



### Supplementary Data for Figure 5

(A) Sox1::GFP cells were maintained in N2B27 and treated with factors as indicated. GFP expression was assessed by flow cytometry and are normalized to 5 days in N2B27. Note ACT and BMP inhibit GFP expression and the longer the exposure to these factors the stronger their inhibitory effects. SB43, XAV939, DMH1 and surprisingly Chi either do not effect or mildly inhibit GFP expression and this inhibition is reduced the longer the exposure to the factors. (B) T::GFP cells were differentiated for 3 days in N2B27 followed by one day in AC and treated with factors as indicated. Results are normalized to 2 days in N2B27 followed by 2 days in AC. Note both BMP and to a lesser extent Chi are able to enhance GFP expression compared to N2B27 over the same period. (C) Sox1::GFP cells were cultured in N2B27, SB43, XAV939 or SB43 + XAV939 for 0-2 days and as indicated days 2-5. GFP expression was analysed by flow cytometry, normalized to 5 days in N2B27 and is presented in the form of a heat map.