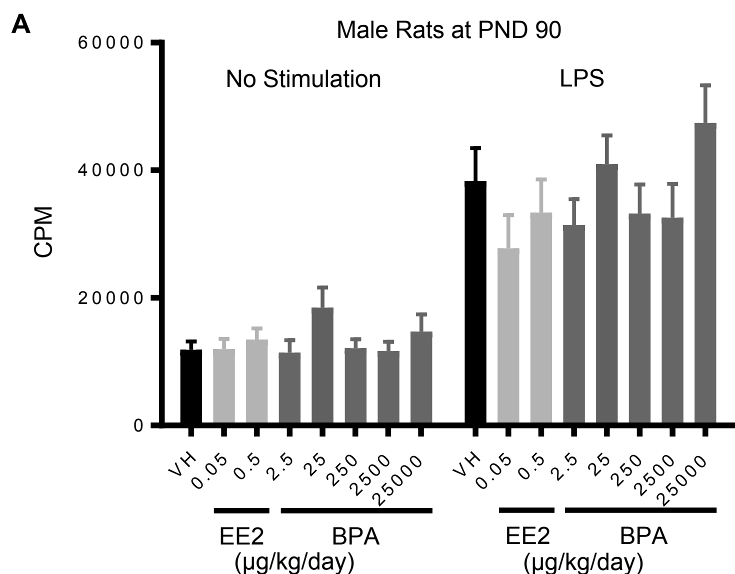
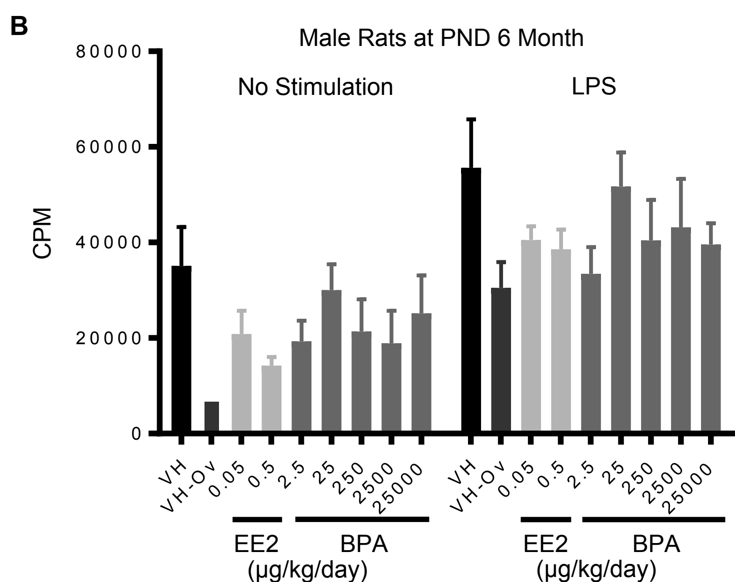
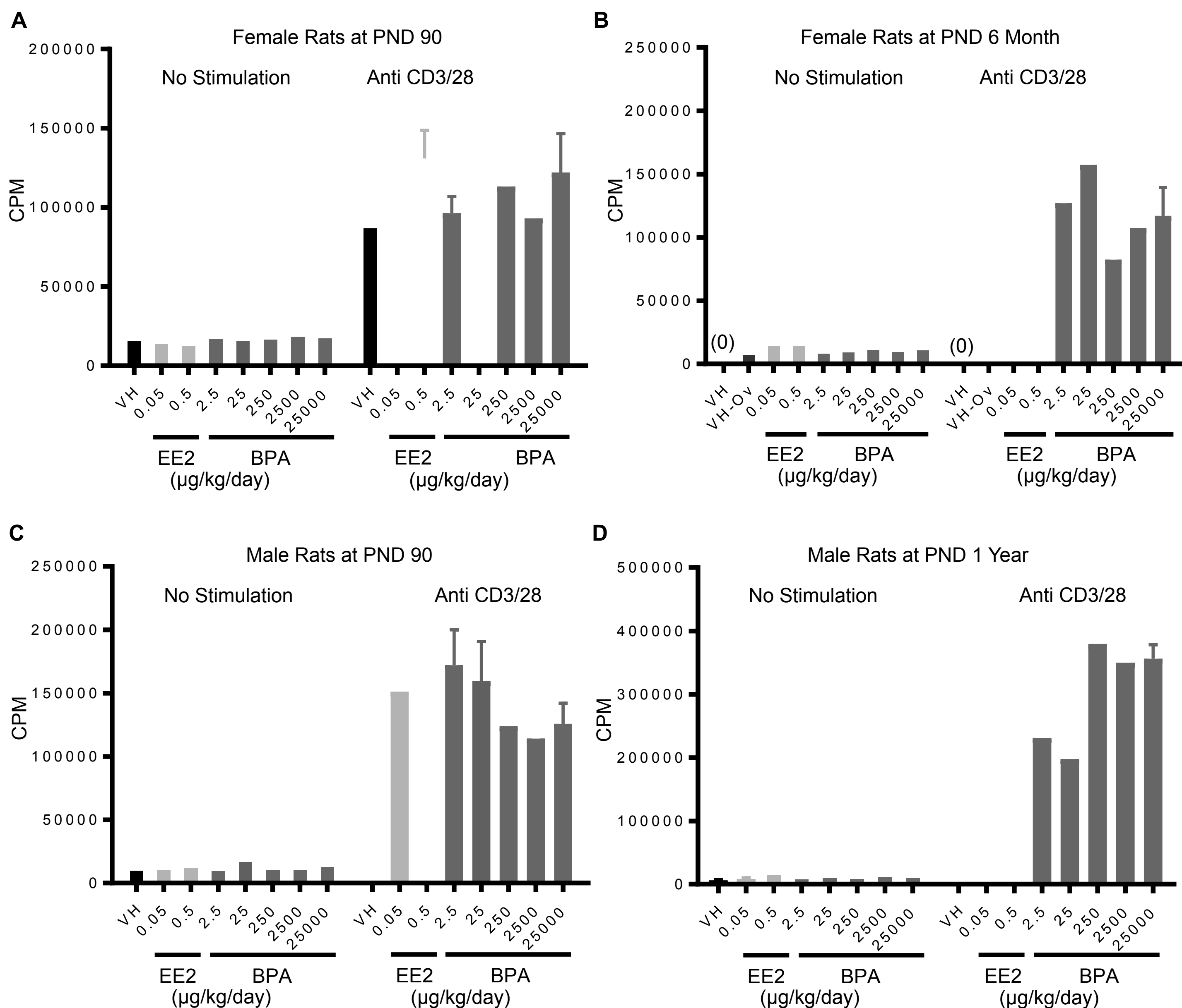
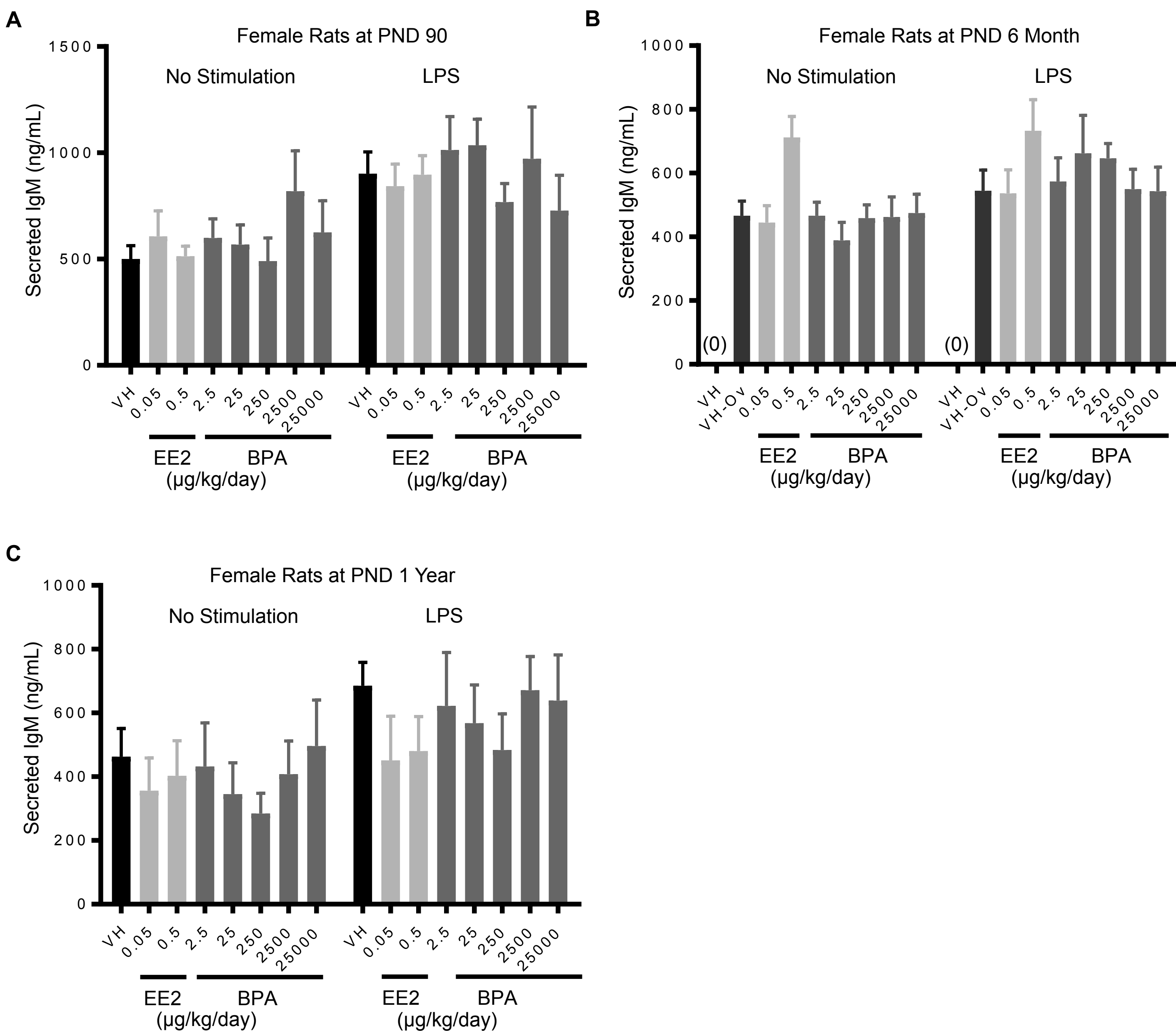




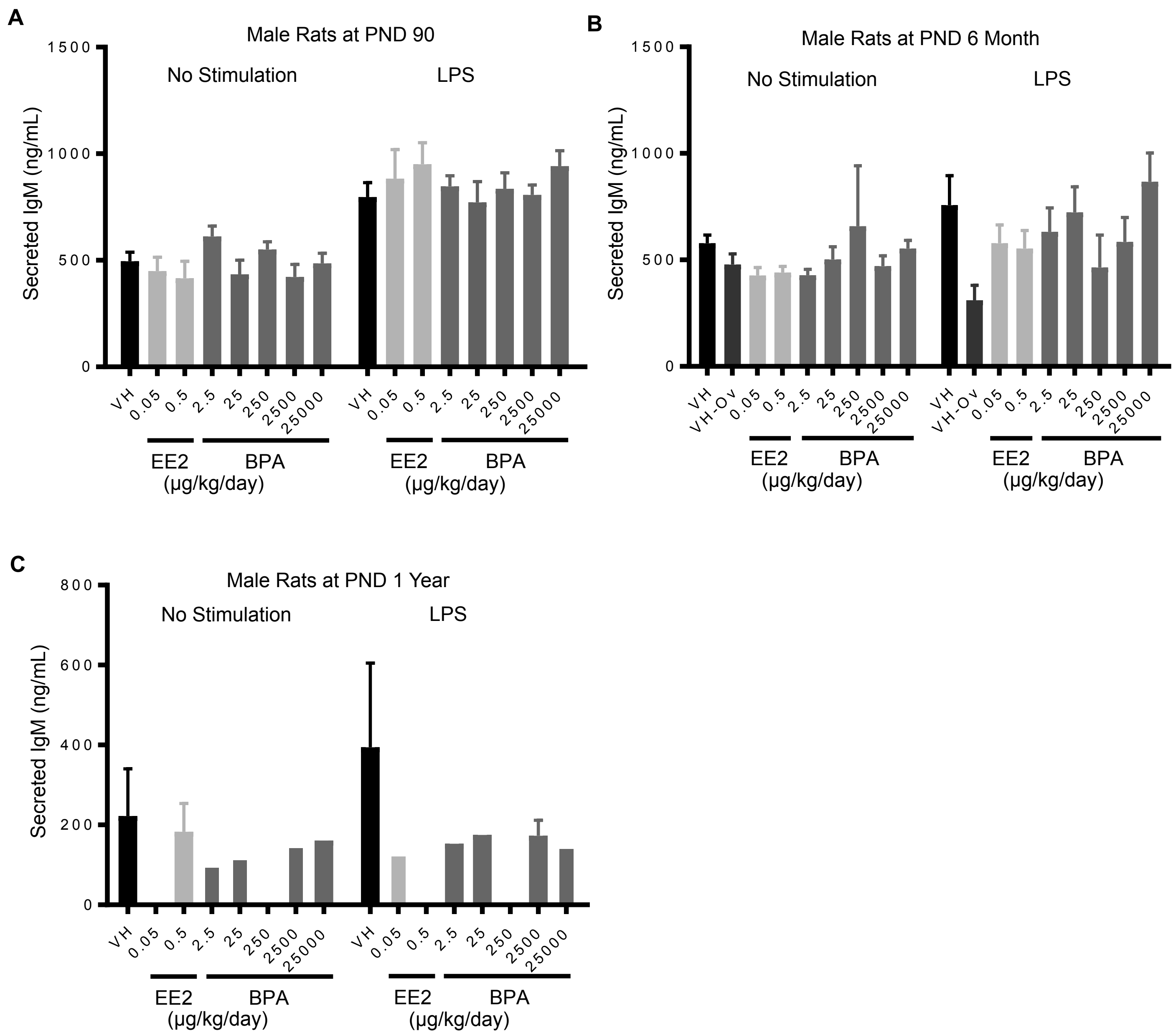
Supplement Figure 1. Quantification of LPS-induced splenocyte proliferation of male rats by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**) or 6 month (**B**). Splenocytes were isolated and treated with LPS for 48 h followed by a 24 h pulse with [^{3}H]-thymidine. Cells were harvested and quantified in counts per minute (CPM) for [^{3}H]-thymidine incorporation using a Tri-Carb 2100 TR scintillation counter. Results are presented as mean ± SE. n = 6-10 rats/treatment group/sex. No significant differences were observed when compared to respective vehicle control group by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



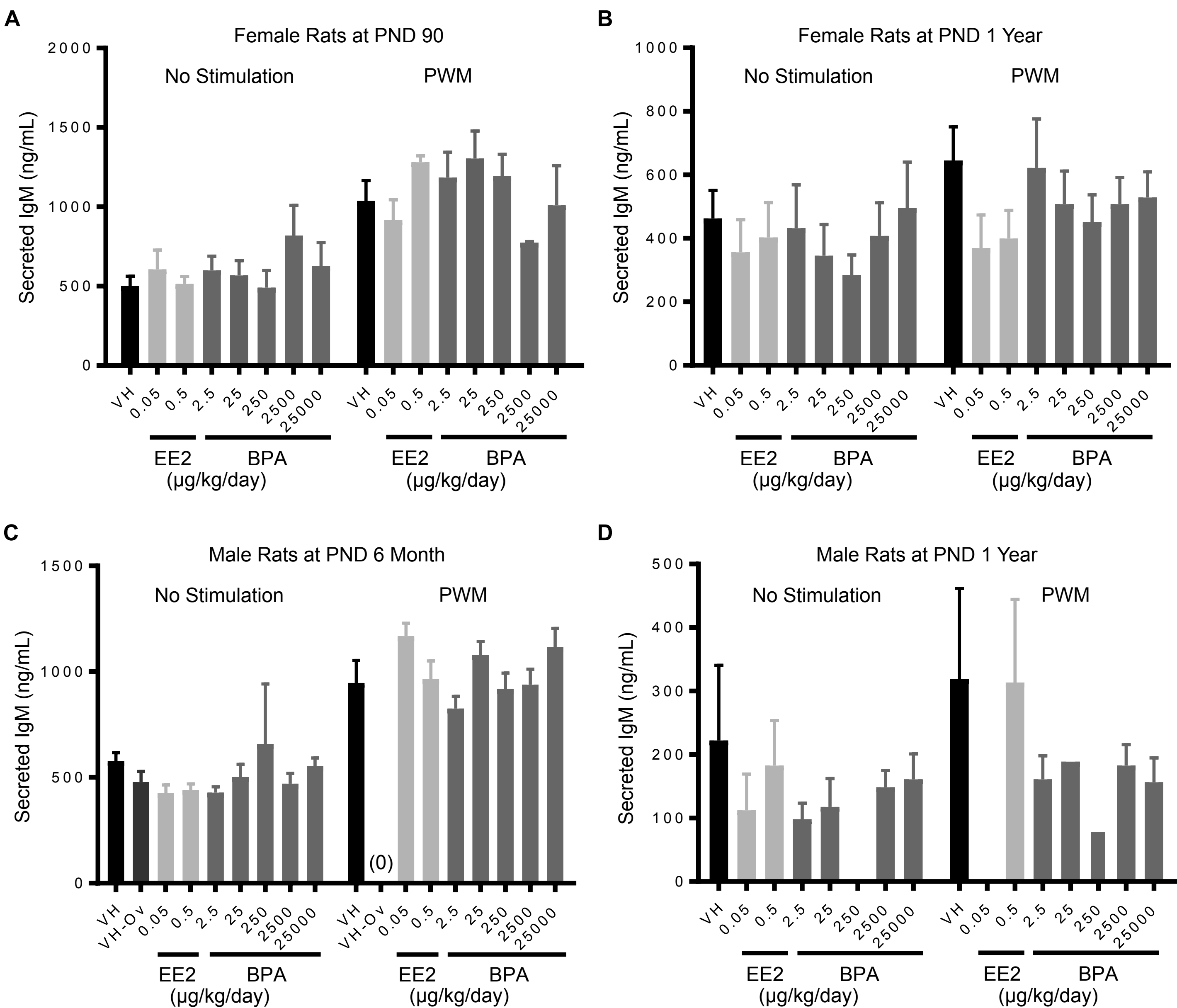
Supplement Figure 2. Quantification of anti-CD3/28-induced splenocyte proliferation of rats by treatment group and sex. Male (**C, D**) and female (**A**, **B**) rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A, C**) 6 month (**B**) and 1 year (**D**). Splenocytes were isolated and treated with anti-CD3/CD28 for 48 h followed by a 24 h pulse with [^{3}H]-thymidine. Cells were harvested and quantified in counts per minute (CPM) for [^{3}H]-thymidine incorporation using a Tri-Carb 2100 TR scintillation counter. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250μg BPA/kg/day treated male group. No significant differences were observed when compared to respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett’s posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



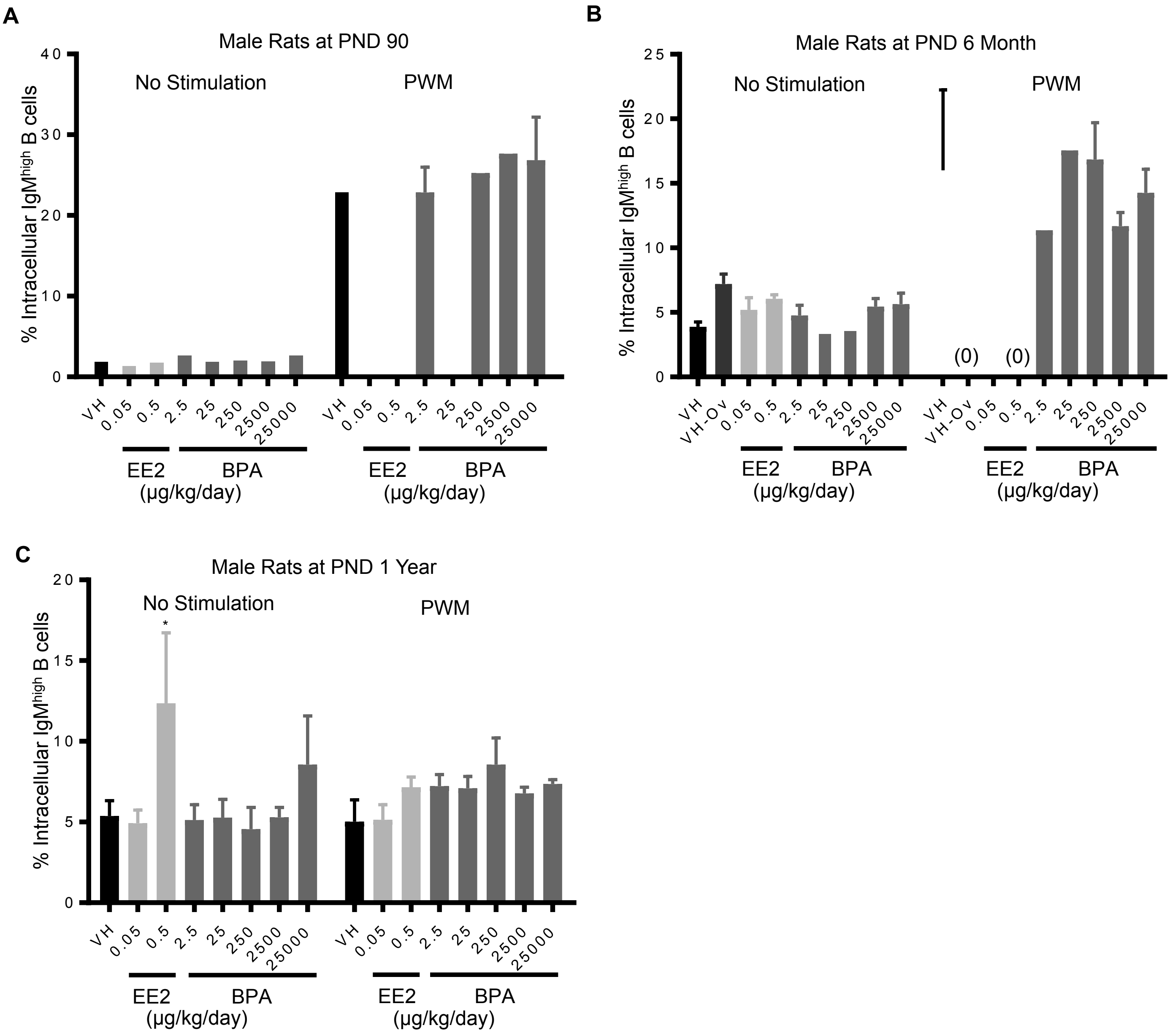
Supplement Figure 3. Quantification of LPS-induced IgM secretion by splenic B cells of female rats by treatment group. Female rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 day (**A**), 6 month (**B**), or 1 year (**C**). Splenocytes were isolated and treated with LPS for 72 h. Post activation, supernatants were collected and the levels of secreted IgM were quantified using ELISA. Results are presented as mean ± SE. n = 6-10 rats/treatment group/sex. No significant differences were observed when compared to the respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



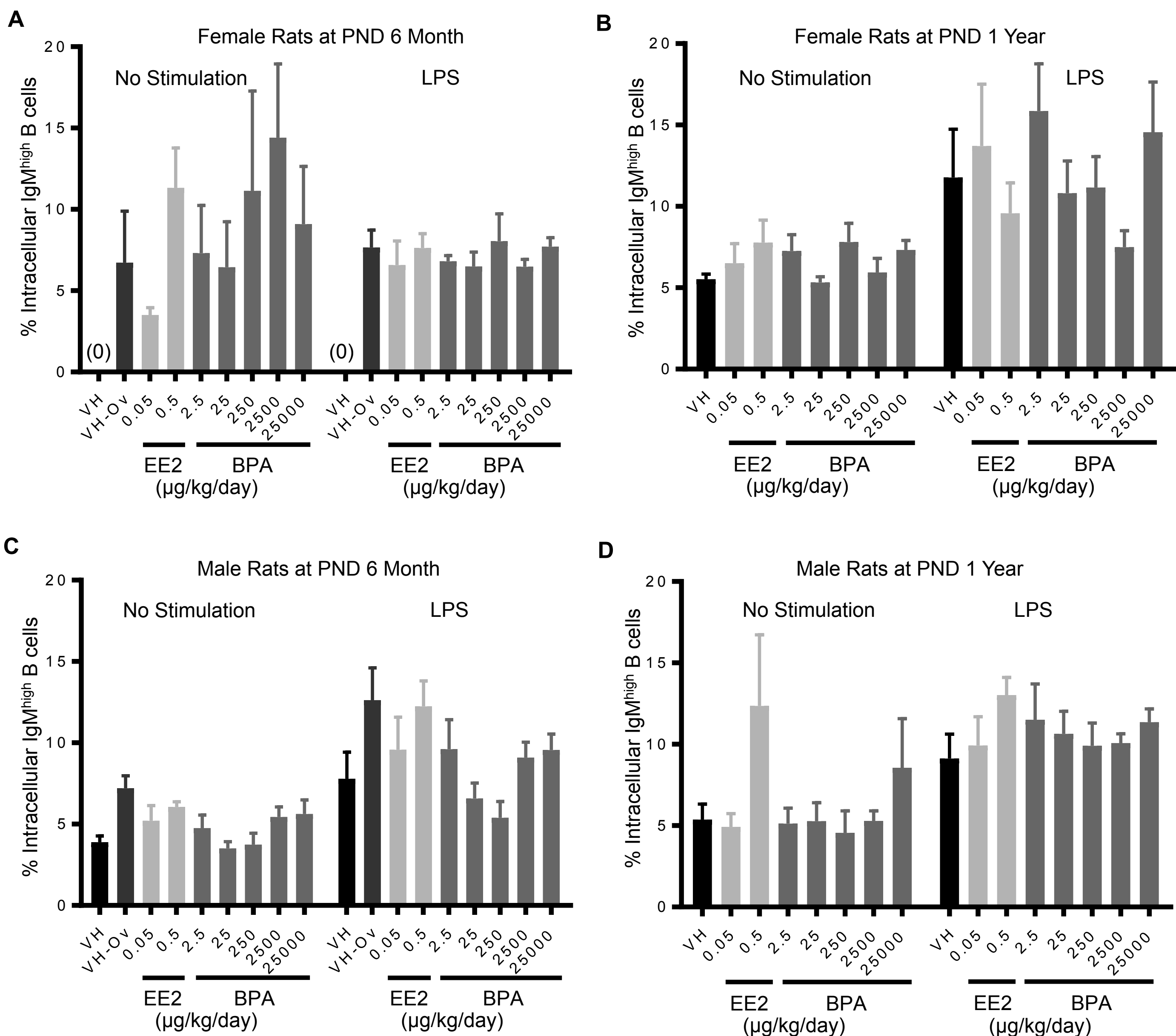
Supplement Figure 4. Quantification of LPS-induced IgM secretion by splenic B cells of male rats by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 day (**A**), 6 month (**B**), or 1 year (**C**). Splenocytes were isolated and treated with LPS for 72 h. Post activation, supernatants were collected and the levels of secreted IgM were quantified using ELISA. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. No significant differences were detected when compared to the respective vehicle control group by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



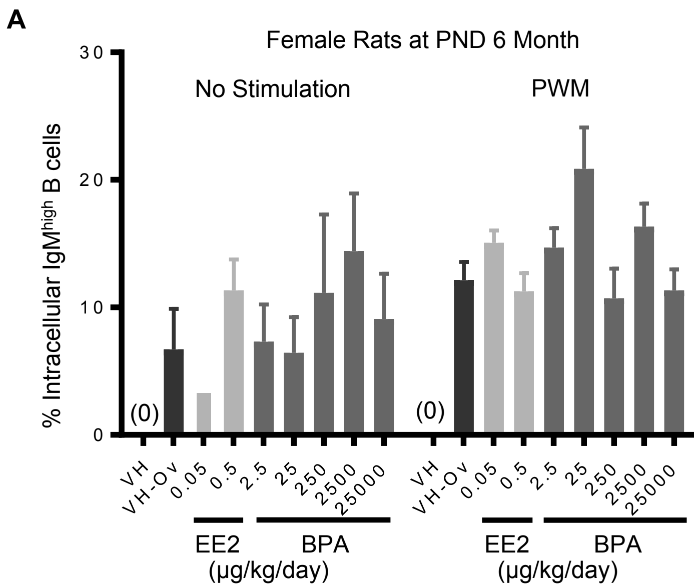
Supplement Figure 5. Quantification of PWM-induced IgM secretion by splenic B cells of rats by treatment group and sex. Male (**C, D**) and female (**A, B**) rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 day (**A**), 6 month (**C**), or 1 year (**B, D**). Splenocytes were isolated and treated with PWM for 72 h. Post activation, supernatants were collected and the levels of secreted IgM were quantified using ELISA. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. No significant differences were observed when compared to respective vehicle control group by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.

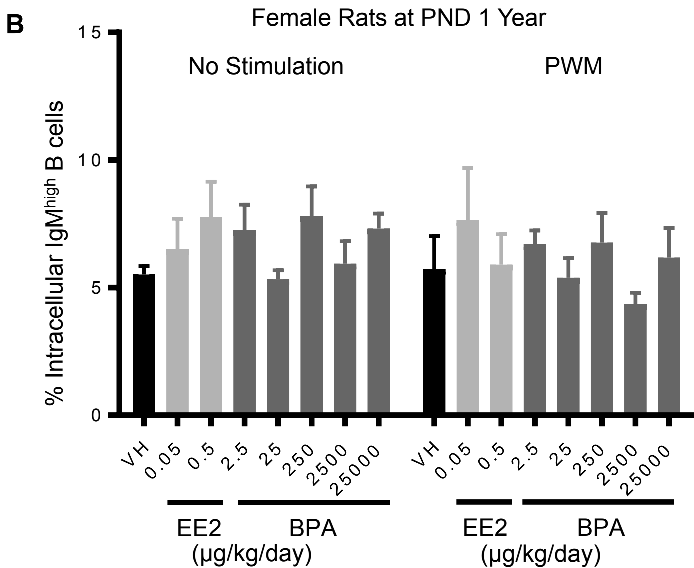


Supplement Figure 6. Percentage of intracellular IgM^{high} B cells in the spleen of male rats post PWM-activation by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 day (**A**), 6 month (**B**), and 1 year (**C**). Splenocytes were isolated and treated with PWM for 72 h. The percentage of IgM^{high} B cells was quantified by flow cytometry. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. Statistic analyses were conducted by comparing to the respective vehicle control group using a two way ANOVA with Dunnett's posttest. *$p < 0.05$. No significant differences were observed post activation. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.

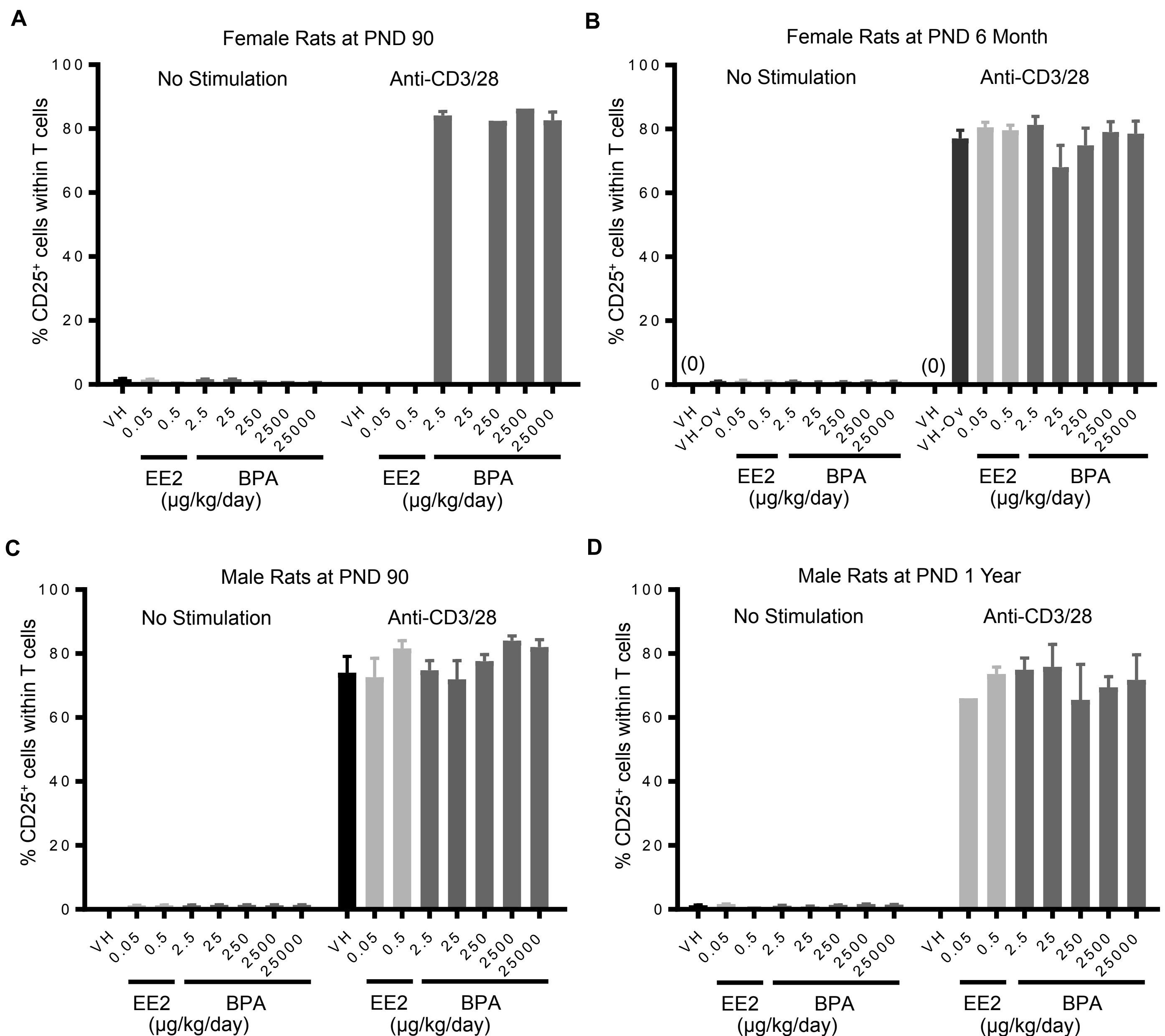


Supplement Figure 7. Percentage of intracellular IgM^{high} B cells in the spleen of rats post LPS-activation by treatment group and sex. Female (**A, B**) and male (**C, D**) rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 6 month (**A, C**) and 1 year (**B, D**). Splenocytes were isolated and treated with LPS for 72 h. The percentage of IgM^{high} B cells was quantified by flow cytometry. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. No significant differences were observed when compared to the respective vehicle control group by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



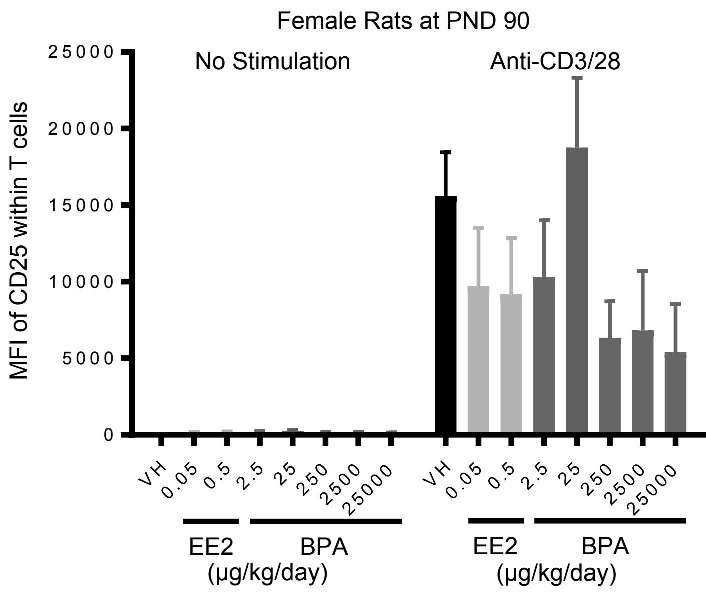


Supplement Figure 8. Percentage of intracellular IgM^{high} B cells in the spleen of female rats post PWM-activation by treatment group. Rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 6 month (**A**) and 1 year (**B**). Splenocytes were isolated and treated with PWM for 72 h. The percentage of IgM^{high} B cells was quantified by flow cytometry. n = 6-10 rats/treatment group/sex. No significant differences were observed when compared to the respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



Supplement Figure 9. Percentage of CD25$^+$ T cells post anti-CD3/28 activation from the spleen of rats by treatment group and sex. Female (**A, B**) and male (**C, D**) rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A, C**), 6 month (**B**), and 1 year (**D**). Splenocytes were isolated and treated with anti-CD3/28 for 48 h. The percentage of CD25$^+$ cells within splenic T cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. No significant differences were observed when compared to the respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.

A



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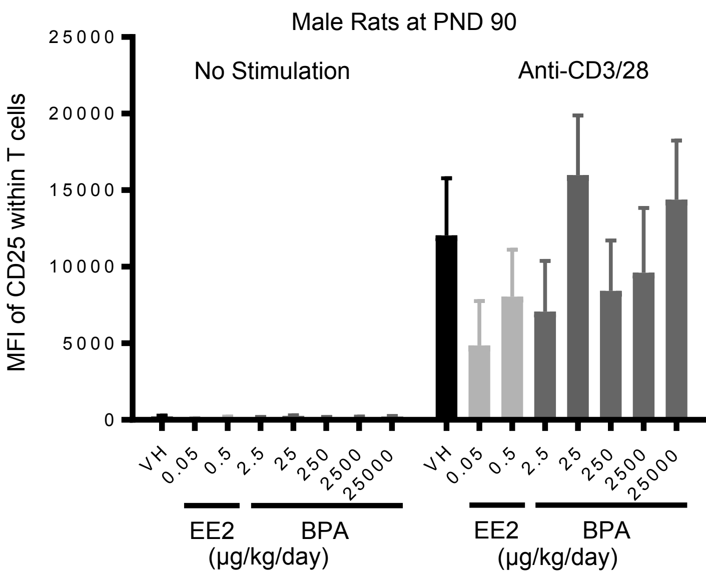
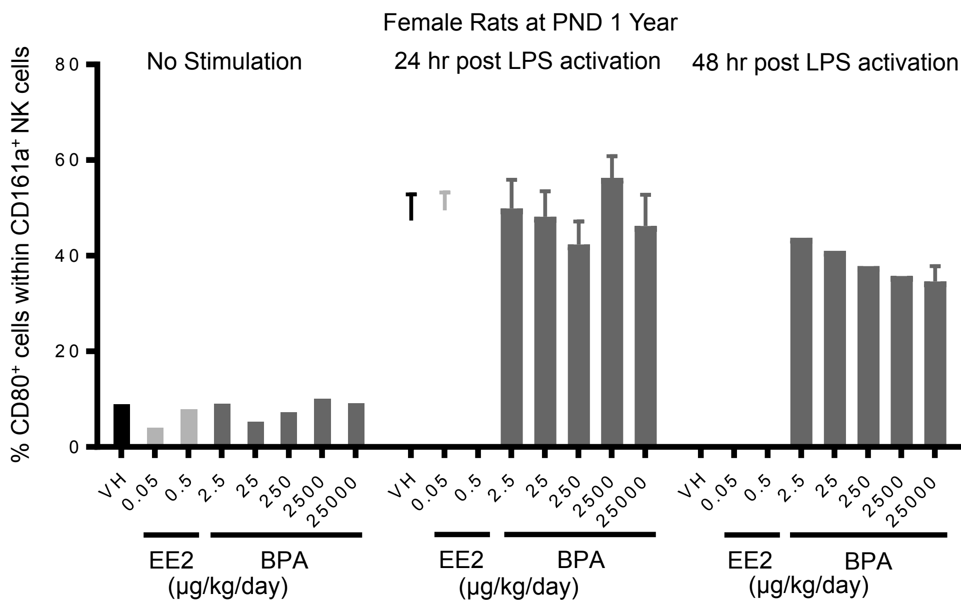
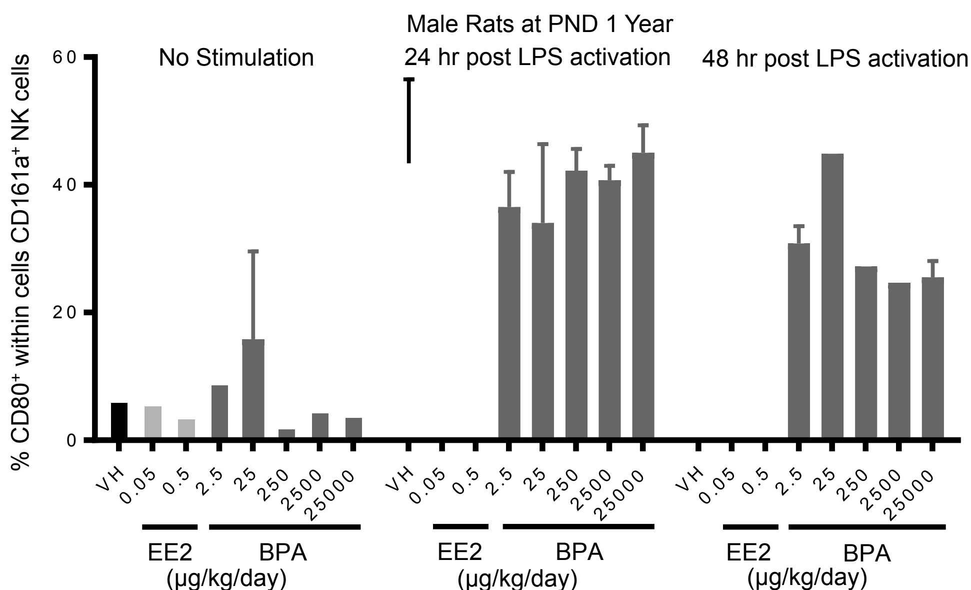
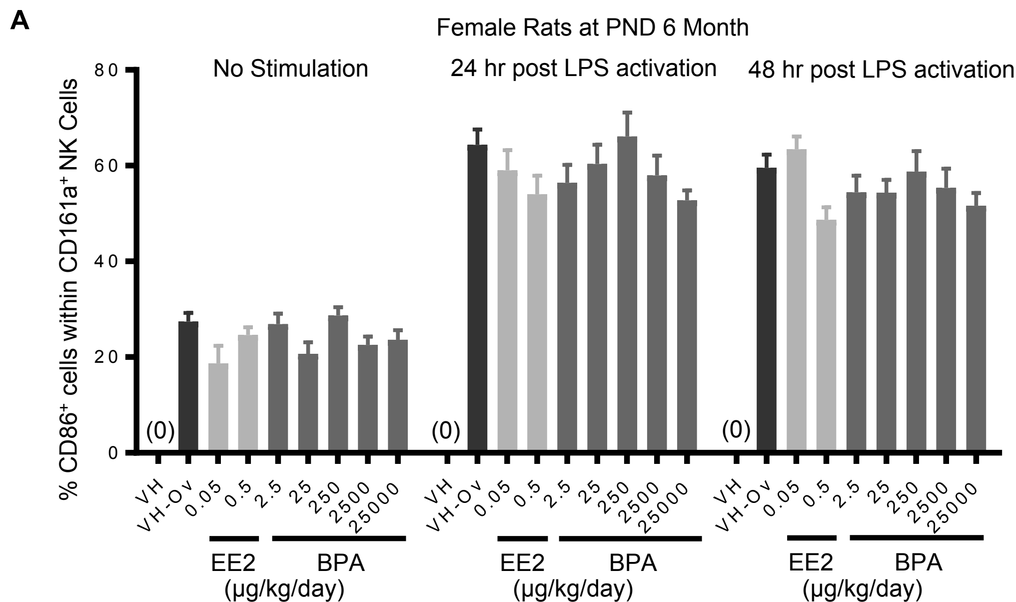


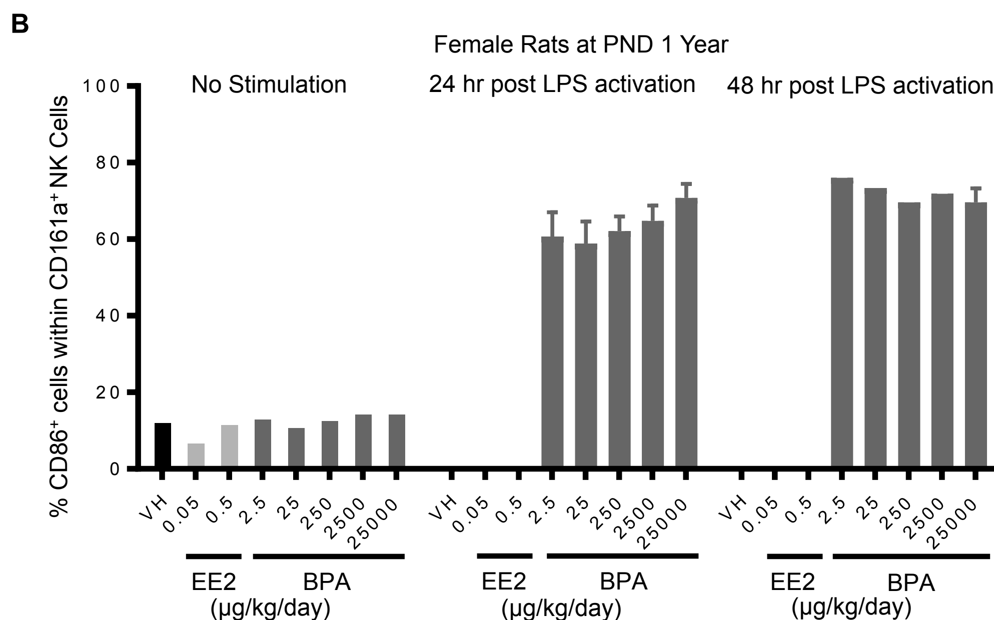
Supplement Figure 10. Quantification of CD25 expression level on T cells post anti-CD3/28 activation from the spleen of rats by treatment group and sex. Female (**A**) and male (**B**) rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90. Splenocytes were isolated and treated with anti-CD3/28 for 48 h. The expression levels of CD25 on splenic T cells, represented as mean fluorescence intensity (MFI), were quantified by flow cytometry. Results are presented as mean ± SE. n = 6-10 rats/treatment group/sex. No significant differences were observed when compared to the respective vehicle control group by a two way ANOVA with Dunnett's posttest.



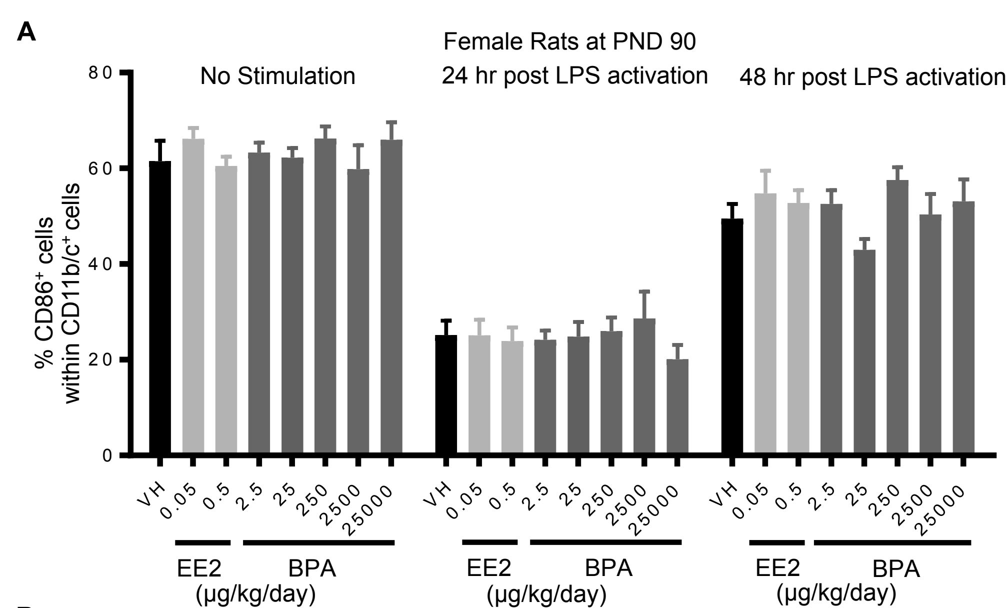


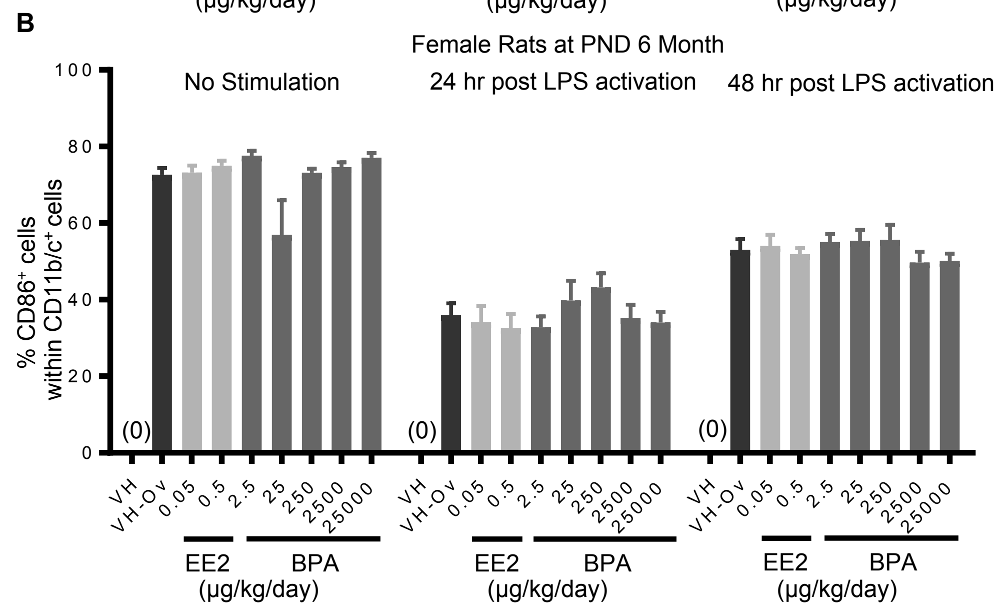
Supplement Figure 11. Percentage of $CD80^+$ cells within NK cells post LPS activation from the spleen of rats by treatment group and sex. Female (**A**) and male (**B**) rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 1 year. Splenocytes were isolated and treated with LPS for up to 48 hours. The percentage of $CD80^+$ cells within $CD161a^+$ NK cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. No significant differences were observed when compared to the respective vehicle control group by a two way ANOVA with Dunnett's posttest.

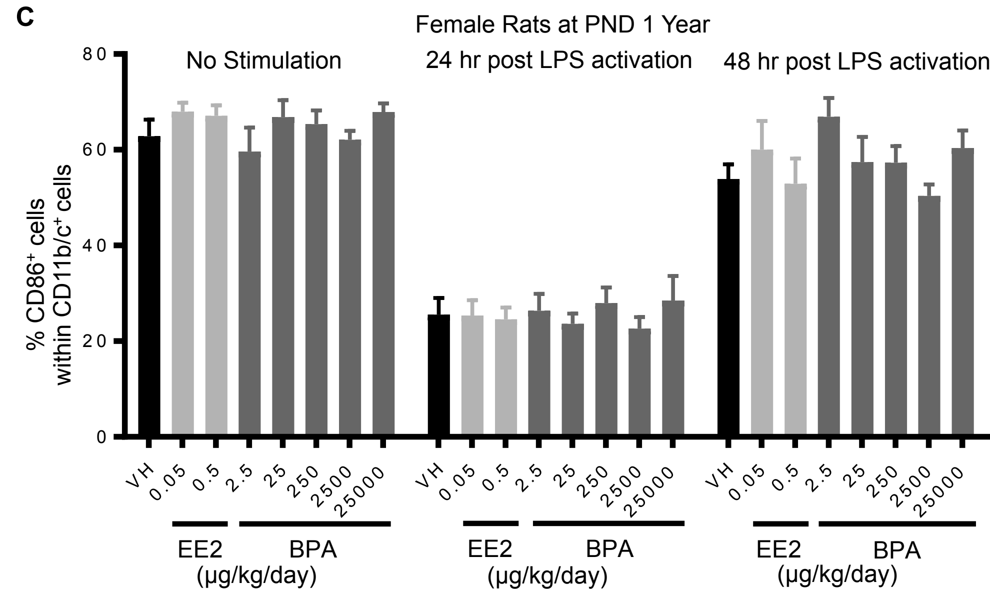




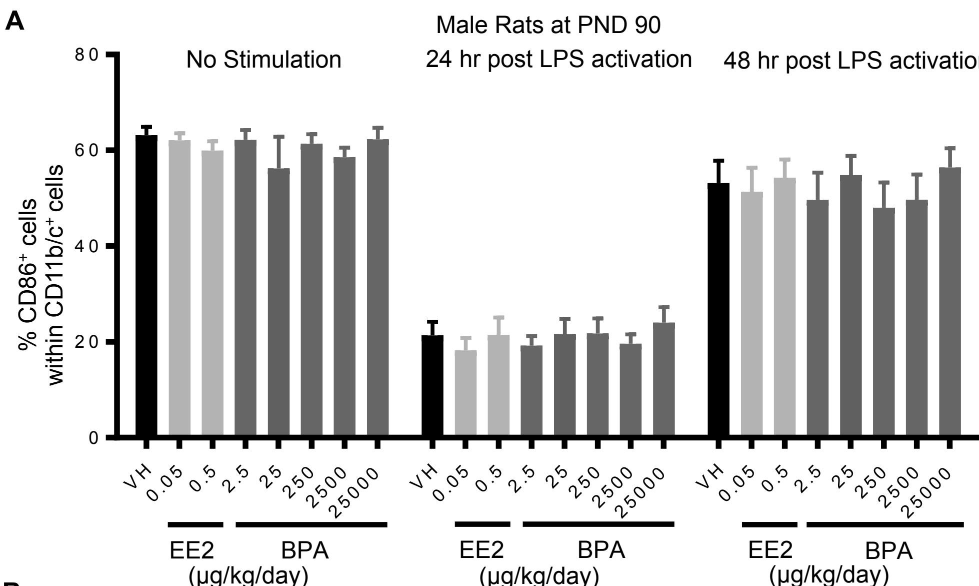
Supplement Figure 12. Percentage of CD86$^+$ cells within NK cells post LPS activation from the spleen of female rats by treatment group. Female rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 6 months (**A**) and 1 year (**B**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of CD86$^+$ cells within CD161a$^+$ NK cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 3-10 rats/ treatment group/sex. No significant differences were observed when compared to the respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett’s posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.

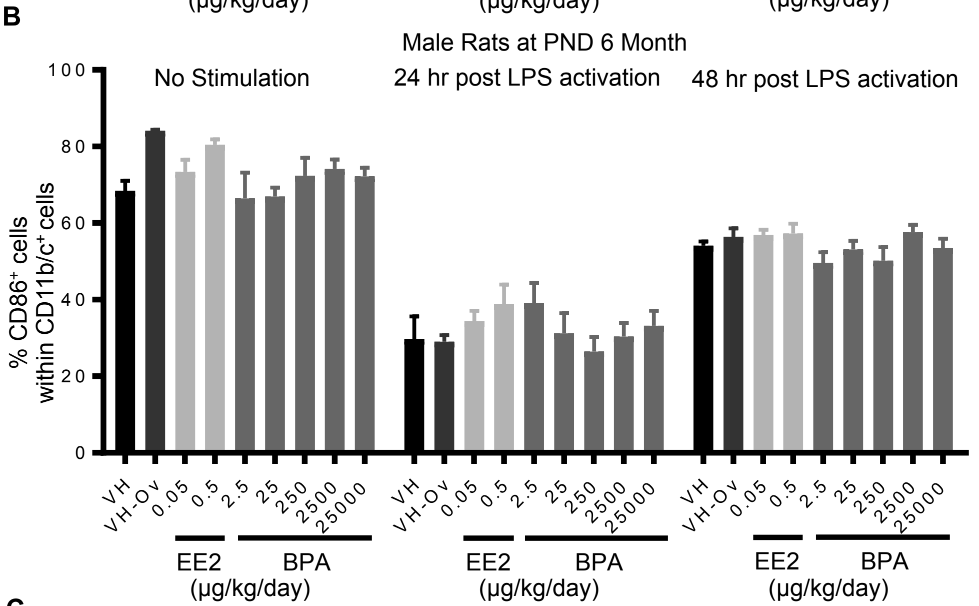


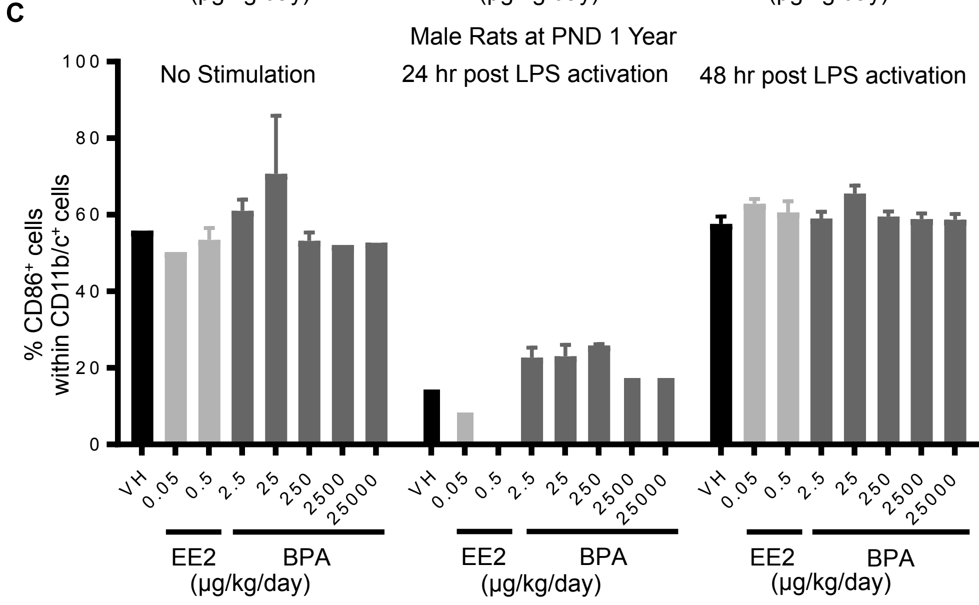




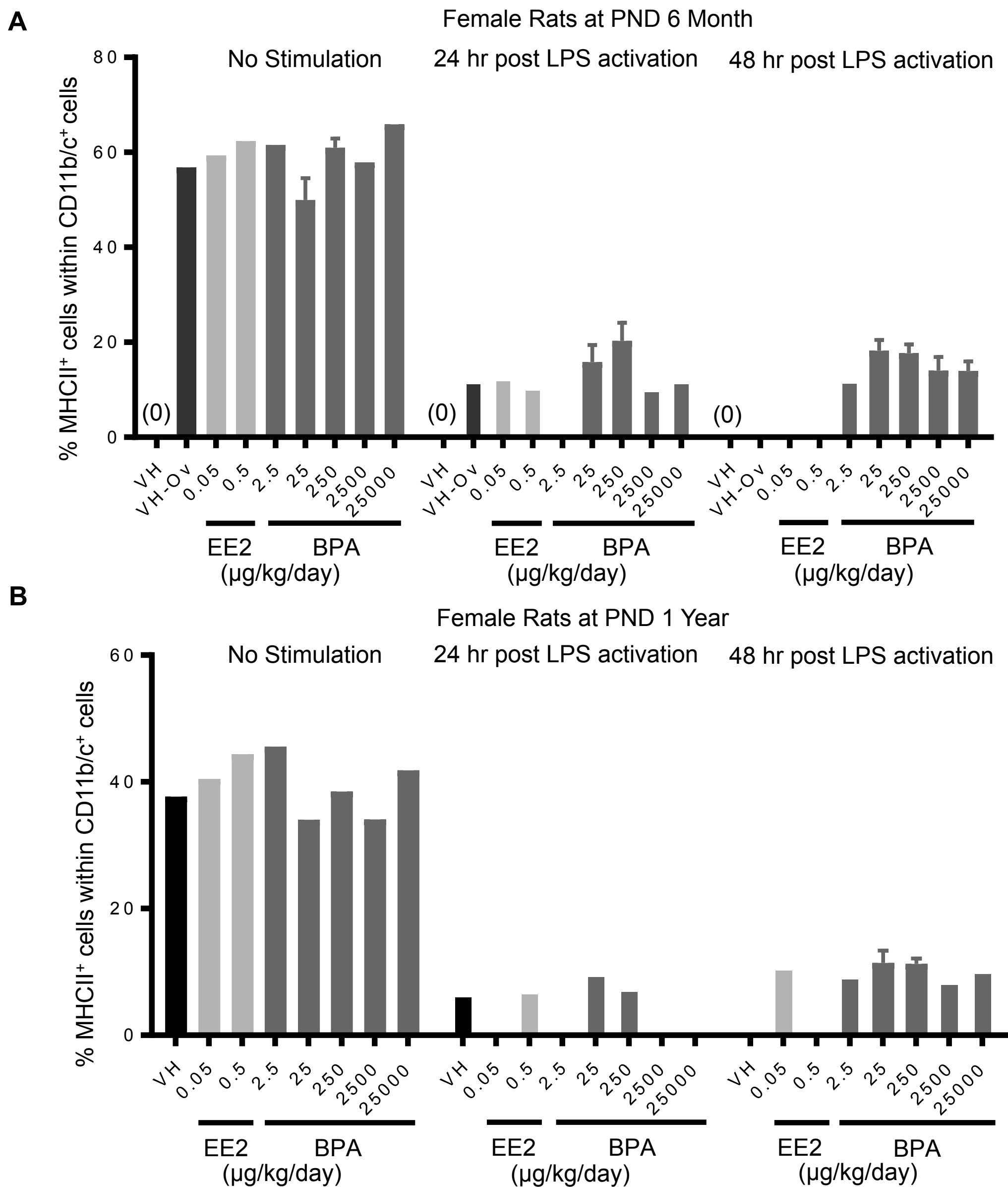
Supplement Figure 13. Percentage of CD86^{+} cells within macrophage/-dendritic cells post LPS activation from the spleen of female rats by treatment group. Female rats were administered vehicle (VH, 0.3% aqueous carboxy-methylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**), 6 month (**B**), and 1 year (**C**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of CD86^{+} cells within CD11b/c $^{+}$ macrophage/ dendritic cells was quantified by flow cytometry. Data are presented as mean ± SE. n = 4-10 rats/treatment group/ sex. No significant differences were observed as compared to the respective vehicle control group (VH-Ov for female rats at the 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



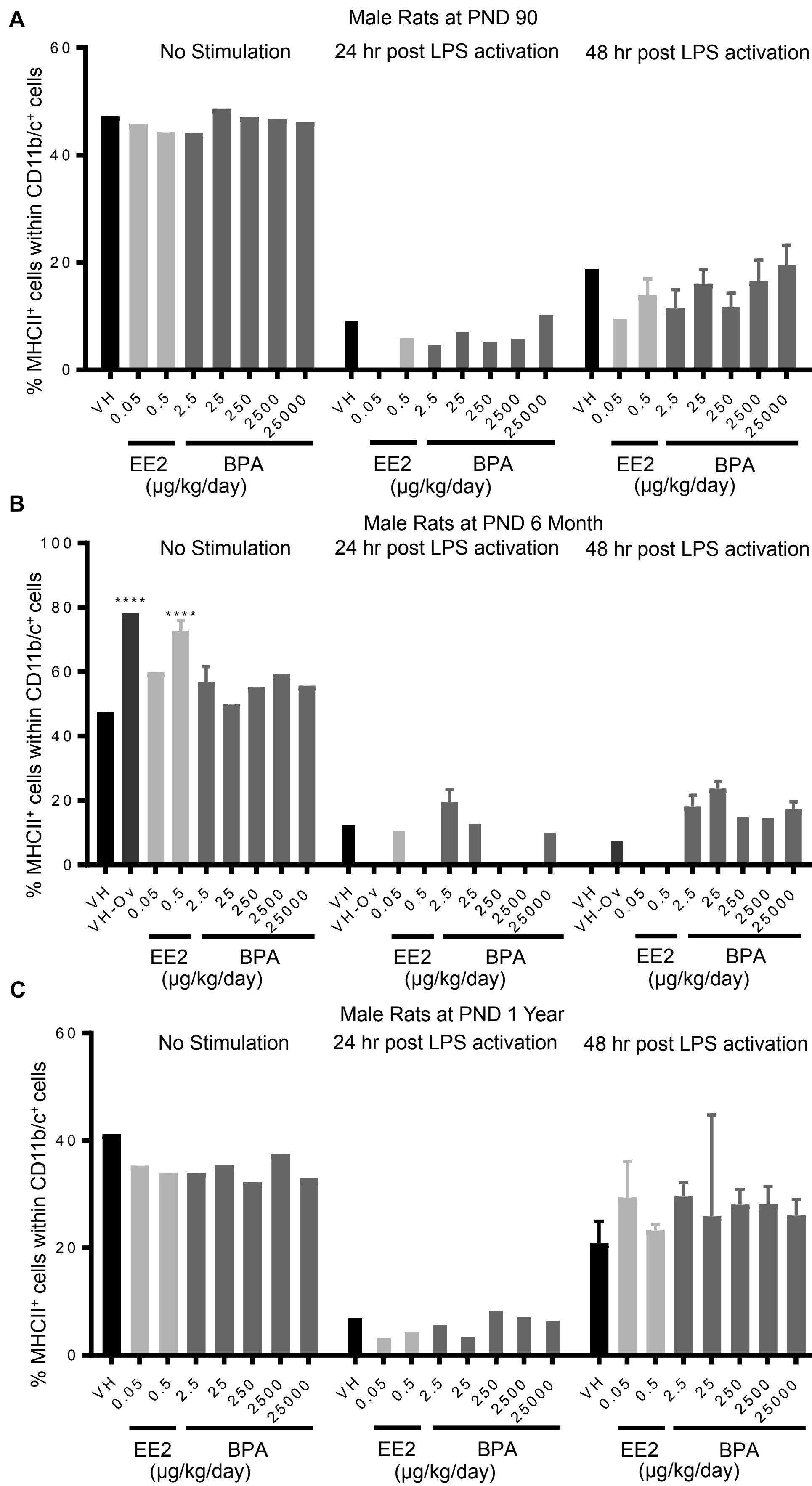




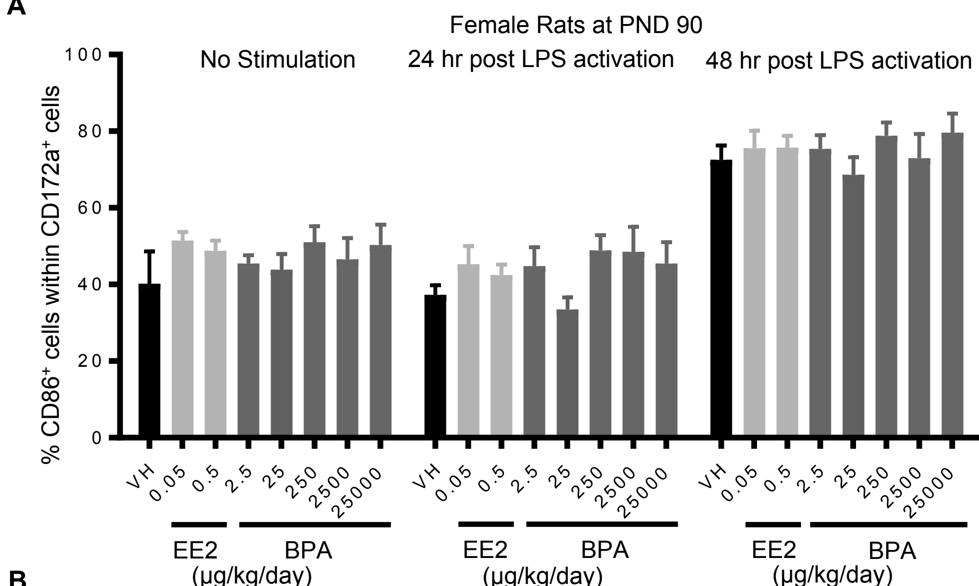
Supplement Figure 14. Percentage of CD86$^+$ cells within macrophage/ dendritic cells post LPS activation from the spleen of male rats by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxy-methylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**), 6 month (**B**), and 1 year (**C**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of CD86$^+$ cells within CD11b/c $^+$ macrophage/ dendritic cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 2-10 rats/treatment group/ sex, n = 2 rats in PND 1 year 250μg BPA/kg/day treated male group. No significant difference was observed when compared to respective vehicle control group by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.

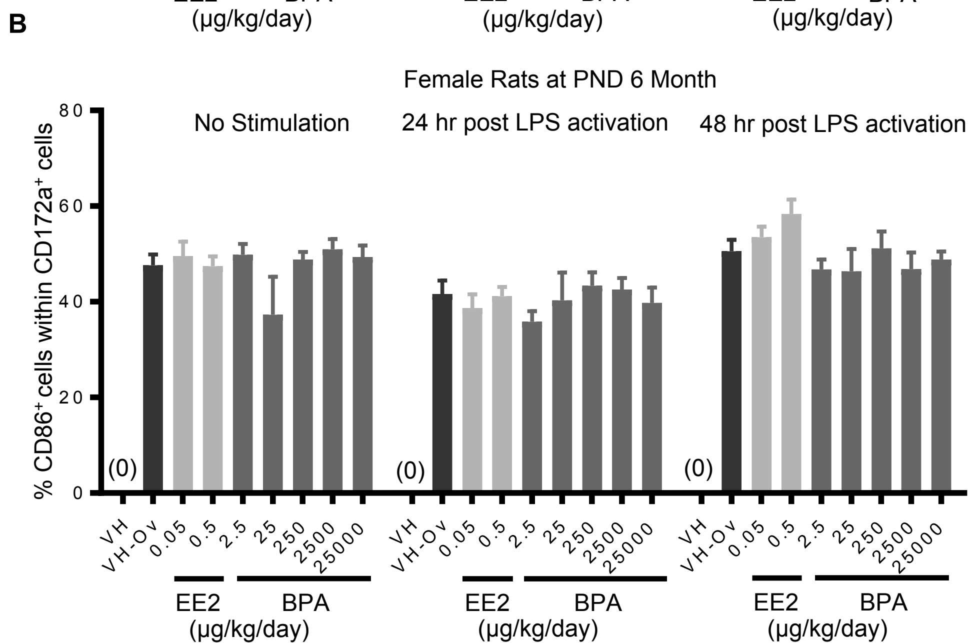


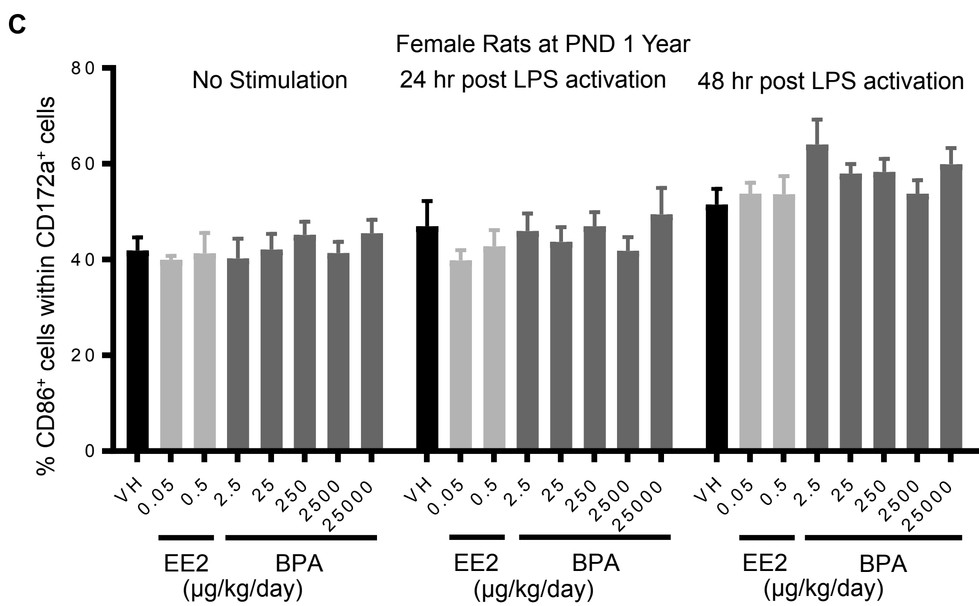
Supplement Figure 15. Percentage of MHCII$^+$ cells within macrophage/dendritic cells post LPS activation from the spleen of female rats by treatment group. Female Rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 6 month (**A**) and 1 year (**B**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of MHCII$^+$ cells within CD11b/c$^+$ macrophage/dendritic cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 4-10 rats/treatment group/sex. No significant differences were observed when compared to the respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



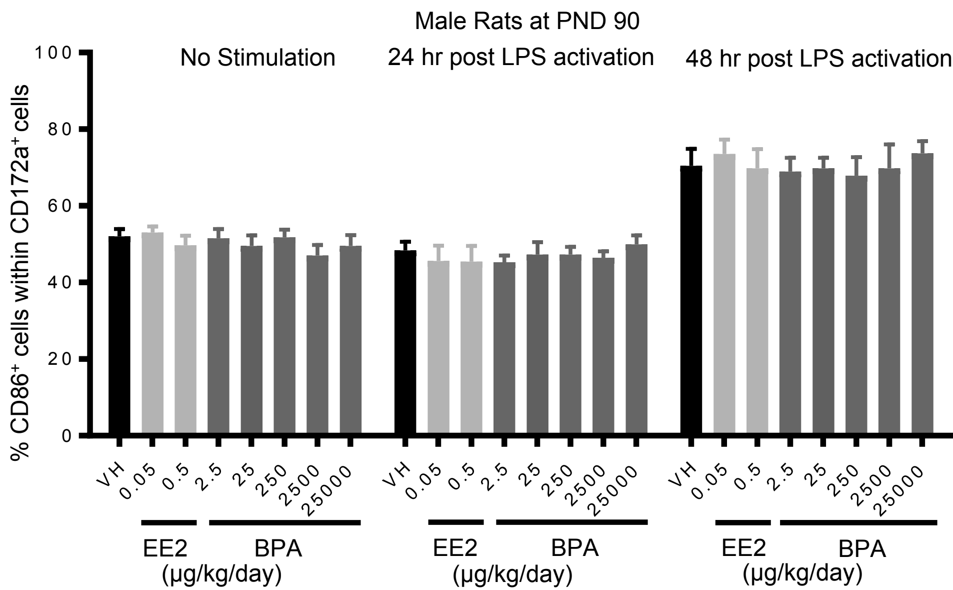
Supplement Figure 16. Percentage of MHCII⁺ cells within macrophage/ dendritic cells post LPS activation from the spleen of male rats by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxy-methylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**), 6 month (**B**), and 1 year (**C**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of MHCII⁺ cells within CD11b/c⁺ macrophage/ dendritic cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in the PND 1 year 250µg BPA/kg/day treated male group. Statistic analyses were conducted by comparing to the respective vehicle control group using a two way ANOVA with Dunnett's posttest.
**** $p < 0.0001$. No significant differences were observed post activation. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



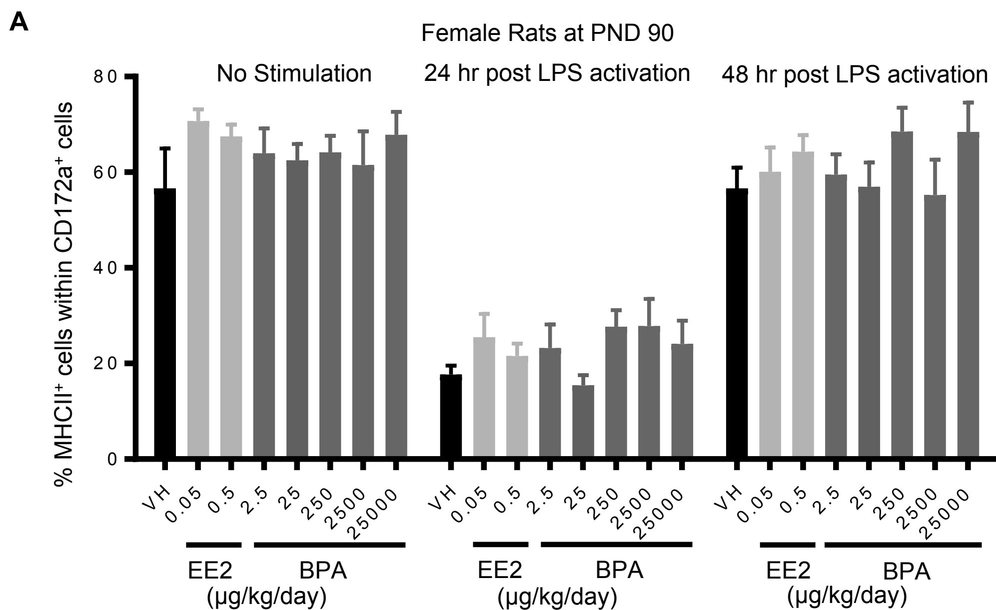


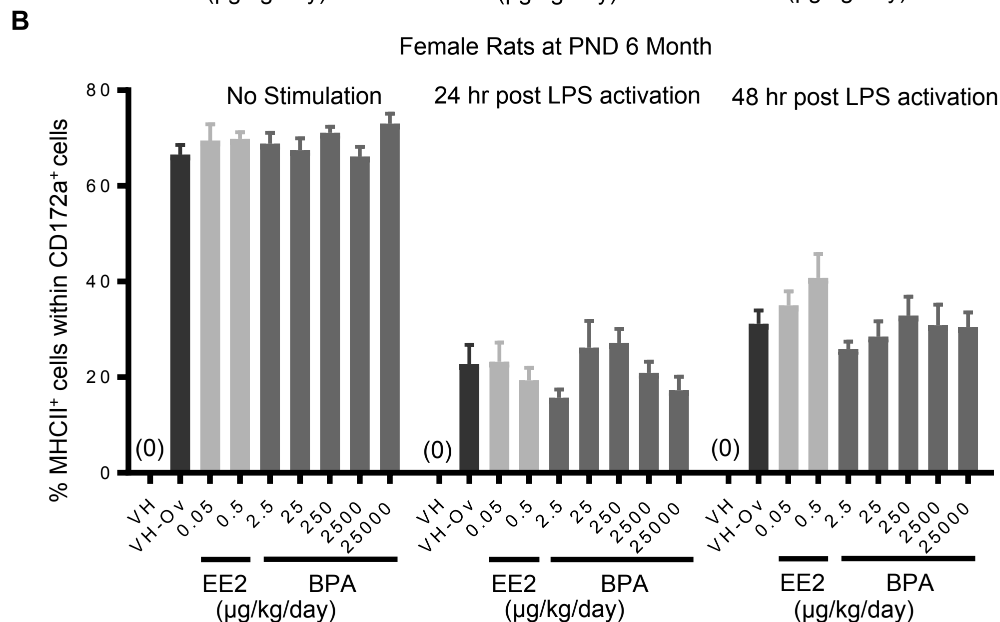


Supplement Figure 17. Percentage of CD86⁺ cells within monocyte/ macrophage/ granulocyte cells post LPS activation from the spleen of female rats by treatment group. Female rats were administered vehicle (VH, 0.3% aqueous carboxy-methylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**), 6 months (**B**), and 1 year (**C**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of $CD86^+$ cells within $CD172a^+$ monocyte/ macrophage/ granulocyte cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 4-10 rats/ treatment group/sex. No significant differences were observed when compared to respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.

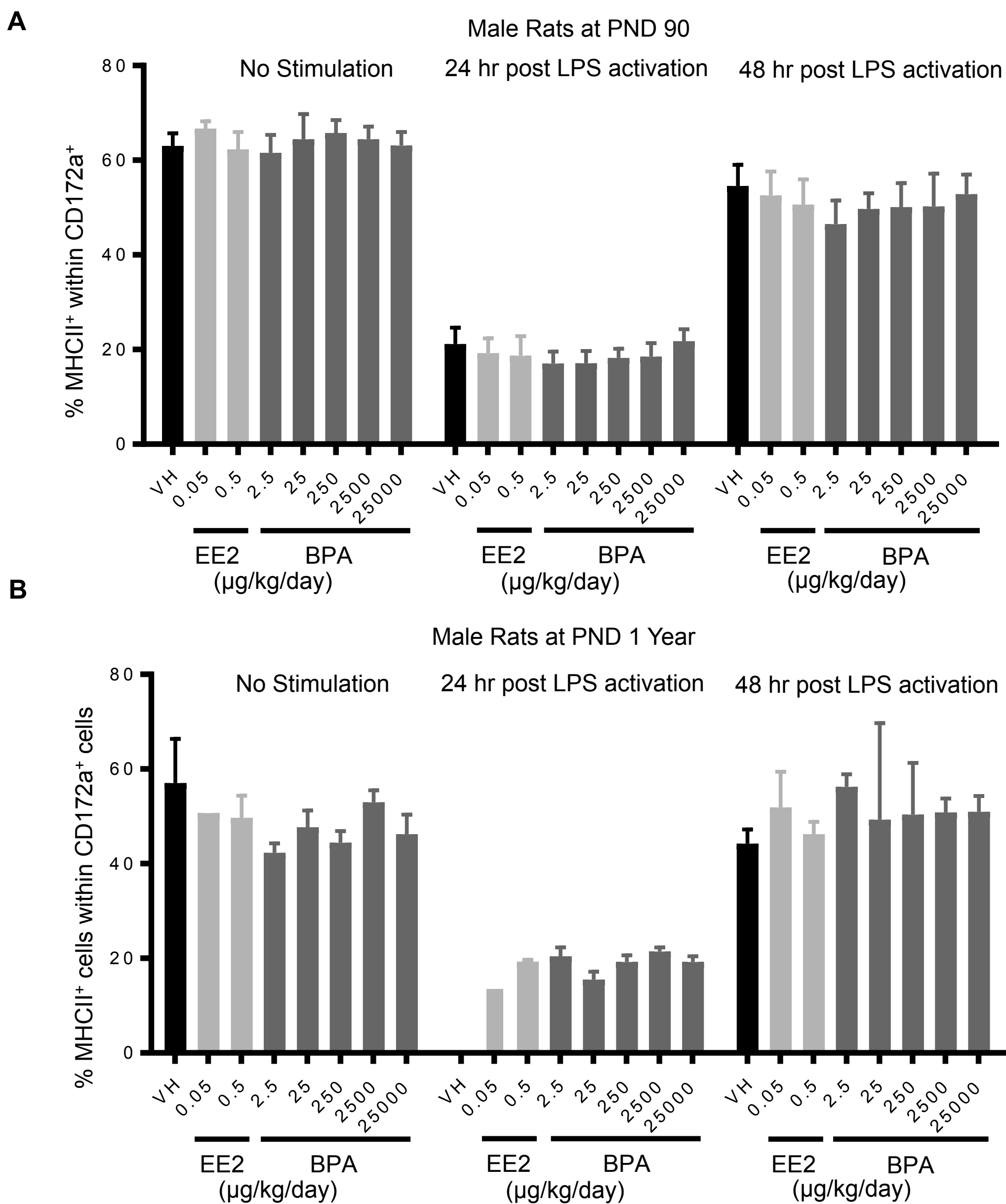


Supplement Figure 18. Percentage of CD86⁺ cells within monocyte/macrophage/granulocyte cells post LPS activation from the spleen of male rats by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90. Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of $CD86^+$ cells within $CD172a^+$ monocyte/macrophage/granulocyte cells was quantified by flow cytometry. Results are presented as mean ± SE. n = 7-10 rats/treatment group/sex. No significant differences were observed when compared to respective vehicle control group by a two way ANOVA with Dunnett's posttest.





Supplement Figure 19. Percentage of MHCII⁺ cells within monocyte/macrophage/granulocyte cells post LPS activation from the spleen of female rats by treatment group. Female rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**) and 6 months (**B**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of $MHCII^+$ cells within $CD172a^+$ monocyte/macrophage/granulocyte cells was quantified by flow cytometry. Data are presented as mean ± SE. n = 4-10 rats/treatment group/sex. No significant differences were observed when compared to the respective vehicle control group (VH-Ov for female rats at 6 month time point) by a two way ANOVA with Dunnett's posttest. For more details about VH-Ov, please see the Statistical Analysis section in the Materials and Methods.



Supplement Figure 20. Percentage of MHCII$^+$ cells within monocyte/macrophage/granulocyte cells post LPS activation from the spleen of male rats by treatment group. Male rats were administered vehicle (VH, 0.3% aqueous carboxymethylcellulose), BPA or estrogen ethinyl estradiol (EE2) at the indicated dose level by oral gavage daily and sacrificed at postnatal day (PND) 90 (**A**) and 1 year (**B**). Splenocytes were isolated and treated with LPS for up to 48 h. The percentage of MHCII$^+$ cells within CD172a$^+$ monocyte/macrophage/granulocyte cells quantified by flow cytometry. Results are presented as mean ± SE. n = 2-10 rats/treatment group/sex, n = 2 rats in PND 1 year 250µg BPA/kg/day treated male group. No significant differences were observed when compared to the respective vehicle control group by a two way ANOVA with Dunnett's posttest.