**Supplementary Figure 2**

(a) Control (2x10^5 cells/ml) Sox1-GFP fluorescence

(b) Dkk1+LeftyA Sox1-GFP fluorescence

(c) Percentage of Sox1-GFP positive cells

(d) SFEB+5%FCS Sox1-GFP fluorescence

(e) SFEB+5%FCS +Dkk1+LeftyA Sox1-GFP fluorescence

(f) Percentage of Sox1-GFP positive cells

(g) Percentage of Sox1-GFP positive cells

(h) Phosphorylated Smad1/5/8 and Total Smad1/5/8

Counts

Counts

Counts

Counts

Counts

Counts

Counts

Counts

Counts
Supplementary Figure 2  Analysis of the effects of Wnt, Nodal and BMP antagonists. (a and b) Flow cytometry profiles of Sox1-GFP fluorescence in SFEB culture in high-cell density conditions with or without Dkk1 and LeftyA proteins. (c) Percentages of Sox1-GFP* cells in the SFEB culture in the high cell density conditions with Dkk1 and LeftyA treatments (day 5). (d and e) Flow cytometry profiles of Sox1-GFP fluorescence of ES cells in suspension culture with a 5% FCS-containing medium. Effects of Dkk1 and LeftyA were analyzed on day 5. (f) Percentages of Sox1-GFP* cells in floating ES cell aggregates cultured in the 5% FCS-containing medium with or without Dkk1 and LeftyA. (g) Treatment with 200 ng/ml BMPRIA-Fc or 500 ng/ml Noggin protein on neural conversion in SFEB. (h) Western blot analysis of amount of phosphorylated Smad1/5/8 in EB5 cells. BMP4-induced increase (0.5 nM, 30 mins) was suppressed by the addition of the BMP antagonists.