHPLC-Orbitrap-MS

Sabitri Lamichhane <sup>1</sup>, Chrysan J. Mohammed <sup>2</sup>, Steven T. Haller <sup>2</sup>, David J. Kennedy <sup>\*2</sup>, Dragan Isailovic <sup>\*1</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, University of Toledo, Toledo, OH 43606,

<sup>2</sup>Department of Medicine, University of Toledo Medical Center, Toledo, OH 43614

#### Equation S1. Extraction recovery calculation

The extraction recovery of an analyte from an aqueous sample or urine is calculated using the equation given below

$$\text{Extraction recovery} = \frac{A}{B} \times 100\% \dots \dots \dots (1)$$

A: Peak area of analyte spiked before extraction

B: Peak area of analyte spiked in water (for aqueous sample)

Peak area of analyte spiked after extraction (for urine sample)

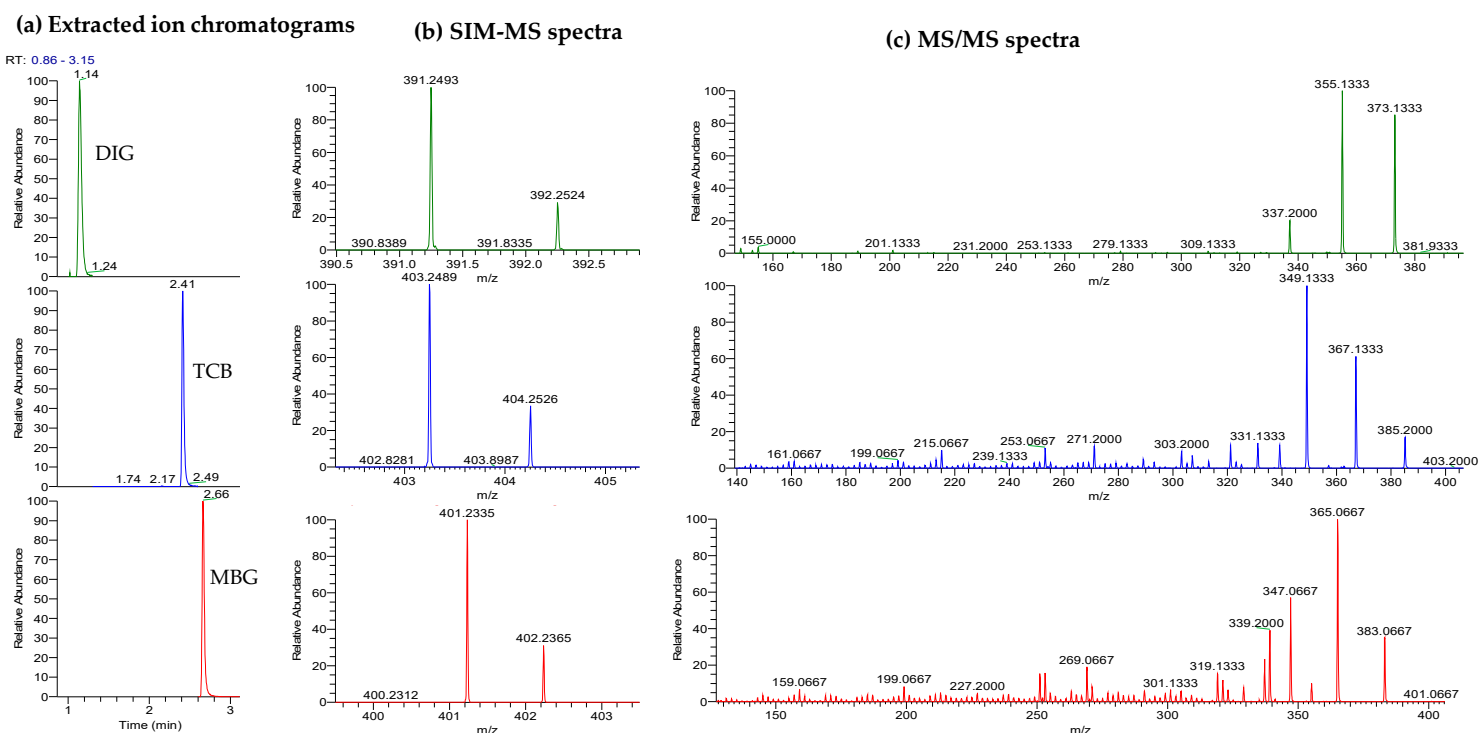
#### Equation S2. Matrix effect calculation

The matrix effect for digoxigenin is calculated using the following equation

$$\text{Matrix effect} = \frac{A - B}{A} \times 100\% \dots \dots \dots (2)$$

A: Peak area of analyte in standard solution

B: Peak area of analyte in urine which has undergone SPE and later spiked with analyte with same concentration as standard sample

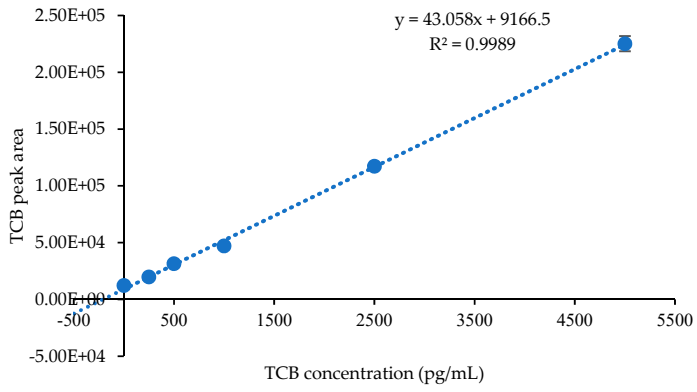


**Figure S1.** Analysis of CTS and DIG by UHPLC-Orbitrap-MS/MS: (a) Extracted-ion chromatograms showing retention times of DIG, TCB and MBG; (b) SIM scan mass spectra, showing protonated DIG, TCB and MBG ions (c) MS/MS spectra of  $[M + H]^+$  monoisotopic precursor ions with  $m/z$  values of 391.2493, 403.2489 and 401.2336.

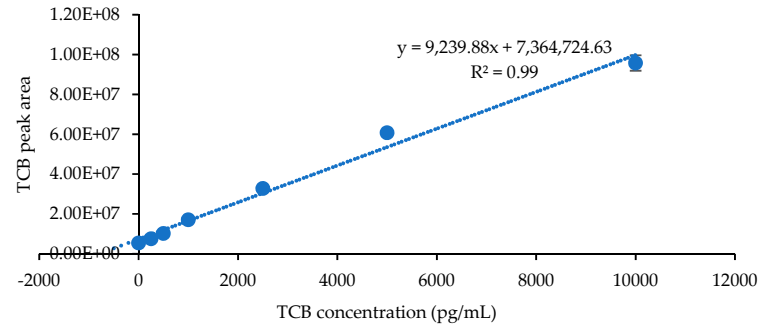
**Table S1.** Mass accuracy calculation

CTS compound	Theoretical $m/z$	Experimental $m/z$	Mass accuracy (ppm)
TCB	403.2479	403.2489	2.5
MBG	401.2323	401.2335	3.2
DIG	391.2479	391.2493	3.6

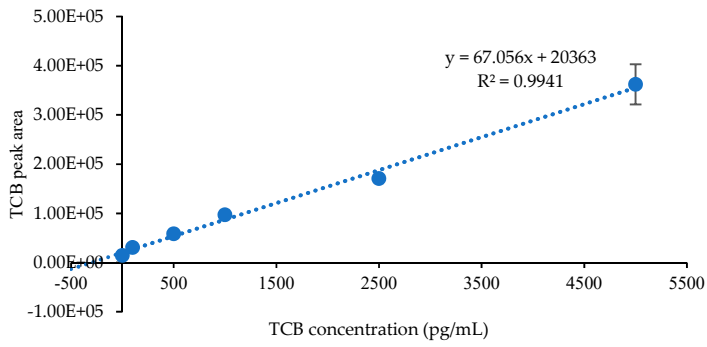
SS-WT NC 346



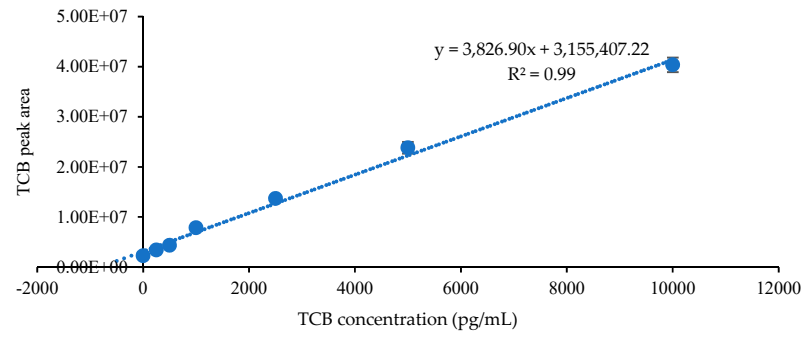
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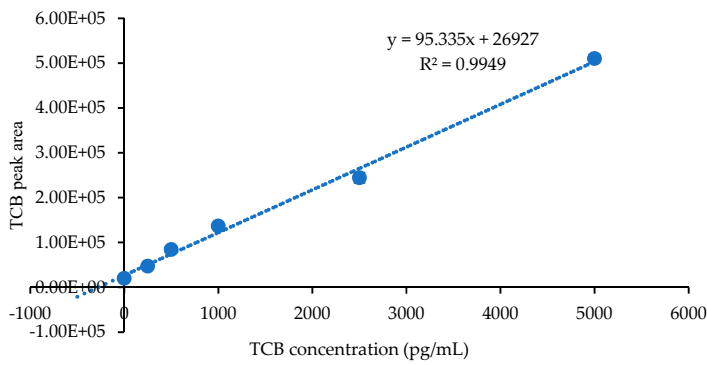
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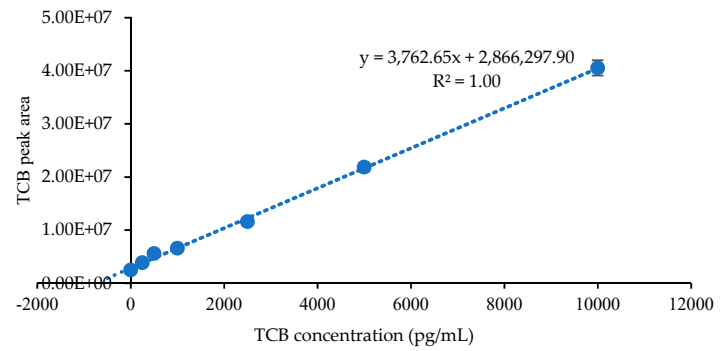
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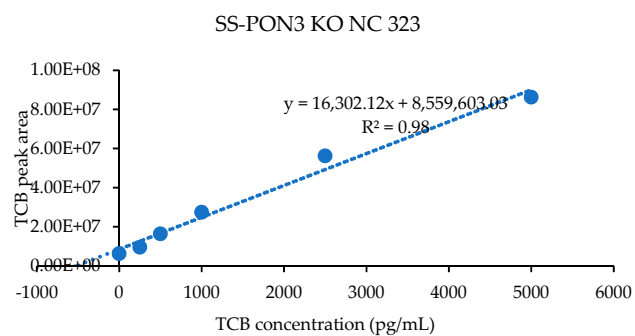
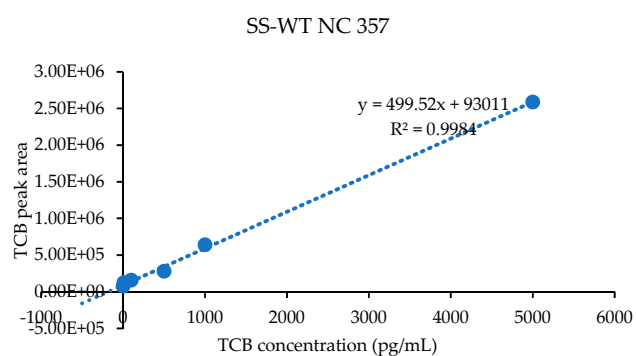
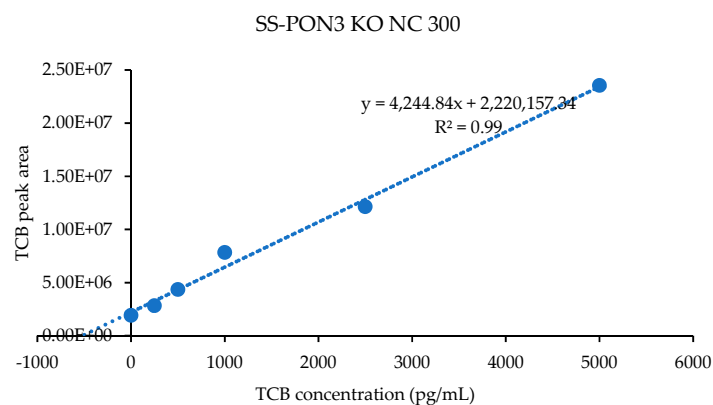
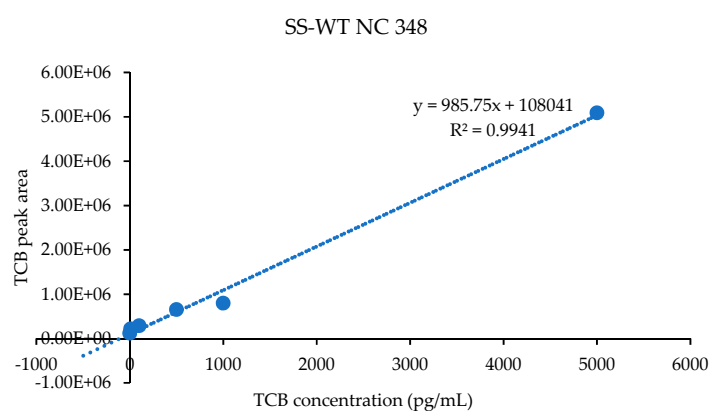


SS-WT NC NT7



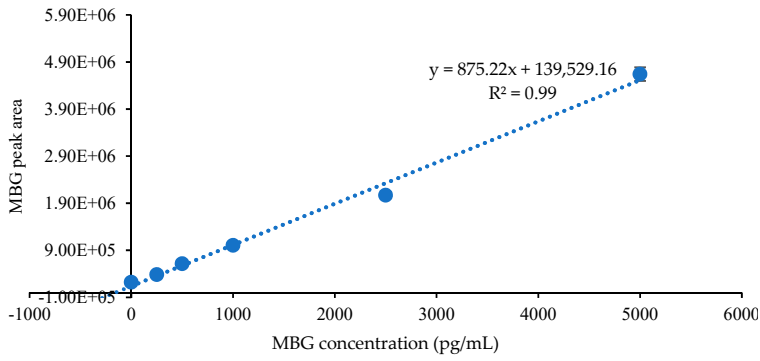
SS- PON3 KO NC 304



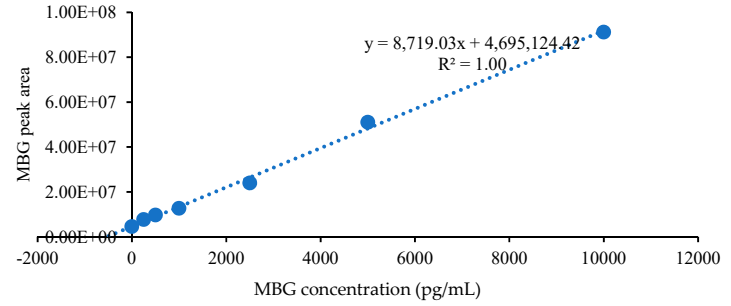


**Figure S2.** Standard addition calibration curves for quantification of TCB in salt-sensitive wild-type (SS-WT) and paraoxonase 3 knockout (SS-PON 3 KO) rat urine samples on a normal chow diet.

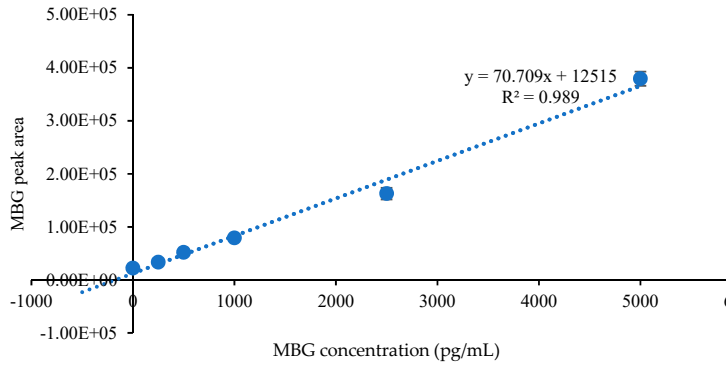
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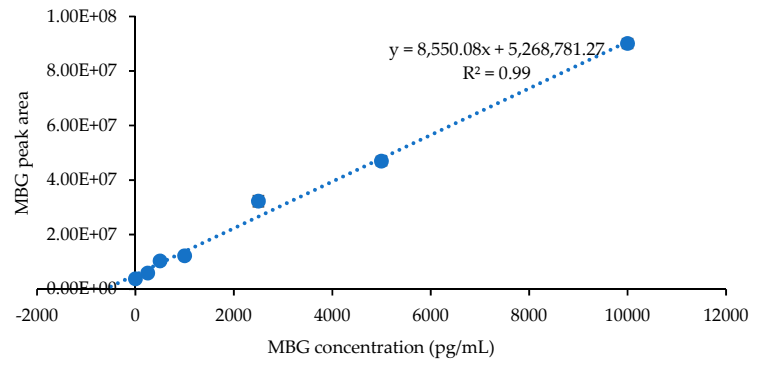
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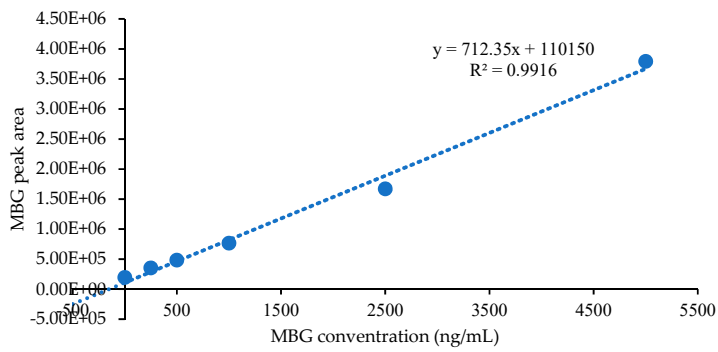
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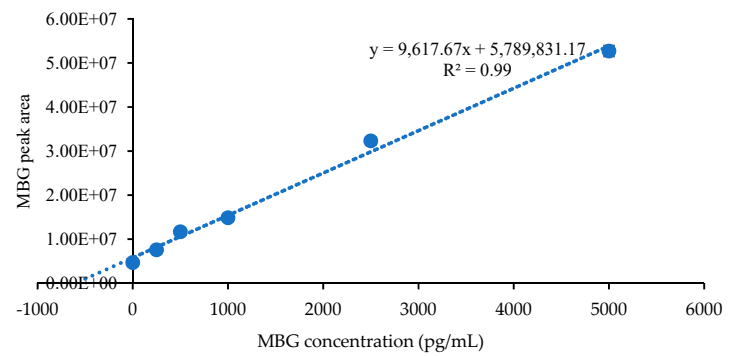
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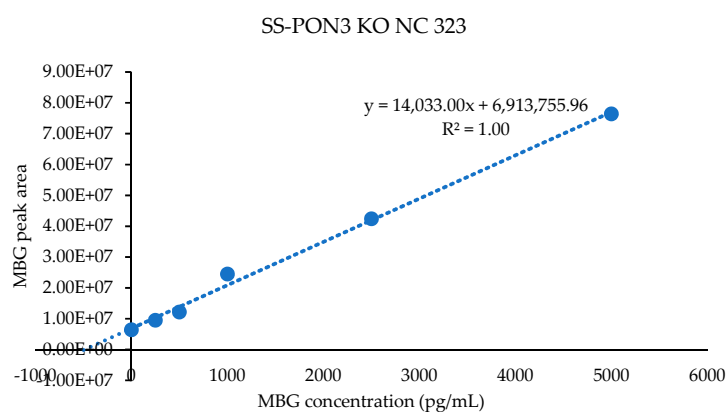
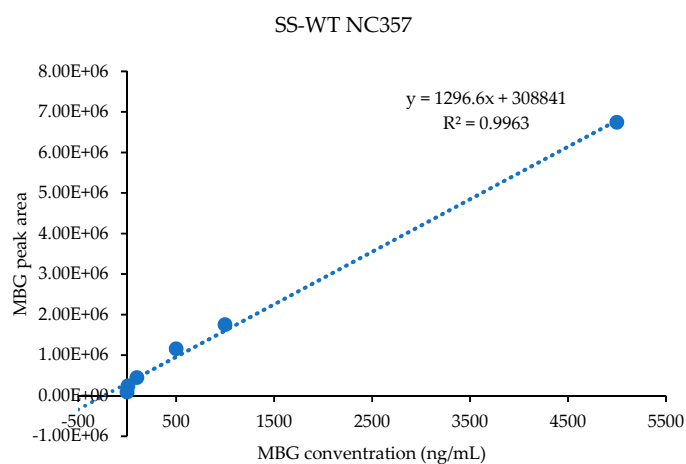
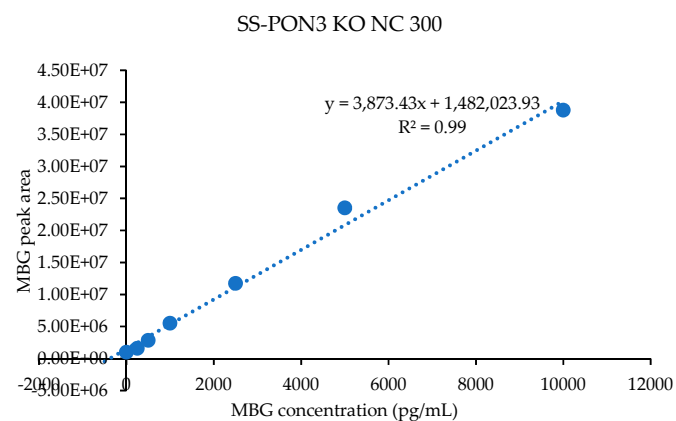
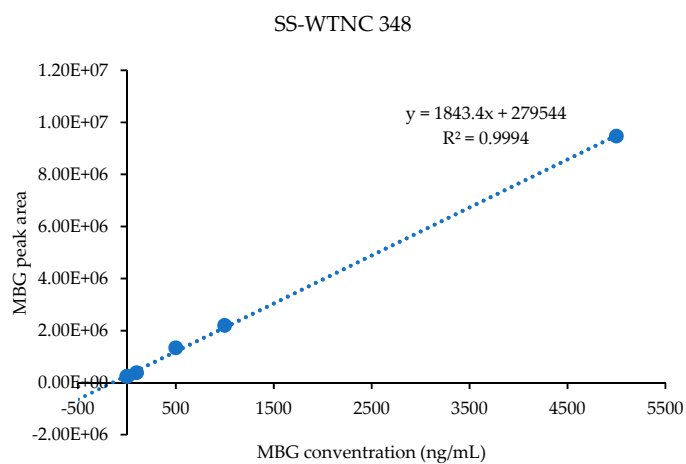


SS-WT NC 346

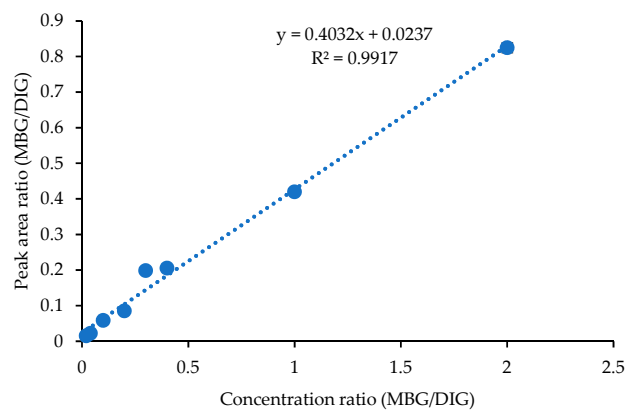
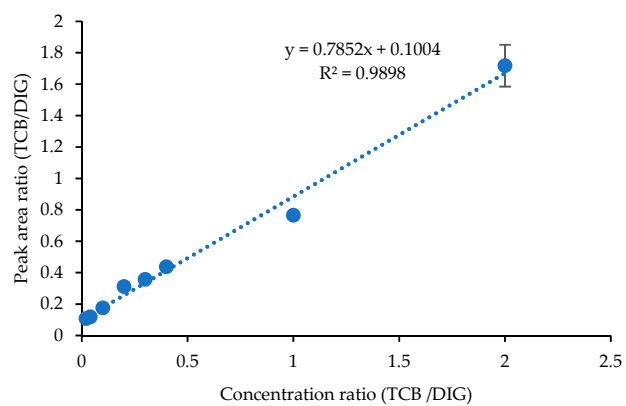


SS-PON3 KO NC 301





**Figure S3.** Standard addition calibration curves for quantification of MBG in SS-WT and SS-PON3 KO rat urine samples on a normal chow diet.



**Figure S4.** Internal standard calibration curves for quantification of TCB and MBG in SS-WT and SS- PON3 KO rat urine samples on a high salt diet.