

# SUPPLEMENTARY INFORMATION

## **Transgenerational inheritance of susceptibility to diabetes-induced male subfertility**

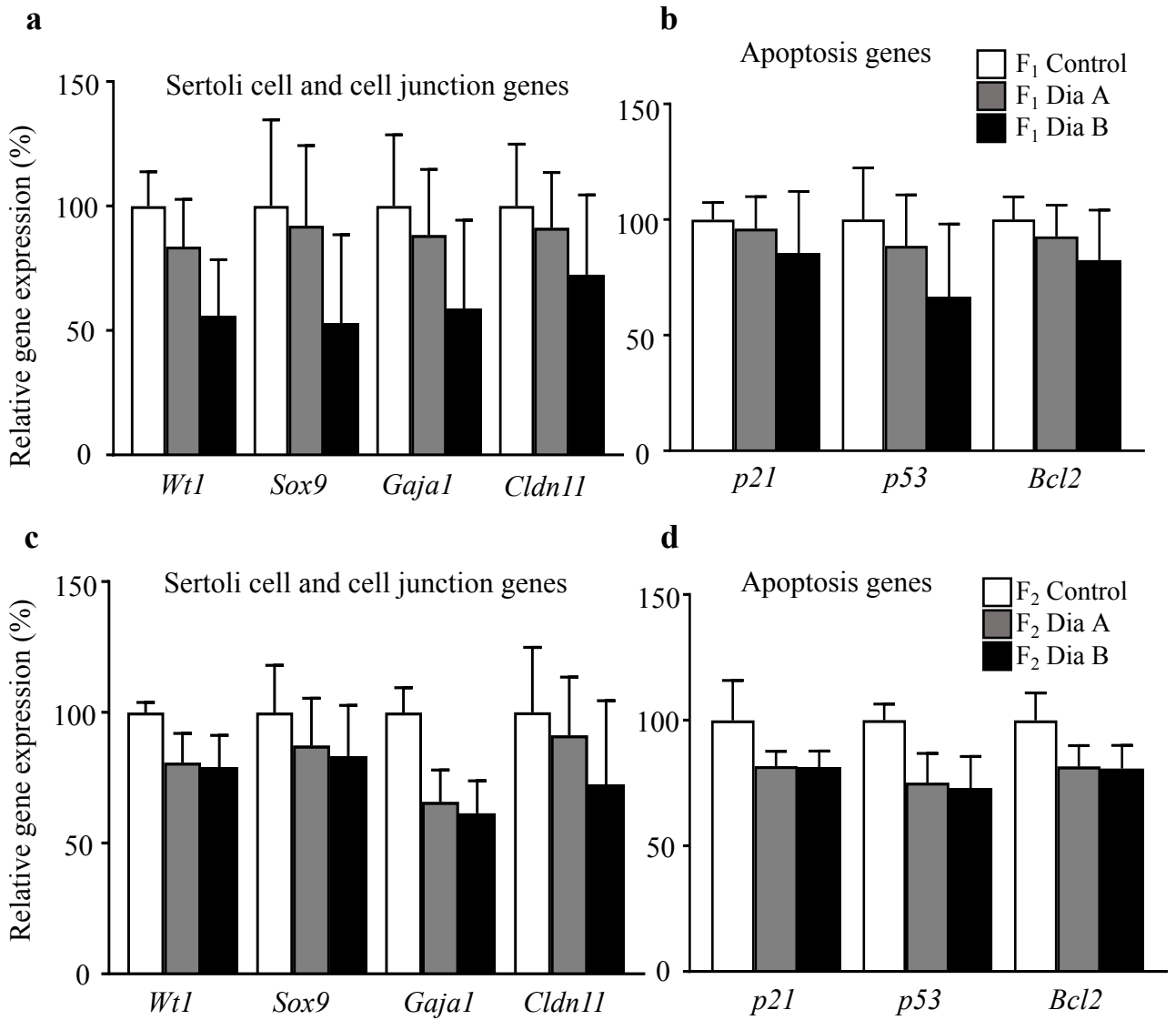
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# SUPPLEMENTARY FIGURE



**Supplementary Figure 1. Transgenerational effects of diabetes on testicular gene expression of the F<sub>1</sub> and F<sub>2</sub> male generations.** The expression levels of genes encoding proteins associated with genes expressed in Sertoli cells: Wilms' tumour 1 (*Wt1*), SRY-box containing gene9 (*Sox9*), gap junction protein alpha 1 (*Gjal1*) and claudin 11 (*Cldn11*); and apoptotic markers: cyclin-dependent kinase inhibitor 1A (*p21*), tumor protein p53 (*p53*), and B cell leukemia/lymphoma 2 (*Bcl2*) are shown (**a**, **b**) for the F<sub>1</sub> males (controls n = 8; diabetic group A n=10; diabetic group B n=5) and (**c**, **d**) for the F<sub>2</sub> males (controls n = 6; diabetic group A n=12; diabetic group B n=11). The graph illustrates the relative expression of the selected genes. The control group represents 100% and the percentage in the experimental groups represents the ratio of gene expression between the treated groups and the control group. The values are means ± SEM.

## SUPPLEMENTARY DATA

**Supplementary Table S1.** List of antibodies with manufacturer reference number

<u>Antibody</u>	<u>Reference</u>	<u>Company</u>
mouse anti-VEGF-A	#sc-7269	Santa Cruz Biotechnology
rabbit anti-WT-1	#CA1026	Calbiochem
rabbit anti-pHH3	#06-570	Merck Millipore
rabbit anti-CX43	#C6219	Sigma
mouse anti-SCP3	#ab97672	Abcam
Alexa Fluor® 488	#115-545-146	Jackson Immuno Research Labs
Alexa Fluor® 594	#111-585-144	Jackson Immuno Research Labs

# SUPPLEMENTARY DATA

**Supplementary Table S2.** List of primer sequences

Gene symbol	Gene name	RefSeq ID	Primer sequence	
<i>Actb</i>	Actin $\beta$	NM_007393.3	F	5' -CGGTTCCGATGCCCTGAGGCTCTT- 3'
			R	5' -CGTCACACTTCATGATGGAATTGA- 3'
<i>Bcl-2</i>	B-cell leukemia/lymphoma	NM_009741.3	F	5' -CAGGGAGATGTCACCCCTGGTGG- 3'
			R	5' -AGGCATCCCAGCCTCCGTTATCC- 3'
<i>Cldn11</i>	Claudin 11	NM_016674.4	F	5' -GGCGACATTAGTGGCCACAGCA- 3'
			R	5' -GCAGCGGCCAGCCAGTAAA- 3'
<i>Gjal</i>	Gap junction alpha-1 protein	NM_010288.3	F	5' -TTTGGCGTGCCGGCTTCACTTT- 3'
			R	5' -CTCCGGCCGTGGAGTAGGCTT- 3'
<i>Ppia</i>	Peptidylprolyl isomerase A	NC_000077.5	F	5' -AGCTCTGAGCACTGGAGAGA- 3'
			R	5' -GCCAGGACCTGTATGCTTTA- 3'
<i>Prm1</i>	Protamine 1	NM_013637.4	F	5' -ACAGGTTGGCTGGCTCGACC- 3'
			R	5' -CGGCAGCATCGGTATCTGGCC- 3'
<i>Prm2</i>	Protamine 2	NM_008933.1	F	5' -CCAGGGGCCTGGACAAGACC- 3'
			R	5' -TCTGTGGTGGTGGTGGCCCC- 3'
<i>p21</i>	Cyclin-dependent kinase inhibitor 1	NM_007669.4	F	5' -CCGCCGCGGTGTCAGAGTCTA- 3'
			R	5' -CTGTGCGGAACAGGTCGGAC- 3'
<i>p53</i>	Tumor protein p53	NM_001127233.1	F	5' -ATGGCTTCCACCTGGGCTTCCTG- 3'
			R	5' -CCACAACAGCACAGGGCACGT- 3'
<i>Sox9</i>	SRY-box containing gene 9	NM_011448.4	F	5' -GCTGGAAGTCGGAGAGCCGAGA- 3'
			R	5' -AGAGAACGAAACCGGGGCCAC- 3'
<i>Sycp1</i>	Synaptonemal Complex Protein 1	NM_011516.2	F	5' -GCCCATGCTCGAACAG GTTGC- 3'
			R	5' -ACAGTCTGCTCATTGGCTCTGAA- 3'
<i>Sycp3</i>	Synaptonemal Complex Protein 3	NM_011517.2	F	5' -GGACAGCGACAGCTCACCGG- 3'
			R	5' -GGTGGCTTCCCAGATTTCCCAGA- 3'
<i>Tnp1</i>	Transition protein 1	NM_009407.2	F	5' -CCGAGCTCCTCACAAG GGCGT- 3'
			R	5' -CAGGGCAGAGCTCATTGCCGC- 3'
<i>Tnp2</i>	Transition protein 2	NM_013694.4	F	5' -CCTGCAAGACCCAGCCACCG- 3'
			R	5' -GTTTCCGCCTCCTGACGGCC- 3'
<i>Wt1</i>	Wilms Tumor 1	NM_144783.2	F	5' -GGCGTTTGAGGGGTCCGAC- 3'
			R	5' -AAAGTGGGCGGAGCACCGAC- 3'