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SUPPLEMENTARY INFORMATION

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BRD4 interacts with NIPBL and *BRD4* is mutated in a Cornelia de Lange–like syndrome

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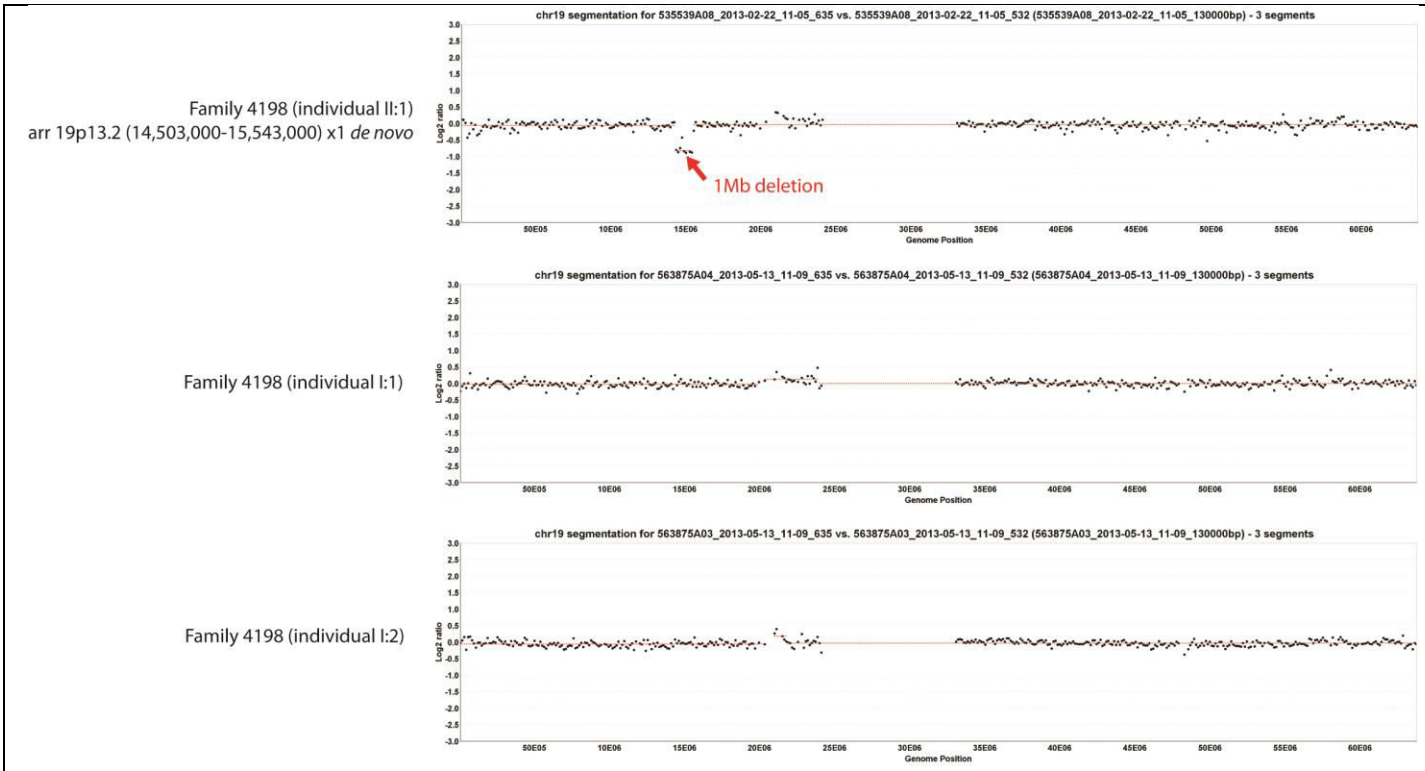
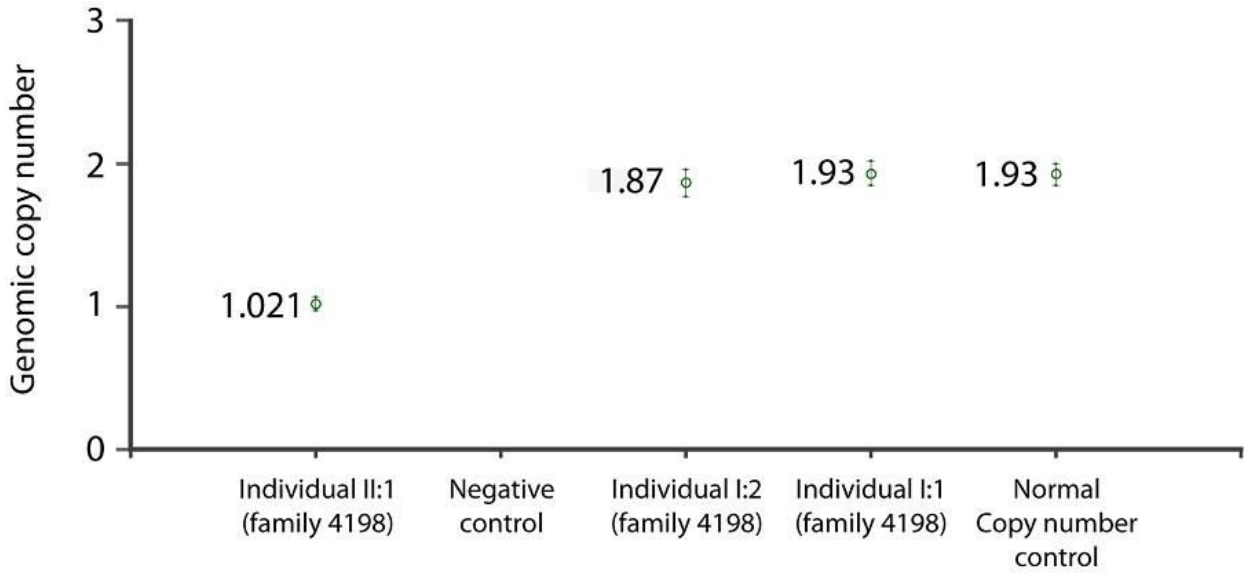


Supplementary Figure 1

A *de novo* heterozygous 1.04Mb deletion in individual II:1 (family 4198; DECIPHER 281165)

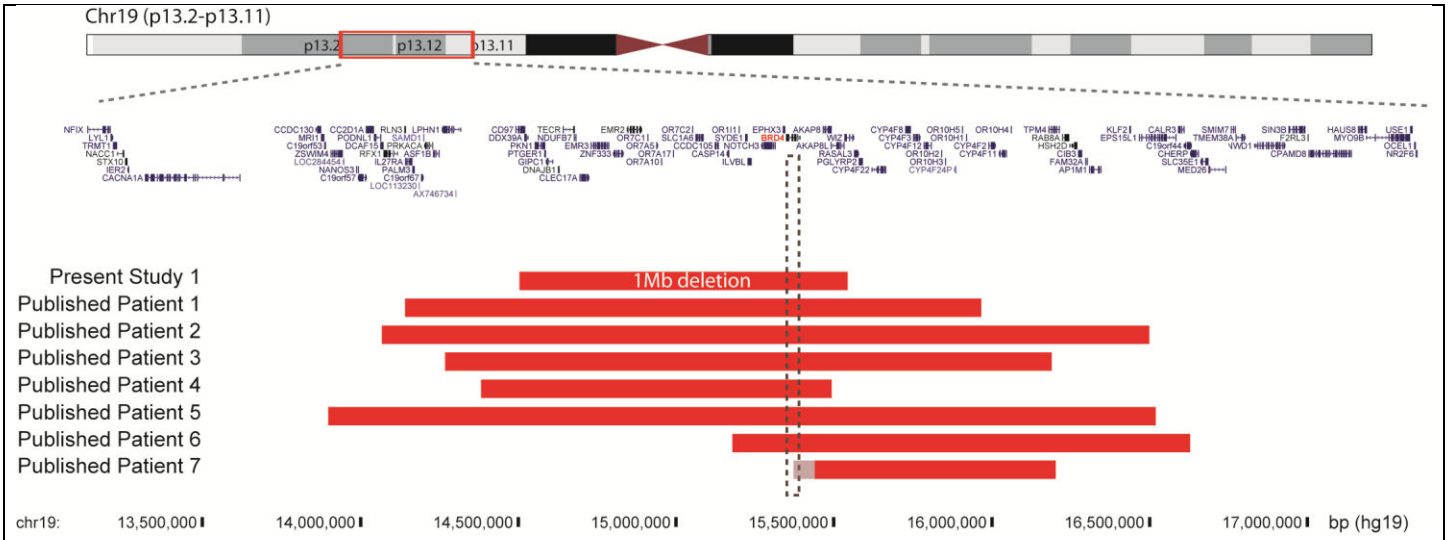
Array CGH identified a 1.04Mb deletion in individual II:1, which is absent in the individual's father (individual I:1) and her mother (individual I:2). The location of the deletion is shown by a red arrow on the log2 ratio plot of the array CGH.



Supplementary Figure 2

Analysis of genomic DNA copy number at the *BRD4* locus using droplet digital PCR (ddPCR)

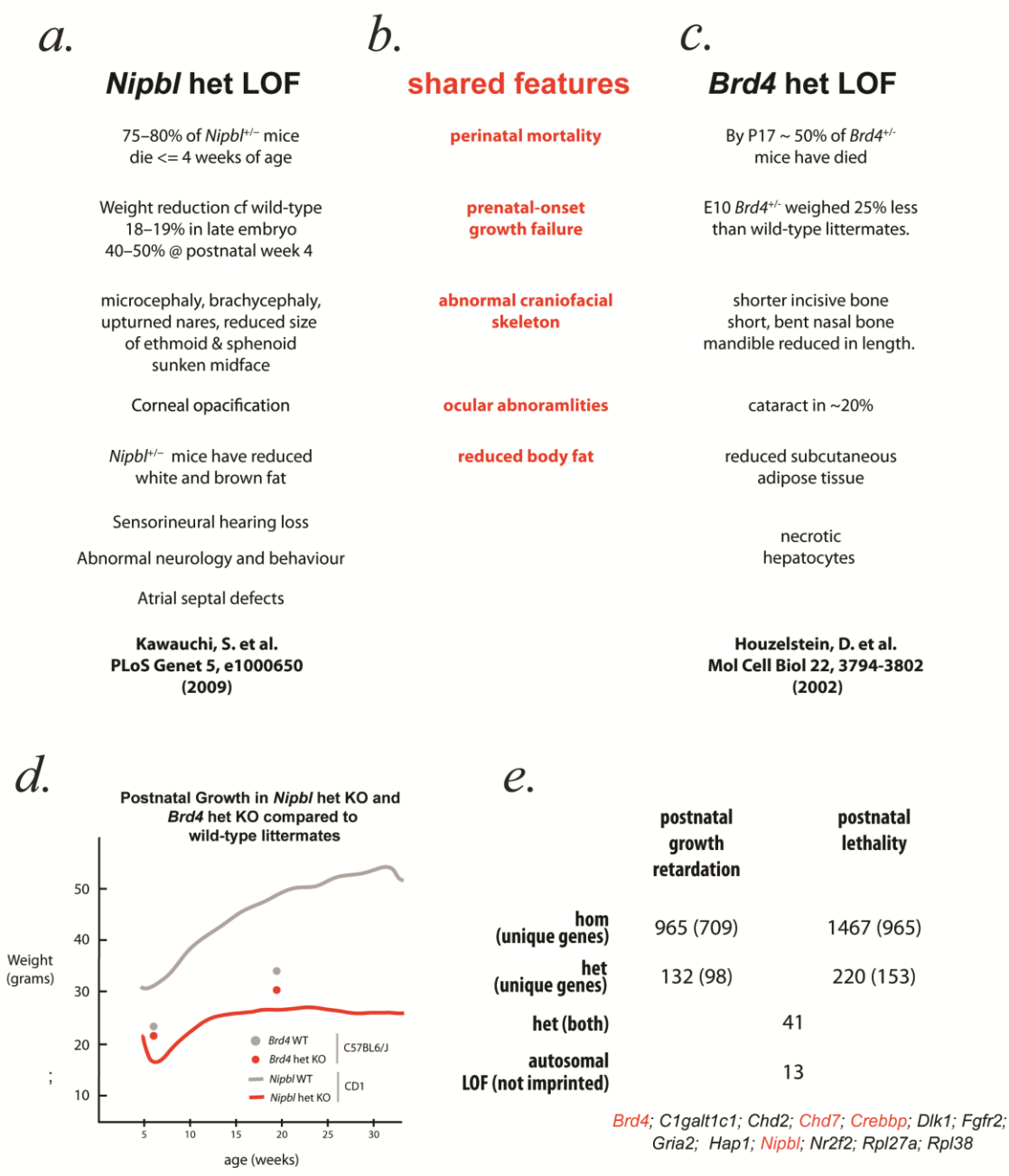
ddPCR was used to confirm the heterozygous deletion encompassing *BRD4* in individual II:1 (family 4198; DECIPHER 281165). Per-well Poisson error bars are presented for each sample.



Supplementary Figure 3

Summary of deletions reported in cases of *BRD4* haploinsufficiency

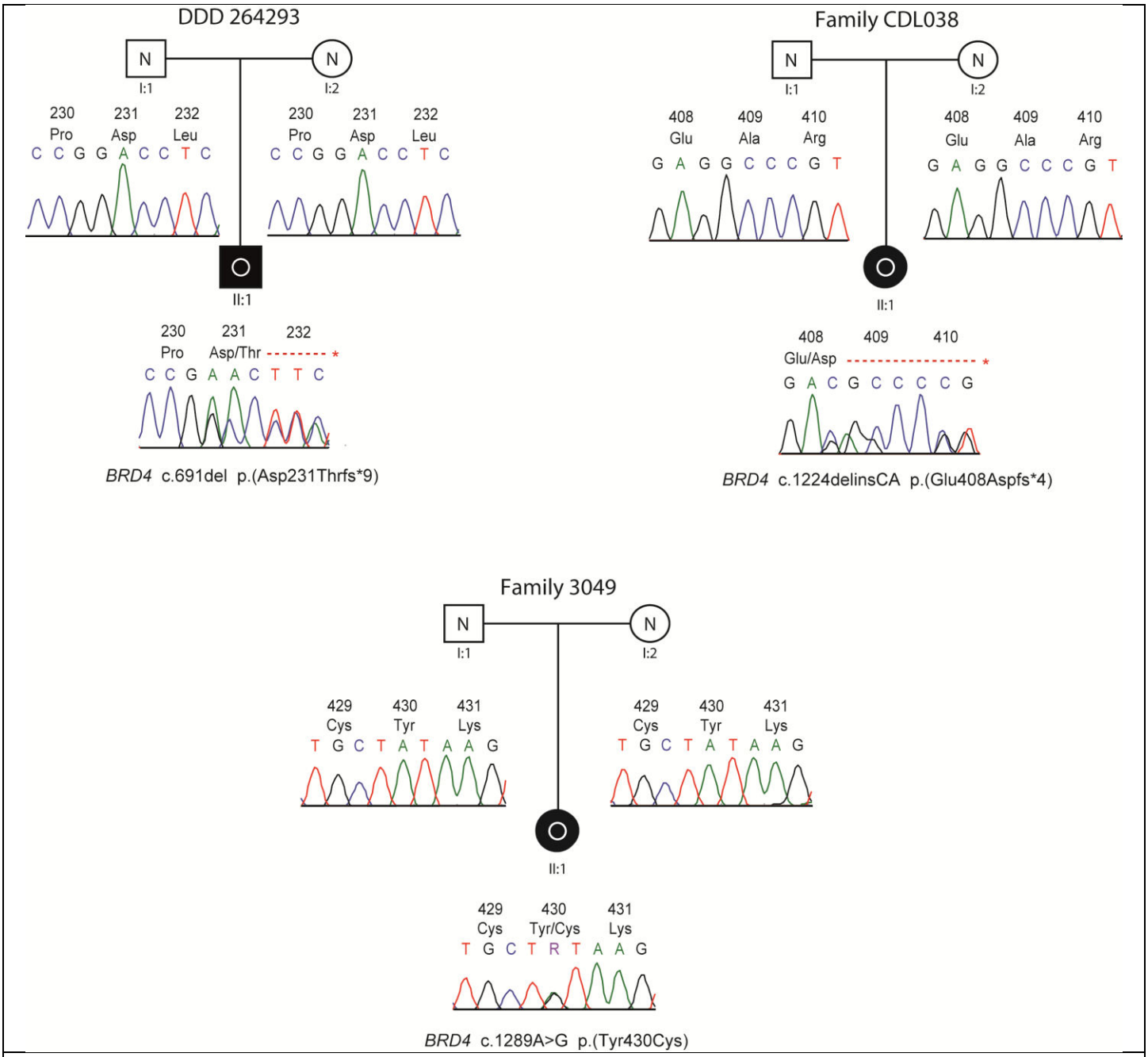
Locations of the deleted regions are shown as red blocks based on the human genome reference assembly hg19. Published Patient 1 (PMID: 17339581, 20648052), Published Patient 2 (PMID: 19215039), Published Patient 3 (PMID: 20648052), Published Patient 4 (PMID: 20570643), Published Patient 5 (PMID: 21994138), Published Patient 6 (PMID: 22750323), Published Patient 7 (PMID: 21815246)



Supplementary Figure 4

Comparison of the phenotype associated with haploinsufficiency for *Nipbl* and *Brd4* in previously reported mouse lines

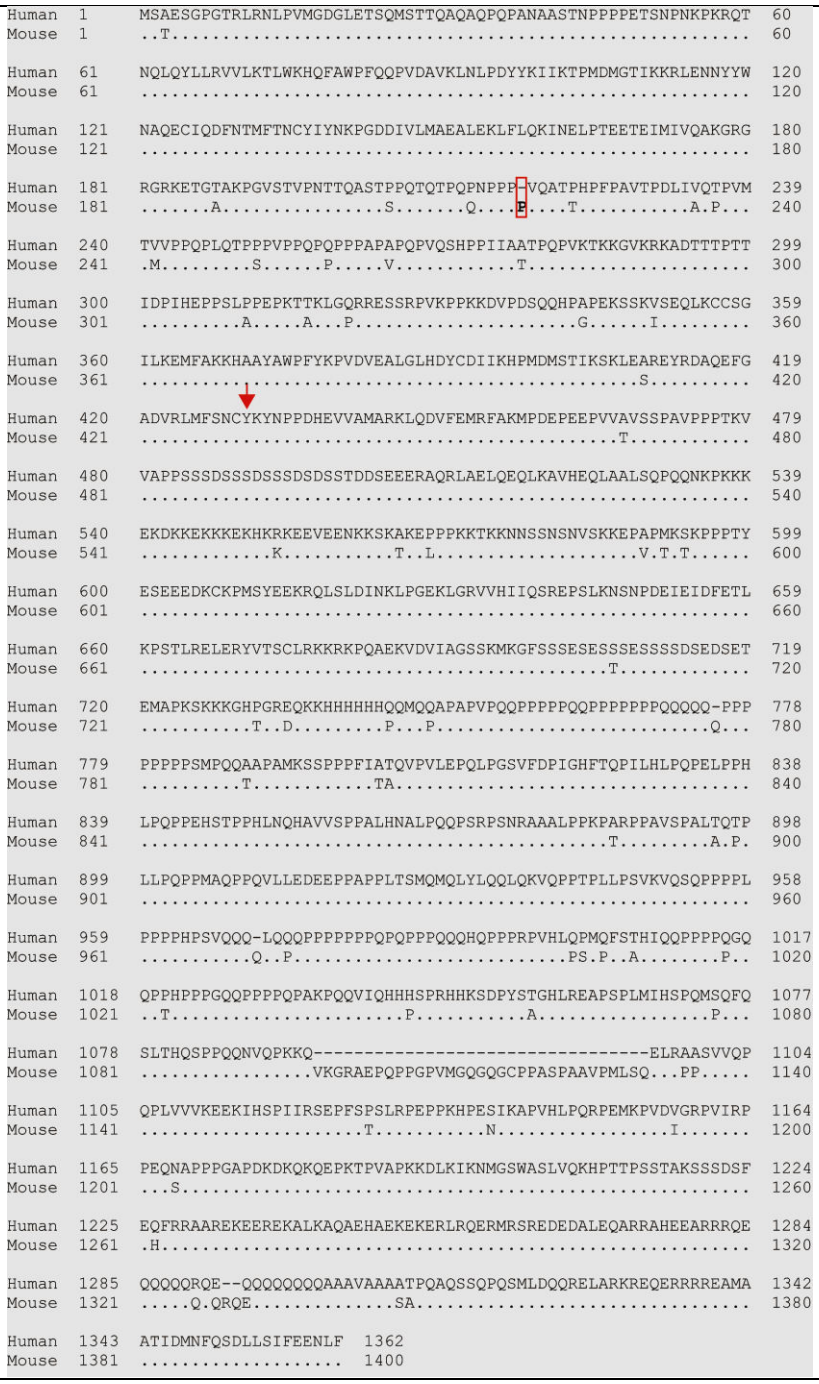
Mouse phenotype terms (a-c) and graphical representation (d) of growth of *Nipbl* (line) and *Brd4* (filled circles) heterozygous loss of function (het LOF) animals (red) with wild-type controls (grey). The shared phenotypic terms are indicated in red text. e. A review of all reported mouse mutant lines (Mouse Genome Informatics; http://www.informatics.jax.org) indicating the 13 het LOF lines sharing postnatal growth retardation and postnatal lethality as features.



Supplementary Figure 5

Pedigrees of individuals with *de novo* intragenic *BRD4* variants

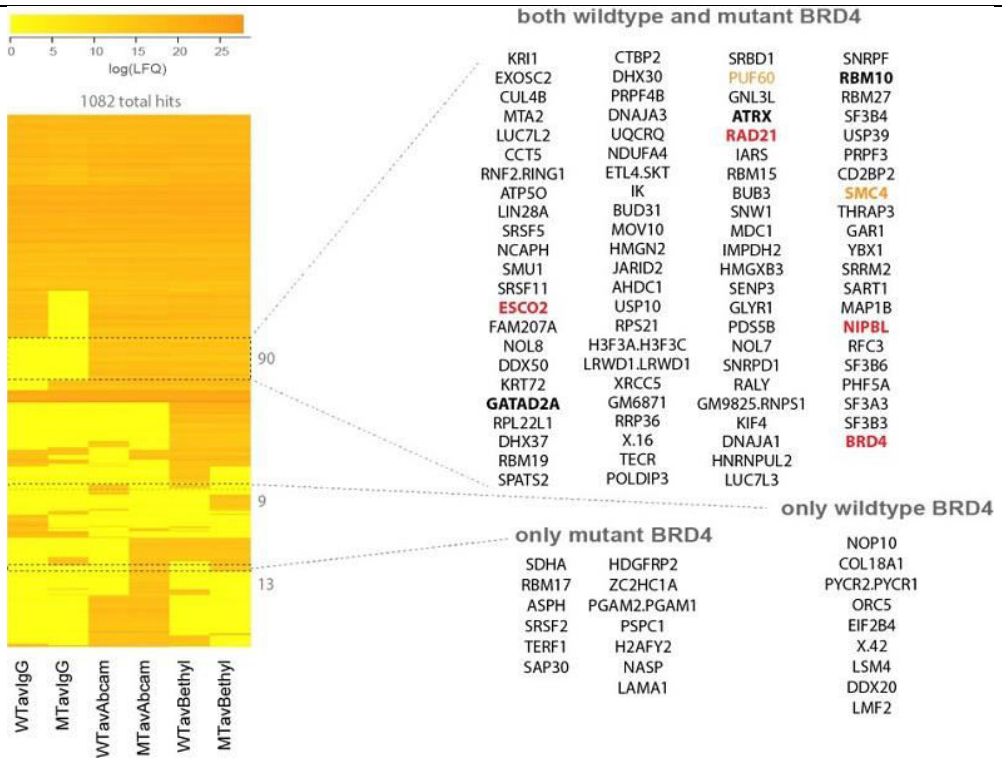
Three intragenic *BRD4* variants were validated by Sanger sequencing, which confirmed that they had occurred as *de novo* events in each of the affected individuals. Sequence accession number NC_000019.10 was used as reference. Variant nomenclature is based on *BRD4* transcript NM_058243.2.



Supplementary Figure 6

Protein sequence alignment of human versus mouse BRD4

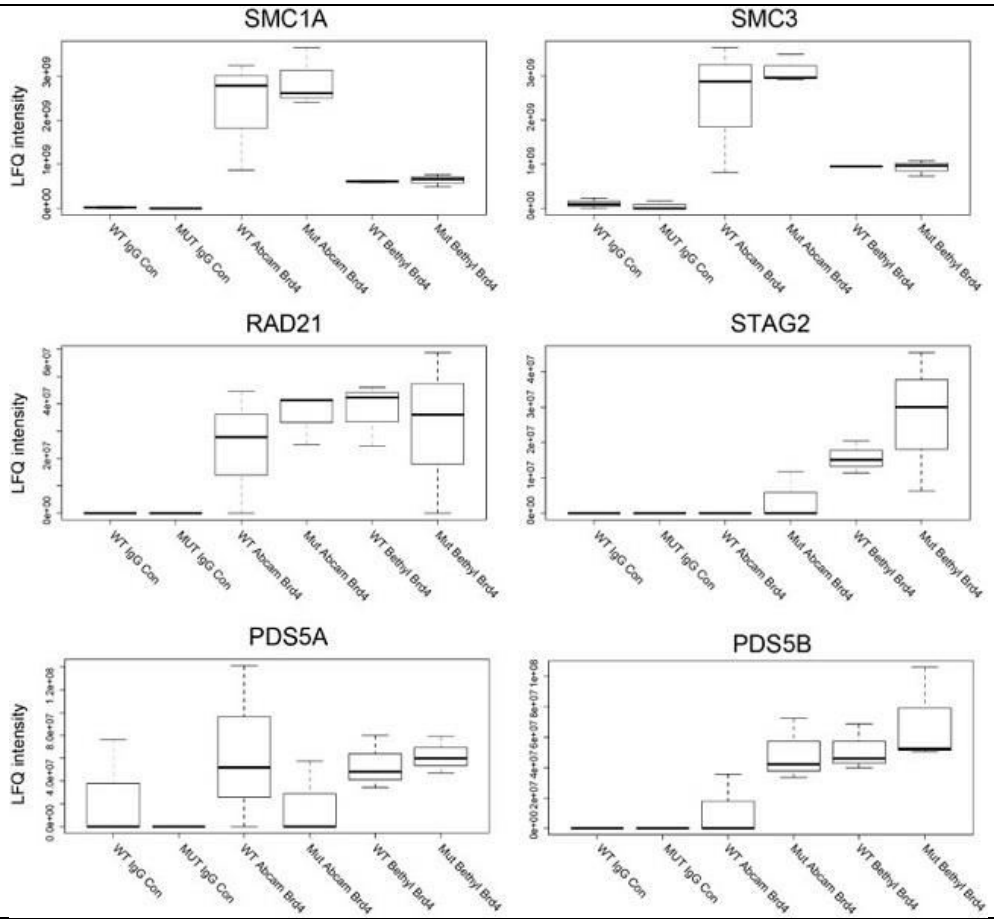
The position of the human p.Tyr430, equivalent to mouse p.Tyr431 is indicated by a red arrow. The presence of an N-terminus proline residue in the mouse sequence (p.Pro218) (red box) results in a shift of one amino acid. Dots denote BRD4 amino acid residues which are identical between human and mouse. Sequence alignment was performed with NCBI BLAST, using sequence accession numbers NP_490597.1 (human) and NP_065254.3 (mouse). For simplicity, and in line with the human data, the mouse p.Tyr431Cys is referred to as *Brd4*^{Y430C} throughout both the main text and the supplementary text and methods.



Supplementary Figure 7

High confidence potential BRD4-interacting proteins indicated in Figure 2C&D

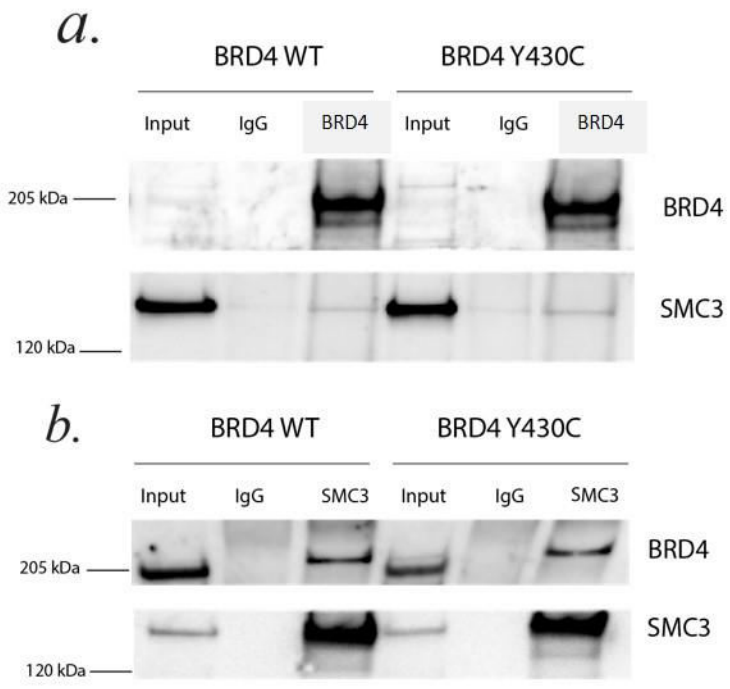
Heatmap of the label-free mass spectrometry quantitative output values (average of triplicates) assigned to each protein following immunoprecipitation from wild-type (WT) and *Brd4*$^{Y430C/Y430C}$ (MT) mESCs using IgG only control or Abcam/Bethyl antibody against BRD4. Proteins that interact with both wildtype and mutant BRD4 (top), wildtype BRD4 only (middle) and mutant BRD4 only (bottom) are indicated by dotted lines and named. Proteins known to be mutated in human cohesinopathies are shown in red, proteins known to be mutated in neurodevelopmental disorders are shown in bold, and those plausibly involved in neurodevelopmental disorders are shown in yellow. Full list of peptide hits available in Supplementary Table 4.



Supplementary Figure 8

Box plots showing details of the mass spectrometry results for members of the cohesin complex

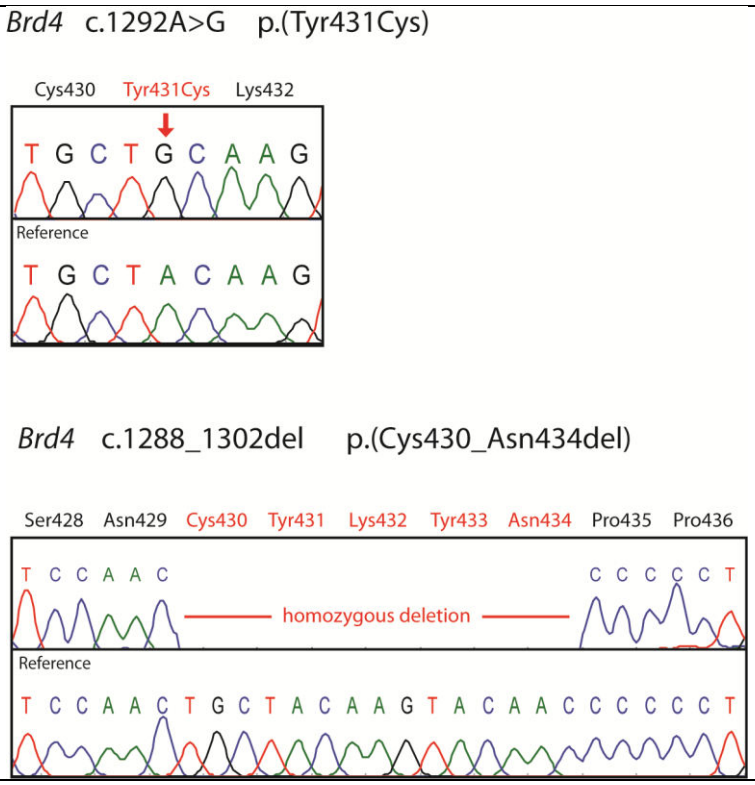
The y-axis represents the label-free quantification (LFQ) intensity and the x-axis the reagent used for the immunoprecipitation in wild-type (WT) and *Brd4*$^{Y430C/\ Y430C}$ (MUT) mESCs. Full list of peptide hits available in Supplementary Table 4.



Supplementary Figure 9

Reciprocal immunoprecipitations (IPs) of BRD4 and SMC3 in wild-type and *Brd4*$^{Y430C/Y430C}$ mouse embryonic stem cells

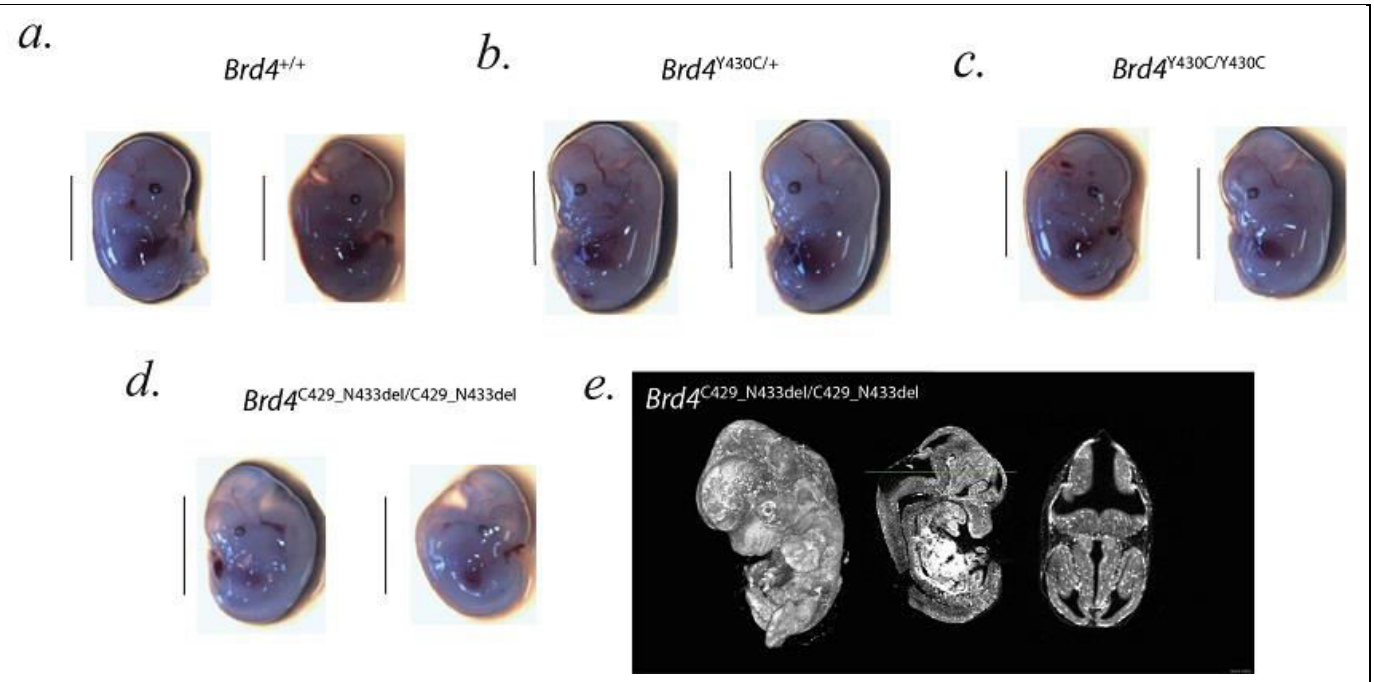
Cropped immunoblots of endogenous BRD4 (a) and SMC3 (b) IPs from wild-type (BRD4 WT) and *Brd4*$^{Y430C/Y430C}$ (BRD4 Y430C) mESCs. Normal rabbit IgG is used as a control. Input =1% of mESC nuclear extract. Antibodies detect BRD4 and SMC3



Supplementary Figure 10

Representative chromatograms showing validation of genome editing in mouse embryos

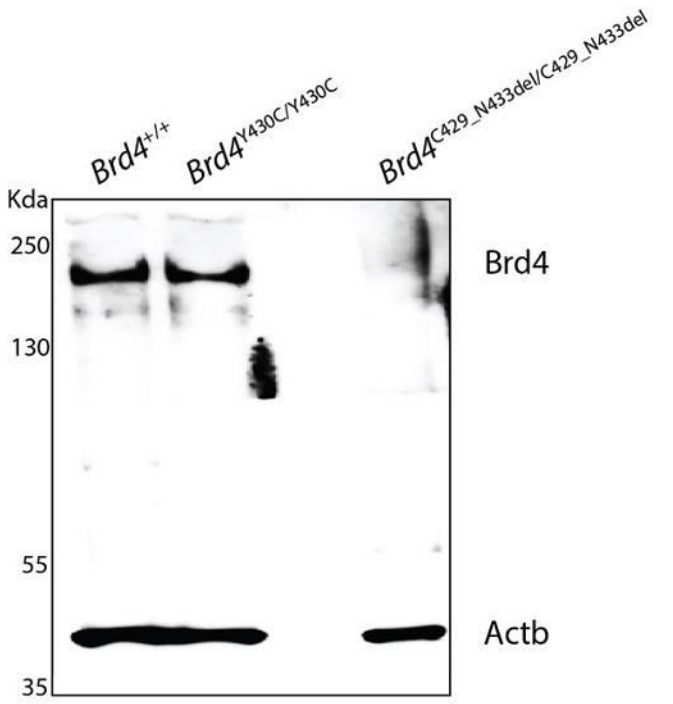
Representative chromatograms showing validation of the homozygous *Brd4* knock-in c.1292A>G, p.(Tyr431Cys)[¥] and the homozygous *Brd4* in-frame deletion c.1288_1302del, p.(Cys430_Asn434del) mice using genomic DNA extracted from mouse embryos. The mutated amino acid residues are shown in red. The mouse *Brd4* sequence accession number NC_000083.6 was used as reference. Variant nomenclature is based on mouse *Brd4* transcript NM_020508.4. [¥] Mouse Brd4 p.Tyr431Cys is equivalent to the human p.Tyr430Cys (see supplementary figure 6). For simplicity, this variant is referred to as Y430C throughout.



Supplementary Figure 11

Images of wild-type and *Brd4* mutant mouse embryos

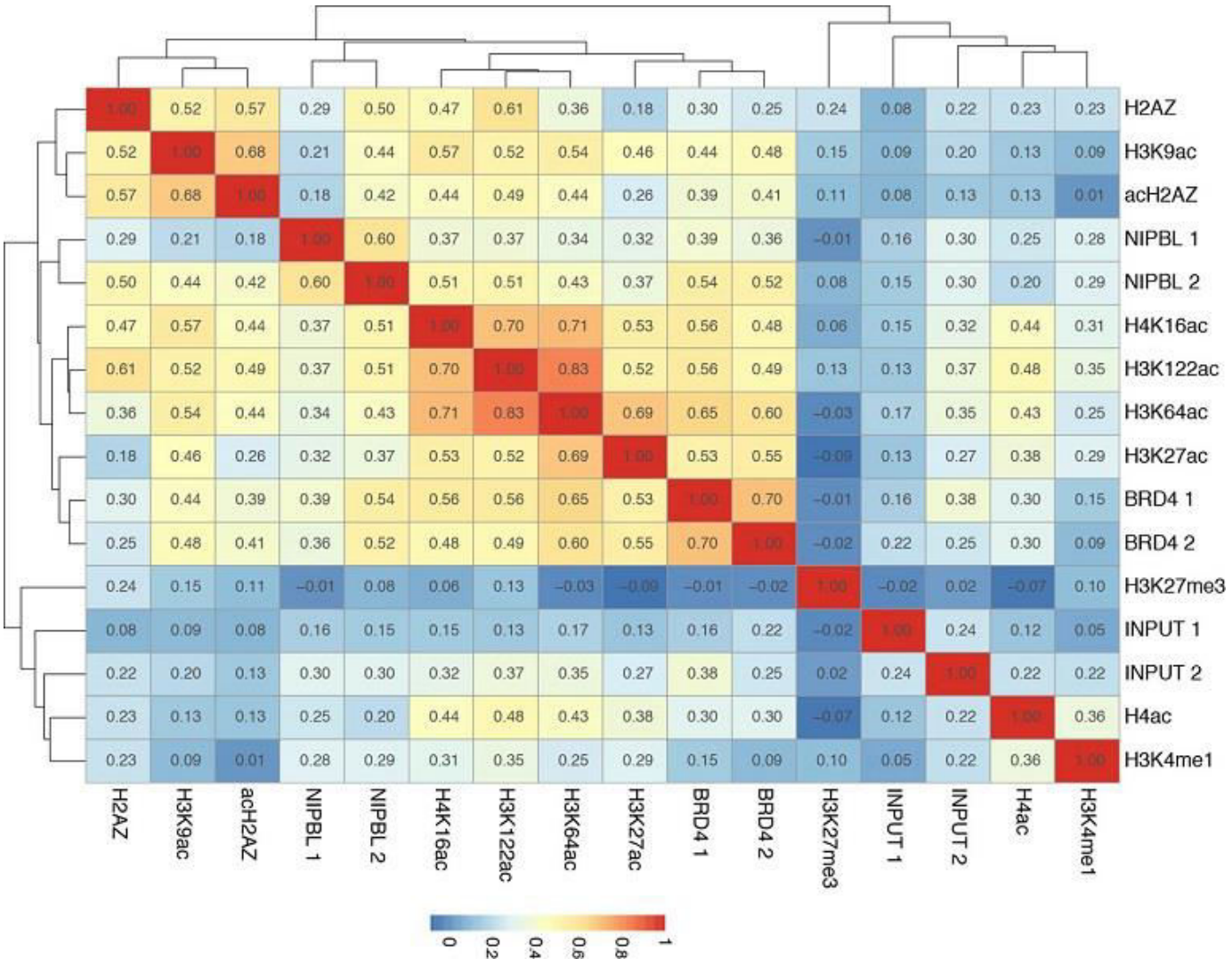
Representative images of wild-type embryos (a), heterozygous and homozygous knock-in of mutant repair template resulting in *Brd4*$^{Y430C/+}$ and *Brd4*$^{Y430C/Y430C}$ embryos respectively (b & c), and (d) *Brd4*$^{C429\_N433del/\ C429\_N433del}$ homozygous in-frame deletion embryos. e. Panel showing optical projection tomography of *Brd4*$^{C429\_N433del/\ C429\_N433del}$ F0 mouse embryo. On the left of the panel is a 3D reconstruction of the whole embryo, in the middle is a sagittal section showing the position of the transverse section of the brain in the right panel. No morphological anomaly was detected other than being small for gestational age.



Supplementary Figure 12

Immunoblot of cell lysate from WT, Y430C mutant and BRD4 deletion MEFs

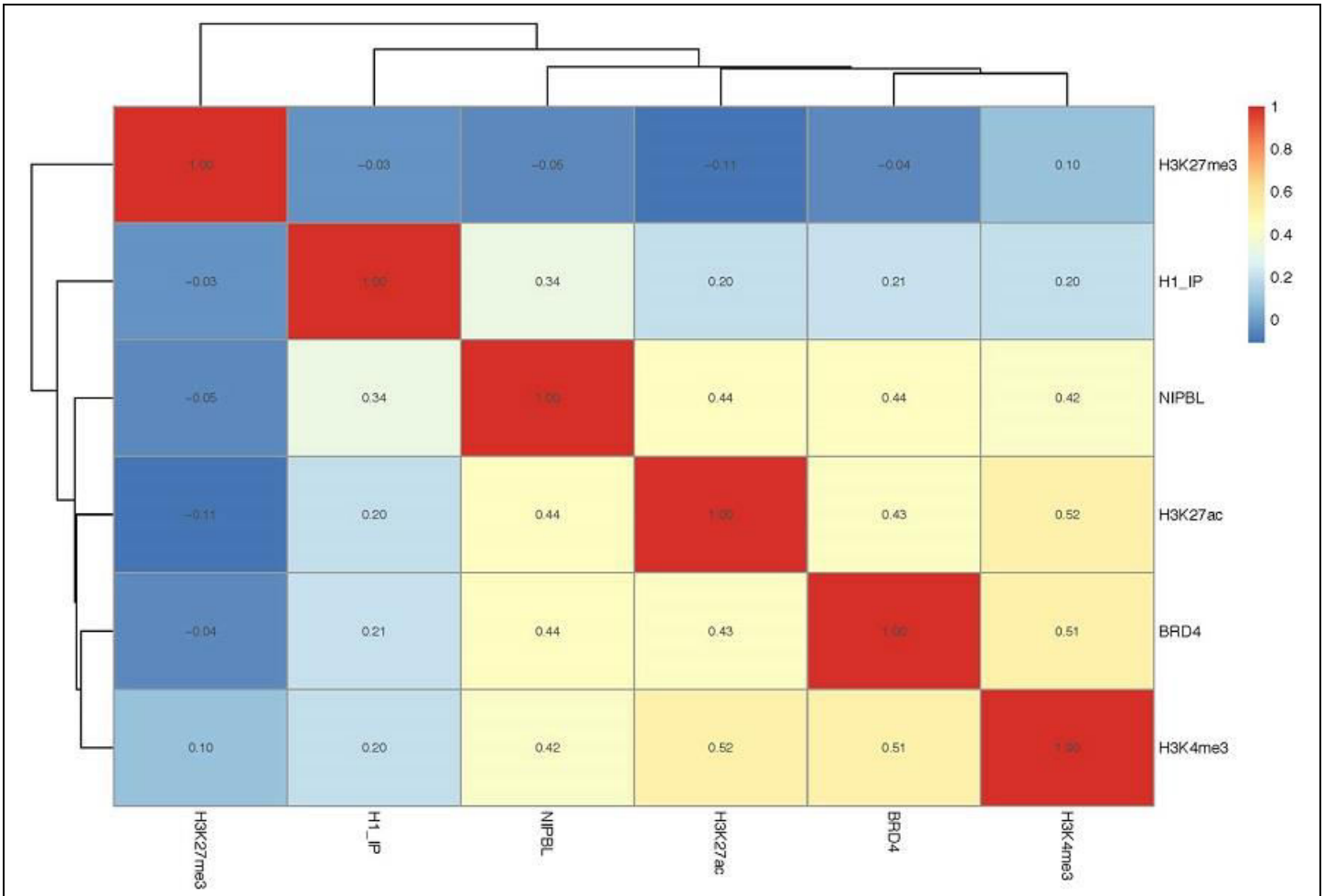
Immunoblot of cell lysate from wild-type (*Brd4*$^{+/+}$), *Brd4*$^{Y430C/Y430C}$, and BRD4 deletion (*Brd4*$^{C429\_N433del/C429\_N433del}$) MEFs. Antibodies detect BRD4 and ACTB (loading control).



Supplementary Figure 13

Clustered heatmap of genome-wide ChIP-seq binding profiles from mouse embryonic stem cells.

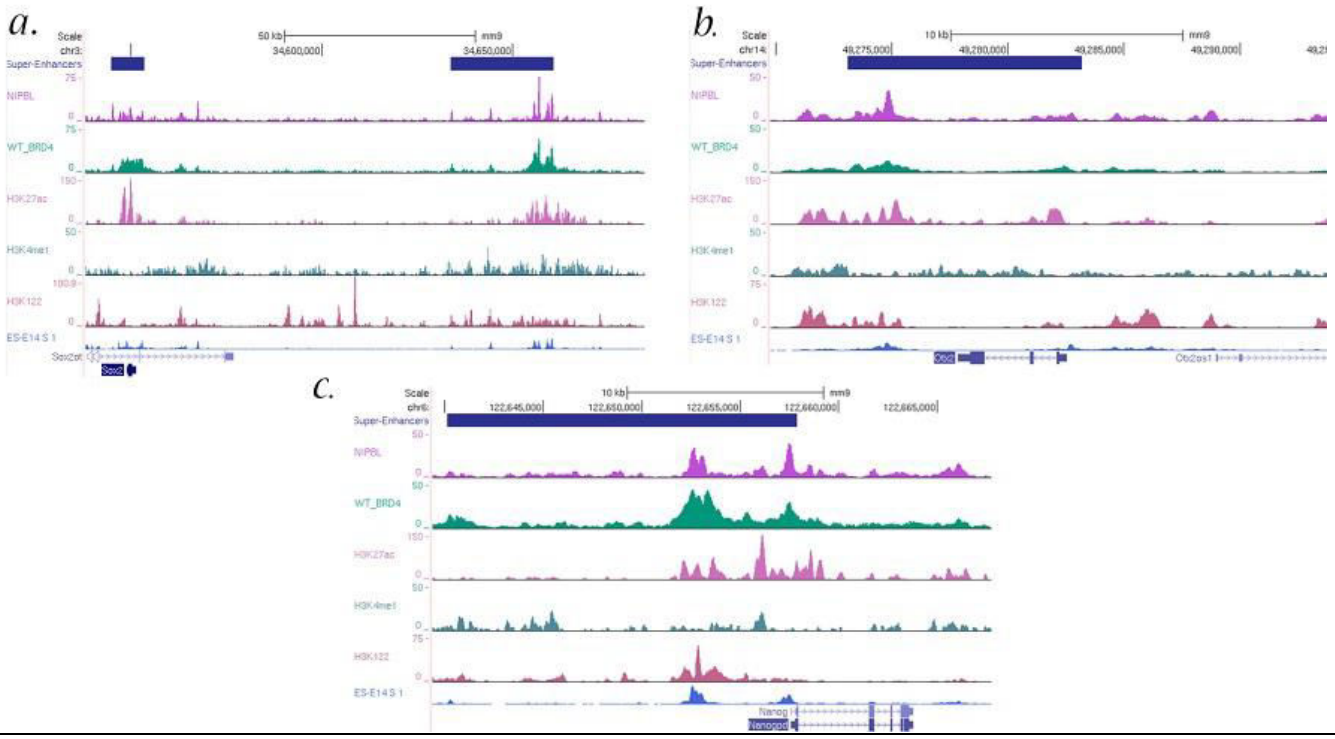
Heatmap was generated using pairwise Pearson's r correlations. The antibodies that were used for the ChIP are indicated on the right and bottom of the heatmap. The accession numbers for each of the datasets is given in Supplementary Table 3



Supplementary Figure 14

Clustered heatmap of genome-wide ChIP-seq binding profiles from human embryonic stem cells

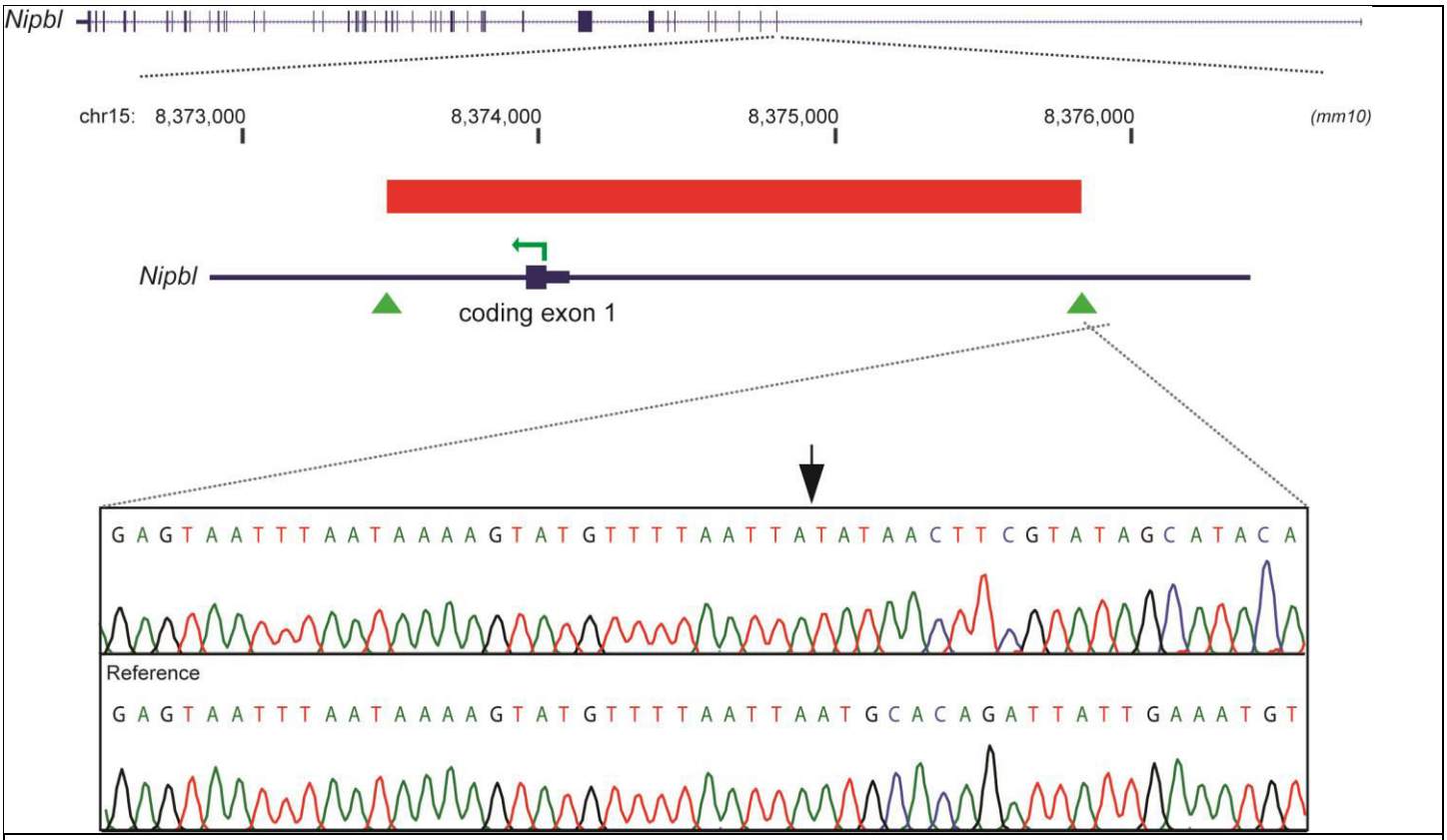
Heatmap was generated using pairwise Pearson's r correlations. The antibodies that were used for the ChIP are indicated on the right and bottom of the heatmap. The accession numbers for each of the datasets is given in Supplementary Table 3



Supplementary Figure 15

Screenshots of UCSC Genome Browser showing colocalisation of BRD4 and NIPBL ChIP-seq peaks over super enhancers

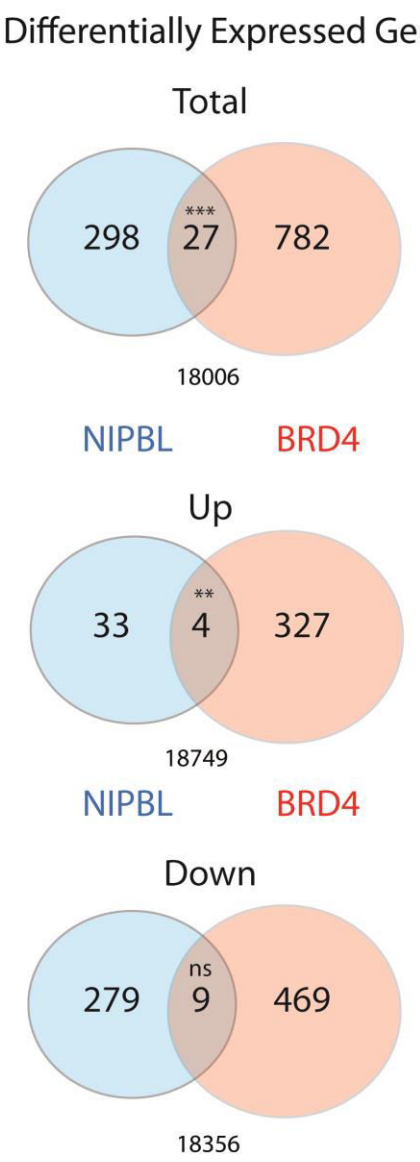
BRD4 and NIPBL ChIP-seq peaks over the super enhancers of *Sox2* (a) *Otx2* (b) and *Nanog* (c). BRD4 ChIP-seq was carried out as described. Other tracks use previously published data: H3K27ac (GSM594578_1/GSE24164), H3K4me1 (GSM594577_1/ GSE24164); H3K122ac, GSM2054689/GSE66023); blue bars show mESC super-enhancers.



Supplementary Figure 16

Schematic representation of the deleted region in *Nipbl* gene in mice with heterozygous LOF of *Nipbl*

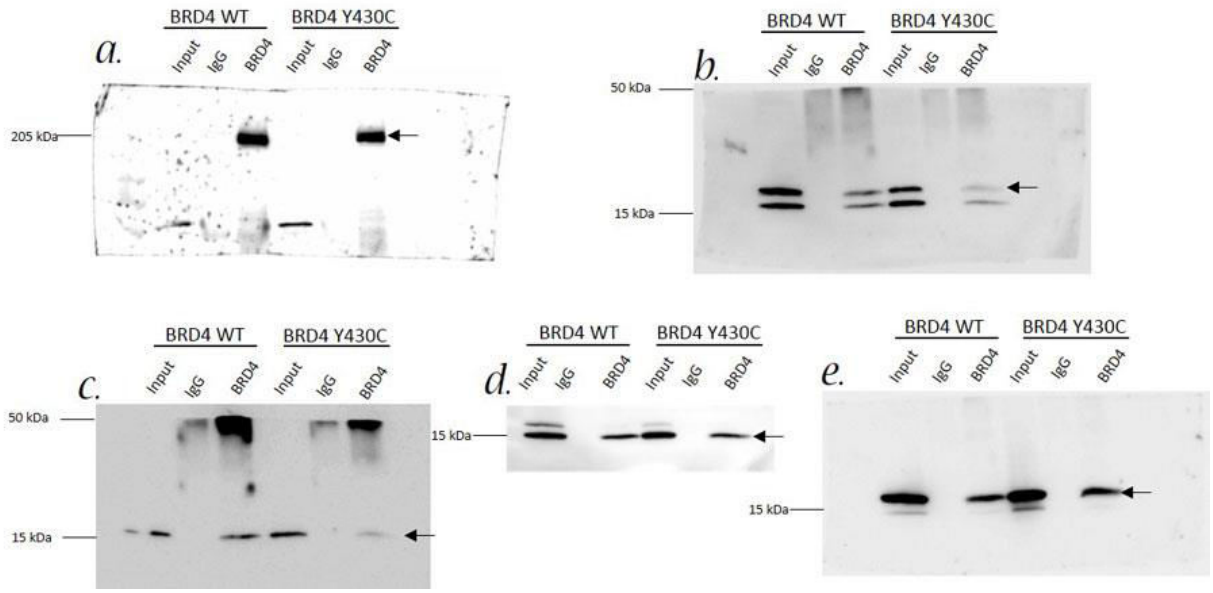
Location of the deleted region is shown as red block. Green triangles mark the boundary of deletion based on the mouse genome reference assembly mm10 (chr15: 8,373,580-8,375,641).Representative chromatogram with nucleotide bases after the breakpoint is marked by black arrow.



Supplementary Figure 17

Venn diagrams showing overlaps between differentially expressed (DE) genes

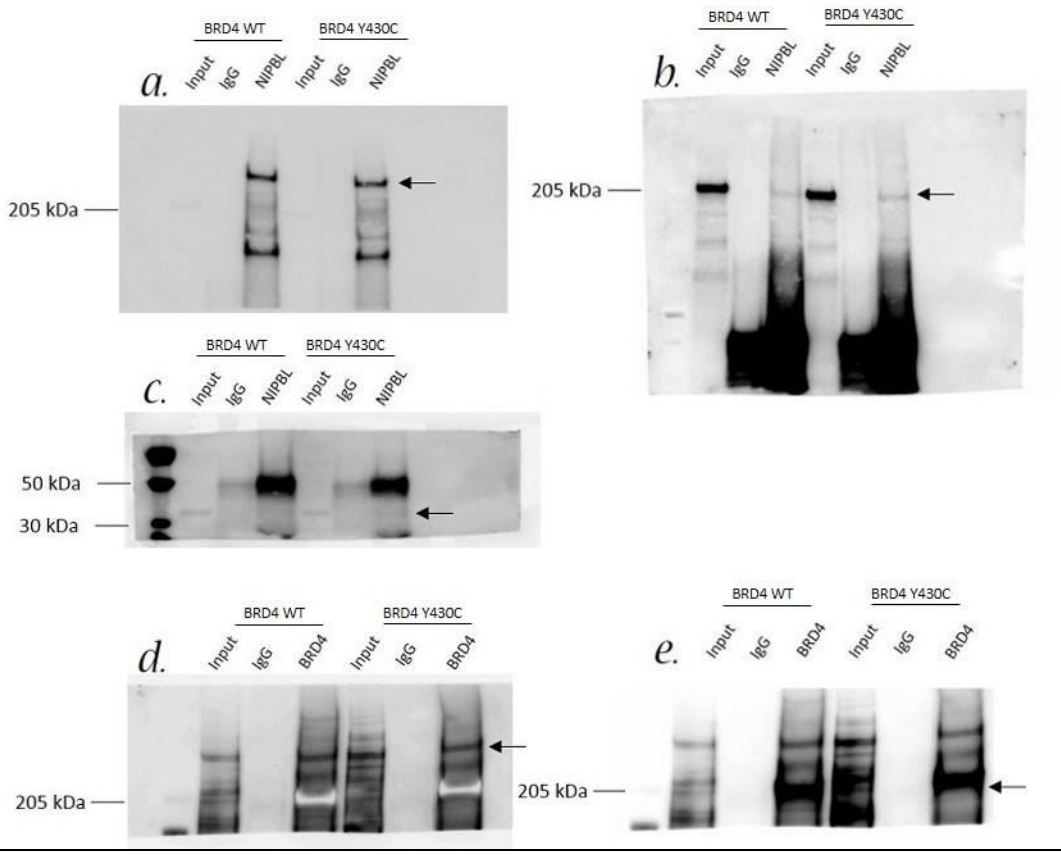
Venn diagrams showing overlaps between differentially expressed (DE) genes from *Brd4*$^{Y430C/Y430C}$ MEFs compared to *Nipbl* heterozygous null MEFs. The total number of genes stays the same in each comparison (19,113). A hypergeometric test was performed on the intersection of the DE genes to determine if DE genes were significantly enriched. *P*-values ***0.00069; ** 0.0037; ns 0.295



Supplementary Figure 18

Uncropped immunoblot images corresponding to figure 2b

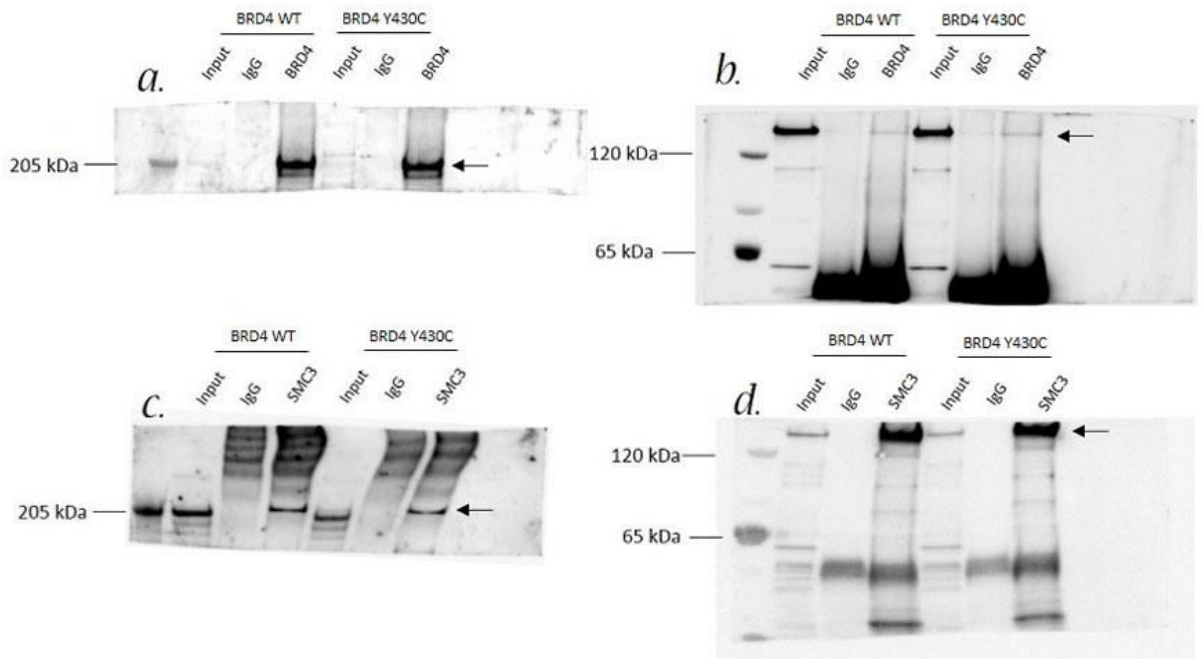
Images show endogenous BRD4 and rabbit normal IgG IPs from wild-type (BRD4 WT) and *Brd4*$^{Y430C/Y430C}$ (BRD4 Y430C) mESCs, with antibodies detecting BRD4 (a), H3K9ac (b), H3K27ac (c), H4K8ac (d), H3 (e). Arrow head identifies band of correct size.



Supplementary Figure 19

Uncropped immunoblot images corresponding to figure 2e

Images a-c show endogenous NIPBL and rabbit normal IgG IPs from wild-type (BRD4 WT) and *Brd4*$^{Y430C/Y430C}$ (BRD4 Y430C) mESCs, with antibodies detecting NIPBL (a), BRD4 (b), SOX2 (c). Images d&e show endogenous BRD4 and rabbit normal IgG IPs from *Brd4* wild-type and *Brd4*$^{Y430C/Y430C}$ mESCs, with antibodies detecting NIPBL (d), BRD4 (e). Arrow head identifies band of correct size.



Supplementary Figure 20

Uncropped immunoblot images corresponding to supplementary figure 9

Images a&b show endogenous BRD4 and rabbit normal IgG IPs from wild-type (BRD4 WT) and *Brd4*$^{Y430C/Y430C}$ (BRD4 Y430C) mESCs, with antibodies detecting BRD4 (a), SMC3 (b). Images c&d show endogenous SMC3 and rabbit normal IgG IPs from *Brd4* wild-type and *Brd4*$^{Y430C/Y430C}$ mESCs, with antibodies detecting BRD4 (c), SMC3 (d). Arrow head identifies band of correct size.

Patient/Study info	Present study 1	Present study 2	Present study 3	Present study 4	Published Patient 1	Published Patient 2	Published Patient 3	Published Patient 4	Published Patient 5	Published Patient 6	Published Patient 7	Summay /Total
PMID	NA	NA	NA	NA	17339581 20648052	19215039	20648052	20570643	21994138	22750323	21815246	
DECIPHER ID	281165	NA	NA	264293				255743		255839?		
Patient ID	4198	3049	CDL038	264293	11/03 and patient 2		patient 1					
Country	UK	UK	UK	UK	Germany	US	Italy	Belgium	US	Denmark	Japan	
DNA mutation/deletion	1.04 Mb deletion at 19p13.12	c.1289A>G; p.Tyr430Cys	c.1224delinsCA p.Glu408Aspfs*4	c.691del; p.Asp231Thrfs*9	~2 Mb deletion at 19p13.12	2.52 Mb deletion at 19p13.12	1.9 Mb deletion at 19p13.12	1.2 Mb deletion at 19p13.12	2.53 Mb deletion at 19p13.11	1.4 Mb deletion at 19p13.1	763 kb deletion at 19p13.12	
hg19	chr19:14503000-15543000	chr19; g.15374283T>C	chr19; g.15374348delinsTG	chr19: g.15376323del	chr19:14141667-15965683	chr19:14069000-16499000	chr19:14269000-16189000	chr19:14382780-15492848	chr19:13899000-16519000	chr19:15178837-16628694	chr19:15439338-16203271	
de novo	yes	yes	yes	yes	unknown	yes	unknown	unknown	yes	yes	yes	
Sex	F	F	F	M	F	F	M	M	F	M	F	7:4
Gestational age (wks)	42	36	40	38	32	40	36	40	36	41	40	
Birth weight (g)	3515	2041	2410			2130	2400	2200	1670	2450	2588	
Birth weight (Z score)	-0.18	-1.42	-2.41	-0.8		-3.18	-0.82	-3.16	-2.34	-2.53	-1.94	-1.88
Length at birth (cm)								46	46	51	46.6	
Length (Z score)								-2.5	-1.5		-1.94	-1.98
Birth OFC (cm)	34						32	32	29		31.5	
Birth OFC (Z score)	-0.44				-2.4		-1.5	-2.5	-3		-2.47	-1.65
Age at evaluation	6 yrs 3 mo	3yrs 1 mo	11yrs 1mo	11.2 years	8.5 yrs	10 yrs	31 yrs	15 yrs	died at 45 days	2 yrs 9 mo	5 yrs	
Weight (kg)	26.5	12.4	37.06				83				16.2	
Weight (Z-score)	1.47	-1.28	0.1	-0.71			1.11				-0.95	-0.04
Height (cm)	115.9	89.5	133.9				150				105.6	
Height (Z-score)	-0.19	-1.55	-1.5	-1.94			-2.2				-0.73	-1.35
OFC (cm)	48	45.3	49				53.5				47.8	
OFC (Zscore)	-3.57	-4.24	-3.9	-1.48	-3.2		-1.4			-3	-3.25	-3.01
Facial photographs available	no	yes	yes	yes	no	yes; 2yrs, 6yrs, 9yrs	yes	yes	no	yes	yes	
Relevant Facial Features	synophrys, arched eyebrows, short nose, long philtrum	synophrys, arched eyebrows, short nose, long philtrum	synophrys,short nose, long philtrum		synophrys, long lashes, mild ptosis, flattened nasal bridge, anteverted nostrils, long philtrum and small mouth with thin upper lip	ptosis, short nose with anteverted nares, long philtrum, flattened vermillion border, and mild micrognathia	synophrys, long philtrum, thin upper lip, short nose	synophrys, thick eyebrows, flat nasal bridge, high palate	Unknown	synophrys, hypotelorism, strabismus	arched eyebrows, depressed nasal bridge, short columella, micrognathia	
CdLS Diagnostic Criteria Checklist Fulfilled?	No	Yes	Yes	No	Yes	Missing data	Yes	Borderline	Missing data	No	No	4/9
Major Criteria	OFC; speech delay; ID; OCD	OFC; ID; DD; absent speech	OFC; DD;	DD	IUGR; small OFC; ID; hyperactivity; absent speech	ID; DD; IUGR	ID; DD; delayed speech; self injury; autistic behaviour; anxiety; hyperactivity	OFC; IUGR; height; DD; speech delay; ID; anxiety; shy	OFC; IUGR	IUGR; OFC; ID; DD; speech delay;	IUGR; OFC; height	
Minor Criteria	short 1st metacarpals	GERD; hip dysplasia; VSD	myopia;blocked tear ducts	VSD	Hearing loss; GERD; PDA	Hearing loss; ASD/VSD	seizures; submucous cleft palate,II and III toe syndactyly; aortic regurgitation; hearing loss	Seizures; hypertrichosis;	Gut malrotation; VSD; seizures; hearing loss	II and III toe syndactyly; hearing loss; hypermetropia	Hearing loss	
Other Relevant Clinical Features	telecanthus; normal height, mild chiari I malformation	None		Cleft lip, inguinal hernia, posteriorly angulated ears, hypertelorism	Preauricular tag	Supernumery nipples; thrombocytopenia	precocious puberty, hypothyroidism, hyperlipidemia	Narrow brain stem, thin corpus callosum, obesity	pontine hypoplasia, thin corpus callosum, preauricular tag	thrombocytopenia; large central incisors	preauricular tag; only mild ID	
Conclusion	Diagnosis of atypical CdLS on basis of facial features, hands and developmental profile	Very typical facial appearance and head growth, relative preservation of height	Atypical CdLS	Unknown	Atypical CdLS	Facial appearance compatable with atypical CdLS: HDAC8-like	Atypical CdLS	Possible atypical CdLS; KBG-like	Unknown	KBG-like; some features of CdLS	Primary microcephaly is the only significant overlap with CdLS	

GERD; gastroesophageal reflux. PDA; patent ductus arteriosis. OFC; occipitofrontal circumference. ID; intellectual disability. DD; developmental delay. IUGR; intrauterine growth retardation. VSD; ventricular septal defect. ASD; atrial septal defect. OCD; obsessive–compulsive disorder. KBG syndrome (MIM 148050).

Supplementary table 1: Clinical Features of individuals with genomic variants affecting *BRD4*

Primers for droplet digital PCR	**Forward (5'-3')**	**Reverse (5'-3')**
	CCAGCCTAGTGCAGAAGCAT	CTGTCGCTGGATGACTTGG
UPL probe	CTCCTCCA	
gRNAs for CRISPR	**gRNA1**	**gRNA2**
	CAACCCCCCTGACCATGAAG	AGCAGTTGGAGAACATCAAT
Primers for ChIP-qPCR	**Forward (5'-3')**	**Reverse (5'-3')**
Klf4 SE chip	CACAATGCCAGCTATGCGAT	TCCTGCCCAAATGTGAGGAT
Myc SE chip	TCCTTGACTGCTTCTCCTGG	AGACAGTAAAGGGCCACACA
Hoxa11 Pro	AGCCCAATGATGGATTTTGA	GAAGGGAGGCTGGAGAAATC
Myc p	CCTAGATAACTCATTCGTTCGTC	CCCTGCGTATATCAGTCACCG
Myc 5'	CCAGAAGCTTTCCCAGCAAGC	CAGCTCAGCCTTGCTTGCTC

Supplementary table 2: Oligonucleotide sequences

Name	Source	Accession	Genome build
BRD4	GEO	GSE33281/GSM823382	hg19
NIPBL	GEO	GSE64758/GSM1579363	hg19
H3K27ac	ENCODE	ENCSR000ANP/ENCFF948TVI	hg19
H3K4me3	ENCODE	ENCSR814XPE/ENCFF195GST	hg19
H3K27me3	GEO	GSE35791/GSM874985	hg19
H1_Input	GEO	GSE64758 /GSM1693959	hg19
hg19 blacklist	ENCODE	ENCSR636HFF	hg19
H1-hESC DNaseI HS Uniform Peaks from ENCODE/Analysis	ENCODE	wgEncodeAwgDnaseUwdukeH1hescUniPk.narrowPeak	hg19
NIPBL 1	GEO	GSE22557/GSM560349	mm9
NIPBL 2	GEO	GSE22557/GSM560350	mm9
H4ac	GEO	GSE76760/GSM2037252	mm9
H4K16ac	GEO	GSE47761/GSM1156617	mm9
H3K27me3	ENCODE	ENCSR000CFN/ENCFF001KDT	mm9
H3K9ac	ENCODE	ENCSR000CGS/ENCFF001KCZ	mm9
H3K122ac	GEO	GSE66023/GSM2054689,GSM2054690	mm9
H3K64ac	GEO	GSE66023/GSM2054691, GSM2054692	mm9
acH2AZ	GEO	GSE34483/GSM849929	mm9
H2AZ	GEO	GSE34483/GSM849929	mm9
H3K4me1	ENCODE	ENCSR000CBF/ENCFF001KDZ	mm9
H3K27ac	ENCODE	ENCSR000CDE/ENCFF001KDH	mm9
H3K4me1	GEO	GSE24164/GSM594577_1	mm9
H3K27ac	GEO	GSE24164/GSM594578_1	mm9
mm9 blacklist	http://mitra.stanford.edu/kundaje/akundaje/release/blacklists/mm9-mouse/mm9-blacklist.bed.gz	http://mitra.stanford.edu/kundaje/akundaje/release/blacklists/mm9-mouse/mm9-blacklist.bed.gz	mm9

Supplementary table 3: list of datasets used and their accession numbers

Supplementary Note

Known disease genes in the deleted interval in individual II:1 (family 4198)

Three genes (*EMR2*, *NOTCH3*, *TERC*) within the deleted interval in individual II:1 (family 4198) have been implicated in human genetic diseases unrelated to CdLS. Monoallelic mutations in *ADGRE2* have been reported as a cause of vibratory urticarial (OMIM 125630). Monoallelic loss of function mutations in *TERC*, a telomerase RNA component, cause autosomal dyskeratosis congenita (OMIM 127550). This condition shows anticipation with telomere shortening becoming more severe with successive generations. Given that these deletions are *de novo*, it seems unlikely that *TERC* haploinsufficiency would cause a childhood onset of the disorder. Heterozygous loss-of-function mutations in *NOTCH3* cause an adult onset neurological disorder Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) Type 1.

Reported p.His304Tyr Variant in *BRD4*

A non-synonymous variant in *BRD4* (c.910C>T, p.His304Tyr) has recently been reported in a single small family with congenital cataracts, macrocephaly, brachydactyly and mild short stature[1]. This variant was identified as one of five co-segregating rare variants identified. The *BRD4* variant was scored as possibly damaging using PolyPhen2 and was unique, although p.His304Pro has been identified once in gnomAD database. This is an interesting observation but we consider the human genetics evidence for association of the variant to the disease (appropriate segregation in three affected and one unaffected individual) to be limited.

URLs

gnomAD database; http://gnomad.broadinstitute.org

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