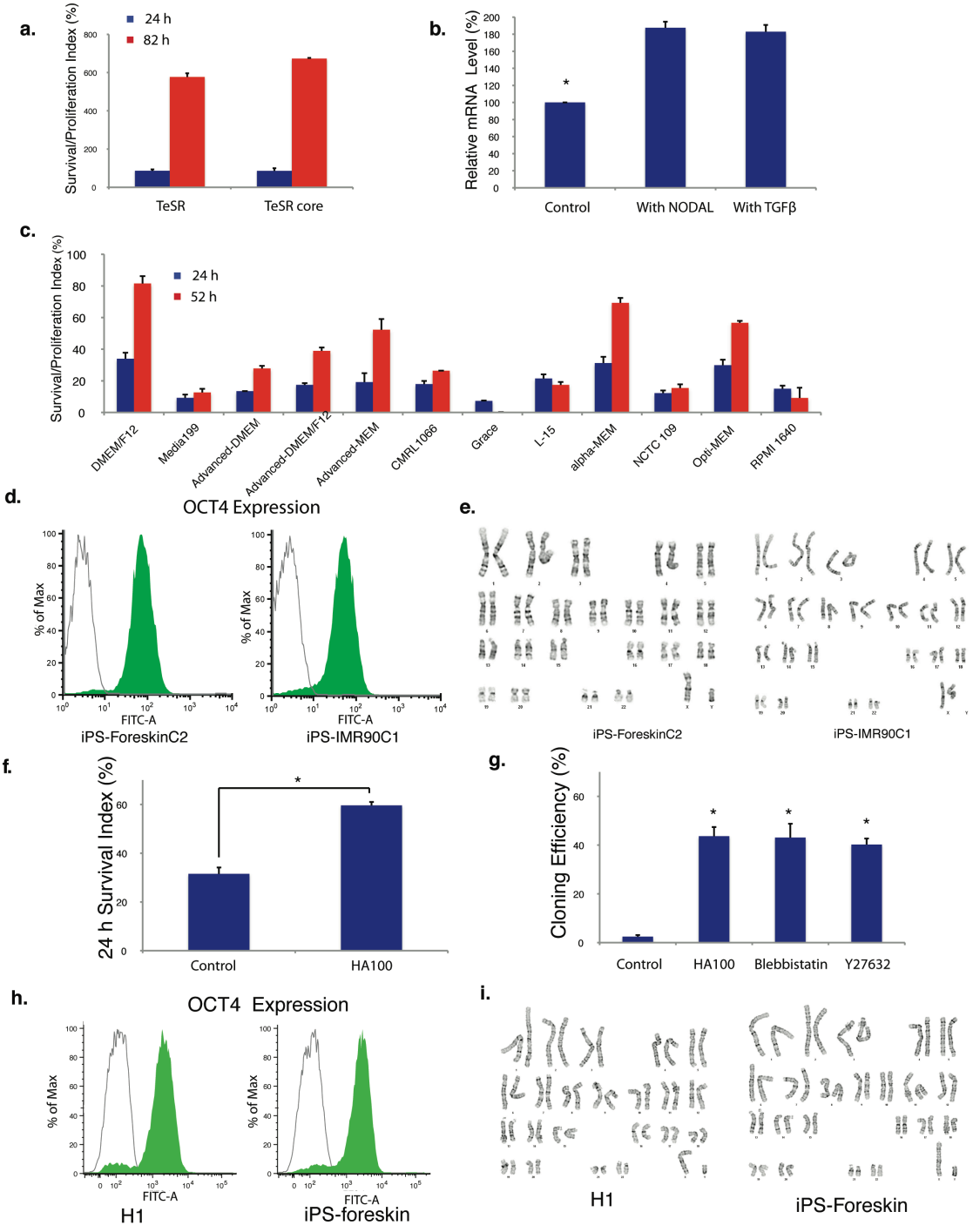
Supplementary Figure 1. Defining essential media components for human pluripotent stem cells.



a. TGFβ, LiCl, Pipecolic acid and GABA are not required for short-term survival and proliferation of human ES cells in TeSR. When those factors were removed from TeSR

(TeSR core), H1 ES cells survived (24 hours, blue columns) and proliferated (82 hours, red columns) as well as in TeSR.

b. Addition of NODAL (100 ng/ml) and TGFß (2 ng/ml) in defined media (DMEM/F12, Insulin, FGF2, LAA and Selenium) maintained significantly higher *NANOG* expression in human ES cells (*$p < 0.05$, $n = 3$). H1 cells were maintained in specific media for 5 days (2 passages), and RNA was purified for qRT-PCR to detect *NANOG* expression relative to GAPDH.

c. DMEM/F12 basic media is among the best basic media in our screen that supports human ES cell survival and proliferation. A variety of basic media were used to make growth media with additional components (Insulin, FGF2, LAA, Selenium and $NaHCO_3$) (**Supplementary Table 1**). Dissociated H1 cells were plated in different media on matrigel-coated plates. Cell survival was measured at 24 hours (blue column), and cell proliferation at 52 hours (red column).

d. Defined media (DMEM/F12, Insulin, FGF2, LAA and Selenium) with NODAL supports pluripotency of human iPS cells. Flow cytometry detected high expression of pluripotency marker OCT4 in two iPS cell lines [1]. Green Peak: OCT4 antibody with Alexa-488 conjugated secondary antibody; Grey peak: mouse IgG control. Similar results were also obtained from H1 and H9 ES cells maintained in the same media.

e. Normal karyotypes were maintained after long-term passage for those iPS cells shown above. Normal karyotypes were also maintained in H1 and H9 ES cells cultured in the same media listed above.

f. ROCK inhibitor HA100 (10 μM) improved cell survival after dissociation in TeSR (*$p < 0.05$,

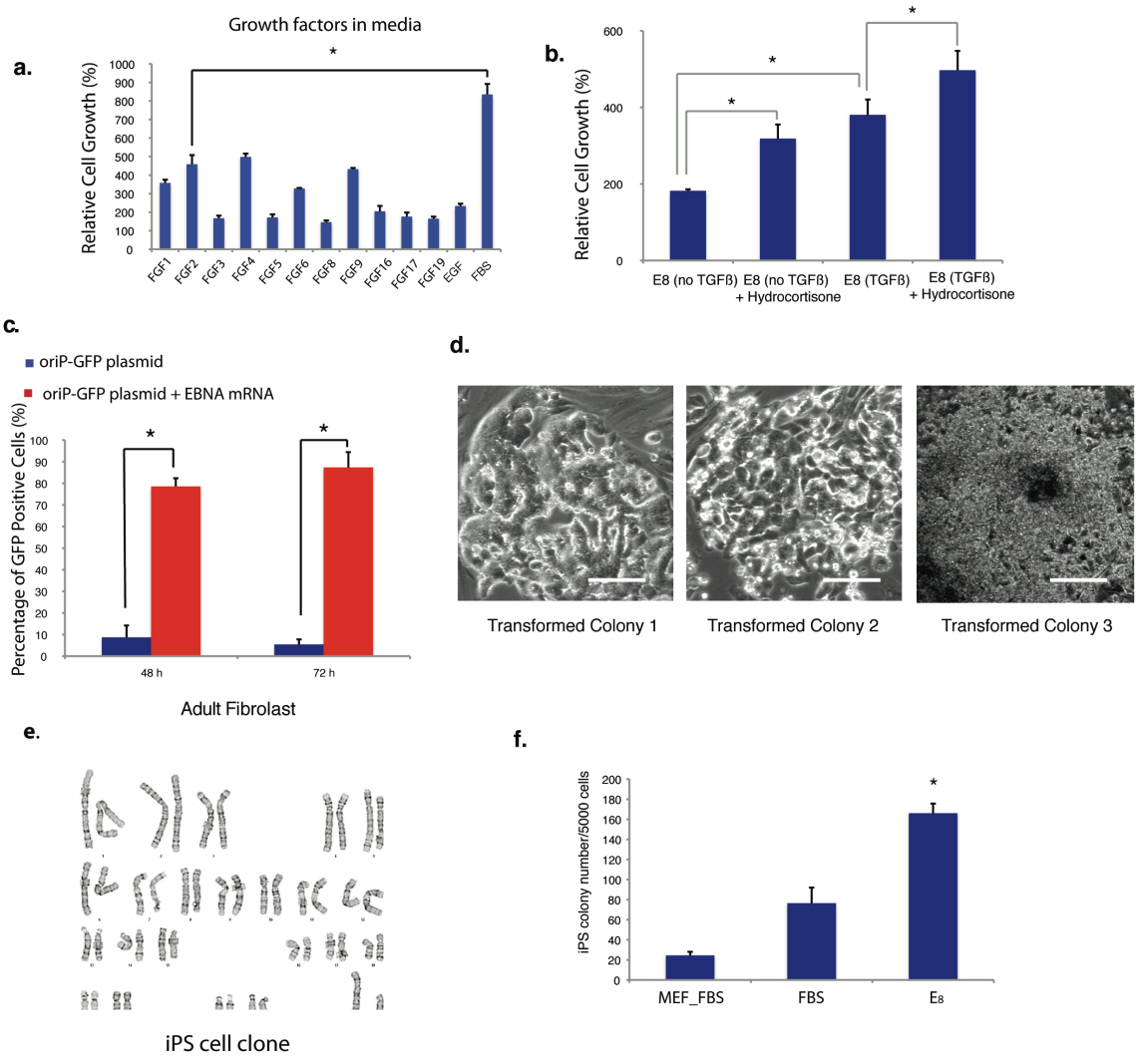
n = 3).

g. HA100 also improved cloning efficiency as efficiently as Y27632 and Blebbistatin in E8 media. Cells were treated with HA100(10 μM) and Y27632(10 μM) for 24 hours, and with blebbistatin (10 μM) for 4 hours (*$p < 0.05$, n = 3).

h. E8(TGFβ) supported proliferation and pluripotency after long-term passage in H1 and iPS cells [2]. High OCT4 expression was detected in H1 and iPS cells. Surface marker SSEA4 is also highly expressed

i. Normal karyotypes were maintained after 25 passages.

Supplementary Figure 2. Optimization of fibroblast reprogramming in defined condition.



a. FGF2 promotes the growth of foreskin cells. Foreskin fibroblast cells were used to screen fibroblast growth factors in E8 based media for cell proliferation in 120 hours, compared with FBS-containing media. (*$p < 0.05$, $n = 3$)

b. Hydrocortisone and TGFβ promotes fibroblast cell growth. Adult cell line PRPF8-2 adult fibroblast cells were cultured in specific media for 90 hours before cell growth was measured.

(*p < 0.05, n = 3)

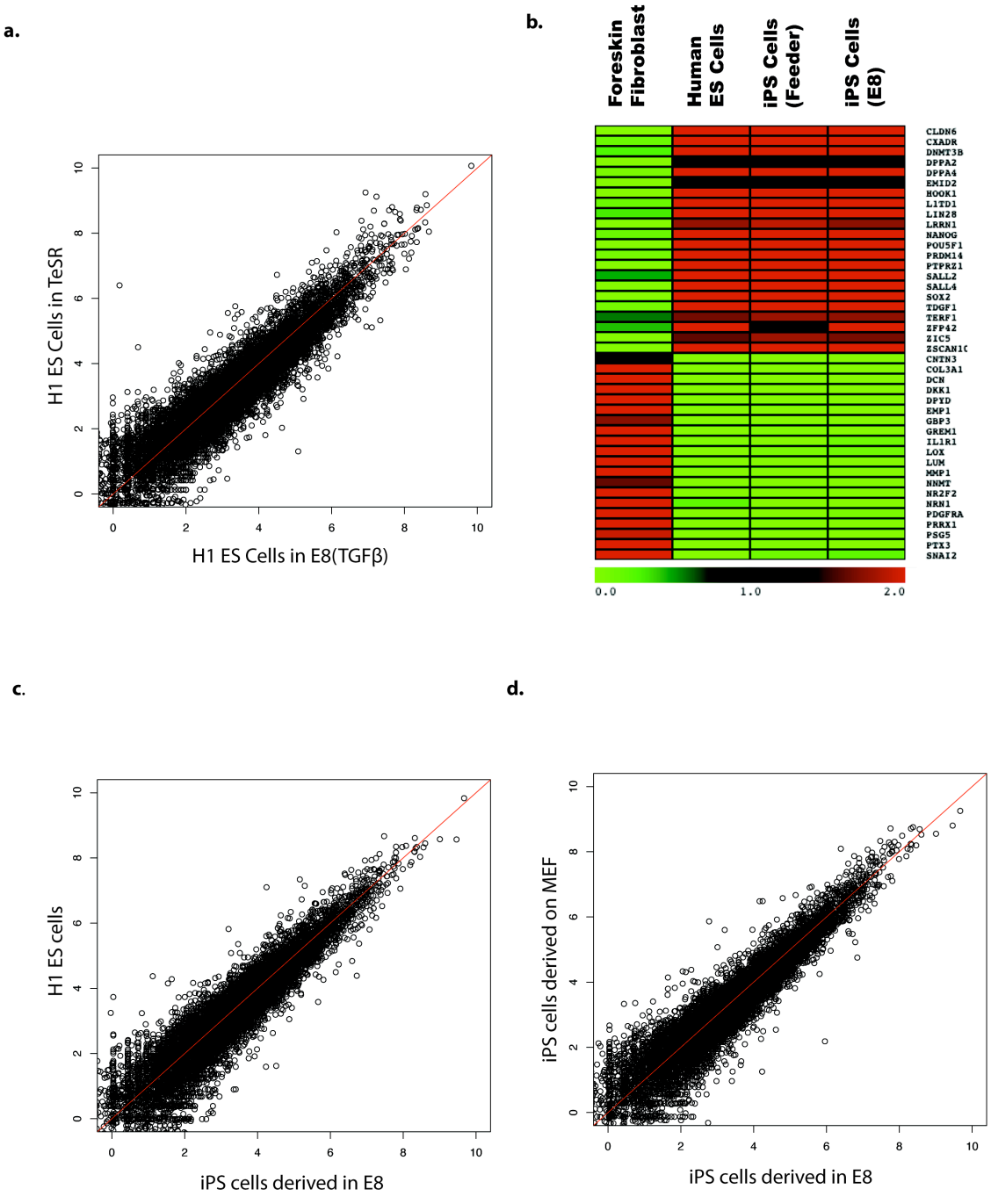
c. EBNA mRNA co-transfection improved transfection efficiency. oriP-plasmid expressing GFP was electroporated into adult fibroblast cell lines. EBNA mRNA significantly improved GFP expression percentage (*p < 0.05, n = 2). The same experiment was repeated in another unrelated adult fibroblast, and similar results were obtained. BioRad Gene Puler II was used for the electroporation (250 V, 1,000 μF) in a 0.4cm cuvette for 1 million cells suspended in Opti-MEM (Invitrogen).

d. During the reprogramming process, many colonies emerged with transformed non-fibroblast morphologies, but they were not iPS cells. The photos demonstrate typical non-iPS colonies after 25 days of reprogramming. Colonies with these morphologies were not counted when determining reprogramming efficiencies. Scale bar = 100 μm.

e. Normal karyotypes were detected for iPS cells that were derived in fully defined conditions (**Fig. 4b**).

f. Defined cell culture condition in Step 2 is critical for reprogramming (**Fig. 4a**). One day after lentivirus transduction, cells were dissociated, and plated into three conditions: feeder cells with FBS media (MEF-FBS), matrigel with FBS media (FBS) and E8 based media (**Fig. 4a**). Five days after plating, all cells were switched to E8 without TGFβ, and iPS cells were scored ~ 25 days after transduction. (*p < 0.05, n = 3)

Supplementary Figure 3. Global gene expression analysis of human ES and iPS cells in defined conditions.



a. ES cell gene expression is similar in cells grown in E8 media compared to those in TeSR. Human ES H1 cells were maintained in either TeSR or E8 (TGFβ) medium for 3

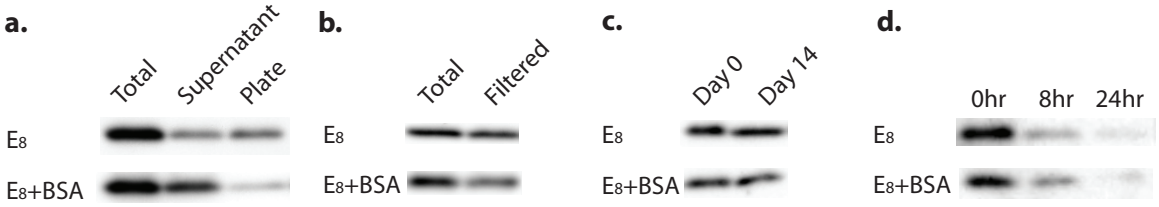
passages, and gene expression was analyzed by RNA-seq with Illumina Genome Analyzer GAIIX. The global gene expression correlation is R = 0.954 (Spearman Correlation).

b. Gene expression of iPS cells is similar to that of ES cells. iPS cells were derived from foreskin fibroblasts in E8 based cell culture (**Figure 4**). H1 ES and iPS cells were maintained in E8 (TGFβ) medium before RNA was harvested for RNA-seq. Pluripotency markers were highly expressed in both ES and iPS cells, while fibroblast specific marker genes were not expressed. Foreskin fibrolasts were maintained in E8 with hydrocortisone. iPS cells from foreskin fibroblasts were derived under defined condition (**Figure 4**, iPS Cells (E8)), or were derivated under undefined conditions on MEF (iPS Cells (Feeder))[2].

c. Global gene expression of human ES and iPS cells is closely correlated (R = 0.955). iPS cells were derived from foreskin fibroblasts on feeder cells [2] or in defined conditions (**Figure 4**), and they and ES cells were maintained in E8 (TGFβ). Gene expression was analyzed by RNA-seq.

d. Global gene expression of iPS cells is closely correlated (R = 0.959), regardless of derivation conditions. iPS cells were derived from foreskin fibroblasts on feeder cells [2] or in defined conditions (**Figure 4**), and they and ES cells were maintained in E8(TGFβ). Gene expression was analyzed by RNA-seq.

Supplementary Figure 4. Growth factor maintenance in E8 media in regular cell culture processes.



E8 media was subjected to different treatments, and FGF2 western blot was performed to detect the impact of BSA on growth factor maintenance in media.

a. Media were added into plate for 24-hour storage at 4^{o}C, and proteins were harvested from supernatant and plate to detect the localization of FGF2.
b. Media were filtered with 0.22 μm filter, and the filter-through was used for FGF2 western.
c. Media were stored at 4^{o}C for 2 weeks, and FGF2 was measured before and after storage.
d. Media were incubated at 37^{o}C, and were harvested at different time points (8-hour and 24-hour) to assay the loss of FGF2.

Supplementary Table 1: Media components used in this study.

Components	TeSR	TeSR core	E8*
DMEM/F12 (liquid)	■	■	■
L-Ascorbic Acid	■	■	■
Selenium	■	■	■
Transferrin	■	■	■
NaHCO3	■	■	■
Glutathione	■	■	
L-Glutamine	■	■	
Defined lipids	■	■	
Thiamine	■	■	
Trace elements B	■	■	
Trace elements C	■	■	
ß-mercaptoethanol	■	■	
Albumin (BSA)	■	■	
Insulin	■	■	■
FGF2	■	■	■
TGFß1	■		▩
Pipecolic acid	■		
LiCl	■		
GABA	■		
H2O	■		
NODAL			▩
Hydrocortisone			
Butyrate			

* In E8 media, NODAL (100 ng/ml) and TGFβ (2 ng/ml) are interchangeable in maintaining ES and iPS cells. When NODAL is used, the media is specified as E8 (NODAL), and vice versa for TGFβ, specified as E8 (TGFβ).

Supplementary Table 2: Pluripotent cells maintained in defined conditions.

Cell lines maintained in E8 media	
ES cells	H1, H9
iPS cells	iPS-imr90[1], iPS-foreskin[1], iPS-DF19[2], and all iPS lines derived in E_8 media
iPS cells derivated and maintained in E8 based media[+]*[%]	
Banked cells	Foreskin-p10 (30-200), OAT-p6 (30), PRPF-p6 (8), PRPF8-2-p6 (4), AG04054-p7 (32)
Derived cells	004-p4 (120), 005-p4 (60), 010A-p4 (~1000), 10B-p4 (100)

[1] iPS cells derived with lentiviral approaches [1].

[2] iPS cells derived with episomal approaches [2].

[+] Only episomal-reprogrammed cells were listed in the table. More cell lines were derived with lentiviral approach in E8 based media.

***** Reprogramming efficiency (iPS cell colonies/10^6 transfected cells) for each cell line was listed in brackets.

[%] Fibroblast culture passage number was placed at the end of cell name. For example, Foreskin-p8 means that foreskin cells were reprogrammed at passage 8.

Supplementary Table 3. Primer set used for RT-qPCR.

Gene names	Forward primer	Reverse primer
GAPDH	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT
NANOG	TTCCTTCCTCCATGGATCTG	TCTGCTGGAGGCTGAGGTAT

Supplemental References

1 Yu, J. Y. *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917-1920 (2007).

2 Yu, J. Y. *et al.* Human Induced Pluripotent Stem Cells Free of Vector and Transgene Sequences. *Science* **324**, 797-801 (2009).