



**Figure S1. BUB1B knockdown prevents longer-term expansion BTICs and SSEA1+ BTIC subpopulations.**

(A) ShRNA competition experiments during BTIC and NSC *in vitro* expansion. Transduced NSC-CB660 and BTIC-G166 cells were mixed in a 9:1 ratio with non-infected cells, and out grown for indicated number of days ( $n=3$ ). ShKIF11 served as a positive control for growth inhibition.

(B) ShRNA competition experiments during NHA and NHA-Ras *in vitro* expansion. Transduced NHA and Ras transformed cells were mixed with non-infected cells, and out grown for indicated number of days ( $n=3$ ). ShKIF11 served as a positive control for growth inhibition.

(C) Assessment of SSEA1+ subpopulations in G166 and 0131 BTICs by FACS. Approximately 5% of G166 and 14% of 0131 cells are SSEA1+ in asynchronously monolayer cultures.

(D) ShRNA competition assay monitoring SSEA1+ BTIC subpopulations. Cells were transduced with shControl or shBUB1B GFP expression vectors, mixed with non-transduced cells at approximate 9:1 ratio at time zero, and expanded *in vitro* in monolayer conditions. Populations were flow analyzed over the course of 22 days during *in vitro* expansion. Similar results to shBUB1B were observed with shKIF11 (data not shown). Linear regression analysis demonstrated significant differences between changes in shBUB1B representation (G166,  $p$ -value=0.002333; 0131,  $p$ -value=0.01099).