Supplemental Material – Archives of Toxicology

Bisphenol A Alteration Of Type 1 Diabetes In Non-obese Diabetic (NOD) Female Mice Is Dependent On Window Of Exposure

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Supplementary Methods.

Library Preparation And Bioinformatics Analysis

For library preparation, DNA was normalized to 5 ng/μL. Locus-specific primers (forward: S-D-Bact-0564-a-S-15, and reverse: S-D-Bact-0785-a-A-21) targeting the V3-V4 region of 16S rRNA and KAPA HiFi HotStart ReadyMix from Kapa Biosystems, Inc. (Boston, MA) were used for the first round of PCR. Next, the PCR amplicon was purified and quality assessed using AMPure beads and a plate fluorometer, respectively. The second round PCR was run using Illumina i5 and i7 dual indices and the KAPA HiFi HotStart ReadyMix. The PCR products were again purified with AMPure beads and quality was assessed with a Qubit and quantitative PCR. Lastly, sequencing was carried out on an Illumina Miseq (Illumina Inc., San Diego, US).

Read 1 and Read 2 sequence files for each sample were merged with Geneious version 10.2.3 (https://www.geneious.com). Bioinformatics analysis was performed as previously described (Huang et al. 2017; Lefever et al. 2016). In brief, merged files were demultiplexed and filtered using a quality score of 1 in 100 error rate. This was then analyzed using Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.1 (Caporaso et al. 2010). The workflows used were *pick_de_novo_otus.py* for OTU picking and then *core_diversity_analysis.py* for beta and alpha diversity analysis. Significantly different taxa were identified using Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis following the website conditions: http://huttenhower.sph.harvard.edu/galaxy and increasing the LDA score to > 3 (Segata et al. 2011).

Supplementary Discussion

For female offspring switched to the soy-based diet, BPA had no effect on T1D, BGLs or GTT. Though, some of the cytokines/chemokines measured on PND218 were decreased including reduced RANTES levels similar to the phytoestrogen-free diet.

Additionally, decreased IL-6 and MIP-2, which are pro-inflammatory and involved in T1D pathogenesis (Haurogné et al. 2015; Hundhausen et al. 2016), suggested lasting protective effects from BPA exposure similar to the phytoestrogen-free diet female offspring. However, the decreased G-CSF, which has protective effects on pancreatic β-cell function (Haller et al. 2016), and lack of effects on immune cells were likely reasons why no effect on T1D or BGLs were observed. This attenuation is consistent with other studies showing genistein, the main phytoestrogen in soy and other legumes, can attenuate BPA's effects such as on macrophage cell cytotoxicity and cytokine/chemokine production *in vitro* and on prostate disease from *in utero* exposure in rats (Bernardo et al. 2015; Chen et al. 2018). While BPA and genistein combinational exposure has also been found to have some additive effects through ER activation, genistein binding to ERs can induce a greater transcriptional response than BPA (Katchy et al. 2014), which may be the reason for the attenuation of BPA's effects on T1D and increase in cytokines/chemokines decreased from BPA exposure in the soy-based diet female offspring.

In males exposed perinatally to BPA on a phytoestrogen-free diet, mice had improved glucose tolerance, which suggests BPA may have some protective effects for T1D in male offspring similar to the female offspring. Consistently, decreased CD40 expression is indicative of protection from T1D development. CD40's involvement in T1D develpment has been indicated from various studies showing blocking CD40 expression can support islet allograft survival in diabetic rhesus macaques, increased CD40 expression in T1D patients and blocking CD40 expression in NOD:SCID mice prevents transfer of diabetes (Badell et al. 2012; Peters et al. 2009). However, male offspring seemed less sensitive to BPA treatment than females, since they did not have alteration of BGLs, diabetic incidence or other immune cells. Previously, perinatal BPA exposure in C57BL6 male mice was shown to cause glucose intolerance and insulin resistance at 3

mo. old, but male offspring became insulin sensitive after 6 mo. of age (Liu et al. 2013). While we did not perform a GTT in our mice at 3 mo. old, our results at 7 mo. old are partly agreeing with the beneficial effects of BPA in 6 mo. old C57BL6 male offspring. Additionally in the male offspring switched to the soy-based diet, BPA had similar effects on the immune cells as the phytoestrogen-free diet female offspring with increased $CD4^+CD8^-$ cells and $B220^+CD40L^-$ B cells, but no changes were observed in their glucose tolerance or BGLs.

BPA had sex-dependent effects on BGLs in perinatally exposed mice. For mice on the phytoestrogen-free diet, BGLs initially were significantly decreased in BPA exposed female offspring compared to males on PND61/58 ($P = 0.001$; PND of female/male, respectively) and PND 75 ($P = 0.0033$), and increased BGLs on PND198/203 ($P = 0.0246$). Additionally, while VH exposure also decreased BGLs on PND 75 ($P = 0.0006$), increased BGLs were observed on PND 148/147 ($P = 0.0019$), PND163/162 ($P = 0.0015$), PND 177/175 ($P = 0.0009$), PND 191 ($P > 0.0001$) and PND198/203 ($P > 0.0001$). For the mice switched to the soy-based diet, the same pattern was seen with BGLs initially decreased on PND61/58 ($P = 0.0017$) and PND 75 ($P = 0.0018$) and increased on PND198/203 ($P = 0.0384$) in BPA exposed female offspring compared to males. VH exposure also decreased BGLs on PND61/58 ($P = 0.0174$) and PND 75 ($P = 0.0247$), and increased BGLs on PND 148/147 ($P = 0.0455$), PND163/162 ($P = 0.0405$), PND 177/175 ($P = 0.0093$), PND 191 ($P > 0.0099$), PND198/203 ($P = 0.0159$) and PND216 ($P = 0.0416$) in females compared to males. The initial sex-dependent decrease in BGLs and later lack on increase of BGLs until PND198/203 in perinatally exposed females compared to males further confirmed that BPA's protective effect on T1D from perinatal exposure was sex-dependent, since the NOD mouse model usually has a greater prevelance of T1D in females than males and higher BGLs are expected in females compared to males (Bao et al. 2002).

Developmental environmental exposures can have differing effects on disease susceptibility in adulthood from genetic and sex hormone differences in pancreatic islets, pancreatic function and immunity (Gannon et al. 2018). Specifically, perinatal BPA exposure has been found to increase β-cell mitochondria defects and β-cell dysfunction from sex-dependent alteration of gene expression from epigenetic mechanisms in male Wistar rats and C57BL6 mice, but not in perinatally exposed females (Bansal et al. 2017; Wei et al. 2011). Unlike these studies, our results showed a greater effect in female offspring than male offspring. This may be due to genetic differences in the mouse model, since NOD mice over 40 gene loci that increased T1D risk similar to humans, T1D development and autoimmunity increased in female NOD mice and/or greater insulin resistence in woman with T1D compared to men with T1D (Al-Awar et al. 2016; Bao et al. 2002; Millstein et al. 2018). Future studies examining genetic and epigenetic differences would help clarify the sex-dependent effects from BPA perinatal exposure.

Table S1. Juvenile female pancreas histopathology score on postnatal day 134

Treatment	Diabetes Status	Histopathology Score
VH	Non-diabetic	1
VH	Non-diabetic	2
VH	Non-diabetic	2
VH	Diabetic	2
VH	Diabetic	2
VH	Non-diabetic	2
30ug/kg	Non-diabetic	2
30ug/kg	Diabetic	2
30ug/kg	Non-diabetic	1
30ug/kg	Diabetic	1
30ug/kg	Diabetic	3

Table S2. Organ weights in female juvenile mice

Absolute Organ Weights (mg)					
Treatment	Spleen	Pancreas	Kidneys	Liver	Thymus
Vehicle	102.8±7.1	178.5±14.4	327.3±16.8	1236.8±76.7	47.8±6.0
30µg/kg BPA	91.8±16.4	173.8±16.1	372.0±30.3	1454.4±126.1	42.0±8.5
Organ to Body Weight (%)					
Treatment	%Spleen	%Pancreas	%Kidneys	%Liver	%Thymus
Vehicle	0.38±0.026	0.66±0.045	1.22±0.06	4.61±0.26	0.18±0.02
30µg/kg BPA	0.37±0.05	0.71±0.041	1.56±0.17	6.02±0.46	0.17±0.03

Data are presented as mean ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxon based on whether equal variance assumption was met as compared to the respective vehicle (VHF) control group. N = 5-6.

Table S3. BPA Modulation of Cytokine/Chemokine Levels in Juvenile and Developmental Females

	Phytoestrogen-free Diet Female Offspring		Soy-based Diet Female Offspring		Juvenile Females	
Cytokine	VHF	300 µg/kg BPA	VHF	300 µg/kg BPA	VHF	30 µg/kg BPA
G-CSF	565.91±263.61	228.59±38.82	267.24±23.85	**170.08±18.14***	209.45±35.05	**359.38±26.19***
EOTAXIN	800.04±137.75	674.78±110.43	1,044.84±156.97	928.36±118.37	961.48±100.62	**651.15±72.90***
GM-CSF	17.41±11.57	13.21±13.21	69.08±36.54	26.10±26.10	2.19±2.19	7.69±7.69
IFN-γ	38.87±20.93	10.79±10.79	52.09±28.01	35.26±32.80	35.69±33.71	8.30±8.30
IL-1α	790.82±110.52	844.76±156.70	887.75±93.39	1,174.07±208.55	500.49±138.87	513.94±47.08
IL-1β	ND	ND	5.35±5.35	7.18±4.40	10.70±6.77	6.42±6.42
IL-2	79.77±38.34	28.24±28.24	67.29±48.27	27.85±21.63	5.42±5.42	ND
IL-3	ND	ND	ND	ND	ND	ND
IL-4	ND	ND	0.26±0.26	ND	ND	ND
IL-5	ND	ND	3.87±3.87	ND	8.76±5.55	**27.01±6.27***
IL-6	9.67±4.77	1.47±1.47	12.36±3.72	**4.50±1.54***	4.53±2.75	4.70±1.42
IL-7	19.11±16.58	77.78±74.69	15.28±6.83	21.06±13.28	14.61±4.17	6.66±3.46
IL-9	683.66±219.30	367.56±148.26	570.10±198.85	396.74±64.28	256.66±42.89	142.08±53.67
IL-10	ND	16.17±16.17	10.25±10.25	16.17±16.17	10.25±10.25	7.01±4.29
IL-12P40	2.92±2.10	ND	3.33±3.33	ND	ND	ND
IL-12P70	15.76±6.24	14.07±5.83	21.00±11.12	3.69±3.69	26.37±8.22	13.57±7.16
LIF	6.64±6.64	0.46±0.46	ND	2.64±2.64	0.20±0.20	ND
IL-13	114.50±42.24	43.75±26.42	151.33±59.62	58.06±26.82	55.38±21.51	33.43±14.75
LIX	12164.80±1291.15	11900.84±1431.80	14,050.93±443.57	13,590.67±839.67	11,987.31±576.65	**14,706.90±785.37***
IL-15	103.58±46.73	620.21±424.72	308.87±19.42	148.89±85.33	242.66±73.73	**68.99±44.95***
IL-17	3.49±2.93	ND	0.72±0.50	ND	ND	ND
IP-10	235.50±22.91	233.86±10.78	286.04±35.93	247.15±30.21	384.97±95.13	291.79±46.20
KC	235.38±48.08	150.41±26.71	129.72±31.30	107.41±15.96	148.35±25.15	140.93±25.14
MCP-1	17.34±17.34	ND	27.96±13.22	ND	30.14±30.14	14.66±8.98
MIP-1α	172.29±75.03	87.39±56.85	219.46±80.65	127.65±53.15	55.22±27.85	66.39±24.51
MIP-1β	47.47±29.19	15.85±15.85	71.75±47.31	40.91±40.91	ND	ND
MCSF	3.00±3.00	3.60±3.60	5.89±5.89	3.60±3.60	ND	7.21±4.41
MIP-2	151.74±37.90	220.43±12.14	263.92±23.48	**172.17±23.10***	203.31±42.50	253.04±15.27
MIG	78.66±15.03	102.92±18.17	107.65±25.30	92.94±34.43	316.62±192.89	197.94±112.36
RANTES	8.60±1.72	**4.05±1.73***	12.48±1.48	**5.96±2.20***	13.08±3.59	7.80±1.41
VEGF	10.04±4.80	3.44±1.32	7.15±2.80	3.60±1.37	2.32±0.38	3.46±2.00
TNF-α	2.01±2.01	ND	ND	ND	2.01±2.01	2.41±2.41

Abbreviations: G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; LIF, leukemia inhibitory factor; IP-10, interferon γ-induced protein 10; KC, keratinocyte chemoattractant; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; VEGF, vascular endothelial growth factor; ND, not detected. Cytokines/chemokines were from serum obtained at euthanization (PND 204, 218 or 134 for phytoestrogen-free diet female offspring, soy-based diet female offspring or juvenile females respectively). Data are presented as mean pg/mL ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxin based on whether equal variance assumption was met as compared to the respective vehicle (VHF) control group. N = 5-6.

Table S4. Alteration of organ weights after adult female BPA exposure in mice on a phytoestrogen-free diet

Absolute Organ Weights (mg)					
Treatment	Spleen	Pancreas	Kidneys	Liver	Thymus
Vehicle	79.3±2.0	152.8±8.0	318.6±9.1	905.0±21.3	28.8±3.7
300µg/kg BPA	**86.3±2.2***	**121.0±15.6***	328.7±17.4	995.8±54.4	24.7±4.7
Organ to Body Weight (%)					
Treatment	%Spleen	%Pancreas	%Kidneys	%Liver	%Thymus
Vehicle	0.30±0.01	0.58±0.03	1.21±0.02	3.43±0.07	0.11±0.01
300µg/kg BPA	0.32±0.01	**0.45±0.06***	1.21±0.04	3.66±0.12	0.09±0.02

Data are presented as mean ± SEM. *, $p < 0.05$ using Dunnett's test or Wilcoxin based on whether equal variance assumption was met as compared to the respective vehicle (VH) control group. N = 9.

Table S5. Organ weights in phytoestrogen-free diet female offspring

Absolute Organ Weights (mg)					
Treatment	Spleen	Pancreas	Kidneys	Liver	Thymus
Vehicle	83.45±6.89	147.45±5.36	352.36±18.95	1,112.00±57.80	31.64±4.47
300µg/kg BPA	81.67±5.12	122.60±12.46	332.07±16.80	1,003.07±38.01	31.67±3.20
Organ to Body Weight (%)					
Treatment	Spleen%	Pancreas%	Kidneys%	Liver%	Thymus%
Vehicle	0.33±0.02	0.59±0.02	1.41±0.08	4.43±0.25	0.13±0.02
300µg/kg BPA	0.32±0.01	0.49±0.05	1.31±0.07	3.95±0.15	0.12±0.01

Data are presented as mean ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxon based on whether equal variance assumption was met as compared to the respective vehicle control group. N = 11-15.

Table S6. Organ weights in soy-based diet female offspring

Absolute Organ Weights (mg)					
Treatment	Spleen	Pancreas	Kidneys	Liver	Thymus
Vehicle	81.67±2.74	169.44±6.50	310.11±12.36	1,085.67±51.62	31.78±2.84
300µg/kg BPA	84.00±3.52	136.22±16.76	312.89±10.55	1,090.67±42.87	36.22±1.39
Organ to Body Weight (%)					
Treatment	Spleen%	Pancreas%	Kidneys%	Liver%	Thymus%
Vehicle	0.31±0.01	0.65±0.02	1.19±0.03	4.16±0.15	0.12±0.01
300µg/kg BPA	0.31±0.01	0.51±0.06	1.17±0.03	4.08±0.13	0.14±0.00

Data are presented as mean ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxon based on whether equal variance assumption was met as compared to the respective vehicle control group. N = 10.

Table S7. Organ weights in phytoestrogen-free diet male offspring

Absolute Organ Weights (mg)					
Treatment	Spleen	Pancreas	Kidneys	Liver	Thymus
Vehicle	98.7±8.6	220.3±13.4	607.7±19.8	1906.1±102.7	20.0±1.3
300μg/kg BPA	87.9±4.1	204.3±14.6	624.7±21.6	1835.7±77.4	20.7±1.7
Organ to Body Weight (%)					
Treatment	Spleen%	Pancreas%	Kidneys%	Liver%	Thymus%
Vehicle	0.25±0.02	0.57±0.04	1.57±0.02	4.89±0.15	0.05±0.00
300μg/kg BPA	0.23±0.01	0.53±0.03	1.63±0.05	4.77±0.11	0.05±0.00

Data are presented as mean ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxon based on whether equal variance assumption was met as compared to the respective vehicle control group. N = 15.

Table S8. Body mass, fat and fluid levels in soy-based diet male offspring

Treatment	Vehicle	300µg/kg BPA
Mass (g)	37.15±1.49	**34.73±1.34***
Fat (g)	6.94±0.28	6.72±0.62
%Fat	18.59±0.74	19.29±1.33
Fluid (g)	2.85±0.10	2.78±0.12
%Fluid	7.62±0.21	**8.13±0.18***
Lean Fat (g)	24.22±0.84	22.31±0.80
%Lean Fat	64.45±0.91	65.44±0.64

Data are presented as mean ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxon based on whether equal variance assumption was met as compared to the respective vehicle control group. N = 13-14.

Table S9. Organ weights in soy-based diet male offspring

Absolute Organ Weights (mg)					
Treatment	Spleen	Pancreas	Kidneys	Liver	Thymus
Vehicle	105.5±9.7	261.4±9.2	613.1±24.8	2005.0±103.3	22.3±1.6
300µg/kg BPA	86.7±25.0	**208.6±60.2***	593.0±171.2	1803.8±520.7	22.0±6.4
Organ to Body Weight (%)					
Treatment	Spleen%	Pancreas%	Kidneys%	Liver%	Thymus%
Vehicle	0.27±0.02	0.68±0.01	1.58±0.03	5.14±0.15	0.06±0.00
300µg/kg BPA	0.24±0.07	**0.56±0.16***	1.63±0.47	4.94±1.43	0.06±0.02

Data are presented as mean ± SEM. *, $p < 0.05$ using Student's t-test or Wilcoxon based on whether equal variance assumption was met as compared to the respective vehicle control group. N = 12-13.

Supplementary Figure 1

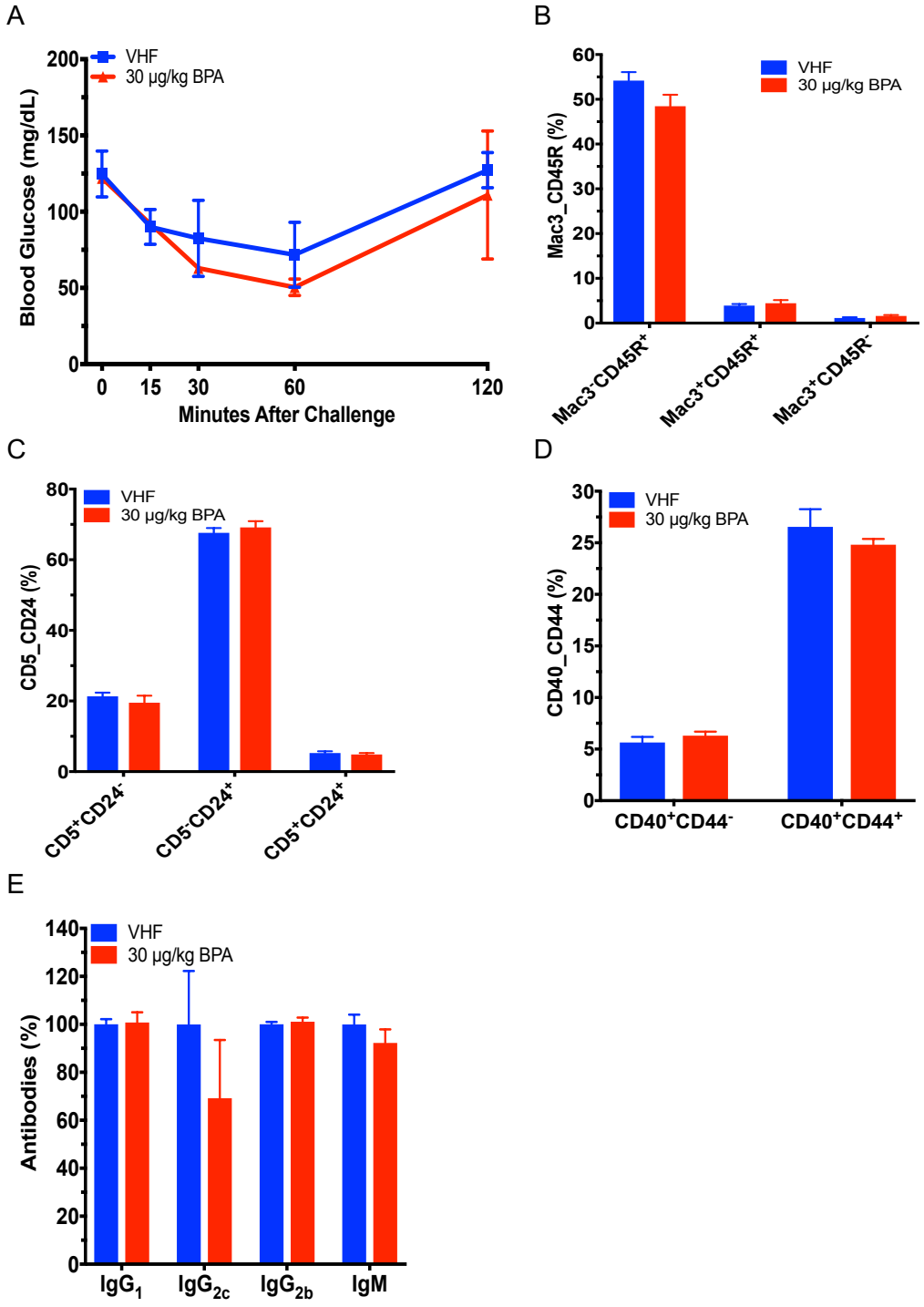

Figure S1. Insulin tolerance test, splenic immune cell populations and antibody levels in BPA exposed juvenile females that were fed with the soy-based PicoLab diet. (a) ITT at 4 mo. old. (b) Splenic %Mac3^{-}CD45R^{+}, Mac3^{+}CD45R^{+} and Mac3^{+}CD45R^{-} cells; (c) Splenic CD5^{+}CD24^{-}, CD5^{-}

$CD24^+$ and $CD5^+CD24^+$ cells; and (d) Splenic $\%CD40^+CD44^-$ and $CD40^+CD44^+$ cells. (e) IgG_1, IgG_{2c}, IgG_{2b} and IgM in NOD males, which were measured at dilutions of 1:2,000, 1:2,000, 1:50 and 1:1,000, respectively, following titration by serial dilution. Measured concentrations were between N = 5-6, except for ITT (b) where N = 2-4. Statistical analysis was not done for ITT due to the low number of nondiabetic BPA mice (N = 2). VHF, vehicle females.

Supplementary Figure 2

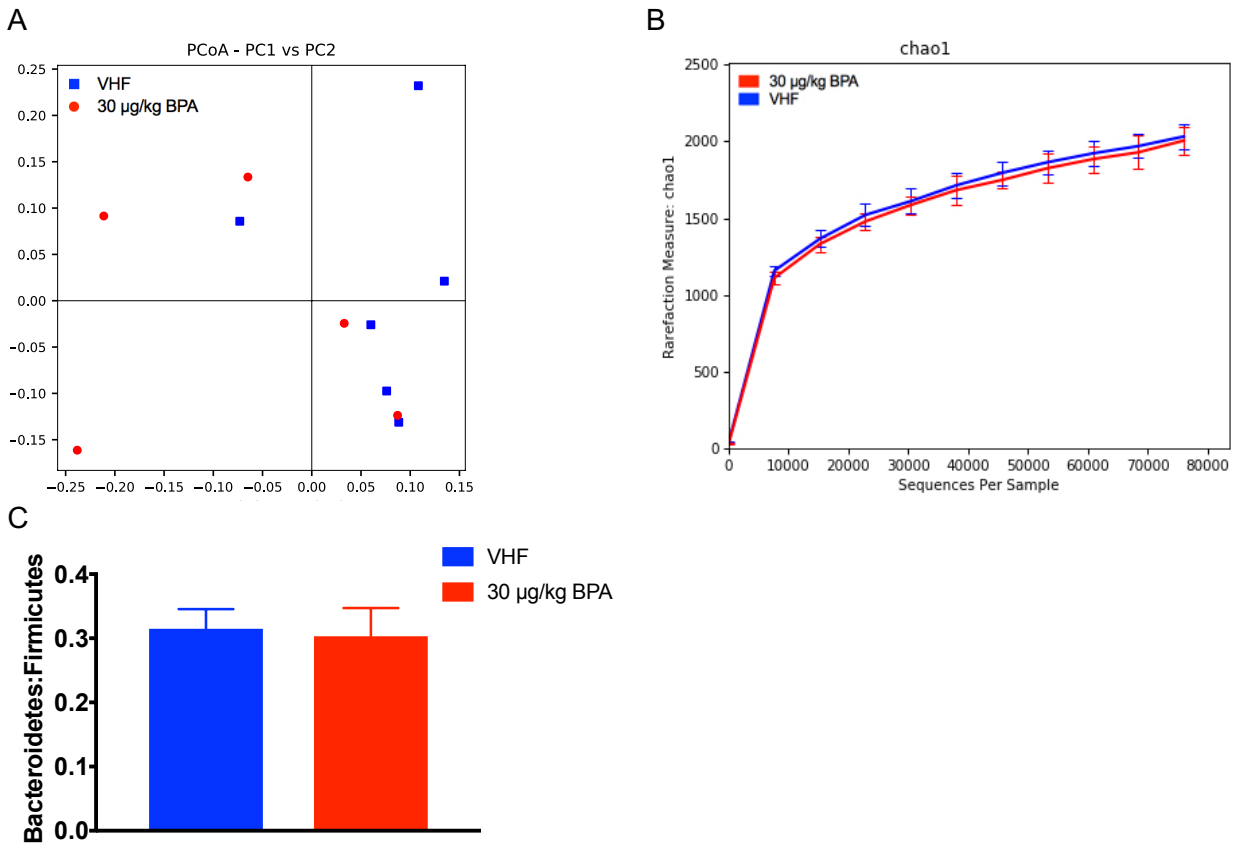

Figure S2. Unweighted beta diversity, alpha diversity and correlation between significantly abundant microbiota and different phenotypic endpoints or BGLs in juvenile exposed females that were fed with the soy-based PicoLab diet. (a) Unweighted UniFrac beta diversity. (b) Chao1 alpha diversity. (c) Bacteroidetes to Firmicutes ratio. N = 5-6. VHF, vehicle females.

Supplementary Figure 3

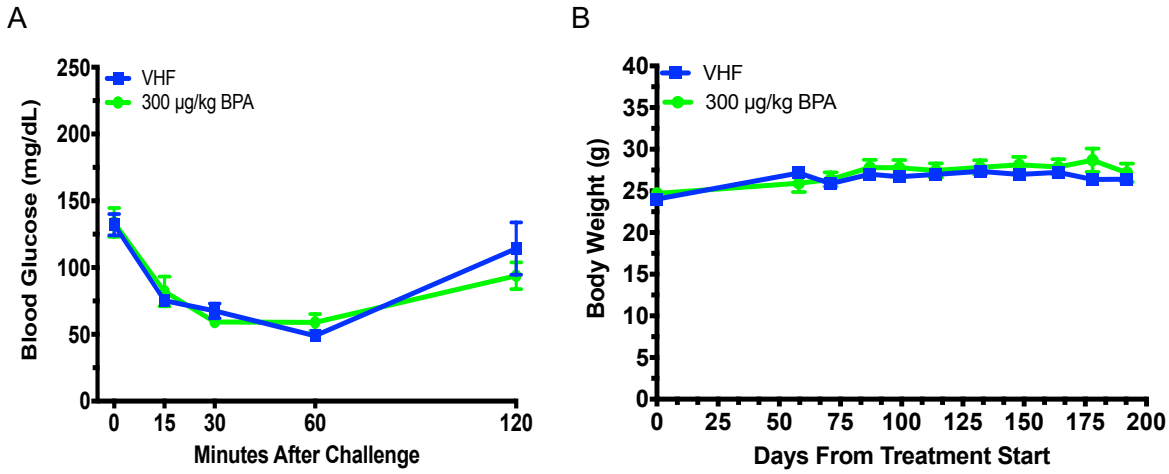

Figure S3. Insulin tolerance tests (ITT) and body weight in adult females on phytoestrogen-free diet. (a) ITT of non-diabetic adult females at 6 mo. after initial dose. (b) Body weight changes over time. The values are presented as mean ± SEM. *, $p < 0.05$ using Dunnett's test or Wilcoxin based on whether equal variance assumption was met as compared to the respective vehicle (VH) control group. N = 8-15. VHF, vehicle females.

Supplementary Figure 4

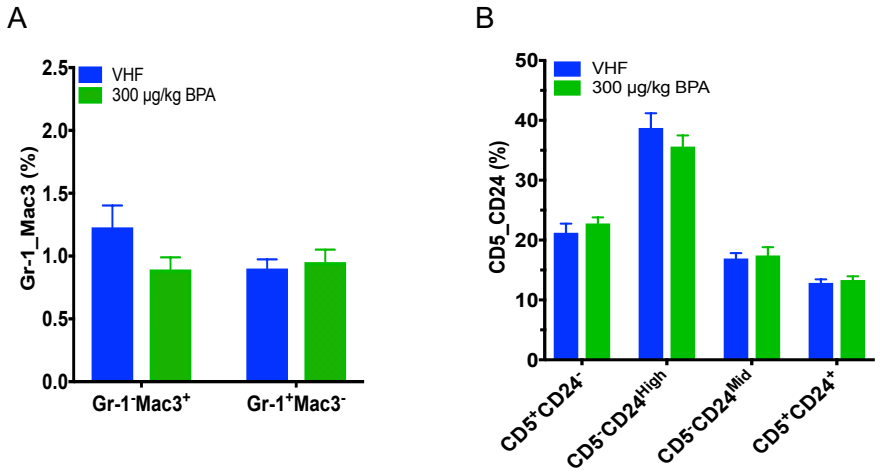

Figure S4. Splenic immune cell populations in BPA exposed adult females on phytoestrogen-free diet. (a) Splenic %Gr-1^{-}Mac3^{+} and Gr-1^{+}Mac3^{-} cells; (b) Splenic CD5^{+}CD24^{-}, CD5^{-}CD24^{+} and CD5^{+}CD24^{+} cells. N = 8-9. VHF, vehicle females.

Supplementary Figure 5

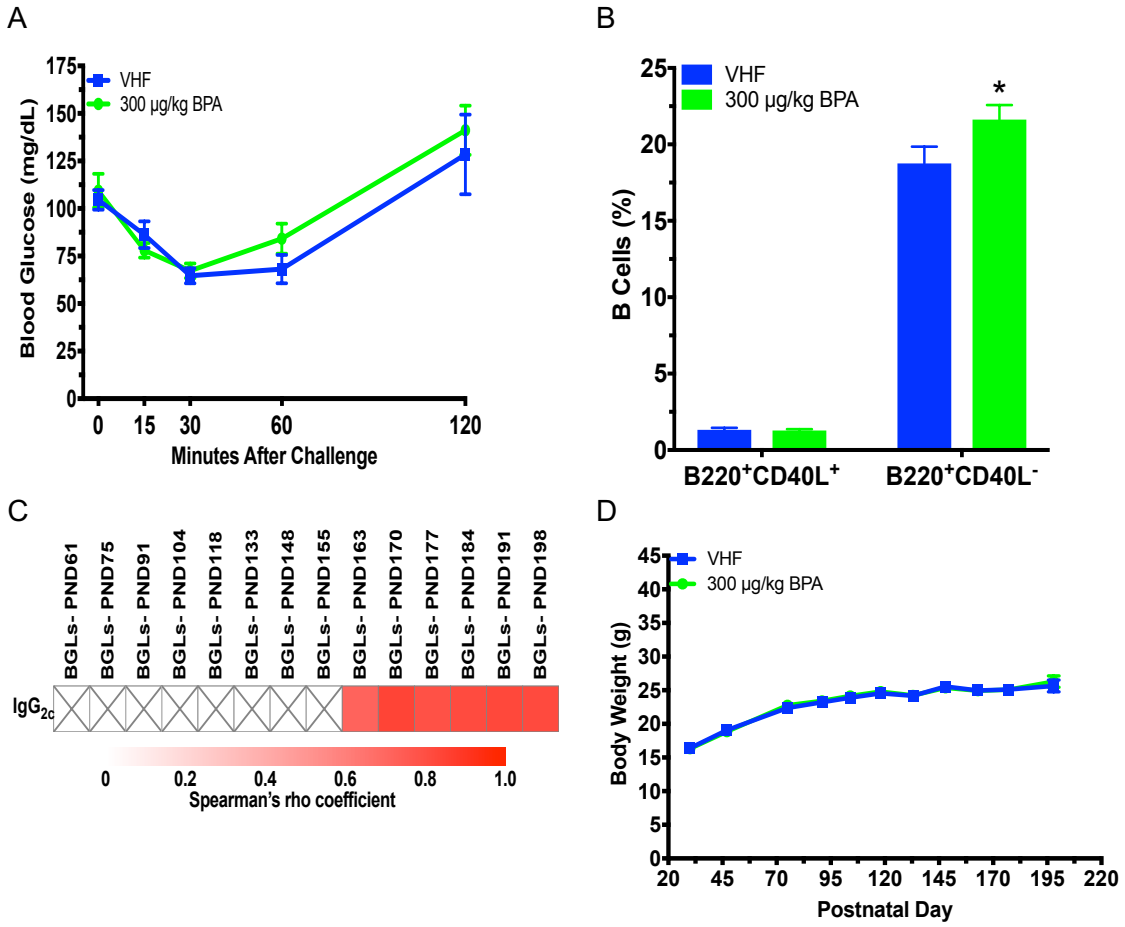

Figure S5. Insulin tolerance tests (ITT), B cells and body weight in female offspring on phytoestrogen-free diet. (a) ITT conducted at 7 mo. old on non-diabetic mice. (VHF, N = 5; BPA, N = 11). (b) %Splenic B cell populations. (N = 11-13) (c) Correlation between significant immune endpoint IgG_{2c} and different timepoints of BGLs represented on a heatmap with Spearman rho's correlation coefficients with red showing significantly positive correlation using Spearman correlation test ($p < 0.05$; N = 6). Blank boxes with an X indicate no significant correlation. (d) Body weight changes over time (N = 11-20). The values are presented as mean ± SE. *, $p<$ 0.05 as compared to the respective vehicle female (VHF) control group.

Supplementary Figure 6

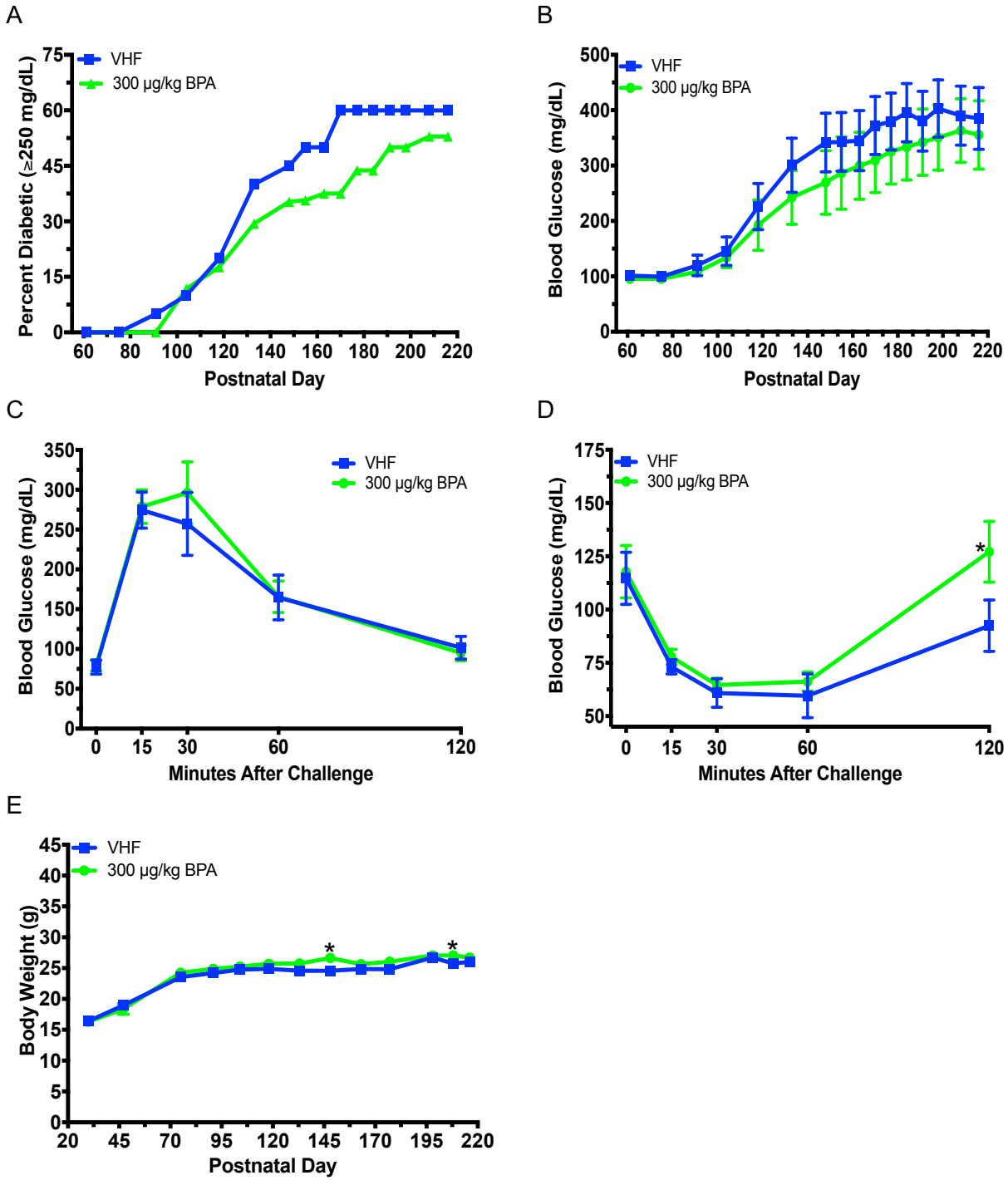

Figure S6. Diabetic incidence, changes in BGLs over time, glucose and insulin tolerance tests (GTT and ITT), and body weight in female offspring on soy-based diet. (a) T1D incidence. Blood

glucose ≥250 mg/dL was considered diabetic (N = 17-20). (b) Time course of BGLs (N = 17-20). (c) GTT was conducted on non-diabetic mice at 5 mo. old (N = 9-10). (d) ITT conducted at 7 mo. old on non-diabetic mice. (N = 7-8). (e) Body weight changes over time (N = 9-20). The values are presented as mean ± SE. *, $p < 0.05$ as compared to the respective vehicle female (VHF) control group.

Supplementary Figure 7

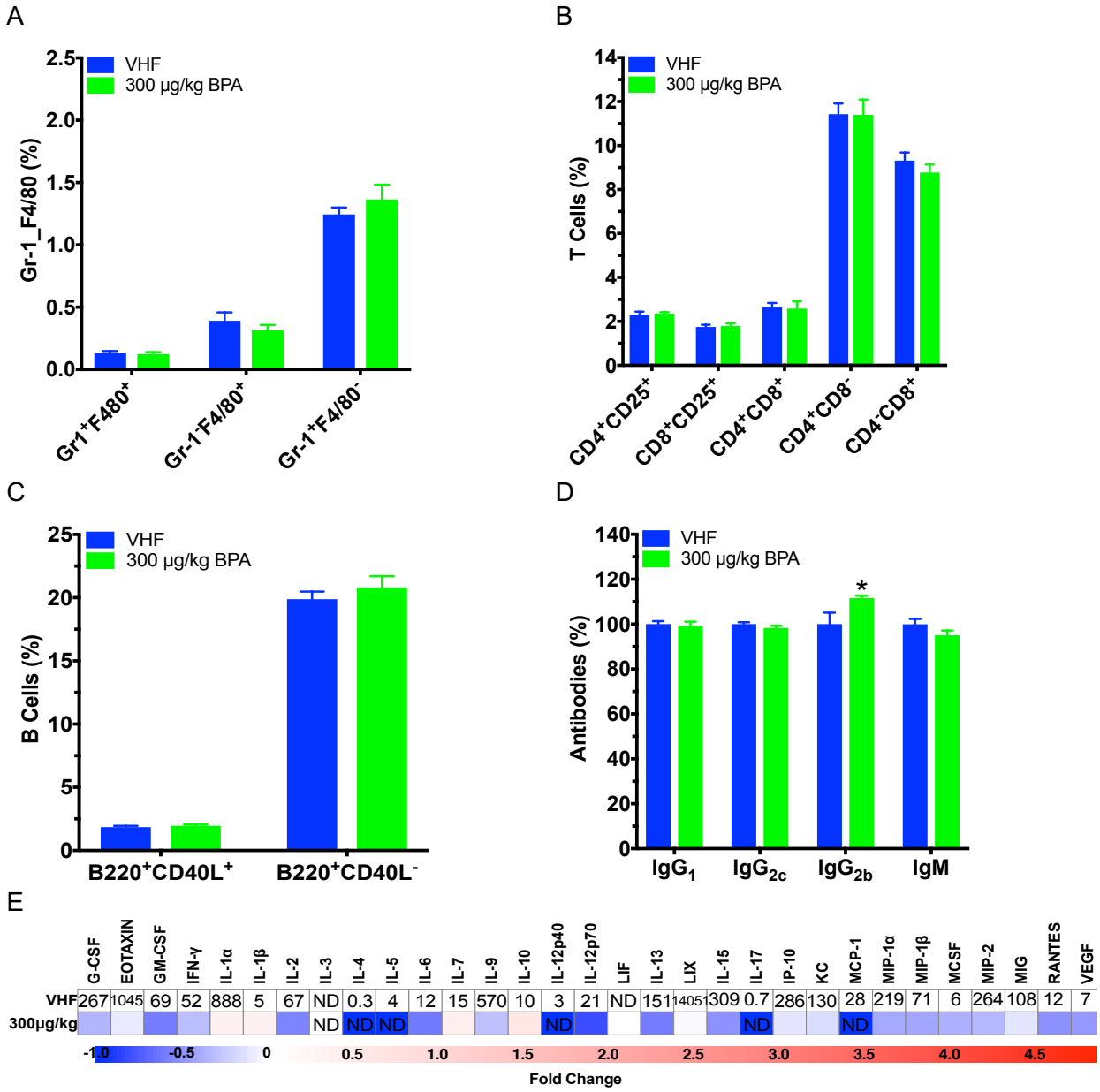

Figure S7. Immune cell alterations in perinatally BPA exposed female NOD offspring on soy-based PicoLab diet. (a) %Gr-1^{+}F4/80^{+}, Gr-1^{-}F4/80^{+} and Gr-1^{+}F4/80^{-} spleen cells. (b) %T cell populations. (c) %B cell populations. (d) IgG_1, IgG_{2c}, IgG_{2b} and IgM were measured at dilutions of 1:2,000, 1:2,000, 1:25 and 1:1,000, respectively, following titration by serial dilution from serum collected at euthanasia (PND218). (e) Heat map of serum cytokine/chemokine changes at time of euthanasia (PND218). Values of VHF are shown as mean pg/mL. ND, not detected. The values

are presented as mean ± SEM. *p< 0.05 as compared to the vehicle female (VHF) control group. N = 9-10 for immune cells; N = 6 for antibodies and cytokines/chemokines.

Supplementary Figure 8

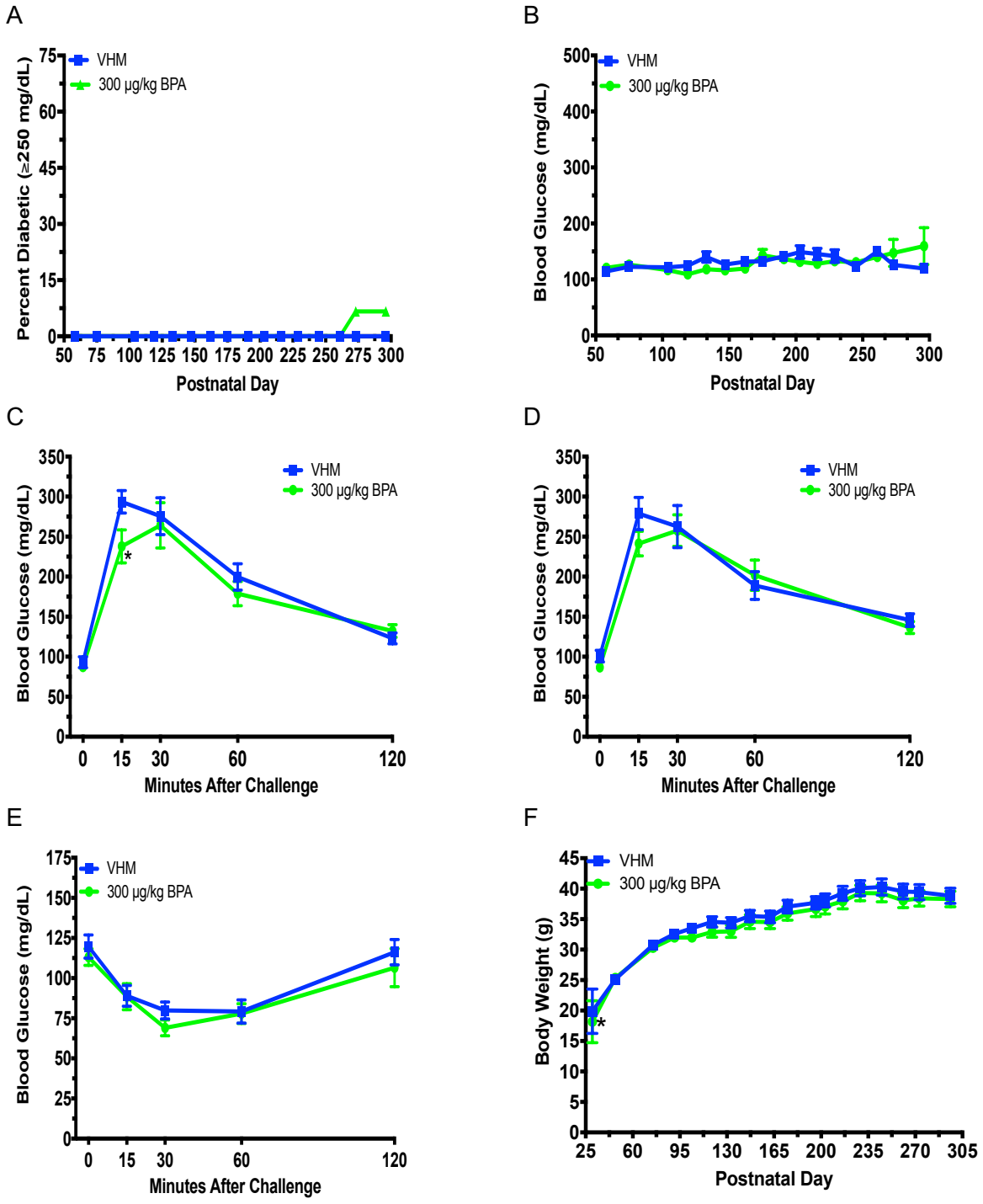

Figure S8. Diabetic incidence, changes in BGLs over time, glucose tolerance tests (GTT), insulin tolerance test (ITT) and body weight showed little effect from perinatal BPA exposure in phytoestrogen-free diet male offspring. (a) Male offspring T1D incidence. Blood glucose ≥250

mg/dL was considered diabetic. Time course of BGLs (b) and GTT at 7 mo. (c) and 9 mo. (d) after initial exposure on non-diabetic mice. (e) ITT in non-diabetic male offspring conducted at 9 mo. old. (f) Body weight changes over time. The values are presented as mean ± SEM. *, $p < 0.05$ as compared to the respective vehicle male (VHM) control group. N = 14-15.

Supplementary Figure 9

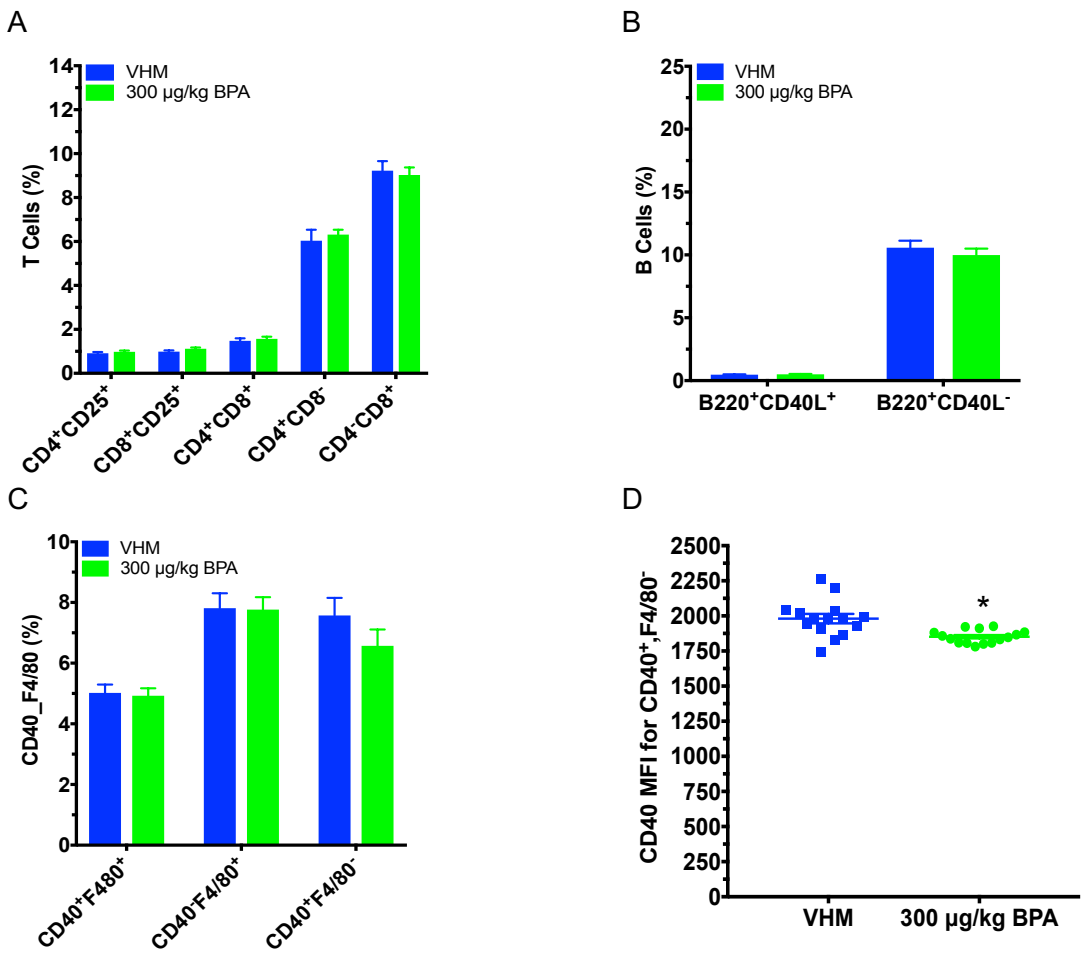

Figure S9. Immune cell populations of perinatally exposed male offspring on the phytoestrogen-free diet. (a) %T cell populations in spleen. (b) %B splenic cell populations. (c) % $CD40^{+}F4/80^{+}$, $CD40^{-}F4/80^{+}$ and $CD40^{+}F4/80^{-}$ spleen cells. (d) CD40 MFI for $CD40^{+}F4/80^{-}$ spleen cells. The values are presented as mean ± SEM. *, $p< 0.05$ as compared to the respective vehicle male (VHM) control group. N = 14-15.

Supplementary Figure 10

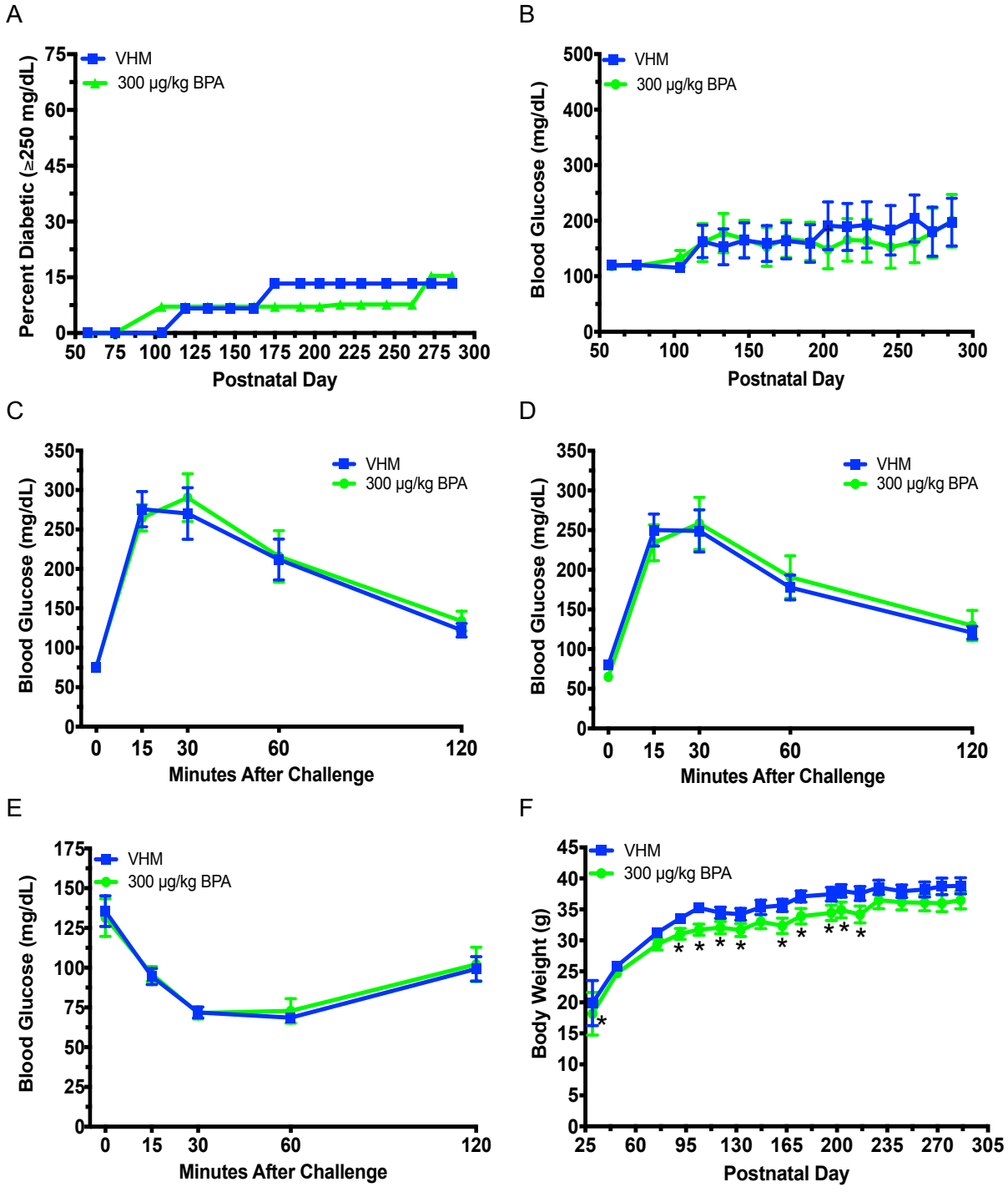

Figure S10. Perinatal BPA exposure in soy-based diet male offspring only decreased body weight, but not diabetic incidence, changes in BGLs over time, glucose tolerance tests (GTT), or insulin tolerance test (ITT). (a) Male offspring T1D incidence. Blood glucose ≥250 mg/dL was considered

diabetic. Time course of BGLs (b) and GTT at 7 mo. (c) and 9 mo. (d) after initial exposure on non-diabetic mice. (e) ITT conducted at 9 mo. old. (f) Body weight changes over time. The values are presented as mean ± SEM. *, $p < 0.05$ as compared to the respective vehicle male (VHM) control group. N = 11-15.

Supplementary Figure 11

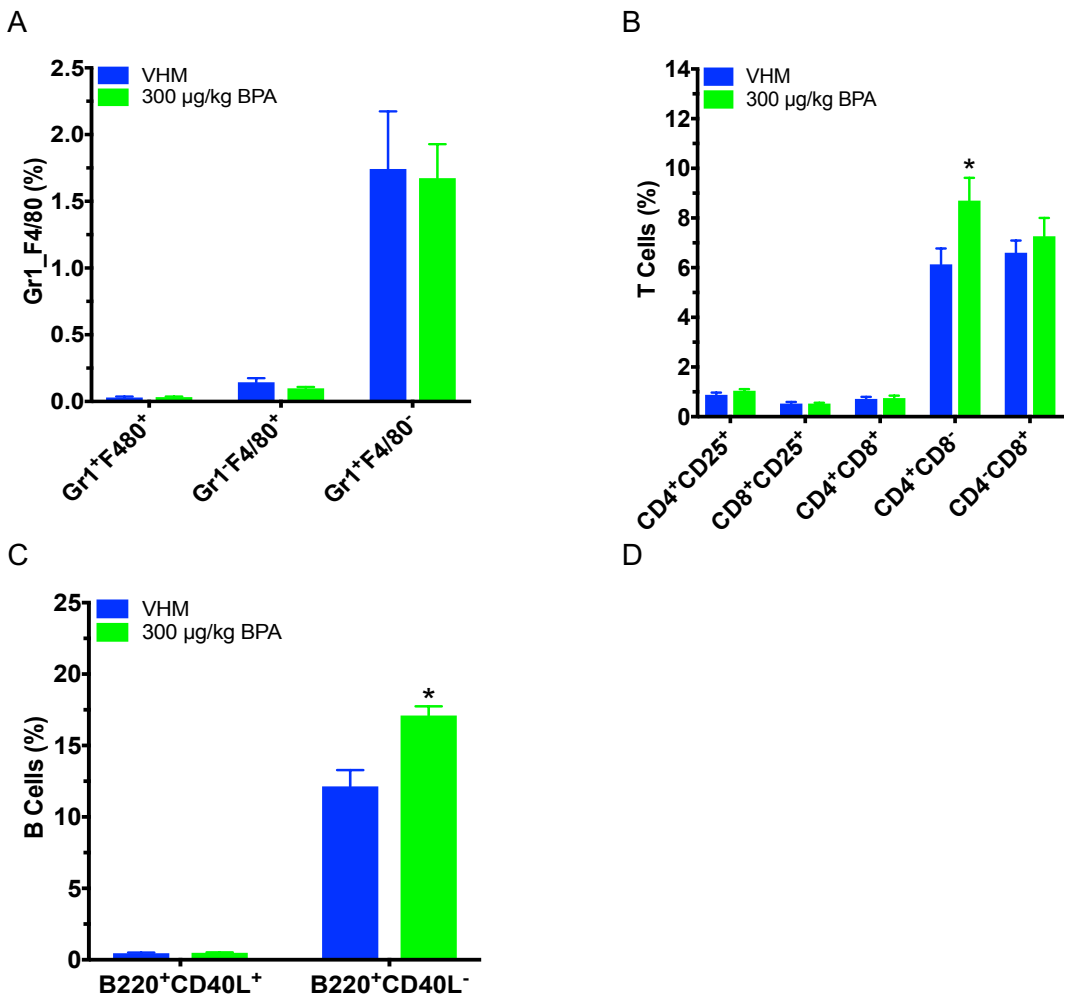

Figure S11. Immune cell populations of perinatally exposed male offspring on soy-based diet. (a) % $Gr1^+F4/80^+$, $Gr1^-F4/80^+$ and $Gr1^+F4/80^-$ spleen cells. (b) %T cell populations in spleen. (c) %B splenic cell populations. The values are presented as mean ± SEM. *, $p< 0.05$ as compared to the respective vehicle male (VHM) control group. N = 9-13.

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