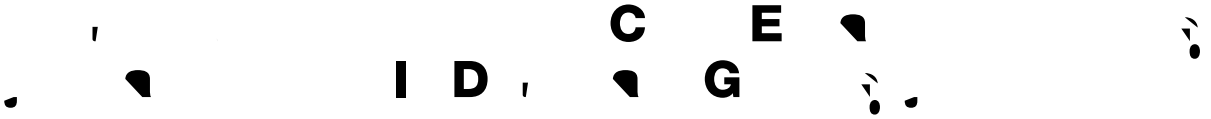
Cell Stem Cell



Graphical Abstract

Highlights

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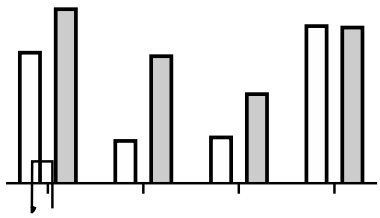
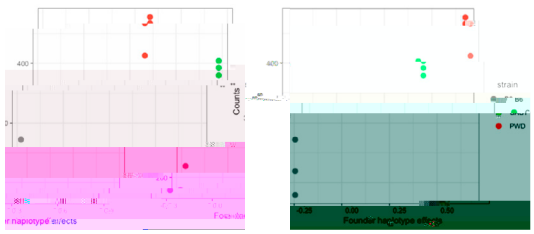
Short Article

However, the resulting lines are often challenging to grow, are genetically variable in some conditions, and require an initial capacitation step prior to multi-lineage differentiation ( ). Therefore, the hypothesis that ground-state culture conditions and the associated epigenetic resetting can overcome phenotypic variability observed in genetically diverse pluripotent stem cell lines remains to be experimentally tested.

Here, we decided to address this issue by taking advantage of 12 inbred mESC lines from four common laboratory strains, along with transcriptome data from 185 outbred mESC lines (Diversity Outbred) from the Collaborative Cross ( ). These lines were assessed for their potential to generate different cell types, and we observed that naive pluripotent stem cells derived from the same strain display a consistent capacity of differentiation. However, cell lines with different genetic back-

expression. On the other hand, addition of the GSK3 inhibitor CHIR99021 and subsequent increase of canonical Wnt b-catenin signaling led to a drastic reduction of OTX2-positive cells, suggesting a delay in the transition toward post-implantation pluripotent states (B). We then asked whether a divergence in canonical Wnt signaling could explain the observed phenotypes during the transition to EpiLCs and assessed the levels of active b-catenin in the different naive mESC lines. These analyses revealed marked differences in the levels of active b-catenin among different genetic backgrounds (B), with PWD mESCs showing the highest level of active b




400
Counts
strain
PWD
0.25
0.00
0.25
0.50

inactivation could inßuence the differentiation capacity of female hPSCs (;). For this reason, we focused our study on male mESC lines, which can contribute efÞciently to chimeras after blastocyst injection. Performing similar studies on female mESCs from different genetic backgrounds could reveal speciÞc variations associated with X-related epigenetic regulation.

Finally, the genetic differences among mouse strains are greater than among human individuals; therefore the results of this study cannot be directly extrapolated but can serve as the basis of similar investigations in the human system.

Detailed methods are provided in the online version of this paper and include the following:

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- d
  - B Lead Contact
  - B Materials Availability
  - B Data and Code Availability
- d
  - B Cell Lines
- d
  - B Cell Line Differentiation
  - B Colony Forming Assay
  - B Flow Cytometry
  - B Cell cycle analysis
  - B Quantitative PCR
  - B Cellular Fractionation
  - B Western Blot
  - B Immunocytochemistry
  - B Luciferase Reporter Assay
  - B ELISA Assay
  - B Bulk RNA-seq on inbred strain mESCs
  - B Expression QTL Mapping
  - B Single Cell analysis
  - B Demultiplexing of 10x Data
  - B Analysis of 10x Data
- d

Supplemental Information can be found online at .

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Further information and requests for resources and reagents should be directed to and will be fulÞlled by the Lead Contact, Ludovic Vallier ( ).

This study did not generate new unique reagents.

The accession number for the Single Cell RNA-Seq data reported in this paper is ArrayExpress: E-MTAB-8844.

the feeder layers. During days 5Ð10, the media were replaced every other day and inner cell mass (ICM) outgrowth was observed.

Single-cell RNA-seq libraries were prepared in the Cancer Research UK Cambridge Institute Genomics Core Facility using the following: Chromium Single Cell 3^0 Library & Gel Bead Kit v3, Chromium Chip B Kit and Chromium Single Cell 3^0 Reagent Kits v3 User Guide (Manual Part CG000183 Rev A; 10X Genomics). Suspensions were loaded on the Chromium instrument with the expectation of collecting up to 4500 gel-beads emulsions containing single cells. RNA from the barcoded cells for each sample was subsequently reverse-transcribed in a C1000 Touch Thermal cycler (Bio-Rad) and all subsequent steps to generate single-cell libraries were performed according to the manufacturerÕs protocol with no modiÞcations (12 cycles used for cDNA ampliÞcation). cDNA quality and quantity was measured with Agilent TapeStation 4200 (High Sensitivity 5000 ScreenTape) after which 25% of material was used for gene expression library preparation.

Library quality was conÞrmed with Agilent TapeStation 4200 (High Sensitivity D1000 ScreenTape to evaluate library sizes) and