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**The Novel, Clinical Stage Soluble Guanylate Cyclase Activator BI 685509 Protects from  
Disease Progression in Models of Renal Injury and Disease**

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## Supplemental Methods

### Telemetry Rat Blood Pressure and Heart Rate Profile

Rats were instrumented with DSI telemetry implants with catheters aseptically placed in the descending aorta as previously described (Fryer et al., 2012). Animals were single housed to record blood pressure and heart rate under conscious conditions.

*Single Dose Study:* BI 685509 was prepared as a nanosuspension in 1% Pluronic F127 in water and administered as single doses of 3, 10, and 30 mg/kg p.o. (5 mL/kg). Hemodynamic values were recorded for 8 hrs post-dose (n=8/group). A blood sample was withdrawn 8 hrs post-dose for analysis of drug plasma concentrations.

*Repeat Dose Study:* Following a baseline period, single-housed, drug-naïve telemetry-instrumented rats were randomized into 3 groups: Vehicle (0.5% methylcellulose and 0.015% Tween 80, 5 mL/kg) and BI 685509 at 60 or 100 mg/kg p.o., QD (n=12/group). Rats were treated for 10 consecutive days; on the 11<sup>th</sup> day, all animals received a challenge dose of BI 685509 at 100 mg/kg p.o. to elicit a maximal hypotensive response and test potential for attenuation. Mean arterial pressure (MAP) and heart rate (HR) were collected continuously throughout the study. Analysis was based on calculating the maximal treatment-induced effect on MAP or HR following each daily dose; values were then averaged across each group and compared each day by one-way ANOVA with Dunnett's post-test vs. vehicle without adjusting for repeated measures.

**Mouse PK:** BI 685509 (suspended in 0.1% Tween and 0.5% Natrosol; 10 mL/kg) was administered as a single oral dose to male mice (5.83 mg/kg; n=3, B6.V-Lep-ob/J, Janvier Labs, Saint-Berthevin Cedex, France, 25g BW) under fed conditions. Blood samples (~25 µL) were collected into EDTA microtainers via retro-orbital sinus bleeds at the following timepoints (hrs) after dosing: 0, 0.25, 0.5, 1, 2, 4, 8, 24 and placed on ice for no longer than 30 minutes prior to centrifugation at 8000 rpm for 5 minutes. Following centrifugation, plasma samples were immediately transferred to Eppendorf tubes and stored frozen at -80°C prior to analysis. Plasma concentrations of BI 685509 were measured by LC-MS/MS as described below.

**Rat PK:** Male rats (n=3/ group, Wistar-Han, Charles River, Raleigh, NC, ~220-230 g BW) were administered a single intravenous (i.v.) or oral dose of BI 685509 under fasted conditions. For i.v. group, BI 685509 was dissolved in 70% PEG 400/30% water and administered as a bolus injection via the femoral cannula at 1 mg/kg (2 mL/kg). For the oral (p.o.) group, BI 685509 was administered as a suspension in 0.5% methyl cellulose/0.015% Tween 80 at 3 mg/kg (5 mL/kg). Blood samples (~200 µL into 1.5 mL polypropylene tubes with prefilled 10 µL 2M citric acid and 23 µL of sodium citrate) were collected via an indwelling jugular catheter at the following timepoints (hrs) after i.v. and p.o. dosing: 0, 0.25, 0.5, 1, 2, 4, 6, 8, 24 and an additional 0.083 hr timepoint was collected after i.v. administration. Following centrifugation, a 100 µL plasma sample was recovered and stored frozen at -40°C until analysis. Plasma concentrations of BI 685509 were measured by LC-MS/MS.

## **Pharmacokinetic Measurements**

Following centrifugation (930 rcf for 10 min), urine samples were diluted in 0.1 N NaOH and protein concentration was determined using Coomassie Plus Reagent (Thermo Fisher, Waltham, MA, #23238).

The quantitation of enalaprilat and BI 685509 in heparin plasma and kidney cortex was performed via liquid chromatography/mass spectrophotometry (LC/MS/MS). An API 5000 triple quadrupole mass spectrometer with Turbo V Ion Source (Applied Biosystems, Toronto, Canada), set to electrospray positive ionization mode, and Analyst 1.4.2 operating software was used. Ion transitions used for quantification were: enalaprilat (349.0-206.0 m/z, CE 25, DP 80V) and BI 685509 (583.2-244.2 m/z, CE 22, DP 80V). The liquid chromatography system was an Agilent 1200 Series pump and column oven (Fullerton, CA, USA) with a LEAP Technologies HTS-PAL Autosampler (Carrboro, NC, US). The analytical column was a Phenomenex Synergi Polar RP, 2.1 x 50 mm, 4 mm (Torrance, CA, US), and the mobile phase consisted of 0.1% formic acid in water (A) and 0.1 formic acid in Acetonitrile (B). Gradient was maintained at 5%B for 0.1 minute, increased to 95% B in 1.8 minutes, held at 95% B for 0.5 minute and decreased to 5% B for 0.2 minute and held at 5% B for a total time of 3 minutes at a flow rate of 0.5 ml/min. The total run time per injection was 3 minutes.

**Sample Preparation:** Enalaprilat and BI 685509 standard stock solutions containing 1 mg/mL in methanol were serially diluted in rat plasma to prepare an 8- point standard curve ranging from 1 to 5000 ng/mL. 20 µL of plasma, calibration standards and blank plasma samples were deproteinized by precipitation with 180 µL of internal standard (100 ng/mL; proprietary small molecule) diluted in acetonitrile: water (85:15). Samples were mixed for 1 min, filtered through AcroPrep Multi-well filter plates (Pall Corporation, Ann Arbor,

MI) using Sciclone ALH 3000 Workstation (Caliper Life Sciences, Hopkinton, MA, USA) and transferred into 96-well injection plate. 5 mL of the solution was then injected into the LC/MS/MS system for analysis.

HbA1c was measured by high-pressure liquid chromatography (DTI Laboratories, Thomasville, GA). Fasting plasma glucose was measured utilizing a glucose meter (Bayer Breeze2; Bayer HealthCare LLC, Mishawaka, IN).

### **Pharmacokinetics/Pharmacodynamics (PK/PD) modeling**

Overall change from baseline in UPE across the study was correlated with BI 685509 individual mean plasma concentrations using an inhibitory effect  $I_{\max}$  model. All analyses were performed using Phoenix WinNonlin Version 6.1 (Pharsight, Cary, NC).

### **Tissue Collection and Processing**

For histological assessment, kidneys were removed, weighed and a mid-organ transverse section of the left kidney was collected and immediately fixed by immersion in 10% phosphate-buffered formalin for 48 hrs. Subsequently, formalin-fixed tissues were washed in phosphate buffer, dehydrated through a graded series of ethanol and xylene, embedded in paraffin, and sectioned at 4  $\mu\text{m}$ .

### **General Assessment of Renal Histopathology**

Kidney tissue sections (4  $\mu\text{m}$ ) were stained with periodic acid methenamine silver (PAM) for the general assessment of incidence of glomerulosclerosis and hematoxylin–eosin (H&E) for the general assessment of total number of foci of interstitial lesions. For the

glomerular lesions, the number of glomeruli showing mild to severe glomerulosclerosis were counted in each kidney section and expressed as a percentage of the total glomerular population. Tubulointerstitial lesions were assessed as the number of foci showing obvious renal damage, which included marked hypercellularity (inflammation or resident myofibroblast increases), fibrosis, tubular hyperplasia, casts, or necrosis. Both assessments were performed under blinded conditions at 10X magnification.

### **Immunohistochemistry and Image Analysis**

Four micrometer kidney sections were air dried overnight at 37°C, dewaxed and rehydrated in graded ethanol to phosphate buffered saline (PBS). Endogenous peroxidase activity was blocked by ImmPRESS (Vector Laboratories, Burlingame, CA) for 20 min at ambient temperature. Sections were then washed and incubated with the primary antibody for  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA; A5228, Sigma, St. Louis, MO) at a dilution of 1:3000, kidney injury molecule-1 (KIM-1; AF3689, R&D Systems, Minneapolis, MN) at a dilution of 1:200 and the rat cellular marker for microglia, monocytes and macrophages (ED-1, MAB1435, Millipore, Temecula, CA) at a dilution of 1:80, and were subsequently incubated with the respective secondary antibodies (ImmPress Reagent Kit, Vector Laboratory, Burlingame, CA) for 30 minutes in a hydration chamber. Immunoperoxidase detection was performed using the avidin-biotin complex method (Vector Laboratories, Burlingame, CA) using 3, 3'-diaminobenzidine tetra hydrochloride as substrate.

Quantitative image analysis of  $\alpha$ -SMA, KIM-1 and ED-1 staining was performed under blinded conditions. Ten random digital images were captured at 10X objective magnification, with each field representing approximately 0.6 mm<sup>2</sup> for a total area of 6.0 mm<sup>2</sup> being

assessed. The percentage of positive staining area per field in the cortex region was measured using computer assisted image analysis with Image-Pro imaging software.

**NGS:** Sequencing libraries were built according to the manufacturer's procedures for the TruSeq polyA kit. Sequencing was performed on an Illumina HiSeq 2500 to a depth ranging from 17 to 25 million reads, with a read length of 50 bases (N=7 for sham and N=9 for drug treated).

The sequencing data were mapped to the reference genome Rnor\_6.0 using the STAR aligner (Dobin 2013). Gene quantitation was performed with RSEM for generation of TPM and feature counts for generation of counts used in downstream analysis. Principal component analysis was used to assess overall sample similarity.

Differential gene expression analysis was performed first independently on the two doses each compared to the vehicle-treated group, using the voom-limma method (Law, 2014). The two doses had a high-degree of overall concordance (Suppl. Fig. 3), so the analysis was repeated by pooling the two dose conditions and comparing them to the vehicle-treated group. This analysis resulted in a list of differentially expressed genes presented in Suppl. Table 5, based on a threshold using a false discovery rate (FDR) threshold of less than 0.05 for statistical significance and a threshold of fold change between groups of  $\pm 2$ .

**Supplemental Table 1. Mean plasma concentration of BI 685509 after oral administration of 5.83 mg/kg BI 685509 in B6.V-Lep-ob/J mice**

<b>Time (Hr)</b>	<b>Plasma Concentration (nM)</b>
0.25	866 ± 82.2
0.5	510 ± 41.9
1	296 ± 29.7
2	165 ± 27.1
4	120 ± 14.2
8	79.1 ± 19.2
24	1.74 ± 0.415

Plasma levels reported as mean ± SEM in nM for BI 685509

**Supplemental Table 2. Mean plasma concentration of BI 685509 after i.v. 1 mg/kg and oral 3 mg/kg administration of BI 685509 in wistar han rats**

<b>Time (Hr)</b>	<b>Plasma Concentration (nM)</b>	
	<b>IV</b>	<b>PO</b>
0.0833	1920 ± 123	-
0.25	-	606 ± 71.6
0.5	346 ± 84.3	502 ± 120
1	94.7 ± 22.1	338 ± 73.9
2	27.9 ± 1.94	297 ± 68.1
4	5.48 ± 1.31	162 ± 45.4
6	5.71 ± 1.17	72.0 ± 23.8
8	1.88 ± 0.98	28.2 ± 10.4
24	0	0.612 ± 0.61

Plasma levels reported as mean ± SEM in nM for BI 685509



**Supplemental Table 3**

Ensg	Symbol	Log2FoldChange	P.Value	Adj.P.Val
ENSRNOG00000016753	Slc14a1	-2.1	2.97E-14	4.67E-10
ENSRNOG00000004030	Nphs2	1.24	4.30E-13	2.25E-09
ENSRNOG000000033609	Irx1	-1.06	1.73E-12	6.80E-09
ENSRNOG000000054360	Tspan11	-1.04	1.53E-11	3.43E-08
ENSRNOG000000061890	Ust5r	-1.35	1.96E-11	3.86E-08
ENSRNOG000000011861	Aadat	-1.7	3.97E-11	5.67E-08
ENSRNOG000000007290	Atp1a2	-1.17	4.80E-11	6.29E-08
ENSRNOG000000009514	Mme	-1.12	1.00E-10	9.84E-08
ENSRNOG000000010388	Slc21a4	-1.73	1.39E-10	1.29E-07
ENSRNOG000000039596	Prob1	-1.05	1.53E-10	1.34E-07
ENSRNOG000000045743	Etnppl	-2.18	1.89E-10	1.48E-07
ENSRNOG000000004018	Tdrd5	1.4	2.53E-10	1.81E-07
ENSRNOG000000030894	Slco1a6	-2.05	2.61E-10	1.81E-07
ENSRNOG000000003616	Grem2	-2.3	4.52E-10	2.67E-07
ENSRNOG000000018420	Slc22a7	-2.13	4.80E-10	2.67E-07
ENSRNOG000000006302	Gclc	-1.51	4.92E-10	2.67E-07
ENSRNOG000000057855	F5	-1.04	5.47E-10	2.78E-07
ENSRNOG000000025848	Sspo	-1.96	6.33E-10	3.11E-07
ENSRNOG000000007932	Gcm1	-2.11	6.55E-10	3.12E-07
ENSRNOG000000018266	Slc23a3	-1.38	7.66E-10	3.43E-07
ENSRNOG000000001159	Tff3	-1.82	8.54E-10	3.53E-07
ENSRNOG000000021771	Trim29	-1.79	8.90E-10	3.58E-07
ENSRNOG000000061695	Slc23a1	-1.07	1.79E-09	6.28E-07
ENSRNOG000000000158	Cdo1	-1.35	1.82E-09	6.28E-07

ENSRNOG00000018445	Agt	-1.45	1.84E-09	6.28E-07
ENSRNOG00000016625	Slc22a2	-1.24	2.43E-09	7.79E-07
ENSRNOG00000005223	Hnmt	-2.14	2.48E-09	7.79E-07
ENSRNOG00000025648	Dhrs7l1	-1.93	2.61E-09	7.88E-07
ENSRNOG00000014948	Osgin1	-1.18	2.70E-09	8.02E-07
ENSRNOG00000057904	LOC102554608	-2.6	4.22E-09	1.16E-06
ENSRNOG00000003620	Fmo3	-1.17	4.37E-09	1.18E-06
ENSRNOG00000028889	Slc51b	-1.24	4.58E-09	1.22E-06
ENSRNOG00000033581	Tnxb	-1	5.04E-09	1.28E-06
ENSRNOG00000057557	Prlr	-1.16	5.30E-09	1.28E-06
ENSRNOG00000012843	Aspg	-1.09	5.38E-09	1.28E-06
ENSRNOG00000015461	Serpine2	-1.01	5.82E-09	1.34E-06
ENSRNOG00000015089	Mcoln2	-1.57	7.34E-09	1.65E-06
ENSRNOG00000004795	Pof1b	-2.46	7.51E-09	1.66E-06
ENSRNOG00000007590	Eya1	-1.03	1.03E-08	2.13E-06
ENSRNOG00000059790	Krt78	-2.33	1.15E-08	2.28E-06
ENSRNOG00000017672	Akr1c14	-1.35	1.42E-08	2.76E-06
ENSRNOG00000009440	Gucy1b2	-1.91	2.41E-08	4.56E-06
ENSRNOG00000050714	Islr2	1.63	2.44E-08	4.56E-06
ENSRNOG00000012575	Fat2	-2.18	4.66E-08	7.94E-06
ENSRNOG00000024903	Slc7a13	-2.14	4.70E-08	7.94E-06
ENSRNOG00000004958	Slc22a22	-1.6	4.85E-08	8.11E-06
ENSRNOG00000033266	Prss30	-1.14	5.87E-08	9.41E-06
ENSRNOG00000016827	Slc38a3	-1.52	8.48E-08	1.26E-05
ENSRNOG00000022711	Slco4c1	-1.41	8.48E-08	1.26E-05
ENSRNOG00000009797	Aqp3	-1.04	1.29E-07	1.75E-05
ENSRNOG00000006120	Shh	-1.54	1.53E-07	1.92E-05

ENSRNOG0000003119	Gc	-1.52	1.64E-07	2.00E-05
ENSRNOG00000019467	Fer1l4	-2.29	1.81E-07	2.14E-05
ENSRNOG00000054378	Aqp2	-1.36	1.86E-07	2.16E-05
ENSRNOG00000025501	Snx31	-2.16	2.43E-07	2.71E-05
ENSRNOG00000007949	Rgn	-1.6	2.66E-07	2.90E-05
ENSRNOG00000029179	Cyp2d1	-1.13	2.85E-07	3.03E-05
ENSRNOG00000007980	Anxa13	-1.63	2.92E-07	3.06E-05
ENSRNOG00000034087	Krt42	-2.1	2.96E-07	3.08E-05
ENSRNOG00000003288	Cacng5	-2.34	3.23E-07	3.27E-05
ENSRNOG00000013704	Cps1	-1.57	3.53E-07	3.47E-05
ENSRNOG00000027739	Cndp1	-1.08	4.22E-07	4.06E-05
ENSRNOG00000027380	Upk1b	-2.31	4.48E-07	4.29E-05
ENSRNOG00000004828	Acvr1c	-1.08	5.58E-07	5.18E-05
ENSRNOG00000022767	Elf1	-1.12	5.64E-07	5.19E-05
ENSRNOG00000015902	Cpxm2	-1.33	5.73E-07	5.24E-05
ENSRNOG00000009388	Sptssb	-2.8	5.76E-07	5.24E-05
ENSRNOG00000019445	Msln	-1.76	5.96E-07	5.35E-05
ENSRNOG00000025059	Nxph4	-1.42	6.16E-07	5.48E-05
ENSRNOG00000024331	Upk1a	-2.32	6.97E-07	6.12E-05
ENSRNOG00000060681	LOC102553472	1.84	1.12E-06	9.40E-05
ENSRNOG00000017786	Acta1	-1.65	1.18E-06	9.70E-05
ENSRNOG00000058571	N/A	-1.46	1.22E-06	1.00E-04
ENSRNOG00000024028	Sprr1a	-2.61	1.58E-06	1.25E-04
ENSRNOG00000021095	Fxyd3	-2.38	1.67E-06	1.30E-04
ENSRNOG00000053494	Mcpt1l1	-2.84	1.87E-06	1.43E-04
ENSRNOG00000010805	Fabp4	-1.78	1.91E-06	1.45E-04
ENSRNOG00000036984	Slco1a1	-2.02	2.14E-06	1.58E-04

ENSRNOG00000031263	Haao	-1.08	2.26E-06	1.66E-04
ENSRNOG00000054508	Foxp2	-1.49	2.30E-06	1.67E-04
ENSRNOG00000029401	Actg2	-1.84	2.48E-06	1.75E-04
ENSRNOG00000016325	F2	-1.24	2.68E-06	1.87E-04
ENSRNOG00000011808	Ghrhr	-1.22	2.93E-06	2.02E-04
ENSRNOG00000000961	Glt1d1	-1.41	2.99E-06	2.05E-04
ENSRNOG00000015682	Kel	1.1	3.10E-06	2.12E-04
ENSRNOG00000029427	Grhl3	-1.39	3.25E-06	2.19E-04
ENSRNOG00000060067	LOC102549636	-1.44	3.44E-06	2.30E-04
ENSRNOG00000050243	Akr1c12l1	-1.29	3.47E-06	2.31E-04
ENSRNOG00000012720	Irx4	-1.2	3.59E-06	2.37E-04
ENSRNOG00000060949	Anxa8	-2.58	3.88E-06	2.44E-04
ENSRNOG00000009314	Ivl	-2.29	3.97E-06	2.46E-04
ENSRNOG00000017872	LOC688778	-2.2	3.97E-06	2.46E-04
ENSRNOG00000056555	N/A	-1.64	4.19E-06	2.56E-04
ENSRNOG00000008015	Fos	1.14	4.41E-06	2.68E-04
ENSRNOG00000006962	Stk32c	-1.57	4.54E-06	2.72E-04
ENSRNOG00000053746	Olr1668	-3.73	4.57E-06	2.73E-04
ENSRNOG00000037080	Adamts17	-1.17	6.50E-06	3.64E-04
ENSRNOG00000033490	Vsig2	-1.81	6.88E-06	3.79E-04
ENSRNOG00000019500	Cyp1a1	-2.97	6.93E-06	3.80E-04
ENSRNOG00000049345	Mep1b	-1.43	9.83E-06	5.07E-04
ENSRNOG00000024899	Cxcl13	1.22	1.05E-05	5.34E-04
ENSRNOG00000004564	Lrriq1	-1.09	1.06E-05	5.39E-04
ENSRNOG00000013593	Upk3a	-2.86	1.08E-05	5.44E-04
ENSRNOG00000054016	N/A	-2.2	1.09E-05	5.47E-04
ENSRNOG00000029964	Tmprss11g	-1.96	1.10E-05	5.53E-04

ENSRNOG00000054957	Sfrp4	-1.43	1.11E-05	5.55E-04
ENSRNOG00000056396	N/A	-1.55	1.17E-05	5.76E-04
ENSRNOG00000003566	Apoh	-1.44	1.21E-05	5.83E-04
ENSRNOG00000000383	Mypn	-1.65	1.22E-05	5.90E-04
ENSRNOG00000032002	Hapln1	-1.57	1.26E-05	6.00E-04
ENSRNOG00000001967	Sult1b1	-1	1.34E-05	6.26E-04
ENSRNOG00000023497	Foxe1	-1.5	1.37E-05	6.33E-04
ENSRNOG00000013036	Epha8	-1.71	1.38E-05	6.34E-04
ENSRNOG00000029128	Cyp2d5	-1.64	1.44E-05	6.54E-04
ENSRNOG00000023686	Upk3b	-1.04	1.80E-05	7.88E-04
ENSRNOG00000011381	Acsbg1	-1.67	1.96E-05	8.49E-04
ENSRNOG00000060413	N/A	-1.69	2.10E-05	8.91E-04
ENSRNOG00000022116	Gjb6	-2.23	2.36E-05	9.67E-04
ENSRNOG00000029911	Cilp	-1.11	2.52E-05	1.03E-03
ENSRNOG00000003899	Krt14	-2.24	2.60E-05	1.05E-03
ENSRNOG00000023561	Ano2	-1.81	2.79E-05	1.11E-03
ENSRNOG00000001691	Cldn14	1.6	2.91E-05	1.14E-03
ENSRNOG00000012906	Bcas1	-1.77	3.34E-05	1.26E-03
ENSRNOG00000042330	Ms4a18	1.4	3.35E-05	1.26E-03
ENSRNOG00000037960	LOC689499	-1.19	3.38E-05	1.26E-03
ENSRNOG00000043324	Upk2	-2.55	3.42E-05	1.27E-03
ENSRNOG00000059314	N/A	-1.35	3.75E-05	1.37E-03
ENSRNOG00000003740	Gpa33	-1.77	3.81E-05	1.39E-03
ENSRNOG00000001984	Kcne1	1.11	4.16E-05	1.50E-03
ENSRNOG00000014099	Krt15	-2.59	4.31E-05	1.55E-03
ENSRNOG00000054898	N/A	-1.97	5.19E-05	1.78E-03
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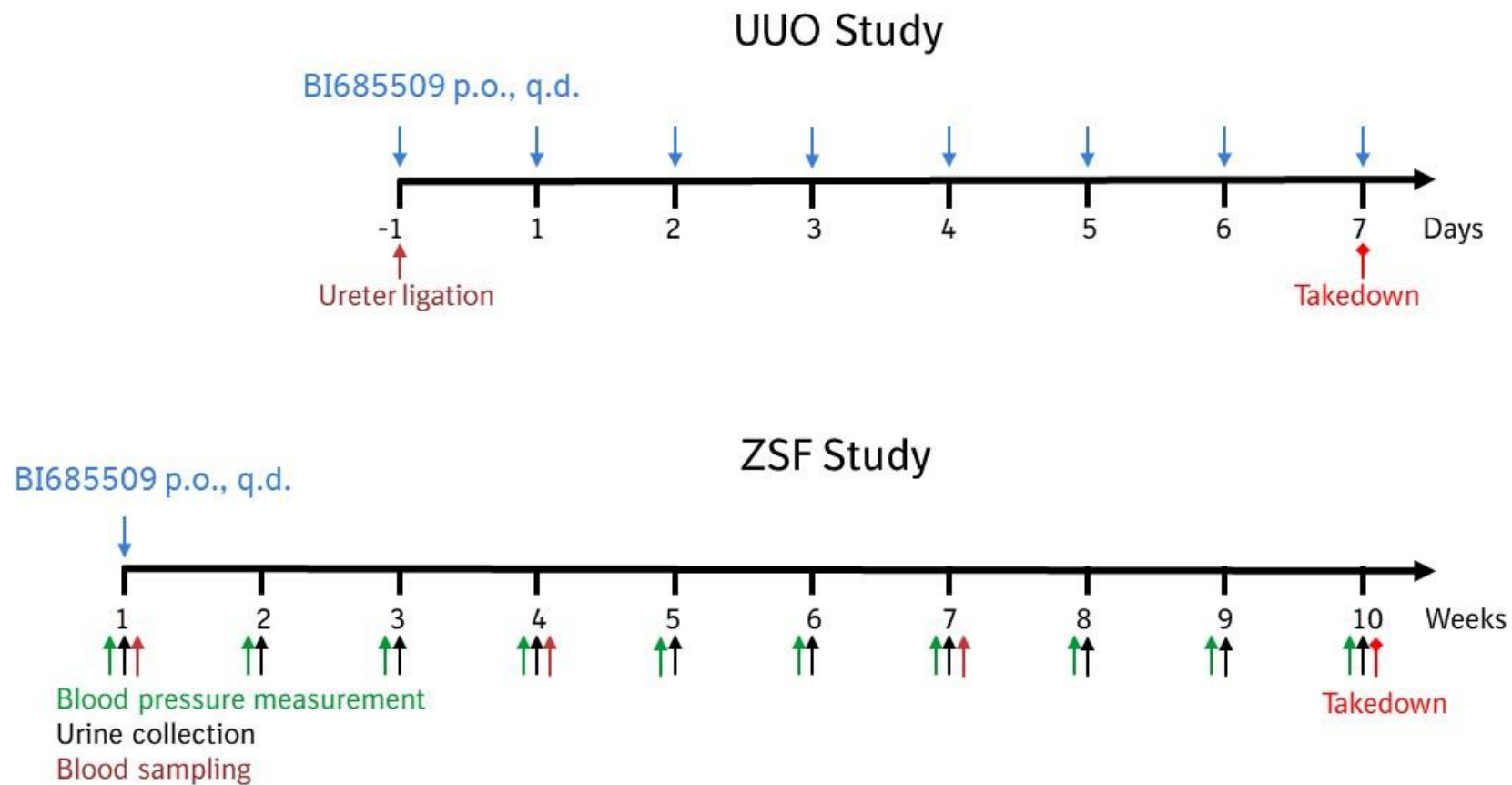
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ENSRNOG00000032871	Mlc1	-1.94	7.59E-05	2.42E-03
ENSRNOG00000060281	N/A	-1.54	7.75E-05	2.46E-03
ENSRNOG00000010031	Vtn	-1.05	7.87E-05	2.49E-03
ENSRNOG00000009284	Foxa1	-1.39	8.23E-05	2.58E-03
ENSRNOG00000037171	Tdgf1	1.74	8.48E-05	2.62E-03
ENSRNOG00000014314	Slc39a4	1.04	9.04E-05	2.77E-03
ENSRNOG00000011706	Mbl1	-1.89	9.26E-05	2.82E-03
ENSRNOG00000029514	Slc26a9	1.07	9.30E-05	2.82E-03
ENSRNOG00000012566	Kcnv2	-1.33	1.00E-04	2.98E-03
ENSRNOG00000009052	Igf2bp3	-1.07	1.01E-04	3.01E-03
ENSRNOG00000034031	Vstm2l	1.15	1.02E-04	3.01E-03
ENSRNOG00000005858	Kap	-1.14	1.12E-04	3.22E-03
ENSRNOG00000007335	Ccl11	-1.47	1.17E-04	3.34E-03
ENSRNOG00000004278	Dlx3	1.02	1.22E-04	3.46E-03
ENSRNOG00000001924	Tp63	-1.86	1.26E-04	3.54E-03
ENSRNOG00000009239	Entpd8	-1.33	1.26E-04	3.54E-03
ENSRNOG00000032959	Adh7	-1.76	1.28E-04	3.59E-03
ENSRNOG00000011575	Adamts18	1.6	1.49E-04	4.03E-03
ENSRNOG00000008219	Ccdc33	1.09	1.56E-04	4.17E-03
ENSRNOG00000050860	Abcb11	-1.55	1.64E-04	4.35E-03
ENSRNOG00000015716	Gp2	-1.06	1.73E-04	4.48E-03
ENSRNOG00000050420	Krt5	-3.01	1.91E-04	4.89E-03
ENSRNOG00000056021	Slc14a2	-2.63	1.94E-04	4.94E-03
ENSRNOG00000053631	Tdrd9	-1.14	1.97E-04	4.99E-03

ENSRNOG00000029147	Rbm11	1.36	2.03E-04	5.07E-03
ENSRNOG00000028985	LOC361914	-1.95	2.22E-04	5.43E-03
ENSRNOG00000010834	Mpped1	-1.02	2.25E-04	5.47E-03
ENSRNOG00000000963	Tmem132c	-1.46	2.44E-04	5.84E-03
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ENSRNOG00000037982	Tmem236	-1.22	4.92E-04	9.93E-03
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ENSRNOG00000031569	Zfp648	-1.64	5.39E-04	1.05E-02
ENSRNOG00000008353	lqch	-1.1	5.44E-04	1.05E-02
ENSRNOG00000042581	Ptcra	-1.09	6.17E-04	1.15E-02
ENSRNOG00000014296	Syt10	-1.57	6.60E-04	1.21E-02
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ENSRNOG00000026060	Arsi	-1.03	7.50E-04	1.33E-02
ENSRNOG00000024259	Tmem54	-1.29	7.65E-04	1.35E-02
ENSRNOG00000028473	LOC691551	-1.47	8.55E-04	1.48E-02
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ENSRNOG00000046403	RGD1564999	-1.47	8.74E-04	1.50E-02
ENSRNOG00000020998	Oosp1	-1.13	9.06E-04	1.54E-02
ENSRNOG00000030285	Epha3	-1.18	9.40E-04	1.57E-02
ENSRNOG00000032669	Serpina1	-1.22	9.77E-04	1.61E-02
ENSRNOG0000002937	Ren	1.12	9.99E-04	1.62E-02

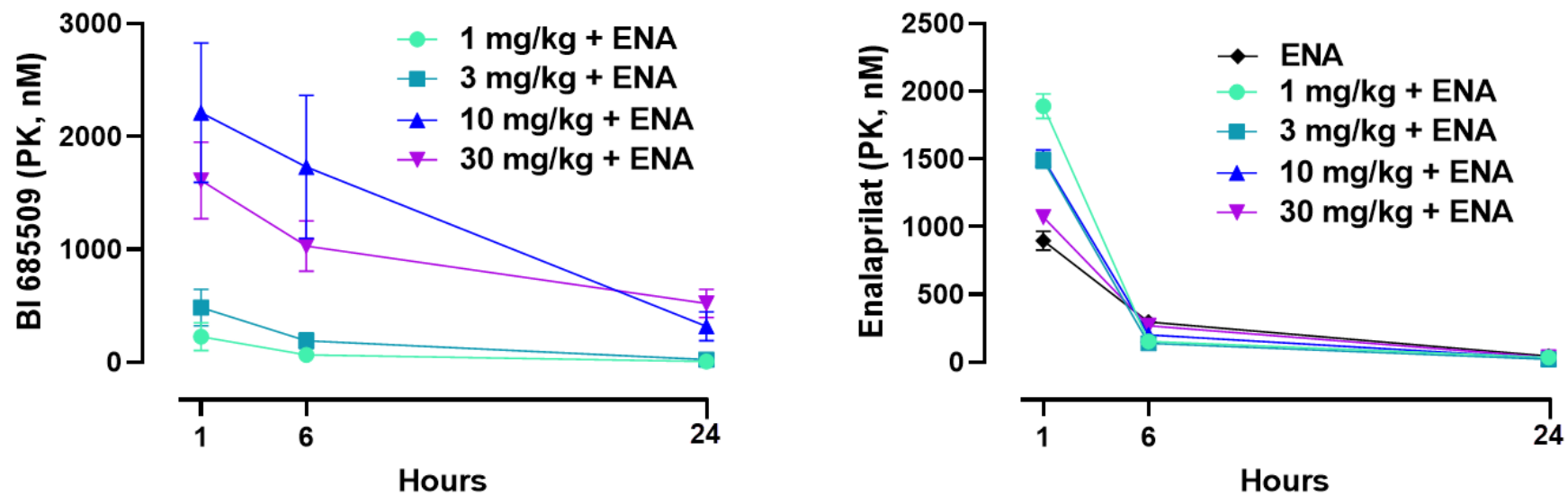
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ENSRNOG000000014231	Pnoc	-1.47	1.13E-03	1.77E-02
ENSRNOG000000057198	N/A	-1.21	1.22E-03	1.86E-02
ENSRNOG000000028082	Tal2	-1.06	1.23E-03	1.87E-02
ENSRNOG000000012404	Thrsp	-1.27	1.28E-03	1.92E-02
ENSRNOG000000002350	Eaf2	-1.1	1.30E-03	1.94E-02
ENSRNOG000000012557	Lgals5	1.36	1.30E-03	1.95E-02
ENSRNOG000000027506	Zp4	1.36	1.32E-03	1.96E-02
ENSRNOG000000005336	Prss27	-1.27	1.46E-03	2.10E-02
ENSRNOG000000008478	Mmp13	-1.39	1.57E-03	2.23E-02
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ENSRNOG000000015518	Rbp4	-1.79	1.68E-03	2.34E-02
ENSRNOG000000010454	Ccno	-1.24	1.73E-03	2.39E-02
ENSRNOG000000054457	N/A	1.02	1.77E-03	2.43E-02
ENSRNOG000000039279	Nhlrc4	1.35	1.77E-03	2.43E-02
ENSRNOG000000046235	LOC686967	-1.74	1.79E-03	2.45E-02
ENSRNOG000000053334	Stmn4	1.17	1.80E-03	2.46E-02
ENSRNOG000000017198	Hif3a	-1.16	1.85E-03	2.49E-02
ENSRNOG000000055499	Grid1	-1.44	1.90E-03	2.54E-02
ENSRNOG000000033830	Slc7a12	-1.3	2.00E-03	2.61E-02
ENSRNOG000000025235	Tmem130	1.1	2.17E-03	2.74E-02
ENSRNOG000000060511	Tcap	-1.17	2.18E-03	2.75E-02
ENSRNOG000000016311	Slc6a2	-1.44	2.21E-03	2.77E-02
ENSRNOG000000011824	Trh	1.24	2.27E-03	2.82E-02
ENSRNOG000000026577	Cpne4	-1.36	2.32E-03	2.87E-02
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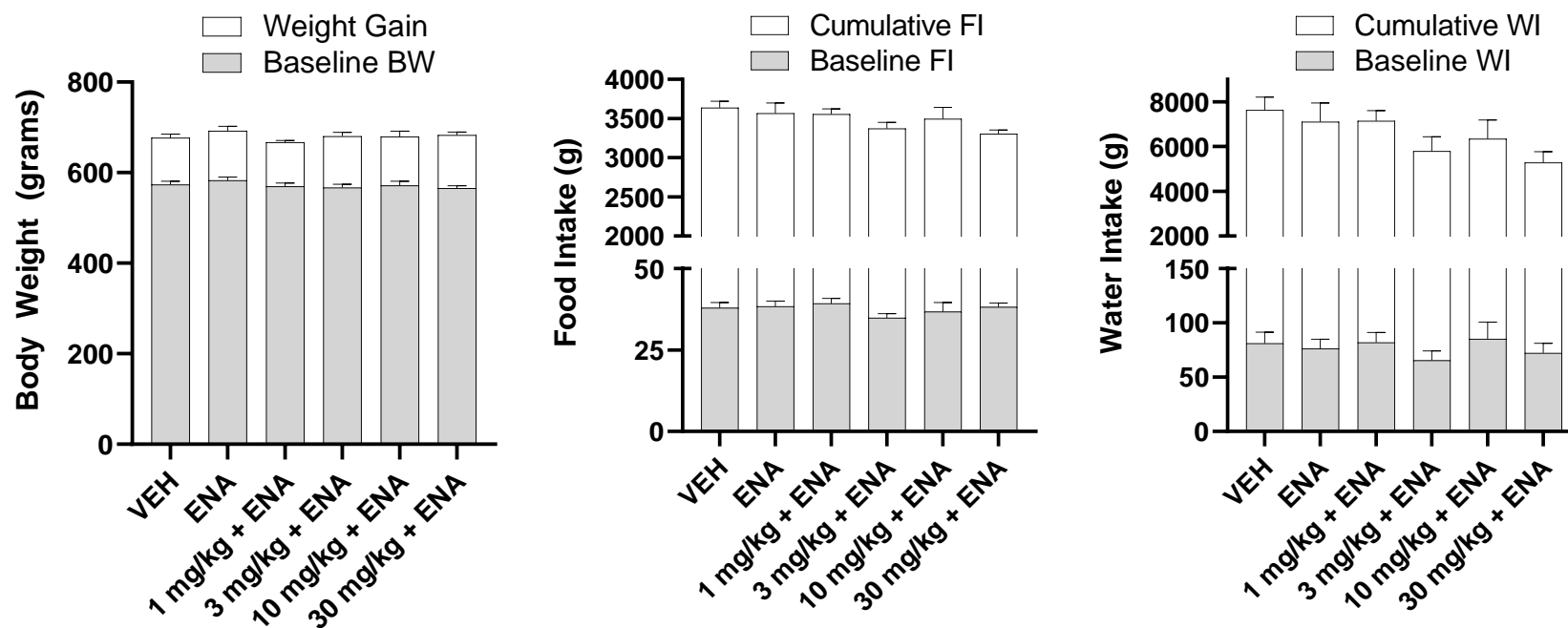
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ENSRNOG00000059540	Gcnt3	-1.09	2.53E-03	3.05E-02
ENSRNOG00000025037	Ankk1	-1.39	2.63E-03	3.13E-02
ENSRNOG00000032508	Acot5	-1.15	2.63E-03	3.13E-02
ENSRNOG00000060211	N/A	1.1	2.72E-03	3.17E-02
ENSRNOG00000015477	Nkx3-1	1.35	3.19E-03	3.54E-02
ENSRNOG00000008337	Gjd2	-1.32	3.27E-03	3.59E-02
ENSRNOG00000015799	LOC103690120	-1.24	3.29E-03	3.61E-02
ENSRNOG00000061299	LOC100134871	1.07	3.40E-03	3.66E-02
ENSRNOG00000033588	Siglec15	-1.34	3.47E-03	3.71E-02
ENSRNOG00000002134	LOC103690086	-1.48	3.90E-03	4.02E-02
ENSRNOG00000053703	N/A	-1.41	4.11E-03	4.19E-02
ENSRNOG00000015086	Plin1	-2	4.11E-03	4.19E-02
ENSRNOG00000060975	N/A	-1.09	4.33E-03	4.35E-02
ENSRNOG00000058442	N/A	-1.33	4.87E-03	4.71E-02
ENSRNOG00000001001	Retn	-1.39	4.94E-03	4.75E-02



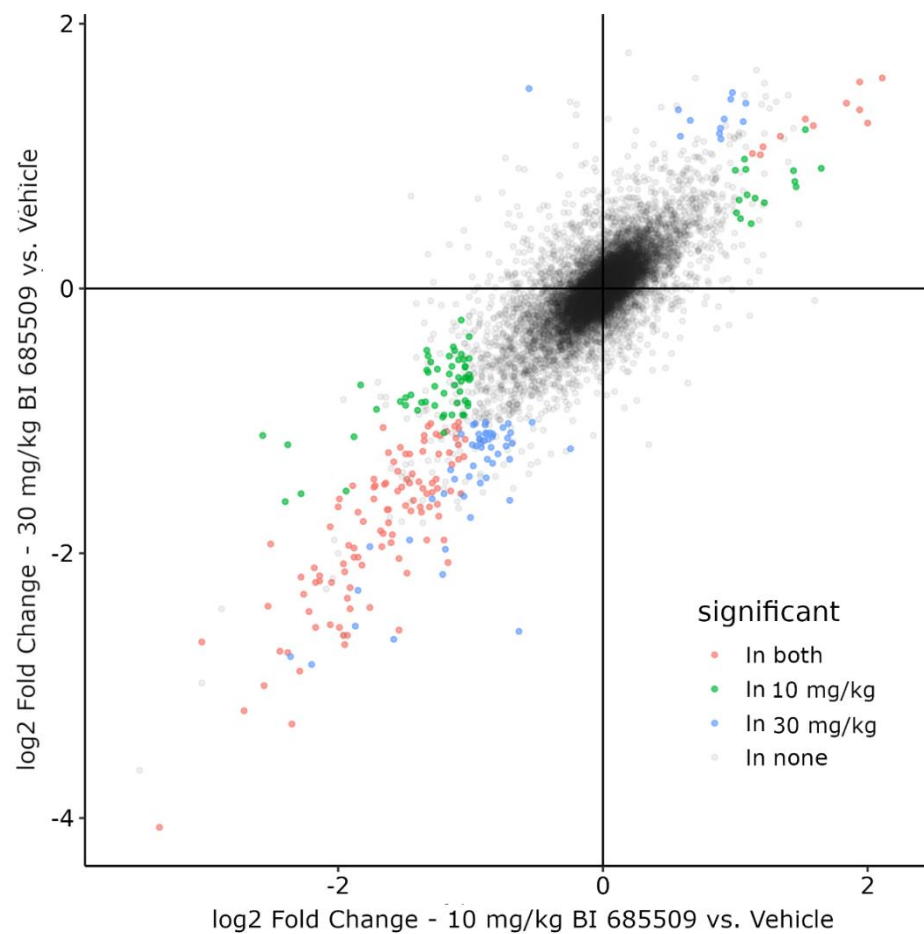
**Supplemental Fig. 1.** General study protocol for UUO and ZSF1 studies indicating relative time for procedures during the course of the 7d or 10wk studies, respectively.



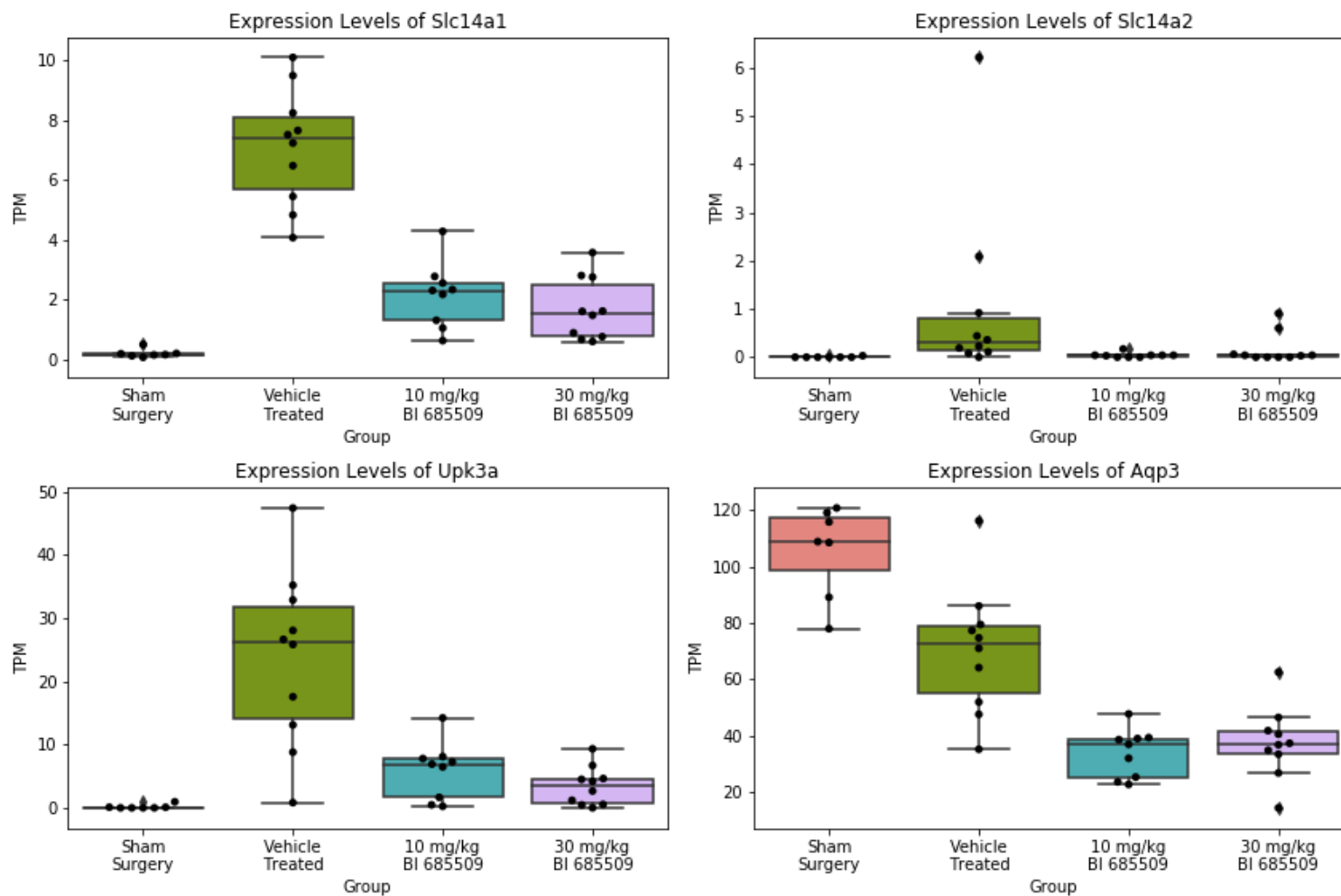
**Supplemental Fig. 2.** Mean plasma concentrations of BI 685509 (A) and enalaprilat (B) in ZSF1 rats. Plasma levels reported as mean  $\pm$  SEM in nM by averaging the timepoints for weeks 1, 4, 7 and 10 per group.



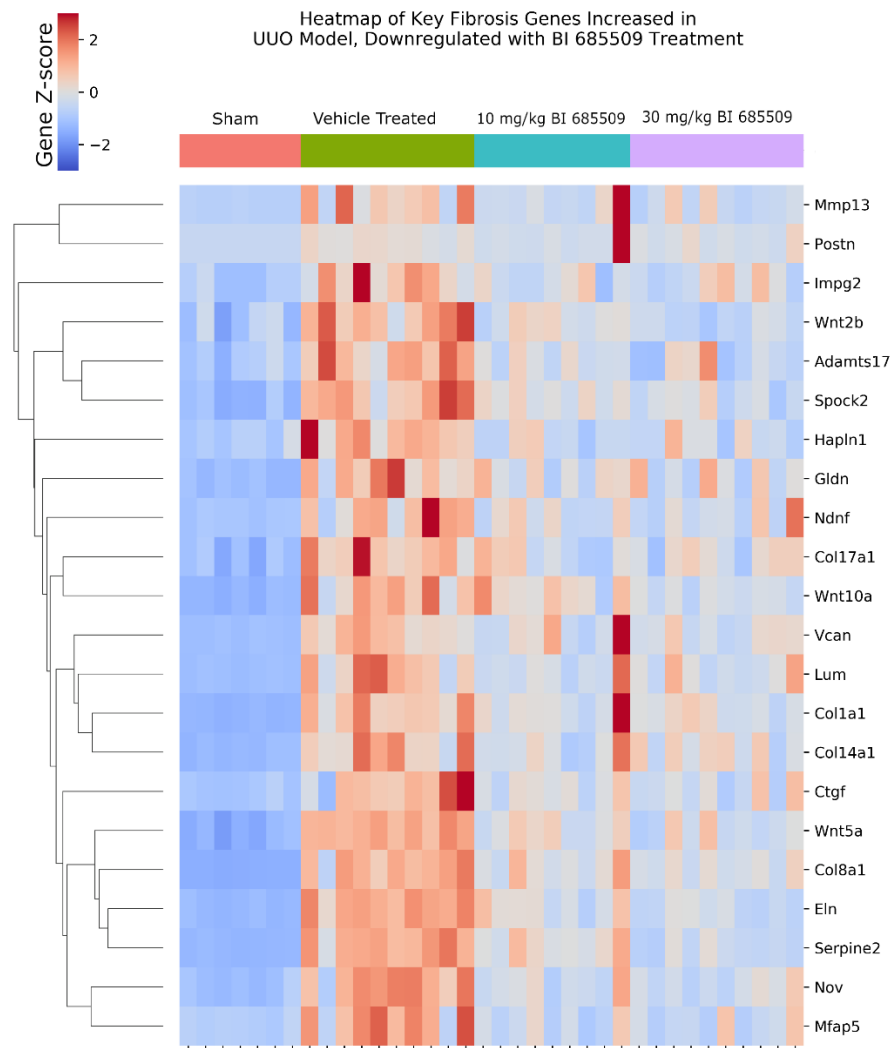
**Supplemental Fig 3:** Effects of BI 685509 and enalapril on body weight and feeding parameters. Parameters of body weight, food intake and water intake shown at baseline (Day 0) and following 10 weeks of treatment. There were no significant differences in these parameters between study groups (one way ANOVA vs. vehicle or enalapril treated groups, Dunnett's post-test).



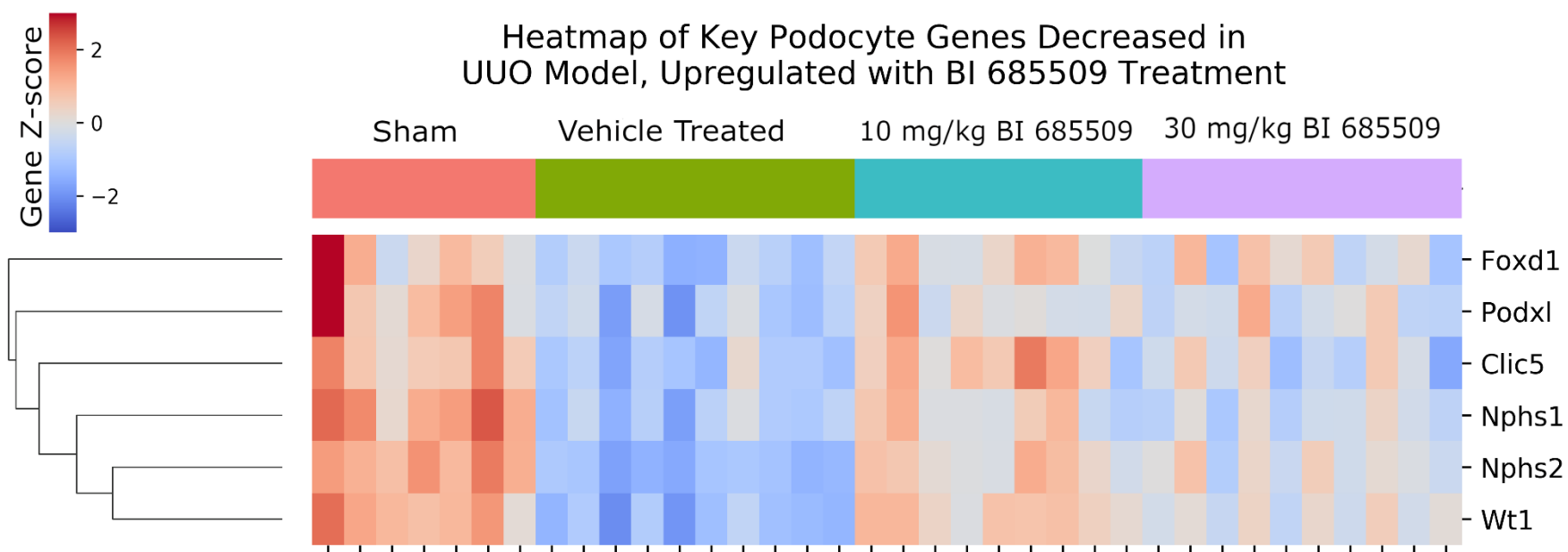
**Supplemental Fig. 4.** Scatterplot of changes of fold-change between BI 685509 and Vehicle-treated groups in UUO model, comparing 10 mg/kg and 30 mg/kg doses.



**Supplemental Fig. 5.** Boxplots of gene expression across groups for urate transporters, Slc14a1, Slc14a2, Upk3a, and Aqp3.

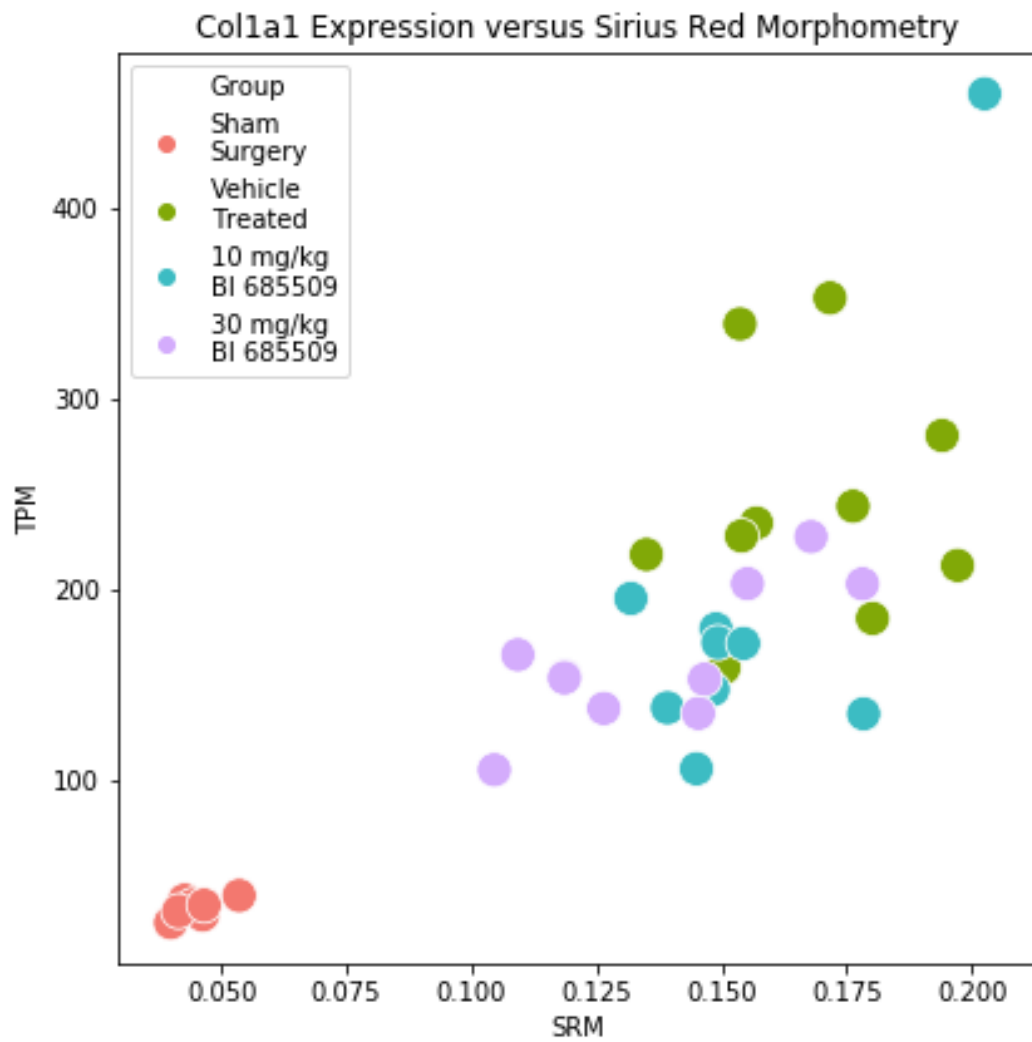


**Supplemental Fig. 6.** Heatmap of extracellular-matrix-associated genes upregulated in UUO model and downregulated in groups treated with 10 mg/kg and 30 mg/kg doses of BI 685509.



**Supplemental Fig. 7.** Heatmap of podocyte-associated genes decreased in UUO model and upregulated in groups treated with 10 mg/kg and 30 mg/kg doses of BI 685509.





**Supplemental Fig. 8.** Scatterplot of the relationship between Col1a1 expression by RNAseq and Sirius Red Morphometry.