

HIF1A-mediated pathways promote euploid cell survival in chromosomally mosaic embryos

Estefania Sanchez-Vasquez, Marianne E Bronner, Magdalena Zernicka-Goetz 

Division of Biology 139-74, California Institute of Technology, Pasadena, United States

 https://en.wikipedia.org/wiki/Open_access

 Copyright information

Reviewed Preprint

v3 • September 24, 2025

Revised by authors

Reviewed Preprint

v2 • June 20, 2025

Reviewed Preprint

v1 • November 21, 2024

eLife Assessment

Sanchez-Vasquez et al establish an innovative approach to induce aneuploidy in preimplantation embryos. This **important** study extends the author's previous publications evaluating the consequences of aneuploidy in the mammalian embryo. In this work, the authors investigate the developmental potential of aneuploid embryos and characterize changes in gene expression profiles under normoxic and hypoxic culture conditions. Using a **solid** methodology they identify sensitivity to Hif1alpha loss in aneuploid embryos, and in further **convincing** experiments they assess how levels of DNA damage and DNA repair are altered under hypoxic and normoxic conditions.

<https://doi.org/10.7554/eLife.101912.3.sa2>

Abstract

Human fertility is suboptimal in part by error-prone divisions during early cleavage stages, which frequently result in chromosomal aneuploidy. Most human pre-implantation embryos are mosaics of euploid and aneuploid cells, yet those with a low proportion of aneuploid cells can develop to term at rates similar to fully euploid embryos. How embryos manage aneuploidy during early development remains poorly understood – yet this knowledge is crucial for improving fertility outcomes and reducing developmental defects. To investigate these mechanisms, we established a new mouse model of chromosome mosaicism to trace the fate of aneuploid cells during pre-implantation development. We previously used the Mps1 inhibitor reversine to induce aneuploidy. Here, we demonstrate that the more specific Mps1 inhibitor AZ3146 similarly disrupts chromosome segregation but supports higher developmental potential than reversine. AZ3146-treated embryos transiently upregulate Hypoxia Inducible-Factor-1A (HIF1A) without triggering p53 activation. Given that pre-implantation embryos develop in a hypoxic environment *in vivo*, we further explored the role of oxygen tension. Hypoxia exposure *in vitro* reduced DNA damage in response to Mps1 inhibition and increased the proportion of euploid cells in mosaic epiblast. Conversely, HIF1A inhibition decreased the proportion of aneuploid cells. Together, these findings uncover a role for hypoxia signaling in modulating the response to chromosomal errors and suggest new strategies to improve the developmental potential of mosaic human embryos.

Introduction

Humans have relatively low fertility compared to other mammals, with only ~30% of conceptions resulting in live birth (Palmerola et al., 2022a [↗](#); Capalbo et al., 2021 [↗](#)). A striking feature of early human development is the high frequency of chromosome segregation errors during cleavage stage divisions, leading to aneuploidy – the gain or loss of chromosomes (Allais & FitzHarris, 2022 [↗](#); Currie et al., 2022 [↗](#); Palmerola et al., 2022b [↗](#); Pauerova et al., 2020 [↗](#); Vanneste et al., 2009 [↗](#)). This high incidence of aneuploidy is thought to underlie low human fecundity and many developmental defects (Palmerola et al., 2022a [↗](#)). Both *in vivo* and *in vitro* fertilization (IVF) frequently results in embryos that are chromosomally mosaic, containing a mixture of diploid and aneuploid cells. It is estimated that ~60% of pre-implantation IVF embryos exhibit this form of mosaicism (Capalbo et al., 2021 [↗](#)). Despite its prevalence, our understanding of how embryos respond to and cope with aneuploidy during early development remains limited.

The incidence of aneuploidy declines as development progresses (Shahbazi et al., 2020 [↗](#); Yang et al., 2021 [↗](#)), but the mechanism underlying this decline remains unclear. Remarkably, mosaic human embryos can develop to term (Capalbo et al., 2021 [↗](#); Greco et al., 2015 [↗](#); Starostik et al., 2020 [↗](#)). Specifically, embryos classified as low- or medium-grade mosaics – defined by presence of 20-30% or 30-50% aneuploid cells in the trophectoderm – have implantation and birth rates comparable to fully euploid embryos (Capalbo et al., 2021 [↗](#)). These observations raise a fundamental question: how do some mosaic embryos maintain developmental potential despite chromosomal abnormalities? Uncovering the mechanisms that support the viability of mosaic embryos is essential for improving reproductive outcomes and embryo selection strategies.

Mouse models of chromosome mosaicism provide a powerful system to investigate mechanisms that cannot be ethically studied in human embryos. Importantly, mouse and human pre-implantation development are highly similar: both undertake cleavage divisions, compaction, blastocyst cavity formation and hatching, albeit with slightly different timings (Mole et al., 2020 [↗](#); Zhu et al., 2021 [↗](#)). During this period, outer cells differentiate into the extra-embryonic trophectoderm (TE), whereas inner cells form the inner cell mass (ICM), which further segregates into the embryonic epiblast (EPI) or extra-embryonic primitive endoderm (PE). The TE will form the placenta, the PE will form the yolk sac and the EPI will form the fetus (Zhu & Zernicka-Goetz, 2020 [↗](#)).

Recently, our group developed the first mouse model of chromosome mosaicism by inducing aneuploidy using the small-molecule inhibitor reversine (Bolton et al., 2016 [↗](#); Singla et al., 2020 [↗](#)). Reversine is a pan-Aurora kinase inhibitor that also antagonizes the A3 adenosine receptor and inhibits mitotic kinase monopolar spindle 1 (MPS1) (D’Alise et al., 2008 [↗](#); Santaguida et al., 2010 [↗](#)). We found that reversine-treated mosaic embryos exhibit widespread aneuploidy and upregulation of p53 (Bolton et al., 2016 [↗](#)). Importantly, aneuploid cells in mosaic embryos are progressively eliminated from the EPI, beginning around the time of implantation. Consistent with findings in human embryos (Capalbo et al., 2021 [↗](#)), murine mosaic embryos containing at least 50% of euploid cells had a similar developmental potential to fully euploid embryos (Bolton et al., 2016 [↗](#); Singla et al., 2020 [↗](#)).

However, because reversine affects p53 and may compromise cellular fitness (D’Alise et al., 2008 [↗](#); Santaguida et al., 2010 [↗](#)), we sought to establish a complementary model using a more specific Mps1 inhibitor, AZ3146 (Hewitt et al., 2010 [↗](#)). Although both AZ3146 and reversine interfere with the spindle assembly checkpoint, they bind to Mps1 in a different manner (Lan & Cleveland, 2010 [↗](#)). In mouse embryos, AZ3146 treatment was shown to double the occurrence of micronuclei, indicative of chromosome segregation defects, but without impairing overall cellular

fitness (Vazquez-Diez et al., 2019). In this study, we use both AZ3146- and reversine-treated embryos to dissect the mechanisms governing aneuploid cell elimination and survival during pre-implantation development.

Results

AZ3146 treatment induces chromosome segregation defects in pre-implantation embryos

To generate distinct models of aneuploidy, we treated 4- to 8-cell stage mouse embryos with AZ3146 (20 μ M) (Vazquez-Diez et al., 2019), as well as with reversine (0.5 μ M) (Bolton et al., 2016) as a positive control, or DMSO (vehicle) as a negative control (Fig. 1A). We evaluated how the different Mps1 inhibitors affect chromosome segregation by detecting nuclei and kinetochores in 8-cell embryos and counted chromosomes *in situ* (Pauerova et al., 2020). Micronuclei were identified as small DAPI-stained chromosomes that were clearly distinct from the nuclei (Fig. S1A). We examined 32 DMSO-treated control embryos and observed only 22 cells with micronuclei out of a total of 256 cells (Fig. S1B). In contrast, we observed 82 cells with micronuclei in a total of 144 individual cells from 18 reversine-treated embryos, and 182 cells in a total of 304 individual cells from 38 AZ3146-treated embryos (Fig. S1A). After normalization, the average of aneuploidy in DMSO-treated 8-cell embryos was 12.5%, compared to 75% in the reversine-treated embryos and 62.5% in the AZ3146-treated embryos (Fig. S1B). We also detected non-dividing nuclei as rounded circles with distinct DAPI intensities in embryos treated with reversine and AZ3146. Specifically, we observed 28 non-dividing cells (19%) in reversine-treated embryos and 24 non-dividing cells (7.9%) in AZ3146-treated blastomeres, but none in DMSO-treated blastomeres (Fig. S1B). The increased frequencies of micronuclei and non-dividing cells in response to Mps1 inhibition are consistent with elevated aneuploidy and chromosomal instability (Daughtry and Chavez, 2016; Vazquez-Diez et al., 2016), as we showed previously for reversine-treated embryos (Bolton et al., 2016).

Given these distinct responses to reversine and AZ3146, we examined how these treatments impact the lineages in the blastocyst. To this end, we treated embryos with DMSO, reversine or AZ3146 from the 4- to 8-cell stage, and then washed and cultured them until the late blastocyst stage (E4.5). We assessed lineages by immunofluorescence (IF) to detect the TE marker CDX2, the EPI marker NANOG, and the PE marker SOX17. We found that all lineages segregated normally, and that blastocyst morphology was similar in all three conditions (Fig. 1B). We quantified the number of cells in the blastocysts and found that DMSO-treated controls had a median of 93 cells in total, whereas reversine- and AZ3146-treated embryos had a median of only 79 and 82 cells, respectively ($n=28$ embryos per treatment collected from three independent experiments. (Fig. 1C). DMSO-treated blastocysts contained a median of 15 EPI, 11 PE, and 67 TE cells, whereas reversine-treated blastocysts contained 12 EPI, 8 PE and 59 TE cells, and AZ3146-treated blastocysts had 11 EPI, 7 PE and 64 TE cells (Fig. 1C). These data suggest that reversine treatment compromises the development of all three lineages, as observed previously (Bolton et al., 2016), whereas AZ3146 mainly compromises ICM (EPI and PE) development (Statistical test: Mann–Whitney U-test).

To compare the developmental potential of blastocysts that had been treated with AZ3146 or reversine, we transfer ~ 7 embryos per treatment into opposite uterine horns of the same mouse and counted decidia, which reflect successful implantation, as well as viable embryos at E9.5. Although two (8%) reversine-treated blastocysts developed decidia (Fig. S1C), none gave rise to viable E9.5 embryos. In contrast, seven (30%) AZ3146-treated blastocysts developed decidia (Fig. S1C) and five (21%) generated viable E9.5 embryos (Fig. S1D). Thus, AZ3146-treated embryos appear to have a higher developmental potential than reversine-treated embryos.

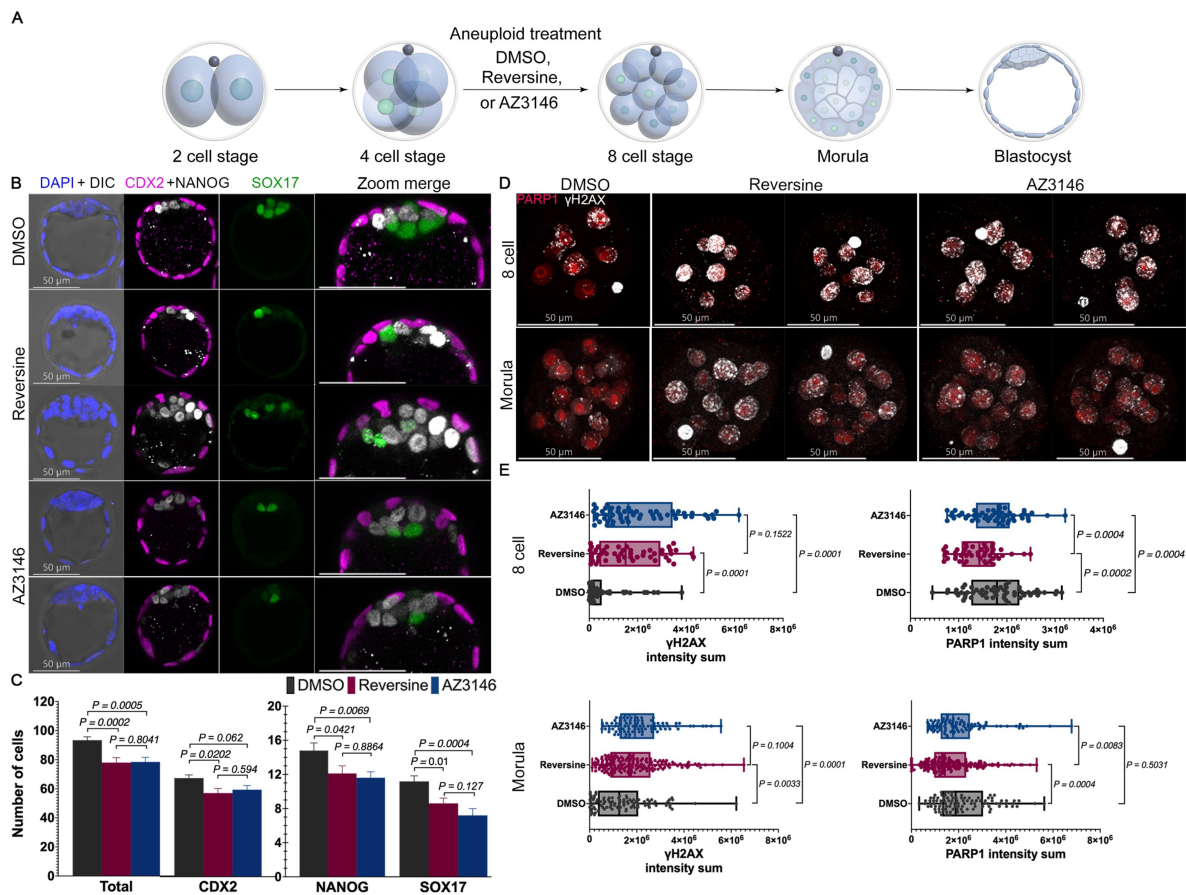


Figure 1

Lineage analysis of aneuploid embryos generated by selective Mps1 inhibitors AZ3146 and reversine.

(A) Graphic representation of 4-cell embryos treated with DMSO (control) or Mps1 inhibitors reversine (0.5 μ M) and AZ3146 (20 μ M) to inactivate the spindle assembly checkpoint (SAC) and induce chromosome segregation errors. After washing, embryos were cultured to the mature blastocyst stage (E4.5) and analyzed for lineage specification. (B) Immunofluorescence imaging of well-known lineage markers CDX2 (TE), NANOG (EPI) and SOX17 (PE) reveals that overall embryonic morphology and cavitation is not affected by Mps1 inhibition. (C) Number of cells in each lineage was quantified to evaluate the effect of drug treatment on blastocyst development. Importantly, both reversine and AZ3146-treatments reduce the number of cells in the ICM, marked by NANOG (EPI) and SOX17 (PE). Whereas the TE, marked by CDX2, is reduced only in the reversine-treated embryos. (n=28 embryos per treatment, cumulative of three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m). (D) Analysis of DNA damage and DNA repair based on immunofluorescence against γ H2AX (phosphorylated form of H2A.X, white) and PARP1 (red), respectively. (E) Intensity analysis shows that reversine and AZ3146 increase DNA damage at the 8-cell stage through morula stage compared with DMSO-treated embryos. Importantly, reversine appears to downregulate PARP1 expression at the 8-cell stage, which extends to morula stage embryos (n=15 embryos per treatment, ~120 cells. Collection was made during three independent experiments. Statistical test: t-test, error bars represent s.e.m).

Considering that cellular fitness and aneuploid stress are related to DNA damage and DNA repair, we first sought to understand if these parameters were affected in our drug treatments. We used IF to quantify the levels of the DNA repair marker PARP1 and of the DNA damage marker, phosphorylated H2A.X (γ H2A.X) at the 8-cell and morula stages (**Fig. 1D**). We observed elevated γ H2A.X levels in reversine- and AZ3146-treated 8-cell embryos compared to controls (**Fig. 1E**) ($n \sim 15$ embryos per treatment, ~ 120 cells. Collection was made during three independent experiments. Statistical test: t-test), which eventually returned to normal in AZ3146-treated blastocysts, but not in reversine-treated blastocysts (**Fig. S2A**).

PARP1 was specifically enriched in the EPI lineage in normal blastocysts, as assessed by IF ($n > 21$ embryos per treatment collected from three independent experiments Statistical test: Mann–Whitney U-test) and re-analysis of published scRNA-seq data (Deng et al., 2014) (**Fig. S2B**). Intriguingly, PARP1 levels were reduced at the 8-cell and morula stages in reversine-treated embryos compared to controls and to AZ3146-treated embryos (**Fig. 1D–E**). Moreover, late morula stage embryos treated with the PARP inhibitor Olaparib (10 μ M) (Hou et al., 2022; Prasad et al., 2017) developed into smaller blastocysts with only 78 total cells, reflecting a relatively low number of ICM cells, only 8 EPI and 4 PE cells in DMSO-treated embryos (**Fig. S2C–E**). Treatment with reversine, but not AZ3146, further reduced the number of cells in the EPI lineage of Olaparib-treated embryos (**Fig. S2E**) ($n = 15$ embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test). Overall, these data suggest that PARP1 is required for proper development of the ICM, and that its reduced levels after reversine treatment may negatively impact development.

Reversine and AZ3146 activate distinct stress response pathways in pre-implantation embryos

Chromosome mis-segregation and aneuploidy are associated with different cellular stress pathways (Zhu et al., 2018). For instance, p53 is frequently activated following DNA damage, which can limit proliferation and trigger apoptosis (Abuetaf et al., 2022; Soto et al., 2017; Thompson & Compton, 2010). The p38 mitogen-activated protein kinase (MAPK) can also be activated following DNA damage (Thompson and Compton, 2010). In aneuploid cells, p38 promotes apoptosis by inhibiting the transcription factor Hif-1 α (Simoes-Sousa et al., 2018), which otherwise promotes cell survival, proliferation, and metabolic changes in aneuploid cells and in response to hypoxia in different contexts (Hu et al., 2003; Simoes-Sousa et al., 2018).

To investigate how treatment with reversine or AZ3146 affect p53 and Hif1 α expression in mouse embryos, we performed RT-qPCR at the morula and blastocyst stages. We normalized to *Ppia* (Peptidylprolyl Isomerase A) mRNA, which is a stable reference gene in diploid and polyploid embryos (Gu et al., 2014). Reversine-treated embryos displayed a significant increase in p53 transcript levels at the morula (7-fold) and blastocyst (1.3-fold) stages compared with DMSO-treated embryos (Sample: 3 biological replicates and 2 technical replicates per experiment with each replicate having a minimum of 16 embryos. Statistical test: Welch's t-test) (**Fig. 2A**), as we showed previously (Singla et al., 2020). In addition, reversine-treated embryos showed reduced expression of Hif1 α at the blastocyst stage, which would be consistent with p38 activation. In contrast, AZ3146-treated embryos did not show upregulation of p53 at either the morula or blastocyst stage (**Fig. 2A**). Moreover, AZ3146-treated embryos showed a transient increase in Hif1 α mRNA levels (3-fold) at the morula stage compared to DMSO- and reversine-treated embryos (**Fig. 2A**), which returned to normal levels at the blastocyst stage. HIF1A protein was present in DMSO- and reversine-treated embryos and elevated at the morula and blastocyst stages in AZ3146-treated embryos ($n \sim 20$ embryos per treatment. Statistical test: Mann–Whitney U-test, error bars represent s.e.m) (**Fig. 2B**). HIF1A appeared to be mostly nuclear in morula, but mostly cytoplasmic in blastocysts, under all three conditions (**Fig. 2B**). Overall, these data suggest that

treatment with reversine, but not AZ3146, induces multiple stress pathways in pre-implantation embryos. These differences may contribute to the increased developmental potential of embryos treated with AZ3146 versus reversine.

HIF1A activity is required for proper blastocyst formation after Mps1 inhibition

It was previously shown that *Hif1a*^{-/-} embryos undergo developmental arrest and lethality by E11 (Iyer et al., 1998 [\[1\]](#)). To assess the role of HIF1A in the embryo's response to reversine and AZ3146, we used a pharmacological approach to inhibit its function. Briefly, we tested two different small molecules that have been shown to inhibit HIF1A activity, PX-478 (Lee & Kim, 2011 [\[2\]](#); Zhao et al., 2015 [\[3\]](#)) and IDF-11774 (Ban et al., 2017 [\[4\]](#)). We treated control zygotes with DMSO, PX-478 (2 μ M), or IDF-11774 (20 μ M) until the blastocyst stage (Fig. S3A [\[5\]](#)). Unlike PX-478, IDF-11774 treatment did not significantly affect the total number of cells in the TE or the whole blastocyst compared to the control (n $\sim$ 12 embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test) (Fig. S3B [\[6\]](#)). Therefore, we used IDF-11774 to inhibit HIF1A in subsequent experiments.

Next, we treated embryos with DMSO, reversine, or AZ3146 from the 4- to 8-cell stage, washed them, and then inhibited HIF1A with IDF-11774 from the 8-cell to blastocyst stage (Fig. 2C [\[7\]](#)). Inhibition of HIF1A did not abolish cavitation but dramatically lowered the number of TE and especially PE cells in AZ3146-treated embryos compared to DMSO-treated controls (Fig. 2D [\[8\]](#)) and compared to AZ3146-treated embryos not exposed to IDF-11884 (Fig. 1C [\[9\]](#)). Inhibition of HIF1A also lowered the number of cells in reversine-treated embryos compared to controls, but the effect was smaller (n=20 embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test) (Fig. 2D [\[10\]](#)). Overall, our data suggest that elevated HIF1A activity from the 8-cell stage onwards is particularly important to promote the survival of aneuploid TE and PE cells after AZ3146 treatment.

Taken together, our data suggest that AZ3146 and reversine have distinct effects on the pre-implantation embryo. Compared to reversine-treated embryos, AZ3146-treated embryos appear to have increased developmental potential, lack upregulation of p53, and show transient upregulation of *Hif1a*. Moreover, AZ3146-treated embryos have a greater dependence on HIF1A activity to form the TE and PE.

Hypoxia exposure attenuates DNA damage and blastomere defects in response to Mps1 inhibition

Pre-implantation development occurs in a hypoxic environment, and many clinics culture human embryos under hypoxic conditions (5%) (Houghton, 2021 [\[11\]](#)). It was reported that physiologic oxygen concentration ($\sim$ 5% oxygen) can improve the yield and quality of mammalian blastocysts (Ciray et al., 2009 [\[12\]](#); Nguyen et al., 2020 [\[13\]](#)) and increase the nuclear translocation of HIF1A in mouse blastocysts (Choi et al., 2021 [\[14\]](#)). To investigate how hypoxia affects the development of euploid and aneuploid mouse embryos, we repeated our experiments performed under normoxia (20% O₂/5% CO₂; control) (Fig. 1A [\[15\]](#)) in hypoxic (5% O₂/5% CO₂) conditions taking into consideration the standard practices in the IVF clinics (Knudtson et al., 2022 [\[16\]](#)) (Fig. 3A [\[17\]](#)). We cultured embryos from the 2-cell stage until the blastocyst stage, treating them with DMSO, reversine, or AZ3146 from the 4-8 cell stage.

First, we examined lineage specification by quantifying the cell numbers in all three lineages at the blastocyst stage. We found that DMSO-treated blastocysts cultured under hypoxia had a median of 74 cells, representing 14 EPI cells, 6 PE cells and 54 TE cells (n=18 embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test)

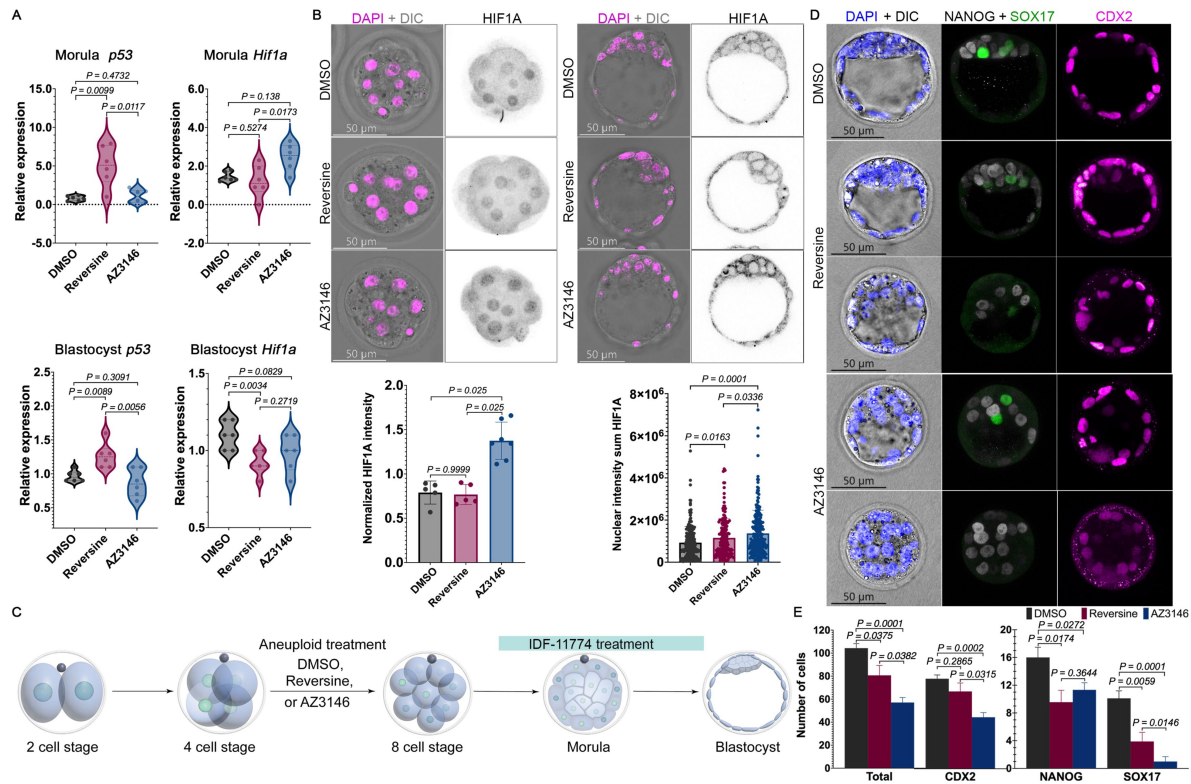


Figure 2

AZ3146-treated embryos elevate HIF1A activity to support formation of the TE and PE.

A) qPCR analysis of *p53* and *Hif1a* mRNA expression at morula and blastocyst stages reveals that *p53* is upregulated in reversine-treated embryos and that *Hif1a* is upregulated in AZ3146-treated embryos at morula stages (Sample: three biological replicates and two technical replicates per experiment with each replicate having a minimum of 16 embryos. Statistical test: Welch's t-test). B) Immunofluorescence against HIF1A (black) shows an increase in nuclear intensity in AZ3146-treated embryos at morula stages. At blastocyst stage, nuclear and cytoplasmic HIF1A are increased in AZ3146-treated embryos; normalization was based on DAPI staining (magenta). (n~20embryos per treatment. Statistical test: Mann-Whitney U-test, error bars represent s.e.m.) (C) Graphic representation of 4-cell embryos treated with DMSO and aneuploid drugs. Downregulation of HIF1A was achieved by treatment with IDF-11774 immediately after wash of the aneuploid drugs. Immunofluorescence analysis of lineage specification in blastocyst cultured with the HIF1A inhibitor IDF-11774 using antibodies against CDX2 (TE), NANOG (EPI) and SOX17 (PE). Importantly, IDF-11774 appears to affect cavitation of some AZ3146-treated embryos. (D) Lineage analysis at the blastocyst stage shows that TE and PE specification are affected by IDF-11774 treatment. Number of cells in each lineage was quantified to evaluate the effect on blastocyst development (n=20 embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m.).

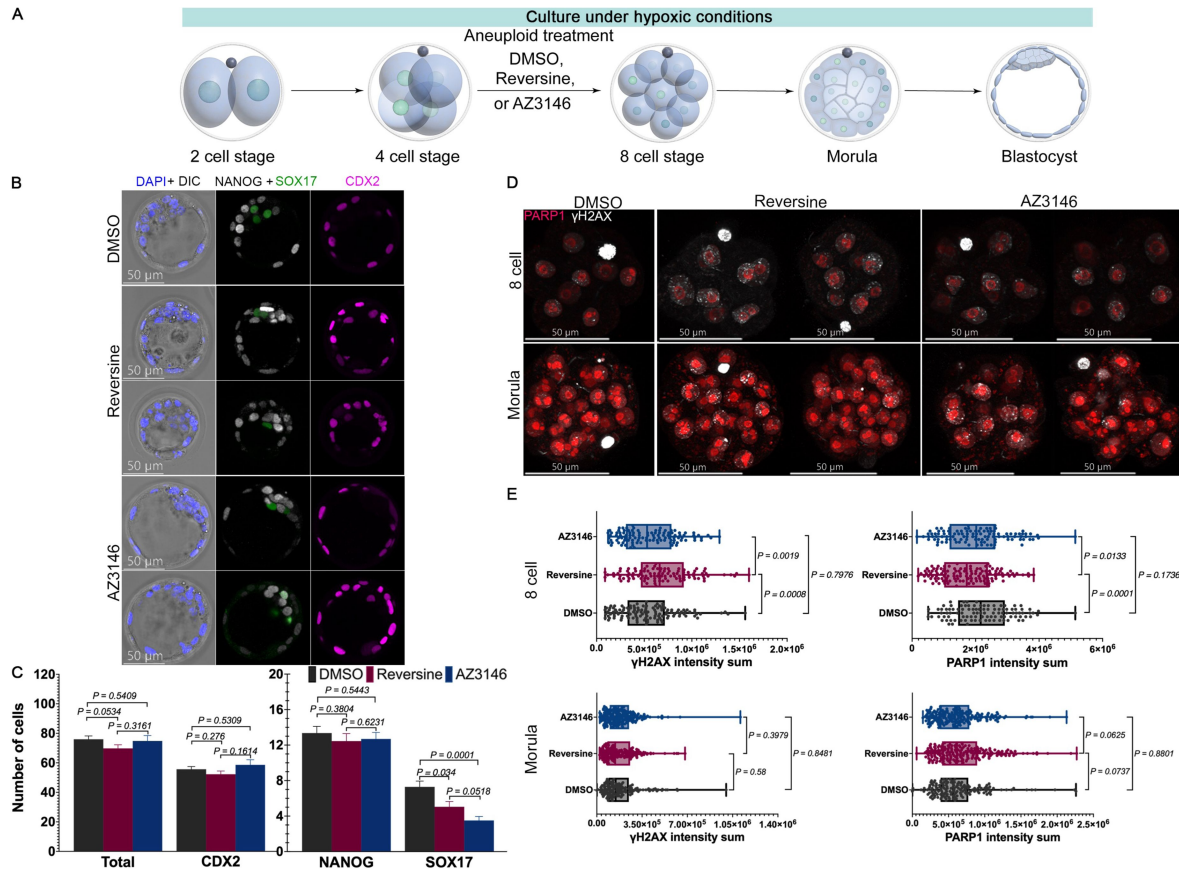


Figure 3

Hypoxia exposure reduces DNA damage and affects lineage proportions in the aneuploid blastocyst.

(A) Graphic representation of the hypoxia experiments. 2-cell embryos were cultured until the blastocyst stage in hypoxia conditions (5% oxygen). As before, 4-cell stage embryos were treated with DMSO or Mps1 inhibitors reversine and AZ3146 until the 8-cell stage. After washing, embryos were cultured to the mature blastocyst stage (E4.5) and analyzed for lineage specification. (B) Immunofluorescence imaging of well-known lineage markers CDX2 (TE), NANOG (EPI) and SOX17 (PE) reveals that overall embryonic morphology and cavitation is not affected by Mps1 inhibition or hypoxia. (C) Lineage analysis at blastocyst stage. Number of cells in each lineage was used to evaluate the effects in blastocyst development (n=18 embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m.). (D) Immunofluorescence against PARP1 (red) and γH2AX (white) in blastocyst after drug treatments. (E) Intensity analysis shows that, under hypoxia, at 8-cell stage, DNA damage is increased after exposure to reversine but not AZ3146. PARP1 expression is altered only at the 8-cell stage in reversine-treated embryos (n=25 embryos per treatment collected from three independent experiments. Statistical test: t-test, error bars represent s.e.m.).

(**Fig. 3B-C**). These data suggest that, under our conditions, the cell number in the pre-implantation mouse blastocyst is reduced when cultured in hypoxia compared to normoxia (**Fig. 3C**, **1C**), particularly in the TE and PE. Notably, reversine and AZ3146 treatment did not further reduce cell numbers for embryos cultured under hypoxia, except for in the PE (**Fig. 3C**).

To investigate how hypoxia exposure affects DNA damage and repair in mouse embryos, we again performed IF for γ H2A.X and PARP1 in 8-cell embryos and morula (**Fig. 3D-E**) (n=25 embryos per treatment collected from three independent experiments. Statistical test: t-test). We found that treated embryos with reversine and AZ3146 under hypoxia generated lower levels of γ H2A.X (**Fig. 3E**) compared with the same treatments under normoxia (**Fig. 1E**). Whereas PARP1 levels at the 8-cell stage are reduced in reversine-treated embryos (**Fig. 3E**), similar to normoxic conditions (**Fig. 1E**). Importantly, at the morula stages, the levels of PARP1 are relatively low compared to morula stage in normoxic conditions (**Fig. 1E**). Overall, these data suggest that hypoxia exposure lowers the accumulation of DNA damage between the 8-cell to morula stages, and results in a reduction of PARP1 at the morula stage.

Hypoxia increases the proportion of euploid cells in the epiblast of mosaic blastocysts

Despite the high incidence of mosaicism in human pre-implantation embryos, the fate of aneuploid cells in mosaic embryos is incompletely understood. We previously used reversine to generate a mouse model of pre-implantation chromosome mosaicism and found that aneuploid (Reversine-treated) cells are eliminated in the EPI of mosaic embryos via apoptosis, starting from the mature blastocyst stage (Bolton et al., 2016). Whether this response reflects reversine treatment specifically, or aneuploidy more generally, is not known. Moreover, how hypoxia versus normoxia affects the outcomes is also not clear.

To address these questions, we created aggregation chimeras at the 8-cell stage that contained a 1:1 ratio of AZ3146-treated and control blastomeres (DMSO/AZ3146 chimeras), which is expected to reflect low-grade mosaicism, or of AZ3146-treated and reversine-treated blastomeres (reversine/AZ3146 chimeras), which is expected to reflect medium-grade mosaicism (**Fig. 4A**). We cultured these chimeric embryos in normoxia and hypoxia, and followed the fate of individual blastomeres by using transgenic mouse lines with the membrane markers mTmG (Muzumdar et al., 2007) and E-cadherin (Christodoulou et al., 2019). DMSO/AZ3146 and reversine/AZ3146 blastocysts displayed proper lineage allocation, embryo morphology and cavitation in both normoxia and hypoxia (**Fig. 4B-C**). In addition, DMSO/AZ3146 and reversine/AZ3146 embryos had comparable total cell numbers in their blastocysts, and blastocysts grown in hypoxia were again smaller than those grown in normoxia (**Fig. 4D-E**). Intriguingly, despite having fewer total cells, DMSO/AZ3146 blastocysts had more EPI cells when they were cultured in hypoxia compared to normoxia (**Fig. 4D-E**).

We quantified the proportion of AZ3146 cells in each lineage for each chimera. In DMSO/AZ3146-treated blastocysts, we found that 46.75% of the TE and 42.88% of the EPI originated from AZ3146-treated blastomeres, compared to only 28.57% of the PE (**Fig. 4F**). In reversine/AZ3146 chimeras, 63% of the TE and 78.4% of the EPI originated from AZ3146-treated cells, compared to only 40% of the PE (**Fig. 4F**). Thus, under normoxia, DMSO cells appear to outcompete AZ3146-treated cells, which in turn outcompete Reversine-treated cells in the TE and EPI. AZ3146-treated cells appeared to be at a competitive disadvantage in the PE in both contexts. Culturing DMSO/AZ3146 chimeras in hypoxia increased the representation of AZ3146-treated blastomeres in the TE (49.06%) and PE (50%) but, strikingly, reduced their representation in the EPI (33.3%) (n>7 embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test) (**Fig. 4G**). These altered frequencies reflect a higher number of DMSO-treated cells in the EPI and a lower number in the PE (**Fig. 4E**). Hypoxia exposure of reversine/AZ3146 embryos lowered the contribution of AZ3146-treated blastomeres to the TE (55.94%) and EPI (50%) but not the PE

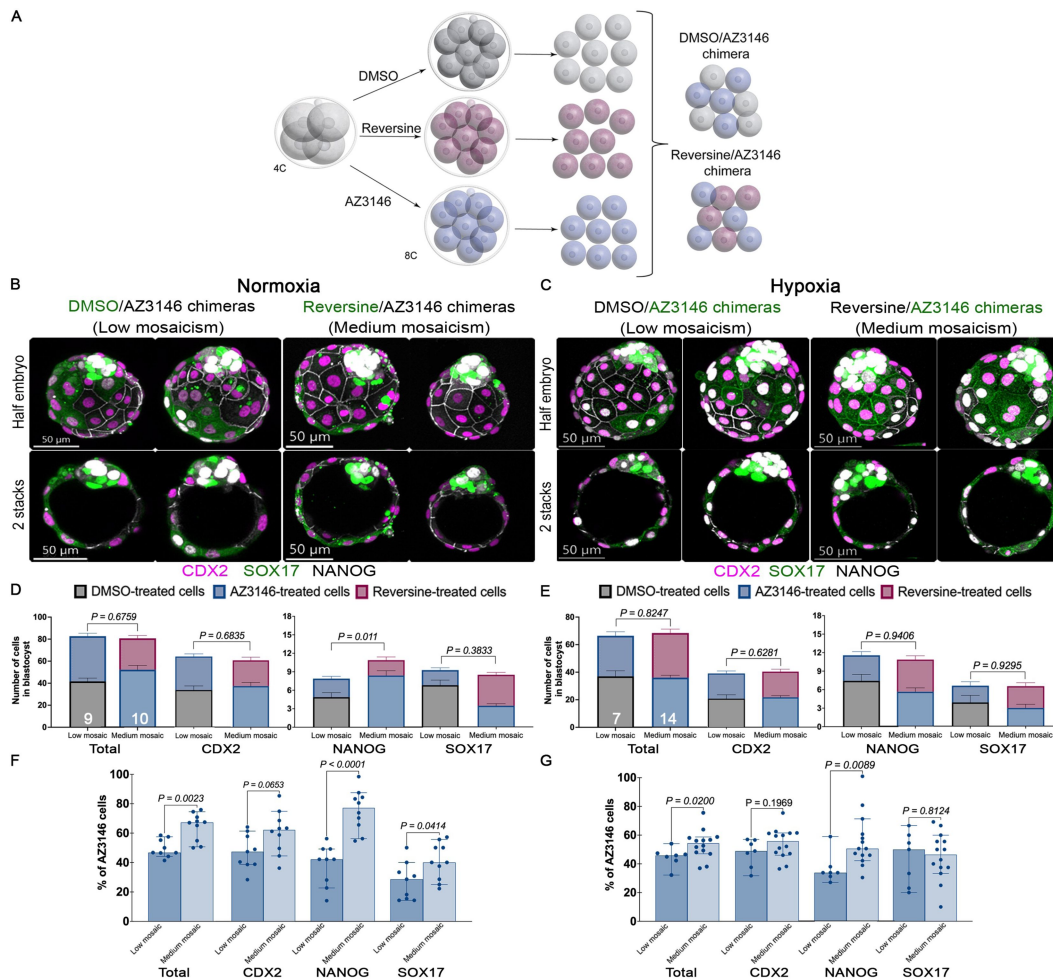


Figure 4

Hypoxia affects cell competition between diploid and aneuploid cells during pre-implantation development.

(A) Graphic representation of the cell competition experiments. Embryos were treated at the 4-cell stage with DMSO or Mps1 inhibitors reversine and AZ3146. After washing, 8-cell stage embryos were disaggregated, and re-aggregated to form chimeras containing a 1:1 ratio of DMSO/AZ314-treated blastomeres and Reversine/AZ314-treated blastomeres. Following aggregation, chimeras were cultured to the mature blastocyst stage (E4.5) and analyzed for lineage specification. For identification of the treatment, we use transgenics lines with membrane markers. Immunofluorescence for CDX2, NANOG, and SOX17 was performed to test lineage specification and allocation during (B) normoxia and (C) hypoxia. Lineage allocation quantification was based on the above markers. Importantly, (D) in normoxia, both chimeras have the same number of cells in all the lineages except the EPI. In addition, AZ3146-treated blastomeres outcompete reversine-treated blastomeres in medium-grade mosaics in the TE and the EPI. (E) Under hypoxia, both chimeras have similar number of cells in all the lineages. Yet, AZ3146-blastomeres do not outcompete reversine-treated blastomeres. Quantification of the contribution of AZ3146-treated blastomeres to the chimeras showed that, (F) under normoxia, compare with DMSO-treated cells, no preferential allocation of aneuploid cells occurs in the TE. In contrast, AZ3146-treated blastomeres increased their contribution when compare with reversine-treated blastomeres, but only for the EPI and the PE. (G) During hypoxia, in DMSO/AZ3146 chimeras no preferential allocation of aneuploid cells occurs in the TE. But preferential allocation of diploid cells to the EPI is observed. Whereas in Reversine/AZ3146 chimeras, AZ3146-treated blastomeres contribute similarly to reversine-blastomeres to the TE and PE but significantly increase contribution to the EPI. These results indicate that hypoxia favors the survival of reversine-induced aneuploid cells compared to their survival in normoxia (n>7 embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test, error bars represent s.e.m).

(46.43%) (**Fig. 4G**). We found that there was no correlation between the proportion of AZ3146-treated cells in the TE and the EPI in DMSO/AZ3146 and reversine/AZ3146 aggregation chimeras, under hypoxia or normoxia, and that hypoxia seemed to have a greater impact on the proportion of AZ3146 cells in reversine/AZ3146 aggregation chimeras (**Fig. S4**). Overall, these data suggest that hypoxia has lineage-specific effects on competitions between aneuploid and euploid cells and increases the contribution of euploid cells to the EPI.

Blastomeres in the 4-cell stage embryo display a lineage bias (Goolam et al., 2016). We considered that mosaicism generated before the 4-cell stage might influence lineage allocation. To test this possibility, we treated zygotes with reversine or AZ3146 during the first cleavage division. Importantly, reversine treatment at the zygote stage seems to strongly affect the morphology of the blastocysts (**Fig. S5A**). Consistent with this change in morphology, reversine-treatment reduced the number of cells in all lineages, particularly in the TE and PE (**Fig. S5B**). In contrast, AZ3146 treatment did not affect morphology or cell number in any of the lineages (**Fig. S5A-B**) ($n \sim 27$ embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test). To evaluate how early generation of aneuploidies in the embryos affect cell competition, we generated DMSO/AZ3146, Reversine/DMSO, and Reversine/AZ3146 aggregation chimeras at the 2-cell stage and cultured them under normoxia until the blastocyst stage (**Fig. S5C**). We found that all three 2-cell stage aggregation chimeras developed into blastocysts with proper lineage segregation and the presence of a cavity (**Fig. S5D**). Interestingly, the reversine-treated cells were extruded from the blastocyst in 54% of the reversine/DMSO chimeras and in 33% of the AZ3146/reversine chimeras, whereas no cell extrusion was observed in the DMSO/AZ3146 chimeras. Quantification of the proportion of AZ3146-treated cells in 2-cell stage derived chimeras (**Fig. S5E**) showed similar results as 8-cell stage derived chimeras (**Fig. 4F-G**). In DMSO/AZ3146-treated blastocysts, we found that 49.28% of the TE, 39% of the EPI and 33.33% of the PE originated from AZ3146-treated blastomeres (**Fig. S5E**). In reversine/AZ3146 chimeras, 68.24% of the TE, 68.83% of the EPI, and 66.67% of the PE originated from AZ3146-treated cells (**Fig. S5E**). In DMSO/reversine chimeras, 22.27% of the TE, 26.98% of the PE, and 31.7% of the EPI originated from reversine-treated cells. Overall, these results suggest that aneuploidy generated at different stages similarly affect the proportion of aneuploid cells in the blastocysts.

HIF1A inhibition reduces the frequency of aneuploid cells in DMSO/AZ3146 mosaic embryos

Our data suggest that hypoxia affects aneuploid-euploid cell competition in mosaic embryos (**Fig. 4**). To further assess the effect of HIF1A and, considering most human embryos are culture under hypoxic conditions, we decided to evaluate the effect of inhibiting HIF1A in mosaic embryos cultured in hypoxic conditions. We assembled DMSO/AZ3146 and Reversine/AZ3146 8-cell stage chimeras, treated them with the HIF1A inhibitor IDF-11774 (**Fig. 5A**, S6A-B), and then assessed lineage allocation in blastocysts. Treatment of DMSO/AZ3146 chimeras with IDF11774 does not affect lineage segregation in the blastocyst but reduces the cavity diameter (**Fig. 5B-C**). Additionally, diminish the contribution of AZ3146-treated cells to the TE, but DMSO-treated cells seem to compensate for this loss in the PE and EPI (**Fig. 5D**, **Fig. S6C**). Treating Reversine/AZ3146 chimeras with IDF11774 compromised blastocyst morphology and cavitation (**Fig. 5E-F**) and considerably increased the number of PE and EPI cells ($n > 8$ embryos per treatment collected from three independent experiments. Statistical test: Mann–Whitney U-test) (**Fig. 5G**), without altering the frequency of AZ3146-treated cells (**Fig. S6D**). Taken together, our data suggest that HIF1A promotes the survival of AZ3146-treated cells in DMSO/AZ3146 chimeras and suggest that inhibiting HIF1A could increase the proportion of karyotypically normal cells in mosaic embryos.

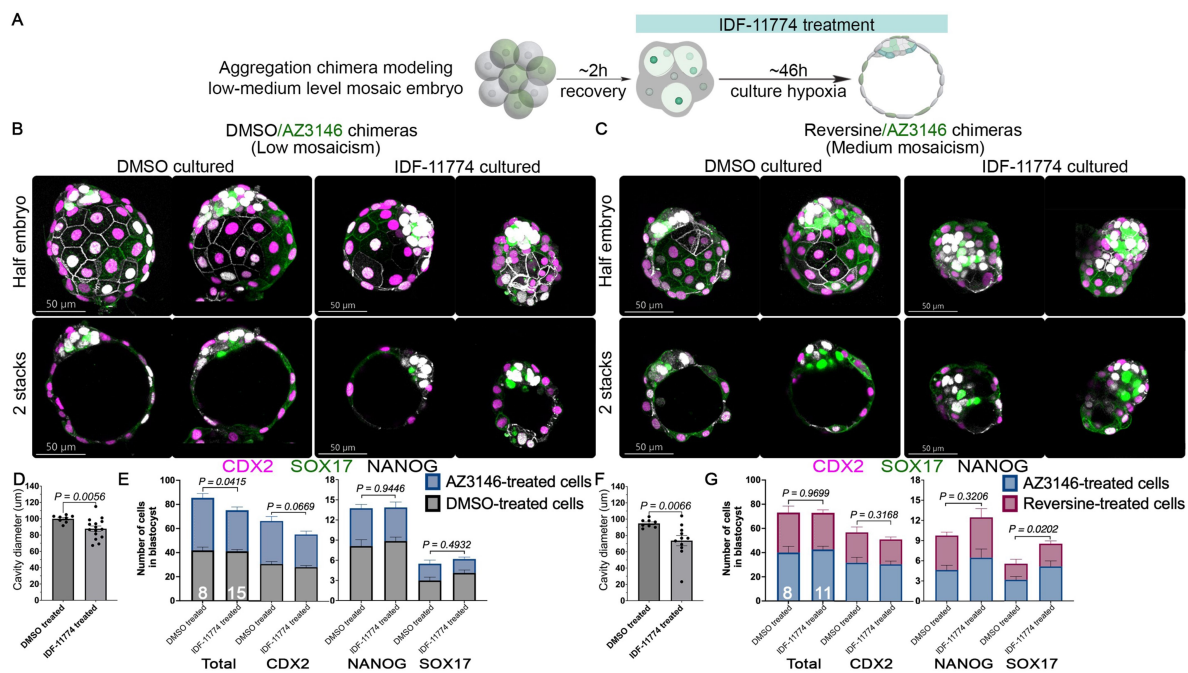


Figure 5

HIF1A inhibition increases the proportion of euploid cells in mosaic embryos.

(A) Schematic of HIF1A inhibition by IDF-11774 in 8-cell stage aggregation chimeras cultured in hypoxia. Immunofluorescence for CDX2, NANOG, and SOX17 was performed to test lineage specification and allocation. (B) HIF1A inhibition in low-grade mosaicism does not affect overall morphology but affects (C) cavity diameter. In addition, (D) lineage allocation quantification reveals a significant reduction of total cell number, as well as a reduction in cell number in the TE but not in the EPI and PE. In contrast, (E) HIF1A inhibition in medium-grade mosaicism affects morphology, and (F) cavitation of the mosaic embryos. However, lineage allocation quantification revealed that total cell number is not affected (G) in medium-grade mosaicism after IDF-11774 treatment. Nevertheless, HIF1A inhibition appears to increase the cell number of the PE. These results show that HIF1A inhibition in hypoxic conditions differentially affect each type of mosaic embryos. (n>8 embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m).

Discussion

Aneuploidy is a frequent outcome of early embryonic divisions (Allais & FitzHarris, 2022 [↗](#); Currie et al., 2022 [↗](#); Palmerola et al., 2022b [↗](#)). Here, we describe complementary strategies for inducing aneuploidy in mouse embryos and reveal how the embryo's response varies depending on the nature of the insult, clonal lineage, and environmental context. Our findings support four key conclusions: 1) the Mps1 inhibitor AZ3146 induces aneuploidy while preserving DNA repair capacity and dampening stress responses, compared to the broader-acting inhibitor reversine; 2) Lineage specific responses to aneuploidy emerge during blastocyst development, consistent with our earlier findings (Bolton et al., 2016 [↗](#); Singla et al., 2020 [↗](#)); 3) HIF1A promotes survival of extraembryonic lineages (TE and PE) in AZ3146-treated embryos, whereas PARP1 is particularly required in the EPI of both AZ3146- and reversine-treated embryos; 4) Hypoxia enhances DNA repair and influences the competitive dynamics between aneuploid and euploid cells in mosaic embryos.

Reversine-treated embryos upregulate mRNA levels of senescent markers p53 and p21 (Singla et al., 2020 [↗](#)) and fail to give rise to viable embryos (Bolton et al., 2016 [↗](#)). Our current results further indicate that reversine downregulates PARP1, consistent with previous findings in glioma cells (Hirakata et al., 2021 [↗](#)), thereby exacerbating DNA damage and limiting DNA repair capacity. In contrast, AZ3146-treated embryos maintain PARP1 expression and exhibit increased HIF1A activity, which appears to support the survival of extra-embryonic lineages and improve overall developmental potential.

These findings intersect with a broader conversation about embryo culture conditions. It has been proposed that standard culture practices shift from normoxia (20% oxygen) to more physiological hypoxia (5% oxygen) to better recapitulate the *in vivo*-like oviductal environment (Alva et al., 2022 [↗](#)). Indeed, hypoxic culture conditions have been shown to improve the quality of mammalian blastocysts (Ciray et al., 2009 [↗](#); Nguyen et al., 2020 [↗](#)), though the underlying mechanisms remain unclear. Studies in somatic cells suggest that hypoxia enhances DNA repair pathways (Marti et al., 2021 [↗](#); Nakamura et al., 2022 [↗](#); Pietrzak et al., 2018 [↗](#)). In line with this, we observed reduced DNA damage in aneuploid embryos cultured under hypoxic conditions, consistent with prior reports of lower γ H2A.X levels in mouse blastocysts recovered from the uterus or cultured in low oxygen (Houghton, 2021 [↗](#); Meuter et al., 2014 [↗](#)). Furthermore, recent work in cancer cells indicates that hypoxia may promote HIF1A-PARP1 interactions, enhancing DNA repair (Marti et al., 2021 [↗](#); Nakamura et al., 2022 [↗](#); Pietrzak et al., 2018 [↗](#)). Together, these findings highlight the interplay between chromosomal stress, lineage context, and the embryonic microenvironment in shaping cell fate during early development. A deeper mechanistic understanding of how DNA repair is regulated at pre-implantation stages will be crucial for elucidating how embryos tolerate or eliminate aneuploid cells — with important implications for improving embryo culture systems and fertility treatments.

Confined placental mosaicism, in which the placenta is aneuploid while the foetus is euploid, is a common phenomenon in human pregnancies (McCoy, 2017 [↗](#)). In previous work, we showed that aneuploid TE cells exhibit prolonged cell cycles and signs of senescence, whereas aneuploid ICM cells have an increased frequency of apoptosis in reversine-treated embryos (Singla et al., 2020 [↗](#)). These findings suggest that abnormal, yet viable TE cells, may contribute to confined placental mosaicism. In the present study, we found that in AZ3146-treated embryos cultured under normoxic conditions, aneuploid cells contributed predominantly to the TE, followed by the EPI, and were especially depleted from the PE. Under hypoxic conditions, however, we observed a shift: aneuploid cells were more frequently eliminated from the EPI and enriched in the PE. These observations suggest that hypoxia modulates lineage-specific cell competition in mosaic embryos, potentially promoting a higher proportion of euploid cells in the EPI relative to the PE and TE.

Moreover, we confirmed that the proportions of aneuploid cells in the TE and EPI do not correlate, consistent with the preferential survival of aneuploid cells in the TE and their selective elimination from the ICM. This is in line with clinical data showing low concordance of aneuploidy between TE and ICM in human mosaic embryos (30–40%) (Dahdouh & Garcia-Velasco, 2021 [↗](#)). Lineage-specific elimination of aneuploid cells by apoptosis has been described in an *in vitro* model of human post-implantation development using reversine (Yang et al., 2021 [↗](#)). Our findings mirror this distinction and further suggest that mechanisms of DNA repair—possibly involving PARP1 and HIF1A—may underlie the differential survival of aneuploid cells across lineages. Investigating how these pathways operate during post-implantation development will be crucial for understanding the developmental trajectories of mosaic embryos and the basis of placental mosaicism.

Since low- and medium-grade mosaic human embryos have similar developmental potential to fully euploid embryos (Capalbo et al., 2021 [↗](#); Greco et al., 2015 [↗](#)), understanding the molecular pathways that govern the response to aneuploidy holds important translational promise. Given that the extent and proportion of aneuploid cells in mosaic embryos impact embryo viability (Capalbo et al., 2021 [↗](#)), strategies that reduce the aneuploid cell burden at the blastocyst stage may enhance the likelihood of a successful pregnancy. In this study, we targeted HIF1A as a potential regulator of aneuploid cell persistence and found that treatment with the HIF1A inhibitor IDF-11774 increased the proportion of euploid cells in mosaic embryos. Taken together, our findings reveal that specific environmental and molecular factors can modulate the composition of mosaic embryos, with potential implications for improving embryo quality and reproductive outcomes.

Materials and methods

Pre-implantation embryo culture

Mice were maintained according to national and international guidelines. Four- to six-week-old B6SJLF1/J female mice were injected with 7.5 IU PMSG followed by 7.5 IU hCG 48 h later, to induce superovulation. The females were then mated with B6CBAF1/J males or were indicated, with E-cadherin GFP or mTmG transgenic males. Pre-implantation mouse embryos were recovered 24 h and 40 h after the hCG injection to obtain zygotes and 2-cell stage embryos, respectively in M2 medium (Sigma, M7167). We incubate the embryos in KSOM until 4-cell stage for the aneuploid treatment, around 53 h post hCG. When recovering zygotes, cumulus cells were removed with 0.3% hyaluronidase (Sigma, H4272) in M2. Embryos were cultured in regular KSOM (Sigma, MR-106) at 37°C under 5% CO₂/air (Normoxia) or with pre-mixed 5% CO₂/ 5% O₂ balance with nitrogen, biologic atmosphere batch (Airgas #Z03NI9022000033) (Hypoxia). Normoxia in our study was defined as the standard atmospheric oxygen levels in culture incubators (Alva et al., 2022 [↗](#)), whereas hypoxia (5% oxygen concentration) was the standard level of oxygen in the IVF clinics and can be used to better model physiological oxygen (physoxia), which varies from ~1.5% to 8% (Alva et al., 2022 [↗](#); Knudtson et al., 2022 [↗](#)). B6SJLF1/J females and B6CBAF1/J males were obtained from JAX laboratories. Females were received at 3–4 weeks of age and were maintained in the CALTECH animal facility, where they were housed with 5 same-sex littermates on a 12 h light/12 h dark cycle with food and water *ad libitum*. The temperature in the facility was controlled and maintained at 21 °C. All experimental procedures involving the use of live animals, or their tissues were performed in accordance with the NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee at the California Institute of Technology (Caltech, protocol number 1772). Reversine (Sigma, R3904), AZ3146 (Sigma, SML1427), PX-478 (Selleckchem, S7612), IDF-11774 (Selleckchem, S8771) and olaparib (Selleckchem, S1060) were dissolved in DMSO (Sigma, D2650) before use to specific concentration. They were respectively used at following final concentrations: 0.5 μM, 20 μM, 2 μM,

20 μ M and 10 μ M. Control embryos were incubated in the equivalent DMSO concentration. Drugs were dissolved in regular KSOM to the concentration of use. DMSO concentration in media should never pass 0.4%.

Immunofluorescence

Embryos were fixed in 4% PFA (Thermo Scientific, AA47340) for 20 min at room temperature (RT). Following by three washes with 0.1% Tween-20 (Sigma, P1379) dissolved in PBS (PBST). The embryos were then permeabilized with 0.3%, Triton X-100 (Sigma, X100) in PBS. Washes were then performed three times in PBST before embryos were transferred to blocking solution (3% BSA in PBS) for at least 3h at RT. Incubation with primary antibodies was performed in blocking solution overnight at 4°C. Next day, washes were performed three times in PBST before incubation with Alexa Fluor secondary antibodies (Thermo Fisher Scientific, 1:500) in blocking solution for 2 h. Washes were performed three times before incubation with DAPI (Thermo Fisher Scientific) for 5 min. Washes after DAPI were performed two times in PBST before the final incubation in M2. Embryos were then mounted in M2 micro-drops on 35 mm glass bottom dish (MATTEK, P35G-1.5-14-C). Confocal imaging was carried out using Leica SP8, 40x objective, 1 μ M Z-step. Image files were viewed and analyzed using ImageJ and IMARIS 9.9 software.

Primary antibodies used: mouse anti-CDX2 (Biogenex, 1:500), rabbit anti-NANOG (1:500; Abcam), goat anti-SOX17 (1:300; R&D Systems), rabbit anti-HIF1A (1:300, Novusbio), mouse anti-PARP1 (1:500, Proteintech) and rabbit anti-Phospho-Histone H2AX (1:500, R&D Systems).

In situ chromosome counting

For determination of aneuploidy, treated 8-cell stage embryos were synchronized in metaphase by 10h treatment with 0.03 μ g/mL colcemid (Cayman, 15364) diluted in KSOM. Following by 1h treatment with 10 μ M Mg132 (Selleckchem, S2619) in KSOM. Finally, 1 h treatment with 5 μ M dimethylenastron (MedChemExpress, HY-19944) and 10 μ M Mg132 in KSOM. Synchronized embryos were then fix in 2% PFA for 20 min, permeabilized with PBST for 15 min and blocked 3h at RT before incubation with human anti-centromere protein antibody (1:300, Antibodiesinc 15-234) overnight at 4°C. Next day, three washes with PBST were performed before 2 h incubation with goat anti-Human secondary antibody, Alexa Fluor 647 (1:400, Invitrogen A-21445) in blocking solution. Washes were performed three times before 20 min incubation with DAPI (1:500) and Alexa Fluor 488 Phalloidin (1:300, Invitrogen A12379). Washes then were performed three times before clearing overnight with AF1 plus (Citifluor, AF1/DAPI-15). Embryos were then mounted in AF1 plus micro-drops on 35 mm glass bottom. Confocal imaging was carried out using Leica SP8, 60x objective, 0.5 μ M Z-step. Image files were viewed and analyzed using ImageJ and IMARIS 9.9 software.

Embryo transfers and post implantation recovery and biopsy

Embryo transfer were performed as described previously (Bermejo-Alvarez et al., 2014 [\[1\]](#)). CD1 females and vasectomized CD1 males were obtained from Charles River. Reversine and AZ3146-treated blastocyst were transfer into 2.5-days pseudopregnant CD1 females. Around 16 AZ3146-treated embryos were transfer in the right uterine horn, whereas around 16 reversine-treated embryos were transfer in the left uterine horn of the same female as control. To evaluate implantation and embryo survival potency, uterine horns were recovered 5 days after surgery. Deciduas were dissected and post implantation embryos at stage E9.5 were recovered into PBST on ice. Embryos were fixed in 4% PFA at RT for 1 h, followed by washes through PBST before imaging. Images were taken using an Olympus LS stereo microscope with a 10x objective. Uterine transfers were performed in accordance with the NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee at the California Institute of Technology (CALTECH).

qRT-PCR

Around 14 to 17 morulas and blastocysts were collected for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Total RNA from morulas was obtained using the NucleoSpin RNA Plus XS kit (Takara, 740990.10). Whereas total RNA from blastocyst was extracted using the Arcturus PicoPure RNA Isolation Kit (Thermo Fisher, KIT0204). qRT-PCR was performed using the Power SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, 4389986) in a StepOne Plus Real-time PCR machine (Applied Biosystems). The following program was used: 30 min 48 °C (reverse-transcription) followed by 10 min 95 °C followed by 45 cycles of 15 s 95 °C (denaturing) and 1 min 60 °C (annealing and extension). The ddCT method was used to determine relative levels of mRNA expression, with Ppia as an endogenous control (Gu et al., 2014 [DOI](#)). Primers were obtained from IDT.

Name	Sequence
p53_Fw	GTCACAGCACATGACGGAGG
p53_Rv	TCTTCCAGATGCTCGGGATAC
Hif1a_Fw	CCTGCACTGAATCAAGAGGTTGC
Hif1a_Rv	CCATCAGAAGGACTTGCTGGCT
Ppia_Fw	GAGCTCTGAGCACTGGAGAGA
Ppia_Rv	CCACCCTGGCACATGAAT

Generation of chimeric embryos

Chimeric embryos were generated following a previous publish protocol (Eakin & Hadjantonakis, 2006 [DOI](#)). Briefly, wild-type, E-cadherin GFP or mTmG 8-cell stage embryos were transfer to micro-drops of M2 after aneuploid treatment. Zona pellucida was removed by treatment with acidic Tyrode's solution (Sigma, T1788). The embryos were then incubated in Ca²⁺/Mg²⁺-free M2 (made in house) for 5 min and then disaggregated into individual blastomeres by gentle mouth pipetting. Mosaic chimeras were form by aggregation of four and four (1:1) of each treatment. Low mosaicism DMSO/AZ3146-treated blastomeres (1:1). Whereas medium mosaicism Reversine/AZ3146-treated blastomeres (1:1). Culture of the chimeras were performed under normoxic and hypoxia condition in KSOM for 48 h to reach blastocyst stage.

Statistical analysis

The statistical tests used are indicated in the corresponding figure legends. Calculations were carried out in Microsoft Excel and data analysis and visualization in Prism 9 software. All graphs show mean values, error bars: s.e.m.

Supplementary figures

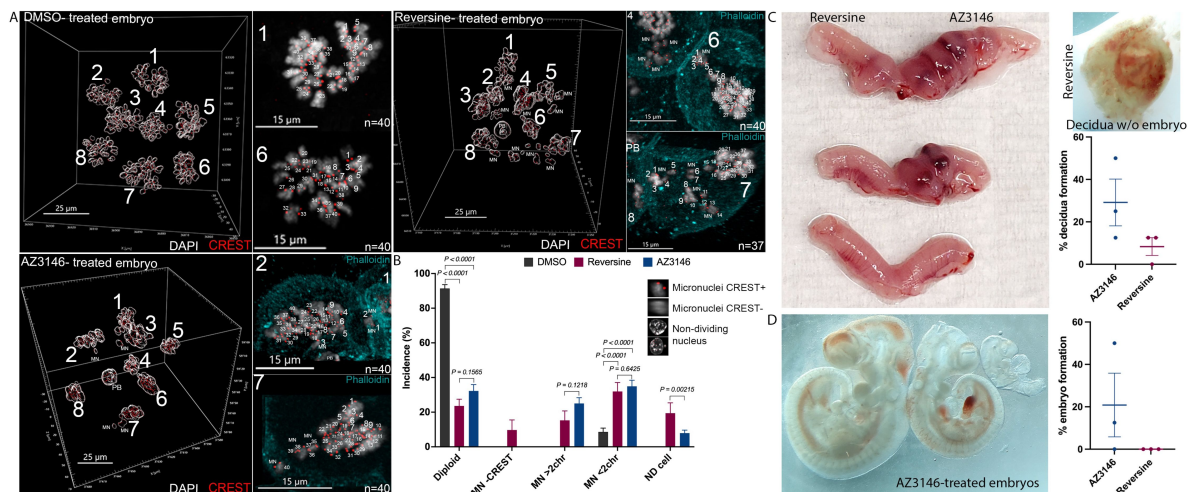


Figure S.1

AZ3146-treated embryos are aneuploid and can develop to post-implantation stages.

(A) *In situ* chromosome counting of 8-cell stage embryos after drug treatments. Representative images of individual embryos in the different treatments. DNA (white) is visualized by DAPI staining and kinetochores (red) by CREST antibody. PB (polar body), MN (micronuclei). Each chromosome presents 2 CREST dots. The large number is the cell ID and the smaller number indicates the number of chromosomes, "n". Diploid blastomeres present 40 pairs of CREST dots. Phalloidin (cyan) was used for cell segmentation. (B) Quantification of the incidence of aneuploid events, designated as those with more than 2 chromosomes (MN>2chr), less than 2 chromosomes (MN<2chr), without kinetochores (MN-CREST) and non-dividing nucleus (ND cell). We observed an increase in MN following reversine and AZ3146 treatment. Importantly, reversine-treated cells have a higher number of ND cells and MN-CREST staining. (n=256 cell DMSO treatment, n=304 cells AZ3146 treatment, n=144 cells reversine treatment, n~20 embryos per treatment. Statistical test: Mann-Whitney U-test, error bars represent s.e.m). (C) Embryo transfer experiments show higher implantation events (% decidua formation) in AZ3146-treated embryos. The left uterine horn was used for transfer of reversine-treated blastocyst and the right uterine horn for AZ3146-treated blastocyst. (n=7 embryos transplanted per uterine horn, repeated experiments two times) (D) Only AZ3146-treated blastocyst can develop post-gastrulation E9.5 fetuses, image representing three out of five surviving embryos (% embryo formation).

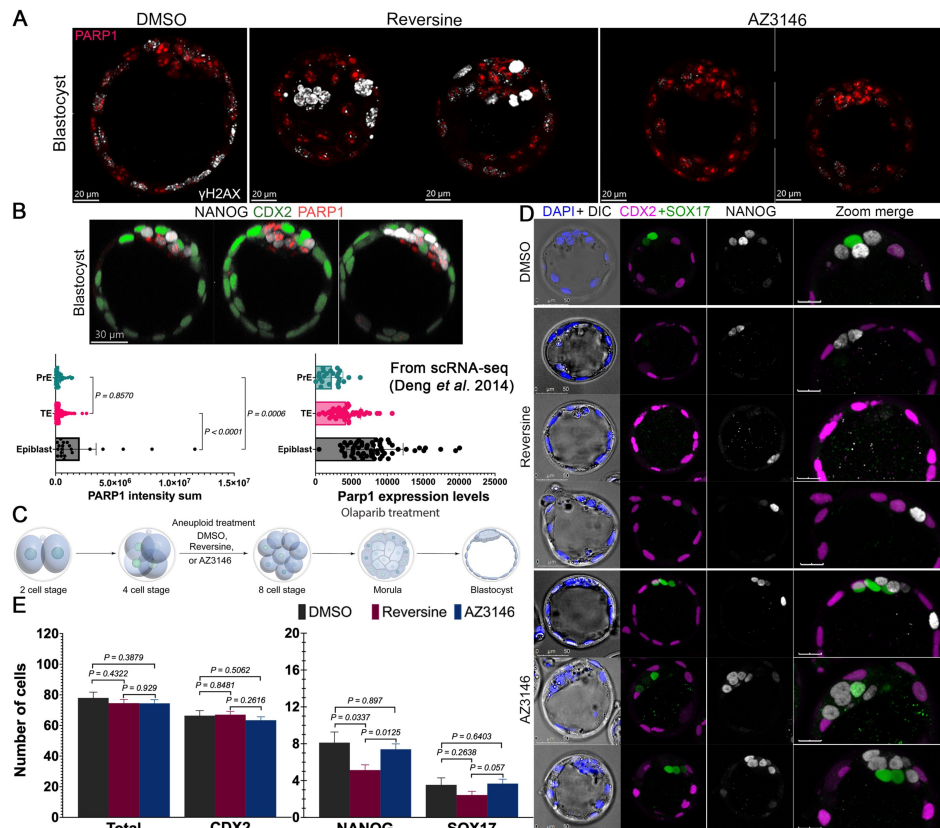


Figure S.2

PARP1 is required for ICM development.

(A) Immunofluorescence against PARP1 (red) and γ H2AX (white) in blastocyst after drug treatments. Notice accumulation of DNA damage in reversine-treated blastocyst (Representative images of $n > 12$ embryos per treatment collected from three independent experiments). (B) Immunofluorescence against PARP1 (red), NANOG (white, epiblast) and CDX2 (green, trophoctoderm) at the blastocyst stage in untreated embryos. Analysis of PARP1 intensity shows an increase in the EPI lineage ($n > 21$ embryos per treatment collected from three independent experiments). Statistical test: Mann-Whitney U-test, error bars represent s.e.m.). To confirm these observation, scRNA-seq (Deng et. al 2014) shows enrichment of *Parp1* mRNA specifically in the EPI. (C) Graphic representation of 4-cell embryos treated with DMSO and aneuploid drugs. Downregulation of PARP1 was achieved by treatment with Olaparib from morula to blastocyst stage. (D) Effects of the inhibition of PARP1 function were assessed by immunofluorescence against CDX2 (TE), NANOG (EPI) and SOX17 (PE) in the different treatments. Importantly, Olaparib treatment does not affect blastocyst morphology. (E) Lineage analysis at blastocyst stage shows a specific effect on the survival of epiblast and PE cells in reversine-treated embryos. Ratio of cells in each lineage in the blastocyst is normalized based on DMSO treatment ($n = 15$ embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m).

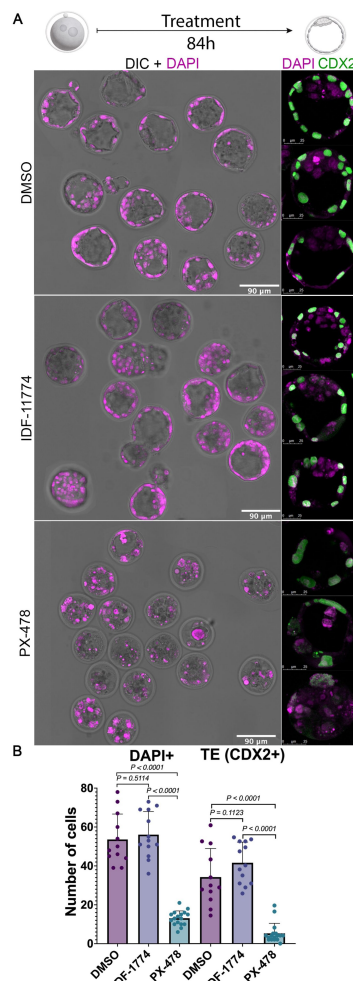


Figure S.3

Pharmacological inhibitors of HIF1A have distinct effects on mouse pre-implantation embryos.

(A) Inhibition of HIF1A from zygotes to blastocyst stage with PX-478 or IDF-11774 in normoxia. Immunofluorescence against CDX2 (TE) and DAPI (magenta) was used to assess blastocyst development. Importantly, IDF-11774 does not compromise pre-implantation development, whereas PX-478 compromised the survival and overall morphology of the blastocyst. (B) Analysis of the number of trophectoderm cells (CDX2) and total number (DAPI) shows a deleterious effect of PX-478 during blastocyst development ($n \sim 12$ embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m).

A

Correlation of AZ3146+ cells between trophectoderm and epiblast lineages

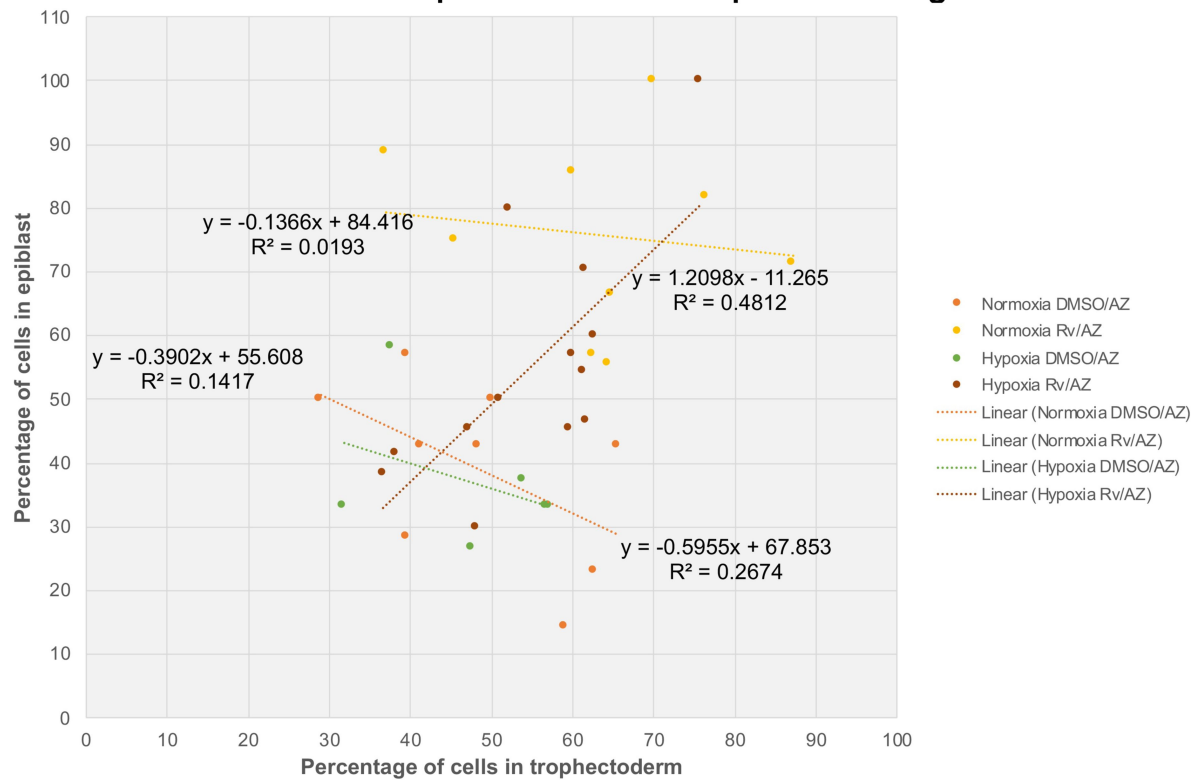


Figure S.4

Correlation plot of AZ3146-treated cells in the epiblast and trophectoderm.

Scatter plot of AZ3146-treated cells in epiblast and trophectoderm in DMSO/AZ3146 and Reversine/AZ3146 mosaic blastocysts cultured under normoxic and hypoxic conditions.

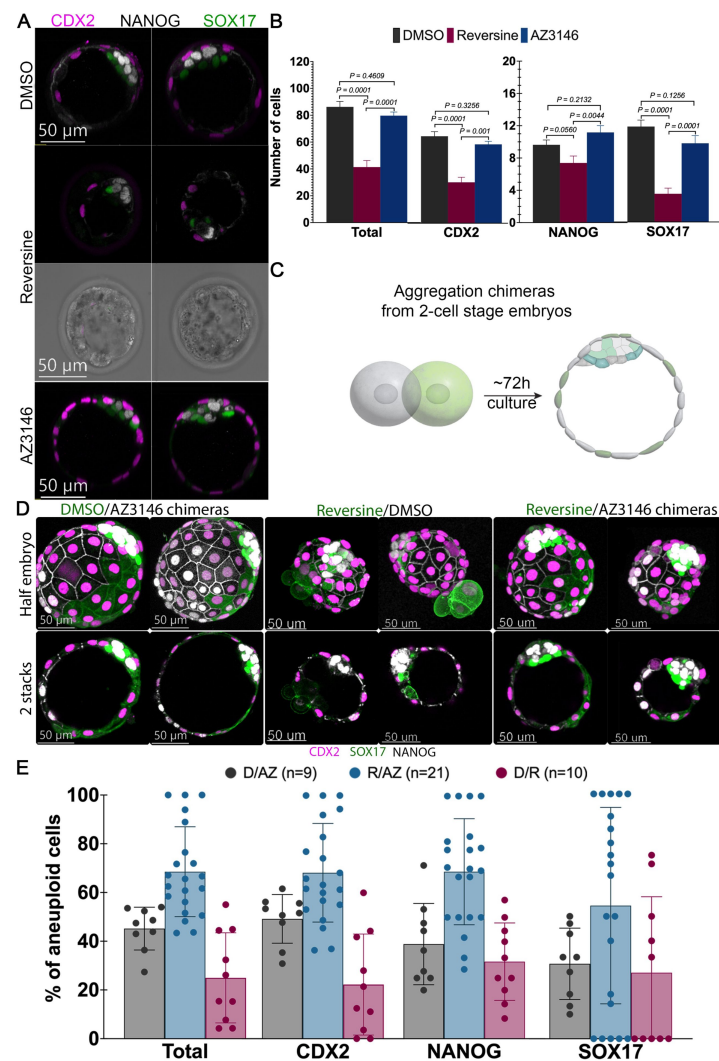


Figure S.5

Aneuploidies generated before the 2-cell stage does not affect lineage specification in mosaic embryos.

(A) Immunofluorescence for CDX2, NANOG, and SOX17 was performed in blastocysts after zygote treatment with Mps1 inhibitors. Importantly, reversine treatment at zygote stages affects morphology of blastocyst. (B) Lineage analysis of blastocyst shows no effect in AZ3146-treated embryos. Whereas reversine treatment reduces the cell number to almost half. Ratio of cells in each lineage in the blastocyst is normalized based on DMSO treatments. (n=27 embryos per treatment collected from three independent experiments. Statistical test: Mann-Whitney U-test, error bars represent s.e.m). (C) Aggregation chimeras at 2-cell stage were created by using transgenics lines with membrane markers: mTmG (green) and E-cadherin (white). Subsequently, (D) immunofluorescence for CDX2, NANOG, and SOX17 was performed to quantify lineage allocation. Our results show no effect on embryo morphology in our chimeras. However, reversine/DMSO (D/R) chimeras seems to have increase events of cell extrusion and reversine/AZ3146 chimeras are smaller in number of cells compare with DMSO/AZ3146 chimeras. (E) Data show the proportion of AZ3146-treated cells in DMSO/AZ3146 mosaic blastocysts (grey) and in reversine/AZ3146 mosaic blastocysts (blue), as well as the proportion of reversine-treated cells in DMSO/reversine mosaic blastocysts (red).

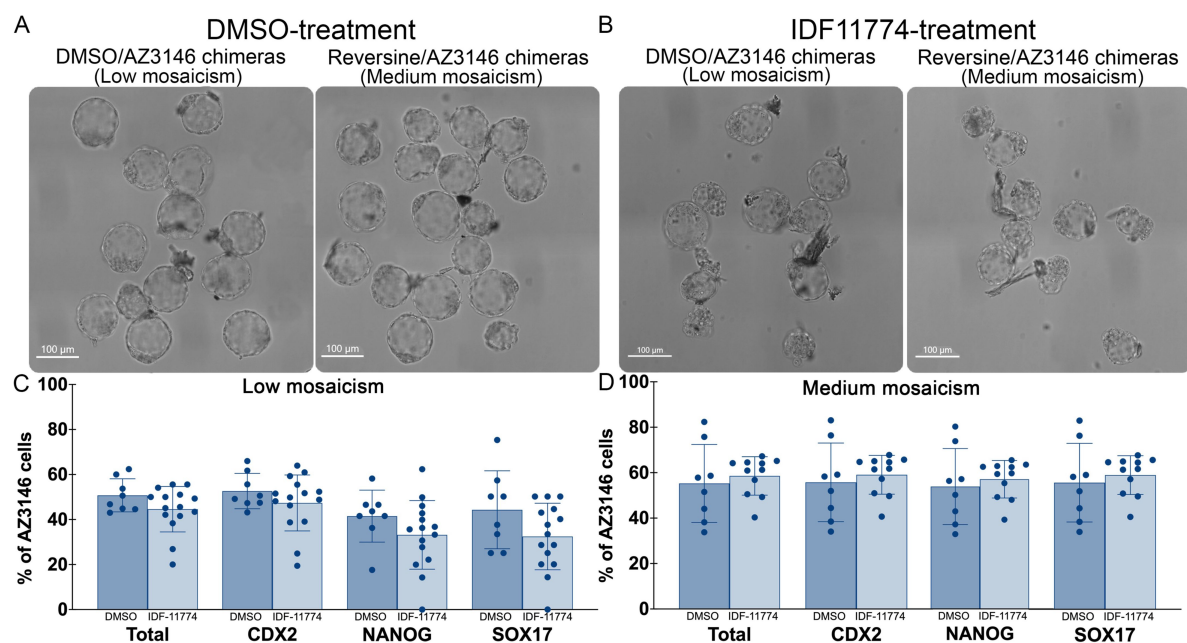


Figure S.6

IDF-11774-mediated inhibition of HIF1A increases the proportion of euploid cells in mosaic embryos.

Brightfield images of low- and medium-mosaic chimeras grown in normoxia and treated with (A) DMSO and (B) IDF-11774. Quantification of the contribution of AZ3146-treated blastomeres to the chimeras showed that, (C) in DMSO/AZ3146 mosaics, IDF-11774 treatment reduces the proportion of AZ3146-treated blastomeres, whereas (D) in reversine/AZ3146 mosaics, IDF-11774 treatment slightly increases the proportion of AZ3146-treated blastomeres. These results may indicate that HIF1A inhibition impairs the less fit population in chimeric embryos.

Acknowledgements

This work was supported by M.Z.G' National Institutes of Health R01 (R01HD101489) grant. E.S.V. is supported by a Pew Latin America fellowship. We thank the Life Science Editors and the Life Science Editors Foundation for invaluable comments and suggestions on the manuscript, and Ariane Helou for copy editing. Data analysis was performed in the Biological Imaging Facility, with the support of the Caltech Beckman Institute and the Arnold and Mabel Beckman Foundation.

Additional information

Author contributions

E.S.V designed and conducted the experiments; analyzed and interpreted the data. E.S.V and M.Z.G conceived the project. M.E.B and M.Z.G supervised the study.

Funding

National Institutes of Health (R01HD101489)

References

- Abuetabh Y., Wu H. H., Chai C., Al Yousef H., Persad S., Sergi C. M., Leng R. (2022) **DNA damage response revisited: the p53 family and its regulators provide endless cancer therapy opportunities** *Exp Mol Med* **54**:1658–1669 <https://doi.org/10.1038/s12276-022-00863-4> | [Google Scholar](#)
- Allais A., FitzHarris G (2022) **Absence of a robust mitotic timer mechanism in early preimplantation mouse embryos leads to chromosome instability** *Development* **149** <https://doi.org/10.1242/dev.200391> | [Google Scholar](#)
- Alva R., Gardner G. L., Liang P., Stuart J. A (2022) **Supraphysiological Oxygen Levels in Mammalian Cell Culture: Current State and Future Perspectives** *Cells* **11** <https://doi.org/10.3390/cells11193123> | [Google Scholar](#)
- Ban H. S., Kim B. K., Lee H., Kim H. M., Harmalkar D., Nam M., Park S. K., Lee K., Park J. T., Kim I., Lee K., Hwang G. S., Won M (2017) **The novel hypoxia-inducible factor-1alpha inhibitor IDF-11774 regulates cancer metabolism, thereby suppressing tumor growth** *Cell Death Dis* **8**:e2843 <https://doi.org/10.1038/cddis.2017.235> | [Google Scholar](#)
- Bermejo-Alvarez P., Park K. E., Telugu B. P (2014) **Utero-tubal embryo transfer and vasectomy in the mouse model** *J Vis Exp* **84**:e51214 <https://doi.org/10.3791/51214> | [Google Scholar](#)
- Bolton H., Graham S. J. L., Van der Aa N., Kumar P., Theunis K., Fernandez Gallardo E., Voet T., Zernicka-Goetz M. (2016) **Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential** *Nat Commun* **7**:11165 <https://doi.org/10.1038/ncomms11165> | [Google Scholar](#)
- Capalbo A., Poli M., Rienzi L., Girardi L., Patassini C., Fabiani M., Cimadomo D., Benini F., Farcomeni A., Cuzzi J., Rubio C., Albani E., Sacchi L., Vaiarelli A., Figliuzzi M., Findikli N., Coban O., Boynukalin F. K., Vogel I., ..., Simon C (2021) **Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial** *Am J Hum Genet* **108**:2238–2247 <https://doi.org/10.1016/j.ajhg.2021.11.002> | [Google Scholar](#)
- Choi J., Kim W., Yoon H., Lee J., Jun J. H (2021) **Dynamic Oxygen Conditions Promote the Translocation of HIF-1alpha to the Nucleus in Mouse Blastocysts** *Biomed Res Int* **2021**:5050527 <https://doi.org/10.1155/2021/5050527> | [Google Scholar](#)
- Christodoulou N., Weberling A., Strathdee D., Anderson K. I., Timpson P., Zernicka-Goetz M (2019) **Morphogenesis of extra-embryonic tissues directs the remodelling of the mouse embryo at implantation** *Nat Commun* **10**:3557 <https://doi.org/10.1038/s41467-019-11482-5> | [Google Scholar](#)
- Ciray H. N., Aksoy T., Yaramanci K., Karayaka I., Bahceci M (2009) **In vitro culture under physiologic oxygen concentration improves blastocyst yield and quality: a prospective randomized survey on sibling oocytes** *Fertil Steril* **91** (4 Suppl):1459–1461 <https://doi.org/10.1016/j.fertnstert.2008.07.1707> | [Google Scholar](#)

- Currie C. E., Ford E., Benham Whyte L., Taylor D. M., Mihalas B. P., Erent M., Marston A. L., Hartshorne G. M., McAinsh A. D (2022) **The first mitotic division of human embryos is highly error prone** *Nat Commun* **13**:6755 <https://doi.org/10.1038/s41467-022-34294-6> | Google Scholar
- D'Alise A. M., Amabile G., Iovino M., Di Giorgio F. P., Bartiromo M., Sessa F., Villa F., Musacchio A., Cortese R. (2008) **Reversine, a novel Aurora kinases inhibitor, inhibits colony formation of human acute myeloid leukemia cells** *Mol Cancer Ther* **7**:1140–1149 <https://doi.org/10.1158/1535-7163.MCT-07-2051> | Google Scholar
- Dahdouh E. M., Garcia-Velasco J. A (2021) **Embryos with mosaic results: busting the myth!** *Reprod Biomed Online* **43**:1–2 <https://doi.org/10.1016/j.rbmo.2021.04.015> | Google Scholar
- Eakin G. S., Hadjantonakis A. K (2006) **Production of chimeras by aggregation of embryonic stem cells with diploid or tetraploid mouse embryos** *Nat Protoc* **1**:1145–1153 <https://doi.org/10.1038/nprot.2006.173> | Google Scholar
- Goolam M., Scialdone A., Graham S. J. L., Macaulay I. C., Jedrusik A., Hupalowska A., Voet T., Marioni J. C., Zernicka-Goetz M (2016) **Heterogeneity in Oct4 and Sox2 Targets Biases Cell Fate in 4-Cell Mouse Embryos** *Cell* **165**:61–74 <https://doi.org/10.1016/j.cell.2016.01.047> | Google Scholar
- Greco E., Minasi M. G., Fiorentino F (2015) **Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts** *N Engl J Med* **373**:2089–2090 <https://doi.org/10.1056/NEJMc1500421> | Google Scholar
- Gu Y., Shen X., Zhou D., Wang Z., Zhang N., Shan Z., Jin L., Lei L (2014) **Selection and expression profiles of reference genes in mouse preimplantation embryos of different ploidies at various developmental stages** *PLoS One* **9**:e98956 <https://doi.org/10.1371/journal.pone.0098956> | Google Scholar
- Hewitt L., Tighe A., Santaguida S., White A. M., Jones C. D., Musacchio A., Green S., Taylor S. S (2010) **Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex** *J Cell Biol* **190**:25–34 <https://doi.org/10.1083/jcb.201002133> | Google Scholar
- Hirakata C., Lima K., De Almeida B. O., De Miranda L. B. L., Florencio K. G. D., Furtado L. C., Costa-Lotufo L. V., Machado-Neto J. A. (2021) **Targeting glioma cells by antineoplastic activity of reversine** *Oncol Lett* **22**:610 <https://doi.org/10.3892/ol.2021.12871> | Google Scholar
- Houghton F. D (2021) **HYPOXIA AND REPRODUCTIVE HEALTH: Hypoxic regulation of preimplantation embryos: lessons from human embryonic stem cells** *Reproduction* **161**:F41–F51 <https://doi.org/10.1530/REP-20-0322> | Google Scholar
- Hu C. J., Wang L. Y., Chodosh L. A., Keith B., Simon M. C (2003) **Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation** *Mol Cell Biol* **23**:9361–9374 <https://doi.org/10.1128/MCB.23.24.9361-9374.2003> | Google Scholar
- Iyer N. V., Kotch L. E., Agani F., Leung S. W., Laughner E., Wenger R. H., Gassmann M., Gearhart J. D., Lawler A. M., Yu A. Y., Semenza G. L (1998) **Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha** *Genes Dev* **12**:149–162 <https://doi.org/10.1101/gad.12.2.149> | Google Scholar

- Knudtson J. F., Robinson R. D., Sparks A. E., Hill M. J., Chang T. A., Van Voorhis B. J. (2022) **Common practices among consistently high-performing in vitro fertilization programs in the United States: 10-year update** *Fertil Steril* **117**:42–50 <https://doi.org/10.1016/j.fertnstert.2021.09.010> | Google Scholar
- Lan W., Cleveland D. W. (2010) **A chemical tool box defines mitotic and interphase roles for Mps1 kinase** *J Cell Biol* **190**:21–24 <https://doi.org/10.1083/jcb.201006080> | Google Scholar
- Lee K., Kim H. M. (2011) **A novel approach to cancer therapy using PX-478 as a HIF-1alpha inhibitor** *Arch Pharm Res* **34**:1583–1585 <https://doi.org/10.1007/s12272-011-1021-3> | Google Scholar
- Marti J. M., Garcia-Diaz A., Delgado-Bellido D., O'Valle F., Gonzalez-Flores A., Carlevaris O., Rodriguez-Vargas J. M., Ame J. C., Dantzer F., King G. L., Dziedzic K., Berra E., de Alava E., Amaral A. T., Hammond E. M., Oliver F. J. (2021) **Selective modulation by PARP-1 of HIF-1alpha-recruitment to chromatin during hypoxia is required for tumor adaptation to hypoxic conditions** *Redox Biol* **41**:101885 <https://doi.org/10.1016/j.redox.2021.101885> | Google Scholar
- McCoy R. C. (2017) **Mosaicism in Preimplantation Human Embryos: When Chromosomal Abnormalities Are the Norm** *Trends Genet* **33**:448–463 <https://doi.org/10.1016/j.tig.2017.04.001> | Google Scholar
- Meuter A., Rogmann L. M., Winterhoff B. J., Tchkonja T., Kirkland J. L., Morbeck D. E. (2014) **Markers of cellular senescence are elevated in murine blastocysts cultured in vitro: molecular consequences of culture in atmospheric oxygen** *J Assist Reprod Genet* **31**:1259–1267 <https://doi.org/10.1007/s10815-014-0299-8> | Google Scholar
- Mole M. A., Weberling A., Zernicka-Goetz M. (2020) **Comparative analysis of human and mouse development: From zygote to pre-gastrulation** *Curr Top Dev Biol* **136**:113–138 <https://doi.org/10.1016/bs.ctdb.2019.10.002> | Google Scholar
- Muzumdar M. D., Tasic B., Miyamichi K., Li L., Luo L. (2007) **A global double-fluorescent Cre reporter mouse** *Genesis* **45**:593–605 <https://doi.org/10.1002/dvg.20335> | Google Scholar
- Nakamura H., Sekine H., Kato H., Masai H., Gradin K., Poellinger L. (2022) **Hypoxia-inducible factor-1alpha and poly [ADP ribose] polymerase 1 cooperatively regulate Notch3 expression under hypoxia via a noncanonical mechanism** *J Biol Chem* **298**:102137 <https://doi.org/10.1016/j.jbc.2022.102137> | Google Scholar
- Nguyen A. Q., Bardua I., Greene B., Wrenzycki C., Wagner U., Ziller V. (2020) **Mouse embryos exposed to oxygen concentrations that mimic changes in the oviduct and uterus show improvement in blastocyst rate, blastocyst size, and accelerated cell division** *Reprod Biol* **20**:147–153 <https://doi.org/10.1016/j.repbio.2020.03.011> | Google Scholar
- Palmerola K. L., Amrane S., De Los Angeles A., Xu S., Wang N., de Pinho J., Zuccaro M. V., Tagliatela A., Massey D. J., Turocy J., Robles A., Subbiah A., Prosser B., Lobo R., Ciccio A., Koren A., Baslan T., Egli D. (2022a) **Replication stress impairs chromosome segregation and preimplantation development in human embryos** *Cell* <https://doi.org/10.1016/j.cell.2022.06.028> | Google Scholar
- Palmerola K. L., Amrane S., De Los Angeles A., Xu S., Wang N., de Pinho J., Zuccaro M. V., Tagliatela A., Massey D. J., Turocy J., Robles A., Subbiah A., Prosser B., Lobo R., Ciccio A., Koren A., Baslan T., Egli D. (2022b) **Replication stress impairs chromosome segregation and**

preimplantation development in human embryos *Cell* **185**:2988–3007 <https://doi.org/10.1016/j.cell.2022.06.028> | Google Scholar

Pauerova T., Radonova L., Kovacovicova K., Novakova L., Skultety M., Anger M (2020) **Aneuploidy during the onset of mouse embryo development** *Reproduction* **160**:773–782 <https://doi.org/10.1530/REP-20-0086> | Google Scholar

Pietrzak J., Spickett C. M., Ploszaj T., Virag L., Robaszkiewicz A (2018) **PARP1 promoter links cell cycle progression with adaptation to oxidative environment** *Redox Biol* **18**:1–5 <https://doi.org/10.1016/j.redox.2018.05.017> | Google Scholar

Santaguida S., Tighe A., D’Alise A. M., Taylor S. S., Musacchio A (2010) **Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine** *J Cell Biol* **190**:73–87 <https://doi.org/10.1083/jcb.201001036> | Google Scholar

Shahbazi M. N., Wang T., Tao X., Weatherbee B. A. T., Sun L., Zhan Y., Keller L., Smith G. D., Pellicer A., Scott R. T., Seli E., Zernicka-Goetz M (2020) **Developmental potential of aneuploid human embryos cultured beyond implantation** *Nat Commun* **11**:3987 <https://doi.org/10.1038/s41467-020-17764-7> | Google Scholar

Simoës-Sousa S., Littler S., Thompson S. L., Minshall P., Whalley H., Bakker B., Belkot K., Moralli D., Bronder D., Tighe A., Spierings D. C. J., Bah N., Graham J., Nelson L., Green C. M., Foijer F., Townsend P. A., Taylor S. S (2018) **The p38alpha Stress Kinase Suppresses Aneuploidy Tolerance by Inhibiting Hif-1alpha** *Cell Rep* **25**:749–760 <https://doi.org/10.1016/j.celrep.2018.09.060> | Google Scholar

Singla S., Iwamoto-Stohl L. K., Zhu M., Zernicka-Goetz M (2020) **Autophagy-mediated apoptosis eliminates aneuploid cells in a mouse model of chromosome mosaicism** *Nat Commun* **11**:2958 <https://doi.org/10.1038/s41467-020-16796-3> | Google Scholar

Soto M., Raaijmakers J. A., Bakker B., Spierings D. C. J., Lansdorp P. M., Foijer F., Medema R. H (2017) **p53 Prohibits Propagation of Chromosome Segregation Errors that Produce Structural Aneuploidies** *Cell Rep* **19**:2423–2431 <https://doi.org/10.1016/j.celrep.2017.05.055> | Google Scholar

Starostik M. R., Sosina O. A., McCoy R. C (2020) **Single-cell analysis of human embryos reveals diverse patterns of aneuploidy and mosaicism** *Genome Res* **30**:814–825 <https://doi.org/10.1101/gr.262774.120> | Google Scholar

Thompson S. L., Compton D. A (2010) **Proliferation of aneuploid human cells is limited by a p53-dependent mechanism** *J Cell Biol* **188**:369–381 <https://doi.org/10.1083/jcb.200905057> | Google Scholar

Vanneste E., Voet T., Le Caignec C., Ampe M., Konings P., Melotte C., Debrock S., Amyere M., Vikkula M., Schuit F., Fryns J. P., Verbeke G., D’Hooghe T., Moreau Y., Vermeesch J. R. (2009) **Chromosome instability is common in human cleavage-stage embryos** *Nat Med* **15**:577–583 <https://doi.org/10.1038/nm.1924> | Google Scholar

Vazquez-Diez C., Paim L. M. G., FitzHarris G (2019) **Cell-Size-Independent Spindle Checkpoint Failure Underlies Chromosome Segregation Error in Mouse Embryos** *Curr Biol* **29**:865–873 <https://doi.org/10.1016/j.cub.2018.12.042> | Google Scholar

Yang M., Rito T., Metzger J., Naftaly J., Soman R., Hu J., Albertini D. F., Barad D. H., Brivanlou A. H., Gleicher N (2021) **Depletion of aneuploid cells in human embryos and gastruloids** *Nat Cell Biol* **23**:314–321 <https://doi.org/10.1038/s41556-021-00660-7> | Google Scholar

Zhao T., Ren H., Jia L., Chen J., Xin W., Yan F., Li J., Wang X., Gao S., Qian D., Huang C., Hao J (2015) **Inhibition of HIF-1alpha by PX-478 enhances the anti-tumor effect of gemcitabine by inducing immunogenic cell death in pancreatic ductal adenocarcinoma** *Oncotarget* **6**:2250–2262 <https://doi.org/10.18632/oncotarget.2948> | Google Scholar

Zhu J., Tsai H. J., Gordon M. R., Li R (2018) **Cellular Stress Associated with Aneuploidy** *Dev Cell* **44**:420–431 <https://doi.org/10.1016/j.devcel.2018.02.002> | Google Scholar

Zhu M., Shahbazi M., Martin A., Zhang C., Sozen B., Borsos M., Mandelbaum R. S., Paulson R. J., Mole M. A., Esbert M., Titus S., Scott R. T., Campbell A., Fishel S., Gradinaru V., Zhao H., Wu K., Chen Z. J., Seli E., ..., Zernicka Goetz M. (2021) **Human embryo polarization requires PLC signaling to mediate trophoctoderm specification** *eLife* **10** <https://doi.org/10.7554/eLife.65068> | Google Scholar

Zhu M., Zernicka-Goetz M (2020) **Principles of Self-Organization of the Mammalian Embryo** *Cell* **183**:1467–1478 <https://doi.org/10.1016/j.cell.2020.11.003> | Google Scholar

Author information

Estefania Sanchez-Vasquez

Division of Biology 139-74, California Institute of Technology, Pasadena, United States

Marianne E Bronner

Division of Biology 139-74, California Institute of Technology, Pasadena, United States
ORCID iD: [0000-0003-4274-1862](https://orcid.org/0000-0003-4274-1862)

Magdalena Zernicka-Goetz

Division of Biology 139-74, California Institute of Technology, Pasadena, United States

For correspondence: magdaz@caltech.edu

Editors

Reviewing Editor

Mark Sharpley

Cedars-Sinai Medical Center, Los Angeles, United States of America

Senior Editor

Utpal Banerjee

University of California, Los Angeles, Los Angeles, United States of America

Reviewer #1 (Public review):

Summary:

This paper developed a model of chromosome mosaicism by using a new aneuploidy-inducing drug (AZ3146), and compared this to their previous work where they used reversine, to demonstrate the fate of aneuploid cells during murine preimplantation embryo development. They found that AZ3146 acts similarly to reversine in inducing aneuploidy in embryos, but interestingly showed that the developmental potential of embryos is higher in AZ3146-treated vs. reversine-treated embryos. This difference was associated with changes in HIF1A, p53 gene regulation, DNA damage, and fate of euploid and aneuploid cells when embryos were cultured in a hypoxic environment.

Strengths:

In the current study, the authors investigate the fate of aneuploid cells in the preimplantation murine embryo using a specific aneuploidy-inducing compound to generate embryos that were chimeras of euploid and aneuploid cells. The strength of the work is that they investigate the developmental potential and changes in gene expression profiles under normoxic and hypoxic culture conditions. Further, they also assessed how levels of DNA damage and DNA repair are altered in these culture conditions. They also assessed the allocation of aneuploid cells to the divergent cell lineages of the blastocyst stage embryo.

<https://doi.org/10.7554/eLife.101912.3.sa1>

Author response:

The following is the authors' response to the previous reviews

Reviewer #1(Public review):

We deeply appreciate the reviewer comments on our manuscript. Following up the revisions, our manuscript has been improved thanks to their insightful remarks. We have proceeded with all the required changes.

Weaknesses:

*The authors have still not addressed the inconsistent/missing description for sample size, the appropriate number of * for each figure panel, and the statistical tests used.*

Description of sample size, specific *P* value and statistical test used has been added it both in the main text, figures and figure legends.

The authors assign 5% oxygen as hypoxia. This is not the case as the in vivo environment is close to this value. 5% is normoxia. Clinical IVF/embryo culture occurs at 5% O₂. Please adjust your narrative around this.

We define in our manuscript “normoxia” as the standard atmospheric oxygen levels in tissue culture incubators, which range from about 20–21% oxygen. Our definition of hypoxia is 5% concentration of oxygen, taking into consideration the standard levels of oxygen in the IVF clinics. Physiological oxygen in mouse varies from ~1.5% to 8% (Alva et al 2022). Considering that these levels of oxygen are the standard levels in tissue culture practices, a paragraph has been added to the discussion and materials and methods for further clarification

Reviewer #2 (Public review):

Weakness:

Given that this is a study on the induction of aneuploidy, it would be meaningful to assess aneuploidy immediately after induction, and then again before implantation. This is also applicable to the competition experiments on page 7/8. What is shown is the competitiveness of treated cells. Because the publication centers around aneuploidy, inclusion of such data in the main figure at all relevant points would strengthen it. There is some evaluation of karyotypes only in the supplemental - why? Would be good not to rely on a single assay that the authors appear to not give much importance.

This is an excellent point. However, due to the stochasticity of the arising of aneuploidies when embryos are treated with AZ3146 and reversine (Bolton et al 2016), every treatment is likely to generate different levels of aneuploidy. Due to this, and to the technical limitations of generating single-cell genomic DNA sequencing at the blastocyst stage, we were unable to determine the karyotype of all cells after different conditions. Nevertheless, Regin et al 2024 (eLife) showed similar results on the overall transcriptome changes of different dosages of aneuploidy: high dosage embryos overexpress p53, like reversine-treated embryos; meanwhile, low dosage embryos overexpress the hypoxic pathway, including HIF1A, similar to embryos treated with AZ3146.

Reviewer #1 (Recommendations for the authors):

Corrections required before final publishing:

Please ensure that the number of asterisks is in alignment with standard convention (<0.05; ** <0.01; *** <0.001; **** <0.0001). If you want to describe an exact P-value it should be presented as P = 0.0004. line 108 *** is <0.0004. line 263 * P<0.0044*

Same issue appears in lines 697, 711, 722, 753, 685

Specific values have been added in the figures and modified in the text.

Line 199: "...viable E9.5 embryos" missing "Figure S1D"

Modified in manuscript

Line 120: "...decidua" please add "Figure S1C"

Modified in manuscript

Line 126-127: Please add a description for the results (morula) in Fig 1D, e.g., It appears that γH2AX persists from 8-cell to morula when treated with Reversine but not AZ3146"

At the morula stage, the levels of γH2A.X in reversine- and AZ3146-treated embryos are similar (Fig. 1E). However, at the blastocyst stage, high levels of γH2A.X are maintained in reversine-treated embryos and reduced in AZ3146-treated embryos, suggesting some level of DNA repair between the morula-to-blastocyst stages (Fig. S2A). In contrast, in hypoxia, the levels of γH2A.X are low in the three treatments at the morula stages, suggesting that DNA repair can be enhanced under hypoxic conditions. Similar results have been reported in somatic cells (Marti et al., 2021; Pietrzak et al., 2018).

Line 213: PARP1 levels were also similar under all conditions; but Fig3E, top right shows PARP1 was significantly lower with Reversine treatment; also please correct me if i am wrong, but does the phrase "all conditions" cross reference γH2AX and PARP1 between Fig 3 and Fig 1 to show the impact of hypoxia? Because from my understanding Fig 1 was done in 20% oxygen, but Fig 3 was done in 5% oxygen – hypoxia.

This is correct. Modification in the manuscript has been performed for clarification

| *Line 264: extra forward dash? "Reversine/AZ3146/ aggregation"*

Modified in manuscript

| *Line 644: you don't have a control for IDF treatment, so how did you differentiate between impact of aneuploid drugs vs IDF treatment alone? Would the impact observed be due to compounding effect of aneuploidy drugs + IDF?*

This is a great observation. We previously demonstrated that IDF-1174 treatments in embryos do not affect pre-implantation development (Fig. S3).

| *Line 681: change their behaviour is a vague statement. Be specific.*

Modified in manuscript

| *Line 676 missing bracket "E")*

Modified in manuscript

| *Line 680: "...significantly on" should be "for"*

Modified in manuscript

| *Line 682-685: "...hypoxia favours the survival of reversine-induced aneuploid cells." does it? the statement before this says in Rev/AZ chimeras, AZ blastomeres contribute similarly to reversine-blastomeres to the TE and PE but significantly increase contributions to the EPI. Wouldn't this mean hypoxia favours survival of AZ aneuploid cells in EPI?*

In normoxic conditions, AZ3146 treated cells in Rev/AZ chimeras contributed mostly to the EPI and TE but not PE. In contrast, in normoxic conditions, Rev-treated cells contributed similarly to all the lineages. This result seems to be due to a better survival of Rev-treated cells under normoxic conditions (Fig. 4D-E)

| *Line 720: (b) shows blastocyst staining from what group? DMSO? Rev/AZ? Or are the 3 blastocysts shown here, 3 separate examples of Reversine-treated blastocysts? Would require labelling Fig S2B, and adding a short description in the corresponding figure legend*

Figure (B) shows the expression pattern of PARP1 at the blastocyst stage. Modified in manuscript

| *Figure 2, Figure S3 and Figure S6: were these experiments performed at 5% or 20% O₂, please add detail.*

Modified in manuscript

| **Reviewer #2 (Recommendations for the authors):**

| *Lines 45-46 understanding of reduction of aneuploidy should mention/discuss the paper of attrition/selection, of the kind by the Brivanlou lab for instance, or others. As well as allocation to specific lineages, including the authors' work.*

A section in the discussion has been added in response to this recommendation. Comparison between models is debatable.

The response does not clarify whether other papers were cited instead, or the authors own work that has shown preferential allocation to TE.

<https://doi.org/10.7554/eLife.101912.3.sa0>