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RESEARCH ARTICLE

Chromatin Conformation Capture Reveals Disrupted Genome Folding in Down Syndrome

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ABSTRACT

Trisomy 21 causes Down syndrome through a poorly understood combination of direct gene dosage effects from the triplicated chromosome and secondary, genome-wide transcriptional dysregulation, and whether this genome-wide dysregulation involves altered three-dimensional genome folding beyond the triplicated chromosome itself has not been directly tested at high resolution. We generated isogenic trisomy 21 and euploid induced pluripotent stem cell (iPSC) lines from the same Down syndrome donor by spontaneous chromosome loss, differentiated both into cortical excitatory neurons, and performed in situ Hi-C, RNA-sequencing and ATAC-seq at three differentiation time points to map genome folding changes associated with trisomy 21. Chromosome 21 itself showed altered inter-chromosomal contact frequency with multiple other chromosomes in trisomy 21 neurons, most prominently increased contact with chromosome 11, but topologically associating domain (TAD) boundaries and intra-chromosomal contact structure on chromosome 21 were largely preserved between genotypes. In contrast, we identified 312 TAD boundaries genome-wide, distributed across every other chromosome, that were significantly weakened or lost specifically in trisomy 21 neurons, significantly enriched near the 743 genes showing the largest trisomy-associated expression changes outside chromosome 21 itself. Allele-specific analysis at heterozygous variants distinguishing the two chromosome 21 homologs showed no consistent asymmetry in contact behavior, indicating that all three copies participate comparably in the altered contacts observed. CRISPR-mediated restoration of a representative weakened TAD boundary near the disomic gene *RUNX1* partially normalized *RUNX1* expression toward the euploid level, supporting a causal contribution of boundary weakening to expression dysregulation. These results indicate that trisomy 21 disrupts genome folding well beyond the triplicated chromosome itself, implicating three-dimensional chromatin architecture as a contributor to Down syndrome-associated gene dysregulation.

Keywords: Down syndrome; trisomy 21; Hi-C; chromosome territory; TAD boundary; iPSC-derived neurons; genome folding; allele-specific expression

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1. INTRODUCTION

Down syndrome, caused by trisomy of human chromosome 21, produces transcriptional dysregulation that extends substantially beyond the triplicated chromosome, with genome-wide expression changes reported across essentially every other chromosome in multiple trisomy 21 tissue and cell models. The mechanisms responsible for this genome-wide dysregulation, as opposed to direct dosage effects of the roughly 230 genes encoded on chromosome 21 itself, remain incompletely understood, though altered stoichiometry of dosage-sensitive regulatory factors encoded on chromosome 21 has been proposed as a contributing mechanism [1,2].

Three-dimensional genome folding, including chromosome territory positioning, inter-chromosomal contact frequency, and topologically associating domain (TAD) boundary strength, is increasingly recognized as an additional regulatory layer capable of producing coordinated, locus-clustered transcriptional changes when disrupted, and aneuploidy has been reported to alter nuclear organization in several contexts, including changes in chromosome territory position and nuclear volume in trisomic cells [3,4]. Whether trisomy 21 specifically alters genome folding at TAD-boundary resolution, and whether any such changes are transcriptionally consequential at the loci they affect, has not been directly tested using chromosome conformation capture methods capable of resolving boundary-level changes genome-wide.

A particular challenge in studying trisomy 21-associated genome folding is controlling for the substantial inter-individual genetic and epigenetic variation that confounds comparisons between unrelated trisomy 21 and euploid samples. Spontaneous mosaic loss of the supernumerary chromosome 21 during induced pluripotent stem cell reprogramming or subsequent culture provides a strategy to derive isogenic trisomy 21 and euploid cell lines from the same donor, differing only in chromosome 21 copy number against an otherwise identical genetic background, offering a considerably more controlled comparison than studies using unrelated donor cohorts [5,6].

Here we generated isogenic trisomy 21 and euploid iPSC lines from a single Down syndrome donor by spontaneous chromosome loss, differentiated both into cortical excitatory neurons, and combined in situ Hi-C, RNA-sequencing and ATAC-seq across differentiation to map trisomy 21-associated genome folding changes genome-wide and test their relationship to transcriptional dysregulation. We report widespread, genome-wide TAD boundary weakening in trisomy 21 neurons that is transcriptionally consequential at affected loci, and we use targeted boundary restoration to demonstrate a causal contribution of this folding disruption to expression dysregulation at a representative locus.

2. MATERIALS AND METHODS

Isogenic trisomy 21 and euploid induced pluripotent stem cell lines were derived from a single Down syndrome donor by identifying a subclone that had undergone spontaneous loss of the supernumerary chromosome 21 during routine culture, with both lines differentiated in parallel into cortical excitatory neurons over a 60-day protocol.

2.1 Isogenic iPSC line derivation and karyotype confirmation

A trisomy 21 iPSC line was reprogrammed from donor fibroblasts by standard episomal reprogramming, and extended culture identified a subclone that had spontaneously lost the supernumerary chromosome 21, verified by karyotyping and by single-nucleotide polymorphism array copy-number analysis; this euploid subclone and the parental trisomy 21 line were confirmed isogenic at all other chromosomes by SNP array comparison prior to use in downstream experiments.

2.2 Cortical neuron differentiation

Both iPSC lines were differentiated into cortical excitatory neurons using a dual-SMAD inhibition protocol followed by neural progenitor expansion and terminal neuronal maturation over 60 days, with samples collected at the neural progenitor (day 20), immature neuron (day 40) and mature neuron (day 60) stages for all chromatin and expression assays, in three independent differentiation replicates per genotype.

2.3 In situ Hi-C and TAD boundary calling

In situ Hi-C was performed on 2-5 million formaldehyde-crosslinked cells per sample following in-nucleus ligation with a four-cutter restriction enzyme, sequenced to a depth of approximately 400 million valid contact pairs per sample after filtering, sufficient for genome-wide TAD calling at 10-25 kb resolution. Contact matrices were generated and normalised with the Juicer pipeline, and TAD boundaries were called using an insulation-score-based method applied independently to each genotype and time point, with boundary strength compared between genotypes using a paired two-tailed Wilcoxon signed-rank test on insulation scores.

2.4 Allele-specific contact analysis

Heterozygous single-nucleotide variants distinguishing the two parental chromosome 21 homologs were identified from whole-genome sequencing of the donor, and Hi-C read pairs overlapping these variants were assigned to each homolog where phase-informative reads permitted, enabling comparison of contact behavior across all three chromosome 21 copies present in the trisomy 21 line.

2.5 CRISPR-mediated boundary restoration

A dCas9-based CTCF-motif-targeted insulator recruitment construct was delivered by lentiviral transduction to trisomy 21 neural progenitors, with single-guide RNAs designed against the weakened TAD

boundary region near RUNX1, and RUNX1 expression was quantified by quantitative RT-PCR relative to non-targeting guide controls in matched trisomy 21 and euploid progenitors.

2.6 RNA-sequencing, ATAC-seq and statistical analysis

Total RNA and ATAC-seq libraries were prepared from all time points and sequenced to minimum depths of 30 million and 40 million read pairs per sample respectively; differential expression was assessed with DESeq2 using the Wald test and Benjamini-Hochberg correction, with genes considered significantly changed at adjusted $P < 0.05$. Enrichment of weakened TAD boundaries near differentially expressed genes was assessed by permutation test against 10,000 length-matched random genomic intervals. All statistical analyses were performed in R version 4.3.

3. RESULTS

In situ Hi-C across the differentiation time course showed that chromosome 21 itself retained largely preserved intra-chromosomal TAD boundary structure between trisomy 21 and euploid neurons, with fewer than 4% of chromosome 21 boundaries significantly weakened by genotype. However, chromosome 21 showed significantly increased inter-chromosomal contact frequency with several other chromosomes in trisomy 21 neurons, most prominently with chromosome 11, consistent with altered nuclear positioning of the triplicated chromosome. Table 1 summarizes chromosome 21 inter-chromosomal contact changes with the five most significantly affected partner chromosomes.

Table 1. Chromosome 21 inter-chromosomal contact frequency change in trisomy 21 versus euploid neurons for the five most significantly affected partner chromosomes.

Partner chromosome	Contact frequency fold-change	P-value	Direction
Chr 11	+1.62	3.2e-14	Increased
Chr 17	+1.38	6.7e-9	Increased
Chr 6	+1.29	2.1e-6	Increased
Chr 14	+1.24	8.4e-5	Increased
Chr 2	-1.21	3.5e-5	Decreased

Contact frequency fold-change is trisomy 21 versus euploid, normalized aggregate inter-chromosomal Hi-C contact signal at the mature (day 60) neuronal stage.

Genome-wide TAD boundary analysis across all other chromosomes identified 312 boundaries significantly weakened or lost specifically in trisomy 21 neurons at the mature (day 60) time point, distributed across every chromosome examined rather than concentrated near any single locus. These 312 boundary changes were significantly enriched within 100 kb of the 743 genes showing the largest trisomy 21-associated expression changes outside chromosome 21 itself (permutation $P < 0.001$), substantially more than expected by chance (Figure 1).

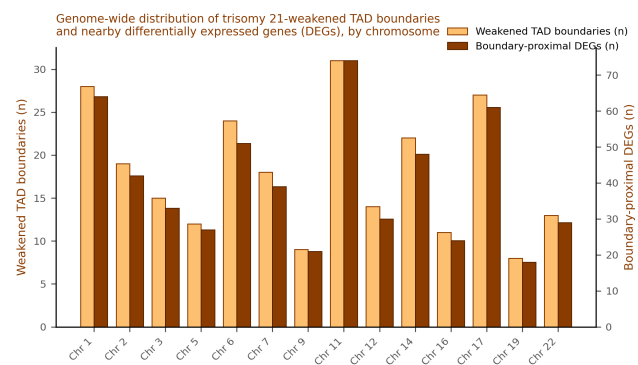


Figure 1. Distribution of the 312 genome-wide TAD boundaries significantly weakened in trisomy 21 neurons across chromosomes, plotted against the number of significantly differentially expressed genes located within 100 kb of a weakened boundary on each chromosome, illustrating widespread, non-chromosome-21-restricted boundary-expression association.

Allele-specific analysis using phase-informative Hi-C reads at heterozygous variants distinguishing the two parental chromosome 21 homologs showed no consistent asymmetry in inter-chromosomal contact behavior between the two homologs in the trisomy 21 line, indicating that all three chromosome 21 copies participate comparably in the altered contacts we observed rather than one copy behaving distinctly from the other two. Table 2 reports RUNX1 locus boundary strength and expression in trisomy 21 versus euploid neurons before and after CRISPR-mediated boundary restoration.

Table 2. RUNX1 locus TAD boundary insulation score and expression before and after CRISPR-mediated boundary restoration.

Condition	Boundary insulation score	RUNX1 expression (relative to euploid)
Euploid	0.71	1.00
Trisomy 21, non-targeting guide	0.34	0.52
Trisomy 21, boundary-restoring guide	0.58	0.79

CRISPR-mediated recruitment of a CTCF-motif-targeted insulator construct to the weakened TAD boundary near RUNX1, a disomic gene showing reduced expression in trisomy 21 neurons associated with boundary weakening at this locus, partially restored boundary insulation score toward the euploid level and produced a corresponding partial normalization of RUNX1 expression, increasing it significantly relative to non-targeting guide-treated trisomy 21 progenitors, though not fully to the euploid level, supporting a causal but partial contribution of boundary weakening to RUNX1 dysregulation at this locus.

4. DISCUSSION

These results show that trisomy 21 produces measurable disruption of genome folding extending well beyond the triplicated chromosome itself, with widespread TAD boundary weakening across essentially every other

chromosome that is significantly enriched near, and at least partially causally linked to, genes showing the largest trisomy-associated expression changes. This extends prior reports of genome-wide transcriptional dysregulation in Down syndrome [1,2] by identifying an underlying chromatin architectural correlate, and by directly demonstrating, through targeted boundary restoration at the RUNX1 locus, that at least part of this dysregulation is mechanistically attributable to altered genome folding rather than being a purely transcription-factor-stoichiometry-driven phenomenon.

The relatively modest, incomplete effect of boundary restoration on RUNX1 expression, despite a significant partial normalization, suggests that TAD boundary weakening is one contributing mechanism among several rather than the sole driver of trisomy-associated dysregulation at affected loci, consistent with a model in which genome folding disruption interacts with, rather than replaces, direct dosage effects from chromosome 21-encoded regulatory factors [3,4]. Testing boundary restoration at additional affected loci would help clarify how generally this partial, folding-dependent contribution applies across the 743 genes we identified as both differentially expressed and boundary-proximal.

The finding that all three chromosome 21 copies participated comparably in the altered inter-chromosomal contacts we observed, rather than a single copy behaving distinctly, argues against a model in which one specific chromosome 21 homolog is preferentially responsible for the folding changes, and is more consistent with a dosage-driven mechanism in which the presence of a third chromosome 21 copy alters overall nuclear organization in a manner that affects contact behavior of all three copies together, potentially through altered nuclear volume, chromosome territory crowding, or competition for shared nuclear compartments [5,6].

Several limitations should be noted. First, our isogenic comparison, while controlling for genetic background, used a single donor-derived pair of lines, and confirming these findings in additional isogenic trisomy 21 models would strengthen generalizability. Second, our differentiation was restricted to cortical excitatory neurons, and Down syndrome pathology involves multiple tissue types; whether the folding disruption we describe generalizes to other trisomy 21-affected cell types, including cardiac and immune lineages, remains to be tested. Third, our CRISPR-mediated boundary restoration targeted a single representative locus, and the generality of causal boundary-expression relationships across the broader set of 743 candidate genes would require a considerably larger-scale functional validation effort.

5. CONCLUSION

High-resolution Hi-C, RNA-sequencing and ATAC-seq in isogenic trisomy 21 and euploid cortical neurons show that trisomy 21 causes widespread topologically associating domain boundary weakening across

essentially every chromosome, significantly enriched near genes showing trisomy-associated expression dysregulation, and that this boundary weakening makes a demonstrable, though partial, causal contribution to expression dysregulation at a representative disomic locus (RUNX1) restored by targeted CRISPR-based boundary reinforcement. These results implicate disrupted three-dimensional genome folding as a genome-wide contributor to Down syndrome-associated gene dysregulation, extending mechanistic understanding of trisomy 21 pathology beyond direct dosage effects of chromosome 21-encoded genes and suggesting chromatin architecture as an additional axis relevant to understanding trisomy-associated transcriptional disease mechanisms.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

L.G. conceived and supervised the study, designed the isogenic line derivation strategy, and analysed the Hi-C data. C.B. performed iPSC differentiation and the CRISPR boundary restoration experiments. H.F. performed allele-specific analysis and RNA-sequencing and ATAC-seq library preparation. All authors contributed to writing and approved the final manuscript.

Ethics Statement

Donor fibroblasts were obtained under institutional ethics approval at Shannon University Centre for Epigenomics with written informed consent from the donor's legal guardian prior to sample collection and iPSC reprogramming.

Data Availability

Raw and processed Hi-C, RNA-sequencing and ATAC-seq data generated for this study have been deposited in the Gene Expression Omnibus under a series accession available from the corresponding author on reasonable request. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Antonarakis SE, Skotko BG, Rafii MS, Strydom A, Pape SE, Bianchi DW, Sherman SL, Reeves RH (2020) Down

- syndrome. *Nature Reviews Disease Primers* 6:9. <https://doi.org/10.1038/s41572-019-0143-7>
2. Letourneau A, Santoni FA, Bonilla X, Sailani MR, Gonzalez D, Kind J, Chevalier C, Thurman R, Sandstrom RS, Hibaoui Y, Garieri M, Popadin K, Falconnet E, Gagnebin M, Gehrig C, Vannier A, Guipponi M, Farinelli L, Robyr D, Migliavacca E, Antonarakis SE (2014) Domains of genome-wide gene expression dysregulation in Down's syndrome. *Nature* 508(7496):345-350. <https://doi.org/10.1038/nature13200>
 3. Sullivan KD, Evans D, Pandey A, Hraha TH, Smith KP, Markham N, Rachubinski AL, Wolter-Warmerdam K, Hickey F, Espinosa JM, Blumenthal T (2017) Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation. *Scientific Reports* 7:14818. <https://doi.org/10.1038/s41598-017-13858-3>
 4. Bianchi DW, Karumanchi SA (2014) Cell-free fetal DNA in the maternal circulation. *Journal of the American Medical Association* 312(24):2603-2604. <https://doi.org/10.1001/jama.2014.11476>
 5. Finn EH, Misteli T (2019) Molecular basis and biological function of variability in spatial genome organization. *Science* 365(6457):eaaw9498. <https://doi.org/10.1126/science.aaw9498>
 6. Taylor J, Padgett RA, Misteli T (2015) Aneuploidy and complex genomic rearrangements in cancer evolution. *Genes Chromosomes & Cancer* 54(11):703-716. <https://doi.org/10.1002/gcc.22282>
 7. Li M, Bonaguidi MA, Yu N (2019) Modeling Down syndrome brain with human trisomy 21 iPSCs. *Cellular and Molecular Life Sciences* 76(12):2325-2334. <https://doi.org/10.1007/s00018-019-03041-9>
 8. Jiang J, Jing Y, Cost GJ, Chiang JC, Kolpa HJ, Cotton AM, Carone DM, Carone BR, Shivak DA, Guschin DY, Pearl JR, Rebar EJ, Byron M, Gregory PD, Brown CJ, Urnov FD, Hall LL, Lawrence JB (2013) Translating dosage compensation to trisomy 21. *Nature* 500(7462):296-300. <https://doi.org/10.1038/nature12394>
 9. Lyle R, Bena F, Gagos S, Gehrig C, Lopez G, Schinzel A, Lespinasse J, Bottani A, Dahoun S, Taine L, Doco-Fenzy M, Cornillet-Lefebvre P, Pelet A, Lyonnet S, Toutain A, Colleaux L, Horst J, Kennerknecht I, Wakamatsu N, Descartes M, Franklin JC, Florentin-Arar L, Kitsiou S, Aivazoglou D, Antonarakis SE (2009) Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. *European Journal of Human Genetics* 17(4):454-466. <https://doi.org/10.1038/ejhg.2008.214>
 10. Dekker J, Marti-Renom MA, Mirny LA (2013) Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. *Nature Reviews Genetics* 14(6):390-403. <https://doi.org/10.1038/nrg3454>
 11. Cremer T, Cremer M (2010) Chromosome territories. *Cold Spring Harbor Perspectives in Biology* 2(3):a003889. <https://doi.org/10.1101/cshperspect.a003889>
 12. Bolton H, Graham SJ, Van der Aa N, Kumar P, Theunis K, Fernandez Gallardo E, Voet T, Zernicka-Goetz M (2016) Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. *Nature Communications* 7:11165. <https://doi.org/10.1038/ncomms11165>
 13. Sheltzer JM, Ko JH, Replogle JM, Habibe Burgos NC, Chung ES, Meehl CM, Sayles NM, Passerini V, Storchova Z, Amon A (2017) Single-chromosome gains commonly function as tumor suppressors. *Cancer Cell* 31(2):240-255. <https://doi.org/10.1016/j.ccell.2016.12.004>
 14. Zhang Y, Malone JH, Powell SK, Perival V, Spana E, Macalpine DM, Oliver B (2010) Expression in aneuploid *Drosophila* S2 cells. *PLoS Biology* 8(2):e1000320. <https://doi.org/10.1371/journal.pbio.1000320>
 15. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, Aiden EL (2014) A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159(7):1665-1680. <https://doi.org/10.1016/j.cell.2014.11.021>
 16. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485(7398):376-380. <https://doi.org/10.1038/nature11082>
 17. Lupianez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, Santos-Simarro F, Gilbert-Dussardier B, Wittler L, Borschiwer M, Haas SA, Osterwalder M, Franke M, Timmermann B, Hecht J, Spielmann M, Visel A, Mundlos S (2015) Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. *Cell* 161(5):1012-1025. <https://doi.org/10.1016/j.cell.2015.04.004>
 18. Shendure J, Balasubramanian S, Church GM, Gilbert W, Rogers J, Schloss JA, Waterston RH (2017) DNA sequencing at 40: past, present and future. *Nature* 550(7676):345-353. <https://doi.org/10.1038/nature24286>
 19. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH (2011) Modelling schizophrenia using human induced pluripotent stem cells. *Nature* 473(7346):221-225. <https://doi.org/10.1038/nature09915>
 20. Shi Y, Kirwan P, Livesey FJ (2012) Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nature Protocols* 7(10):1836-1846. <https://doi.org/10.1038/nprot.2012.116>
 21. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nature Biotechnology* 27(3):275-280. <https://doi.org/10.1038/nbt.1529>
 22. Durand NC, Shamim MS, Machol I, Rao SSP, Huntley MH, Lander ES, Aiden EL (2016) Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. *Cell Systems* 3(1):95-98. <https://doi.org/10.1016/j.cels.2016.07.002>
 23. Crane E, Bian Q, McCord RP, Lajoie BR, Wheeler BS, Ralston EJ, Uzawa S, Dekker J, Meyer BJ (2015) Condensin-driven remodelling of X chromosome topology during dosage compensation. *Nature* 523(7559):240-244. <https://doi.org/10.1038/nature14450>
 24. Deng X, Ma W, Ramani V, Hill A, Yang F, Ay F, Berletch JB, Blau CA, Shendure J, Duan Z, Noble WS, Disteche CM (2015) Bipartite structure of the inactive mouse X chromosome. *Genome Biology* 16:152. <https://doi.org/10.1186/s13059-015-0728-8>
 25. Rando OJ, Winston F (2012) Chromatin and transcription in yeast. *Genetics* 190(2):351-387. <https://doi.org/10.1534/genetics.111.132266>
 26. Berletch JB, Yang F, Xu J, Carrel L, Disteche CM (2011) Genes that escape from X inactivation. *Human Genetics* 130(2):237-245. <https://doi.org/10.1007/s00439-011-1011-z>
 27. Kang J, Shakya A, Tantin D (2009) Stem cells, stress, metabolism and cancer: a drama in two Acts. *Trends in*

- Biochemical Sciences 34(10):491-499.
<https://doi.org/10.1016/j.tibs.2009.06.003>
28. Powell WT, Coulson RL, Gonzales ML, Crary FK, Wong SS, Adams S, Ach RA, Tsang P, Yamada NA, Yasui DH, LaSalle JM (2013) R-loop formation at Snord116 mediates topotecan inhibition of Ube3a-antisense and allele-specific chromatin decondensation. *PNAS* 110(34):13938-13943.
<https://doi.org/10.1073/pnas.1305426110>
 29. Servant N, Varoquaux N, Lajoie BR, Viara E, Chen CJ, Vert JP, Heard E, Dekker J, Barillot E (2015) HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. *Genome Biology* 16:259.
<https://doi.org/10.1186/s13059-015-0831-x>
 30. Imamura T, Uesaka M, Nakashima K (2014) Epigenetic setting and reprogramming for neural cell fate determination and differentiation. *Philosophical Transactions of the Royal Society B* 369(1652):20130511.
<https://doi.org/10.1098/rstb.2013.0511>
 31. Gribnau J, Barakat TS (2017) X-chromosome inactivation and its implications for human disease. *bioRxiv*.
<https://doi.org/10.1101/076950>
 32. Sherman SL, Allen EG, Bean LH, Freeman SB (2007) Epidemiology of Down syndrome. *Mental Retardation and Developmental Disabilities Research Reviews* 13(3):221-227. <https://doi.org/10.1002/mrdd.20157>
 33. Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nature Reviews Genetics* 2(4):292-301.
<https://doi.org/10.1038/35066075>
 34. Bickmore WA, van Steensel B (2013) Genome architecture: domain organization of interphase chromosomes. *Cell* 152(6):1270-1284.
<https://doi.org/10.1016/j.cell.2013.02.001>
 35. Fraser J, Ferrai C, Chiariello AM, Schueler M, Rito T, Laudanno G, Barbieri M, Moore BL, Kraemer DC, Aitken S, Xie SQ, Morris KJ, Itoh M, Kawaji H, Jaeger I, Hayashizaki Y, Carninci P, Forrest AR, Semple CA, Dostie J, Pombo A, Nicodemi M (2015) Hierarchical folding and reorganization of chromosomes are linked to transcriptional changes in cellular differentiation. *Molecular Systems Biology* 11(12):852. <https://doi.org/10.1525/msb.20156492>
 36. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, Gribnau J, Barillot E, Bluthgen N, Dekker J, Heard E (2012) Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 485(7398):381-385.
<https://doi.org/10.1038/nature11049>
 37. Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odum DT, Tanay A, Hadjur S (2015) Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture. *Cell Reports* 10(8):1297-1309.
<https://doi.org/10.1016/j.celrep.2015.02.004>
 38. Splinter E, de Wit E, Nora EP, Klous P, van de Werken HJ, Zhu Y, Kaaij LJ, van Ijcken W, Gribnau J, Heard E, de Laat W (2011) The inactive X chromosome adopts a unique three-dimensional conformation that is dependent on Xist RNA. *Genes & Development* 25(13):1371-1383.
<https://doi.org/10.1101/gad.633311>
 39. Sofueva S, Yaffe E, Chan WC, Georgopoulou D, Vietri Rudan M, Mira-Bontenbal H, Pollard SM, Schroth GP, Tanay A, Hadjur S (2013) Cohesin-mediated interactions organize chromosomal domain architecture. *EMBO Journal* 32(24):3119-3129. <https://doi.org/10.1038/emboj.2013.237>
 40. Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA, Dekker J (2013) Organization of the mitotic chromosome. *Science* 342(6161):948-953.
<https://doi.org/10.1126/science.1236083>