SUPPLEMENTAL MATERIAL AND METHODS

Cell lines and culture conditions

Human fibroblasts were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen), supplemented with 20% fetal bovine serum (Gibco) and 1% Glutamax (Invitrogen). H9 and H13B (hereafter referred as H13) hES cells (1) and human iPS cell lines 8.11 and F were grown on gelatin-coated plates containing mitotically inactivated SNL feeder cells (2) or on plates coated with growth-factor-reduced Matrigel in the presence of hES culture medium conditioned (CM) by mouse embryonic fibroblasts (Millipore). hES cell culture medium consists of KO-DMEM F12 (Invitrogen), supplemented with 10 ng/ml basic fibroblast growth factor (b-FGF) (R&D Systems), 20% knockout serum replacement (Invitrogen), 1% Glutamax (Invitrogen), 50 mM β-mercaptoethanol, and 0.1 mM nonessential amino acids (Invitrogen). CM was generated by plating mouse embryonic fibroblasts on gelatin-coated plates in complete hES culture medium; the medium was harvested every day for 10 days, filtered (0.22 μM), stored at –80ºC, and supplemented with b-FGF (4 ng/ml) before use. Cells were passaged by Accutase treatment (Millipore) and then cultured in CM with 10 μM ROCK-inhibitor (Sigma) for 24 h. The medium was replaced daily with CM. iPSC line iAND-4 was maintained on inactivated human feeder fibroblasts in hES culture medium supplement with 50 ng/ml b-FGF. Frozen stocks of karyotyped normal hES and iPS cells were used for approximately 30 passages.

Lentiviral production and infection and isolation of iPSCs

The iPS cell lines 8.11 and F were derived as described (3) using four factors (Oct3/4, Sox2, Klf-4, and c-Myc) expressed from retroviral constructs. The complete characterization of the iPSC lines 8.11 and F will be described in a forthcoming publication (Collins *et al*., in preparation). For the generation of the iPS cells iAND-4, lentiviral virus-like particles were produced using standard procedures. For reprogramming, human foreskin fibroblasts (ATCC, CRL-2429) were seeded in six-well plates ($1x10^5$ cells/well) in fibroblast medium consisting of

Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin. The next day, cells were transduced with 400 µl of the virus-like particles and 8 µg/µl polybrene and resuspended in 1 ml of fresh medium. The medium was changed 5.5 h and 24 h after transduction with hES culture medium supplemented with 50 ng/ml b-FGF. After 10–20 days, iPSC-like colonies were manually picked and expanded on irradiated feeder cells in iPS cell medium as described (4, 5). iPS cells were expanded and frozen as described (4, 5). iAND-4 cells have been deposited at the National Repository Stem Cell Bank in Spain (http://www.isciii.es/htdocs/terapia/terapia_lineas.jsp#iPS).

Transfection of hES and iPS cells, retrotransposition assay, and FACS analysis

L1 retrotransposition assays using the L1 *mneoI* indicator cassette were performed as follows. Cells ($4x10^6$) were nucleofected with an Amaxa nucleofector, using 4 µg of plasmid DNA, hES cell solution 2, and the A-23 program. The transfected cells were cultured in iPS cell medium, supplemented with 10 µM ROCK-inhibitor (6). Cells were selected with 50 µg/ml G418 for 7 days and 100 µg/ml G418 for an additional 7 days, fixed in PBS containing 0.2% glutaraldehyde and 2% formaldehyde, and stained with 0.1% crystal violet to visualize foci. For *mEGFPI*-based retrotransposition assays, cells were nucleofected with V-Kit solution (Amaxa) and the A-23 program. Cells ($2x10^6$) grown on Matrigel-coated plates were detached with Accutase (Millipore) for 5 min at 37°C, washed twice with CM, and nucleofected, according to the manufacturer's instructions. Cells were recovered in RPMI 1640 for 30 min and seeded onto Matrigel-coated plates with CM + 10 µM ROCK-inhibitor. Cells were re-fed daily. Cells nucleofected with *pLRE3-EF1-mEGFP(∆intron)* were harvested 2 days after nucleofection at the peak of EGFP expression; cells nucleofected with *pLRE3-EF1-mEGFPI* were harvested 4 days after nucleofection. Cells were detached with Accutase, washed with PBS, and fixed in 1% paraformaldehyde for 30 min. FACS analysis was performed on an LSR-II (BD Biosciences). For each sample, 1–2 x 10^6 hES or iPS cells or ~0.25–0.5 x 10^6 HDFs were analyzed.

Autofluorescent cells were eliminated by gating against an empty channel before analysis. Data were analyzed with FlowJo software.

L1 promoter methylation studies

Whole genome bisulfite analysis was performed as described (7). Briefly, genomic DNA was extracted using DNA easy kit (Qiagen). Bisulfite conversion was performed using the Epitect kit (Qiagen) according to the manufacturer's instructions. PCR was performed to amplify a 363-bp region in the 5' UTR harboring 20 CpG dinucleotides (2 min at 95ºC, 35 cycles of 30 sec at 94ºC followed by 30 sec at 54ºC and 60 sec at 72ºC, and a final extension of 5 min at 72ºC, primers are listed in Table S2). The obtained PCR products were subcloned into the pCR2.1 (Invitrogen) or the pGEM-T Easy Vector (Promega). The clones were analyzed with the online software program "QUantification tool for Methylation Analysis, QUMA" (quma.cdb.riken.jp)(8). Only sequences with a CpG conversion rate >95% were accepted for analysis. The final analysis included the 10 clones with the highest sequence similarity to a hot L1 element (L1.3, accession number L19088.1 (9)). Bisulfite analysis for the "hot" L1 promoter regions were performed with genomic DNA extracted from the iPS-F cell line. Genomic DNA was extracted using DNA easy kit (Qiagen) and bisulfite conversion was performed using the Epitect kit (Qiagen). To analyze specific gene loci L1s, we first amplify a ~900-1200-bp region, including the first 700 bp of L1 and a 200-500-bp region upstream (3 min at 95ºC, 35 cycles of 30 sec at 94ºC, followed by 30 sec at 50ºC and 2 min at 72ºC, and a final extension of 7 min at 72ºC; primers are listed in Table S2). Two microliters of the PCR product were used as template for the second round of PCR, using the same primer and conditions as for the whole genome bisulfite analysis. The obtained PCR products were subcloned into the pCR2.1 (Invitrogen) and analyzed with the online software program QUMA (quma.cdb.riken.jp)(8). Only sequences with a CpG conversion rate >95% were accepted for analysis.

Real-time RT-PCR

Total RNA was extracted with Trizol (Invitrogen), treated with Turbo DNA-free (Ambion), and further enriched with an RNeasy kit (Qiagen). Purified RNA was reverse transcribed with sense-strand-specific L1 primer and the SuperScript III first-strand synthesis system (Invitrogen). Real-time PCR was performed with QuantiTect Probe PCR kit (Qiagen) on a 7900HT fast real-time RT-PCR system (Applied Biosystems). Thermal cycling consisted of a 15-min denaturation at 94ºC, followed by 50 cycles of 30 sec at 94ºC, and 60 sec at 60ºC. To control for the amount of input per reaction, the purified RNA was also reverse transcribed with random hexamer primer. A control real-time RT-PCR was performed using a GAPDH specific primer/probe pair utilizing the above described conditions. Standard curves were prepared using known DNA concentrations. For Supplemental Figure 1, the RNA was extracted with the RNeasy Mini Kit (Qiagen), cDNA synthesis was performed using the high-capacity cDNA reverse transcription kit (Applied Biosystems). Unless otherwise indicated, PCR was carried out for 2 min at 95ºC, 35 cycles of 30 sec at 94ºC, followed by 30 sec at 60ºC, and 60 sec at 72ºC, with a final extension of 5 min at 72ºC. All primers and probes used in this study are listed in Table S2.

Northern blot analysis

For northern blot analysis, ~0.5 x10^8 cells were harvested, washed once with 1xPBS, lysed with 1 ml of ice-cold RNL buffer (50 mM Tris/HCl, pH 8.0, 140 mM NaCl, 1.5 mM $MgCl_2$, 0.5% NP-40, 1000 U/ml RNase Out; Invitrogen), and incubated on ice for 10 min. Cell debris was removed by centrifugation at 3000 *g* at 4°C for 10 min. RNA from the supernatant was extracted with RNA STAT 50 LS (Tel-Test), according to the manufacturer's protocol. PolyA Tract mRNA isolation system III (Promega) was used to extract the poly(A)+ RNA. For each sample, 2.5 µg of cytoplasmic poly(A) selected RNA was fractionated with a 1% agarose-formaldehyde gel. The RNA was transferred onto a Hybond-N+-Nylon membrane (GE Healthcare Life Sciences) by capillary transfer in a 3 M NaCl, 0.01 N NaOH solution overnight. After UV crosslinking, the

membrane was prehybridized in ExpressHyb hybridization solution (Clontech) for 30 min at 68°C. Hybridization with the strand-specific probe (alpha-^{32}P-labeled probe, final concentration 1–2 x 10^7 cpm/ml) diluted in the hybridization solution was carried out overnight at 68°C, followed by two washes with low-stringency buffer (2x sodium chloride/sodium citrate solution (SSC), 0.1% SDS) at room temperature for 10 min. and two washes with high-stringency buffer (0.1x SSC, 0.1% sodium dodecyl sulfate) at 68°C for 20 min. The result of the northern blot analysis was visualized by autoradiography. To generate an L1 strand-specific northern blot probe, a 500-bp sequence of LRE3 5'UTR was cloned into a pBluescript SK+ plasmid (primer Table S2). The plasmid was linearized with *Bam*H1 and used as a template in the MAXIscript T3 system (Ambion). To generate the actin RNA probe, pTri-β-actin125 human antisense control template (Ambion) was used for the *in vitro* transcription; transcription was performed in the presence of 0.156 μM/μl UTP [α-^{32}P]- 800 Ci/mmol, 10 mCi/ml (Perkin-Elmer).

Analysis of expressed L1 mRNAs

For analysis of the iPS-F cell line, total RNA was extracted with Trizol (Invitrogen), treated with Turbo DNA-free (Ambion), and further enriched with an RNeasy kit (Qiagen). Purified RNA was reverse transcribed with sense-strand-specific L1 primer and the SuperScript III first-strand synthesis system (Invitrogen). The cDNA was used in an RT-PCR reaction with primers L1 5'UTR-16s and L1 ORF1-2072as. The obtained 2000-bp PCR product was purified, subcloned into the pCR2.1 (Invitrogen), and sequenced with an M13F or M13R primer. A 750-bp region in the ORF1 was analyzed with Repeatmasker (http://www.repeatmasker.org/).

For analysis of iPS iAND-4 and MSUH001 cell lines, total RNA was extracted and purified from iPSCs with the RNeasy Mini Kit (Qiagen). cDNAs were synthesize with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Next, 1 μl of cDNA was used in a PCR reaction with primers ORF1-851as and ORF1-615s as described (Primers listed in Table S2)(4, 10, 11). The 236-bp band was excised, purified, and cloned in pGEMT-Easy (Promega). The inserts

were sequenced with an M13F primer. Sequences were then analyzed with Repeatmasker (http://www.repeatmasker.org/).

Isolation of ribonucleoprotein particles and western blot analysis

Ribonucleoprotein particles were isolated and analyzed as described (4). For Fig. 1J, rabbit polyclonal anti-ORF1p antibody (a kind gift from Dr. John Goodier) (12) was used at a 1:1000 dilution, followed by a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (GE Healthcare Life Sciences; 1:25,000). Rabbit polyclonal anti-RPS6 antibody (ab40820, Abcam; 1:1000) was used as loading control. For Fig. 1K, we used a purified rabbit polyclonal antibody against a C-terminal peptide of L1.3-ORF1p (CERNNRYQPLQNHAKM; 1:1000) (kindly provided by Dr. Gael Cristofari, CNRS, France), and a mouse monoclonal anti-β-actin antibody (Sigma; 1:20,000). Goat anti-rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibodies were used at 1:20,000.

***In vitro* differentiation of iPSCs and histological analysis**

Embryoid bodies were formed by harvesting iPSCs with collagenase IV (Roche). The floating colonies generated were transferred to low-attachment plates in KO-DMEM supplemented with 20% fetal bovine serum (Invitrogen), 1% nonessential amino acids, 1 mM L-glutamine and 0.1 mM β-mercaptoethanol. Medium was changed every 4 days, and after 21 days, the EBs were spun down, fixed with 4% paraformaldehyde for 10 min, and embedded in paraffin as described (5), and cut into 3-μm sections. The sections were incubated for 1 h at room temperature with the following primary antibodies: anti-smooth muscle α-actin (Chemicon, 1:50), anti-pancreatic cytokeratin (Dako, 1:100), and anti-GFAP (Dako, 1:500). Slides were incubated with a biotinylated secondary antibody for 30 min at room temperature, and signal was detected with a streptavidin-peroxidase complex (Vector Laboratories) for 30 min at room temperature. Immunostaining was visualized with diaminobenzidine, and the sections were counterstained with hematoxylin (5).

In vivo differentiation of iPSCs and histological analysis

Animal protocols were approved by the Local University Hospital Council on Animal Care and Experimentation. iPS cells colonies were grown on Matrigel (4, 5) and harvested with collagenase IV. iPS cell suspensions were injected into the testicular capsule and subcutaneously into flank of immunodeficient mice (NOD/SCID IL2Rγ$^{-/-}$) (5, 13, 14). After 4 weeks, teratomas were extracted, fixed, embedded in paraffin (5, 14), cut into 3 μm sections, and stained with hematoxylin/eosin. In addition, we used immunohistochemistry and the following antibodies: anti-smooth muscle α-actin (Chemicon, 1:50), anti-pancreatic cytokeratin (Dako, 1:100), and anti-βIII-tubulin (Chemicon, 1:100). As above, slides were incubated with a biotinylated secondary antibody for 30 min at room temperature, and signal was detected with streptavidin-peroxidase complex for 30 min at room temperature (5).

Karyotype analysis

G-banding karyotype. Karyotyping was performed as described (5). Briefly, the cells were treated with 0.1 mg/ml colcemid (Biological Industries) for 3 h, washed in PBS, trypsinized, and pelleted. Cells were resuspended in a KCl hypotonic solution (0.075 mol/l), rinsed to remove cytoplasm, and fixed in Carnoy's solution (methanol/glacial acetic acid 3:1) three consecutive times. Finally, the pellet was resuspended in a final volume of 1 ml of Carnoy's solution and dropped on glass slides. Chromosomes were visualized with modified Wright's staining solution. At least 20 metaphases were analyzed with a conventional microscope (Leica DM 5500B). Karyotype analysis was performed with IKAROS version 5.1 software designed for chromosome analysis (Metasystems, Altlussheim, Germany).

Spectral karyotyping (SKY-FISH). Traditional harvesting and dropping techniques were implemented using a 0.06 M KCl hypotonic solution and Carnoy's 3:1 methanol:glacial acetic acid fixative. A SkyPaint kit (Applied Spectral Imaging) was used to hybridize SKY probes. For SKY analysis, fluorescently labeled chromosomes were imaged with the SpectraCube imaging

system (Applied Spectral Imaging) mounted on a Leica DM 5500 microscope using a custom-designed optical filter. Twenty metaphase chrmosomes were analyzed for each sample. SKY is limited in breakpoint determination and in identifying intrachromosomal changes, such as duplications, deletions, and inversions. As a result, breakpoints on the SKY-painted chromosomes were determined by comparison with the G-banding karyotype.

Isolation of genomic DNA from hES cells after L1 retrotransposition assay

Cells were harvested, and genomic DNA was isolated with DNeasy kits (Qiagen). PCRs were performed with AccuPrime Taq SuperMix (Invitrogen) with an initial step at 94°C for 3 min and then 30 amplification cycles (94°C for 30 sec, 61°C for 30 sec, and 72°C for 1 min/kb). Primers are listed in Table S2.

Insertion characterization by inverse PCR (iPCR)

iPCR was carried out as described (4, 15). Briefly, genomic DNA was isolated from clonal iAND-4 iPSC lines using a DNeasy kit (Qiagen); 2 μg of DNA were digested overnight with a molar excess of *Xba* I, *Ssp* I or *Bgl* II (NEB). Digested DNAs were ligated at 16°C for 14 h in a final volume of 1000 μl using T4-DNA ligase (NEB). Ligated DNA was precipitated and dissolved in 20 μl of purified deionized water (Gibco); 3–6 μl of ligated DNA were used to set up a PCR reaction with primers Neo 210as and Neo 1700s (listed in Table S2) using Expand Long Template Taq (Roche) and the following conditions: initial step 94°C for 5 min, 30 amplification cycles (94°C for 15 sec, 64°C for 30 sec, 68°C for 15 min), and final extension at 72°C for 15 min. Five microliters of the PCR reaction product were used as a template in a second PCR with primers Neo 173as and Neo 1808s under the same cycling conditions. Amplified products were resolved in 0.8% agarose gels and bands excised, purified using a QiaQuick kit (Qiagen), and cloned in Topo-XL (Invitrogen). Independent colonies were sequenced, and BLAT was used to

map the insertion site in the human genome (16). Conventional long-range PCR and sequencing were used when iPCR clones did not contain the 5´ end of the insertion.

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Characterization of the iPS iAND-4 cell line. **(A)** Structure of the lentiviral construct ThOKSIM (17) used to reprogram human foreskin fibroblasts. The human EF1α promoter drives the expression of a polycistronic cassette containing the cDNA sequence of the human genes Oct4, Klf4, Sox2 (separated by a self-cleaving 2A element), and c-Myc (separated by an internal ribosome entry site (IRES) sequence). LTR, long terminal repeats. **(B)** Light microscopy images of the parental HDF cells and the iPS cell line iAND-4 (grown on feeder cells). Magnification 10x. **(C** and **D)** Expression of pluripotent markers in iAND-4 iPS cells. (C) Immunofluorescence of iAND-4 cells stained with antibodies for Oct4 (green), SSEA4 (red), TRA1-60 (green), and TRA1-81 (green); nuclei were stained with DAPI (blue). All images were captured with an inverted microscope at a 10x magnification. (D) RT-PCR analysis of *Nanog*, *Oct4*, *Rex1,* and *Sox2* expression in iAND-4 cells. A reaction without reverse transcriptase served as a negative control (RT– lanes). **(E)** Lentiviral insertion silencing in iAND-4 cells. RT-PCR experiments show incomplete silencing of the lentiviral polycistron in iAND-4 cells (Oct4-Klf4 lanes). RT-PCR reactions were conducted with 25 (left side) or 30 cycles (right side) of elongation. Sox2 served as a positive control; the ThOKSIM plasmid served as a second positive control. MW, molecular weight markers. **(F–I**) Karyotype analysis of parental HDF and iPS-iAND-4 cells revealed an aneuploid karyotype (46,XY,+der(1),t(1;17)), resulting in partial trisomy of chromosome 1, which is not present in the parental HDFs. Thus, this abnormality was acquired during or after the reprogramming. In addition, the iPS iAND-4 cell line is mosaic for trisomy of chromosome 5 (I), with 50% of the cells harboring this aneuploidy**.** (F) Representative G-banding karyotype (at least 20 metaphases were scored) of the parental HDFs and the iAND-4 cells at passage 10 (G). (H) Representative spectral karyotyping (SKY)–fluorescence *in situ* hybridization (FISH) analysis of iAND-4 iPS cells at passage 16. Shown are the DAPI-banded chromosomes (left) next to their false-colored chromosome (right), confirming the chromosome 17 to 1 translocation and the trisomy 5. (I) Two representative G-banding karyograms of the iPS cell line iAND-4 at

passage 16 (at least 20 metaphases were scored) indicating the presence of a mosaic trisomy 5.

Figure S2. iAND-4 cells differentiate into the three major germ layers. To determine whether iAND-4 cells can differentiate into the three major embryonic germ layers, we conducted an *in vitro* EB assay and an *in vivo* teratoma differentiation assay (3). We analyzed the expression of endoderm (PanCK, pancreatic cytokeratin), mesoderm (α-actin, alpha smooth muscle actin) and ectoderm (βIII-tubulin *in vivo* and GFAP, glial fibrillary acidic protein *in vitro*) markers in sections from teratomas (left column) or EBs (right column). Nuclei were stained with hematoxylin. Magnification, 20x.

Figure S3. Karyotype analysis of MSUH001 iPSCs (17) revealed a normal euploid karyotype. (46,XX). Shown is a representative G-banding karyotype (at least 20 metaphases were scored) of MSUH001 iPSCs.

Figure S4. Analysis of CpG methylation of the L1 5'UTR promoter in several HDFs and pluripotent cell lines. Analysis of five HDF lines, three iPS cell lines, and two hES cell lines revealed significant hypomethylation in the L1 5'UTR promoter region in pluripotent cells. Overall, a higher percentage of methylation in the 5' region of the studied CpG island was observed. The greatest differences between somatic and pluripotent cells were in the 3' region of the CpG island.

Figure S5. Verification of the *pLRE3-EF1-mEGFPI* L1 reporter in hESCs. **(A)** L1 retrotransposition in H9 hESCs is inhibited by treatment with the reverse transcriptase inhibitor lamivudine (3TC). The frequency of new retrotransposition events yielding $EGFP^+$ cells was analyzed by flow cytometry; on average, ~10^6 cells/sample were analyzed. **(B)** Schematic of the *pLRE3-EF1-mEGFP(Δintron)* control plasmid and the *pLRE3-EF1-mEGFPI* reporter construct. The control plasmid is identical to the L1 reporter construct except that it lacks an intron in the

mEGFPI gene, thereby allowing EGFP expression in the absence of splicing, reverse transcription, and integration. **(C)** L1 *retrotransposition events are* stably integrated into the genome of hESCs. Genomic DNA isolated from EGFP-positive colonies after nucleofection served as a PCR template. The 343-bp PCR product indicates the spliced tagged L1 (insertion); the 1243-bp product contains the intron (vector). **(D)** Cells were cultured in the presence or absence of 100 μM 3TC 5 h before nucleofection and subsequently postnucleofection. Two days (*pLRE3-EF1-mEGFP(Δintron)*) or 4 days (*pLRE3-EF1-mEGFPI*) after nucleofection, the frequency of EGFP-positive cells was analyzed by flow cytometry; on average, ~1 x 10^6 cells per sample were analyzed. Results were normalized to those in untreated controls.

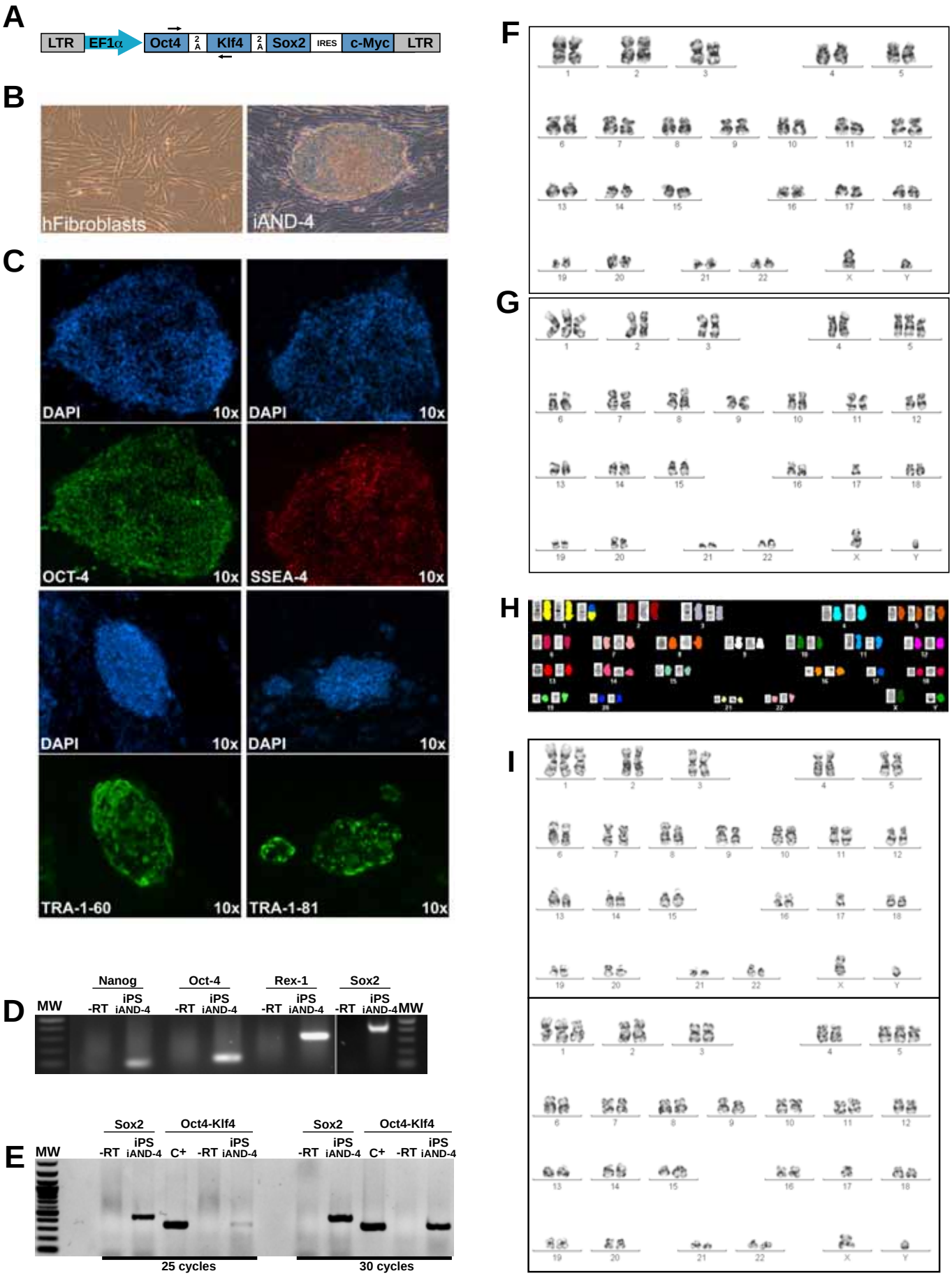
Figure S6. Characterization of L1 retrotransposition events in iAND-4 iPSCs. **(A)** Scheme of the retrotransposition assay as described (18). The schematic shows plasmid *pKUB102/L1.3-sv+* (see Methods), where the expression of a L1.3 element (lacking its 5´UTR) is driven by an exogenous human UBC promoter and contains an *mneoI* retrotransposition indicator cassette. This arrangement ensures that the NEO-phosphotransferase gene (*NEO-PT*) is transcribed only after successful retrotransposition. The engineered L1 plasmid was transfected into iAND-4 iPSCs, and cells were treated with G418 to select for retrotransposition events. **(B–D)** Characterization of the insertion site in clonal cell lines iAND4-2 (B), iAND4-6 (C), and iAND4-7 (D). The chromosomal location of each insertion is indicated, as are insertions into known genes. Each panel displays a scheme of the retrotransposed L1 element (indicated below each cartoon). Blue arrowheads indicate target site duplications (TSD) flanking the insertion, and the length of the poly (A) tail is indicated. Numbering indicates the truncation site in L1, and is based on the L1.3 reference sequence (L19088.1) (9). In each insertion, the sequences of the pre- and post-integration sites are indicated. The L1 endonuclease bottom-strand cleavage site is shown in red text.

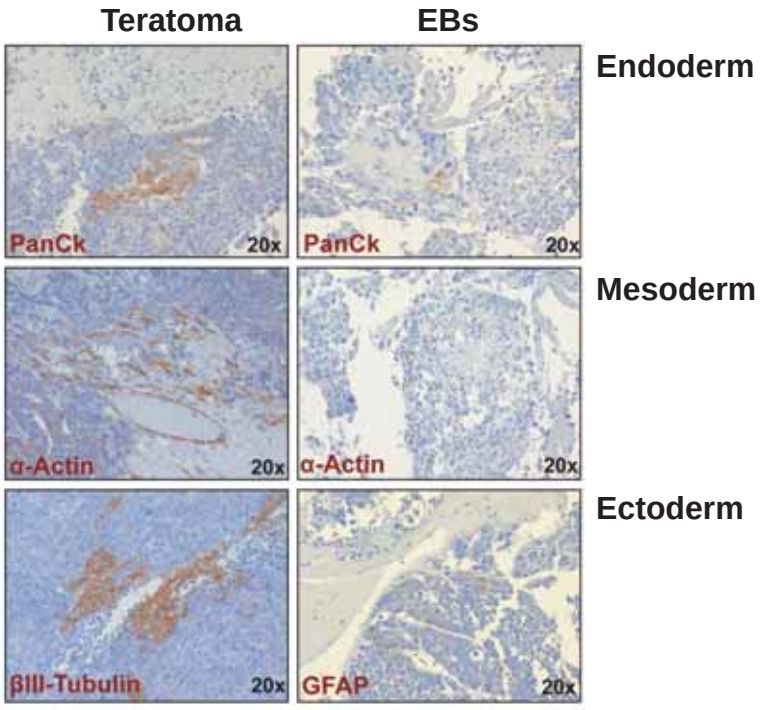
Table S1: Cell lines used in this study

	Name	Karyotype	Description
Fibroblast lines	**HDF-F**	46X,del(x)(q24)	adult human dermal fibroblasts obtained by skin biopsy
	HDF-8.11	46,XY	adult human dermal fibroblasts obtained by skin biopsy
	iAND-4 HDF	46,XY	neonatal foreskin fibroblasts (ATCC, CRL-2429)
	HDF 2134	46,XY	adult human dermal fibroblasts (Cell Application, Inc.)
	HDF neo.	46,XY	neonatal foreskin fibroblasts (Invitrogen)
iPS cell lines	**iPS-F**	46X,del(x)(q24)	iPSCs generated by reprogramming of HDF-F cells with retroviruses expressing *Sox2*, *Oct3/4*, *Klf4*, and *c-Myc*
	iPS-8.11	46,XY	iPSCs generated by reprogramming HDF-8.11 cells with retroviruses expressing *Sox2*, *Oct3/4*, *Klf4*, and *c-Myc*.
	iAND-4 iPS	46,XY,+der(1),t(1;17)	iPSCs generated by reprogramming newborn foreskin fibroblasts (ATCC, CRL-2429) with a polycistronic lentiviral vector expressing *Sox2*, *Oct3/4*, *Klf4*, and *c-Myc*.
	MSUH001-iPS	46,XX	iPSCs generated by reprogramming of adult human dermal fibroblasts with a polycistronic lentiviral vector expressing *Sox2*, *Oct3/4*, *Klf4*, and *Lin28*. (17)
hES cell lines	**H9**	46,XX	human embryonic stem cell lines, WiCell (WA09)
	H13	46,XY	human embryonic stem cell lines, WiCell H13.B. (WA13)

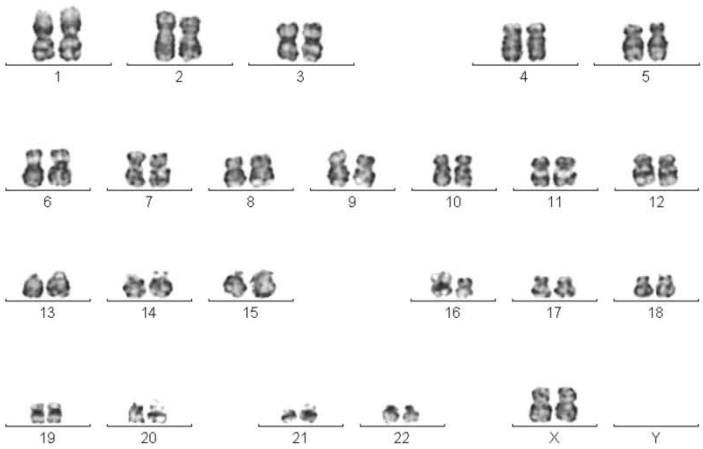
Table S2: Primer and Probes used in this study

L1 Northern probe 5'UTR forward	5'-CAGTGAATTACAAGATGGCCGAATAGGAACAGC-3'	
L1 Northern probe 5'UTR reverse	5'-CAGTGGATCCGTTTGATCTCAGACTGCTGTG-3'	
L1 RT primer	5'-ACATGTGCACATTGTGCAGGTTAG-3'	
L1 5'UTR forward	5'-ACGGAATCTCGCTGATTGCTA-3'	
L1 5'UTR reverse	5'-AAGCAAGCCTGGGCAATG-3'	
L1 5'UTR probe	5'- FAM-AAACTGCAAGGCGGCAA-MGB-3'	
L1 ORF1 forward	5'-TCAAAGGAAAGCCCATCAGACTA-3'	
L1 ORF1 reverse	5'-TTGGCCCCCACTCTCTTCT-3'	
L1 ORF1 probe	5'- FAM-CAGCGGATCTCTCGG-MGB-3'	
LINE-1 ORF2 forward	5'-GAGAGGATGCGGAGAAATAGGA-3'	(19)
LINE-1 ORF2 reverse	5'-GGATGGCTGGGTCAAATGGT-3'	
LINE-1 ORF2 probe	5'-FAM-CAACCATTGTGGAAGTCAGTGTGGCG-TAMRA-3'	
eGFP968 forward	5'-GCACCATCTTCTTCAAGGACGAC-3'	(20)
eGFP1013 reverse	5'-TCTTTGCTCAGGGCGGACTG-3'	
Neo 1808 s	5'-CATTGAACAAGATGGATTGCACGC-3'	(18)
Neo 437 as	5'-CAGCCCCTGATGCTCTTCGTCC-3'	
Oct3/4 forward	5'-TCTGCAGAAAGAACTCGAGCAA-3'	
Oct3/4 reverse	5'-AGATGGTCGTTTGGCTGAACAC-3'	
Nanog forward	5'-TGCAGTTCCAGCCAAATTCTC-3'	
Nanog reverse	5'-CCTAGTGGTCTGCTGTATTACATTAAGG-3'	
Sox2 forward	5'-CCCCCGGCGGCAATAGCA-3'	
Sox2 reverse	5'-TCGGCGCCGGGGAGATACAT	
Rex-1 forward	5'-CAGATCCTAAACAGCTCGCAGAAT-3'	
Rex-1 reverse	5'-GCGTACGCAAATTAAAGTCCAGA-3'	
Neo 210as	5'- GACCGCTTCCTCGTGCTTTACG -3'	(4, 15)
Neo 1700s	5'- TGCGCTGACAGCCGGAACACG -3'	
Neo 173as	5'- CATCGCCTTCTATCGCCTTCTTG -3'	
ORF1-851as	5'- GCTGGATATGAAATTCTGGGTTGA -3'	(4)
ORF1615s	5'- AGGAAATACAGAGAACGCCACAA -3'	
Oct3/4 Fw polycistron	5'-GCCTTTCCCCCTGTCTCCGTC-3'	
Klf4 Rv polycistron	5'-GGCTCCGCCGCTCTCCAGGTCTG-3'	
L1-ORF1-2072as	5'-GTCTTGACTCTTTATCCAACTTGC-3'	
L1-5'UTR-16s	5'-GATGGCCGAATAGGAACAGCT-3'	
CpG-L1.2 (AC217064.2)s	5'-GGTTTTTGTTGTTGTATTTGTGTGT-3'	
CpG-AL512428s	5'-AAATTAGTTGGGTATGATGGTAGGT-3'	
CpG-AL137845s	5'-GGAAATTGAATAATTTGTTTTTGAAT-3'	
CpG L1-HS reverse	5'-CAAAAACCCACTTAAAAAAACAATC-3'	
CpG analysis For	5'-AAGGGGTTAGGGAGTTTTTTT-3'	(7)
CpG analysis Rev	5'-TATCTATACCCTACCCCCAAAA-3'	

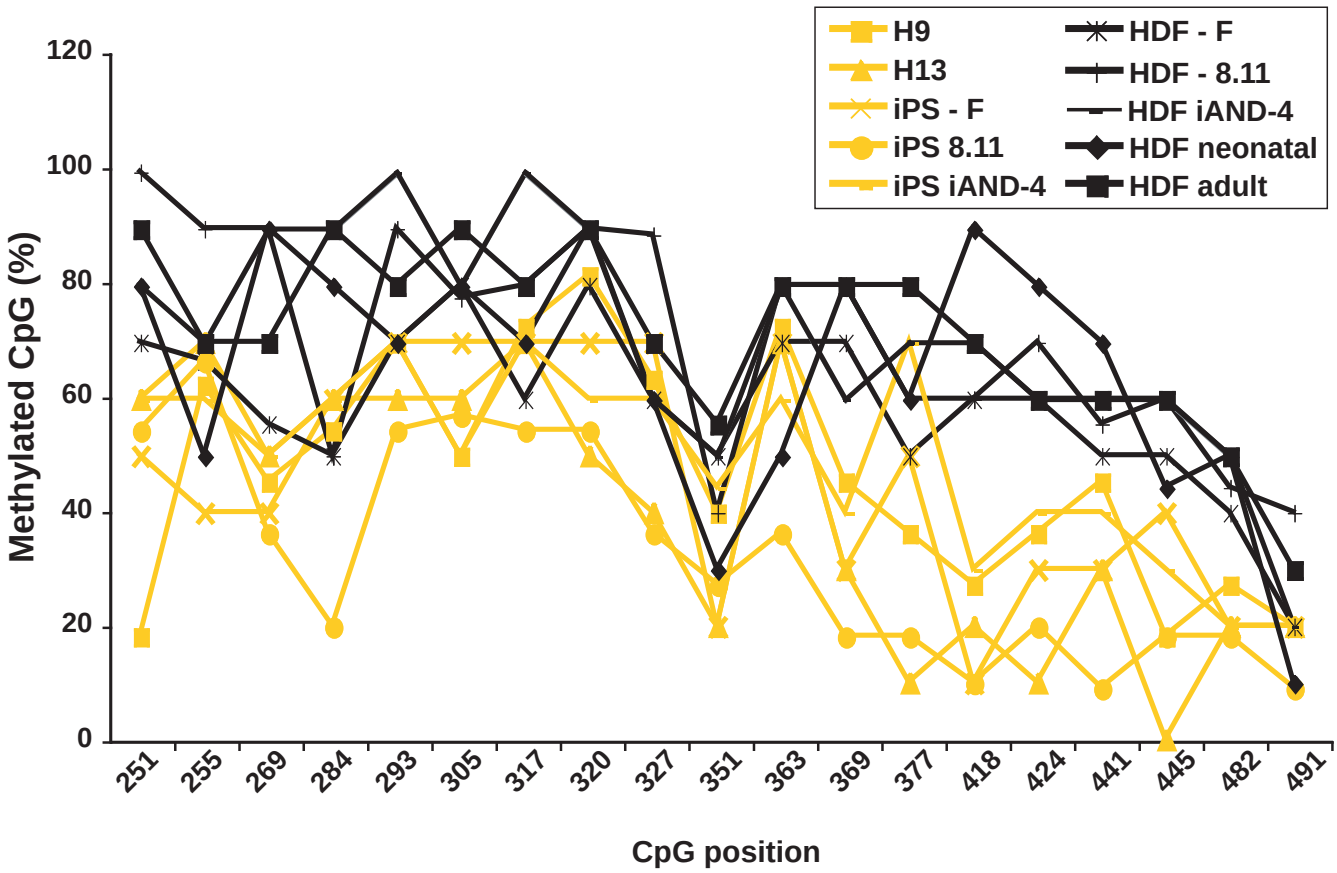

A
LTR
EF1α
Oct4
2A
Klf4
2A
Sox2
IRES
c-Myc
LTR
B
hFibroblasts
iAND-4
C
DAPI 10x
DAPI 10x
OCT-4 10x
SSEA-4 10x
DAPI 10x
DAPI 10x
TRA-1-60 10x
TRA-1-81 10x
D
MW
Nanog
Oct-4
Rex-1
Sox2
-RT
iPS iAND-4
MW
E
MW
Sox2
Oct4-Klf4
-RT
iPS iAND-4
C+
25 cycles
30 cycles
F
G
H
I

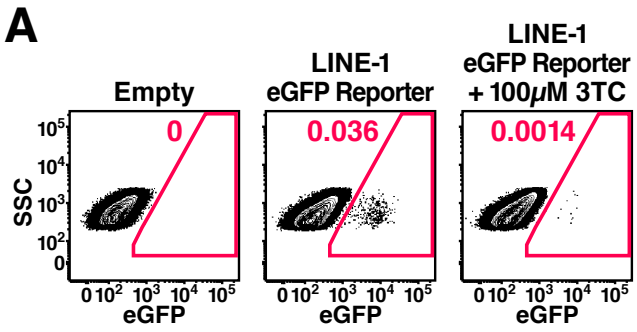

Teratoma
EBs
Endoderm
Mesoderm
Ectoderm
PanCk
20x
PanCk
20x
α-Actin
20x
α-Actin
20x
βIII-Tubulin
20x
GFAP
20x

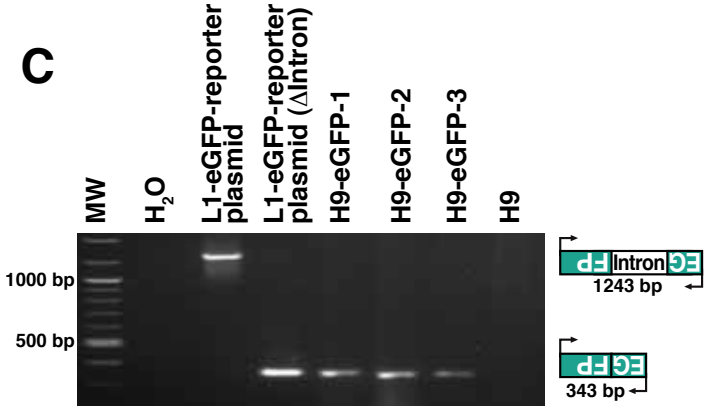
Wissing et al, Figure S3

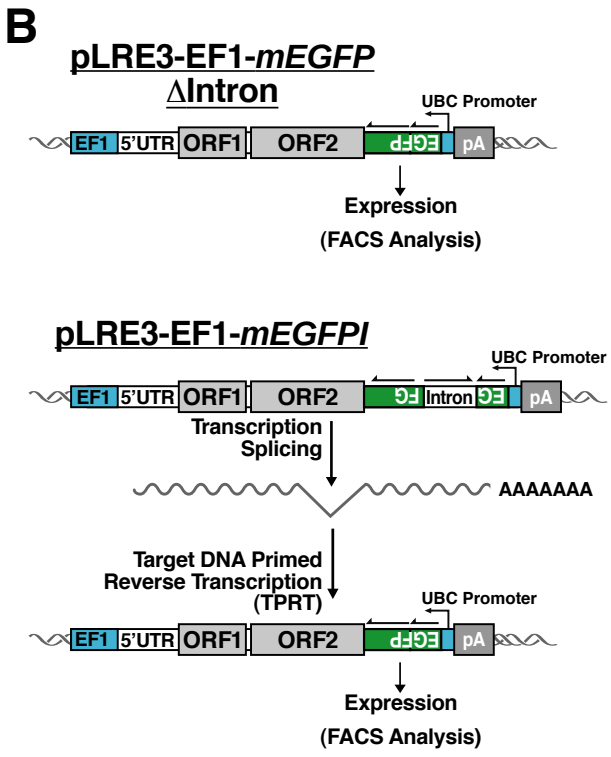


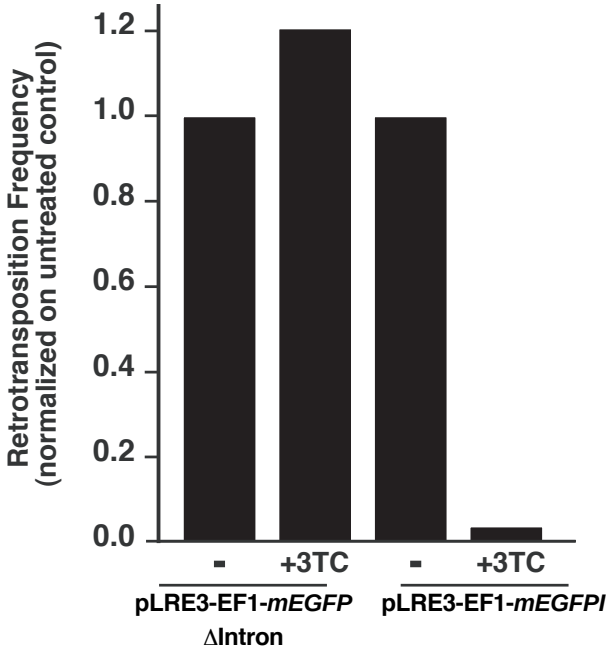
Wissing et al, Figure S4



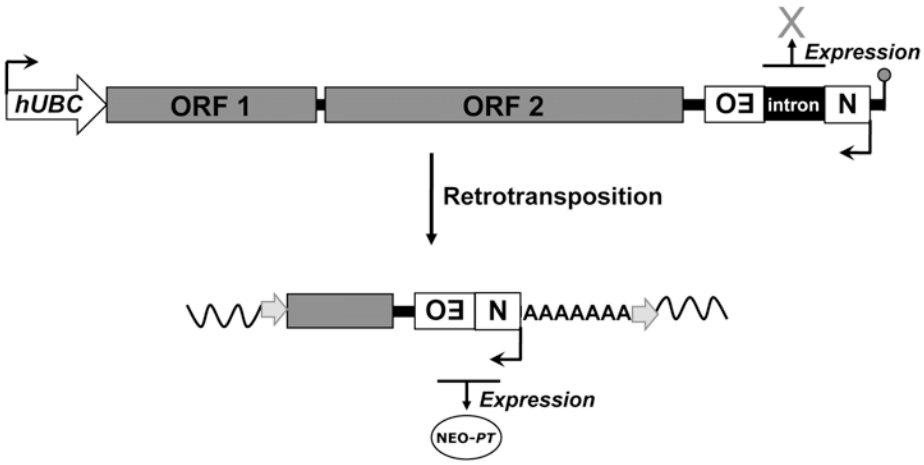

A
Empty
LINE-1
eGFP Reporter
LINE-1
eGFP Reporter
+ 100μM 3TC
0
0.036
0.0014
SSC
eGFP


C
MW
H2O
L1-eGFP-reporter plasmid
L1-eGFP-reporter plasmid (ΔIntron)
H9-eGFP-1
H9-eGFP-2
H9-eGFP-3
H9
1000 bp
500 bp
Intron
1243 bp
343 bp

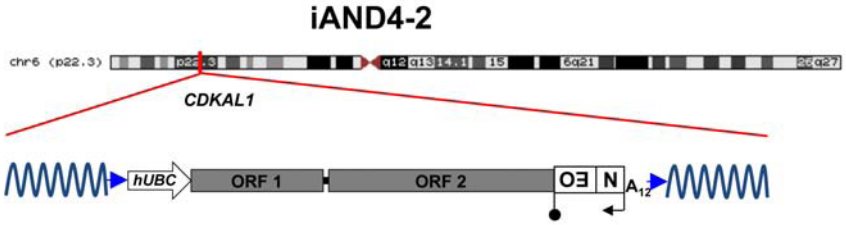

B
pLRE3-EF1-mEGFP
ΔIntron
UBC Promoter
EF1
5'UTR
ORF1
ORF2
pA
Expression
(FACS Analysis)
pLRE3-EF1-mEGFPI
Intron
Transcription
Splicing
AAAAAAA
Target DNA Primed
Reverse Transcription
(TPRT)


D
Retrotransposition Frequency
(normalized on untreated control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-
+3TC
-
+3TC
pLRE3-EF1-mEGFP
ΔIntron
pLRE3-EF1-mEGFPI

A



B



Pre-Integration Site:

```
Chr6 (20782135)  5'.....TTAGGTGCTC CCTGTGATTT AAGAAACAGA AGCCCTTCAT CTCATCAAAT .....3'
                 3'.....AATCCACGAG GGACACTAAA TTCTTTGTCT TCGGGAAGTA GAGTAGTTTA .....5'
```

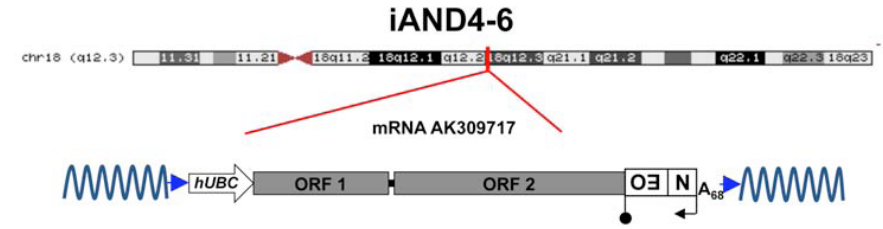
Post-Integration Site:

```
Chr6 (20782135)  5'.....TTAGGTGCTCCCTGTGATTTAAGAAACAGA (L1-NEO)A12 GAAACAGAAGCCCTTCATCTCATCAAAT .....3'
                 3'.....AATCCACGAGGGACACTAAATTCTTTGTCT (L1-NEO)T12 CTTTGTCTTCGGGAAGTAGAGTAGTTTA .....5'
```

8bp TSD; EN cleavage: 5' TTTC/T

Within Intron 6 *CDKAL1* (CDK5 regulatory subunit associated protein).

C



Pre-Integration Site:

```
Chr18 (37472029) 5'..... ATTAAGCAGAGGTTATCTAACATCATATTTAAAAAGAAAGCATATGTATT .....3'
                 3'..... TAATTCGTCTCCAATAGATTGTAGTATAAATTTTTCTTTCGTATACATAA .....5'
```

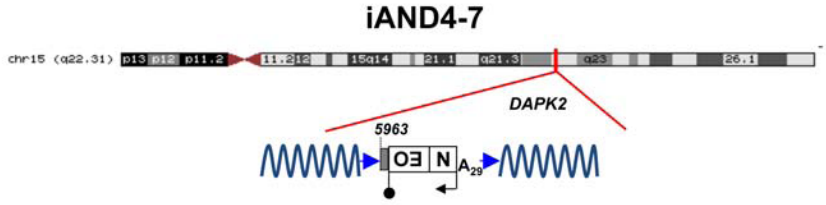
Post-Integration Site:

```
Chr18 (37472029) 5'..... ATTAAGCAGAGGTTATCTAACATCATATTTAAAAAGAAAGCATA (L1-NEO)A68 AAAAAGAAAGCATATGTATT .....3'
                 3'..... TAATTCGTCTCCAATAGATTGTAGTATAAATTTTTCTTTCGTAT (L1-NEO)T68 TTTTTCTTTCGTATACATAA .....5'
```

14 bp TSD; EN cleavage: 5'TTTT/A

Within human mRNA AK309717 (FLJ99758).

D



Pre-Integration Site:

```
Chr15 (64272957) 5'..... ATACGGCCGCATTGGGAATTAAATTTCAATGCGAGTTTGTAGGGCACAAA .....3'
                 3'..... TATGCCGGCGTAACCCTTAATTTAAAGTTACGCTCAAACATCCCGTGTTT .....5'
```

Post-Integration Site:

```
Chr15 (64272957) 5'..... ATACGGCCGCATTGGGAATTAAATTTCAATGCGA (L1-NEO)A29  AAATTTCAATGCGAGTTTGTAGGGCACAAA .....3'
                 3'..... TATGCCGGCGTAACCCTTAAAATTTCAATGCGA (L1-NEO)T29  TTTAAAGTTACGCTCAAACATCCCGTGTTT .....5'
```

14 bp TSD; EN cleavage: 5'ATTT/A

Within Intron 2 *DAPK2* (death-associated kinase 2).