Dietary nitrate attenuates renal ischemia-reperfusion injuries by modulation of immune responses and reduction of oxidative stress

Ting Yang^{1*#}, Xing-Mei Zhang², Laura Tarnawski³, Maria Peleli¹, Zhengbing Zhuge ¹, Niccolo Terrando^{1*}, Robert A. Harris², Peder S. Olofsson³, Erik Larsson⁴, A. Erik G. Persson^{1,5}, Jon O. Lundberg¹, Eddie Weitzberg¹, Mattias Carlstrom^{1#}

*Authors current institution: Dept. of Medicine, Div. of Nephrology (T.Y.), Dept. of Anesthesiology (N.T.), Duke University Medical Center, Durham, NC, USA # Corresponding authors

Correspondence to:

Ting Yang (MD, PhD)

Department of Physiology and Pharmacology, Karolinska Institutet

Nanna Svartz Väg 2, 17177 Stockholm, Sweden

Email: ting.yang2@duke.edu

Mattias Carlström (PharmD, PhD)

Associate Professor of Physiology

Department of Physiology and Pharmacology, Karolinska Institutet

Nanna Svartz Väg 2, 17177 Stockholm, Sweden

Email: mattias.carlstrom@ki.se

¹Dept. of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

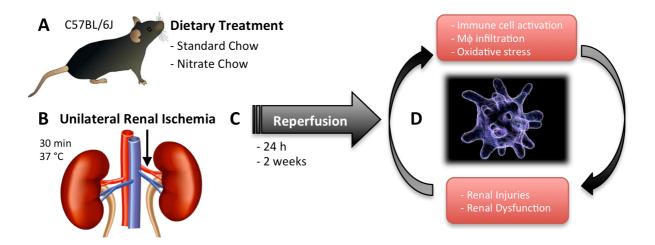
² Dept. of Clinical Neuroscience, Karolinska Institutet, Center for Molecular Medicine, Karolinska University Hospital Solna, Stockholm, Sweden

³ Dept. of Medicine, Center for Molecular Medicine, Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

⁴ Dept. of Immunology, Genetics and Pathology, Uppsala University, Sweden

⁵ Dept. of Medical Cell Biology, Uppsala University, Sweden

Figure S1. Experimental protocol



Adult male mice (C57BL/6J) were fed with regular or nitrate-supplemented chow for two weeks ($\bf A$), which was followed by unilateral ischemia of the left kidney ($\bf B$). The reperfusion time, before termination, was 24 hours or two weeks ($\bf C$). Renal function determined by renal plasma flow (RPF) and glomerular filtration rate (GFR) was assessed *in vivo*, and at the end of the reperfusion period plasma, tissues and bone-marrow-derived macrophages were collected for analyses of kidney injuries, oxidative stress (NADPH oxidase-derived $O_2^{\bullet-}$ generation), immune cell activation (cytokines in plasma and in kidney) and infiltration of macrophages (M Φ) in the kidney, and finally the phenotype of bone marrow-derived macrophages was characterized ($\bf D$).