**Additional File 1**

**Transcriptome analysis of *Shank3*-overexpressing mice reveals unique molecular changes in the hypothalamus**

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**Materials and Methods**

**Mice**

The *EGFP* (enhanced green fluorescent protein)*-Shank3* transgenic (TG) mice used in this study have been described previously [[1](#_ENREF_1), [2](#_ENREF_2)]. The male wild-type (WT) and TG mice were bred and maintained in a C57BL/6J background according to the Korea University College of Medicine Research Requirements, and all the experimental procedures were approved by the Committees on Animal Research at the Korea University College of Medicine (KOREA-2016-0096). The mice were fed and had access to water *ad libitum* and were housed under a 12-h light-dark cycle.

**RNA sequencing and analysis**

The mice (10 to 12-week-old male WT and *Shank3* TG) were deeply anesthetized with isoflurane and decapitated. The hypothalamus was dissected from each brain, immediately placed in RNAlater solution (Ambion), and stored at 4 °C overnight. The hypothalamus from 2 mice of same genotype were pooled to make one RNA sample, and a total three pairs of RNA samples (three WT and three *Shank3* TG, thus total six mice per each genotype) were processed for RNA sequencing. RNA extraction, library preparation, cluster generation, and sequencing were performed by Macrogen Inc. (Seoul, Korea). RNA samples for sequencing were prepared using a TruSeq Stranded mRNA LT Sample Prep Kit (Illumina) according to the manufacturer’s instructions. An Illumina’s HiSeq 2000 was used for sequencing to generate 101-bp paired-end reads (Additional file 2, Table 1). Raw data were submitted to the GEO (Gene Expression Omnibus) repository under accession number GSE120609.

Transcript abundance was estimated with Salmon (v0.9.1) [[3](#_ENREF_3)] in Quasi-mapping-based mode onto the Mus musculus genome (GRCm38) with GC bias correction (--gcBias). Quantified gene-level abundance data was imported to R (v.3.6.0) with the tximport [[4](#_ENREF_4)] package and differential gene expression analysis was carried out using R/Bioconductor DEseq2 (v1.19.11) [[5](#_ENREF_5)]. Normalized read counts were computed by dividing the raw read counts by size factors and fitted to a negative binomial distribution. The *P* values were first corrected by applying an empirical estimation of the null distribution using the R fdrtool (v.1.2.15) package and then adjusted for multiple testing with the Benjamini–Hochberg correction. Genes with an adjusted *P* value of less than 0.05 were considered as differentially expressed. Volcano plots were generated using the R ggplot2 (v.2.2.1) package.

The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using DAVID software (version 6.8) [[6](#_ENREF_6)]. Mouse gene names were converted to human homologs using the Mouse Genome Informatics (MGI) database (<http://www.informatics.jax.org/homology.shtml>).

Gene Set Enrichment Analysis (GSEA) (http://software.broadinstitute.org/gsea) [[7](#_ENREF_7)] was used to determine whether a *priori*-defined gene sets would show statistically significant differences in expression between *Shank3* TG and WT mice. Enrichment analysis was performed using GSEAPreranked (gsea-3.0.jar) module on gene set collections H (Hallmark gene sets; 50 gene sets) and CP (KEGG; 186 gene sets) downloaded from Molecular Signature Database (MSigDB) v6.1 (http://software.broadinstitute.org/gsea/msigdb). GSEAPreranked was applied using the list of all genes expressed, ranked by the fold change and multiplied by the inverse of the *P* value with recommended default settings (1,000 permutations and a classic scoring scheme). The False Discovery Rate (FDR) was estimated to control the false positive finding of a given Normalized Enrichment Score (NES) by comparing the tails of the observed and null distributions derived from 1000 gene set permutations. The gene sets with an FDR of less than 0.05 were considered as significantly enriched.

**RNA purification and qRT-PCR**

Real-time quantitative reverse transcription PCR (qRT-PCR) was performed as described previously [[2](#_ENREF_2), [8](#_ENREF_8)]. Briefly, total RNA was extracted from the brain regions of WT and *Shank3* TG mice using an miRNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Two micrograms of total RNA was used for cDNA synthesis using iScript™ cDNA Synthesis Kit (Bio-Rad). Target mRNAs were detected and quantified by a real-time PCR instrument (CFX96 Touch, Bio-Rad) using SYBR Green master mix (Bio-Rad). The results were analyzed using the comparative Ct method normalized against the housekeeping gene *Gapdh*. The primer sequences for real-time PCR are as follows:

Mouse *Shank3* forward 5’ TGGTTGGCAAGAGATCCAT 3’ (exon 6 of 22),

reverse 5’ TTGGCCCCATAGAACAAAAG 3’ (exon 7 of 22)

Mouse *Gpr85* forward 5’ ATGCAGCCGACAACATTTTGC 3’,

reverse 5’ CAGGTGGAGCCATTTTTGACA 3’

Mouse *Cav2* forward 5’ CTCAAGCTAGGCTTCGAGGA 3’,

reverse 5’ ACAGGATACCCGCAATGAAG 3’

Mouse *Gapdh* forward 5’ GGCATTGCTCTCAATGACAA 3’,

reverse 5’ CCCTGTTGCTGTAGCCGTAT 3’

**Biochemistry and antibodies for Western blotting**

Whole lysates of the mouse brain were prepared as described previously [[9](#_ENREF_9), [10](#_ENREF_10)]. Briefly, the hypothalamus of 12-week-old mice were homogenized in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate) with freshly added protease and phosphatase inhibitors (Roche). Protein concentration was measured using Bradford Protein Assay (Bio-Rad). Brain lysates were heated in 1x NuPAGE LDS sample buffer (Invitrogen) containing a 1x NuPAGE reducing agent (Invitrogen). From each sample, 10~20 g of proteins were loaded for Western blotting. The antibodies used for Western blotting were GAPDH (Cell Signaling, #2118), Homer1b/c (Santa Cruz, sc-20807), PSD-95 (NeuroMab, 75-028), Shank3 (Santa Cruz Biotechnology, sc-30193). Western blot images were acquired by ChemiDoc Touch Imaging System (Bio-Rad).

**References**

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