Figure S1. Treatment with JQ1 inhibits cell viability and proliferation of CSC1589 cells. (A) Cell viability of CSC1589 was detected by a Cell Counting Kit-8 assay. CSC1589 cells were treated with JQ1 for 24 and 48 h (error bars represent standard error of the mean). (B) Cell proliferation was assessed by cell growth counting assay. CSC1589 were treated with JQ1. After seeding cells into plates for 24 h, the relative the number of cells was counted for the next 3 days. (error bars represent standard error of the mean). (C) Macroscopic images and the electron microscopy images of colonies of CSC1589 cells. Scale bars=20 μm. (D) Bar graph of the colony numbers of CSC1589 (error bars represent standard error of the mean). *P<0.05, **P<0.01 vs. control.

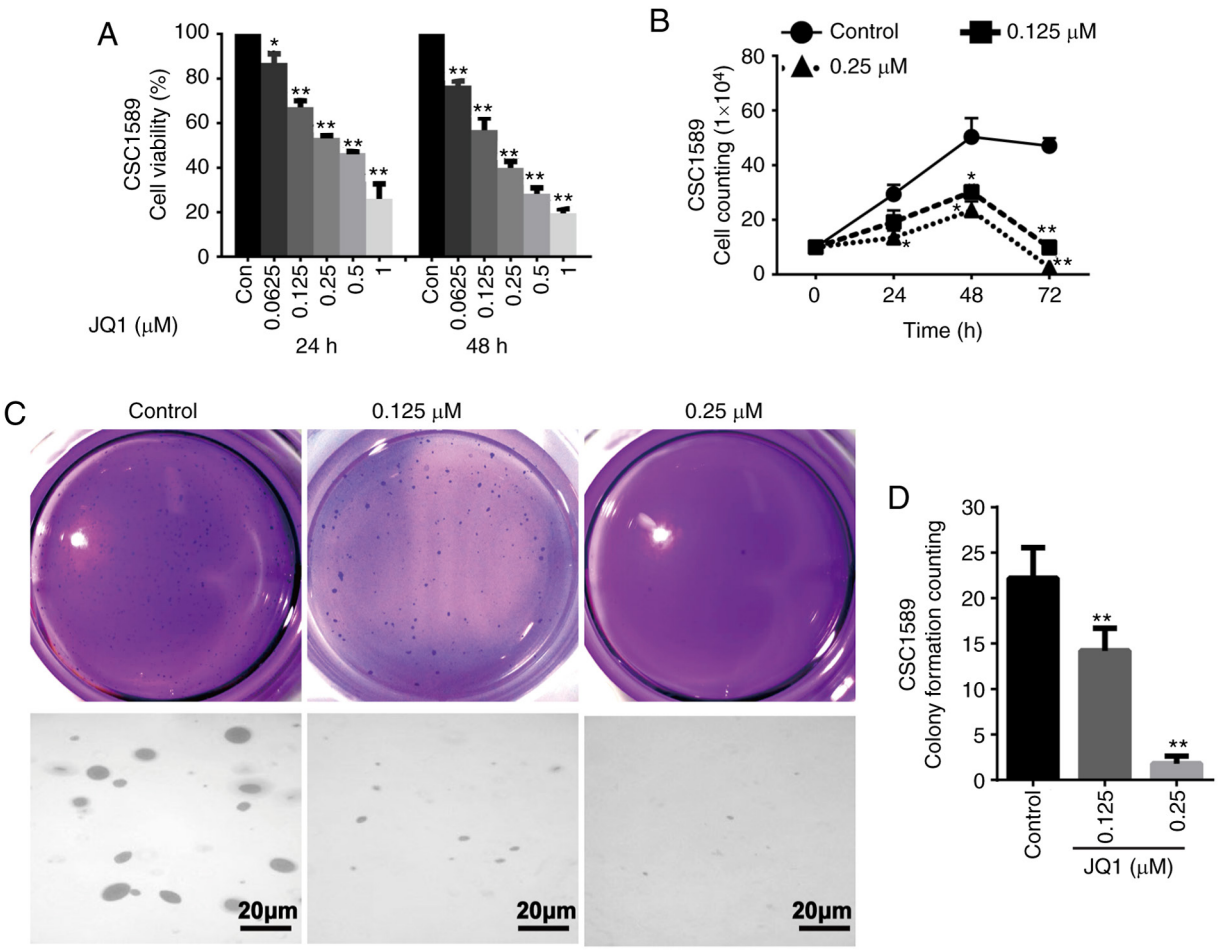


Figure S2. Treatment with JQ1 or siBrd4 reduced proliferation and self-renewal in CSC1589, CSC2078 and TS543 cells. (A) Macroscopic images of colonies of CSC2078 and TS543 cells treated with JQ1 or siBrd4. (B and C) Neural spheres formation of CSC1589 were measured in the presence of JQ1 at indicated concentrations (means ± standard error of the mean of five fields). Scale bar=50 μm. (D) Electron microscopy analysis of neural spheres of CSC2078 and TS543 cells treated with JQ1. Scale bar=50 μm. (E) Electron microscopy analysis of neural spheres of CSC2078 cells treated with siBrd4 (Scale bar=100 μm). **P<0.01, vs. Con. Con, control; siBrd4, small interfering RNA against bromodomain-containing protein 4.

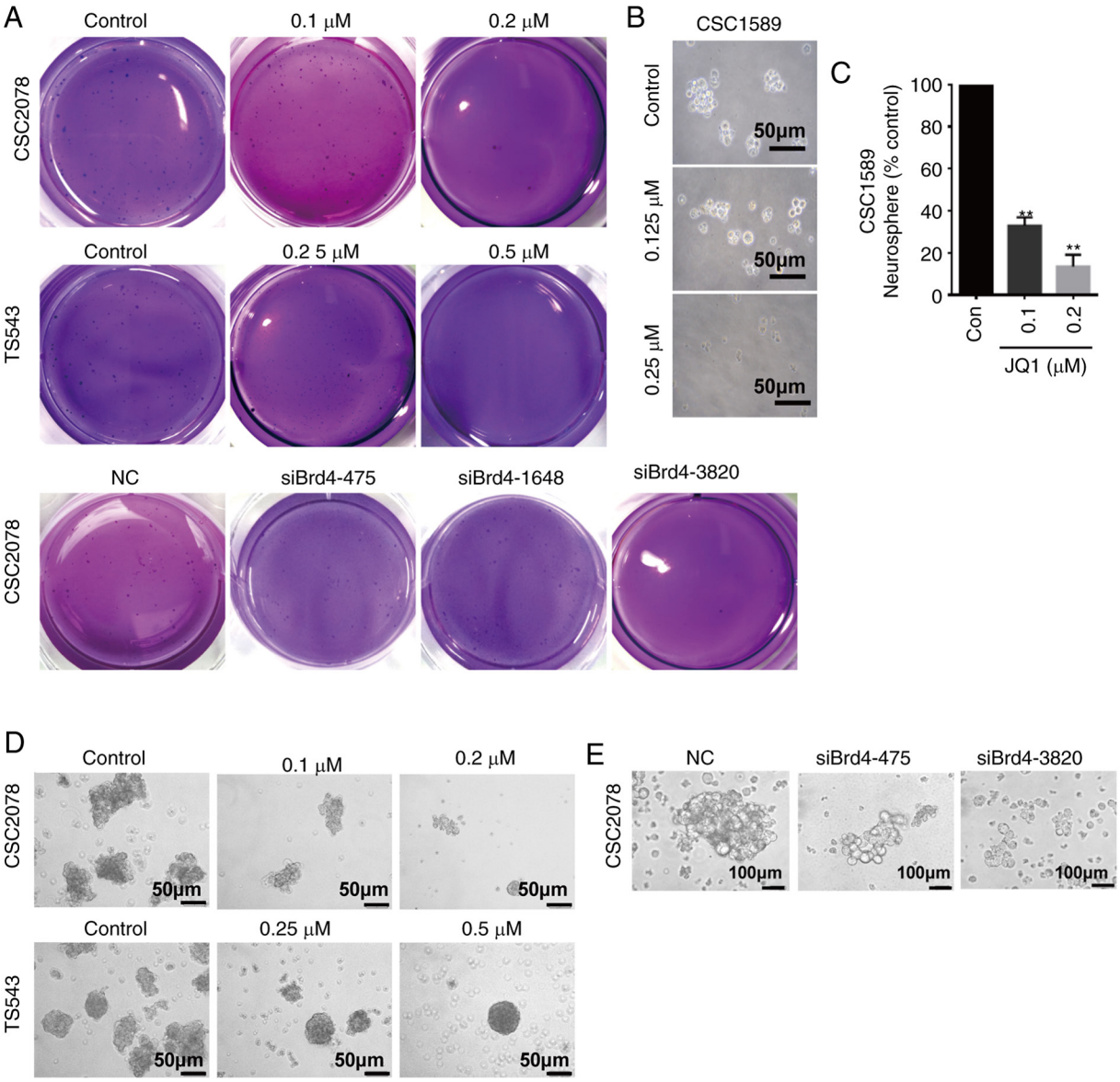


Figure S3. JQ1 or siBrd4 induces cell cycle arrest in glioma stem cells. CSC2078 and TS543 cells were treated with JQ1 for 24 h. CSC2078 cells were transfected with si-NC or siBrd4 for 48 h. Then subjected to flow cytometry analysis of cell cycle using PI staining. NC, negative control; PI, propidium iodide; siBrd4, small interfering RNA against bromodomain-containing protein 4.

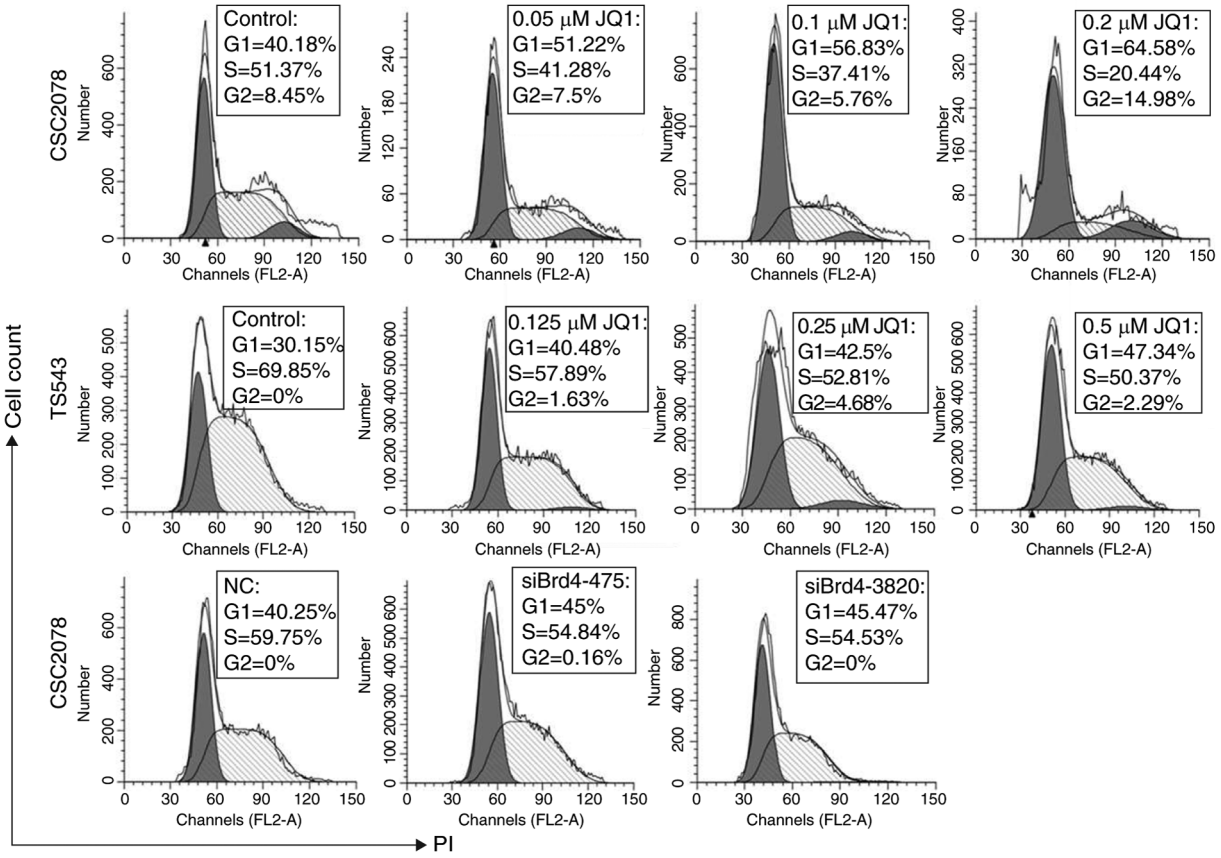


Figure S4. JQ1 or siBrd4 induces cell apoptosis in CSC2078 and TS543 cells. CSC2078 and TS543 cells were treated with JQ1 for 24 h. CSC2078 cells were transfected with si-NCor siBrd4 for 48 h. Cell apoptosis was evaluated in CSC2078 and TS543 by flow cytometry with double staining of Annexin V and PI. FITC, fluorescein isothiocyanate; PI, propidium iodide; siBrd4, small interfering RNA against bromodomain-containing protein 4.

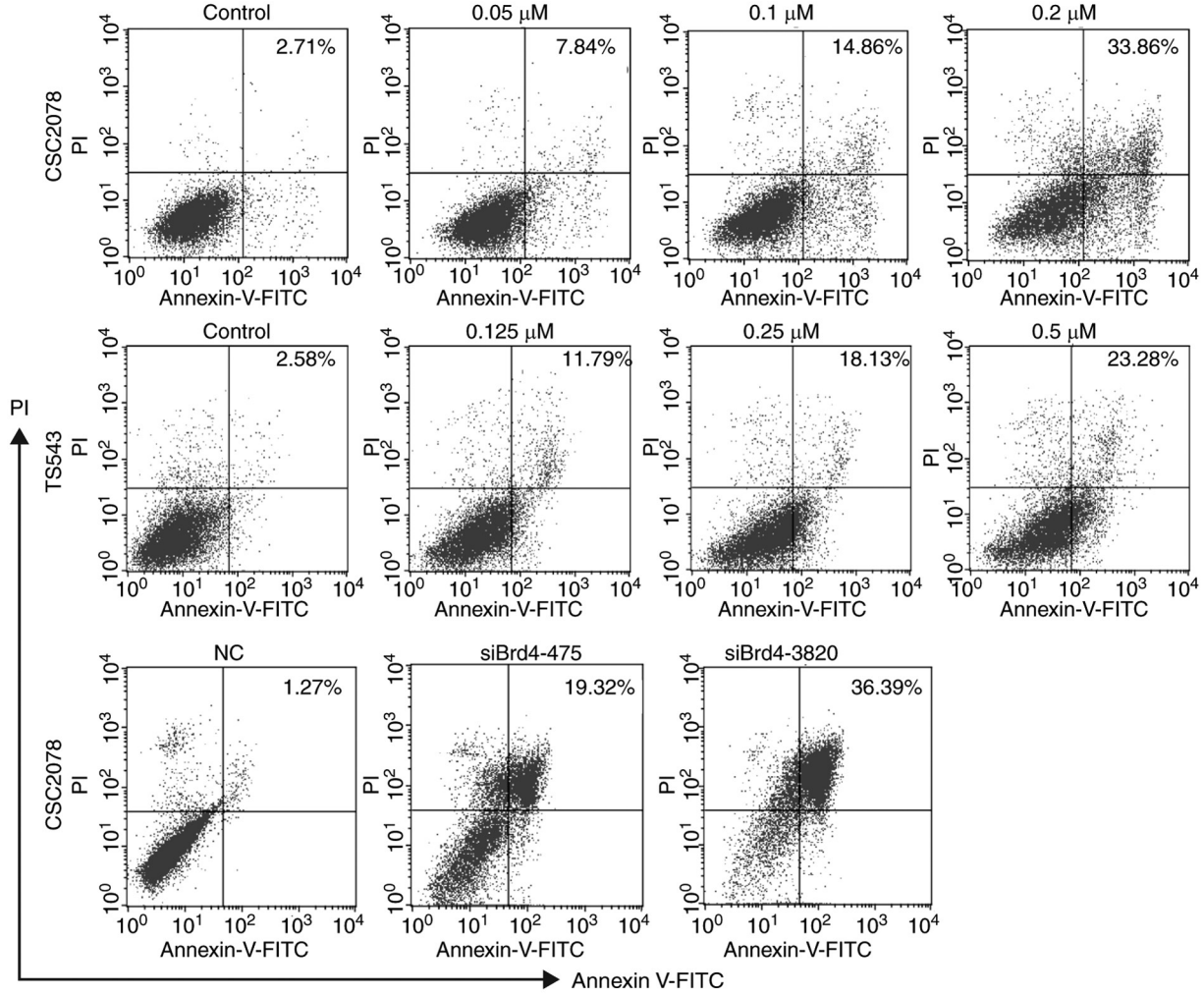


Figure S5. JQ1 induces cell apoptosis in CSC1589 and CSC2078 cells. (A) Hochest33342 staining of CSC1589 cells validated the pro-apoptotic effect of different concentrations of JQ1. Scale bar=20 μm. (B) Statistical analysis (error bars represent standard error of the mean). (C) The pro-apoptotic effect of JQ1 on CSC2078 was demonstrated via with Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling staining. Scale bar=20 μm. (D) Quantitative analysis of apoptotic index (error bars represent standard error of the mean). *P<0.05, **P<0.01 vs. Con. Con, control.

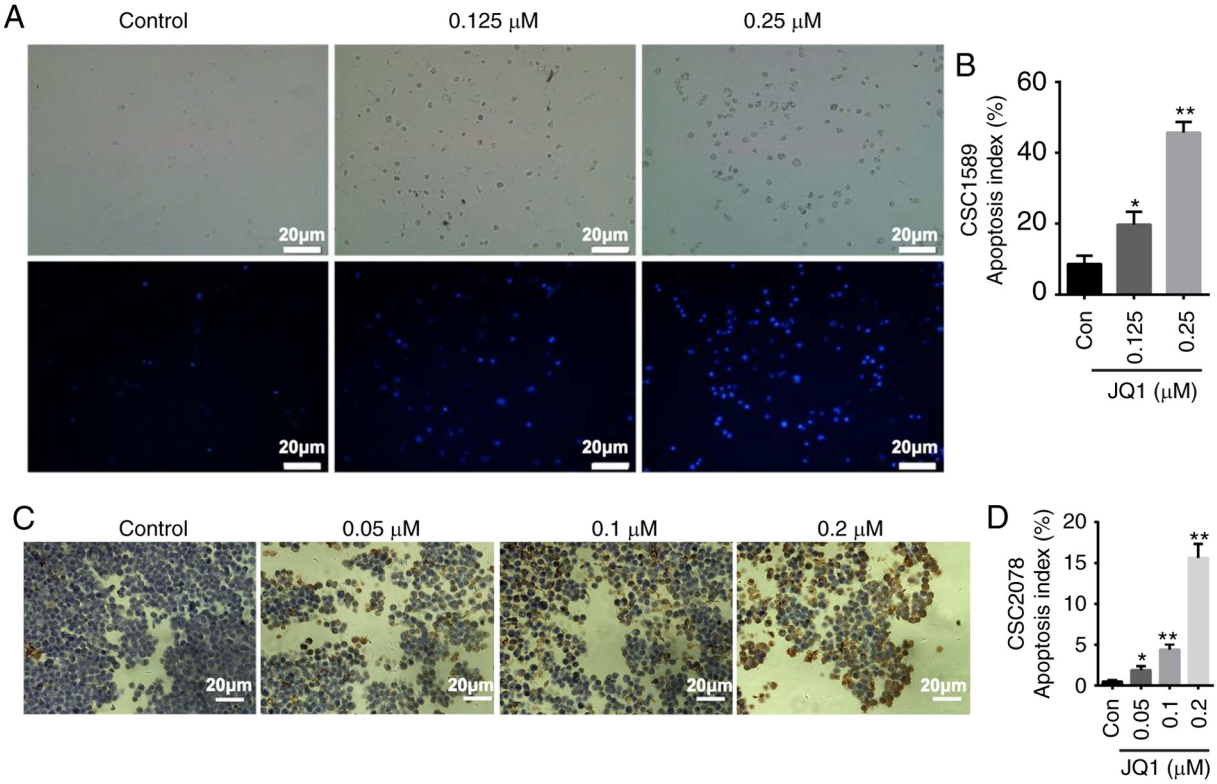


Figure S6. JQ1 has the potential to the promote differentiation of glioma stem cells. (A) GO analysis of mRNAs according to biological process. (B) Fold change in nerve cell markers of CSC2078 was analyzed by RNA-Seq. (C and D) Immunofluorescent analysis of stem cell marker Nestin in CSC1589 cells treated with JQ1 for 48 h (scale bar=20 μm). (E) Reverse transcription-quantitative polymerase chain reaction of Nestin expression in CSC2078 cells treated with JQ1 for 24 h (error bars represent standard error of the mean). *P<0.05, **P<0.01 vs. Con. Con, control; Cntf, ciliary neurotrophic factor; GO, Gene Ontology; PDGFα, platelet-derived growth factor α; Tuj1, class III β-tubulin.

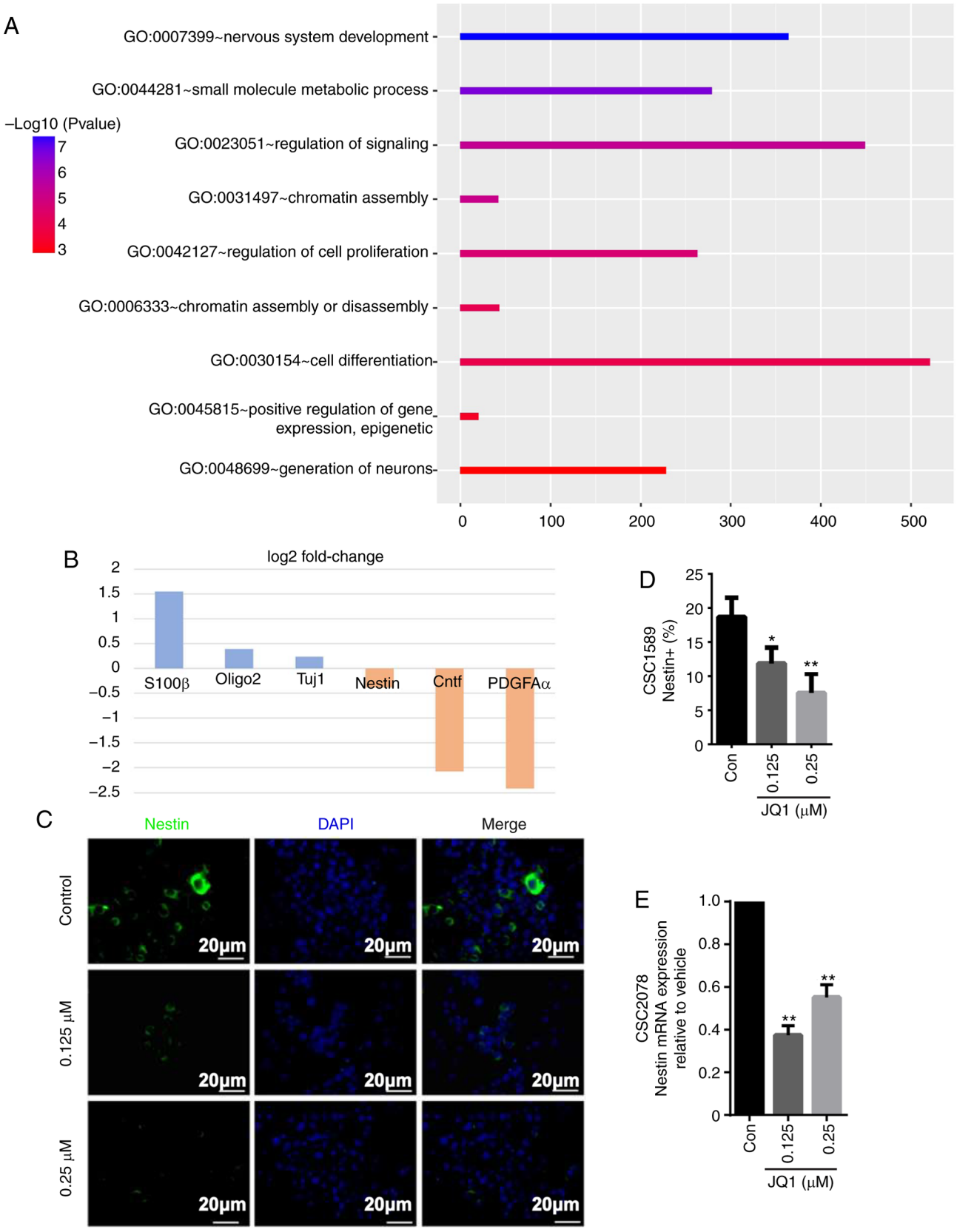


Figure S7. Primary data of CSC2078 apoptosis analysis. (A) JQ1 induced cell apoptosis in CSC2078 in control. (B) Apoptosis of CSC2078 treated with JQ1 (0.05 μM). (C) Cell apoptosis of CSC2078 treated by JQ1 (0.1 μM). (D) Cell apoptosis of CSC2078 treated by JQ1 (0.2 μM).

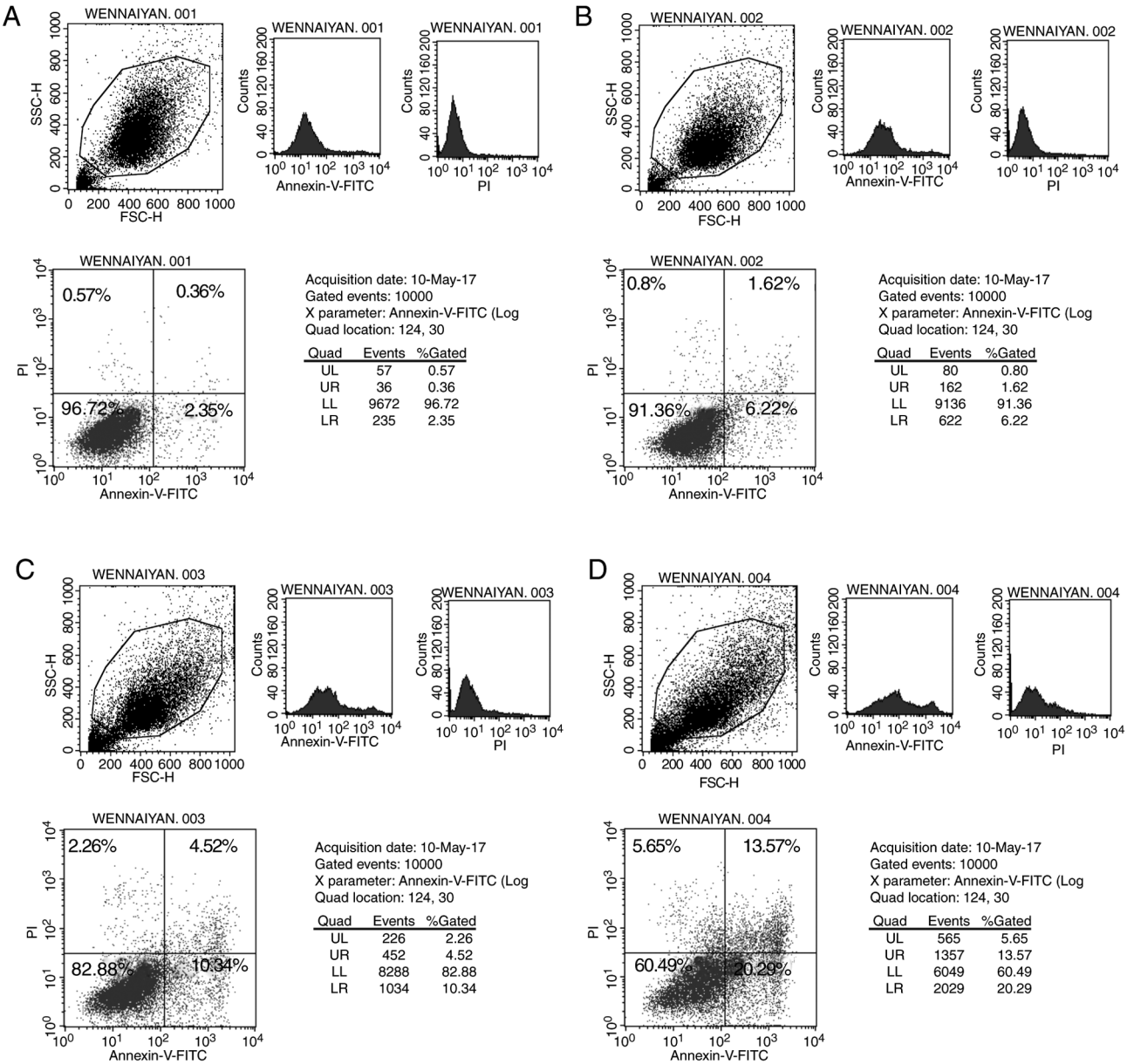


Figure S8. Primary data of TS543 and CSC2078 apoptosis. (A) JQ1 induced cell apoptosis in TS543 in control. (B) Cell apoptosis of TS543 treated with JQ1 (0.125 μM). (C) Cell apoptosis of TS543 treated with JQ1 (0.25 μM). (D) Cell apoptosis of TS543 treated with JQ1 (0.5 μM). (E) Cell apoptosis of CSC2078 transfected with si-NC. (F) Apoptosis of CSC2078 transfected with siBrd4-475. (G) Apoptosis of CSC2078 transfected with siBrd4-3820. Con, control; NC, negative control; siBrd4, small interfering RNA against bromodomain-containing protein 4.

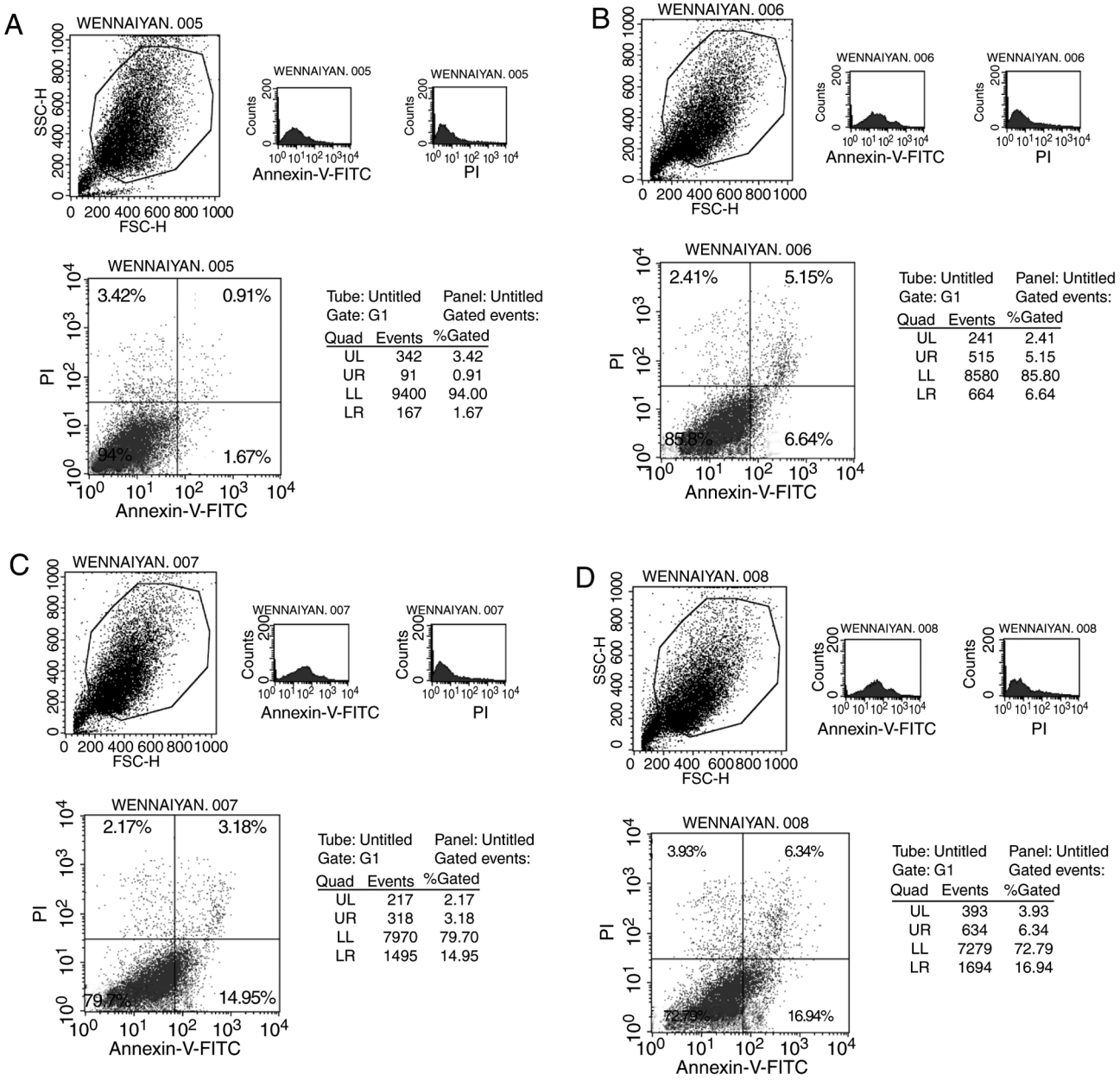


Figure S8. Continued.

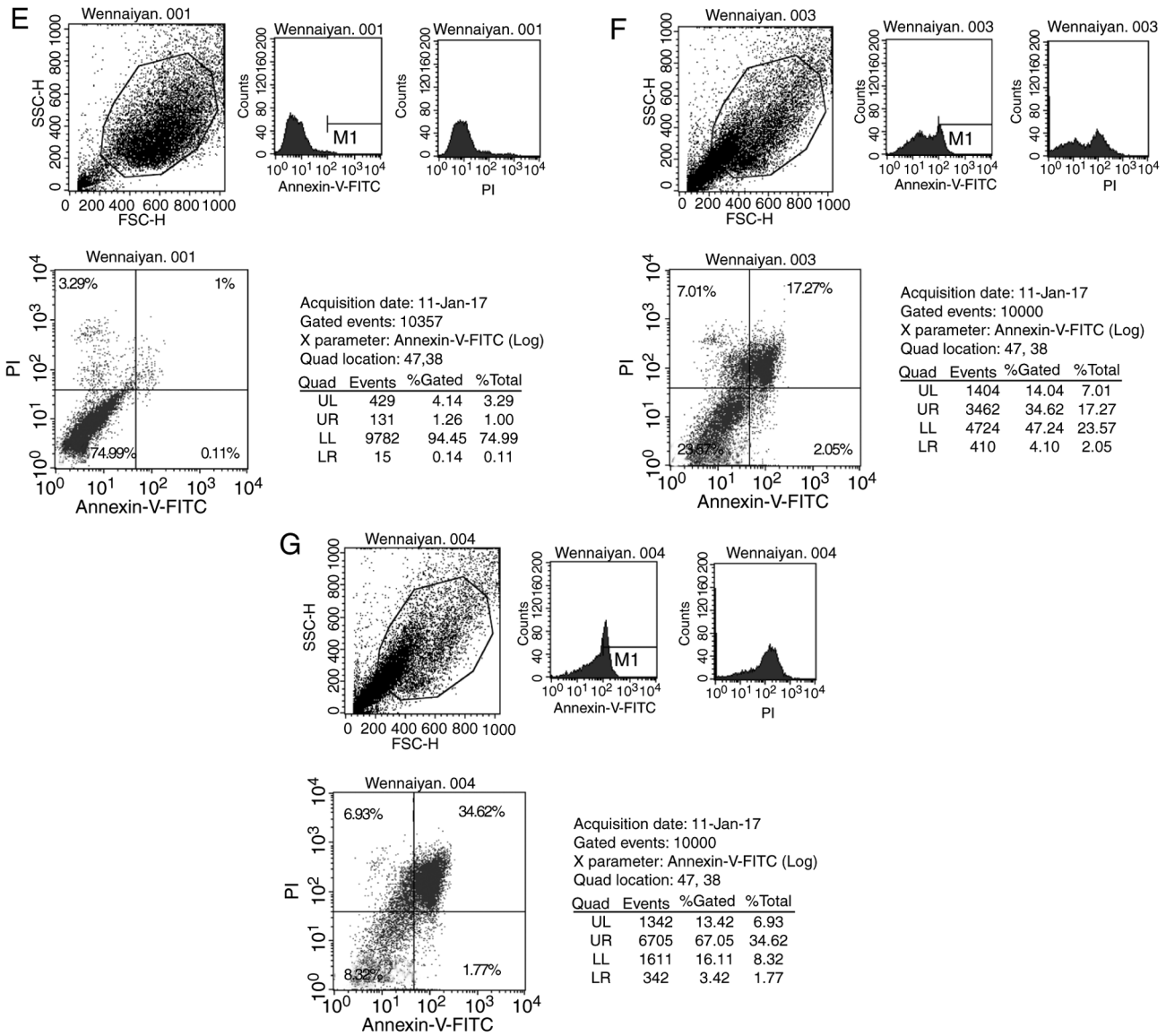
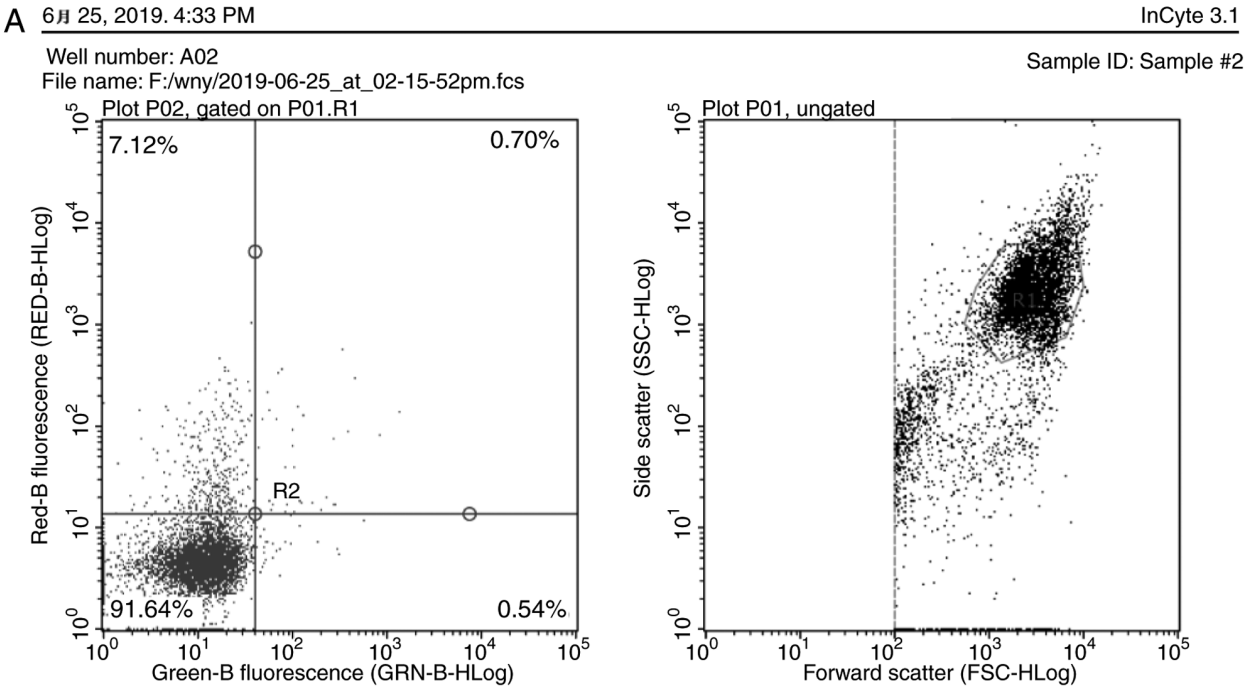


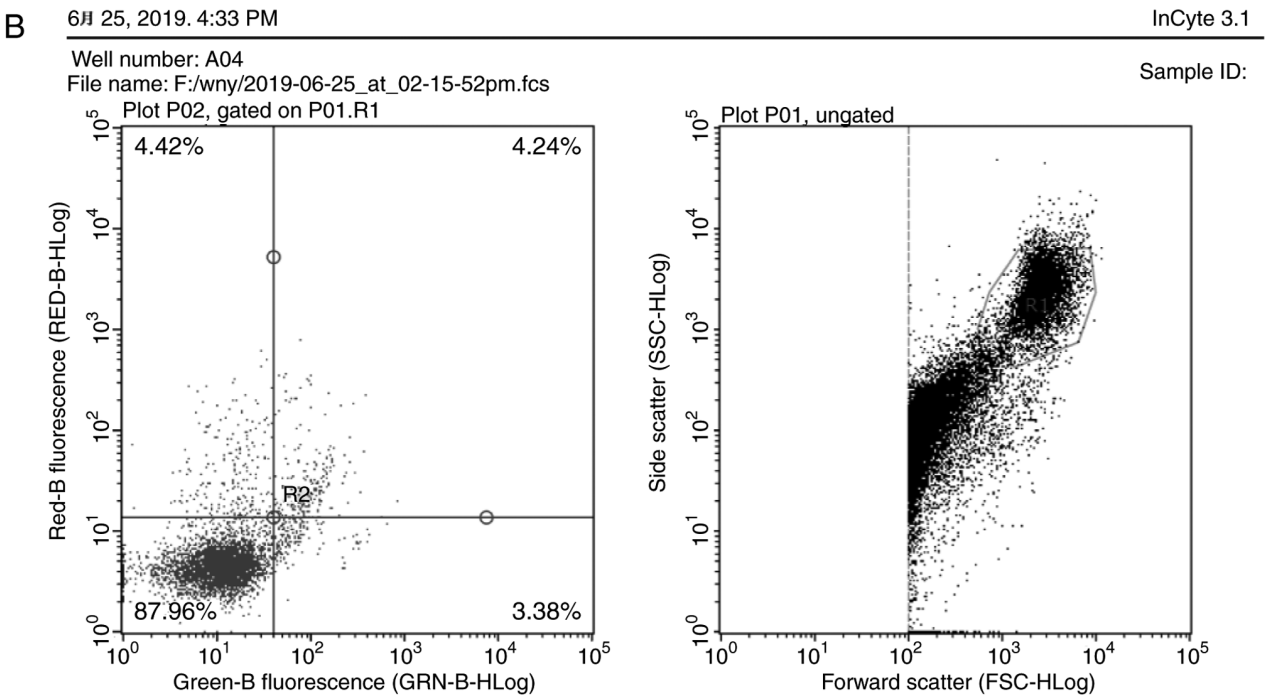
Figure S9. Primary data of CSC2078 apoptosis. (A) Apoptosis of CSC2078 cells in the control. (B) Apoptosis of CSC2078 treated with JQ1. (C) Cell apoptosis of CSC2078 treated by linifanib. (D) Apoptosis of CSC2078 cells treated with linifanib and JQ1.

A



Well	Sample ID	Date	R2.Percent.UL Percent for R2 gated by P01.R1 (%)	R2.Percent.UR Percent for R2 gated by P01.R1 (%)	R2.Percent.LL Percent for R2 gated by P01.R1 (%)	R2.Percent.LR Percent for R2 gated by P01.R1 (%)
A02	Sample #2	06.25.2019	7.12	0.70	91.64	0.54

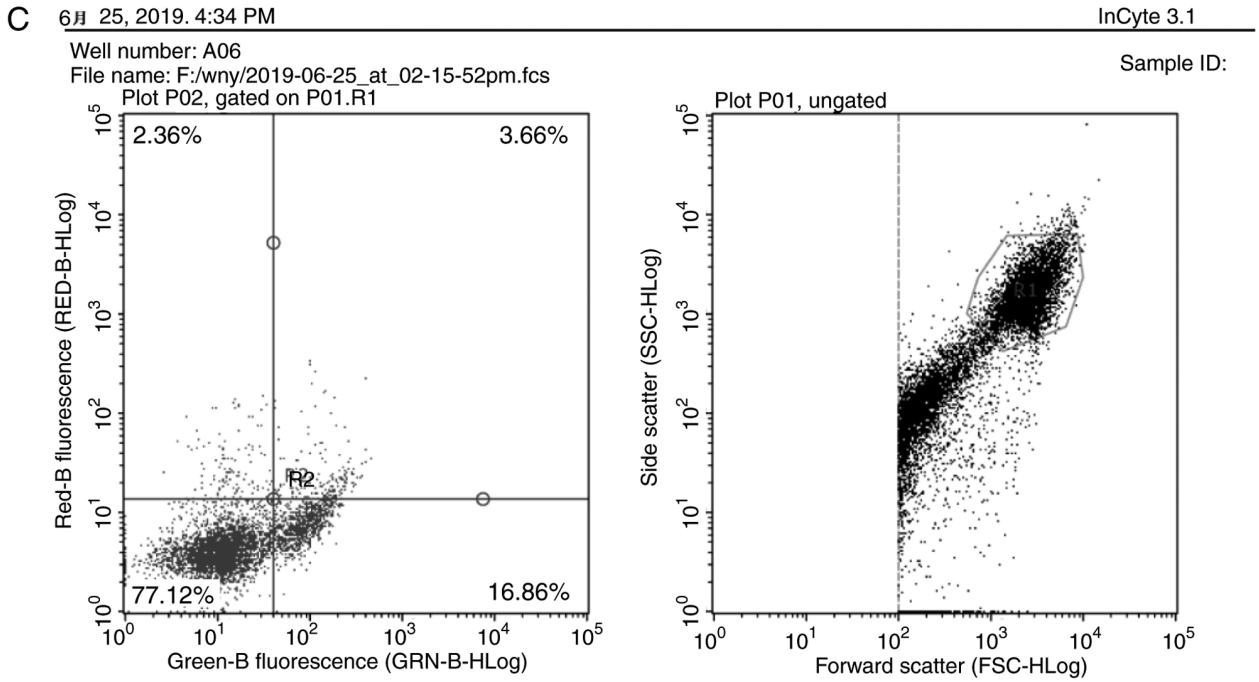
B



Well	Sample ID	Date	R2.Percent.UL Percent for R2 gated by P01.R1 (%)	R2.Percent.UR Percent for R2 gated by P01.R1 (%)	R2.Percent.LL Percent for R2 gated by P01.R1 (%)	R2.Percent.LR Percent for R2 gated by P01.R1 (%)
A04		06.25.2019	4.42	4.24	87.96	3.38

Figure S9. Continued.

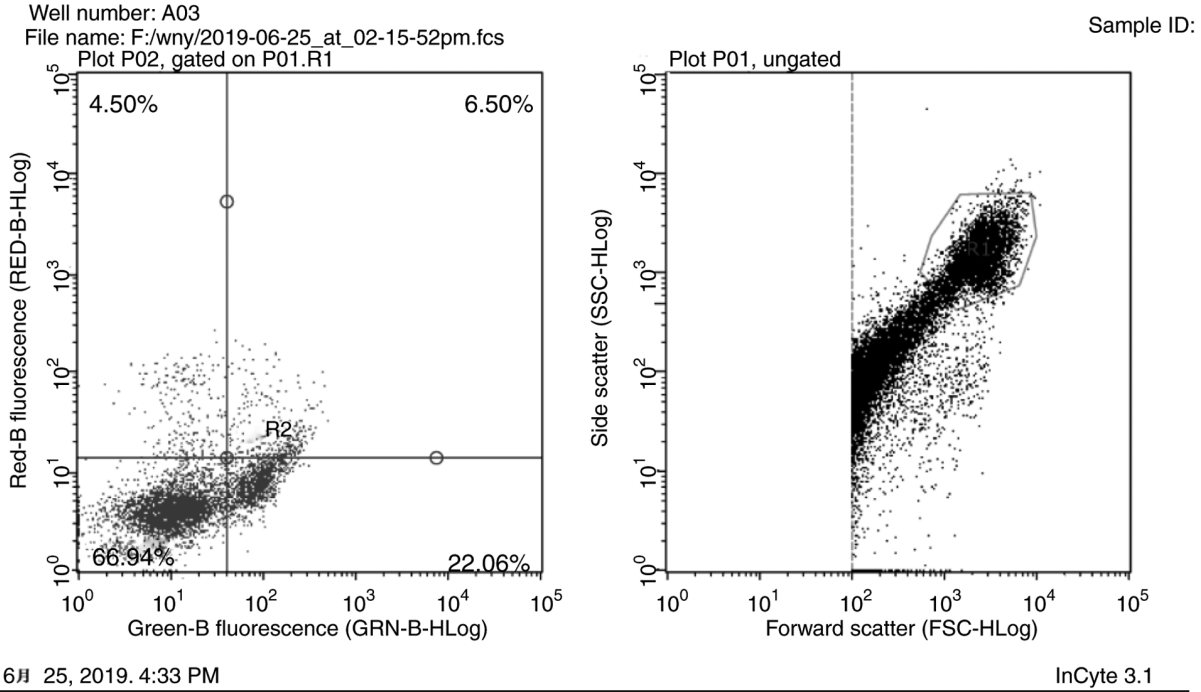
C



6月 25, 2019. 4:34 PM InCyte 3.1

Well	Sample ID	Date	R2.Percent.UL Percent for R2 gated by P01.R1 (%)	R2.Percent.UR Percent for R2 gated by P01.R1 (%)	R2.Percent.LL Percent for R2 gated by P01.R1 (%)	R2.Percent.LR Percent for R2 gated by P01.R1 (%)
A06		06.25.2019	2.36	3.66	77.12	16.86

D



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Well	Sample ID	Date	R2.Percent.UL Percent for R2 gated by P01.R1 (%)	R2.Percent.UR Percent for R2 gated by P01.R1 (%)	R2.Percent.LL Percent for R2 gated by P01.R1 (%)	R2.Percent.LR Percent for R2 gated by P01.R1 (%)
A03		06.25.2019	4.50	6.50	66.94	22.06

Table SI. siRNA sequences.

siRNA	Sense (5'-3')	Antisense (5'-3')
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
Brd4-mus-475	GCUCAAGACACUAUGGAAATT	UUUCCAUAGUGUCUUGAGCTT
Brd4-mus-1648	GCCUGAAGAGCCAGUUGUUTT	AACAACUGGCUCUUCAGGCTT
Brd4-mus-3820	GCCUGAGAUGAAGCCUGUATT	UACAGGCUUCAUCUCAGGCTT

siRNA, small interfering RNA.

Table SII. Primer sequences (*Mus musculus*).

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
c-Myc	ATGCCCCTCAACGTGAACTTC	GTCGCAGATGAAATAGGGCTG
Cyclin D1	GCGTACCCTGACACCAATCTC	ACTTGAAGTAAGATACGGAGGGC
P21	CGAGAACGGTGGAACTTTG	CCAGGGCTCAGGTAGACCTT
P27	TCAAACGTGAGAGTGTCTAACG	CCGGGCCGAAGAGATTTCTG
BIM	GACAGAACCGCAAGACAGGAG	GGACTTGGGGTTTGTGTTGAC
BAX	AGACAGGGGCCTTTTTGCTAC	AATTCGCCGGAGACACTCG
BAK	GCCCTGTACGTCTACCAGC	TGGCGATGTAATGATGCAGTATG
BCL-2	GAGAGCGTCAACAGGGAGATG	CCAGCCTCCGTTATCCTGGA
BCL-XL	ACATCCCAGCTTCACATAACCC	CCATCCCGAAAGAGTTCATTCAC

BAX, Bcl-2-associated X protein; Bak, Bcl2 antagonist/killer 1; BCL-XL, Bcl-extra large; Bim, Bcl2 like 11.

Table SIII. Primer sequences (*Homo sapiens*).

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
c-Myc	TCCCTCCACTCGGAAGGAC	CTGGTGCATTTTCGGTTGTTG
CyclinD1	GCTGCGAAGTGGAAACCATC	CCTCCTTCTGCACACATTTGAA
P21	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
P27	ATGAGCCGCAAACTGGGTC	AGAGCCGAACTCCACAATCTC
BIM	TAAGTTCTGAGTGTGACCGAGA	GCTCTGTCTGTAGGGAGGTAGG
BAX	CCCGAGAGGTCTTTTTCCGAG	CCAGCCCATGATGGTTCTGAT
BAK	ATGGTCACCTTACCTCTGCAA	TCATAGCGTCGGTTGATGTCG
BCL-2	GGTGGGGTCATGTGTGTGG	CGGTTCAGGTACTCAGTCATCC

BAX, Bcl-2-associated X protein; Bak, Bcl2 antagonist/killer 1; BCL-XL, Bcl-extra large; Bim, Bcl2 like 11.