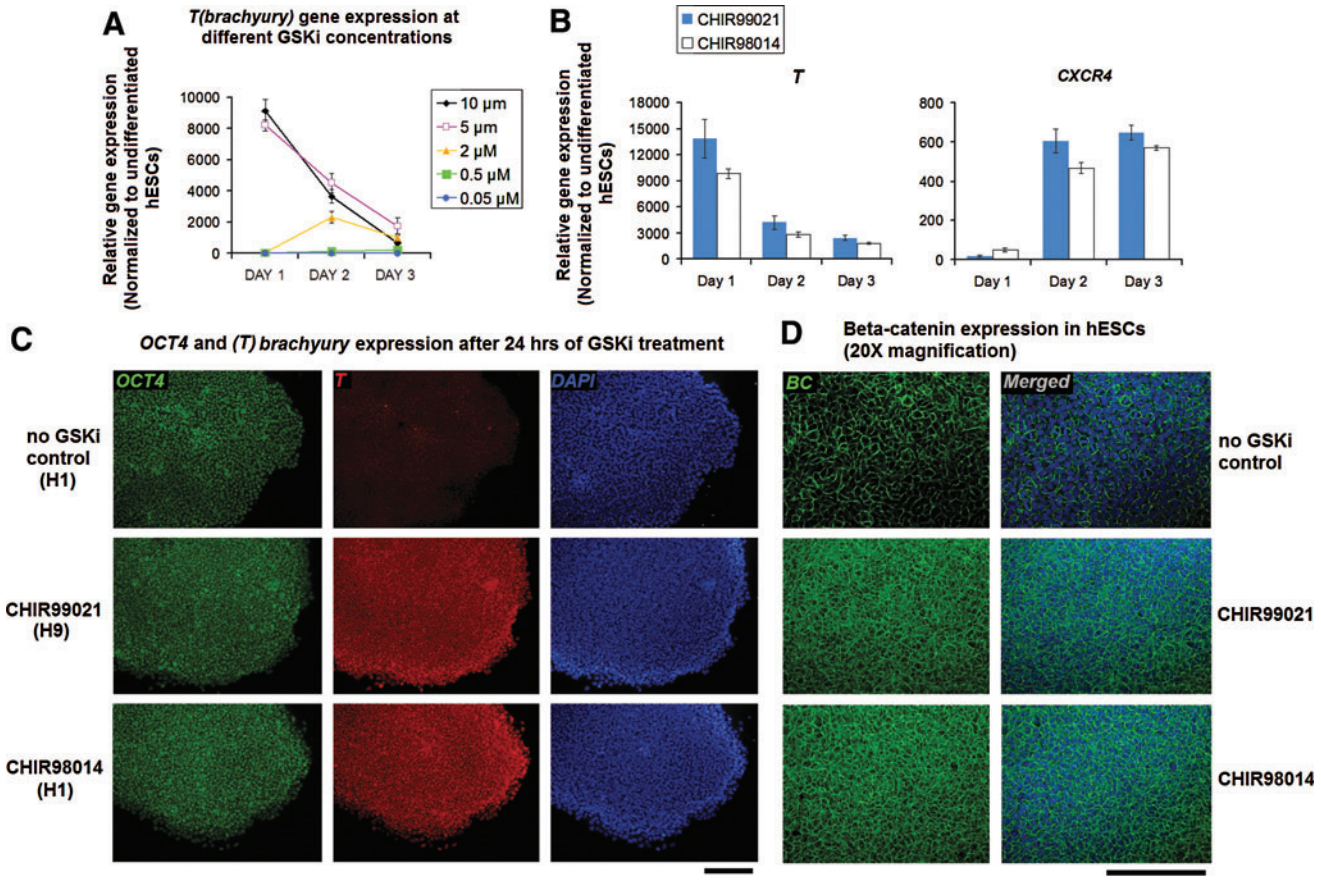

SUPPLEMENTARY FIG. S2. *T (Brachyury)* and *β-catenin* expression in hESCs after GSKi treatment. **(A)** H1-hESCs were differentiated in different concentrations of GSKi (CHIR99021) to assess its effect on the PS/early mesoderm marker, *T*. qRT-PCR analysis revealed up-regulation at day 1 using concentrations of 5 μM and higher. While *T* begins to up-regulate only at day 2 at 2 μM, lower concentrations resulted in little or no up-regulation. **(B)** Comparison of transcription profiles for *T* and *CXCR4* in hESCs treated using CHIR99021 at 5 μM and a different GSKi, CHIR98014 at 2 μM. Although gene expression levels were different, the transcription profiles for both genes were approximately similar for both GSKis with *T* up-regulating and peaking at day 1, while *CXCR4* begins up-regulating at day 2. **(C)** Immunofluorescence analysis of *OCT4* and *brachyury* in hESCs after 24 h of GSKi treatment. *Top row*: H1-hESCs treated with basal media alone without any GSKi, *middle row*: H9 hESCs treated with CHIR99021, *bottom row*: H1-hESCs treated with 2 μM CHIR98014. **(D)** 20× magnification of *β-catenin* expression in H1-hESCs after 24 h of GSKi treatment. *Top row*: hESCs treated with basal media alone without any GSKi, *middle row*: hESCs treated with CHIR99021, *bottom row*: hESCs treated with CHIR98014. All scale bars represent 200 μm. GSK, glycogen synthase kinase; GSKi, GSK inhibitor; PS, primitive streak; qRT-PCR, quantitative real-time PCR.