

**3D Genome Architecture Under Stress: A Survey of Ionizing
Radiation, Progeria, and Osmotic Stress**

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DEDICATION

This is to those who have loved and supported me throughout my journey.

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ABSTRACT

The human nucleus contains 2 meters of DNA which is intricately folded into a three-dimensional (3D) structure. It has become increasingly clear that this 3D structure plays an important role in the expression of genes. Proper gene expression is necessary for cellular homeostasis, cell state, and response to environmental/physical perturbations. Faithful repair of damage DNA damage is necessary to prevent genomic aberrations, such as translocations, which may lead to misregulation of gene expression. Hi-C, a sequencing technique that labels proximal chromatin interactions, provides a clearer picture of how the genome is spatially organized within the nucleus. Here, we discuss the effects that DNA damage has on 3D genome structure. Chapters 1 and 2 discuss the effects of ionizing radiation by X-rays and how it influences 3D genome architecture in wild- type and DNA repair deficient cells. Chapter 3 analyzes the 3D genome structure of Hutchinson-Gilford Progeria Syndrome patient cells and how it influences gene expression. Finally, Chapter 4 discusses the effects of osmotic stress on 3D genome organization. These findings suggest that different stressors which activate DNA damage pathways result in changes in 3D genome organization specific to the stress induced.

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INTRODUCTION

The human nucleus is crowded with macromolecules and membraneless organelles contained within a nuclear lamina^{1,2}. DNA is an important molecule that fills the space of the nucleus. The spatial organization of chromatin is important for proper gene expression. The human cell folds about 2 meters of DNA into a 10 μm nucleus. DNA is also known to interact with a variety of molecules within the nucleus. These interactions facilitate a variety of mechanisms which function in cell division, intracellular communication, and nuclear structural stability^{3,4,5}.

Three-Dimensional Hierarchy of the Genome

Chromatin folding has been a topic of interest for decades, but recent technological advances have provided greater detail into the structure of chromatin within the nucleus. Chromatin is organized in a variety of structures which include chromosomal territories, active and inactive compartments, topologically associating domains (TADs), and chromatin loops^{6,7,8,9}. Chromosomes are known to be spatially segregated into chromosome territories and rarely interact with other chromosomes^{10,11}. Chromatin compartments are separated by active and inactive regions of chromatin which are generally thought to be heterochromatin and euchromatin^{7,12}. TADs are regions of DNA which interact with each other more frequently. TADs are known to be separated at boundaries by CTCF and other boundary elements. Loss of boundary elements or intermingling of TADs can result in improper enhancer-promoter looping which can lead to phenotypic defects^{6,13}. Chromatin loops are necessary to mediate gene regulation. Looping may occur within a short genomic distance or over long stretches of DNA. Looping may be

accomplished through loop extrusion mediated by cohesin and can bring together enhancer-promoter interactions.^{14,15} Furthermore, improper folding or damage of chromatin in these various structural states can result in improper gene regulation and disease^{16,17}. To date the majority of 3D genome organization data was gathered in wild-type unstressed cells. The goal of the work presented here is to understand how different stressors affect genome organization. More importantly, the stressors presented in these studies induce DNA damage. Therefore, the data presented here can provide useful information on how specific stressors and the resulting DNA repair can alter genome organization.

Cellular Response to Ionizing Radiation

Damage to chromatin can be caused exogenously or endogenously. One of the most common forms of exogenous DNA damage is caused by ionizing radiation (IR). The most common form of radiation that affects humans are caused by background levels of radon that can be found in the ground or in building materials¹⁸. However, advances in medicine and technology have led to the introduction of man-made IR sources. The introduction of low- and high-linear energy transfer (LET) in X-ray equipment or common mechanical equipment such as televisions and computer screens have become common sources of man-made sources of radiation. Additionally, processing of nuclear fuel generates man-made sources of radiation¹⁸.

Due to the rise in radiation exposure, the effects of IR and their biological effects have been well studied. Response to IR exposure can be different

depending on the type of radiation event^{19,20}. IR has recently been used as a medical therapy to treat cancer and Alzheimer's disease^{21,22,23}. However, the effects of radiation on cellular processes and survival have resulted in the decrease of IR medical therapies due to their potential oncogenic effects. The type of IR can lead to a variety of effects depending on the tissue and track energy²⁴. IR exposure that damages DNA can cause lasting effects or induce apoptosis. Failure to repair double-stranded breaks (DSBs) caused by IR can result in genomic aberrations and mutations that can persist and become oncogenic. DSBs occurring in the G0/G1 cell cycle stage may result in the formation of dicentric chromosomes, the formation of two proximal chromosomes fused together^{25,26}. Dicentric chromosome formations are also accompanied by acentric chromosomal fragments that can be lost before cell division is complete²⁷. High doses of radiation (>5 Gy), such as those experienced by workers exposed at the Chernobyl, can have deadly effects caused by radiation sickness²⁸. Cells exposed to IR undergo an adaptive response leading to the activation of DNA damage response (DDR) pathways to prevent long-term effects^{29,30,31}. Though the potential to repair chromatin after exposure to IR is possible, the long-lasting effects to the genome can persist and ultimately result in genome organization changes.

Hutchinson-Gilford Progeria Syndrome

Hutchinson-Gilford Progeria Syndrome, or Progeria, is a debilitating premature aging disease present in twenty million births worldwide. Progeria was originally described by Jonathan Hutchinson in 1886 and later by Hastings Gilford

in 1897^{32,33}. Both studies documented symptoms of premature aging in Progeria patients, however, the mechanisms that contribute to Progeria phenotypes have only recently been investigated. Children affected by this disease experience symptoms that include short stature, skin wrinkling, alopecia, decreased bone density, and joint pain. Progeria patients also suffer from atherosclerosis which lead to their premature death by heart disease or stroke at an average age of 14.6 years^{34,35}. The cause of Progeria has been identified as a mutation in the *LMNA* gene which produces progerin, a mutated form of lamin A. This mutation results in a dominant negative phenotype that increases in severity during aging due to subsequent rounds of cellular division^{34,35}.

Structure of Lamins

The lamin family of proteins are a key component of the nucleus by forming the nuclear lamina³⁶. Lamins are a type V intermediate filament and were identified based on sequence homology with other known intermediate filaments^{37,38}. Lamins are prevalent in metazoans and are specifically expressed in animals. No homologs have been found to exist in lower eukaryotes or plants, based on sequence homology. However, not all animals express all types of lamins. The lamin family is composed of two types of lamins, A and B. The *LMNA* gene is responsible for encoding lamin A and C, proteins which contain identical sequences in the first 566 amino acids. Lamin C contains a unique 8 amino acid sequence at its C-terminus end and does not contain the 98 amino acid sequence found in the carboxy-terminus of preprocessed lamin A³⁹. The *LMNB* gene encodes

lamin B1 and lamin B2⁴⁰. Each member of the lamin family has specific functions, differing binding partners, and expression patterns. Both lamin A and B form a meshwork attached to the nuclear envelope and have direct interactions with nuclear envelope proteins and nuclear pore complexes^{36,41}. All lamins are derived from precursor proteins, undergoing post translational modifications that lead to the development of each type of lamin. Lamins share a tripartite structure which is found in other intermediate filaments. The basic structure of lamins include a head group, an α -helical rod domain, and a C-terminal tail domain. The α -helical rod domain allows lamins to dimerize allowing for the formation of a complex lamin network and higher order structures⁴². The C terminal domain is critical for post-translational modification and function, which is specific for each lamin type and contains a nuclear localization signal, an Ig domain, and a CAAX motif^{39,40}. The Ig domain, which is also found in many proteins, facilitates protein-protein interactions^{43,44}. The CAAX domain, essential for the post-translational addition of a farnesyl group, is composed of four amino acids where C stands for cysteine, A is any aliphatic amino acid, and X is any amino acid. Lamin C differs from lamin A, B1, and B2 in that it does not contain a CAAX motif⁴⁵. The role of farnesylation is to direct proteins to the nuclear membrane. The addition of farnesyl groups takes place in three steps. First, the cysteine residue is farnesylated by a farnesyltransferase. Next, the AAX sequence is proteolytically cleaved by the metalloprotease Rce1 (also known as ZMPSTE24), which allows for the carboxy-methylation of the farnesylated cysteine once it is incorporated in the nuclear

membrane the CAAX motif must be removed from prelamin A^{46,47,48}. Lamins B1 and B2 maintain their farnesylation after incorporating into the nuclear membrane, while Lamin C localizes to the nuclear envelope, but does not contain a farnesyl group. Lamin A can be found at the nuclear envelope and in the nucleoplasm, although the function of nucleoplasmic lamin A is not fully understood. In addition to its role in nuclear structure, lamin A is an important regulator of the genome. Lamins are known to interact with regions of the DNA, termed lamin associated domains (LADs). These regions typically contain heterochromatic DNA, which is generally thought to contain mostly silenced genes⁴⁹.

Since Hutchinson-Gilford Progeria Syndrome (HGPS) is caused by mutations in the *LMNA* gene, it is classified as a laminopathy. The most common mutation in HGPS is a *de novo* point mutation at position 1824 which contains a C to T nucleotide replacement. This point mutation activates a cryptic splice site, that does not change the encoded amino acid, but leads to the translation of a defective lamin A protein, termed progerin. Progerin carries a deletion of 150 nucleotides which contain the cleavage site for ZMPSTE24 and in turn results in the preservation of 50 amino acids in the C-terminus and a permanently farnesylated lamin A^{34,35}. In addition to defects in the structural stability of lamin, progerin also interacts with regions of the genome and proteins causing aberrant gene expression and damaged proteins which are thought to lead to defects that are observed in HGPS patients^{50,51}.

Effects of Progerin Expression

The effects of progerin expression are not initially observed in children suffering from HGPS, carrying the LMNA mutation; the disease does not manifest until the children are around the age of two. This is due to the fact that, unlike *LMNB* which is expressed in stem cells, LMNA is only expressed in differentiated cells⁵². The most noticeable defect observed in Progeria cells is a defect in nuclear morphology. Progeria nuclei contain characteristic blebbing (formation of round protrusions that project from the nucleus), invaginations, and wrinkling which increase throughout passaging of cell culture models and with aging in Progeria. Microscopy studies have shown that the lamin meshwork is incorrectly formed, due to the incorporation of progerin^{53,54}. Lamin B and C proteins are not affected in HGPS patients and can be visualized through antibody staining or live cell imaging, localizing to their wild-type locations. In contrast, Lamin A and progerin, localize to nuclear blebs that do not contain lamin B. Previous drug trials have used the loss of nuclear blebbing in HGPS patients as a sign of phenotype rescue^{55,56}. Nuclei of HGPS patients also display an increased stiffness that increases as Progeria fibroblasts undergo serial cell division⁵⁷. Interestingly, altered mechanosensitivity was observed in Progeria nuclei before nuclear stiffness occurred⁵⁷. This suggests that HGPS nuclei experience structural changes prior to displaying morphological changes such as nuclear blebbing and increased stiffness. Progeria cells subjected to biaxial strain also experienced an increase in apoptosis.

Progeria cells are subject to increased DNA damage. An increase in γ -H2AX has been observed in nuclei expressing progerin, suggesting that DNA damage is increased in Progeria cells^{58,59}. The accumulation of DNA damage is believed to lead to an impaired DNA damage repair pathway, by overwhelming the existent pathways. Of the known repair pathways responsible for sensing and regulating DNA damage, Progeria fibroblasts present with constitutively upregulated ATM and ataxia telangiectasia mutated and Rad3-related (ATR). The constant activation of these proteins leads to the phosphorylation of downstream targets Chk1 and Chk2 and other downstream response factors⁶⁰. Increased DNA damage can have a major impact on the global organization of the genome. Recent studies have revealed that the epigenetic landscape is compromised in Progeria cells. Similar to aged individuals, Progeria cells have decreased levels of tri-methylation of histone 3 lysine 9 (H3K9me3) and tri-methylation of histone 3 lysine 27 (H3K27me3) marks that are associated with heterochromatic regions of the genome^{61,62}. The loss of heterochromatin in aging and Progeria cells has yet to be elucidated but seems to be a common factor which correlates with ageing related phenotypes. McCord and colleagues have observed a loss of spatial compartments with increasing passages of Progeria cells⁶¹. Therefore, genome organization is altered when progerin is expressed. However, data supporting this claim was collected in the infancy of Hi-C, and high-resolution structures such as TADs were not discussed. Moreover, the effects of 3D genome folding and its effects on gene expression remain unclear.

Nuclear Response to Osmotic Stress

Osmolarity within cells is a tightly regulated process. While most cells remain in a constant osmotic environment, exposure to changes in osmolarity can occur in certain cell types, such as epithelial cells found in the urinary tract and intestines^{63,64}. Osmotic stress results in a change in nuclear volume which can disrupt the actin cytoskeleton^{65,66}. Hyper-osmotic stress results in nuclear shrinkage and stress on the nuclear membrane^{66,67,68}. The consequences of these changes are poorly understood but may lead to transcriptional and nucleocytoplasmic transport changes. Mechanical stress caused by changes in the nuclear structure by stretching has shown that certain genes can be activated⁶⁹. In rapid osmotic stress events in cultured cells, chromatin condenses and resulting in regions void of chromatin and DNA damage^{66,67,68}. These changes in chromatin may be the result of the removal of chromatin binding proteins, such as insulator proteins and cohesin, from the DNA. The removal of these proteins causes them to aggregate to form nuclear bodies and visibly alters chromatin structure⁷⁰. Together, these results indicate that alterations in nuclear shape and DNA damage may result in changes in 3D genome structure.

DNA Damage Repair Pathways

DNA damage is an inevitable consequence in living cells that can occur from endogenous from reactive oxygen species (ROS) as a result of metabolic byproducts and exogenous factors, such as X-ray or UV radiation^{71,72,73}. Unrepaired DNA can result in mutations which can become oncogenic. To mitigate

the effects of unrepaired DNA, cells either undergo apoptosis or initiate DDR pathways to repair damaged chromatin⁷⁴. The resulting repair pathway depends on the type of break and the timing of when the break is detected. DSBs that occur in G0 and G1 phase lead to the activation of the non-homologous end joining (NHEJ) pathway⁷⁵. DSBs that are detected in S and G2 phase are repaired by the homologous recombination (HR) pathway⁷⁶. These repair pathways are key to the viability of the cell and prevent the passing of mutated genetic material to daughter cells.

The detection of DSBs leads to the recruitment of the ataxia-telangiectasia mutated (ATM) kinase which phosphorylates the histone variant H2AX on serine 1397^{77,78}. This phosphorylation event results in binding sites for repair factors to bind and begin the repair process in both NHEJ and HR. Although each pathway requires ATM to sense DSBs, the downstream mechanism requires distinct proteins for each pathway. NHEJ, an error prone mechanism, utilizes microhomologies to directly ligate ends of broken strands of DNA. The first step to NHEJ requires the Mre11-Rad50-Nbs1 (MRN) complex to bridge broken ends. In mammalian cells, Ku70 and Ku80 are recruited to form a complex with DNA-PKcs which serves as a binding site for downstream proteins⁷⁹. Pol λ and Pol μ are then recruited for end processing to fill the gaps between broken ends. Finally, ligation occurs with DNA ligase IV (Lig4) and XRCC4 to complete repair⁸⁰. HR is more complex and requires a similar DNA sequence to repair DSBs. Like NHEJ, the MRN complex is required to bridge the broken ends. Resection then occurs by

removing 5' ends to generate a 3' overhang. The 3' end then forms a Holliday junction with the homologous chromosome. DNA synthesis and ligation then occur to resolve broken ends⁸¹.

The studies reported here probe the effects of DNA damage on 3D genome organization in three different perturbations that either directly cause DNA damage or result in secondary DNA damage effects. We find that, although DNA damage is present in these perturbations, the organization of the genome is different based on the stressor. These results provide direct evidence that the 3D genome organization can be altered to respond to a variety of stresses and the response is may be mediated by the nuclear processes that occur either directly from DNA damage repair or by other mechanisms that respond to nuclear stress.

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CHAPTER I
RADIATION-INDUCED DNA DAMAGE AND REPAIR EFFECTS ON
3D GENOME ORGANIZATION

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ABSTRACT

The three-dimensional structure of chromosomes plays an important role in gene expression regulation and also influences the repair of radiation-induced DNA damage. Genomic aberrations that disrupt chromosome spatial domains can lead to diseases including cancer, but how the 3D genome structure responds to DNA damage is poorly understood. Here, we investigate the impact of DNA damage response and repair on 3D genome folding using Hi-C experiments on wild-type cells and ataxia telangiectasia mutated (*ATM*) patient cells. We irradiate fibroblasts, lymphoblasts, and *ATM*-deficient fibroblasts with 5 Gy X-rays and perform Hi-C at 30 minutes, 24 hours, or 5 days after irradiation. We observe that 3D genome changes after irradiation are cell type-specific, with lymphoblastoid cells generally showing more contact changes than irradiated fibroblasts. However, all tested repair-proficient cell types exhibit an increased segregation of topologically associating domains (TADs). This TAD boundary strengthening after irradiation is not observed in *ATM* deficient fibroblasts and may indicate the

presence of a mechanism to protect 3D genome structure integrity during DNA damage repair.

INTRODUCTION

Ionizing radiation (IR) is a well-known carcinogen, and IR exposure inflicts a wide spectrum of DNA lesions among which the double strand break (DSB) is the most lethal lesion. Mis-rejoining of DSBs leads to chromosome translocations, some of which may be oncogenic in nature. Therefore, efficient DNA repair is necessary to maintain genome integrity by suppressing chromosome translocations and genomic alterations¹. Most studies thus far have focused on IR-induced DNA damage and repair at the level of the linear DNA sequence with a special emphasis on mutations, cancer induction, and downstream effects for cell death and organ/tissue damage. But the human genome is not only a linear DNA sequence, but is also organized in a three-dimensional (3D) structure which influences the proper regulation of transcription, replication, and repair^{2,3,4,5}. Considering the importance of 3D genome structure in various cellular activities, it is important to understand whether IR-induced DNA damage alters the genome architecture and whether the 3D genome architecture is restored after DNA repair.

Translocations, deletions, and other genomic aberrations that may follow DNA damage can lead to cancer by directly mutating genes or altering their regulation^{6,7}. Recently, it has become clear that the disruption of 3D genome domains can also be oncogenic⁸. Does the process of DNA repair protect the

3D folding of the genome as well as the linear DNA sequence? Certain cell types are considered to be more radiosensitive than others, but little is known about what contributes to their radiosensitivity. It has been previously demonstrated that chromosome folding states and chromosome positioning can have a significant impact on which translocations occur^{7,9}. Such initial 3D genome factors and local chromatin states may also influence DNA repair after IR^{10,11}. Thus, it is possible that pre-existing 3D genome structure states help to explain why certain cell types are more sensitive to radiation

Previous studies suggest that DNA repair efficiency may differ for heterochromatin and euchromatin^{12,13}. Heterochromatic regions may be more mobile and move to DNA repair sites, where they decondense^{14,15}. Condensation or decondensation of specific chromatin regions may not be determined by their preexisting histone modifications, suggesting that other factors may contribute to changes in 3D genome structure after DNA damage¹⁴. One previous study demonstrated spatial clustering of DSBs in active genes by inducing specific breaks and measuring their interactions with Capture Hi-C¹⁶. This suggests that changes in the structure of local genome domains may happen at a broader scale after IR. Additionally, CCCTC-binding factor (CTCF) and cohesin have been shown to be early responders to DNA damage induced by IR^{17,18,19,20}. These proteins have also been recently demonstrated to play significant roles in chromosome folding, contributing to the formation of topologically associating domains (TADs)^{21,22,23,24}. These genomic domains

interact more frequently within themselves than with other regions and appear to help bring genes into contact with appropriate regulatory elements^{25,26}. The disruption of TAD boundaries can lead to developmental diseases and cancer^{27,28}. The prevailing model of TAD formation proposes that CTCF helps to establish TAD boundaries by blocking the loop extruding activity of cohesin²³. Rapid recruitment of CTCF and cohesin to the sites of DNA damage may indicate that preservation of 3D genome architecture after DNA repair completion is important for genome integrity.

The above-mentioned studies suggest that changes occur in the 3D genome structure at multiple levels of its hierarchical organization in response to IR. However, the few studies that have examined chromosome contact changes after DNA damage thus far have focused on changes around specific induced break sites^{16,29}. While this is very valuable for studying local detailed structure mechanisms, various evidence suggests that DNA damage at one location can affect chromosome structures far away from the site of damage^{11,30}. Further, different 3D genome structure responses may occur when breaks happen in different genomic locations. Therefore, we seek to capture general principles of how widespread and random IR-induced damage and the subsequent repair of DNA influences genome organization across length scales in a cell type-specific manner. To accomplish this, we employ chromosome conformation capture followed by high throughput sequencing (Hi-C) to characterize genome organization across a population of cells before

and after exposure to X-rays (5 Gy)³¹. In a previous study, we used multi-color fluorescence in situ hybridization (mFISH) and low-resolution Hi-C experiments on human blood cells and lymphoblasts to examine changes in the contacts and positioning of whole chromosomes at 24 hours post-irradiation. We found that while individual cells harbored a variety of chromosomal translocations as observed by mFISH, at a population level, no recurrent translocations were evident in the Hi-C data⁶. Here, with additional cell types and repair mutants at more timepoints and higher resolution, we assay the cell type-specific effects of IR on the hierarchical layers of genome organization. We perform Hi-C on fibroblasts and lymphoblastoids at 30 min and 24 h after exposure to 5 Gy X-rays. Changes in the overall genome organization are observed in the irradiated human fibroblasts and lymphoblastoid cells when compared to their non-irradiated counterparts. Human lymphoblastoid cells (GM12878) exhibited more evident changes in genome wide 3D organization when compared to fibroblasts. However, both cell types exhibited a strengthening of TAD insulation, suggesting that TADs are more segregated after X-ray exposure. This effect persists at 5 days post-IR. To determine whether increased TAD insulation after IR is linked to the DNA damage/repair pathway, we examine the 3D genome organization changes in cells with compromised double strand break repair: human fibroblasts deficient in the *ataxia telangiectasia mutated* (*ATM*) gene. We observe that the TAD strengthening that occurs in several repair-proficient cell types after IR does not occur in ATM deficient cells,

suggesting that this genome structure change is dependent on the ATM DNA repair pathway.

RESULTS

Skin fibroblasts exhibit subtle changes in genome architecture after IR

To determine how X-ray irradiation affects genome organization, we first irradiated confluent BJ-5ta foreskin fibroblasts with 5 Gy X-rays and analyzed cells at 30 minutes and 24 hours post-irradiation. The majority of cells exposed to 5 Gy X-rays show chromosomal damage and translocations⁶. Because cells at different cell cycle phases (G1, S and G2/M) respond differently to IR exposure, we grew the fibroblasts to a confluent, contact-inhibited state in which most cells are in G0/G1 phase (Figure 1)^{32,33}. To confirm the induction of DSBs by IR, immunofluorescence and western blot analyses were performed for phosphorylated-H2AX (γ -H2AX), a surrogate marker of DSBs^{34,35}. We observed that X-ray irradiated BJ-5ta cells showed an elevated level of γ -H2AX protein at 30 minutes post-exposure followed by a decline at 24 hours post-IR, indicating an efficient repair of DNA DSBs (Figure 2a and Figure 3a-b). There are remaining unrepaired foci at 24 h after IR, some of which are slowly repaired by 96 h post-IR, but others of which remain unrepaired until cells are passaged and allowed to proceed through cell division (Figure 4).

To measure changes in genome-wide chromosome contacts after IR, Hi-C was performed immediately before IR exposure and at 30 minutes and 24 hours

after exposure (see Methods and Table 1 for statistics, processing, and replicate information). We perform Hi-C on populations of 5 million cells, and since each cell is likely to harbor 100-200 DSBs after 5 Gy radiation, we are measuring aggregate genome structure effects of a set of ~1 billion DSBs across the cell population, along with other forms of non-DSB damage that can be created by IR^{36,37}. Strikingly, despite the observation of obvious DSB induction and repair events detected by γ -H2AX, only subtle changes were detected in whole genome contact maps after 30 minutes (Figure 2b) or 24 hours (Figure 5a) after IR exposure when compared with non-irradiated control cells. Chromosome territories were maintained at 30 minutes, and there is no evidence of recurrent translocations, insertions, or deletions in the cell population at 30 min or 24 h post-IR. However, the log ratio of contact frequency at 30 minutes post-irradiation compared to control shows a decrease in interactions between chromosome arms which suggests a separation of chromosome arms after irradiation which could indicate a loss of a Rabl-like configuration (Figure 2c)³⁸. The decrease in interactions between chromosome arms persists at 24 hours, accompanied by an even greater loss of interactions between telomeres and some increase in interaction between telomeres and centromeres (Figure 2d). Hi-C experiments were also carried out in BJ-1 hTERT cells as an independent biological replicate. This is also an immortalized BJ fibroblast cell line made from a different clonal population than BJ-5ta. The BJ1 Hi-C results cluster well with BJ-5ta experimental data (Figure 6), and we observe similar changes to interaction patterns in these cells, specifically

with the loss of interactions between chromosome arms (Figure 5c), as well as subtle changes at 30 minutes becoming more apparent at 24 hours post-IR (Figure 5b-c).

Lymphoblastoid cells exhibit more evident genome architecture changes after IR

We next examined whether cell type specific differences exist in IR induced organizational changes by comparing the results just discussed for human fibroblasts with results for a lymphoblastoid cell line (GM12878). As stated before, X-ray induced DSB induction and repair were monitored at 30 min and 24 hours post IR exposure by measuring γ -H2AX using immunofluorescent and western blot techniques. As in BJ-5ta, an elevated level of γ -H2AX protein was observed in GM12878 cells at 30 minutes post-IR with a subsequent decline to baseline level at 24 hours post-IR (Figure 7a and Figure 8a-b). We then performed Hi-C on GM12878 cells exposed to 5 Gy X-ray after 30 minutes and 24 hours. While Hi-C replicates showed high correlation (Figure 6), we chose to treat replicates independently throughout subsequent analyses to provide independent corroboration of potential genome structure changes. Indeed, as discussed below, some features of contact changes were reproducible and others were not, suggesting that validating observations separately in multiple replicates is important, and that Hi-C datasets should not always be pooled immediately after a single reproducibility statistic is calculated.

Much like the BJ-5ta cells, GM12878 did not exhibit any recurrent translocations, insertions, or deletions in genome structure (Figure 7b and Figure 9a). However, GM12878 cells exhibit a more variable response at 30 minutes post-irradiation. While there was an increase in the intrachromosomal interactions in both replicates, patterns of interactions observed were not consistent (Figure 7c and Figure 9b). Both replicates exhibited a loss of intrachromosomal interactions at 24 hours of post-exposure, and this loss of interactions was more pronounced in lymphoblastoid cells relative to fibroblasts. Also, GM12878 cells compared to fibroblasts showed more patterns of localized loss of intrachromosomal interactions at specific regions after 24 hours of IR exposure (Figure 7d). These patterns of lost contacts were consistent between independent replicates (Figure 8c).

Interestingly, many of the regions which show increased interactions at 30 minutes decreased in interactions after 24 hours. Some of the regions involved in contact loss are notably gene dense, but further work will be needed to investigate whether other properties, like combinations of histone modifications, help explain these regions of contact change after IR.

Compartment identity is unchanged despite decrease in distal interactions after IR exposure

Since we observed changes in intrachromosomal interactions in whole genome contact maps, we next examined higher resolution contact maps of

individual chromosomes. These maps showed a depletion of interactions as distance from the diagonal increases after X-ray irradiation. This loss of distal interactions was quantified by scaling plots which showed a loss of mid-to-long range interactions after 24 hours in both the BJ-5ta and GM12878 cell lines (Figure 10a-b and Figure 9d).

Individual chromosome contact maps showed no apparent changes in the plaid contact patterns between IR and control conditions, suggesting that A/B compartment identity is not affected by IR (Figure 10c-d)³¹. The A and B compartment pattern captures the spatial segregation of euchromatin and heterochromatin within the nucleus. It has been previously shown that heterochromatin and euchromatin are differentially affected by DNA damaging irradiation and have different DNA repair responses^{12,13}. Despite this, we observed few instances of compartment identity switches (<~1%) in both cell types and conditions as shown in both contact maps and eigenvector compartment tracks (Figure 10c-f and Figure 11a- b). Even if compartment identity does not change, the strength of interactions within existing compartments can change, so we assessed changes in compartment strength after X-ray irradiation.

Saddle plots of compartment strength changes show varied and generally weak changes in compartment strength that were not consistent between replicates of the experiment (Figure 12). Therefore, we concluded that the strength of interactions within compartments was not a feature consistently affected by IR and DNA repair.

TAD boundary strength and CTCF loops increase after exposure to IR

The chromosome architectural proteins CTCF and cohesin are recruited to sites of DNA damage¹⁹. CTCF occupancy increases both at binding sites flanking γ -H2AX domains and at sites of DNA lesions after DNA damage is induced. In addition to their role in the DNA damage response, CTCF and cohesin are known to be important for the formation of TADs, and alterations of CTCF and cohesin can lead to an overall change in local genome topology^{17,18,39}. As previously mentioned, TADs have been identified as important for the spatial organization of gene regulation: enhancers and promoters are more likely to communicate within the same TAD and less likely to interact across TAD boundaries^{27,40}. The current model for TAD formation is that loop extrusion by the cohesin complex dynamically brings together genomic regions within TADs until extrusion is blocked by a boundary protein such as CTCF^{22,23}. But, it has not been investigated whether DNA damage and repair would weaken TAD boundaries as damaged sites rearrange or randomly diffuse, strengthen TAD boundaries, or leave them unchanged.

To assess whether TADs are affected after IR, we identified TAD boundaries and calculated the boundary strength (degree of contact separation between TADs) for each boundary using both the InsulationScore and the Hicratio method. Both methods determined that TAD boundary strength increases in both BJ-5ta and GM12878 cells 24 hours after IR (Figure 13a-b and Figure 14a-b). One inconsistency that was observed between the two methods

was the response at 30 minutes post IR in GM12878. In contrast to BJ-5ta, which had an increase in boundary strength in both methods after 30 minutes, GM12878 shows an increase as measured by the InsulationScore method but a decrease by the Hicratio method^{41,42}. This may be due to a subtle difference in the way that the algorithm detects TADs, so we focus on results that were consistent between both methods. To visualize the alterations in contacts around TAD boundaries, we plotted the average interaction profile around TAD boundaries called by the InsulationScore and Hicratio method (Figure 13c-d and 15a-d). We observe a decrease in interactions across TAD boundaries (indicating increased TAD separation) after exposure to IR in all conditions except, again, in GM12878 at 30 minutes post IR. This divergence at 30 minutes between cell types may reflect different rates of DNA damage response and repair in different cell types, but the consistent effects on TAD strength at 24 hours across cell types and replicates suggests that this reflects a more fundamental feature of DNA damage response. The TAD boundary strengthening is observed not only in aggregate, but is also detectable at specific loci (Figure 13e and Figure 16). TAD boundary strengthening also is detectable throughout the genome, not only in specific regions or types of chromatin. To test the hypothesis that TAD boundary strengthening might occur primarily in euchromatin or heterochromatin, we analyzed A or B compartment regions of the genome separately. We observe that both compartments exhibit increases in TAD boundary strength after IR (Figure 17).

Notably, we observe of a similar increase in TAD boundary strength 24 hours after IR in both lung fibroblasts (MRC-5) and an independent line of foreskin fibroblasts (BJ1-hTERT) (Figure 14a-b). This supports the idea that the increase of TAD boundary strength is a general phenomenon, perhaps related to the dual roles of CTCF and cohesin in DNA damage response and genome architecture. The observation of this phenomenon in both fibroblasts and lymphoblasts also makes it less likely that the TAD strengthening observed is simply an effect of cell cycle state. BJ cells, as noted, are predominantly in G1/G0 phase before irradiation and become more uniform after irradiation.

In contrast, GM12878 cells are still cycling at the time of irradiation and maintain similar distributions of cells in G1 and G2 at 24 h post-IR (Figure 1b). Despite this difference, TAD strengthening is observed in both cell types. In contrast, the TAD strengthening observed in BJ-5ta fibroblasts can be blocked by treatment with KU-55933, an ATM kinase inhibitor (Figure 13a-b). As explored further below, this result suggests DNA repair pathways lead to TAD boundary strengthening (Figure 13a-b). If TAD boundaries are reinforced after IR because of increased recruitment of CTCF after DNA damage, we would expect that this might also increase contact frequency within CTCF-mediated loops. To test this hypothesis, we used the previously annotated set of loops from high resolution GM12878 Hi- C data, specifically analyzing those with CTCF motifs at both loop anchors⁴³. We aggregated the interaction patterns across all loop regions and found that CTCF loops indeed show an increase in

average strength after exposure to IR in both fibroblasts and lymphoblasts (Figure 13f-g).

ATM is necessary for IR-induced genome architecture changes

Changes observed in the 3D genome structure after IR exposure could be a passive side effect of DNA DSBs. However, the TAD boundary and CTCF loop strengthening that we observe are the opposite of what would be expected from a passive mixing of broken chromosomes, so we hypothesized that these changes are dependent on the cell's active response to DNA damage. Ataxia telangiectasia mutated (ATM), a PI3K-like protein kinase, plays a key role in the repair of DSBs through phosphorylation of number of downstream targets^{44,45,46}. Patients with mutations in the *ATM* gene have an increased likelihood of several types of cancer, including breast cancer, leukemias, and lymphomas^{47,48}. ATM phosphorylates the histone variant H2AX which results in the recruitment of proteins involved in DNA damage response, including genome architecture proteins CTCF and cohesin⁴⁹. Interestingly, the observation that γ -H2AX foci are bound by CTCF and are approximately the size of TADs has led several groups to hypothesize that ATM-mediated repair is restricted to TADs^{29,50,51}. To evaluate the role of ATM in the 3D genome response to X-ray irradiation, we performed similar IR experiments on ATM mutant fibroblasts immortalized with human telomerase reverse transcriptase (ATM-hTERT) as we did for previous cell types. Immunofluorescence imaging confirmed the faulty DNA repair pathway in these cells, shown by a lower mean fluorescence intensity of γ -H2AX after IR than was observed in BJ-5ta fibroblasts

(Figure 18a and Figure 19). The presence of γ -H2AX at all after IR in the ATM mutants suggests that an alternate mechanism, likely the ATR pathway, in part compensates for the deficiency in ATM⁵².

We performed Hi-C on ATM-hTERT cells 24 hours post irradiation as a comparison to the condition in which the largest changes in genome organization occurred in BJ-5ta and GM12878 cells. Like both repair-proficient cell types, no recurrent translocations were observed in ATM-hTERT cells at 24 hours post IR (Figure 18b). The log ratio of Hi-C contact maps 24 hours post-IR vs. control showed only modest changes in contacts after IR in these ATM mutant cells (Figure 18c). We observed no change between post-IR and control scaling plots suggesting that the reduction of distal interactions in BJ-5ta and GM12878 cells is dependent on DNA damage response and repair. As in BJ-5ta cells, A/B compartment identities in ATM mutant cells are maintained 24 hours after exposure to IR (Figure 18e). To evaluate TAD strength changes in ATM mutant cells after X-ray, we again used both the InsulationScore and Hicratio methods. In contrast to the increased TAD boundary strength observed in all DNA repair proficient cells after X-ray, we observe a slight decrease in TAD boundary strength in ATM mutant cells after IR (Figure 18f). We confirmed that there was an increase in interactions on average across TAD boundaries after X-ray in the ATM mutant cells by plotting the average interaction profile around TAD boundaries (Figure 18g). Additionally, in contrast with BJ-5ta and GM12878, aggregate interaction patterns showed no increase of CTCF loops in ATM mutant cells (Figure 18h).

These results suggest that ATM is necessary for the increased segregation of TADs after IR, and that in the absence of ATM, genomic regions have a higher chance of interacting across TAD boundaries. To ensure that these results are attributable to ATM and not an artifact of TERT immortalization or specific to one cell line, we repeated the same set of experiments using primary human fibroblasts derived from an AT patient (GM02052). We confirmed that in the absence of functional ATM, insulation between TADs remained the same or decreased (Figure 13a-b).

As a final confirmation of the importance of ATM to the 3D genome structure response to IR, we compared effects of IR in a syngeneic system, treating BJ-5ta cells with 10 μ M KU-55933 (an ATM inhibitor) for one hour and then irradiating with 5 Gy X-rays. This treatment abrogates the γ -H2AX signal after IR, as expected (Figure 20 and 21a). We find that all the major effects of IR on untreated BJ-5ta cells are lost after ATM inhibition in this same cell line: there is no loss of Rabl-like chromosome arm contacts, no decrease in distal chromosome contacts, and no increase in TAD boundary or CTCF loop strength.

3D genome response to IR is delayed in ATM mutants and persists in wild-type fibroblasts

The persistence of changes observed in the TAD strength at 24 hours after IR exposure in DNA repair proficient cells is striking, given that most DNA damage is repaired/misrepaired by this time point. Over an even longer time course after IR, cells that have failed to repair properly will often die and be eliminated from the

population, selecting for cells that can continue to proliferate after repair. To determine the long-term effects of IR and the subsequent repair of DSBs DNA on the 3D genome, we irradiated BJ-5ta and ATM-hTERT fibroblasts with 5 Gy X-rays and then split the cells into sub- confluent densities after 24 hours. Cells were allowed to recover and proliferate until confluent (~4-5 days). Hi-C experiments on the recovery condition from both cell lines showed that BJ-5ta fibroblasts maintained the loss of interactions at telomeric regions in large chromosomes observed at 30 minutes and 24 hours post-IR but showed a decrease in interactions at the centromeric regions among most chromosomes. Interactions that changed 24 hours post-irradiation are even more apparent in recovery (Figure 22a). In ATM mutant cells, genome wide contacts remain largely unaffected after 5 days recovery, as at 24 h after IR. The notable exception was that one replicate showed the emergence of two new translocations after 5 days of recovery: between chromosomes 17 and 21 and 19 and 21 (Figure 22b). This is consistent with the repair-deficient nature of ATM mutant cells. As at 24 hours post-IR, mid-to-long range interactions remained decreased in BJ-5ta recovery as compared to the control (Figure 22c). In addition, ATM mutants also had a decrease in mid-to-long range interactions after recovery (Figure 22c), which differs from the lack of change observed after 24 hours in these mutant cells (Figure 18d). A/B compartment identity is maintained in both BJ-5ta and ATM mutant cells after recovery, which is consistent with data obtained from 30 minutes and 24 hours IR responses (Figure 22d).

TAD boundary strength measured by both InsulationScore and Hicratio methods remains higher than control 5 days after IR in BJ-5ta, suggesting that TADs maintain increased segregation long term after IR (Figure 22e). Consistent with this result, the average TAD profile comparison showed a decrease in interactions across BJ-5ta TAD boundaries in 5-day recovery vs. control (Figure 22f). Interestingly, ATM mutant cells also show some evidence of increased segregation of TADs after 5 days of recovery. The effect is not as strong or consistent, however, and varies by the quantification approach used (Figure 22e). This again suggests that IR effects on TAD boundaries in ATM mutant cells are not the same as in repair- proficient cells but indicates that there could be a longer-term convergence, in which the ATM mutant cells that survive radiation long-term eventually show increased boundary strength. (Figure 22f).

DISCUSSION

The 3D architecture of the genome influences various DNA metabolic activities, including DNA repair to ensure the maintenance of genomic stability. Although IR-induced gene mutations and chromosomal alterations have been well studied in various cell and animal model systems, IR-induced structural changes at the level of higher order chromatin organization have been given less attention.

In this study, we have identified notable and lasting effects of irradiation on genome organization within the cell population. Overall, the changes in 3D genome contacts that we observe after irradiation provide a new platform from which to consider the interplay between local chromatin structure, genome architecture

proteins, TAD strength, and DNA repair after irradiation. Changes that are evident in population-averaged Hi-C data must have been occurring consistently, or at least in a substantial minority of cells, across the cell population. Our analysis methods also required that changes occur consistently across completely independent biological replicates. On the one hand, this is a potential weakness in the sense that we do not see the changes that are only occurring in certain individual cells, nor can we link a given change to a certain specific location of DNA damage. Even if we mapped genome wide sites of DNA damage across the population of cells, we would not expect there to be specific hotspots of damage that we could correlate with our 3D genome effects⁵³. But, this requirement for signals to rise above the inherent variability in each cell means that the changes we observe report consistent features of the genome structure's response to irradiation. Such features are likely to reflect biologically controlled and significant responses. It is important to note that we cannot distinguish whether these consistent changes are the aggregate of many local responses or a widespread long-distance propagating response to single breaks. However, by comparing our data to future work on the 3D genome response to individual breaks, we will be better able to distinguish these possibilities²⁹. Our results, combined with other published data, do indicate that we are observing 3D genome responses to DNA damage specifically rather than a generic cellular stress response. First, we know that these genome structure changes require ATM, a DNA damage response factor. Second, published results show that other non-DNA damage stresses

either have no effect on TAD strength (as in heat shock), or cause TAD boundary weakening (as in osmotic stress)^{54,55}.

We found that the effects of the same dose of radiation on genome organization vary between different cell types, causing different specific patterns of interaction change. This observation relates to key questions about the human health implications of radiation: What factors determine the downstream severity of radiation impacts (radiosensitivity) of exposed cells and tissues⁵⁶? Why are certain cell types, genetic profiles, or disease states more radiosensitive than others⁵⁷? Under highly similar culture and IR exposure conditions, we see that fibroblasts show an overall loss of chromosome arm contacts after IR while lymphoblasts show loss of interactions between specific compartment-scale genomic regions. We hypothesize that variations in initial chromosome structure and local chromatin state are a good candidate to explain some of the variations in the response to IR of different cells under different conditions. Since notable variations in initial chromosome structure occur across different cell types, in disease, and in response to different external conditions, our results support future work investigating the contribution of initial chromatin state to the changes we observe^{54,55,58}.

Most strikingly, this study revealed that an increase of TAD boundary strength after exposure to IR is consistent across cell types and that this phenomenon is dependent on functional ATM activity. Considering this observation in the light of previous research suggests that this TAD strengthening

after IR may arise from CTCF and cohesin's dual roles in genome architecture and DNA damage response. Cohesin has long been known to be important in the DNA damage response and is a target of ATM^{20,59}. ATM signaling also increases CTCF recruitment to chromatin, CTCF sites are vulnerable to DNA breaks, and CTCF depletion increases genome instability^{17,53}. Meanwhile, increasing evidence supports the idea that cohesin and CTCF participate in the formation of TADs by loop extrusion^{2,23,24,39}. Thus, we propose that more cohesin complexes recruited to sites of DNA damage bring regions within TADs together more frequently as they extrude loops. As they do so, they would make H2AX phosphorylation within TADs more likely, potentially explaining the apparent coincidence of γ H2AX foci and TADs^{14,50}. The extruding complexes would then be blocked by increased CTCF recruitment, further decreasing interactions between TADs and leading to the boundary strength increases we see across cell types.

Why would increased TAD strength matter biologically to the cells exposed to radiation? Such increased genome structure segregation in response to DNA damage could be an important part of the cell's ability to maintain genome integrity and prevent deleterious translocations. It is known that translocations which occur across TAD boundaries can result in the misregulation of genes and be oncogenic, and it is known that translocation frequency is influenced by pre-existing genome contact frequency^{7,8}. Thus, decreasing contacts across TAD boundaries in response to DNA damage would be one way to decrease the frequency of translocation events that disrupt TAD boundaries. We observe long-term

persistence of TAD strengthening after IR (even at 5 days after IR, when cells have been allowed to proliferate). Notably, the cells that survive to divide are those that have not been eliminated by mitotic catastrophe due to deleterious translocations. If TAD strengthening is protective, we would predict that exposure to and recovery from one round of IR would make the cell population more resistant to deleterious effects of a second round of IR. While it is difficult to quantitate what degree of boundary strength change would have a meaningful biological impact, previous work has suggested a role for similar changes in boundary elements in biological processes such as gene dosage compensation⁴¹.

Future work will be needed to clarify the role of chromatin state, cell cycle state, and genome architecture proteins in the responses to IR that we observe. Perturbations of chromatin state before IR exposure would reveal the impact of different initial structures on downstream effects of radiation. Similarly, measuring the effect of irradiation on cells with altered levels of CTCF or cohesin will determine whether these factors indeed affect the spread of γ -H2AX. The consistent responses of GM12878, which have sub-populations in G1, G2, and S phase, and BJ cells, which are primarily in G1, suggests that effects we observe are not entirely dependent on cell cycle state. But, future work repeating these experiments on cells synchronized in different phases of the cell cycle, where different repair pathways are favored, would better clarify the role of different repair pathways in the effects we observe. Our data supports a model in which ATM is required for the reinforcement of 3D genome organization after exposure to IR.

But, the possibility remains that not only ATM, but also the subsequent signaling cascade involved in DNA repair downstream of ATM may be necessary to elicit the genome structure responses we observe in healthy cells. To understand more clearly how DNA damage pathways influence the 3D genome architecture, the role of proteins such as DNA ligases and ATR must be interrogated more thoroughly^{60,61,62,63,64,65}.

Additionally, future work is needed to identify the biological importance of TAD strengthening after IR. It is possible that the TAD structure effects we observe are indeed a byproduct of repair processes, but not an important or controlled component of protecting the genome from aberrations. In the future, artificially altering TAD strength, by altering concentrations of architecture proteins, would allow a more direct perturbation of the effect of TAD strength on cellular radiosensitivity and downstream effects of radiation.

METHODS

Cell culture

Fibroblast cell lines include: BJ-5ta (ATCC, CRL-4001), BJ1-hTERT (kind gift from Vilhelm A. Bohr, Laboratory of Molecular Genetics, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, MD), MRC-5 (kind gift from Tim Sparer, University of Tennessee, Knoxville, Knoxville, TN), AG04405 (ATM-hTERT) (kind gift from Peter Lansdorp, University of British Columbia, Vancouver, Canada), and GM02052 (Coriell). The lymphoblastoid cell line GM12878 (Coriell) was also used. Information about cell

lines and media formulations is summarized in Table 2. All growth media and fetal bovine serum was purchased from Corning, except for GM12878 growth media from Gibco. All fibroblast lines were passaged at a density of 80%. For irradiation experiments, fibroblast lines were grown to confluency prior to X-ray exposure. For the GM12878 suspension cell line, cells were passaged at a density around 500,000 cells per 1 mL of medium. For irradiation experiments, GM12878 cells were grown to a density of 1 million cells per 1 mL of medium prior to X-ray exposure.

X-ray irradiation

Cells were irradiated with 5 Gy X-rays using a RadSource RS-2000 Biological Irradiator. X-rays were emitted with a concentration of 2Gy min^{-1} with cells being exposed for 2 minutes and 30 seconds (160.9 kV; 25.1 mA). After irradiation, cells were returned to an incubation chamber and kept at 37°C with 5% CO_2 . Cells were collected at 0 minutes, 30 minutes, or 24 hours post-irradiation and fixed for the respective downstream application.

Immunofluorescence staining and image analysis

Fibroblast cells were seeded on poly-D-lysine (PDL) coated 35 mm coverslip dishes prior to X-ray exposure. For GM12878 cells, coverslips were sterilized and coated in 0.1 mg /mL^{-1} PDL. GM12878 cells were X-rayed while in suspension and centrifuged onto coverslips at $800\times g$ for 10 minutes immediately before fixation. At the appropriate time points, all cells were fixed in 4% formaldehyde for 20 minutes at room temperature, washed in PBS for 5 minutes

three times, and then concurrently permeabilized and blocked in blocking buffer (10% goat serum, 0.5% triton in 1X PBS) for 1 hour at room temperature. The primary antibody to γ -H2AX (Abcam- ab26350, 1:500) was diluted in a 1:1 mixture of blocking buffer and PBS, and incubated overnight at 4°C. Samples were then washed three times in PBS for 5 minutes and incubated in secondary antibody (Alexa Fluor 488 goat anti-mouse, Invitrogen; R37120) for 30 minutes at room temperature, in the dark. Samples were washed as previously described and exposed to 0.1 mg/mL DAPI for one minute, followed by washing. All PBS was removed by aspiration and samples were briefly air dried. Coverslips were mounted on slides with Slow fade Diamond Antifade Mountant (Molecular Probes, S36963).

Images were acquired using a Leica SP8 confocal microscope equipped with a 63 $\times$ oil immersion objective (Leica) using the same settings. Image quantification was carried out by measuring the mean fluorescence intensity of γ -H2AX inside the nucleus divided by area of the nucleus in ImageJ. γ -H2AX foci were counted by normalizing fluorescence to remove background and using the analyze particle function to select foci. An ordinary one-way ANOVA test was used to determine statistical significance for all image quantification.

Flow cytometry and cell cycle analysis

Cells were fixed before or after irradiation and fixed with 70% ethanol for 30 minutes at 4°C. Cells were then centrifuged for 10 minutes at 800 $\times$ g for 10 minutes and resuspended with Guava® Cell Cycle Reagent (Luminex, 4500-0220). 10,000

events were collected for each condition on an Attune Nxt Acoustic Focusing Cytometer using software version 2.1. Cell cycle analysis was done using Flowing Software 2.5.1 (Terho, P. Cell Imaging Core, Centre for Biotechnology, University of Turku, Finland).

Hi-C

To prepare the Hi-C libraries and prepare for sequencing, at the appropriate time points, Hi-C experiments were performed according to the protocol detailed in Gollosi et al.⁶⁶. Briefly, 5-20 million cells were fixed with 1% formaldehyde, suspended in cell lysis buffer for permeabilization, and homogenized by douncing. Crosslinked chromatin was digested overnight with HindIII. Sticky ends were filled in with biotin-dCTP, and the blunt ends of interacting fragments were ligated together. DNA was purified by two phenol-chloroform extractions and ethanol precipitation. Biotin-dCTP at unligated ends was removed, and the DNA was sheared to a target size of 200-400 bp by a Covaris sonicator (Covaris, M220). DNA between 100-400 bp was selected for using AMPure XP beads (Beckman Coulter). Biotinylated DNA was pulled down using streptavidin coated magnetic beads and prepared for multiplex sequencing on an Illumina platform using the NEBNext Ultra II kit (NEB, E7645S). All end preparation, adaptor ligation, and PCR amplification steps were carried out on bead bound DNA libraries.

Sequencing was carried out at the Oklahoma Medical Research Foundation Clinical Genomics Facility on an Illumina HiSeq 3000 platform with 75 bp paired end reads or a NovaSeq 6000 with 50 bp paired end reads. Sequencing reads

were mapped to the reference human genome hg19, filtered, and iteratively corrected using the pipeline detailed in Imakaev et al.⁶⁷ and available on github (dekkerlab-cMapping). All processed Hi-C contact maps were scaled to a sum of 1 million to allow comparisons between conditions for subsequent analyses.

ATMi Treatment

BJ-5ta fibroblasts were grown to confluency and supplemented with 10µM KU-55933 (Selleck Chemicals, S1092) for 1 hour before irradiation. For microscopy experiments, cells were fixed before and 30 minutes after 5 Gy X-Rays exposure consistent with previous microscopy experiments. Cells were collected for Hi-C before exposure to 5 Gy X-rays and 24 hours after exposure with the previously stated Hi-C protocol.

Hi-C Reproducibility Assessment

A spearman correlation-based metric was used to assess reproducibility across all Hi-C datasets. After taking the log of iteratively corrected interaction counts, binned at 2.5 Mb across the entire genome, correlation maps were calculated for each individual matrix. Each entry in such a Hi-C data correlation matrix represents the correlation between the full row and column of data at that position in the matrix. This is like the calculation typically performed to emphasize interaction patterns over the average distance decay for compartment analysis. Then, each genome-wide correlation matrix was flattened into a vector (concatenating all columns into one single column), and all vectorized Hi-C

matrices were compared with pairwise Spearman correlation to obtain the final reproducibility metric between each pair of datasets. The results from this approach match well with the published GenomeDISCO approach⁶⁸.

Interaction vs. Distance Scaling Plots

Using 250 kb binned iteratively corrected and scaled contact matrices, we extracted contact frequencies between bins at each genomic distance, excluding the diagonal bin (zero distance). We then used a loess fit to find a smooth curve describing interaction decay vs. distance. Loess curves for each chromosome were averaged together to generate a single scaling curve representing the whole genome. The interaction frequencies were then normalized to set the maximum value (loess fit value for the minimum distance) for each dataset to 1 and then plotted on a log scale.

Compartment analysis

Principle components analysis was performed using the matrix2compartment perl script in the cworld-dekker pipeline available on github on 250 kb binned matrices. The eigen1 value, which typically represents the compartment profile, was used to determine A/B compartments.

TAD boundary and insulation analysis

Topologically associating domain (TAD) boundaries were called on 40 kb binned matrices using two methods: the InsulationScore method, as detailed in Crane et al., available as matrix2insulation in the cworld-dekker github package, and the Hicratio method, as described in Gong et al.^{36,37}. For the InsulationScore,

a 500 kb insulation square size was used, and for the Hicratio method, we used $d = 400$ kb. Insulation contact maps in main figures were generated by averaging all called TAD boundaries with an insulation score greater than one as determined by the InsulationScore method. We plotted 500 kb away from the diagonal and 500 kb on either side of the TAD boundary. To remove biases introduced by the contact matrix diagonal, we excluded the 30 kb closest to the diagonal. Boxplots were generated using boundary strength scores determined by both InsulationScore and Hicratio methods. All TAD boundaries were plotted for the InsulationScore boxplots. For Hicratio boxplots, outliers were removed by filtering out TAD boundaries with a Hicratio score greater than twelve or less than one.

Western blot

At the appropriate time points, cells were trypsinized using standard protocols, and up to 5 million cells were collected by centrifugation (1000 rcf, 5 min, room temperature). After carefully removing the media from the pellet, cells were resuspended in 300 μ l of RIPA buffer (Thermo Scientific, 189900), supplemented with protease inhibitors/EDTA (GenDepot 50-101-5485), and phosphatase inhibitors (GenDepot 50-101-5488). Cells were lysed by repeated pipetting followed by incubation, on ice, for 10 minutes. DNA was degraded by adding 1.5 μ l of micrococcal nuclease (0.5 U/ μ l⁻¹ stock, Thermo Scientific, FEREN0181) and 3 μ l of calcium chloride (100 mM stock) to each lysate, followed by incubation at 37°C for 15 min. Nuclease was inactivated by subsequent

incubations at 68 °C for 15 min, and ice for 10 min. Cell debris was removed by centrifugation at 14,000 rcf for 15 minutes. Protein samples were stored at -80 °C until use. Protein concentration was measured using the BCA Protein Assay Kit. Thermo (234225), according to the manufacturer's instructions. Denatured protein samples were resolved using a mini gel tank (Invitrogen, A25977) for 35 min at 200V, using 4-12% Bis-Tris Plus gels (Invitrogen, NW04120BOX) for all other targets. Protein was transferred to low fluorescence PVDF membranes using the mini Bolt Blotting System (Invitrogen, B1000), along with system-specific reagents. Blotting was performed using the Odyssey TBS blocking system (Licor, 927-400000), according to the manufacturer's protocol. Briefly, membranes were activated with methanol after transfer (1 min, RT), washed twice with milliQ water (5 min), twice with TBS (2 min), and blocked with Odyssey TBS blocking solution for 1 hour at RT. Primary antibodies were diluted in blocking buffer (containing 0.2% Tween), and incubated over night at 4°C as follows: γ -H2AX (1:1000, mouse monoclonal [9F3], Abcam). Beta actin was used as a loading control, using the appropriate antibody (1:10,000; rabbit polyclonal PA1-16889, Thermo Fisher or 1:10,000; mouse monoclonal MA1-140, Thermo Fisher) concomitantly with targets of interest. Detection was carried out using the following secondary antibodies: goat anti-rabbit (1:10,000; IRDye 680RD [92568071]; Licor) and goat anti-mouse (1:10,000, IRDye 800CW [95-32210]; Licor). Secondary antibodies were diluted in Odyssey TBS blocking buffer containing 0.2% Tween and 0.01% SDS.

Membranes were incubated in secondary antibody for 1 hour at RT, followed by three washes (TBST, 5 min ea.).

Images of near infrared fluorescent signal were acquired using an Odyssey scanner (Licor) in both the red and green channels. Signal was quantitated using the Image Studio software (Licor).

Data Availability

All relevant data supporting the key findings of this study are available within the article and its Supplementary Information files or from the corresponding author on reasonable request. The Hi-C data generated in this study have been deposited in Gene Expression Omnibus (GEO) under accession number GSE136899. Source data are provided with this paper.

Code Availability

The majority of software and code used for the analyses presented are available as previously published public github packages, as described in the Methods section. Custom scripts for calculating HiC Ratio TAD boundaries and measuring Hi-C reproducibility are available from [<https://github.com/rpmccordlab/XrayHiCAnalysis>].

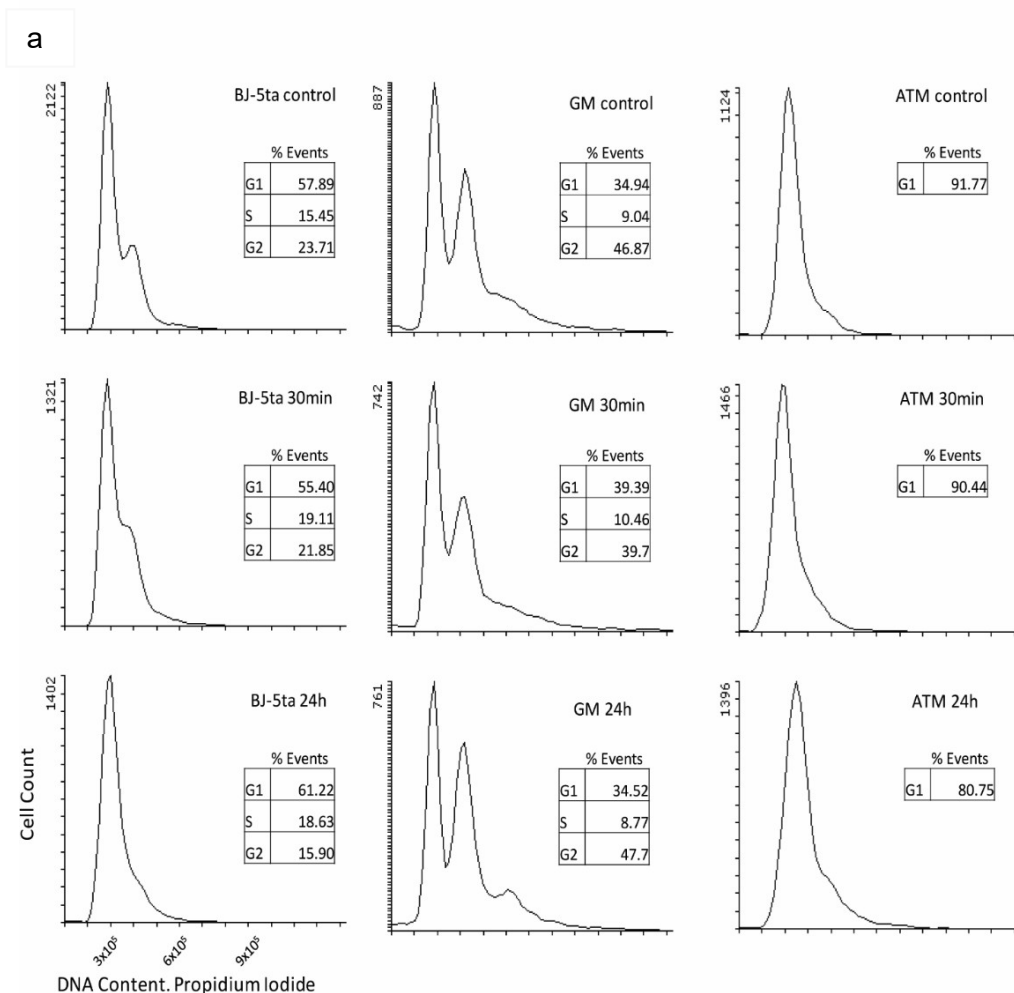


Figure 1. Flow cytometry analysis of BJ-5ta, GM12878, and ATM-hTERT cells before and after irradiation.

a Y-axis shows cell counts while x-axis represents the fluorescence measurement of DNA content. BJ-5ta cells are predominantly in G1 in all conditions but contain a small population of G2 in control and 30 minutes post IR. GM12878 cells maintain a very similar cell cycle state distribution before and after IR. ATM-hTERT cells are mostly G1 stalled in all conditions.

a BJ-5ta stained with γ -H2AX (green) and DAPI (blue) before (Control) or after (30 min and 24 hr) exposure to 5 Gy X-rays. Scale bar: 10 μ m. **b** Genome wide contact heatmap for BJ-5ta non-irradiated (lower left) or 30 minutes after IR (upper right) in 2.5 Mb bins. **c** $\log_2$ (30 minutes post IR/Control) contact heatmap in 2.5 Mb bins. **d** $\log_2$ (24 hours post IR/Control) contact heatmap in 2.5 Mb bins. Inset reveals loss of interactions between chromosome arms and decrease in telomere-telomere interactions on chromosomes 1 and 2.

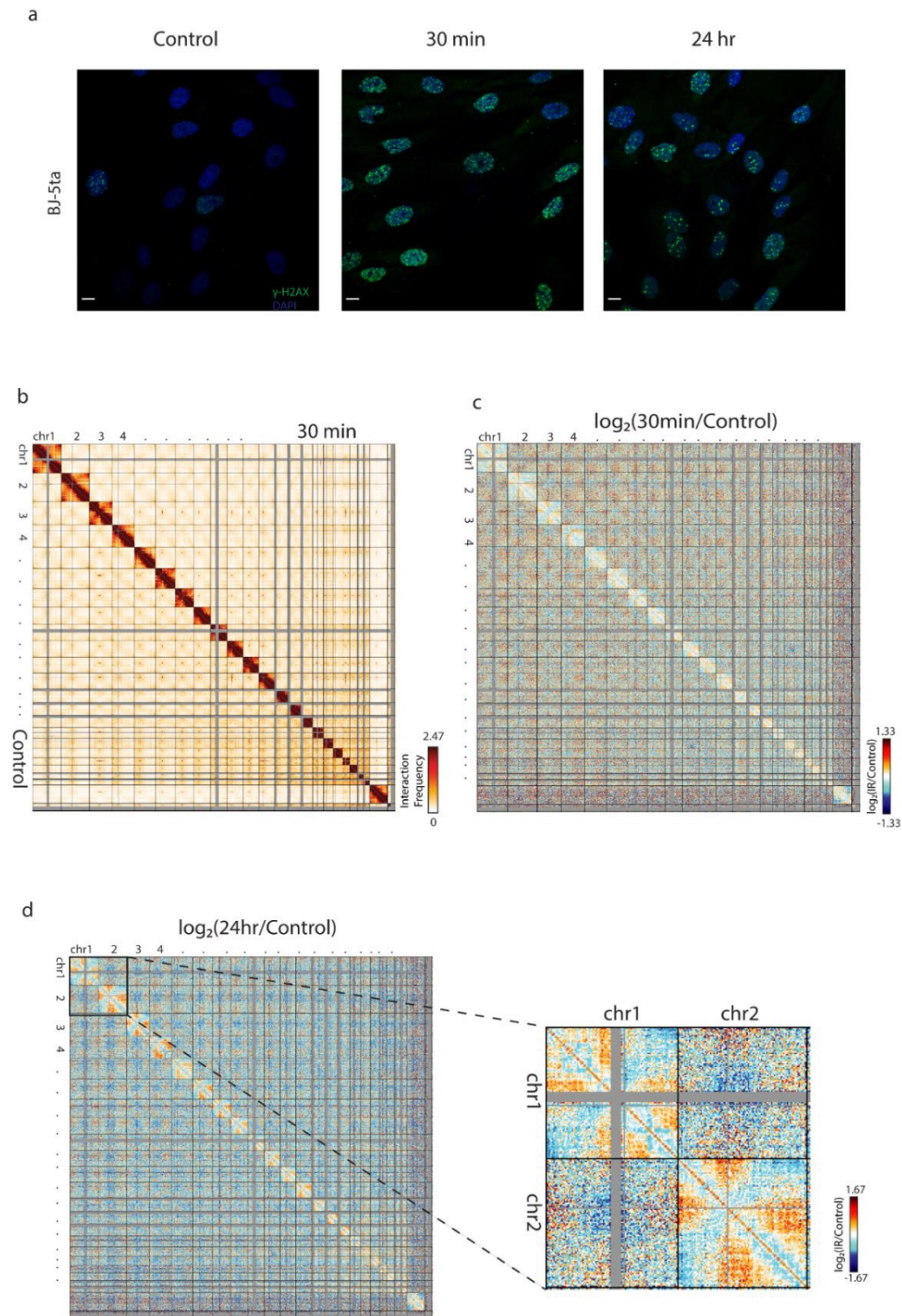
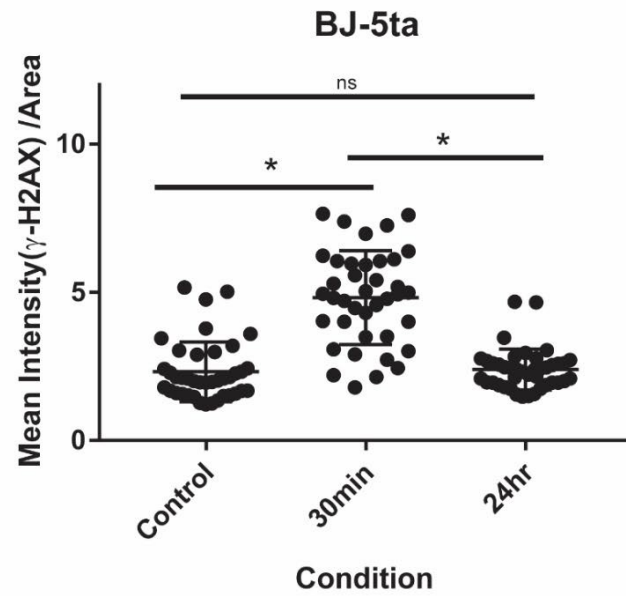


Figure 2. Subtle changes to genome organization in BJ-5ta skin fibroblasts exposed to IR.

a Quantification of mean γ -H2AX fluorescence intensity per nucleus area in BJ-5ta cells after exposure to IR (n=40 cells, * p = <0.0001, one way ANOVA with Tukey's multiple comparison test. Data is presented as the mean of SD with the 25th (bottom line) and 75th (Top line) percentile shown). **b** Western blot for β -actin (red) and γ H2AX (green). Bands around ~15 kDa indicate the presence of γ H2AX.

a



b

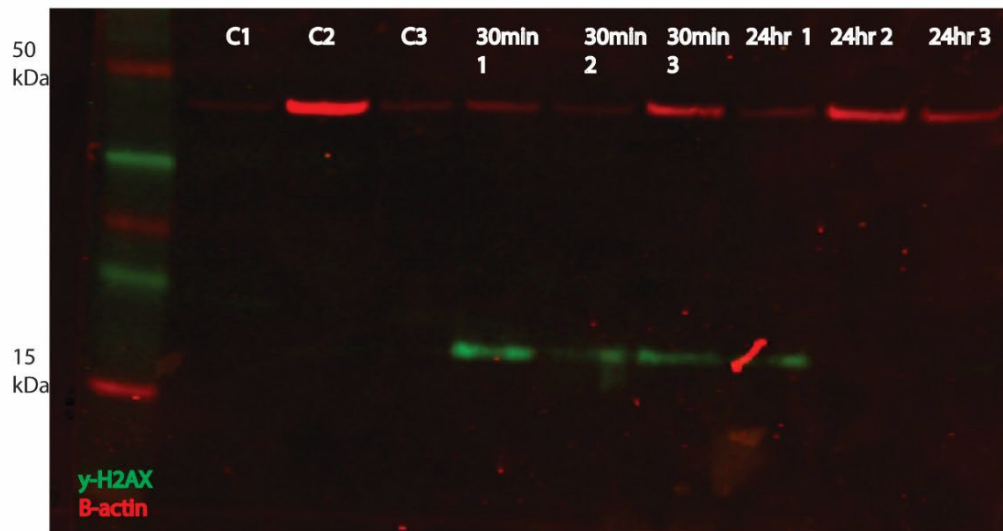


Figure 3. γ -H2AX levels increase in BJ-5ta after exposure to IR.

a Schematic of experimental procedure. BJ-5ta were irradiated with 5 Gy X-rays and then grown for up to 120 hours in the same dish (b) or passaged 24 hours after irradiation and split into separate dishes and grown for up to 120 hours (c). **b** BJ-5ta stained with γ H2AX (green) and DAPI (blue) after exposure to 5 Gy X-rays in a confluent contact-inhibited culture. Scale bar: 20 μ m. **c** BJ-5ta stained with γ H2AX (green) and DAPI (blue) in cultures passaged 24 h after exposure to 5 Gy X-rays and then allowed to continue growing for indicated total times after X-ray. Scale bar: 20 μ m. **d** Quantification of γ H2AX foci count per nucleus for confluent culture over time (as in b) (n=15 cells, * $p < 0.02$ ** $p < 0.005$, one-way ANOVA. Data is presented as the mean of SD with the 25th (bottom line) and 75th (Top line) percentile shown). **e** Quantification of γ H2AX foci count per nucleus for cells passaged after exposure to X-ray (as in c). Time reports time after passaging (so 24 h after split = 48 h total after Xray) (n=19 cells for 24hr, 72hr, and 96hr. n=24 cells for 48hr, one way ANOVA. Data is presented as the mean of SD with the 25th (bottom line) and 75th (Top line) percentile shown).

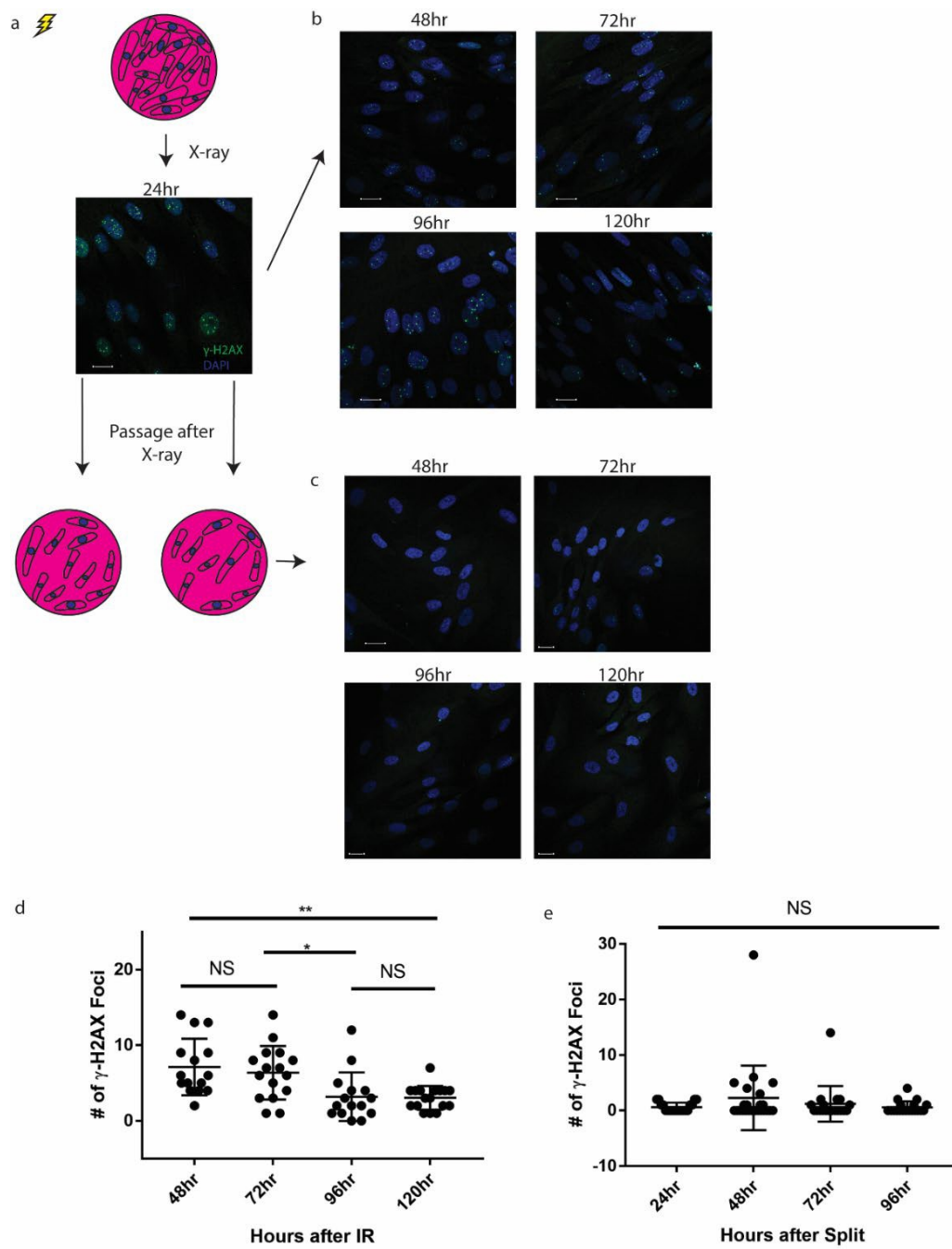


Figure 4. Unrepaired γ -H2AX foci remain until after passaging BJ-5ta cells.

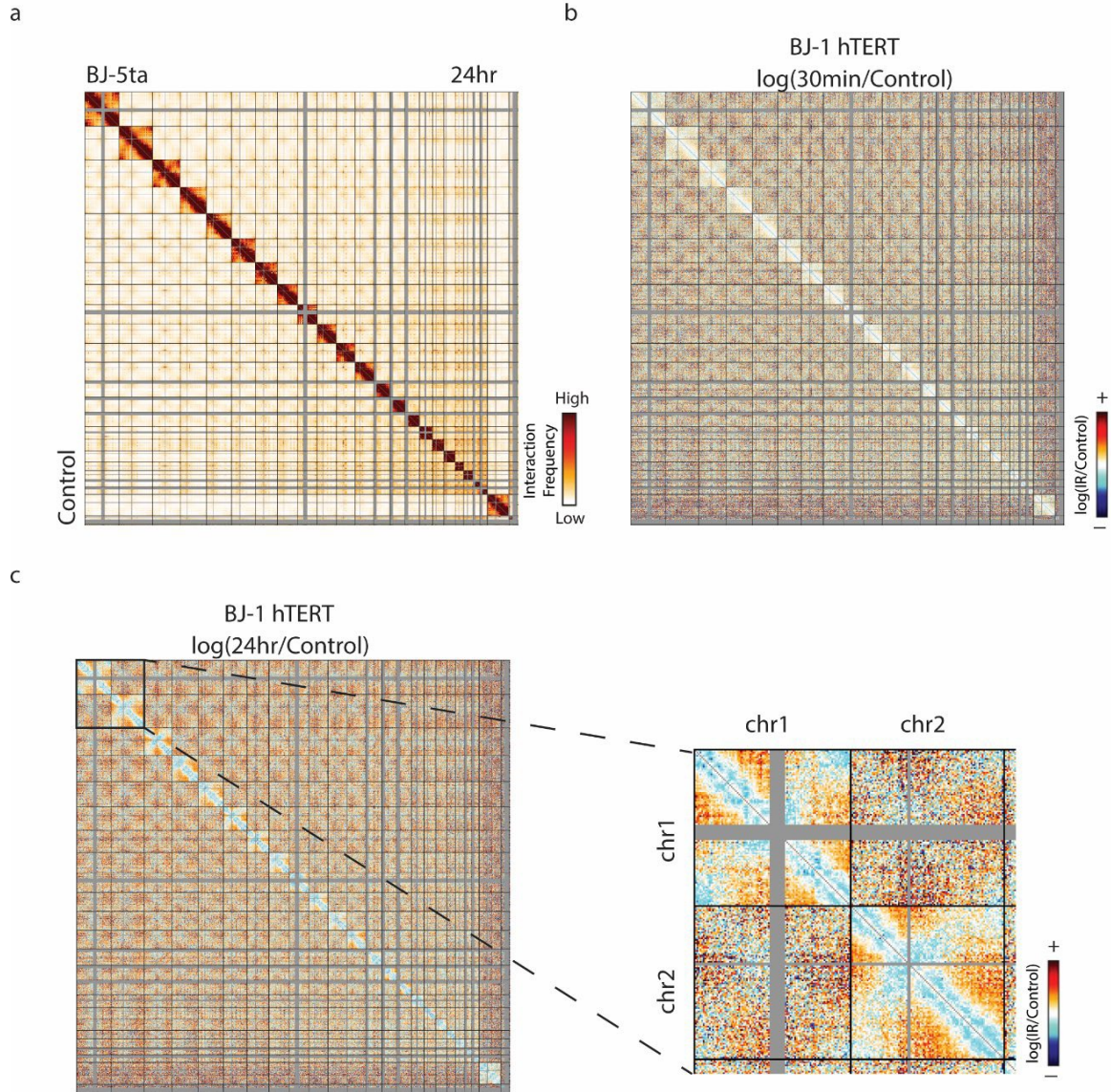


Figure 5. BJ-5ta and BJ1-hTERT share similar responses to IR.

a Genome wide contact map of non-irradiated BJ-5ta (left) and 24 hours after irradiation (right). **b** $\log_2(30 \text{ minutes post IR}/\text{Control})$ BJ1-hTERT contact heatmap in 2.5 Mb bins. **c** $\log_2(24 \text{ hours post IR}/\text{Control})$ BJ1-hTERT contact heatmap in 2.5 Mb bins. Inset reveals loss of interactions between telomeres after IR in chromosomes 1 and 2 analogous to Fig. 1d.

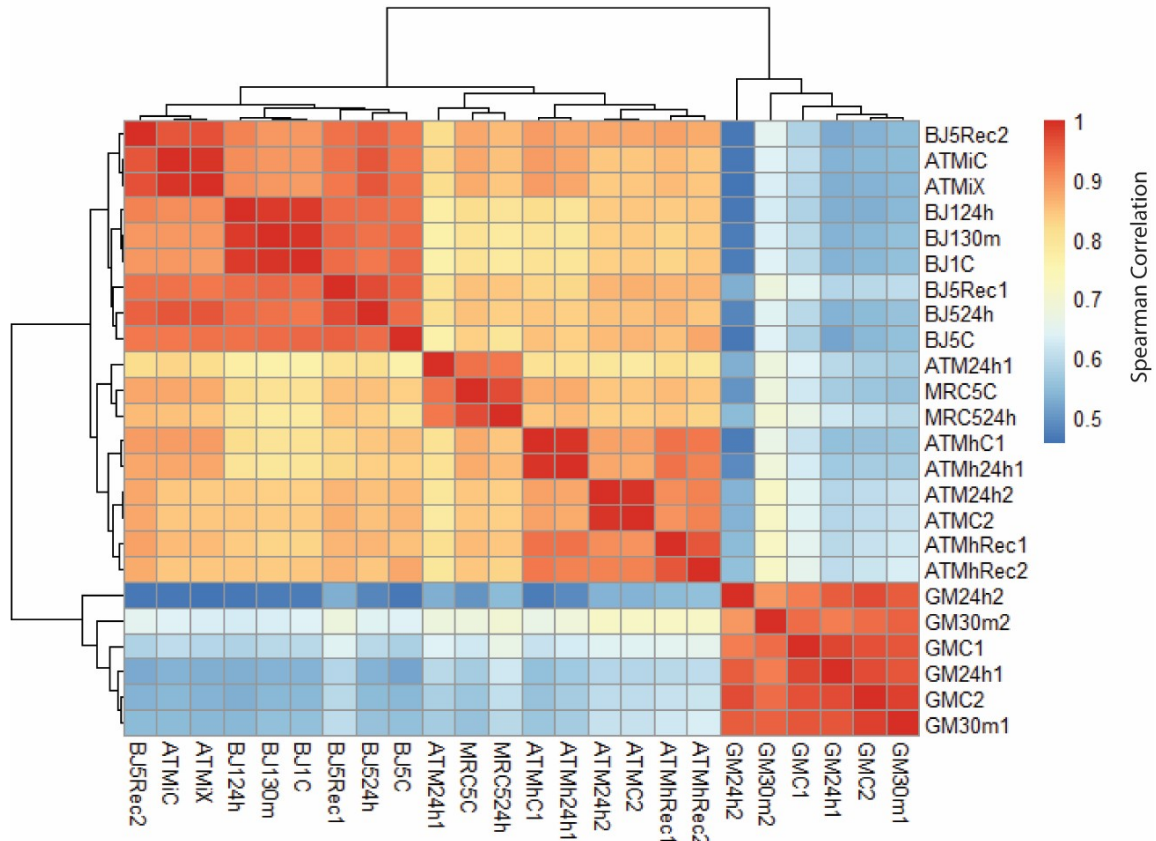


Figure 6. Reproducibility correlation plot for all Hi-C experiments.

Spearman correlation-based reproducibility is calculated as described in the Methods. ATM proficient fibroblasts mostly cluster together and separately from ATM deficient fibroblasts. GM12878 cells cluster separately from fibroblasts. C= Control, X=Xray, Rec=5 day recovery, 1 and 2 = replicate numbers, ATMi = ATM inhibitor treatment of BJ5-ta, ATMh = ATM mutant cells.

a GM12878 stained with γ -H2AX (green) and DAPI (blue) before or after exposure to 5 Gy X-rays. Scale bar: 10 μ m. **b** Genome wide contact heatmap of GM12878 in non-irradiated (left) or 30 minutes after IR (right) in 2.5 Mb bins. **c** $\log_2$ (30 minutes post IR/Control) contact heatmap in 2.5 Mb bins. **d** $\log_2$ (24 hours post IR/Control) contact heatmap in 2.5 Mb bins. Inset reveals example patterns of intrachromosomal interactions that are lost after exposure to IR.

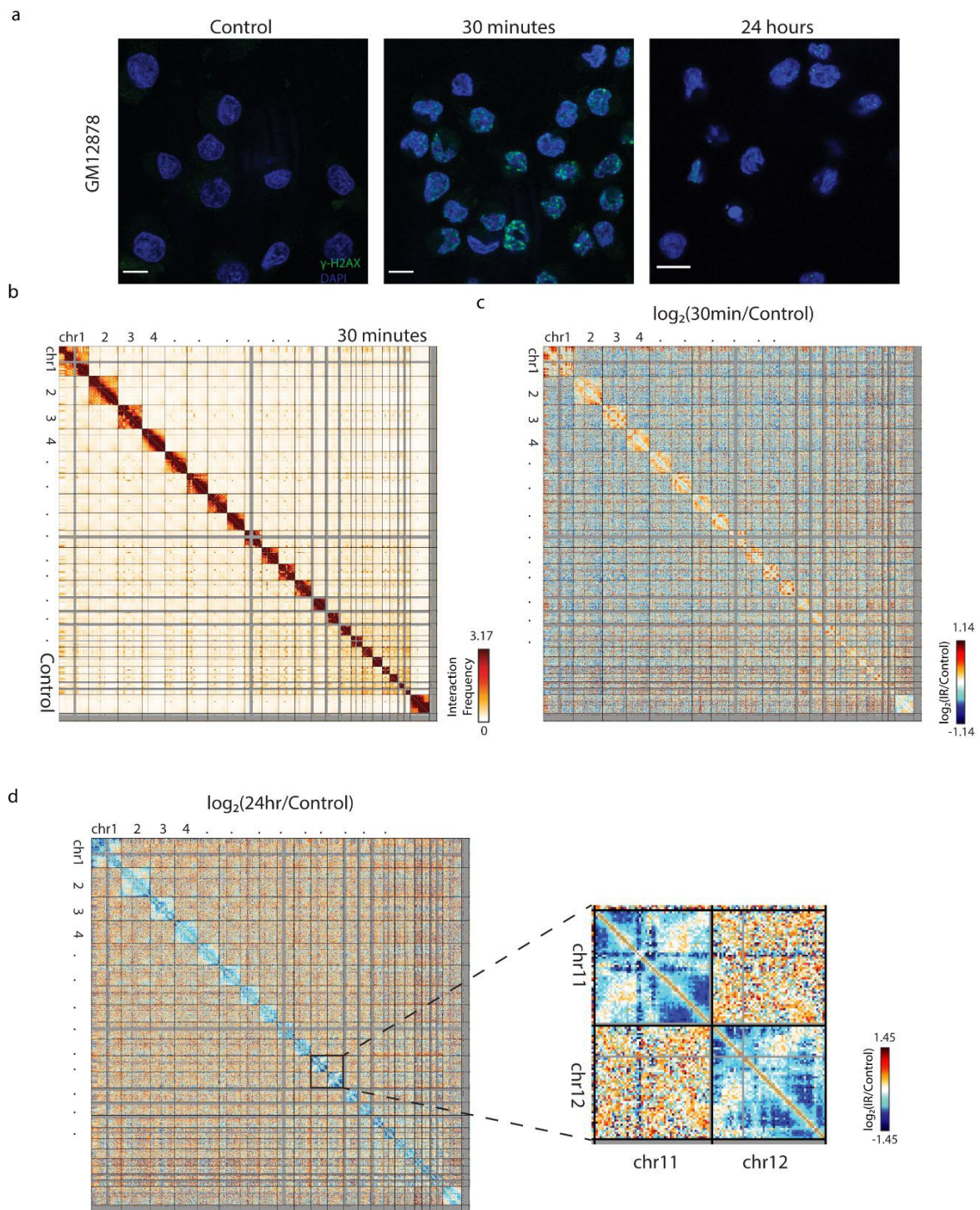
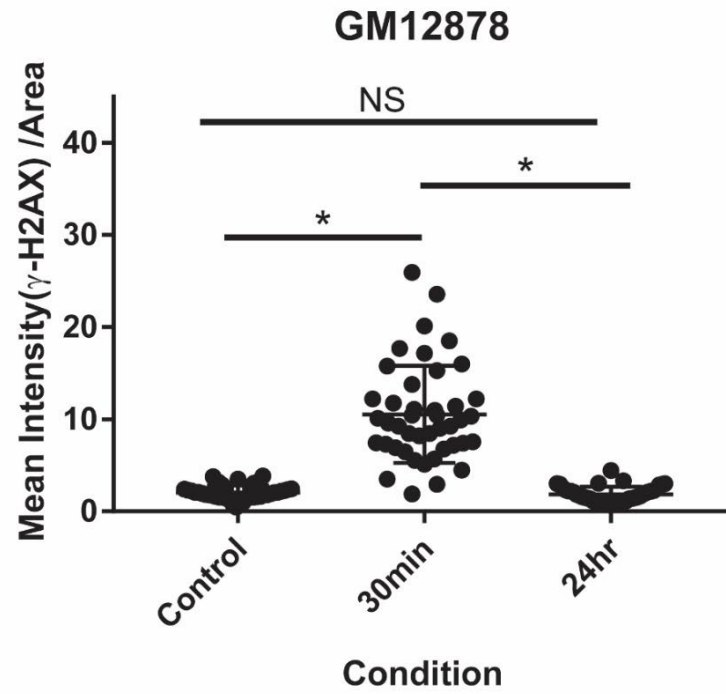


Figure 7. GM12878 exhibits more evident changes to genome organization after exposure to IR.

a Quantification of mean γ -H2AX fluorescence intensity per nucleus area in GM12878 cells after exposure to IR (n=40 cells, * p = 0.0001, one-way ANOVA. Data is presented as the mean of SD with the 25th (bottom line) and 75th (Top line) percentile shown). **b** Western blot analyses for β -actin (red) and γ H2AX (green). Bands around ~15 kDa indicate the presence of γ H2AX. GM12878 contains more γ H2AX in control cells than BJ-5ta, but this may be due to ongoing cell division, including replication stress.

a



b

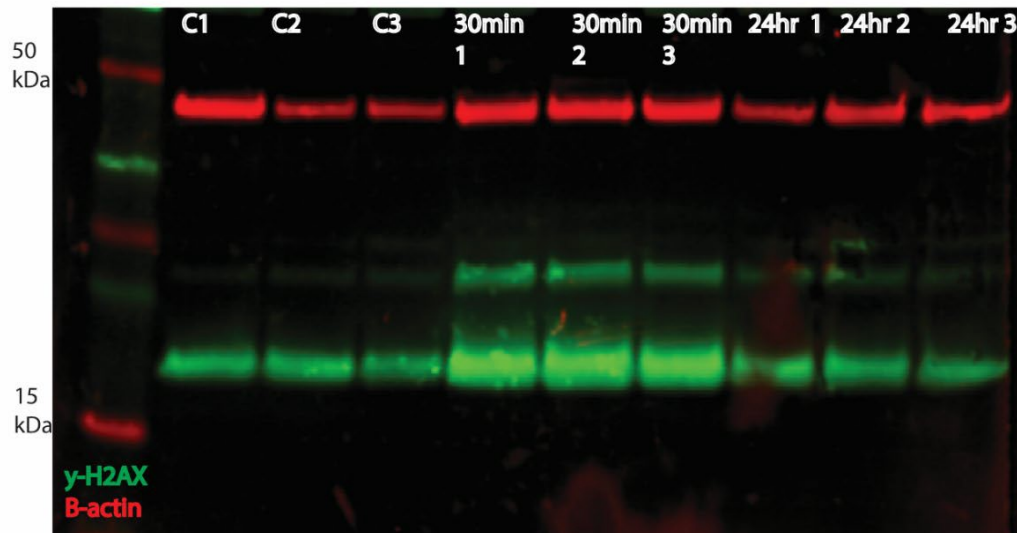


Figure 8. γ -H2AX levels increase in GM12878 after exposure to IR.

a Genome wide contact map of non-irradiated GM12878 (bottom left) and 24 hours after irradiation (upper right). **b** $\log_2$ (30 minutes post IR/Control) GM12878 contact heatmap in 2.5 Mb bins. **c** $\log_2$ (24 hours post IR/Control) GM12878 contact heatmap in 2.5 Mb bins. Inset reveals similar specific changes within chr11 and chr12 here in Replicate 1 as shown in main Figure 2 (Replicate 2). **d** Scaling plots showing average decay of contacts with distance across all chromosomes at a 250 kb bin size for GM12878 (R1) before and after IR.

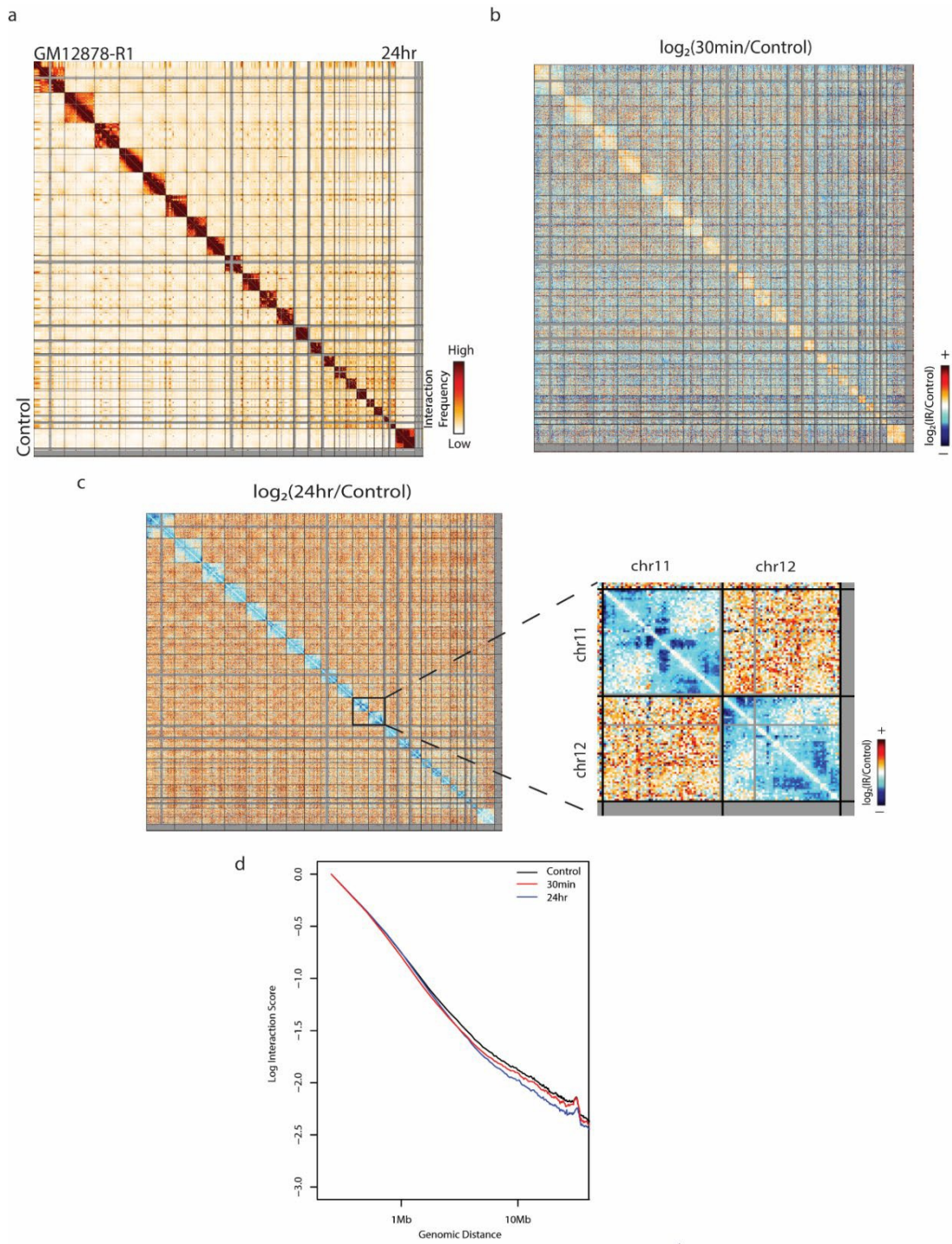


Figure 9. Similar major features of GM12878 genome structure change after IR are observed in a biological replicate.

a, b Scaling plots showing average decay of contacts with distance across all chromosomes at a 250 kb bin size in BJ-5ta (**a**) or GM12878 (**b**) cells for control, 30 minutes post IR, and 24 hours post IR. **c, d** 250kb binned Hi-C interaction heatmaps for chromosome 4 in BJ-5ta (**c**) and GM12878 (**d**) cells in control (Top), 30 minutes post IR (Middle), and 24 hours post IR (Bottom). **e,f** Plots of the first eigenvector from principal component analysis of chromosome 4 in BJ-5ta (**e**) and GM12878 (**f**) cells in control (Top), 30 minutes post IR (Middle), and 24 hours post IR (Bottom).

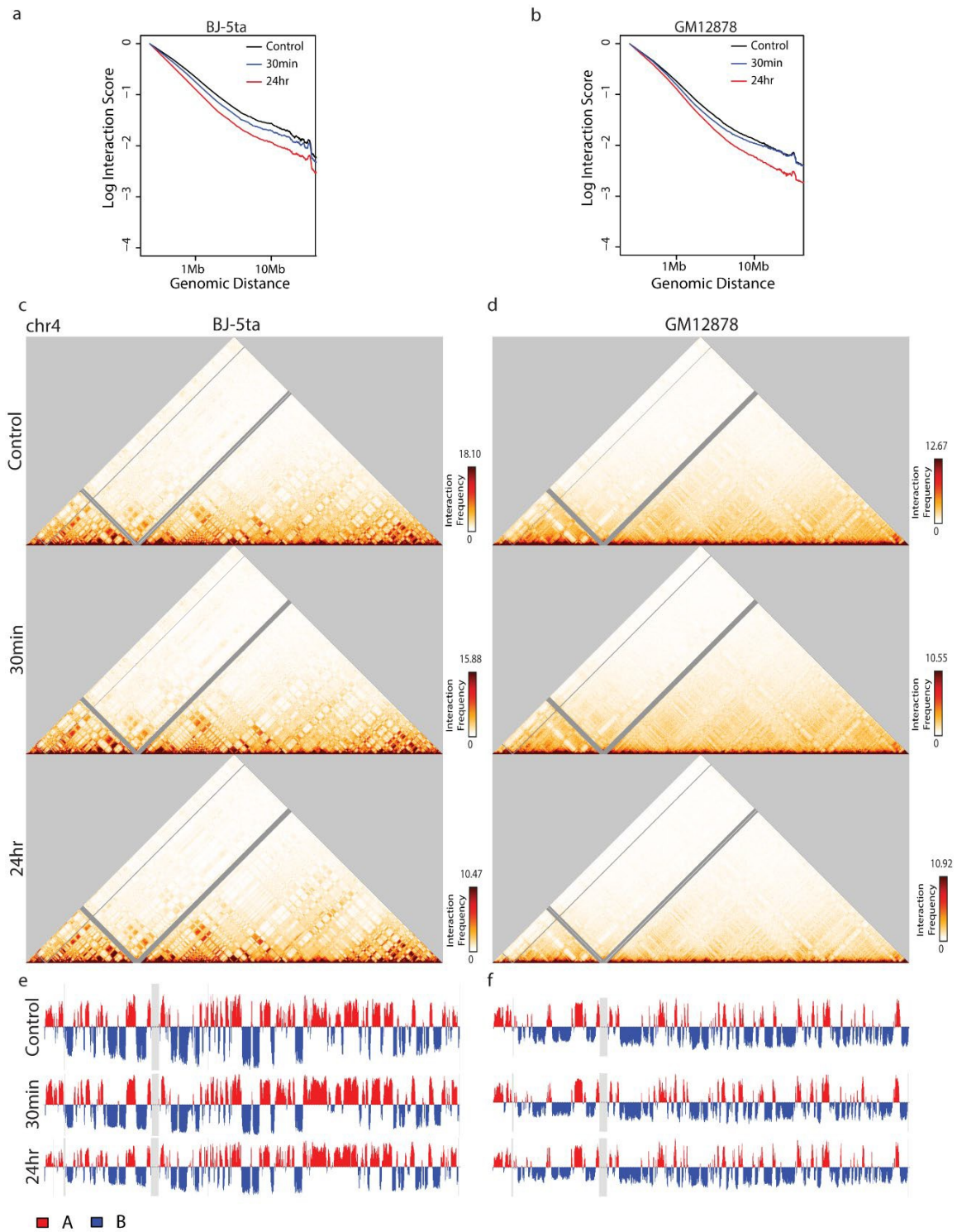


Figure 10. Healthy fibroblast and lymphoblast cell lines experience some loss of distal contacts, but maintain compartments after exposure to IR.

a 250kb bin size Hi-C interaction heatmaps (top) and plots of the first eigenvector from principal component analysis (bottom) for chromosome 16 in healthy fibroblasts: BJ1-hTERT (left) and MRC-5 (right). **b** 250kb bin size Hi-C interaction heatmaps (top) and plots of the first eigenvector from principal component analysis (bottom) for chromosome 16 in healthy lymphoblastoids: GM12878 Replicate 1. **c** 250kb bin size Hi-C interaction heatmaps (top) and plots of the first eigenvector from principal component analysis (bottom) for chromosome 16 in ATM mutant fibroblasts: ATM-hTERT Replicate 2 (left) and GM02052 (right).

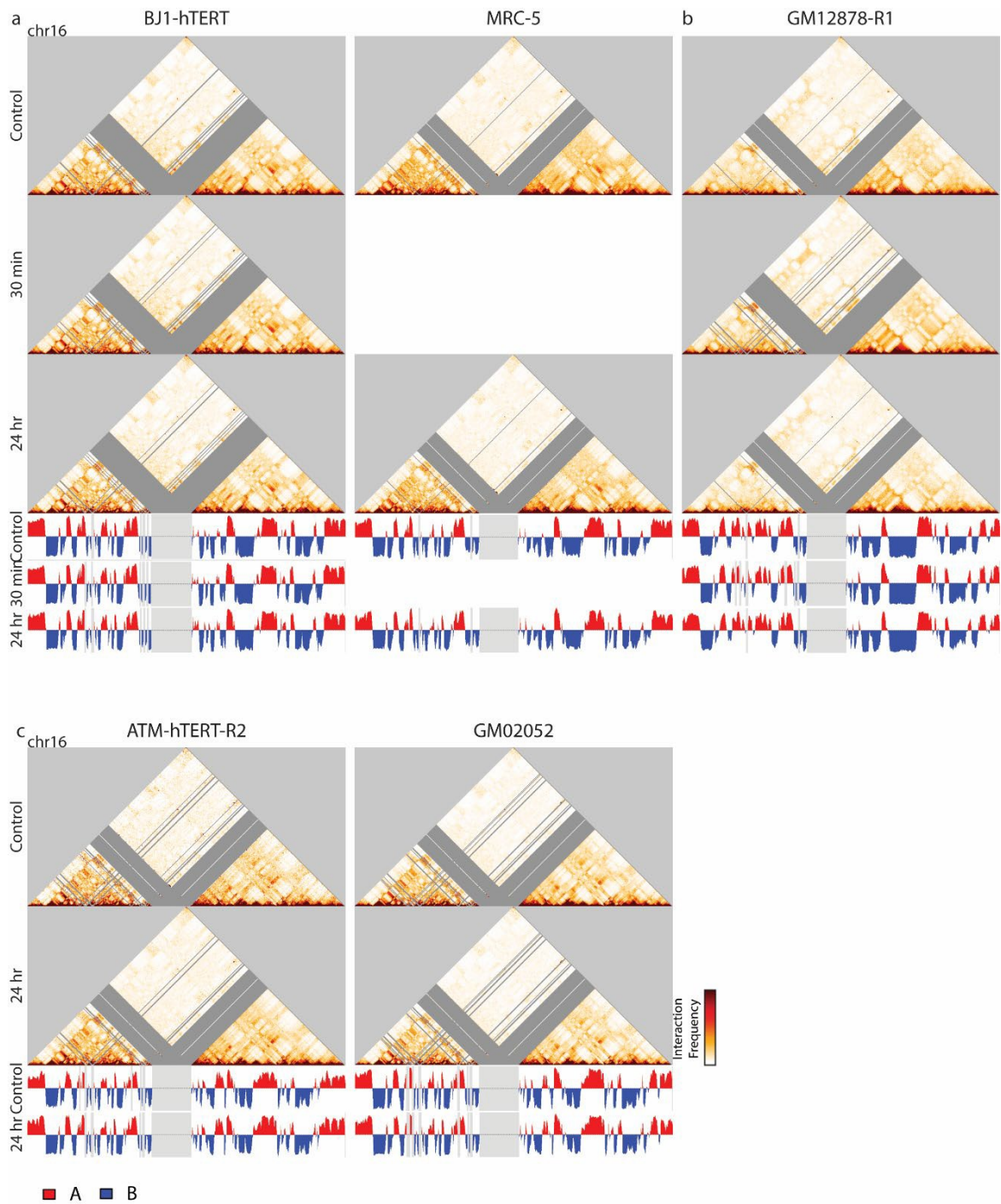


Figure 11. A/B compartment identity is robust to changes post-IR in healthy cell lines and ATM mutant cell lines.

Subtracted saddle plots (showing interaction Z-score differences from Control ordered from strongest B compartment to strongest A compartment, binned and smoothed at 500 kb) for **(a)** BJ-5ta 30 minutes (left), 24 hours (middle), Recovery (right) **(b)** BJ1-hTERT 30 minutes (left) and 24 hours (middle) **(c)** GM12878-R1 (top) and GM12878-R2 (bottom) 30 minutes (left) and 24 hours (middle) **(d)** ATM-hTERT-R1 (top) and ATMhTERT-R2 (bottom) 24 hours (left) and Recovery (middle).

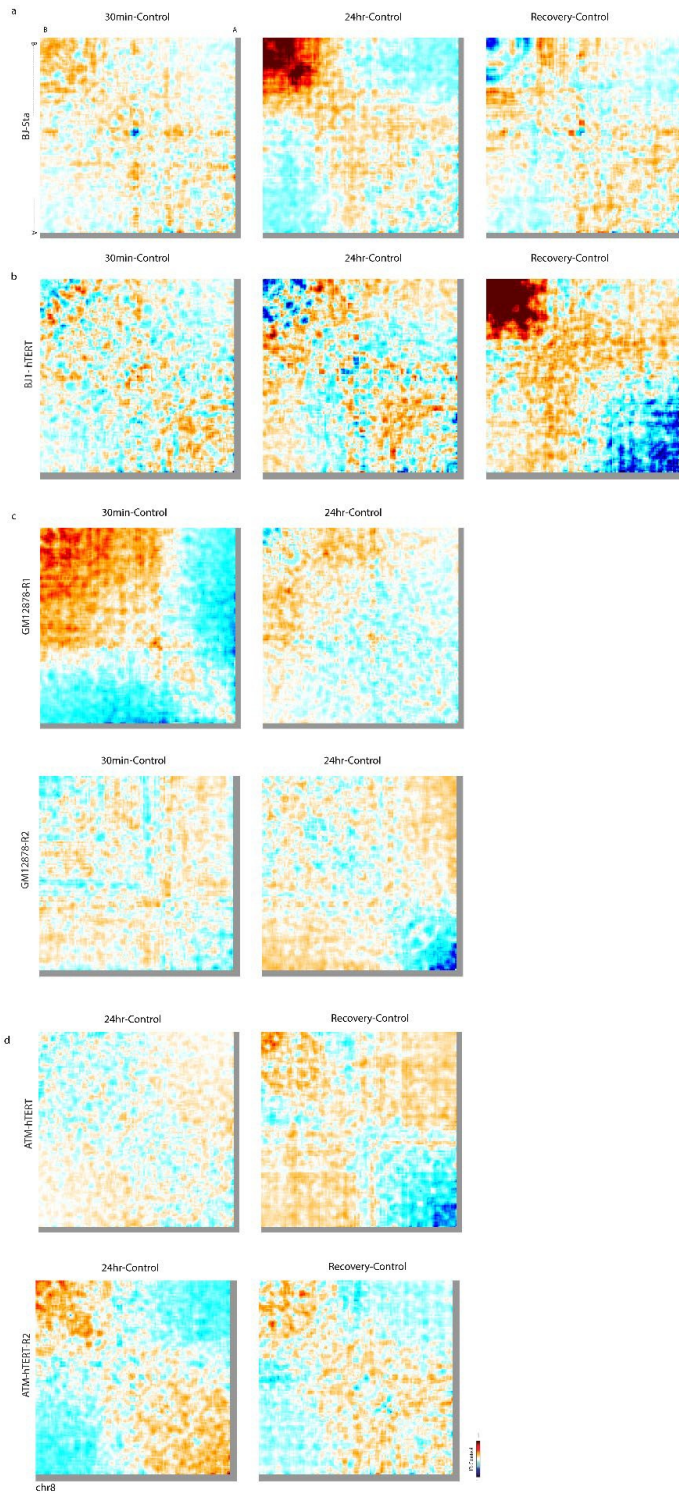


Figure 12. Compartment strength changes are subtle and inconsistent between replicates after X-ray.

a, b TAD boundary strength boxplots calculated using both the InsulationScore (a) (Number of TAD boundaries (N) BJ-5ta = 1232, BJ-5ta – ATMi = 2010, GM12878 = 1091) and Hicratio (Number of TAD boundaries (N) BJ-5ta = 1298, BJ-5ta -ATMi = 2868, GM12878 = 1878) (b) methods for both BJ-5ta (blue) and GM12878 (red) cells. Grey represents the lack of TAD strength increase at 24 h after IR when BJ-5ta cells were treated with ATM inhibitor. Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend 1.5×IQR beyond the upper quartile, and lower whiskers extend either 1.5×IQR below the lower quartile or to the end of the dataset. A Wilcoxon signed rank test with continuity correction determined that both BJ-5ta and GM12878 control boundary strength distributions were statistically less than their 30 minutes and 24 hour counterparts ($p < 2.2 \times 10^{-16}$). **c, d** Aggregate contact maps (40 kb bins) at TAD boundaries called by the InsulationScore method with strength greater than 1 in BJ-5ta (c) and GM12878 (d): Control (Left), $\log_2(30\text{minutes}/\text{control})$ (Middle), and $\log_2(24\text{hours}/\text{Control})$ (Right). **e** Example of strengthened TAD boundary. Top- BJ-5ta control Hi-C contacts around a TAD boundary from chr2. Bottom- InsulationScore profile across the region showing the sharper dip in 24 hr (dotted) vs. control (solid) at the TAD boundary. **f, g** Subtracted heatmaps for 24 hours minus Control aggregate interactions around loop anchors with CTCF sites (3,851 total loops) for BJ-5ta (f) and GM12878 (g).

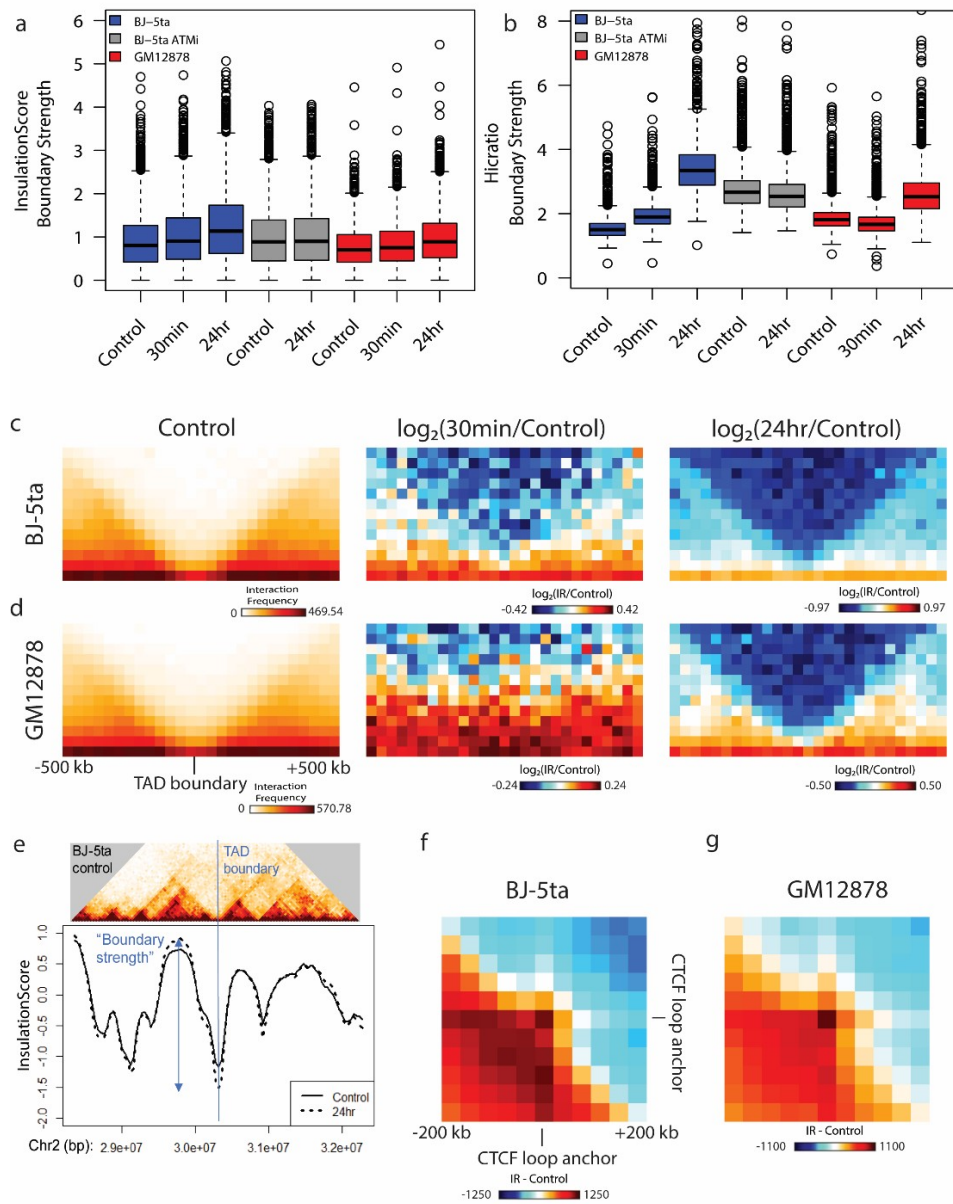
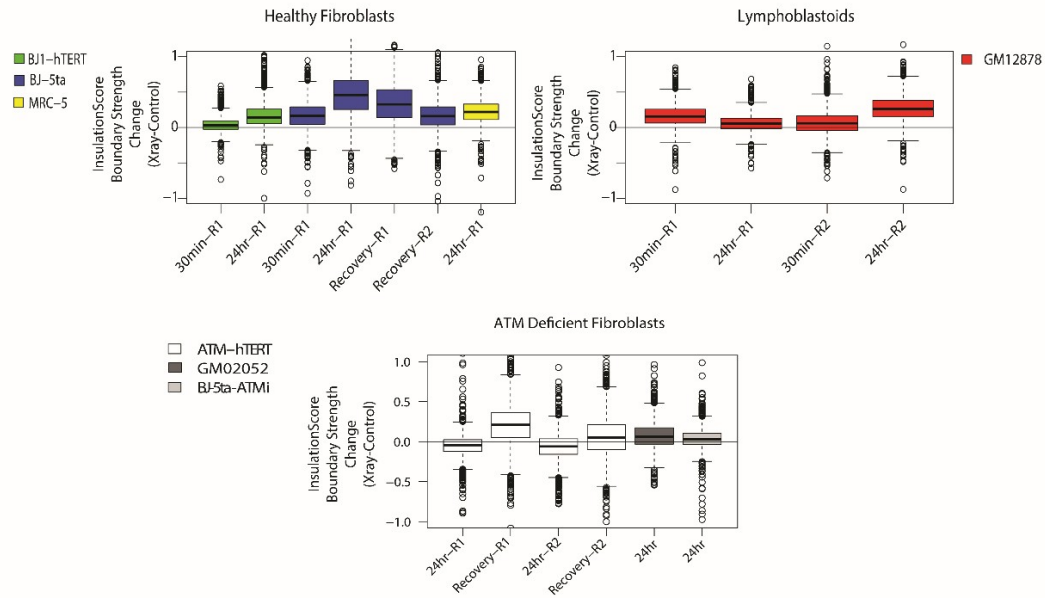


Figure 13. TAD boundary strength and CTCF loop strength both increase after IR.

a The change in strength for each TAD boundary shared between each cell type's control and IR condition was calculated by InsulationScore method and the distribution of changes are represented in boxplots for all replicates of all cell lines used in the study. Healthy fibroblast lines: BJ1-hTERT (green), BJ-5ta (blue), and MRC-5 (yellow); Lymphoblastoid line: GM12878 (red); ATM deficient fibroblast lines: ATM-hTERT (white), GM02052 (dark gray), BJ5-ta + ATM inhibitor (light gray). Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend $1.5 \times \text{IQR}$ beyond the upper quartile, and lower whiskers extend either $1.5 \times \text{IQR}$ below the lower quartile or to the end of the dataset (Number of TAD boundaries (N) BJ-5ta = 1232, BJ1-hTERT = 1310, MRC-5 = 1869, GM12878-R1 = 1371, GM12878-R2 = 1091, ATM-hTERT -R1= 1502, ATM-hTERT-R2 = 1311, GM02052 = 2693, BJ-5ta-ATMi = 2010). **b** Boxplots of changes in TAD boundary strength relative to pre-IR calculated by Hicratio method for all replicates of all cell lines used in study. Healthy fibroblast lines: BJ1-hTERT (green), BJ-5ta (blue), and MRC-5 (yellow); Lymphoblastoid line: GM12878 (red); ATM mutant fibroblast lines: ATM- hTERT (white) and GM02052 (gray). Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend $1.5 \times \text{IQR}$ beyond the upper quartile, and lower whiskers extend either $1.5 \times \text{IQR}$ below the lower quartile or to the end of the dataset (Number of TAD boundaries (N) BJ-5ta = 1298, BJ1-hTERT = 1661, MRC-5 = 2471, GM12878-R1 = 1371, GM12878-R2 = 1878, ATM-hTERT -R1= 1971, ATM-hTERT-R2 = 1674, GM02052 = 3258, BJ-5ta-ATMi = 2868).

a



b

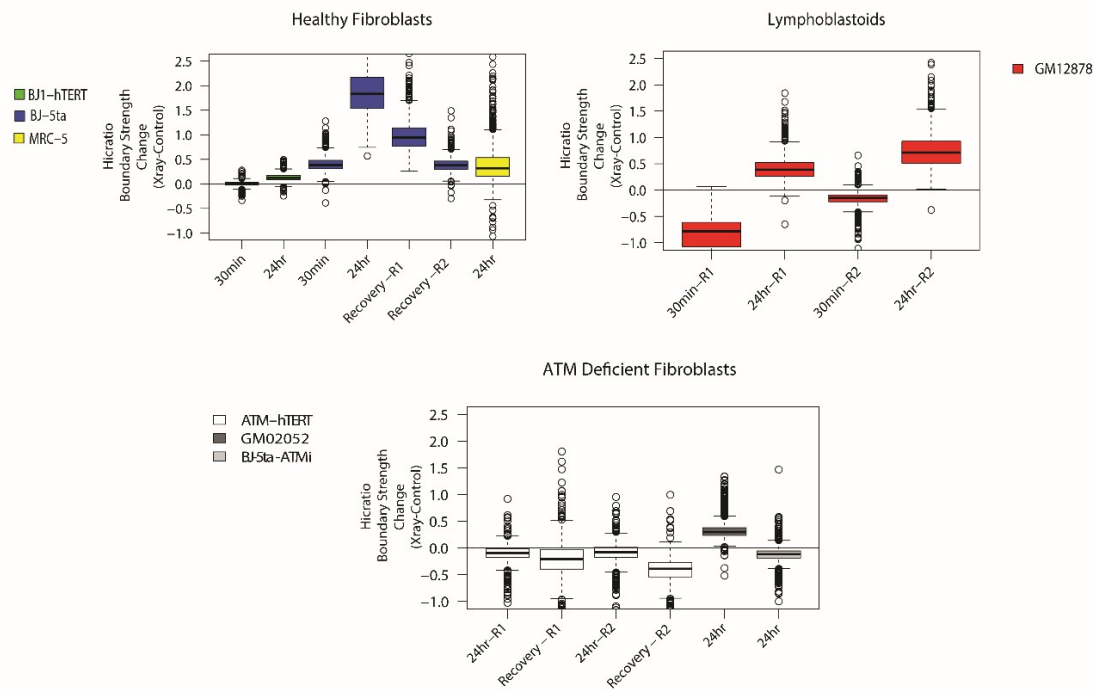


Figure 14. TAD boundary strength changes after exposure to IR are consistent between replicates and among similar cell lines.

Aggregate contact maps of averaged TAD boundaries called by Hicratio method in **a** BJ-5ta, **c** GM12878, and **d** ATM-hTERT. **b** Aggregate contact map of averaged TAD boundaries called by InsulationScore method in GM12878-R1 (other replicate not represented in main figure).

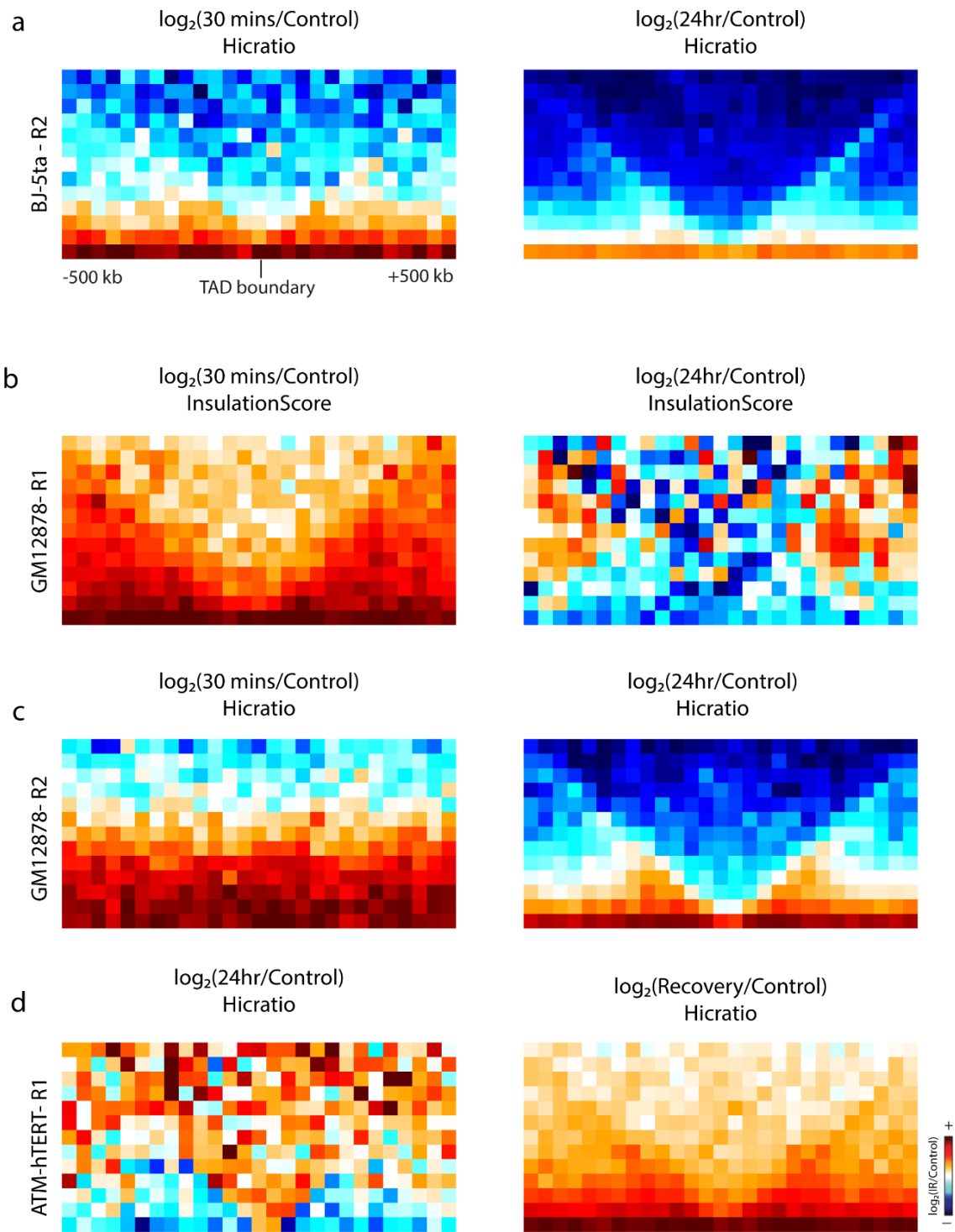


Figure 15. Average interactions across TAD boundaries detected using InsulationScore or Hicratio approaches.

a 40 kb heatmaps of BJ-5ta control (top), 30 minutes (middle), and 24 hours (bottom). InsulationScore line plot shows the increase in boundary strength (visible as a decrease in interactions calculated by the insulationScore) between samples.

b 40 kb heatmaps of GM12878 control (top), 30 minutes (middle), and 24 hours (bottom).

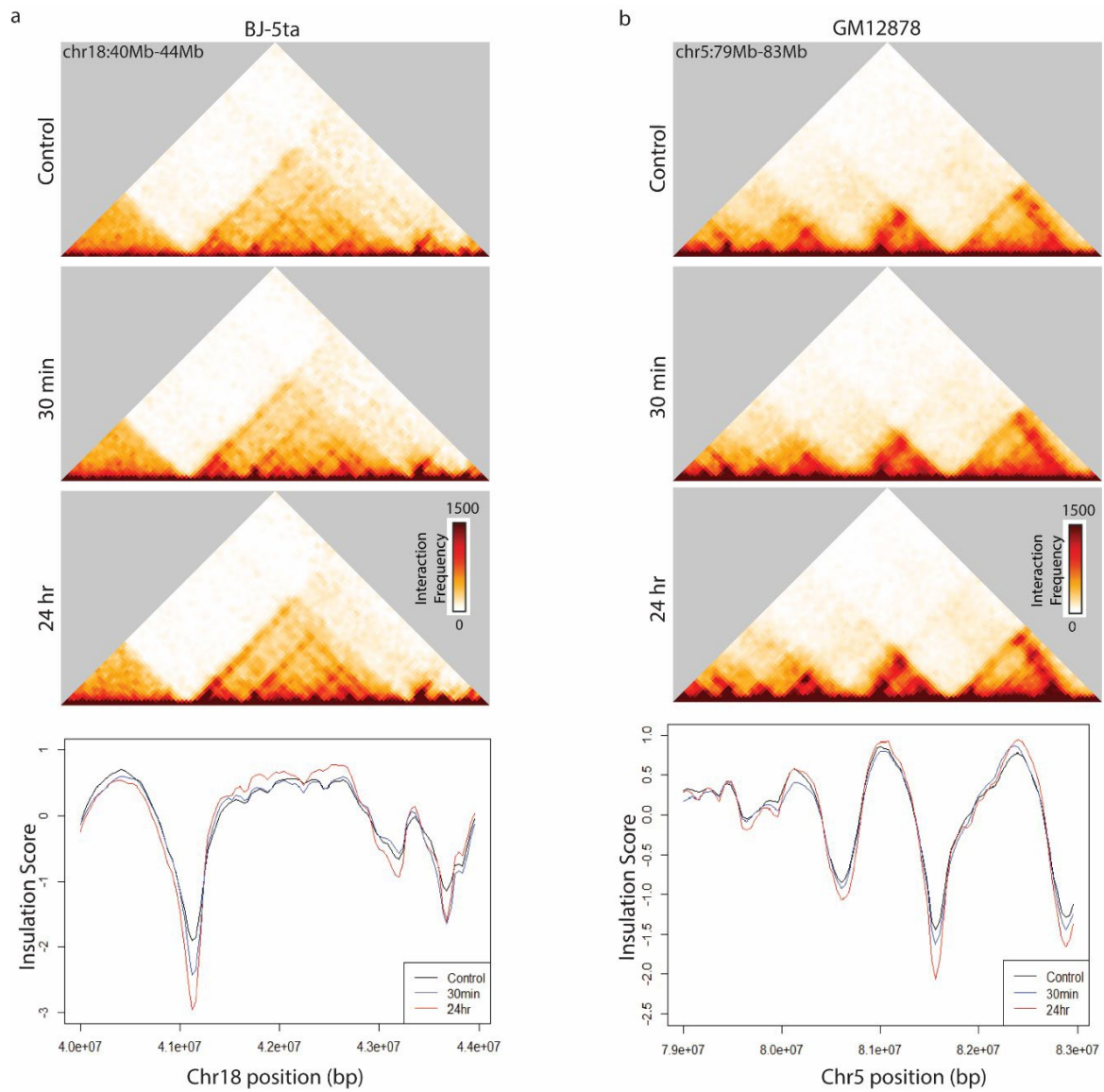


Figure 16. Examples of TAD boundary increase.

TAD boundary strength distribution calculated by InsulationScore method for A (red) and B (blue) compartment regions separately for **(a)** BJ-5ta (Number of TAD boundaries (N) BJ-5ta A compartment = 493, B compartment = 434), **(b)** GM12878 (Number of TAD boundaries (N) ATM-hTERT A compartment = 462, B compartment = 496), and **(c)** ATM-hTERT (Number of TAD boundaries (N) ATM-hTERT A compartment = 705, B compartment = 637). Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend $1.5 \times \text{IQR}$ beyond the upper quartile, and lower whiskers extend either $1.5 \times \text{IQR}$ below the lower quartile or to the end of the dataset.

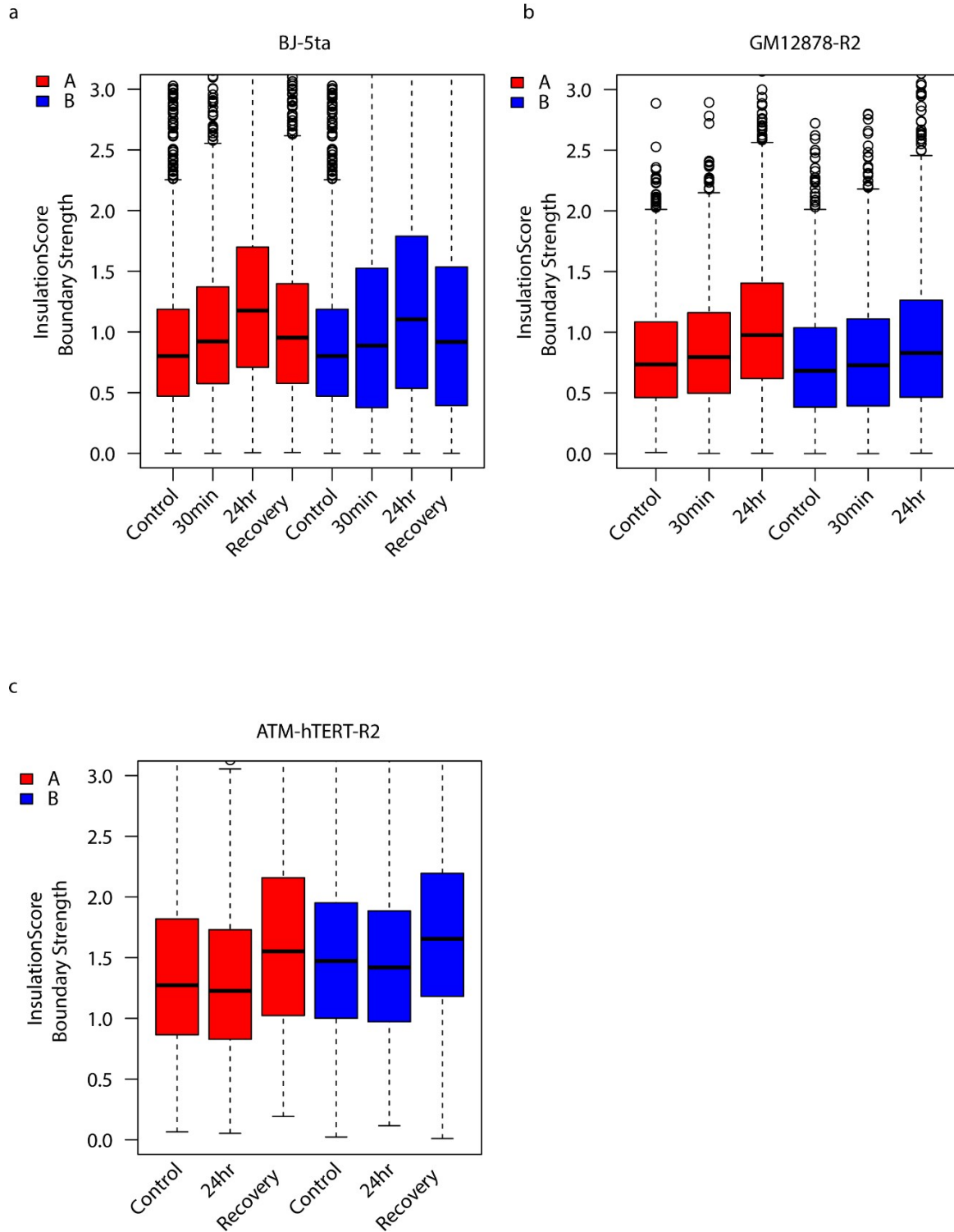


Figure 17. TAD boundary strength changes are consistent in both A and B compartments.

a ATM mutant cells stained with γ -H2AX (green) and DAPI (blue) after before (Control) or after exposure to 5 Gy X-rays. Scale bar: 10 μ m. **b** Genome wide contact heatmap of ATM mutant cells in non-irradiated (lower left) or 24 h after IR (upper right) in 2.5 Mb bins. **c** $\log_2$ (24 hours post IR/Control) contact heatmap in 2.5 Mb bins. **d** Scaling plots showing average decay of contacts with distance across all chromosomes at a 250 kb bin size in ATM mutant cells for control and 24 hours post IR. **e** 250kb bin size Hi-C interaction heatmaps for chromosome 4 in ATM mutant cells in control (Top) and 24 hours post IR (Bottom) show little difference in compartment as evidenced by little change to A (red) and B (blue) compartmentalization in the first principle component (below heatmaps). **f** TAD boundary strength boxplots calculated using both the InsulationScore (Number of TAD boundaries (N) ATM-hTERT = 1311) and Hicratio (Number of TAD boundaries (N) ATM-hTERT = 1674) methods for ATM mutant cells. A one-tailed Wilcoxon signed rank test showed that the 24 hour sample boundaries were not statistically increased compared to controls Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend 1.5 $\times$ IQR beyond the upper quartile, and lower whiskers extend either 1.5 $\times$ IQR below the lower quartile or to the end of the dataset. **g** Contact maps were aggregated at TAD boundaries called by InsulationScore with strength greater than 1 and then these maps are compared by $\log_2$ (24 h post IR/Control). **h** Subtraction of 24 hours minus Control aggregate CTCF-anchored loop contact maps in these ATM mutant cells.

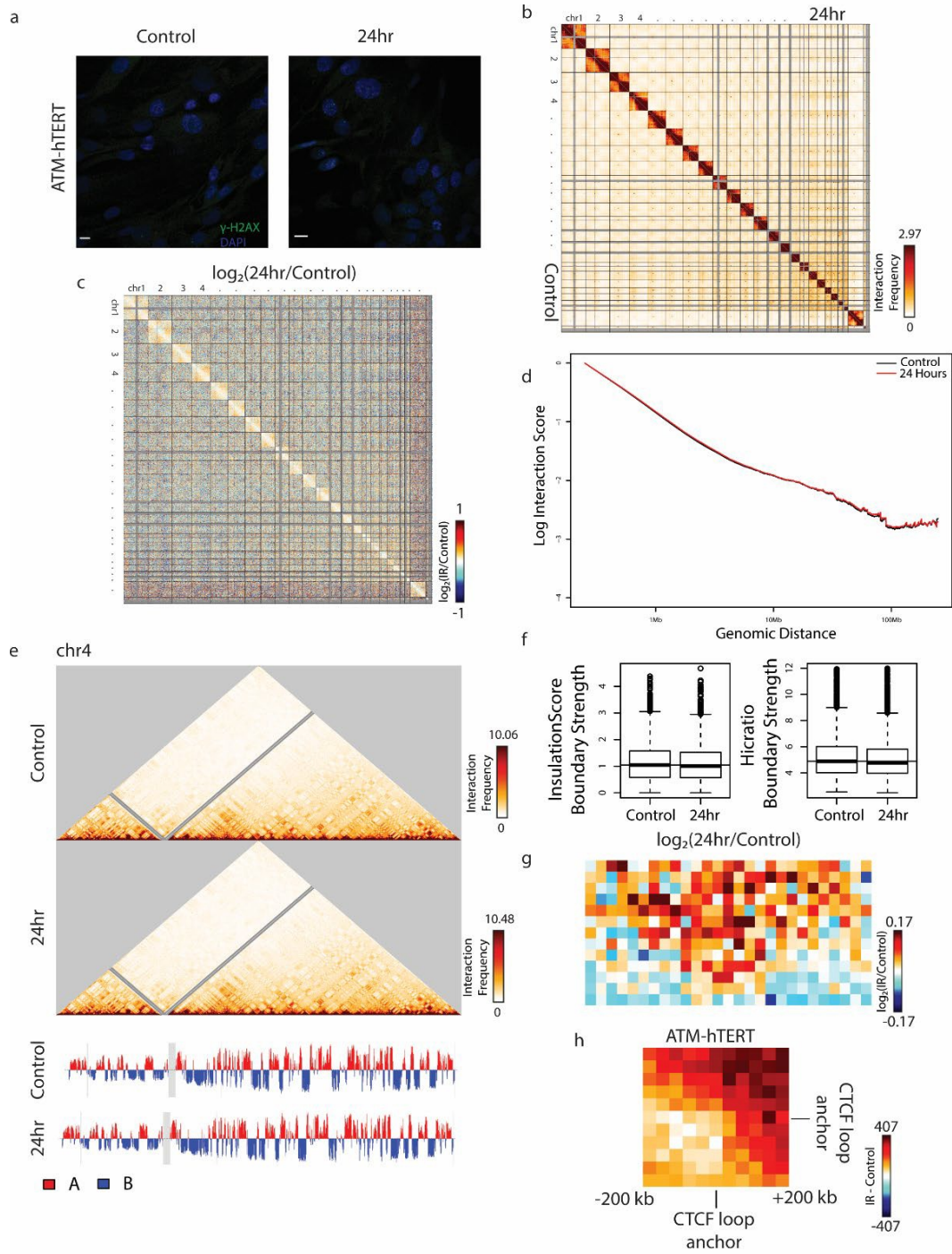


Figure 18. ATM is necessary for 3D genome organization change after IR exposure.

a ATM-hTERT fibroblasts stained with γ -H2AX (green) and DAPI (blue) 30 minutes after exposure to 5 Gy X-rays. Scale bar: 10 μ m. **b** Quantification of mean fluorescence intensity in ATM-hTERT compared to BJ-5ta data in Figure 3a (n = 40 cells, * p <0.0020, ** p<0.0001, one way ANOVA with Tukey's multiple comparison test). γ H2AX levels are significantly lower in ATM mutants than BJ-5ta but maintain a similar pattern of increase at 30 mins (p = <0.0001) and return to initial levels at 24hrs (p = ns).

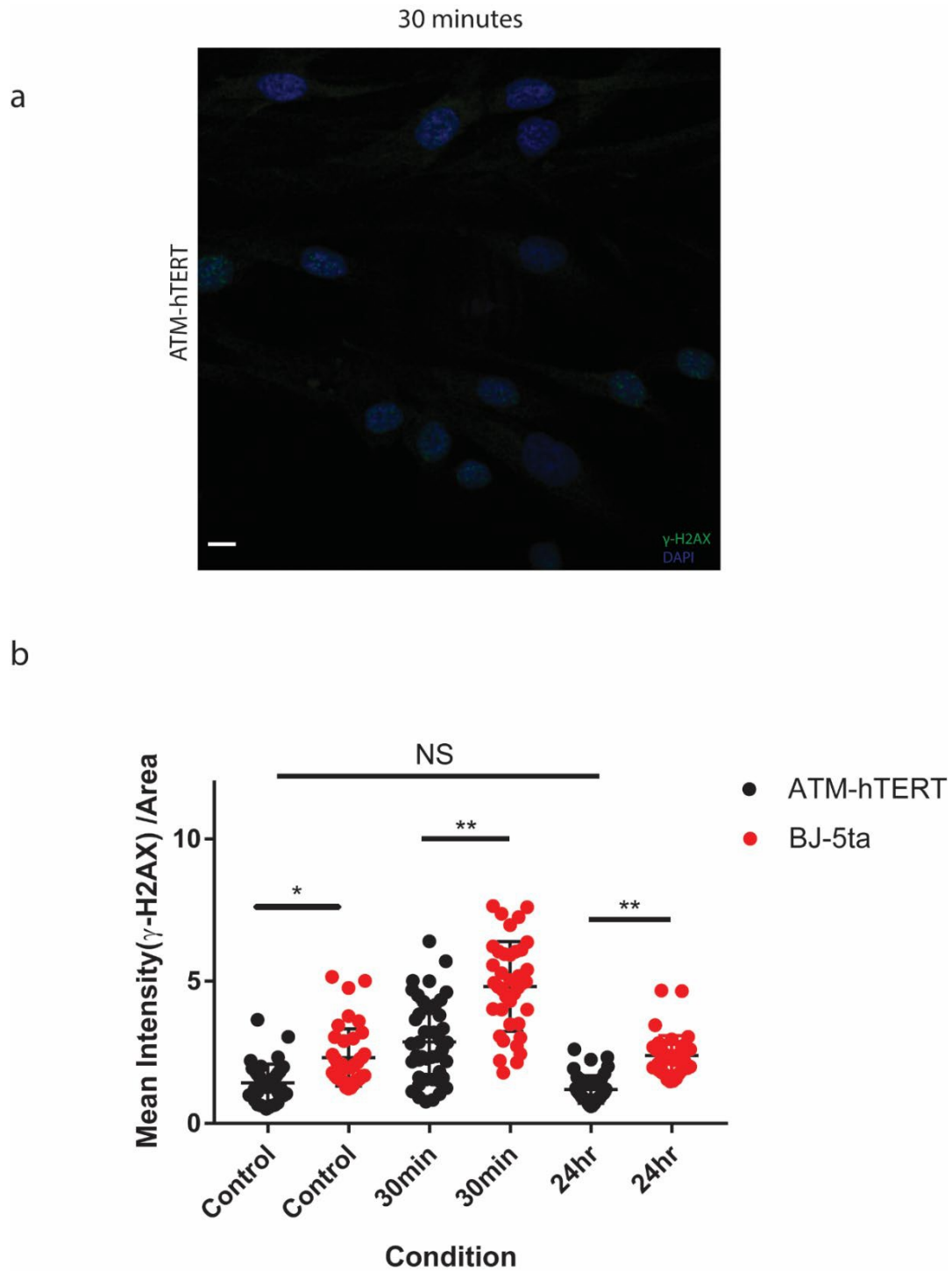


Figure 19. γ -H2AX levels increase in ATM-hTERT cells, but have a weaker response than BJ-5ta.

a ATMi treated BJ-5ta fibroblasts stained with γ -H2AX (green) and DAPI (blue) before and after exposure to 5 Gy X-rays. Scale bar: 10 μ m. **b** Genome wide contact heatmap of ATMi treated BJ-5ta fibroblasts in non-irradiated (bottom left) or 24 h after IR (top right) in 2.5 Mb bins. **c** $\log_2$ (24 hours post IR/Control) contact heatmap in 2.5 Mb bins. **d** Scaling plots showing average decay of contacts with distance across all chromosomes at a 250 kb bin size in ATMi treated BJ-5ta fibroblasts for control and 24 hours post IR. **e** 250kb bin size Hi-C interaction heatmaps for chromosome 4 in ATMi treated BJ-5ta fibroblasts in control (Top) and 24 hours post IR (Bottom) show little difference in compartment as evidenced by little change to A (red) and B (blue) compartmentalization in the first principle component tracks below heatmaps. **f** TAD boundary strength boxplots calculated using both the InsulationScore (Number of TAD boundaries (N) BJ-5ta-ATMi = 2010). and Hicratio (Number of TAD boundaries (N) BJ-5ta-ATMi = 2868) methods for ATMi treated BJ-5ta fibroblasts. A one-tailed Wilcoxon signed rank test showed that the 24 hour sample boundaries were not statistically increased compared to controls. **g** Contact maps were aggregated at TAD boundaries called by InsulationScore with strength greater than 1 and then these maps are compared by $\log_2$ (24 h post IR/Control). **h** Subtraction of 24 hours minus Control aggregate CTCF-anchored loop contact maps in ATMi treated BJ-5ta fibroblasts. Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend 1.5 $\times$ IQR beyond the upper quartile, and lower whiskers extend either 1.5 $\times$ IQR below the lower quartile or to the end of the dataset.

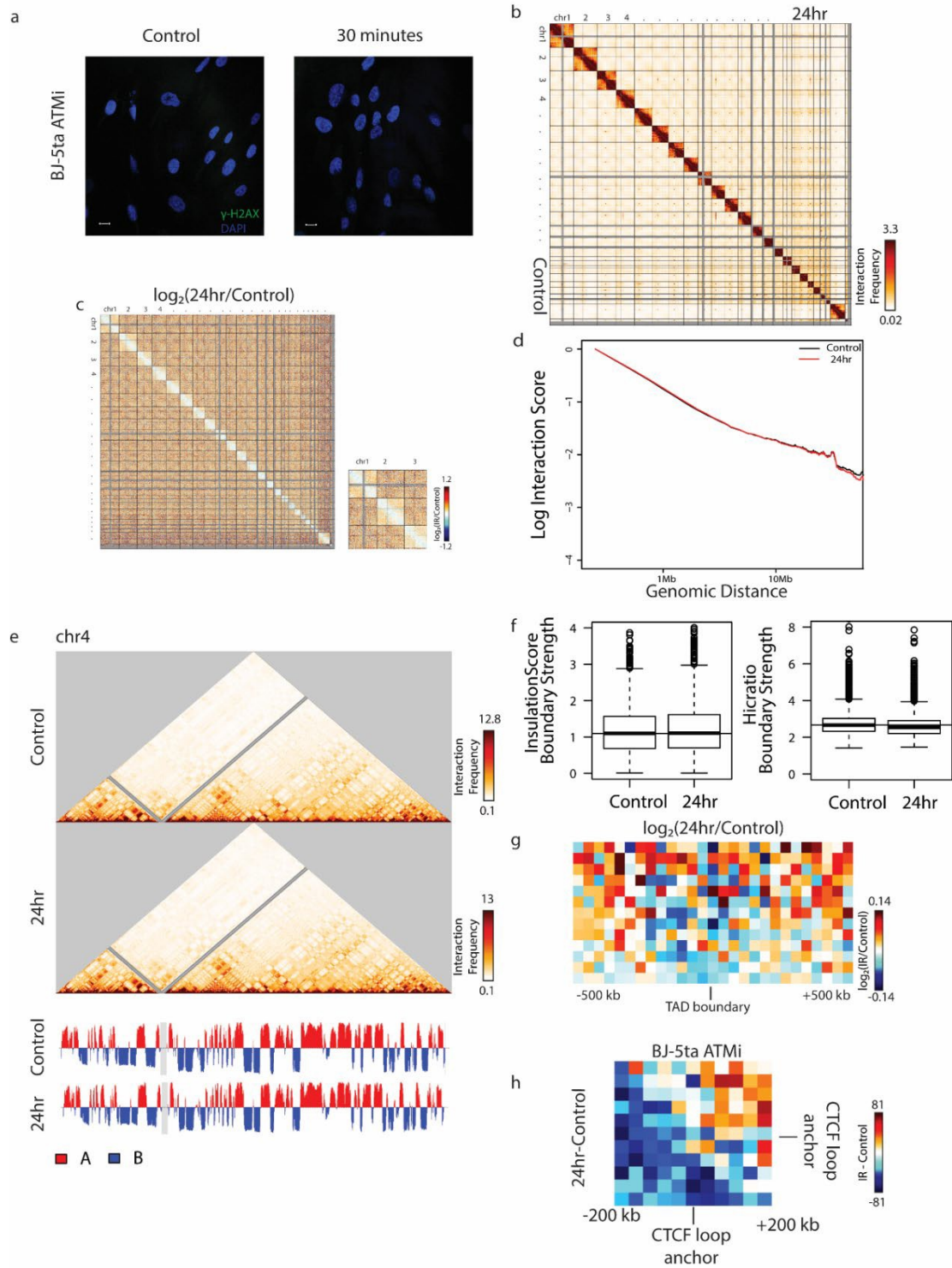


Figure 20. ATMi-treated BJ-5ta fibroblasts mimic the effects of ATM deficient patient fibroblasts.

BJ-5ta fibroblasts were exposed to **(a)** 10 μ M DMSO or **(b)** 10 μ M KU-55933 for one hour and then irradiated with 5 Gy X-rays and imaged at 30 minutes after IR. Each were stained with γ -H2AX (green) and DAPI (blue) after before or after exposure to 5 Gy X-rays. Scale bar: 10 μ m. **c** Quantification of ATMi or DMSO treated BJ-5ta fibroblasts mean fluorescence intensity in BJ-5ta cells before and after exposure to IR (n=12 cells, * p <0.0001, one way ANOVA with Tukey's multiple comparison test. Data is presented as the mean of SD with the 25th (bottom line) and 75th (Top line) percentile shown).

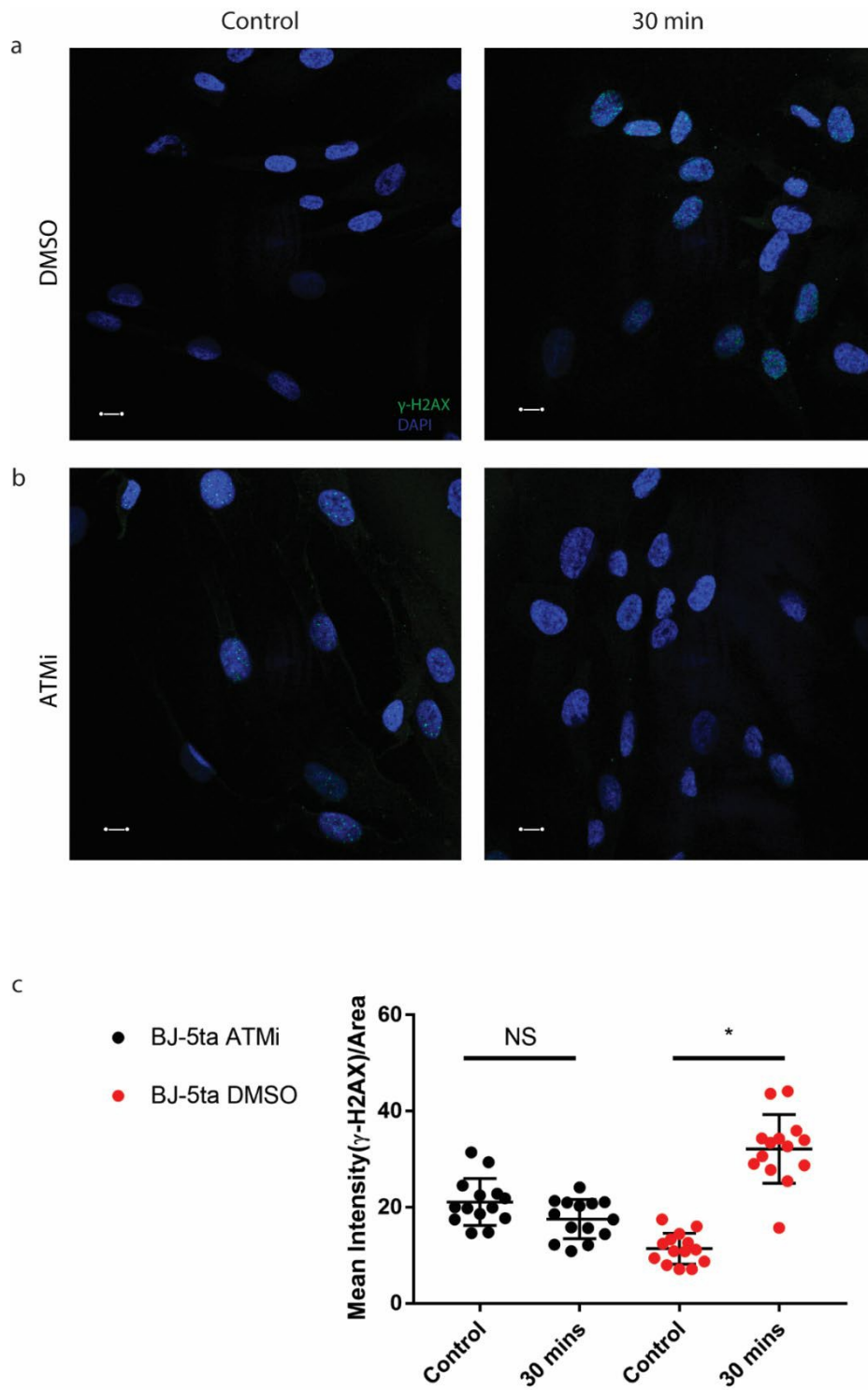


Figure 21. ATMi results in decreased γ -H2AX foci.

a $\log_2(\text{Recovery}/\text{Control})$ contact heatmap for BJ-5ta binned at 2.5 Mb. Inset reveals the loss of interactions between chromosome arms and decrease in telomere-telomere interactions on chromosomes 1 and 2 persists. **b** $\log_2(\text{Recovery}/\text{Control})$ contact heatmap in ATM mutant cells binned at 2.5 Mb. Inset indicates translocations observed between chromosomes 17 and 19 and 19 and 21 at 5 days post IR that were not in the control ATM mutant cells. **c** Scaling plots showing average decay of contacts with distance across all chromosomes at a 250 kb bin size in BJ-5ta (left) and ATM mutant (right) cells before X-ray IR and after 5 days recovery. **d** 250kb bin size Hi-C interaction heatmaps for chromosome 4 in BJ-5ta control (top left), BJ-5ta recovery (bottom left) ATM mutant control (top right) and ATM recovery (bottom right). PC1 compartment identity values (below heatmaps) remain relatively unchanged 5 days post IR. **e** The change in strength for each TAD boundary shared between each cell type's control and IR recovery condition was calculated by InsulationScore method (left) (Number of TAD boundaries (N) BJ-5ta = 1232, ATM-hTERT = 1311) or HiCRatio (right) (Number of TAD boundaries (N) BJ-5ta = 1298, ATM-hTERT = 1674) and the distribution of changes are represented in boxplots for BJ-5ta (blue) and ATM mutant cells (white). A Wilcoxon signed rank test with continuity correction determined that both BJ-5ta and ATM mutant control distributions were significantly lower than the recovery samples ($p < 2.2 \times 10^{-16}$) for the InsulationScore approach. **f** $\log_2(\text{Recovery}/\text{Control})$ heatmap of InsulationScore called TAD boundaries with

strength greater than 1 averaged across the genome in BJ-5ta (top) and ATM mutant cells (bottom).

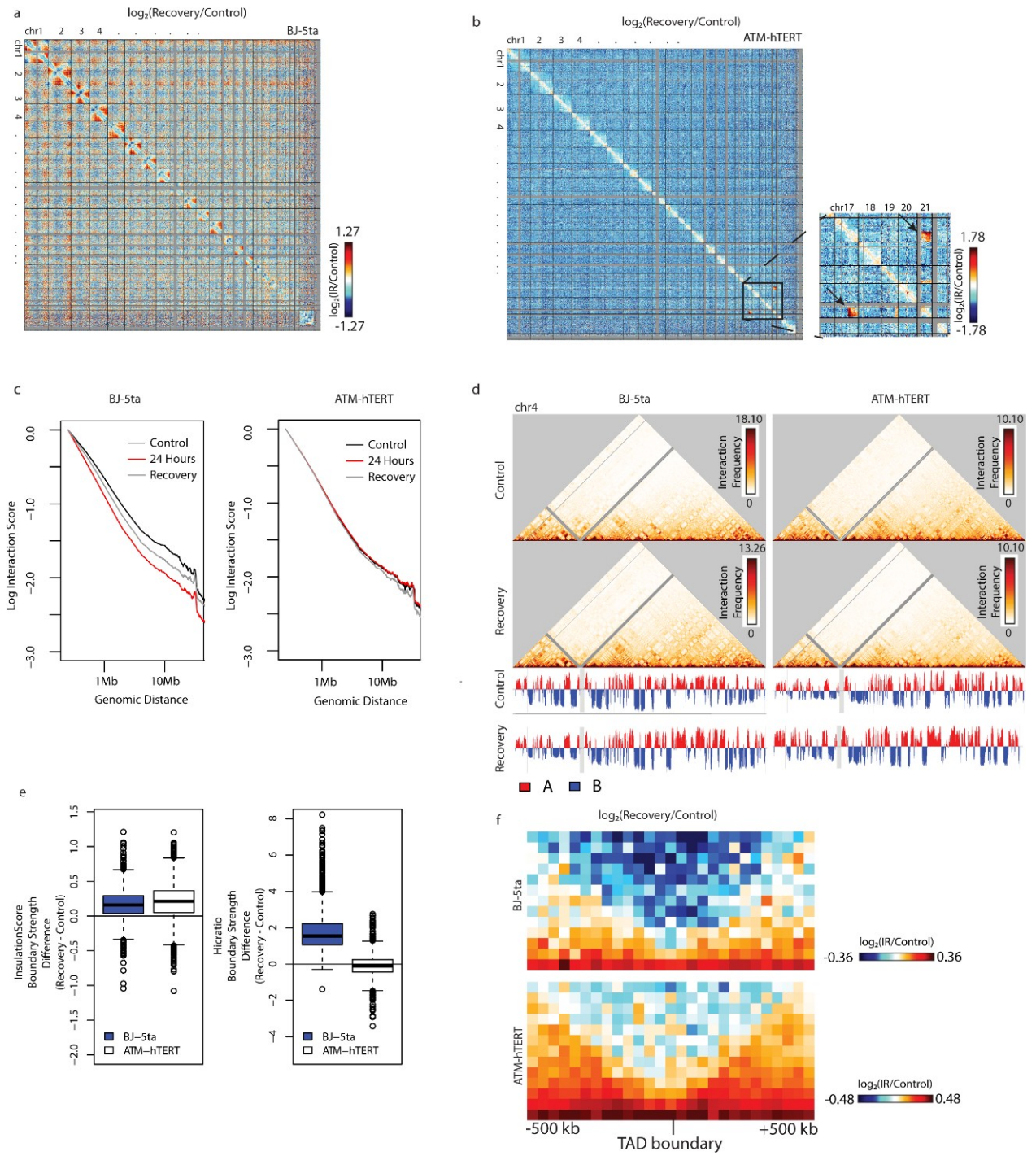


Figure 22. Similarities and differences in 3D genome organization changes after 5 days recovery.

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Appendix

Table 1: Hi-C Mapping Statistics

Cell Line	Condition	Replicate	Raw # Reads	Valid Pairs	Unique Valid Pairs	% Cis
BJ-5ta	Control	R1	180,650,273	98,769,926	62,194,681	74.4
BJ-5ta	30 mins X	R1	172,469,514	101,168,488	76,520,452	77.9
BJ-5ta	24 hr X	R1	439,993,397	285,966,444	212,551,030	86.2
BJ-5ta	Recovery	R1	387,242,758	242,377,386	141,227,429	86.2
BJ-5ta	Recovery	R2	283,878,060	165,142,153	104,333,341	77.6
BJ-1 hTERT	Control	R1	246,979,150	166,441,060	129,865,596	84.4
BJ-1 hTERT	30 min X	R1	231,840,475	155,628,339	125,629,148	83.8
BJ-1 hTERT	24 hr X	R1	205,760,134	134,110,303	109,198,540	84.8
ATM-hTERT	Control	R1	204,830,725	141,109,197	124,465,656	78
ATM-hTERT	Recovery	R1	389,931,788	243,088,728	125,064,421	87.4
ATM-hTERT	24 hr X	R1	179,977,928	121,284,841	111,110,533	78.6
ATM-hTERT	Control	R2	60,818,985	34,216,181	31,768,043	76.9
ATM-hTERT	24 hr X	R2	198,588,290	110,691,110	102,849,684	77.8
ATM-hTERT	Recovery	R2	98,687,970	59,208,561	48,238,983	82.9
GM12878	Control	R1	169,041,926	111,380,425	95,881,352	77.7
GM12878	30 min X	R1	404,673,351	248,835,472	175,309,206	80
GM12878	24 hr X	R1	192,011,866	122,355,226	107,876,878	75.2
GM12878	Control	R2	198,416,626	138,034,618	124,040,375	77.4
GM12878	30 mins X	R2	242,425,056	146,862,286	115,111,914	79.9
GM12878	24 hr X	R2	190,188,740	131,529,834	106,197,889	83
MRC5	Control	R1	128,868,956	90,160,337	66,947,233	73.1
MRC5	24 hr X	R1	95,372,972	52,976,419	43,945,126	82.7
GM02052	Control	R2	402,772,517	224,921,471	154,220,231	83.7
GM02052	24 hr X	R2	384,201,796	221,226,346	139,824,607	84.7
GM02052	Control	R1	91,382,917	18,121,300	14,742,265	88.7
GM02052	24 hr X	R1	123,383,470	51,729,101	39,730,700	86.9

Table 2. Cell Media Recipes

Cell Line	Medium	FBS Content	Additional Supplements
BJ-5ta	4:1 DMEM:Medium 199	10%	Hygromycin B (0.01 mg/mL)
BJ1-hTERT	4:1 DMEM:Medium 199	10%	Hygromycin B (0.01 mg/mL)
GM12878	RPMI 1640	15%	
MRC-5	MEM	10%	
AG04405 (ATM-hTERT)	DMEM	15%	1X Non-essential amino acids
GM02052	MEM	10%	1X Non-essential amino acids

CHAPTER II

3D GENOME FOLDING IN LIGASE REPAIR MUTANTS

ABSTRACT

Repair of damaged DNA from external factors is necessary for the prevention of long-term effects. ATM-mediated DNA damage repair pathways resolve DSBs to prevent chromosomal aberrations. IR-induced DNA damage results in the formation of DSBs. In response to DSBs, 3D genome organization is altered. Changes in genome organization requires ATM, but the role of downstream repair proteins in this process has yet to be elucidated. We perform Hi-C on ligase I and ligase IV mutants to determine if alterations in genome structure is ATM independent. We observe similar phenotypes with both ligase mutants when compared to ATM mutants. Most notably, we observe no TAD boundary strength increase which is observed in repair-proficient cells.

INTRODUCTION

DNA damage repair is necessary to prevent chromosomal aberrations which can lead to oncogenic effects^{1,2}. DDR pathways resolve DNA breaks to prevent the propagation of unrepaired DNA. IR leads to the formation of random DSBs which can lead to the formation of translocations¹. 3D genome organization changes by IR-induced damage occur in repair-proficient cell lines. These changes, however, are not observed in ATM mutant fibroblasts³. ATM, a phosphatidylinositol 3-kinase-related kinases, serves as a DNA damage sensor^{4,5,6}. ATM binds to sites of DSBs leading to the recruitment of downstream repair factors, such as DNA ligase I (Lig1) and IV (Lig4)^{7,8,9}.

Lig1 is responsible for repairing DNA during replication and the final step of base excision repair¹⁰. Lig1 is necessary to excise and replace damaged bases caused by IR. Mutations in Lig1 show a decrease in repair proficiency and an increased sensitivity to IR. Repair by Lig1, like many DNA ligases, requires ATP to perform its function. During replication, Lig1 joins Okazaki fragments formed during DNA synthesis¹¹. Three steps are required to prevent DSBs after replication and include 1) adenylation 2) adenosine monophosphate (AMP) transfer to DNA 3) nick sealing¹². Recent evidence points to the involvement of Lig1 in the alternative non-homologous end joining (a-NHEJ) pathway^{13,14}. To date, only one known case of a Lig1 deficiency in patients has been reported. The patient exhibited signs of sensitivity to sunlight, delayed development, and retarded growth. This patient died at the age of 19 from pneumonia¹⁰.

Lig4 is necessary to resolve DSBs by the classical non-homologous end joining pathways (c-NHEJ). Forming a complex with XRCC4 and XLF at DSBs, Lig4 facilitates repair broken ends^{15,16}. Similar to Lig1, Lig4 requires the adenylation, AMP transfer, and nick sealing to repair breaks¹². Additionally, Lig4 mutations lead to increased sensitivity to hazardous DNA-damaging agents. Patients with Lig4 syndrome experience retarded growth, microcephaly, and higher occurrences of leukemia¹⁷.

Previous studies from our lab determined that ATM is required for 3D genome organization changes to occur in response to IR-induced DNA damage³. Here , we report that Lig1 and Lig4 share a similar lack of response to IR-induced

damaged when compared to repair proficient fibroblasts. We find that minor changes to overall genome structure occurs after IR. Increases, as seen in repair proficient fibroblasts, in TAD boundary strength does not occur in ligase mutants. So far, 5 patients have been identified to have a Lig4 deficiency and present symptoms of microencephaly, skin abnormalities, development delays, and immunodeficiencies¹⁰.

RESULTS

The 3D genome organization of ligase mutants remain relatively unchanged after IR

Previous studies from our lab indicate that cells exposed to IR results in cell type specific changes to the 3D organization of the genome when a functional ATM is present, however, these changes are not observed when ATM is not functional. While ATM is necessary to initiate the canonical DNA damage response pathway, the subsequent repair process relies on multiple proteins to resolve DSBs generated by IR. To determine if changes to the genome structure are the result of a failure to respond to DNA damage or the repair of damaged DNA, we sought to understand if proteins involved in the repair of DNA result in a similar phenotype as ATM-deficient cells or have a repair specific phenotype. Ligases are responsible for the ligation of DNA by adding adenosine monophosphate to the broken strands of DNA and are involved in the ending stages of DNA repair. To measure changes in genome-wide chromosome contacts in ligase mutants after IR, Hi-C was performed immediately before IR exposure and 24 hours after exposure on Lig1

and Lig4 mutant fibroblasts. Similar to ATM-deficient fibroblasts, irradiation of both ligase mutants resulted in modest changes to the overall genome structure (Figure 24). Interestingly, one replicate of the Lig4 mutants contains a recurrent translocation, however, the initial population also contained the translocation. This is of note because both replicates were obtained within weeks of each other. This suggests that Lig4 mutants may more likely to have issues with repair. In contrast, the translocation may have been present in a small number of cells and were selected over time resulting in the translocation being more apparent in the second replicate.

A/B compartmentalization is maintained in ligase mutants after IR

While spatial compartments are maintained in both ATM-proficient and deficient fibroblasts, we sought to determine if ligase mutants maintained their compartment identities. The individual chromosome contact maps also showed no apparent changes in the plaid patterns between IR and control conditions, which suggests A/B compartment identity is not affected in ligase mutants (data not shown). While the A/B compartments remain relatively unchanged, the loss of long-range interactions were observed in ATM-proficient cells. The generation of scaling plots reveal that Lig1 mutants do not lose long range interactions similar to what was observed in ATM mutants. Scaling plots generated for Lig4 mutants were inconsistent with one replicate resulting in a loss of interactions and the other a gain of long-range interactions (Figure 25). It is worth noting that controls for both Lig4 mutants shared a similar pattern. These inconsistencies may be the result of

noise in the data, however, the possibility remains that the inconsistencies could be of biological relevance.

Ligase mutants do not exhibit TAD boundary strength increase after IR

The observation of TAD boundary strengthening in ATM-proficient fibroblasts, but not ATM-deficient fibroblasts, may result a be a result of the ATM-mediated DNA response mechanism. However, the observed effect may be the result of downstream targets. To determine if TAD boundary strength increase is a result of the loss of functional ATM or downstream targets, we calculated the average TAD boundary strength and determined that both ligase mutants do not exhibit an increase in TAD boundary strength (Figure 26). To observe the genomic landscape of the called TAD boundaries, we generated TAD boundary heatmaps which shows a similar phenotype as ATM-deficient fibroblasts (data not shown). This result suggests that the TAD boundary strength is a result of the repair of the DNA repair process and not the DNA damage response.

DISCUSSION

IR-induced damage can lead to tumorigenic effects if left unrepaired. The data presented in this study builds upon our previously published data which shows the DNA damage sensor, ATM, is necessary for the IR-induced genome architectural response. Here, we show that downstream targets of the c-NHEJ and a-NHEJ pathways can also influence the 3D genome organization in response to IR-induced DNA damage. We report that ligase I and ligase IV mutants share similar responses to IR as ATM. Most notably, TAD boundary strength is not

affected as seen in DNA repair-proficient cells. These data suggest that the changes in genome organization are a result of DNA repair.

We observe no changes in TAD boundary strength in both ligase mutant fibroblasts. Recent hypotheses suggest that ATM may be constricted by TADs and boundaries restrict repair from crossing TAD boundaries. Our recent results suggest that the repair machinery is necessary for the increase in boundary strength. This indicates that the repair machinery is the cause of TAD boundary strength as ATM is normally expressed in these ligase mutants. However, it is unclear how repair machinery may dictate changes in 3D genome organization. Additionally, we observe that Lig4, involved in c-NHEJ, and Lig1, involved in the a-NHEJ pathway, respond similarly to stress. This observation suggests some crosstalk between repair pathways resulting in similar phenotypes being observed. The fact still remains that boundary elements, such as CTCF and cohesin, may be responsible for TAD boundary strength increases, however, the possible mechanism that would lead to their increase at boundaries is unclear.

Another potential cause for the similarities between ATM and ligase mutants may be the complexes formed to respond to damage. While the c-NHEJ complex has been well studied, the a-NHEJ pathway is less clear. Lig1 mutants serve a primary role in ligating Okazaki fragments during DNA replication. Although c-NHEJ and a-NHEJ pathways are error prone, a-NHEJ repair is considered more error prone and may be the source of chromosomal translocations. While each pathway utilizes specific proteins for repair, a-NHEJ can utilize c-NHEJ proteins.

Current models suggest two potential choices for DNA repair. One possible mechanism being equal choice in NHEJ repair pathways. The other possibility remains that a-NHEJ proceeds with DNA damage repair only after c-NHEJ failure¹⁸. Further understanding of proteins involved in both pathways or competition between these pathways is necessary to tease out 3D genome organization responses.

METHODS

Cell culture and irradiation

Ligase cell lines include: GM16088 (Coriell, Ligase IV) and GM16096 (Coriell, Ligase I). Both cell lines were grown in EMEM (Corning, MT10010CV) growth media and supplemented with 15% fetal bovine serum (Corning, 35010CV), 1% Pen-strep, and 1% L-Glutamine. For irradiation experiments, fibroblast lines were grown to confluency prior to X-ray exposure.

Cells were irradiated with 5 Gy X-rays using a RadSource RS-2000 Biological Irradiator. X-rays were emitted with a concentration of 2Gy min⁻¹ with cells being exposed for 2 minutes and 30 seconds (160.9 kV; 25.1 mA). After irradiation, cells were returned to an incubation chamber and kept at 37°C with 5% CO₂. Cells were collected at 0 minutes or 24 hours post-irradiation and fixed for the respective downstream application.

Hi-C

To prepare the Hi-C libraries and prepare for sequencing, at the appropriate time points, Hi-C experiments were performed according to the protocol detailed

in Gollosi et al.¹⁹. Briefly, 5-20 million cells were fixed with 1% formaldehyde, suspended in cell lysis buffer for permeabilization, and homogenized by douncing. Crosslinked chromatin was digested overnight with HindIII. Sticky ends were filled in with biotin-dCTP, and the blunt ends of interacting fragments were ligated together. DNA was purified by two phenol-chloroform extractions and ethanol precipitation. Biotin-dCTP at unligated ends was removed, and the DNA was sheared to a target size of 200-400 bp by a Covaris sonicator (Covaris, M220). DNA between 100-400 bp was selected for using AMPure XP beads (Beckman Coulter). Biotinylated DNA was pulled down using streptavidin coated magnetic beads and prepared for multiplex sequencing on an Illumina platform using the NEBNext Ultra II kit (NEB, E7645S). All end preparation, adaptor ligation, and PCR amplification steps were carried out on bead bound DNA libraries.

Sequencing was carried out at the Oklahoma Medical Research Foundation Clinical Genomics Facility on an Illumina HiSeq 3000 platform with 75 bp paired end reads or a NovaSeq 6000 with 50 bp paired end reads. Sequencing reads were mapped to the reference human genome hg19, filtered, and iteratively corrected using the pipeline detailed in Imakaev et al. and available on github (dekkerlab-cMapping)²⁰.

Interaction vs. Distance Scaling Plots

Using 250 kb binned iteratively corrected and scaled contact matrices, we extracted contact frequencies between bins at each genomic distance, excluding the diagonal bin (zero distance). We then used a loess fit to find a smooth curve

describing interaction decay vs. distance. Loess curves for each chromosome were averaged together to generate a single scaling curve representing the whole genome. The interaction frequencies were then normalized to set the maximum value (loess fit value for the minimum distance) for each dataset to 1 and then plotted on a log scale.

TAD boundary and insulation analysis

Topologically associating domain (TAD) boundaries were called on 40 kb binned matrices using two methods: the InsulationScore method, as detailed in Crane et al., available as matrix2insulation in the cworld-dekker github package, and the Hicratio method, as described in Gong et al.^{21,22}. For the InsulationScore, a 500 kb insulation square size was used, and for the Hicratio method, we used $d = 400$ kb. Boxplots were generated using boundary strength scores determined by both InsulationScore and Hicratio methods. All TAD boundaries were plotted for the InsulationScore boxplots. For Hicratio boxplots, outliers were removed by filtering out TAD boundaries with a Hicratio score greater than twelve or less than one.

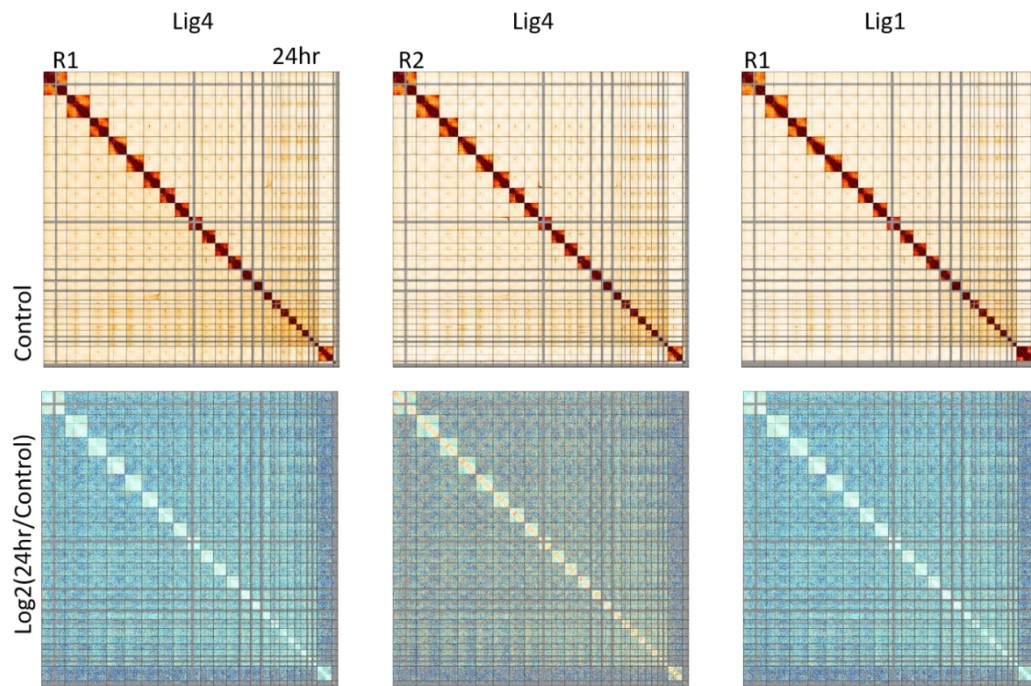


Figure 23. Ligase mutants show subtle changes to genome organization after exposure to IR.

2.5 Mb heatmaps of Ligase IV (left and center) and Ligase I (right) in top panel.

Log2ratio differences between 24 hr after IR exposure vs Control in Ligase IV (left and center) and Ligase I (right) in bottom panel.

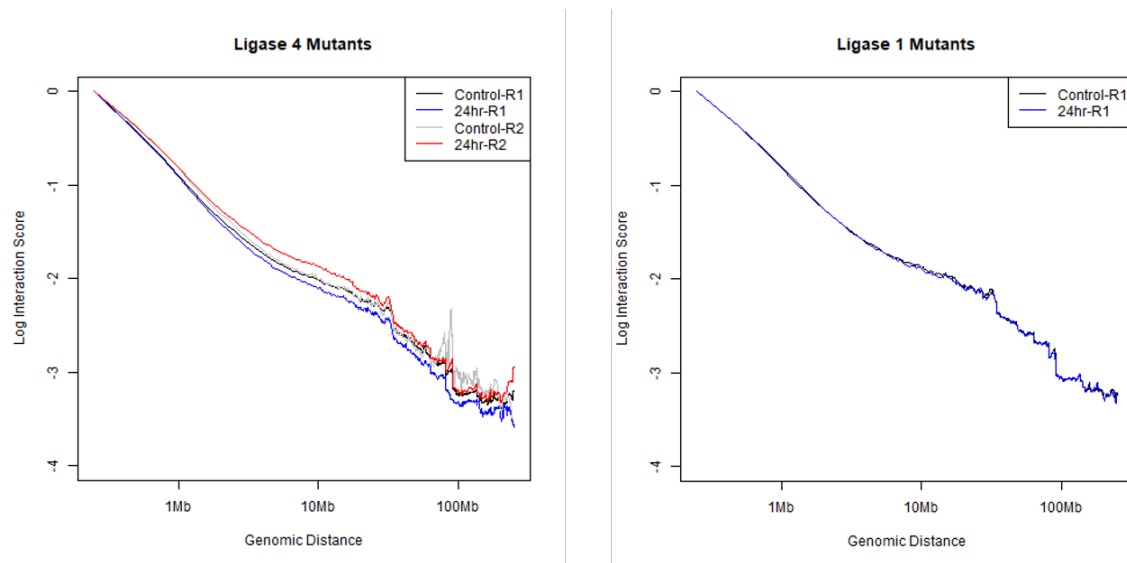


Figure 24. Ligase mutants exhibit similar features of ATM fibroblasts.

Scaling plots of Ligase IV (left) and Ligase I (right).

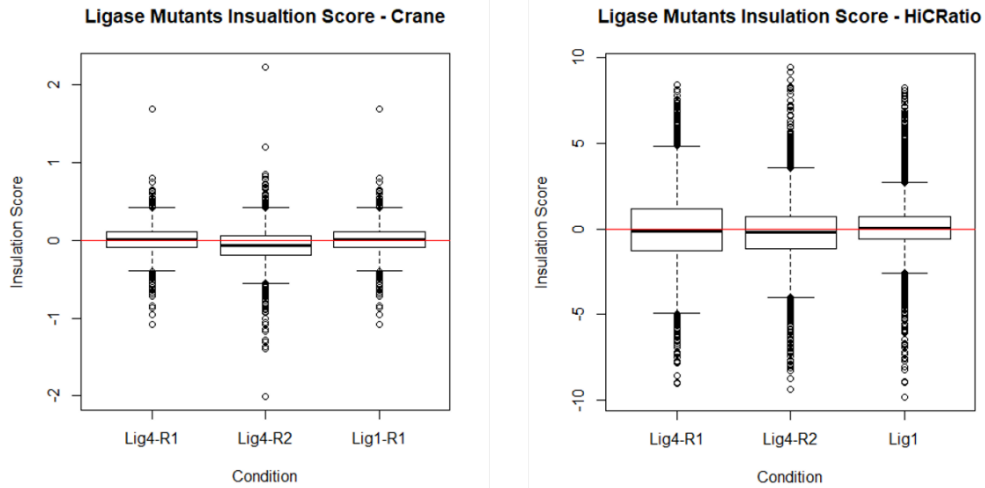


Figure 25. Ligase mutants TAD boundary strength does not increase after IR exposure.

TAD boundary strength boxplots calculated using both the InsulationScore (left) and Hicratio (right) methods for 24 hour post-IR ligase IV replicates and ligase I cells subtracted by control. Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend $1.5 \times \text{IQR}$ beyond the upper quartile, and lower whiskers extend either $1.5 \times \text{IQR}$ below the lower quartile or to the end of the dataset.

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CHAPTER III
UNDERSTANDING THE EFFECT OF PROGERIN ON GENOME
ARCHITECTURE AND GENE EXPRESSION

ABSTRACT

The three-dimensional folding of the human genome is important for gene expression, cellular division, and DNA repair. Lamins play a major role in organizing chromatin by providing nuclear stability and structure. Hutchinson-Gilford Progeria Syndrome results in a mutation in *LMNA*, resulting in the mutant protein termed progerin. Progerin expression leads to alterations in genome architecture, nuclear morphology, and epigenetic states. To understand the effects that progerin has on genome architecture, we employed Hi-C on early and late passage Progeria fibroblasts. Here, we report that late passage Progeria fibroblasts exhibit a global loss of interactions at a genome wide scale when compared to early passage Progeria fibroblasts. Contrary to previous reports, A/B compartments are not completely lost, but are weaker in late passage Progeria cells. In addition, we report that TADs are maintained and are unexpectedly more defined in late passage Progeria cells.

INTRODUCTION

Lamins, which interact with chromatin, are crucial proteins that function in the cell division, intracellular communication, and nuclear structural stability^{1,2,3}. Lamins localize to the periphery of the nucleus and interact with a variety of membrane proteins to form the nuclear lamina^{4,5}. Recent evidence points to lamin associated domains (LADs) which contain heterochromatic regions of chromatin that bind to lamins⁶. The lamin protein family is composed of two types of lamins, lamin A/C and lamin B. Although both lamins form a meshwork, their functions are

mostly independent of each other and mutations cause different phenotypic effects⁷. Mutations in the lamin A gene have been shown to cause a multitude of diseases termed laminopathies which can cause a variety of diseases. One major disease caused by a mutation in *LMNA* is Hutchinson-Gilford Progeria syndrome (HGPS)^{8,9}. This disease affects 1 in 20 million children and it is estimated that about 300-400 children are affected by this disease worldwide. Children affected by this mutation exhibit premature aging phenotypes which include hair loss, brittle bones, and atherosclerosis^{10,11}. These symptoms lead to premature death at the average age of 13 years old. The most common mutation is a point mutation at position 1824 which contains a C to T nucleotide substitution. Although this nucleotide change does not change the amino acid that is transcribed, it creates a cryptic splice site that leads to a defective lamin A protein, termed progerin, to be translated. Progerin contains a permanently farnesylated CAAX motif which is typically cleaved off in the production of healthy lamin A^{8,9}. This leads to a disruption of the normal lamin meshwork, leading to deformed nuclei and nuclear stiffening^{12,13}. The production of progerin also causes an inversion of heterochromatin and euchromatin¹⁴. This inversion can lead to other issues within the cell as it is thought that heterochromatin is on the periphery of the cell to protect gene rich euchromatin typically found in the interior. The most comprehensive study investigating genome structure provided evidence that dramatic changes occur in aging progeria cells. These changes include compartment breakdown and changes in DNA-*LMNA* and H3K27me3 patterns¹⁵.

Few studies have investigated 3D genome structure in progeria cells. Thus, further understanding of the 3D genome organization in HGPS cells may provide deeper understanding and methods to improve the quality of life for HGPS patients. Here, we report high resolution Hi-C data on two Progeria cell lines to further understand the effects that progerin has on genome organization. We find that Progeria cell lines, both early and late passages, exhibit features reminiscent of X-ray irradiated cells. We find that Progeria cells have a loss of *Rabl* structure and increased TAD boundary strength. We also report while compartments are maintained, however, A/B compartmentalization strength is lost. Additionally, we analyze 24, both classical and atypical, Progeria gene expression datasets to determine common genes affected across Progeria cell lines. We find that gene expression datasets are not correlated with local 3D genome structure changes.

RESULTS

Progeria patient cells share similarities to irradiated fibroblasts

Previously reported described the effects of Progeria on the 3D genome organization, the data was not high resolution. Due to the fact that the data was obtained in the infancy of Hi-C, we decided to repeat Hi-C on Progeria patient fibroblasts and the matching parental line. Using passage 19 Progeria fibroblasts (HGADFN167) vs a passage 16 normal parental line (HGADFN168) we observe features that are similar to that of irradiated fibroblasts. These features include a loss of telomere-telomere interactions and a gain of interactions along the centromere (Figure 26). This indicates that DNA damage, despite how the damage

is caused, can be observed in the 3D genome organization. To determine if these features were a feature of late passage Progeria fibroblasts, we performed Hi-C on passage 7 Progeria fibroblasts (AG11513) vs a normal fibroblast (AG03257) and find that similar features persist, however, these features are less obvious (Figure 26).

Compartments become weaker in early and late passage Progeria fibroblasts

Previous studies from our lab indicated that compartments are lost in the Progeria fibroblast HGADFN167. However, due to the improvement of the Hi-C technique we can now report that while compartments are not completely lost, they are weakened in both early and late passage Progeria fibroblasts. This is in agreeance with senescence studies that show the weakening of compartments.

To observe regions that were weakening we generated heatmaps of Z-score matrices binned at 250 kb. Our data shows that when late passage data are subtracted from early passage data both A and B compartments are decreased (Figure 27). We also generated saddle plots which show that both A and B compartments are lost in late passage Progeria fibroblasts.

TAD boundary strength increases in late passage Progeria cells

While compartments had been previously addressed in our past publication, TADs had not been described in Progeria cells. Therefore, we sought to observe the genomic landscape of TADs. Due to the activation of ATM in Progeria cells, we calculated the average TAD boundary strength in early and late passage Progeria fibroblasts. We observe an increase in TAD boundary strength in early

and late passage Progeria cells (Figure 28). This increase in TAD boundary strength is consistent with reported irradiation data and suggests that the increase in damage observed in Progeria cells may be caused by ATM recruitment and DNA repair within TADs. We also observed the average TAD size in P12 and P19 Progeria cells to determine if TADs are maintained.

Gene expression is variable across Progeria patient cells

The observation of 3D genome folding changes in Progeria cells led us to probe if gene expression changes occur as well. To determine if changes in genome organization influence gene expression, we analyzed 17 gene expression datasets generated in our lab or from publicly available data. Upon looking at genes found to be upregulated in 90% of the cell lines, we were unable to find local changes to genome structure in the HGADNF167 or AG11513. We analyzed these datasets against 4 healthy adults and 4 healthy children. We find that when compared to adults, 32 genes are upregulated, and 337 genes downregulated when analyzing at differentially expressed gene changes contained in 75% of the cell lines. Gene ontology (GO) terms for the upregulated genes include organogenesis and differentiation pathways, whereas, downregulated pathways include calcium ion channel regulation. When compared to children, we find that 183 genes are upregulated and 522 downregulated when analyzing differentially expressed gene changes contained in 75% of the cell lines. The GO terms for upregulated genes include epidermis and valve development, whereas, downregulated genes include cell proliferation.

To add more complexity to our gene expression data sets, we added gene expression datasets from atypical Progeria patients. When analyzing genes differentially expressed in at least 75% of the cell lines, we find that 19 genes are upregulated and 276 genes are downregulated when compared to adults. Further analysis to determine potential genes that may be involved in phenotypical defects in Progeria patients must be conducted.

DISCUSSION

In this recent study, we are continuing to attempt to understand how accelerated aging in Progeria affects their 3D genome organization. In addition to understanding how aging affects the genome, this study will elucidate the role that the lamina has in maintaining genome organization. Studies have consistently reported that defects in the lamin family of proteins leads to increased DNA damage, suggesting that it has some role in the maintenance of the genome. In conjunction with our irradiation study, we hope to tease out the contribution that the lamina has on maintaining the overall genome organization. To address the differences between DNA damage and lamina associated changes to the genome, we will irradiate Progeria cells in an attempt to find the differences in genome structure caused by DNA damage and the lamina.

Taken together, these studies provide evidence of the severe compromise in the mesenchymal differentiation commitment of progerin-producing cells, even fibroblasts. To address our hypothesis, our eventual aim is to elucidate the 3D genome structure of progeria MSCs compared to normal controls. Thus, we

propose using CRISPR mediated gene editing to introduce the cytidine to thymidine mutation at position 608 of exon 11 (GGC>GGT) into established, commercially available, MSC cell lines. By compiling this 3D architecture with gene expression data, we can evaluate how current therapeutics (Lonafarnib) affect MSC fate and differentiation, by treating the gene-edited cells *in vitro*. Generating isogenic cell lines will also allow us to probe genes uncovered in our RNA-seq analysis. Recent advancements in techniques have provided other means of understanding what changes are occurring as Progeria cells age. In future experiments we hope to integrate ATAC-seq data to assess the open regions of the genome and how that leads to changes in the 3D genome.

METHODS

Cell culture

HGADFN167 and HGADFN168 fibroblasts were purchased from the Progeria Research Foundation and AG11513 and AG03257 fibroblasts were purchased from Coriell. Cells were grown in DMEM supplemented with 15% FBS, 1% Pen-strep, and 1% L-glutamine and passaged at a density of 80%.

Hi-C

To prepare the Hi-C libraries and prepare for sequencing, at the appropriate time points, Hi-C experiments were performed according to the protocol detailed in Gollosi et al.¹⁶. Briefly, ~5 million cells were fixed with 1% formaldehyde, suspended in cell lysis buffer for permeabilization, and homogenized by douncing.

Crosslinked chromatin was digested overnight with DpnII. Sticky ends were filled in with biotin-dATP, and the blunt ends of interacting fragments were ligated together. DNA was purified by two phenol-chloroform extractions and ethanol precipitation. Biotin-dATP at unligated ends was removed, and the DNA was sheared to a target size of 200-400 bp by a Covaris sonicator (Covaris, M220). DNA between 100-400 bp was selected for using AMPure XP beads (Beckman Coulter). Biotinylated DNA was pulled down using streptavidin coated magnetic beads and prepared for multiplex sequencing on an Illumina platform using the NEBNext Ultra II kit (NEB, E7645S). All end preparation, adaptor ligation, and PCR amplification steps were carried out on bead bound DNA libraries.

Sequencing was carried out at the Oklahoma Medical Research Foundation Clinical Genomics Facility on an Illumina HiSeq 3000 platform with 75 bp paired end reads or a NovaSeq 6000 with 50 bp paired end reads. Sequencing reads were mapped to the reference human genome hg19, filtered, and iteratively corrected using the pipeline detailed in Imakaev et al. and available on github (dekkerlab-cMapping)¹⁷.

Compartment analysis

Principle components analysis was performed using the matrix2compartment perl script in the cworld- dekker pipeline available on github on 250 kb binned matrices. The eigen1 value, which typically represents the compartment profile, was used to determine A/B compartments.

Interaction vs. Distance Scaling Plots

Using 250 kb binned iteratively corrected and scaled contact matrices, we extracted contact frequencies between bins at each genomic distance. We then used a loess fit to find a smooth curve describing interaction decay vs. distance. Loess curves for each chromosome were averaged together to generate a single scaling curve representing the whole genome. The interaction frequencies were then normalized to set the maximum value (loess fit value for the minimum distance) for each dataset to 1 and then plotted on a log scale.

TAD boundary and insulation analysis

Topologically associating domain (TAD) boundaries were called on 40 kb binned matrices using the InsulationScore method, as detailed in Crane et al., available as matrix2insulation in the cworld-dekker github package¹⁸. Boxplots were generated using boundary strength scores determined by InsulationScore analysis. All TAD boundaries were plotted for the InsulationScore boxplots.

RNA Sample Collection

RNA for AG01178, AG01972, AG07493, AG06917, AG08466, AG10586, AG10677, AG03199, AG03513, AG11513, AG11572 was purchased from Coriell Institute for Medical Research.

RNA Sequencing

RNA for AG01178, AG01972, AG07493, AG06917, AG08466, AG10586, AG10677, AG03199, AG03513, AG11513, AG11572 were sequenced at Genewiz

using a NovaSeq sequencer. rRNA was depleted to capture mRNA and long noncoding RNA.

RNA-seq Analysis

Data for HGADFN122, HGADFN127, HGADFN143, HGADFN164, HGADFN167, HGADFN1169, HGADFN178, HGADFN188, and HGADFN367 was downloaded from NCBI series GSE113957¹⁹. Data for HGADFN155 was downloaded from NCBI series GSE150137²⁰. All RNA data was uploaded to Galaxy and mapped files were generated using RNA Star^{21,22}. Differential expression results were generated using edgeR by comparing Progeria samples to 4 healthy adult individual RNA profiles GM00499, GM00726, GM03651, GM03652 or 4 healthy child RNA profiles GM05069, GM00969, GM00038, GM08398 in the NCBI series GS113957^{19,23}.

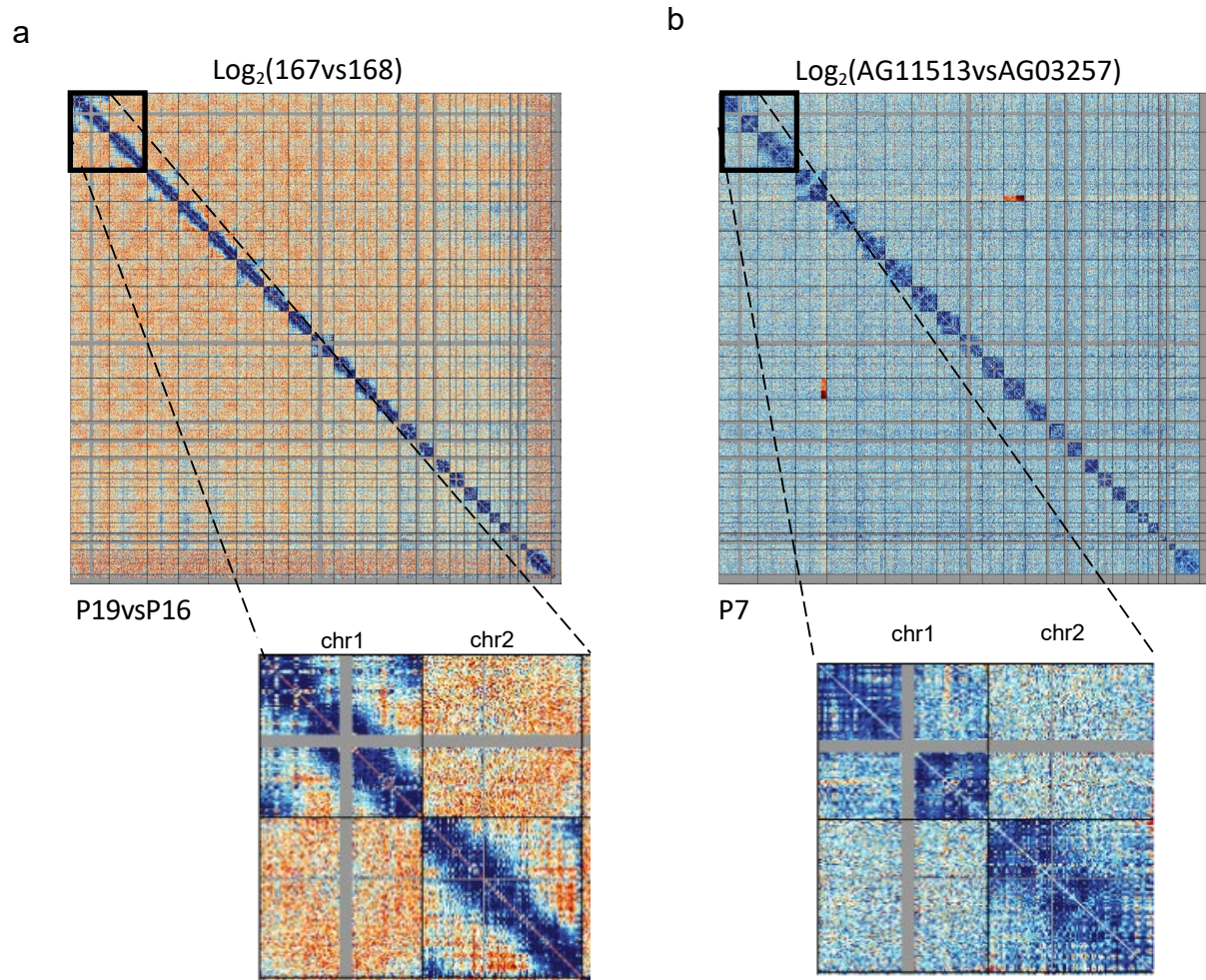


Figure 26. Progeria fibroblasts exhibit 3D genome organization features of DNA damage.

a 2.5 Mb log_2 ratio whole genome heatmaps of HGADFN167 (Passage 19) vs. HGADFN168 (Passage 16). **b** AG11513 (Passage 7) vs AG03257 (Passage 7). Inserts of chromosome 1 and 2 is below their respective heatmap and which highlights the decrease in *Rabl* structure¹⁵.

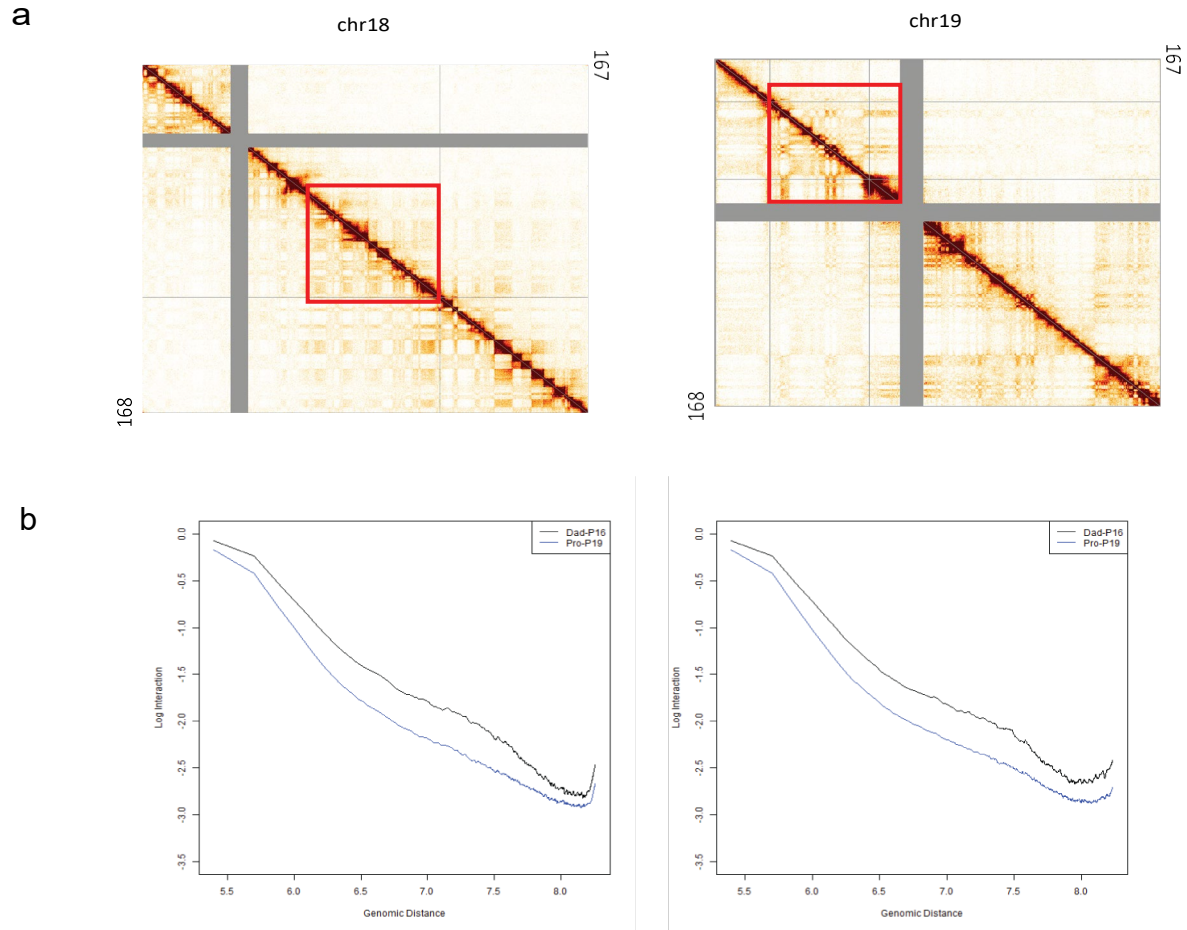


Fig. 27. Compartments weaken in late passage Progeria cells.

a 250 kb heatmaps of chromosome 18 (left) and 19 (right) in HGADFN167 (top right triangle) and HGADFN168 (bottom left triangle) Red inserts indicate visible regions of compartment loss. **b** Scaling plots showing average decay of contacts with distance across all chromosomes at a 250 kb bin size in HGADFN167 and HGADFN168 fibroblasts.

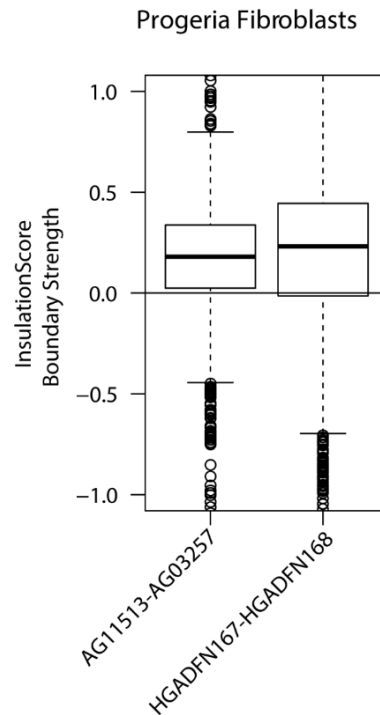


Figure 28. TAD boundary strength increases early and late passage Progeria fibroblasts.

TAD boundary strength boxplots calculated using InsulationScore data between early (left) and late (right) passage Progeria cells minus their respective controls. Boxes represent the upper and lower quartiles with the center line as the median. Upper whiskers extend $1.5 \times \text{IQR}$ beyond the upper quartile, and lower whiskers extend either $1.5 \times \text{IQR}$ below the lower quartile or to the end of the dataset.

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Appendix

Table 3. Progeria RNA-seq Data

Comparison	Upregulated	Downregulated	GO Terms of Interest
Classical Adult	37	336	organogenesis, differentiation pathways, calcium ion channel regulation
Atypical Adult	19	277	skeletal system morphogenesis, piRNA metabolic processing
Classical Child	183	522	epidermis and valve development, cell proliferation
Atypical Child	128	435	sensory organ development, regulation of bone remodeling

CHAPTER IV

Osmotic Stress Effects on Genome Organization

ABSTRACT

Increases in osmolarity causes nuclear condensation and loss of nuclear volume. Hyper-osmotic stress results in a physical change in the appearance of chromosomes. Osmotic stress leads to the removal of insulator proteins, such as CTCF and cohesin, from chromatin. To address the effects of osmotic stress on genome folding we conducted Hi-C on A375 cells exposed to 250mM NaCl and their recovery by returning to an isotonic condition. Here we confirm and add more detailed data to the previously reported osmotic stress Hi-C study. We find that distinction between compartments are less noticeable under osmotic stress. We find that Hi-C data adds additional support to condensation of chromatin. Lastly, we find that TAD boundary strength decreases in osmotic stress cells.

INTRODUCTION

Hyperosmolarity is a process that has been well studied in renal cells but is poorly understood in other human cell types. Recent evidence points to hyperosmolarity being involved in a multitude of human diseases, such as eye and liver disease^{1,2,3}. Additionally, osmotic stress may play a role in cardiovascular disease⁴. To understand why these tissues may be prone to osmotic stress, understanding the effects that it has on the cell is important. Osmotic imbalance induces physical changes to the physiological condition of the cell resulting in a decrease in nuclear volume. Decreases in nuclear size and changes in shape directly affect the size of the cell^{5,6}. Modifications in cell shape by the actin cytoskeleton result in changes in the nuclear lamina⁷. Hyper-osmotic stress results

in a physical change in the appearance of chromosomes resulting in “vermicelli” chromosomes reminiscent of WAPL depleted chromosomes and chromosomal voids in the nucleus^{8,9}.

Increases in osmolarity have profound changes on chromosomes. *Drosophila* S2 cells exposed to high concentrations of NaCl exhibit condensed chromosomes¹⁰. Additionally, insulator proteins bound to chromatin are removed and coalesce into insulator bodies in the nucleoplasm¹⁰. While this phenomenon has not been observed in human interphase cells, the removal of insulator proteins by NaCl in mitotic cells has recently been reported¹¹. The removal of these proteins from chromatin is not clearly understood, but changes in gene expression for stress related proteins, such as heat shock factor 1, has been reported in response to osmotic stress¹². Therefore, the importance of understanding how salt stress can influence cellular and nuclear behavior is important.

Like many stressors that affect cells, hyperosmolarity activates DNA damage pathways to prevent DNA breaks from occurring. Hypertonicity leads to the activation of G1 and G2 checkpoints¹³. ATM-mediated phosphorylation of p53 are associated with increased osmolarity. Apoptosis and autophagy are triggered in response to hyperosmolarity and share characteristic cell shrinkage phenotypes¹⁴. ATM is associated with the nuclear translocation of NFAT5, which may have an important role in regulation genes during osmotic stress¹⁵.

Changes in chromatin organization after hyper-osmotic stress led us to investigate how genome organization is affected. Here we show that hyper-

osmotic stress leads to and overall loss of compartment strength and compaction of chromatin. Changes in compartment strength are resolved upon isotonic conditions. We also observe changes in constitutive lamin associated domains (cLADs) in hyper-osmotic stress conditions which recover upon media replacement.

RESULTS

Osmotic stress induces genome-wide organizational changes which return upon recovery

Salt stress causes chromatin condensation in human cells. To understand how an increase in salt affects genome organization, we performed Hi-C on A375 cells before salt introduction, with 250 mM salt, and recovery by returning cells to normal media. We observe a dramatic change in control and salt stressed cells. We find that our results confirm previously published data with less salt (Figure 29a)¹⁶. We find that an increase in salt results and more defined changes in compartments (Figure 29a). Scaling plots show an increase in long-range interactions in salt stressed cells (Figure 29b). We find that replacing media with proper amounts of salt results in the recovery of this phenotype.

TAD boundary strength decreases after osmotic stress

Our lab has previously published data that shows an increase in topological associating domain (TAD) boundary strength after DNA damage. To assess whether DNA damage caused by salt stress leads to an increase in TAD boundary strength, we calculated the average TAD boundary strength between control, salt stressed, and recovery conditions. We find that in contrast to IR induced DNA damage, cells exposed to

high concentrations of salt have a decrease in TAD boundary strength (Figure 30). This is likely due to the removal of CTCF and cohesin which is normally found at TAD boundaries. TAD boundary strength is returned to its control strength upon return to control state.

Chromatin is more condensed upon osmotic stress

The visualization of chromatin condensing by microscopy events is evident. To determine how condensed chromatin is after osmotic stress, we measured the distal-to-local ratio in cells after osmotic stress and recovery¹⁷. Distal-to-local ratio measures interactions greater than 3 Mb divided by interactions less than 3 Mb multiplied by log2. We observe that upon osmotic stress chromatin interactions are indeed more condensed. In contrast, recovery by normal media results in a return to normal chromatin interaction states.

Osmotic stress changes LAD interactions

Increases in osmolarity cause a major strain on cytoskeletal interactions with the nuclear lamina. Chromatin condensation along with strain on the nuclear lamina results increased nuclear volume. Additionally, the addition of salt in drosophila results in chromatin pulling away from the nuclear laminate (data not shown). To determine if chromatin interactions with the lamina change after the introduction of high salt conditions, we analyzed the interactions of chromatin with known LADs. We find that cells exposed to high salt have a decrease in constitutive LADs (cLADs) when they have DLR score below 0 (less compact chromatin) and an increase in both facultative and constitutive interLADs. However, cLADS

increase in cells that have a DLR score above 0 (more compact chromatin). This result would suggest that interactions with the lamina are indeed changing due to salt stress.

DISCUSSION

Osmotic stress leads to changes in genome structure by condensing chromatin and nuclear volume. We expand upon recent Hi-C osmotic stress results by assessing higher salt concentrations and by performing further analyses on high resolution structures. We find that using 250mM NaCl on A375 cells that compartments become less distinguished. It is worth noting that compartment strength exhibits only minor changes. We determine, that unlike other DNA damage results, TAD boundary strength decreases after salt stress. The observation of chromatin condensation under microscopy conditions are confirmed by distal-to-local ratio measurements. This evidence is also strengthened by the increase in mid-to-long range interactions. We also show that interactions with LADs are altered upon induction of osmotic stress.

It is not surprising to see, that although DNA damage is occurring in osmotic stressed cells, a decrease in TAD boundary strength is observed. This is likely due to insulator proteins being removed from chromatin at a rapid. CTCF and cohesin which mediate TAD and compartmentalization have been shown to form insulator bodies under osmotic stress¹⁰. While we do not see this phenomenon under microscopy conditions, we feel that this is most likely the case. While not as dramatic, these results are more similar Hi-C experiments that remove cohesin.

We also note that other DNA damage pathways may be activated to manage breaks in DNA. Ataxia telangiectasia and Rad3-related protein (ATR) is a kinase known to be activated upon osmotic stress to resolve single stranded breaks. ATR is also responsible for mediating checkpoints in response to mechanical stress¹⁹. Future experiments that investigate the role ATR has on 3D genome organization is needed to understand whether these changes would occur.

METHODS

Cell culture and salt stress

A375 malignant melanoma cells were purchased from ATCC (ATCC® CRL-1619™). Cells were grown in DMEM supplemented with 10% FBS, 1% Pen-strep, and 1% L-glutamine and passaged at a density of 80%. For salt stress experiments, A375 cells were incubated with media supplemented with 250mM NaCl for one hour or 250mM NaCl for one hour and reintroduced to normal A375 media for one hour. Cells were then collected for Hi-C.

Hi-C

To prepare the Hi-C libraries and prepare for sequencing, at the appropriate time points, Hi-C experiments were performed according to the protocol detailed in Gollosi et al.²⁰. Briefly, ~5 million cells were fixed with 1% formaldehyde, suspended in cell lysis buffer for permeabilization, and homogenized by douncing. Crosslinked chromatin was digested overnight with HindIII. Sticky ends were filled in with biotin-dCTP, and the blunt ends of interacting fragments were ligated together. DNA was purified by two phenol-chloroform extractions and ethanol

precipitation. Biotin-dCTP at unligated ends was removed, and the DNA was sheared to a target size of 200-400 bp by a Covaris sonicator (Covaris, M220). DNA between 100-400 bp was selected for using AMPure XP beads (Beckman Coulter). Biotinylated DNA was pulled down using streptavidin coated magnetic beads and prepared for multiplex sequencing on an Illumina platform using the NEBNext Ultra II kit (NEB, E7645S). All end preparation, adaptor ligation, and PCR amplification steps were carried out on bead bound DNA libraries.

Sequencing was carried out at the Oklahoma Medical Research Foundation Clinical Genomics Facility on an Illumina HiSeq 3000 platform with 75 bp paired end reads or a NovaSeq 6000 with 50 bp paired end reads. Sequencing reads were mapped to the reference human genome hg19, filtered, and iteratively corrected using the pipeline detailed in Imakaev et al.⁶⁷ and available on github (dekkerlab-cMapping).

Compartment analysis

Principle components analysis was performed using the matrix2compartment perl script in the cworld- dekker pipeline available on github on 250 kb binned matrices. The eigen1 value, which typically represents the compartment profile, was used to determine A/B compartments.

TAD boundary and insulation analysis

Topologically associating domain (TAD) boundaries were called on 40 kb binned matrices using two methods: the InsulationScore method, as detailed in Crane et al., available as matrix2insulation in the cworld-

dekker github package²¹. All TAD boundary strengths were plotted for the InsulationScore boxplots.

Distal to Local Ratio

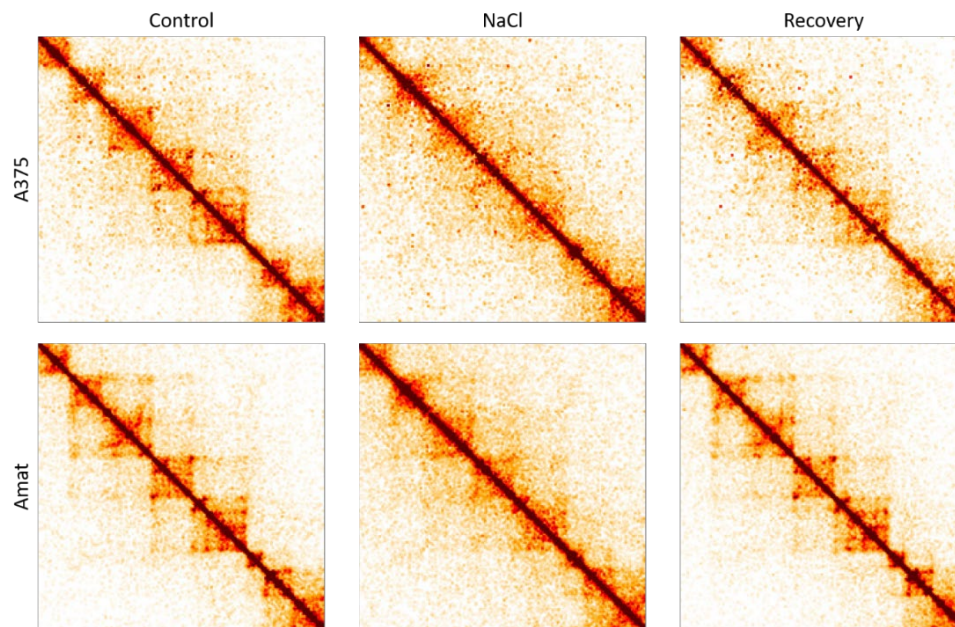
Distal to local ratio as calculated as indicated in the equations below. Δ DLR was calculated as previously described in Heinz et.al¹⁷.

$$DDDDDD = UUUU2 * \frac{DDDDDDDDUU \text{ IIITDDIIITDDIITDDUUIDD } (> 3MMM)}{DDUUIDDU \text{ IIITDDIIITDDIITDDUUIDD } (< 3MMM)}$$

$$\Delta DDDDD = DDDDDDDUUIDDUUUUU - DDDDDDDUUIDDUUUU$$

a 20 kb control, NaCl stressed, and recovery heatmaps of A375 cells (top) and T47D cells (bottom) on chromosome 4:71-75 Mb. A375 cells were exposed to 250mM NaCl and T47D cells were exposed to 110mM NaCl. **b** Scaling plots of control, 250mM NaCl, and recovery cells, show a gain of long-range interactions.

a



b

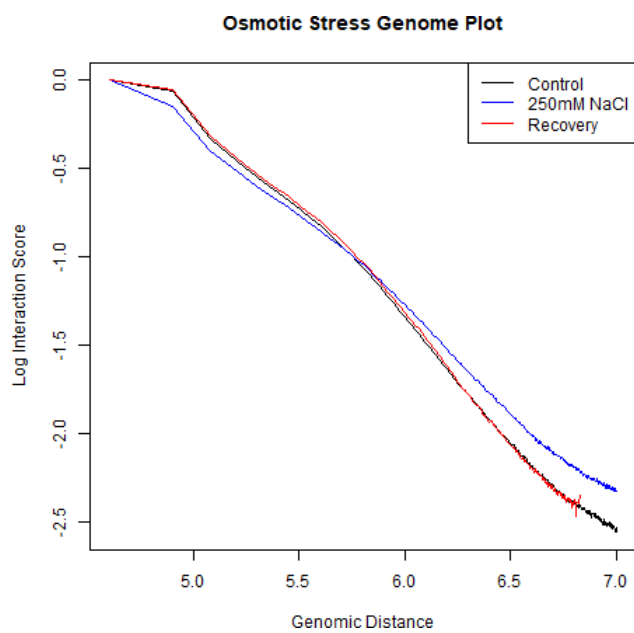


Figure 29. Compartments become less distinct in osmotic stressed cells.

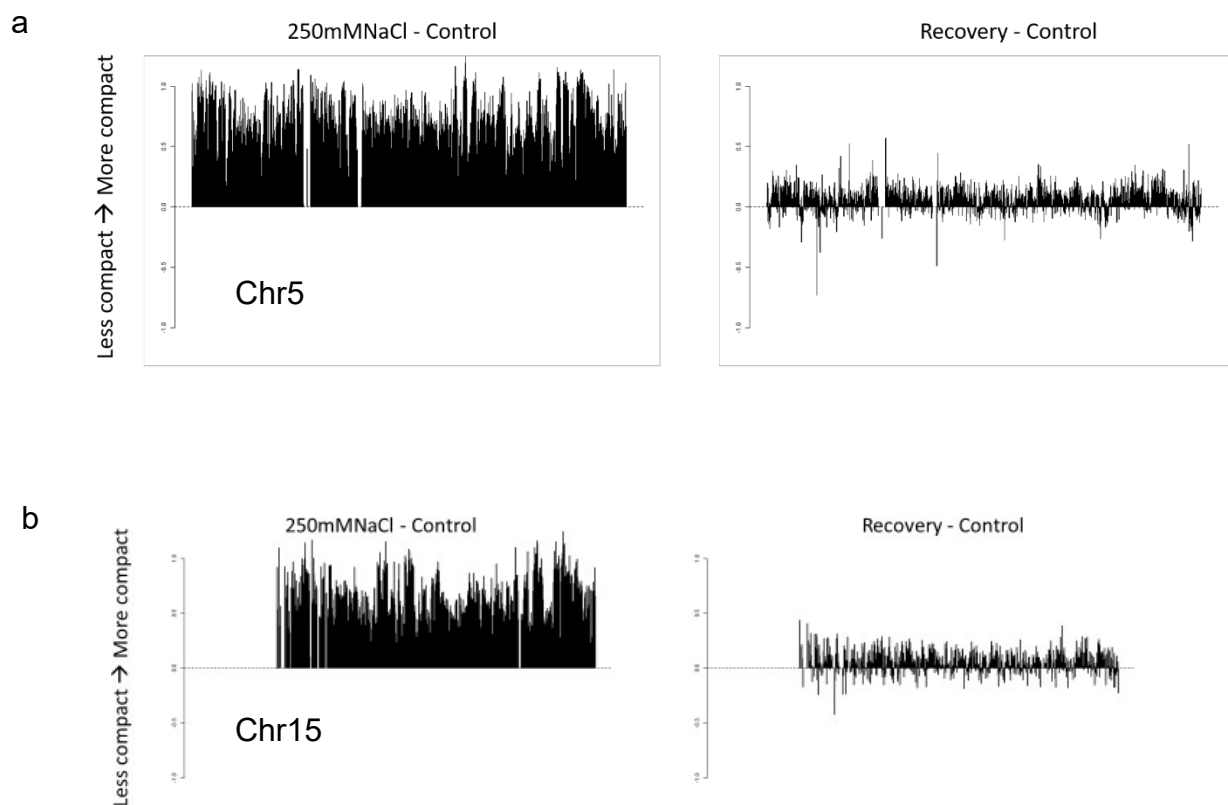


Figure 30. Osmotic stress compacts chromatin.

a Δ DLR measurement of chr5 in 250mM NaCl – Control (left) and Recovery - Control (right). **b** Δ DLR measurement of chr15 in 250mM NaCl – Control (left) and Recovery -Control (right).

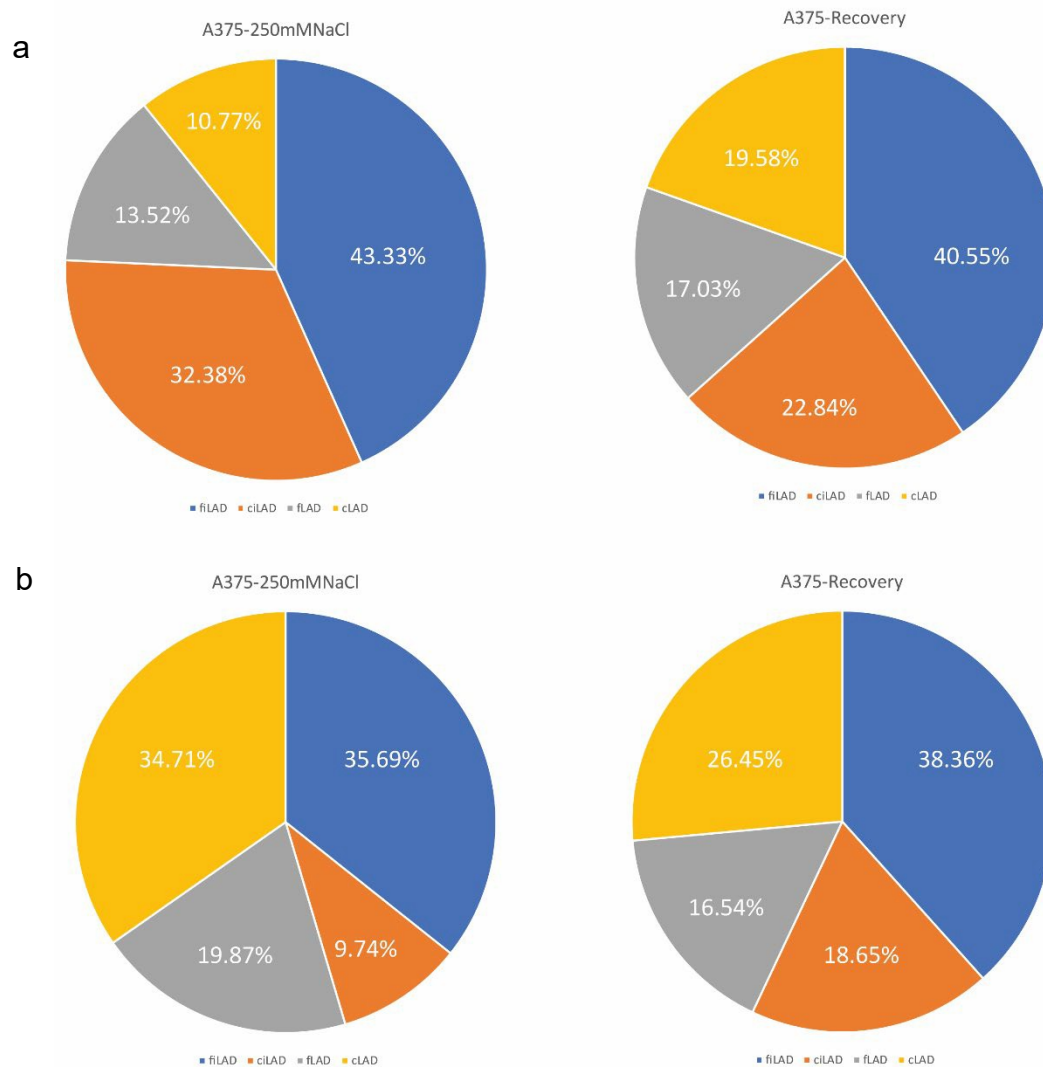


Figure 31. Lamin interactions change after exposure to 250mM NaCl.

a Interactions with LADs in chromatin interactions with a Δ DLR score of <0 . **b** Interactions with LADs in chromatin interactions with a Δ DLR score of >0 . The biggest changes are observed in cLADs suggesting that chromatin bound to the lamina is changing interaction partners.

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CONCLUSION

The studies presented here provide a collection of work to probe the effects of DNA damage on genome organization caused by IR, progerin expression, and osmotic stress. Previous studies have focused primarily using classical cellular techniques to identify proteins involved in DNA repair or molecular techniques to identify changes in gene expression or chromosome location. Here, we utilize Hi-C to identify chromatin interactions in nuclei experiencing increased DNA damage. Interestingly, we find that the response to specific stressors results in differing responses to chromatin interactions.

Chapters I and II focus on the 3D genome folding response in repair-proficient and repair-deficient before and after the exposure of IR caused by X-rays. Here, we find cell-type specific changes that occur in both fibroblasts and lymphoblastoids at the chromosomal territory level. Although there is much debate on repair kinetics in heterochromatin and euchromatin, we report that little change occurs in compartmentalization. However, we report that, regardless of cell-type, TAD boundaries become strengthened after exposure to IR. We find that CTCF mediated loops are also increased after IR exposure which may provide a potential mechanism as to why TAD boundaries are strengthened. In contrast, DNA repair-deficient fibroblasts do not show changes in genome structure after IR exposure. Using ATM, Lig1, and Lig4 mutants, we find that 3D genome organization remains relatively unchanged. We do find that the progression of ATM into the later stages of the cell cycle results in similar responses.

Chapter III investigates the influence that the lamin A mutation, progerin has on 3D genome structure. We provide evidence that while compartment strength is decreased in Progeria fibroblasts, it is not completely lost as was previously reported. We determined that Progeria fibroblasts contain features that we observe in IR-induced DNA damage datasets which include loss of *Rabl* configuration and an increase in TAD boundary strength. We also have begun the process of understanding the variability of gene expression changes among multiple Progeria cells. Heretofore, the majority of Progeria gene expression datasets contain less than 3 cell lines. We endeavor to analyze this data further to mine potential genes that may influence phenotypic defects in Progeria patients.

Chapter IV discusses the effects of osmotic stress on 3D genome folding. We confirm previously reported data that compartments become less clear. When compared to this study, our data is more evident which can be attributed to higher salt concentrations used in our study. We find that chromatin is more condensed as evidenced by distal-to-local measurements and scaling decay plot data. We find that interactions with the nuclear lamina is also altered after exposure to high salt concentrations.

VITA

Jacob Tyler Sanders was born in Nashville, TN to Theresa and Thomas Sanders. He graduated from Glenclyff Comprehensive High school in 2008. He obtained a Bachelor of Science in Biological Sciences at Tennessee State University. Afterward, he obtained a Master of Science in Cell Biology at Middle Tennessee State University (MTSU). While at MTSU, he studied under the direction of Dr. Lynn Boyd investigating nuclear ubiquitin in *C. elegans* exposed to environmental stressors. He also received an NSF GK-12 fellowship which allowed him to train high schoolers at Stewarts Creek High School to design and carry out experiments. Continuing his interest in nuclear stress, he joined the lab of Dr. Rachel Patton McCord at the University of Tennessee, Knoxville in 2015.