

## SUPPLEMENTARY INFORMATION

### **Biophysical phenotypes and determinants of anterior vs. posterior primitive streak cells derived from human pluripotent stem cells**

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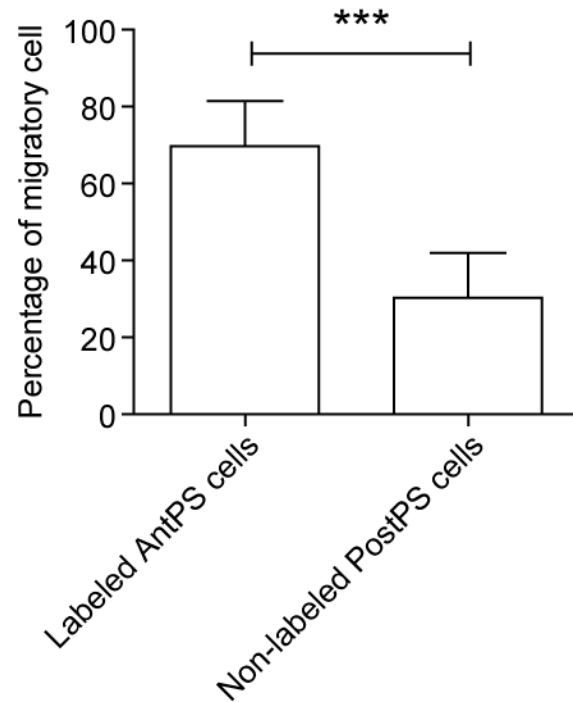
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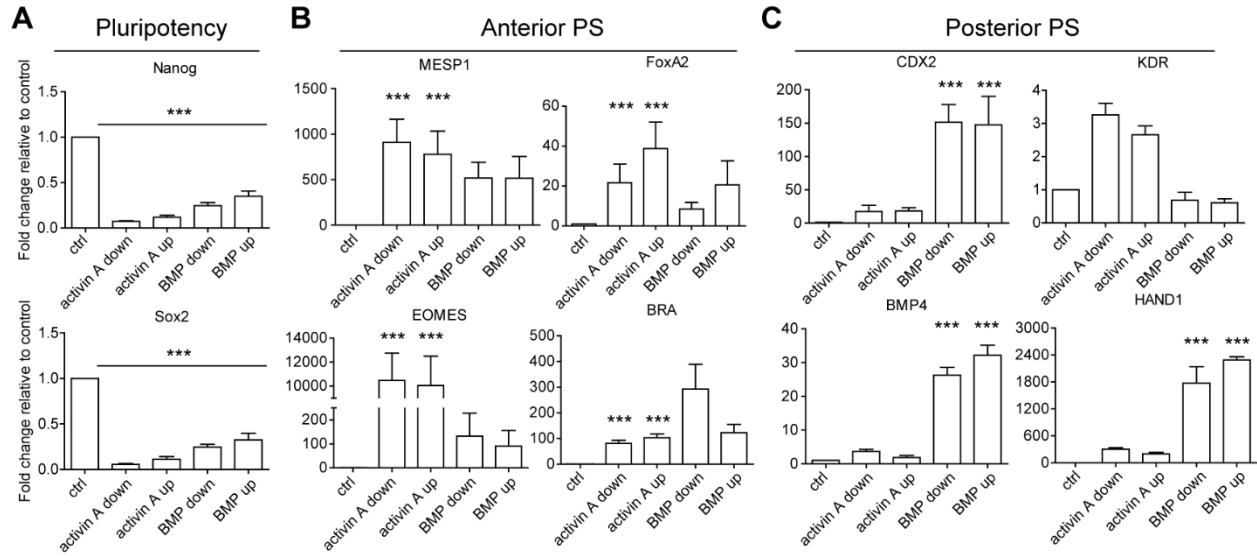
## SUPPLEMENTARY FIGURES

### Supplementary Figure 1



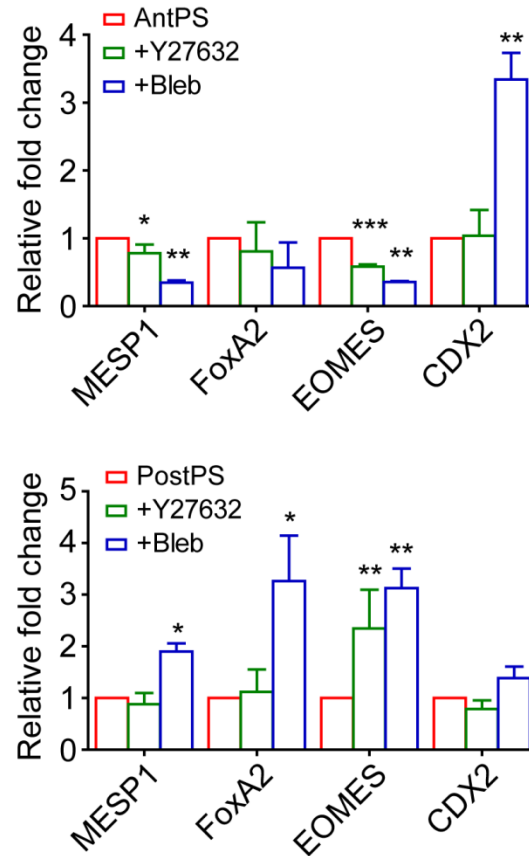
**Figure S1. Quantitative data of percentages of migratory AntPS and PostPS cells away from 3D cell spheres.** Bars represent the mean  $\pm$  SD calculated from 23 spheres in one experiment that is representative of three independent experiments. *P* values were calculated using one-way ANOVA followed by *t*-test analysis. \*\*\*, *P* < 0.001.

## Supplementary Figure 2



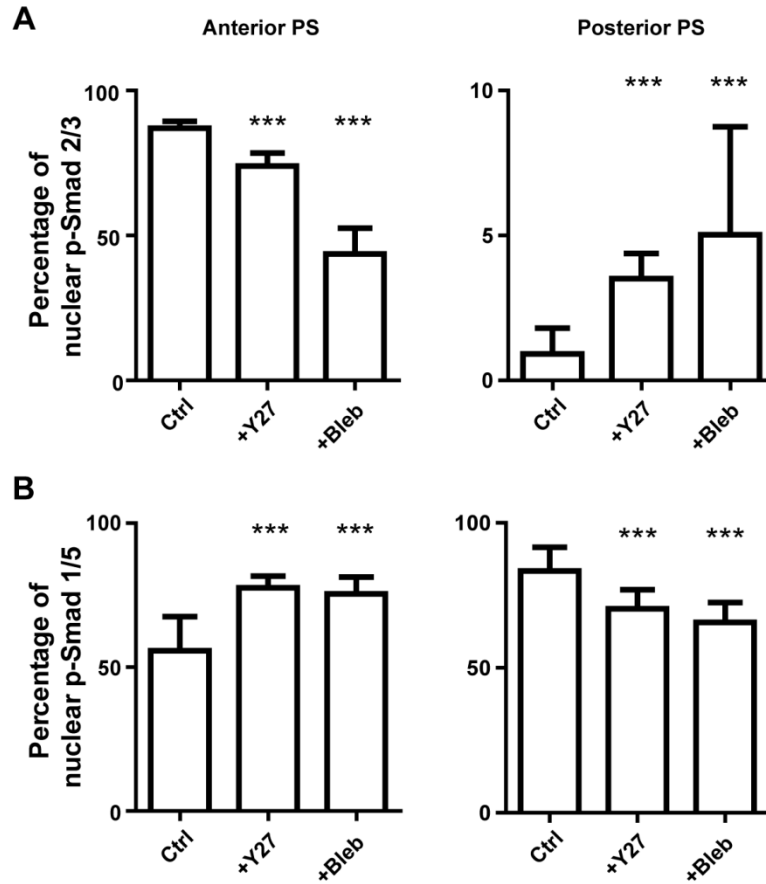
**Figure S2. qRT-PCR analysis of pluripotency markers (A; *Nanog* and *Sox2*), AntPS cell markers (B; *MESP1*, *FoxA2*, *Eomes*, and *BRACHYURY* or *BRA*), and PostPS cell markers (C; *CDX2*, *KDR*, *BMP4*, and *HAND1*) for hPSCs cultured under indicated conditions. Fold changes of expression levels of these markers were calculated against to that of *GAPDH* for hPSCs cultured under indicated conditions and normalized to that of hPSCs maintained in mTeSR1 medium. Data represent the mean  $\pm$  SD with  $n = 3$ .  $P$  values were calculated using one-way ANOVA followed by t-test analysis. \*\*\*,  $P < 0.001$ .**

### Supplementary Figure 3



**Figure S3. qRT-PCR analysis of AntPS and PostPS cell markers under indicated conditions.** Fold changes of expression levels of these markers were calculated against to that of *GAPDH* for hPSCs cultured under indicated conditions and normalized to that of hPSCs maintained in mTeSR1 medium. Data represent the mean  $\pm$  SD with  $n = 3$ .  $P$  values were calculated using one-way ANOVA followed by t-test analysis. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ .

## Supplementary Figure 4



### Figure S4. Effect of inhibiting cell contraction on the activation of Activin A and BMP4

**signaling.** (A) Bar plots showing percentages of cells with nuclear p-Smad 2/3 in anterior PS (left) and posterior PS (right) cells either with or without inhibition of cytoskeletal contraction.

(B) Bar plots showing percentages of cells with nuclear p-Smad 1/5 in anterior PS (left) and posterior PS (right) cells either with or without inhibition of cytoskeletal contraction. Data represent

the mean  $\pm$  SD calculated from at least 10 random fields in one experiment that is representative of three independent experiments. *P* values were calculated using one-way ANOVA followed by t-test analysis. \*\*\*, *P* < 0.001.

## SUPPLEMENTARY TABLES

**Supplementary Table 1.** List of antibodies used in Western blotting and immunocytochemistry.

<b>Protein</b>	<b>Vendor</b>	<b>Catalog number</b>	<b>Dilution</b>
<b>CDX2</b>	Biogenex	MU392AUC	1:500 (WB); 1:200 (ICC)
<b>FoxA2</b>	Seven Hills Bioreagents	WRAB-1200	1:200 (ICC)
<b>Eomes</b>	Abcam	ab23345	1:200 (WB); 1:100 (ICC)
<b>p- Smad 1/5/8</b>	Millipore	AB3848	1:100 (ICC)
<b>p-Smad 2/3</b>	Cell Signaling	3108s	1:100 (ICC)
<b>GAPDH</b>	Santa-Cruz Biotechnology	sc-25778	1:200 (WB)

**Supplementary Table 2.** List of primers used in qRT-PCR. Primers for SYBR green PCR were designed using NCBI-primer BLAST. Sequences were as follows:

Gene	Forward	Reverse
<b>KDR</b>	CCCCAGAAATAAAAATGG- TATAAAAATG	TTTCACTCACTTCCATAATCGTCA
<b>MESP1</b>	GAAGTGGTTCCTTGGCAGAC	TCCTGCTTGCCTCAAAGTGT
<b>EOMES</b>	CACATTGTAGTGGGCAGTGG	CGCCACCAAACCTGAGATGAT
<b>HAND1</b>	CCAAGGATGCACAGTCTGG	AGGAGGAAAACCTTCGTGCTG
<b>Nanog</b>	GATTTGTGGGCCTGAAGAAA	ATGGAGGAGGGAAGAGGAGA
<b>BRACH- YURY</b>	TGCTGCAATCCCATGACA	CGTTGCTCACAGACCACA
<b>BMP4</b>	TCCACAGCACTGGTCTTGAG	GGGATGTTCTCCAGATGTTCTT
<b>CDX2</b>	GACGTGAGCATGTACCCTAGC	GCGTAGCCATTCCAGTCCT
<b>FoxA2</b>	CGACTGGAGCAGCTACTATGC	TACGTGTTTCATGCCGTTTCAT
<b>Sox2</b>	GCTTAGCCTCGTCGATGAAC	AACCCCAAGATGCACAACCTC
<b>GAPDH</b>	CTCTGCTCCTCCTGTTTCGAC	TTAAAAGCAGCCCTGGTGAC