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Supplemental Material

Sex- and Dose-Specific Effects of Maternal Bisphenol A Exposure on Pancreatic Islets of First- and Second-Generation Adult Mice Offspring

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Table S3. Changes in cytokine/chemokine levels on a LUMINEX assay in pancreatic lysates of F1 adult male offspring. N=3 to 4 litters per group. Data are normalized to total protein concentration as pg of cytokine or chemokine per μg of total protein, and presented as mean \pm SEM. Decimal places are represented by E notation, where E represents base 10, followed by the power of 10 (example, E-2 = 10^{-2} , E-3 = 10^{-3} , E-4 = 10^{-4}). P values are from Dunnett's test performed on log-transformed data, where required (g-csf, IL9, IL15, and RANTES). Control: 7% corn oil diet; LowerB: Lower BPA (10 $\mu\text{g}/\text{kg}/\text{day}$); UpperB: Upper BPA (10 $\text{mg}/\text{kg}/\text{day}$).

Table S4. Changes in cytokine/chemokine levels on a LUMINEX assay in pancreatic lysates of F2 adult male offspring. N=3 to 4 litters per group. Data are normalized to total protein concentration as pg of cytokine or chemokine per μg of total protein, and presented as mean (SEM). Decimal places are represented by E notation, where E represents base 10, followed by the power of 10 (example, E-2 = 10^{-2} , E-3 = 10^{-3} , E-4 = 10^{-4}). p values are from Dunnett's test performed on log-transformed data, where required (IL2, IL9, IL10, IL12p40, IL15, IP10, and MIP2). Control: 7% corn oil diet; LowerB: Lower BPA (10 $\mu\text{g}/\text{kg}/\text{day}$); UpperB: Upper BPA (10 $\text{mg}/\text{kg}/\text{day}$).

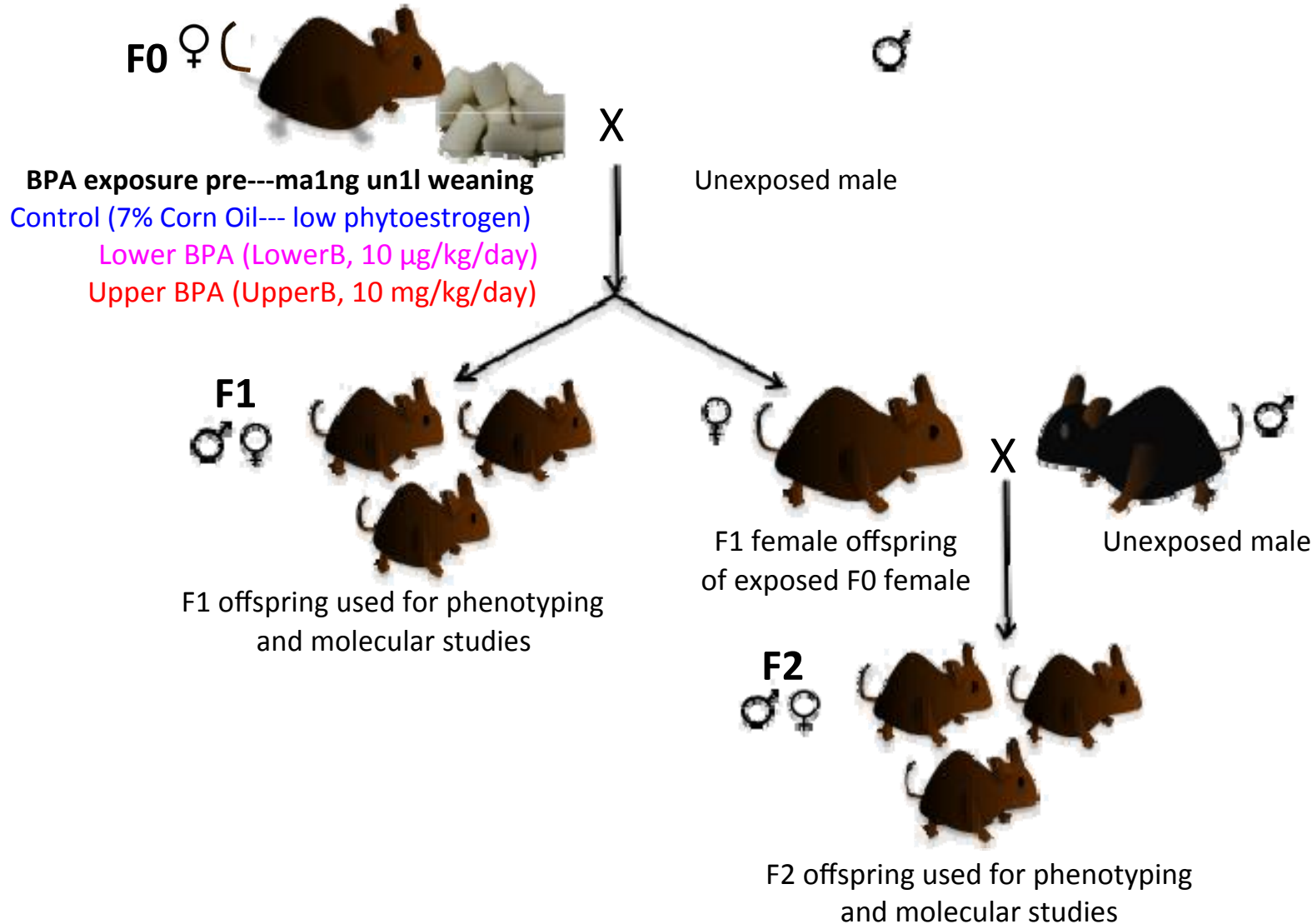


Fig.S1. Experimental paradigm of maternal BPA exposure study. C57BL/6 virgin female mice (F0) were randomly assigned to the following three diets: 7% corn oil (Control), 10 µg/kg/day (LowerB), and 10 mg/kg/day (UpperB) BPA. After two weeks, while continuing on these diets, the females were time-mated to unexposed C57BL/6J males and once pregnant designated as the “F0”. The females were allowed to deliver and raise their offspring (F1) until weaning. At postnatal day (PND) 21, all F1 mice were weaned on to the control diet, so the exposure to BPA was limited to gestation and lactation only. All subsequent generations were maintained on the control diet as well. A subset of F1 females were time-mated to unexposed C57BL/6J males and allowed to deliver and raise their offspring (F2) until weaning. The only time when F2 offspring were exposed to BPA was as ovulum of the F1 females during gestation and lactation.

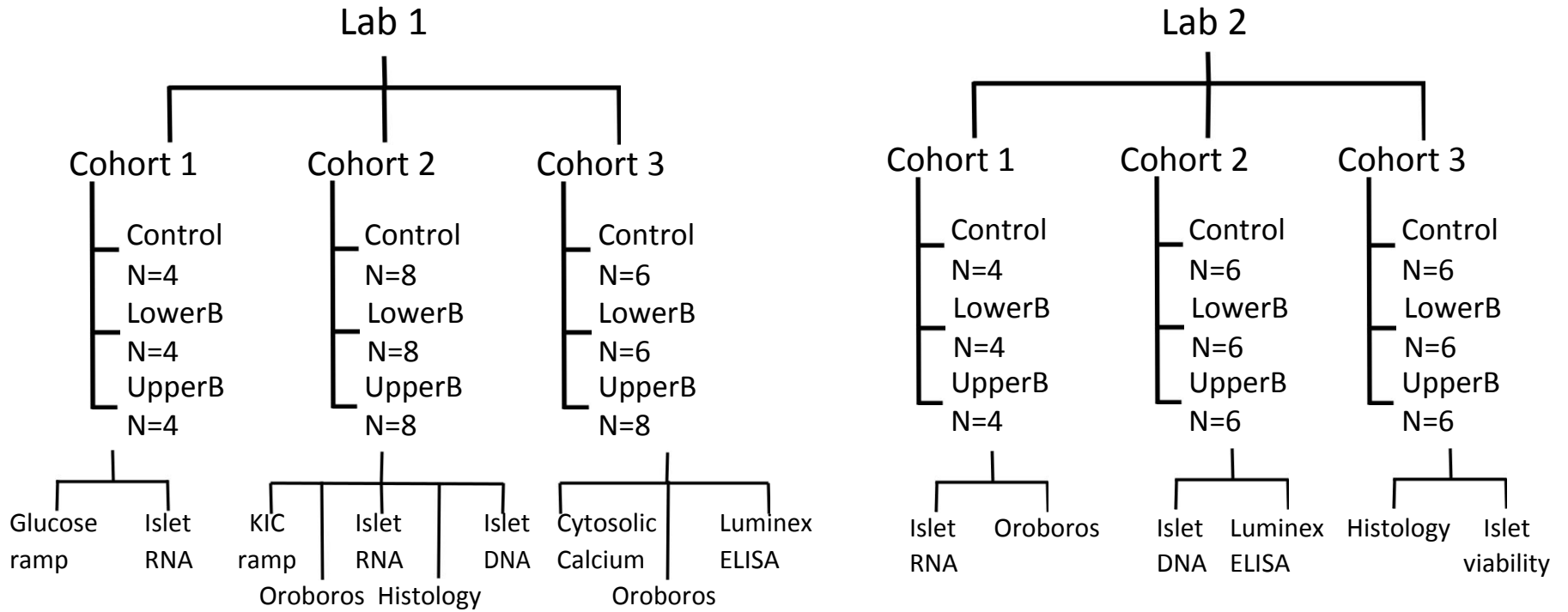


Fig.S2. Flowchart of animal usage from multiple cohorts generated in two different animal care facilities. N are number of litters. 2 to 5 male offspring and 2 to 5 female offspring per litter. KIC: α -keto isocaproate.

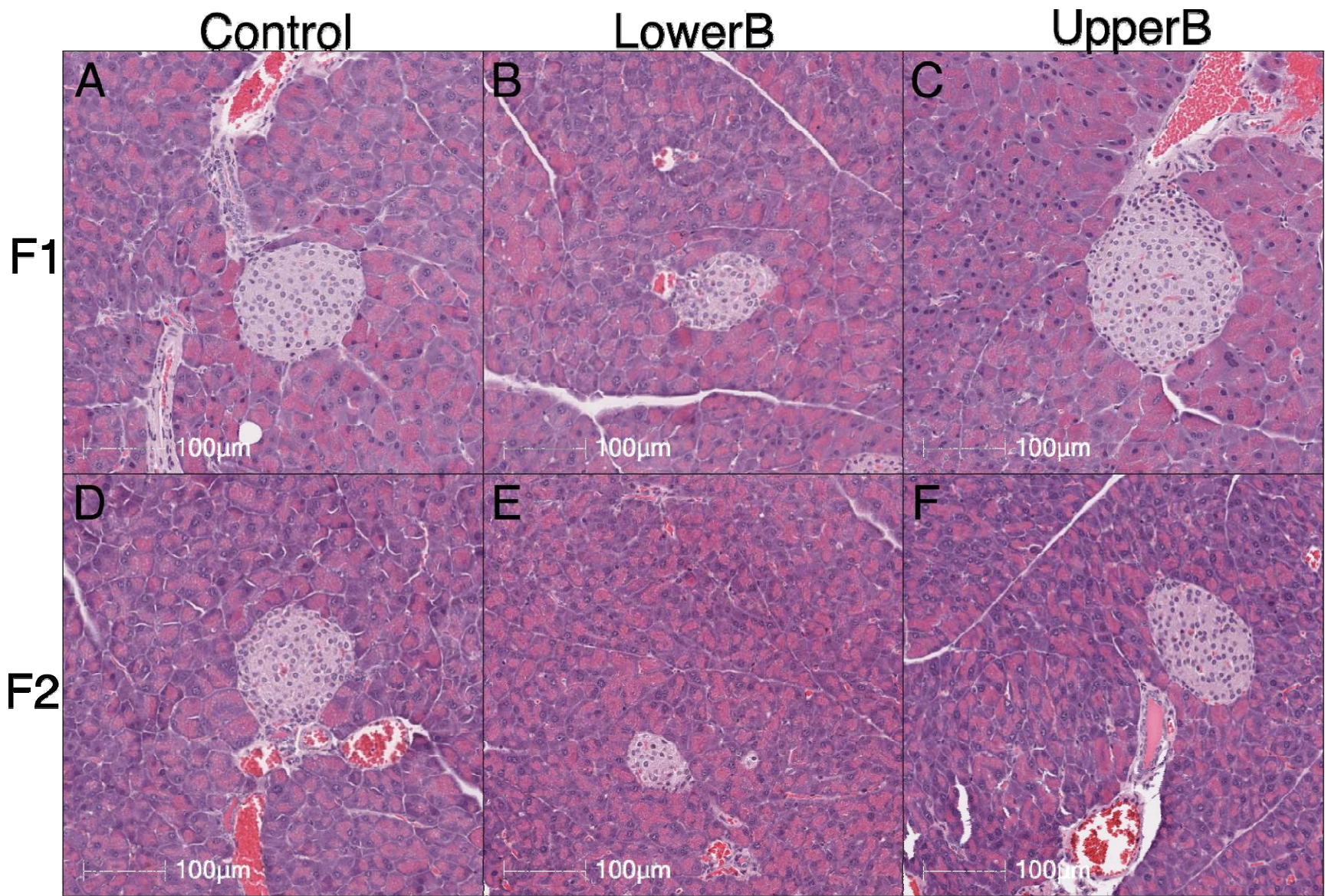


Fig.S3. Representative photos of H&E stained pancreatic sections from F1 and F2 males. (A-C) F1 males, and (D-F) F2 males.

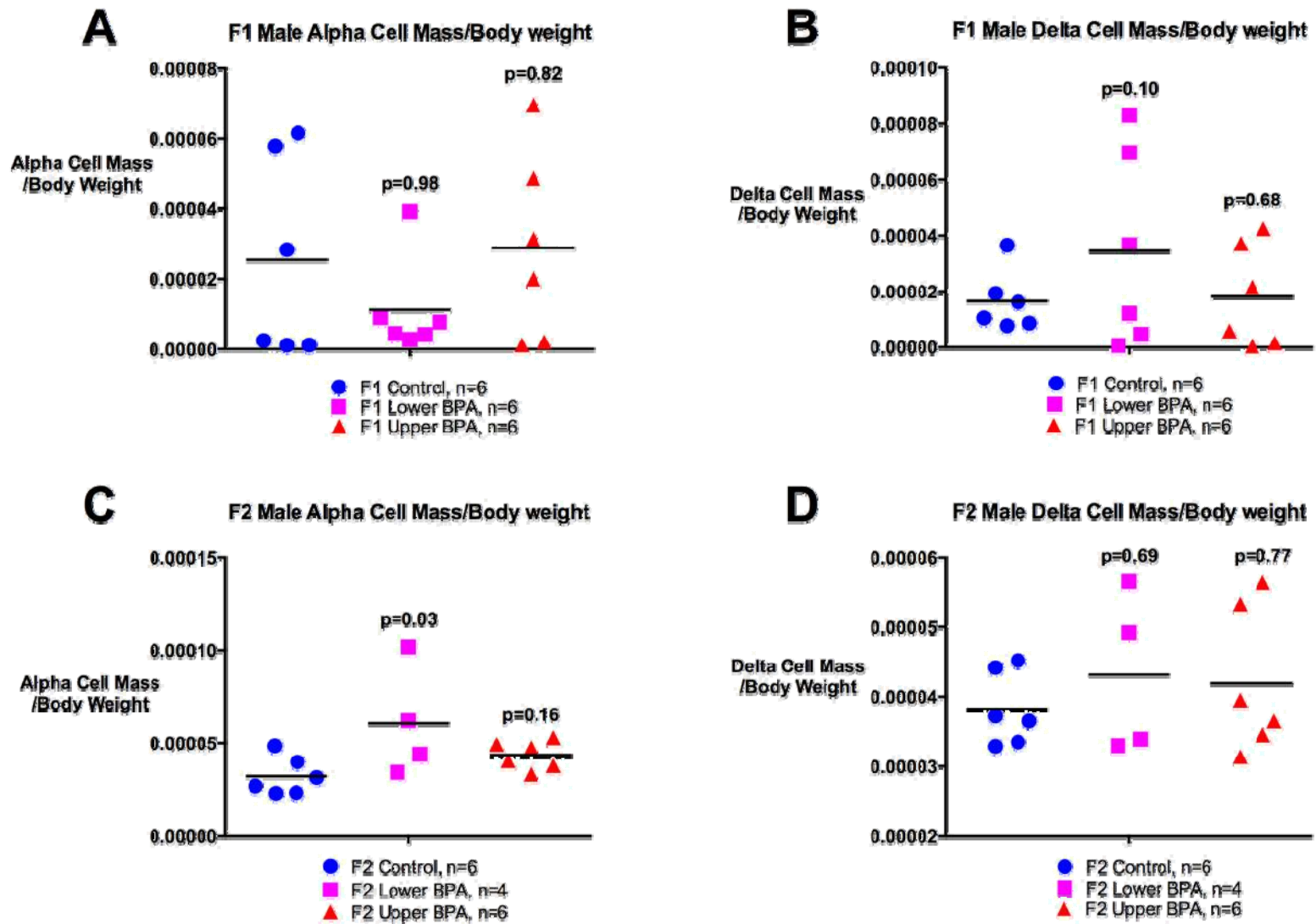


Fig. S4. Alpha and Delta mass adjusted for body weight in F1 and F2 males: (A-B) F1 males, and (C-D) F2 males. Data are individual litter data (one animal per litter) with mean superimposed. Data were analyzed using Dunnett's test performed on log-transformed data, where required (F1 alpha and delta, F2 alpha and delta); P values are relative to Control.

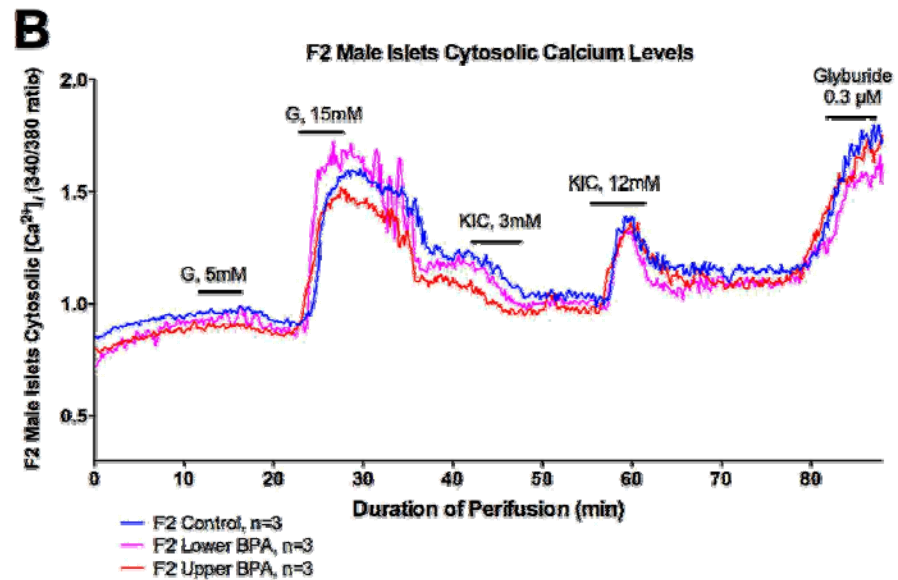
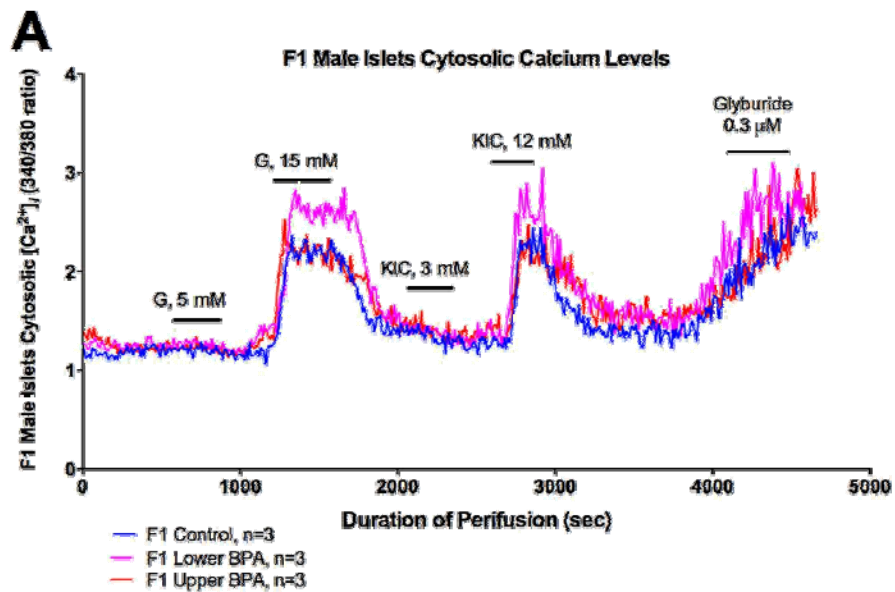


Fig. S5. Islet cytosolic calcium levels in (A) F1 males, and (B) F2 males. Data are expressed as mean values. G: glucose; KIC: α -ketoisocaproate.

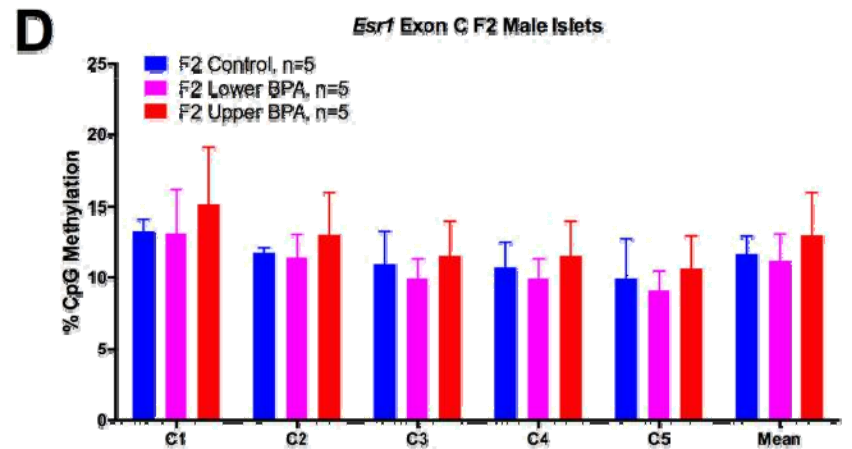
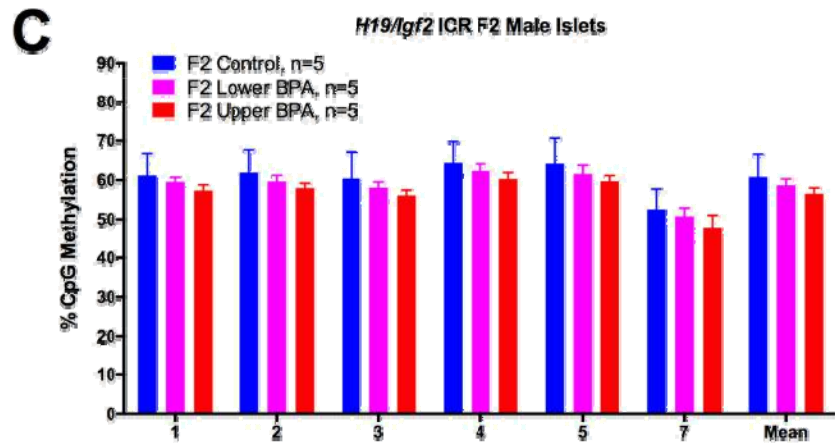
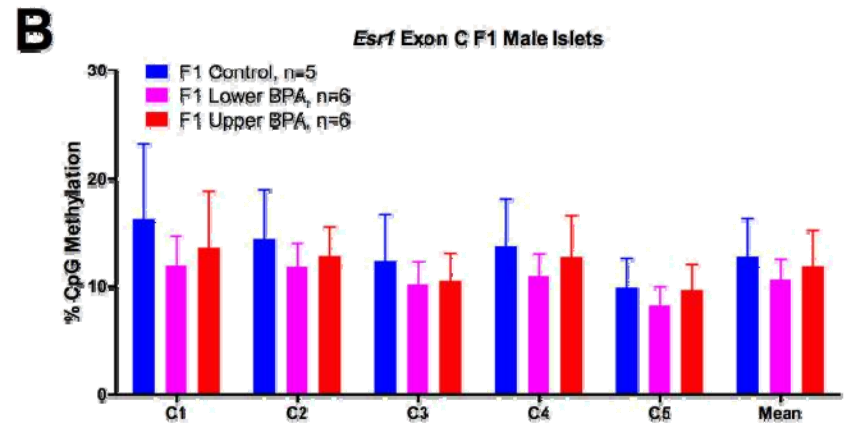
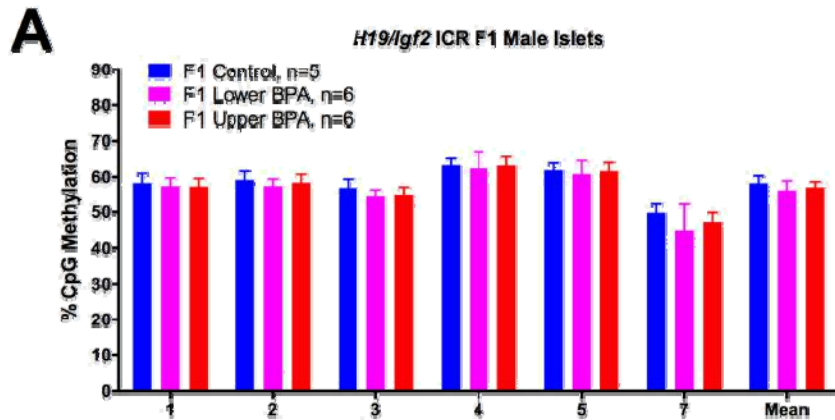


Fig.S6. Percent DNA methylation at *H19/Igf2* ICR (*H19* 1524JH) and *Esr1* Exon C in islets: (A) F1 and (B) F2 adult male offspring. Data are percent CpG methylation values from individual litter (islets pooled from 2-3 males per litter) and presented as mean + SEM. Data were analyzed using Dunnett's test. For %methylation at all CpG cites as well as mean methylation across all sites, p values were >0.1 relative to Control.

Table S1. Custom designed TaqMan Primers and Probes Assays for qPCR.

Serial #	Gene Name	Species	Assay ID	Amplicon Length (bp)	Assay Design
1	<i>Pdx1</i>	Species: Mouse	Mm00435565_m1	74	Probe spans exons
2	<i>Igf1</i>	Species: Mouse	Mm00439560_m1	77	Probe spans exons
3	<i>Igf2</i>	Species: Mouse	Mm00439564_m1	107	Probe spans exons
4	<i>Esr1</i>	Species: Mouse	Mm00433147_m1	99	Probe spans exons
5	<i>Hnf1a</i>	Species: Mouse	Mm00493434_m1	134	Probe spans exons
6	<i>Ucp2</i>	Species: Mouse	Mm00627599_m1	137	Probe spans exons
7	<i>Ogdh</i>	Species: Mouse	Mm00803119_m1	90	Probe spans exons
8	<i>Kcnj11</i>	Species: Mouse	Mm00440050_s1	129	Both primers and probe map within a single exon
9	<i>Abcc8</i>	Species: Mouse	Mm00803458_g1	106	Probe spans exons
10	<i>Snap25</i>	Species: Mouse	Mm00456922_m1	90	Probe spans exons
11	<i>Beta actin</i>	Species: Mouse	Mm00607939_s1	115	Both primers and probe map within a single exon
12	<i>Cyclopilin A</i>	Species: Mouse	Mm02342430_g1	148	Probe spans exons
13	<i>Rpl19</i>	Species: Mouse	Mm02601633_g1	69	Probe spans exons
14	<i>Hprt</i>	Species: Mouse	Mm01545399_m1	81	Probe spans exons

Table S2. PCR and Pyrosequencing Primers for *Igf2* DMR1, *H19/Igf2* ICR, and *Esr1*

Genes	Primer Type	Primer Sequence	CpG sites
<i>Igf2</i> DMR1	PCR forward primer	5'- TGAGGTTAGATTAGGTTGTAAGTT-3'	
	PCR reverse-biotinylated primer	5'- /5Biosg/CTTCCCTACCCCTTAAACC -3'	
	Pyrosequencing primer S1	5'- GGATTTTGTTAGGTAGGA -3'	1 and 2
<i>H19/Igf2</i> ICR	PCR forward primer	5'- GGGTAGGATATATGTATTTTTTTAGGTTG -3'	
	PCR reverse-biotinylated primer	5'- /5Biosg/CTCATAAAACCCATAACTATAAAATCAT -3'	
	Pyrosequencing primer	5'- TGTAAGATTAGGGTTGT- 3'	1, 2, 3, 4, 5, and 7
<i>Esr1</i> Exon A	PCR forward primer	5'- TGGGTTATTTGTGTTTTGTAGGATAG -3'	
	PCR reverse-biotinylated primer	5'- /5Biosg/CTTAAATCTAATACAACAAAACCATTC -3'	
	Pyrosequencing primer F1	5'- GGTAGGGTTAGGGTTAGTAT -3'	A1-A4
	Pyrosequencing primer F2	5'- AGGTTTTATTTTTTTTTTTTAGGTGG -3'	A5-A11
<i>Esr1</i> Exon C	PCR forward primer	5'- TATGGGTTTGTAGAAGTTAAGGGTTGAG -3'	
	PCR reverse-biotinylated primer	5'- /5Biosg/CCAAATACCCTACCTACTAACTACTTCC -3'	
	Pyrosequencing primer F1	5'- GAAGTTAAGGGTTGAGATA -3'	C1-C5

Table S3. Changes in cytokine/chemokine levels on a LUMINEX assay in pancreatic lysates of F1 adult male offspring. N=3 to 4 litters per group. Data are normalized to total protein concentration as pg of cytokine or chemokine per μg of total protein, and presented as mean \pm SEM. Decimal places are represented by E notation, where E represents base 10, followed by the power of 10 (example, E-2 = 10^{-2} , E-3 = 10^{-3} , E-4 = 10^{-4}). P values are from Dunnett's test performed on log-transformed data, where required (g-csf, IL9, IL15, and RANTES). Control: 7% corn oil diet; LowerB: Lower BPA (10 $\mu\text{g}/\text{kg}/\text{day}$); UpperB: Upper BPA (10 $\text{mg}/\text{kg}/\text{day}$).

Cytokine/ Chemokine	Produced By	Respond To	Control pg/ μ g	LowerB pg/ μ g	UpperB pg/ μ g	LowerB p value	UpperB p value
G-CSF	Neutrophil	growth	1E-3 \pm 4E-4	1E-3 \pm 5E-3	2E-3 \pm 5E-4	0.99	0.04
GM-CSF	Monocyte growth		2E-3 \pm 5E-4	2E-3 \pm 6E-4	3E-3 \pm 6E-4	0.96	0.49
IL1a	Macrophages, Neutrophils, Epithelial and Endothelial cells	cells with IL1a receptor	6E-3 \pm 2E-3	3E-3 \pm 2E-3	5E-3 \pm 2E-3	0.32	0.86
IL2		Immature T cells \nearrow Treg	13E-4 \pm 2E-4	13E-4 \pm 3E-4	12E-4 \pm 3E-4	0.99	0.94
IL9	CD4 ⁺ cells	Cells that have IL9R, \times co-stimulatory molecules of Macrophages, \times B-cells, \times NFkB	6E-3 \pm 4E-3	7E-3 \pm 6E-3	18E-3 \pm 5E-3	0.94	0.07
IL10	mostly by Monocytes, less commonly by T _H 2, Mast and T _{Reg} cells	\times T _H 1, \times MHCII	6E-3 \pm 2E-3	11E-3 \pm 2E-3	5E-3 \pm 2E-3	0.29	0.78
IL12p40	activated Macrophages	T _H 1, Natural killer cells	1E-3 \pm 1E-3	2E-3 \pm 2E-3	5E-3 \pm 2E-3	0.89	0.25
IL13	T _H 2	Same cells that respond to IL4	2E-3 \pm 5E-4	1E-3 \pm 6E-4	1E-3 \pm 6E-4	0.34	0.15
IL15	Macrophages	\times T cell growth	2E-3 \pm 7E-4	3E-3 \pm 8E-4	5E-3 \pm 8E-4	0.88	0.12
IP10	Macrophages Endothelial cells, Fibroblast	attracts Macrophages, T-cells, Natural killer cells, Dendritic cells,	3E-3 \pm 1E-3	2E-3 \pm 1E-3	4E-3 \pm 1E-3	0.53	0.81
MIP2	Macrophages	Neutrophils, Stem cells	5E-3 \pm 1E-3	4E-3 \pm 1E-3	7E-3 \pm 1E-3	0.99	0.21
RANTES	+ CD8 ⁺ cells	Chemoattractant, T cells, Eosinophils, Basophils, Natural killer cell proliferation	5E-4 \pm 2E-4	3E-4 \pm 2E-4	4E-4 \pm 2E-4	0.75	0.99

Table S4. Changes in cytokine/chemokine levels on a LUMINEX assay in pancreatic lysates of F2 adult male offspring. N=3 to 4 litters per group. Data are normalized to total protein concentration as pg of cytokine or chemokine per μg of total protein, and presented as mean (SEM). Decimal places are represented by E notation, where E represents base 10, followed by the power of 10 (example, E-2 = 10^{-2} , E-3 = 10^{-3} , E-4 = 10^{-4}). p values are from Dunnett's test performed on log-transformed data, where required (IL2, IL9, IL10, IL12p40, IL15, IP10, and MIP2). Control: 7% corn oil diet; LowerB: Lower BPA (10 $\mu\text{g}/\text{kg}/\text{day}$); UpperB: Upper BPA (10 $\text{mg}/\text{kg}/\text{day}$).

Cytokine/ Chemokine	Produced By	Respond To	Control pg/ μ g	LowerB pg/ μ g	UpperB pg/ μ g	LowerB p value	UpperB p value
G-CSF	Neutrophil growth		1E-3 \pm 5E-4	2E-3 \pm 5E-4	2E-3 \pm 5E-4	0.36	0.19
GM-CSF	Monocyte growth		1E-3 \pm 6E-4	2E-3 \pm 6E-4	2E-3 \pm 6E-4	0.59	0.74
IL1a	Macrophages, Neutrophils, Epithelial and Endothelial cells	cells with IL1a receptor	1E-3 \pm 2E-2	1E-3 \pm 2E-2	25E-3 \pm 1E-2	0.99	0.75
IL2		Immature T cells \rightarrow Treg	1E-3 \pm 2E-3	1E-3 \pm 2E-3	3E-3 \pm 2E-3	0.86	0.88
IL9	CD4 ⁺ cells	Cells that have IL9R, \rightarrow co-stimulatory molecules of Macrophages, \rightarrow B-cells, \rightarrow NFkB	23E-3 \pm 5E-2	78E-3 \pm 5E-2	92E-3 \pm 5E-2	0.79	0.95
IL10	mostly by Monocytes, less commonly by T _h 2, <small>Mast and T_{reg} cells</small>	\rightarrow T _h 1, \rightarrow MHCII	13E-3 \pm 1E-2	7E-3 \pm 1E-2	17E-3 \pm 1E-2	0.85	0.72
IL12p40	activated Macrophages	T _h 1, Natural killer cells	2E-3 \pm 2E-3	2E-3 \pm 2E-3	4E-3 \pm 1E-3	0.73	0.55
IL13	T _h 2	Same cells that respond to IL4	1E-3 \pm 3E-4	1E-3 \pm 3E-4	1E-3 \pm 3E-4	0.39	0.99
IL15	Macrophages	\rightarrow T cell growth	3E-3 \pm 5E-3	7E-3 \pm 5E-3	11E-3 \pm 4E-3	0.51	0.50
IP10	Macrophages Endothelial cells, Fibroblast	attracts Macrophages, T-cells, Natural killer cells, Dendritic cells,	3E-3 \pm 3E-3	3E-3 \pm 3E-3	6E-3 \pm 3E-3	0.91	0.83
MIP2	Macrophages	Neutrophils, Stem cells	1E-2 \pm 1E-2	14E-3 \pm 1E-2	24E-3 \pm 1E-2	0.75	0.48
RANTES	+ CD8 cells	Chemoattractant, T cells, Eosinophils, Basophils, Natural killer cell proliferation	1E-3 \pm 2E-4	1E-3 \pm 2E-4	1E-4 \pm 2E-4	0.35	0.24