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| **Supplemental Table S1A.** Other gene expression differences in the hypothalamus of females across treatment groups. Data shown as mean, with lower and upper 95% confidence intervals shown in parenthesis. | | | | | | |
| **Brain Region** | **Transcript** | **EE**  **Fold Change\*** | **BPA**  **Fold Change** | **p-value\*\***  **Vehicle vs. EE\*\*\*** | **p-value**  **Vehicle vs. BPA** | **p-value**  **EE vs. BPA** | |
| Hypothalamus | *Dnmt3a* | 1.44 (0.38, 5.51) | 1.40 (0.37, 5.32) | 0.599 | 0.624 | 0.970 | |
| *Avp* | 0.87 (0.33, 2.32) | 0.85 (0.32, 2.25) | 0.784 | 0.747 | 0.964 | |
| *Ar* | 0.74 (0.18, 3.11) | 0.57 (0.14, 2.39) | 0.683 | 0.450 | 0.732 | |
| *Oxt* | 1.47 (0.31, 7.01) | 0.74 (0.16, 3.47) | 0.634 | 0.700 | 0.393 | |
| \*Fold change refers to the gene expression level relative to that in the vehicle control group using the 2-ΔΔCT method.  \*\*Statistical significance was determined using the ΔCT values | | | | | | | |

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| **Supplemental Table S1B.** Other gene expression differences in the hypothalamus of males across treatment groups. Data shown as mean, with lower and upper 95% confidence intervals shown in parenthesis. | | | | | | |
| **Brain Region** | **Transcript** | **EE**  **Fold Change** | **BPA**  **Fold Change\*** | **p-value\*\***  **Vehicle vs. EE\*\*\*** | **p-value**  **Vehicle vs. BPA** | **p-value**  **EE vs. BPA** | |
| Hypothalamus | *Dnmt3a* | 0.28 (0.07, 1.07) | 0.46 (0.12, 1.75) | 0.071 | 0.262 | 0.470 | |
| *Avp* | 0.38 (0.14, 1.02) | 0.65 (0.25, 1.72) | 0.064 | 0.392 | 0.299 | |
| *Ar* | 0.16 (0.04, 0.67) | 0.41 (0.10, 1.71) | 0.018 | 0.229 | 0.205 | |
| *Oxt* | 0.31 (0.07, 1.49) | 0.31 (0.07, 1.45) | 0.154 | 0.146 | 0.986 | |
| \*Fold change refers to the gene expression level relative to that in the vehicle control group using the 2-ΔΔCT method.  \*\*Statistical significance was determined using the ΔCT values | | | | | | |

**Supplemental Table S2**. Comparison of hypothalamic gene expression differences between females and males within the same treatment group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Brain Region** | **Transcript** | **Female vs. Male \*** | | |
| **p-value \*\*** | | |
| **Control Females vs. Control Males \*\*\*** | **EE Females vs. EE males** | **BPA females vs. BPA males** |
| Hypothalamus | *Dnmt3a* | 0.015 (M > F) | 0.813 | 0.522 |
| *Dnmt3b* | 0.0001 (M > F) | 0.902 | 0.623 |
| *Dnmt1* | 0.0001 (M > F) | 0.833 | 0.030 (M > F) |
| *Esr1* | 0.002 (M > F) | 0.015 (F > M) | 0.417 |
| *Esr2* | 0.0001 (M > F) | 0.193 | 0.112 |
| *Avp* | 0.410 | 0.284 | 0.877 |
| *Ar* | 0.014 (M > F) | 0.943 | *0.051 (M > F)* |
| *Otr* | 0.0001 (M > F) | 0.224 | 0.133 |
| *Oxt* | 0.090 | 0.470 | 0.778 |
| *Bdnf* | 0.0001 (M > F) | 0.167 | 0.253 |

\* F, female; M, male; >, higher expression (lower ΔCT) than the other sex

\*\* Statistical significance was determined using the ΔCT values

\*\*\*Comparisons with a significant difference (p < 0.05) are highlighted in grey and in bold, while those with p < 0.06 are highlighted in italic

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| --- | --- | --- | --- |
| Supplemental Table S3. Primer sequences used for gene expression and bisulfite sequencing analyses. | | | |
| Gene | **Direction** | **Primer Sequence (5’ 🡪 3’)** | **Amplicon Size** |
| *For gene expression* | | | |
| *Rpl19* | Forward | AGCACATCCACAAACTGAAGGCAG | 168 bp |
|  | Reverse | TACAGACACGAGGGACGCTTCATT |  |
| *Dnmt3a* | Forward | TTCAGCAAAGTGAGGACC AT | 121 bp |
|  | Reverse | GGACAGGGAAGCCAAACA |  |
| *Avp* | Forward | CCTCACCTCTGCCTGCTACTT | 442 bp |
|  | Reverse | GGGGGCGATGGCTCAGTAGAC |  |
| *Ar* | Forward | GTAGAGCAAGACTGGAGAG | 89 bp |
|  | Reverse | GACGAAGAAGTTGAAGTAGAC |  |
| *Dnmt3b* | Forward | TGTGCAGAGTCCATTGCTGTAGGA | 104 bp |
|  | Reverse | GCTTCCGCCAATCACCAAGTCAAA |  |
| *Esr2* | Forward | AGGATGTACCACCGAATGCCAAGT | 63 bp |
|  | Reverse | TCCAAGTGGGCAAGGAGACAGAAA |  |
| *Dnmt1* | Forward | AAGCCAGCTATGCGACTTGGAAAC | 121 bp |
|  | Reverse | ACAACCGTTGGCTTTCTGAGTGAG |  |
| *Esr1* | Forward | GATGGTCAGTGCCTTATTGGATGC | 453 bp |
|  | Reverse | GCAGGTTCATCATGCGGAATCGA |  |
| *Otr* | Forward | CGATTGCTGGGCGGTCTT | 143 bp |
|  | Reverse | CCGCCGCTGCCGTCTTGA |  |
| *Oxt* | Forward | gaggagaactacctgccctc | 170 bp |
|  | Reverse | ggtatcatcacaaagcgggc |  |
| *Bdnf* | Forward | TGGCTGACACTTTTGAGCAC | 161 bp |
|  | Reverse | TTCCTCCAGCAGAAAGAGCA |  |
|  | | | |
| *For bisulfite sequencing* | | | |
| *Bdnf* | Forward | TGGAAAGGGTTTTATTAATATGTGAT | 450 bp |
|  | Reverse | ACCAAAAATCTATTCCAACCTACAC |  |
| *Dnmt3b* | Forward | GGGAGGGATTTTAAATTTTTTT | 664 bp |
|  | Reverse | AACACTAAATCCTAAAAACAACCTCTACT |  |
| *Esr1* | Forward | TAGTTTAAGATGTTTATGGAGAGGGTTTT | 661 bp |
|  | Reverse | TAAATCTAACTCTCCCACAAAATAACTAC |  |

**Supplemental Table S4.** Summary of male and female offspring and litters tested in the study.

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment group | Total number of offspring (Number of litters) | | |
|  | Males | Females | Males and Females |
| Vehicle Control | 5 (5) | 5 (5) | 10 (8) |
| EE | 5 (5) | 5 (5) | 10 (9) |
| BPA | 5 (5) | 5 (5) | 10 (8) |

**Supplemental Figure Legends**

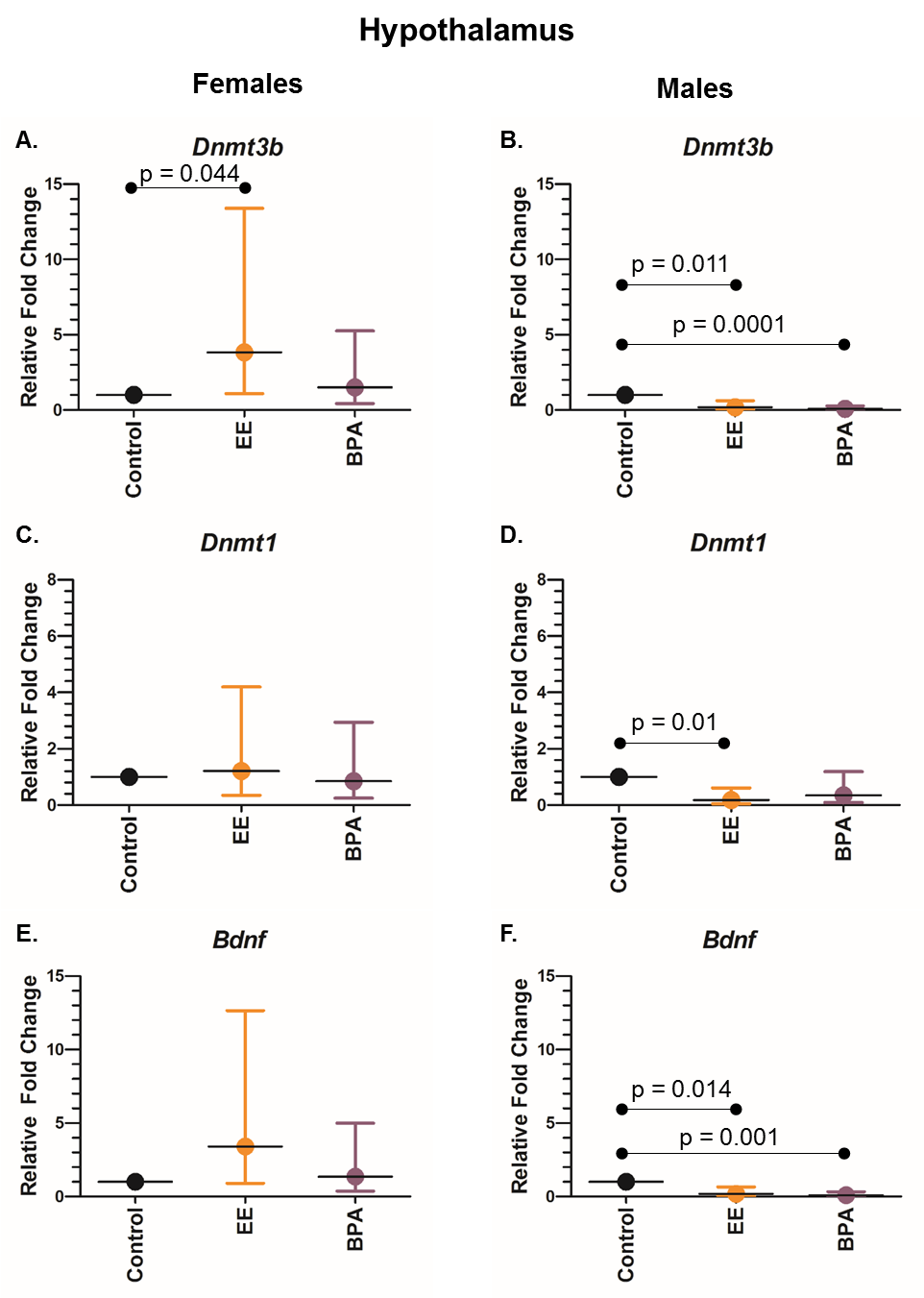
**Supplemental Figure S1.** Gene expression differences in the hypothalamus of female and male rats developmentally exposed to BPA or EE. (A) *Dnmt3b* expression in females. (B) *Dnmt3b* expression in males. (C) *Dnmt1* expression in females. (D) *Dnmt1* expression in males. (E) *Bdnf* expression in females. (F) *Bdnf* expression in males. P values are shown in the individual panels. Fold change refers to the gene expression level relative to that in the vehicle control group using the 2-ΔΔCT method. Statistical significance was determined using the ΔCT values. The graphs depict the mean ± upper and lower 95% confidence interval.

**Supplemental Figure S2.** Additional gene expression differences in the hypothalamus of female and male rats developmentally exposed to BPA or EE. (A) *Esr1* expression in females. (B) *Esr1* expression in males. (C) *Esr2* expression in females. (D) *Esr2* expression in males. (E) *Otr* expression in females. (F) *Otr* expression in males. P value differences are shown in the individual panels. Fold change refers to the gene expression level relative to that in the vehicle control group using the 2-ΔΔCT method. Statistical significance was determined using the ΔCT values. The graphs depict the mean ± upper and lower 95% confidence interval.

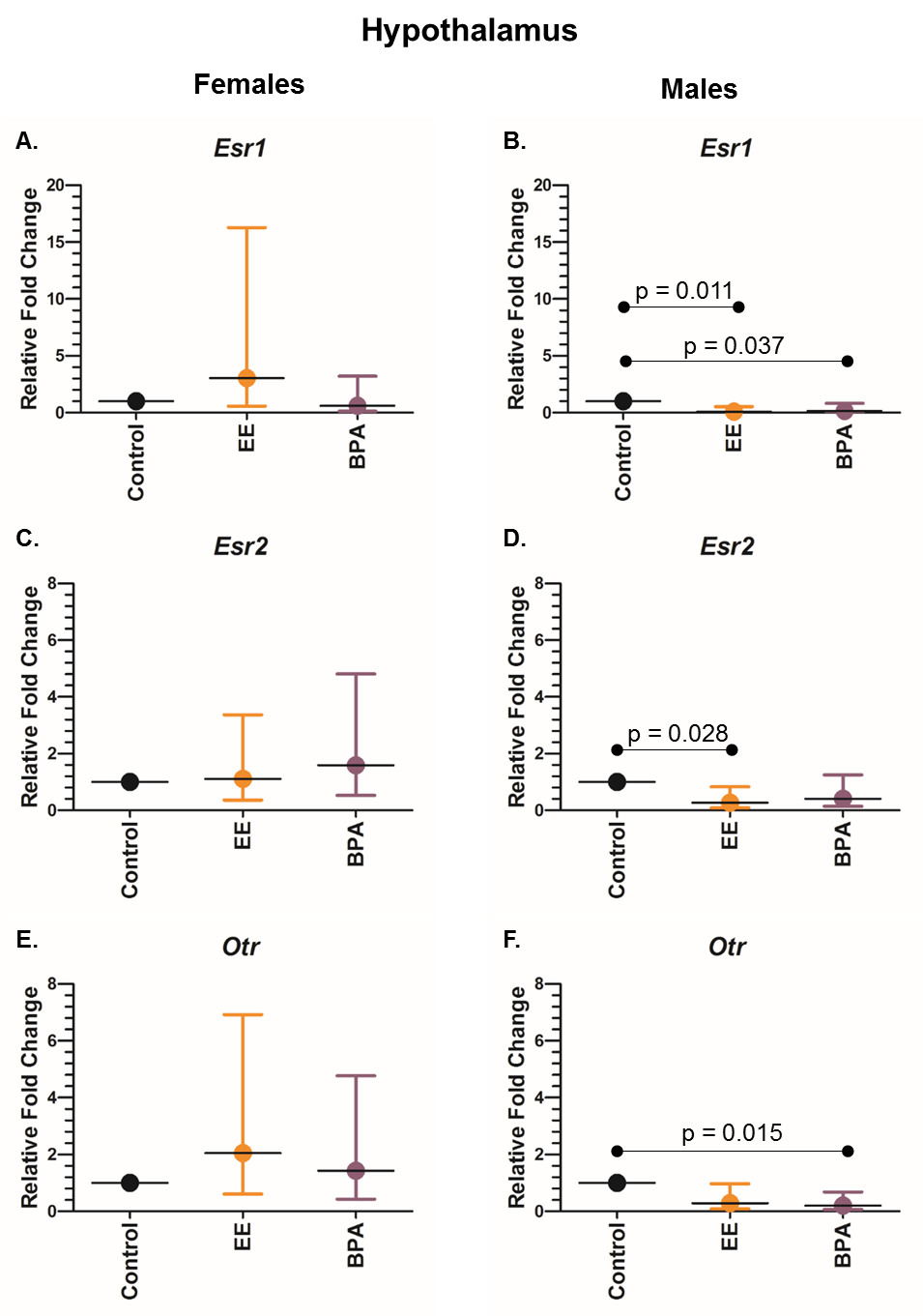
**Supplemental Figure S3.** Effect of treatment with 5-aza-cytidine on the expression of (A) *Bdnf*, (B) *Esr1*, and (C) *Dnmt3b* in rat prostate epithelial cell lines by qPCR. A normal prostate epithelial cell line NbE-1 (left), and a cancerous rat prostate epithelial cell line AIT (right) were treated with DMSO (Veh), or 0.5 µM or 1 µM 5-aza-2-deoxycytidine, a DNA methylation inhibitor, every two days for 8 days. Since the expression level of *Bdnf* was very low in AIT cells, it was not shown in this figure. Data are expressed as mean ± SEM. \*\*p<0.01 and \*\*\*p<0.001 vs Vehicle by One-way ANOVA and Tukey’s multiple test comparison.

**Supplemental Figure S4.** Effect of early-life exposure to EE and BPA on the methylation and expression of *Dnmt3b* in the adult rat hypothalamus. (A) Schematic diagram showing the presence of a CG-rich region (green) at the promoter region of *Dnmt3b*, which spans over the exon 1. Figure with annotation modified from UCSC Genome Browser. TSS, transcription start site. Region used for primer design (300 bp upstream and 300bp downstream of the CG-rich region) is highlighted with a red line. (B) Primer design using MethPrimer. The blue highlighted region under the curve indicates the CG-rich region within the sequence used for primer design. A blue line indicates the target region for bisulfite sequencing. Methylation pattern of the CG-rich region for *Dnmt3b* in the hypothalamus of (C) females and males, (D) females, and (E) males with early-life exposure to vehicle control (white), 0.5 μg EE/kg BW (EE; orange), and 2500 μg BPA /kg BW (BPA; violet). Data are expressed as mean ± SEM. Each circle represents the average % methylation of each corresponding CG site from 5-7 individual colonies. (N=5 males and N=5 females per group). For methylation analysis, \*p<0.05 vs Vehicle by Two-way ANOVA and Tukey’s multiple test comparison. Gene expression levels are expressed as gene expression level in treatment groups relative to the control group using the 2-ΔΔCT method. For the gene expression graphs, \*p<0.05; p<0.0001.

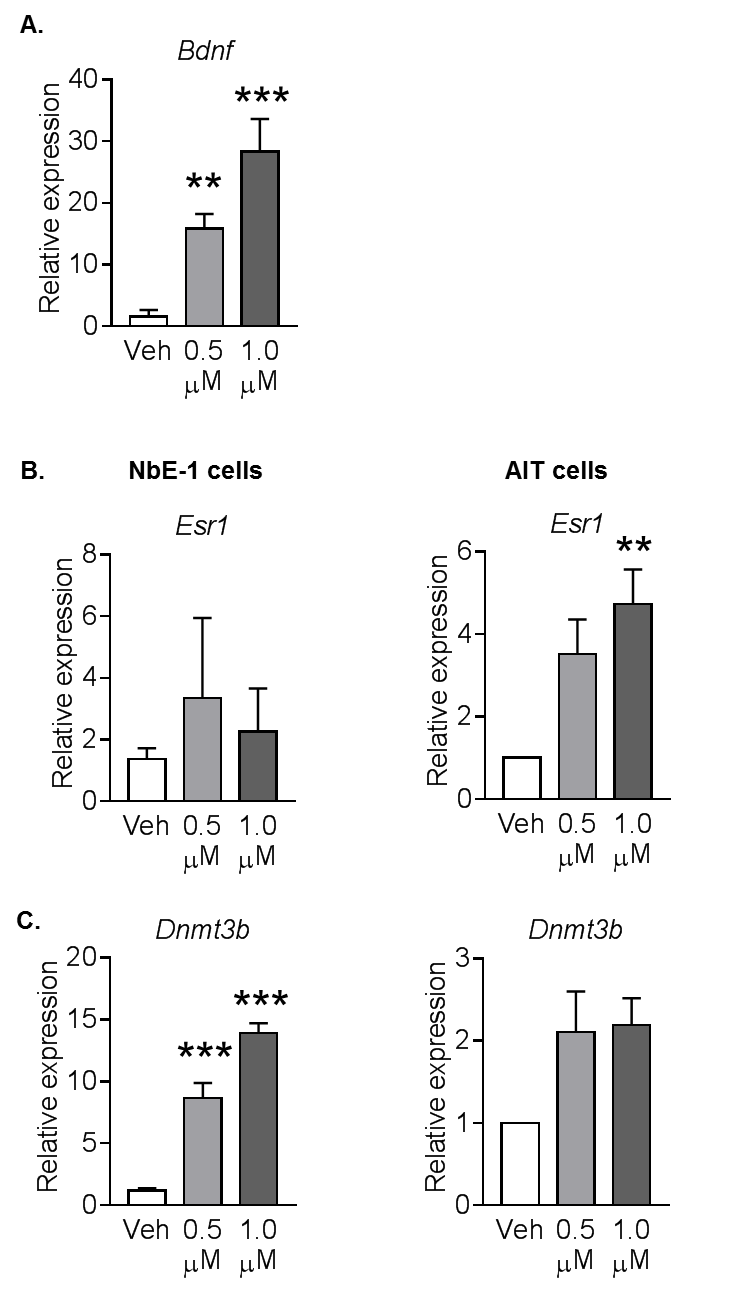
**Supplemental Figure S5**. Effect of early-life exposure to EE and BPA on the methylation and expression of *Esr1* in the adult rat hypothalamus. (A) Schematic diagram showing the presence of a CG-rich region (green) within exon1 of *Esr1*. Figure with annotation modified from UCSC Genome Browser. Region used for primer design (1,000 bp upstream and 1,000bp downstream of the CG-rich region) is highlighted with a red line. (B) Primer design using MethPrimer. The blue highlighted region under the curve indicates the CG-rich region within the sequence used for primer design. A blue line indicates the target region for bisulfite sequencing. Methylation pattern of the CG-rich region for *Esr1* in the hypothalamus of (C) females and males, (D) females, and (E) males with early-life exposure to vehicle control (white), 0.5 μg EE/kg BW (EE; orange), and 2500 μg BPA /kg BW (BPA; violet). Data expressed as mean ± SEM. Each circle represents the average % methylation of each corresponding CG site from 6-7 individual colonies. (N=5 males and N=5 females per group). For methylation analysis, ap<0.05, EE vs Vehicle, and bp<0.05, BPA vs Veh, and cp<0.05, EE/BPA vs Vehicle, by Two-way ANOVA and Tukey’s multiple test comparison. Gene expression levels are expressed as gene expression level in treatment groups relative to the control group using the 2-ΔΔCT method. For the gene expression graphs, \*p<0.05.



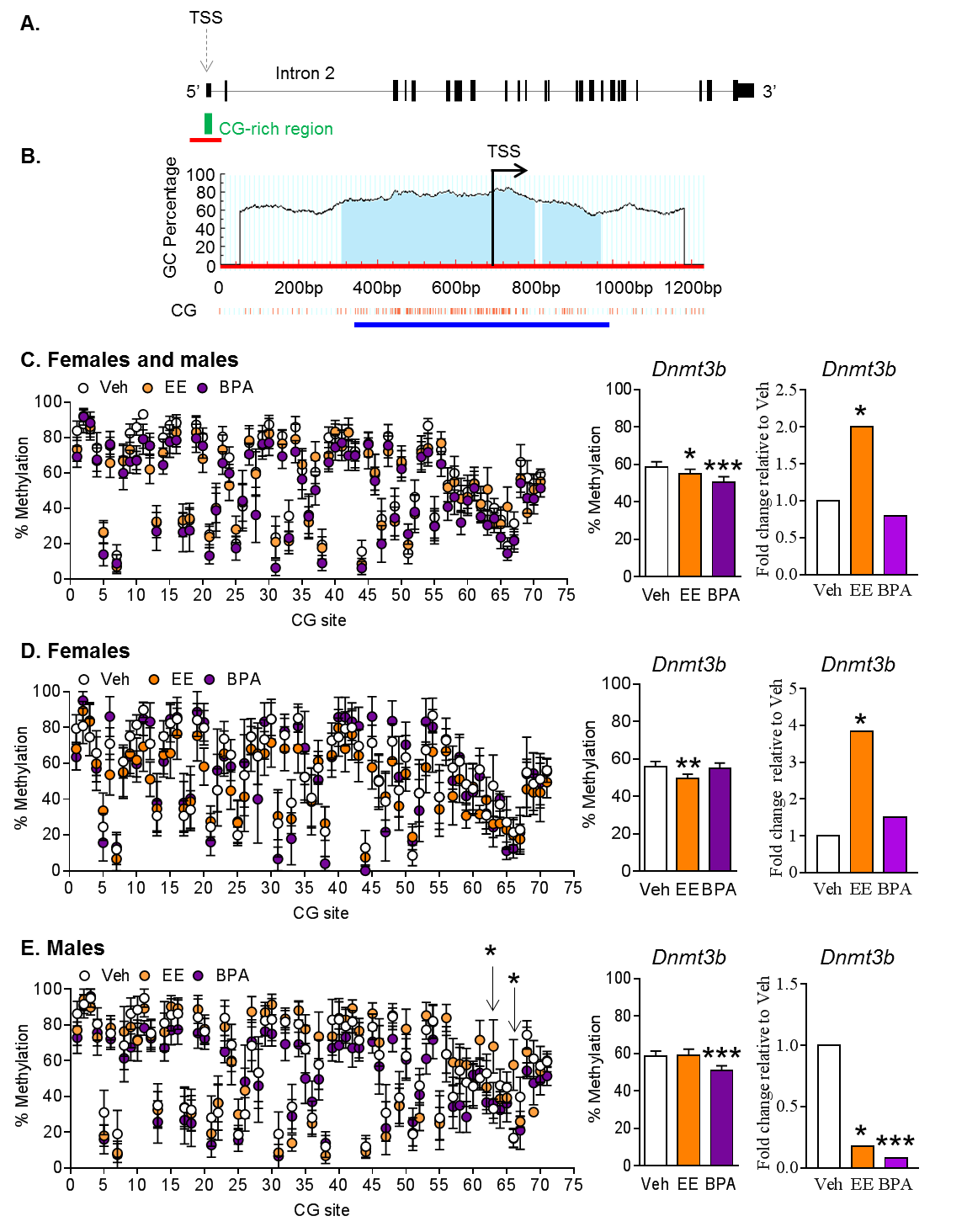
Supplemental Figure S1



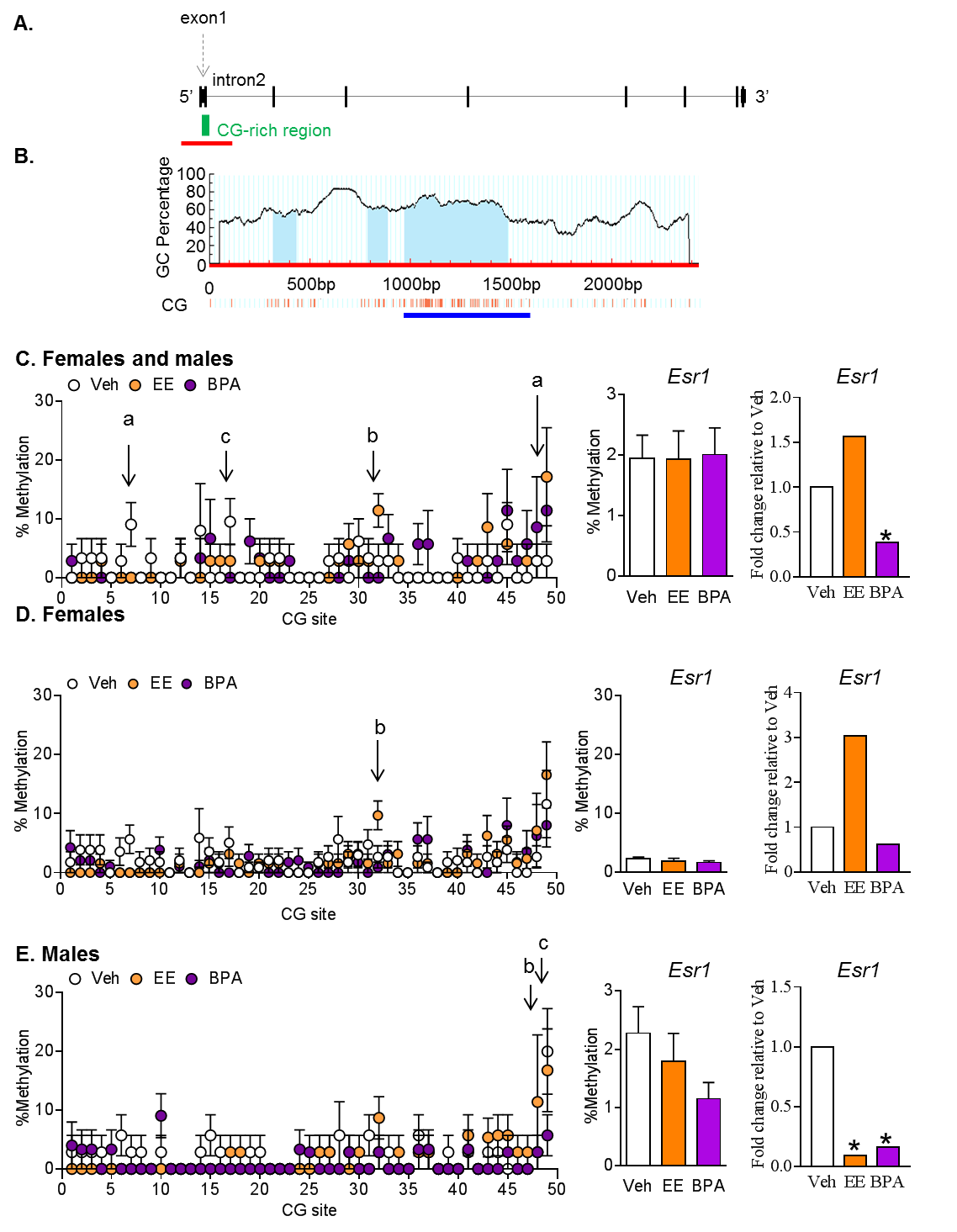
Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5