Data Supplement for Luo and Zhang, Down-Regulation of *SIRT1* Gene Expression in Major Depressive Disorder. Am J Psychiatry (doi: 10.1176/appi.ajp.2016.16040394)

MDD diagnosis and detailed demographic information

Diagnosis of MDD was based on criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Assessments of the Hamilton Rating Scale for Depression -17 (HRSD-17) were performed independently by two experienced psychiatrists (interrater reliability, kappa=0.84). Detailed demographic information about age, sex, smoking status, alcoholic abuse, duration of illness prior to admission, number of episode, family history of mood disorders are listed in **Supplementary Table 1**. All of the subjects are Han Chinese and this study was approved by Institutional Review Boards of Shanghai Mental Health Center.

Supplementary Table 1. Demographics of MDD cases and controls for expression analyses

	Cases	Controls	*P*
Number of subjects (n)	50	50	
Age (years), mean (SD)	29.2 (6.0)	30.8 (6.1)	0.19
Gender, male n (%)	17 (34.0)	21 (42.0)	0.54
Smoking status, n (%)	13 (26.0)	15 (30.0)	0.82
Alcoholic abuse, n (%)	0 (0)	0 (0)	
Duration of illness (month) a, mean (SD)	2.9 (1.0)	N/A[c]	
HRSD-17 b, mean (SD)	24.9 (2.2)	N/A	
Number of episode, mean (SD)	1.4 (0.4)	N/A	
Family history of mood disorders, n (%)	3 (6.0)	N/A	

Note: [a]Duration of illness prior to admission

[b]HRSD-17 on admission

[c]Not Available

Blood collection and RNA extraction

Peripheral blood were collected from fasting patients and healthy controls (between 07:00 am and 09:00 am). RNA was extracted from fresh peripheral blood using the QIAamp RNA blood Mini Kit (Qiagen, Chatsworth, California, USA). If the RNA is not extracted immediately from fresh

peripheral blood, to avoid the degradation of RNA, buffer BR1 (a long-term preservation solution for RNA extraction from peripheral blood) (lifefeng BioTec: www.lifefeng.com) was added immediately into the extracted peripheral blood. After thorough mixture, the peripheral blood that contained buffer BR1 was stored at -80 ^{o}C. RNA in this mixture solution is stable for at least two years (www.lifefeng.com). RNA was extracted from frozen blood (contained buffer BR1) using RK-206-02 RNA extraction kit (www.lifefeng.com). RNA concentration and purity were determined with a NanoDrop ND 2000 spectrophotometer (Thermo Scientific).

DNA extraction

Genomic DNA was extracted from 300 µl whole blood using phenol/chloroform method as described in our previous study (1). DNA concentration was determined by a NanoDrop ND 2000 spectrophotometer.

Reverse transcription and real-time PCR

Before performing reverse transcription assay, equal amount total RNA (200 ng) was treated with DNase I (Fermentas) to remove the potential DNA contamination. After treatment with DNase I, RNA was reverse transcribed into cDNA using oligo-$dT_{(15)}$ primers and GoScript Reverse Transcriptase (Promega). The real-time quantitative PCR primers were selected from previous publications (2, 3) and the sequences are as follows: *GAPDH*-F: 5'-ATCCCATCACCATCTTCCA-3'; *GAPDH*-R: 5'-TGGACTCCACGACGTACTCA-3'; *SIRT1*-F: 5'-TCGCAACTATACCCAGAACATAGACA-3'; *SIRT1*-R: 5'-CTGTTGCAAAGGAACCATGACA-3'. It is important to select proper internal control gene in quantitative real-time PCR (qPCR). Previous study (4) has shown that *GAPDH* is one of the

best genes for qPCR when using human blood cells. Spinsanti et al (4) investigated four internal control genes (including *GAPDH*, *ACTB*, *HPRT1*, and *hCyPB*) and found that *GAPDH* and *ACTB* are the most suitable internal control genes for qPCR when human blood cells were used to extract RNA. We used human peripheral blood to extract RNA in our study, therefore, we utilized *GAPDH* as internal control as recommended by Spinsanti et al (4). In addition, we also checked the stability of *GAPDH* and found that *GAPDH* is stably expressed in all of studied subjects (**Figure S1**). These consistent evidence suggest that *GAPDH* is suitable for qPRC in our study. We verified the specificity of these primers using agrose gel electrophoresis and melting curve analysis. Quantitative PCR was performed by using the SYBR Green master mix and the Bio-Rad CFX Real-Time PCR Systems (BioRad). Quantitative PCR data was analyzed using the ΔC_t values and fold change was determined by $2^{-\Delta\Delta Ct}$ method (5).

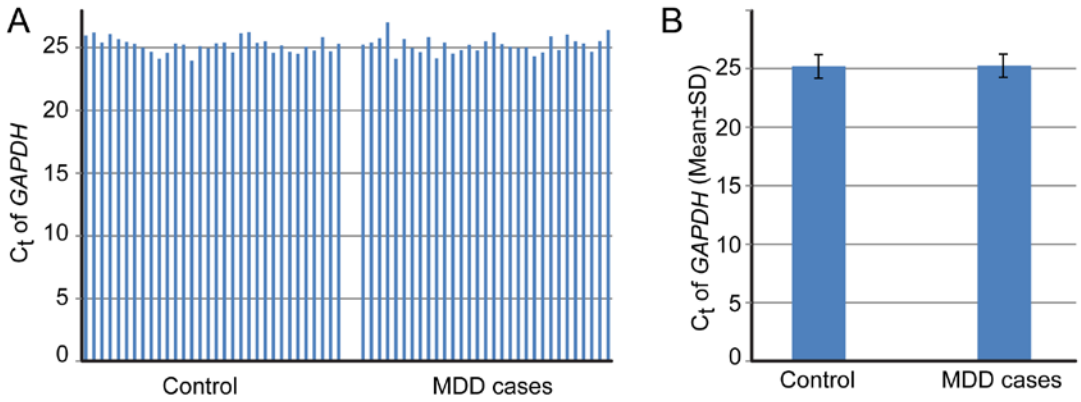

Figure S1. *GAPDH* is stably expressed in controls and MDD cases. (A), Ct value of *GAPDH* in controls and MDD cases. (B), The average Ct of *GAPDH* in controls and MDD cases. Ct, cycle threshold.

Genotyping of rs12415800

SNP rs12415800 was genotyped using direct sequencing (Sanger sequencing) on an ABI 3730 sequencer (Applied Biosystems, Foster City, California, USA). Briefly, genomic sequence contains SNP rs12415800 was amplified first. The PCR products were purified and sequencing

reaction was conducted using ABI Big Dye sequencing kit (Applied Biosystems). Then the genotype of rs12415800 was determined by ABI 3730 sequencer through direct sequencing.

Statistical analyses

Significance test

Student t-test was used to determine if *SIRT1* was significantly down-regulated in MDD cases compared with controls. Real-time quantitative assays were performed in triplicate (for each subject, three real-time quantitative assays (technical replicate) were performed, and the mean of three replicates was used as the Ct value of this individual) and the relative expression of *SIRT1* are expressed as median and the first and third quartiles (Box plot). The five basic elements of a box plot are as follows (**Figure S2**): (1) The median; (2) The first quartile (Q1); (3) The third quartile (Q3); (4) The minimum point (Q1-1.5×IQR); (5) The maximum point (Q3+1.5×IQR). IQR: interquartile range.

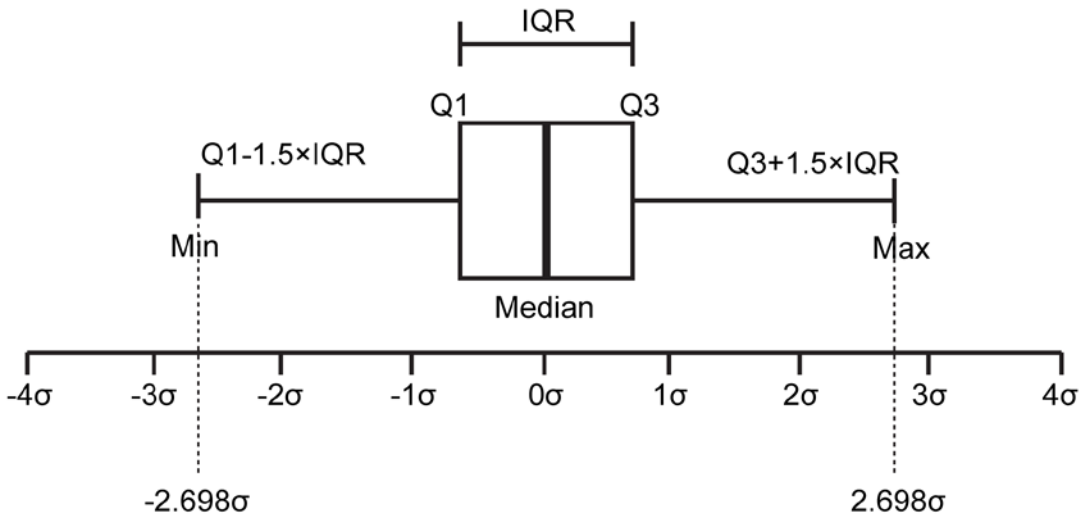

Figure S2. Five basic elements of a box plot. Q1, The first quartile; IQR: interquartile range; σ, standard deviation.

Outliers detection and sensitivity analyses

We performed sensitivity test to determine if the extreme p value comes from outliers of over-expressed gene in MDD cases. There are two scenarios that may lead to the extreme p value observed in our test. First, several outliers have extreme high expression level of *SIRT1* in control group. Second, several outliers have extreme low expression level of *SIRT1* in case group. To determine if the extreme p value comes from these two scenarios, we performed sensitivity test. We first determined the outliers in controls using SPSS statistic software (Version 16.0). After removing the outliers that have extreme high expression level of *SIRT1* in control group (5 subjects), we found that *SIRT1* is still significantly down-regulated in MDD cases ($P = 7.95\times10^{-7}$). Similarly, after removing the outliers that have extreme low expression level of *SIRT1* in case group (5 subjects), *SIRT1* is still significantly down-regulated in MDD cases ($P = 8.06\times10^{-5}$). We also used box plot (Implemented in R software package (https://www.r-project.org/)) to detect the outliers (individuals with *SIRT1* expression level lower than Q1-1.5×IQR or higher than Q3+1.5×IQR are defined as outliers). Again, we found that *SIRT1* is still significantly down-regulated in MDD cases. These results suggest that the extreme p value is unlikely comes from outliers.

Power calculation

The detailed procedure are as follows: First, we used a two-group independent sample t-test to determine if *SIRT1* is significantly down-regulated in MDD cases. Second, the difference between expression means in control group and case group is 0.37 (as *SIRT1* is decreased by 37% in MDD cases compared with controls). Third, the standard deviation (s.d.) of *SIRT1* expression in control

group is 0.764, and the standard deviation of *SIRT1* expression in case group is 0.213. Fourth, the pooled standard deviation is 0.56 (pooled s.d. = sqrt ((s.d.$^2_{control}$ + s.d.$^2_{case}$)/2)). And the default significance level (alpha level) is 0.05. After setting these parameters, we used "pwr" package implemented in R (Version 2.14.0) (https://www.r-project.org/) to calculate the power. Power estimation showed that our current sample size has a relatively high power (0.92).

eQTL analysis

Expression quantitative analysis was performed as performed previously described (6). Briefly, relative expression level of *SIRT1* (*GAPDH* was used as internal control) in each subject was determined using qPCR (as described above) and genotype of each individual was determined using Sanger sequencing (a total of 78 subjects was genotyped). *SIRT1* expression was regressed on the number of minor allele (0, 1 or 2) of rs12415800 to compute the effect of allele dosage on *SIRT1* expression level. PLINK (v1.07) (7) was used to conduct association between the SNP rs12415800 and *SIRT1* expression. *SIRT1* expression is considered to be dependent and additive genetic model was used as described previously (6).

References

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