REVIEW

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Hallmarks of pluripotency

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Stem cells self-renew and generate specialized progeny through differentiation, but vary in the range of cells and tissues they generate, a property called developmental potency. Pluripotent stem cells produce all cells of an organism, while multipotent or unipotent stem cells regenerate only specific lineages or tissues. Defining stem-cell potency relies upon functional assays and diagnostic transcriptional, epigenetic and metabolic states. Here we describe functional and molecular hallmarks of pluripotent stem cells, propose a checklist for their evaluation, and illustrate how forensic genomics can validate their provenance.

Stem cells, defined by dual hallmark features of self-renewal and differentiation potential, can be derived from embryonic and postnatal animal tissues and are classified according to their developmental potency (Fig. 1). The zygote and blastomeres are totipotent[1], denoting potential to give rise to all embryonic and extra-embryonic tissues, but their developmental potential has not been captured *in vitro*. Mouse embryonic stem cells exemplify a quintessential pluripotent stem (PS) cell that can form all tissues of the body, but provides only limited contributions to the extra-embryonic membranes or placenta. As described in greater detail below, PS cells manifest distinct functional properties depending upon the conditions under which they are derived and cultured. Multipotent stem cells, such as the paradigmatic haematopoietic stem cell, are restricted to generating the mature cell types of their tissue of origin, but under normal physiologic circumstances will not differentiate into unrelated lineages. Unipotent stem cells, such as spermatogonial stem cells (SSCs), share the capacity for self-renewal yet exhibit limited developmental potential, giving rise to only a single cell type, such as sperm.

Human PS cells correspond to a stable state allowing propagation of immortal pluripotent cells that can generate any cell within the body. Nuclear reprogramming, via somatic cell nuclear transfer and transcription factor transduction, demonstrates that the specialized state of a somatic cell can be reversed to a totipotent or pluripotent state, respectively[2,3]. The generation of induced pluripotent stem (iPS) cells from somatic cells via transcription factor expression constitutes a facile route to generate patient-specific PS cells, and has opened new paths to model diseases and new prospects for regenerative medicine. Given their versatility for medical applications, PS cells command considerable attention; therefore, defining the hallmarks of pluripotency has practical as well as fundamental value to biomedical research.

In this technical review, we describe the hallmark characteristics of PS cells, propose a checklist of assays for assessing the function and molecular state of pluripotency, and outline forensic genomic approaches to validate the provenance of reprogrammed cell lines.

Defining pluripotent stem cells

PS cells are self-renewing cells with the capacity to form representative tissues of all three germ layers of the developing embryo—ectoderm, mesoderm and endoderm, as well as the germ lineage, but typically provide little or no contribution to the trophoblast layers of placenta. PS cells can be derived from numerous sources (Table 1). The first PS cells cultured *in vitro* were derived from teratocarcinomas, a tumour of germ cell origin[4]. Later, derivation of PS cells from the murine blastocysts proved that pluripotent cells could be propagated as immortalized, non-transformed cell lines[5,6]. PS cells have also been derived from non-human primate and human embryos[7,8], and from various stages of development, including the post-implantation epiblast and the germ line[9–14]. Finally, somatic cells can be reprogrammed to pluripotency by ectopic expression of select sets of transcription factors[3].

PS cells manifest distinct properties depending on derivation and maintenance conditions. PS cells established from pre-implantation embryos are known as ES cells, whereas those generated from slightly later embryonic epiblast stages are called epiblast stem cells (EpiSCs)[9,10]. Their distinct culture requirements, gene expression programs and epigenetic features may reflect the dynamic development of pluripotency in the embryo. The terms 'naive' and 'primed' were introduced to describe early and late phases of epiblast ontogeny and respective ES cell and EpiSC derivatives[15]. PS cells from various sources have been classified accordingly (Table 1). Conventional human PS cells exhibit molecular attributes similar to EpiSCs and are classified as 'primed'. Evaluation of naive pluripotency in humans by formation of human chimaeras is restricted on ethical grounds in many jurisdictions, but as conventional non-human primate ES cells fail to chimaerize pre-implantation embryos, traditional human ES cells are also probably primed by this criterion[16].

Molecular hallmarks of pluripotency

PS cells are characterized by molecular mechanisms that sustain self-renewal and suppress differentiation while maintaining key differentiation genes in a quiescent yet 'poised' state reflective of their incipient developmental potential.

A select set of core transcription factors in combination governs and thereby defines pluripotency: OCT4 (also known as POU5F1), SOX2 and NANOG (collectively, OSN). OCT4 and NANOG are designated as

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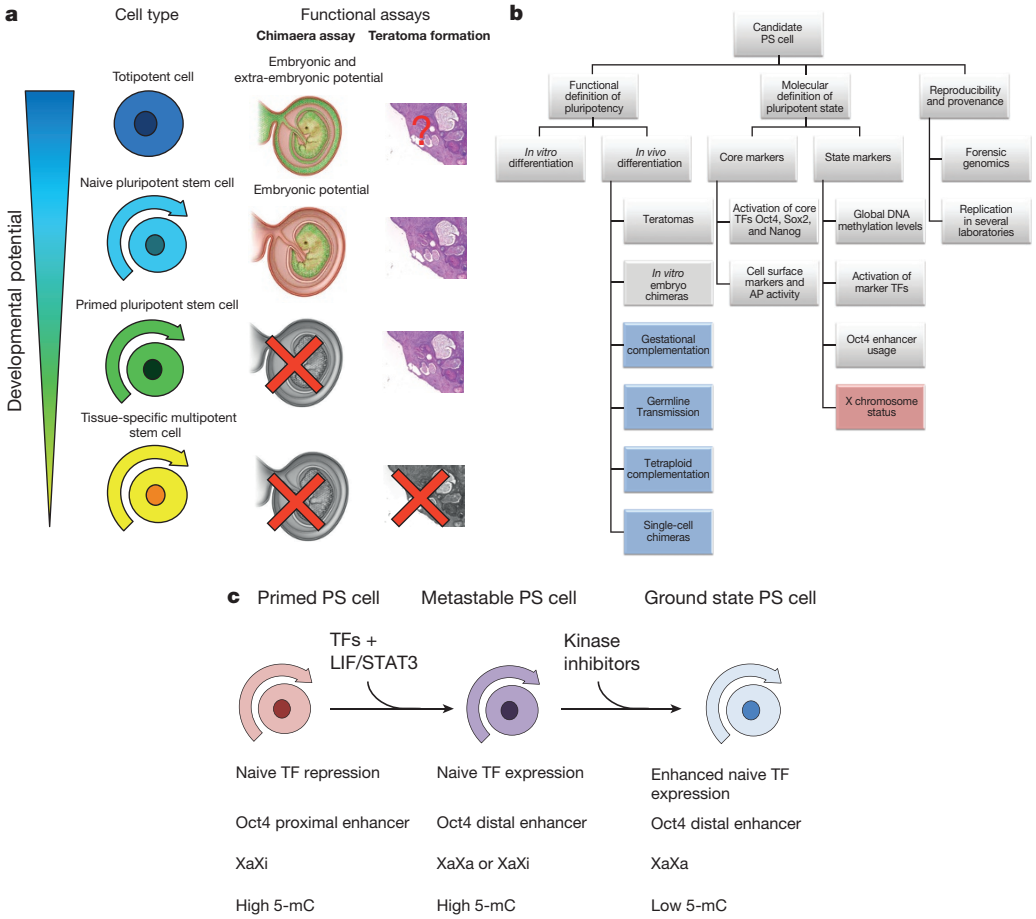


Figure 1 | Stem-cell potency. **a**, Two cardinal assays for assessing PS-cell potency are blastocyst chimaerism and teratoma formation. Performance in these assays allows classification of totipotent, naive pluripotent, primed pluripotent, and multipotent developmental potentials. Totipotency is defined by the capacity to develop and form all tissues of the organism, including extra-embryonic tissues. Naive PS cells are distinguished by the capacity to form a teratoma and a chimaeric animal following introduction into pre-implantation embryos, whereas primed PS cells form teratomas but do not efficiently form chimaeras following introduction into pre-implantation embryos. Tissue-specific multipotent stem cells form cell types related to their tissue-of-origin, but do not form teratomas or chimaeras. Primed EpiSCs do not efficiently form chimaeras when introduced into blastocysts, but can contribute to non-viable post-implantation chimaeras. Therefore, EpiSCs also exhibit pluripotency when introduced into post-implantation embryos. A strict criterion for potency is the demonstration that a single cell can differentiate into the different cell types via single-cell transplantation or by genetically labelling test cells and demonstrating that the daughters of a single cell contribute to different lineages. For human PS cells, teratoma formation remains the gold standard functional assay. Although single-cell-derived teratomas have not been directly generated from diploid human PS cells, clonal-cell-line-derived teratomas provide indirect evidence for the developmental potential of human PS cells at a single-cell level. **b**, Checklist for assessing the function and state of candidate PS cells. Validating the pluripotency of novel PS cells involves assessment of 'function' by measuring self-renewal capacity and developmental potential, and validating pluripotency as a 'state' by measuring the activation of core pluripotency transcription factors (TFs) OCT4, SOX2 and NANOG, and characterization of state markers, such as marker transcription factors and DNA methylation levels. For example, human ground state PS cells are anticipated to exhibit global DNA hypomethylation and reactivation of transcription factors expressed during pre-implantation development. For novel claims of PS cells, when possible, forensic-genomics-based approaches and independent reproduction in an independent laboratory should validate the provenance and reproducibility of pluripotent phenomena. The blue boxes indicate *in vivo* differentiation assays that should not be assessed in human cells; the red box indicates the uncertain relevance of X chromosome reactivation as a criterion for human ground state PS cells owing to the unresolved interpretation of X chromosome status in human naive pluripotency. AP activity, alkaline phosphatase activity. **c**, Resetting to ground state pluripotency. Primed PS cells exhibit high levels of DNA methylation, cannot chimaerize pre-implantation blastocysts, and female primed PS cells exhibit post-X-chromosome-inactivation status. Xa, active X chromosome; Xi, inactivated X chromosome. To overcome the differentiation barrier between naive and primed PS cells, transcription factors (TFs) are introduced into primed PS cells to initiate resetting. Transcription-factor-induced PS cells or metastable PS cells cultivated in ground state culture conditions will be reset to ground state pluripotency, demarcated by homogeneous expression of naive transcription factors and global DNA hypomethylation (low 5-mC) reminiscent of pre-implantation embryo cells. Globally hypomethylated genomes in ground state mouse ES cells resemble pre-implantation blastocysts, whereas serum-cultivated mouse ES cells and primed EpiSCs possess a hypermethylated genome reminiscent of post-implantation epiblasts and somatic cells. The methylation state of altered human PS cells is undefined, but reset cells generated by the Smith laboratory exhibit DNA methylation level changes closer to ground state mouse ES cells[55].

core transcription factors based on their specific expression pattern in PS cells and early embryos, and genetic screens identifying their essential role in establishing pluripotency in mice and humans[3,17–26]. OCT4 functions as a heterodimer with SOX2, placing SOX2 among the core regulators[22]. The generation of mouse and human iPS cells by ectopic expression of OCT4 and SOX2 highlights the pre-eminent role of OCT4/SOX2 in establishing pluripotency. Although NANOG is not required for mouse PS-cell maintenance[25], and is expressed at low or absent levels in mouse EpiSCs, it stabilizes PS cells, is necessary for *in vivo* pluripotency to develop in the inner cell mass (ICM)[26], and extensively co-localizes with OCT4 and SOX2 throughout the mouse and human PS cell genome. While the core transcription factors define and govern pluripotency, in special circumstances PS cells can tolerate loss of SOX2 or NANOG or substitution with other factors, suggesting flexibility in pluripotency governance. Among the core

Table 1 | Different PS cell types and their developmental potentials

Starting cells	Pluripotent stem cell	Criteria for pluripotency						Ref.
		In vitro differentiation	Teratoma	Postnatal chimaera	Germ line transmission	4*n* complementation	State of pluripotency	
Mouse germline tumour	ECCs	Yes	Yes	Yes	Yes	No	Naive	4
Mouse oocyte	Parthenogenetic ES cells	Yes	Yes	Yes	Yes	No	Naive	101
Mouse blastomere	ES cells	Yes	Yes	Yes	Yes	Yes	Naive	102
Mouse ICM	ES cells	Yes	Yes	Yes	Yes	Yes	Naive	5,6
Mouse Epiblast	EpiSCs	Yes	Yes	No	No		Primed	9,10
Mouse primordial germ cell	Embryonic germ cells	Yes	Yes	Yes	Yes	?	Naive	11,103
Mouse SSCs	GS cells, gPS cells; MASC	GS cells, gPS cells, MASC	GS cells, gPS cells, MASC	GS cells, gPS cells	GS cells, gPS cells	?	Naive (GS cells, gPS cells) Primed (MASCs)	12,104
Mouse somatic cells	iPS cells	Yes	Yes	Yes	Yes	Yes	Naive	3
Mouse somatic cells	Nuclear-transfer ES cells	Yes	Yes	Yes	Yes	Yes	Naive	105
Human germline tumour	ECCs	Yes	Yes	No	No	No	?	106
Human oocyte	Parthenogenetic ES cells	Yes	Yes	N/A	N/A	N/A	Primed	107
Human blastomere	ES cells	Yes	Yes	N/A	N/A	N/A	Primed	108
Human ICM	ES cells	Yes	Yes	N/A	N/A	N/A	Primed	7
Human somatic cells	iPS cells	Yes	Yes	N/A	N/A	N/A	Primed	109
Human somatic cells	Nuclear transfer ES cells	Yes	Yes	N/A	N/A	N/A	Primed	95

All cells listed are able to differentiate *in vitro*. Mouse oocyte-derived, blastocyst-derived ES cells, primordial germ-cell-derived embryonic germ cells, embryonic carcinoma cells (ECCs), SSC-derived cells, and iPS cells are able to generate chimaeras and contribute to the germ line. N/A, not applicable; ?, unknown. GS cells, germline stem cells; MASC, multipotent adult spermatogonial-derived stem cells; gPS cells, germline-derived pluripotent stem cells.

transcription factors, OCT4 has proven most indispensable and remains the preeminent pluripotency factor.

Mapping of OSN targets supports a model of regulatory control whereby OSN sustains self-renewal while restricting differentiation. OSN cooperatively bind their own promoters, forming an interconnected auto-regulatory loop[17,18]. OSN activate a substantial fraction of protein-coding, miRNA, and non-coding RNA genes in ES cells, while also occupying genes encoding lineage-specific regulators[27,28]. The promoters of many lineage regulators harbour both active (H3K4me3) and repressive (H3K27me3) histone marks, a bivalent state thought to facilitate activation of development genes upon exit from pluripotency[29]. The capacity of OSN to activate genes necessary for maintaining ES cells, while repressing lineage-specifying regulators, chiefly accounts for the dual hallmark features of self-renewal and differentiation potential.

While OCT4 and SOX2 are expressed in all PS cells, PS cells can be classified into different states of pluripotency based on a complement of diagnostic molecular signatures that delineate proximity to the pre-implantation ICM or post-implantation epiblast, respectively (Fig. 1c). In mice, four key distinctions amongst the various pluripotent states have been described to date: (1) X chromosome status in female cells; (2) global levels of DNA methylation; (3) Oct4 enhancer utilization; and (4) expression levels of a select group of regulators designated as 'naive' transcription factors: Klf4, Klf2, Esrrb, Tfcp2l1, Tbx3 and Gbx2 (refs 10, 26, 30–33). These naive transcription factors, along with Nanog, are expressed at low levels or are absent in primed PS cells and can reset primed PS cells in conjunction with naive pluripotency culture conditions. The capacity of 'naive' transcription factors to reset primed PS cells suggests a regulatory intersection between naive transcriptional circuitry and epigenetic resetting of the DNA methylome and X chromosome.

A molecular 'ground state' in mouse ES cells can be enforced by cultivating cells in leukaemia inhibitory factor and small molecule inhibitors of Mek and Gsk3 kinases (2i/LIF conditions), which stabilizes the diagnostic signatures of pluripotency in the pre-implantation blastocyst[30,34,35]. Ground state ES cells exhibit two active X chromosomes in female cells, low levels of DNA methylation, preferential utilization of the Oct4 distal enhancer, and naive transcription factor expression. In contrast, an alternative primed state is favoured by cultivation in FGF/ACTIVIN. Primed EpiSCs exhibit X-chromosome inactivation in female cells, high levels of DNA methylation, preferential utilization of the Oct4 proximal enhancer, and naive transcription factor repression. The molecular changes observed when ground state ES cells transition to primed EpiSCs *in vitro* appear to mirror changes during maturation of pre-implantation epiblast to post-implantation epiblast *in vivo*[14,36].

Both naive and primed PS cells exhibit heterogeneity at the level of state markers and single cells, which we briefly discuss below. While serum-cultivated mouse PS cells form chimaeras capable of germline transmission (a functional hallmark of naive pluripotency), such PS cells also bear high DNA methylation levels reminiscent of post-implantation epiblast[30]. EpiSCs also exhibit heterogeneity that can be altered via signalling pathway modulation. For example, region-specific EpiSCs (rsEpiSCs) preferentially engraft into posterior epiblasts and bear diagnostic markers of the post-implantation state, consistent with their status as primed PS cells[37]. Yet, rsEpiSCs possess higher cloning efficiency, a feature typically associated with naive PS cells. Thus, like serum-cultivated ES cells, rsEpiSCs manifest features associated with different phases of pluripotency. Cumulatively, these observations suggest mouse pluripotency encompasses a spectrum of functional and molecular states, highlighting the imprecision of nomenclature in the face of biological complexity.

A caveat to the concept of ground state PS cells arises from single-cell studies suggesting inherent metastability in PS cells. Heterogeneous single-cell gene expression profiles, flow cytometry, and replating experiments indicate the coexistence of distinct molecular and functional states in serum-cultivated mouse ES cells[38]. Even individual cells in more homogeneous ground state cultures have been reported to exhibit variable pluripotency transcription factor expression[39] and, while the origin and consequence of such heterogeneity are yet to be elucidated, the dynamic nature of pluripotency cannot be disregarded when classifying PS cell states. The markers distinguishing ground state from alternative PS cells remain relevant for evaluating novel PS cell types, especially claims of ground state human PS cells.

Adding additional nuance to the definitions of pluripotency, functional and molecular states are not always correlated. Mouse PS cells maintain molecular features of pluripotency, including expression of the core transcription factors, even when DNA methylation and H3K27 methylation are ablated[40–43], but cannot differentiate, and thereby lack functional pluripotency[44]. Thus, while molecular signatures can suggest pluripotency, only functional tests can establish the true developmental potential of a cell. Unlike mouse ES cells, conventional 'primed' human ES cells cannot tolerate *DNMT1* deletion, emphasizing the functional differences between mouse and human ES cells, which we discuss in detail below[45]. The observation that naive cells tolerate depletion of epigenetic regulators supports the concept of naive pluripotency as a

configuration with a reduced requirement for epigenetic repression compared to primed PS cells and somatic cells.

Functional assessment of pluripotency

A range of assays can be employed to reveal the developmental potential of PS cells: (1) *in vitro* differentiation; (2) teratoma formation; (3) chimaera formation; (4) germline transmission; (5) tetraploid complementation; and (6) single-cell chimaera formation. A summary of these assays along with their advantages and disadvantages is provided in Box 1.

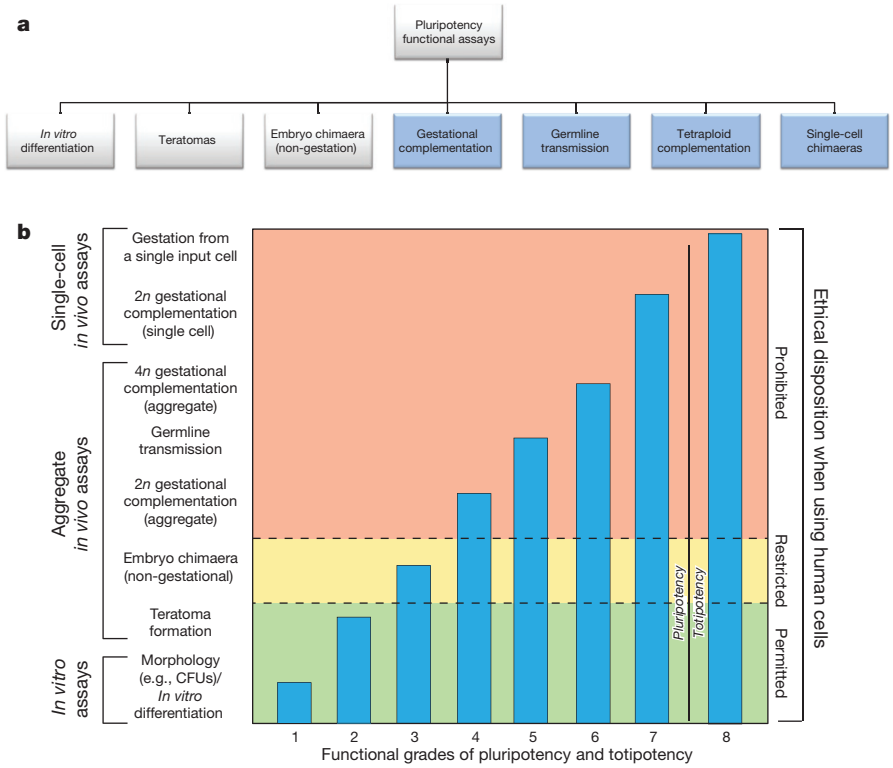
In vitro differentiation to derivatives of all three embryonic germ layers—ectoderm, mesoderm and endoderm—represents the lowest hurdle for establishing pluripotency. Typically, culture conditions that maintain pluripotency are replaced by cocktails of differentiation-inducing cytokines, morphogens or chemicals, and markers of specific target tissues are then surveyed.

The teratoma formation assay assesses the spontaneous generation of differentiated tissues from the three germ layers following the injection of cells into immune-compromised mice. Histologic analysis of teratomas is neither quantitative nor capable of assessing every possible cell type. Incompletely reprogrammed cells can generate masses that superficially resemble teratomas yet lack terminal three-germ-layer differentiation, potentially leading to misinterpretation[46]. Moreover, co-injection with matrices or scaffolds can elicit inflammatory or foreign-body reactions that can be misinterpreted as evidence of tissue differentiation, necessitating the use of lineage tracing or marker analysis to distinguish donor cells from reactive host tissue[93]. Because teratomas are not generated from single cells, the teratoma assay assesses developmental potency at a population-based level.

A third differentiation assay, blastocyst chimaera formation, measures whether test cells can re-enter development when introduced into host embryos at either of two pre-implantation stages: by aggregation with cleavage-stage morulas or by injection into blastocysts[47]. High-quality PS cells support normal development and generate high-grade chimaeras with extensive colonization of all embryonic tissues including the germ line, whereas less-potent PS cells produce either low chimaerism or reduced embryo viability.

BOX 1

Functional assays for pluripotency.



a, Overview of functional tests to assess developmental potency of PS cells. Blue boxes indicate assays that are restricted using human cells.
b, Functional assays for pluripotency, their grades of functional stringency, and ethical permissibility when using human cells. Analysis of *in vitro* characteristics, such as self-renewal capacity, colony morphology (CFU, colony-forming unit), and differentiation capacity *in vitro*, comprise a basic layer of pluripotency characterization. *In vivo* assays that measure differentiation capacity are taken as more robust indicators of potency.
Mouse PS-cell potency evaluation includes aggregate *in vivo* assays (that is, teratoma formation, embryo chimaeras (non-gestation), germline transmission, 2*n*/4*n* gestational complementation) and single-cell *in vivo* assays (that is, single-cell chimaeras and single-cell input gestations). 4*n* tetraploid complementation and single-cell chimaera formation are taken as more stringent functional assays for pluripotency.
The teratoma assay is the gold standard functional assay for assessing human PS-cell developmental potential. Chimaerism assays of human PS cells in murine embryos, as well as formation of primary human embryo chimaeras (non-gestation), are permissible under international stem-cell research guidelines[110] after rigorous scientific and ethical review. Potency evaluation of primary human chimaeras by *in vivo* gestational complementation in humans is ethically impermissible.
The assays for totipotency are: (1) gestation from a single input cell; and (2) gestational complementation experiments from a single cell that demonstrate contribution to all tissues of the body and high-grade placenta contribution. Note that it is not necessarily the case that if a test cell performs well in a more stringent test, that it will definitely pass a less stringent test. For example, it is unclear if totipotent cells form teratomas.

A fourth assay, germline transmission, entails breeding chimaeras to produce all-donor PS cell-derived offspring, which thus demonstrates the capacity of test cells to generate functional gametes. The integration of donor cells into all tissues of viable late-stage embryos, postnatal or adult mice, followed by germline transmission, is a robust indicator of chromosomal integrity and of functional pluripotency.

A fifth assay applied to mouse cells, tetraploid complementation, measures the capacity of test PS cells to direct development of an entire organism. Donor PS cells are introduced into tetraploid (4*n*) host blastocysts, which are generated by electrofusion of blastomeres at the two-cell stage. Because 4*n* blastocysts cannot sustain normal embryonic development beyond mid-gestation, while tetraploid extra-embryonic tissues develop normally and support donor cells[48], any resulting embryos are derived essentially entirely from donor PS cells.

A sixth, highly stringent assay is to inject single-donor mouse PS cells into a morula or blastocyst[49]. Genuine pluripotency is a property of a single cell and therefore chimaeras with widespread contribution from a single injected cell provide the clarity of clonal analysis. Both single-cell chimaerism and tetraploid complementation assays suffer from higher failure rates, but can be interpreted as the most definitive ways of demonstrating pluripotency.

Finally, while primed EpiSCs generate tri-lineage differentiation *in vitro* and form teratomas, EpiSCs rarely form chimaeras upon introduction into pre-implantation blastocysts. However, EpiSCs contribute to all germ layers when introduced into early post-implantation embryos in whole-embryo culture[37,50], although pluripotency of single cells has not yet been demonstrated.

Human pluripotent stem cells

Conventional human PS cells exhibit molecular hallmarks of primed state pluripotency, including preferential utilization of the OCT4 proximal enhancer, pronounced levels of DNA methylation, and a propensity for X chromosome inactivation in female cell lines[51]. Reports of human naive PS cells prompted some groups to attempt to assess potency by blastocyst chimaerism[52–54], constrained by the widespread acceptance that culture of human embryos for more than 14 days of development *in vitro*, or past the point of primitive streak formation (whichever is first), is ethically impermissible. Nevertheless, both primed and altered human PS cells have been introduced into mouse pre-implantation embryos[52–55]. Human naive PS cells engraft into the mouse ICM[52,54], although contribution to cross-species chimaeras has been minimal[52] or not detectable[53,54]. By contrast, region-specific human PS cells engraft into the posterior epiblast of cultured murine post-implantation embryos, indicating limited cross-species chimaerism[37].

More compelling evidence for cross-species blastocyst chimaerism has been reported following injection of primate naive iPS cells into mouse blastocysts, leading to clonal contribution to solid tissues[56]. Whereas primate ICM cells have thus far failed to form blastocyst chimaeras, unlike mouse ICM cells[16], aggregation of primate blastomeres (totipotent cells) does produce chimaerism[16]. Nonetheless, a recent study described altered primate PS cells that can incorporate into host embryos and develop into chimaeric fetuses with low-grade contribution to all three germ layers and early germ cell progenitors[57]. As in mice, high-grade contribution and germline transmission remain as more stringent tests to demonstrate naive pluripotency in primate ES cells.

Given the distinct behaviour of primate PS cells in chimaera studies, and lingering uncertainties about interspecies chimaerism, injecting human cells into mouse embryos needs additional validation before being accepted as a routine assay for stem-cell potency. Lacking robust functional assays for human stem-cell potency, transcriptional and epigenetic similarity of hypothetical ground state PS cells to the pluripotent cells in human pre-implantation embryos will remain the molecular standard for designation of human ground state PS cells (Fig. 1).

Erasure and resetting of DNA methylation is a molecular hallmark in mammalian pre-implantation and germline development. Human pre-implantation embryos have hypomethylated genomes. In contrast, ICM outgrowths undergo genomic remethylation and established human ES cells maintain pronounced DNA hypermethylation, similar to mouse primed PS cells[58,59]. Such epigenetic resetting appears to be controlled by a unique regulatory network present in pre-implantation embryos and the germ line. KLF4, TFCP2L1, ESRRB, TBX3 and GBX2, transcription factors implicated in mouse naive pluripotency, have been detected in human pre-implantation epiblast and are transcriptionally repressed in derived human ES cells, similarly to mouse EpiSCs[60]. However, the transcripts of certain murine naive transcription factors, such as KLF2, have not been detected in the human pre-implantation epiblast, revealing complexity. Additional species-specific differences also remain unresolved. The timing of X chromosome inactivation in human embryos is contentious[61,62] and 'epigenetic erosion' of the X chromosome in primed human ES cells complicates our understanding of X chromosome regulation[63,64]. Therefore, by current standards, we identify human ground state or naive PS cells according to molecular criteria used to delineate mouse ground state pluripotency, accepting that these criteria are tentative and subject to revision.

Acknowledging such caveats, a growing number of studies have demonstrated the feasibility of altering human PS cells towards a 'metastable' naive state of pluripotency[52,65–67]. More convincingly, PS cells generated by the Jaenisch and Smith laboratories express transcription factors implicated in the governance of mouse ground state ES cells[53,54]. While the X chromosome was inactive in human PS cells generated in the Jaenisch laboratory, we note again the uncertain significance of X chromosome status in human pluripotency[52,53,61–64]. Cells 'reset' in the Smith laboratory exhibit a meaningful reduction in DNA methylation to levels approaching human pre-implantation embryos. However, the unclear activation of the OCT4 distal enhancer, and lack of detailed characterization of transgene-independent cell lines leaves open the question of whether the reset state is stable[54].

More experimental understanding of the transition from totipotency to pluripotency in the intact human or primate embryo will be needed to truly define the human ground state PS cell. Direct derivation of ground state ES cells from human embryos would be a landmark, highlighting the continued relevance of human ES cell research.

Potency in native somatic cells

As an organism progresses from the earliest embryonic stages to adulthood its cells become progressively restricted in developmental potency, and acquire epigenetic modifications that present barriers to dedifferentiation. However, germ cells, responsible for perpetuating the species, retain a unique chromatin state receptive to reprogramming to a naive pluripotent state by signalling pathway modulation alone. Cultivation of primordial germ cells in 2i/LIF, among other culture conditions, generates chimaera-competent naive pluripotent cells[68].

By contrast, acquisition of naive pluripotency from somatic cells requires the prolonged, combinatorial action of reprogramming transcription factors and ES cell growth conditions[3]. An exception to this principle is chemical reprogramming, suggesting that culture conditions alone can fully reverse the differentiated state to pluripotency[69]. Notably, the final stage of chemical reprogramming is also induced by 2i/LIF. In contrast to mouse cells, our current capacity to generate human PS cells by signalling pathway modulation alone is more limited. The pluripotency of human embryonic germ cells and adult testis-derived human PS cells, both generated by culture of human germ cells, remains contentious, and small-molecule-based reprogramming of human somatic cells to pluripotency has not yet been demonstrated[70–72].

Alterations in cellular identity can accompany human disease. Chronic exposure to stomach acid from gastro-oesophageal reflux converts stratified squamous epithelium of the distal oesophagus to goblet-cell containing columnar epithelia more typical of the intestine, a condition termed Barrett's oesophagus, which predisposes to adenocarcinoma. Metaplasia and other forms of tissue ectopias, where aberrant tissues form in unusual locations, suggest cell identity conversion occurs in the body. Thus it is intriguing to consider various claims of pluripotency for cells isolated from

perinatal or somatic tissues, such as multipotent adult progenitor cells[73,74], very small embryonic-like cells[75], multi-lineage differentiating stress-enduring cells[76], and endogenous pluripotent stem cells[77]. When considering novel claims of expanded potency a strict criterion is demonstration that a single cell can differentiate into different cell types, a standard of clonal analysis lacking in most studies.

Evaluating totipotency features

A robust, bidirectional capacity to form both embryonic lineages and extra-embryonic trophoblast layers of the placenta, as well as yolk sac derivatives, distinguishes totipotency from pluripotency. While somatic cells are reset to totipotency following nuclear transfer into oocytes, to date no lab (to our knowledge) has claimed to propagate *in vitro* cells with totipotency equivalent to zygotes or blastomeres. Below, we briefly review previous claims of placental differentiation capacity in PS cells and propose how one might evaluate claims of totipotency (Table 2).

The most stringent demonstration of totipotency requires that a single cell produce a term birth under experimental conditions[78–81], a standard achieved in rodents and in non-human primates for single blastomeres extracted from pre-implantation embryos[1,81]. Later-stage blastomeres may contribute to all embryonic and extra-embryonic tissues, and yet fail to support a viable conceptus because of reduced cell numbers at the blastocyst stage. Thus, an alternate and less stringent test of totipotency is the potential of genetically marked single cells to contribute extensively to both embryonic and extra-embryonic lineages after introducing donor cells into pre-blastocyst-stage embryos. In the mouse, for example, only isolated two-cell blastomeres can generate an entire conceptus[82], but single blastomeres at the eight-cell stage still manifest totipotency in aggregation chimaeras[1]. Sister blastomeres of a four-cell stage human embryo can develop individually into blastocysts with ICM and trophectoderm cells[83]. An essential feature of these functional tests of totipotency is demonstration of developmental capacity at the single-cell level.

Mouse PS cells with bidirectional developmental capacity for extra-embryonic and somatic fates have been claimed following specific genetic (for example, *Dnmt1* knockout[84]) or cell culture modifications (for example, ground state[39,85]) (summarized in Table 2). '*In vivo* reprogrammed' iPS cells purportedly contribute to the placenta, unlike ES cells or *in vitro* reprogrammed iPS cells[86]. These studies reported differentiation into trophoblast-stem-like cells and the formation of blastocyst-like structures. However, the *in vivo* chimaera potential of trophoblast-stem-like cells was not assessed. Further, single cells did not yield robust high-grade contribution to the placenta[39]. Thus, the definitive functional criterion for establishing totipotency, single-cell contribution to the trophoblast and ICM lineages, has not yet been demonstrated. The molecular changes associated with acquisition of totipotent-like developmental potential have differed across studies and include the expression of '2C-specific' genes, morula-specific genes, and extra-embryonic transcription factors. Therefore, by current standards, accepting that the relevance of these molecular criteria are tentative and subject to revision, the essential criterion of totipotency remains functional, whereby a single cell generates both ICM and trophectoderm fates in a transplantation assay. Ideally, detailed assessment of embryonic and extra-embryonic tissues should be made late in gestation, so that extensive and functional contribution can be demonstrated.

Conventional primed human PS cells reportedly form both trophectoderm and primitive endoderm-like derivatives *in vitro*[87]. However, confirmation of the identity of these derivatives has proven challenging[88]. Injection of human naive PS cells into mouse embryos has not resulted in contribution to ICM and trophectoderm lineages. Future claims of mouse totipotent stem cells will require stringent functional and molecular validation, while in humans, molecular criteria and comparison to primate species will have to suffice to establish plausibility.

Assessing provenance and potency via genomics

Advanced sequencing platforms have allowed researchers to generate a multitude of genomic and epigenomic data (for example, RNA sequencing (RNA-seq), chromatin immunoprecipitation sequencing (ChIP-seq) and bisulfite sequencing), enabling a more comprehensive description of cellular identity. Systems-level analyses have confirmed that direct reprogramming of somatic cells largely re-establishes molecular signatures associated with ES cells[89,90]. These analyses also detected low-fidelity reprogramming, such as in intermediates and cells with epigenetic memory[89,90]. Recently, genomic analyses have proven instrumental in defining ground state pluripotency. Thus, while not required for routine characterization of PS cells, genomic analyses play a critical role for benchmarking novel claims of reprogramming and PS cells (Box 2).

DNA sequencing also provides genetic fingerprints that can eliminate cell contamination as a confounder of reported results. Because cell line contamination is widespread, applying such genotyping methods to confirm cell line provenance is appropriate[91]. In the case of the STAP cell phenomenon, the authors reported acid-reprogrammed PS cells with features of totipotency. Our re-analysis of genomic data revealed unexpected mismatches in sex and genotype between donor somatic cells and converted STAP cells[92]. Further analysis of a STAP-derived cell line, Fgf4-induced stem cells, revealed a mixture that contained trophoblast stem cells, explaining the high-grade placenta colonization reported for Fgf4-induced stem cells. These findings are consistent with and extend the results of an extensive whole-genome sequencing analysis of STAP-related samples for the RIKEN investigation[93], which found contamination of purported STAP stem-cell lines with embryonic stem cells of a different genetic background[94].

By contrast, forensic genomics applied to sequencing data from two reports of nuclear-transfer-derived human ES cells (NT-hESCs) have confirmed cell line provenance[95,96]. Inferred genome-wide single nuc-

Table 2 | Stem cells with reported bidirectional developmental potential

	Critera for totipotency								
Cells	Genetic manipulation	Embryonic contribution	Placenta contribution	Yolk sac contribution	*In vitro* differentiation into trophoblast	Trophoblast stem-cell derivation	Single-cell injection	Molecular features	Ref.
Dnmt1 KO ES cells	*Dnmt1* KO ES cells	?	Yes	?	Yes	No	No	Co-expression of Oct4 and Cdx2 upon 'differentiation'; hypomethylation of Elf5 promoter	84
'2C' ES cells/Kdm1a ES cells	2C reporter	Yes	Yes	Yes	Not tested/No	Not tested/No	No	Activation of 2C genes.	85
Hex + 2i ES cells	Hex reporter	Yes	Yes	Yes	$Cdx2^+$ trophoblast differentiation	Not tested/No	Yes	Co-expression of embryonic and extra-embryonic genes	39
In vivo reprogrammed iPS cells	OSKM cassette	Yes	Yes	Yes	Yes	TSC-like cells, but no *in vivo* chimaeric placenta	No	Morula gene signature	86

KO, knockout; TSC, trophoblast stem cell; ?, unknown.

BOX 2

Forensic genomics for potency and provenance of PS cells.

Evaluating potency via genomic analyses.

Transcriptome analysis. Computational analysis can quantify the extent to which an experimental protocol converts a parental cell towards the target cell. For example, PluriTest[111] defines a pluripotency-specific signature based on a compendium of expression data sets from pluripotent and non-pluripotent cells and evaluates the presence of this signature in a given sample. CellNet is a bioinformatics algorithm that assesses the fidelity of cell fate conversion using cell- and tissue-specific gene regulatory networks[112].

Epigenomic characterization. Genome-wide profiling of chromatin features (for example, histone modifications, transcription factor binding, DNase I hypersensitivity, and DNA methylation) captures the epigenetic landscape of PS cells and the transitions that occur during reprogramming or differentiation. Combining gene expression and epigenetic maps can provide mechanistic insights into the fidelity of reprogrammed PS cells. For example, some mouse iPS cells fail to re-establish bivalent domains at developmental loci, which might reduce developmental competence for all tissue types[89]. This failure could only be detected via epigenetic analysis.

Genomic integrity. Whole-genome sequencing (WGS) allows comprehensive identification of single nucleotide polymorphisms (SNPs) and a wide range of structural variations including copy number variants, copy-neutral events (such as translocations and inversions), and viral insertions, at base-pair resolution. Comparison of genomic variants before and after reprogramming will locate genomic alterations that may occur during reprogramming and potentially impact cellular function.

Genotyping for cell line provenance and contamination.

Provenance. Comparing the genotype of reprogrammed PS cells to parental cells enables verification of provenance. Genome-wide SNP arrays characterize a known set of SNPs and are sufficient for matching two samples. Based on the intensities of the probe hybridization reaction for each SNP and the ratio of the intensities between the two alleles, it is possible to estimate allele-specific copy numbers in addition to SNP genotypes. Sequencing-based assays such as exome or whole-genome sequencing can provide a more comprehensive characterization of SNPs; genotype information can also be inferred from RNA-seq and possibly other functional genomics data. Analyses of other types of genome variation such as microsatellites can also be used as a form of genetic fingerprinting. Microsatellite profiling, for example, is recommended by the International Cell Line Authentication Committee (ICLAC) for cultured cell lines[113].

Contamination. Genome-wide SNP data can also be used to examine genetic heterogeneity of cell line cultures and to detect contamination with another cell line. For a homogeneous population, we expect to see sharp allele frequency (alternative over reference allele frequency ratio) distributions with a dominant peak near 0 (homogyzous reference) and smaller peaks at ~0.5 (heterozygous) and 1 (homozygous alternative). When there is contamination by cells from different individuals or strains, we expect to observe small peaks at low allele frequencies (for example, at 0.05 and 0.1 if there is 10% contamination), corresponding to the alternative alleles from the second population of cells. With sequencing data, these estimates can be derived with greater precision, using both annotated SNPs and novel single-nucleotide variants. Sample contamination was detected in the STAP data with this analysis.[93]

leotide variants (SNVs) from exome sequencing data classified samples generated in the Egli laboratory as genetically similar or dissimilar (Fig. 2). Parental donor fibroblasts and NT-hESCs possessed similar SNV profiles, consistent with nuclear transfer origin. Independently sourced *in vitro*-fertilization-derived ES cells and parthenogenetic ES cells manifest distinct genetic provenance from parental donor fibroblasts and NT-hESCs, as expected. SNV genotyping also confirmed previously reported patterns of recombination in human parthenogenetic ES cells, concordant with observations in mouse parthenogenetic ES cells[97,98]. Matching genotypes between parental fibroblasts and reprogrammed NT-hESCs were also confirmed in RNA-seq data generated in the Mitalipov laboratory (Supplementary Fig. 1). Collectively, these analyses support appropriate provenance of NT-hESCs and exclude a parthenogenetic origin for NT-hESCs.

Reproducibility of computational analyses

As genomic analyses can validate the provenance and confirm molecular signatures of novel PS cells, we advocate posting of relevant genomic data, metadata, and full details of computational analysis upon manuscript publication. Deposition of sequencing data to public repositories such as the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and Short Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra) is required by most peer-reviewed journals, but enforcement of sharing policies is highly variable, and complicated by the complexities of experimental design and data. Consequently, verification that full data and associated metadata have been deposited often requires expertise and time beyond what is available during peer review. Greater compliance by the stem-cell community in depositing all relevant genomic data and metadata as well as consistent enforcement by journals will promote reproducibility of results. We also recommend deposition of 'intermediate' data, the key steps and results obtained in the data analysis process. For full reproducibility of computational analysis, we also advocate release of the computer code, through a supplementary website or open source code management tools. We note that genomic analysis and availability of data, metadata, and methods are especially important for novel claims of reprogramming and altered stem-cell states.

Conclusion and future prospects

Here, we articulate a consensus definition of pluripotency predicated on both functional assessments of differentiation potential and diagnostic molecular signatures. Such an integration of functional and molecular hallmarks of pluripotency provides for a robust set of criteria against which to validate claims of pluripotency achieved by novel experimental strategies. Given the central role of core transcription factors in reprogramming somatic cells and maintaining the pluripotent state, failure to observe ES-cell-like levels of these transcription factors in studies asserting functional pluripotency from novel sources should merit scepticism and should be accompanied by strong evidence for alternative gene regulatory networks and mechanisms that maintain the unique pluripotent state of the mammalian genome. Another example of uncoupling between molecular and functional hallmarks is a report that overexpression of cell adhesion molecules such as E-cadherin can endow primed PS cells with the capacity to chimaerize the pre-blastocyst, with no evidence of resetting to naive pluripotency[99]. Conversely, recent reports suggesting that reprogramming transitions through a transient state that molecularly resembles naive pluripotency, but without functional hallmarks of naive pluripotency, might not comprise bona fide naive pluripotency[100]. While most labs deriving PS cells for routine use need not employ the comprehensive set of assays reviewed here, claims of novel states of potency or new means of deriving PS cells necessitate more comprehensive characterization and documentation.

Documentation of PS-cell states that span the continuum between ground state pluripotency and primed pluripotency provokes the question of how to define the human ground state. Further, reports that human PS cells can be 'reset' imply the feasibility of generating PS cells with bona fide totipotency. Ultimately, refined molecular benchmarking of reprogramming and more predictable experimental capture of altered

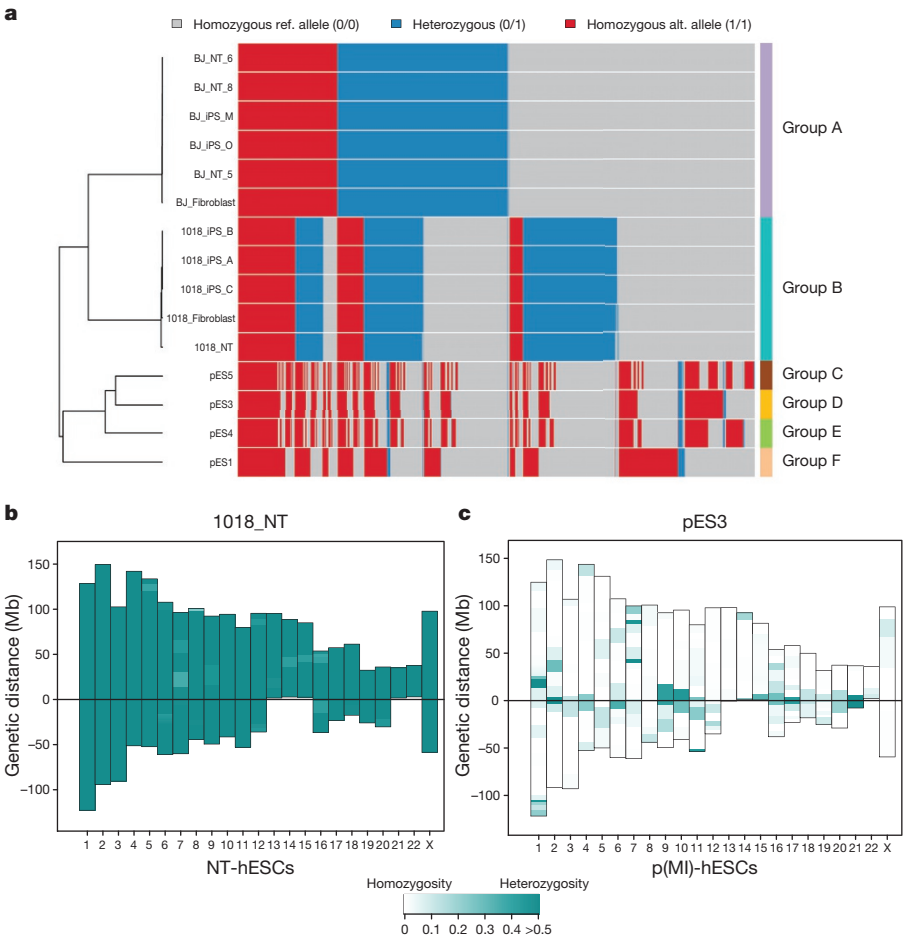


Figure 2 | Genomic provenance of nuclear transfer human embryonic stem cells (NT-hESCs). **a**, Single nucleotide sequence variants (SNVs) inferred using exome sequencing data using the human reference genome GRch37. The selected SNVs are classified as homozygous for reference allele (0/0 genotype), homozygous for alternative allele (1/1 genotype) or heterozygous (0/1 genotype). Samples are clustered based on the sum of the edit distance between each SNV. The six different genotypes in three groups can be discerned: group A (BJ fibroblast and BJ fibroblast-reprogrammed human pluripotent stem-cell lines); group B (1018 fibroblast and 1018 fibroblast-reprogrammed human pluripotent stem-cell lines); and groups C–F (human parthenogenetic embryonic stem cells). **b**, Genome-wide SNP genotyping of a representative clone of NT-hESCs (Egli laboratory exome sequencing data) excluding parthenogenetic origin. Panels show genotypes for each chromosome, from centromere to telomere revealing blocks or haplotypes of markers. Mb, megabases. **c**, Genome-wide SNP genotyping of a representative clone of parthenogenetic (meiosis I) human embryonic stem cells (p(MI)-hES cells) (Egli laboratory exome sequencing data). Panels show genotypes for each chromosome, from centromere to telomere, revealing blocks or haplotypes of markers. Pericentromeric heterozygosity is consistent with a meiosis I parthenogenetic ES cell.

pluripotent states requires a more sophisticated understanding of human pre-implantation development.

For lasting scientific impact, claims of reprogramming and altered states of pluripotency should be broadly applicable to more than one experimental model and be independently replicated by multiple laboratories. Before publication, we encourage that researchers claiming landmark reprogramming advances first demonstrate replication by independent laboratories and incorporate forensic genomic analyses to confirm appropriate cell provenance. Science is ultimately a self-correcting process where the scientific community plays a crucial and collective role.

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1. Kelly, S. J. Studies of the developmental potential of 4- and 8-cell stage blastomeres. *J. Exp. Zool.* **200,** 365–376 (1977).
2. Gurdon, J. B. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* **10,** 622–640 (1962).
   A pioneering study that demonstrated that somatic cells can be reset to an early embryonic state via nuclear transplantation into eggs.
3. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126,** 663–676 (2006).
   The landmark paper establishing that four transcription factors can reprogram somatic cells to a pluripotent state.
4. Stevens, L. C. Studies on transplantable testicular teratomas of strain 129 mice. *J. Natl. Cancer Inst.* **20,** 1257–1275 (1958).
5. Evans, M. J. & Kaufman, M. H. Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292,** 154–156 (1981).
   A paper establishing that pluripotent stem cells can be isolated from mouse blastocysts and be propagated *in vitro* as continuously growing cell lines.
6. Martin, G. R. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl Acad. Sci. USA* **78,** 7634–7638 (1981).
   A paper establishing that pluripotent stem cells can be isolated from mouse blastocysts and be propagated *in vitro* as continuously growing cell lines.
7. Thomson, J. A. *et al.* Embryonic stem cells derived from human blastocysts. *Science* **282,** 1145–1147 (1998).
   The landmark paper establishing that pluripotent stem cells can be isolated from human blastocysts.
8. Thomson, J. A. *et al.* Isolation of a primate embryonic stem cell line. *Proc. Natl Acad. Sci. USA* **92,** 7844–7848 (1995).
9. Brons, I. G. *et al.* Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* **448,** 191–195 (2007).
   One of two pioneering studies that established that an alternative pluripotent state can be isolated from post-implantation mouse embryos resembling conventional human ES cells, suggesting that human ES cells might correspond to a post-implantation state.
10. Tesar, P. J. *et al.* New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448,** 196–199 (2007).
    One of two pioneering studies that established an alternative pluripotent state from post-implantation mouse embryos that resembles conventional human ES cells, suggesting that human ES cells might correspond to a post-implantation state.
11. Matsui, Y., Zsebo, K. & Hogan, B. L. M. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* **70,** 841–847 (1992).
12. Kanatsu-Shinohara, M. *et al.* Generation of pluripotent stem cells from neonatal mouse testis. *Cell* **119,** 1001–1012 (2004).
13. Ko, K. *et al.* Induction of pluripotency in adult unipotent germline stem cells. *Cell Stem Cell* **5,** 87–96 (2009).
14. Boroviak, T. *et al.* The ability of inner-cell-mass cells to self-renew as embryonic stem cells following epiblast specification. *Nature Cell Bio.* **16,** 516–528 (2014).
15. Nichols, J. & Smith, A. Naive and primed pluripotent states. *Cell Stem Cell* **4,** 487–492 (2009).
16. Tachibana, M. *et al.* Generation of chimeric rhesus monkeys. *Cell* **148,** 285–295 (2012).
17. Boyer, L. A. *et al.* Core regulatory circuitry in human embryonic stem cells. *Cell* **122,** 947–956 (2005).
    A study that affirmed the principle that OCT4, SOX2, and NANOG constitute a core regulatory circuitry that explains the self-renewal and differentiation capacity of PS cells.
18. Loh, Y. H. *et al.* The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genet.* **38,** 431–440 (2006).
19. Nichols, J. *et al.* Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95,** 379–391 (1998).

20. Chambers, I. *et al.* Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* **113,** 643–655 (2003).
21. Mitsui, K. *et al.* The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **113,** 631–642 (2003).
22. Avilion, A. A. *et al.* Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **17,** 126–140 (2003).
23. Masui, S. *et al.* Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nature Cell Biol.* **9,** 625–635 (2007).
24. Wang, Z., Oron, E., Nelson, B., Razis, S. & Ivanova, N. Distinct lineage specification roles for NANOG, OCT4, and SOX2 in human embryonic stem cells. *Cell Stem Cell* **10,** 440–454 (2012).
25. Chambers, I. *et al.* Nanog safeguards pluripotency and mediates germline development. *Nature* **450,** 1230–1234 (2007).
26. Silva, J. *et al.* Nanog is the gateway to the pluripotent ground state. *Cell* **138,** 722–737 (2009).
27. Chen, X. *et al.* Integration of external signaling pathway with the core transcriptional network in embryonic stem cells. *Cell* **133,** 1106–1117 (2008).
28. Kim, J., Chu, J., Shen, X., Wang, J. & Orkin, S. H. An extended transcriptional network for pluripotency of embryonic stem cells. *Cell* **132,** 1049–1061 (2008).
29. Bernstein, B. E. *et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125,** 315–326 (2006).
30. Leitch, H. G. *et al.* Naive pluripotency is associated with global DNA hypomethylation. *Nature Struct. Mol. Biol.* **20,** 311–316 (2013).
The first study to describe the association between 2i cultivation and DNA hypomethylation, linking *in vitro* naive pluripotency with the DNA hypomethylation observed in pre-implantation embryos and the germ line.
31. Guo, G. *et al.* Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development* **136,** 1063–1069 (2009).
The first paper to convert EpiSCs to naive pluripotency, affirming the concept that the naive and primed states of pluripotency are interconvertible.
32. Bao, S. *et al.* Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature* **461,** 1292–1295 (2009).
33. Dunn, S. J., Martello, G., Yordanov, B., Emmott, S. & Smith, A. G. Defining an essential transcription factor program for naive pluripotency. *Science* **344,** 1156–1160 (2014).
34. Ying, Q. L. *et al.* The ground state of embryonic stem cell self-renewal. *Nature* **453,** 519–523 (2008).
The first study to report the remarkable synergism between MEK and GSK3 inhibition and the first articulation of the concept of a pluripotent 'ground state'.
35. Marks, H. *et al.* The transcriptional and epigenomic foundations of ground state pluripotency. *Cell* **149,** 590–604 (2012).
36. Smith, Z. D. *et al.* A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484,** 339–344 (2012).
37. Wu, J. *et al.* An alternative pluripotent state confers interspecies chimeric competency. *Nature* **521,** 316–321 (2015).
38. Kumar, R. M. *et al.* Deconstructing transcriptional heterogeneity in pluripotent stem cells. *Nature* **516,** 56–61 (2014).
39. Morgani, S. M. *et al.* Totipotent embryonic stem cells arise in ground-state culture conditions. *Cell Rep.* **3,** 1945–1957 (2013).
40. Chamberlain, S. J., Yee, D. & Magnuson, T. Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. *Stem Cells* **26,** 1496–1505 (2008).
41. Okano, M., Bell, D. W., Habeer, D. A. & Li, E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99,** 247–257 (1999).
42. Li, E., Bestor, T. H. & Jaenisch, R. Target mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69,** 915–926 (1992).
43. Tsumura, A. *et al.* Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a, and Dnmt3b. *Genes Cells* **11,** 805–814 (2006).
44. Beard, C., Li, E. & Jaenisch, R. Loss of methylation activates XIST in somatic but not in embryonic cells. *Genes Dev.* **9,** 2325–2334 (1995).
45. Liao, J. *et al.* Targeted disruption of DNMT1, DNMT3A, and DNMT3B in human embryonic stem cells. *Nature Genet.* **47,** 469–478 (2015).
46. Chan, E. M. *et al.* Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells. *Nature Biotechnol.* **27,** 1033–1037 (2009).
47. Nagy, A., Gocza, E. *et al.* Embryonic stem cells alone are able to support fetal development in the mouse. *Development* **110,** 815–821 (1990).
48. Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W. & Roder, J. C. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc. Natl Acad. Sci. USA* **90,** 8424–8428 (1993).
49. Wang, Z. & Jaenisch, R. At most three ES cells contribute to the somatic lineages of chimeric mice produced by ES-tetraploid complementation. *Dev. Biol.* **275,** 192–201 (2004).
50. Huang, Y., Osorno, R., Tsakiridis, A. & Wilson, V. *In vivo* differentiation potential of epiblast stem cells revealed by chimeric embryo formation. *Cell Rep.* **2,** 1571–1578 (2012).
51. Hanna, J. *et al.* Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. *Proc. Natl Acad. Sci. USA* **107,** 9222–9227 (2010).
52. Gafni, O. *et al.* Derivation of novel human ground state naive pluripotent stem cells. *Nature* **504,** 282–286 (2013).
53. Theunissen, T. W. *et al.* Systematic identification of culture conditions for induction and maintenance of naive human pluriptoency. *Cell Stem Cell* **15,** 471–487 (2014).
54. Takashima, Y. *et al.* Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell* **158,** 1254–1269 (2014).
55. James, D., Noggle, S. A., Swigut, T. & Brivanlou, A. H. Contribution of human embryonic stem cells to mouse blastocyst. *Dev. Biol.* **295,** 90–102 (2006).
56. Fang, R. *et al.* Generation of naive induced pluripotent stem cells from rhesus monkey fibroblasts. *Cell Stem Cell* **15,** 488–496 (2014).
57. Chen, Y. *et al.* Generation of cynomolgus monkey chimeric fetuses using embryonic stem cells. *Cell Stem Cell* **17,** 116–124 (2015).
58. Guo, H. *et al.* The DNA methylation landscape of human early embryos. *Nature* **511,** 606–610 (2014).
59. Smith, Z. D. *et al.* DNA methylation dynamics of the human preimplantation embryo. *Nature* **511,** 611–615 (2014).
60. Yan, L. *et al.* Single-cell RNA-seq profiling of human preimplantation embryos and embryonic stem cells. *Nature Struct. Mol. Biol.* **20,** 1131–1139 (2013).
61. Okamoto, I. *et al.* Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. *Nature* **472,** 370–374 (2011).
62. O'Leary, T. *et al.* Tracking the progression of the human inner cell mass during embryonic stem cell derivation. *Nature Biotechnol.* **30,** 278–282 (2012).
63. Silva, S. S., Rowntree, R. K., Mekhoubad, S. & Lee, J. T. X-chromosome inactivation and epigenetic fluidity in human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **105,** 4820–4825 (2008).
64. Anguera, M. C. *et al.* Molecular signatures of human induced pluripotent stem cells highlight sex differences and cancer genes. *Cell Stem Cell* **11,** 75–90 (2012).
65. Chan, Y. S. *et al.* Induction of a human pluripotent state with distinct regulatory circuitry that resembles preimplantation epiblast. *Cell Stem Cell* **13,** 663–675 (2013).
66. Ware, C. B. *et al.* Derivation of naive human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **111,** 4484–4489 (2014).
67. Valamehr, B. *et al.* Platform for induction and maintenance of transgene-free hiPSCs resembling ground state pluripotent stem cells. *Stem Cell Rep.* **2,** 366–381 (2014).
68. Leitch, H. G. *et al.* Rebuilding pluripotency from primordial germ cells. *Stem Cell Rep.* **1,** 66–78 (2013).
69. Hou, P. *et al.* Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* **341,** 651–654 (2013).
70. Shamblott, M. J. *et al.* Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc. Natl Acad. Sci. USA* **95,** 13726–13731 (1998).
71. Conrad, S. *et al.* Generation of pluripotent stem cells from adult human testis. *Nature* 456, 344–349 (2008); retraction **512,** 338 (2014).
72. Ko, K. *et al.* Human adult germline stem cells in question. *Nature* **465,** E1 (2010).
73. Reyes, M. & Verfaillie, C. M. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. *Ann. NY Acad. Sci.* **938,** 231–235 (2001).
74. Jiang, Y. *et al.* Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418,** 41–49 (2002).
75. Kucia, M. *et al.* A population of very small embryonic-like (VSEL) $CXCR4^+SSEA\text{-}1^+Oct4^+$ stem cells identified in adult bone marrow. *Leukemia* **20,** 857–869 (2006).
76. Kuroda, Y. *et al.* Isolation, culture, and evaluation of multilineage-differentiating stress-enduring (Muse) cells. *Nature Protocols* **8,** 1391–1415 (2013).
77. Roy, S. *et al.* Rare somatic cells from human breast tissue exhibit extensive lineage plasticity. *Proc. Natl Acad. Sci. USA* **110,** 4598–4603 (2013).
78. Nicholas, J. & Hall, B. Experiments on developing rats: II. The development of isolated blastomeres and fused eggs. *J. Exp. Zool.* **90,** 441–459 (1942).
79. Tarkowski, A. K. Experiments on the development of isolated blastomeres of mouse eggs. *Nature* **184,** 1286–1287 (1959).
80. Willadsen, S. M. & Polge, C. Attempts to produce monozygotic quadruplets in cattle by blastomere separation. *Vet. Rec.* **108,** 211–213 (1981).
81. Mitalipov, S. M. *et al.* Monozygotic twinning in rhesus monkeys by manipulation of *in vitro*-derived embryos. *Biol. Reprod.* **66,** 1449–1455 (2002).
82. Rossant, J. Postimplantation development of blastomeres isolated from 4- and 8-cell mouse eggs. *J. Embryol. Exp. Morphol.* **36,** 283–290 (1976).
83. Van de Velde, H., Cauffman, G., Tournaye, H., Devroey, P. & Liebaers, I. The four blastomeres of a 4-cell stage human embryo are able to develop individually into blastocysts with inner cell mass and trophectoderm. *Hum. Reprod.* **23,** 1742–1747 (2008).
84. Ng, R. K. *et al.* Epigenetic restriction of embryonic cell lineage fate by methylation of Elf5. *Nature Cell Biol.* **10,** 1280–1290 (2008).
85. Macfarlan, T. S. *et al.* Embryonic stem cell potency fluctuates with endogenous retrovirus activity. *Nature* **487,** 57–63 (2012).
86. Abad, M. *et al.* Reprogramming *in vivo* produces teratomas and iPS cells with totipotency features. *Nature* **502,** 340–345 (2013).
87. Xu, R. H. *et al.* BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nature Biotechnol.* **20,** 1261–1264 (2002).
88. Bernardo, A. S. *et al.* BRACHYURY and CDX2 mediate BMP-induced differentiation of human and mouse pluripotent stem cells into embryonic and extraembryonic lineages. *Cell Stem Cell* **9,** 144–155 (2011).
89. Mikkelsen, T. S. *et al.* Dissecting direct reprogramming through integrative genomic analysis. *Nature* **454,** 49–55 (2008).
90. Kim, K. *et al.* Epigenetic memory in induced pluripotent stem cells. *Nature* **467,** 285–290 (2010).
91. Yu, M. *et al.* A resource for cell line authentication, annotation, and quality control. *Nature* **520,** 307–311 (2015).
92. De Los Angeles, A. *et al.* Failure to replicate the STAP cell phenomenon. *Nature* http://dx.doi.org/10.1038/nature15513 (this issue).
93. RIKEN. Report on STAP Cell Research Paper Investigation http://www3.riken.jp/stap/e/c13document52.pdf (2014).

94. Konno, D. *et al.* STAP cells are derived from ES cells. *Nature* http://dx.doi.org/10.1038/nature15366 (this issue).
95. Tachibana, M. *et al.* Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* **153,** 1228–1238 (2013).
This study was the first to demonstrate the feasibility of somatic cell nuclear transfer to reset human cells to totipotency.
96. Ma, H. *et al.* Human oocytes reprogram adult somatic nuclei of a type I diabetic to diploid pluripotent stem cells. *Nature* **511,** 177–183 (2014).
97. Kim, K. *et al.* Histocompatible embryonic stem cells by parthenogenesis. *Science* **315,** 482–486 (2007).
98. Kim, K. *et al.* Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer. *Cell Stem Cell* **1,** 346–352 (2007).
99. Ohtsuka, S., Nishikawa-Torikai, S. & Niwa, H. E-cadherin promotes incorporation of mouse epiblast stem cells into normal development. *PLoS ONE* **7,** e45220 (2012).
100. Cacchiarelli, D. *et al.* Integrative analyses of human reprogramming reveal dynamic nature of induced pluripotency. *Cell* **162,** 412–424 (2015).
101. Narasimha, M., Barton, S. C. & Surani, M. A. The role of the paternal genome in the development of the mouse germ line. *Curr. Biol.* **7,** 881–884 (1997).
102. Wakayama, S. *et al.* Efficient establishment of mouse embryonic stem cell lines from single blastomeres and polar bodies. *Stem Cells* **25,** 986–993 (2007).
103. Resnick, J. L., Bixler, L. S., Cheng, L. & Donovan, P. J. Long-term proliferation of mouse germ cells in culture. *Nature* **359,** 550–551 (1992).
104. Seandel, M. *et al.* Generation of functional multipotent adult stem cells from GPR125^{+} germline progenitors. *Nature* **449,** 346–350 (2007).
105. Munsie, M. J. *et al.* Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei. *Curr. Biol.* **10,** 989–992 (2000).
106. Andrews, P. W., Bronson, D. L., Benham, F., Strickland, S. & Knwles, B. B. A comparative study of eight cell lines derived from human testicular teratocarcinoma. *Int. J. Cancer* **26,** 269–280 (1980).
107. Revazova, E. S. *et al.* Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* **9,** 432–439 (2007).
108. Klimanskaya, I., Chung, Y., Becker, S., Lu, S. J. & Lanza, R. Human embryonic stem cell lines derived from single blastomeres. *Nature* **444,** 481–485 (2006).
109. Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131,** 861–872 (2007).
110. ISSCR. Guidelines for the conduct of human embryonic stem cell research http://www.isscr.org/docs/default-source/hesc-guidelines/isscrhescguidelines2006.pdf (2006).
111. Müller, F.-J. *et al.* A bioinformatics assay for pluripotency in human cells. *Nature Methods* **8,** 315–317 (2011).
112. Cahan, P. *et al.* CellNet: network biology applied to stem cell engeineering. *Cell* **158,** 903–915 (2014).
113. International Cell Line Authentication Committee. Guide to human cell line authentication (2012 http://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac_/Authentication_SOP.pdf.

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

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Author Information Reprints and permissions information is available at 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 G.Q.D. (george.daley@childrens.harvard.edu).

CORRIGENDUM

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Corrigendum: Hallmarks of pluripotency

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In this Review, a sentence was added at proof stages and we inadvertently omitted a citation to a study from the laboratory of Jacob Hanna[1]. This reference citation should have appeared associated with the sentence: "The observation that naive cells tolerate depletion of epigenetic regulators supports the concept of naive pluripotency as a configuration with a reduced requirement for epigenetic repression compared to primed PS cells and somatic cells[1]."

1. Geula, S. *et al.* m^{6}A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation. *Science* **347,** 1002–1006 (2015).