A Genetic Screen Identifies Etl4-Deficiency Capable of Stabilizing the Haploidy in Embryonic Stem Cells

Guozhong Zhang,1,2,3 Xiaowen Li,1,2,3 Yi Sun,1,2 Xue Wang,1,2 Guang Liu,1,2,* and Yue Huang1,2,*
1State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, China
2Department of Medical Genetics, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, China
3These authors contributed equally
*Correspondence: liuguang@ibms.pumc.edu.cn (G.L.), huangyue@pumc.edu.cn (Y.H.)
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SUMMARY

Mammalian haploid embryonic stem cells (haESCs) hold great promise for functional genetic studies and forward screening. However, all established haploid cells are prone to spontaneous diploidization during long-term culture, rendering application challenging. Here, we report a genome-wide loss-of-function screening that identified gene mutations that could significantly reduce the rate of self-diploidization in haESCs. We further demonstrated that CRISPR/Cas9-mediated Etl4 knockout (KO) stabilizes the haploid state in different haESC lines. More interestingly, Etl4 deficiency increases mitochondrial oxidative phosphorylation (OXPHOS) capacity and decreases glycolysis in haESCs. Mimicking this effect by regulating the energy metabolism with drugs decreased the rate of self-diploidization. Collectively, our study identified Etl4 as a novel haploidy-related factor linked to an energy metabolism transition occurring during self-diploidization of haESCs.

INTRODUCTION

For most metazoans, haploidy is restricted to their gametes, which are terminally differentiated cells and cannot proliferate further. Recently, haploid embryonic stem cells (haESCs) have been generated in various organisms ranging from mouse to human (Elling et al., 2011; Leeb and Wutz, 2011; Li et al., 2012; Sagi et al., 2016; Yang et al., 2012). As they possess only one set of chromosomes, it is quite convenient to generate loss-of-function mutations in haploid cells, and this method holds great promise for forward genetic screening (Cui et al., 2020; Yilmaz et al., 2016).

In spite of their multiple scope of application, haESCs have the major disadvantage to undergo spontaneous diploidization during long-term culture (Elling et al., 2011; Leeb and Wutz, 2011). Until now, several groups focused their research on elucidating the mechanisms of self-diploidization with the aim to prolong haploidy in long-term culture. Recent studies have demonstrated that the mitotic process in haploid cells is different from that in diploid cells, the main differences laying in prolonged G2/M phase (Li et al., 2017) and metaphase (Guo et al., 2017), mitotic slippage (He et al., 2017), or failed cytokinesis (Leng et al., 2017). It was also reported that overexpression of Dnmt3b (He et al., 2018), or deletion of Ps3 (Olbrich et al., 2017) or P73 (Zhang et al., 2020) in mouse haESCs could decrease self-diploidization. Another study showed that 10-Deacetyl-baccatin-III (DAB), a precursor of Taxol, selectively favors haploidy in mammalian cells (Olbrich et al., 2019). Other works led to contradictory results, as both activation (Takahashi et al., 2014) and inhibition (He et al., 2017) of CDK1, a cyclin involved in diploidy control, could reduce the rate of self-diploidization. Therefore, more efforts are needed to elucidate the mechanisms underlying self-diploidization.

Here, we used a genome-wide mutant library to screen for possible haploidy-maintaining factors. This strategy revealed that the loss of Etl4 function promotes the maintenance of haploidy in different haESC lines. Moreover, RNA-Seq data showed that Etl4-knockout (KO) haESCs exhibit a distinct metabolism state directly correlated with the reduction of self-diploidization. Our study provides a new general strategy for maintaining haploid state during cell cultures via the modulation of cell metabolism.

RESULTS

Genetic Screening to Identify Mutations Stabilizing Haploidy in Mouse haESCs

To uncover genes related to the self-diploidization of haESCs, we conducted a high-throughput screening on a previously described genome-wide haploid mutant library that contains ~70,000 individual AGH-OG-3 (OG3) cell colonies covering 18,841 different genes mutated by the insertion of PiggyBac transposon-based gene trap vector (Liu et al., 2017). The mutant library was first replicated into three copies. One copy was used as Initial Mutant Library (IML). The second copy was used for enrichment in desired mutant haESCs via a multiple-round “cell culture-sorting” strategy. Consecutive cell cultures and five
times of fluorescence-activated cell sorting (FACS) for haploid cells resulted in the collection of a Screened Mutation Library (SML). To eliminate possible artifacts due to faster proliferation of some mutants, the third copy was cultured continuously for 30 passages without FACS and served as the Control Mutant Library (CML). SML retained a high haploidy ratio, but this ratio gradually decreased in CML during long-term culture without FACS (Figure S1).

***Figure 1. A Genetic Screen to Identify Factors Stabilizing Haploidy in Mouse haESCs***

(A) Schematic representation of the genetic screening procedure.

(B) Box plot of average read frequencies of transposon insertions in IML, SML, and CML. Dots represent the top 1% insertions. The upper vertical line represents the 75%–99% percentile group. Boxes indicate the distribution of 25%–75% of insertions. The lower vertical line represents the 1%–25% percentile group.

(C) The reads for the top 50 hits in SML and CML were increased compared with IML.

(D) Top 10 candidates obtained from the next generation sequencing (NGS) of SML and CML. S and C refer to SML and CML, respectively. Candidate genes obtained from IML, SML, and CML are listed in Table S3.

(E) Validation of the NGS results via Sp-PCR. The ratio represents the percentage of each insertion site to the total in Sp-PCR. The $f$ (NGS) means the percentage of each insertion site to the total in NGS. The primers of Sp-PCR are listed in Table S2.
Figure 2. Etl4 Deficiency Facilitates the Maintenance of Mouse haESCs
(A) Schematic diagram of the strategy to knockout Etl4 and Adam12 in OdG haESCs by using CRISPR/Cas9 system. Boxes indicate the exon of the genes and the triangles represent the single guide RNA (sgRNA). The sequences of the sgRNAs are listed in Table S1.
(B) Validation of Etl4 and Adam12 expression level in mutant and WT OdG haESCs by RT-qPCR. The expression level was normalized by Gapdh (n = 3 independent experiments).
(C) Chromosome spreads of Etl4-KO OdG haESCs showing normal haploidy. The right panel depicts the self-diploidized Etl4-KO cells.
(D) Morphology of Etl4 and Adam12-deficient OdG haESCs cultured in M2iL medium. Scale bars, 100 μm.
(E and F) Flow cytometry analysis of the ratio of haploid cells in (E) Etl4-KO, Adam12-KO and (F) two corresponding WT haESC lines during cell passages.
(G) Expressional level of Etl4 in OG3, Etl4-GT, RVT-1, and RVT-2 haESCs tested by RT-qPCR (n = 3 independent experiments).
(H) Percentages of haploid cells in OG3, Etl4-GT, RVT-1, and RVT-2 haESCs after 12 days of culture (n = 3 independent experiments).
(I) Alkaline phosphatase activity in Etl4-KO and WT OdG haESCs. Scale bars, 50 μm.

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Through Splinkerette (Sp)-PCR combined with next generation sequencing (NGS) of the three mutant libraries, self-diploidization-related gene candidates were identified (Figure 1A).

In comparison with IML, the ratios of the reads obtained for the top 50 hits in SML and CML were higher, confirming the effectiveness of the screening strategy (Figures 1B and 1C). Not surprisingly, among the top 10 hits in CML (Figure 1D) were a few genes previously reported to be involved in cell proliferation, such as Ankrd44 (La Ferla et al., 2019) and Lipa (Zhao et al., 2015). Interestingly, Ankrd44 also appeared among the gene selection from SML (Figure 1D), indicating that proliferation-related genes also could be enriched with continuous haploidy sorting and cell passages.

To confirm the NGS results, 96 individual clones were randomly picked up from SML and the transposon-host junctions were amplified by Sp-PCR and submitted to Sanger sequencing. After mapping to the mouse reference genome (GRCm38.p5), 26 insertions were located in Et4 locus and 23 in Adam12 locus, in keeping with their frequency distribution in the NGS analysis (Figure 1E). Therefore, Et4 and Adam12 were selected for further study.

**Et4-Deficiency Reduces the Self-Diploidization Rate of Different Haploid ES Cell Lines**

To verify the effects of these two candidates on haploidy maintenance, we used CRISPR/Cas9 technology to delete critical exons of Et4 and Adam12 in the OdG haESC line (Figure 2A), derived from OG3 (Yang et al., 2012) cells by deletion of the EGFP cassette. Two Et4-KO (Odg\textsubscript{Et4\textendash KO}-1 and Odg\textsubscript{Et4\textendash KO}-2) and two Adam12-KO (Odg\textsubscript{Adam12\textendash KO}-1 and Odg\textsubscript{Adam12\textendash KO}-2) clones were verified by genomic PCR sequencing (Figure S2A) and RT-qPCR (Figure 2B). Standard karyotyping showed that these KO cells contained 20 chromosomes (Figure 2C). Study of their morphology revealed no difference between mutant and wild type (WT) haESCs (Figures 2D and S2B).

Then, we assessed the effects of Et4 or Adam12 deficiencies on haploidy maintenance. Both Et4 and Adam12-deficient OdG cell cultures had a higher proportion of haploid cells than the WT during continuous cell passages (Figure 2E). To exclude any effects of the genetic background, we tested AGH-EG-1 (EG1) cells, another haESC line that has a faster self-diploidization rate than OG3 (Yang et al., 2012). Et4 and Adam12-deficient EG1 haESCs were also generated by CRISPR/Cas9 technology (Figure S2C). At 38 days of culture, approximately 20% of Et4-deficient EG1 cells remained haploid, whereas the proportion of haploid cells in both WT and Adam12-deficient EG1 cells decreased to less than 10% (Figures 2F and S2D), suggesting that Adam12-deficiency stabilizes haploidy in a cell line–dependent manner. Because the Et4-deficiency could reduce the rate of self-diploidization, we wondered if rescuing Et4 expression would accelerate the rate of self-diploidization. We generated two revertant clones, RVT-1 and RVT-2, by reexpressing PiggyBac transposase in the Et4 gene-trapped OG3 haESC clones (Et4-GT). RT-qPCR analysis confirmed that the expression of Et4 in RVT-1 and RVT-2 was restored compared with the Et4-GT haESCs (Figure 2G). After 12 days of cultures started with 100% of haploid cells, the ratio of haploid cells was lower in revertant than in Et4-GT haESCs (Figure 2H).

Next, we assessed the possible effect of Et4-deficiency on basic properties of OdG haESCs. Et4-deficient haESCs formed alkaline phosphatase (AP)-positive dome-shaped colonies similar to those of the WT cells (Figure 2I), and expressed pluripotency markers-Oct4, Sox2, and Nanog at normal levels (Figures 2 and 2K). In addition, the loss of Et4 did not affect the proliferation rate of OdG haESCs (Figure 2L). Similarly, Et4-deficiency had no obvious impact on colony morphology, pluripotency, and cell proliferation of EG1 haESCs (Figures S2E–S2I). Altogether, these data suggest that Et4 is an obstacle to haploidy maintenance, but does not influence the self-renewing ability of ES cells.

**Transcriptome Analysis of Et4-Deficient haESCs**

To elucidate the mechanism underlying haploidy maintenance in Et4-deficiency, we analyzed the transcriptomes of Et4-deficient and WT haESCs. Et4-deficient OdG cells exhibited a slightly different expression pattern compared with WT haESCs (Figure 3A). MA plots of normalized mean counts versus fold change (log2 scale) showed that 1,100 genes were upregulated and 1,658 genes were downregulated in the Et4-deficient haESCs (Figure 3B). Gene ontology (GO) analysis indicated that genes related to non-coding RNA metabolic process, methylation, chromosome segregation, and mitotic cell cycle phase transition were upregulated (Figure 3C), whereas some metabolic processes were downregulated in Et4-deficient haESCs (Figure 3D). Similar results were found in EG1\textsubscript{Et4\textendash KO} haESCs (Figures S3A and S3B). A recent study showed that overexpression of DNA methyltransferases 3b (Dnmt3b) effectively improves DNA methylation level...
and reduces the rate of self-diploidization in androgenetic haESCs (He et al., 2018). Consistently, our RNA-Seq data revealed that Dmnt1 and Dmnt3b were substantially upregulated in Et4-deficient haESCs (Figures 3E and 3SC). This result was confirmed by western blot analysis (Figure 3SD). Moreover, the 5-methylcytosine (5-mC) level in Et4-KO haESCs was higher than in WT haESCs (Figures 3SE and 3SF), which is consistent with a previous report (He et al., 2018). Several works reported that abnormal cell cycle was the major reason for self-diploidization, and that inducing G2/M phase transition or promoting metaphase/anaphase transition could partially maintain haploid state (He et al., 2017; Takahashi et al., 2014). Similarly, G2/M phase transition-related genes, such as Chek1, Chek2, and Bub3 (Figures 3F and 3G), and the core components of Rho A pathway, which is a central player in the assembly of the contractile ring during cytokinesis, were upregulated in Et4-deficient haESCs (Figure 3G). Although these results suggested that Et4-deficiency maintains haploid state through methyltransferases and cell cycle-related genes, to clarify whether other mechanisms operate requires further investigation.

Furthermore, both GO (Figures 3D and 3B) and KEGG enrichment analysis (Figures 3H and 3H) showed a significant enrichment in terms related to amino acid and carbohydrate metabolic processes in Et4-deficient haESCs. Gene set enrichment analysis (GSEA) also indicated that anaerobic glycolysis gene set was predominantly reduced in Et4-deficient haESCs (Figures 3I and 3J). The energy metabolism is closely linked to many important biological processes, including stem cell self-renewal (Ito and Ito, 2016), cell reprogramming (Cliff and Dalton, 2017), and cell cycle (Icard et al., 2019). Because Et4-deficient haESCs provoked an increase in OXPHOS level and decrease of glycolysis, we hypothesized that promoting OXPHOS or inhibiting the glycolysis in haESCs would reduce the rate of self-diploidization. To test our conjecture, haploid cells collected by FACS were treated with two classes of energy metabolism–modifying drugs during long-term cultures. Both 1,25-(OH)2D3, an OXPHOS agonist (Ferreira et al., 2015), and 3-Bromopyruvic acid (3-BrPA), a glycolysis inhibitor (Fang et al., 2019), could maintain haploidy to a certain extent in both OdG and EG1 haESCs.

Enhancement of OXPHOS or Suppression of Glycolysis Diminishes the Rate of Self-Diploidization

To further explore the metabolic changes in Et4-deficient haESCs, we measured the respiratory capacity using a Seahorse Extracellular Flux XF24 Analyzer. As indicated by the oxygen consumption rate curves, OdG Et4-KO-1 and OdG Et4-KO-2 haESCs showed a significant increment in the basal and maximal mitochondrial respiratory capacities, and in ATP production (Figures 4A and 4B). These results were consistent with the RNA-Seq data indicating a higher expression level of oxidative phosphorylation (OXPHOS)-related genes in Et4-deficient haESCs (Figure 3J). In addition, we monitored the extracellular acidification rate (ECAR) in Et4-KO and WT OdG haESCs (Figure 4C). The rate of glycolysis, glycolytic capacity, and glycolytic reserve were decreased in Et4-deficient haESCs, whereas no differences were observed for nonglycolytic acidification between Et4-KO and WT cells (Figure 4D). Next, we tested the changes of metabolism in Et4-GT and RVT haESCs. The restoration of Et4 level decreased OHPXOS capacity and increased the glycolysis in haESCs (Figures 4A–4D). Collectively, our results demonstrate that there is a shift from glycolysis to OXPHOS when Et4 is deficient, with no obvious changes in mitochondria morphology (Figure 4E).

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**Figure 3. Transcriptome of Et4-deficient OdG haESCs**

(A) Hierarchical clustering of gene expression profiles from two biologically independent samples based on Pearson correlation coefficient in two Et4-KO and WT haES clones. Colors from green to red indicate weak to strong correlations.

(B) MA plots showing gene expression changes in Et4-KO haESCs. Red dots indicate upregulated genes and blue dots indicate downregulated genes.

(C and D) GO analysis of upregulated genes (C) and downregulated genes (D) for biological processes in Et4-KO OdG haESCs.

(E–G) Heatmap showing the expression of methylation related genes (E), cell-cycle–related genes (F), and Rho A pathway (G) in Et4-KO and WT OdG haESCs.

(H) Significantly enriched KEGG pathways for genes expressed differentially in Et4-KO OdG haESCs.

(I) GSEA for anaerobic glycolysis gene set in WT OdG and Et4-KO haESCs. For the x axis, genes were ranked based on the ratio of Et4-KO versus WT haESCs.

(J) Heatmap showing changes in RNA expression levels for various enzymes and regulators of central carbon metabolism in Et4-KO and WT OdG haESCs. The scale represents Z score.
(Figures 4E and 4F). Furthermore, neither of the two drugs affected the proliferation (Figure S4F) or pluripotency of the haESCs (Figures S4G and S4H). Collectively, here we provide a tractable solution to maintain the haploid state of haESCs through enhancement of OXPHOS or suppression of glycolysis.

**DISCUSSION**

Here, we report a genome-wide genetic screening and the identification of Etl4-deficiency as a significant reducer of self-diploidization. Etl4 (enhancer trap locus 4, also named Skt) is required for normal development of the intervertebral disc (Semba et al., 2006) and anorectum (Suda et al., 2011). ETL4 protein is cytoplasmic and contains a proline-rich region in its N terminus and a coiled-coil domain in its middle (Semba et al., 2006). The exact function of ETL4 protein is not clear, but the proline-rich region has been shown to be important for proper folding of cytochrome P450s (Kusano et al., 2001), which catalyzes many metabolic reactions. A previous study also showed that the proline-rich region may play a role in the subcellular localization and enzymatic function (Arreaza and Deutsch, 1999). Thus, here we defined a novel function carried out by Etl4, consisting...
of the regulation of self-diploidization in haESCs through a metabolic control.

Glycolysis and OXPHOS are two key cellular pathways for energy production. Most cells can switch between these two pathways to adapt to changes in the environment. OXPHOS, taking place in mitochondria, and the different subunits of the OXPHOS complexes are encoded by both mitochondrial (mtDNA) and nuclear DNA (nucDNA) (Quiros et al., 2016). Because haESCs have one-half of the nucDNA, they have a higher ratio of mtDNA to nucDNA compared with diploid cells. In response to the difference in mtDNA/nucDNA ratio, the expression of some OXPHOS-related genes encoded by the nucleus is upregulated in haploid cells (Sagi and Benvenisty, 2017). The relatively higher level of OXPHOS in haESCs may serve the increase in energy demands and be beneficial to haploidy maintenance. Our results clearly show that Etil4-deficiency increases the mitochondrial OXPHOS capacity and decreases glycolysis in haESCs, although the detailed mechanism linking the two phenomena needs to be further investigated.

Previous studies showed that deficient phosphorylation of mitochondrial respiration chain complex I (CI) subunits by Cdk1 results in a loss of G2/M-associated enhancement of mitochondrial activity, and delays G2/M progression (Wang et al., 2014). Loss of mitochondrial function during prolonged mitotic arrest results in the upregulation of glycolysis (Salazar-Roa and Malumbres, 2017). Progression through the cell cycle, including phase transition and structural rearrangements for cell division are energy-demanding (Pederson, 2003). Combined with our finding that both 1,25-(OH)2D3 and 3-BrPA help maintain haploidy in haESCs, it can be proposed that Etil4 deficiency causes cell cycle changes and maintains the haploidy state by increasing mitochondrial metabolism.

Overall, our work identified Etil4 as a novel haploidy-related factor, whose deficiency significantly reduces the rate of self-diploidization in haESCs through increasing OXPHOS or inhibiting glycolysis. Moreover, our study suggests that elucidating the interplay between metabolic changes and haploid state stabilization would provide interesting insights for future hESCs applications.

EXPERIMENTAL PROCEDURES

Cell Cultures and Drug Treatment
Mouse haESC lines AGH-OG-3 and AGH-EG-1 were kindly provided by Prof. Jinsong Li (Institute of Biochemistry and Cell Biology, Shanghai). Cells were cultured in M2iL mediums, which containing knockout DMEM (10,829-018, Gibco), 15% fetal bovine serum (SH30396.03, HyClone), 1% GlutaMAX (35,050-061, Gibco), 1% nonessential amino acids (11140050, Gibco), 100 µM β-mercaptoethanol (21985-023, Gibco), 100 U Leukemia inhibitory factor (ESG1107, Merck Millipore), 3µM CHIR99021 (S2924, Selleck), and 1µM PD0325901 (S1036, Selleck). The cells were cultured at 37°C with 5% CO2 in a humidified environment. 1,25-(OH)2D3 (Aladdin, C120126) and 3-BrPA (Selleck, S5426) were used at a final concentration of 12.5 µg/mL and 5µM, respectively.

FACS and DNA Content Analysis
For FACS, haESCs were trypsinized and stained with Hoechst33342 (5 µg/mL) (B2261, Sigma) for 30 min at 37°C. After filtering with a 40-µm cell strainer, the haploids in 1n peak were purified with a Beckman MoFlo-XDP cell sorter. Diploid (2n) ES cells were used as a control.

For DNA content analysis, haESCs were treated with 0.2 µg/mL Colchicine (D1925, Sigma) for 6 h and then trypsinized into single cells followed by fixation with 75% ethanol at 4°C for more than 2 h. Fixed cells were treated with 25 µg/mL RNase at 37°C for 30 min and then stained with 5 µg/mL propidium iodide (P4170, Sigma) at 4°C for 30 min. Flow cytometry data were recorded by using BD Accuri C6. The percentage of the haploid cells was calculated as hap/ (hap + dip), where hap indicates haploid cells, and dip indicates diploid cells.

Data and Code Availability
The accession number for the RNA-Seq data in this paper is GEO: GSE150215.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.stemcr.2020.11.016.

AUTHOR CONTRIBUTIONS
Y.H. and G.L. conceived and designed the study; G.Z., Y.S., G.L., and X.W. performed the experiments; X.L. performed bio-informatic analyses; Y.H., G.Z., X.L., Y.S., and G.L. wrote the manuscript.

CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

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REFERENCES

Arreaza, G., and Deutsch, D.G. (1999). Deletion of a proline-rich region and a transmembrane domain in fatty acid amide hydrolase. FEBS Lett. 454, 57–60.

Cliff, T.S., and Dalton, S. (2017). Metabolic switching and cell fate decisions: implications for pluripotency, reprogramming and development. Curr. Opin. Genet. Dev. 46, 44–49.

Cui, T., Li, Z., Zhou, Q., and Li, W. (2020). Current advances in haploid stem cells. Protein Cell 11, 23–33.

Elling, U., Taubenschmid, J., Wirsberger, G., O’Malley, R., Domers, S.P., Vanhaelen, Q., Shukalyuk, A.I., Schmauss, G., Schrammek, D., Schnuetgen, F., et al. (2011). Forward and reverse genetics through derivation of haploid mouse embryonic stem cells. Cell Stem Cell 9, 563–574.

Fang, Y., Shen, Z.Y., Zhan, Y.Z., Feng, X.C., Chen, K.L., Li, Y.S., Deng, H.J., Fan, S.M., Wu, D.H., and Ding, Y. (2019). CD36 inhibits beta-catenin/c-myc-mediated glycolysis through ubiquitination of GPC4 to repress colorectal tumorigenesis. Nat. Commun. 10, 3981.

Ferreira, G.B., Vanherwegem, A.S., Eelen, G., Gutierrez, A.C.F., Van Lommel, L., Marchal, K., Verlinden, L., Verstuyf, A., Nogueira, T., Georgiadou, M., et al. (2015). Vitamin D3 induces tolerance in human dendritic cells by activation of intracellular metabolic pathways. Cell Rep. 10, 711–725.

Guo, A., Huang, S., Yu, J., Wang, H., Li, H., Pei, G., and Shen, L. (2017). Single-cell dynamic analysis of mitosis in haploid embryonic stem cells shows the prolonged metaphase and its association with self-diploidization. Stem Cell Rep. 8, 1124–1134.

He, W., Zhang, X., Zhang, Y., Zheng, W., Xiong, Z., Hu, X., Wang, M., Zhang, L., Zhao, K., Qiao, Z., et al. (2018). Reduced self-diploidization and improved survival of semi-cloned mice produced from androgenetic haploid embryonic stem cells through overexpression of Dnmt3b. Stem Cell Rep. 10, 477–493.

He, Z.Q., Xia, B.L., Wang, Y.K., Li, J., Feng, G.H., Zhang, L.L., Li, Y.H., Wan, H.F., Li, T.D., Xu, K., et al. (2017). Generation of mouse haploid somatic cells by small molecules for genome-wide genetic screening. Cell Rep. 20, 2227–2237.

Icard, P., Fournel, L., Wu, Z., Alifano, M., and Lincet, H. (2019). Interconnection between metabolism and cell cycle in cancer. Trends Biochem. Sci. 44, 490–501.

Ito, K., and Ito, K. (2016). Metabolism and the control of cell fate decisions and stem cell renewal. Annu. Rev. Cell Dev. Biol. 32, 399–409.

Kusano, K., Kaga, N., Sakaguchi, M., Omura, T., and Waterman, M.R. (2001). Importance of a proline-rich sequence in the amino-terminal region for correct folding of mitochondrial and soluble microbial p450s. J. Biochem. 129, 271–277.

La Ferla, M., Lessi, F., Aretini, P., Pellegrini, D., Franceschi, S., Tantillo, E., Menicagli, M., Marchetti, L., Scopelliti, C., Civita, P., et al. (2019). ANKRD44 gene silencing: a putative role in trastuzumab resistance in her2-like breast cancer. Front. Oncol. 9, 547.

Leeb, M., and Wutz, A. (2011). Derivation of haploid embryonic stem cells from mouse embryos. Nature 479, 131–134.
respiration for cell-cycle G2/M progression. Dev. Cell 29, 217–232.

Yang, H., Shi, L., Wang, B.A., Liang, D., Zhong, C., Liu, W., Nie, Y., Liu, J., Zhao, J., Gao, X., et al. (2012). Generation of genetically modified mice by oocyte injection of androgenetic haploid embryonic stem cells. Cell 149, 605–617.

Yilmaz, A., Peretz, M., Sagi, I., and Benvenisty, N. (2016). Haploid human embryonic stem cells: half the genome, double the value. Cell Stem Cell 19, 569–572.

Zhang, W., Tian, Y., Gao, Q., Li, X., Li, Y., Zhang, J., Yao, C., Wang, Y., Wang, H., Zhao, Y., et al. (2020). Inhibition of apoptosis reduces diploidization of haploid mouse embryonic stem cells during differentiation. Stem Cell Rep. 15, 185–197.

Zhao, T., Du, H., Ding, X., Walls, K., and Yan, C. (2015). Activation of mTOR pathway in myeloid-derived suppressor cells stimulates cancer cell proliferation and metastasis in lal(-/-) mice. Oncogene 34, 1938–1948.