

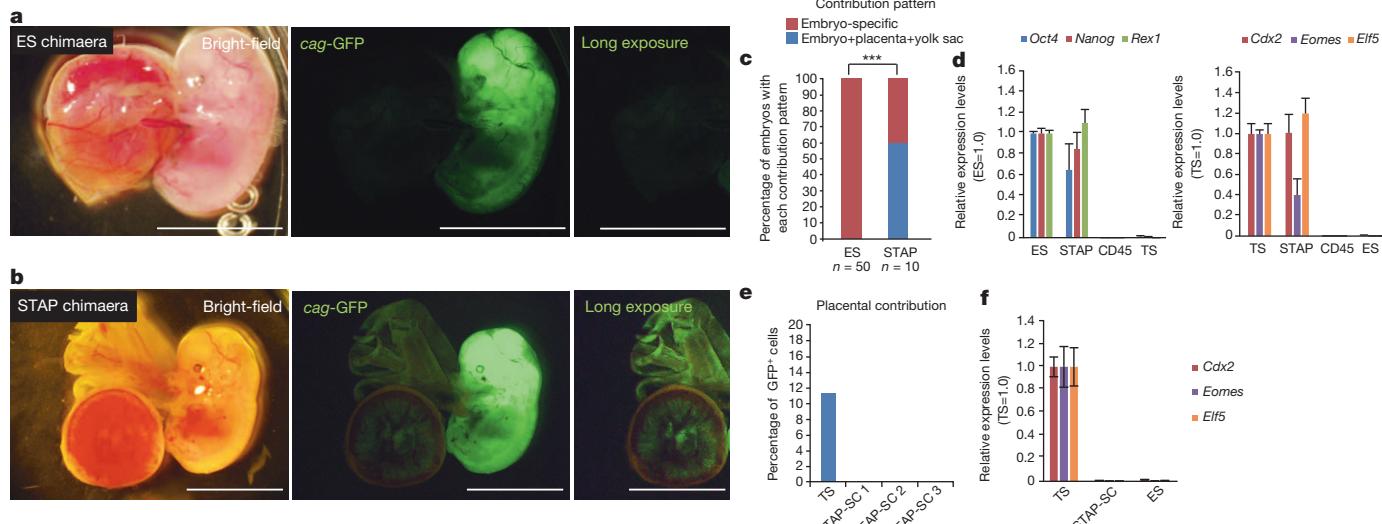
# Bidirectional developmental potential in reprogrammed cells with acquired pluripotency

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We recently discovered an unexpected phenomenon of somatic cell reprogramming into pluripotent cells by exposure to sublethal stimuli, which we call stimulus-triggered acquisition of pluripotency (STAP)<sup>1</sup>. This reprogramming does not require nuclear transfer<sup>2,3</sup> or genetic manipulation<sup>4</sup>. Here we report that reprogrammed STAP cells, unlike embryonic stem (ES) cells, can contribute to both embryonic and placental tissues, as seen in a blastocyst injection assay. Mouse STAP cells lose the ability to contribute to the placenta as well as trophoblast marker expression on converting into ES-like stem cells by treatment with adrenocorticotropic hormone (ACTH) and leukaemia inhibitory factor (LIF). In contrast, when cultured with Fgf4, STAP cells give rise to proliferative stem cells with enhanced trophoblastic characteristics. Notably, unlike conventional trophoblast stem cells, the Fgf4-induced stem cells from STAP cells contribute to both embryonic and placental tissues *in vivo* and transform into ES-like cells when cultured with LIF-containing medium. Taken

together, the developmental potential of STAP cells, shown by chimaera formation and *in vitro* cell conversion, indicates that they represent a unique state of pluripotency.

We recently discovered an intriguing phenomenon of cellular fate conversion: somatic cells regain pluripotency after experiencing sublethal stimuli such as a low-pH exposure<sup>1</sup>. When splenic CD45<sup>+</sup> lymphocytes are exposed to pH 5.7 for 30 min and subsequently cultured in the presence of LIF, a substantial portion of surviving cells start to express the pluripotent cell marker Oct4 (also called Pou5f1) at day 2. By day 7, pluripotent cell clusters form with a bona fide pluripotency marker profile and acquire the competence for three-germ-layer differentiation as shown by teratoma formation. These STAP cells can also efficiently contribute to chimaeric mice and undergo germline transmission using a blastocyst injection assay<sup>1</sup>. Although these characteristics resemble those of ES cells, STAP cells seem to differ from ES cells in their limited capacity for self-renewal (typically, for only a few



**Figure 1 | STAP cells contribute to both embryonic and placental tissues *in vivo*.** **a, b**, E12.5 embryos from blastocysts injected with ES cells (**a**) and STAP cells (**b**). Both cells are genetically labelled with GFP driven by a constitutive promoter. Progeny of STAP cells also contributed to placental tissues and fetal membranes (**b**), whereas ES-cell-derived cells were not found in these tissues (**a**). Scale bar, 5.0 mm. **c**, Percentages of fetuses in which injected cells contributed only to the embryonic portion (red) or also to placental and yolk sac tissues (blue). \*\*\*P<0.001 with Fisher's exact test. **d, e**, qPCR analysis of FACS-sorted Oct4-GFP-strong STAP cells for pluripotent marker genes (left) and trophoblast marker genes (right). Values are shown as ratio to the expression level in ES cells. Error bars represent s.d. **e**, Contribution to placental tissues. Unlike parental STAP cells and trophoblast stem (TS) cells, STAP stem cells (STAP-SCs) did not retain the ability for placental contributions. Three independent lines were tested and all showed substantial contributions to the embryonic portions. **f**, qPCR analysis of trophoblast marker gene expression in STAP stem cells. Error bars represent s.d.

analysis of FACS-sorted Oct4-GFP-strong STAP cells for pluripotent marker genes (left) and trophoblast marker genes (right). Values are shown as ratio to the expression level in ES cells. Error bars represent s.d. **e**, Contribution to placental tissues. Unlike parental STAP cells and trophoblast stem (TS) cells, STAP stem cells (STAP-SCs) did not retain the ability for placental contributions. Three independent lines were tested and all showed substantial contributions to the embryonic portions. **f**, qPCR analysis of trophoblast marker gene expression in STAP stem cells. Error bars represent s.d.

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passages) and in their vulnerability to dissociation<sup>1</sup>. However, when cultured in the presence of ACTH and LIF for 7 days, STAP cells, at a moderate frequency, further convert into pluripotent ‘stem’ cells that robustly proliferate (STAP stem cells).

Here we have investigated the unique nature of STAP cells, focusing on their differentiation potential into the two major categories (embryonic and placental lineages) of cells in the blastocyst<sup>5–8</sup>. We became particularly interested in this question after a blastocyst injection assay revealed an unexpected finding. In general, progeny of injected ES cells are found in the embryonic portion of the chimaera, but rarely in the placental portion<sup>5,7</sup> (Fig. 1a; shown with Rosa26-GFP). Surprisingly, injected STAP cells contributed not only to the embryo but also to the placenta and fetal membranes (Fig. 1b and Extended Data Fig. 1a–c) in 60% of the chimaeric embryos (Fig. 1c).

In quantitative polymerase chain reaction (qPCR) analysis, STAP cells (sorted for strong Oct4-GFP signals) expressed not only pluripotency marker genes but also trophoblast marker genes such as Cdx2 (Fig. 1d and Supplementary Table 1 for primers), unlike ES cells. Therefore, the blastocyst injection result is not easily explained by the idea that STAP cells are composed of a simple mixture of pluripotent cells (Oct4<sup>+</sup>Cdx2<sup>-</sup>) and trophoblast-stem-like cells (Oct4<sup>-</sup>Cdx2<sup>+</sup>).

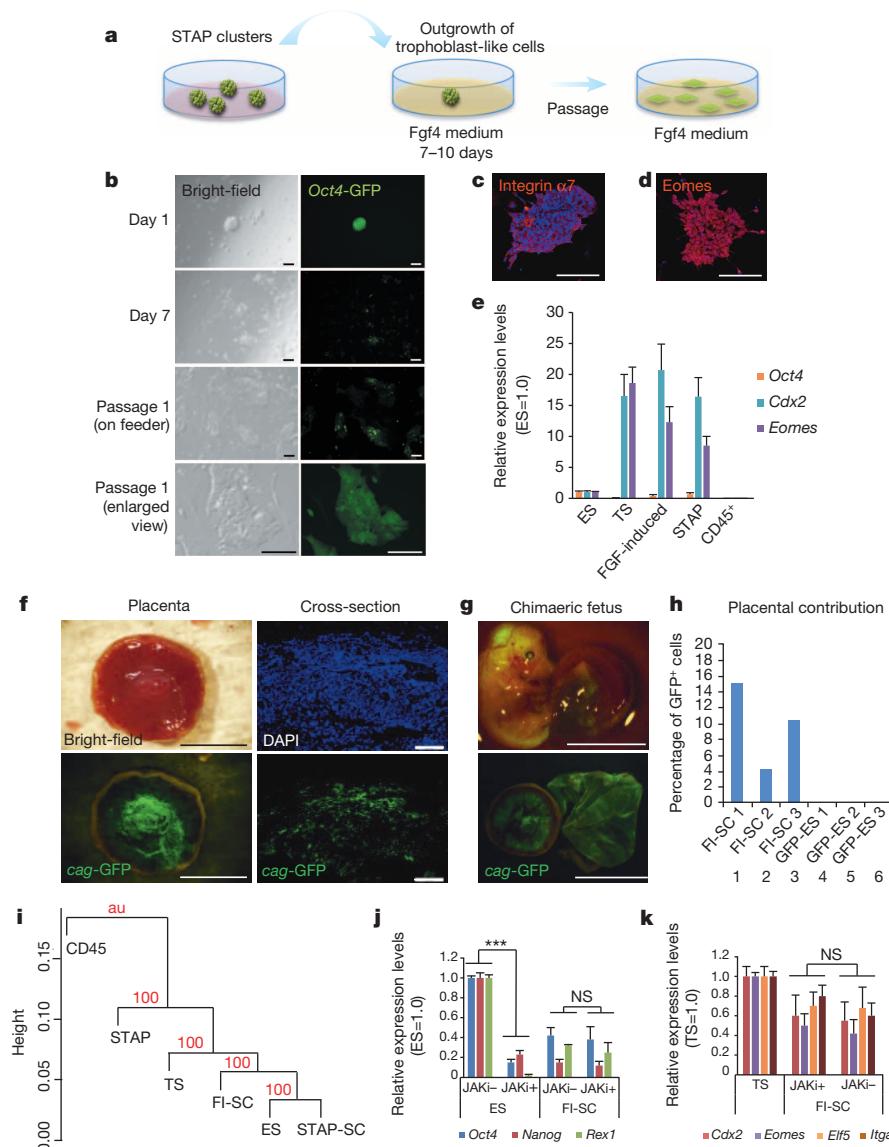
In contrast to STAP cells, STAP stem cells did not show the ability to contribute to placental tissues (Fig. 1e, lanes 2–4), indicating that the

derivation of STAP stem cells from STAP cells involves the loss of competence to differentiate into placental lineages. Consistent with this idea, STAP stem cells show little expression of trophoblast marker genes (Fig. 1f).

We next examined whether an alteration in culture conditions could induce *in vitro* conversion of STAP cells into cells similar to trophoblast stem cells<sup>8,9</sup>, which can be derived from blastocysts during prolonged adhesion culture in the presence of Fgf4. When we cultured STAP cell clusters under similar conditions (Fig. 2a; one cluster per well in a 96-well plate), flat cell colonies grew out by days 7–10 (Fig. 2b, left; typically in ~30% of wells). The Fgf4-induced cells strongly expressed the trophoblast marker proteins<sup>9–12</sup> integrin  $\alpha$ 7 (Itga7) and eomesodermin (Eomes) (Fig. 2c, d) and marker genes (for example, Cdx2; Fig. 2e).

These Fgf4-induced cells with trophoblast marker expression could be expanded efficiently in the presence of Fgf4 by passaging for more than 30 passages with trypsin digestion every third day. Hereafter, these proliferative cells induced from STAP cells by Fgf4 treatment are referred to as Fgf4-induced stem cells. This type of derivation into trophoblast-stem-like cells is not common with ES cells (unless genetically manipulated)<sup>13</sup> or STAP stem cells.

In the blastocyst injection assay, unlike STAP stem cells, the placental contribution of Fgf4-induced stem cells (*cag*-GFP-labelled) was observed with 53% of embryos (Fig. 2f, g; n = 60). In the chimaeric



**Figure 2 | Fgf4 treatment induces some trophoblast-lineage character in STAP cells.**

**a**, Schematic of Fgf4 treatment to induce Fgf4-induced stem cells from STAP cells. **b**, Fgf4 treatment promoted the generation of flat cell clusters that expressed Oct4-GFP at moderate levels (right). Top and middle: days 1 and 7 of culture with Fgf4, respectively. Bottom: culture after the first passage. Scale bar, 50  $\mu$ m. **c**, **d**, Immunostaining of Fgf4-induced cells with the trophoblast stem cell markers integrin  $\alpha$ 7 (c) and eomesodermin (d). Scale bar, 50  $\mu$ m. **e**, qPCR analysis of marker expression. **f**, **g**, Placental contribution of Fgf4-induced stem cells (Fl-SCs) (genetically labelled with constitutive GFP expression). Scale bars: 5.0 mm (f, left panel) and g); 50  $\mu$ m (f, right panel). In addition to placental contribution, Fgf4-induced stem cells contributed to the embryonic portion at a moderate level (g). **h**, Quantification of placental contribution by FACS analysis. Unlike Fgf4-induced cells, ES cells did not contribute to placental tissues at a detectable level. **i**, Cluster tree diagram from hierarchical clustering of global expression profiles. Red, approximately unbiased P values. **j**, qPCR analysis of Fgf4-induced cells (cultured under feeder-free conditions) with or without JAK inhibitor (JAKi) treatment for pluripotent marker genes. **k**, qPCR analysis of Fl-SCs with or without JAK inhibitor (JAKi) treatment for trophoblast marker genes. Values are shown as ratio to the expression level in ES cells (j) or trophoblast stem cells (k). \*\*\*P < 0.001; NS, not significant; t-test for each gene between groups with and without JAK inhibitor treatment. n = 3. Statistical significance was all the same with three pluripotency markers. None of the trophoblast marker genes showed statistical significance. Error bars represent s.d.

placentae, Fgf4-induced stem cells typically contributed to ~10% of total placental cells (Fig. 2h and Extended Data Fig. 2a, b).

Despite their similarities, we noted that Fgf4-induced stem cells also possessed some critical differences compared with blastocyst-derived trophoblast stem cells. First, Fgf4-induced stem cells exhibited moderate GFP signals and expressed a moderate level of *Oct4* (Fig. 2b; moderate and low levels of immunostaining signals were also seen for Oct4 and Nanog proteins, respectively; Extended Data Fig. 2c), unlike conventional trophoblast stem cells<sup>9</sup> that have little *Oct4* expression (Fig. 2e). Second, unlike trophoblast stem cells, blastocyst-injected Fgf4-induced stem cells also contributed to embryonic tissues (in all cases that involved chimaeric placentae;  $n = 32$ ), although the extent of contribution was generally modest (Fig. 2g). Third, immunostaining revealed that the level of Cdx2 protein accumulation in the nuclei of Fgf4-induced stem cells was marginal as compared to the cytoplasmic level, although the transcript expression level was substantial (Fig. 2e). This may suggest complex and dynamic post-transcriptional regulations for this key transcription factor in Fgf4-induced stem cells (a similar situation was seen for STAP cells, in which clear nuclear localization was not observed for either Cdx2 or Eomes, despite substantial expression of their transcripts). Fourth, in the absence of Fgf4, Fgf4-induced stem cells gradually died in 7–10 days and did not differentiate into large and multi-nuclear cells, unlike trophoblast stem cells (Extended Data Fig. 2d).

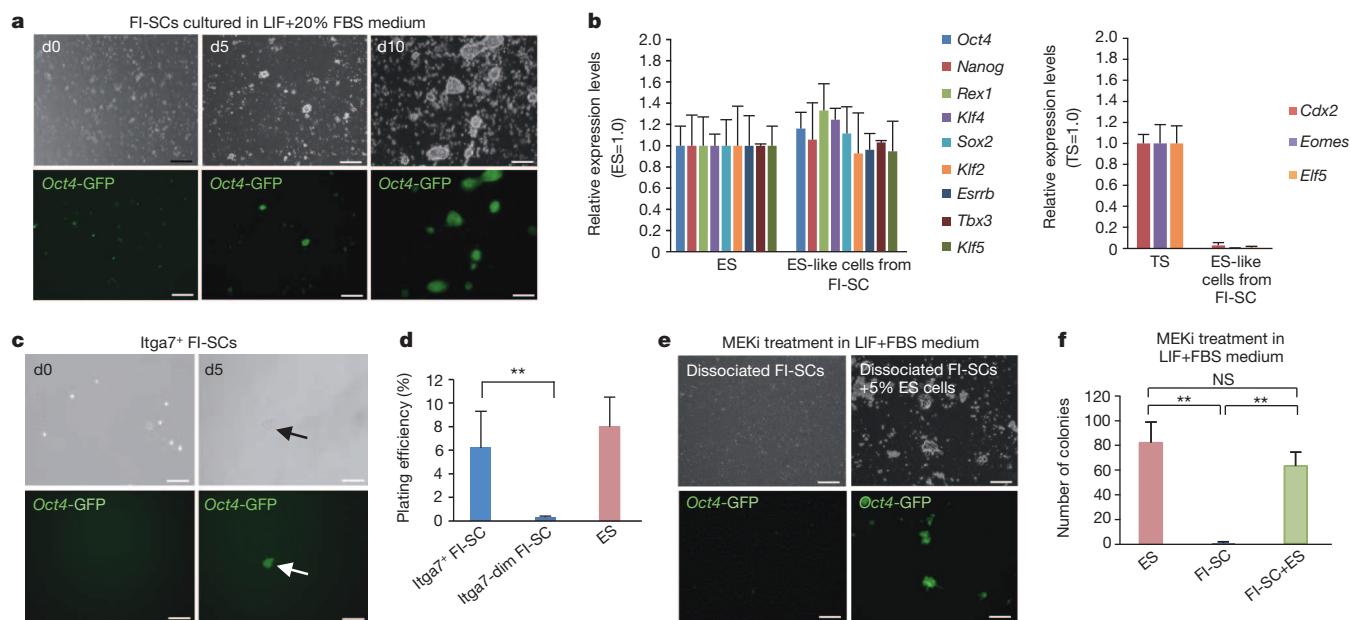
To investigate the relationship among STAP cells, STAP stem cells, Fgf4-induced stem cells, ES cells and trophoblast stem cells, we performed genome-wide RNA-seq analysis (Fig. 2i for dendrogram; Extended Data Figs 3 and 4 for expression analyses of representative genes<sup>14,15</sup>; Supplementary Tables 2 and 3 for analysis conditions). Whereas STAP cells formed a cluster with STAP stem cells, Fgf4-induced stem cells, ES cells and trophoblast stem cells and not with the parental CD45<sup>+</sup> cells, STAP cells were an outlier to the rest of the cell types in the cluster. In contrast, STAP stem cells were closely clustered with ES cells. Fgf4-induced stem cells formed a cluster with a sub-cluster of ES cells and

STAP stem cells, whereas trophoblast stem cells comprised an outlier to this cluster, indicating a close relationship of Fgf4-induced stem cells with these pluripotent cells.

However, as Fgf4-induced stem cells lay between STAP stem cells and trophoblast stem cells in the dendrogram, the possibility of contamination of STAP stem cells in the Fgf4-induced stem-cell population cannot be ruled out. Previous studies have indicated that inner cell mass (ICM)-type pluripotent cells can be removed from culture by treating the culture with a JAK inhibitor<sup>16</sup> (Extended Data Fig. 5a, b). In contrast, the JAK inhibitor treatment had no substantial effect on *Oct4*-GFP expression in Fgf4-induced stem-cell culture (Extended Data Fig. 5c, d; see Extended Data Fig. 5e, f for control). Expression of neither pluripotency markers (Fig. 2j) nor trophoblast markers (Fig. 2k) was substantially affected, indicating that pluripotency marker expression is unlikely to reflect contaminating STAP stem cells (ICM-type). Consistent with this idea, Fgf4-induced stem cells that were strongly positive for the trophoblast marker Itga7 (a surface marker for trophoblasts but not ES cells) also expressed high levels of *Oct4*-GFP (Extended Data Fig. 5g).

Notably, when cultured in LIF + FBS-containing medium for 4 days, Fgf4-induced stem cells underwent substantial changes in morphology and started to form ES-cell-like compact colonies with strong GFP signals (Fig. 3a). These cells showed expression of pluripotency makers, but not trophoblast markers (Fig. 3b and Extended Data Fig. 6a), and formed teratomas in mice (Extended Data Fig. 6b). These ES-like cells were generated from Fgf4-induced stem cells sorted for strong expression of the trophoblast marker Itga7, but rarely from Itga7-dim cells (Fig. 3c, d).

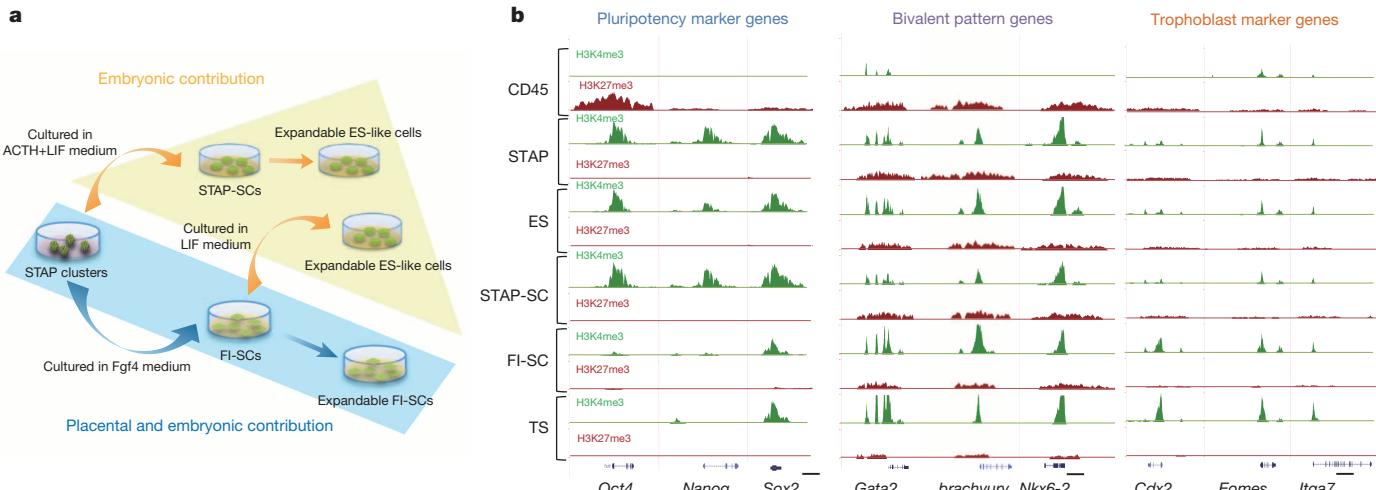
To confirm further that Fgf4-induced stem cells with a trophoblast-like nature were converted into ES-like cells, rather than just selecting ES-like cells pre-existing in the Fgf4-induced stem cell culture, we examined the effect of the MEK inhibitor PD0325901 on the ES-like cell generation from Fgf4-induced stem cells. Like trophoblast stem cells, Fgf4-induced stem-cell survival is dependent on FGF-MEK signals, and



**Figure 3 |** Fgf4 treatment induces some trophoblast-lineage character in STAP cells. **a**, Culture of *Oct4*-GFP Fgf4-induced cells in LIF + 20% FBS medium. **b**, qPCR analysis of ES-like cells derived from Fgf4-induced cells for pluripotent marker genes (left) and trophoblast marker genes (right). Values are shown as ratio to the expression level in ES cells (left) or trophoblast stem (TS) cells (right). **c, d**, Culture of *Oct4*-GFP Fgf4-induced cells sorted by FACS for strong integrin  $\alpha$ 7 (Itga7) expression in LIF + 20% FBS medium.

**d**, Formation frequency (shown by percentage) of *Oct4*-GFP<sup>+</sup> colonies from cells plated on gelatin-coated dishes at a clonal density. \*\*P < 0.01; t-test; n = 3. **e, f**, Culture of *Oct4*-GFP Fgf4-induced cells (dissociated) in LIF + 20%

FBS medium with MEK inhibitor. \*\*P < 0.01; NS, not significant; Tukey's test; n = 3. **e**, No substantial formation of *Oct4*-GFP<sup>+</sup> colonies was seen from Fgf4-induced cells in the presence of MEK inhibitor (left), whereas colonies frequently formed when cells were co-plated with *Oct4*-GFP ES cells (right; plated cells were 1/20 of Fgf4-induced cells). **f**, Quantification of colony formation per plated cells ( $1 \times 10^3$  Fgf4-induced cells and/or  $1 \times 10^3$  ES cells). Unlike Fgf4-induced cells, ES cells formed colonies (regardless of co-plating with FI-SCs) in the presence of MEK inhibitor. Bars and error bars represent mean values and s.d., respectively (b, d, f). Scale bars: 100 μm (a, c, e).



**Figure 4 | Differentiation potential and epigenetic state of STAP and STAP-derived stem cells.** **a**, Schematic diagram of stem-cell conversion cultures from STAP cells under different conditions. **b**, ChIP-seq results of histone H3K4 (green) and H3K27 (red) trimethylation at the loci

of pluripotent marker genes (left), bivalent pattern genes (middle) and trophoblast marker genes (right). Scale bars indicate 10 kb for pluripotency marker genes and trophoblast marker genes, and 20 kb for bivalent pattern genes.

the inhibition of MEK activity caused massive cell death (Extended Data Fig. 6c). However, PD0325901 is also known to be a main effector in 2i medium<sup>17</sup> and to promote ES cell maintenance. Addition of PD0325901 to LIF + FBS-containing medium strongly inhibited the formation of ES-like colonies from Fgf4-induced stem cells (Fig. 3e, left, and Fig. 3f). This inhibition was unlikely to be due to secondary toxic effects from massive cell death of Fgf4-induced stem cells, as colonies formed in the presence of PD0325901 when ES cells were co-plated in the same culture with Fgf4-induced stem cells (Fig. 3e, right, and Fig. 3f).

Collectively, these findings demonstrate that STAP-derived Fgf4-induced stem cells not only express both pluripotency markers and trophoblast genes but also have the potential to convert into ES-like cells when cultured in LIF + FBS-containing medium (Fig. 4a).

Here we demonstrate that STAP cells, which have a limited self-renewal ability, can be induced to generate two distinct types of robustly self-renewing stem cells—STAP stem cells and Fgf4-induced stem cells—under different culture conditions. Chromatin immunoprecipitation (ChIP) sequencing analysis showed distinct accumulation patterns of modified histone H3 in the two types of STAP-cell-derived stem cells (Fig. 4b). STAP stem cells (as well as STAP cells) had accumulation patterns of H3K4 and H3K27 trimethylation that resembled those of ES cells at the loci of pluripotency marker genes (*Oct4*, *Nanog*, *Sox2*), bivalent pattern genes<sup>18</sup> (*Gata2*, *brachyury*, *Nkx6-2*) and trophoblast marker genes (*Cdx2*, *Eomes*, *Itga7*). In contrast, the accumulation patterns in Fgf4-induced stem cells at these loci matched more closely those of trophoblast stem cells, except that low levels of accumulation of H3K4 trimethylation in *Oct4* and *Nanog* and of H3K27 trimethylation in the trophoblast marker genes were observed in Fgf4-induced stem cells but not trophoblast stem cells.

Recent studies have also begun to reveal dynamic regulations in multiple cellular states related to pluripotency. These include reports of co-expression of *Oct4* and *Cdx2* in rat ES cells maintained in the presence of a GSK-3β inhibitor<sup>19,20</sup> and of *Oct4* expression in rat extra-embryonic precursors<sup>21</sup>. Another recent study has indicated that conventional ES cell culture also contains a very minor population of *Oct4*<sup>-</sup> cells with features resembling those of very early-stage embryos<sup>22</sup>, including bidirectional potential. However, these cells are dissimilar to STAP cells as they are *Oct4*<sup>-</sup>, unlike STAP cells and Fgf4-induced stem cells. Our preliminary genome-wide RNA-sequencing analysis indicated that both morulae and blastocysts are outliers to the cluster of STAP and ES cells (Extended Data Fig. 6d-f and Supplementary Tables 4 and 5).

A key conclusion drawn from this study is that the reprogramming in STAP conversion goes beyond the pluripotent state of ES cells and

involves the acquisition of a wider developmental potential related to both ICM- and trophoectoderm-like states. Because of the inability to clone STAP cells from single cells, we must await future technical advancement to examine whether their dual-directional differentiation potential at the population level may reflect one totipotent state at the single-cell level or two different states of STAP cells coexisting (or fluctuating between them) in culture. As for STAP-cell-derived Fgf4-induced stem cells, which can also contribute to both embryonic and placental tissues, our *in vitro* conversion study combined with inhibitor treatments clearly indicate that the bidirectional potential of Fgf4-induced stem cells is unlikely to reflect the co-presence of separate subpopulations of ES-like and trophoblast-stem-like cells in the culture. Collectively, our study indicates that STAP-based conversion can reprogram somatic cells to acquire not only pluripotency but also the ability of trophoblast differentiation.

## METHODS SUMMARY

**Cell culture.** STAP cells were generated from mouse splenic CD45<sup>+</sup> cells by a transient exposure to low-pH solution, followed by culture in B27 + LIF medium<sup>1</sup>. For establishment of the Fgf4-induced stem-cell line, STAP cell clusters were transferred to Fgf4 (25 ng  $\mu$ l)-containing trophoblast stem-cell medium<sup>9</sup> on MEF feeder cells in 96-well plates. The cells were subjected to the first passage during days 7–10 using a conventional trypsin method. For inducing conversion from Fgf4-induced stem cells into ES-like cells, Fgf4-induced stem cells were trypsinized, and suspended cells were plated in ES maintenance medium containing LIF and 20% FBS. For the establishment of STAP stem-cell lines, STAP spheres were transferred to ACTH-containing medium<sup>15</sup> on a MEF feeder or gelatin-coated dish. Four to seven days later, the cells were subjected to the first passage using a conventional trypsin method, and suspended cells were plated in ES maintain medium containing 5% FBS and 1% KSR.

**Chimaeric mice generation and analyses.** For injection of STAP stem cells, Fgf4-induced stem cells and ES cells, a conventional blastocyst injection method was used. For STAP cell injection, STAP cell clusters were injected en bloc, because trypsin treatment caused low chimaerism. STAP spherical colonies were cut into small pieces using a microknife under microscopy, then small clusters of STAP colony were injected into day-4.5 blastocysts by large pipette. The next day, the chimaeric blastocysts were transferred into day-2.5 pseudopregnant females.

**Online Content** Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** H.O. and Y.S. wrote the manuscript. H.O., Y.S., M.K., M.A., N.T., S.Y. and T.W. performed experiments, and M.T. and Y.T. assisted with H.O.'s experiments. H.O., Y.S., H.N., C.A.V. and T.W. designed the project.

**Author Information** RNA-seq and ChIP-seq files have been submitted to the NCBI BioSample databases under accessions SAMN02393426, SAMN02393427, SAMN02393428, SAMN02393429, SAMN02393430, SAMN02393431, SAMN02393432, SAMN02393433, SAMN02393434 and SAMN02393435. Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.O. (obokata@cdb.riken.jp), T.W. (teru@cdb.riken.jp) or Y.S. (yoshikisasai@cdb.riken.jp).

## METHODS

**Animal studies.** Research involving animals complied with protocols approved by the Harvard Medical School/Brigham and Women's Hospital Committee on Animal Care, and the Institutional Committee of Laboratory Animal Experimentation of the RIKEN Center for Developmental Biology.

**Cell culture.** STAP cells were generated from low-pH-treated CD45<sup>+</sup> cells, followed by culture in B27 + LIF medium for 7 days, as described<sup>1</sup>. For Fgf4-induced stem-cell line establishment, STAP cell clusters were transferred to Fgf4-containing trophoblast stem-cell medium<sup>9</sup> on MEF feeder cells in 96-well plates. In most cases (40 out of 50 experiments), colonies grew in 10–50% of wells in 96-well plates. In minor cases (10 out of 50 experiments), no colony growth was observed and/or only fibroblast-like cells appeared. The cells were subjected to the first passage during days 7–10 using a conventional trypsin method. Subsequent passages were performed at a split ratio of 1:4 every third day before they reached subconfluence.

STAP stem-cell lines were established as described<sup>1</sup>. STAP spheres were transferred to ACTH-containing medium<sup>1</sup> on MEF feeder cells (several spheres, up to a dozen spheres, per well of 96-well plates). Four to seven days later, the cells were subjected to the first passage using a conventional trypsin method, and suspended cells were plated in ES maintain medium containing 20% FBS. Subsequent passaging was performed at a split ratio of 1:10 every second day before they reached subconfluence.

**Chimaera mouse generation and analyses.** For production of diploid and tetraploid chimaeras with STAP cells, STAP stem cells and Fgf4-induced stem cells, diploid embryos were obtained from ICR strain females. Tetraploid embryos were produced by electrofusion of 2-cell embryos. Because trypsin treatment of donor samples turned out to cause low chimaerism, STAP spherical colonies were cut into small pieces using a microknife under microscopy, and small clusters of STAP cells were then injected into day-4.5 blastocysts by a large pipette. Next day, the chimaeric blastocysts were transferred into day-2.5 pseudopregnant females.

**In vivo differentiation assay.**  $1 \times 10^5$  cells of Fgf4-induced stem-cell-derived ES-like cells were injected subcutaneously into the dorsal flanks of 4-week-old NOD/SCID mice. Six weeks later, the implants were collected and histologically analysed. The implants were fixed with 10% formaldehyde, embedded in paraffin, and routinely processed into 4-μm-thick sections. Sections were stained with haematoxylin and eosin. So far, we have not investigated whether Fgf4-induced stem cells form tumours such as teratomas and yolk sac tumours *in vivo*.

**Immunostaining.** Cells were fixed with 4% PFA for 15 min and, after permeabilization, with 0.5% Triton X-100 and then incubated with primary antibodies: anti-H3K27me3 (Millipore; 1:300), anti-Oct3/4 (Santa Cruz Biotechnology; 1:300), anti-Nanog (eBioscience; 1:300), anti-KLF4 (R&D System; 1:300), anti-Esrrβ (R&D System; 1:300) and integrin α7 antibody (R&D system; 1:200). After overnight incubation, bound antibodies were visualized with a secondary antibody conjugated to Alexa546 (Molecular Probes). Nuclei were stained with DAPI (Molecular Probes).

**RNA preparation and RT-PCR analysis.** RNA was isolated with the RNeasy Mini kit (Qiagen). Reverse transcription was performed with the SuperScript III First Strand Synthesis kit (Invitrogen). Power SYBR Green Mix (Roche Diagnostics) was used for PCR amplification, and samples were run on a Lightcycler-II Instrument (Roche Diagnostics). The primer sets for each gene are listed in Supplementary Table 1.

**Inhibitor assay.** For JAK inhibitor assay, Fgf4-induced stem cells were cultured without feeders for 48 h in trophoblast stem-cell culture medium supplemented with 0.6 μM JAK inhibitor (CalBiochem, 420097). As a control, ES cells were also cultured for 48 h in ES medium supplemented with 0.6 μM JAK inhibitor. After the JAK inhibitor treatment, cells were collected and their gene expression was analysed by RT-PCR. For MEK inhibitor assay, dissociated Fgf4-induced stem cells were plated in either LIF containing ES medium supplemented with 1 μM MEK inhibitor (PD025901) or FGF4 containing trophoblast stem cell medium supplemented with 1 μM MEK inhibitor for 48 h. As controls, dissociated Fgf4-induced stem cells were co-plated with 5% or 50% of ES cells into the same culture conditions.

After the MEK inhibitor treatment, colonies that formed in each culture condition were counted.

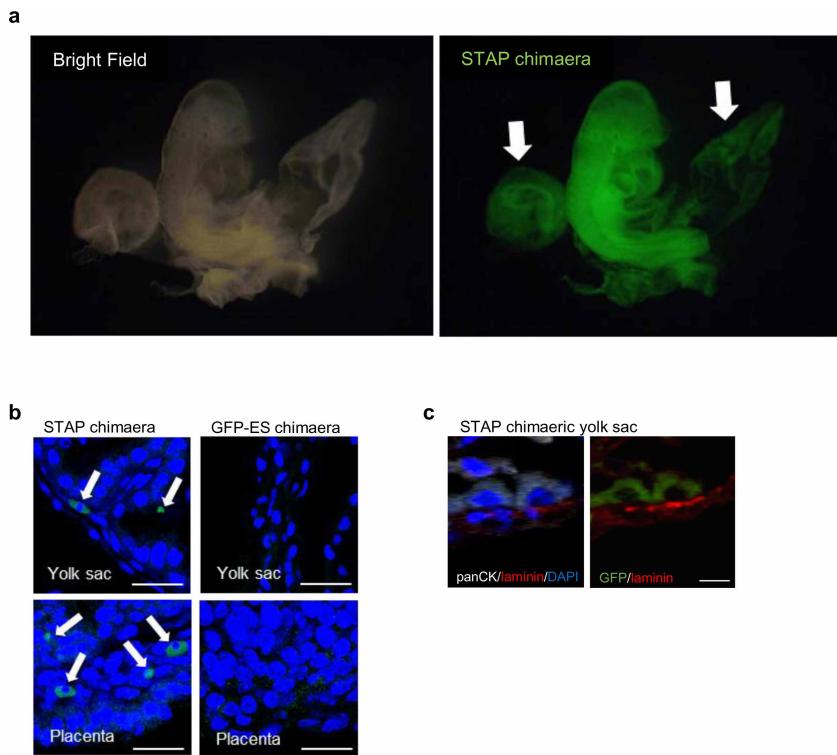
**FACS sorting.** Fgf4-induced stem cells were dissociated into single cells and were suspended in 0.5% BSA PBS. Suspended cells were Fc-blocked by treatment with 1 μg of mouse IgG per  $10^5$  cells for 15 min at room temperature. PE-conjugated integrin α7 antibody (R&D system, FAB3518P, dilution at 1:10) was added into cell suspension, and cells were incubated for 30 min on ice. Finally, cells were rinsed with PBS three times and propidium iodide was added for dead cell elimination. As a control, Fgf4-induced stem cells in a separate tube were treated with PE-labelled rat IgG<sub>2B</sub> antibody. Integrin α7-positive and -dim cells were sorted by FACS aria II (BD).

**RNA sequencing and ChIP sequencing analyses.** RNA-seq sequencing of cell lines was performed with biological duplicate samples. Total RNA was extracted from T cells by the RNasy mini kit (Qiagen). RNA-seq libraries were prepared from 1 μg total RNAs following the protocol of the TruSeq RNA Sample Prep kit (Illumina) and subjected to the deep sequencing analysis with Illumina Hi-Seq1000. A cluster tree diagram of various cell types was obtained from hierarchical clustering of global expression profiles ( $\log_2$  FPKM of all transcripts; FPKM, fragments per kilobase of transcript per million mapped reads). Complete linkage method applied to  $1 - r$  ( $r$  = Pearson's correlation between profiles) was used for generating the tree and 1,000 cycles of bootstrap resampling were carried out to obtain statistical confidence score in % units (also called AU *P* values). For the analysis that included morula and blastocyst embryos (only small amounts of RNA can be obtained from them), we used pre-amplification with the SMARTer Ultra Low RNA kit for Illumina Sequencing (Clontech Laboratories). Differentially expressed genes were identified by the DESeq package<sup>23</sup>.

ChIP-seq libraries were prepared from 20 ng input DNAs, 1 ng H3K4me3 ChIP DNAs, or 5 ng H3K27me3 ChIP DNAs using the KAPA Library Preparation kit (KAPA Biosystems). TruSeq adaptors were prepared in-house by annealing a TruSeq universal oligonucleotide and each of index oligonucleotides (5'-AATGATACG GCGACCACCGAGATCTACACTTTCCCTACACGACGCTTCCGATC T-3', and 5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXA TCTCGTATGCCGTCTCTGCTTG-3'; where X represents index sequences).

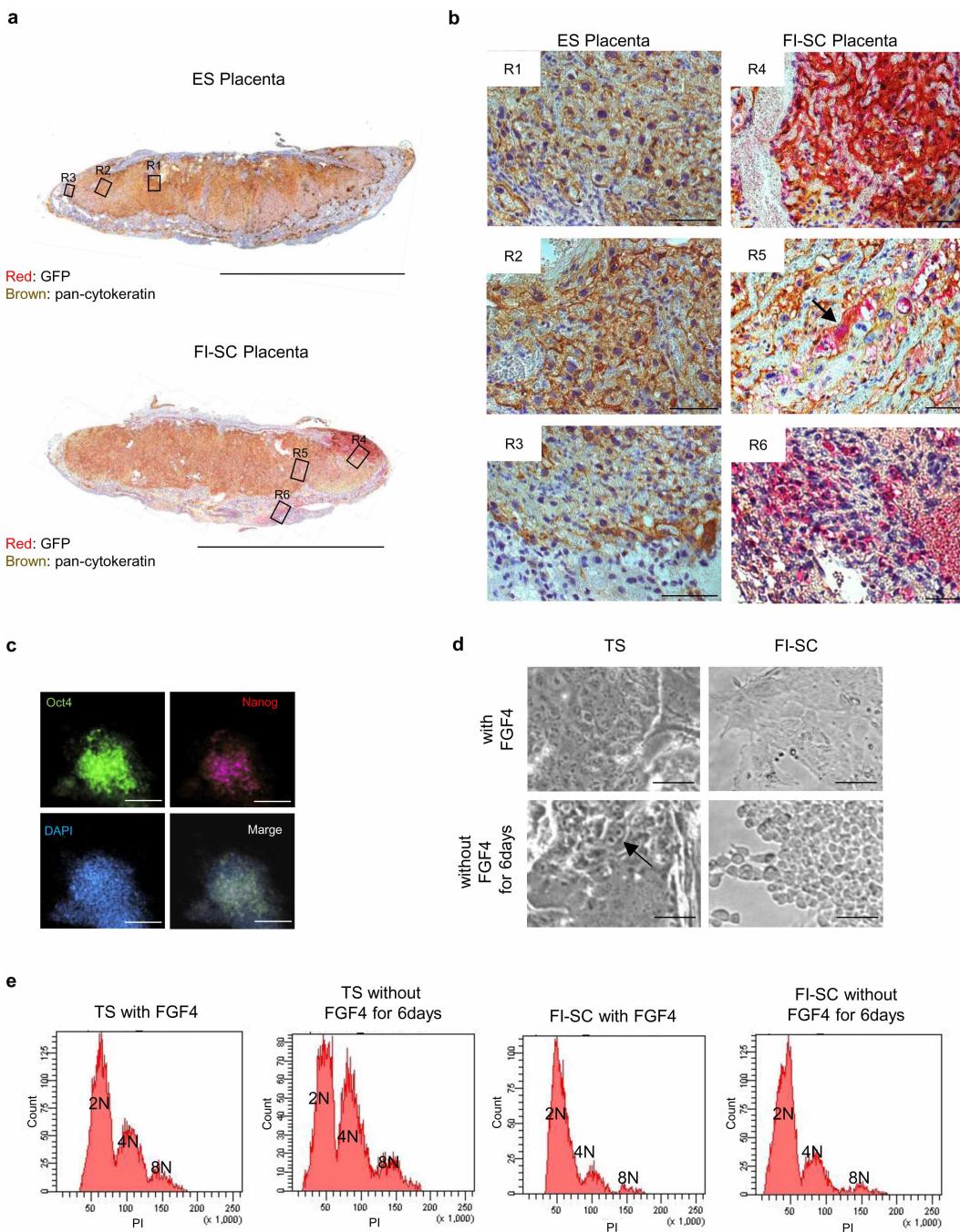
Chromatin immunoprecipitation was performed as follows. Cells were fixed in PBS(-) containing 1% formaldehyde for 10 min at room temperature. Glycine was added to a final concentration of 0.25 M to stop the fixation. After washing the cells twice in ice-cold PBS(-), cells were further washed in LB1 (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100) and LB2 (10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA). Cells were then re-suspended in lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS). Lysates were prepared by sonication using COVARISS220 in a mini tube at duty cycle = 5%, PIP = 70, cycles per burst = 200, and the treatment time of 20 min. Lysates from  $2 \times 10^6$  cells were diluted in ChIP dilution buffer (16.7 mM Tris-HCl pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100, 0.01% SDS). ChIP was performed using sheep anti-mouse IgG beads (Invitrogen) or protein A beads (Invitrogen) coupled with anti-histone H3K4me3 antibody (Wako, catalogue no. 307-34813) or anti-histone H3K27me3 antibody (CST, catalogue no. 9733), respectively. After 4–6 h of incubation in a rotator at 4 °C, beads were washed five times in low-salt wash buffer (20 mM Tris HCl pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), and three times in high-salt wash buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS). Target chromatin was eluted off the beads in elution buffer (10 mM Tris-HCl pH 8.0, 300 mM NaCl, 5 mM EDTA, 1% SDS) at room temperature for 20 min. Crosslink was reversed at 65 °C, and then samples were treated with RNaseA and proteinase K. The prepared DNA samples were purified by phenol-chloroform extraction followed by ethanol precipitation and dissolved in TE buffer.

23. Anders, S. & Huber, W. Differential expression analysis for sequence count data. *Genome Biol.* **11**, R106 (2010).



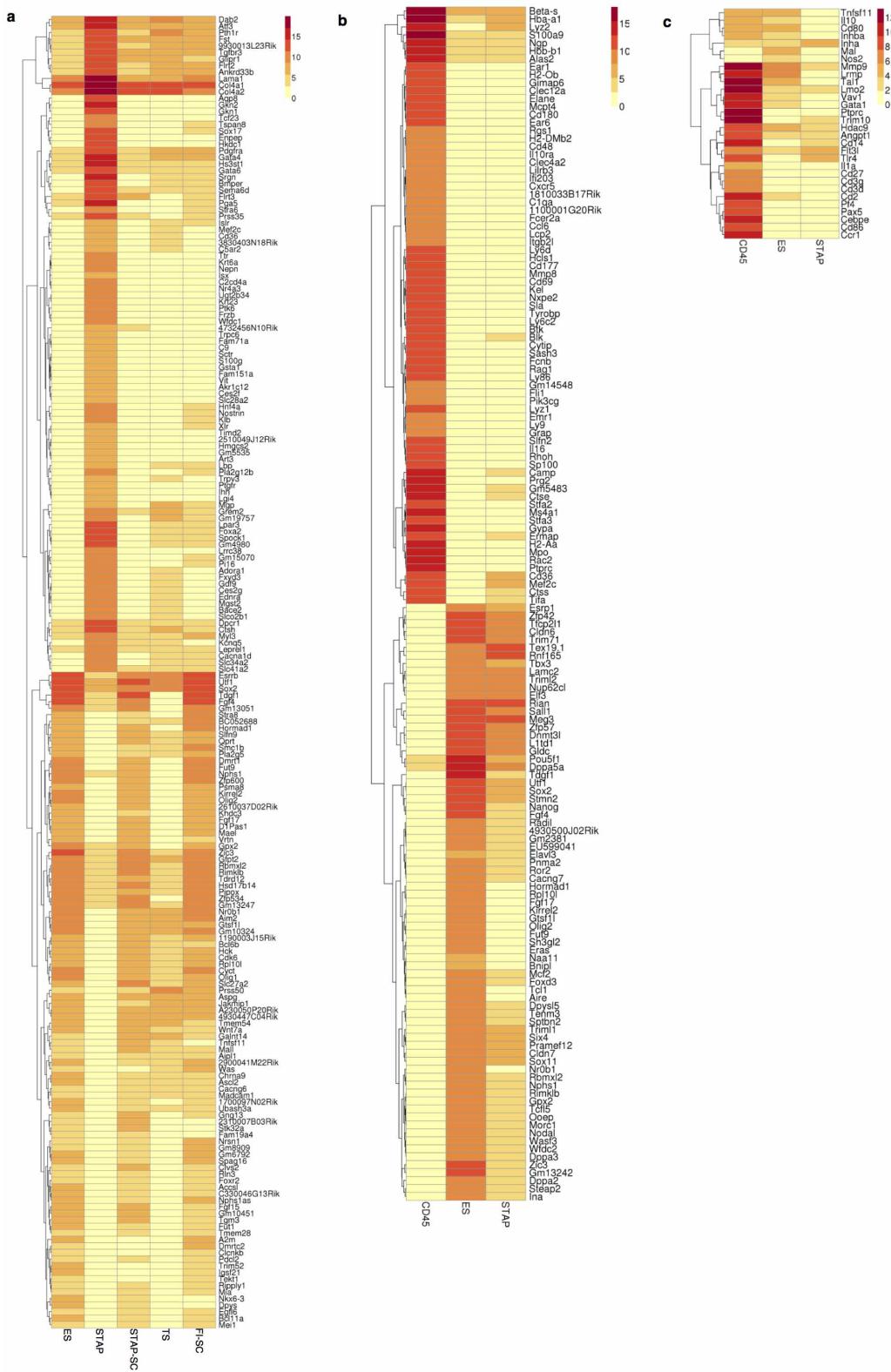
**Extended Data Figure 1 | Placental contribution of STAP cells.** **a**, Chimaeric mouse with STAP cells derived from CD45<sup>+</sup> cells of B6GFP × 129/Sv mice (B6GFP, C57BL/6 line with *cag-gfp* transgene). Arrows indicate a placenta and a yolk sac. **b**, Cross-sections of yolk sac (top) and placenta (bottom). GFP-positive cells (arrows) were seen only in yolk sac and placenta of the STAP

cell chimaera. Scale bars, 50 µm. **c**, Co-immunostaining showed that these GFP-positive cells (right) were found in the extra-embryonic endoderm-derived epithelial cells (pan-cytokeratin<sup>+</sup> and overlying laminin<sup>+</sup> basement membrane; left) of the yolk sac. Scale bar, 10 µm.



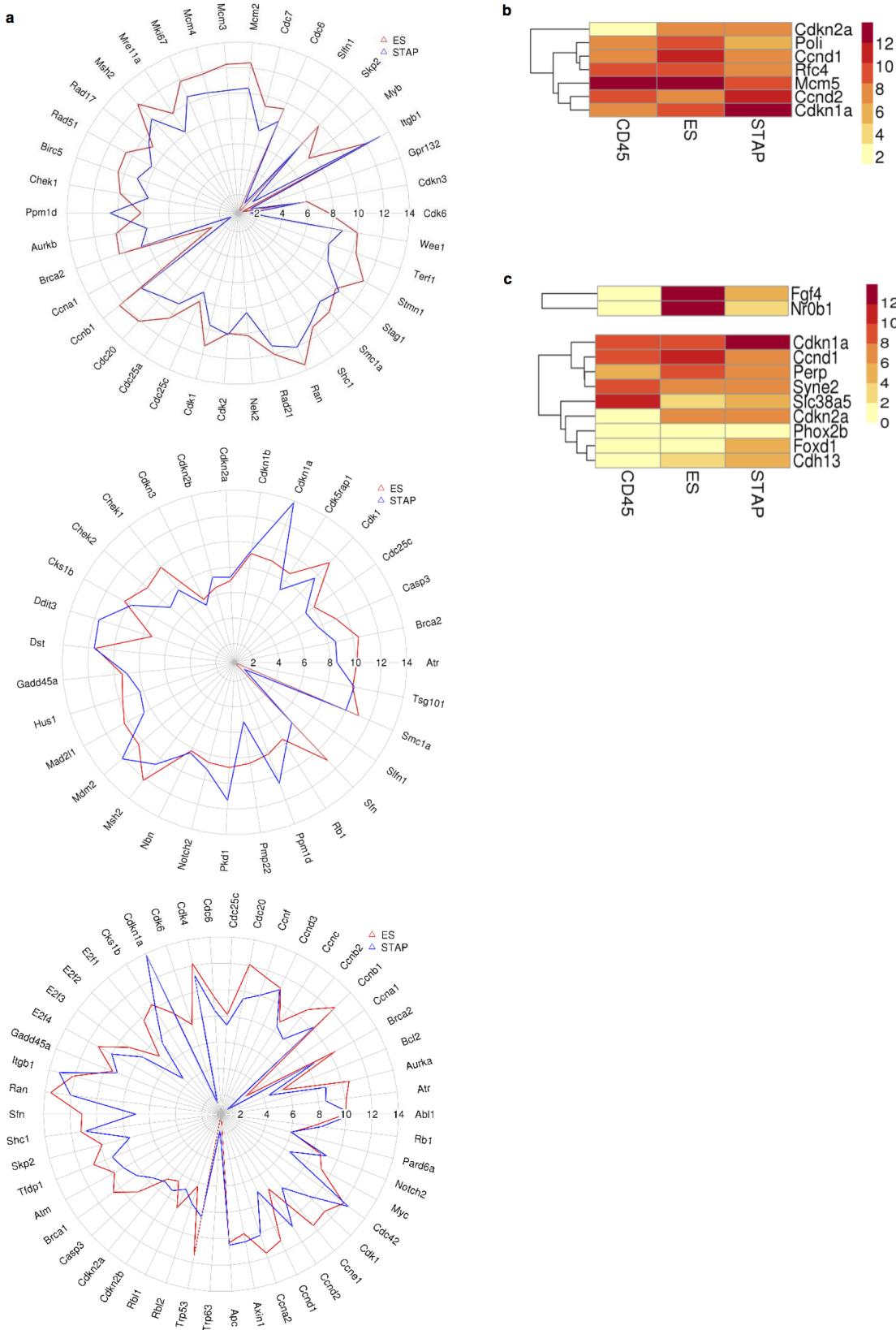
**Extended Data Figure 2 | Trophoblast differentiation potential of Fgf4-induced stem cells.** **a, b,** Immunostaining (cross-section) of placentae obtained in the blastocyst injection assay with GFP (constitutive)-labelled ES cells (upper) or Fgf4-induced stem cells (bottom). Brown shows pan-cytokeratin and red shows GFP (ES cell or Fgf4-induced stem cell contribution). Regions indicated in **a** are shown in **b**. Fgf4-induced stem cells contributed to all layers of placentae, whereas no contribution was observed with ES cells. **a,** Scale bars, 5 mm. **b,** Scale bars, 50  $\mu$ m. **c,** Pluripotent marker expression of Fgf4-induced stem cells. Scale bars, 50  $\mu$ m. **d, e,** Effects of Fgf4 withdrawal from

Fgf4-induced stem cell culture. Unlike trophoblast stem cells (**d**, left), which generated multi-nucleated large cells (arrow) in the absence of Fgf4, Fgf4-induced stem cells (**d**, right) simply stopped proliferation and gradually died on Fgf4 withdrawal. Scale bars, 50  $\mu$ m. This finding suggests that placental differentiation of Fgf4-induced stem cells *in vivo* may involve more than just Fgf4 signal suppression. **e,** The number of 4N and 8N cells increased within 6 days of Fgf4 withdrawal in trophoblast stem cells but not in Fgf4-induced stem cells.



**Extended Data Figure 3 | Transcriptome analyses of STAP cells shown by heat maps.** **a**, Heat maps of expression profiles of top-ranked up- and downregulated genes in STAP cells (*Oct4*-GFP<sup>+</sup> clusters converted from CD45<sup>+</sup> cells) compared to ES cells. Their respective expression levels in STAP stem cells, trophoblast stem cells and Fgf4-induced stem cells are shown. Absolute expression values are scaled by log<sub>2</sub>. The genes expressed differentially between ES cells and STAP cells tended to show more similar expression profiles to ES cells in STAP stem cells and Fgf4-induced stem cells than in trophoblast stem cells. Expression of some early endodermal lineage genes such as *Gata4* and *Sox17* was moderately elevated in STAP cells as compared to ES

cells, whereas its biological significance remains elusive (these genes are shown to be strongly expressed in *Oct4*-GFP-dim cells<sup>1</sup>). **b**, Heat maps of expression profiles of top-ranked up- and downregulated genes in ES cells compared to CD45<sup>+</sup> cells and their respective expression levels in STAP cells. The genes expressed differentially between CD45<sup>+</sup> and ES cells tended to show similar expression profiles in ES cells and STAP cells. **c**, Heat maps of expression profiles of representative genes implicated in haematopoietic lineage development in CD45<sup>+</sup>, ES and STAP cells. No strong correlation was seen between CD45<sup>+</sup> cells and STAP cells in their expression profiles (a similar tendency of no correlation was seen for the data in **b**).

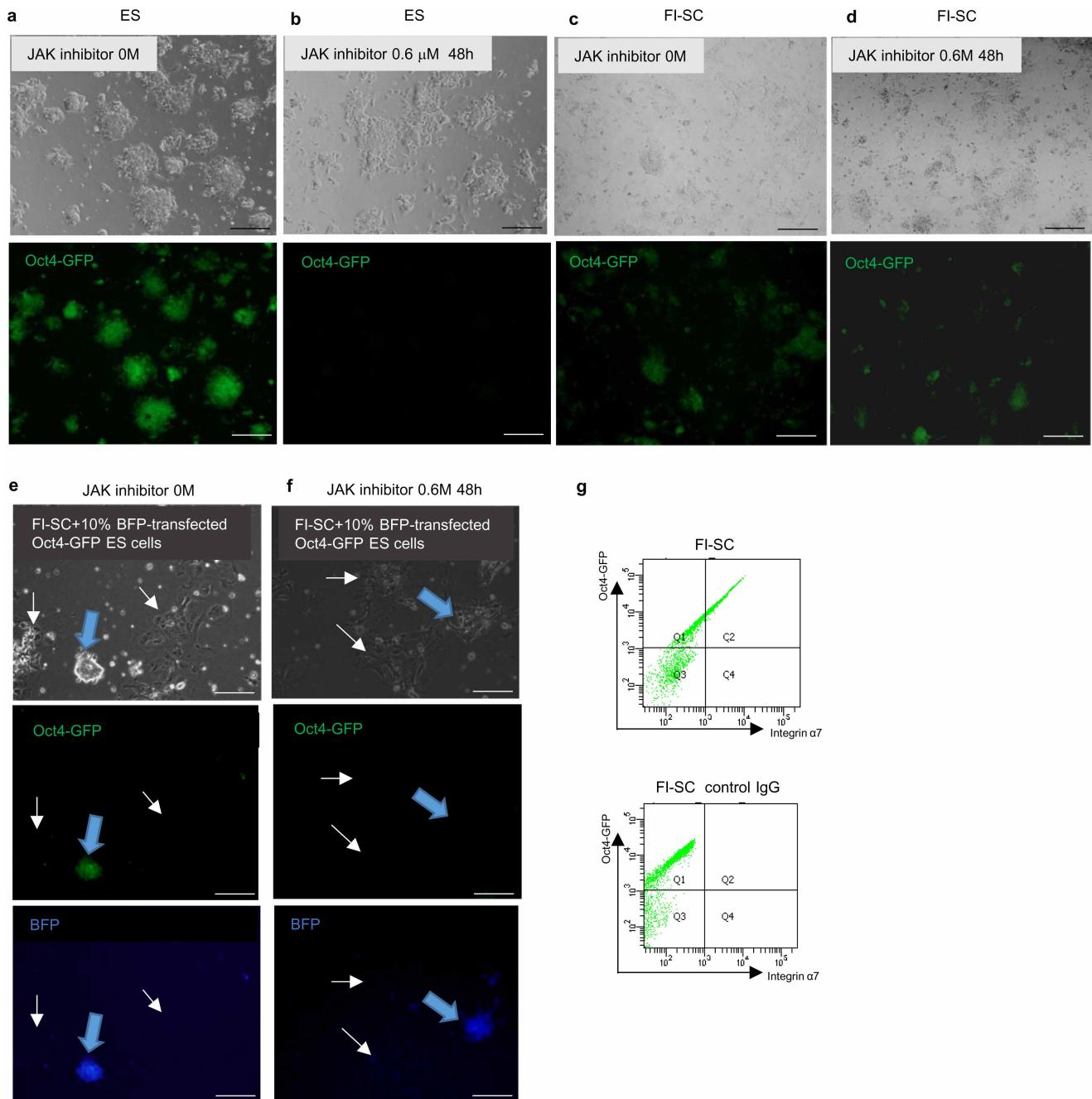


**Extended Data Figure 4 | Transcriptome analyses for genes implicated in cell-cycle control and induced pluripotent stem-cell conversion.**

**a**, Comparison of expression values of genes involved in cell-cycle control in ES and STAP cells; the G to M cell cycle phases (upper), the cell cycle checkpoint and cell cycle arrest (middle), and the cell cycle regulation (bottom) are shown. Expression level was measured by  $\log_2$  of mean normalized counts.

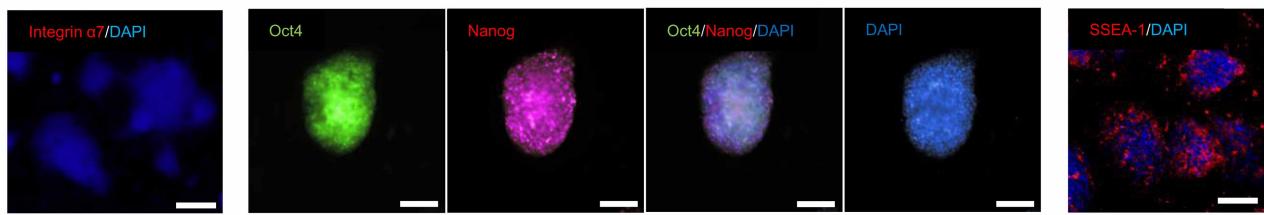
**b**, Heat map for upregulated genes in cells undergoing reprogramming by

'Yamanaka factors'<sup>14</sup>. **c**, Heat maps for upregulated genes in pre-iPS cells<sup>15</sup> (top) and in partially reprogrammed cells by Yamanaka factors (bottom)<sup>14</sup>. Expression level was measured by  $\log_2$  of mean normalized counts. Differentially expressed genes were identified by the DESeq package<sup>21</sup> and only genes with a false discovery rate of 1% were selected for comparison, unless mentioned otherwise.

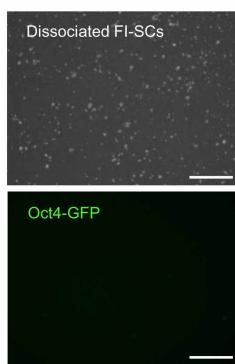
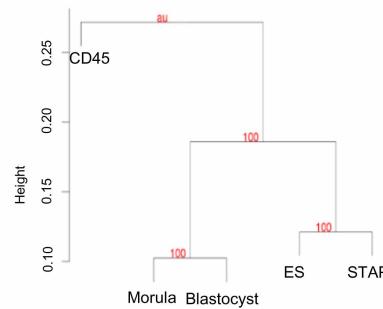
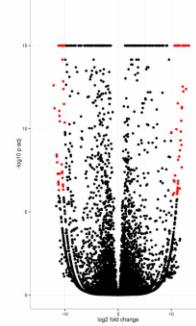
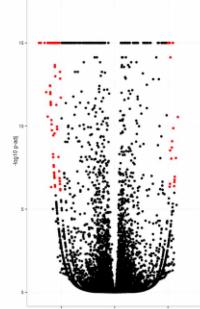


**Extended Data Figure 5 | Responses of Fgf4-induced stem cells to signal modifications.** **a–f**, JAK inhibitor treatment assay for Fgf4-induced stem cells. Fgf4-induced stem cells were cultured under feeder-free conditions and treated with 0.6  $\mu$ M JAK inhibitor for 48 h. JAK inhibitor treatment assay eliminated ES cells ( $Oct4$ -GFP $^+$ ) from the culture (**a**, **b**). The level of  $Oct4$ -GFP expression in Fgf4-induced stem cells, which was moderate, was maintained even after JAK inhibitor treatment (**c**, **d**; three independent experiments). Scale bar, 100  $\mu$ m. **e**, **f**, For an additional control, Fgf4-induced stem cells were plated in trophoblast stem-cell medium containing Fgf4 together with  $Oct4$ -GFP ES cells that constitutively expressed BFP (the number of plated cells

was one-tenth of that of plated Fgf4-induced stem cells). Whereas BFP-expressing colonies (ES-cell-derived) still expressed  $Oct4$ -GFP in trophoblast stem-cell culture medium after 2 days (**e**), no  $Oct4$ -GFP $^+$  colonies from BFP-expressing ES cells were observed in the JAK-inhibitor-treated culture (**f**). **g**, FACS analysis of integrin  $\alpha$ 7 expression in Fgf4-induced stem cells. Over 40% of Fgf4-induced stem cells strongly expressed both the pluripotency marker  $Oct4$ -GFP and the trophoblast marker integrin  $\alpha$ 7. The bottom panel shows an isotype control for integrin  $\alpha$ 7 antibody. In ES cells, integrin- $\alpha$ 7-expressing cells were less than 0.1% (data not shown; three independent ES cell lines were examined).

**a****b****c**

MEKi treatment in TS medium

**d****e****f**

### Extended Data Figure 6 | Characterization of ES-like cells converted from Fgf4-induced stem cells and comparison of STAP cells with early embryos.

**a**, Immunohistochemistry of ES-like cells for trophoblast and pluripotency markers. ES-like cells converted from Fgf4-induced stem cells no longer expressed the trophoblast marker (integrin alpha 7), but they did express the pluripotency markers (Oct4, Nanog and SSEA-1). Scale bar, 100  $\mu$ m. **b**, Pluripotency of ES-like cells converted from Fgf4-induced stem cells as shown by teratoma formation. Those cells successfully formed teratomas containing tissues from all three germ layers: neuroepithelium (left, arrow indicates), muscle tissue (middle, arrow indicates) and bronchial-like epithelium (right). Scale bar, 100  $\mu$ m. **c**, MEK inhibitor treatment assay for

*Oct4-gfp* Fgf4-induced stem cells in trophoblast stem-cell medium containing Fgf4. No substantial formation of *Oct4-GFP*<sup>+</sup> colonies was observed from dissociated Fgf4-induced stem cells in MEK-inhibitor-containing medium. Scale bar, 100  $\mu$ m. **d**, Cluster tree diagram from hierarchical clustering of global expression profiles. Red, AU P values. As this analysis included morula and blastocyst embryos from which only small amounts of RNA could be obtained, we used pre-amplification with the SMARTer Ultra Low RNA kit for Illumina Sequencing (Clontech Laboratories). **e, f**, Volcano plot of the expression profile of STAP cells compared to the morula (e) and blastocyst (f). Genes showing greater than 10-fold change and  $P$  value  $1.0 \times 10^{-6}$  are highlighted in red and are considered up- (or down-) regulated in the STAP cells.