Tumor cell membrane-coated continuous electrochemical sensor for GLUT1 inhibitor screening

Jiaqian Zhao[a,b], Yuqiao Liu[a], Ling Zhu[a], Junmin Li[a], Yanhui Liu[a], Jiarui Luo[a], Tian Xie[a,b*], Dajing Chen[a**]
a, School of Pharmacy, Hangzhou Normal University, China.
b, Innovation Research Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, China.

1 Materials and Methods

1.1 Materials

Glucose oxides (GOx), chitosan (CS), polyvinyl alcohol (PVA), glucose, ascorbic acid (AA, ≥99.7%), uric acid (UA, ≥99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-α-phosphatidylcholine (PC), RNAiMAX transfection agent, BAY-876, curcumin, apigenin, phloretin, baicalin and Dil were purchased from Sigma-Aldrich (St. Louis, USA). siRNA were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Baicalein, wogonin, wogonoside, epigallocatechin gallate (EGCG), catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC) and epicatechin gallate (ECG) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). GLUT1 and β-actin antibody were purchased from ImmunoWay (Plano, USA). HRP conjugated IgG antibody and BCA kit were purchased from Bioshap (Hefei, China). Phosphate buffer solution (PBS), polycarbonate membrane and PVDF membrane were purchased from HyClone (Waltham, USA). Glucose update assay kit was purchased from Abcam (Cambridge, US). All the chemicals used in this experiment are analytical grade and commercially available. All the aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm, Millipore).

1.2 Small interfering RNA synthesis and transfection

We used small interfering RNA (siRNA) to knockdown GLUT1 in H1299 and MGC803 cells. Three siRNA sequences were synthesized by Shanghai Sangon Biotech as follows: siRNA1: 5’-GGACCTCAAATTTCATTGT-3’, siRNA2:

5’-GTCCCTACGTCTTCATCAT-3, siRNA3: 5’-GTGCCATACTCATGACCAT-3’. An equal volume of siRNAs and RNAiMAX transfection agent were added to the cell culture medium and incubated for 60 h to ensure the siRNA entered the cells.

RNA was isolated from the transfected cells by Trizol reagent, 1 μg RNA was reversed transcribed to cDNA. Then 0.1 μg cDNA was used as a template to perform quantitative reverse transcription polymerase chain reaction (RT-qPCR). The mRNA expression level of GLUT1 was normalized to that of GAPDH. The primer pairs for genes were: GLUT1 forward 5’-GGCCAAGAGTGTGCTAAAGAA-3’ and reverse 5’-ACAGCGTTGATGCCAGACAG-3’, GAPDH forward 5’-ATGGGGAAGGTGAA GGTCG and reverse 5’-GGGGTCATTGATGGCAACAATA. Each sample was analyzed in triplicate.

1.3 Fluorescent recovery after photobleaching (FRAP) test

FRAP test was prepared according to our previous work [22]. Briefly, cell membrane and lipophilic fluorescent dye Dil (5 μg/mL) were incubated for 15 min at ambient temperature and sonicated for 20 min to form fluorescent labelled cell membrane vesicles. 100 μL cell membrane vesicles were dropped on the piranha solution treated coverslip and washed by water to remove unfused cell membrane vesicles. The fluorescence was quenched by a laser confocal microscope at 561 nm wavelength with 90% laser power. The fluorescence recovery time was recorded to characterize lipid mobility in the membrane.

1.4 Western blot

Cells were lysed with RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.1% SDS, pH=7.4) for 30 min on ice and centrifuged at 15000 r/min for 30 min. The total protein content of the collected supernatant was measured by BCA Protein Assay Kit. 20 μg total protein samples were dissolved by SDS-PAGE and transferred onto PVDF membrane. After washed by PBST, PVDF membrane was incubated with GLUT1 primary antibody (1:2000) overnight at 4 ℃ and subsequently incubated with secondary HRP conjugated antibody (1:5000). Finally, the PVDF membrane was exposed in Chemiluminescence imaging system to obtain protein bands. β-actin was selected as the reference protein in this experiment.

1.5 Docking study

The GLUT1 homology modeled (4PYP) and grid preparations were conducted in the Glide module (First Discovery, Schrodinger). The bounding box encompassing the entire channel was centered in the middle of the GLUT1 channel. The GLUT1 inhibitors and drug molecules structures were imported to GLUT1 model and docked using Glide. The structural interaction between inhibitors and GLUT1 were selected based on the Glide-calculated E_{model} value.

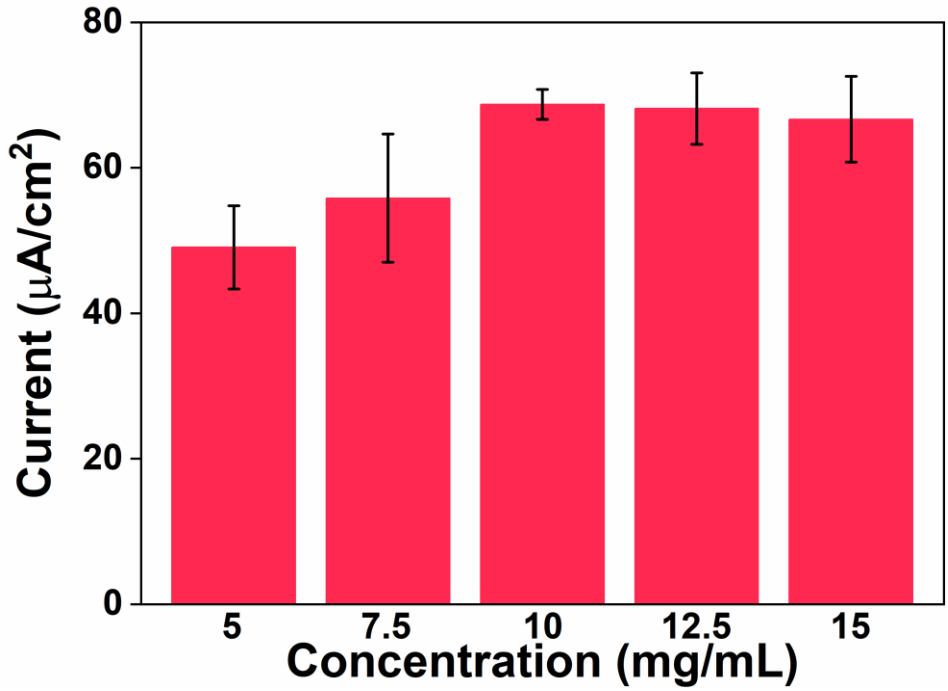

Figure S1. The current of glucose tested with different concentrations of immobilized glucose oxidase (GOx) (added glucose concentration: 10 mM).

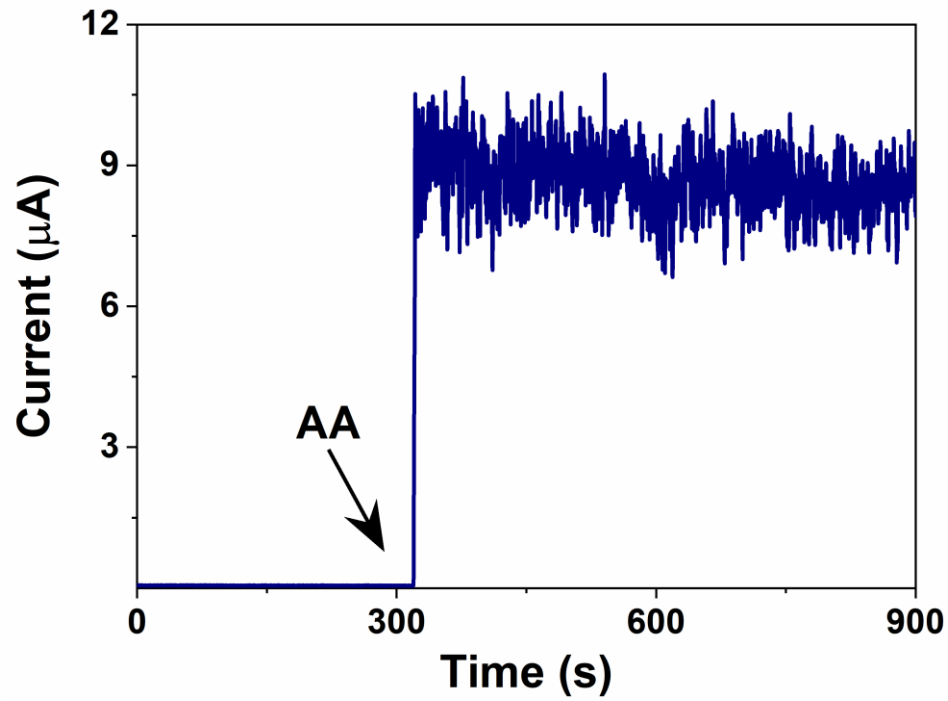

Figure S2. Current response of the bare electrode to 250 μM ascorbic acid (AA).

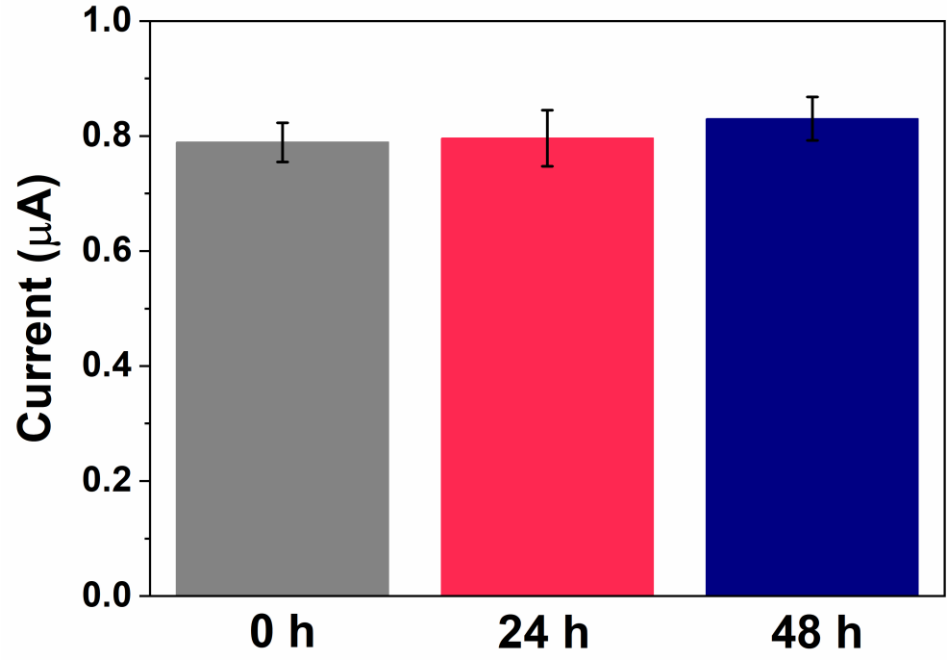

Figure S3. Stability test of cell membrane-based glucose sensor (CMGS) at 24 h and 48 h.

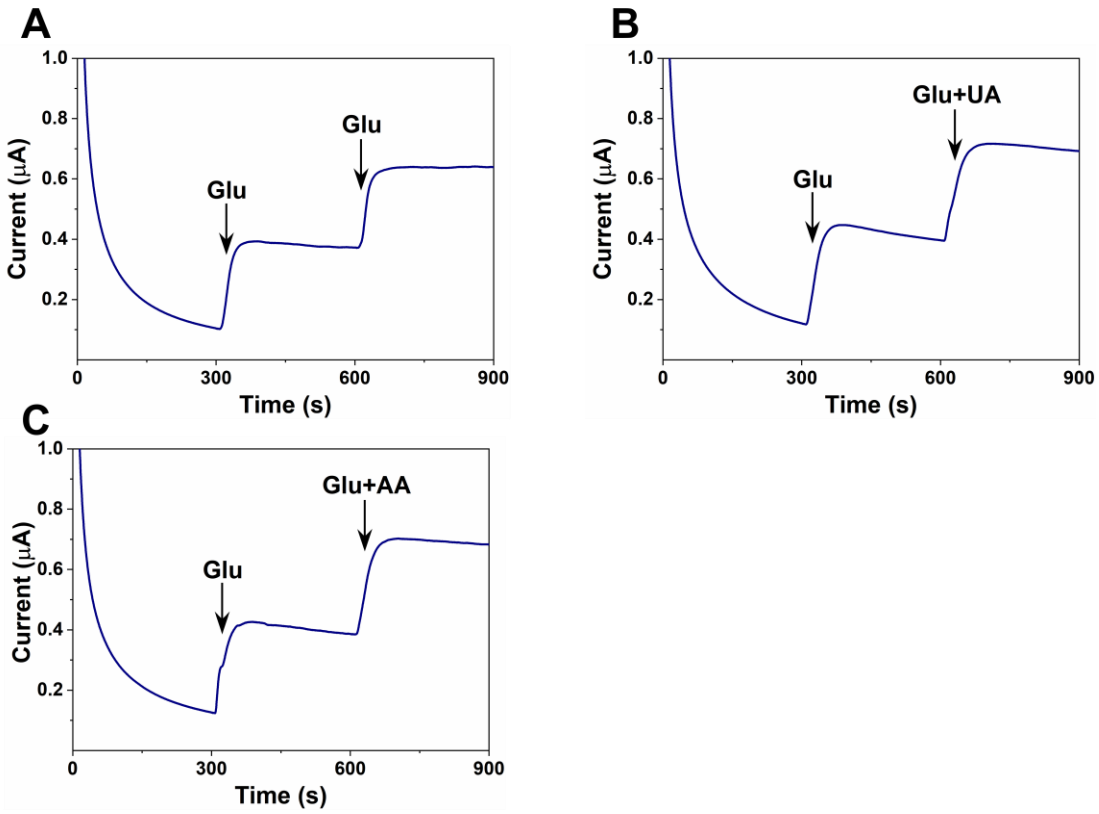

Figure S4. Selective testing of cell membrane-based glucose sensor (CMGS). Current response of CMGS to (A) 2 mM glucose + 2 mM glucose, (B) 2 mM glucose + 0.1 mM UA, (C) 2 mM glucose + 0.25 mM AA.

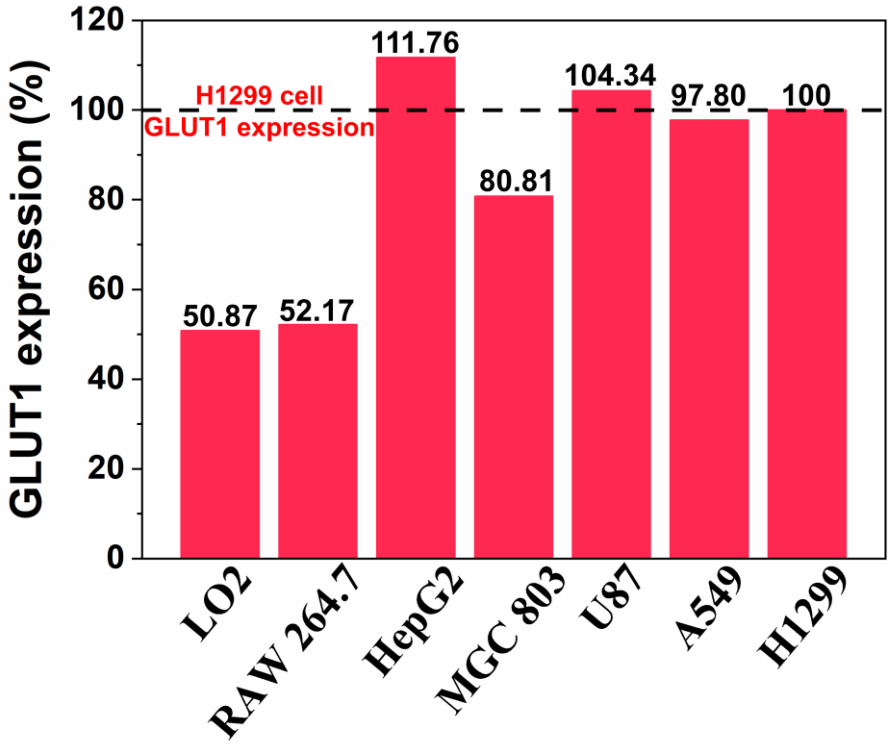

Figure S5. The expression ratio of glucose transporter 1 (GLUT1) in different cells (the ratio of all cells to H1299 cells).

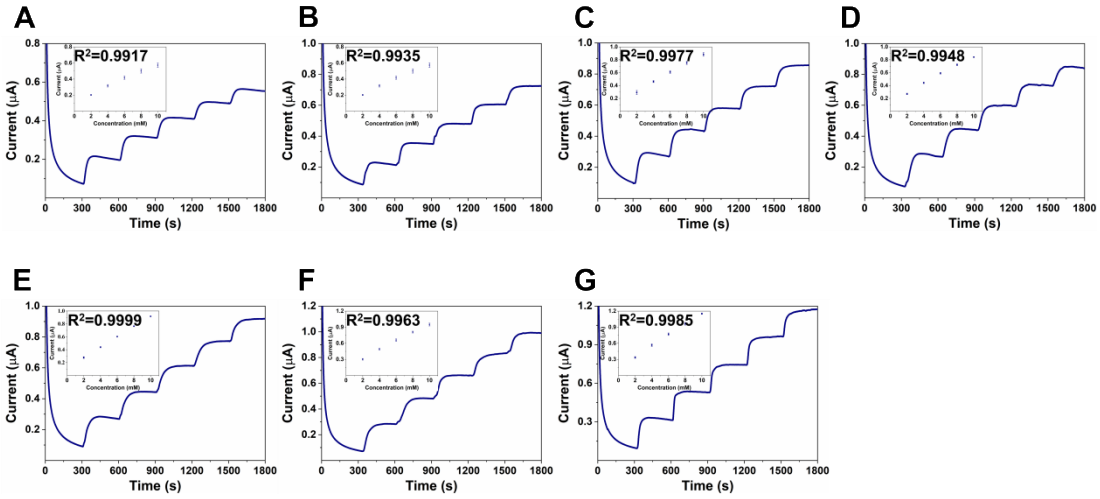

Figure S6. Current response curve and linear relationship of (A) LO2, (B) RAW 264.7, (C) H1299, (D) HepG2, (E) U87, (F) MGC803 and (G) A549 cell membrane glucose sensor to 10 mM glucose.

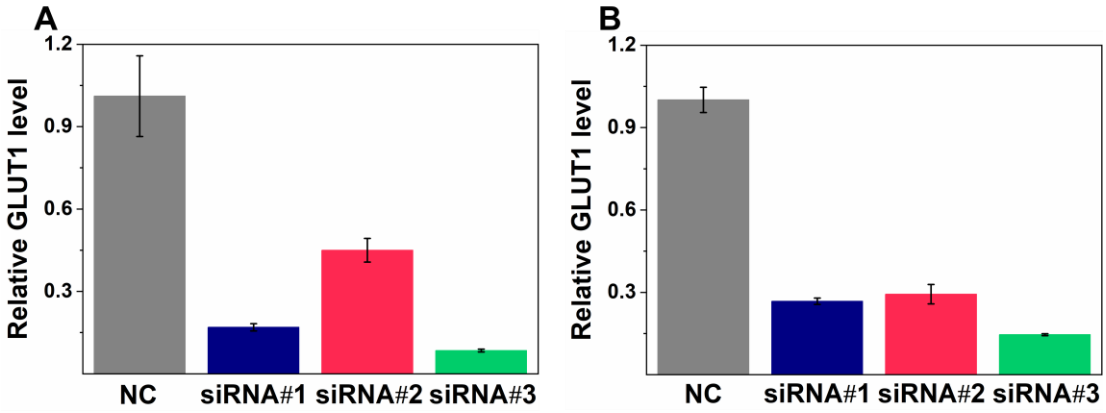

Figure S7. Small interfering RNA (siRNA) decreased glucose transporter 1 (GLUT1) mRNA levels on (A) H1299 cells and (B) MGC803 cells by quantitative reverse transcription polymerase chain reaction.

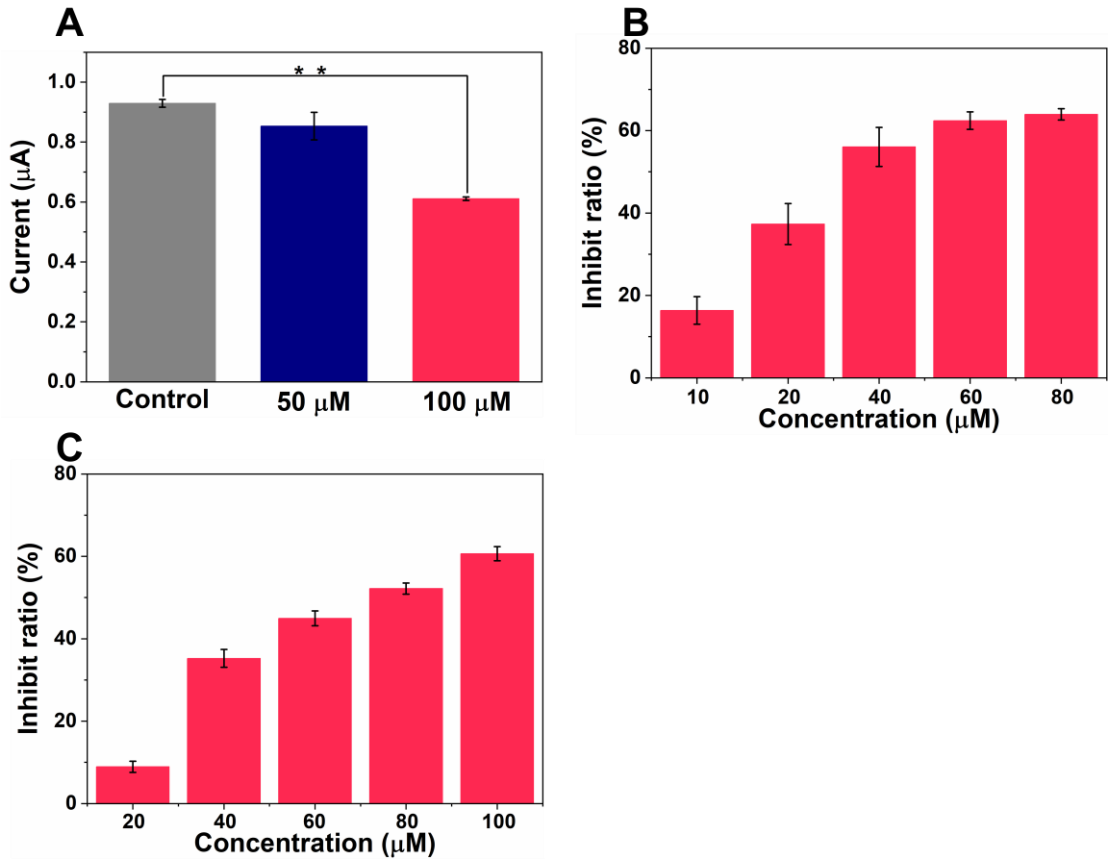

Figure S8. (A) Current responses of cell membrane-based glucose sensor (CMGS) treated with 50 μM and 100 μM phloretin to 10 mM glucose. (B) The inhibit rate of H1299 cells treated with different concentration apigenin in 24 h. (C) The inhibit rate of MGC803 cells treated with different concentration phloretin in 48 h.

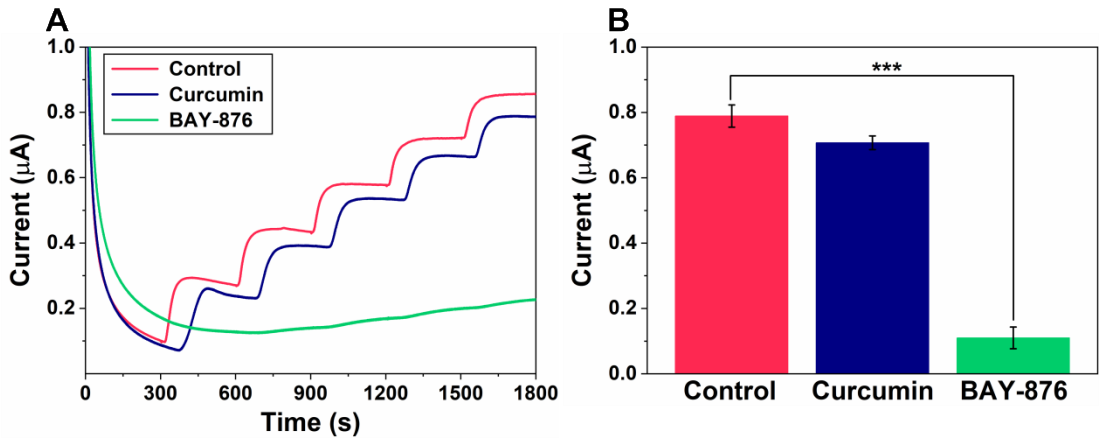

Fig S9. (A) Current response curves of BAY-876-treated and curcumin-treated cell membrane-based glucose sensor (CMGS) to 10 mM glucose. (B) Current responses of CMGS treated with BAY-876 and curcumin to 10 mM glucose.

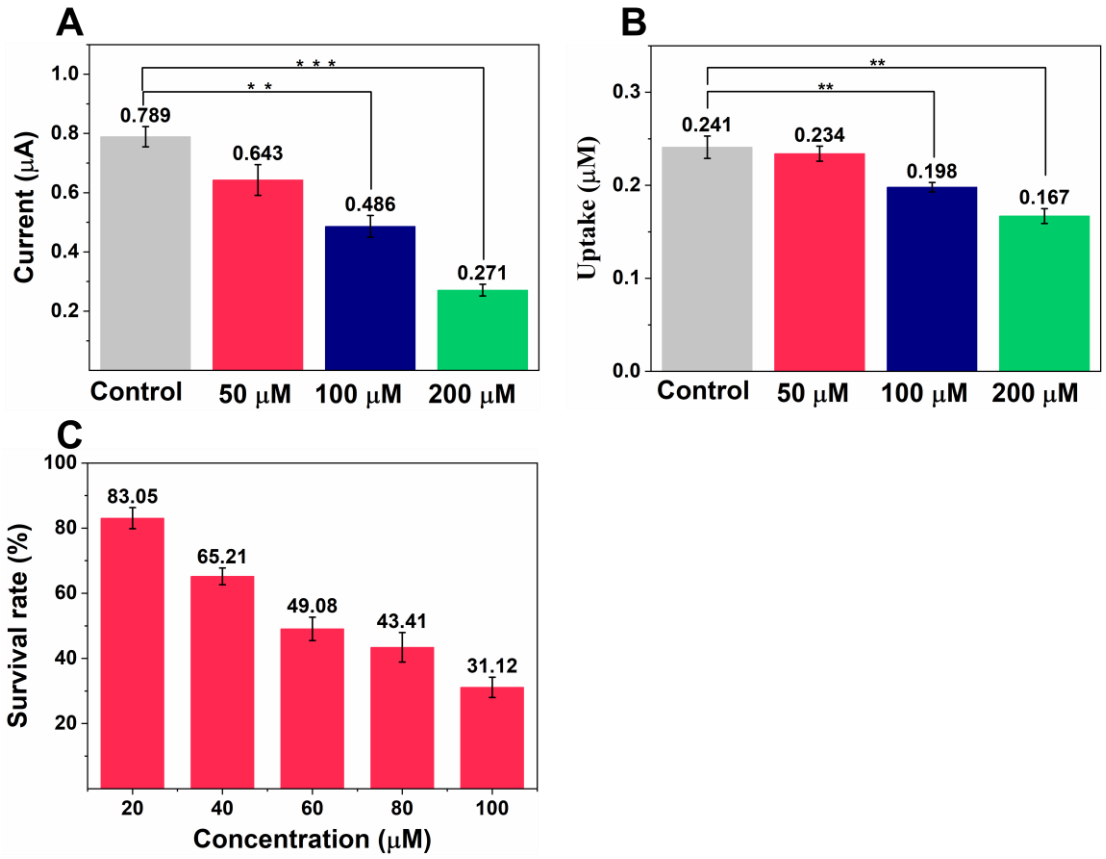

Fig S10. (A) Current responses of cell membrane-based glucose sensor (CMGS) treated with different concentration baicalin to 10 mM glucose. (B) 2-deoxyglucose (2-DG) uptake concentration of different concentration baicalin treated with H1299 cells. (C) The survival rate of H1299 cells treated with different concentration baicalin in 24 h.

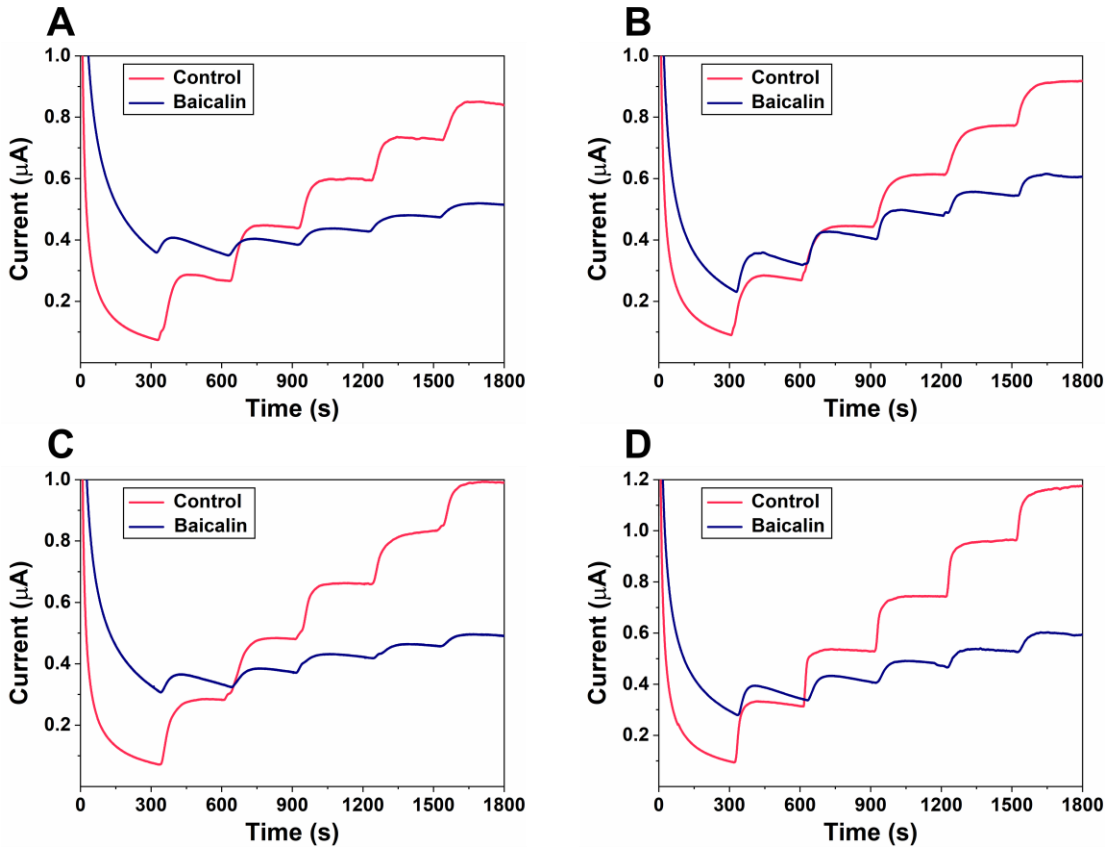

Fig S11. Current response curves of 200 μM baicalin-treated (A) HepG2, (B) U87, (C) MGC803 and (D) A549 cell membrane glucose sensor to 10 mM glucose.

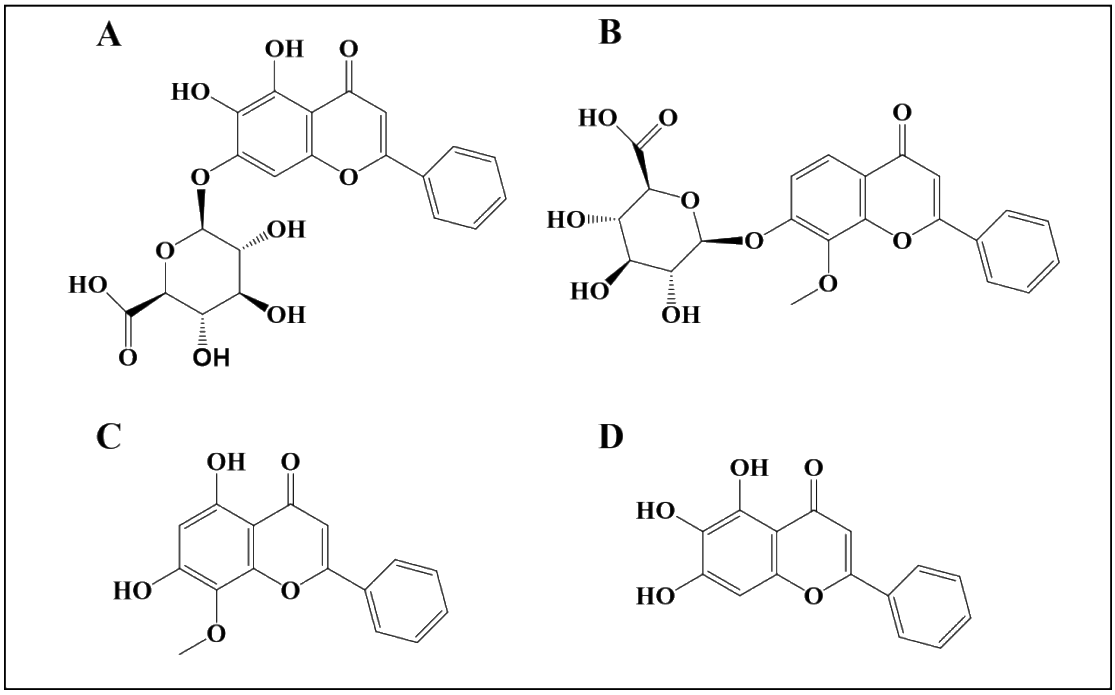

Fig S12. The molecular structure of (A) baicalin, (B) wogonoside, (C) wogonin, (D) baicalein.

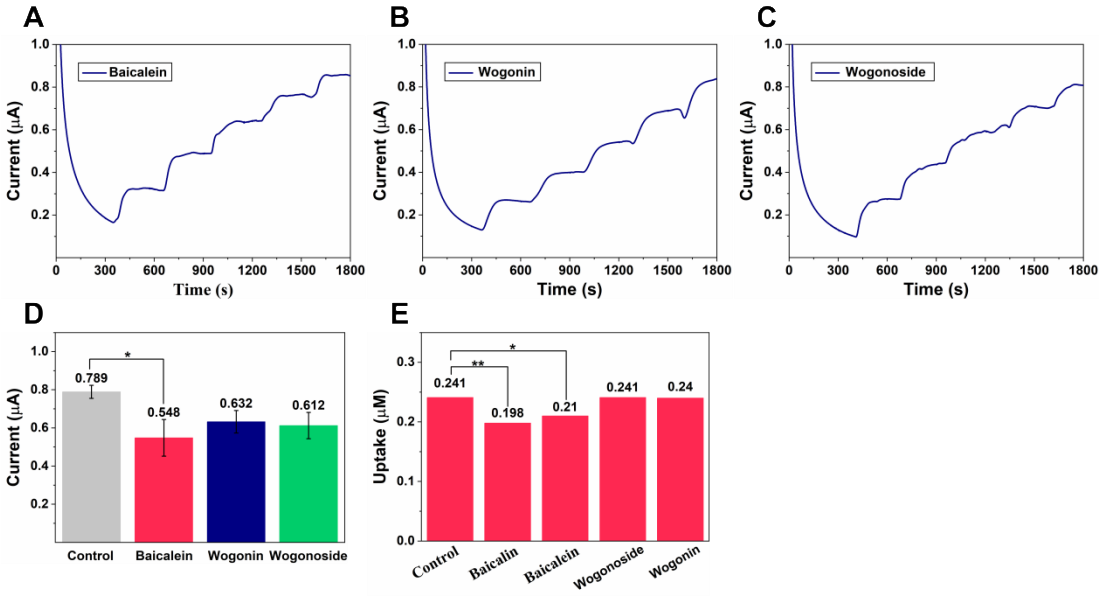

Fig S13. Current response curves of cell membrane-based glucose sensor (CMGS) treated with (A) 100 μM baicalein, (B) 100 μM wogonin and (C) 100 μM wogonoside to 10 mM glucose. (D) Current responses of CMGS treated with baicalein, wogonin, and wogonoside to 10 mM glucose. (E) 2-deoxyglucose (2-DG) uptake concentration of 200 μM baicalin, baicalein, wogonin, and wogonoside treated with H1299 cells.

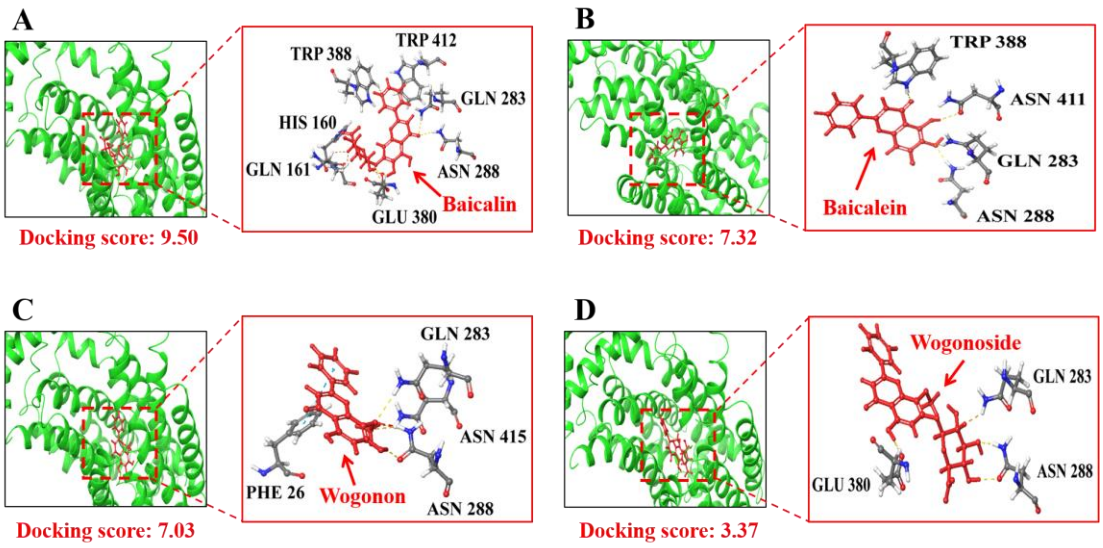

Fig S14. Docking study of (A) baicalin, (B) baicalein, (C) wogonon and (D) wogonoside binding to glucose transporter 1 (GLUT1).

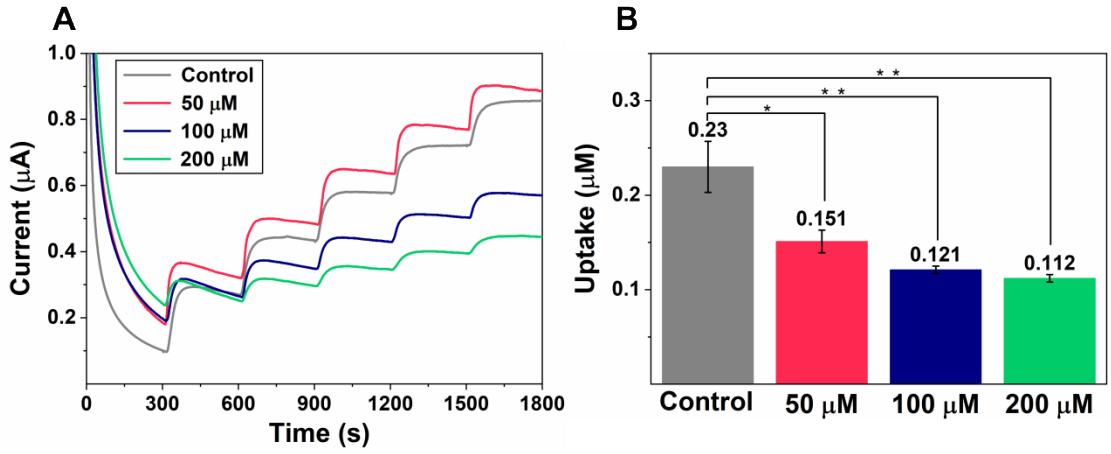

Fig S15. (A) Current response curves of different concentration epigallocatechin gallate (EGCG)-treated cell membrane-based glucose sensor (CMGS) to 10 mM glucose. (B) 2-deoxyglucose (2-DG) uptake concentration of different concentration EGCG treated with H1299 cells.

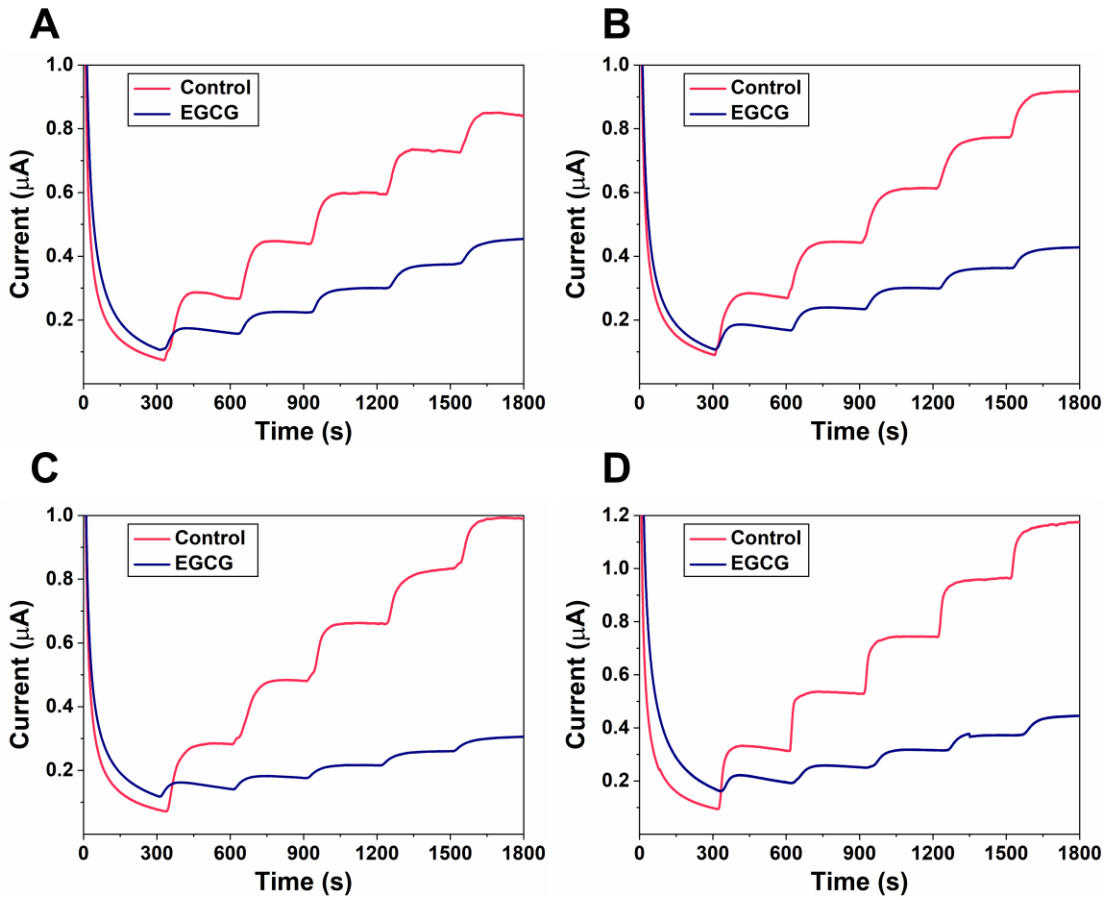

Fig S16. Current response curves of 200 μM epigallocatechin gallate (EGCG)-treated (A) HepG2, (B) U87, (C) MGC803 and (D) A549 cell membrane glucose sensor to 10 mM glucose.

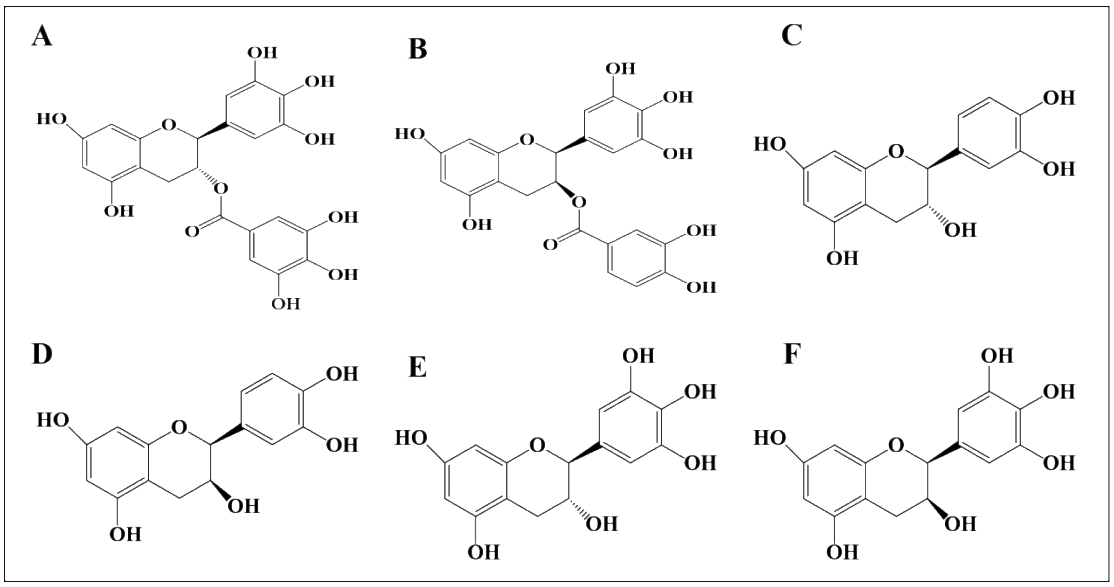

Fig S17. The molecular structure of (A) epigallocatechin gallate (EGCG), (B) epicatechin gallate (ECG), (C) catechin (C), (D) epicatechin (EC), (E) gallocatechin (GC) and (F) epigallocatechin (EGC).

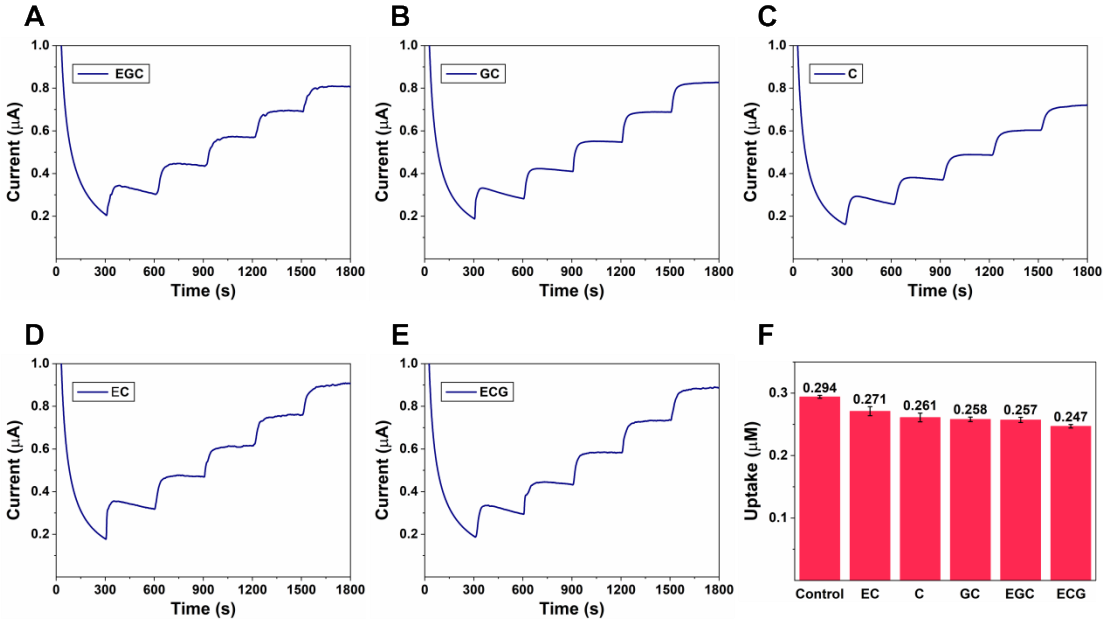

Fig S18. Current response curves of cell membrane-based glucose sensor (CMGS) treated with (A) 200 μM epigallocatechin (EGC), (B) gallocatechin (GC), (C) catechin (C), (D) epicatechin (EC) and (E) epicatechin gallate (ECG) to 10 mM glucose. (F) 2-deoxyglucose (2-DG) uptake concentration of 200 μM EGC, GC, C, EC and ECG treated with H1299 cells.

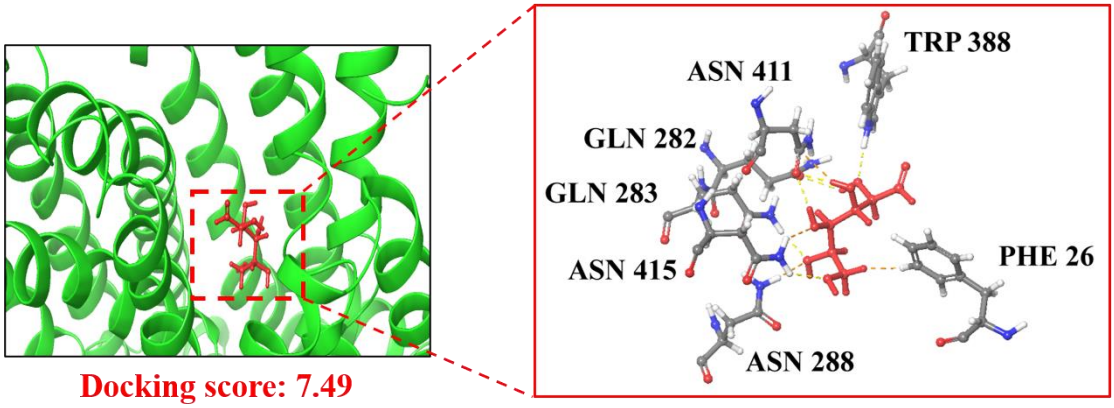

Fig S19. Docking study of glucose binding to glucose transporter 1 (GLUT1).