Supplementary information, Data S1 Material and methods

Cell culture

Human embryonic stem cells were cultured in E8 medium on Matrigel coated plates[1]. Cells were split every 4 to 5 days with 0.5mM EDTA in 1xPBS[2]. Human 293FT cells and human foreskin fibroblast (HFF) cells were cultured in DMEM/F12 medium supplemented with 10% FBS.

Whole cell SELEX

The aptamer library used in the selection and the selection procedure is the same as previously reported[3] with the following exceptions. Human ESC cell line H1 was individualized using Accutase (Life Technologies) and washed with medium once before use. The number of cells used in the selection decreased in each round, approximately $1.2x10^7$ cells were used in the first round, $3.7x10^6$ used in the second round, $1.3x10^6$ in the third round, $1x10^6$ in the fourth round and $6.5x10^5$ in the fifth round. No negative selection was performed for the SELEX with hESCs. Single stranded DNA eluted from the cells was amplified with 3 cycles of PCR before submitting for high throughput sequencing.

Next generation sequencing of aptamer libraries

Sequencing of aptamer libraries was done according to previously published procedures. Briefly, indexed and double-stranded DNA aptamers were generated using TruSeq DNA sample preparation kit v.2 (Illumina). We then amplified the library with 15 cycles of PCR. The final library was then run on HiSeq2000 (Illumina) with a Single Read 80 Base Pair

program to obtain the whole aptamer sequence and the index. FASTQ files were generated using Illumina's CASAVA software (v1.8.2). We transformed the aptamers (29nt) to sense strand based on matching the primer regions (forward or reverse) allowing one mismatch. All the aptamers can be found via the link (http://www.morgridge.net/Aptamer/).

Affinity measurement of truncated aptamers

Biotinylated aptamers with different truncations in the constant regions were synthesized by IDT. Right before use, the aptamers were diluted in aptamer binding buffer (dPBS ($+Ca^{2+}/Mg^{2+}$), 5mM $MgCl_2$, 0.45% glucose, 0.1% BSA) to 50nM and heated to 95°C for 10min and snap cooled on ice for at least 10min. Human ESC were individualized with TrypLE (Life Technologies), washed with binding buffer and resuspended with diluted aptamer solution to $1x10^6$ cells/ml and incubated for 1hr at 4°C. After incubation, cells were washed 3 times with aptamer buffer and then resuspended in aptamer buffer with 1:500 dilution of Alexa Fluor 594 conjugated streptavidin (Life Technologies) and incubated at 4°C for 30min. Cells were then washed 3 times and analyzed with flow cytometry. The affinity of each aptamer against hESCs was represented by the mean fluorescence value of intact cells measured by the flow cytometer.

Pluripotent stem cell sorting using Apt19S

Apt19S and Apt19S-SC were synthesized with 5' Biotin by IDT. Aptamers were diluted in binding buffer (Hank's Balanced Salt Solution (HBSS, Life Technologies) with 5mM $MgCl_2$) to 200nM, heated to 95°C for 10min and cooled on ice for at least 10min. BSA was added

to 1% final concentration before use. Individualized human ESC and HFF cells were mixed at 1:4 ratio and resuspended in diluted aptamer solution at $5x10^5$ cells per ml concentration. AlexaFluor488 labeled anti-SSEA4 antibody was added at 1:100 dilution. The reactions were incubated at 4°C for 20min. Cells were then pelleted, washed twice with binding buffer and resuspended in 0.5ml binding buffer with 5µl Streptavidin labeled C1 dynabeads (10mg/ml, Life Technologies). The suspension was incubated at 4°C for 20min and the dynabeads were separated from the rest of the cell suspension on a magnet and washed 3 times with binding buffer. Portions of the unbound cells and the cells bound to the magnetic beads were analyzed by flow cytometry. The rest of the cells bound to the magnetic beads were then resuspended in E8 medium supplemented with 10µM Y27632 and plated onto Matrigel (BD Biosciences) coated tissue culture plates for further expansion. Cells were fixed after 7 days of culture and stained for pluripotency markers Pou5f1 and Nanog.

iPS cell enrichment using Apt19S

Reprogramming was done using CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Life Technologies). Briefly, human foreskin fibroblast cells were plated at $7x10^3$ per well in a 24-well plate one day before virus addition. Virus was added according to manufacture suggested ratios. Culturing media was changed 4 days after virus addition to E8 with 100µM sodium butyrate without TGFβ1. Media was then changed everyday. Cells were individualized using Accutase (Life Technologies) 14 days after virus addition. iPS cell enrichment using aptamers was then carried out as described in the pluripotent stem cell

enrichment section. Sorted cells were analyzed with flow cytometry for SSEA4 expression after 5 days of culture and POU5F1 and NANOG expression using immunostaining after 8 days of culture.

Cell differentiation and RNA sequencing analysis

Differentiation of hESCs to neural progenitor cells and trophoblast cells was done according to previously published protocols[4]. The RNA sequencing data sets were from the same study.

ALPL knock down with siRNA

siRNAs targeting ALPL and BMP4 were purchased from Dharmacon. Transfection was done using DharmaFECT formulation 1 according to manufacture recommended procedures. Cells were assayed after 48-72 hours of transfection. ALPL knock-down efficiency was monitored with a non-competing anti-ALPL antibody (TRA-2-39 from Santa Cruz).

Antibody competition with aptamer

Competing anti-ALPL antibody was purchased from Biolegend (cat number 327307). For competition assays, cells are incubated with APC labeled anti-ALPL antibody at 1:100 dilution and increasing concentrations of Apt19S. The binding of the antibody was then analyzed on a FACSCanto II flow cytometer (BD Biosciences).

Overexpression of ALPL and PROM1 in 293 FT cells

Plasmids that overexpress ALPL (EX-C0757-M02) or PROM1 (EX-M0038-M02) were purchased from Genecopia. These plasmids were transfected into 293FT cells using FugeneHD (Promega) with 1µg plasmid and 3µl transfection reagent per well of a 12 well plate. Cells were used after 48 hours of transfection.

Circular dichroism (CD) analysis of Apt19S and Apt19S-SC

The analysis was carried out at Alliance Protein Laboratories (San Diego CA USA). Briefly, both oligos were dissolved at 1mg/ml and subjected to CD measurements using 0.05 cm cell and the following parameters: 10 nm/min, 4 sec time constant, 0.1 nm data pitch, 1 nm band width, 5 accumulations. CD measurements were carried out at room temperature on a Jasco J-715 spectrophotometer.

Supplemental references

1 Chen G, Gulbranson DR, Hou Z *et al.* Chemically defined conditions for human iPSC derivation and culture. *Nat Methods* 2011; **8**:424-429.

2 Beers J, Gulbranson DR, George N *et al.* Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. *Nat Protoc* 2012; **7**:2029-2040.

3 Meyer S, Maufort JP, Nie J *et al.* Development of an efficient targeted cell-SELEX procedure for DNA aptamer reagents. *PLoS One* 2013; **8**:e71798.

4 Xie W, Schultz MD, Lister R *et al.* Epigenomic analysis of multilineage differentiation of human embryonic stem cells. *Cell* 2013; **153**:1134-1148.