Exposure of pregnant mouse females to sevoflurane results in alterations of transcription factor occupancy in sperm and transgenerational inheritance of autism-like behaviors

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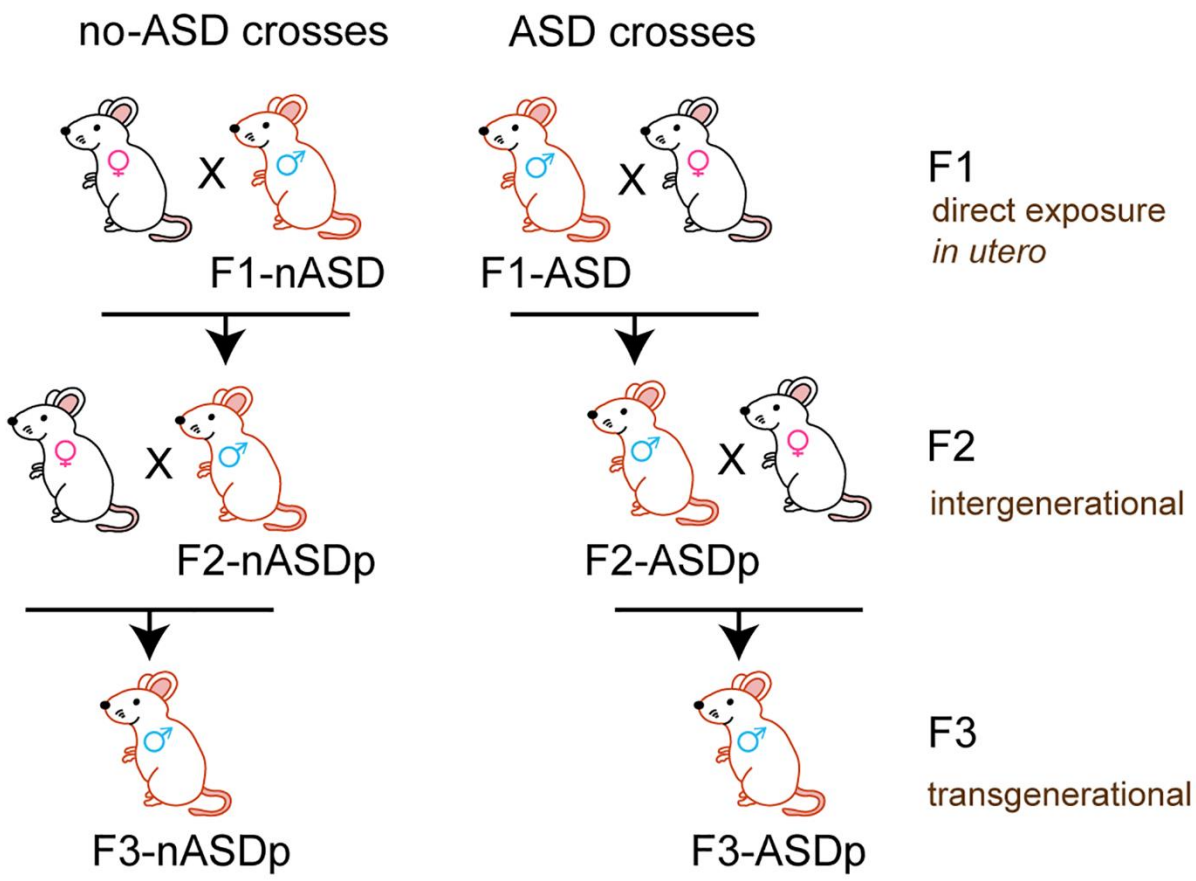
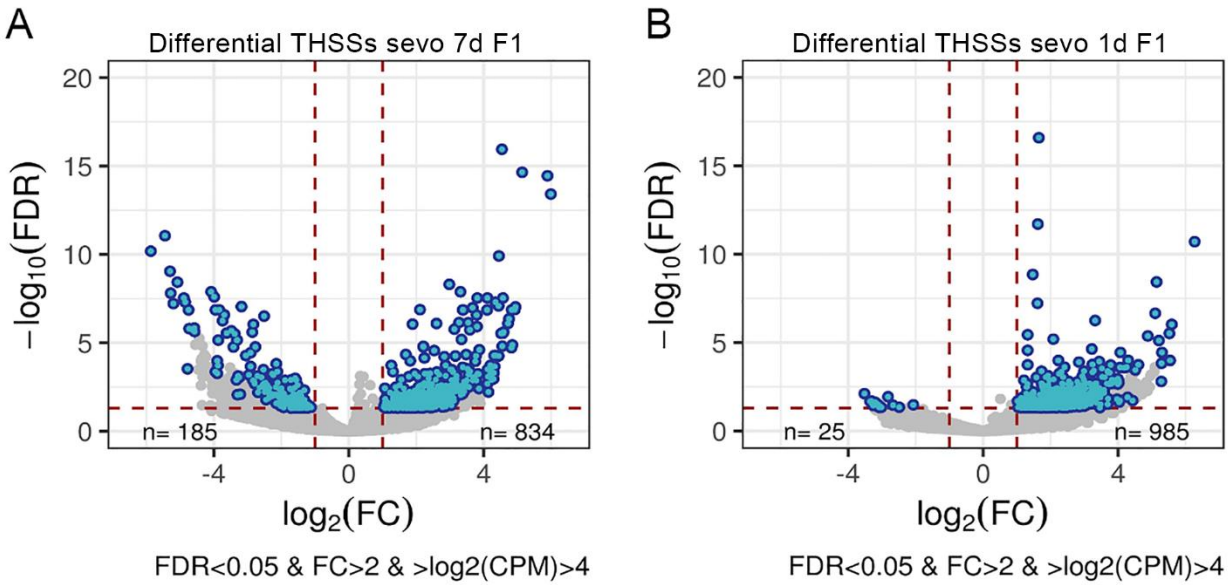
Supplemental Figures

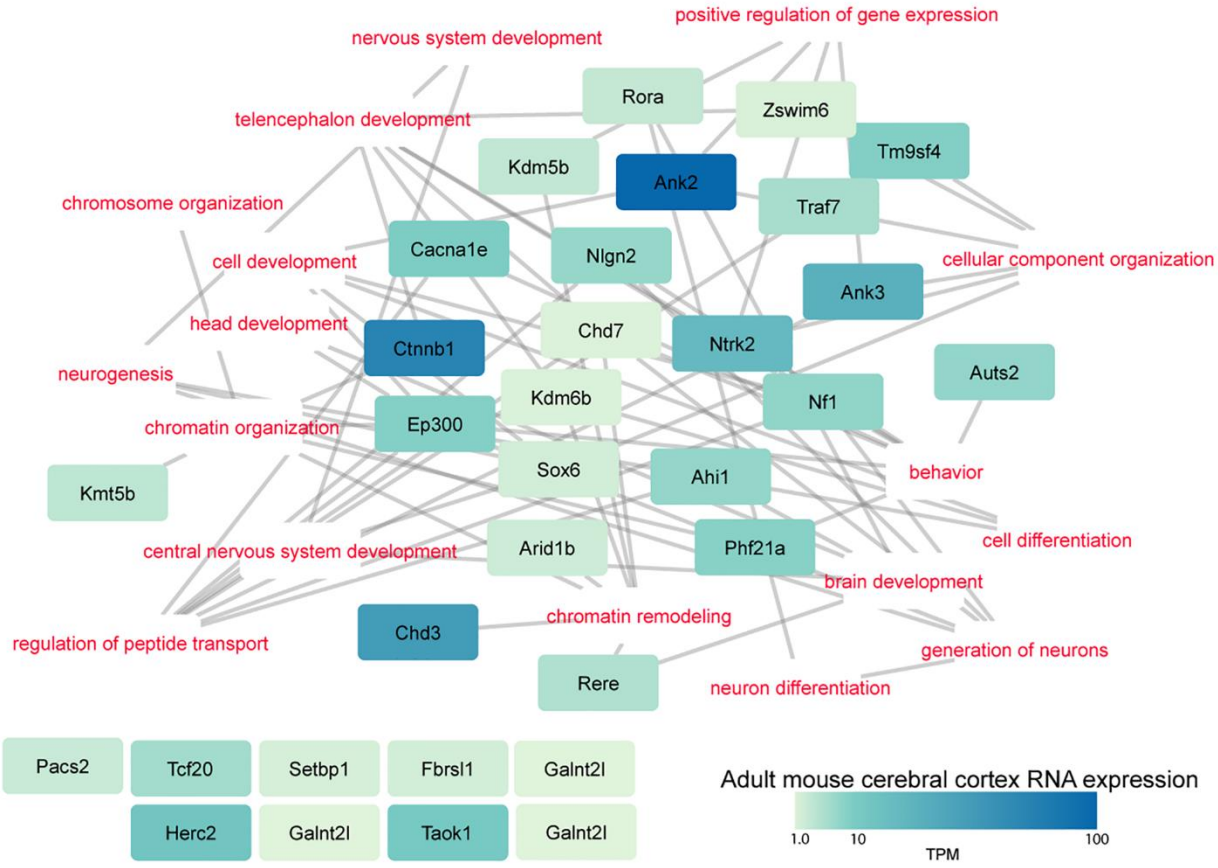


Supplemental Figure S1. Breeding schemes of sevoflurane-exposed mice. F1 males directly exposed to sevoflurane *in utero* were crossed with control females to generate F2 animals. F2 is the inter-generational group, where the germline that gave rise to F2 was directly exposed to sevoflurane in the F1 embryo. Two separate crosses, each with two biological replicates, were performed to generate F2 animals that are sired by either F1 exposed male mice showing ASD phenotypes or showing normal behavior. Similarly, two separate crosses, each with two biological replicates, were performed to generate F3 animals that are either sired by no-ASD F2 or ASD F2 animals. F3 is the first trans-generational group that was never directly exposed to sevoflurane.



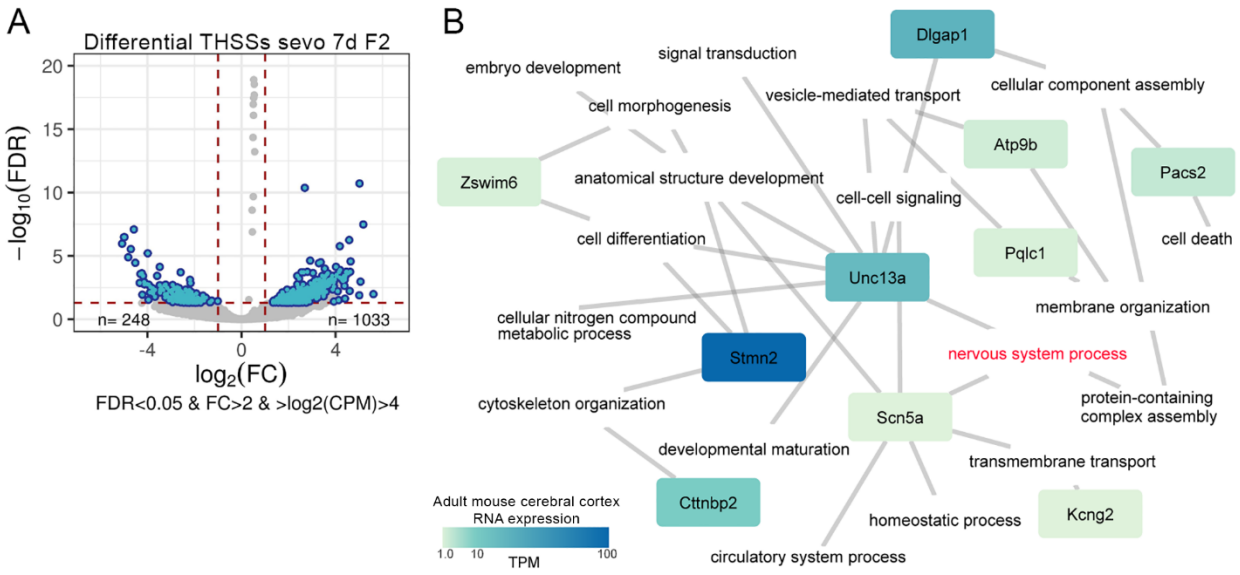
Supplemental Figure S2. Analysis of differential THSSs between sevoflurane exposed F1 and control animals.

Volcano plots showing pair-wised comparison between sperm THSSs datasets of 7-day exposed F1 (A) and 1-day exposed F1 (B) compared to control animals. The x-axis indicates the $\log_2$(fold change) and the y-axis shows the $-\log_{10}$(FDR). Differential THSSs with FDR<0.05, fold change (FC) >2 and $\log_2$(counts per million, CPM>4) are shown in blue.



Supplemental Figure S3. Genes implicated in ASD are enriched in/around differential THSSs between F1 exposed to sevoflurane and control.

Gene ontology network analysis of genes adjacent to differential THSSs. Each node of the network contains genes previously shown to be mutated in ASD patients and enriched in/around THSSs differentially occupied in sperm of F1 sevoflurane exposed versus control males. Colors indicate levels of RNA expression in the mouse cortex in transcripts per million, TPM. All ASD genes are connected based on enrichment in gene ontology biological processes terms (marked in red). Genes with no shared ontology are indicated at the bottom left.



Supplemental Figure S4. Genes involved in mental disorders and synapsis function are highly associated with differential THSSs shared between F1 and F2 mouse sperm.

(A) Analysis of THSSs present in the sperm of F1 and F2 sevoflurane ASD males but not in control. Volcano plot showing pairwise comparison. The x-axis shows the log2(fold change) and the y-axis indicates the log10(FDR). Significant THSSs with FDR<0.05, fold change (FC) >2 and log2(CPM)>4 are shown in blue. (B) Mental disorders and synapsis function genes enriched in/around differential conserved THSSs are shown in each node of the network. Color intensity indicates RNA expression level in mouse brain cortex in transcript per million, TPM. All ASD genes are connected by gene ontology biological process terms. Nervous system processes term is marked in red.