Supplementary Table 1. **Detailed Methods For Determining Metformin In Serum**

Reagents and Materials. Metformin (1,1 Dimethylbiguanide HCl), ammonium acetate and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Internal standard (N,N-Dimethly-d6-diguanide HCL) was purchased from CDN Isotopes (Toronto, Canada). HPLC-grade acetonitrile was purchased from Fisher Scientific (Pittsburg, PA,USA). HPLC-grade water and methanol were purchased from EMD Chemicals (Gibbstown, NJ, USA). Protein crash plates and 96 well collection plates were purchased from Chromtech (Apple Valley, MN, USA). Rat plasma collected in EDTA from Sprague Dawley females was purchased from Valley Biomedical Products and Services (Winchester, VA)

LC/MS/MS Instrumentation. The LC/MS/MS system consisted of a Shimadzu liquid chromatograph (Wood Dale, IL, USA) with two LC-10ADvp pumps (flow rate 0.200 mL/min), and a SIL-10ADvp autoinjector (injection volume 50 μL) coupled to a Quattro Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA) fitted with a switching valve and an electrospray ionization probe operating in the positive mode. Metformin detection was accomplished by MS/MS using the parent ion m/z of 130.1 and the daughter ion m/z of 59.8. The dwell time, cone voltage, and collision energy values were 0.3 sec, 23 volts, 13 eV, respectively. The internal standard (d6-Metformin) was detected by MS/MS using parent ion signal of m/z 136.2 and daughter ion signal of m/z 59.9. The dwell time, cone voltage and collision energy values were 0.3 sec, 22 volts, and 13 eV, respectively. The source temperature, desolvation temperature, cone gas flow and desolvation gas flow were 120°C, 350°C, 25 L/hr and 650 L/hr, respectively. LC/MS/MS data were collected from 7 to 10 minutes after sample injection.

Chromatographic Conditions. Separation of metformin and d6-metformin (IS) was achieved

using a Thermo BDS-C18 precolumn (20 x 2.1 mm i.d., 5 □m) (Chrom Tech, Apple Valley, MN,

USA) and a Phenomenex Luna CN analytical column (15 cm x 3.0 mm, 100Å, 3□m)

(Phenomenex, Torrance, CA, USA). The elution program was an isocratic flow of 15:85, A:B at

0.4ml/minute. The flow was switched to waste from 0 to 7 minutes and again from 10 to 11

minutes. Solvent A consists of HPLC grade water with 0.1% formic acid and 15mM ammonium

acetate. Solvent B consists of acetonitrile with 0.1% formic acid.

Sample Preparation. Stock solutions of metformin (10mg/ml) and d6-metformin (10mg/ml)

were prepared in water and were stored at -20°C. Working standard solutions were prepared in

methanol:water, 1:1. Plasma and urine standards (1-1000 ng/ml) containing metformin were

prepared by adding 5ul of a 20X working standard solution to plasma or urine (90 ul) that had

been added to wells of a protein crash plate. Five ul of internal standard d6-Metformin (final

concentration 100ng/ml) was added to all samples. Metformin was isolated from plasma by

adding 300ul to each crash plate well, shaking at 1100 RPM for 20 minutes on an Eppendorf

Thermomixer R (Brinkmann Instruments, Westbury, New York) followed by filtering into a 96

well collection plate by use of a filter plate apparatus and in house vacuum. Ten microliters of

the filtrates were injected directly on the HLPC/mass spec.

Drug Administration and Specimen Collection. Metformin 150 mg/kg body weight/day was

administered by oral gavage for 1 day (Group 1, N=15) or 14 days (Group 2, N=15) to female

Spague-Dawley rats (Harlan, City, State) when the rats were 53 days of age and fed standard

laboratory chow during treatment. Blood samples (1 mL) were collected via the jugular vein into

heparin-containing tubes at the following times (N=5 rats/time) before treatment; 1, 2, 4, 8 and

24 hours after the end treatment. Plasma was separated by centrifugation (10,000 rpm x 3

min), transferred to micro centrifuge tubes and then immediately frozen. Urine was collected

prior to gavaging metformin and overnight from 5 rats in Group 1 and from 5 rats in Group 2 after their last dose.

Data Analysis. Plasma concentration-time data were analyzed by standard non-compartmental methods using the program WinNonlin Version 4.1 (Pharsight Corporation, Mountainview, CA).