

Consequences of Both Cocksackievirus B4 and Type 1 Diabetes on Female Non-Obese Diabetic Mouse Kidneys

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Supplementary Materials

Supplementary Table S1. SYBR green primer names, sequences and references.

Gene Name	Full Name	Strand	Sequence	Reference
CVB4	Coxsackievirus B4	+ −	CCCACAGGACGCTCTAATA CAGAGTTACCCGTTACGACA	[1]
Tlr3	Toll Like Receptor 3	+ −	AATCCTTGCGTTGCGAAGTG GGTTCAGTTGGGCGTTGTTC	[2]
Ifnb1	Interferon Beta	+ −	ATAAGCAGCTCCAGCTCCAA CTGTCTGCTGGTGGAGTTCA	[2]
Tnfa	Tumor Necrosis Factor Alpha	+ −	CGGTCCCCAAAGGGATGAG CCTTGAAGAGAACCCTGGGAGTA	[2]
Tgfb1	Transforming Growth Factor Beta	+ −	TTGCTTCAGCTCCACAGAGA CGGGTTGTGTTGGTTGTAGA	[2]
Fn1	Fibronectin	+ −	CTTTGGCAGTGGTCATTTC TGGTAGGTCTTCCCATCGTC	[2]
Havcr1	Hepatitis A Virus Cellular Receptor 1	+ −	TCCACACATGTACCAACATCAA TGCTCATGGGGACAAAATG	[2]
Il6	Interleukin 6	+ −	GATGGATGCTACCAAACTGGA GGAAATTGGGGTAGGAAGGA	[2]
Gapdh	Glyceraldehyde 3-phosphate dehydrogenase	+ −	TGTGTCCGTCGTGGATCTGA CCTGCTTCACCACCTTCTTGA	[3]
Actg1	Actin Gamma 1	+ −	ACCAACAGCAGACTTCCAGGAT AGACTGGCAAGAAGGAGTGG-TAA	[4]
Cd68	CD68 Molecule	+ −	CTTCCCACAGGCAGCACAG AATGATGAGAGGCAGCAA-GAGG	[3]

Supplementary Table S2. TaqMan primer names and catalog numbers.

Gene Name	Full Name	Supplier	Catalog Number
<i>Spp1</i>	Osteopontin	Thermo Fisher, Waltham, MA, USA	Mm00436767_m1
<i>Agt</i>	Angiotensinogen	Thermo Fisher, Waltham, MA, USA	Mm00599662_m1
<i>Vegfa</i>	Vascular Endothelial Growth Factor A	Thermo Fisher, Waltham, MA, USA	Mm01281449_m1
<i>Il18</i>	Interleukin 18	Thermo Fisher, Waltham, MA, USA	Mm00434226_m1
<i>Tlr4</i>	Toll Like Receptor 4	Thermo Fisher, Waltham, MA, USA	Mm00445273_m1
<i>Ccl2</i>	X-C Motif Chemokine Ligand 2	Thermo Fisher, Waltham, MA, USA	Mm00441242_m1
<i>Lcn2</i>	Lipocalin 2	Thermo Fisher, Waltham, MA, USA	Mm01324470_m1
<i>Cxcl10</i>	C-X-C Motif Chemokine Ligand 10	Thermo Fisher, Waltham, MA, USA	Mm00445235_m1
<i>Nos2</i>	Nitric Oxide Synthase 2	Thermo Fisher, Waltham, MA, USA	Mm00468487_m1
<i>Mhc1</i>	Major Histocompatibility Complex 1	Thermo Fisher, Waltham, MA, USA	Mm01309902_m1

Supplementary Table S3. Sample size and duration of diabetes per NOD mouse group at euthanasia.

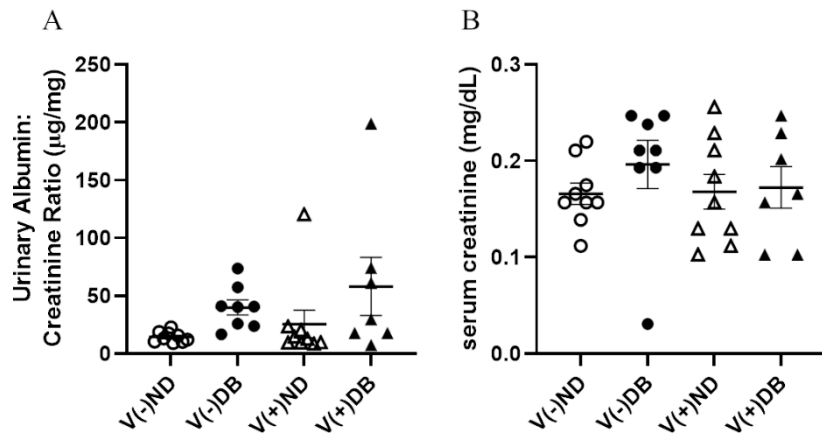
Group†	7 Weeks Post Exposure	Weeks Diabetic‡	12 Weeks Post Exposure	Weeks Diabetic‡	17 Weeks Post Exposure	Weeks Diabetic‡
V(-) ND	3		3		3	
V(-) DB	3	2,3,7	3	1,6,7	2	3,3
V(+) ND	3		3		3	
V(+) DB	1	5	4	5,7,8,9	2	14,14

† V(-) ND = Non-Exposed/Non-Diabetic; V(-) DB = Non-Exposed/Diabetic; V(+) ND = Virus-Exposed/Non-Diabetic; V(+) DB = Virus-Exposed/Diabetic.‡ Measured by the number of glucose readings above 250mg/dL prior to euthanasia

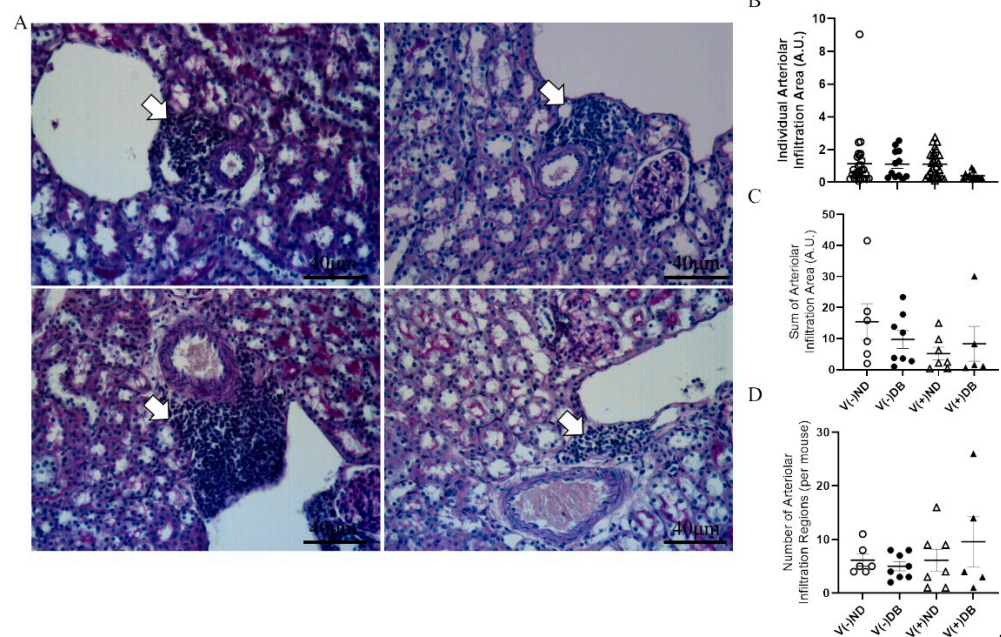
Supplementary Table S4. NMDS1 and NMDS2 species scores for each parameter.

Gene Name	MDS1	MDS2
<i>Fn1</i>	0.078974	-0.10507
<i>Havcr1</i>	0.094793	-0.09177
<i>Vegfa</i>	0.008206	-0.04695
Body Weight	-0.07569	-0.04397
<i>Spp1</i>	-0.04766	-0.04079
<i>Tlr4</i>	0.025125	-0.03933
<i>Tlr3</i>	0.003621	-0.03766
<i>Tgfb</i>	0.063486	-0.03173
<i>Nos2</i>	0.057767	-0.02846
<i>Il18</i>	0.004907	-0.02832
Kidney Weight	-0.12466	-0.00782
SrCr	-0.09726	0.010299
<i>Cd68</i>	0.073767	0.01456
<i>Ccl2</i>	0.197826	0.027797
Normalized Kidney Weight	-0.11768	0.030018
<i>Ifnb</i>	0.054015	0.031058

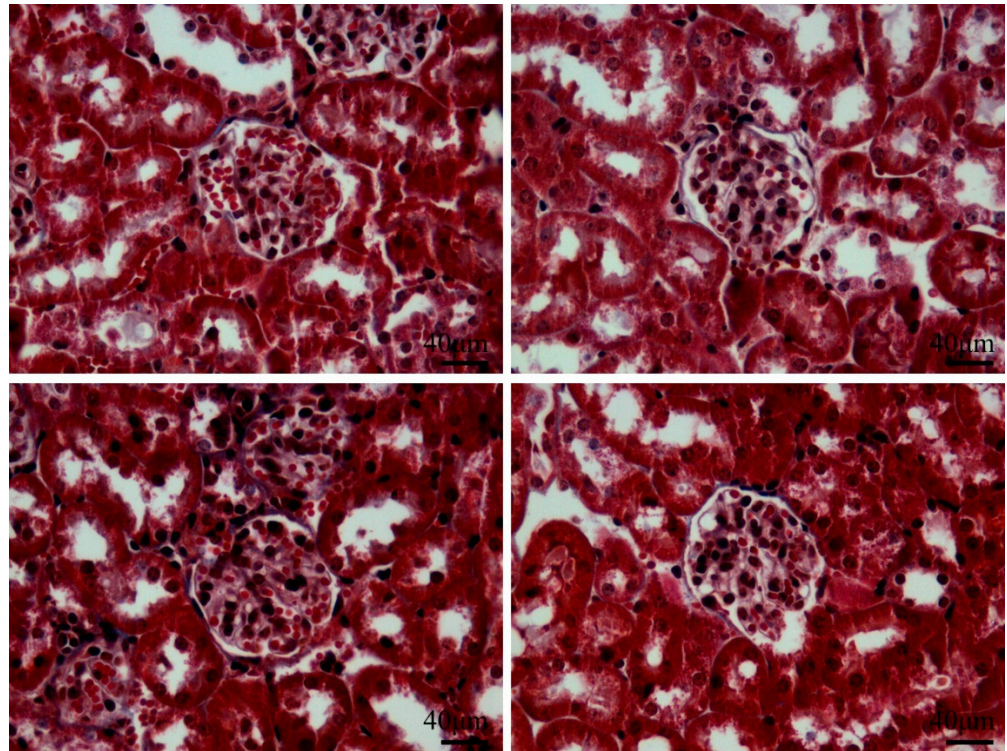
<i>Mhc1</i>	0.016621	0.043313
<i>Il6</i>	0.181237	0.043341
<i>Cxcl10</i>	-0.00561	0.096404
<i>Agt</i>	-0.15425	0.11908
<i>Lcn2</i>	0.125138	0.124897
<i>Tnfa</i>	0.291052	0.143158
UACR	-0.1869	0.158651



Supplementary Figure S1. Urine albumin and creatinine excretion and serum creatinine levels. (A) UACR and (B) serum creatinine were measured and compared across all four NOD mouse groups ($n = 7-9$; \circ V(-)ND, \bullet V(-)DB, \triangle V(+)ND, \blacktriangle V(+)DB).



Supplementary Figure S2. Immune Cell Infiltration. (A) Representative images of Periodic acid-Schiff staining of non-exposed (upper two panels) and CVB4-exposed (lower two panels) NOD mouse kidneys without (left two panels) and with diabetes (right two panels). Plots of infiltration (B) individual areas, (C) area sum and (D) number of locations per three kidney sections per mouse. Arrowheads point to immune cell infiltration. Images taken at 200 \times magnification ($n = 30-65$; \circ V(-)ND, \bullet V(-)DB, \triangle V(+)ND, \blacktriangle V(+)DB).



Supplementary Figure S3. Fibrosis evaluation following CVB4 exposure. Representative images of Masson's Trichrome stain of non-exposed (upper two panels) and CVB4-exposed (lower two panels) NOD mouse kidneys without (left two panels) and with diabetes (right two panels). Images taken at 400× magnification.

Reference

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