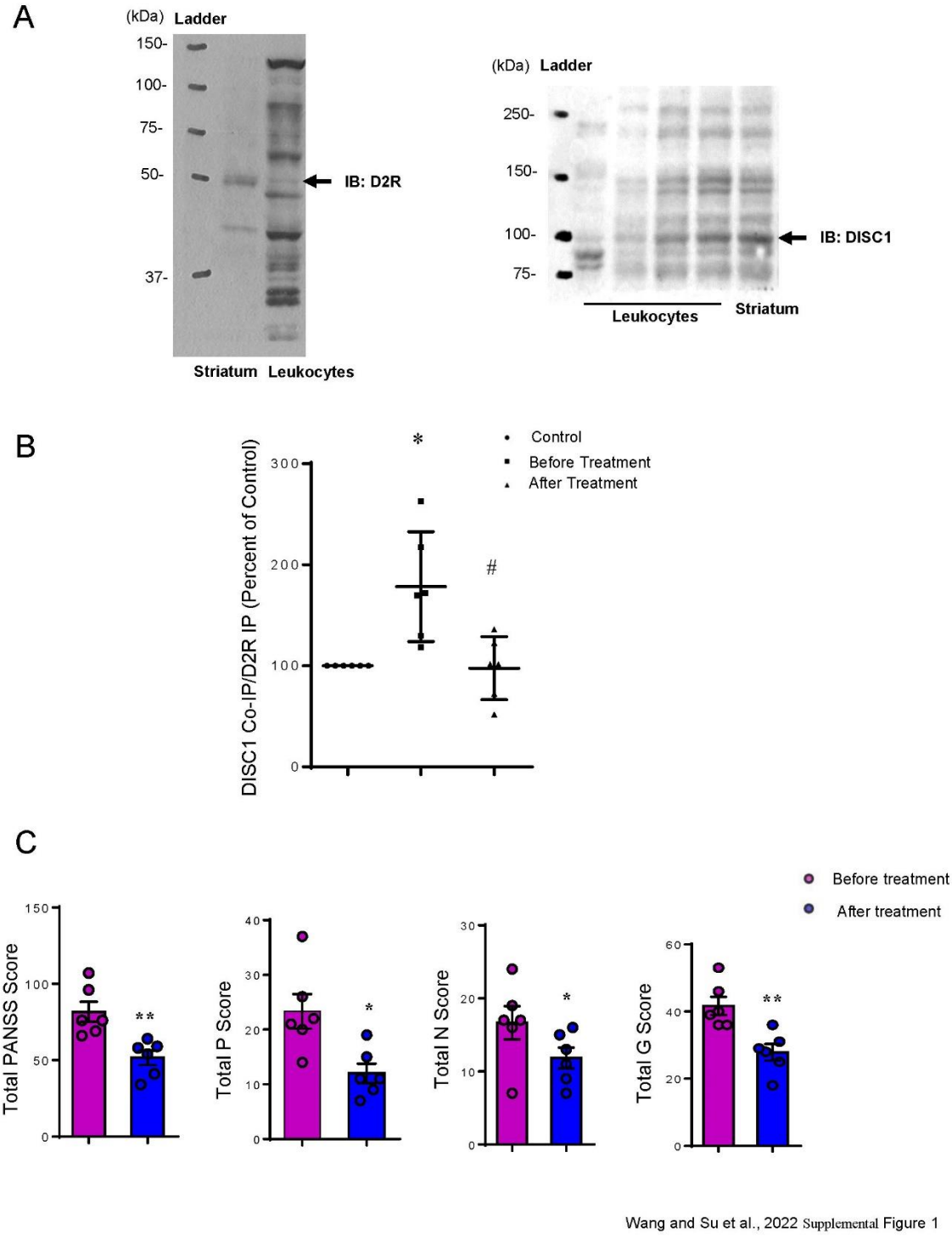
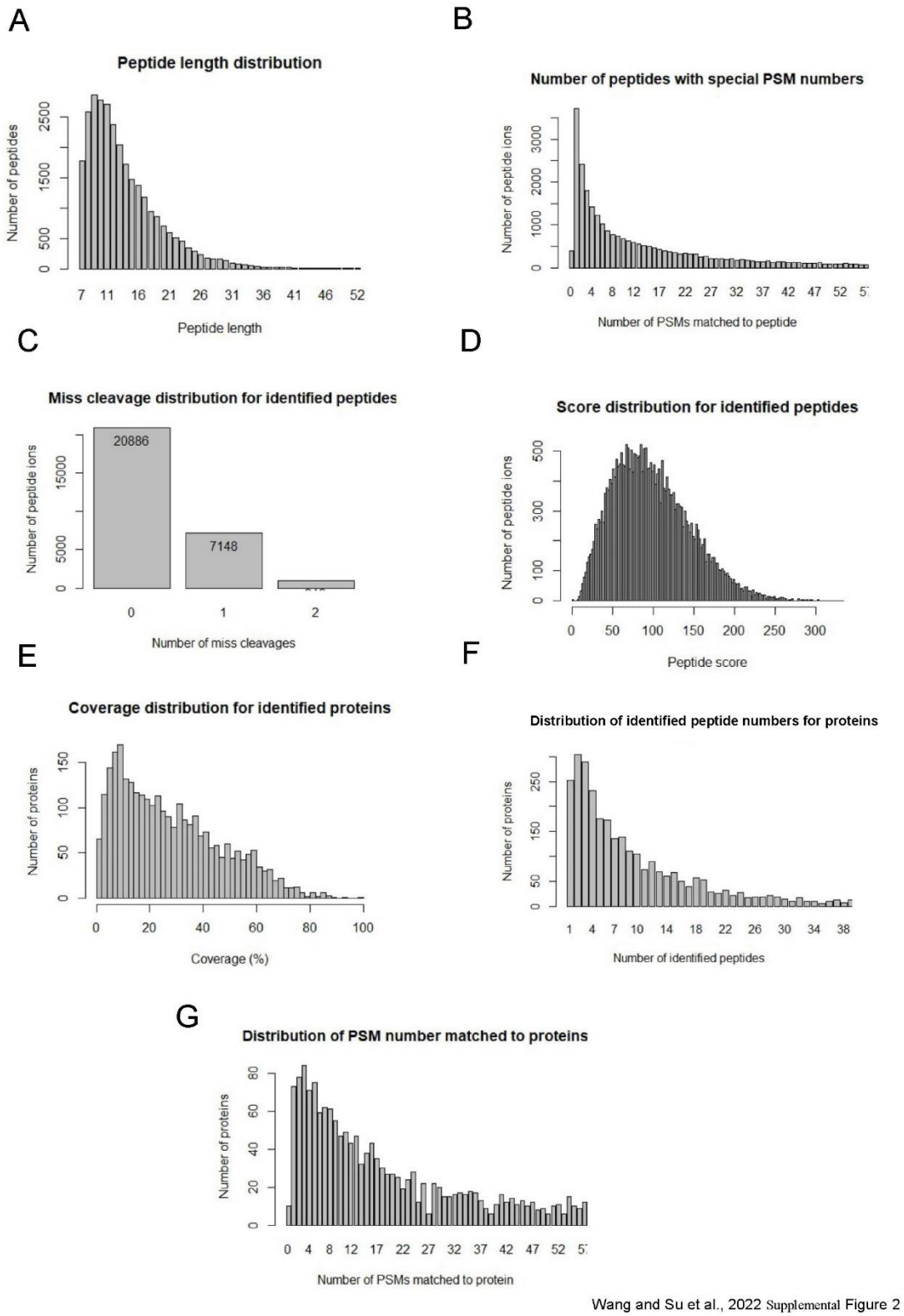
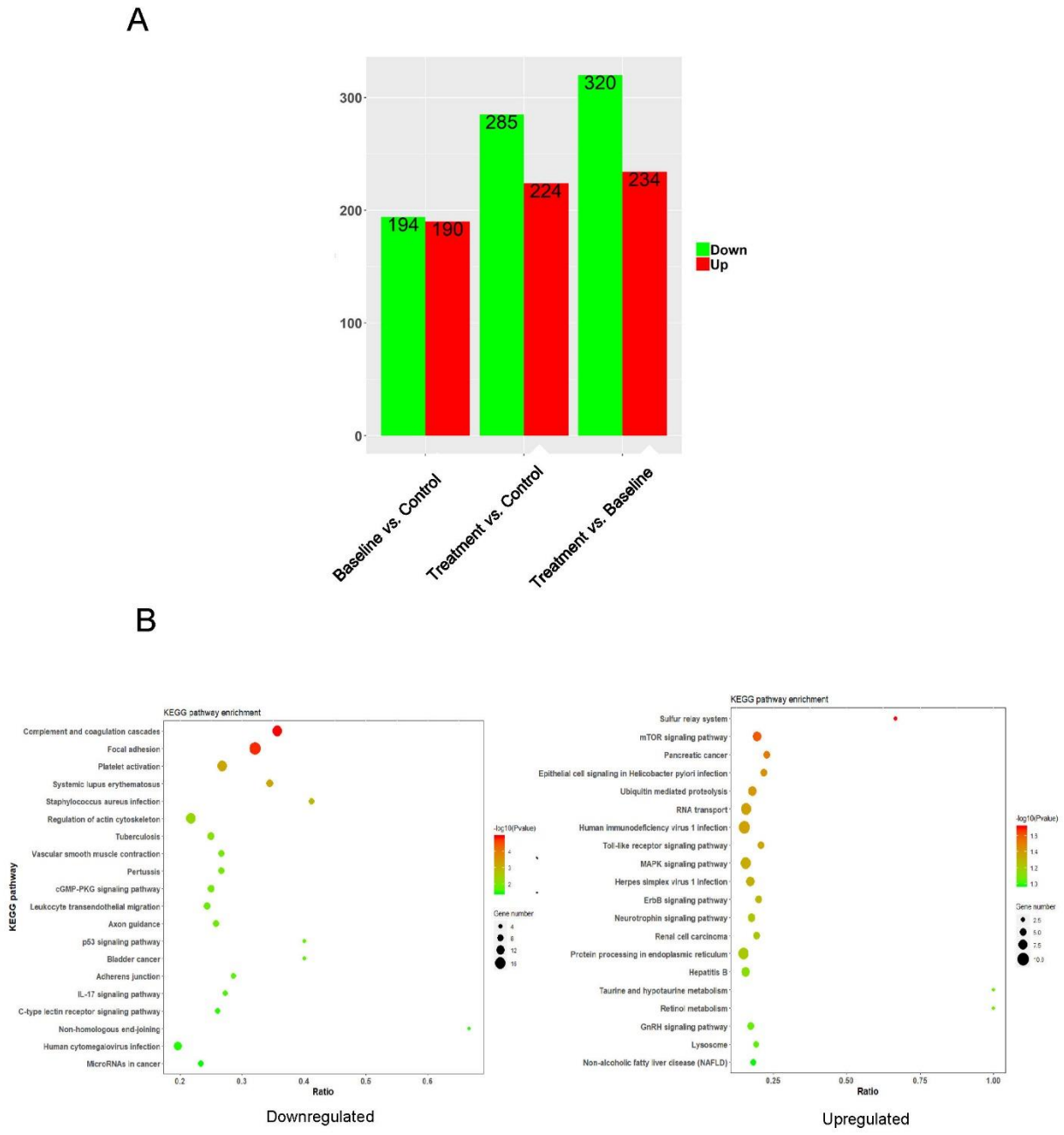


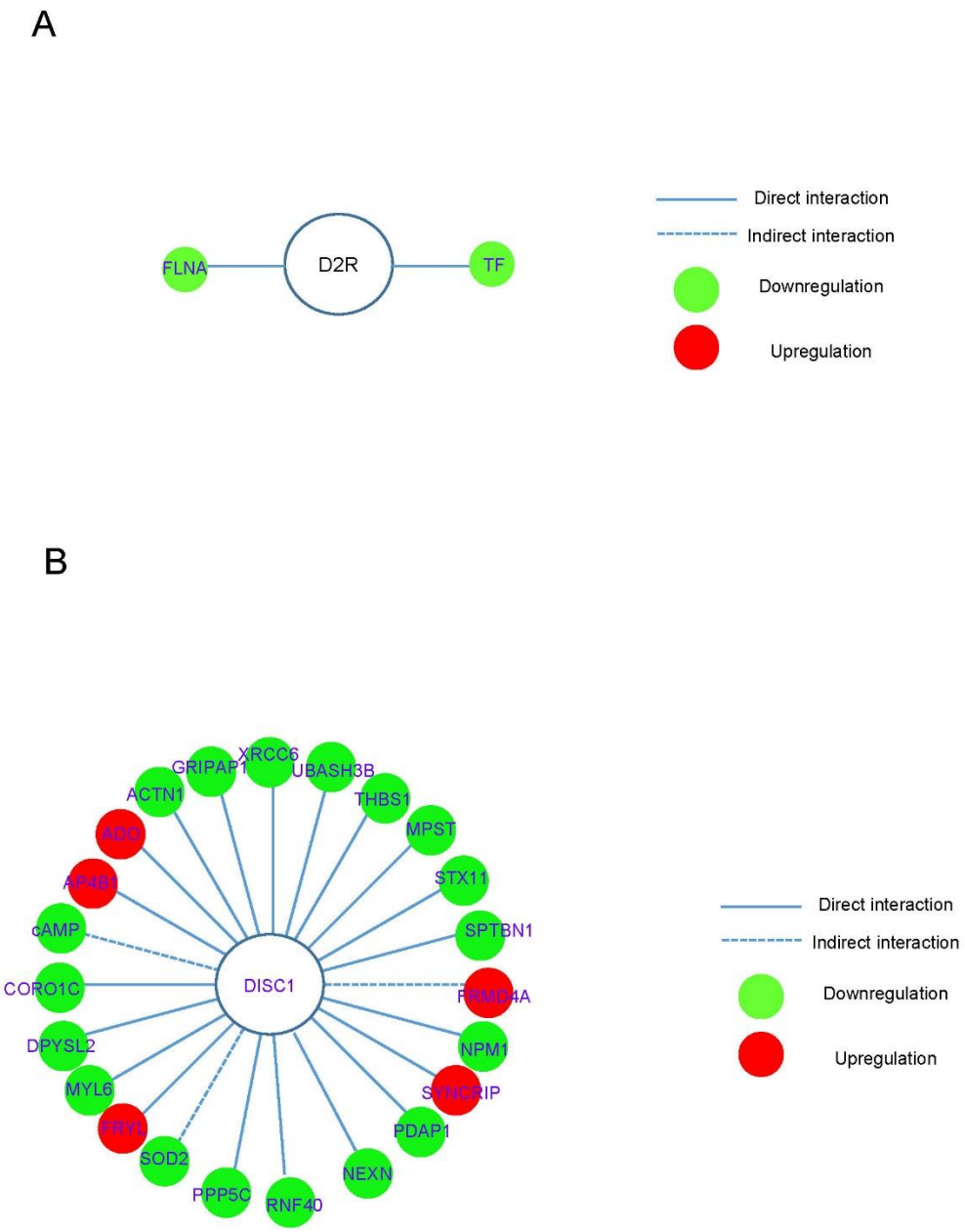
Wang and Su et al., 2022 Supplemental Figure 1



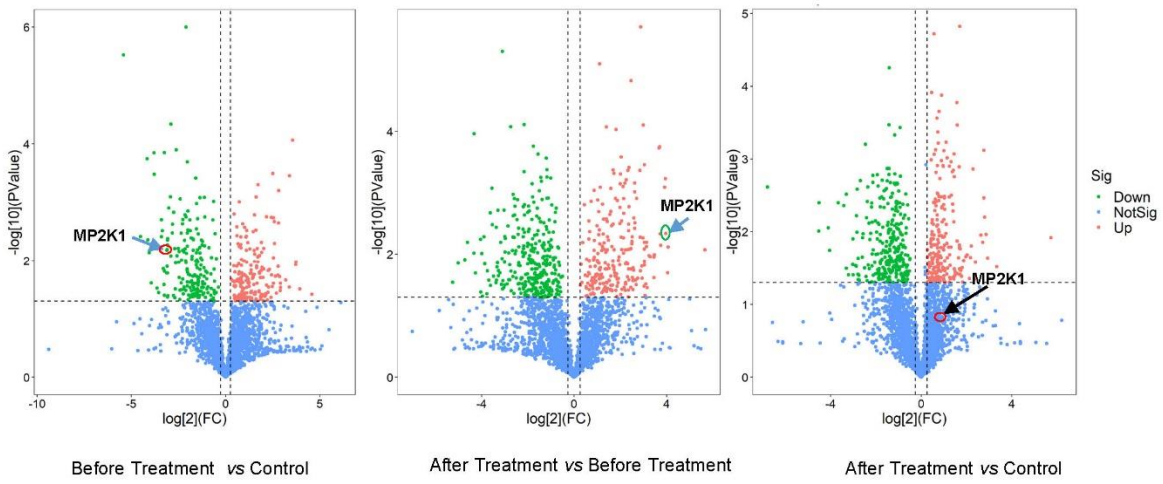
Wang and Su et al., 2022 Supplemental Figure 2



Wang and Su et al., 2022 Supplemental Figure 3



Wang and Su et al., 2022 Supplemental Figure 4



Before Treatment *vs* Control

After Treatment *vs* Before Treatment

After Treatment *vs* Control

Wang and Su et al., 2022 Supplemental Figure 5

Antipsychotic medications information of patients in the first cohort.

Patient No.	Mean Daily dosage in terms of Olanzapine (mg/day)	Mean Daily dosage in terms of CPZ (mg/day)	Patient No.	Mean Daily dosage in terms of Olanzapine (mg/day)	Mean Daily dosage in terms of CPZ (mg/day)
P1	10	300	P18	5	150
P2	10	300	P19	10	300
P3	12.5	375	P20	10.03	300.9
P4	14.5	435	P21	10	300
P5	12.5	375	P22	3.36	100.8
P6	17.4	522	P23	10	300
P7	13.33	399.9	P24	6.71	201.3
P8	28.33	849.9	P25	5.8	174
P9	14.77	443.1	P26	10	300
P10	22.91	687.3	P27	11.6	348
P11	10	300	P28	10	300
P12	5.8	174	P29	13.3	399
P13	20.92	627.6	P30	20	600
P14	26.67	800.1	P31	13.33	399.9
P15	2.9	87	P32	11.6	348
P16	6.71	201.3	P33	28.42	852.6
P17	23.42	702.6	P34	5	150

Wang and Su et al., 2022 Supplemental Table 1

Demographic and clinical information of schizophrenia patients and control subjects (34 subjects)

Variables	Control (N = 34)	Schizophrenia (N = 34)	P
Age (years) *	22.24 ± 1.18	22.21 ± 1.13	0.9856
Sex	23 Female + 11 Male	23 Female + 11 Male	
Education (years) *	12.29 ± 0.52	12.65 ± 0.58	0.6524
Disease Duration (yrs) *	N/A	0.995 ± 0.21	
Race-ethnicity	34 Asian	34 Asian	
PANSS total score *	N/A	87.59 ± 3.14	

* Mean ± SEM

Wang and Su et al., 2022 Supplemental Table 2

Antipsychotic medications information of patients in the second cohort.

Patient No.	Mean Daily dosage in terms of Olanzapine (mg/day)	Mean Daily dosage in terms of CPZ (mg/day)	Patient No.	Mean Daily dosage in terms of Olanzapine (mg/day)	Mean Daily dosage in terms of CPZ (mg/day)
P1	30.07	902.1	P15	13.32	399.6
P2	8.39	251.7	P16	16.67	500.1
P3	10.8	324	P17	20	600
P4	20	600	P18	23.2	696
P5	10	300	P19	10.07	302.1
P6	16.71	501.3	P20	13.42	402.6
P7	13.33	399.9	P21	13.33	399.9
P8	15	450	P22	12.5	375
P9	5.8	174	P23	20	600
P10	8.33	249.9	P24	23.38	701.4
P11	12.5	375	P25	20.04	601.2
P12	22.02	660.6	P26	3.36	100.8
P13	30.73	921.9	P27	17.4	522
P14	12.5	375	P28	10	300

Wang and Su et al., 2022 Supplemental Table 3

Demographic and samples' clinical information of subjects recruited from Shanghai

Variables	CTRL (N = 20)	Schizophrenia (N = 28)	P
Age (years) *	22.6 ± 1.90	23.14 ± 1.27	0.8064
Sex (female/male)	11 Female + 9 Male	17 Female + 11 Male	
Education* (completed post-secondary)	12 ± 0.55	11.86 ± 0.70	0.8808
Disease Duration (yrs) *	N/A	0.5 ± 0.07	
Onset of Disease (age) *	N/A	22 ± 1.24	
BPRS score *	N/A	50.36 ± 2.11	

* Mean ± SEM

Wang and Su et al., 2022 Supplemental Table 4

Antipsychotic medications information of patients in the third cohort.

Patient No.	Mean Daily dosage in terms of Olanzapine (mg/day)	Mean Daily dosage in terms of CPZ (mg/day)	Patient No.	Mean Daily dosage in terms of Olanzapine (mg/day)	Mean Daily dosage in terms of CPZ (mg/day)
P1	29.80	894.05	P17	32.48	974.48
P2	26.23	786.76	P18	27.66	829.91
P3	28.08	842.40	P19	18.90	567.04
P4	36.86	1105.94	P20	23.23	696.77
P5	21.20	636.05	P21	13.93	417.86
P6	28.61	858.29	P22	17.40	522.04
P7	23.19	695.57	P23	17.54	526.32
P8	33.81	1014.18	P24	25.66	769.68
P9	28.00	840.07	P25	21.55	646.48
P10	25.02	750.68	P26	21.73	652.00
P11	35.58	1067.29	P27	23.94	718.18
P12	29.71	891.33	P28	23.33	700.00
P13	22.40	672.05	P29	21.27	638.05
P14	24.35	730.40	P30	18.33	550.00
P15	27.81	834.24	P31	8.10	242.86
P16	32.18	965.40			

Wang and Su et al., 2022 Supplemental Table 5

Demographic and samples' clinical information of subjects recruited from Beijing

Variables	CTRL (N = 12)	Schizophrenia (N = 31)	P
Age (years) *	31.53 ± 1.55	31.43 ± 1.65	0.9715
Sex (female/male)	5 Female + 7 Male	1 Female + 30 Male	
Education			0.8808
Lower than Undergraduate	7 (58.33%)	20 (64.52%)	
Undergraduate	3 (25.00%)	8 (25.81%)	
Granduate	2 (16.67%)	3 (9.68%)	
Disease Duration (yrs) *	N/A	6 ± 1.17	
Onset of Disease (age) *	N/A	25 ± 1.36	
Other Diseases (subjects)	0	7	
PANSS total score *	N/A	79.06 ± 3.87	

*Mean ± SEM

Wang and Su et al., 2022 Supplemental Table 6

Demographic and clinical information of schizophrenia patients and control subjects (mass spectrometry)

Variables	Control (N = 6)	Schizophrenia (N = 6)	P
Age (years) *	17.00 ± 1.63	17.00 ± 1.55	>0.9999
Sex	4 Female + 2 Male	4 Female + 2 Male	
Education (years) *	10.17 ± 1.35	10.33 ± 1.15	0.9269
Disease Duration (yrs) *	N/A	0.79 ± 0.54	
PANSS total score *	N/A	81.67 ± 6.63	
Race-ethnicity	6 Asian	6 Asian	

* Mean ± SEM

Wang and Su et al., 2022 Supplemental Table 7

Supplemental Figure 1. A. Both D2R and DISC1 are expressed in leukocytes of peripheral blood samples. **B.** Densitometric analysis of levels of D2R-DISC1 complex in patients with schizophrenia and control subjects for mass spectrometry analysis. * $p<0.05$ compared to controls. # $p<0.05$ compared to patients with schizophrenia before treatment. One-way ANOVA followed by Tukey's *post hoc* test. n = 6. C. PANSS scores of patients with schizophrenia before and after treatment. n = 6, t-test, *$p < 0.05$, **$p < 0.01$ as compared to before treatment group.

Supplemental Figure 2. Quality control assessment of the proteomic data detected by mass spectrometry in blood samples from patients with schizophrenia before and after treatment as well as unaffected control subjects. A. Peptide length distribution**; B.** Number of peptides with special PSM numbers; **C.** Miss cleavage distribution for identified peptides; **D.** Score distribution for identified peptides; **E.** Coverage distribution for identified proteins; **F.** Distribution of peptide numbers for proteins; **G.** Distribution of PSM number matched to proteins.

Supplemental Figure 3. Protein expression and KEGG pathway analysis of proteins detected by mass spectrometry in blood samples from patients with schizophrenia before and after treatment as well as unaffected control subjects. A. Number of proteins that were expressed with significantly different levels in each comparation. Increased proteins are shown in red; decreased proteins are shown in green. **B.** Analysis of the top 20 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways. These differentially expressed proteins were grouped into pathways using pathway enrichment analysis with the KEGG database. The size of the circle is proportional to the number of enriched proteins.

Supplemental Figure 4. D2R- and DISC1-interacting proteins that are significantly expressed in peripheral blood samples from patients with schizophrenia after antipsychotic treatment as compared to those before treatment detected in mass spectrometry analysis. A.

Two D2R-interacting proteins show significantly different expression levels in peripheral blood samples from patients with schizophrenia after antipsychotic treatment as compared to those before treatment. **B**. 23 proteins that directly or indirectly interact with DISC1 show significantly different expression levels in peripheral blood samples from patients with schizophrenia after antipsychotic treatment as compared to those before treatment.

Supplemental Figure 5. Volcano plots of proteins detected in mass spectrometry. The proteins are presented in the volcano plot using $\log_2$ (fold change) (log[2](FC)) on the x-axis and $-\log_{10}$ (P-value) (-log[10](PValue)) on the y-axis. The increased proteins are in red, and decreased proteins are in green. Proteins without significant differences are in blue.

Supplemental Table 1. Antipsychotic medication information for patients with schizophrenia in the Shanghai cohort 1.

Supplemental Table 2. Demographic and clinical information for patients with schizophrenia and control subjects (Shanghai cohort 1).

Supplemental Table 3. Antipsychotic medications information for patients with schizophrenia in the Shanghai cohort 2.

Supplemental Table 4. Demographic and clinical information for subjects recruited from Shanghai (Shanghai cohort 2).

Supplemental Table 5. Antipsychotic medications information for patients with schizophrenia in the third cohort (Beijing).

Supplemental Table 6. Demographic and clinical information for subjects recruited from Beijing (third cohort).

Supplemental Table 7. Demographic and clinical information for patients with schizophrenia and control subjects for mass spectrometry analysis (specific cohort selected from Shanghai cohort 1).

Supplemental Experimental Procedures

Co-immunoprecipitation and Western blot

Co-immunoprecipitation and Western blot analysis were performed as previously described (1, 2). For samples from subjects recruited in Shanghai Mental Health Center, total proteins were extracted from frozen blood cells using Hemoglobin Depletion and Protein Enrichment kit (Biotech Support Group, Monmouth Junction, NJ). For samples from subjects recruited in Beijing AnDing Hospital, total proteins were extracted from peripheral blood mononuclear cells using lysis buffer (50 mM Tris-Cl, 150 mM NaCl, 2 mM EDTA, 0.5% sodium deoxycholate, 1% NP-40, 1% Triton X-100, 0.1% SDS, Protease inhibitor cocktail (1:100; Sigma-Aldrich)). For co-immunoprecipitation experiments, 500 ~ 700 µg solubilized protein was incubated with 25 µl of protein A/G plus agarose (Santa Cruz Biotechnology) for 1 hour at 4ºC, followed by replacement of the agarose with 25 µl new agarose in the presence of primary antibody or control IgG (1 ~ 2 µg) for 12 h at 4ºC with gentle shaking. Pellets were washed, boiled for 5 min in SDS sample buffer and subjected to SDS-PAGE. 10 ~ 50µg of extracted protein was used as control in each experiment. After transfer of proteins onto nitrocellulose, membranes were Western blotted with the primary antibodies. The antibodies used were D2R (Proteintech, rabbit) for immunoprecipitation, and anti-D2R (Santa Cruz Biotechnology, mouse) for Western blot; DISC1 (Invitrogen, rabbit), anti-MEK1/2 (Cell Signaling Technology, rabbit), and anti-β-actin (Cell Signaling Technology, rabbit). The secondary antibodies were HRP-conjugated anti-rabbit IgG (Cell Signaling Technology), HRP-conjugated anti-mouse IgG (Sigma-Aldrich) and anti-

mouse IgG for IP (HRP) (Abcam). Densitometric analysis of the co-immunoprecipitation and western blot data was conducted using ImageJ (NIH).

Isolation of leukocytes from peripheral blood samples

Peripheral blood samples were collected in EDTA whole blood tubes (BD Biosciences), and the leukocytes were isolated using Ficoll-Paque PLUS (GE Healthcare) according to the manufacturer's instruction. Briefly, each blood sample was diluted with an equal volume of balanced salt solution. 3 ml of Ficoll-Paque PLUS was added into a centrifuge tube, and 4 ml of the diluted blood sample was carefully layered on Ficoll-Paque PLUS, and centrifuged at 400 g for 30 minutes at 18-20ºC. The leukocyte layer was carefully transferred to a clean centrifuge tube using a clean Pasteur pipette, and was washed twice with at least 3 volumes of balanced salt solution, and centrifuged at 100 g for 10 minutes at 18-20ºC. The supernatant was removed, and the leukocytes were stored at -80ºC for further experiments.

Mass spectrometry analysis

Total protein (120 μg) extracted from samples of the first cohort in Shanghai Mental Health Center were prepared and submitted to National Center for Protein Science Shanghai for mass spectrometry analysis. Further analysis of Mass spectrometry data was carried out by Beijing Qinglian Biotech Co., Ltd.

Protein precipitation and digestion: Proteins were precipitated with acetone. The protein pellet was dried by using a Speedvac for 1−2 min. The pellet was subsequently dissolved in 8 M urea, 100 mM Tris-HCl, pH 8.5. TCEP (final concentration is 5 mM) (Thermo Scientific) and iodoacetamide (final concentration is 10mM) (Sigma) for reduction and alkylation, respectively, were added to the solution and incubated at room temperature for 30 minutes. The protein mixture

was diluted four times and digested overnight with Trypsin at 1:50 (w/w) (Promega, http://www.promega.com/). The resulting solution was desalted using a MonoSpin™ C18 column (GL Science, Tokyo, Japan) and dried with a SpeedVac.

LC/ tandem MS (MS/MS) analysis of peptide: The peptide mixture was reconstituted with 0.1% formic acid (FA) and then equal amount of MassPREP ADH Digestion Standard (Waters Corporation, Milford, MA) was added to each sample before loading to the mass spectrometer. The samples were analyzed using a modified MudPIT separation that has been described previously (3) on an Easy-nLC 1000 nano HPLC (Thermo Scientific, San Jose, CA) equipped with a SCX column (5 μm, Whatman, Clifton, NJ, USA) and a home-made 25 cm-long pulled-tip analytical column (75 μm ID packed with 3 μm, Aqua, Phenomenex, Torrance, CA). The analytical column temperature was set at 55 ℃ during the experiments. A fully automated five-step MudPIT run was performed on each sample using two mobile phase system consisting of buffer A (0.1% formic acid in water), buffer B (0.1% formic acid in 80% acetonitrile). The sample was loaded on the SCX column at the first step which consisted a 240 min gradient as follows: 0-1 min, 5%-10% B; 1-201 min, 10-40% B; 201-219 min, 40-60% B; 219-220 min, 60-100% B; 221-240 min, 100% B. 10 μl 50 mM ammonium acetate was injected to fraction the sample at the second step with the following profile: 0-1 min, 5%-10% B; 1-361 min, 10-40% B; 261-279 min, 40-60% B; 279-280 min, 60-100% B; 280-300 min, 100% B. 10 μl 100 mM or 300 mM ammonium acetate was then injected to further fraction the sample, comprising steps 3-4 with the same gradient as in the first step. Finally, 450 mM ammonium acetate in 10% acetonitrile was injected at the last step with the same gradient as the second step. The flow rate was set as 300 nl/min.

Mass Spectrometry: Data-dependent tandem mass spectrometry (MS/MS) analysis was performed with an Orbitrap Elite ETD mass spectrometer (Thermo Scientific, San Jose, CA) equipped with

a nano electrospray ionization source using a distal 2-kV spray voltage. A cycle of one full-scan MS spectrum (m/z 300-1800) was acquired followed by top 20 MS/MS events, sequentially generated on the first to the twentieth most intense ions selected from the full MS spectrum at a 35% normalized collision energy. Full scan resolution was set to 60,000 and MS/MS scan resolution was set to 15,000. MS scan functions and LC solvent gradients were controlled by the Xcalibur data system (Thermo Scientific).

Data analysis: Carbamidomethylation (+57.021) of cysteine was set as a static modification.

Identification and quantitation of proteins

The MS/MS spectra from each LC-MS/MS run were searched against the human. FASTA data from UniProt (4) using Maxquant (5) (Computational Systems Biochemistry under Prof. Jürgen Cox·DEU). The search criteria were as follows: full tryptic specificity was required; two missed cleavages were allowed; carbamidomethylation (C) were set as the fixed modifications; The oxidation (M) is set as the variable modification; precursor ion mass tolerances were set at 15 ppm for all MS acquired in an orbitrap mass analyzer; and the fragment ion mass tolerance was set at 20mmu for all MS2 spectra acquired. The peptide false discovery rate (FDR) was calculated using Target Decoy PSM Validator provided by Maxquant. FDR was determined based on PSMs when searched against the reverse, decoy database. Peptides only assigned to a given protein group were considered as unique. The false discovery rate (FDR) was also set to 0.01 for protein identifications.

The resulting spectra from each fraction were searched separately in the Uniprot database by the search engines: Maxquant 1.5.2.8. identification was set as follows: precursor ion mass tolerance, ± 15 ppm; fragment ion mass tolerance, ± 20 ppm Da; max missed cleavages, 2; static modification, carbox yamidomethylation (57.021 Da) of Cys residues; dynamic modifications,

oxidation modification (+15.995 Da) of Met residues. According to the p-value of primary data, the data with $p \leqslant 0.05$ and difference ratio $\geqslant 1.2$ were selected for further analysis.

The functional analysis of proteins

The hierarchical clustering analysis were used to assess the batch effects in the proteomic data regarding sample groups, PANSS P score, and levels of D2R-DISC1 complex. Samples exhibited a high similarity within the same group, while different groups of samples were different obviously.

Gene Ontology (GO) and InterPro (IPR) analysis were conducted using the interproscan-5 program against the non-redundant protein database, and the databases COG (Clusters of Orthologous Groups) and KEGG (Kyoto Encyclopedia of Genes and Genomes) were used to analyze the protein families and pathways. The probable interacting partners were predicted using the STRING-db server (http://string.embl.de/) based on the related species. STRING is a database of both known and predicted protein-protein interactions. The enrichment pipeline was used to perform the enrichment analysis of GO, and KEGG, respectively. The PPI network was generated using Cytoscape_3.8.0 (6).

Quality control for the mass spectrometry analysis and proteomic data

To check the quality of the mass spectrometry, equal amounts of MassPREP ADH Digestion Standard (Waters Corporation, Milford, MA) were added to each sample before loading in the mass spectrometer. For identification and quantification of proteins, the search criteria were as follows: full tryptic specificity was required; two missed cleavages were allowed; carbamidomethylation (C) were set as the fixed modifications; oxidation (M) was set as the variable modification; precursor ion mass tolerances were set at 15 ppm for all MS acquired in an orbitrap mass analyzer; and the fragment ion mass tolerance was set at 20mmu for all MS2 spectra

acquired. The peptide false discovery rate (FDR) was calculated using Target Decoy PSM Validator provided by Maxquant. FDR was determined based on PSMs when searched against the reverse, decoy database. Peptides assigned only to a given protein group were considered as unique. The false discovery rate (FDR) was set to 0.01 for protein identifications.

Multiple linear regression approach with difference score as the outcome

Difference scores were calculated by simply subtracting after treatment values from before treatment values. Difference in psychiatric symptom scores were used as the outcome, with difference in DISC1-D2 complex levels, antipsychotic dose, and their interaction, cohort (where applicable), and before treatment psychiatric symptom scores as predictors. Simple slopes analysis was used to examine significant interactions.

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