



## Supplementary Materials

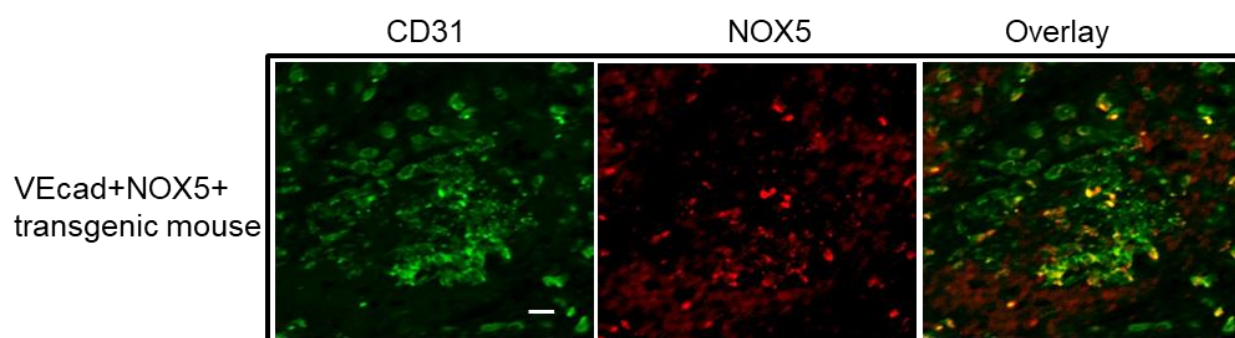
Article

# Endothelial NOX5 Obliterates the Reno-Protective Effect of Nox4 Deletion by Promoting Renal Fibrosis via Activation of EMT and ROS-Sensitive Pathways in Diabetes

Karin A. M. Jandeleit-Dahm <sup>1,2</sup>, Haritha R. Kankanamalage <sup>1</sup>, Aozhi Dai <sup>1</sup>, Jaroslawna Meister <sup>2</sup>, Sara Lopez-Trevino <sup>1</sup>, Mark E. Cooper <sup>1</sup>, Rhian M. Touyz <sup>3</sup>, Christopher R. J. Kennedy <sup>4</sup> and Jay C. Jha <sup>1,\*</sup>

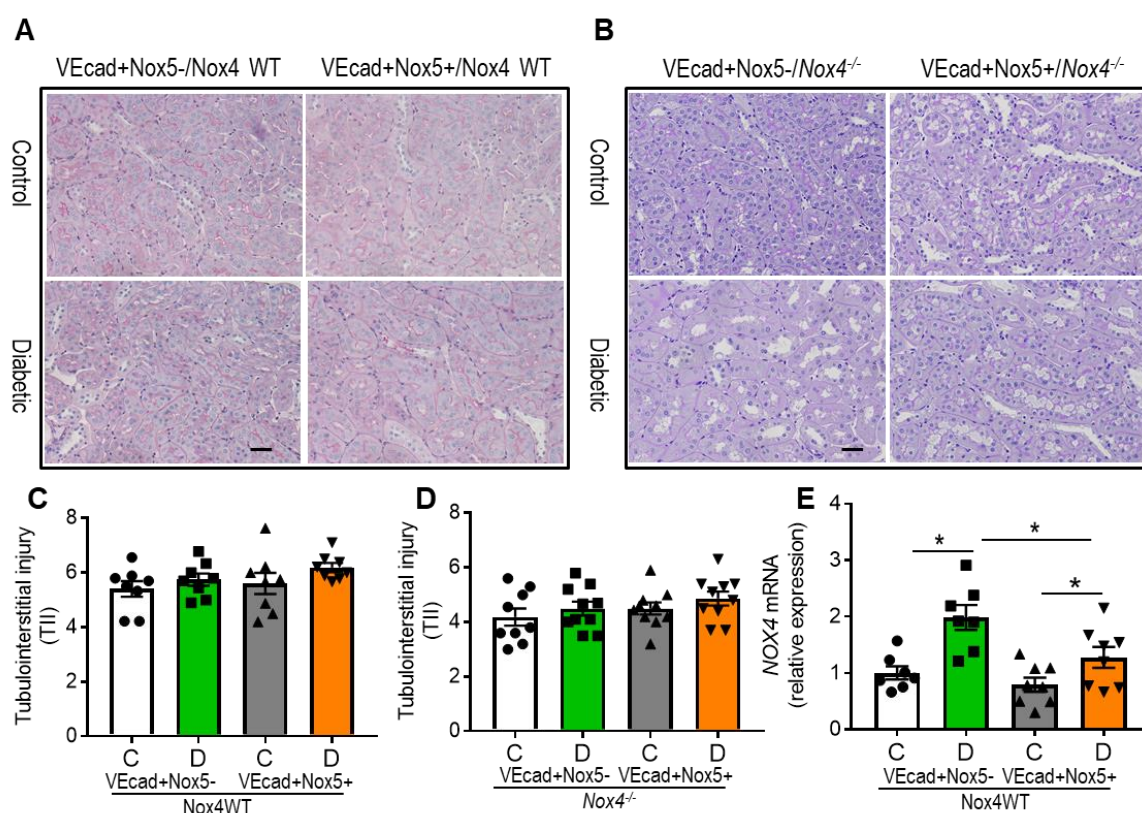
- 1 Department of Diabetes, School of Translational Medicine, Monash University, Alfred Medical Research & Education Precinct, Melbourne, VIC 3004, Australia
- 2 Institute for Clinical Diabetology, German Diabetes Centre, Leibniz Centre for Diabetes Research at Heinrich Heine University, 40225 Düsseldorf, Germany
- 3 Research Institute of the McGill University Health Centre, McGill University, Montreal, QC H3H 2R9, Canada
- 4 Department of Medicine, Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON K1Y 4E9, Canada

\* Correspondence: jay.jha@monash.edu




**Supplementary Figure S1:** NOX5 transgenic mice expressing human NOX5 in endothelial cells (VEcad+NOX5+) in the glomerulus. Co-localisation of CD31 (a marker of endothelial cells; green staining) and NOX5 (red staining) in frozen kidney sections of control VEcad+NOX5+ transgenic mouse. Scale bar, 20  $\mu$ m in all photomicrographs.

**Immunofluorescence:** Immunostaining for the co-localisation of NOX5 and CD31 was performed. Double staining for NOX5 and CD31 in mouse frozen kidney sections (5  $\mu$ m) was performed by incubation with primary antibody to NOX5 (rabbit polyclonal, catalogue no. ab191010; Abcam, Cambridge, MA, USA) and CD31 (goat polyclonal, catalogue no. AF3628; R&D Systems, Minneapolis, MN, USA), followed by incubation with secondary antibody Alexa Fluor 568 (donkey anti-rabbit; Invitrogen, Eugene, OR, USA) and Alexa Fluor 488 (donkey anti-goat; Invitrogen). All stained sections were examined and images were captured using a Nikon eclipse-Ni (Tokyo, Japan) fluorescence microscope.



**Supplementary Figure S2:** Periodic Acid-Schiff staining (A and B) and the scoring of tubulointerstitial injury, TII (C and D) in all groups of wild type and Nox4 knockout (*Nox4*<sup>-/-</sup>) mice with and without NOX5 expression after 10 weeks of STZ- diabetes. Scale bar, 50  $\mu$ m in all photomicrographs (n = 7–10/gp). Renal cortical gene expression of *Nox4* (E) in wild type mice with and without NOX5 expression after 10 weeks of STZ- diabetes. Data are shown as mean  $\pm$  SEM. Asterisks represent p-values for comparisons of the indicated groups: \* < 0.05.

**Supplementary Table S1. Mouse probes and primers for RT-PCR.**

Genes	Gene accession no.	Probe Sequence (6-FAM 5'-3')	Forward Primer 5'-3'	Reverse Primer 5'-3'
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<i>Nox2</i>	U43384	CAACTGGACAGGAACCT	AGTGC GTGTGCTCGACAAG	CCAAGCTACCATCTTATGGAAAGT
<i>Mcp-1</i>	NM_011333	AATGGGTCCAGACATAC	GTCTGTGCTGACCCCAAGAAG	TGGTCCGATCCAGGTTTTTA
<i>Egr-1</i>	NM_007913	SYBER	TCGGCTCCTTCTCACTCA	GCATCATCTCTCCAGTTTGG
<i>Vimentin</i>	NM_011701	CCGCACCAACGAGA	CGCCATCAACACTGAGTTCAA	TGGCAAAGCGGTCAATCA
<i>Pkc-α</i>	X52685	CGATCCCAGTCCCAG	AGACAAAGACCGCGACTGT	TTAGCTCTGAGACACCAAGGAAA
<i>Nox4</i>	NM_015760	CATTTTGCTATTTTCATCAA	AAAAATATCACACTGAATTCGAGACT	TGGGTCCAGCAGAAAACCTC
<i>Ki-67</i>	NM_001081117	SYBER	CAAAAGCGGAAGTGGAGCTT	TGTTTCGCAACTTTCGTTTGTG
<i>Enos</i>	BC052636	CCAGAGCTACGCACAGC	AACCATCTGTATGGCTCTGAGACT	CTTAGGGACACCACATCACTCAT
<i>α-SMA</i>	NM_007392	TGCCAGATCTTTTCC	GACGCTGAAGTATCCGATAGAACA	GGCCACACGAAGCTCGTTAT
<i>Ctgf</i>	BC006783	ACTGCCTGGTCCAGAC	GCTGCCTACCGACTGGAAGA	CTTAGAACAGCGCTCCACTCT
<i>Vegf</i>	M95200	CTGTACCTCCACCATGC	GCACTGGACCTGGCTTTACT	ATGGGACTTCTGCTCTCTTCTG
<i>Fn1</i>	M10905	CCCCGTCAAGGCTTA	ACATGGCTTTAGGCGGACAA	ACATTCGGCAGGTATGGTCTTG
<i>Col4</i>	J04694	CAGTGCCCTAACGGT	GGCGGTACACAGTCAGACCAT	GGAATAGCCGATCCACAGTGA
<i>Tlr4</i>	NM_021297	AAACCTACCTGGAATGG	GCAGCAGGTGGAATTGTATCG	TGTGCCTCCCCAGAGGATT
<i>Nrf2</i>	NM_001399226	CACAGTGCTCCTATGC	AGCCTCTGTACCAGCTCAAG	TGTTGATTTTCACATTGGGATTCA

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