

Figure S1. Anti-proliferative effects of MLN8237 on pGBM cell lines. Paired monolayer (*Mono*) and neurosphere (*NS*) of IC-4687GBM, IC-3752GBM and IC-R0315GBM were seeded at 2,000 cells/well and exposed to MLN8237 (1 - 4,000 nM) and anti-proliferative effect was assessed by staining with 250 μ g/mL of MTT for 4 hrs at day 11. Viable cells were stained with dark colored intracellular crystal. (Bar=100 μ m)

In vitro treatment with MLN8327 (Day 11)



Figure S2. FCM analysis of cell cycle distribution in vitro and in vivo.

- A. In vitro analysis was performed in paired monolayer (*Mono*) and neurosphere (*NS*) cells of IC-4687GBM, IC-3752GBM and IC-R0315GBM. Both dose-responses (on day 7) (*left panel*) and time-course change (at 62.5 nM) (*right panel*) were examined and presented as percentages of G₀/G₁, S and G₂/M phase in alive cells. [†]*P*<0.05, ^{*}*P*<0.01 compared to the untreated (0 nM) cells.
- B. In vivo changes of cell cycle distribution were analyzed in IC-4687GBM cells treated by MLN8237 (30 mg/kg/day for 12 days by gavage). In addition to the recurrent tumors (*Recurrent*) harvested when the mice became moribund in the survival group, a separate group of tumors harvested 1 hr post the last treatment of MLN8237 (30 mg/kg/day for 12 days by gavage) (*End of Treatment* samples) were also included. DNA/RNA were stained with Hoechst 33342/Pyronin Y followed by mouse antibody cocktail staining (to gate out mouse cells) and analyzed by FCM. Representative DNA/RNA profiles (*upper panel*) and percentage of G₀, G₁, S and G₂/M phase in cell cycle (*lower panel*) were presented.



