Supporting information

Polysaccharide from Ganoderma lucidum Alleviates Congnitive Impairment in a Mice Model of Chronic Cerebral Hypoperfusion by Regulating $CD4^+CD25^+Foxp3^+$ regulatory T cells

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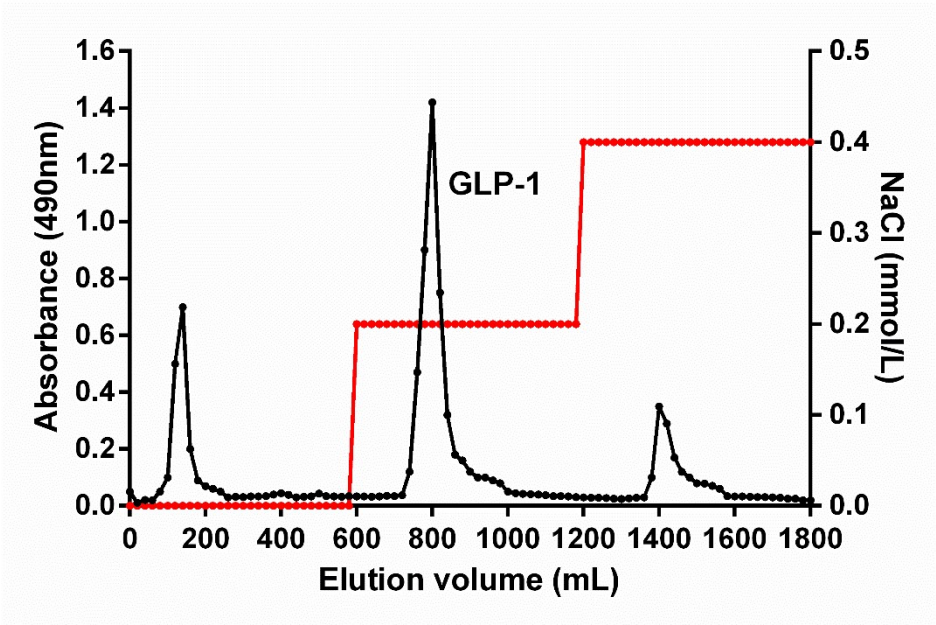

Figure S1. the elution curve of GLP-1 purification process.

Immunofluorescence staining and flow cytometry of spleen cells

Splenocytes suspension were prepared to 1×10^7/mL. Fluorescently antibodies of anti-mouse CD4（0.25μL）and CD25(0.5μL) were added into 100μL splenocytes suspension. After incubated for 30 minutes protecting from light, 2mL 1×flow cytometry staining butter was added, then samples were centrifuged at 300-400 xg for 5 minutes. After discarding the supernatant, 1mL 1×fixation/permeabilization working solution was added. After incubated for 30 minutes protecting from light, 2mL 1×fixation/permeabilization butter was added, then samples were centrifuged at 300-400 xg for 5 minutes. After discarding the supernatant, 1×fixation/permeabilization butter was added to 100mL samples, After incubated for 15 minutes protecting from light, fluorescently antibody of anti-mouse Foxp3（2.5μL）was added. After incubated for 30 minutes protecting from light, resuspended stained cells in an appropriate volume of flow cytometry staining buffer and acquired samples on a flow cytometer.

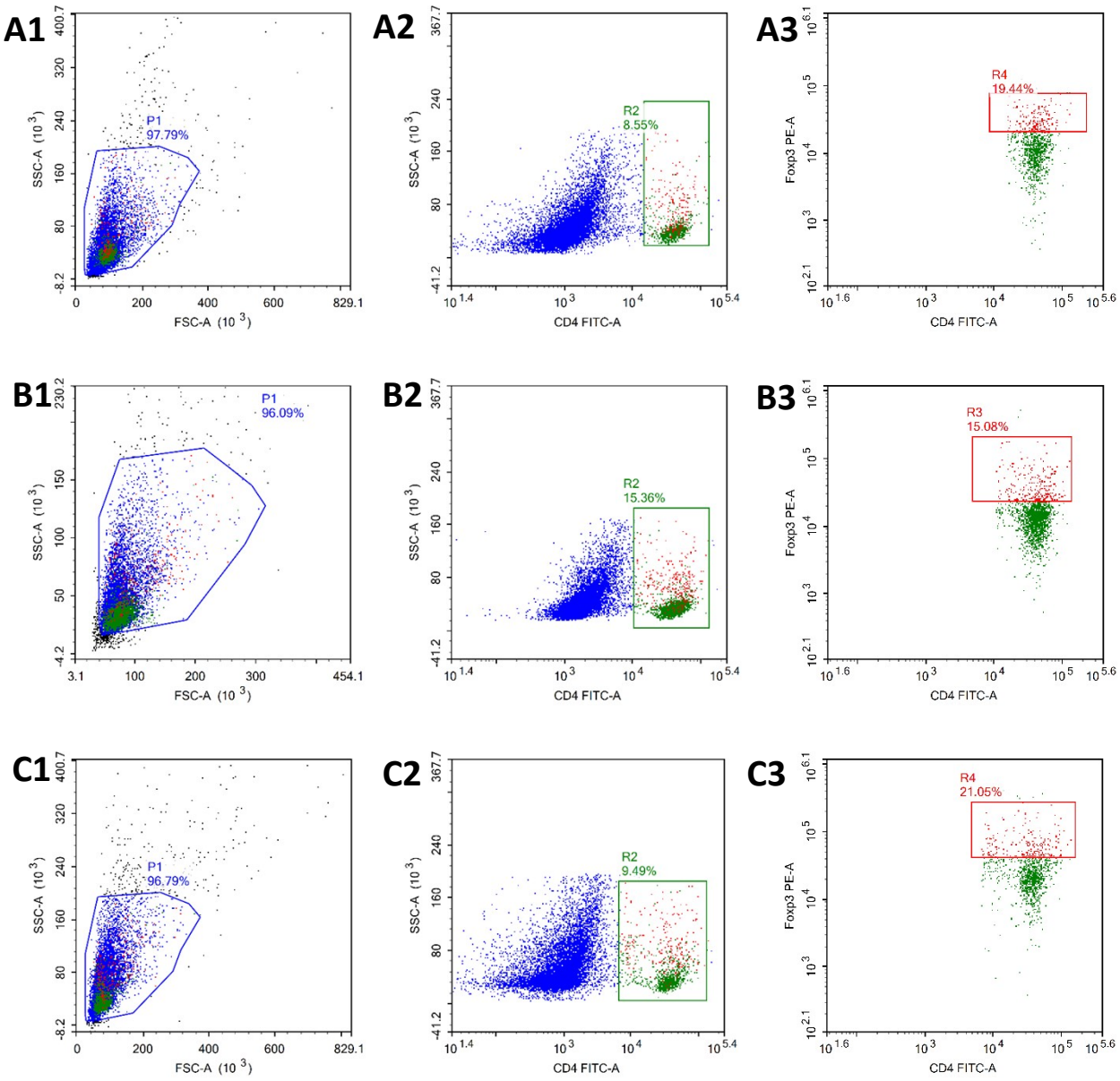

Figure S2. The proportion of $CD4^+CD25^+Foxp3^+$T cells of spleen were analyzed by flow cytometry. Gate information of lymphocytes of sham group (A1), CCH group (B1) and CCH+GLP-1 group (C1). Gate information of $CD4^+$T cells of sham group (A2), CCH group (B2) and CCH+GLP-1 group (C2).The proportion of $Foxp3^+$T cells on gated $CD4^+$T cells of sham group (A3), CCH group (B3) and CCH+GLP-1 group (C3).

Immunofluorescence staining and flow cytometry of peripheral blood

Fluorescently antibodies of anti-mouse CD4（0.25μL）and CD25(0.5μL) were added into 100μL anticoagulated peripheral blood. After incubated for 15 minutes protecting from light, 2mL 1×red blood cell lysate was added, then samples were centrifuged at 300-400 xg for 5 minutes. After discarding the supernatant，2mL 1×flow cytometry staining butter was added, then samples were centrifuged at 300-400 xg for 5 minutes. After discarding the supernatant, 1mL 1×fixation/permeabilization working solution was added. After incubated for 20-60 minutes protecting from light, 2mL 1×fixation/permeabilization butter was added, then samples were centrifuged at 300-400 xg for 5 minutes. After discarding the supernatant, 1×fixation/permeabilization butter was added to 100mL samples, After incubated for 15 minutes protecting from light, fluorescently antibody of anti-mouse Foxp3（2.5μL）was added. After incubated for 30 minutes protecting from light, resuspended stained cells in an appropriate volume of flow cytometry staining buffer and acquired samples on a flow cytometer.

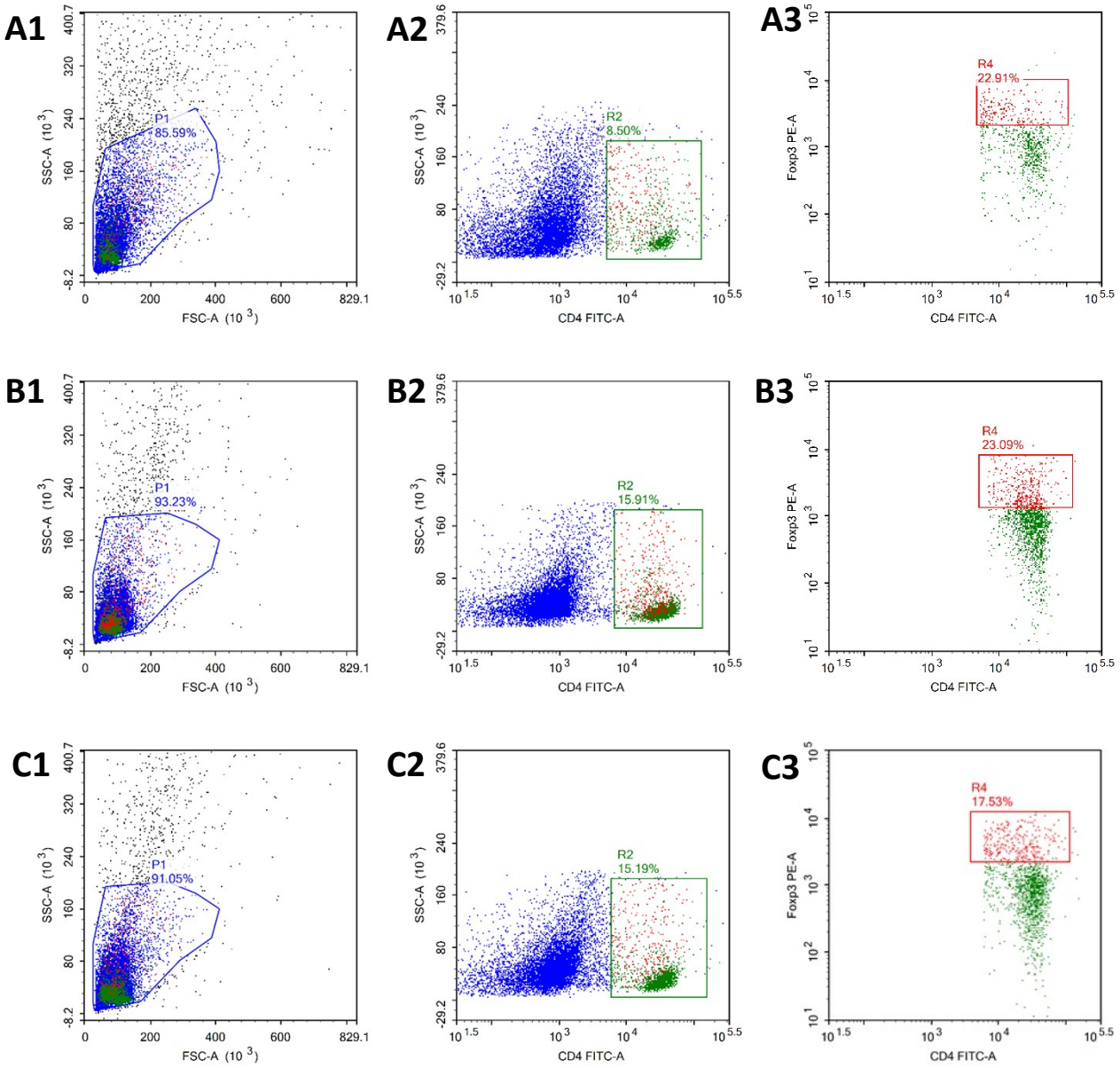

Figure S3. The proportion of $CD4^+CD25^+Foxp3^+$T cells of peripheral blood were analyzed by flow cytometry. Gate information of lymphocytes of sham group (A1), CCH group (B1) and CCH+GLP-1 group (C1). Gate information of $CD4^+$T cells of sham group (A2), CCH group (B2) and CCH+GLP-1 group (C2).The proportion of $Foxp3^+$T cells on gated $CD4^+$T cells of sham group (A3), CCH group (B3) and CCH+GLP-1 group (C3).

Metabolomic analysis

GC-MS raw data and TIC (Figure S3) were acquired as metabolic profiling via GC-MS Postrum Analysis (Shimadzu, Tokyo, Japan). GC-MS raw data (CDF format) were deconvoluted and analysis by XCMS Online. Perform principal component analysis (PCA), the orthogonal partial least squares discriminant analysis (O-PLS-DA) and S-plot analysis were carried out by Simca-P 13.0. PCA analysis were shown in Figure S4. Separating tendency was observed in the PCA score plots of 3 groups, indicating that group difference was more remarkable than individual difference. O-PLS-DA and S-plot analysis were shown in Figure S5. O-PLS-DA results showed that either sham and CCH groups or CCH+GLP-1 and CCH groups were definitely divided into two classes. S-plot analysis showed coefficients vs. VIP. The VIP value more than 1.00 was considered as potential biomarkers. Metabolites of variable importance in the projection (VIP) value more than 1.00 were considered as potential biomarkers. Databases such as HMDB, NIST and KEGG were used to identify biomarkers. Pathway analysis were carried out by MetaboAnalyst 5.0. Pathways, impact value more than 0.10 and P value less than 0.05, were screened as mainly impacted pathways.

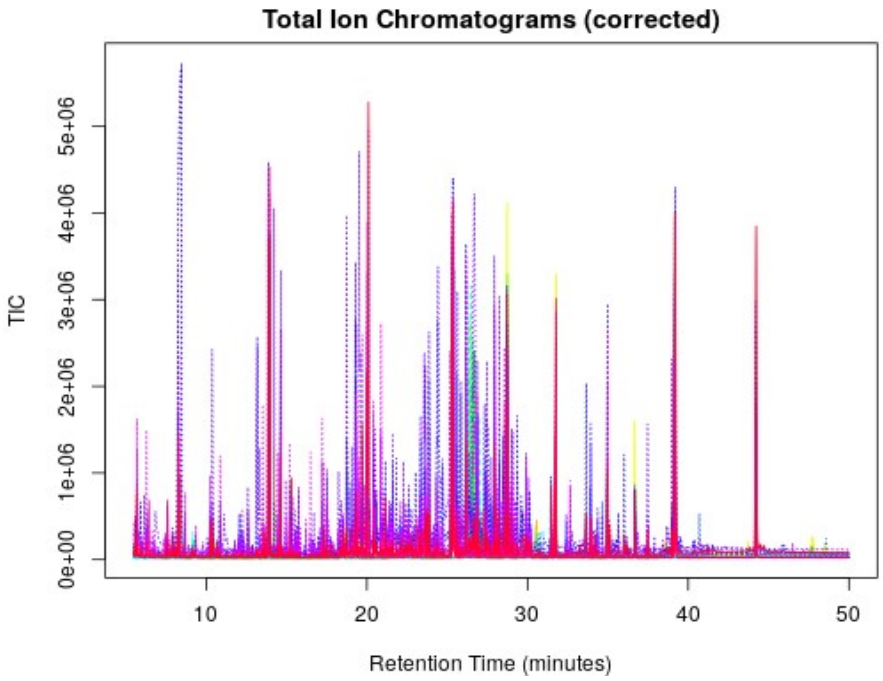

Figure S4. Corrected TIC of sham group, CCH group and CCH+GLP-1 group.

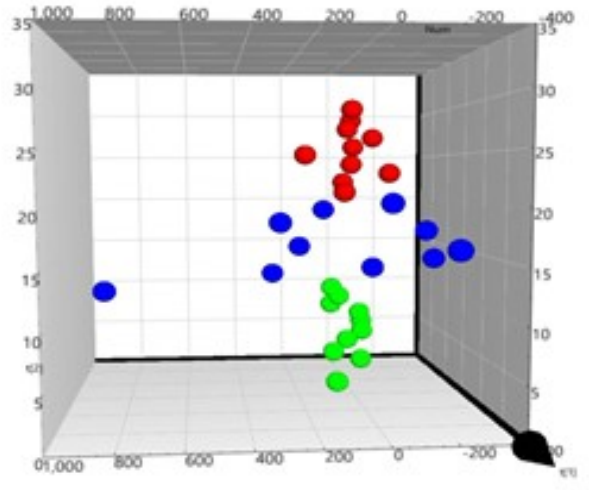

Figure S5. PCA score plots of metabolic profiling of sham group (●), CCH group (●) and CCH+GLP-1 group (●).

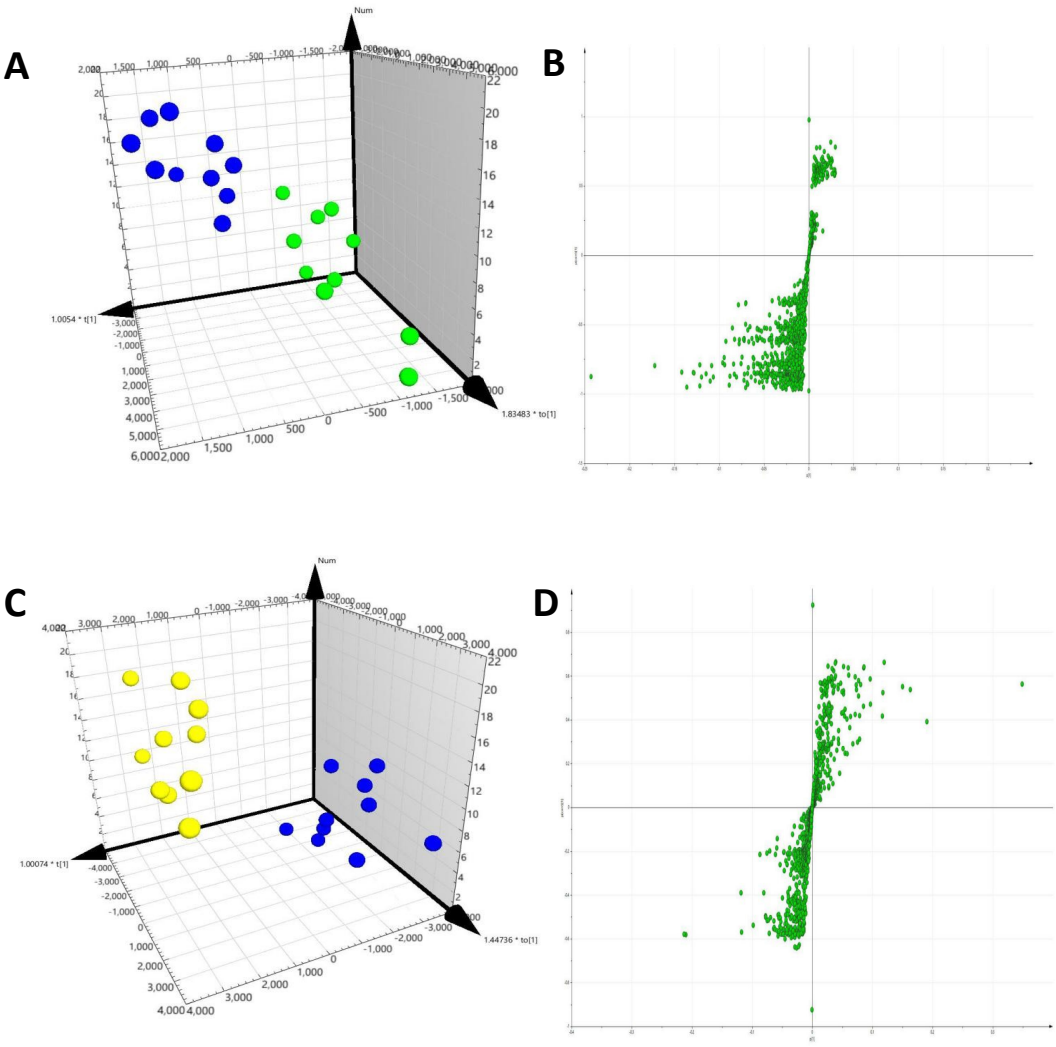

Figure S6. O-PLS-DA score plots and S-plot of metabonomic profiles of sham group (●), CCH group (●) and CCH+GLP-1 group (●). O-PLS-DA score plots of sham group vs CCH group (A), S-plots of sham group vs CCH group (B), O-PLS-DA score plots of CCH+GLP-1 group vs CCH group (C),S-plots of CCH+GLP-1 group vs CCH group (D).