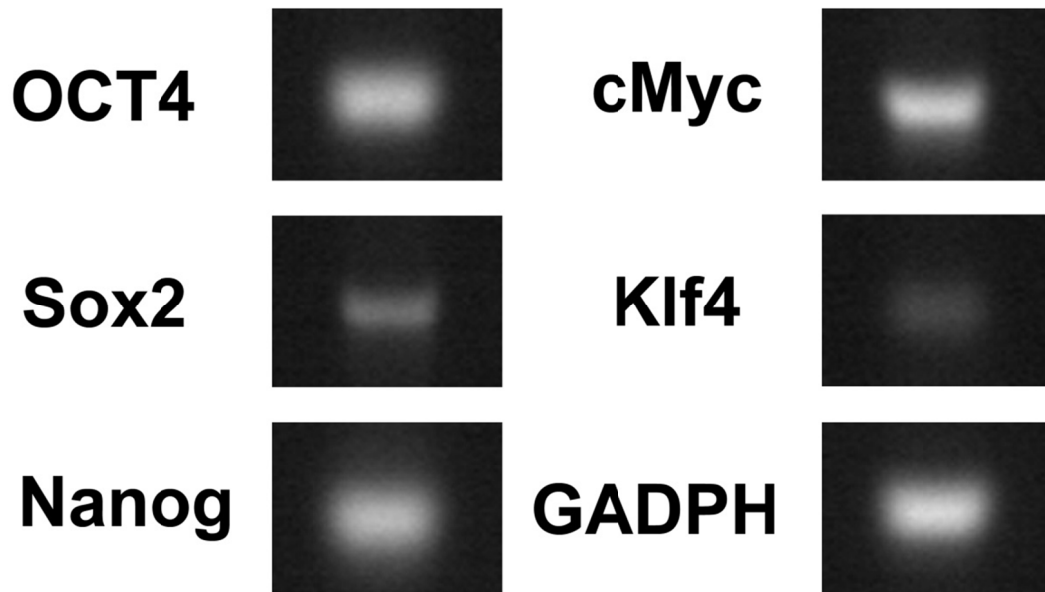


## Supplementary information

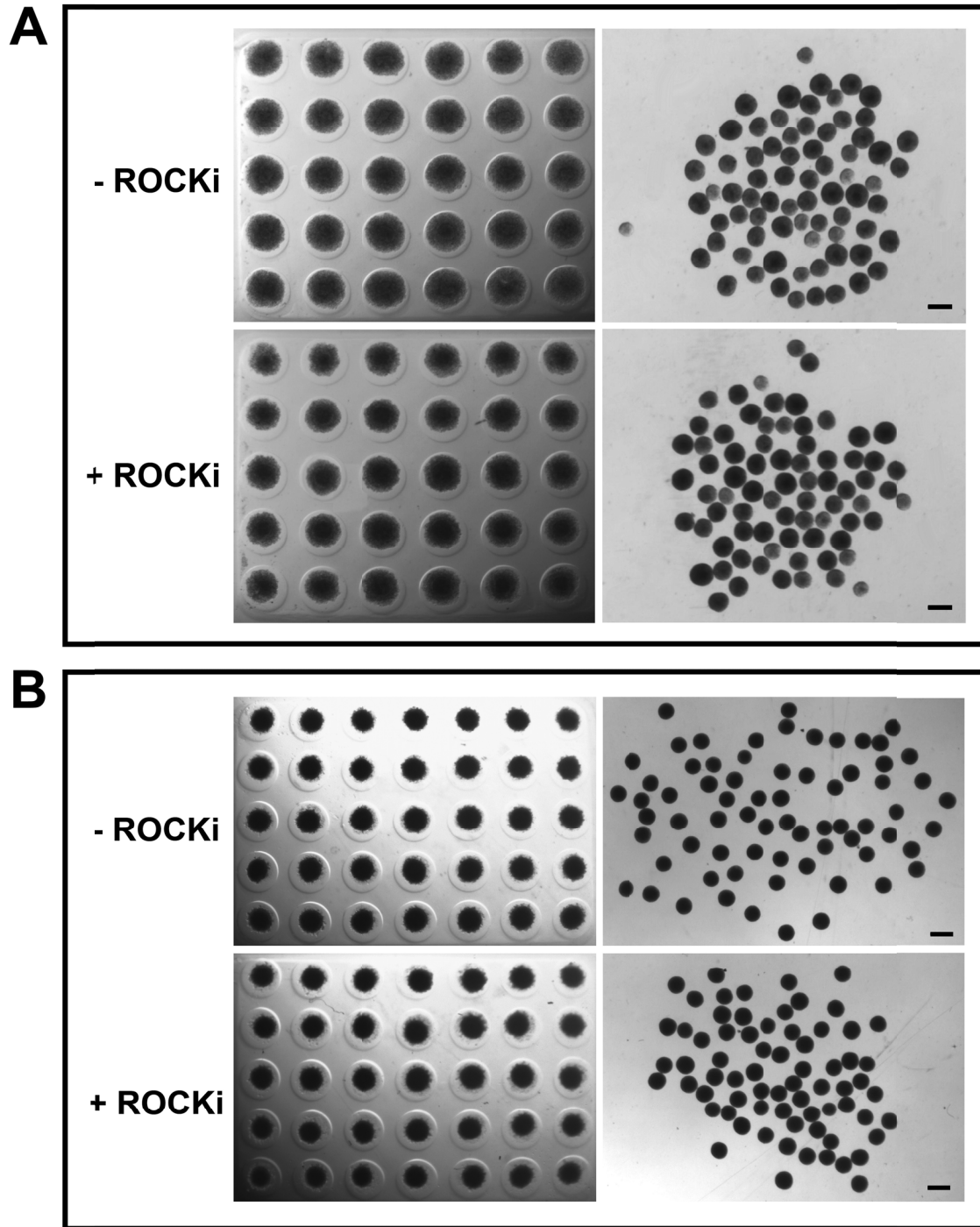
### Formation of Well-defined Embryoid Bodies from Dissociated Human Induced Pluripotent Stem Cells using Microfabricated Cell-repellent Microwell Arrays

Giuseppe Pettinato, Xuejun Wen \*, Ning Zhang \*

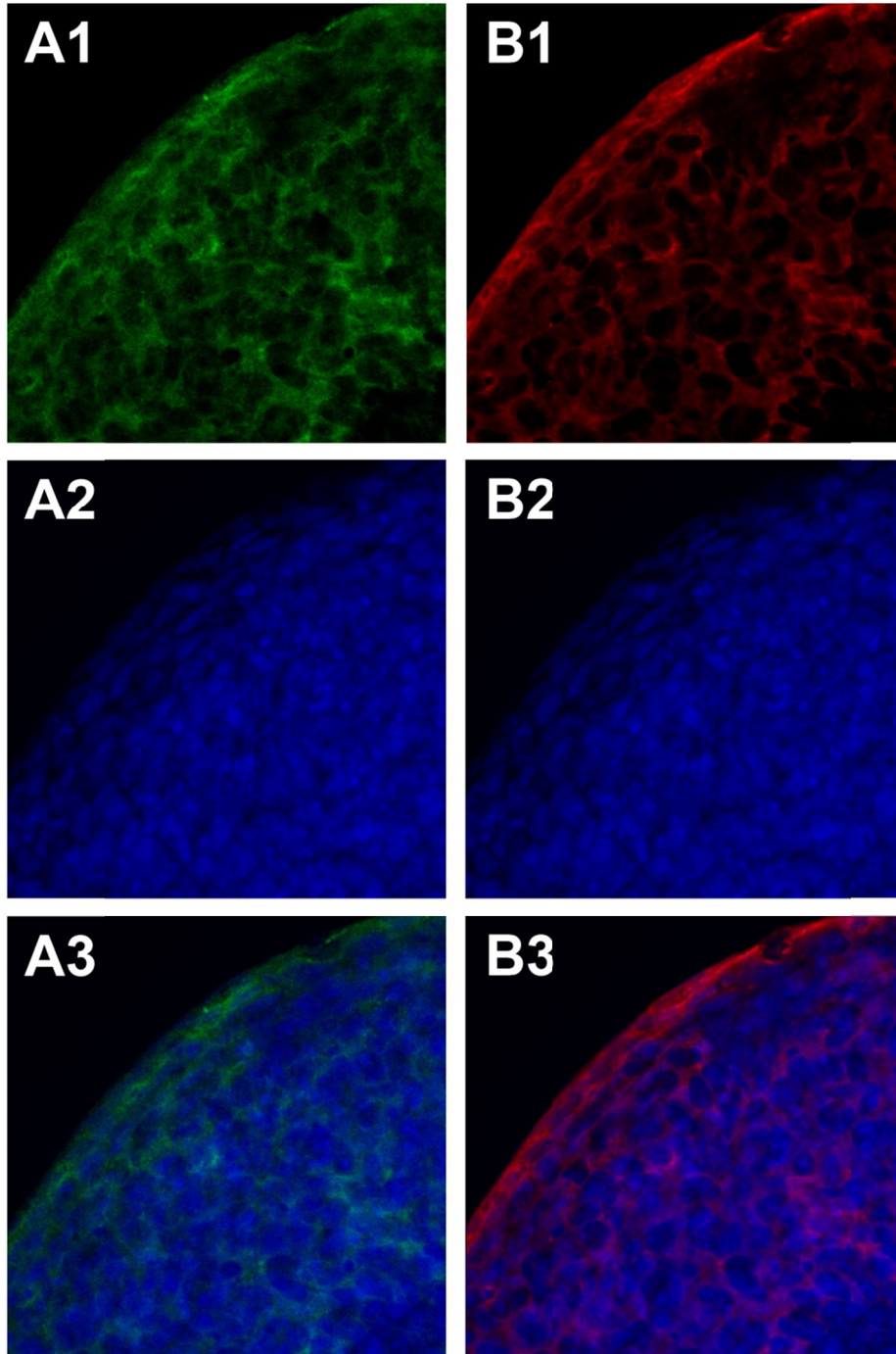
## Undifferentiation genes



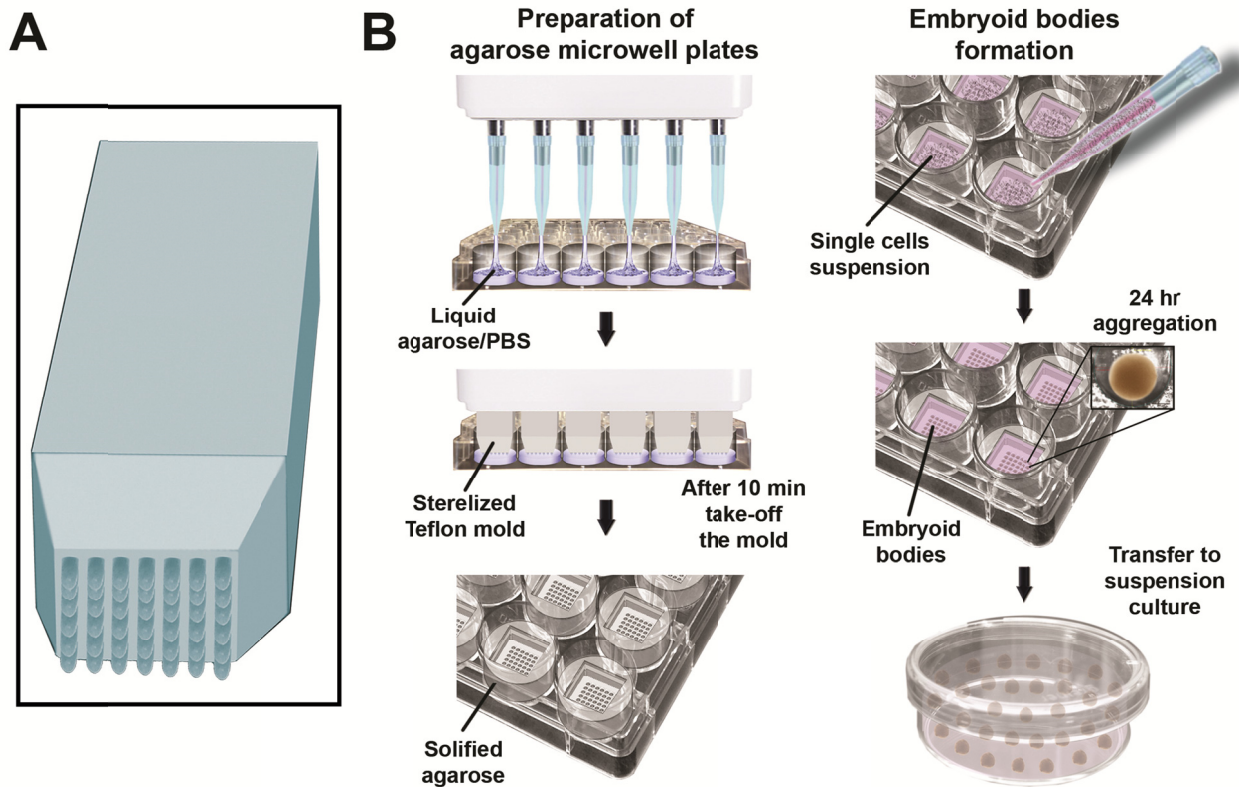
**Supplementary figure 1:** RT-PCR analysis for genes associated with pluripotency of hiPSCs used in this study (full-length gel and blot are included in the supplementary information).



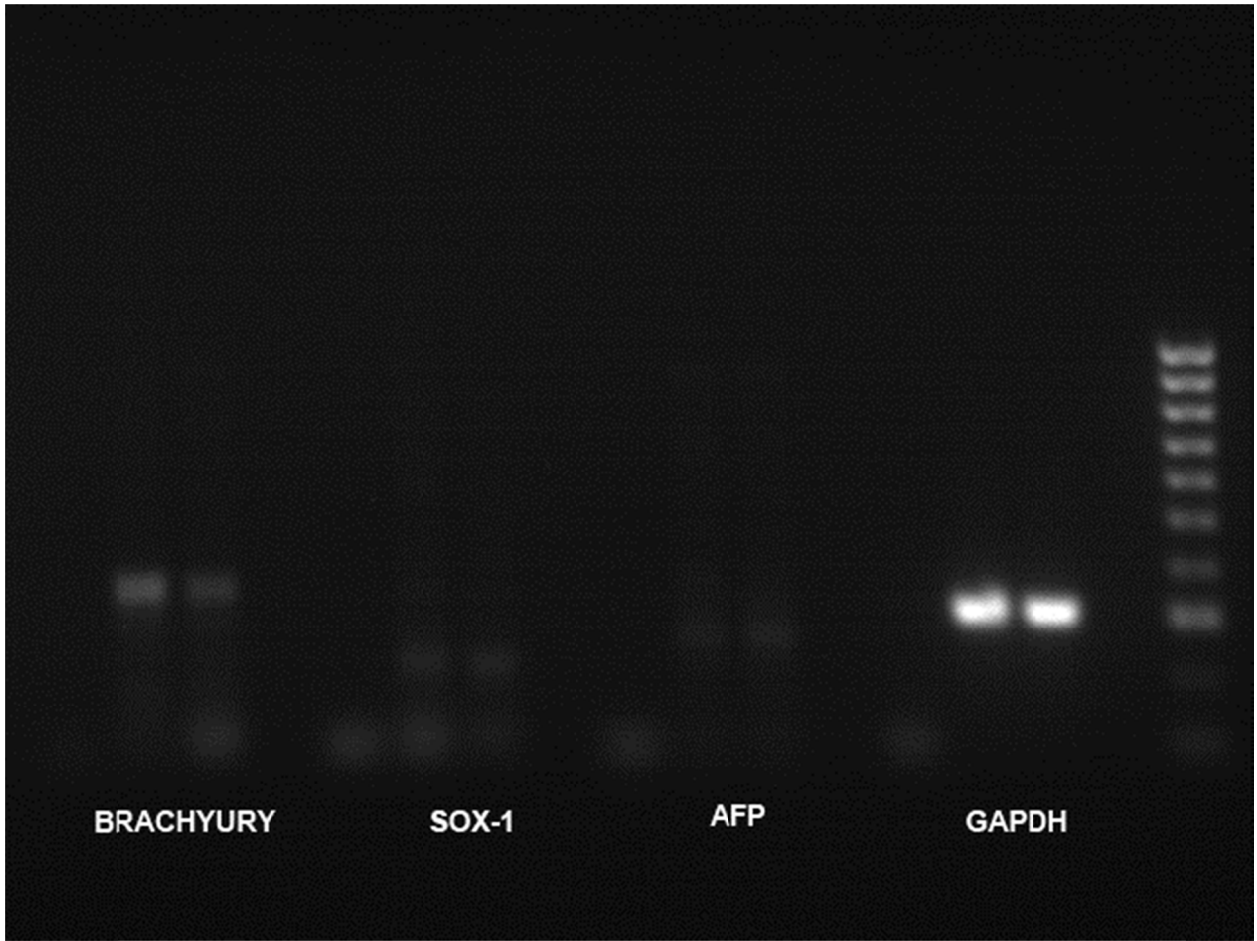
**Supplementary Figure 2:** Gross morphology of EBs from hiPSC (A) and hES (B) formed in the agarose micro-wells after 24 hrs of incubation. hiPSCs seeding density was 35,000 cells per micro-well. hES seeding density was 15,000 cells per micro-well. -ROCKi: hEBs formed under the no-ROCKi condition; +ROCKi: hEBs formed in the presence of ROCKi. Scale bar 500  $\mu$ m.



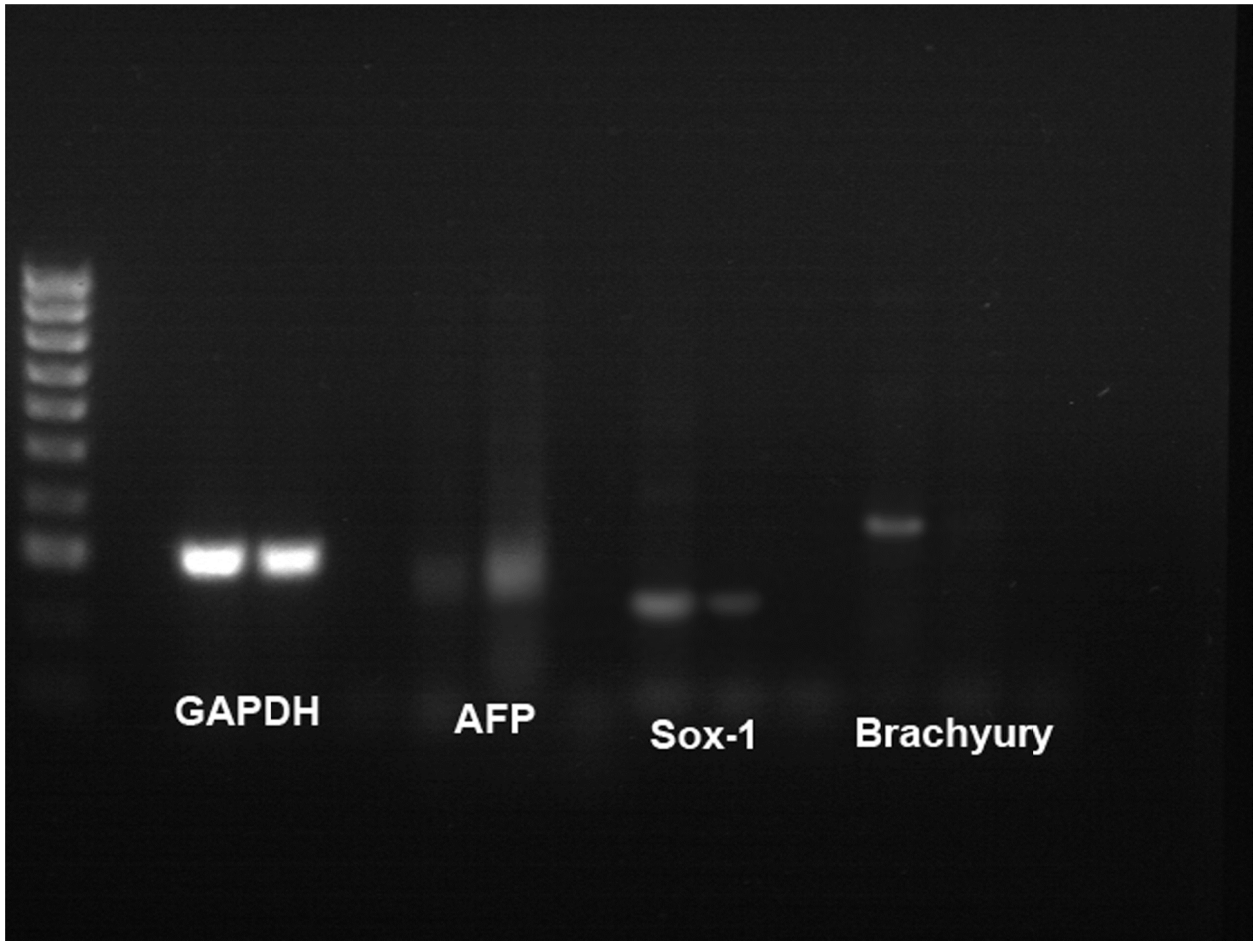
**Supplementary Figure 3:** Representative microscopic fields showing insulin (green) and C-peptide (red) immunoreactivity in a cell cluster derived from hEBs formed in our microwell system under no-ROCK-i condition.



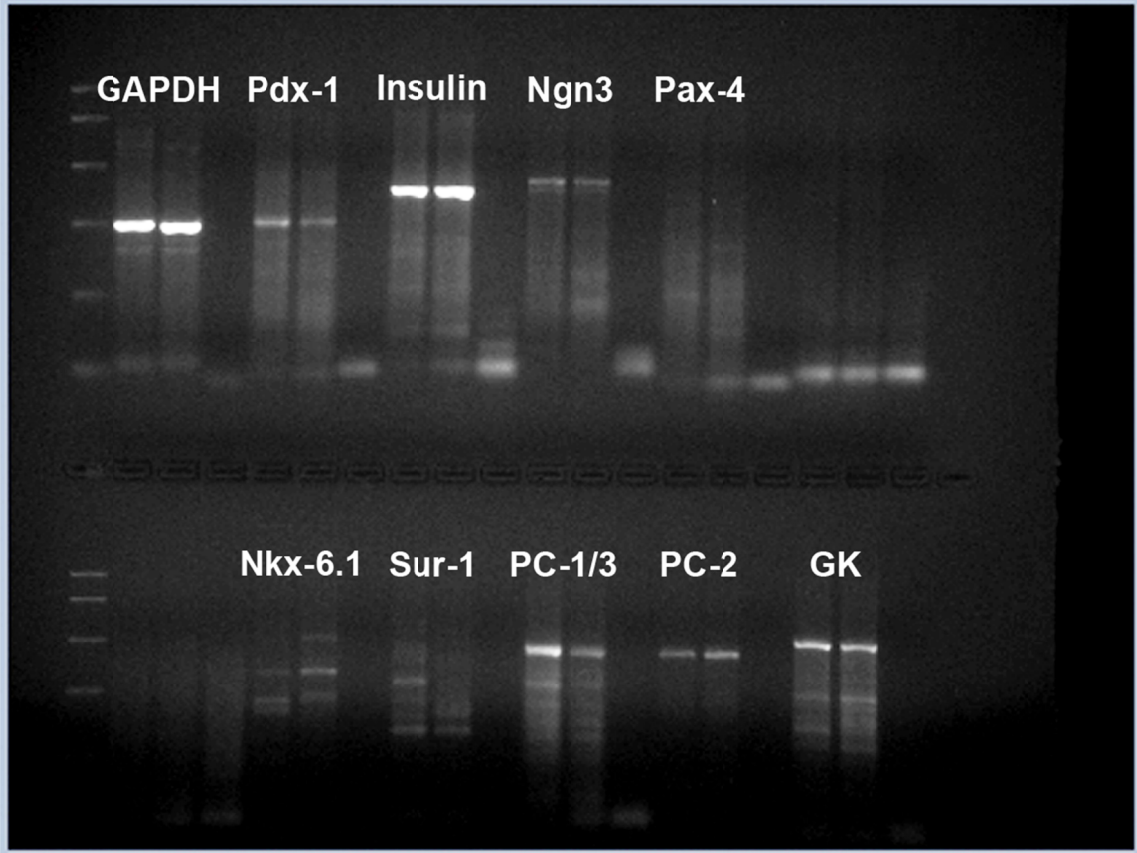
**Supplementary Figure 4:** (A) Image of a Teflon stamp with microneedle arrays that was used to create the hydrogel microwells for the hEB formation; (B) Low-melting-point agarose was used to make microwells to form hEBs. The agarose was dissolved in 1X phosphate buffered saline (PBS) at 100°C and pipetted into the culture ware. After approximately 10 min, the agarose gelled, the Teflon microneedle stamp was withdrawn, and hydrogel microwells with replicative geometry of the microneedle stamp were formed into the gel; A 50  $\mu$ l dissociated single-cell suspension of hiPSCs was dispensed into each mold to evenly fill all the microwells. After 24 hours of incubation, the formed hEBs were taken out from microwells, imaged, and transferred to suspension culture.



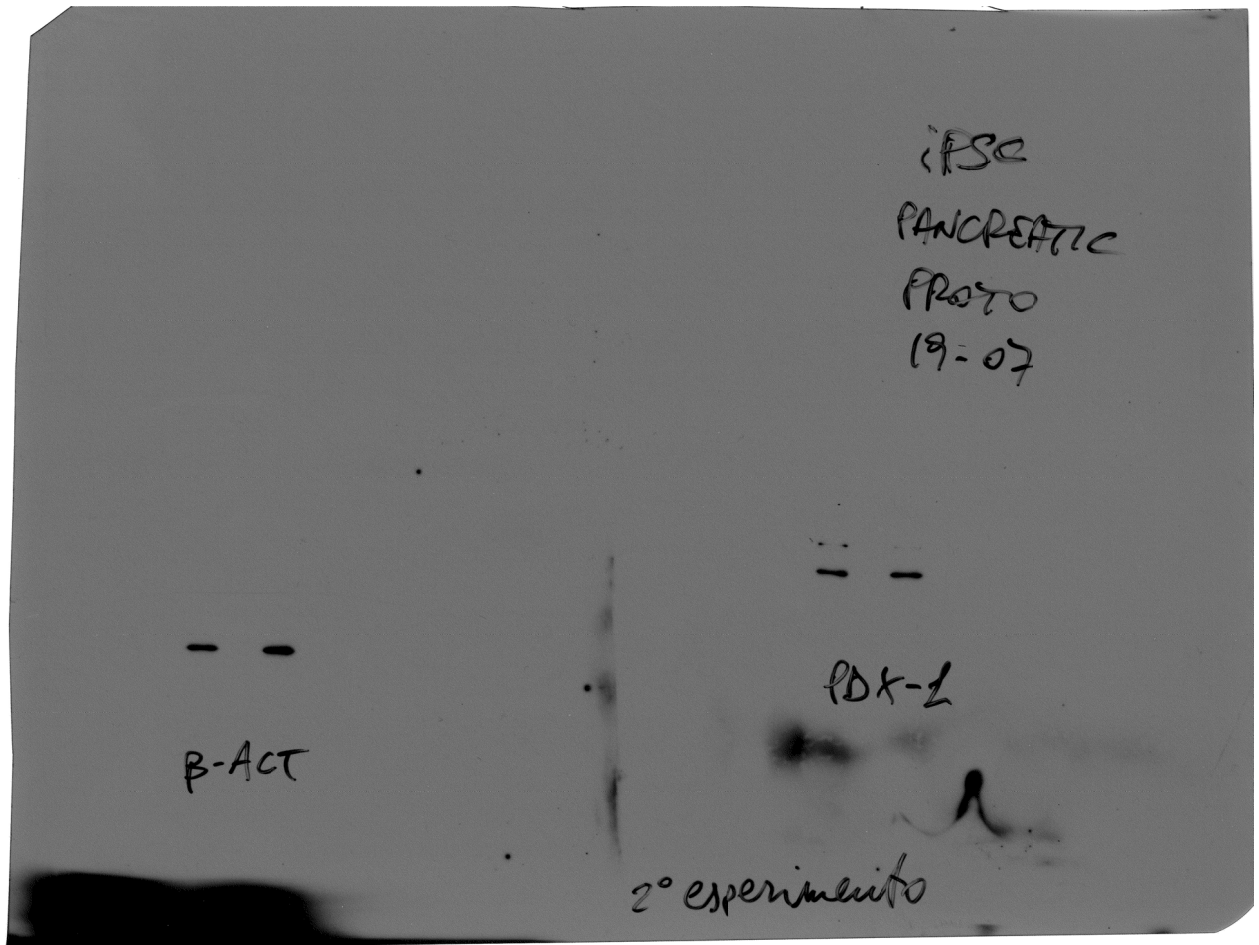
Full-length gel and blot for the Figure 5A1



Full-length gel and blot for the Figure 5B1

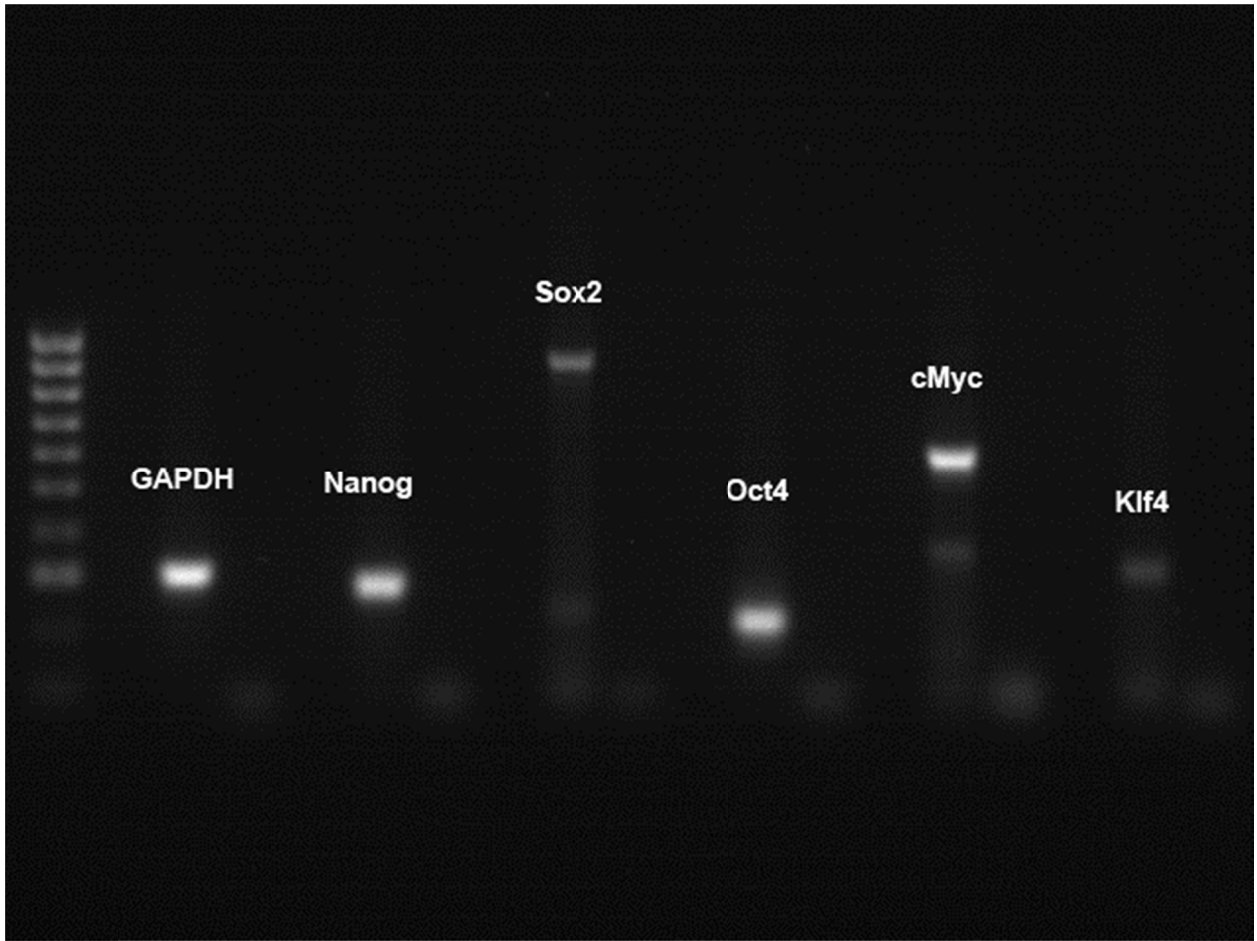


Full-length gel and blot for the Figure 6C



Full-length gel and blot for the Figure 6D





Full-length gel and blot for the Supplementary Figure 1