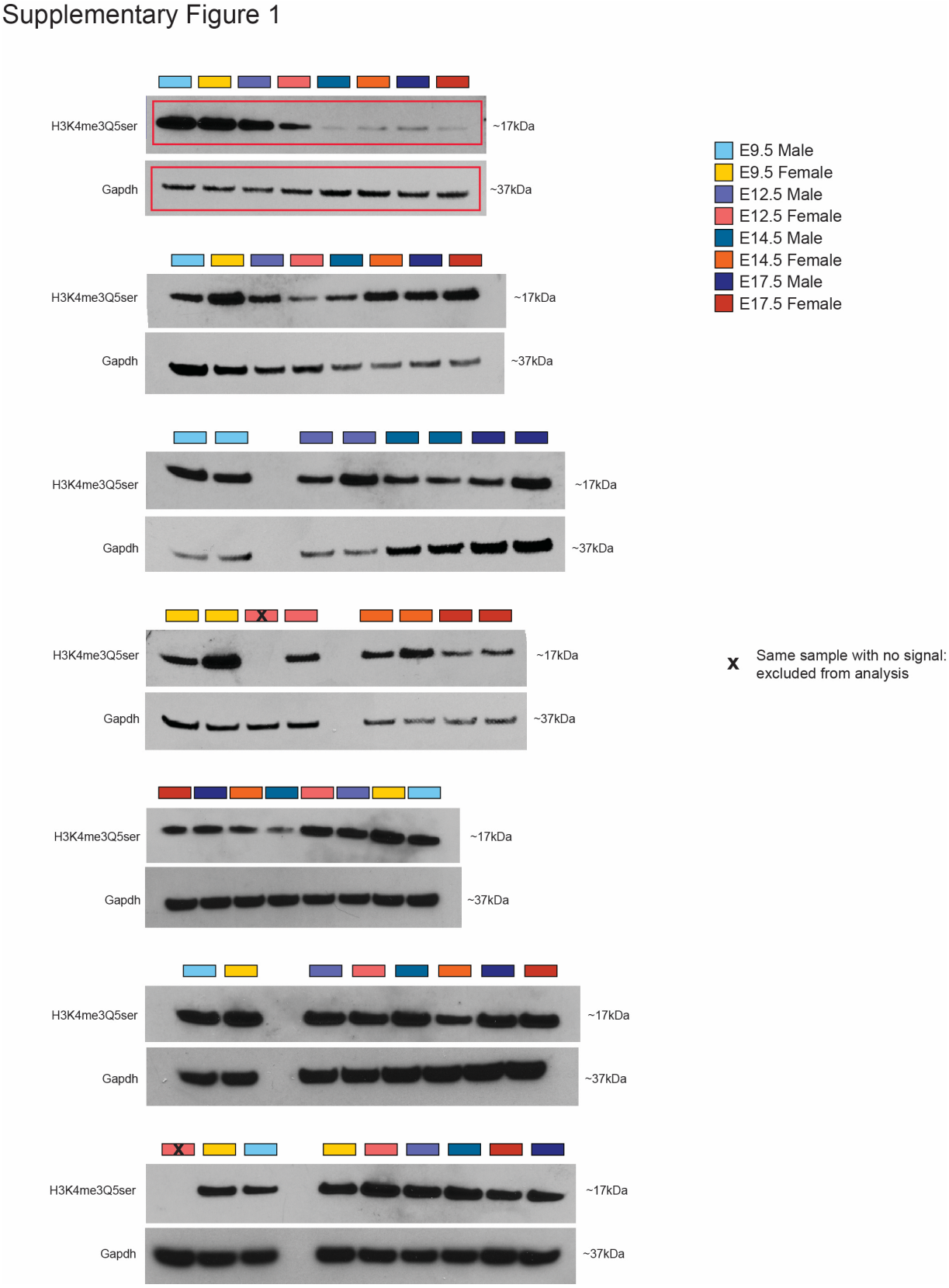
Supplementary Figure Captions

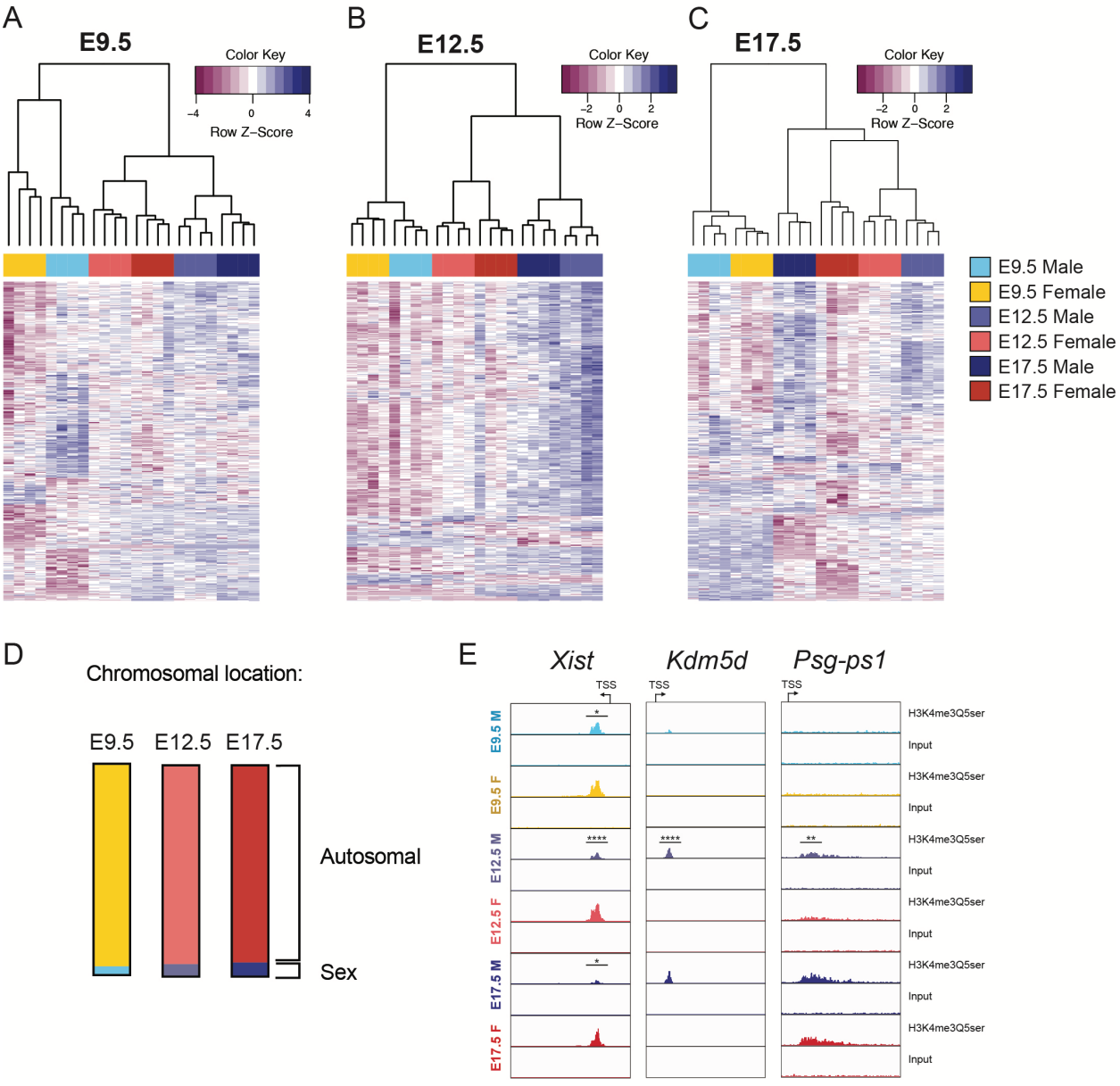
Supplementary Figure 1



Supplementary Figure 1. Western blots used for the quantification of the placental H3K4me3Q5ser signal in Figure 1C

Red rectangles indicate the representative blots displayed in the main figure. One sample (run two times) was excluded due to lack of H3K4me3Q5ser signal (indicated by X).

Supplementary Figure 2



Supplementary Figure 2. Sex differences in placental H3K4me3Q5ser

(A-C) Heatmaps of top 500 differential peaks from Diffbind analysis ($p < 0.05$) based on fold change comparing male vs. female placental tissues within age at **(A)** E9.5, **(B)** E12.5, and **(C)** E17.5 with hierarchical clustering (N=4 samples/sex/age). **(D)** Chromosomal location of all significant ($p < 0.05$) sex different peaks per age, where ~95% of peaks occurred on autosomal

665 chromosomes and 4-6% of peaks occurred on the X or Y chromosomes. **(E)** Representative
666 genome browser tracks of sex different H3K4me3Q5ser peaks (vs respective DNA input on the X
667 chromosome (*Xist*: $p < 0.05$ relative to male at E9.5 and E17.5, $p < 0.0001$ relative to male at
668 E12.5), Y chromosome (*Kdm5d*: $p < 0.0001$ relative to male at E12.5), and on chromosome 7 (*Psg-*
669 *ps1*: $p < 0.01$ relative to male at E12.5). Each track represents merged signal for 4 samples.
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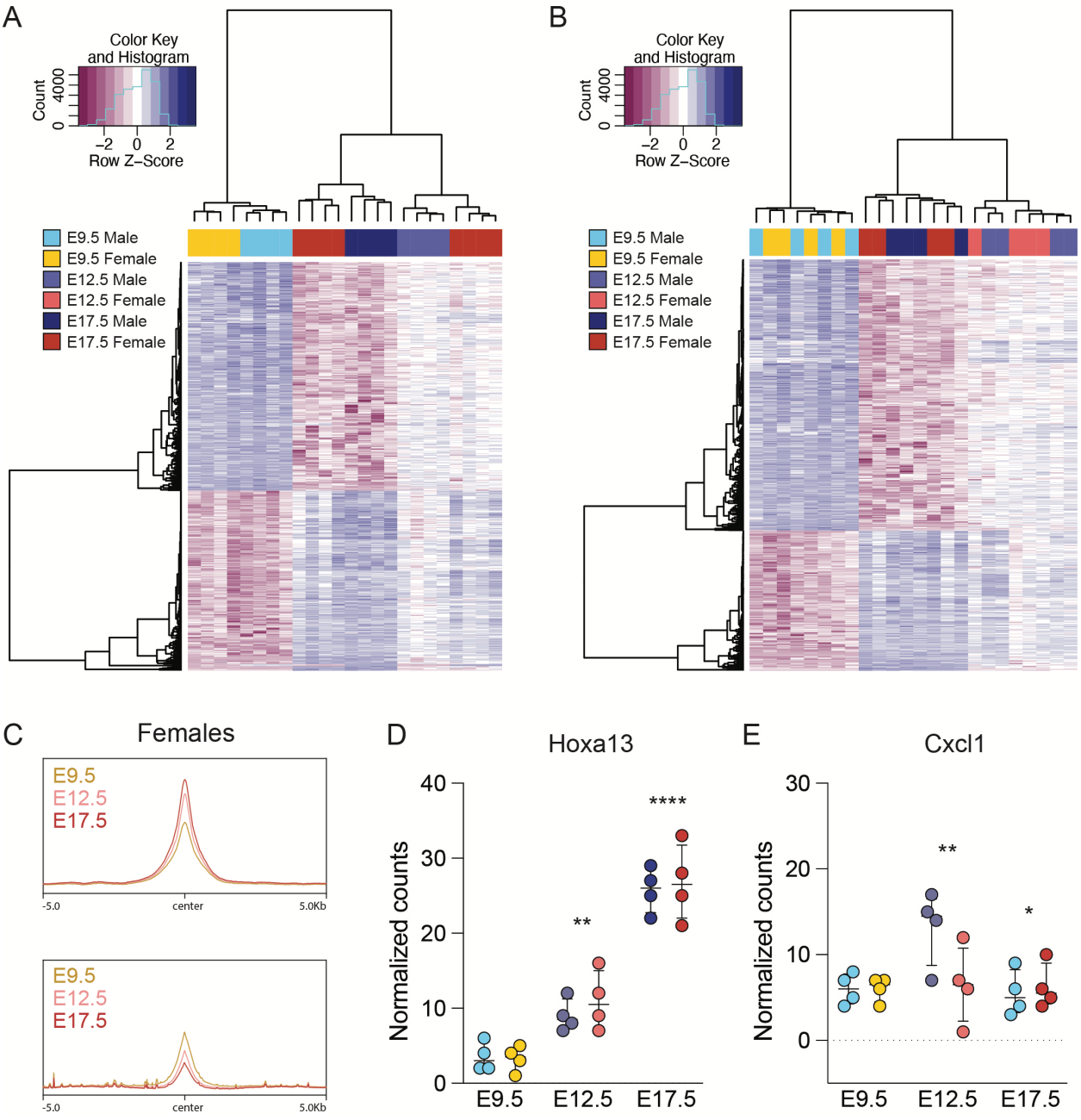
Supplementary Figure 3



Supplementary Figure 3. Differential H3K4me3Q5ser enrichment in male and female placenta

(A-B) Heatmaps of top 1000 differential peaks from Diffbind analysis ($p < 0.05$) based on fold change comparing E9.5 vs E17.5 in male **(A)** and female **(B)** placental tissues with hierarchical clustering, showing developmental changes are largely conserved across sexes (N=4

680 samples/sex/age). **(C)** Profiles of differential peaks from E9.5 vs E17.5 comparisons, separated by
681 directionality and centered on genomic regions in females. **(D)** *Hoxa13* gene expression increases
682 across development, corresponding with H3K4me3Q5ser increases (two-way ANOVA, effect of
683 age: $F(2,18) = 1108$, $p < 0.0001$; Tukey's post-hoc, E9.5 vs E12.5: $^{**}p = 0.0016$; E12.5 vs E17.5:
684 $^{****}p < 0.0001$). **(E)** *Cxcl1* gene expression decreases from E12.5 to E17.5, similarly to
685 H3K4me3Q5ser decreases at the same time points (two-way ANOVA, effect of age: $F(2,18) =$
686 4.244, $p = 0.0309$; Tukey's post-hoc; E12.5 vs E17.5: $^{*}p = 0.049$). N = 4/sex/age. Data are median
687 ± interquartile range.
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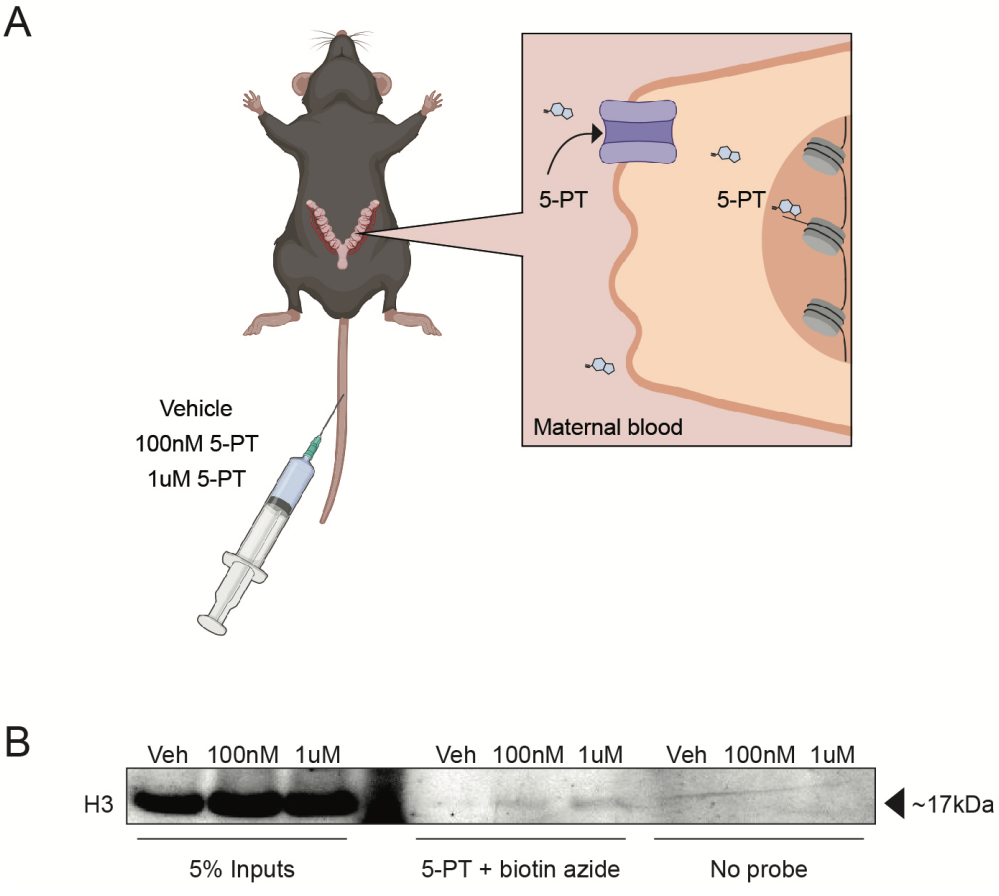
Supplementary Figure 4

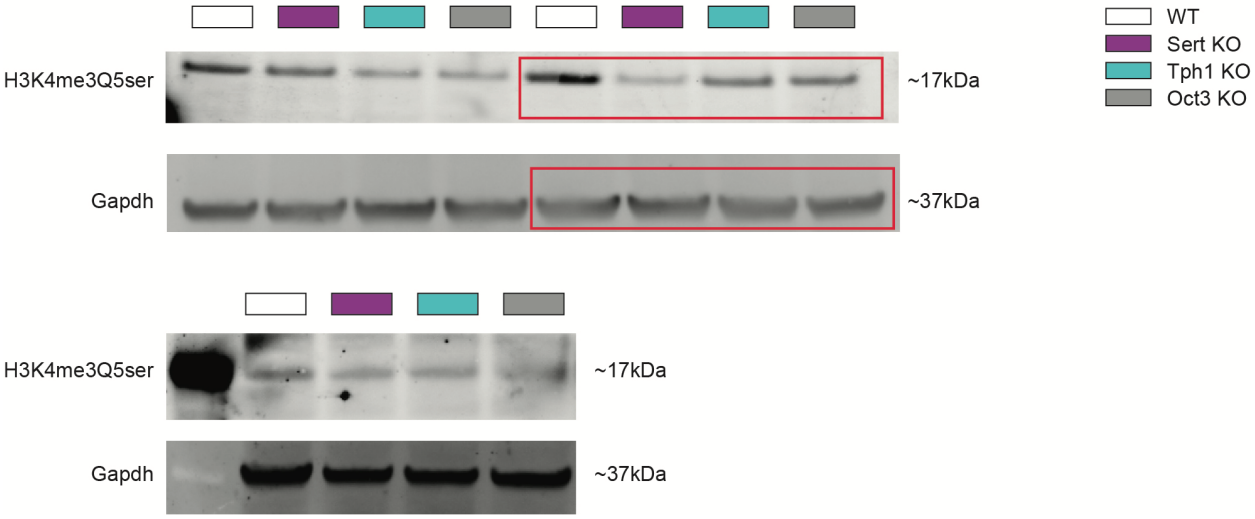


Supplementary Figure 4. Detection of circulating propargylated 5-HT (5-PT) on placental histone H3

(A) Schematics of experimental design and expected results. The alkyne-functionalized 5-HT analogue, 5-PT, was injected into pregnant E12.5 mice at 100nM or 1μM (vs. vehicle). Transporter-mediated mechanisms within the apical membrane of the placenta facing maternal circulation would directly take up 5-PT into cells, increasing 5-PT addition to histone H3 as proxy for how H3 serotonylation might be regulated. One hour post-injection, placental tissues were collected and subjected to copper-click chemistry using biotin azide (vs. no probe). 5-PT-ylated proteins were immunoprecipitated with streptavidin beads, followed by western blotting for H3.

(B) Western blot showing H3 is enriched in 5-PT-treated samples in dose-dependent manner (compared to vehicle) only in click reaction conditions.

Supplementary Figure 5



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704 **Supplementary Figure 5. Western blots used for the quantification in Figure 2F.** Western

705 blots of placental tissues at E12.5, showing H3K4me3Q5ser in WT, Sert KO, Tph1 KO and Oct3

706 KO tissues. Red rectangles indicate the representative blots displayed in the main figure.

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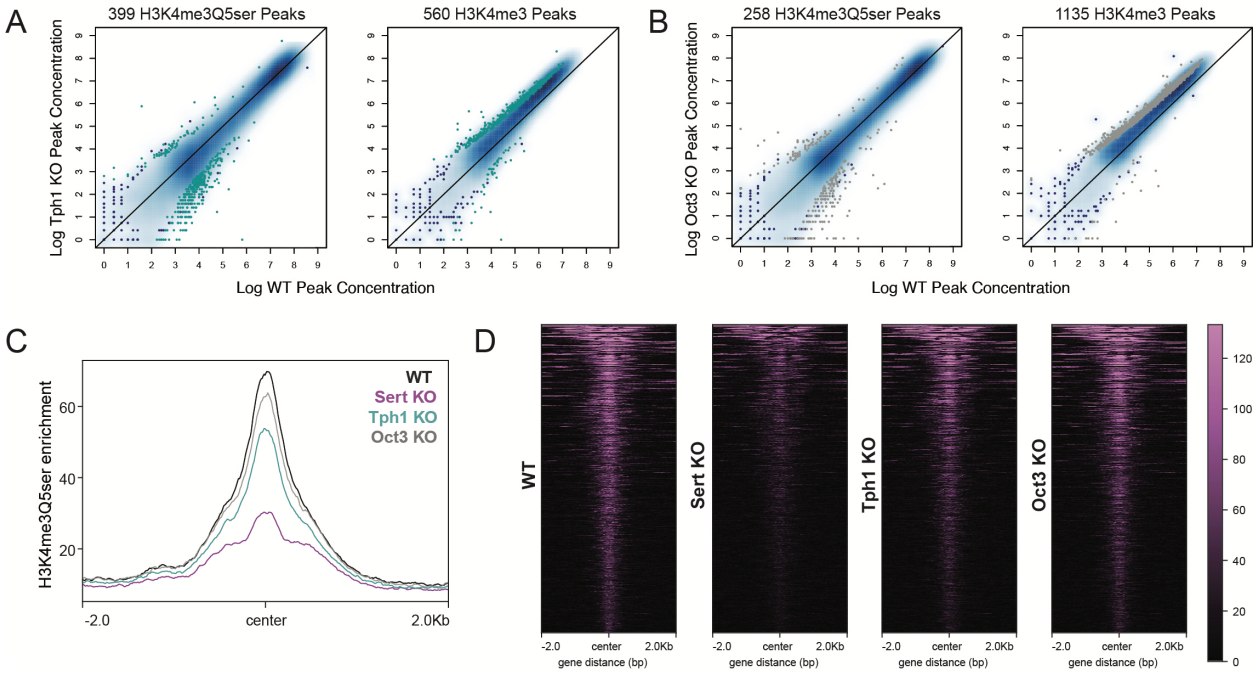
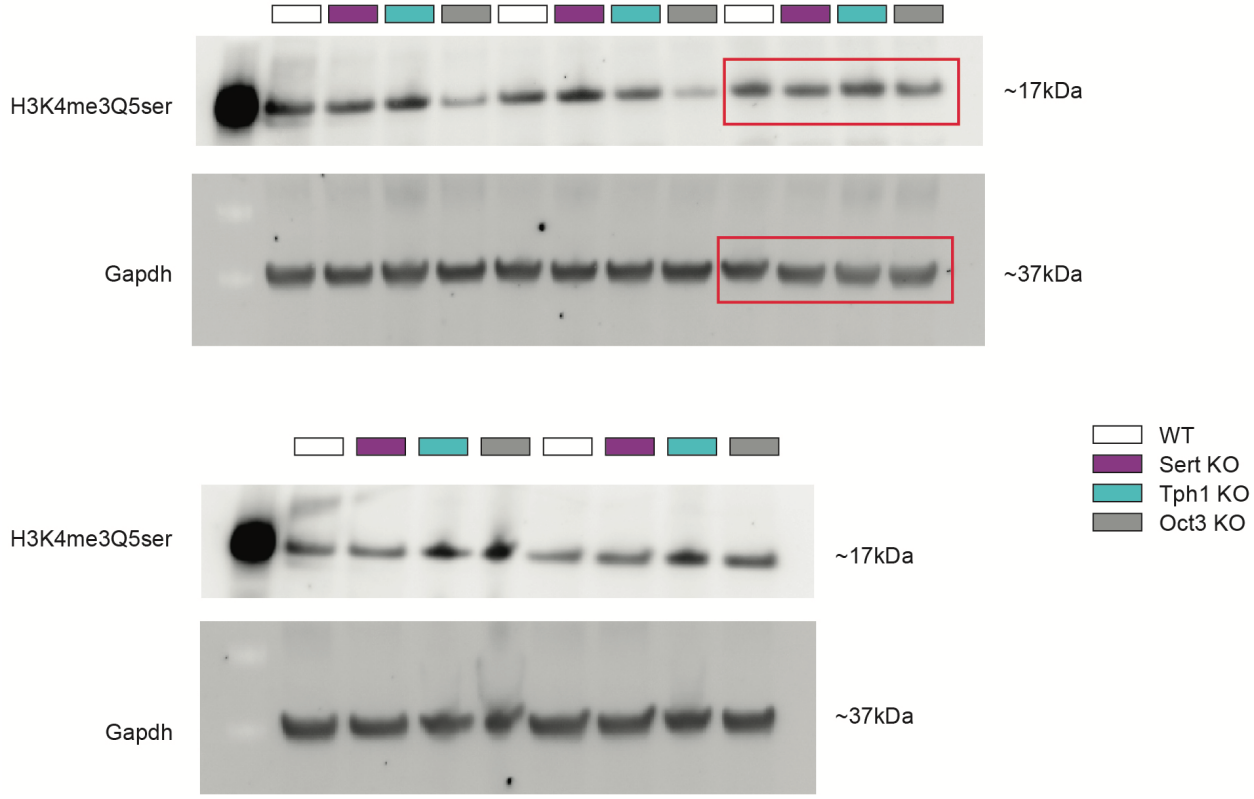
Supplementary Figure 6



Supplementary Figure 6. Histone serotonylation reductions are most affected by deletion of SERT

(A-B) Scatterplots of differential H3K4me3Q5ser and H3K4me3 peaks in **(A)** Tph1 KO and **(B)** Oct3 KO E12.5 placentas relative to WT ($p < 0.05$). **(C)** Profiles and **(D)** heatmaps of all downregulated differential H3K4me3Q5ser loci comparing WT vs Sert KO placental tissues ($p < 0.05$), showing Sert KO has the greatest impact on histone serotonylation peak reductions compared to Tph1 KO and Oct3 KO, consistent with reductions in 5-HT levels.

Supplementary Figure 7



Supplementary Figure 7. Western blots used for the quantification of H3K4me3Q5ser in E12.5 brain tissues of WT, Tph1 KO, Sert KO, and Oct3 KO in Figure 4D. Red rectangles indicate the representative blots displayed in the main figure.

Supplementary Table Captions

Excel file including Supplementary Tables 1-28, which contain analyses of ChIP-seq and RNA-seq from placental and brain tissues.

Supplementary Table 1: Developmental placenta H3K4me3Q5ser ChIP-seq, DiffBind results (E9.5 male vs E17.5 male): Fig. 1E-J, Supplementary Fig. 3A

Supplementary Table 2: Developmental placenta H3K4me3Q5ser ChIP-seq, DiffBind results (E9.5 female vs E17.5 female): Fig. 1E, H, I, Supplementary Fig. 3B-C

Supplementary Table 3: Developmental placenta H3K4me3Q5ser ChIP-seq, DiffBind results (E9.5 male vs E9.5 female): Fig. 1E, Supplementary Fig. 2A

Supplementary Table 4: Developmental placenta H3K4me3Q5ser ChIP-seq, DiffBind results (E12.5 male vs E12.5 female): Fig. 1E, Supplementary Fig. 2B

Supplementary Table 5: Developmental placenta H3K4me3Q5ser ChIP-seq, DiffBind results (E17.5 male vs E17.5 female): Fig. 1E, Supplementary Fig. 2C

Supplementary Table 6: Developmental placenta bulk RNA-seq, normalized counts table

Supplementary Table 7: Developmental placenta bulk RNA-seq, DESeq2 results (E9.5 male vs E17.5 male): Fig. 1I, Supplementary Fig. 3D-E

Supplementary Table 8: Developmental placenta bulk RNA-seq, DESeq2 results (E9.5 female vs E17.5 female): Fig. 1I, Supplementary Fig. 3D-E

Supplementary Table 9: Developmental placenta functional annotation analysis, Gene Ontology Biological Processes (E9.5 male vs E17.5 male): Fig. 1K

Supplementary Table 10: Developmental placenta functional annotation analysis, Reactome (E9.5 female vs E17.5 female): Fig. 1K

Supplementary Table 11: Transgenic placenta H3K4me3Q5ser ChIP-seq, DiffBind results (WT vs Sert KO): Fig. 3A-C, Supplementary Fig. 7C-D

Supplementary Table 12: Transgenic placenta H3K4me3 ChIP-seq, DiffBind results (WT vs Sert KO): Fig. 3A, 3C

Supplementary Table 13: Transgenic placenta H3K4me3Q5ser ChIP-seq, DiffBind results (WT vs Tph1 KO): Fig. 3A, 3C, Supplementary Fig. 7A

Supplementary Table 14: Transgenic placenta H3K4me3 ChIP-seq, DiffBind results (WT vs Tph1 KO): Fig. 3A, 3C, Supplementary Fig. 7A

Supplementary Table 15: Transgenic placenta H3K4me3Q5ser ChIP-seq, DiffBind results (WT vs Oct3 KO): Fig. 3A, 3C, Supplementary Fig. 7B

Supplementary Table 16: Transgenic placenta H3K4me3 ChIP-seq, DiffBind results (WT vs Oct3 KO): Fig. 3A, 3C, Supplementary Fig. 7B

Supplementary Table 17: Transgenic placenta functional annotation analysis, Gene Ontology Biological Processes (WT vs Sert KO): Fig. 3G

Supplementary Table 18: Transgenic placenta functional annotation analysis, Gene Ontology Biological Processes (WT vs Tph1 KO): Fig. 3G

Supplementary Table 19: Transgenic placenta functional annotation analysis, Gene Ontology Biological Processes (WT vs Oct3 KO): Fig. 3G

Supplementary Table 20: Transgenic brain bulk RNA-seq, normalized counts table

Supplementary Table 21: Transgenic brain bulk RNA-seq, DESeq2 results (WT vs Sert KO): Fig. 4E-F

Supplementary Table 22: Transgenic brain bulk RNA-seq, DESeq2 results (WT vs Tph1 KO): Fig. 4E-F

Supplementary Table 23: Transgenic brain bulk RNA-seq, DESeq2 results (WT vs Oct3 KO): Fig. 4E-F

Supplementary Table 24: Transgenic brain bulk RNA-seq functional annotation analysis, Gene Ontology Biological Processes (WT vs Sert KO): Fig. 4G

Supplementary Table 25: Transgenic brain bulk RNA-seq functional annotation analysis, Reactome (WT vs Sert KO): Fig. 4G

Supplementary Table 26: Transgenic brain bulk RNA-seq functional annotation analysis, Gene Ontology Biological Processes (WT vs Oct3 KO): Fig. 4G

Supplementary Table 27: Transgenic brain bulk RNA-seq functional annotation analysis, Reactome (WT vs Oct3 KO): Fig. 4G

Supplementary Table 28: Transgenic brain bulk RNA-seq functional annotation analysis, Reactome (WT vs Tph1 KO): Fig. 4G