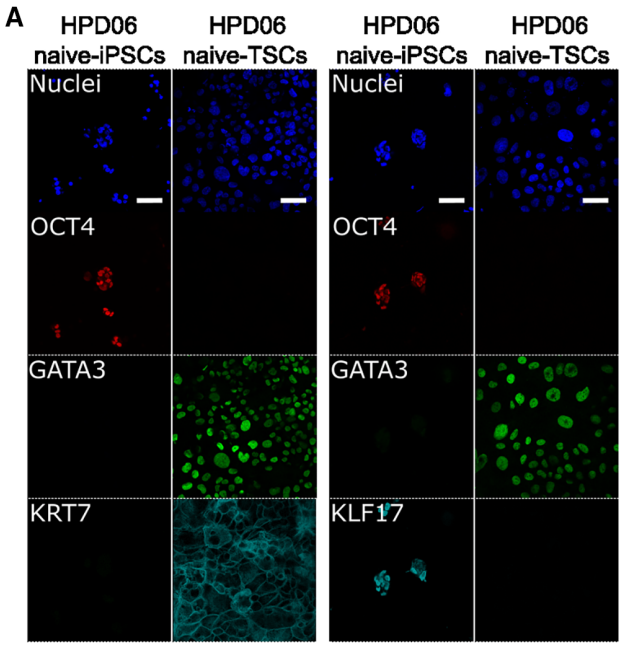
Expanded View Figures



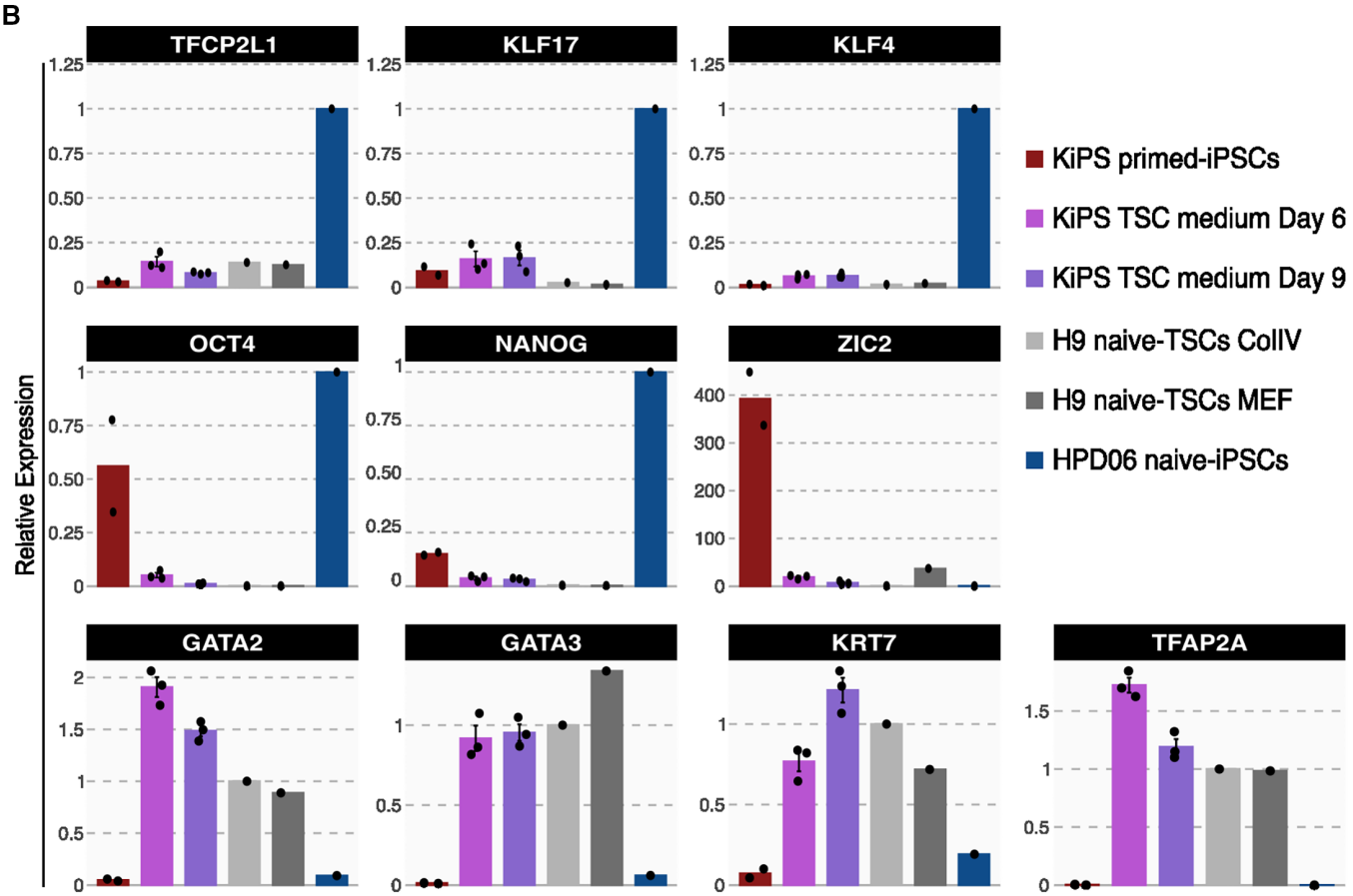


Figure EV1.

Figure EV1. Characterisation of naive-TSCs and conversion of conventional PSCs.

A Immunostaining for the pluripotency markers OCT4, KLF17 and the TSC markers GATA3 and KRT7 of HPD06 naive-iPSC and TSC cells derived from naive-iPSCs (HPD06 naive-TSCs). Scale bars: 30 μm.

B Gene expression analysis by RT–qPCR of KiPS primed-iPSCs, KiPS in TSC medium at day 6 and 9, H9 naive-TSCs cultured on ColIV or MEF, and HPD06 naive iPSCs.

Data information: (A) Representative images of $n = 3$ biological replicates are shown. (B) Dots indicate biological replicates, and bars indicate the mean ± SEM of $n = 3$ biological replicates. Top and middle: naive, primed and general pluripotency markers, expression was normalised to HPD06 naive-iPSCs; bottom: trophoblast markers, expression was normalised to H9 naive-TSCs.

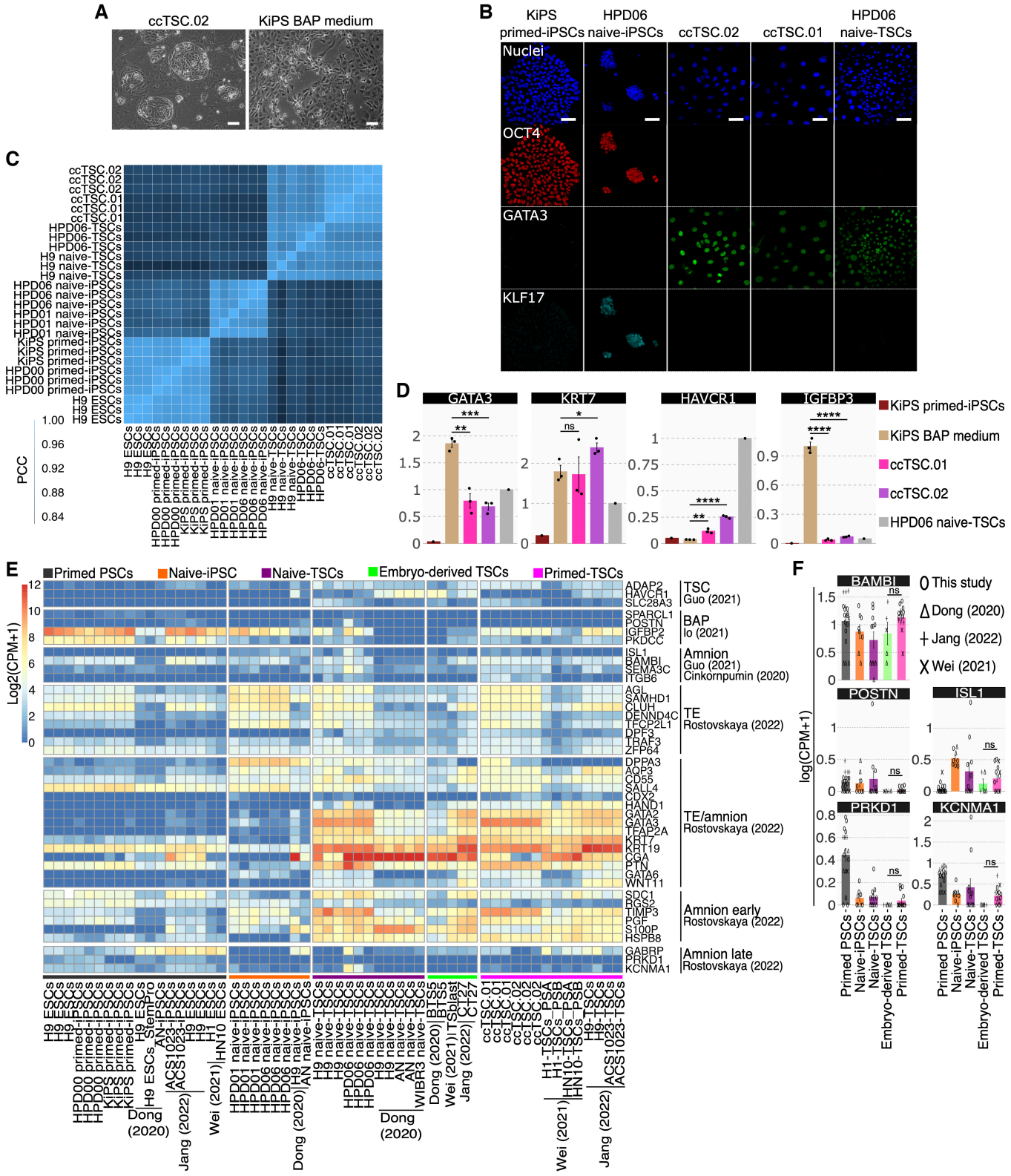


Figure EV2.

Figure EV2. Transcriptional analysis of BAP, ccTSCs and naive-TSCs.

A Phase-contrast images of ccTSC.02 at passage 5 and of BAP-treated KiPS after 3 days of treatment. Scale bar: 200 μm.

B Immunostaining for the pluripotency markers OCT4, KLF17 and the TSC marker GATA3 of KiPS primed-iPSCs, HPD06 naive-iPSCs, ccTSC.02 at passage 4, ccTSC.01 at passage 4 and HPD06 naive-TSCs. Scale bars: 30 μm.

C Correlation plot of primed PSCs (H9, HPD00 and KiPS), naive-iPSCs (HPD01 and HPD06), and TSC cells derived from naive-iPSCs (H9 naive-TSCs and HPD06 naive-TSCs) and ccTSCs (ccTSC.01 and ccTSC.02).

D Gene expression analysis by qPCR of KiPS primed-iPSCs, KiPS in BAP medium, KiPS ccTSC.01, KiPS ccTSC.02 and HPD06 naive-TSCs.

E Heatmap of TSC, BAP, amnion, TE, TE/amnion, amnion early, amnion late specific genes in primed PSCs, naive-iPSC, naive-TSCs, Embryo-derived TSCs and primed-TSCs from our study or from published data.

F Barplots showing the absolute expression as log(CPM + 1) of amnion markers in the reported conditions.

Data information: (A and B) Representative images of $n = 3$ biological replicates are shown. (D) Dots indicate biological replicates and bars indicate the mean ± SEM of $n = 3$ biological replicates. For trophoblast markers, the expression was normalised to HPD06 naive-TSCs, for amnion markers the expression was normalised to the mean of KiPS in BAP medium samples. (F) Bars indicate the mean ± SEM of at least $n = 5$ biological replicates from at least two independent studies shown with different symbols. (D–F) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns no significance (Student's t-test).

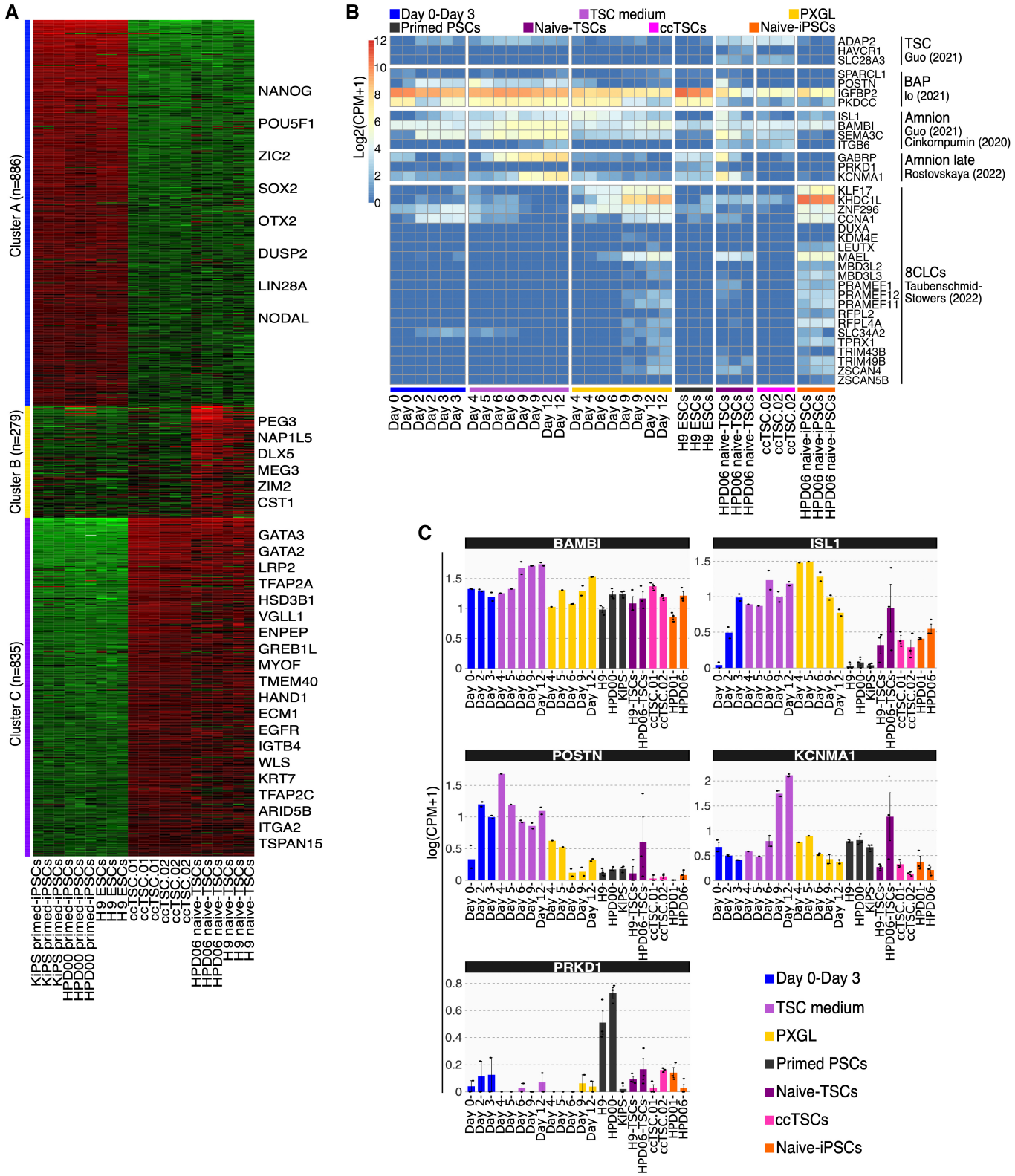


Figure EV3.

Figure EV3. Analysis of amnion and 8CLC markers during conversion.

A K-means clustering of the 2000 most variable genes in primed-iPSCs (KiPS primed-iPSCs, HPD00 primed-iPSCs and H9 ESCs), ccTSCs (ccTSC.01 and ccTSC.02), and naive-TSCs (HPD06 naive-TSC and H9 naive-TSCs). Red and green indicate high and low expression, respectively. Representative genes of each cluster are shown.

B Heatmap of TSC, BAP, amnion, amnion late and 8CLCs specific genes during the conversion of conventional PSCs into TSCs or naive-iPSCs, with primed-ESCs (H9), TSCs derived from naive-iPSCs (HPD06 naive-TSCs), ccTSC.02 and naive-iPSCs (HPD06).

C Barplots showing the absolute expression as log(CPM + 1) of amnion markers (BAMBI, ISL1, POSTN, KCNMAI and PRKD1) in the reported conditions highlighted in different colours.

Data information: (C) Dots indicate biological replicates, and bars indicate the mean ± SEM of $n = 3$ biological replicates.