Supplemental Figure 1. Aurora-A inhibitor effects on proliferation and metabolic activity in GB neurosphere cells, and apoptosis in normal primary astrocytes. A. GB9, GB30 and GB169 neurospheres were grown 7 days in the presence of the indicated concentrations of the Aurora-A inhibitor TC-A2317 and counted. **B.** Primary human astrocytes or GB169 cells were treated with 200 nM alisertib for 5 days and apoptosis was measured by Annexin V staining. Data are means ± standard deviations of triplicate wells. Two independent experiments provided similar results. **C.** GB9 cells were treated with 200 nM alisertib or DMSO for 3 to 12 days, recovered for 3 days and counted as described for Fig. 1D. **D.** GB169 neurosphere cells were treated for 5 days with the indicated concentrations of alisertib and washed with PBS. Each culture was then spit into 2 new cultures: one treated with alisertib for an additional 3 days (the continuously-treated group) and one cultured in drug-free media for additional 3 days (the recovered group). Cellular metabolic activity was then assessed by MTT assay. This pattern was repeated in separate cultures representing 2 and 3 weeks of treatment. Note that unlike in Fig. 1D where cells were continuously exposed to alisertib for up to 12 days before subjecting them to 3 days of continued drug treatment or recovery, here alisertib-treated cells were switched to drug-free media for 2 days after each 5 days of alisertib exposure prior to starting the next week of drug exposure. For instance, for week 3 cells were treated with alisertib 5 days, then cultured 2 days without drug, then exposed to drug for 5 more days, then 2 days without drug, then drug for an additional 5 days and then split into continuously-treated and recovered cultures for 3 days followed by MTT assay. This *in vitro* treatment schedule was originally designed to more closely mimic the *in vivo* dosing, Data are expressed as the difference between recovered cells and continuously treated cells as an indication of recovery from cytotoxicity. After 3 weeks, little difference remained between the metabolic activity of cells treated with 3 cycles of this alisertib regimen and recovered cells consistent with no cellular proliferation occurring in recovered cultures.

Supplemental Figure 2. Morphologic changes of GB neurosphere cells treated with different Aurora-A inhibitors. Glioblastoma neurosphere cells were grown in the presence of DMSO alone (0.008%) or Aurora-A inhibitors (200 nM alisertib or 2 μM TC-A2317) for 2 weeks. Photomicrographs were then taken, all at equal magnification.

Supplemental Figure 3. Additional effects of Aurora-A inhibition on senescence, apoptosis and stem markers in GB neurospheres. A. Glioblastoma neurosphere cells were treated with 0.008% DMSO vehicle alone (C) or 1 μM TC-A2317 (T) for 12 days. Lysates were immunoblotted for markers of apoptosis, stemness, differentiation and senescence. **B.** GB9 neurosphere cells were treated with DMSO vehicle or 200 nM alisertib for 5 days and cytospin preparations were immunostained for β-catenin.