December 25, 2014

To: Ryoji Noyori, RIKEN President

Report on STAP Cell Research Paper Investigation

Research Publication Investigative Committee
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1. Background

In response to doubts raised about two papers on STAP cells published in *Nature*, the first investigative committee, established by RIKEN, looked into six allegations of research misconduct related to Obokata et al., Nature 505: 641-647 (2014) and Obokata et al., Nature 505: 676-680 (2014). On March 31, 2014, the first investigative committee confirmed that there were two instances of research misconduct and, following an appeal by Obokata, on May 8 RIKEN instructed her to retract Nature 505: 641-647. In a separate investigation convened by the Center for Developmental Biology (CDB), further questions were raised concerning figures in the papers, in addition to the six items addressed by the first investigative committee. On July 2, the two STAP papers were retracted by *Nature* at the request of the authors.

Genetic analyses of the samples used in the STAP cell research have raised additional questions regarding the STAP cell phenomenon and the origins of the mice used in the experiments. Given the complexity and diversity of these allegations, RIKEN launched a preliminary inquiry on June 30, in line with its Regulations on the Prevention of Research Misconduct (2012, Reg. 61), to determine whether another full investigation was warranted. This preliminary inquiry confirmed there were grounds for several of the allegations, and in view of their seriousness, RIKEN deemed a full investigation was required. On September 3, a committee of seven outside experts, chaired by Isao Katsura, was convened to review the new allegations.

2. Content of the investigation

2-1. Objective

To determine whether there were any instances of research misconduct in the following three papers, as defined in Article 2, paragraph 2 of RIKEN's Regulations on the Prevention of Research Misconduct and, in the event of such misconduct, to identify the persons(s) responsible.

Obokata et al., *Nature* 505: 641–647 (2014) (hereafter referred to as *Article*)
Obokata et al., *Nature* 505: 676–680 (2014) (hereafter referred to as *Letter*)
Obokata et al., *Protocol Exchange* (2014) doi: 10. 1038/protex.2014.008 (hereafter referred to as *Protocol Exchange*)

2-2. Duration and methods of investigation
2-2-1. Duration and methods

The investigative committee convened its first meeting on September 22, 2014, and met a total of 15 times through December 23.

The investigation began by reexamining the items addressed by the preliminary inquiry, evaluating the data that had given rise to the allegations, and considering methods to be used in the investigation. The committee next collected and examined the original data from the experiments presented in the papers, electronic files relating to the preparation of the manuscripts, the laboratory notebooks and progress reports made by those involved in the research in question, and other documents, email messages, and materials submitted by those involved. The persons under investigation and others involved were sent questionnaires or interviewed. In the process of the investigation, RIKEN was asked to make further analyses of items that were found to require scientific verification. The results of these investigations were deliberated by the committee and compiled in this report.

2-2-2. Subjects of the investigation

Haruko Obokata
Visiting Scientist, CDB Laboratory for Genomic Reprogramming, April 6, 2011 to February 28, 2013
Unit Leader, CDB Cellular Reprogramming Research Unit, March 1, 2013 to November 20, 2014
Research Scientist, Office for the Prevention of Research Misconduct, Verification Experiment Team, November 20, 2014 to December 21, 2014

Teruhiko Wakayama
Team Leader, CDB Laboratory for Genomic Reprogramming, April 1, 2001 to March 31, 2012
Research Team Leader (part-time), CDB Laboratory for Genomic Reprogramming, April 1, 2012 to March 31, 2013
Senior Visiting Scientist, CDB Division of Human Stem Cell Technology, April 1, 2013 to November 20, 2014
Senior Visiting Scientist, CDB Organ Development Research Team, November 21, 2014 to present
Professor, Faculty of Life and Environmental Sciences, University of Yamanashi, April 1, 2012 to present

Hitoshi Niwa
Team Leader, CDB Laboratory for Pluripotent Cell Studies, February 9, 2001 to September 30, 2009
Project Leader, CDB Laboratory for Pluripotent Stem Cell Studies, October 1, 2009 to November 20, 2014
Team Leader, CDB Laboratory for Pluripotent Stem Cell Studies, November 21, 2014 to present

2-3. Results of the investigation and opinions

2-3-1. Investigation of new doubts raised by results of scientific verification

2-3-1-1. Results of investigation of STAP cell-related cell lines, chimera mice, and teratomas

(a) STAP cell-related cell lines used for investigations described in sections (b) through (d) below

STAP cell-related cell lines used for genome analysis by RIKEN are shown in the table below.

Table of STAP cell-related cell lines

Name	Cell type	GFP insertion (by NGS)	Sex	Genetic background[1]	Characteristic deletion, etc.	Established[2]
FLS1~8	STAP stem cell	*Acr/CAG* (heterozygous)[3]	♂	129X1SLC♀/ B6N SLC♂	Chr 3/8	2012 1/31–2/2
CTS-1, 11~13	FI stem cell	*Acr/CAG* (heterozygous)[3]	♂	129X1SLC♀/ B6N SLC♂	Chr 3/8	2012 5/25, 7/9
GLS-1~13	STAP stem cell	*Oct4*	♀	B6	X chromosome + X chromosome fragment	2012 1/31
AC129-1, 2	STAP stem cell	*CAG* (homozygous)	♂	129X1SLC♀/ B6N SLC♂[4]	Chr 1/4/10/19	2012 8/13
FLS-T1, T2	STAP stem cell	*CAG* (homozygous)	♂	129X1SLC♀/ B6N SLC♂	Chr 1/4/10/19	2013 2/22
GOF-ES	Nuclear transfer ES cell	*Oct4*	♀	B6	X chromosome + X chromosome fragment	2011 5/26–10/31
129B6 F1 ES1[5]	Fertilized egg ES cell	*CAG* (homozygous)	♂	129X1SLC♀/ B6N SLC♂	Chr 1/4/10/19	2012 4/19
129/GFP ES	Unknown	*Acr/CAG* (heterozygous)	♂	129X1SLC♀/ B6N SLC♂	Chr 3/8	Unknown
FES1[6]	Fertilized egg ES cell	*Acr/CAG* (heterozygous)	♂	129X1SLC♀/ B6N SLC♂	Chr 3/8	2005 12/7
FES2[6]	Fertilized egg ES cell	*Acr/CAG* (heterozygous)	♂	129X1SLC♀/ B6N SLC♂	—	2005 12/7
ntESG1[6]	Nuclear transfer ES cell	*Acr/CAG* (heterozygous)	♂	B6N SLC♀/ 129^{+}Ter CLEA♂	—	2007 8/3
ntESG2[6]	Nuclear transfer ES cell	*Acr/CAG* (heterozygous)	♂	B6N SLC♀/ 129^{+}Ter CLEA♂	—	2005 1/20

1. Judged by comparing the SNPs of these cell lines with those of parental mice. B6 stands for C57BL/6.
2. Date of start of cultivation. In the case of FES1, FES2, ntESG1, and ntESG2, date of making frozen stock.
3. Described by the developer as "*CAG-GFP* (homozygous)".
4. Described by the developer as "129 *CAG-GFP* (homozygous)".
5. 129B6 F1ES1 through 6 were generated as controls for the STAP stem cells, and 129B6 F1 ES1 is most closely

related to this investigation.

6. Full names: FES1: 129B6GFP1 FES♂; FES2: 129B6GFP2 FES♂; ntESG1: 129B6F1G1; ntESG2: 129B6F1G2.

The first eight cell lines in the Table are STAP stem cells, FI stem cells, and related ES cells kept by the Cellular Reprogramming Research Unit of RIKEN CDB (hereinafter referred to as the Obokata lab) and by Teruhiko Wakayama's laboratory in Faculty of Life and Environmental Sciences, University of Yamanashi (hereinafter referred to as the Wakayama university lab). After determining that the STAP stem cell line FLS shows a co-insertion of *Acr-GFP* and *CAG-GFP*, which respectively express GFP under the control of Acrosin gene promoter and *CAG* promoter, the last four cell lines in the Table (generated by the Genome Reprogramming Research Team of RIKEN CDB, hereinafter referred to as the Wakayama CDB lab), which also shows co-insertion of *Acr-GFP/CAG-GFP*, were obtained and analyzed. Genome analyses of some of the mouse strains used for generating STAP cells were also performed.

(b) STAP stem cell line FLS and FI stem cell line CTS were derived from ES cell line FES1

Results of investigation

The STAP stem cell line FLS was used for measurement of cell growth in *Article* Fig. 5c (based on interviews with Obokata), generation of chimera mice in *Article* Fig. 5j–l and *Article* Extended Fig. 8i–j, and analysis of DNA methylation in *Article* Extended Fig. 8d. FI stem cell line CTS was used for generation of chimera mice in *Letter* Fig. 2f, g and *Letter* Extended Fig. 2a, b. Genomic analysis by RIKEN confirmed that FLS and CTS contain the insertion of *Acr-GFP/CAG-GFP*. Genome analyses of the ES cell lines, FES1, FES2, ntESG1, and ntESG2, which were generated in the Wakayama CDB lab and also contain insertions of *Acr-GFP/CAG-GFP*, and related mouse strains were carried out, yielding the conclusions described above. The basis for these conclusions is summarized in the following four points.

1) Chromosomal insertion position, copy number, DNA sequence surrounding the insertion site of *Acr-GFP/CAG-GFP*
2) Results of single nucleotide polymorphism (SNP) data analysis
3) Results of analysis by next-generation sequencing
4) Deletion mutations on chromosomes 3 and 8

These four points are explained below.

1) Chromosomal insertion position, copy number, DNA sequence surrounding the insertion site of *Acr-GFP/CAG-GFP*

Whole-genome analyses of 11 of the 12 cell lines in the Table, with the exception of STAP stem cell line FLS-T, and of mouse strains 129/Sv and C57BL/6 ((hereafter referred to as 129 and B6, respectively), which were used for the generation of those stem cells, were performed using a next-generation sequencer. The results of this analysis indicate that seven cell lines—STAP stem cell line FLS3, FI stem cell line CTS1, ES cell lines FES1, FES2, ntES1, and ntES2, and 129/GFP ES—all show co-insertion of *Acr-GFP/CAG-GFP* at the same site on chromosome 3. It was also determined that *Acr-GFP* was inserted into only a single allele of chromosome 3 (confirmed by FISH) in all seven cell lines, that the copy number of *Acr-GFP* was ~20, that an approximately 20 kb region surrounding the GFP insertion site on chromosome 3

was duplicated, and that a 20 kb region from chromosome 4 was inversely inserted adjacent to the *GFP* insertion site. These features are identical to those of the *Acr-GFP/CAG-GFP* mice which the Wakayama CDB lab had obtained from Prof. Masaru Okabe's lab at Osaka University in 2003.

2) Results of SNP data analysis

To clarify the genetic background of the 12 cell lines in the Table, SNP analysis using TaqMan PCR method was performed by RIKEN. The results of RIKEN's genetic analysis indicated that cell lines containing the co-insertion of *Acr-GFP* and *CAG-GFP* needed to be analyzed selectively. Based on this, the 12 cell lines described above, and mouse strains 129, B6, which were used to generate those cell lines, and their sub-strains (total of 14 strains) were investigated.

Comparison of SNPs capable of discriminating the 129 and B6 strains revealed the following.

(1) Based on SNP analysis of the sex chromosome in the STAP stem cell line FLS3, FI stem cell line CTS1, ES cell lines FES1and FES2, the genetic backgrounds of the maternal and paternal founders were 129 and B6, respectively. These results are consistent with the FLS3 and CTS1 cells generated from F_1 mice by crossing of 129 and B6 (129 x B6). ES cell line 129/GFP ES also exhibited the same SNP distribution of sex chromosomes as these three cell lines. The genetic background of the X chromosome in ES cell lines ntESG1 and ntESG2, which had the same *Acr-GFP/CAG-GFP* insertion, was B6. Since this is different from FLS3 and CTS1, those two ES cell lines were excluded from the comparison control.

(2) The SNPs on autosomes were similarly analyzed. Results indicated that STAP stem cell line FLS3, FI stem cell line CTS1, ES cell lines FES1 and FES2, and ES cell line 129/GFP ES in the stock of the Obokata lab had nearly the same genetic background as 129 X1/SvJmsSlc x C57BL/6NCrSlc. Although this indicates that all SNPs should be heterozygous, four of the 99 SNPs analyzed, other than the ES cell line FES2, were found to be homozygous for 129. This suggests that the genetic background of the mice used for generation of these stem cell lines were heterogeneous, or that mutations had occurred at those four sites. In fact, the *Acr-GFP/CAG-GFP* mice maintained in the Wakayama CDB lab showed heterogeneity in the genetic background of B6 (see 3. Results of analysis using next-generation sequencer).

3) Results of next-generation sequencing (NGS) analysis

Whole genome SNP distribution was analyzed using 11 of the 12 stem cell lines in the Table, except for FLS-T, and mouse strains 129 and B6, which were used for generation of these stem cell lines. Results indicated that STAP stem cell line FLS3 and FI stem cell line CTS1, and the ES cell line labeled 129/GFP ES found in the freezer of Obokata's lab was very similar to the ES cell lines FES1 and FES2 which were generated from fertilized eggs in Wakayama's CDB lab in 2005. Precise analysis of the SNP distribution of those five cell lines indicated that the especially problematic four cell lines, STAP stem cell line FLS3, FI stem cell line CTS1, ES cell line 129/GFP ES, and FES1, have very similar SNP distribution. On the other hand, ES cell line FES2,

which was established at the same time as FES1, shows a SNP distribution very similar to that of FES1, but contains different SNP clusters on parts of chromosomes 6, 11, and 12. In those three regions, the B6/B6-type and B6/129-type SNP distribution in FES2 was replaced by the B6/129-type and 129/129-type SNP distribution in FES1, respectively. This suggests that when FES1 and FES2 were established, the parental mice did not have homogeneous genetic backgrounds, that the B6-type and 129-type SNPs might co-exist on three regions of chromosomes 6, 11, 12, and that FES1 and FES2 may have retained this heterogeneity when they were established. STAP stem cell line FLS3, FI stem cell line CTS1, and the ES cell line 129/GFP ES of unknown origin maintained in the Obokata lab had nearly the same SNP distribution in those three regions as FES1, but not FES2.

Results of a comparison of 1,290 SNPs other than those on chromosomes 6, 11, and 12 described above indicates that STAP stem cell line FLS3, FI stem cell line CTS1, and ES cell line 129/GFP ES exhibited nearly the same SNP distributions, suggesting that these cell lines were derived from the same cell. We therefore conclude that FLS3, CTS1, and 129/GFP ES cell lines were derived from the ES cell line FES1.

4) Deletion mutations on chromosomes 3 and 8

Whole genome analysis of the 11 STAP-related cell lines indicated that the 5 kb deletion on chromosome 3 and the 17 kb deletion on chromosome 8 (chromosomes 3 and 8 had the genetic background of 129 and B6, respectively) were found only in STAP cell line FLS3, FI stem cell line CTS1, and the ES cell lines FES1 and 129/GFP ES. By determining the DNA sequences of PCR products, it was confirmed that these two deletions are found in all the sublines of STAP stem cell line FLS and FI stem cell line CTS. On the other hand, those two deletions were not found in the sub-strains of 129, 129X1/SvJJmsSlc(SLC) or 129^{+}Ter/SvJcl(CLEA). The 5 kb deletion on chromosome 3 was also not found in commercially available sub-strains of C57BL/6, C57BL/6JJmsSlc (SLC), C57BL/6NCrSlc (SLC), C57BL/6J (Charles River), C57BL/6NCrl (Charles River), C57BL/6JJcl (CLEA), C57BL/6NJcl (CLEA), and in the *Acr-GFP/CAG-GFP* mice, which were cryopreserved as fertilized eggs in the Wakayama CDB lab in 2010.

If these cells were in fact derived from the (129 x B6)F_1 (hereafter, referred to as 129B6F1) mice as described in the publications, then these deletions should be present in either or both the 129 or B6 lineages, as it is highly improbable that both deletions would spontaneously occur during the 2–3-year period of the STAP research. Given these findings, the committee concludes that these four cell types are not directly derived from the 129B6F1 mice described in the publications.

These two deletions are unique chromosomal anomalies that are commonly found in FLS STAP stem cell lines, CTS FI stem cell lines, the ES cell line, 129/GFP ES of unknown origin in the Obokata lab stock and the ES cell line FES1, but not in FES2 (nor in any tested mouse strains). Thus, these deletions, in addition to the 3 SNP clusters in chromosome 6, 11, and 12, provide strong evidence that these cell lines were derived from the ES cell line FES1 established in 2005.

(c) STAP stem cell line GLS is derived from the ES cell line, GOF-ES

Results of investigation

In the *Article*, Fig. 5 and Extended Data Fig. 8, STAP cells were reported to convert into ES-like cells able to proliferate when cultured in the presence of ACTH and LIF. According to Wakayama's lab notebook, Wakayama established STAP stem cell lines, GLS1 and GLS11–13, on January 31, 2012, using STAP cells that Obokata had prepared from GOF mice bearing *GFP* transgenes under the control of the *Oct4* promoter.

Independently, a member of the Wakayama CDB lab had established an ES cell line, GOF-ES, from GOF mice given to the lab by another CDB team during the period between May 26 and October 31, 2011. During this period, Obokata requested this Wakayama lab member to let her use the GOF-ES line as control cells that expressed GFP, and the member provided culture dishes of these cells to Obokata.

This raises the possibility that the GLS STAP stem cell lines were in fact derived from GOF-ES cells that contaminated the STAP cell dishes during the culture procedure. To clarify this, the whole genome sequences of GLS1 (one chosen from the four GLS STAP stem cell lines) and the GOF-ES cell line, were determined and compared.

As a result, the following points were revealed regarding genomes of GLS1 and GOF-ES.

1) Both cell lines are identical in SNP distribution over the entire genome.
2) Both cell lines are identical in the type of inserted *GFP* gene, copy number, and the detailed sequences of the genomic regions in which the transgenes are tandemly inserted.
3) Both cell lines were derived from female mice.
4) The two cell lines share characteristic structural abnormalities on the X chromosome; one of two X chromosomes is significantly deleted, with terminal inverted repeats

These findings indicate that the GLS1 STAP stem cell is almost identical with GOF-ES.

In addition, the following points were revealed.

5) An X chromosome deletion as large as that described above is not stably maintained through successive generations in the mouse.
6) The structural abnormalities found in GOF-ES were not present in the parental GOF mice.
7) SNP distribution along the entire GOF mouse genome was different from those of GOF-ES and the STAP stem cell GLS1.
8) All the independent GLS lines, which were established at the same time as GLS1, share exactly the same structural abnormalities.

From the above, it is unlikely that the STAP stem cell line GLS1 and other GLS lines were established from STAP stem cells prepared from GOF mice. The Wakayama CDB lab member established the GOF-ES cell line at CDB between May 26 and October 31, 2011, and Wakayama established the GLS STAP stem cells from STAP cells provided by Obokata on January 31, 2012. These time lines are compatible with the possibility that the STAP stem cell line GLS was derived from GOF-ES cells.

It should also be noted that the STAP stem cell GLS1 exhibits trisomy on chromosome 8, while the GOF-ES cells and GOF mice do not. While trisomy of

chromosome 8 is lethal in mice, it is known to occur frequently during ES cell culture. Thus, this trisomy in GLS1 is likely to have occurred during or after the GLS STAP stem cell preparations were contaminated with GOF-ES cells.

From the above, the investigating committee determined that STAP stem cell GLS and GOF-ES cells are of the same origin. The committee also concluded that (i) structural abnormalities of the X chromosome occurred during the process of establishing GOF-ES cells from GOF mice; (ii) GOF-ES cells were contaminated when GLS STAP stem cell lines were established from GOF mouse STAP cells; and (iii) Nature *Article* Fig. 5 and Extended Data Fig.8 show experimental results using the GLS STAP stem cells contaminated with GOF-ES cells.

(d) STAP stem cell AC129 is derived from ES cells established from 129B6F1 fertilized eggs

Results of investigation

From summer to autumn of 2012, Wakayama investigated the effects of genetic background on the efficiency of STAP stem cell establishment. In these experiments, Wakayama established two independent STAP stem cell lines, AC129-1 and AC129-2, from STAP cells that Obokata had prepared from $CD45^+$ splenic cells of 129-*CAG-GFP* mice (which have *CAG-GFP* genes inserted homozygously in chromosome 8) provided by Wakayama. Stocks of these cell lines were stored in deep freezers both at the Obokata lab in CDB and at the Wakayama university lab after Wakayama moved to Yamanashi University. The STAP stem cell AC129-1 was investigated using the TaqMan PCR method to identify SNP markers that distinguish parental mouse sub-strains; the whole genome sequence of AC129-1 was also determined by next-generation sequencing (NGS). One of the ES cell lines (129B6F1 ES6) was investigated in the same manner. Because the ES cell lines 129B6F1 ES1 through 6 were established from fertilized eggs derived from a cross of 129 *CAG-GFP* mice and B6 *CAG-GFP* mice, these serve as control ES cell lines for the FLS STAP stem cell lines that are supposed to have the genotype of 129B6F1 *CAG-GFP*. The following points were revealed by comparing these data with results obtained for other cell lines and with the data deposited in the database when the *Nature* papers were published.

1) Results of SNP analysis

The STAP stem cell AC129-1 was derived from F_1 (male) generated from a cross of 129 *CAG-GFP* mice and B6 *CAG-GFP* mice. Characterization of 197 SNPs (TaqMan PCR analysis) revealed that the STAP stem cell AC129-1 had the genetic background of 129B6F1 instead of 129/Sv. This was reconfirmed by whole-genome DNA sequence analysis. Because the genetic background of AC129-1 was different from what was expected from mice used for the establishment of the cell line, it is suspected that some errors were made in the process of conducting the experiments.

2) Results of NGS analysis

AC129-1 had a single copy of the *GFP* gene in chromosome 18 (base position 46,261,277). This *GFP* insertion site was identical with that in *CAG-GFP* mice (129 and B6 backgrounds), which had been established at the Wakayama CDB lab. In addition, AC129-1 turned out to be homozygous for the insertion of the *CAG-GFP*

transgene.

3) There was a domain of B6-homozygous SNPs at the central region of chromosome 6 in the STAP stem cell AC129-1

While the analysis described above indicated that the STAP stem cell AC129-1 was derived from an F_1 mouse line generated by the outcross of 129 *CAG-GFP* and B6 *CAG-GFP* mice, it was determined that there was a chromosomal domain in which all the SNPs were homozygous with those of B6 in the central region of chromosome 6. The committee investigated why this peculiar chromosomal domain is present in the AC129-1 genome by examining the genome of the parental 129 *CAG-GFP* mouse. This mouse strain was generated by a series of backcrosses of B6 *CAG-GFP* mice with 129X1/Sv. SNP analysis of the whole genome of a 129 *CAG-GFP* mouse revealed that the genetic background of the 129 *CAG-GFP* mice currently maintained in the Wakayama university lab, did not become completely homogeneous to the 129 genetic background. Specifically, there is a genomic domain of approximately 30 Mb that is heterozygous for both 129 and B6-homozygous SNPs, which covers the domain of B6-homozygous SNPs found in AC129-1. This heterogeneity in the genetic background in the parental 129 *CAG-GFP* mice accounts for the B6-homozygous SNP domain in chromosome 6 of AC129-1.

4) Heterogeneity of genetic backgrounds in other cell lines

The heterogeneity in the 129 *CAG-GFP* genetic background also resulted in the heterogeneous genetic background of other cell lines that were derived from this mouse strain. As described above, Wakayama established six independent ES cell lines from different fertilized eggs of 129B6F1 *CAG-GFP* (these ES lines, 129B6F1 ES1 through 6, were established in May, 2012). All of these ES cell lines also had a domain of B6-homozogous SNPs in the central region of chromosome 6. The boundary between the B6-homozygous domain and the adjacent 129/B6-heterozygous domain differed from line to line among 129B6F1 ES1 through 6. It is highly likely that this variation in the boundary of this chromosomal domain arose from the recombination of B6 and 129 domains during the meiotic process from which the gametes of the parental 129 *CAG-GFP* male mice were generated. Nevertheless, there is also the possibility that mitotic recombination during the establishment of these ES cell lines from embryos contributed to this chromosomal variation.

5) Characteristic structural anomalies in the genome of the STAP stem cell line AC129-1

AC129-1 contains chromosomal anomalies (four deletions and one duplication) that were specifically found in this line, but not in the other STAP stem cell lines. Deletion #1 lacks a DNA sequence of approximately 9 kb in chromosome 19; Deletion #2, approximately 5 kb in chromosome 1; Deletion #3, 16 kb in chromosome 4; Deletion #4, 2 kb in chromosome 10. Duplication #1 was the repeat of a genomic sequence of approximately 2.5 kb in chromosome 1.Among these structural anomalies, Deletion #2 was confirmed by PCR to exist homozygously in the genome of B6 *CAG-GFP* (but not 129 *CAG-GFP*) mice that are currently maintained at the Wakayama university lab. The other structural anomalies (deletions #1, 3, 4 and duplication #1) were not found in the parental B6 *CAG-GFP* or 129 *CAG-GFP* mice. As described above, independent ES

lines, 129B6F1 ES1 through 6, were all simultaneously established from different blastocysts generated from the same combination of parental mouse strains. These ES cell lines, while sharing deletions #1 and #2, were of non-homogeneous sexual backgrounds, and possessed different combinations of the other three chromosomal anomalies. Therefore, it is highly probable that these three chromosomal anomalies were present heterozygously in the parental mouse strains that were used to establish those ES cells lines.

Of these ES cell lines, 129B6F1 ES1 was identical with the STAP stem cell AC129-1 in sex and all chromosomal anomalies described above (deletions #1 through #5, and duplication #1). Furthermore, 129B6F1 ES1 and 129AC129-1 shared the boundary of B6 –homozygous SNP domain of chromosome 6 (the region that is described in the previous section 4).

On the other hand, the ES cell line 129B6F1 ES6, which was subjected to NGS, differed from AC129-1 in some of the above chromosomal anomalies; 129B6F1 ES6 did not share deletion #4 and duplication #1 with AC129-1, but had deletions #1 through #3. In addition, the boundary of B6 SNP domain was different between 129B6F1 ES6 and AC129-1.

129B6F1 ES1 turned out to be identical with the other STAP stem cell lines, AC129-2, FLS-T1 and FLS-T2, which carry *CAG-GFP*, in all the above chromosomal characteristics. The latter two STAP stem cell lines were exceptional in that Wakayama established those two lines from the STAP cells that Wakayama himself (not Obokata) had prepared under direct instruction by Obokata.

In summary, 129B6F1 ES1 and the STAP stem cell lines AC129 (AC129-1 and AC129-2) and FLS-T (FLS-T1 and FLS-T2) turned out to share the same sex and 4 different chromosomal anomalies (deletions #3, #4, duplication #1, and the same B6-homozygous SNP domain in chromosome 6). Although these five cell lines were supposed to have been established independently, it is highly unlikely that they would share these five characteristic features heterozygously in the chromosome by chance. Therefore, the investigating committee concluded that STAP stem cell lines AC129-1, AC129-2, FLS-T1 and FLS-T2 were not independent from each other, and were instead derived from the 129B6F1 ES1 that was the earliest of the 5 cell lines to be established.

Because AC129 STAP stem cell lines were established on August 13, 2012, the investigative committee considered it likely that they were used in the experiments that were carried out after this date. Based on a re-examination of the samples for the ChIP-seq experiments, it is highly probable that AC129 was used for the ChIP-seq experiments described in the Nature *Letter* Fig. 4. AC129 is also likely to have been used for *Letter* Fig. 2i (a hierarchical clustering analysis of global expression profiles). However, the investigative committee was unable to specify in which experiments AC129 was used due to defects in the experiment records.

It should be also noted that, although there is a statement in the *Nature Article* Methods section that STAP stem cells capable of forming chimeric mice were established from 129 mice carrying *Rosa26-GFP*, this statement seems to be a error in the text of the manuscript. There is no record at CDB regarding the introduction or breeding of a 129 strain carrying *Rosa26-GFP*. According to Wakayama's explanation, it is probable that the STAP stem cells described here were actually AC129 (which was supposed to be 129 carrying *CAG-GFP*).

(e) It is highly probable that the chimera mice claimed to be developed from STAP cells were actually developed from FES1 ES cells.

1) It was reported in *Article* Fig. 4 and Extended data Fig. 7 that 2N chimeric mice from STAP cells derived from 129 x B6*(CAG-GFP)*F1 mice were established, and that these mice gave birth through germline transmission.

Nine DNA samples labeled as "offspring of callus chimera #1" through "offspring of callus chimera #9" were found in a freezer in Obokata's lab. Because STAP cells were referred to as 'animal callus cells' in the Wakayama CDB lab from 2011 to 2012, it is likely that these DNA samples were derived from offspring of chimeric mice. In fact, Obokata confirmed during an interview that these DNA samples were derived from the offspring of chimeric mice reported in *Article* Extended Fig.7. According to Wakayama's laboratory notebook, these chimeric mice were generated between late January and early February 2012.

These DNA samples were analyzed by PCR in RIKEN. The integration of *Acr-GFP* into chromosome 3, which is present in FES1 ES cells, was observed in three DNA samples, a deletion (approximately 5 kb) in chromosome 3 specifically observed in FES1 ES cells was detected in four DNA samples, and a deletion (approximately 17 kb) in chromosome 8 was found in two DNA samples. The deletions specifically observed in FES1 are not found in the mouse strain used to establish FES1, or in FES2, which was independently established from FES1. It is thus highly probable that these DNA samples were derived from FES1 ES cells.

2) *Article* Fig. 5k described 4N chimeric mice derived from STAP stem cells. There were eight DNA samples labeled "4N-1" through "4N-8", which appear to be derived from 4N chimeric mice. Face-to-face interviews and questionnaires revealed that these DNA samples were prepared from 4N chimeric mice by a member of the Wakayama CDB lab on April 6, 2012. It is believed that these 4N chimeric mice were established from STAP stem cell FLS between February 15 and 22, 2012. Obokata also indicated that these DNA samples were derived from 4N chimeric mice. The Wakayama CDB lab notebook and a record made on March 11, 2012, in a microscope logbook confirms that these 4N chimeric mice were the mice described in *Article* Fig. 5k.

Analysis of these DNA samples in RIKEN showed that insertion of *CAG-GFP* on chromosome 18 in mice purportedly used for the generation of STAP stem cells was not detected; instead, an insertion of *Acr-GFP* in chromosome 3 present in FES1 ES cells was observed. It is thus probable that the 4N chimeric mice were derived from FES1 ES cells.

(f) It is highly probable that the teratomas reported as having been derived from STAP cells were actually derived from FES1 ES cells

Results of investigation

It was claimed that the STAP cell-derived teratomas reported in *Article* Fig 2e and its Extended Data Fig. 4a-c were derived from cell aggregates of day 7 *Oct4*-GFP^{+} cells. However, the following tests reveal that these teratomas,

(1) expressed *Acr*-GFP but not *Oct4*-GFP,

(2) were shown by PCR analysis to have two deletions specifically observed in FES1 ES cells, and
(3) have one X chromosome and one Y chromosome detected by FISH and chromosome-painting technique, which is consistent with the fact that FES1 ES cells are derived from male mice. It is thus probable that the STAP cell-derived teratomas presented in the figure were derived from FES1 ES cells.

1) Identification of samples used for the preparation of *Article* Fig. 2e and its Extended Data Fig. 4a-c

Pictures of STAP cell-derived teratomas presented in *Article* Fig. 2e and its Extended Data Fig. 4a-c were taken from slide glass specimens labeled "6weeks+PGA 12/27 transplanted Haruko," left in CDB. It was revealed by comparing the shapes of the slide glass specimens and the paraffin blocks that these slide glass specimens were derived from a paraffin block labeled, "CD45 callus teratomas".

2) Analysis by quantitative PCR

After trimming the aforementioned paraffin block to avoid contamination, 10 5μm sections were prepared and 2.2 μg DNA was extracted from these sections ("callus teratomas #1"). 6.1 μg DNA was separately extracted from another 20 5μm sections from the same paraffin block ("callus teratomas #2"). Quantitative PCR was performed using DNA samples of "callus teratomas #1" and "callus teratomas #2" to examine copy numbers of transgenes and deletions on chromosomes 3 and 8 specifically observed in FES1 ES cells. Details of deletion characteristics are shown in the Table of STAP cell-related cell lines given at the beginning of this report and in 3-2-1-1 (b).

Given the likelihood that DNA was fragmented as a result of formalin fixation, DNA fragments of < 100 b.p. were used for quantification by PCR. To avoid contamination, the experiment for "callus teratomas #2" was performed in a separate room with new reagents and equipment.

Experimental groups consisted of DNA samples from "callus teratomas #1" and "callus teratomas #2". DNA was derived from three STAP stem cell lines were used as positive controls: FLS4 derived from FES1 ES cells containing approximately 24 copies/genome of *Acr-GFP* insertion, 129B6F1 ES5 containing two copies/genome *CAG-GFP* insertion, and GLS13 containing approximately 28 copies/genome *Oct4-GFP* insertion. Tail DNA derived from wild type C57BL/6NSlc that does not have a *GFP* insertion was used as a negative control. Autosomal *IL-2* gene was selected as an internal control and the fold amplification value of DNA from the *IL-2* gene was considered to be two copies/genome to calculate the copy numbers of amplified DNA from the samples of experimental groups. Copy numbers of experimental groups were determined by multiplying that of the *IL-2* gene and fold amplification of a sample of interest. Note that these are semi-quantitative values that do not compensate for differences in PCR amplification of each gene segment. It was confirmed that primers for *GFP* worked well with good reproducibility. The results showed that samples from two teratoma DNA samples contained 20–30 copies of *GFP* insertions/genome. Expected copy numbers were detected from positive control samples and no *GFP* insertion was detected in a negative control sample derived from tail DNA prepared from C57BL/6NSlc mouse.

Next, to distinguish *Oct4-GFP* and *Acr-GFP* insertions, distinct primer sets were designed to detect borders of the promoter sequence and the *GFP* gene within the inserted transgenes, and quantitative PCR analyses were performed. Primers for *Acr-GFP* insertion detected approximately 30 and 20 copies of insertions from "CD45 callus teratomas" and the STAP cell line FLS4, respectively. Again, note that these are also semi-quantitative without compensation.

Furthermore, PCR analysis was performed to detect deletions on chromosomes 3 and 8 that are specifically observed in FES1 ES cells. It was found that two deletions were observed in samples from "CD45 callus teratomas #1", "CD45 callus teratomas #2" and STAP stem cell line FLS4 that is derived from FES1 ES cells (based on the results shown in 2-3-1-1 (b)), but not in other samples. It is thus highly probable that samples from the paraffin block "CD45 callus teratomas" were contaminated with FES1 ES cells.

3) Verification by FISH analysis of tissue sections

The paraffin block "CD45 callus teratomas" was examined by FISH analysis with probes specific for Y chromosome and chromosome painting analysis with probes specific for X chromosome. It was found that regions likely derived from cells containing *Acr-GFP/CAG-GFP* insertions possessed male chromosomes (XY), which is consistent with the conclusion that the teratoma samples are derived from male-derived FES1 ES cells.

4) Distinction between teratoma-derived tissues and host mouse-derived tissues

Because "CD45 callus teratomas" tissues are derived from *Acr-GFP/CAG-GFP* cells constitutively expressing GFP, immunohistochemical analysis was performed to distinguish tissues derived from transplanted cells and those of the host mouse. Many GFP positive cells were observed in teratoma regions likely derived from transplanted cells. In contrast, the intestinal epithelia-like tissue shown in *Article* Fig. 2e, right, and pancreas-like tissue shown in *Article* Extended Data Fig. 4c were GFP negative, indicating that those tissues were not derived from teratomas, but were of host mouse origin.

Collectively, the results of these analyses suggest the following.

Based on the results that "CD45 callus teratomas" contained *Acr-GFP/CAG-GFP* insertions and deletions on chromosomes 3 and 8 specifically observed in FES1 ES cells, it is likely that these teratoma tissues are not derived from STAP cells but from FES1 ES cells. This interpretation was consistent with the detection of the Y chromosome in most of the cells in the teratomas. Differentiated tissues that were reported as derived from teratomas were of host mouse origin. These results collectively reject the probative value of teratoma assays in determining the pluripotency of STAP cells.

(g) Evaluation of 2-3-1-1

1) Was there contamination by ES cells in the process of creating the STAP stem cells? Is it possible to identify the person who put in the ES cells? Does this warrant a finding of research misconduct?

(1) Evidence for contamination by ES cells

This investigation has confirmed that the three lines of STAP stem cells (FLS, GLS, AC129) reported in the STAP cell papers were in fact generated from ES cells FES1, GOF-ES, and 129B6 F1 ES1, respectively. In addition, it is highly probable that the FI stem cell CTS was derived from the ES cell FES1. The logic by which the source cells were identified is as follows.

It is possible to determine which mouse strain gave rise to each cultured cell line by analyzing the insertion locus of the *GFP*-fused gene and specific SNPs. Sequence analysis by NGS makes it possible to validate the origins of the cell lines with a high degree of confidence, and further detailed analyses of SNPs and inserted and/or deleted DNA can identify the cell lines in the same mouse strains. New mutations, such as deletions and single nucleotide substitutions frequently occur randomly during the establishment of cultured mouse cell lines, while mutations such as deletions preexisting in the parent mice are randomly segregated into the offspring during gamete development. It is therefore possible to determine whether two cell lines are independently established from the same mouse strain or derived from the same cultured cell line by checking for the presence of these mutations. Although a small number of mutations are gradually generated after establishment of the cultured cell lines, the probability of spontaneous generation of mutations at the same positions is extremely low. Thus, if the mutations that are not present in the parental mice, are found at multiple positions in both cell lines, it can be concluded that they are derived from the same cultured cell line. On the other hand, it is known that trisomy of chromosome 8 occurs at a certain frequency in cultured cell lines, but the probability of this occurring in different cell lines independently is much higher than that of spontaneous mutations at the same positions, even when the trisomy is not observed in the mice due to its lethality.

By this logic, we can conclude that the STAP stem cells and FI stem cells were in fact derived from ES cells. There are two possible scenarios to account for this. One is that the STAP cell cultures were contaminated by ES cells during the process of generating STAP stem cells or FI stem cells. The other is that the ES cell cultures were contaminated by STAP stem cells or FI stem cells. Given that the establishment of ES cells preceded the establishment of the STAP stem cells or FI stem cells, it is likely that the ES cells were mixed in the cell culture during the generation of the STAP stem cells or FI stem cells.

In addition, the chimera mice and the teratomas reportedly generated from STAP cells or STAP stem cells, have now been shown to have actually been generated from ES cell lines, based on the analysis of specific DNA sequences unique to the above ES cells in the remaining samples.

(2) Is it possible to identify the person who put in the ES cells?

Although contamination due to careless experimental manipulation is possible, given that there were so many instances of contamination, it is difficult to eliminate the suspicion that some person or persons might have intentionally contaminated the STAP cell culture with the ES cells. The investigative committee looked into who would have had the opportunity to do this. According to the testimonies of Obokata and Wakayama, the persons in charge

of each experimental step were as follows.
Mice for STAP cell generation: Wakayama produced the mice for the STAP cell generation by crossing, and transferred these to Obokata. As for the STAP cells expressing *Oct4*-GFP, however, Obokata selected and utilized neonatal infants from GOF mice in cages that had been prepared by a member of the Wakayama CDB lab and were maintained by the Wakayama CDB lab.
STAP cell generation: All STAP cells used in the generation of STAP stem cells, FI stem cells, chimera mice, and teratomas were generated by Obokata. Although many members of the Wakayama CDB lab attempted to generate STAP cells, no one other than Obokata was able to do so, with the exception of Wakayama who reportedly succeeded in STAP cell generation a single time when Obokata was present to instruct him. From these STAP cells Wakayama generated STAP stem cells (FLS-T1 and T2 in the Table) which were not reported in the papers.
Generation of STAP stem cells, FI stem cells, chimeric mice, and teratomas: Wakayama dissected the STAP cell aggregates, which had been brought to Wakayama on petri dish lids by Obokata, into small pieces, and transplanted them into mouse embryos to generate chimera mice. Wakayama generated STAP stem cells and/or FI stem cells from the remaining STAP cells used for chimera mouse generation. According to an interview with Obokata, she was able to develop FI stem cells by herself only once, but they were not used for further analysis and were not stored. In addition, Obokata testified that she attempted to generate STAP stem cells independently, but failed.
Teratoma formation: Obokata generated teratomas in all the cases reported. Therefore, it is likely that all the experiments relating to teratoma formation were performed by Obokata alone.

Based on the experimental procedures described above, it would appear that only Obokata and Wakayama (and only Obokata for teratoma development from STAP cell generation) had the opportunity to contaminate the STAP cell cultures. However, this is not necessarily the case, given the situation of the Wakayama CDB lab during that period. For STAP cell generation, it is necessary to keep the cell culture in an incubator for seven days. The cell culture room in which the incubator was located was isolated from other rooms (laboratory office, experimental space, and embryo manipulation space), and people rarely entered the room. Still, many people could have entered the room at that time, especially at night, according to Wakayama, and thus anyone with access to the incubator or the freezer in the room who might have recognized the STAP cell culture petri dishes would have had the opportunity to contaminate them.

Another unanswered question relating to the ES cell contamination is how the FES1 ES cells happened to be in the Wakayama CDB lab at that time. FES1 ES cells which had been established by a member of the Wakayama CDB lab in 2005, were not used for research in the Wakayama CDB lab. When this lab member left in March 2010, the member took all the frozen FES1 cell samples, leaving none in the Wakayama CDB lab. This was prior to the start of the STAP cell research. Despite questionnaires and interviews of the Wakayama CDB lab members, as well as examinations of their laboratory notebooks, it was not possible to identify anyone else who might have used the FES1 ES cells other

than the lab member who had originally established them.

On the other hand, a sample labeled “129/GFP ES” was found in a freezer in the Obokata lab, and genome analysis revealed it to be nearly identical with FES1. However, neither Wakayama nor Obokata, nor the other members of the Wakayama CDB lab could explain the presence of this sample. Therefore, the mystery of how the FES1 ES cells were incorporated into the STAP cell cultures remains unsolved.

Although we investigated all the persons potentially involved in the ES cell contamination, everyone, including Obokata unequivocally denied that they had been involved in any way in intentional or negligent contamination. We further analyzed remaining samples, experimental records, emails exchanged between the related parties, and other objective samples, but could not find any evidence identifying who might have been responsible for the contamination. Without any witnesses or evidence, the committee was forced conclude that the person or persons responsible could not be identified.

(3) Intent or negligence?

Whether an act is intentional or the result of negligence can only be decided on the basis of a comprehensive analysis of objective and subjective factors. Given that it has not been possible to identify the person or persons responsible for the ES cell contamination, it is hard to conclude whether the contamination was intentional or the result of negligence. As far as can be judged by the evidence gathered through this investigation, there is not enough basis to conclude that there has been research misconduct.

2-3-1-2. Doubts concerning publicly archived data (ChIP-seq, RNA-seq, etc.)

From analyses of the NGS data used in the STAP papers (RNA-seq and ChIP-seq input data deposited in the public International Nucleotide Sequence Database) and RNA-seq data not used in the papers but referenced to NGS data newly generated for this investigation (each type of genomic and STAP ChIP-seq input data), the following issues became clear.

1) There are inconsistencies in the information on cell lines and mouse strains used in the RNA-seq and ChIP-seq analyses (described in the papers and deposited in public databases)

Results of investigation

Based on the descriptions in the *Nature* papers and the RNA-seq and ChIP-seq data deposited in public archives, cells/cell lines used in these NGS analyses are of 129B6F1 mouse strain genetic background (except $CD45^+$ and TS cells) and contain the CAG- or *Oct4*-type *GFP* transgene (*CAG*-GFP^+ or *Oct4*-GFP^+). However, analysis of ChIP-seq input data strongly indicated that FI stem cells were *Acr*-GFP/*CAG*-GFP^+ and 129B6F1 background, $CD45^+$ cells were *Oct4*-GFP^+ and B6 background, and the STAP cells and STAP stem cells were *CAG*-GFP^+ and 129B6F1 background, respectively. Analysis of RNA-seq (TruSeq) data, in contrast, suggested that the FI stem cells were *Oct4*-GFP^+ and B6-homozygous background, the $CD45^+$ and STAP cells were *CAG*-GFP^+ and

129B6F1 background, and the STAP stem cells were *Acr*-GFP/*CAG*-GFP^{+} and 129B6F1 background, respectively.

Thus, the genetic background of FI stem cells used in the RNA-seq analyses was incorrectly reported in the papers, and *Acr*-GFP/*CAG*-GFP^{+} cells which were not mentioned in the papers were utilized in the ChIP-seq analysis of FI and STAP stem cells. Since Obokata prepared all the samples for the RNA-seq and ChIP-seq analyses, the investigative committee interviewed her with a specific focus on how she prepared the samples. She only explained that she harvested cells and prepared sequencing samples. There are no laboratory notes on these experiments, making it impossible to confirm further details.

2) RNA-seq data of FI stem cell is generated from a sample containing two kinds of cell species

Results of investigation
Detailed investigation of FI stem cell RNA-seq and SNPs data constructed from comparative analysis of different mouse strain genome sequencing (NGS) data sets clarified that SNPs of the FI stem cell RNA-seq data set consist primarily of B6 type alleles and a 5–10% population of different strain alleles. This raises the possibility that a significant portion of FI stem cell RNA-seq data was generated from B6 type cells with minor contamination of different mouse strain cell RNA (the profile of the non B6 type SNPs alleles in the FI stem cell RNA-seq data is highly similar to that of TS cell RNA-seq data (CD1 strain)).

3) STAP cell ChIP-seq (input) samples were derived from 129B6 F1 ES1

Results of investigation
STAP cell ChIP-seq (input) samples which had been kept by the CDB Genome Resource and Analysis Unit (GRAS) and originally deposited by Obokata were re-analyzed. This NGS re-analysis showed that STAP cell ChIP-seq input data was generated from 129B6F1 cells containing the *CAG-GFP* transgene. Furthermore, SNP analysis in combination with specific genomic deletion/mutation analysis clarified that the STAP cell ChIP-seq input data is nearly identical to that derived from 129B6F1 ES1 cells possessing the *CAG-GFP* transgene.

4) Investigation of unpublished RNA-seq data set (not reported in the papers) demonstrates that cell/mouse lines used in the unpublished RNA-seq analysis are different from the published RNA-seq, and *Letter* Fig.2i is not supported by the unpublished RNA-seq data set

Results of investigation
Regarding the RNA-seq analyses of TS cell and FI stem cell, multiple samples were sequenced for each cell type, and one of each was used in the paper. However, only one sample was analyzed with RNA-seq for the other cell types. Thus, the committee examined the unutilized RNA-seq data and investigated the reasons why multiple samples were analyzed for these two cell types.

In August 2012, Obokata prepared RNA-seq samples for TS cells (TS1) and FI stem cells (FI-SC1) and deposited them with GRAS for sequencing for the first time. The results of the analysis of the original RNA-seq data left at GRAS strongly suggest that TS1 and FI-SC1 were both prepared from cells containing 129B6F1mouse background with origins of *CAG*-GFP$^+$ TS cells and *Acr*-GFP/*CAG*-GFP$^+$ FI stem cells, respectively.

Since the results of the first RNA-seq data analysis were unexpected, Obokata prepared more RNA-seq samples (one TS cell sample (TS2) and two FI stem cell samples (FI-SC2 and FI-SC3)) and deposited them with GRAS in January and June 2013, for another sequencing analysis. The analysis of those FI-SC RNA-seq data suggested that FI-SC2 were prepared from cells containing *Acr*-GFP/*CAG*-GFP$^+$ and 129B6F1 hetero background. The FI-SC3 RNA-seq data set which was used in the *Letter* was prepared from *Oct4*-GFP$^+$ and B6 type cells with up to 10% contamination of different mouse strain (most likely CD1 strain) cells. With regard to the RNA-seq data for the two TS and three FI stem cell lines, we confirmed that the tree diagram showing hierarchical clustering of global expression profiles presented in *Letter* Figure 2i would differ depending on the TS and FI stem cell RNA-seq data used. Obokata and Sasai decided which TS and FI stem cell RNA-seq data would be used for publication. Obokata explained that the reason for this selection was that they wanted to show the intermediate phenotype.

Evaluation

It is clear that Obokata prepared samples from various different genetic background cells and performed RNA-seq and ChIP-seq analyses. Given that Obokata used different cell lines or *GFP*-infused mice, from those given in the papers or deposited in the public open database, and otherwise employed data that would normally not be used for comparison, it is reasonable to suspect her of research misconduct. Our interviews with her however led us to the conclusion that it was highly probable that she was unaware of a basic research principle, i.e., the importance of proper preparation of experimental conditions, and there is no evidence suggesting anything more than negligence.

In the case of the FI stem cell data set, when the first RNA-seq analysis did not yield the expected results, Obokata performed an additional analysis. There were different results, including different mouse genetic backgrounds between the two RNA-seq analyses, and it is highly probable that one sample was derived from a mixture of multiple cell types (although it is unclear whether this act was negligent or intentional), suggesting that there may have been research misconduct in this instance. However, other than in Obokata's memory, there is no record of how she prepared the samples, making it impossible for the committee to conclusively judge this to be a case of intentional fabrication.

Obokata prepared RNA samples before giving them to GRAS to make the RNA-seq libraries. The investigative committee was able to confirm that the original data left at GRAS was not contaminated in the computer after the sequencing process, so it is reasonable to consider the libraries to have already been contaminated before they were deposited with GRAS.

2-3-2. Investigation of suspicions regarding figures and text of the STAP papers

1) *Article* Fig. 5c
The graph showing the rate of cell growth for ES and STAP stem cells is incongruous.

Results of investigation
Obokata prepared this figure, and in three interviews with her, the committee clarified the following points.

(1) Regarding the discrepancy in dates between the STAP stem cell and ES cell growth curve data, Obokata explained that this was because the cell growth experiments for these two cell types were conducted separately. She also explained that the STAP stem cell line used in this study was FLS, but that she did not remember which ES cell line was used. There are no experimental notes on this. In the interviews, she said the ES cell experiments were conducted in the spring to summer of 2011 and that the STAP cell cultures were started between the end of January and February 2012. However, Obokata's work attendance records for these periods show no time at which she could have conducted the experiments once every three days.

(2) Regarding the procedure used to measure cell growth, Obokata explained that she first counted the cells before starting the cell culture, and then waited until the culture was confluent and re-plated before treating it with trypsin. Since the total number of 129B6F1 ES1 was $1x10^7$ when confluent, she also defined the total number of the confluent cells in this experiment as $1x10^7$, and then prepared a graph based on the time it took for the cells to become confluent again. Furthermore, she diluted the cells by 3–5 fold at each re-plating, and in most cases re-plating was conducted once every three days. But she also controlled the timing of confluence by changing the dilution rates whenever it was impossible to re-plate every three days due to business trips or other reasons. Obokata's explanation made it clear that she did not correctly measure the cell numbers at each re-plating, even though she recognized that counting the cell numbers was important for this study.

(3) In the interviews, Obokata repeatedly stated that she made this figure because Wakayama requested her to make a figure similar to that in Fig. 1d of the Yamanaka & Takahashi paper (*Cell*, 126:4, 663–676), and Wakayama confirmed this in his interview with the committee. Obokata also claimed that she reported the cell growth rate measurement graph to Wakayama, but he said that although he heard from her that she had completed this experiment, he knew nothing about the contents of the graph.

Evaluation
There are no notes on this experiment. Obokata's work attendance record also does not support that the cell number measurements were made every three days as shown in *Article* Fig. 5c. Obokata's explanation indicates that she understands the principle and method of counting cell numbers, which is a necessary and fundamental technique in cell biology for measuring cell growth rates. Initially she followed the correct procedure in this study, but later she defined confluent cells as having multiplied $1x10^7$ and skipped the counting process in the middle of the culture experiment. In particular,

Obokata said that she did not measure the correct cell number at each re-plating when she created *Article* Fig. 5c. If this is the case, Fig. 5c is meaningless as a measure of cell growth rate. Obokata did not follow the most basic procedure of making a cell number count, her statement regarding the dilution rate for re-plating changed from 1/5 to between 1/8 and 1/16, and it is impossible to validate this figure as no original data set is available. The committee can only conclude that she fabricated the data. Obokata acknowledges that only she performed the cell number measurement and that only she created the cell growth rate measurement graph. The actions taken by Obokata completely undermine the credibility of the data. There is no doubt that she was fully aware of this risk, and we therefore conclude that this was an act of research misconduct involving fabrication.

Wakayama suggested to Obokata that she should make this cell growth rate measurement figure. As laboratory head and supervisor of senior researcher Obokata and as a collaborator, Wakayama had a responsibility to check the validity and accuracy of the data. He was negligent in allowing this kind of fabrication, but this does not extend to confirmation of his direct involvement in the cell number measurement and figure preparation. Nonetheless, he bears heavy responsibility given his leadership position and his failure to supervise and verify the validity and accuracy of the data.

2) *Article* Fig.2c

- DNA methylation status of CpG dinucleotides at *Oct4* promoter in *Oct4*-GFP$^+$cells are shown. Filled and open circles represent methylated and unmethylated status, respectively. Circles are irregularly aligned.
- Results of DNA methylation status at *Oct4* locus in CD45$^+$ and cultured CD45$^+$ cells are extremely similar. This is also the case at *Nanog* locus between ES cells and cultured CD45$^+$ cells and between CD45$^+$ and cultured CD45$^+$ cells.
- Some data do not represent original data.

Results of investigation

The committee investigated the progress report (PR) files submitted by the Wakayama CDB lab, the figures used in submitted manuscripts at different time points, experimental records provided by Wakayama CDB lab members who participated the research, and electronic data left on the server at GRAS, and examined the credibility of the data in the PR files and manuscript figures. We also asked Obokata how she handled original data and generated figures from them. As a result, the following points became clear.

(1) We observed the following temporal changes in the figures showing DNA methylation status. DNA methylation data at *Oct4* and *Nanog* promoter regions in untreated cells, Oct4$^+$ spheres and ES cells first appear in the Wakayama lab PR files on 22 Sep 2011. Very similar data appears a second time in November 2011. These two instances were understood to come from the same experiment, but we could not find experimental records or lab notebooks to validate this data. We also found that the same results were differently labeled on these two occasions, once as “ES cells” and once as “sphere”.

The Wakayama lab PR file for April 12, 2012, shows results from completely different experiments. These were used in the manuscript submitted

to *Nature* in April 2012, as well as the manuscript later submitted to *Cell*, which was ultimately published in *Nature* as *Article* Fig. 2c. In this figure, we found irregular alignment of closed circles that represent methylated CpG dinucleotides that are likely the result of manipulation rather than computational alignment from the original data. In addition, the methylation patterns seen in "$CD45^+$" and "Cultured $CD45^+$" cells turned out to be extraordinarily similar.

DNA methylation analysis was also performed for STAP stem cells. These results first appeared in the first version of the *Letter* submitted in March 2013 and eventually published as Extended Data Fig. 8d in the *Article*. The DNA methylation status of *Oct4* and *Nanog* promoters in $CD45^+$ cells were also shown in this figure. It is noteworthy that the methylation levels shown in this figure were lower than those shown in *Article* Fig. 2c.

We strongly speculate that Article Extended Data Fig.8d was generated from the data obtained from materials that a Wakayama lab member submitted to GRAS, based on our analysis of sequence data stored on the GRAS server. During the assembly of these data to generate Article Extended Data Fig.8d, we found there was selective use and misuse of data that may or may not have been intentional.

(2) Original data for *Article* Fig. 2c could not be identified in the notebook. We, however, found that Obokata submitted three sets of samples designated as "bisulfite" to GRAS for sequencing, one on October 27, 2011 and two on November 17, 2011. The two samples submitted on November 17, 2011 were also designated as "*Oct4*" and "*Nanog*", respectively. We conclude that these represent the results for DNA methylation status. We found that 96 clones were sequenced for "*Oct4*" and highly reliable data that could be used for publication were obtained from 74 clones. However, we found considerable inconsistencies between the results from these 74 clones and the data shown in Fig. 2c. For example, in Fig. 2c, 18 clones were shown to possess < 1 methylated CpG of 11 CpG in the *Oct4* promoter, but we observed only 3 of the 74 clones exhibited such a pattern. Even if the unreliable data were included, the DNA pattern shown in Fig. 2c could not be replicated from the data left on the GRAS server. The same was also true for the "*Nanog*" data. Here, 40 of 96 sequenced clones gave highly reliable results and qualified for publication, but these data were again inconsistent with those shown in Fig. 2c. For example, we found 15 fully methylated clones in Fig. 2c but only 7 in the sequenced data. Again, even including the unreliable clones, we could not replicate the "*Nanog*" DNA methylation data shown in Fig. 2c from these sequence data.

(3) In an interview with Obokata, we confirmed that she intentionally selected DNA sequence data and *E. coli* clones that supported the authors' hypothesis and assembled them to generate Fig. 2c. Obokata realized this was not normal practice and said she was ashamed of having manipulated the data.

Evaluation

We found that data storage and handling by Obokata was inappropriate and, as a result, there was room to make mistakes and the traceability of the research was

tremendously lessened. On top of that, as Obokata realized, only a part of the data was intentionally selected and assembled to generate panels that supported the authors' model and intentionally mislead readers into accepting the authors' conclusions. We conclude that this assembly process taken by Obokata to generate *Article* Fig. 2c can be deemed to be fabrication.

We further speculate that the excessive demand for supporting evidence by Obokata's colleagues could have been taken as a cue to fabricate DNA methylation data. Wakayama in particular bore responsibility for avoiding such misconduct because he was expected to give scientific guidance to Obokata and to check the accuracy and validity of individual data. Although we did not identify specific evidence suggesting the involvement of Wakayama in the biased data selection and assembly, we nevertheless found that Wakayama failed to sufficiently fulfill his roles as a supervisor and colleague to keep Obokata's research on a normal track. The failure of Wakayama to guide and monitor Obokata can be considered as part of the reason for her actions even though Wakayama did not actively participate in this misconduct.

3) *Article* Fig. 2e and Extended Data Fig. 4a-c

Histological images of teratoma sections in *Article* Fig. 2e and Extended Data Fig. 4a–c were described as being derived from *Oct4*-GFP^{+} and *Oct4*-GFP-dim cells, respectively. However, we found inconsistencies between this description and the DNA sequence data of these teratoma samples.

Results of investigation

Both of the section images shown in *Article* Fig. 2e and Extended Data Fig. 4a-c were found on microscopic inspection to be the same section designated as "6weeks+PGA 12/27 implanted Haruko". DNA sequence analysis for DNA extracted from this section revealed that the teratoma was derived from FES1 ES cells.

Evaluation

Two different images obtained from a single specimen could have been fabricated to appear as if they had different origins. We, however, could not exclude the possibility of an innocent mistake due to simple mislabeling of images or other reasons and therefore could not conclude that this was a case of misconduct.

4) *Letter* Extended Data Fig. 1a

- It is suspected that this image does not show 2N chimera, but rather 4N chimera, which is identical to that shown in *Article* Extended Data Fig. 7d (retraction note point 2). (This correction was made by the authors on May 10, 2014 and reported by the media on May 21, 2014.)
- A part of the embryos in this image was misidentified as placenta.

Results of investigation

This image was identified as showing 4N chimera, based on records on the PC connected to the microscope (images were taken on November 28, 2011) and in

Wakayama's notebook. According to the figure legend, one arrow represents placenta and another yolk sac. But experts suggest that both likely represent yolk sac.

Evaluation

Since the question of whether the images showed 2N or 4N chimeras was not central to the main findings of the papers, we conclude that this may have been the result of a simple error. The contribution of STAP-derived cells to placenta, however, is a critical issue for distinguishing STAP cells from ES cells, and the authors may thus have intentionally misidentified the yolk sac as placenta. We, however, could not find evidence to suggest there was misconduct in this case. The figure legend, "B6GFPx129/Sv", is incorrect in view of standard genetics nomenclature. This is clearly a simple mistake.

5) *Letter* Fig. 1a and 1b

- The images in *Letter* Fig. 1a and 1b are extraordinarily similar and an image in Fig. 1a is speculated to represent STAP cell chimera rather than ES cell chimera (Retraction note point 1).
- *Letter* Fig.1a is described as representing a long exposure image of placenta but it is doubtful that it is a long exposure (Retraction note point 3).

Results of investigation

Letter Fig. 1a was confirmed to represent STAP cell chimera as was the case with Fig. 1b by examining images taken on 17 July 2012 and stored in the hard disk connected to the fluorescence microscope and by Wakayama's experiment records. We could not find any long exposure images in this hard disk, and speculate that the description is incorrect. We did not find signs that the electronic records had been manipulated.

Evaluation

It is clear that the descriptions of Fig. 1a and 1b are incorrect. As mentioned above, the contribution of STAP-derived cells to placenta is a critical point for the authors and they may have intentionally misidentified embryonic tissue as placenta. We, however, could not exclude the possibility that this was simply a mistake and therefore do not conclude that this is a case of misconduct.

6) *Article* Fig. 3b

- Images in "Control" and "Low-pH-treated cells" panels should not be compared because these images seem to have been prepared under different conditions.
- In the control panel, the position of cells in the bright field view and fluorescence signals that were only visible after contrast adjustment in the *Oct4*-GFP panels did not overlap. It is suspected that the bright field and fluorescent view images do not represent the same sample and/or field.

Results of investigation

Despite repeated requests, Obokata did not submit any original data. An attempt was made to locate original data on every hard disk attached to fluorescent microscopes in the CDB and the Wakayama lab, but none was found.

Evaluation
It is clear that the bright field and fluorescent view images of Fig. 3b do not show the same sample. We further speculate that the *Oct4*-GFP images for "Control" and "Low-pH-treated cells" were taken under different conditions (i.e., exposure time, camera sensitivity, etc.) or processed in different ways, although they must be prepared in the same way in normal practice. These could be instances of inappropriate manipulation of images, but we cannot exclude the possibility of simple errors due to ignorance of imaging technology or mixing up of images. As far as what we could find in our investigation, there was insufficient evidence to deem this a case of misconduct.

7) *Article* Extended Data Fig. 2f
It is suspected that the bright field and fluorescent view images do not represent the same sample and/or field.

Results of investigation
Despite repeated requests, Obokata did not submit any original data.

Evaluation
We could not judge whether this represents misconduct as the materials needed were not provided by Obokata.

8) *Article* Extended Data Fig. 5f and *Article* Extended Data Fig. 8k.
The signal intensity of H3K27me staining in R channel of the RGB images was unusually low, raising the possibility that deletion or excessive image manipulation of the R channel image of a specific subset of cells was performed.

Results of investigation
Obokata did not submit the original data requested by the investigative committee.

Evaluation
Since Obokata failed to provide the original data to verify her claim, it was not possible to confirm whether inappropriate image manipulation had occurred. The investigative committee was unable to conclude whether the irregularities in image presentation constitute misconduct.

9) Irregularities in error bars of the graphs presented in *Article* Fig. 2b, 3d, 3g, Extended Data Fig. 1a, and Extended Data Fig. 6d.

Results of investigation
The investigative committee confirmed that the graph in *Article* Extended Data Fig. 1a differs from the corresponding data in the past versions of the manuscript. The size of the error bar and the value of data differed in different version of the graph. The investigative committee asked in its interview of Obokata whether she was able to account for how such variations had been introduced, such as modification with graphic software. She stated that she did not modify the graph, but admitted that such variations in error bars are unusual. Obokata explained that the variation might have been

introduced due to a fault in the spreadsheet program. The investigative committee asked Obokata for the original data that was supposedly stored in her computer, but she failed to submit the data.

Evaluation
Obokata acknowledged that the graph was inaccurate. She should have made a best effort to handle the data accurately with proper use of software. Although the possibility of such errors being introduced inadvertently due to a lack of knowledge in software operation cannot be excluded, the investigative committee considers this possibility unlikely. Nevertheless, in the absence of original data, the present evidence was not sufficient to conclude that these problems constitute misconduct.

10) FACS sorting data shown in *Letter* Extended Data Fig. 5g, *Letter* Fig. 3c–d.
The FACS data showed unusual diagonal distribution of the signals that appear to indicate that fluorescence crosstalk between *Oct4*-GFP and integrin alpha7 channels were not properly compensated for, or that detector sensitivity was set differently for different samples. This raises a question of the reliability of the data shown in *Letter* Fig. 3c and 3d, which reports characterization of FI-SC that was sorted by this procedure.

Results of investigation
Based on interviews with Obokata and other CDB staff, it was learned that Obokata mostly used the cell sorter (FACS Aria) installed in CDB. It was confirmed that Obokata did not have sufficient training in the operation of the equipment and cell sorting technique at the time she performed the experiment.

The majority of the cells in the two FACS plots shown in *Letter* Extended Data Fig. 5g were distributed in upward diagonal lines. This pattern typically occurs when dead cells are not excluded from analysis, or when fluorescence crosstalk occurs between fluorescence of GFP and integrin alpha7 staining, and suggests that the sorting conditions were not optimal.

GFP signal distribution differed greatly between the top panel (integrin alpha7 staining) and the bottom panel (control IgG staining). The data are unusual because GFP signal distribution should be the same if the integrin alpha7 and control staining were conducted on aliquots of the same cell preparation.

Obokata explained that the collaborators did not express any concern regarding these problematic FACS data.

The investigative committee searched for the original FACS data in the computer used for the experiment but was unable to find any.

Evaluation
In FACS experiment using multiple fluorescence, extreme care should be taken to avoid fluorescence crosstalk and to exclude dead cell signals. In general, if a strong correlation between two fluorescence signals was observed, as represented by diagonal plots of *Letter* Extended Data Fig. 5g, the presence of dead cell signal or the signal of one fluorescent probe leaking into another channel should be suspected. This problem can be minimized by estimating the background fluorescence by analyzing singly labeled control samples and using this data for compensation in each FACS experiment.

Based on the presented data and interviews with Obokata, we conclude that she had

insufficient knowledge of the correct use of the FACS machine and performed FACS experiments without the required control and compensation procedures. Furthermore, since the upper and lower panels in *Letter* Extended Data Fig. 5g differed significantly, the data were likely to have been obtained by some improper experimental procedure. In addition to Obokata, other coauthors were inexperienced in the FACS procedure, thereby casting doubt on whether other FACS data (*Article* Fig. 1c, *Letter* Fig. 3c–d) were obtained by proper procedures. However, since the original data was not provided, the present evidence was not sufficient for concluding that these problems constitute misconduct.

11) Presence of FI-stem cell with *Oct4*-GFP described in *Letter* Fig.2b-e, Fig. 3, Extended Data Fig. 5, Fig. 6 was not confirmed. All of FI-SC stocks in the Obokata and Wakayama labs contained *Acr-GFP/CAG-GFP*. No FI-SC containing *Oct4*-GFP was identified, bringing into question the claim of establishing FI-SC with *Oct4*-GFP.

Results of investigation
Based on email inquiries of Obokata and Wakayama, it was confirmed that the FI-SC strain CTS1 was established by Wakayama (culture started on May 21, 2012 and ended on May 28) from STAP cells provided by Obokata. She generated the STAP cells from 129X1xB6N F1 mice carrying CAG-GFP provided by Wakayama. Wakayama established additional FI-SC strains on July 9, 2012 (based on his notebook entries). Genetic background of those strains was unknown because the genotype of the mouse strain used for the experiment was not recorded. However, Wakayama stated that he was not aware that he has ever established FI-SC from mice other than 129B6F1. Wakayama prepared all the cultures for FI-SC generation. Obokata did not perform the FI-SC derivation described in the papers.

The two sets of FI-SCs (Call TS-1, Call-TS11-TS13) were found in a freezer in CDB Building A. The description of the cells was consistent with the records in Wakayama's notebook describing derivation of one line on May 25, 2012 and three lines on July 9, 2012.

Whole-genome sequencing analysis of FI-SC CTS1 (Call TS-1) performed by RIKEN revealed that *Oct4*-GFP was not detected in the genome of this cell line. Instead, this strain carried *Acr-GFP/CAG-GFP*. Furthermore, the genome sequence of CTS1 matched perfectly with the genome sequences of ES cell line FES1 (derived from *Acr-GFP/CAG-GFP* mouse in 2005) and STAP-SC line FLS3 (derived from *Acr-GFP/CAG-GFP* mouse between January 28 and February 2, 2012).

Obokata's response to the email inquiry revealed that she did not know the genotype of mice she used for STAP cell induction. She misunderstood that she was given *Oct4*-GFP (GOF) mice by Wakayama.

Evaluation
The FI-SC strain CTS1 used in the *Letter* was found not to contain *Oct4*-GFP. No case of FI-SC with *Oct4*-GFP derivation was confirmed.

Since the parental mouse genotype for the second FI-SC derivation (July 9, 2012) was not recorded in Wakayama's notebook, the possibility remains that in this experiment FI-SC strains (Call-TS11-TS13) derived from *Oct4*-GFP (GOF) mice were

contaminated with ES cell line FES1, and that the latter was retained in the current cell stocks.

In summary, the committee did not find any evidence supporting the derivation of FI-SC carrying *Oct4*-GFP as described in the *Letter*. Therefore, it is possible that the data described in *Letter* Fig. 2b–e, Fig. 3, Extended Data Fig. 5, Fig. 6 was obtained not with FI-SC carrying *Oct4*-GFP, but with FI-SC carrying *Acr-GFP/CAG-GFP* or a mixture of FI-SC expressing *Oct4*-GFP and the ES cell line FES1 expressing *Acr-GFP/CAG-GFP*.

However, available evidence was insufficient to identify the person responsible for the ES cell contamination, and we thus cannot conclude that these problems constitute misconduct.

12) *Letter* Fig. 2i, Extended Data Fig. 6d. Omission of a part of original data and sample mislabeling

Letter Fig. 2i is a tree diagram of cells based on similarity of gene expression profiles presumably deduced from RNA-seq data obtained using a TruSeq kit in 2012. A subset of the original data was omitted in the final data set.

Letter Extended Data Fig. 6d is a tree diagram of cells based on similarity of gene expression profiles presumably deduced from RNA-seq data obtained using a SMARTER kit in 2012. Data of the sample labeled Callus1 (equivalent to "STAP cell 1", according to the 2012 Wakayama lab nomenclature) upon submission was labeled $CD45^+$ in the Figure.

Results of investigation

RNA-Seq analyses and Figure preparation were performed by the members of the CDB Functional Genomics Unit (FG) andGRAS. Mapping of RNA sequence was repeated after the release of the new version of mouse genome assembly and tree diagram was prepared. Initial tree diagram (original for *Letter* Fig. 2i) included the samples Obokata labeled Callus1 and Callus2, but not the sample labeled $CD45^+$. When resubmitting the paper to Nature in March 2013, Obokata requested the lab members to change the style of the Figure, and to replace the sample labels by explaining that Callus1 and Callus2 corresponded to STAP cell and $CD45^+$ cells, but this change did not actually appear until the revised *Letter* submitted in September 2013.

The variation of RNA sequences of multiple TS and FI-SC samples was larger than the authors expected. Obokata explained that she chose one of the three FI-SC data placed in the intermediate positions in the tree diagram. The variation of TS cell data was considered to be due to cell differentiation in the culture. Therefore, new data were taken from RNA samples of other TS cells prepared by a member of the Niwa laboratory and these were used for the tree diagram.

Evaluation

The term "Callus" used in the Wakayama laboratory referred to what were later called STAP cells. $CD45^+$ was the type of cell used for Callus/STAP induction. Labeling $CD45^+$ cell samples with the name of Callus and submitting for analysis to the support units is misleading and confusing. The Wakayama lab often used very similar names for different samples, and this habit might have influenced the confusing action taken by Obokata. She requested changes of Figure format without providing any detail of the

project and did not ask for discussions with the staff of FG or GRAS. This was inappropriate for a scientist engaged in research collaboration. Nevertheless, the present evidence was not sufficient to conclude that these problems constitute misconduct.

13) As a result of the preliminary inquiry, the following issues were previously assumed to need no further investigation. The committee confirmed that they involved no misconduct.

(1) Fig. 1h in the *Article*
The upper part of the histogram is missing and accurate evaluation of the data is impossible.

(2) Fig. 2b of the Extended Data in the *Article*
It was impossible to determine whether the 24-h experiment tracked the CD45-immunoreactivity of the same cell or not.

(3) Fig. 5g in Extended Data of the *Article*
Between data 3 and data 4, there is an unaccounted for gap suggesting the two graphs had been spliced together.

(4) Fig. 8e in Extended Data of the *Article*
The photos of STAP stem cells and ES cells are suspected to have been mistaken for each other.

(5) Fig. 4b in the *Letter*
STAP cells and ES cells are suspected to have been mislabeled (reason 4 for the paper's retraction).

(6) Fig. 1c of the Extended Data in the *Letter*
Left and right photos are of different sizes and they do not overlap each other.

2-3-3. Investigation of doubtful points in the manuscript preparation process

1) Possible concealment of inconsistent data on the TCR recombination

Results of investigation
Obokata started her experiments for detection of TCR recombination, and reported for the first time to the Wakayama lab that the recombination was detected in a cell mass containing STAP cells or a portion of STAP stem cells. However, when the co-authors attempted to confirm this, no recombination could be confirmed in eight STAP stem cell lines. These eight cell lines had been continuously sub-cultured by Obokata herself.

Thereafter, Obokata asked a member of the Wakayama lab to conduct experiments to confirm TCR recombination,, but according to the lab journals of this member, no recombination was detected.

From the above, it appears obvious that there were inconsistencies in the experimental data; Obokata reported TCR recombination based on her first experiment,

whereas the later experiments by Obokata herself as well as those by the Wakayama's lab member could not confirm this finding.

According to Niwa's explanations, he was told when he joined the authorship in January 2013 that the eight STAP stem cell lines sub-cloned by Obokata did not show any TCR recombination. Therefore, Niwa insisted to Sasai that they should be very careful about the inclusion of the recombination data in the manuscript.

To explain the unsuccessful detection of the recombination, Sasai and the other co-authors assumed that the cells were initially heterogeneous, and those with the recombination would probably have disappeared through the long-term sub-cloning. In the *Article*, TCR recombination was described in the STAP cell mass, but not in the STAP stem cells per se.

Meanwhile, Niwa was anxious right after the publication of *Nature* article that readers would immediately complain of not being able to reproduce the experiment. Niwa explained that he felt Obokata's original protocol was insufficient and needed to be elaborated and published as soon as possible. He further explained that Obokata and Sasai were then very busy preparing a corrigendum, and Niwa was the only person who could write the Protocol Exchange while communicating with the editors.

There is a statement referring to the absence of the TCR recombination in the section marked "IMPORTANT, (iii), 2. After 4–7 days of…" in the "STAP stem-cell conversion culture" of the elaborated protocol published in the *Protocol Exchange* on March 5, 2014.

Also, in Niwa's own words, "Wakayama says that he heard from Obokata that there was TCR recombination in the early passages of the STAP stem cells".

Evaluation

Regarding the TCR recombination, it was first reported to have been confirmed by Obokata, but could not be confirmed by later experiments conducted by a member of Wakayama's lab or by Obokata herself. That notwithstanding, the authors chose to present only those data consistent with the hypothesis. Yet, the absence of the evidence of recombination (in eight lines of STAP stem cells) was later described in the Protocol Exchange. Taking Niwa's explanation into consideration, it cannot be unequivocally determined that there was an intentional concealment or other kind of scientific misconduct.

2) Inconsistency in the protocol for establishing STAP cells (no description of ATP for low pH treatment)

Results of investigation

The *Article* describes only the usage of HCl for the low-pH treatment of cells for obtainment of STAP cells. However, it became clear in the interviews with Obokata and Wakayama, that ATP, instead of HCl, was the reagent mainly used for the treatment.

In this regard, Obokata explained that HCl can also generate STAP cells and that a part of the published experiments were performed with HCl. Similarly, Wakayama said that ATP and HCl have nearly equivalent effect, and that ATP was only slightly more effective than HCl. Niwa, who wrote the *Protocol Exchange*, said he only described the use of HCl since he had heard from Obokata that HCl was the only reagent used in the experiments for the *Article*.

The usage of HCl is described in the revised *Article* manuscript submitted to *Nature* in September 2013, and no description of the reagents used can be found in earlier manuscripts.

Evaluation
While there is inconsistency in the explanations given by the above three authors, this apparently stems from Obokata's assertion that she used HCl in the experiments for the *Article*. However, since Obokata has not specified the experiments that were based on HCl nor provided the original data, there is no objective evidence that her statements are false. Nor is the evidence gathered in the investigation sufficient to prove scientific misconduct.

3) Did the authors not try to clarify the inconsistencies in the experiments, even though they knew that the GFP insertion pattern was not homo- but heterozygous in the STAP stem cell FLS?
(Note: According to Wakayama who actually performed the crossing of mice, the labels such as B6GFP x 129/Sv, 129/Sv x B6GFP found in the papers are errors.)

Results of investigation
Mice with no GFP were found among the litter obtained by the backcross of the 4N chimera produced from the STAP stem cell FLS.

This contradicts Wakayama's understanding that the STAP stem cell FLS originated from an F1 crossing of 129 (*CAG-GFP* homozygotic) and B6 (*CAG-GFP* homozygotic). However, neither Wakayama nor Obokata tried further investigations to clarify this inconsistency.

To explain this, Wakayama replied to the committee's letter of inquiry saying, "At that time, I believed in the existence of STAP cells 100%, and I thought that was simply due to my own mistake made in the crossing of mice."

He concluded, "When you are helping with someone's experiments and inexplicable things happen, I think it is only natural to first doubt the part you are in charge of."

Evaluation
From the situation described above and from Wakayama's explanations as well, it can be assumed that Wakayama was in charge of the mouse rearing and maintenance management and was helped by some of his own lab members.

Also, management of mouse lines was designed such that that there could be no cross-contamination among strains by separate racks and rooms, for example.

Obokata, on the other hand, was entirely dependent on Wakayama in terms of mouse management, and thus her responsibility for this problem is apparently minor.

From these points, it appears apparent that the failure to clarify the inconsistency can be ascribed as a mistake on Wakayama's part, which he should not have made as a scientist.

Yet, as far as evidence acquired in the investigation is concerned, this mistake cannot be considered overt scientific misconduct.

3. Conclusion

This investigative committee was established on the basis of RIKEN's Regulations on the Prevention of Research Misconduct (Sep 13, 2012, Reg. 61), and acted in accordance with the provisions of these Regulations. The committee also took into consideration the MEXT guidelines on the handling of research misconduct issued by the MEXT Minister on Aug 26, 2014. RIKEN's Regulations were revised after the committee was convened (the revised version took effect on Nov 25, 2014), but, as provided for in the Supplementary Regulations (Oct 30, 2014, Reg. 74), the definitions of research misconduct set forth in the original Regulations prior to the revision were used in this investigation.

The Regulations define research misconduct as "fabrication, falsification, or plagiarism". This investigative committee concluded that Obokata fabricated data in Article Fig. 5c, showing the STAP stem cell growth curve, and Article Fig. 2c, showing DNA methylation. Research misconduct of this sort represents a significant impediment to the pursuit and development of robust science. Wakayama and Niwa were not found to have been involved in any research misconduct.

However, when we expand our view to encompass the problems identified in the STAP papers and the research on which they were based, the research misconduct that has been confirmed to date is only the tip of the iceberg. For example, the following four points clearly indicate that there are significant additional problems with the papers.

First, the investigation's findings refute the primary conclusion of the papers that STAP cells are pluripotent. The STAP stem cells, FI stem cells, chimera mice and teratomas that would support the papers' findings were all found to be either derived from cultures contaminated with ES cells, or under circumstances that could be scientifically explained in terms of such contamination. This effectively refutes the core finding of the STAP papers. It is difficult to eliminate thesuspicion that contamination by ES cells to such a great extent was intentional and not the result of negligence. Unfortunately, however, we were unable to gather sufficient evidence to make a conclusive determination of misconduct with regard to this aspect of the research. We consider this to be due to the limitations of our abilities as well as our authority.

Second, there was little to no original data for the figures in the papers, particularly those prepared by Obokata, with the exception of image data saved in an external hard drive attached to one of the microscopes used in the study, indicating a serious lapse of research integrity. As Obokata prepared the final images for the papers, we find that the primary responsibility for this lapse rests with her. Furthermore, Obokata carried out most of the analyses of the STAP stem cells, FI stem cells, chimera mice, and teratomas after they were established or formed, yet there are few to no records of the experiments she conducted. In fact, there are several experiments for which there is no evidence that they were carried out, such as the measurement of cell proliferation and the generation of *Oct4*-GFP FI stem cells.

Third, there were numerous instances of errors in the papers, including incorrect figures and improper manipulation of images, as well as basic errors in the use of instruments and in the manner of carrying out experiments, for which, again, the responsibility rests primarily with Obokata, as she was the one who prepared the images and carried out the experiments.

Fourth, the collaborators and co-authors overlooked or ignored the lack of

experiment records and original data, and the presence of clearly suspect images. Moreover, no effort was made to conduct further experiments when the data for the STAP stem cells and chimera mice was so clearly problematic. For this, Wakayama who headed the laboratory in which Obokata worked for much of the study, and Sasai, who played a major role in preparing the final manuscripts of the STAP cell papers, both bear heavy responsibility.

We would like to examine this last problem in more detail. Obokata's failure to maintain experiment records and her numerous errors would not have been overlooked if the laboratory was managed properly and if the progress reports were examined in detail. Co-authors have a responsibility to read through the final version of any paper they submit, but did all of the co-authors fulfill this responsibility? Wakayama explained the sudden success to create STAP stem cells efficiently as being due to a change in procedure from enzymatically dispersing STAP cell aggregates to physically cutting them into small pieces before injection. However, if a control experiment with dispersed cells had been carried out at this point, it may have been possible to discover the contamination by ES cells. Again, when the *GFP* insertion in a mouse was found not to be homozygous when it should have been (see section (3) of 2-3-3 above), no additional experiments were performed to determine the cause of this discrepancy. We cannot help but think that these failures to follow up when there were questions may have been due to the sense of urgency to publish rapidly. Securing patents and research funds, and getting papers published in prestigious journals are not inherently negative, but perhaps in the eagerness to achieve these goals, not enough attention was being paid to the actual content of the research. If appropriate action had been taken in any one of the above cases, the STAP problem probably would not have evolved into such a major crisis.

While most researchers were not in a position to collaborate with Obokata, we must all consider what we would have done under similar circumstances. Now, we must ask, how can we minimize the risk of this kind of misconduct occurring again? The MEXT guidelines referred to above state: "Research misconduct must be dealt with as an issue of researcher ethics and social responsibility. Preventing research misconduct depends on researchers' self-discipline, and the autonomous ability of the science community and the research organization to maintain its integrity." The massive amount of verification data collected for this investigation represents the dedication and effort of RIKEN's researchers, and is an indication that the STAP issue has triggered an appropriate self-regulating response from within RIKEN. All researchers, not only those in RIKEN, should realize that the STAP problem could have just as easily occurred in their own laboratories, and should make even more conscientious efforts to educate their staff and manage their laboratories. In order to ensure that measures to prevent research misconduct take hold and are implemented as intended, fabrication, falsification and plagiarism must be recognized as the major violations that they are. Even more so, however, there is a need for education in research ethics that encompasses broad perspectives covering responsible conduct of research and research integrity. Responsible and fair research is not measured by the impact factor of published papers, the amount of research funding, or even the number of Nobel Prizes, but by the joy of unraveling the mysteries of nature and the mind to contribute to society.

The problems with the STAP publications have pierced the scientific community like an arrow in the side. We may pull out the arrow, but it will take the collective effort of the community as a whole to heal the wound and restore its health.

We conclude by thanking the following people, and the many, many others, who assisted us in our investigation by carrying out genetic analyses, checking the images in the STAP papers, submitting documentation and materials, and in general giving of their invaluable time and energy: Piero Carninci, Kosuke Hashimoto and Takeya Kasukawa, Division of Genomics Technologies, RIKEN Center for Life Science Technologies (CLST); the staff of the Genome Network Analysis Support Facility (GeNAS); Shigehiro Kuraku, Phyloinformatics Unit, Biosystem Dynamics Group, Division of Bio-Function Dynamics Imaging, CLST; Masashi Matsuda and Tomoyuki Ishikura, Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences (IMS); Masaki Okano, RIKEN Center for Developmental Biology (CDB); Daijiro Konno and Taeko Suetsugu, Laboratory for Cell Asymmetry, CDB; and Shigeo Hayashi, Laboratory for Morphogenetic Signaling, CDB.

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