BRACHYURY SOX17 CXCR4 SOX7 All 8 cell lines 800 200 induce robust expression of SOX17 to levels 40 600 150 that range from 60-fold to 750-fold over those 30 400 1.0 found in the respective hESC culture prior to 100 CyT25 20 differentiation. CXCR4 gene expression is 200 50 10 also robustly expressed and generally shows a pattern slightly delayed from that of SOX17. 1d 2d 5d 0d 1d 2d 5d 0d The 8 hESCs lines varied with regard to their 800 gene expression profiles and based on these 100 40 profiles were subdivided into groups 1 and 2. 600 75 30 Group 1 cell lines exhibit a peak in brachyury 400 50 20 expression at day 1 of differentiation after CyT203 200 25 which brachyury is down-regulated to at or 10 below hESC levels. Group 2 cell lines show 0 0d 1d 2d 5d 2d 1d 2d 0d 1d 2d 5d 04 14 a delayed course of brachyury expression Group ' and after 5 days differentiation levels remain 1000 2000 10- to 30-fold above that found in the hESCs. 750 1500 We suggest that either rapid disappearance or prolonged maintenance of brachyury may HUES7 30 500 1000 predict the relative proportion of endoderm 15 250 500 versus mesoderm that is produced from each cell line using this high activin/low FBS 1d 3d 5d 1d 3d 0d 1d 3d 5d 0d 1d differentiation protocol. X-axes indicate days of differentiation. 100 400 1.5 50 Important note: 40 75 300 1.0 Differentiation of the H7, H9, and HUES7 Н9 30 50 200 hESC lines was kindly provided by 20 collaborators working from a blinded protocol. 25 100 Relative expression 10 Cell lysates were sent to CyThera for analysis of gene expression using real-time PCR. 1d 2d 5d 1d 2d 1d 2d 5d 0d 0d 0d Differentiation of H7 and H9 hESC lines was 150 50 5.0 carried out by the laboratory of Melissa 120 40 4.0 Carpenter at The Robarts Institute, Ontario, 90 30 3.0 Canada. Differentiation of HUES7 was H7 40 60 20 2.0 carried out by Gillian Beattie at the UCSD 20 30 10 1.0 Whittier Institute, San Diego, CA. 0.0 2d 1d 2d 2d 0d 600 320 90 5.0 75 4.0 450 240 60 3.0 45 300 160 **BG01** 2.0 30 150 80 1.0 1d 2d 1d 2d 5d 2d 1d 2d 5d 1d Group 250 50 400 6.0 40 200 300 150 30 **BG02** 4.0 200 20 100 20 100 50 10 0 2d 1d 2d 2d 5d 50 400 4.0 40 60 300 3.0 30 40 BG03 200 2.0 20 20

0d 1d 2d

Eight hESC lines exhibit PS-like gene expression dynamics during DE formation.

Supplementary Fig. 7

0d 1d 2d

0d 1d 2d 5d