Signal transducer and activator of transcription 3 (STAT3) regulates diverse cellular processes including cell growth, differentiation, and apoptosis, and is frequently activated during tumorigenesis. Recently, putative glioblastoma stem cells (GBM-SC) have been isolated and characterized. These cells can self-renew indefinitely in culture, are highly tumorigenic, and retain the ability to differentiate in culture. We have found that treatment of GBM-SC with two chemically distinct small molecule inhibitors of STAT3 DNA-binding inhibits cell proliferation and the formation of new neurospheres from single cells. Genetic knockdown of STAT3 using an shSTAT3-containing lentivirus also inhibits GBM-SC proliferation and neurosphere formation, confirming that these effects are specific to STAT3. While STAT3 inhibition can induce apoptosis in serum-derived GBM cell lines, this effect was not observed in GBM-SC grown in stem cell media. Markers of neural stem cell multipotency also decrease upon STAT3 inhibition, suggesting that STAT3 is required for maintenance of the stem-like characteristics of these cells. Strikingly, even a transient inhibition of STAT3 leads to irreversible growth arrest and inhibition of neurosphere formation. These data suggest that STAT3 regulates the growth and self-renewal of GBM-SC and is thus a potential target for cancer stem cell-directed therapy of glioblastoma multiforme.