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Supporting Information

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Supporting Information

Expanded potential stem cells from human embryos have an open chromatin configuration with enhanced trophoblast differentiation ability

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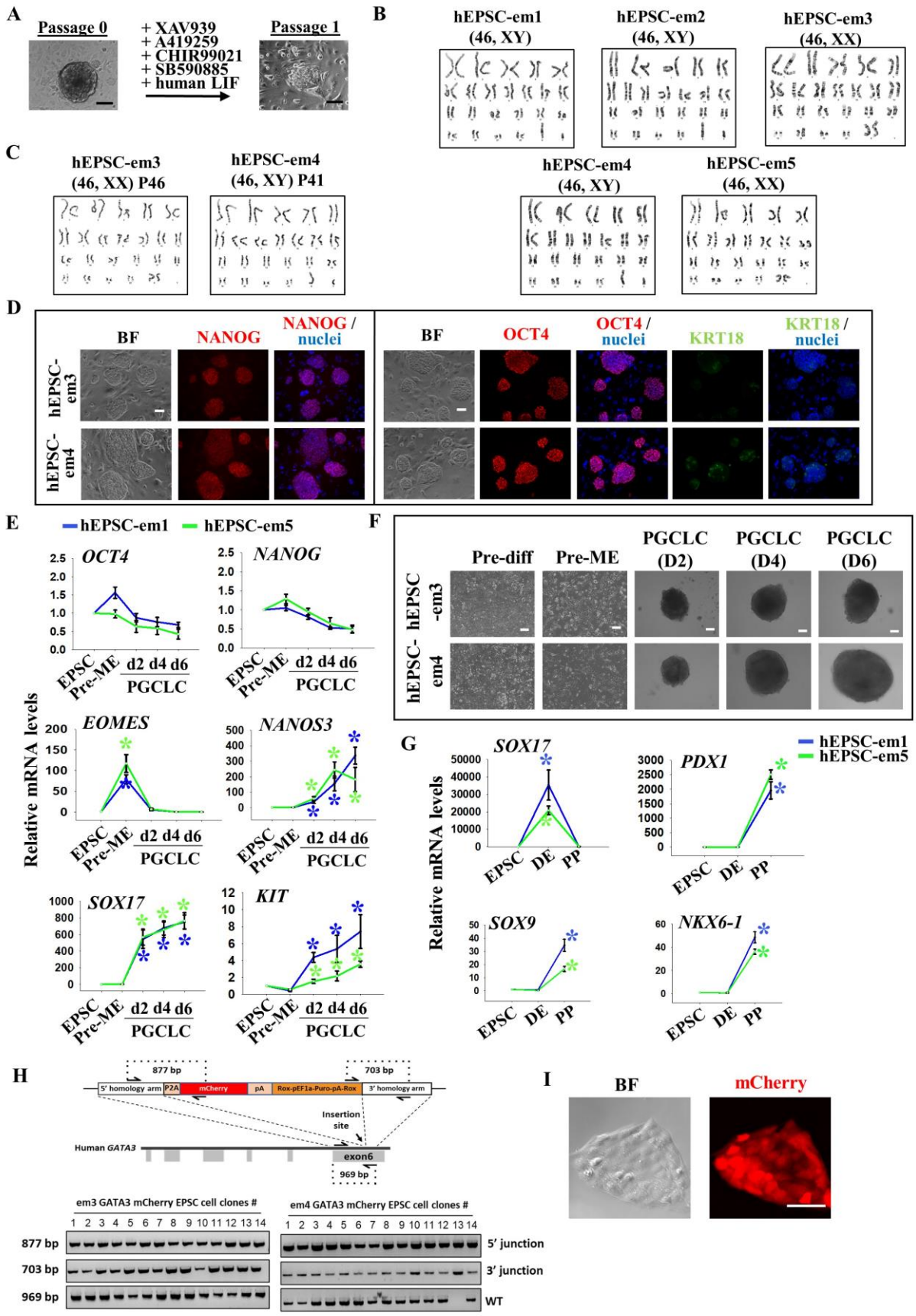

Fig. S1. Establishment of EPSC from human pre-implantation embryos (hEPSC-em)

(A): Images of donated human embryos at morula stage (left) and mechanically dissected colony (right) during the derivation of hEPSC-em. Scale bar: 100 μm. **(B):** Karyotyping of hEPSC-em-1, -2, -3, -4 and -5. **(C):** Karyotyping of hEPSC-em-3 and hEPSC-em-4 after long-term culture (>40 passages). **(D):** Immunofluorescent staining of OCT4, NANOG and KRT18 in undifferentiated hEPSC-em-3 and hEPSC-em-4. The nuclei were stained with Hoest33258. Scale bar: 100 μm. **(E):** RT-qPCR analysis of *OCT4*, *NANOG*, *EOMES*, *NANOS3*, *SOX17* and *KIT* during PGCLC formation from hEPSC-em1 and hEPSC-em5. *: $p<0.05$ compared to EPSC control, t-test; n=3. **(F):** Photos showing different stages of PGCLC differentiation from hEPSC-em. Pre-diff: pre-differentiation, Pre-ME: pre-mesendoderm. Scale bar: 100μm. **(G):** RT-qPCR analysis of DE marker (*SOX17*) and PP markers (*PDX1*, *SOX9* and *NKX6-1*) during pancreatic differentiation from hEPSC-em1 and hEPSC-em5. *: $p<0.05$ compared to EPSC control; t-test; n=3. **(H):** Schematic diagram showing knock-in of the mCherry reporter cassette after exon 6 of the *GATA3* locus (top). Arrow indicated the location of PCR primers for amplifying the 5' and 3' inserted regions, and the wildtype *GATA3* locus. PCR amplification of the 5'and 3' inserted regions, and the wild-type GATA3 locus (bottom). **(I):** Images of mCherry reporter fluorescent signal in hTSC-em. Scale bar: 100μm.

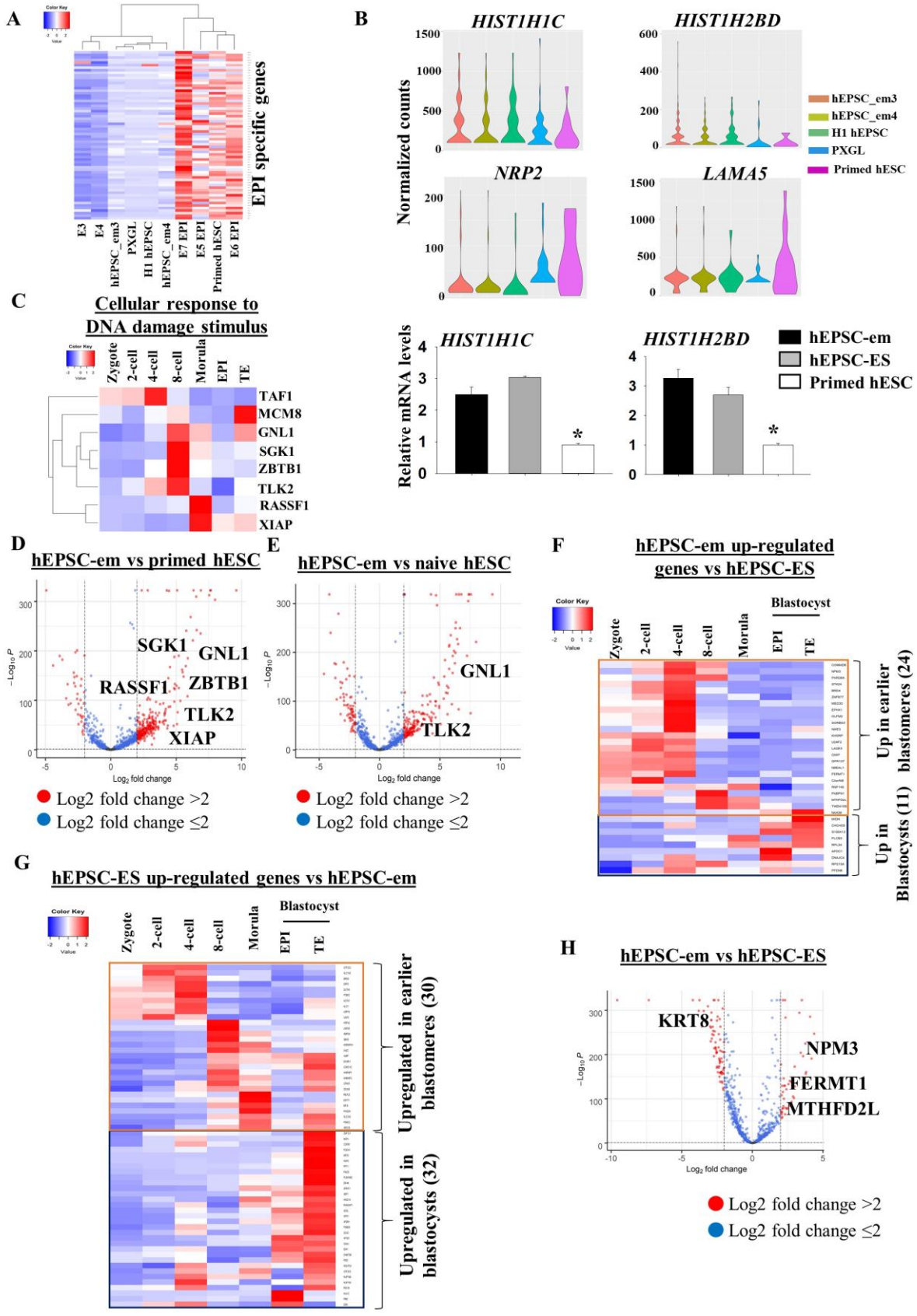

Fig. S2. Single cell RNA sequencing analysis of hEPSC-em transcriptomes

(A): Heatmap clustering showing the expression values of epiblast specific genes among hEPSC-em3, hEPSC-em4, hEPSC-ES, naive hESC, primed hESC, and human pre-implantation embryos. **(B):** The violin plots showing the expression patterns of histone cluster genes (*HIST1H1C, HIST1H2BD*) and stem cell differentiation genes (*NRP2, LAMA5*) between hEPSC-em, hEPSC-ES, primed hESC and naive hESC (top). RT-qPCR analysis of *HIST1H1C* and *HIST1H2BD* between hEPSC-em3, hEPSC-em4, hEPSC-ES and primed hESC. *: $p<0.05$ compared to EPSC control, t-test; n=4 (bottom). **(C)**: Heatmap showing the expression levels of genes under cellular response to DNA damage stimulus in human pre-implantation embryos. **(D)**: Volcano plot indicating the differentially expressed genes between hEPSC-em and primed hESC. *SGK1, GNL1, RASSF1, ZBTB1, TLK2* and *XIAP* were specifically labeled. **(E)**: Volcano plot indicating the differentially expressed genes between hEPSC-em and naive hESC. *GNL1* and *TLK2* were specifically labeled. **F-G**: Heatmap showing the expression levels of genes differentially upregulated in hEPSC-em **(F)** or in hEPSC-ES **(G)** in human pre-implantation embryos. **(H)**: Volcano plot indicating the differentially expressed genes between hEPSC-em and hEPSC-ES. *KRT8, NPM3, FERMT1* and *MTHFD2L* were specifically labeled. Published scRNA-seq data sets of human pre-implantation embryos (12), primed hESC (12), naive hESC (14) and hEPSC-ES (4) were used for the analysis.

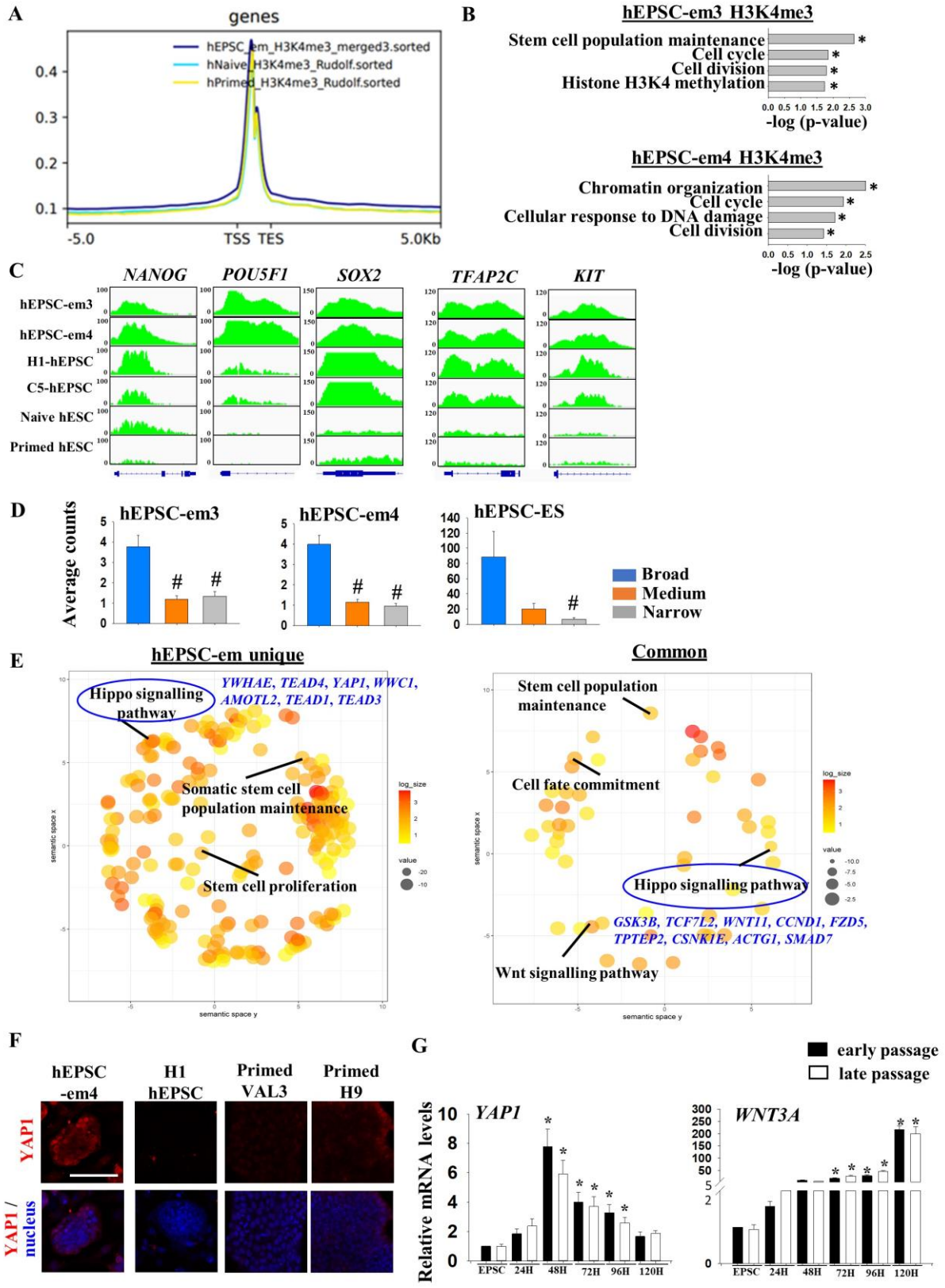

Fig. S3. hEPSC-em had unique broad H3K4me3 conformation

(A): Peak coverage plot from chromatin immunoprecipitation-sequencing (ChIP-seq) analysis of H3K4me3 marks in hEPSC-em3, hEPSC-em4, primed and naive hESC. The plot covered peaks around the transcription start site (TSS ± 5000bp). The ChIP-seq data of hEPSC-ES and

primed hESC were from Ref. 5 and 19 respectively. (**B):** Gene ontology analysis for H3K4me3 marked gene promoters in hEPSC-em3 and hEPSC-em4. (**C):** The tracks showing H3K4me3 peaks at the gene loci (*NANOG*, *POU5F1*, *SOX2*, *TFAP2C*, *KIT*) in hEPSC-em, hEPSC-ES, primed and naive hESC. (**D):** Average counts of genes with broad (>5kb), medium (1-5kb) and narrow (<1kb) H3K4me3 domains between hEPSC-em and hEPSC-ES. #: $p<0.001$ compared to broad domain, t-test. scRNA-seq data of hEPSC-ES was from Ref. 5. (**E):** REVIGO analysis of gene ontology enrichment in H3K4me3 broad domain unique in hEPSC-em (left) and common among different cell types (right). The genes of the Hippo signaling pathways were shown. **(F):** Immunofluorescent staining of YAP1 in undifferentiated hEPSC-em4, hEPSC-ES, naive and primed hESCs. The nuclei were stained with Hoest33258. Scale bar: 100 μm. **(G):** RT-qPCR analysis of *YAP1* and *WNT3A* upon trophoblast differentiation from hEPSC-em4 at early (20-40) and late (>50) passages. *: $p<0.05$ compared to EPSC control, t-test; n=3.

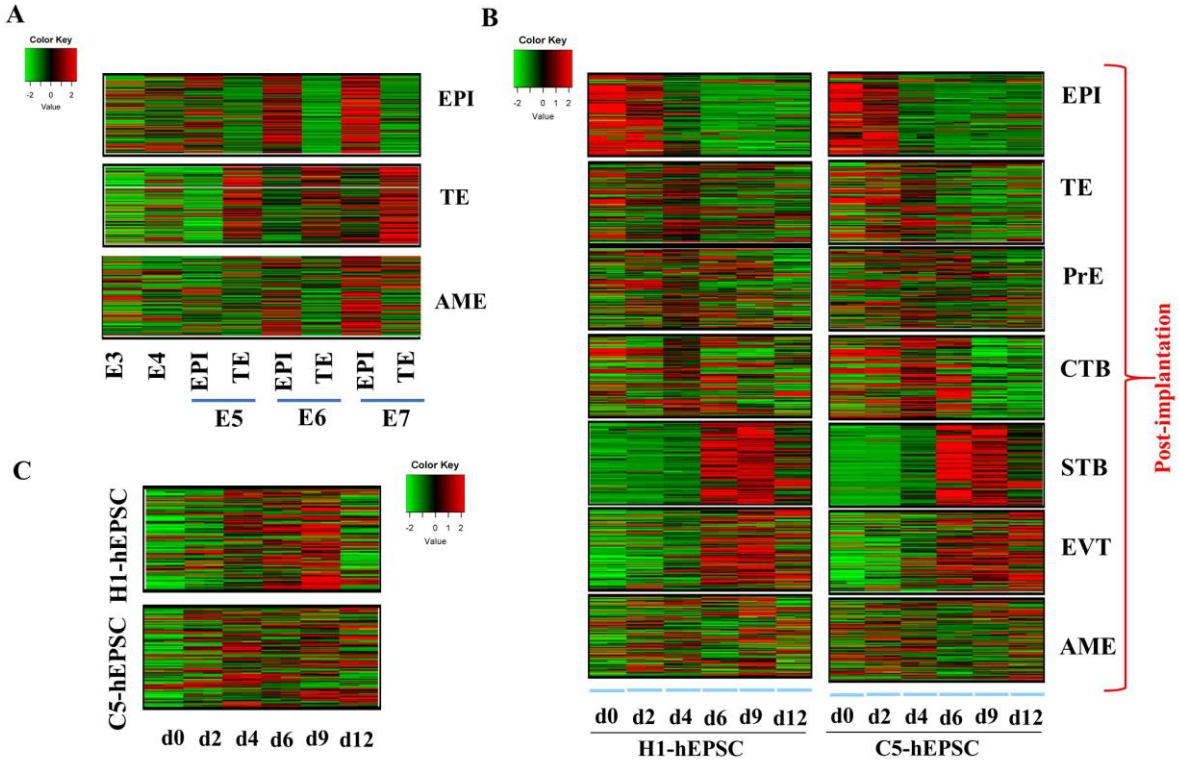

Fig. S4. Trophoblast differentiation from hEPSC-ES corresponded to trophoblast rather than amnion cells

(A): Heatmap showing the gene expression patterns in human pre-implantation embryos (Ref. 13) under the category of EPI, TE and AME in post-implantation embryos (Ref. 23). (**B):** Heatmap showing the gene expression patterns during trophoblast differentiation from hEPSC-

ES (left: H1-hEPSC, right: C5-hEPSC) under the category of EPI, TE, Primitive endoderm (PrE), CTB, STB, EVT, AME in human post-implantation embryos from Ref. 23 after cell re-annotation as described in Ref. 24. (**C):** Heatmap showing the gene expression patterns during trophoblast differentiation from hEPSC-ES (top: H1-hEPSC, bottom: C5-hEPSC) under the category of cynomolgus AME from Ref. 26 after cell re-annotation as described in Ref. 24.

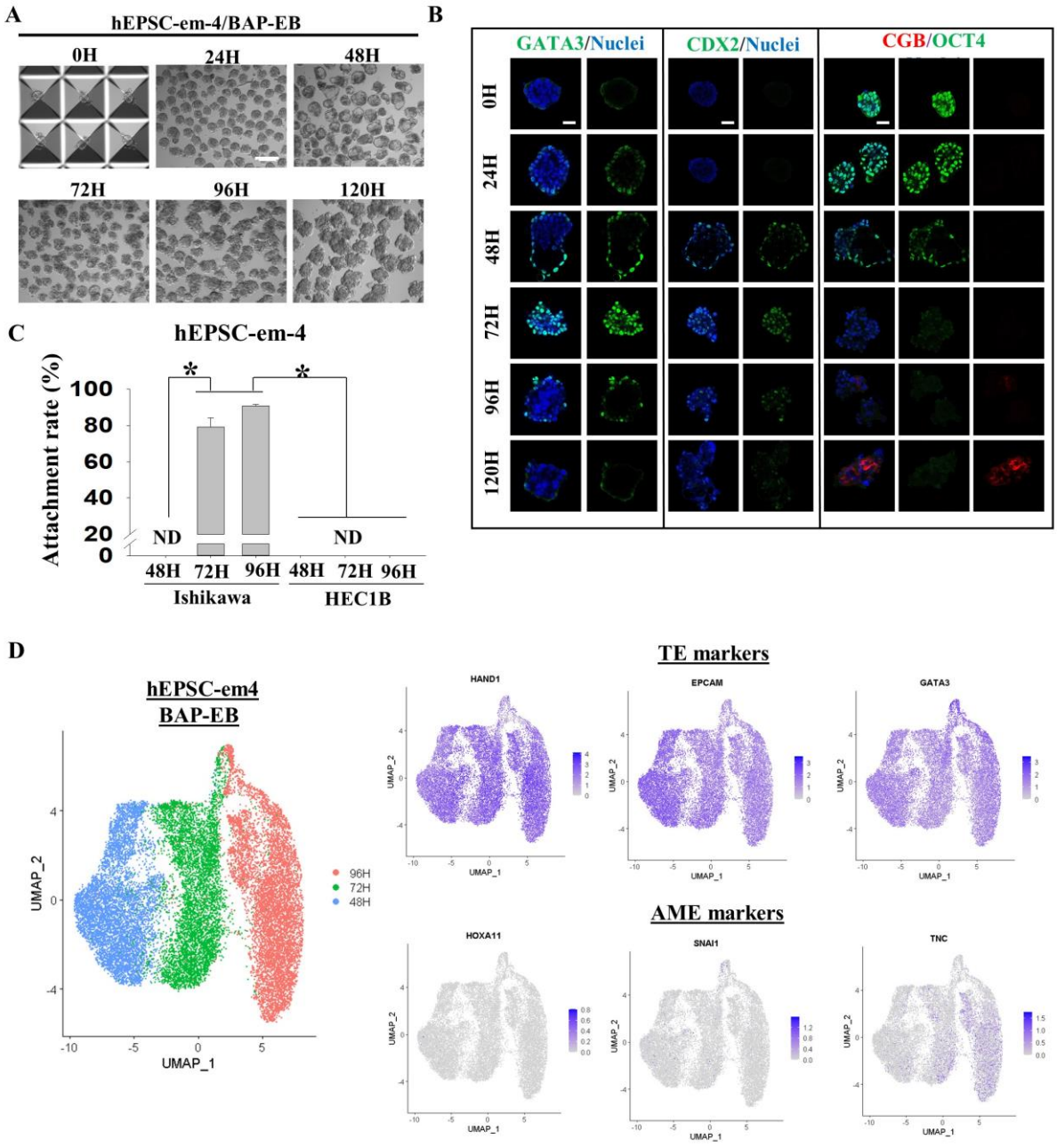

Fig. S5. BAP-EB formation from hEPSC-em

(A): Representative figures showing the trophoblast spheroids (BAP-EB) differentiated from hEPSC-em4 at different time points. Cystic structures were observed at 48H. Scale bar: 100μm. (**B):** Immunofluorescent staining of GATA3, CDX2, OCT4 and HCG of BAP-EB at different time points of BAP-EB differentiation from hEPSC-em4. The nuclei were stained with Hoest33258. Scale bar: 100μm. **(C)**: The attachment rate of hEPSC-em4 BAP-EB onto Ishikawa and HEC1B cells. *: $p<0.05$, t-test; n=4. **(D):** single-cell RNA sequencing analysis on TE markers (*HAND1*, *EPCAM* and *GATA3*) and AME markers (*HOXA11*, *SNAI1* and *TNC*) in BAP-EB derived from hEPSC-em4.

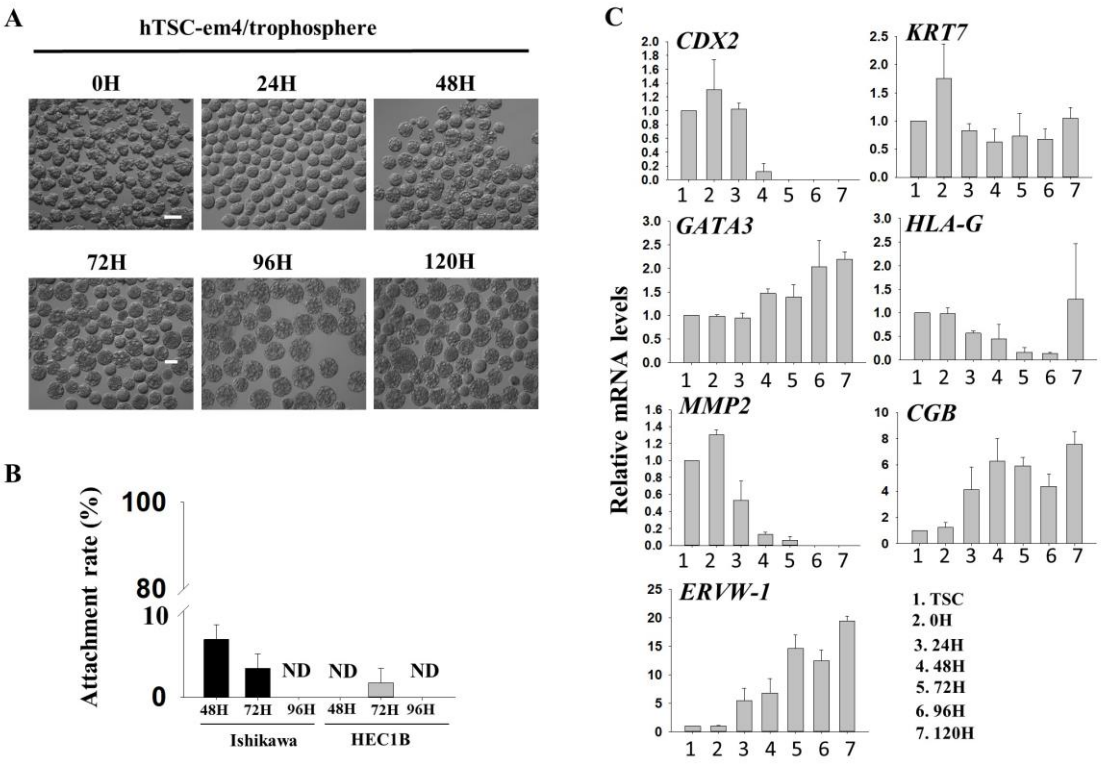

Fig. S6. Trophospheres formed from hTSC-em

(A): Representative figures showing the trophospheres formed from hTSC-em4 at different time points. Scale bar: 100μm. (**B):** The attachment rates of trophospheres -48h, -72h and -96h on receptive Ishikawa cells or non-receptive HEC-1B cells. n=4. ND: not detected. **(C):** RT-qPCR analysis of *CDX2*, *KRT7*, *GATA3*, *HLA-G*, *MMP2 CGB* and *ERVW-1* gene expressions in trophospheres formed from hTSC-em4; n=4.

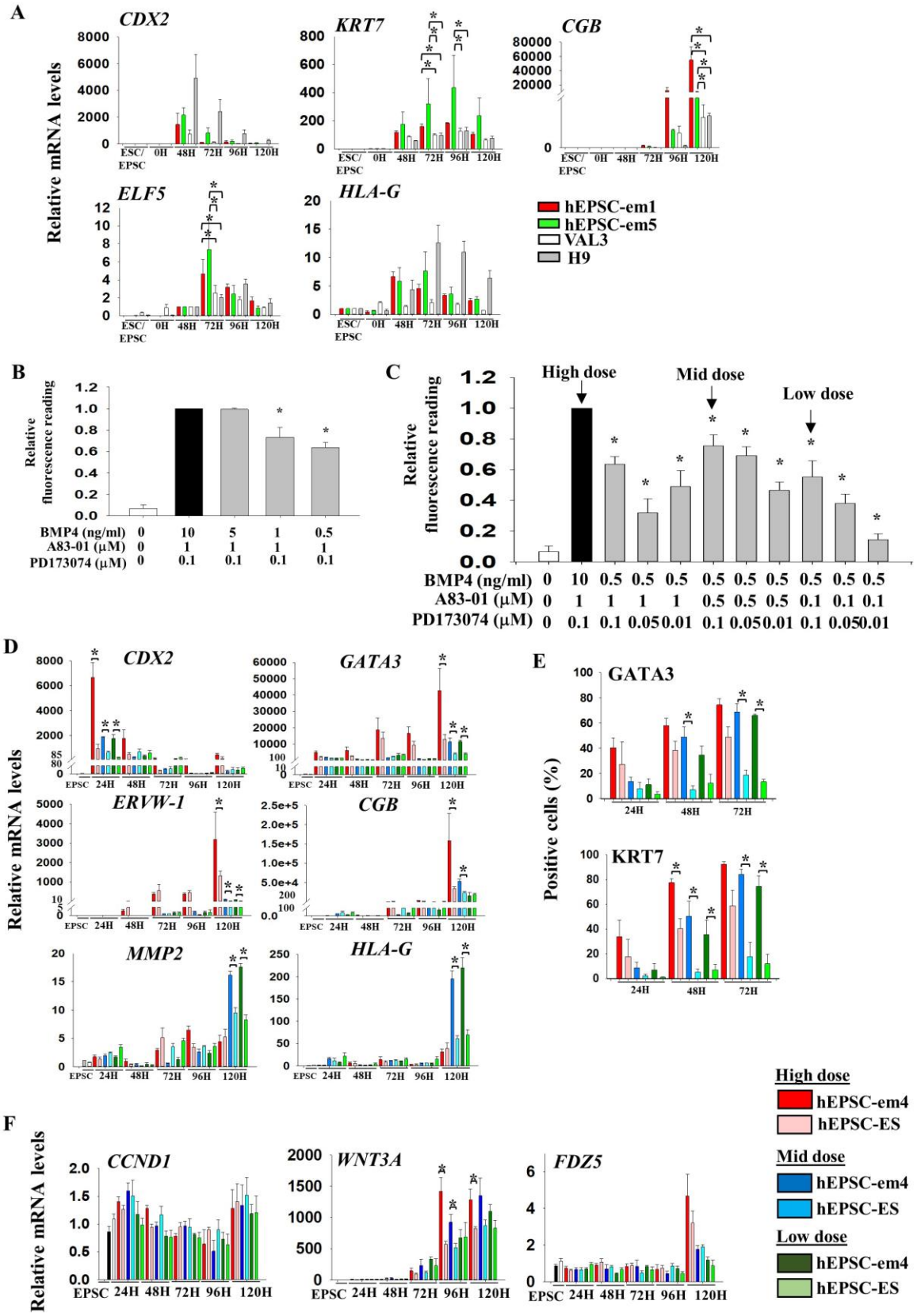

Fig. S7. hEPSC-em had enhanced trophoblast differentiation potency than hEPSC-ES

(**A):** RT-qPCR analysis of *CDX2*, *KRT7*, *CGB*, *ELF5* and *HLA-G* gene expressions in BAP-EB formed from hEPSC-em1 and hEPSC-em5; n=3. (**B):** Relative fluorescent reading of

GATA2-Venus reporter from hiPSC-EPSC upon trophoblast differentiation for 120H under different doses of BMP4 (0.5 ng/ml – 10 ng/ml) with constant A83-01 (1 μM) and PD173074 (0.1 μM). *: $p<0.05$ compared to high dose BAP (black bar); t-test; n=3. (**C):** Relative fluorescent reading of *GATA2*-Venus reporter from hiPSC-EPSC upon trophoblast differentiation for 120H under different doses of BAP. *: $p<0.05$ compared to high dose BAP (black bar); t-test; n=3. **(D):** RT-qPCR analysis of *CDX2*, *GATA3, ERVW-1, CGB, MMP2* and *HLA-G* for trophoblast differentiation from hEPSC-em-4 and hEPSC-ES at different time points under different doses of BAP: high dose (BMP4: 10 ng/ml, A83-01: 1 μM, PD173074: 0.1 μM), mid dose (BMP4: 0.5ng/ml, A83-01: 0.5 μM, PD173074: 0.1 μM) and low dose (BMP4: 0.5 ng/ml, A83-01: 0.1 μM, PD173074: 0.1 μM). *: $p<0.05$ comparing between experimental groups; t-test; n=3. (**E):** FACS analysis showing proportion of GATA3- and KRT7-positive cells after trophoblast differentiation from hEPSC-em-4 and hEPSC-ES at different time points under different doses of BAP. *: $p<0.05$ comparing between experimental groups; t-test; n=3. **(F):** RT-qPCR analysis of *CCND1*, *WNT3A* and *FDZ5* for trophoblast differentiation from hEPSC-em-4 and hEPSC-ES at different time points under different doses of BAP. *: $p<0.05$ comparing between experimental groups; t-test; n=3.

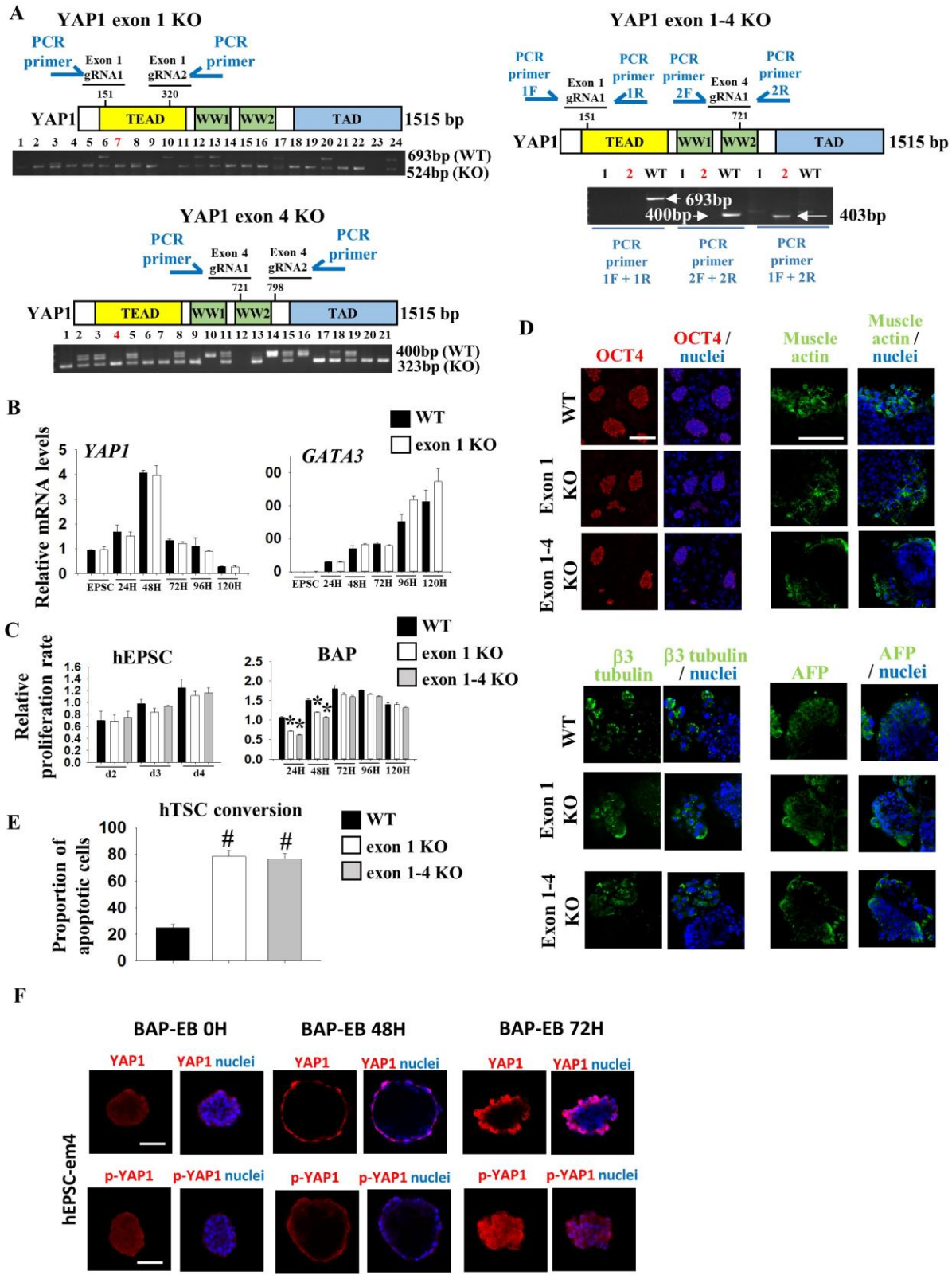

Fig. S8. YAP1 knockout inhibited trophoblast differentiation from hEPSC

(A): Schematic diagram showing the positions of gRNAs and PCR primers at exon 1, exon 4, and exon 1-4 of human *YAP1* gene. PCR analyses for validation of *YAP1* knockout were shown. The clones selected for subsequent studies were highlighted in red. (**B):** RT-qPCR analysis of

YAP1 and *GATA3* for trophoblast differentiation from hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 4. n=3. (**C):** Proliferation rates of hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 1 and from exon 1-4 in non-differentiated states (hEPSC) and during trophoblast differentiation (BAP differentiation) *: $p<0.05$ comparing with wild-type; t-test; n=3. (**D):** Immunofluorescent staining of OCT4 in hEPSC-em-4 wild-type and *YAP1* knockout hEPSC. Immunofluorescent staining of muscle actin (mesoderm); βIII tubulin (ectoderm); and alpha-fetoprotein (endoderm) in embryoid bodies derived from hEPSC-em-4 wild-type and *YAP1* knockout hEPSC-em. The nuclei were stained with Hoest33258. Scale bar: 100μm. (**E):** FACS analysis showing proportion of apoptotic cells after hTSC formation from hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 1 and from exon 1-4. *: $p<0.05$ comparing with wild-type; t-test; n=4. **(F):** Immunofluorescent staining of YAP1 and phospho-YAP1 in BAP-EB from hEPSC-em-4 wild-type and *YAP1* knockout hEPSC. The nuclei were stained with Hoest33258. Scale bar: 100μm.

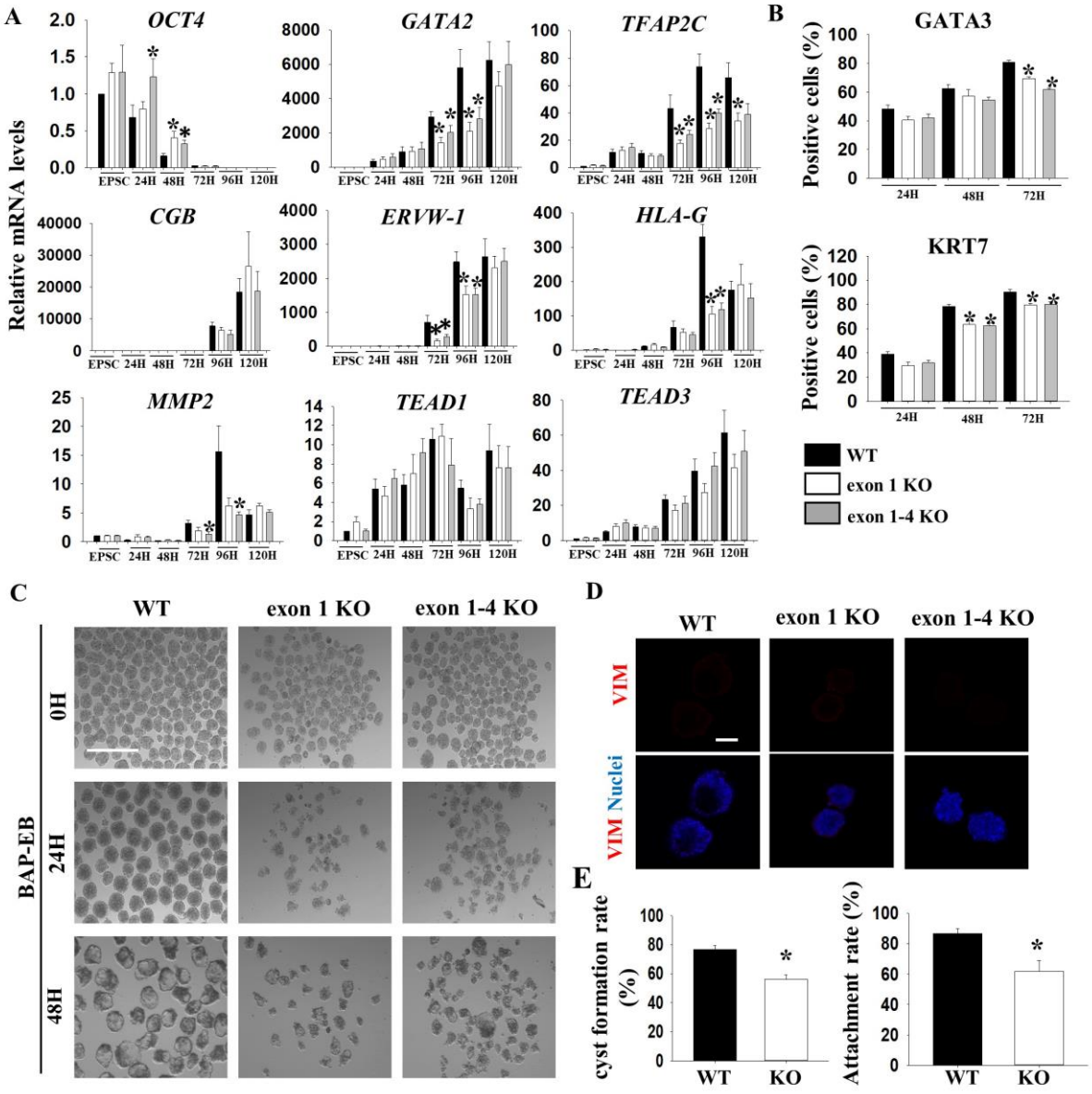

Fig. S9. YAP1 knockout inhibited trophoblast spheroid formation from hEPSC

(**A):** RT-qPCR analysis of *OCT4*, *GATA2*, *TFAP2C*, *CGB*, *ERVW-1*, *HLA-G*, *MMP2*, *TEAD1* and *TEAD3* for trophoblast differentiation from hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 1 and from exon 1-4. *: $p<0.05$ comparing with wild-type; t-test; n=5. (**B):** FACS analysis showing proportion of GATA3- and KRT7-positive cells after trophoblast differentiation from hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 1 and from exon 1-4. *: $p<0.05$ comparing with wild-type; t-test; n=5. **(C):** Representative figures showing BAP-EB differentiated from hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 1 and from exon 1-4 at different time points. Scale bar: 100μm. (**D):** Immunofluorescent staining of VIM in 48H BAP-EB derived from hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 1 and from exon 1-4. The nuclei were stained with Hoest33258. Scale bar: 100μm. (**E):**

Proportion of cystic BAP-EB formation (left) and the endometrial Ishikawa cell line attachment rates (right) between hEPSC-em-4 wild-type and *YAP1* knockout cells at exon 4. *: $p<0.05$ comparing with wild-type; t-test; n=3.