

Figure S1. CYLD expression is significantly reduced by CYLD-specific siRNA transfection, whereas it is markedly increased by transfection with wild-type CYLD expression plasmid. U215MG cells were transfected with (A) control siRNA (siN) or CYLD-specific siRNA (siCYLD), and with (B) control plasmid (pcDNA3; Control) or wild-type CYLD expression plasmid (WT-CYLD). CYLD mRNA expression was assessed by reverse transcription-quantitative PCR, as follows. Total RNA extraction from U215MG cells was performed by phenol chloroform extraction method using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse-transcribed to cDNA by the PrimeScript RT reagent (Takara Bio Inc.). For quantification of mRNA, each PCR assay was performed using the LightCycler System with SYBR Premix DimerEraser (Takara Bio Inc). Relative expression levels were determined using the $2^{-\Delta\Delta Cq}$ method. Human 18S ribosomal RNA (rRNA) was used as an internal control. Human CYLD sense, 5'-TCAGGCTTATGGAGCCAAGAA-3' and antisense, 5'-ACTTCCCTTCGGTACTTTAAGGA-3'; human 18s rRNA sense, 5'-CGGCTACCACATCCAAGGAA-3' and antisense, 5'-GCTGGAATTACCGCGGCT-3'. Values are expressed as the means \pm SD of triplicate samples. * $P < 0.05$ via Student's t-test.

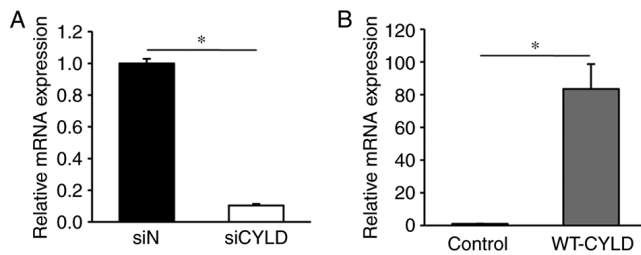


Figure S2. Temozolomide does not affect the proliferation, migration, and GBM stem-like cell formation in CYLD-silenced GBM cells. (A) Cells were treated with temozolomide, and the cell survival rate was assessed 72 h after treatment. The cell viability of the control group of siN and siCYLD was considered as 100%. Values are expressed as the mean \pm SD of triplicate samples. (B) Cells were transfected with siRNA, reseeded into the Transwell insert with 200 μ M temozolomide or DMSO (control) for 24 h, and migrating cells were stained with crystal violet. Scale bars, 200 μ m. (C) A limiting dilution assay was performed. Cells were transfected and reseeded in a cell suspension using a serial dilution method with 10 μ M temozolomide or DMSO (control). Cells were reseeded in 4 wells in each group in a 96-well plate. The percentage of wells in which a cell mass \geq 50 μ m was formed was calculated. GBM, glioblastoma; CYLD, cylindromatosis; siRNA or si, small interfering RNA; DMSO, dimethyl sulfoxide; n.s., not significant.

