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SUPPLEMENTARY MATERIAL

corresponding to:

Definitive endoderm differentiation is promoted in suspension cultured human iPS-derived spheroids more than in adherent cells

SHIGEHARU G. YABE*, JUNKO NISHIDA, SATSUKI FUKUDA, FUJIE TAKEDA, KIYOKO NASHIRO, MASATO IBUKI and HITOSHI OKOCHI*

Department of Regenerative Medicine, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan

***Address correspondence to:** Shigeharu G. Yabe (syabe@ri.ncgm.go.jp) or Hitoshi Okochi (hokochi@ri.ncgm.go.jp). Department of Regenerative Medicine, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama Shinjuku-ku, Tokyo, Japan 162-8655. TEL: 81-3-3202-7181 Fax: 81-3- 3202-7192
https://orcid.org/0000-0001-5377-5131 (Yabe) and https://orcid.org/0000-0001-6938-7993 (Okochi).

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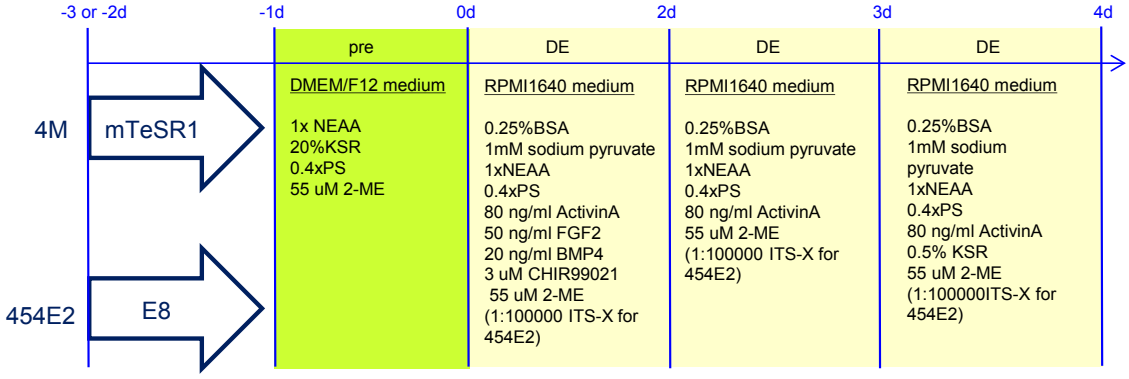
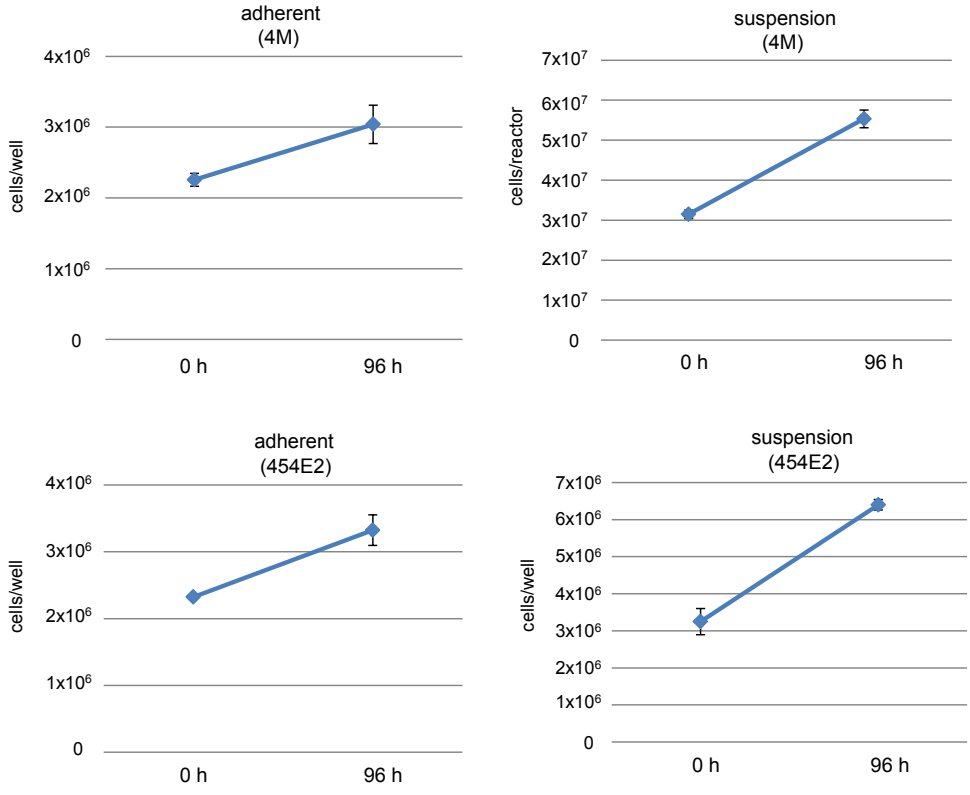
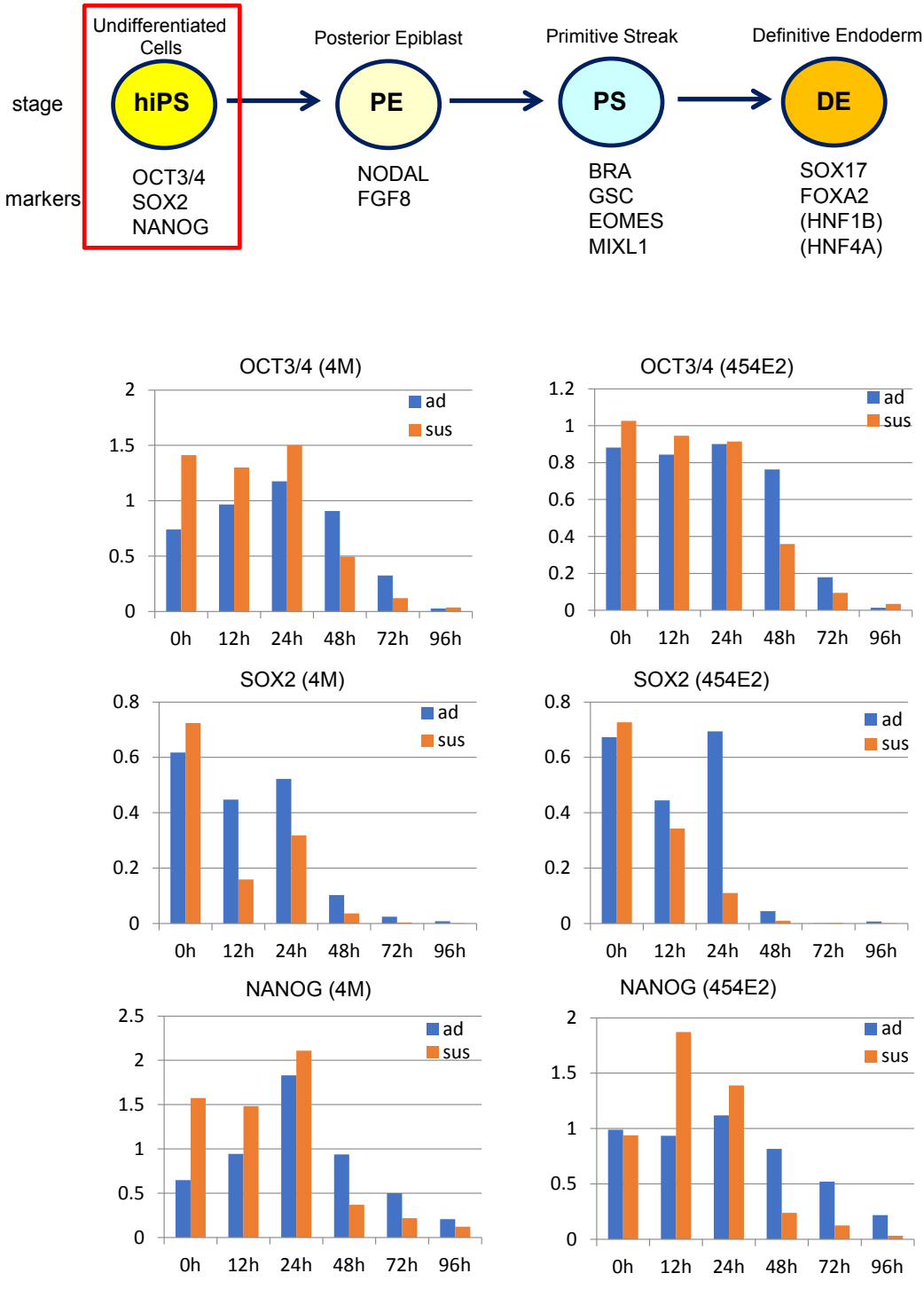


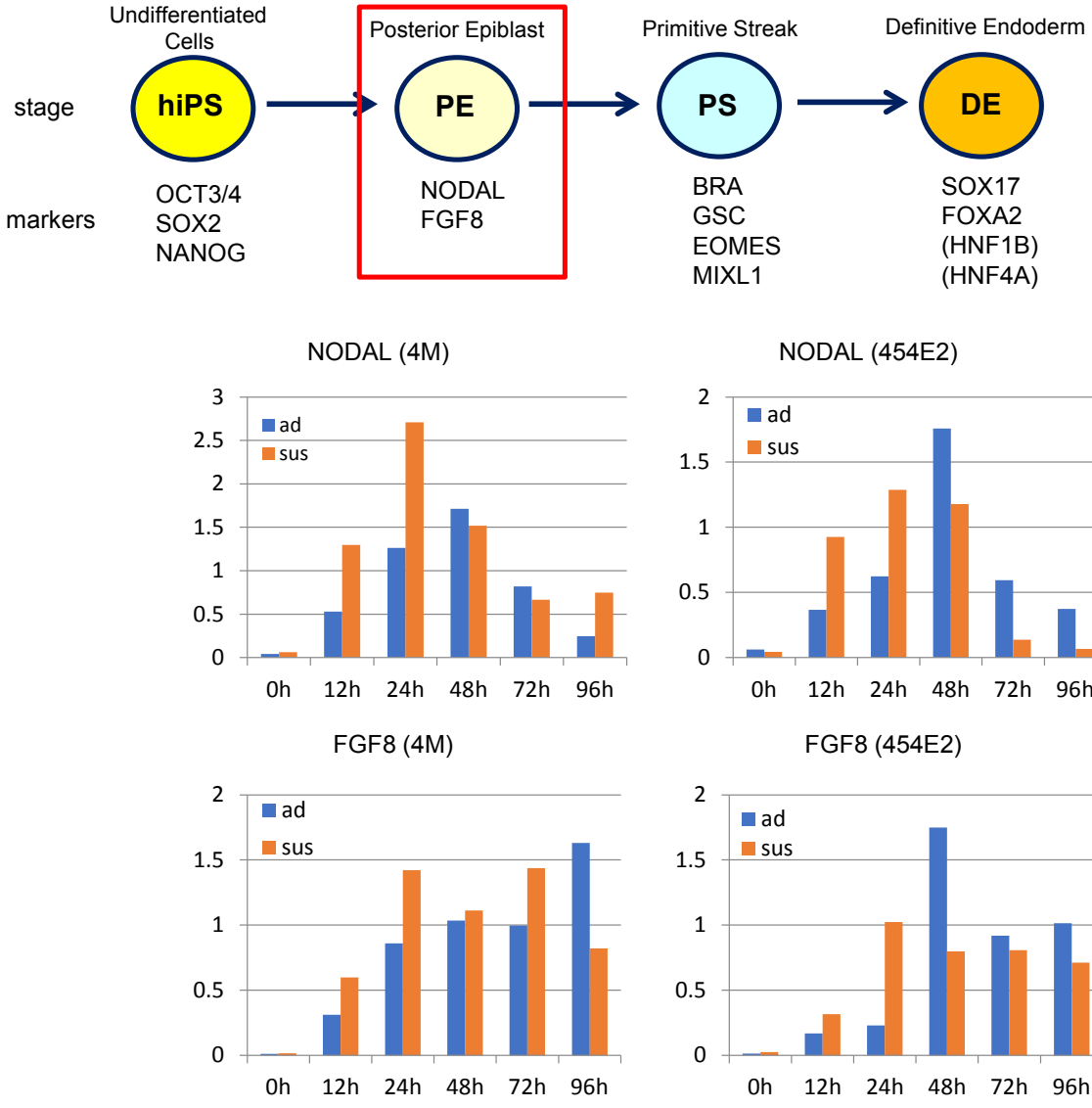
Sup. Fig. S1. Scheme of definitive endoderm (DE) differentiation protocol. *Although the culture media were different between 4M and 454E2 before differentiation, media for DE differentiation were the same afterwards except for ITS-X.*



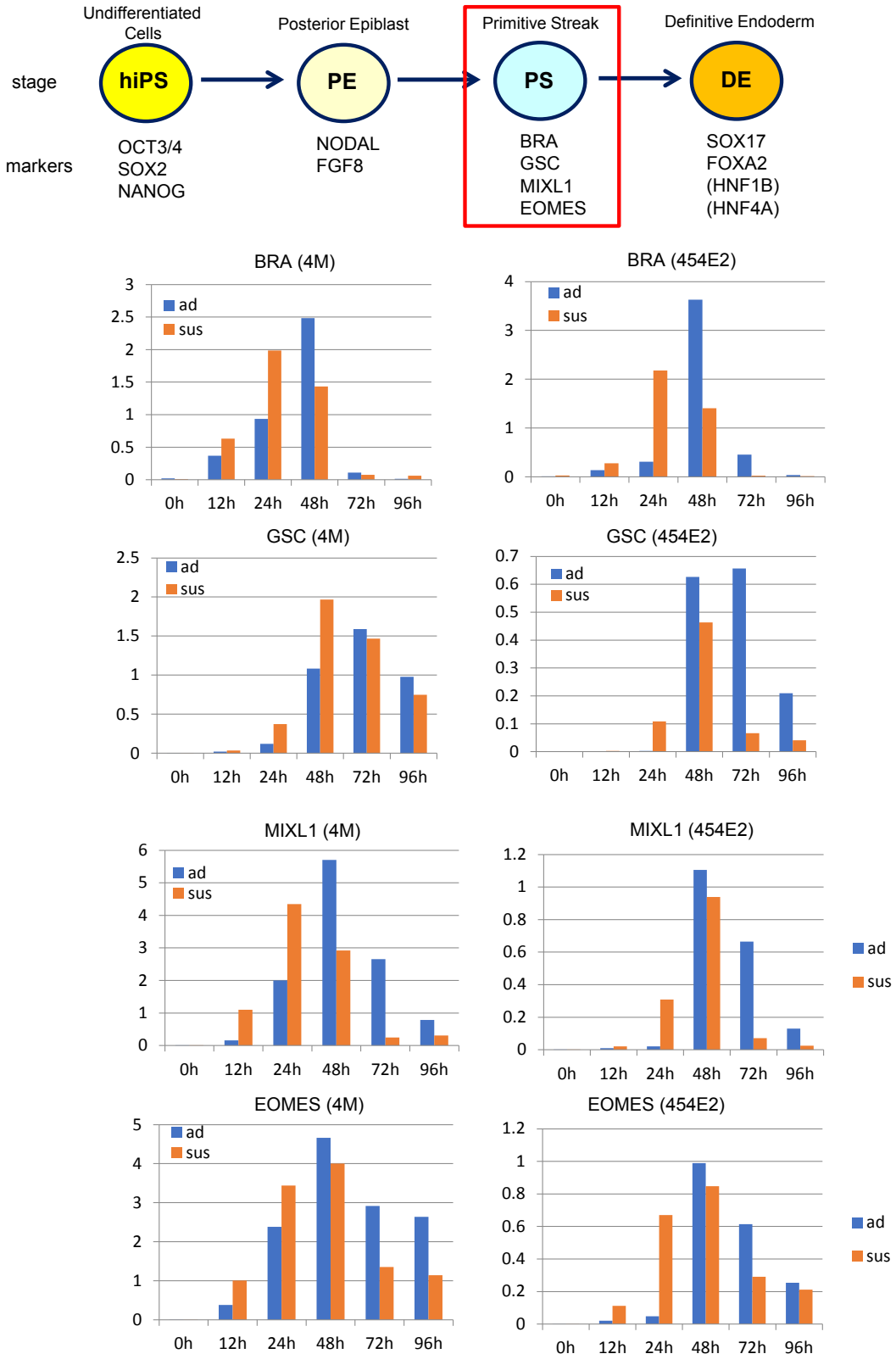
Sup. Fig. S2. Growth curve of 4M and 454E2 in definitive endoderm (DE) differentiation. *Adherent or suspension cultured cells were dissociated into single cells using accutase and counted at 0 and 96 h. For adherent cultured cells (4M and 454E2) and suspension cultured cells (454E2), cell numbers are an average of 3 wells. For suspension cultured cells (4M), cell numbers are an average of 3 bioreactors. Scale bars are based on the standard deviation of the mean (SEM) from three technical replicates. Adherent cultured cells increased about 1.5-fold between 0 and 96 h; suspension cultured cells increased about 2-fold between 0 and 96 h.*



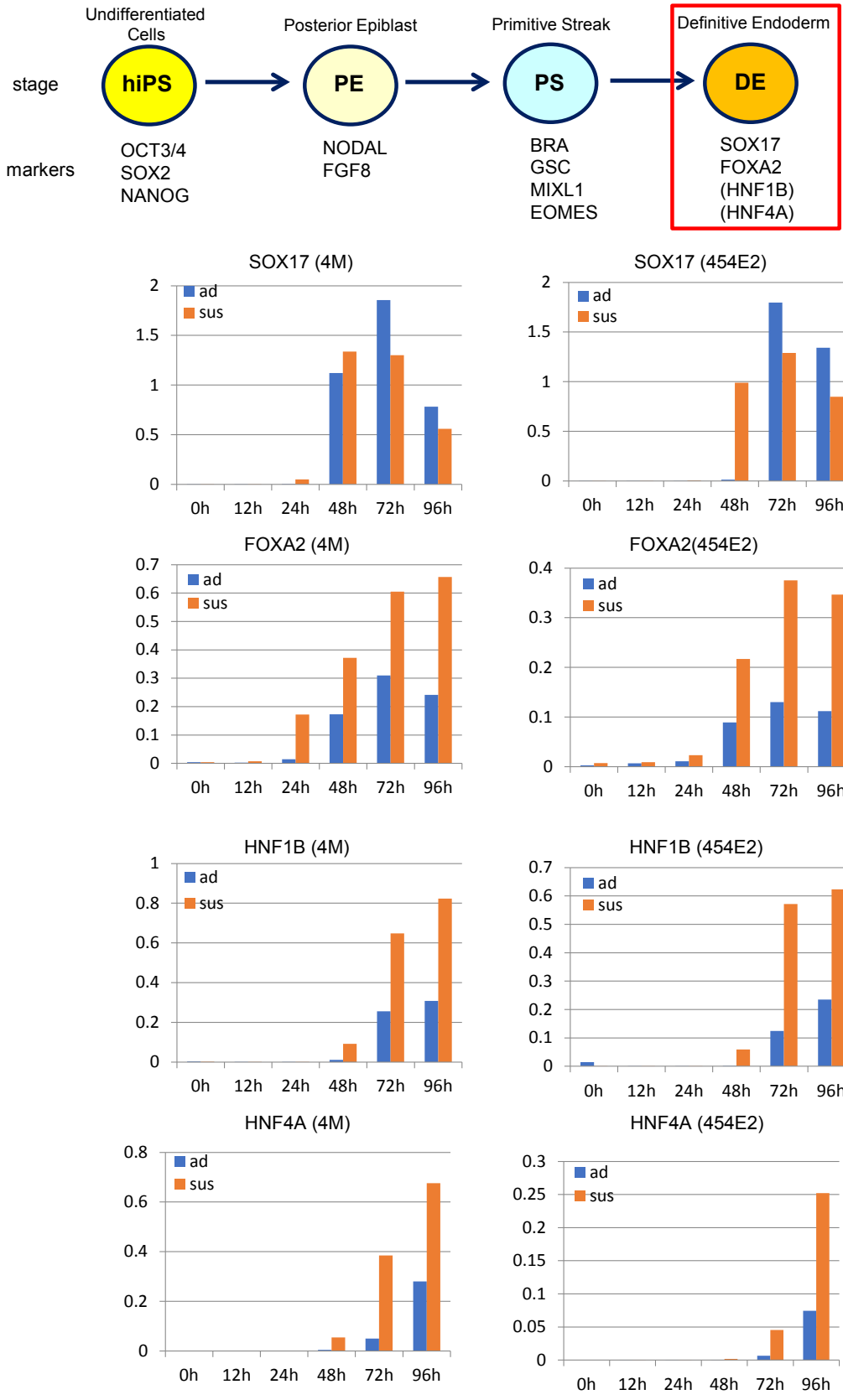
Sup. Fig. S3. Expression pattern of undifferentiated human induced pluripotent stem cell (hiPSC) markers (representative data). OCT3/4, SOX2 *and* NANOG *expressions were examined by qPCR at 0, 12, 24, 48, 72, 96 h in suspension or adherent culture using two hiPSCs lines (4M and 454E2).Y-axis indicates relative gene expression normalized to the* OAZ1. *ad, adherent; sus, suspension.*



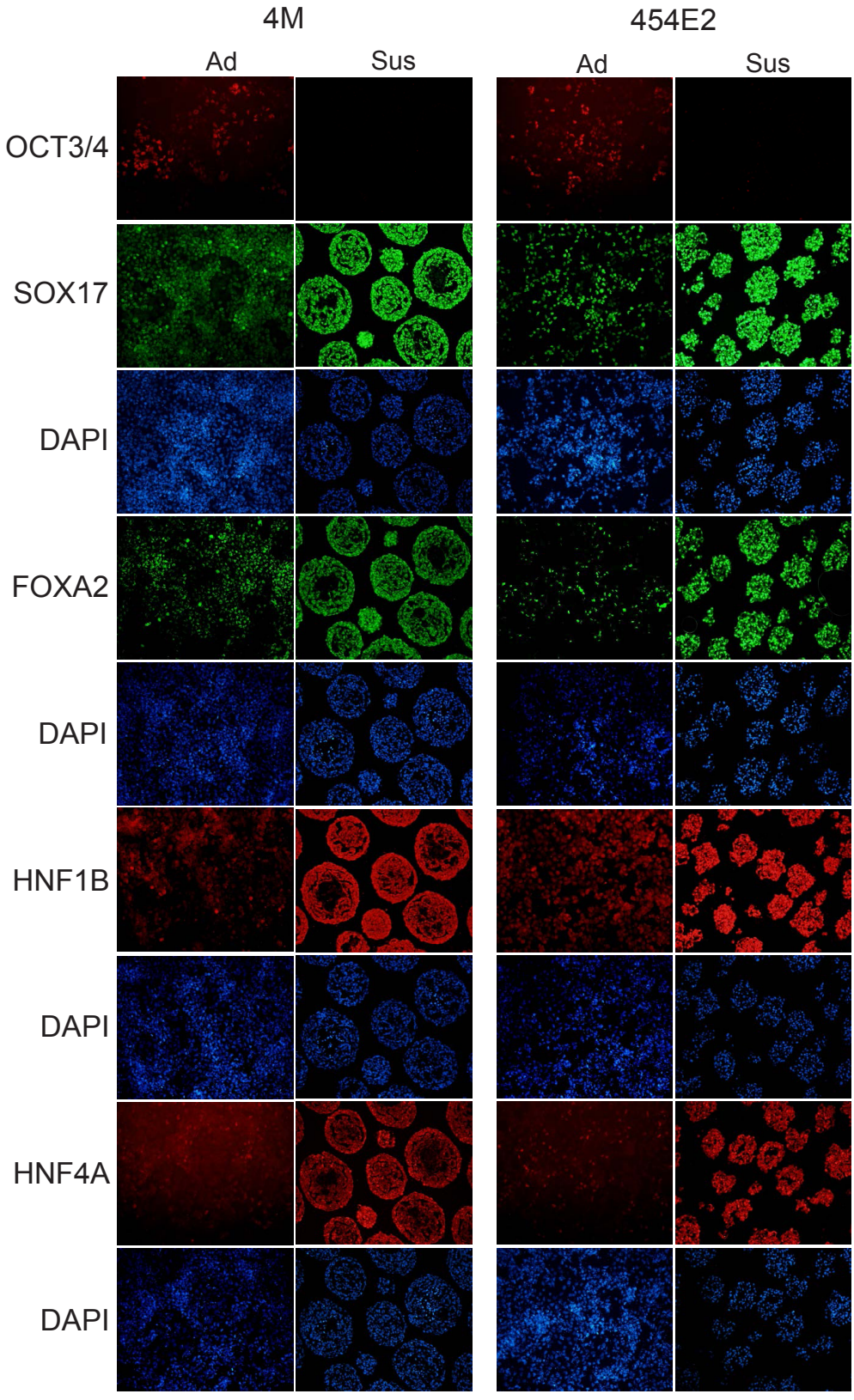
Sup. Fig. S4. Expression pattern of posterior epiblast markers (Representative data). NODAL *and* FGF8 *expressions were examined in the same way as Sup. Fig. 1. Y-axis indicates relative gene expression normalized to the* OAZ1*. ad, adherent; sus, suspension.*



Sup. Fig. S5. Expression pattern of posterior primitive streak markers (Representative data). BRA, GSC, MIXL1 *and* EOMES *expressions were examined in the same way as Sup. Fig.1. Y-axis indicates relative gene expression normalized to* OAZ1. *ad, adherent; sus, suspension*



Sup. Fig. S6. Expression pattern of definitive endoderm and primitive gut tube markers (Representative data). SOX17, FOXA2, HNF1B *and* HNF4A *expressions were examined. Y-axis indicates relative gene expression normalized to* OAZ1*. ad, adherent; sus, suspension.*



Sup. Fig. S7. Immunocytochemistry for representative markers. *Immunocytochemistry was carried out at 96 h according to Yabe* et al.*, 2017. OCT3/4 was used as undifferentiated marker; SOX17 and FOXA2 were used as DE markers; HNF1B and HNF4A were used as a PGT marker.ad, adherent; sus, suspension.*