Supplementary materials

Methods

Rats

Adult two female were mated with one male Sprague-Dawley (Jackson Labs) rats per facility to generate male and female offspring used in our experiments. The pregnant female rats 1-3 days prior to giving birth were singly housed to give birth. The delivery day was designated as postnatal day (PND) 0, and offspring were weaned at postnatal PND 21, then male and female offspring were separated into cages of 3 rats, keeping littermates together. All animals were maintained under a 12 hours light/dark cycle at 23℃ with free access to water and food.

Behavioral procedures

All behavioral testing were conducted during the light cycle between 9:00 a.m. and 6:00 p.m. Animals were habituated to the behavioral testing room for at least 1 hour and the experimenter was blind to animal treatment. To eliminate odor cues, each apparatus was thoroughly cleaned with 75% ethanol after each animal. All data were analyzed with video tracking software (Ethovision, Noldus, Netherlands).

Open field test (OFT)

Rats were placed in the center of an open field arena (60 × 60 × 60 cm) and were

allowed to freely explore their surroundings. The animals' movement trajectories were recorded for 10 min, and the time spent in the center (30 × 30 cm) and periphery of the test arena were analyzed as index of anxiety. The area was cleaned with 75% ethanol after each test to remove olfactory cues from the apparatus.

Elevated plus maze (EPM)

The maze is a grey plus-crossed-shaped apparatus, with two open arms (50 × 15cm) and two closed arms (50 × 15cm), linked by a central platform and located 50 cm above the floor. Rats were individually put in the center of the maze facing an open arm and were allowed to explore the maze for 5 min. The time spent in each arm and the distance moved were automatically measured using Noldus software. Rats that fell off the open arms were excluded from analyses. The apparatus was thoroughly cleaned with 75% ethanol after each animal.

Sucrose Preference Test (SPT)

Sucrose preference, as an index of anhedonia-like behavior in rodents, was conducted in two-bottle choice test. Before the test began, rats were trained to two bottles (200-mL cylindrical tubes) in the homecage for two days with free access to food and water. On the first day both bottles were filled with normal drinking water, and the following day both bottles were replaced with 1% sucrose (prepared in drinking

water). After the two-days training, the sucrose preference test began. Both bottles were taken away at 13:00 on the test day. After 6-h water deprivation, rat was allowed 3 h access to normal drinking water and 1% sucrose water from 19:00 to 22:00. Sucrose water consumption was recorded. During recording, the positions of 1% sucrose and normal drinking water were changed at 20:30 to avoid position preference. Both bottles were weighed before and after the test to calculate sugar water preference: surcose preference (%) = (surcose consumption / (sucrose consumption + normal water consumption)) *100%

Primers in reverse transcription quantitative PCR (qRT-PCR)

Sets of specific primers are showed: Uba52 F: GTCTTCGCGGTGGCATCATC R: GTGGCCGCACTTCTTCTTGC; Fau F: ATTGCCCCCGAAGATCAAGT R: CCAGCTACCTCCAGAGTGGT; Rps6 F: GAGAGGAAGCGCAAGTCTGT R: CGAGGCACAGTGGTATCTGT; Rsl1d1l1 F: GCACACAGCCGAAAGTTACAG R: CTGGAGTCTCTGTAGCCGGT; Rpl8 F: TCCTCTGCTAACCGAGCTGT R: GTTACCACCGCCAAAGGGAT; Rpl19 F: CGTCCTCCGCTGTGGTAAA R: TCCGGATGATCAGGCCATCT; Pten F: ATACCAGGACCAGAGGAAACCT R: GTCAGGGTGAGCACAAGATACT; GAPDH F: AAGGTCGGTGTGAACGGATT R: TGAACTTGCCGTGGGTAGAG.

Library generation protocol, sequencing parameters, quality control and bioinformatics analysis of RNA-seq

The RNA samples are subjected to strict quality control. The quality control standards mainly include the following aspects: (1) 1% agarose electrophoresis to detect whether the RNA sample has degradation and impurities; (2) Qubit2.0 Fluorometer: precise quantification of RNA concentration; (3) Qsep nucleic acid detector (BiOptic Inc.) detects the integrity and concentration of RNA samples.

Library generation protocol: After the total RNA sample is qualified, for eukaryotes, use magnetic beads with Oligo (dT) to enrich mRNA, for prokaryotes, use a kit to remove rRNA, and add fragmentation buffer to the obtained mRNA to make the fragments into short fragments, , and then use the fragmented mRNA as a template to synthesize the first strand of cDNA with six-base random primers, and add buffer, dNTPs, RNaseH and DNA Polymerase I to synthesize the second strand of cDNA, which is purified by QIAQuick PCR kit and buffered with EB liquid elution. The purified double-stranded cDNA is then subjected to end repair, base A, and sequencing adapters, and then the target size fragments are recovered by agarose gel electrophoresis and PCR amplification is performed to complete the entire library preparation.

Sequencing parameters: After the library inspection is qualified, the different libraries are pooled according to the requirements of effective concentration and target data

volume, and then Illumina sequencing is performed. The basic principle of sequencing is Sequencing by Synthesis. Four fluorescently labeled dNTPs, DNA polymerases, and adapter primers are added to the sequencing flow cell for amplification. When each sequencing cluster extends the complementary chain, each fluorescently labeled dNTP can release the corresponding fluorescence, the sequencer captures the fluorescent signal and converts the light signal into a sequencing peak through computer software to obtain the sequence information of the fragment to be detected. Quality control: In order to ensure the quality of subsequent analysis, we will strictly control the screening criteria of clean data. The specific criteria are as follows: (1) Remove reads with adapters; (2) Remove reads containing N (N means that the base information cannot be determined); (3) Remove low-quality reads (reads whose bases with quality value Qphred <= 20 account for more than 50% of the entire read). Pair-wise differential expression comparisons were performed using Voom Limma. A nominal significance threshold of $p < 0.05$ and fold change > 2.0 was used. Full lists of transcriptomic changes are in Supplementary Table S2-4. Enrichment between gene lists were analyzed using the DAVID (2021 Update). Heatmap analysis and volcano plot of expression levels were analyzed using R4.2.0.

Figure legends

Supplementary Figure. 1 Differential gene expression analysis between female

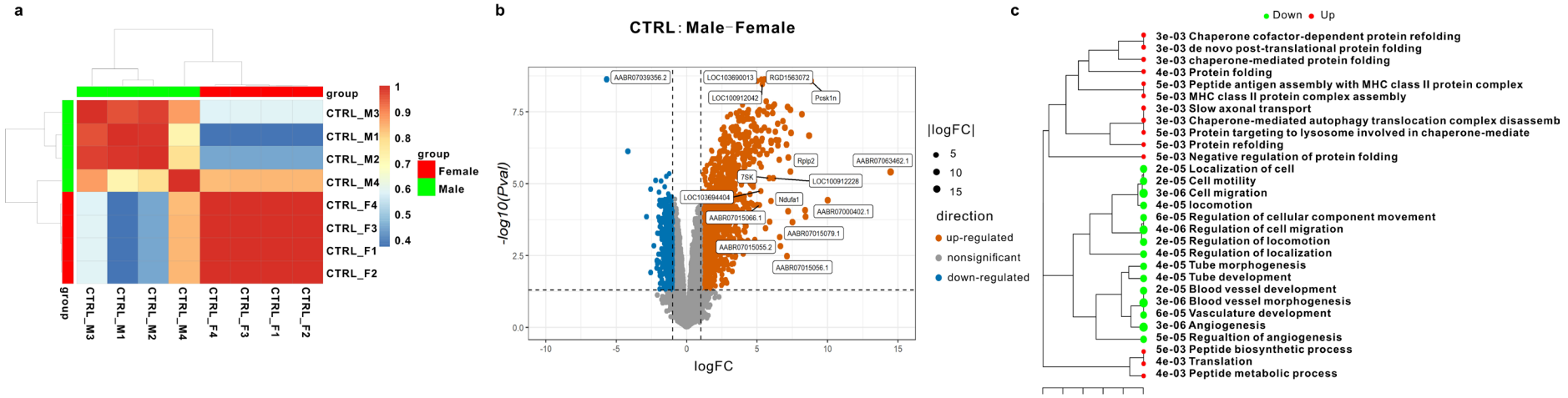
and male in control rats.

a, Heatmap analysis. **b**, Volcano plot of expression levels mPFC region in control rats. 624 genes were found downregulated in male rats compared to female subjects. Female rats have 1321 genes declines compared to male rats (FDR ≤ 0.05, Fold change ≥ 2). **c**, network enrichment analysis for differential expressed genes (DEG) using DAVID (2021 Update). Connected gene sets share more genes. Color of node correspond to adjusted Pvalues (adj.Pval). Two pathways (nodes) are connected if they share 30% (default, adjustable) or more genes. Green and red represents down- and up-regulated pathways. Darker nodes are more significantly enriched gene sets. Bigger nodes represent larger gene sets. Thicker edges represent more overlapped genes.

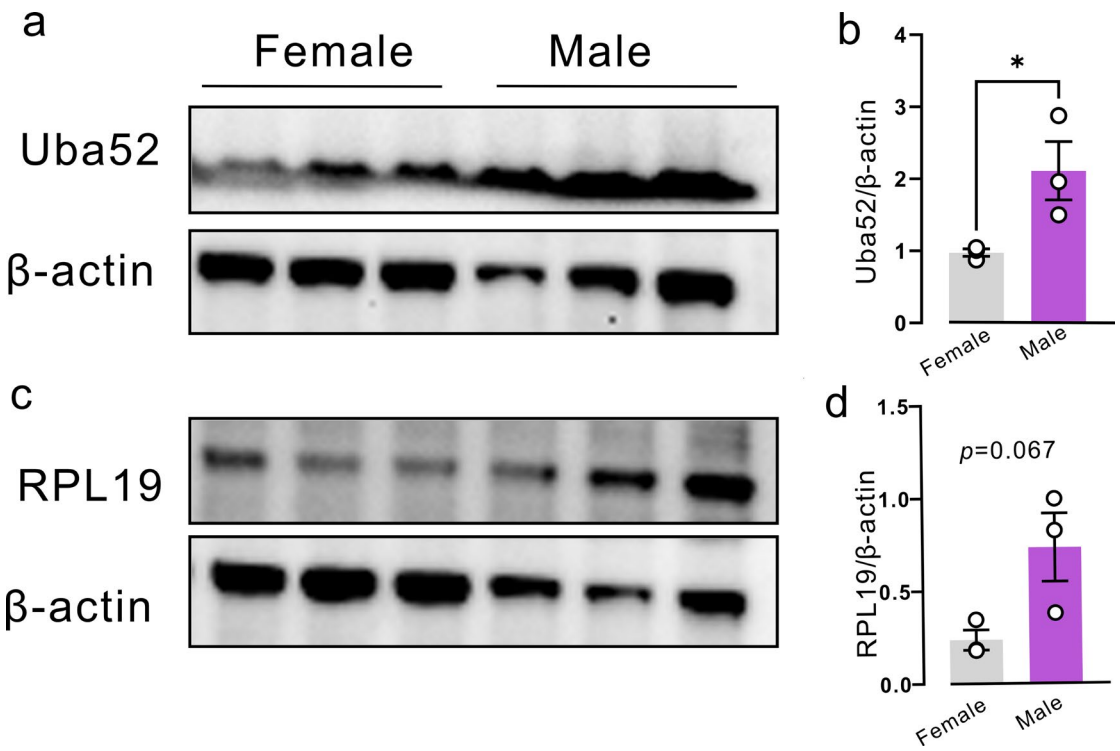
Supplementary Figure. 2 The proteins related to ribosomal signaling are higher in control male rats compared to females.

a-b, Comparisons of expression of Uba52 in control females and males. (a) Representative western blot image and (b) analysis of Uba52 expression in mPFC. The expression of Uba52 in control male rats is higher than females (two tailed unpaired t-test p= 0.0495). c-d, Comparisons of expression of RPL19 in control females and males. (c) Representative western blot image and (d) analysis of RPL19 expression in mPFC. The expression of RPL19 tend higher in control male rats

relative to females (two tailed unpaired t-test p= 0.0607).



Supplementary Figure. 1



Supplementary Figure. 2

Table legends

Table S1. Sequencing quality control measures. Sample name, total sequenced reads, and mapping rate for uniquely mapped reads for each RNA-seq sample.

Table S2 DEG genes of Ctrl male vs female.

Table S3 DEG genes of UMS male vs female.

Table S4 DEG genes of RS male vs female.

Table S5. Common differentially expressed genes. Subset of genes differentially expressed by sex.

Table S1. Sequencing quality control measures.

Sample name, total sequenced reads, and mapping rate for uniquely mapped reads for each RNA-seq sample.

RNA-seq Sample	Total Reads	Mapping rate (%)
Ctrl-Female1	39621936	91.35%
Ctrl-Female2	42852348	90.22
Ctrl-Female3	44498260	89.94
Ctrl-Female4	38878372	89.34
Ctrl-Male1	53534656	83.28
Ctrl-Male2	54973760	82.72
Ctrl-Male3	52393340	84.11
Ctrl-Male4	43210966	83.43
UMS-Female1	40124920	86.40
UMS-Female2	42779936	89.75
UMS-Female3	45300696	89.55
UMS-Female4	38212794	89.34
UMS-Male1	48802504	82.68
UMS-Male2	47015904	82.72
UMS-Male3	42386968	84.93
UMS-Male4	45993592	83.87
RS-Female1	38952674	89.96
RS-Female2	42040202	90.05
RS-Female3	55918068	90.04
RS-Female4	64342688	90.06
RS-Male1	60019213	86.83
RS-Male2	40070125	85.90
RS-Male3	50071098	89.39
RS-Male4	45990940	83.83

Table S5. Common differentially expressed genes.

Subset of genes differentially expressed by sex.

Diff Group	Diff Number (up)	Diff Number (down)
CTRL: Male-vs-Female	1321	624
UMS: Male-vs-Female	1380	759
RS: Male-vs-Female	1278	417