



Supplementary Material

# Telocinobufagin, a Novel Cardiotonic Steroid, Promotes Renal Fibrosis via Na<sup>+</sup>/K<sup>+</sup>-ATPase Profibrotic Signaling Pathways

David J. Kennedy <sup>1</sup>, Fatimah K. Khalaf <sup>1</sup>, Brendan Sheehy <sup>2</sup>, Malory E. Weber <sup>2</sup>, Brendan Agatista-Boyle <sup>2</sup>, Julijana Conic <sup>2</sup>, Kayla Hauser <sup>2</sup>, Charles M. Medert <sup>2</sup>, Kristen Westfall <sup>2</sup>, Philip Bucur <sup>2</sup>, Olga Fedorova <sup>3</sup>, Alexei Y. Bagrov <sup>4</sup>, W. H. Wilson Tang <sup>2,5,6,\*</sup>

<sup>1</sup> Department of Medicine, University of Toledo College of Medicine, Toledo, Ohio

<sup>2</sup> Department of Cellular and Molecular Medicine, Lerner Research Institute Cleveland Clinic, Cleveland, Ohio

<sup>3</sup> Laboratory of Cardiovascular Science, National Institute on Aging, National Institutes of Health, Baltimore, Maryland

<sup>4</sup> Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

<sup>5</sup> Center for Cardiovascular Diagnostics and Prevention, Lerner Research Institute Cleveland Clinic, Cleveland, Ohio

<sup>6</sup> Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic, Cleveland, Ohio

\* Correspondence: 9500 Euclid Avenue, J3-4, Cleveland, OH 44195; E-mail: tangw@ccf.org; Tel.: +216-444-2121; Fax: +216-445-6165

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**Table S1.** Qiagen RT<sup>2</sup> Profiler™ Mouse Nephrotoxicity PCR array comparing renal gene expression from TCB treated wild type and NKA  $\alpha$ -1<sup>+/−</sup> mice.

| Gene                           | Wild Type (Relative Gene Expression, Mean ± SD) | NKA $\alpha$ -1 <sup>+/−</sup> (Relative Gene Expression, Mean ± SD) | p value      |
|--------------------------------|---|--|--------------|
| <b>Apoptosis</b>               |   |  |              |
| Anxa5                          | 1 ± 0.4   | 3.21 ± 1.24  | <b>0.042</b> |
| Ghr                            | 1 ± 0.19  | 0.54 ± 0.17  | 0.059        |
| Igfbp3                         | 1 ± 0.45  | 0.29 ± 0.29  | 0.078        |
| Rtn4                           | 1 ± 1.02  | 0.51 ± 0.02  | 0.443        |
| Bmp4                           | 1 ± 0.91  | 0.38 ± 0.31  | 0.318        |
| Cd44                           | 1 ± 1.02  | 0.83 ± 0.37  | 0.787        |
| Bmp1                           | 1 ± 0.36  | 1.03 ± 0.06  | 0.909        |
| Cdkn1a                         | 1 ± 1.03  | 1.17 ± 0.44  | 0.812        |
| Btg2                           | 1 ± 0.69  | 1.84 ± 0.58  | 0.184        |
| Cd24a                          | 1 ± 0.53  | 0.27 ± 0.19  | 0.085        |
| Angptl4                        | 1 ± 1   | 1.19 ± 0.3   | 0.769        |
| Clu                            | 1 ± 1.15  | 0.65 ± 0.15  | 0.622        |
| Nqo1                           | 1 ± 1.72  | 0.04 ± 0.03  | 0.383        |
| Cat                            | 1 ± 1.45  | 0.11 ± 0.05  | 0.346        |
| <b>Cell cycle</b>              |   |  |              |
| Ccng1                          | 1 ± 1.41  | 0.19 ± 0.17  | 0.379        |
| Ccnd1                          | 1 ± 1.07  | 0.35 ± 0.08  | 0.353        |
| Egf                            | 1 ± 1.48  | 1.8 ± 1.78   | 0.584        |
| <b>Cell Proliferation</b>      |   |  |              |
| Nox4                           | 1 ± 0.76  | 0.54 ± 0.46  | 0.418        |
| Uchl1                          | 1 ± 0.17  | 0.79 ± 0.15  | 0.172        |
| Vcam1                          | 1 ± 0.34  | 0.71 ± 0.1   | 0.223        |
| Timp1                          | 1 ± 0.86  | 0.6 ± 0.03   | 0.461        |
| <b>Cytoskeleton Regulators</b> |   |  |              |

|  |          |             |              |
|--|----------|-------------|--------------|
| Sprr1a                                 | 1 ± 1.26 | 1 ± 0.6     | 0.997        |
| Tmsb10                                 | 1 ± 0.62 | 0.89 ± 0.01 | 0.768        |
| <b>Extracellular matrix</b>            |          |             |              |
| Lgals3                                 | 1 ± 0.86 | 0.03 ± 0.03 | 0.118        |
| Mgp                                    | 1 ± 0.21 | 1.09 ± 0.21 | 0.647        |
| Cyr61                                  | 1 ± 0.27 | 0.04 ± 0.01 | <b>0.004</b> |
| Mt1                                    | 1 ± 0.19 | 0.75 ± 0.11 | 0.103        |
| Ccl3                                   | 1 ± 0.46 | 0.27 ± 0.11 | 0.051        |
| <b>Genes related to nephrotoxicity</b> |          |             |              |
| A2m                                    | 1 ± 0.43 | 0.26 ± 0.02 | <b>0.039</b> |
| Aass                                   | 1 ± 1.57 | 0.15 ± 0.14 | 0.399        |
| Aldh1a1                                | 1 ± 1.68 | 0.02 ± 0.01 | 0.367        |
| Calb1                                  | 1 ± 1.35 | 0.02 ± 0.01 | 0.273        |
| G6pc                                   | 1 ± 0.71 | 0.43 ± 0.01 | 0.221        |
| Ipmk                                   | 1 ± 1    | 0.14 ± 0.01 | 0.210        |
| Klk1                                   | 1 ± 0.96 | 0.11 ± 0.01 | 0.181        |
| Ugt1a1                                 | 1 ± 1.12 | 1.43 ± 0.16 | 0.552        |
| <b>Metal ion binding</b>               |          |             |              |
| Ccs                                    | 1 ± 0.63 | 0.78 ± 0.24 | 0.598        |
| Ctss                                   | 1 ± 0.45 | 0.7 ± 0.13  | 0.323        |
| Rgn                                    | 1 ± 1.69 | 0.54 ± 0.54 | 0.674        |
| Scd1                                   | 1 ± 0.66 | 0.63 ± 0.21 | 0.403        |
| <b>Oxidative stress</b>                |          |             |              |
| Gatm                                   | 1 ± 1.74 | 0.01 ± 0.01 | 0.374        |
| G6pdx                                  | 1 ± 1.34 | 0.09 ± 0.09 | 0.304        |
| <b>Tissue remodeling</b>               |          |             |              |
| Fn1                                    | 1 ± 1.01 | 0.61 ± 0.61 | 0.590        |
| Igfbp1                                 | 1 ± 0.45 | 4.56 ± 3.54 | 0.160        |
| Cst3                                   | 1 ± 0.13 | 0.54 ± 0.01 | <b>0.003</b> |
| <b>Transporters</b>                    |          |             |              |
| Abcb1a                                 | 1 ± 0.09 | 0.78 ± 0.42 | 0.414        |
| Abcc2                                  | 1 ± 0.47 | 0.71 ± 0.17 | 0.364        |
| Slc22a1                                | 1 ± 0.67 | 0.73 ± 0.25 | 0.539        |
| Slc22a5                                | 1 ± 1.2  | 0.27 ± 0.25 | 0.355        |
| <b>Xenobiotic metabolism</b>           |          |             |              |
| Cyp2d22                                | 1 ± 0.45 | 0.07 ± 0.03 | <b>0.022</b> |
| Fmo2                                   | 1 ± 1.26 | 0.14 ± 0.02 | 0.299        |

Arrays were run with kidney cDNA from  $n = 2-3$  pooled samples per array and  $n = 3$  arrays per group. Reference sequence number, Gene abbreviation, and Gene description for genes included in the Qiagen RT<sup>2</sup> Profiler™ PCR Array for Mouse Nephrotoxicity are listed below:

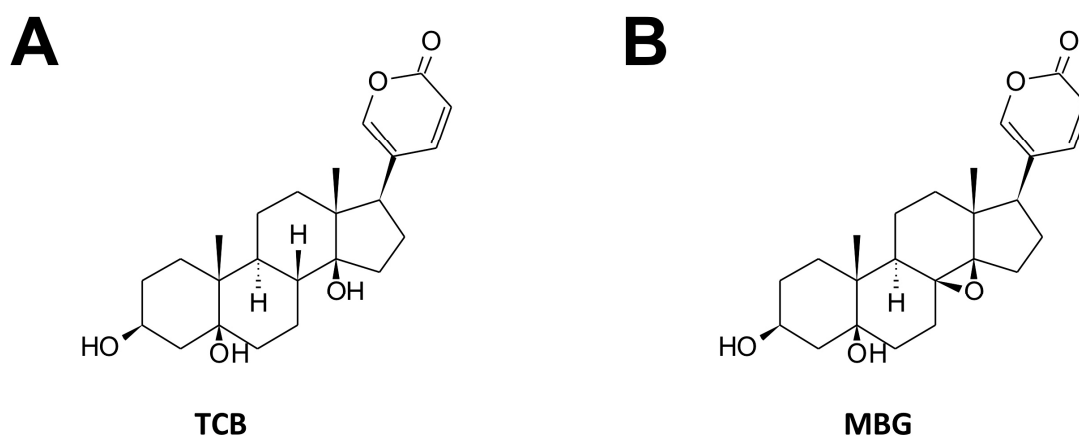
|                   |   |
|-------------------|---|
| NM_175628 A2m     | Alpha-2-macroglobulin                                   |
| NM_013930 Aass    | Amino adipate-semialdehyde synthase                     |
| NM_011076 Abcb1a  | ATP-binding cassette, sub-family B (MDR/TAP), member 1A |
| NM_013806 Abcc2   | ATP-binding cassette, sub-family C (CFTR/MRP), member 2 |
| NM_013467 Aldh1a1 | Aldehyde dehydrogenase family 1, subfamily A1           |
| NM_020581 Angptl4 | Angiopoietin-like 4                                     |
| NM_009673 Anxa5   | Annexin A5  |
| NM_007498 Atf3    | Activating transcription factor 3                       |
| NM_016668 Bhmt    | Betaine-homocysteine methyltransferase                  |
| NM_009755 Bmp1    | Bone morphogenetic protein 1                            |
| NM_007554 Bmp4    | Bone morphogenetic protein 4                            |
| NM_007570 Btg2    | B-cell translocation gene 2, anti-proliferative         |
| NM_009788 Calb1   | Calbindin 1   |
| NM_009804 Cat     | Catalase  |
| NM_011337 Ccl3    | Chemokine (C-C motif) ligand 3                          |
| NM_007631 Ccnd1   | Cyclin D1   |

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|                    |  |
|--------------------|--|
| NM_009831 Ccng1    | Cyclin G1  |
| NM_016892 Ccs      | Copper chaperone for superoxide dismutase  |
| NM_009846 Cd24a    | CD24a antigen  |
| NM_009851 Cd44     | CD44 antigen   |
| NM_007669 Cdkn1a   | Cyclin-dependent kinase inhibitor 1A (P21)   |
| NM_013492 Clu      | Clusterin  |
| NM_007752 Cp       | Ceruloplasmin  |
| NM_009976 Cst3     | Cystatin C   |
| NM_021281 Ctss     | Cathepsin S  |
| NM_021274 Cxcl10   | Chemokine (C-X-C motif) ligand 10  |
| NM_203320 Cxcl3    | Chemokine (C-X-C motif) ligand 3   |
| NM_206537 Cyp2c54  | Cytochrome P450, family 2, subfamily c, polypeptide 54   |
| NM_019823 Cyp2d22  | Cytochrome P450, family 2, subfamily d, polypeptide 22   |
| NM_010516 Cyr61    | Cysteine rich protein 61   |
| NM_010113 Egf      | Epidermal growth factor  |
| NM_181849 Fgb      | Fibrinogen beta chain  |
| NM_018881 Fmo2     | Flavin containing monooxygenase 2  |
| NM_010233 Fn1      | Fibronectin 1  |
| NM_008061 G6pc     | Glucose-6-phosphatase, catalytic   |
| NM_008062 G6pdx    | Glucose-6-phosphate dehydrogenase X-linked   |
| NM_007836 Gadd45a  | Growth arrest and DNA-damage-inducible 45 alpha  |
| NM_010255 Gamt     | Guanidinoacetate methyltransferase   |
| NM_025961 Gatm     | Glycine amidinotransferase (L-arginine:glycine amidinotransferase)                               |
| NM_008096 Gc       | Group specific component   |
| NM_010284 Ghr      | Growth hormone receptor  |
| NM_008131 Glul     | Glutamate-ammonia ligase (glutamine synthetase)  |
| NM_053110 Gpnmb    | Glycoprotein (transmembrane) nmb   |
| NM_027127 Gpx8     | Glutathione peroxidase 8 (putative)  |
| NM_029555 Gstk1    | Glutathione S-transferase kappa 1  |
| NM_013541 Gstp1    | Glutathione S-transferase, pi 1  |
| NM_134248 Havcr1   | Hepatitis A virus cellular receptor 1  |
| NM_010442 Hmox1    | Heme oxygenase (decycling) 1   |
| NM_010443 Hmox2    | Heme oxygenase (decycling) 2   |
| NM_010480 Hsp90aa1 | Heat shock protein 90, alpha (cytosolic), class A member 1                                       |
| NM_010497 Idh1     | Isocitrate dehydrogenase 1 (NADP+), soluble  |
| NM_008341 Igfbp1   | Insulin-like growth factor binding protein 1   |
| NM_008343 Igfbp3   | Insulin-like growth factor binding protein 3   |
| NM_027184 Ipmk     | Inositol polyphosphate multikinase   |
| NM_010639 Klk1     | Kallikrein 1   |
| NM_008491 Lcn2     | Lipocalin 2  |
| NM_010705 Lgals3   | Lectin, galactose binding, soluble 3   |
| NM_008567 Mcm6     | Minichromosome maintenance deficient 6 (MIS5 homolog, <i>S. pombe</i> ) ( <i>S. cerevisiae</i> ) |
| NM_008597 Mgp      | Matrix Gla protein   |
| NM_013602 Mt1      | Metallothionein 1  |
| NM_001285833 Nox4  | NADPH oxidase 4  |
| NM_130456 Nphs2    | Nephrosis 2 homolog, podocin (human)   |
| NM_008706 Nqo1     | NAD(P)H dehydrogenase, quinone 1   |
| NM_016978 Oat      | Ornithine aminotransferase   |
| NM_013614 Odc1     | Ornithine decarboxylase, structural 1  |
| NM_009060 Rgn      | Regucalcin   |
| NM_194053 Rtn4     | Reticulon 4  |
| NM_009127 Scd1     | Stearoyl-Coenzyme A desaturase 1   |
| NM_009202 Slc22a1  | Solute carrier family 22 (organic cation transporter), member 1                                  |
| NM_011396 Slc22a5  | Solute carrier family 22 (organic cation transporter), member 5                                  |
| NM_008766 Slc22a6  | Solute carrier family 22 (organic anion transporter), member 6                                   |
| NM_007707 Socs3    | Suppressor of cytokine signaling 3   |
| NM_013671 Sod2     | Superoxide dismutase 2, mitochondrial  |
| NM_011435 Sod3     | Superoxide dismutase 3, extracellular  |

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|                     |   |
|---------------------|---|
| NM_009263 Spp1      | Secreted phosphoprotein 1                               |
| NM_009264 Sprr1a    | Small proline-rich protein 1A                           |
| NM_011593 Timp1     | Tissue inhibitor of metalloproteinase 1                 |
| NM_025284 Tmsb10    | Thymosin, beta 10                                       |
| NM_013749 Tnfrsf12a | Tumor necrosis factor receptor superfamily, member 12a  |
| NM_011670 Uchl1     | Ubiquitin carboxy-terminal hydrolase L1                 |
| NM_201645 Ugt1a1    | UDP glucuronosyltransferase 1 family, polypeptide A1    |
| NM_145079 Ugt1a6a   | UDP glucuronosyltransferase 1 family, polypeptide A6A   |
| NM_011693 Vcam1     | Vascular cell adhesion molecule 1                       |
| NM_011701 Vim       | Vimentin  |
| NM_007393 Actb      | Actin, beta   |
| NM_009735 B2m       | Beta-2 microglobulin                                    |
| NM_008084 Gapdh     | Glyceraldehyde-3-phosphate dehydrogenase                |
| NM_010368 Gusb      | Glucuronidase, beta                                     |
| NM_008302 Hsp90ab1  | Heat shock protein 90 alpha (cytosolic), class B member |



**Figure S1.** Structural comparison of (A) Telocinobufagin and (B) Marinobufagenin.