A reporter model to visualize imprinting stability at the *Dlk1* locus during mouse development and in pluripotent cells

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Summary statement

Genomic imprinting is a paradigmatic epigenetic process that is central to mammalian development. Here, we report a mouse model to define and prospectively isolate cells based on Dlk1 imprinting status.
Abstract
Genomic imprinting results in the monoallelic expression of genes that encode important regulators of growth and proliferation. Dysregulation of imprinted genes, such as those within the Dlk1-Dio3 locus, is associated with developmental syndromes and specific diseases. Our ability to interrogate causes of imprinting instability has been hindered by the absence of suitable model systems. Here, we describe a Dlk1 knockin reporter mouse that enables single-cell visualization of allele-specific expression and prospective isolation of cells, simultaneously. We show that this “imprinting reporter mouse” can be used to detect tissue-specific Dlk1 expression patterns in developing embryos. We also apply this system to pluripotent cell culture and demonstrate that it faithfully indicates DNA methylation changes induced upon cellular reprogramming. Finally, the reporter system reveals a role of elevated oxygen levels in eroding imprinted Dlk1 expression during prolonged culture and in vitro differentiation. The possibility to study allele-specific expression in different contexts makes our reporter system a useful tool to dissect the regulation of genomic imprinting in normal development and disease.
INTRODUCTION
More than 100 mammalian genes are expressed in predominantly monoallelic fashion, a paradigmatic epigenetic event referred to as genomic imprinting (Bartolomei and Ferguson-Smith, 2011). Imprinted genes, such as those within the commonly studied Dlk1-Dio3 gene cluster, are regulated by gender-specific DNA methylation marks at imprinting control regions (ICRs) (Duffie and Bourc'his, 2013; Sanli and Feil, 2015). Failure to preserve allele-specific imprinted gene expression, such as by the acquisition of hypermethylation at the ICR (referred to as loss-of-imprinting or LOI), can have detrimental developmental consequences and is a hallmark of cancer (Peters, 2014). The factors that contribute to the epigenetic instability of imprinted genes remain largely elusive, partly due to the absence of suitable model systems. At present, imprinting is typically studied by assessing DNA methylation levels or nucleotide polymorphisms in imprinted transcripts. These tools, however, are restricted to either bulk populations (Babak et al., 2015; Hammoud et al., 2013) and/or retrospective analysis (Ginart et al., 2016).

Dlk1 is a paternally-expressed protein coding gene within Dlk1-Dio3 (da Rocha et al., 2008) that regulates fetal growth. We and others have previously shown that the ICR controlling Dlk1-Dio3, called the intergenic differentially methylated region (IG-DMR), frequently becomes hypermethylated in a context-dependent manner during in vitro reprogramming of somatic cells (Carey et al., 2011; Stadtfeld et al., 2010). This yields induced pluripotent stem cells (iPSCs) with LOI and upregulation of Dlk1 that is indicative of expression from both alleles without paternal bias (Stadtfeld et al., 2010). Together, these observations suggested that the insertion of fluorescent reporter genes into the endogenous Dlk1 locus would enable us to develop novel approaches to study imprinted gene expression in living cells and tissues.

RESULTS AND DISCUSSION
A reporter model for allele-specific expression of Dlk1
To generate a mouse reporter system for imprinted Dlk1 expression, we inserted the coding sequence for the green/yellow fluorescent protein, Venus, or the red fluorescent protein, tdTomato (Tomato), into the three prime untranslated region (3'UTR) of
endogenous *Dlk1* (Fig. 1A). In accordance with widespread *Dlk1* expression at perinatal stages (da Rocha et al., 2007), embryonic day 16.5 (E16.5) mice that had inherited reporter alleles from the father exhibited strong fluorescence (Fig. 1B). Reporter gene expression from the maternal allele was greatly diminished but detectable above background levels (Fig. 1B). The brightness of the paternal reporter allele allowed direct visualization of *Dlk1* expression in living pups. This revealed expression in the growing limbs and the trunk until postnatal day 2, when it rapidly declined and became nearly undetectable by day 4 (Fig. 1C). These findings are in accordance with previous studies (Lui et al., 2008), indicating that our reporter model recapitulates expression hallmarks of *Dlk1*.

**Identification of organ-specific expression patterns at single-cell level**

We next sought to test whether the dual-reporter system could provide insight into allele-specific *Dlk1* expression during development. Therefore, we analyzed E16.5 lung, skin and liver, representing tissues for which a developmental function of *Dlk1* has been reported (Driskell et al., 2013; Tanimizu et al., 2003; Weng et al., 2009; Wu et al., 2008). Flow cytometric analysis revealed strong paternal reporter expression and detectable levels of maternal expression in some cells (Fig. 2A). The number of such bi-allelic cells ranged from rare (~4% of Dlk1⁺ cells) in lung to predominant (>95%) in liver (Fig. 2A,B). Importantly, the reporter insertions did not alter the expression levels of *Dlk1* or the reciprocally expressed *Gtl2* gene in the tissues analyzed (Fig. S1). Of note, while the reporter alleles revealed relatively homogenous expression profiles in liver and lung, two distinct populations could be defined based on allele-specific *Dlk1* expression in skin: one with paternal and one with bi-allelic expression (Fig. 2A). This expression dichotomy has not previously been identified (Driskell et al., 2013) and suggests that the dual-reporter could aid in the identification of novel cell populations. In addition, comparison of reporter activity in B6 background and B6x129 F1 mice revealed similar expression patterns but subtle differences in the abundance of expressing cells, suggesting the model might be useful to study strain-specific aspects of *Dlk1* regulation (Fig. S2).
As expected, we did not detect cells expressing only maternal $Dlk1$ within the analyzed tissues (Fig. 2A) and the intensity of the maternal reporter in bi-allelic cells remained comparatively weak in both reporter configurations (Fig. 2C; Fig. S3). Quantification of fluorescent intensity suggested that maternal expression levels of the skin and liver range between 7-14% of total $Dlk1$ expression within the bi-allelic cell populations (Fig. 2D). This observation suggests relaxation of imprinting (ROI), defined by incomplete silencing of the maternal allele, rather than LOI, as the paternal expression bias is still apparent. In support of this conclusion, we did not detect elevated levels of DNA methylation at the IG-DMR in skin or liver cells isolated based on maternal $Dlk1$ expression (Fig. 2E). Overall, the pattern of $Dlk1$ reporter activity is consistent with studies that report maternal expression in liver but fail to detect it in lung (da Rocha et al., 2007; Sato et al., 2011). Analysis of allele-specific $Dlk1$ expression by quantitative PCR in whole tissue confirmed that the activity of the maternal allele is not altered by the reporter insertion (Fig. S4). These results suggest that the $Dlk1$ reporter model allows sensitive and reliable detection of tissue-specific expression patterns.

Detection of loss-of-imprinting upon cellular reprogramming

Next, we set out to test whether the reporter system can faithfully detect instances of bona fide LOI. For this, we took advantage of the observation that reprogramming of murine fibroblasts by the transcription factors, Oct4, Klf4, Sox2 and Myc (OKSM), frequently yields induced pluripotent stem cells (iPSCs) with DNA hypermethylation of the IG-DMR and upregulation of $Dlk1$ (Liu et al., 2010; Stadtfeld et al., 2010). This abnormality results in reduced developmental potential but can be prevented by addition of ascorbic acid (AA) to the reprogramming media (Stadtfeld et al., 2012). We therefore anticipated that reprogramming of $Dlk1$ reporter fibroblasts in basal conditions would predominantly yield cells with equal paternal and maternal expression, while reprogramming in presence of AA would prevent the occurrence of such cells (Fig. 3A).

In agreement with low levels of $Dlk1$ transcription in pluripotent cells (Kota et al., 2014), no reporter gene expression could be detected in naïve iPSCs. However, exposure to the differentiation-inducing agent retinoic acid (RA) yielded cells with readily detectable reporter gene fluorescence. As shown in Fig. 3B, cells derived in basal
conditions almost exclusively expressed both reporter alleles, while monoallelic fluorescence was prevalent in cells obtained in presence of AA. In contrast to our observations in the embryo, paternal and maternal expression levels in cells generated in basal reprogramming conditions were indistinguishable (compare Fig. 2C and Fig. 3C). As this is consistent with imprinting loss, we will refer to such cells as “LOI” cells, while we call those with paternal-only or bi-allelic with low maternal expression “MOI” cells (see Fig. 3B). In agreement with this categorization, iPSCs with high-degree of LOI expression showed strongly elevated levels of DNA methylation at the IG-DMR (Fig. 3E; Fig. S5).

These observations suggest that the \textit{Dlk1} reporters accurately reflect changes in imprinting status during reprogramming. We also evaluated \textit{Dlk1} imprinting in iPSCs derived by combined modulation of TGF\textbeta and Wnt signaling in presence of AA (referred to as “3c” conditions) (Vidal et al., 2014), which increases reprogramming efficiencies 10-20 fold (Fig. 3D). We found that differentiation of iPSCs derived in 3c mirrored the results obtained in AA conditions, with the majority of cells exhibiting normal DNA methylation levels and MOI expression of \textit{Dlk1} (Fig. 3E; Fig. S5; Fig. S6). This suggests that this highly efficient reprogramming condition might provide a tractable method to study factors that contribute to imprinting maintenance.

**Modulation of allele-specific \textit{Dlk1} expression by oxygen levels during iPSC culture and differentiation**

Despite the striking difference in maternal reporter expression between cells derived in basal and 3c conditions, we noticed that differentiation of AA and 3c iPSCs frequently yielded a small subset of LOI cells (Fig. 3B). Such cells were rare when differentiating freshly generated iPSCs but became more frequent with iPSCs at higher passage, along with a number of cells with maternal-only expression (Fig. 4A). All \textit{in vitro} experiments described thus far were conducted in standard culture conditions, including 20% oxygen levels. Due to the reported impact of molecular oxygen on epigenetic processes in pluripotent cells, including imprinting (Xie et al., 2014) and X chromosome inactivation (Lengner et al., 2010), we tested the impact of oxygen concentration on allele-specific \textit{Dlk1} expression. Taking advantage of the 3c system, which enables
analysis at earlier passage, we compared iPSCs that were expanded and differentiated in atmospheric (20%) and physiological (4%) oxygen levels. We observed no significant difference with early passage iPSCs, but a gradual increase in cells with LOI and maternal-only reporter expression in 20% oxygen conditions at higher passage (Fig. 4B,C). In contrast, predominantly MOI expression was maintained in 4% oxygen (Fig. 4B,C). When cells expanded in 20% oxygen were differentiated in 4% oxygen, the frequency of cells with LOI and maternal-only expression remained low, while their numbers strongly increased in the reverse conditions (expansion in 4%, followed by differentiation in 20% oxygen) (Fig. 4C; Fig. S7). This indicates an unexpected susceptibility of the maternal Dlk1 allele to become reactivated upon prolonged expansion and differentiation of iPSCs at elevated oxygen levels. Due to the fact that imprint dysregulation has been recognized as a concern for the quality of pluripotent cell lines (Greenberg and Bourc'his, 2015), we anticipate that the Dlk1 reporter system will be useful in optimizing stem cell derivation and culture conditions.

In summary, we have developed a reporter model that serves as a sensitive indicator for allele-specific expression of Dlk1 and allows prospective isolation of even small subsets of cells with paternal, bi-allelic, LOI or maternal-only expression patterns. Our observations are consistent with tight control of Dlk1 imprinting during early developmental stages in the tissues analyzed, but suggest frequent erosion during in vitro manipulations. The system described here should therefore provide novel means to systematically study Dlk1-Dio3 regulation in diseased tissues, pluripotent cell culture and during development (Fig. 4E). This should aid in refining our understanding of the molecular processes involved in imprint establishment and maintenance.
MATERIALS AND METHODS

Transgenic mice
The generation of Dlk1 reporter mice is described in the Supplementary Information. Unless otherwise indicated, all animals were on a B6 background. For reprogramming experiments, Dlk1 reporter mice were crossed with animals carrying an inducible OKSM transgene (Stadtfeld et al., 2010). All animal experiments were done in accordance with the guidelines of the NYU School of Medicine IACUC.

Cell culture and reprogramming
Pluripotent cell culture and MEF reprogramming was conducted as previously described procedures (Vidal et al., 2014) (see also Supplementary Information). Cells were reprogrammed for 12 days (basal conditions), 10 days (AA) or 6 days (3c) in the same oxygen condition in which they were later expanded (4% or 20%).

Cell differentiation and flow cytometry
Trypsinized iPSCs were pre-plated for 30 minutes to remove feeder cells and seeded onto gelatinized plates at a density of 30,000 cells/cm². The next day, fibroblast medium with 0.4 μg/ml retinoic acid was added, followed by daily media changes and imaging using a Nikon Eclipse TiE inverted microscope with filters to detect Venus (Ex 500/20; Em 535/30) and Tomato (Ex 545/30; Em 620/60). For quantification of Dlk1 reporter expression, dissociated cultures were acquired on an LSRII cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star Inc.).

Tissue isolation and reporter detection
Mouse embryos isolated at E16.5 were imaged using a Nikon SMZ1500 Stereo Fluorescence Microscope. For flow cytometry, isolated tissues were incubated for 30 minutes at 37° in 0.25% trypsin. During this time, tissues were disassociated using progressively smaller pipet tips (1000 μl – 200 μl), followed by analysis on an LSRII or sorting on a FACSARia (BD Biosciences).
DNA methylation analysis
Genomic DNA was isolated using proteinase K in lysis buffer, pH 8 (100 mM Tris-HCl, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) and reconstituted in TE buffer, pH 7.5 (10 mM Tris-HCl, 1 mM EDTA). DNA pyrosequencing and next generation bisulfite sequencing was conducted using the ADS1452 assay, which covers the IG-DMR (chr12:109,528,253-109,528,471 in mm10) (EpigenDX).

Gene expression analysis
RNA isolated using the miRNeasy kit (Qiagen) was used for cDNA preparation with the Transcriptor HIFI cDNA synthesis kit (Roche). Samples were run on a LightCycler 480 Real-Time PCR System (Roche). See Supplementary Information for primer sequences.

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COMPETING INTERESTS
No competing interests declared.

AUTHOR CONTRIBUTIONS
E.S. and M.S. conceived the study, designed experiments, interpreted results and wrote the manuscript. E.S. conducted all experiments.

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REFERENCES


Fig. 1. Reporter knock-in alleles capture imprinted *Dlk1* expression *in vivo*. (A) Genetic configuration of *Dlk1* reporter mice with coding sequences of Tomato (Tom) and Venus (Ven) inserted into the paternal (Pat) and maternal (Mat) allele of *Dlk1*: *Dlk1*<sup>MatVen/PatTom</sup> (upper panel) or *Dlk1*<sup>MatTom/PatVen</sup> (lower panel). Lollipops indicate methylation status of the IG-DMR and an arrow at exon 1 indicates imprinted expression from the paternal allele. (B) Fluorescent images of E16.5 mouse embryos of the corresponding genotypes. Scale bar = 1mm. (C) Whole body images of *Dlk1*<sup>MatWT/PatTom</sup> mice at postnatal days (P) 0 to 4. Scale bar = 2 mm.
**Fig. 2. Quantification of allele-specific expression in developing tissues.** (A) Flow cytometric analysis of fetal tissues from *Dlk1*\textsuperscript{MatWT/PatVen} (left column) control mice or *Dlk1*\textsuperscript{MatTom/PatVen} (right column) dual-reporter mice at E16.5. (B) Percentage of *Dlk1*\textsuperscript{+} cells with paternal-only and bi-allelic expression in fetal lung, skin and liver. (C) Mean fluorescent intensity of paternal and maternal *Dlk1*\textsuperscript{Tom} in bi-allelic fetal skin and liver cells. (D) Quantification of relative strength of maternal to total *Dlk1* in bi-allelic cells as measured by flow cytometry. Error bars indicate standard error (n=3 embryos). (E) DNA methylation at the IG-DMR in FACS sorted fetal skin and liver cells with active maternal *Dlk1* allele from two embryos. Methylation levels in iPSCs with established LOI or MOI were used as controls. Error bars indicate standard error (28 CpGs analyzed).
Fig. 3. Detection of LOI induced by cellular reprogramming. (A) Transgenic alleles in fibroblasts isolated from reprogrammable imprinting reporter animals. The expected behavior of the reporter genes in cells derived upon reprogramming in basal or AA conditions is illustrated. (B) Representative FACS plots of RA differentiated iPSCs derived via basal reprogramming (left) or in AA conditions (right). Yellow and green gates indicate LOI and MOI expressing cells, respectively (C) Mean fluorescence intensity of Tomato in the indicated genotypes. (D) Reprogramming efficiency measured as percent stable iPSC colonies per input MEFs in basal conditions, AA or 3c. (****) indicates p<0.00005 with a one-way ANOVA and Turkey’s multiple comparison test. Error bars indicate standard error (n=3). (E) Degree of LOI-level Dlk1 expression (upper panel) and DNA methylation at the IG-DMR (lower panel) in iPSC cultures derived in the indicated conditions.
A

Basal  3c (p0)  3c (p5)

Venus (pat)

Tomato (mat)

B

20% oxygen

4% oxygen

LOI in disease models

Characterization of developmental role

Identify regulators

Test culture conditions

E

20% oxygen

4% oxygen

MOI

LOI

Mat

% expression

in vivo

Development • Advance article

% expression

in vitro

Development • Advance article
Fig. 4. Instability of allele-specific expression during culture and *in vitro* differentiation of pluripotent cells. (A) Allele-specific reporter expression in differentiating cultures of early passage *Dlk1*<sup>MatTom/PatVen</sup> iPSCs derived in basal conditions (left column) or of iPSCs derived in 3c at either early (P0, middle column) or higher (P5, third column) passage. Scale bars = 100 μm. (B) Gating strategy to define cells based on their *Dlk1* expression pattern in 3c iPSCs maintained and differentiated in the indicated conditions. p5 cultures are shown. Red gates indicate maternal-only expression (mat), yellow gates show LOI expression and green gates indicate MOI. (C) Relative abundance of MOI (green), LOI (yellow) or maternal-only (red) cells in cultures expanded and differentiated at the indicated passage number in either 20% (upper) or 4% (lower) oxygen. (D) Same samples that are represented in (C) but iPSCs expanded in 20% oxygen were differentiated in 4% oxygen (upper panel) and iPSCs expanded in 4% oxygen were differentiated in 20% oxygen (lower panel). Error bars indicate standard error (n=3). (E) Possible applications of the *Dlk1* reporter model to study genomic imprinting.
SUPPLEMENTARY METHODS

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Emily Swanzey and Matthias Stadtfeld

Generation of Dlk1 reporter mice
To generate targeting vectors for insertion of reporter genes into the 3’ UTR of Dlk1, a 6.7kb EcoRV fragment was isolated from BAC RP23-117C15 and cloned into pBluescript together with a floxed PGK-neoR cassette. In the resulting pBS-Dlk1, a SnaBI site was introduced at the site corresponding to position chr12:109,460,514 in mm10 using site-directed mutagenesis (Agilent QuikChange II). This site was used to insert the coding sequence of IRES-Venus or IRES-Tomato. V6.5 ESCs were electroporated with linearized (by SacII digestion) targeting vectors, followed by selection in G418-containing media. Correctly targeted clones were identified by Southern blotting of AseI-digested genomic DNA, using a 770bp HincII/AseI fragment probe isolated from pBS-Dlk1. Following confirmation, the PGK-neoR cassette was removed by transient transduction with Adeno-Cre. Resultant ESCs were injected into diploid blastocysts and transgenic chimeric mice bred with B6 animals to establish homozygous Dlk1 reporter mouse strains.

Cell culture
ESCs and iPSCs were cultured in KO-DMEM with 15% FBS, L-Glutamine, penicillin-streptomycin, non-essential amino acids, 2-Mercaptoethanol and 1000 U/ml LIF on irradiated feeder cells. MEFs were cultured in DMEM with 10% FBS, L-Glutamine, penicillin, streptomycin, non-essential amino acids and 2-Mercaptoethanol. For reprogramming, MEFs were seeded in ESC media on a layer of irradiated feeder cells in presence of 1 µg/ml doxycycline. If applicable, L-Ascorbic acid (50 µg/ml), CHIR99021 (3 µM) and TGFβ RI Kinase Inhibitor II (0.25 µM) were added.
Primers for quantitative PCR

*Dlk1*: GCGTGCTGTCCTGTGTGC and TGTCAGCCTCGCAGAATCCATAC

*Gtl2*: TTGCACATTTCCCTGTGGGAC and AAGCACCATGAGCCACTAGG

*Dlk1 3' UTR*: CCCCAGGCCCTTCTCTATTA and GTAGCATGGCACACAGCAAC

*Venus*: GGGACGTTGTTTTCTTCTTGA and AGATCAGCTTCAGGGTCAGC

*GAPDH*: AGGTCGGTGTGAACGGATTTG and TGTAGACCATGTAGTTGAGGTCA

Calculation of relative allele-specific expression by qRT-PCR

Allele-specific expression was calculated from *Dlk1*\(^{\text{MatWT/PatVen}}\) and *Dlk1*\(^{\text{MatVen/PatWT}}\) samples. WT expression was measured from the WT primer amplification of the unmodified alleles and Venus expression was detected from Venus primer amplification. Ct values were normalized to GAPDH, followed by calculation of MatWT expression as a percentage of total WT (MatWT + PatWT) or MatVen expression as a percentage of total Venus (MatVen + PatVen).
SUPPLEMENTARY FIGURES

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Emily Swanzey and Matthias Stadtfeld

Fig. S1. Assessment of the impact of reporter insertion on *Dlk1* and *Gtl2* expression.

(A, B) Quantification by qRT-PCR of (A) *Dlk1* and (B) *Gtl2* expression in skin, liver and lung isolated from E16.5 WT and *Dlk1*<sup>MatTom/PatVen</sup> mice. Error bars represent standard error (n=3 samples). Values are normalized to results obtained with WT cells for the respective tissue.
Fig. S2. Quantification of allele-specific Dlk1 expression in different mouse strains. (A) Flow cytometric analysis of E16.5 mouse lung (first row), skin (middle) and liver (bottom) of Dlk1<sup>MatWT/PatTom</sup> and Dlk1<sup>MatTom/PatWT</sup> reporter mice in a C57BL/6 (B6) background, compared to F1 crosses with 129/SV-E (129) mice. Gating to identify Tomato<sup>+</sup> cells is shown. (B) Quantification of Tomato<sup>+</sup> cells identified as shown in A. (*) indicates p<0.05 and (**) p<0.005 with a one-way ANOVA and Bonferroni multiple comparison test (n≥3 samples).
Fig. S3. Measurement of allele-specific Dlk1 reporter gene activity by flow cytometry. Histograms showing fluorescent intensities of paternal and maternal Dlk1-Venus expression in E16.5 skin (left panel) and liver (right panel) cells (compare to the results obtained with the Tomato reporter configuration shown in Fig. 2C).
Fig. S4. Assessment of the impact of reporter insertion on allele-specific *Dlk1* expression. (A) Schematic showing the strategy to measure allele-specific *Dlk1* expression with qRT-PCR, in the presence or absence of reporter insertion. The black box represents the 3’ UTR; a white bar, the reporter insertion site; grey arrows, the primer positions to measure the *Dlk1<sup>WT</sup>* allele; and green arrows, primers to measure the *Dlk1<sup>Ven</sup>* allele. In *Dlk1<sup>MatWT/PatVen</sup>* mice, paternal expression can be detected with primers that are specific to the Venus insertion and maternal expression can be detected with WT primers that flank the insertion site. The WT primers are unable to amplify the reporter insertion sequence under qRT-PCR conditions, due to its size. In *Dlk1<sup>MatVen/PatWT</sup>* mice, the WT primers detect paternal expression and the Venus primers detect maternal expression. (B) Representative qRT-PCR measurements showing the effectiveness of the strategy outlined in C in liver cells. Note that WT primers are unable to detect any transcript in *Dlk1<sup>MatVen/PatVen</sup>* (V/V) cells while Venus primer are unable to detect transcript in *Dlk1<sup>WT/WT</sup>* (-/-) cells. (n = 3 samples). (C) Maternal *Dlk1* expression represented as a percentage of total expression in E16.5 skin, liver and lung by qRT-PCR. Error bars represent standard error (n≥3 samples).
Fig. S5. Statistical assessment of allele-specific expression and DNA methylation differences. Mean percentage of LOI Dlk1 expression (upper panel) and mean DNA methylation at the IG-DMR (lower panel) in matching, independent iPSC cultures derived in basal conditions, with only AA or with AA, iAlk5 and CHIR99021 (3c). (*) indicates p<0.05 with a one-way ANOVA and Turkey’s multiple comparison test. Error bars represent standard error (n≥3).
Fig. S6. Quantification of DNA methylation at the IG-DMR in iPSCs derived in basal and 3c conditions. (A) Representation of next generation bisulfite sequencing reads for iPSCs derived by reprogrammed MEFs in basal (left) and 3c (right) conditions. Each row represents a single read and each column represents a CpG. Methylated CpGs are indicated by black boxes and unmethylated are white. For clarity purposes, only the first 60 reads obtained from each sample are shown. (B) Quantification of the percentage of sequencing reads with high (>75% CpGs methylated), intermediate (25-75%) or low (<25%) methylation at the IG-DMR. The even distribution of predominantly methylated and unmethylated DNA in the 3c sample suggests that most cells in this condition maintained their germline imprinting status. (C) Quantification of the percentage of cells with LOI in the samples shown in (A) and (B) by flow cytometry.
Fig. S7. Effect of oxygen levels on allele-specific Dlk1 expression during differentiation. (A) Representative FACS plots of retinoic acid-differentiated Dlk1MatTom/PatVen iPSCs at p8 that were derived in 3c conditions and cultured in 20% oxygen and differentiated in 4% (upper panel) or cultured in 4% oxygen and differentiated in 20% (lower panel). Red gates indicate maternal-only expression (mat), yellow gates show LOI expression and green indicates MOI. Note the elevated levels of maternal Dlk1 expression in cultures differentiated in 20% oxygen. (B) Representative images of cultures corresponding to FACS plots shown in (A), with iPSCs cultured in 20% oxygen and differentiated in 4% (upper row) and cultured in 4% and differentiated in 20% (bottom row). A yellow arrow points to a group of LOI cells and a red arrow indicates maternal-only expression. Scale bar = 100 μm.