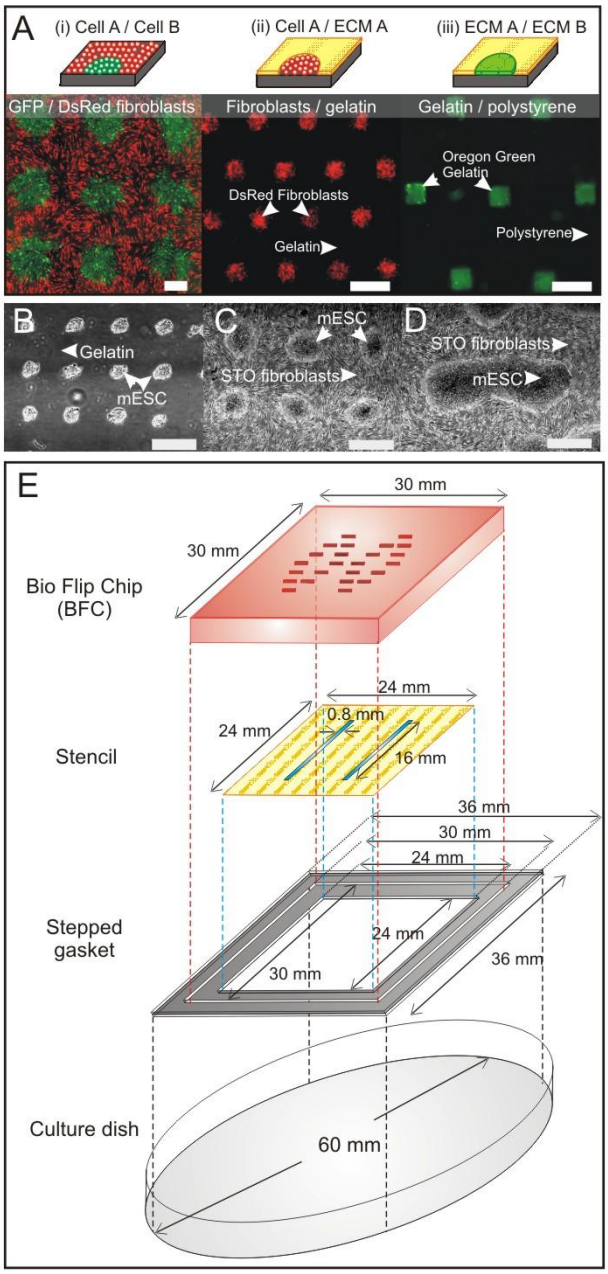
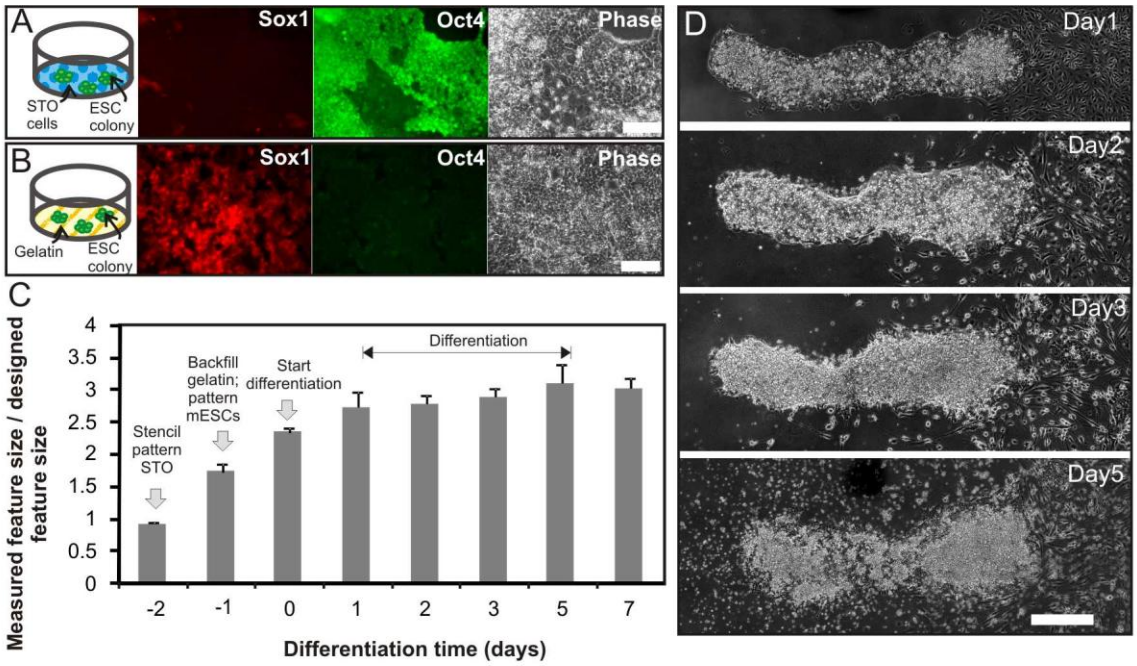
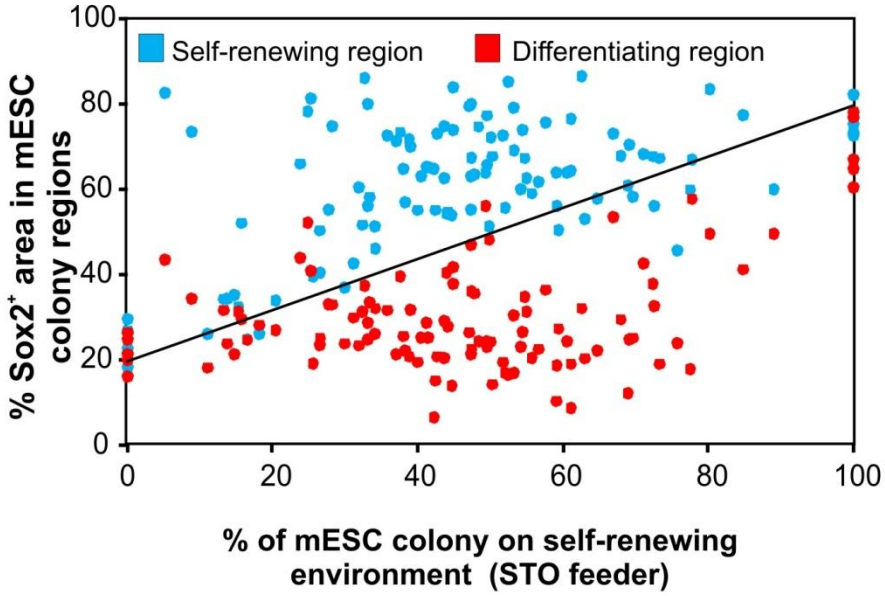
**Electronic Supplementary Data**




**SI Fig. 1** Characterization of Differential Environmental Spatial Patterning (δESP). **(A)** Images of dual microenvironments patterned in first step using a stencil: (i) two different cell types, (ii) one cell type and one ECM, or (iii) two different ECMs. **(B-D)** Images of cell colonies patterned in the second step via BFC: square and rectangular mouse embryonic stem cell (mESC) colonies of varying sizes. **(E)** Schematic showing the dimensions and assembly alignments of δESP. Scale bars = 500 μm.

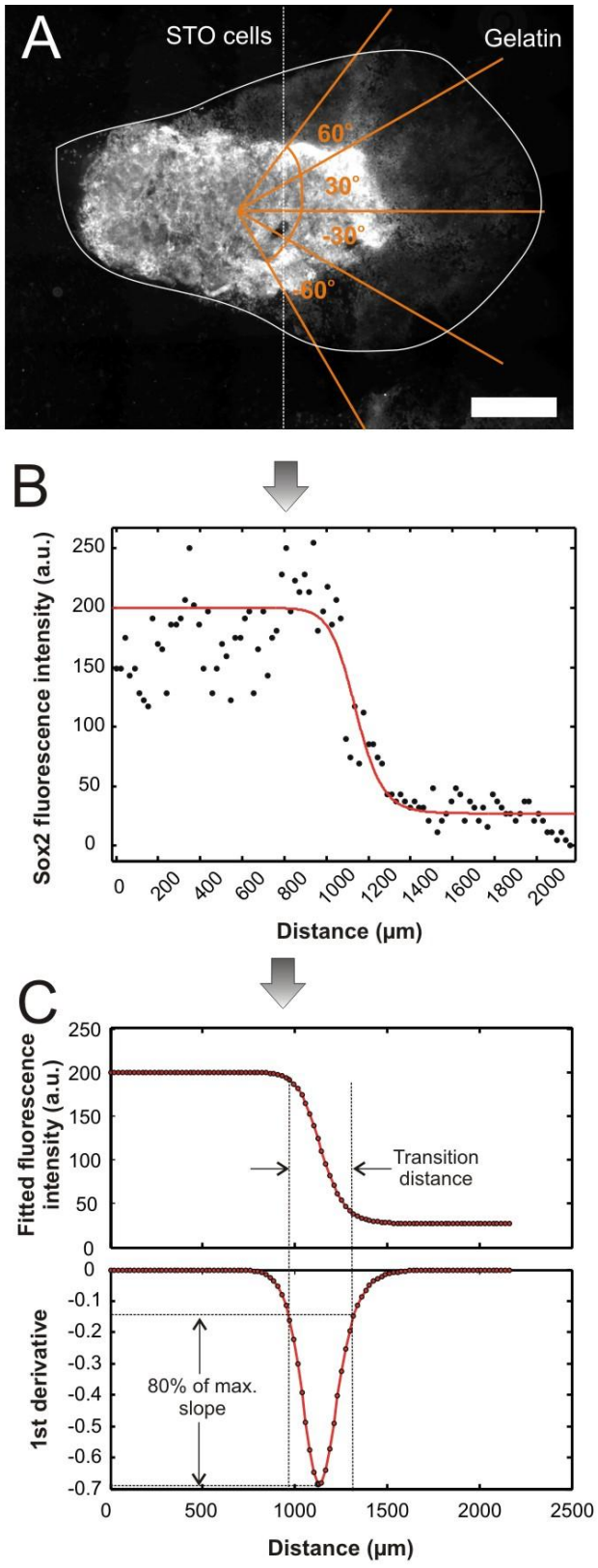



**SI Fig. 2** Organization of dual self-renewing and neuronal differentiating microenvironments around mouse embryonic stem cell (mESC) colonies. **(A-B)** Self-renewing ($Oct4^+$) and differentiating ($Sox1^+$) microenvironments were maintained by **(A)** STO fibroblasts and **(B)** gelatin respectively in serum free medium (N2B27) for 7 days. Scale bars = 100 μm. **(C)** Measurement of the feature sizes of stencil-patterned STO fibroblasts in N2B27 medium. STO fibroblasts were patterned as 1000 x 1000 μm squares, treated with 10 μg/ml mitomycin for 3 hours and maintained in N2B27 for 9 days. The stenciled STO features were imaged with an inverted microscope (Nikon) and measured with image analysis software (Nikon Elements AR 3.00). Data are average ± s.e.m of 6 features. The corresponding time points in the reciprocal self-renewal/neuronal differentiating δESP experiment is indicated. **(D)** Phase images of a mESC colony patterned on STO-gelatin interface over different time points, illustrating that the extent of contact between STO and mESCs stabilizes on Day 2 of differentiation. Scale bar = 500 μm.

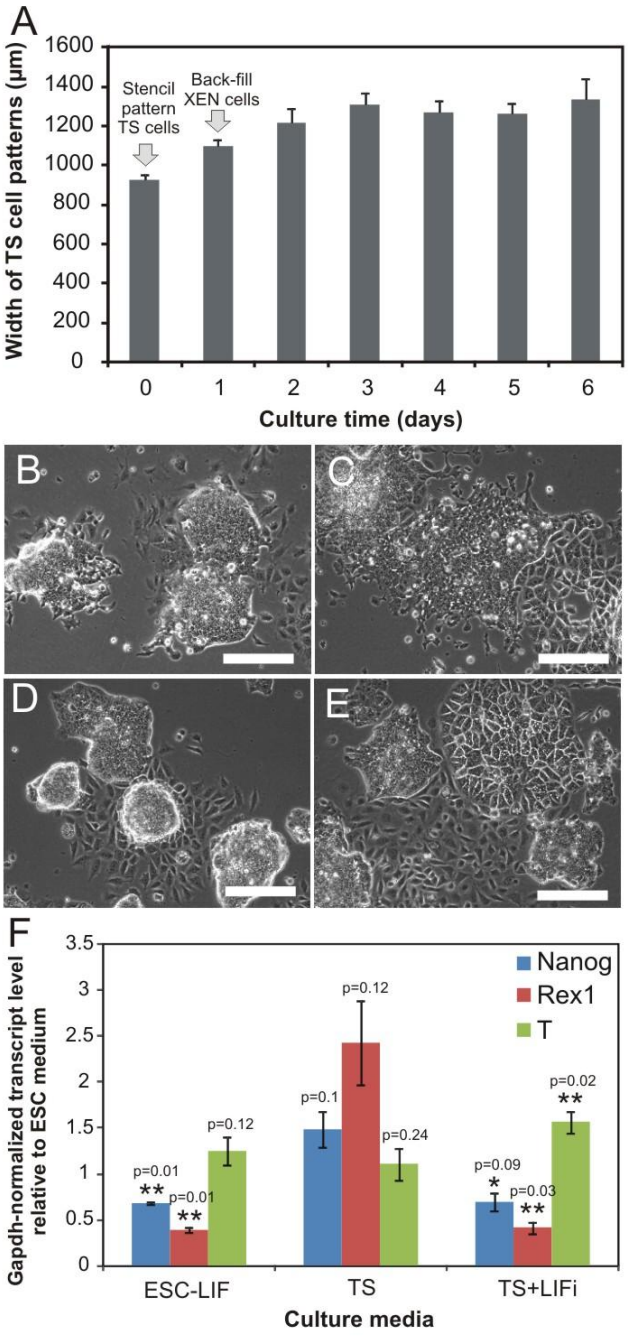



**SI Fig. 3** Sox2 expression in self-renewing (blue) and differentiating (red) regions of mESC colonies, which were patterned on varying ratios of dual microenvironments. To examine the amount of asymmetry in the colonies in the limit that the colony was entirely on gelatin ($x$=0) or on STO fibroblasts ($x$=100), we divided those colonies equally into two regions that were arbitrarily designated as self-renewing and differentiating regions and processed them similarly as the other colonies. Black line indicates average Sox2 expression of the entire colony. The region of the mESC colony that was in a self-renewing environment (on STO cells) consistently expressed higher percentage of Sox2-positive cells than the juxtaposed differentiating region (on gelatin), regardless of the ratio of STO cells to gelatin being presented. In colonies that were entirely on gelatin or STO, the designated self-renewing (blue) and differentiating (red) halves of the colonies expressed similar levels of Sox2 expression, which is indicative that there was no asymmetry in Sox2 expression.

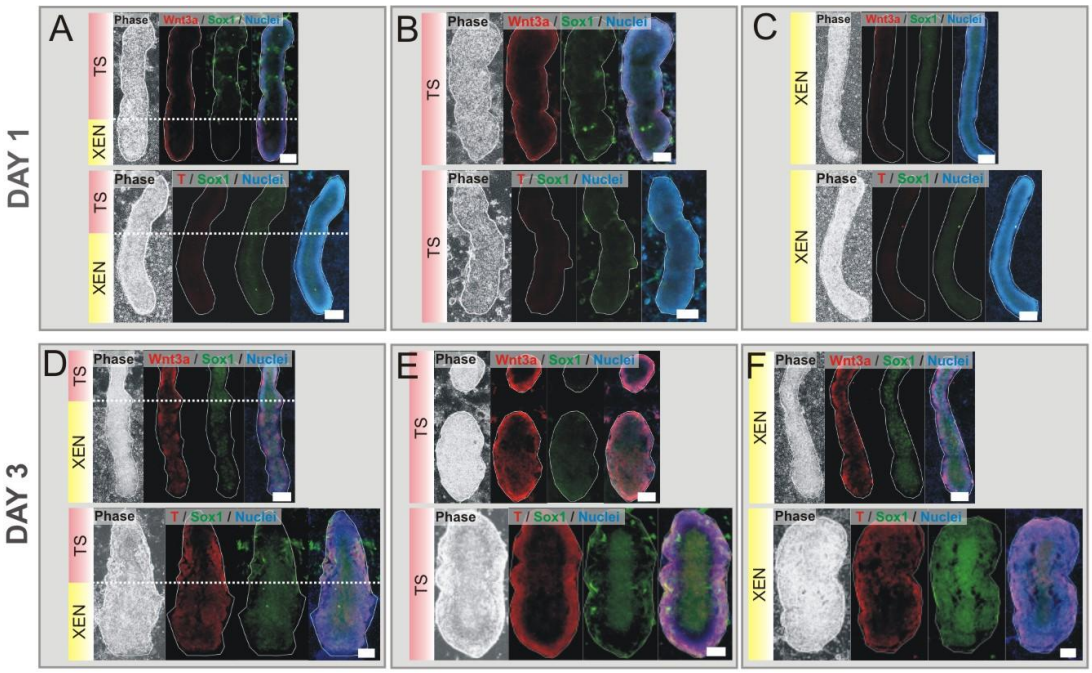
Electronic Supplementary Material (ESI) for Integrative Biology



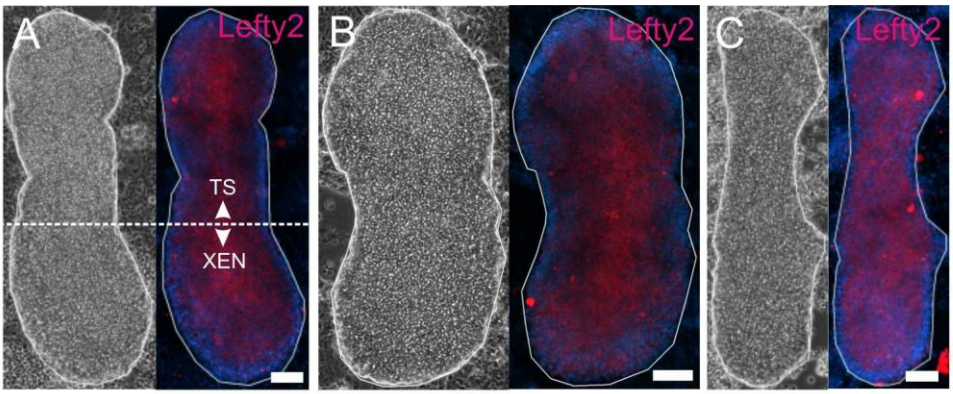

**SI Fig. 4** Quantitative measurements of transition distance over which phenotypic change occurs in a mESC colony being presented with dual self-renewing (on STO cells) and differentiating (on gelatin) microenvironments. **(A)** Profiles of Sox2 expression are obtained in multiple directions. Scale bar = 500 μm. **(B)** The data of a representative intensity profile (0° line in (A)) are then fitted to a 4-parameter logistic function. **(C)** The 1st derivative (*i.e.,* slope) of the fitted profile is then calculated and the distance spanning 80% of the maximum slope is taken to be the transition distance.



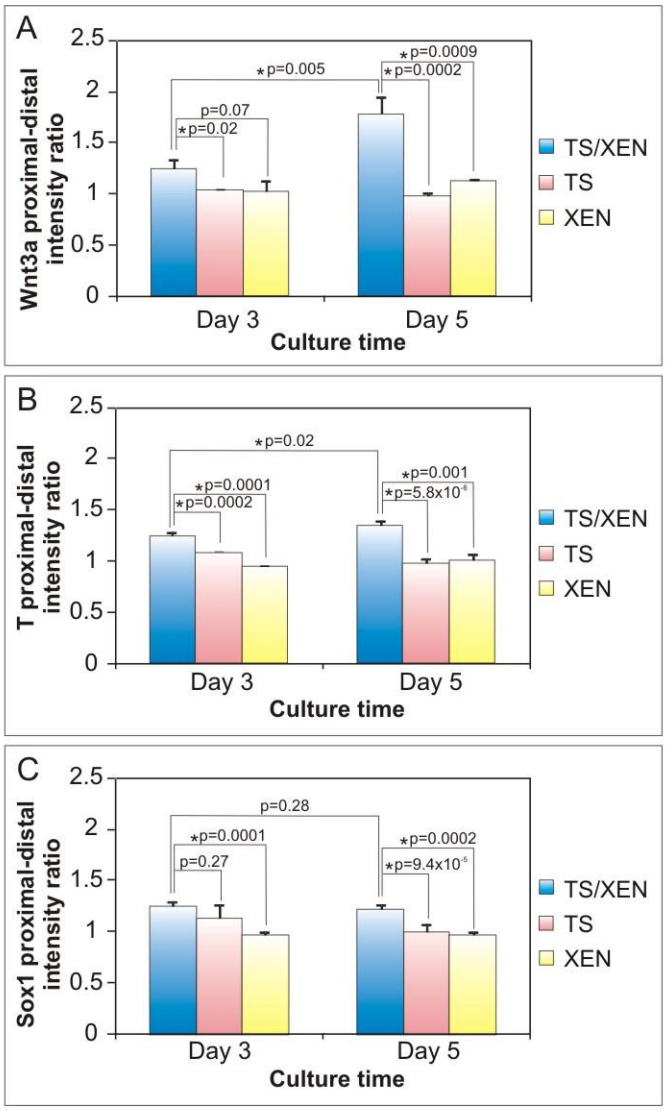

**SI Fig. 5** Establishment of an *in vitro* PD embryonic patterning with δESP. **(A)** Measurement of the feature sizes of stencil-patterned TS/XEN cells. TS cells were patterned as 800 µm (width) x 16 mm (length) strips and the remaining substrate was back-filled with XEN cells after 24 hours. The TS/XEN co-culture was maintained in TS cell culture medium for 5 days. The width of the stencil-patterned TS cell strips were measured daily by phase imaging and image analysis (Nikon Elements AR 3.00) as an indicator of the stability of the TS/XEN cel interface. Data are average ± s.e.m of 6 features. **(B-F)** Characterization of TS/XEN/mESC co-culture medium (TS medium with LIF inhibitor) to allow for mESCs differentiation and maintenance of TS and XEN cells. 1 µg/ml of recombinant mouse LIF (mLIF) antibody was used to block LIF present in TS cell culture medium (owing to 70% mouse embryonic fibroblast (MEF) conditioned medium in the formulation) to allow for mESC differentiation. D3 mESCs after 3 days culture in (B) normal ESC medium, (C) ESC medium without LIF (ESC-LIF), (D) complete TS cell culture medium (TS) and (E) complete TS cell culture medium with 1 µg/ml mLIF antibody (TS+LIFi). Scalebars = 200 µm (F) Expression of self-renewal (Nanog, Rex1) and differentiation (Brachyury, T) markers in mESCs after 5 days culture in different media. Data are average ± s.e.m of 2 biological replicates. Asterisks indicate statistical significance compared to ESC medium; p-value for each condition is indicated at top of the bars (Student's t-test, *$p<0.1$ **$p<0.05$).



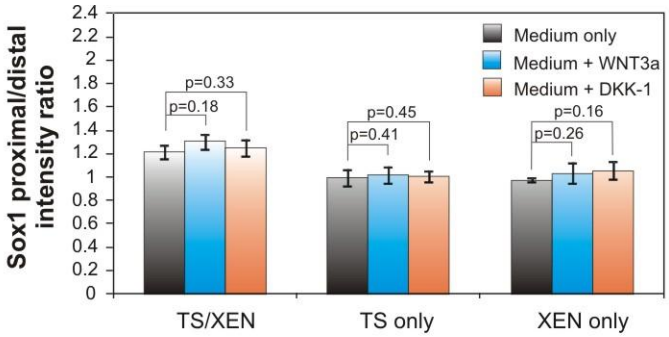

**SI Fig. 6** Expression of Wnt 3a, Brachyury (T) and Sox1 in mESC colonies at **(A-C)** 1 day, and **(D-F)** 3 days post-patterning with δESP. mESC colonies were patterned on (A, D) both TS and XEN cells in designated proximal-distal orientation, (B, E) only TS cells and (C, F) only XEN cells. Immunofluorescence for Wnt3a and T are shown in red while GFP-Sox1is in green. Scale bars = 200 μm




**SI Fig. 7** Expression of Lefty2 (in red) in mESC colony patterned on **(A)** TS-XEN cells interface. (**B**) TS cells alone and (**C**) XEN cells alone. Cell nuclei are indicated in blue and corresponding phase images are shown in the left panels. Scale bars = 100 μm.




**SI Fig. 8** Quantitative temporal development of **(A)** Wnt3a, **(B)** Brachyury (T) and **(C)** Sox1 proximal-distal (PD) asymmetries in mESC colonies patterned on TS-XEN cells interface or on TS or XEN cells alone. PD asymmetry is indicated by the ratio of fluorescent intensities of the respective markers in the proximal (on TS cells) and distal (on XEN cells) colony regions. Data are average of >10 colonies (on TS/XEN cells) or >3 colonies (on TS or XEN cells alone) from 2 independent experiments ± s.e.m. Pariwise comparisons (t-test) are indicated by connecting lines with the respective p-values.*indicates statistical significance ($p<0.05$).




**SI Fig. 9** Assessment of Sox1 proximal-distal (PD) asymmetry in mESC colonies after 5 days culture in co-culture medium alone or co-culture medium supplemented with 50 ng/ml WNT3a or 200 ng/ml DKK-1 to disrupt Wnt-signaling. Colonies were presented with both TS/XEN cells in a PD orientation and with either TS or XEN cells alone. PD asymmetry is indicated by the ratio of fluorescent intensities of Sox1 in the proximal (on TS cells) and distal (on XEN cells) colony regions. Data are average of >10 colonies (on TS/XEN cells) or >3 colonies (on TS or XEN cells alone) from 2 independent experiments ± s.e.m. Pairwise comparisons (t-test) are indicated by connecting lines with the respective p-values.