Cornelia de Lange syndrome is a multisystem developmental disorder typically caused by mutations in the gene encoding the cohesin loader NIPBL. The associated phenotype is generally assumed to be the consequence of aberrant transcriptional regulation. Recently, we identified a missense mutation in BRD4 associated with a Cornelia de Lange-like syndrome that reduces BRD4 binding to acetylated histones. Here we show that, although this mutation reduces BRD4-occupancy at enhancers it does not affect transcription of the pluripotency network in mouse embryonic stem cells. Rather, it delays the cell cycle, increases DNA damage signalling, and perturbs regulation of DNA repair in mutant cells. This uncovers a role for BRD4 in DNA repair pathway choice. Furthermore, we find evidence of a similar increase in DNA damage signalling in cells derived from NIPBL-deficient individuals, suggesting that defective DNA damage signalling and repair is also a feature of typical Cornelia de Lange syndrome.
Cornelia de Lange syndrome (CdLS) is a clinically distinctive neurodevelopmental disorder (OMIM:122470). Disease severity varies greatly and patients can suffer from a range of symptoms including: a characteristic facial appearance, upper limb abnormalities, intellectual disability and delayed growth. CdLS is described as a 'cohesinopathy'—most cases can be attributed to heterozygous loss of function mutation in NIPBL encoding a protein involved in loading of the cohesin complex onto chromatin. Mutation in genes encoding cohesin complex proteins SMC1, SMC3 and RAD21, or HDAC8 (SMC8 deacteylase), have also been identified in CdLS-like probands. However cells from CdLS patients have no obvious defects in sister chromatid cohesion, and individuals with mutations in SMC1, SMC3 and RAD21 are often considered ‘atypical’ in terms of facial appearance and growth, and are less likely to have limb defects than those with NIPBL mutations.

Dysregulated gene expression has been proposed to be the main mechanism underlying CDLS. Mutations in genes encoding chromatin regulators unrelated to cohesin, such as ANKRD11, KMT2A, AFF4 and the bromodomains and extra-terminal domain (BET) protein BRD4, have been reported to cause CdLS-like phenotypes suggesting that chromatin dysregulation may play a role in CdLS as well. Additionally, increased sensitivity to DNA damage has been reported in CdLS patient cells, but the mechanism underlying this defect is unknown and its participation in the disease aetiology remains unclear.

Recently, we described de novo deletion and missense mutations in BRD4 associated with a clinical phenotype overlapping CdLS. BRD4 binds acetylated lysine residues in histones H3 and H4 through its two N-terminal bromodomains (BD). BRD4 localises to promoters and enhancers of active genes and is particularly enriched at super enhancers (SEs). BRD4 is a key regulator of transcription; through its C-terminal domain it recruits positive transcription elongation factor (P-TEFb) and the Mediator complex to promoters and enhancers, whilst its extra-terminal domain confers transcriptional activation through the recruitment of CHD4, JMJD6 and NSD3.

The CdLS-associated BRD4 missense mutation is in the second bromodomain (BD2) (NM_058243.2:c.1289A > G, p.(Tyr430C), termed here as Y430C (Fig. 1a), and results in decreased binding to acetylated histones. To gain further insights into the mechanisms underlying CdLS, and the role of BRD4, we investigated the phenotype of mouse embryonic stem cells (mESCs) homozygous for the orthologous amino acid substitution in mouse Brd4 (actually p.Tyr431Cys but for simplicity here termed Brd4Y430C). Here we show that the decreased affinity for acetylated lysines results in diminished occupancy of BRD4Y430C at cis regulatory elements (CREs) across the genome, including the super-enhancers of pluripotency genes. However, we find no evidence of altered transcription of the pluripotency network in these cells. Instead, we report increased and more persistent DNA damage signalling and cell cycle checkpoint activation in Brd4Y430C mESCs. We show increased persistent foci of the DNA damage response (DDR) protein 53BP1 upon double-strand break (DSB) induction in Brd4Y430C mutant cells. 53BP1 is a key factor in the regulation of DNA repair pathway choice that inhibits repair by homologous recombination (HR). We also show increased foci of the downstream effectors of 53BP1, Rif1 and the Mad2L2 (Rev7) subunit of the shieldin complex in the mutant cells and decreased recruitment of RAD51, suggesting impaired HR repair. Further, we show that cells from CdLS patients harbouring mutations in NIPBL have a similar DDR phenotype, indicating there may be a previously under-appreciated role for the DNA damage response in the aetiology of CdLS.

Results

Reduced occupancy of Y430C-BRD4 at cis-regulatory elements. Our previous work suggested that the Y430C mutation abrogates BRD4 binding to acetylated histones in vitro and in vivo. To determine the genome-wide effect of this reduced affinity we carried out Brd4 ChIP-seq in two independently generated mESCs lines engineered by CRISPR-Cas9 to carry the Y430C mutation on both alleles of Brd4. Immunoblotting showed that the Y430C mutant BRD4 protein was present at levels equivalent to that of Brd4 in wild-type cells (Supplementary Fig. 1a) and was efficiently immunoprecipitated by anti-Brd4 antibodies (Supplementary Fig. 1b). As expected, BRD4 was enriched over CREs (SEs, typical enhancers and promoters) in both wild-type (WT) and Y430C cells (Fig. 1b, Supplementary Fig. 1c). However, consistent with a lowered affinity for acetyl-lysines, there was a general decrease in BRD4 occupancy in both clones of Y430C cells, most striking at enhancers and super-enhancers (SE) (Fig. 1c, d, Supplementary Fig. 1c–d). In mESCs, BRD4 binding to SEs regulates the transcription of stem cell identity genes. As BRD4Y430C occupancy is decreased at the SEs of a number of stem cell identity genes, this suggests that there might be decreased transcription of these genes in mutant cells.

Decreased occupancy of mutant BRD4 at CREs does not have major effects on transcription in mESCs. The use of inhibitors that competitively bind the acetyl-lysine binding pockets of BET proteins has shown that loss of BRD4 binding disrupts the expression of target genes, especially genes regulated by SEs. Consistent with this, we observed decreased expression of the SE associated genes Nanog, Myc, Klf4 and Oct4 in both WT and Y430C mESCs after treatment with JQ1 (Fig. 2a). However, we did not observe any decrease in levels of Klf4, Nanog and Oct4 mRNAs in Y430C compared to WT mESCs by RT-qPCR (Fig. 2b).

To determine whether mRNA stability was masking an effect on transcription per se, we performed 4-thiouridine sequencing (4SU-seq) to assay nascent transcription. Transcription was surprisingly similar between WT and Y430C mESCs (Pearson correlation coefficient = 0.98) (Fig. 2c, d and data from an independent Y430C in Supplementary Fig. 2a). In particular, decreased BRD4 binding at SEs did not lead to transcriptional changes at stem cell identity genes (Fig. 2c–e, replicate in Supplementary Fig. 2b), or of eRNAs at the SEs themselves (Fig. 2f, Supplementary Fig. 2c). Due to normalisation, these experiments could not rule out that transcription is not globally decreased in the mutant ESCs. We therefore performed a spike-in RNA-seq experiment, using RNA from Drosophila cells for normalisation. Again, we did not observe any major transcriptional differences between WT and Y430C cells (Supplementary Fig. 2d, e). We conclude that the decreased occupancy of BRD4Y430C at CREs in mESCs is not sufficient to affect the transcription of associated genes.

This result is surprising, given BRD4’s well-documented roles in transcriptional regulation. Therefore, we also analysed gene expression in embryonic fibroblasts (MEFs) that had been derived from either wild-type or Brd4Y430C homozygous embryos. In contrast to homozygous null Brd4 embryos, which show significant growth retardation at E13.5 of embryonic development, Brd4Y430C homozygous embryos are reported to be morphologically indistinguishable from wild-type at this stage suggesting the absence of major developmental gene dysregulation. However, analysis of gene expression shows significant alteration of gene expression between wild-type and mutant MEFs (Supplementary Fig. 3). We consider that this difference in the transcriptional consequences of Brd4Y430C between ESCs and
MEFS may reflect the differing dependencies on epigenetic regulators at different stages of development.

**Y430C-BRD4 mESCs have a delayed cell cycle and increased cell cycle checkpoint activation.** We noted that BRD4<sup>Y430C</sup> mESCs grew slower and showed an accumulation of cells in G2/M (33.7%), compared to their WT counterparts (27.8%) (Fig. 3a, b, Supplementary Fig. 4a and Supplementary Fig. 9). This observation, together with the recently reported roles for BRD4 in the DDR and DNA repair<sup>23–26</sup> led us to investigate potential DDR defects in mutant cells.

The DDR allows coordination between DNA repair and cell cycle progression. Recognition of DNA damage by sensor proteins initiates a cascade that results in the phosphorylation and activation of the checkpoint kinases CHK1 and CHK2, delaying or blocking cell cycle progression<sup>27</sup>. CHK1 is the main kinase required for delay at G2/M<sup>27</sup>. To determine whether the altered cell cycle in BRD4<sup>Y430C</sup> cells is associated with increased activation of the G2/M checkpoint, we analysed CHK1 phosphorylation (CHK1-P) after treatment with neocarzinostatin (NCS), a radiomimetic drug which induces mainly DSBs. CHK1-P is increased in both WT and Y430C mESCs cell lines 1 h post NCS treatment, which is resolved by 16 h. However, the levels of CHK1-P are higher in BRD4<sup>Y430C</sup> mESCs (Fig. 3c, Supplementary Fig. 4b), suggesting an increased checkpoint activation. These results suggest a defect in DNA repair or signalling caused by BRD4<sup>Y430C</sup>. BRD4 has been shown to be directly involved in DNA repair through the transcriptional regulation of DNA repair proteins<sup>24,25,28</sup>. However, 4SU-seq showed that transcription of genes encoding DNA repair proteins was unaffected in BRD4<sup>Y430C</sup> mESCs (Supplementary Fig. 4c, d) and immunoblotting showed that overall levels of 53BP1 and Rad51 were not significantly altered in the mutant cells (Supplementary Fig. 4e).

**Y430C-BRD4 mESCs have increased DDR signalling.** Although not being directly recruited to DSBs<sup>29</sup> (Supplementary Fig. 5a), BRD4 restricts the DDR and depletion of BRD4 isoform B leads to

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**Fig. 1 Decreased binding of BRD4 at CREs in Y430C mESCs.**

- **a** Cartoon of BRD4 showing location of the Y430C mutation in the second bromodomain (BD2).
- **b** Heatmaps show enrichment of wild-type (WT) and Y430C BRD4 ChIP over super enhancers (SE), typical enhancers, promoters and gene bodies.
- **c** UCSC genome browser screenshot showing reads per 10 million over the Klf4, extended Nanog and Sox2 loci for BRD4 ChIP-seq in WT and Y430C mESCs. Extent of SEs are shown in blue. Below are shown previously published ChIP-seq data for H3K27ac (ENCSR000CDE), H3K9ac (ENCSR000CGS), H3K122ac (GSE66023) and DNase I hypersensitivity (DHS). Genome co-ordinates (Mb) are from the mm9 assembly of the mouse genome. Biological replicate from an independent Y430C clone are in Supplementary Fig. 1.
- **d** ChIP-qPCR measuring concentration of BRD4 ChIP DNA relative to input across the SEs of Oct4, Klf4, Nanog, and Sox2; in WT and Y430C mESCs. Data are represented as mean ± SEM from 3 technical replicates.
increased DDR signalling\textsuperscript{23}. We therefore tested whether BRD4\textsuperscript{Y430C} affects DNA damage signalling. mESCs have constitutively high levels of γH2AX, even in the absence of a DNA damaging stimulus\textsuperscript{30}. We therefore used 53BP1 as a marker of DDR. 53BP1 is recruited to DSBs, spreads to form microscopically visible foci and acts as a scaffold for the recruitment of further DSB response proteins, to regulate the choice of DNA repair pathway and to promote cell cycle checkpoint signalling\textsuperscript{31}. Immunoﬂuorescence showed formation of multiple 53BP1 foci, representing DNA damage sites, upon DSB induction (1 h
after NCS treatment). These foci are only present at low levels prior to NCS treatment and decrease in number at 16 and 20 h post treatment, as cells repair the damage (Fig. 4a). Supporting the hypothesis that the Y430C mutation impairs the role of BRD4 in DDR restriction, we observed that 53BP1 foci are larger in BRD4Y430C mESCs than in WT (Fig. 4a, b). In addition, whilst the number of 53BP1 foci in WT cells returns to pre-treatment levels at 16 and 20 h time points, the number of 53BP1 foci in BRD4Y430C cells remains higher (Fig. 4a, c, Supplementary Fig. 5b, c), suggesting that DNA repair itself could be impaired.

During our analysis of transcription, we found potential evidence for aneuploidy for chromosome 11 in BRD4Y430C cells. Concerned that the DNA repair defects we observed in BRD4Y430C cells were caused by aneuploidy rather than the BRD4 mutation, we repeated the 53BP1 staining using WT and BRD4Y430C mESCs that had been confirmed to have a diploid karyotype. This analysis confirmed that the increased number and size of 53BP1 foci after NCS treatment is specific to cells carrying the BRD4Y430C mutation, and not an additional chromosome 11 (Supplementary Fig. 6).

Given the accumulation of BRD4Y430C cells in S/G2 (Fig. 3), one possible origin of 53BP1 foci is the formation of Oct1/PTF transcription (OPT) domains arising as a consequence of problems encountered in S-phase and propagated through mitosis into the subsequent G1. Indeed inhibition of BRD4 by BET inhibitors has been reported to induce replication stress. However, immunofluorescence showed that 53BP1 foci present in BRD4Y430C cells, either with or without NCS treatment, do not co-localise with OPT domains marked by Oct1 (Supplementary Fig. 7).

Defective DSB repair in Y430C-BRD4 cells. For the most part, DSBs are repaired by either non-homologous end-joining (NHEJ) or HR. Use of the appropriate pathway is important for faithful repair and is determined by antagonistic recruitment of 53BP1 and BRCA1. 53BP1 inhibits DSB end resection, the initial step of HR, thereby promoting NHEJ and inhibiting HR. Downstream effectors of 53BP1 in the regulation of resection include RIF1 and the recently identified shieldin complex (SHLD1, SHLD2, SHLD3 and MAD2L2). If timely repair does not occur by NHEJ, BRCA1 promotes the release of RIF1, leading to end-resection and HR.

As BRD4Y430C mESCs show increased numbers and size of 53BP1 foci compared to WT cells, we reasoned that there may also be increased recruitment of the downstream effectors of 53BP1 such as RIF1 and MAD2L2. Indeed, we observed an increased number of RIF1 (Fig. 5a, b, Supplementary Fig. 5d, e) and MAD2L2 (Fig. 5c, d, Supplementary Fig. 5f, h) foci in BRD4Y430C compared to WT cells at all time-points, similar to 53BP1. Conversely, we observed a significant decrease in the number of foci of RAD51, a protein necessary for HR repair, in mutant cells at 1 h post NCS (Fig. 6a, b, Supplementary Fig. 5h, i), suggesting a repression of HR. Given our cell cycle analysis (Fig. 3b) this cannot be simply explained by fewer cells in S/G2. Given the role of the shieldin complex in protecting DSB
end-resection, we propose that the Y430C BRD4 mutation leads to an altered balance between NHEJ and HR, consistent with the synthetic lethality observed between BRD4 and PARP inhibitors.

Increased number and size of 53BP1 foci in NIPBL mutant lymphoblastoid cell lines. To see if the DDR defect that we have observed in the presence of the BRD4 Y430C would also be apparent in cells carrying other CdLS mutations, we utilised two lymphoblastoid cell lines (LCL) previously derived from CdLS patients with heterozygous mutations in NIPBL, Ile1206del and Arg2298His. These LCLs have significantly more, and larger, 53BP1 foci per cell compared to a WT LCL, in the absence of any exogenous damage (Fig. 6c–e, Supplementary Fig. 8a, b). This suggests that increased DDR signalling and/or impaired DNA repair pathway choice balance may be a common mechanism underlying CdLS caused by BRD4 and NIPBL.

Discussion
We previously showed that a Y430C-BRD4 mutation, and BRD4 haploinsufficiency, cause a CdLS-like syndrome. The severe developmental phenotypes associated with CdLS are generally thought to be due to aberrant gene regulation. Here, however, we show that BRD4 Y430C, whilst lowering the affinity of BRD4 to acetylated lysine residues and decreasing its occupancy at enhancers and SEs, causes minor changes in transcription in
mESCs, in contrast to the major transcriptional changes caused by the profound loss of BRD4 binding induced by BET inhibitors. Instead, we provide evidence that the BRD4Y430C hypomorph causes increased G2/M checkpoint activation, aberrant DDR signalling and an altered focal accumulation of proteins that promote NHEJ and inhibit HR—53BP1 and the shieldin complex. Conversely there is a depletion of foci containing HR proteins (Rad51), suggesting a defect in HR. Our results suggest a role for BRD4 in the regulation of DNA repair pathway choice and are consistent with recent results from an siRNA screen in human

**Fig. 5 Increased RIF1 and MAD2L2 foci after DSB induction in Y430C mESCs.** a Representative images of wild-type and Y430C mESCs upon RIF1 immunofluorescence and DAPI staining after treatment with NCS. Scale bars: 2 μm. The experiment was repeated three times with similar results. b Box-plot shows number of RIF1 foci per cell, respectively, in WT and Y430C cells after treatment with NCS in one representative experiment. Horizontal lines within boxes show medians, boxes are inter-quartile ranges and whiskers are range. P-values were calculated with Mann–Whitney one-sided U test. *<0.05, **<0.01, ***<0.001. 50 cells were examined per condition. c Representative images of wild-type and Y430C mESCs upon MAD2L2 immunofluorescence and DAPI staining after treatment with NCS. Scale bars: 2 μm. The experiment was repeated three times with similar results. d Box-plot shows number of MAD2L2 foci per cell, respectively, in WT and Y430C cells after treatment with NCS in one representative experiment. Horizontal lines within boxes show medians, boxes are inter-quartile ranges and whiskers are range. P-values were calculated with Mann–Whitney one-sided U test. *<0.05, **<0.01, ***<0.001. 50 cells were examined per condition.
cell lines that suggest that BRD4 deficiency leads to less repair by HR and more by NHEJ38. Whether BRD4 mutation affects repair by HR at specific regions in the genome, or globally, remains to be investigated. For example, different levels of histone acetylation in different chromatin environments—e.g. heterochromatin vs euchromatin—upon DNA damage may recruit different amounts of BRD439,40. Alternatively, as well as interacting with NIPBL, Brd4 has been reported to interact with a number of proteins involved in DNA damage sensing and repair, including Rif1 of the Sheildin complex.8,41 We cannot exclude that impaired BRD4 function in BRD4Y430C cells affects the formation of DSBs themselves, for example by preventing the accumulation of R-loops23,38,42.

We observed a similar focal accumulation of 53BP1 in cells from CdLS patients with mutations in NIPBL. Could aberrant DDR and DNA repair choice, or perhaps elevated DNA damage

Fig. 6 Evidence for DNA repair defects in CdLS. Representative images of wild-type and Y430C mESCs upon RAD51 immunofluorescence and DAPI staining after treatment with NCS. Scale bar = 5 μm. The experiment was repeated three times with similar results. b Box-plot shows number of RAD51 foci per cell, respectively, in WT and Y430C cells after treatment with NCS in one representative experiment. Horizontal lines within boxes show medians, boxes are inter-quartile ranges and whiskers are range. P-values were calculated with Mann-Whitney one-sided U test. *<0.05, **<0.01, ***<0.001. 50 cells were examined per condition. c Representative images of wild-type, R2298 and I1206del LCLs upon 53BP1 and DAPI immunofluorescence. Scale bar = 5 μm. The experiment was repeated three times with similar results. d, e Box-plots show number of 53BP1 foci per cell and area of 53BP1 foci (μm²), respectively, in WT, R2298H and I1206del LCLs in one representative experiment. Horizontal lines within boxes show medians, boxes are inter-quartile ranges and whiskers are range. P-values were calculated with Mann-Whitney one-sided U test. *<0.05, **<0.01, ***<0.001. 50 cells were examined per condition.
per se, therefore account for some of the phenotypes associated with CdLS. Congenital mutation in many different genes involved in cell cycle progression and DNA repair, are—like CdLS—generally associated with intraterine growth retardation and short stature\(^5\). Similarly, microcephaly also results from mutation in genes associated with S phase progression (ATR, ATRIP, ChIP—Seckel syndrome; DNA ligase IV—lig4 syndrome; XRC4—microcephalic primordial dwarfism\(^44\),\(^45\)). Clinically, there is strongest phenotypic overlap between CdLS and Rubinstein–Taybi syndromes (RTS)—including arched eyebrows and other shared distinctive facial features. RTS is caused by mutations in \(p300\) or CREBBP. These lysine acetyltransferases are also both involved in DNA damage signalling and repair\(^48\) and DNA damage sensitivity\(^49\). Even though we cannot discount that BRD4 mutation in CdLS cases—Y430C, or heterogeneous deletions, cause aberrant transcriptional regulation in cell types other than ESCs, our results suggest that dysregulation of DDR and repair may contribute to the aetiology of CdLS.

**Methods**

Cell culture. Y430C-BRD4 mutant and corresponding wild-type mouse embryonic stem cells (mESCs) were generated by CRISPR Cas9 genome editing in 46 C.\(^{36,37}\) mESCs were described previously\(^3\). NIPBL, I206N and R298H lymphoblastoid cell lines (LCLs) were obtained from patients’ mESC. LCLs were cultured in GEMM medium (GBICO; 11710035) supplemented with 10% Fetal Calf Serum (FCS), 5% penicillin-streptomycin, 1 mM sodium pyruvate (GBICO; 11360007), 1X non-essential amino acids (GBICO; 11440050), 50 mM 2-mercaptopethanol (GBICO; 31350010), 2 mM L-glutamine and 500U/mL Leukemia Inhibitory Factor (in-house). Lymphoblastoid cell lines (LCLs) were grown in RPMI 1640 medium (GBICO; 11875093) supplemented with 15% FCS and 2 mM L-glutamine. All cells were grown at 37°C in a 5% CO\(_2\) humidified atmosphere.

ChIP-qPCR. Cells were harvested by trypsinising and fixed with 1% formaldehyde (Thermo Fisher; 28906) in media (25°C, 10 min). This reaction was quenched with 0.125 M glycine for 5 min. ChIP-qPCR was performed as described previously\(^8\) (see Table\(1\) for antibodies). DNA was purified using the QiAquick PCR Purification Kit (Qiagen; 28104). Input samples were diluted to 1%, and all samples diluted a further 10-fold, in ddH\(_2\)O. SYBR-green based qPCR reactions were performed in a final volume of 20 µl containing diluted ChIP DNA, SYBR select master mix (ThermoFisher Scientific; 4472908) and 0.25 µM/L of each primer (see Table\(1\)). Concentration of IPs are relative to 1% input.

ChIP-seq. ChIP was carried out as above. After purification, DNA was eluted in 20 µl and libraries were prepared for ChIP and input samples as previously described\(^9\). Samples were sequenced at BGI (Hong Kong; 50-bp single-end reads) using the HiSeq 4000 system (Illumina). Fastq files were quality controlled using FastQC and mapped to the mm9 genome using Bowtie2 (parameters: default). Sam files were converted to bam files and sorted using SamTools. Homer was used to make tagdirectories (makeTagDirectory, parameters: -unique -sspe -unique, fragLength 150) and these were used to plot heatmaps (plotHeatmap, parameters: -colormap RdBuRyl reverse).

RT-PCR. RNA was extracted from cells using the RNeasy Mini Kit (Qiagen; 74104) using spin technology, with an additional on-column DNA digestion using the RNAse Free DNase Set (Qiagen; 79254). cDNA was synthesised from 1 µg RNA using SuperScript II Reverse Transcriptase (ThermoFisher Scientific; 18064-014) as per manufacturer’s instructions. cDNA was diluted 1 in 25 for qPCR analysis. SYBR green-based qPCR reactions were performed in a final volume of 20 µl containing diluted cDNA, SYBR select master mix (ThermoFisher Scientific; 4472908) and 0.5 µM of region specific intron-spanning primer pairs.

4S-Useq. 4S-U RNA was generated and isolated as described previously\(^9\), with the following changes: cells were incubated at 37°C with 4S-U-supplemented medium for 20 min. The reaction was incubated with Biotin-HPPD with rotation for 1.5 h at RT. For recovery of biotinylated 4S-U RNA, 1 µl of streptavidin beads was added per µg of RNA. Columns were washed using 900 µl washing buffer and RNA was eluted using 100 µl Elution Buffer (Invitrogen; 15506052) for 10 min. RNA fragmentation was then carried out for 90 min at 50°C and eluates combined. RNA was further purified using the RNAeasy MinElute Clean-up kit (Qiagen; 74204) according to the manufacturer’s guidelines, eluting in 20 µl water. 1 µl of 4S-U-labelled RNA was quality-checked by running on a 2100 Bioanalyzer Instrument (Agilent).

Growth assay. WT and Y430C mESCs were each seeded in 4 wells of a 6 well plate. 2 million mESCs were seeded at a density of 2 × 10\(^5\) cells/ml and seeded at a density of 4 × 10\(^5\) cells/ml. Cells were grown at 28°C in a 5% CO\(_2\) humidified atmosphere. Cells were frozen at a density of 1 × 10\(^5\) cells/ml in 45% conditioned Schneider’s Drosophila Medium media (containing 10% FCS), 45% fresh Schneider’s Drosophila Medium supplemented with 10% FCS, and 10% DMSO, and stored in liquid nitrogen.

RT-PCR: Stability and Recovery. Cells and s2 cells were heat-shocked and counted. 0.2 million s2 cells were mixed with 10 million mESCs, and RNA was extracted using the RNeasy Mini Kit (Qiagen; 74104) using spin technology, with an additional on-column DNA digestion using the RNAse-Free DNase Set (Qiagen; 79254). RNA was depleted of rRNA and RNA-seq libraries prepared as for the 4S-U-seq.

Growth assay. WT and Y430C mESCs were each seeded in 4 wells of a 6 well plate (1 × 10\(^5\) cells/well). WT and Y430C cells from 1 well were trypsinised and counted at 24, 48, 72 and 96 h post seeding. Counting was carried out manually using a haemocytometer. The addition of trypan blue dye allowed for the exclusion of dead cells.

Weight assay. 2 million mESCs were fixed in 70% ethanol (in PBS) at 4°C for 1 h. Fixed cells were centrifuged at 3000 g at 4°C for 5 min, washed twice with PBS and resuspended in 500 µl PBS. 20 µg RNAase A was added and cells were incubated at 37°C for 10 min. Cells were stained with propium iodide at a final concentration of 50 µg/ml. Acquisition was carried out on a BD LSFortessa cell analyser, collecting 25,000 events per sample. Results were analysed using BD FACSDiviva 8.0.1 and gated cells were manually categorised into cell cycle stages G0/G1, S and G2/M.

NCS treatment and CHK1 inhibitor protein Western blots. Cells were incubated with mESC media supplemented with necarozistatin (Sigma; N9162) (NCS), to a final concentration of 25 ng/ml, for 15 min at 37°C. Cells were then washed with PBS and fresh, non-supplemented media was added. Protein was either extracted directly from the media or for incubations at 37°C for 4 h after incubation. cDNA was synthesised from 1 µg RNA using SuperScript II Reverse Transcriptase (ThermoFisher Scientific; 18064-014) as per manufacturer’s instructions. cDNA was diluted 1 in 25 for qPCR analysis. qPCR reactions were performed as above (see Table\(1\) for primers). Concentration of Q1 + cDNA was calculated relative to Q1- (arbitrarily set to 1).
and resuspended in PBS to $1.8 \times 10^5$ cells/ml. 500 µl of cell suspension was added to gelatinised coverslips and LCLs were grown in suspension. LCLs were harvested and blocked in 1% BSA in PBS for 30 min at RT, incubated with primary antibody overnight and washed 3 × 3 min in PBS. Cells were next incubated with secondary antibody (see Table 1) diluted in 1% BSA for 45 min at RT, washed 3 × 3 min in PBS, incubated with DAPI in PBS (250 ng/ml) for 2 min, and washed 3 × 3 min in PBS. Cover slips were mounted on slides in Vectashield (Vector; H1000) mounting medium for fluorescence imaging. Images were taken using confocal microscopy.

**Deposited Data**

| Deposited Data | Source | Identifier |
|---------------|--------|------------|
| BRD4-WT ChiP-seq | This paper | GSE130659 |
| BRD4-Y430C ChiP-seq | This paper | GSE130659 |
| WT 4sU-seq | This paper | GSE130659 |
| Y430C 4sU-seq | This paper | GSE130659 |
| WT Spike-in RNAseq | This paper | GSE130659 |
| Y430C Spike-in RNAseq | This paper | GSE130659 |

**Table 1 Key resources.**

| Antibodies | Source | Identifier |
|-----------|--------|------------|
| BRD4 | Bethyl | Cat# A301-985A-M |
| 53BP1 | Novus | Cat# NB100-304 |
| Normal Rabbit IgG | Santa Cruz | Cat# sc-2025 |
| CHK1 | Abcam | Cat# ab47574 |
| CHK1-p | Cell signalling technologies | Cat# 2348 |
| Lamin B | Santa Cruz | Cat# sc-374015 |
| MAD2L2 | Abcam | Cat# ab180579 |
| RIF1 | A kind gift from Sara Buonomo | Rabbit anti-mouse Rif1 serum 1240 |
| RAD51 for IF | Calbiochem | Cat# PC130 |
| RAD51 for WB | Merck-Millipore | Cat# 05-636 |
| γH2AX | Abcam | Cat# Ab1136 |
| Goat anti-Rabbit IgG, secondary, Alexa Fluor 488 | Invitrogen | Cat# A10134 |
| Donkey anti-Rabbit IgG, secondary, Alexa Fluor 586 | Invitrogen | Cat# A10042 |

**Primers**

| Primers | Forward | Reverse |
|---------|---------|---------|
| ChIP-qPCR | TAGAGGAAAGGAGCTGAGAGA | AAGGAAAAGAGAGGGAGG |
| Sox2 SE | CCAATGCGAGGTGGAACAA | TCCTCCCCAAAGTGTG |
| Kif4 SE | GTGAATGTTGTTGCTGAGG | GTGTCCTTCACCCACCTCAG |
| Nanog SE | CTTGCGTTCAGAGCAGTGTG | GAGCCCTACCTGAAACTCC |
| Oct4 SE | CTTGCGTTCAGAGCAGTGTG | GAGCCCTACCTGAAACTCC |
| Kif4 | GTGAGCTCTGAGAGCAGTAAAC | AGCCAGGTTGGAAAGAGATAAGT |
| Myc | CCTGCTGCGTGGAGTGGGAA | CTGTGCTGGTGAGT |
| Oct4 | CGAGAAGAGAAGAGGTGAA | CTTCTCTAAGAGAGTC |
| Nanog | TGAGTCTGGAGAGAGGTGAA | CAGGGCTGAGGAGAGGG |

**Software and algorithms**

|Bowtie2| Langmed and Salzberg, 2012 | [https://bowtie-bio.sourceforge.net/bowtie2/index.shtml](https://bowtie-bio.sourceforge.net/bowtie2/index.shtml) |
|MACS2| BD Bioscience | [http://cole-trapnell-lab.github.io/MACS](http://cole-trapnell-lab.github.io/MACS) |
|FACS/siva software| TopHat | [http://deepblue.readthedocs.io/en/latest/index.shtml](http://deepblue.readthedocs.io/en/latest/index.shtml) |
|Cufflinks| Trapnell et al., 2012 | [http://deepblue.readthedocs.io/en/latest/index.shtml](http://deepblue.readthedocs.io/en/latest/index.shtml) |
|DeepTools2| Ramírez et al., 2016 | [http://samtools.sourceforge.net](http://samtools.sourceforge.net) |

**ChIP-seq**

BRD4-Y430C 4sU-seq This paper GSE130659

WT 4sU-seq This paper GSE130659

BRD4-WT ChIP-seq This paper GSE130659

**Data Availability**

Data supporting the findings of this study have been deposited in GEO with accession number GSE130659. All data is available from the authors upon reasonable request. Source data are provided with this paper.
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Acknowledgements

We thank the individuals with CdLS and their families for generously donating their samples and genetic information and for consenting to their use in research studies. We thank Tom Strachan (Newcastle) and Matt Deardorff and Ian Krantz (Children’s Hospital of Philadelphia) for their gifts of CdLS LCRs. We thank the Institute of Genetics and Cancer (IGC) Advanced Imaging Resource for assistance with imaging and fIly
Flaimer for his help with deposition of data on GEO. We are indebted to Iain Williamson
and Shelagh Boyle (IGMM) for mESC karyotyping. We also thank the ImagoSeine core
facility (Institut Jacques Monod, France-BioImaging ANR-10-INBS-04) for confocal
microscopy and the imaging platform of the Epigenetics and Cell Fate Center for epi-
fluorescence microscopy for the micro-irradiation experiments. We thank Andrew
Jackson (IGMM, Edinburgh) for feedback on the manuscript. We thank Valérie Doye
(IJM, Paris) for advice during the revisions of the manuscript. G.O. was supported by a
PhD studentship from the Medical Research Council (MRC). M.M.P. is supported by the
UKRI/MRC grant (MR/T000783/1) and Barts charity (MGIU0475) grants. D.R.F is
supported by MRC University Unit grant (MC_UU_00007/3) and by the Simons
Initiative for the Developing Brain. W.A.B. is supported by MRC University Unit grant
(MC_UU_00007/2). C.B. was supported by a H2020 Marie-Curie Individual Fellowship
(655350-NPCRChr), a Bettencourt-Schueller foundation prize for young researchers, a
grant from the Fondation ARC (Projet Fondation ARC PJA 20191209584) and a grant
from the LIGUE contre le cancer île de France (RS20/75-53). S.P. and S.E.P. are sup-
ported by the French National Research Agency (ANR-18-CE12-0017-01). S.E.P. is an
EMBO Young Investigator.

Author contributions
W.A.B., M.M.P. and C.B. conceived and designed the experiments with input from D.R.
F. G.O. conducted most of the experiments with help from M.M.P. for ChIPseq and
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MAD2L2. G.R.G. provided bioinformatic analysis of expression data. S.E.P. and S.P.
performed the laser micro-irradiation experiment. G.O., W.A.B. and C.B. wrote the
manuscript with input from all authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material
available at https://doi.org/10.1038/s41467-021-23500-6.

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Peer review information Nature Communications thanks the anonymous reviewer(s) for
their contribution to the peer review of this work.

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