CTNNBL1 facilitates the association of CWC15 with CDC5L and is required to maintain the abundance of the Prp19 spliceosomal complex

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SUPPLEMENTARY DATA

Supplementary Methods

Expression vectors. Plasmids with CTNNBL1 and its mutants have been described previously (1, 2). Full length and truncated versions of CWC15 and CDC5L were PCR amplified and cloned using the primers and enzymes listed in Table S1. pOPTM was a kind gift of Dr. O. Perisic, Cambridge.

Protein crosslinking. After purification, 10 mM of each protein was mixed in crosslinking buffer (see buffer list), crosslinking was initiated by addition of a mix of BS3-d0 and BS3-d4 (Bis(sulfosuccinimidyl) suberate deuterated and non-deuterated crosslinking reagent mix, BS3-d4/d0 -with a spacer arm of 11.4 Å) up to 0.5 mM, and terminated with a final concentration of 50 mM Tris pH7.5 after 0, 15 and 30 min. Complexes were separated by SDS-PAGE and gel bands were cut out and digested with trypsin. The resulting peptides were analysed by nano-scale capillary LC-MS/MS using an Ultimate U3000 UPLC (ThermoScientific Dionex) to deliver a flow of approximately 300 nL/min. A C18 Acclaim PepMap100 5 μm, 100 μm x 20 mm nanoViper trapped the peptides prior to separation on a C18 Acclaim PepMap100 3 μm, 75 μm x 250 mm nanoViper (both from ThermoScientific Dionex). Peptides were eluted with a gradient of acetonitrile. The analytical column outlet was directly interfaced with a LTQ-Velos Orbitrap mass spectrometer (ThermoScientific) operated in DDA (Data Dependent Acquisition) mode, where an initial MS scan is followed by fragmentation of the top 10 ions. The resulting data were analysed using Hekate cross-linking software (3) and all potential cross-links were manually validated.

Hydrogen deuterium exchange mass spectrometry (HDX-MS). Proteins were dialysed against exchange buffer but with MPW instead of D_2O (see buffer table) and then diluted as single proteins or combined ([CWC15 + CDC5L], [CWC15 + CTNNBL1] or [CWC15 + CDC5L + CTNNBL1]) to 1 mg/ml for His-CTNNBL1 and 0.5 mg/ml for CWC15 and CDC5L in a volume of 10 µl. The protein solutions were mixed with 40 µl of exchange buffer (with D_2O) for 3 seconds on ice and 3, 30, 300 and 3000 seconds at room temperature (22°C) before quenching by the addition of 20 µl ice cold quench buffer (buffer list) and snap freezing in liquid nitrogen. Each time point and protein combination was done in triplicate.

The quenched protein samples were rapidly thawed and subjected to proteolytic cleavage by pepsin followed by reversed phase HPLC separation essentially as previously described (4). The

protein was passed through an immobilized pepsin column, 2.1 x 30 mm (Porozyme, ABI) at 230 μ l/min for 3 min and the peptic peptides trapped and desalted on a 2.1 x 5 mm C18 trap column (Acuity BEH C18 Van-guard pre-column, 1.7 μ m, Waters). Trapped peptides were subsequently eluted over 12 min using a 5-36% gradient of acetonitrile in 0.1%v/v formic acid at 40 μ l/min. Peptides were separated on a reverse phase column (Aquity UPLC BEH C18 column 1.7 μ m, 100 mm x 1 mm (Waters). Peptides were detected on a Xevo G2 Q-TOF (Waters) acquiring over a mass range from 300 to 1400 m/z, with the standard electrospray ionization (ESI) source and lock mass calibration using [Glu1]-fibrino peptide B (500 fmol/ μ l). The mass spectrometer was operated at a source temperature of 80°C, and a spray voltage of 2.6 kV. Spectra were collected in positive ion mode.

Peptide identification was performed by data dependent acquisition using a 3-35% gradient of acetonitrile in 0.1%v/v formic acid over 120 min with a Xevo G2 Q-TOF (Waters). This was supplemented with a shorter 20 min gradient separation to identify and correct the retention time for all peptide samples. The resulting MS/MS data was analysed using the Mascot database search engine (Matrix Science) using an MS tolerance of 5 ppm and a MS/MS tolerance of 0.15 Da. Mass analysis of the peptide centroids was performed as described previously, using the software HD-Examiner (Sierra Analytics) (4). Only peptides with a Mascot score >15 were considered. The first round of analysis and identification were performed automatically by the HD-Examiner software, however, all peptides (deuterated and non-deuterated) were manually verified at every state and time point for the correct charge state, m/z range, presence of overlapping peptides, and the expected retention time. Deuterium incorporation was not corrected for back-exchange and represents relative, rather than absolute changes in deuterium levels. Changes in H/D amide exchange in any peptide may be due to a single amide or a number of amides within that peptide. All time points in this study were prepared at the same time. Individual time points were acquired on the mass spectrometer on the same day.

Buffer list

Triton lysis buffer

20 mM Tris-Cl pH8, 125 mM NaCl, 1 mM MgCl₂, 10% glycerol

Bacterial protein expression and purification buffers

Lysis Buffer: As wash buffer, but supplemented with: Lysozyme, 1 mg/ml (Sigma Aldrich), EDTA-free protease inhibitors (Roche) and Benzonase, 100 U/ml (Merck Millipore) Wash Buffer for CTNNBL1: 50 mM Na₂HPO₄/NaH₂PO₄ pH7.4, 500 mM NaCl, 1 mM MgCl₂. Wash Buffer for CDC5L and CWC15: 50 mM Tris pH7.4, 150 mM NaCl, 5mM MgCl₂, 5 mM 2-Mercaptoethanol, 0.1% Triton X-100.

lon exchange buffers:

For CTNNBL1: (Buffer A) 20 mM Tris pH8.0, 50 mM NaCl, 5 mM 2-Mercaptoethanol and (Buffer B) As buffer (A) but 1M NaCl For CDC5L: (Buffer A) 50 mM Tris pH7.0, 150 mM NaCl, 5mM MgCl₂, 5 mM 2-Mercaptoethanol and (Buffer B) As buffer (A) but 1M NaCl For CWC15: (Buffer A) 50 mM Tris pH8.0, 150 mM NaCl, 5mM MgCl₂, 5 mM 2-Mercaptoethanol and (Buffer B) As buffer A, but 1M NaCl

Pull down buffer

20 mM Hepes pH8.0, 110 mM KAc, 2 mM MgAc, 20 mM Imidazole, 10% glycerol, 0.2% Tween 20, 5 mM 2-Mercaptoethanol

Crosslinking buffer/SEC-MALS buffer

20 mM Hepes pH8.0, 110 mM KAc, 1 mM DTT

HDX - Exchange buffer

10 mM Hepes pH8.0, 50 mM NaCl, 1 mM DTT, in 99% Deuterium

3.5x HDX - Quench buffer

2 M Guanidinium HCl, 2.6% formic acid in Millipore deionised water

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Supplementary Tables

Table S1.

List of plasmids and primers used.

Primer	Construct name/Primer sequence	Vector	Enzyme
	HA-CWC15	pcDNA3.1-3HA	
	GST-CWC15	pGEX-6P-1	
CWC15[1-229]_fw	ATAT-GGATCC-ATGACAACAGCAGCCAGGC		BamHI
CWC15[1-229]_rev	ATAT-GAATTC-		EcoRI
	CTATTTAATATATTTCTCCATGAACTTTTTGTG		
	HA-CWC15[1-79]	pcDNA3.1-3HA	
	GST-CWC15[1-79]	pGEX-6P-1	
CWC15[1-229]_fw	ATAT-GAATTC-		BamHI
	CTATTTAATATATTTCTCCATGAACTTTTTGTG		
CWC15[1-79] rev	ATAT-GAATTC-		FcoRI
	CTATGGACGATCCCTATTTTCTCTC		Loon
	HA-CWC15[80-229]	pcDNA3 1-3HA	
	GST-CWC15[80-229]	pGEX-6P-1	
CWC15[80-229] fw	ATAT-GGATCC-ACCCGAGAACATACAACCTCC		BamHI
			Bainin
CWC15[1-229]_rev	ATAT-GAATTC-		EcoRI
	СТАТТТААТАТАТТТСТССАТБААСТТТТТБТБ		
	MBP-CDC5L[1-500]	рОРТМ	
CDC5L[1-500]_fw	AAT-CATATG-CCTCGAATTATGATCAAG		Ndel
CDC5L[1-500]_rev			Mlul
	MBP-CDC5L[1-271]	рОРТМ	
CDC5L[1-500]_fw	AAT-CATATG-CCTCGAATTATGATCAAG		Ndel
CDC5L[1-271]_rev	AAT-ACGCGT-		Mlul
	TCAGAATAGCTGATGGTAAATCAG		
	MBP-CDC5L[1-195]	pOPTM	
CDC5L[1-500]_fw	AAT-CATATG-CCTCGAATTATGATCAAG		Ndel
	ΔΔΤ.ΔΟΩΟΩΤ.ΤΟΔΩΟΟΔΩΟΤΩΟΤΟΩΔΔΩΤΤΟΤΟ		NALUI
			wiiui
		μομινί	Ndal
	AAT-CATATG-CCTCGAATTATGATCAAG		indel

CDC5L[1-165]_rev	AAT-ACGCGT-	Mlul
	TCACTTCTTTCCCTGAGTATTAGCC	

Table S2.

List of primers used for quantitative PCR.

Primer	Primer sequence	
CDC5L		
_fw	CCAGTGCTTGGAACACTATGAG	exon 3
_rev	CATCCATGTCTATCGGGTCA	exon 5
B2M		
_fw	GTCGCGGTCGCTTCAGTCGT	exon 1
_rev	TCCGGTGGGTGGCGTGAGTA	exon 2
ACTB		
_fw	CTGTCGAGTCGCGTCCACCC	exon 1
_rev1	ACATGCCGGAGCCGTTGTCG	exon 2
_rev2	CACCCGCCCATGGTGTCCG	intron 1-2
CDK4		
_fw	CACCTCCAGAGCTCTTAGCC	exon 1
_rev1	GATCTCGGGCTTTGTACACC	exon 2
_rev2	TCCAAAATCACACCCTTGC	intron 1-2
MYC		
_fw	CTCCTCGAGCTGTTTGAAGG	exon 1
_rev1	AATAGGGCTGTACGGAGTCG	exon 2
_rev2	TGTCAAAAGCACAGGAATGG	intron 1-2

Table S3

Species nomenclature for components of the mammalian Prp19 complex and spliceosome proteins referred to in the text. The known protein homologues in yeast are included for reference.

	Homo Sapiens	Mus Musculus	Schizosaccharomyce	Saccharomyces
			s pombe	cerevisiae
CTNNBL1	CTNNBL1	CTNNBL1 ^{EL}	SPAC1952.06c [№]	not present
CWC15	CWC15/AD002	CWC15	Cwf15/	CWC15/
			SPBC337.06c*	YDR163W ^{NE}
CDC5L	CDC5L	CDC5L	Cdc5/	CEF1/NTC85/
			SPAC644.12*	YMR213W*
PRPF19	PRPF19/PSO4/	PRPF19 ^{EL}	Prp19/Cwf8/	PRP19/PSO4/
	SNEV		SPAC29A4.08c*	YLL036C*
PLRG1	PLRG1/PLR1	PLRG1 ^{EL}	Prp5/Cwf1/	PRP46/NTC50

			SPBP22H7.07*	YPL151C*
SPF27	SPF27/BCAS2/	SPF27/BCAS2	Cwf7/Spf27/	SNT309/NTC25/
	DAM1		SPBC28F2.04c*	YPR101W ^{NE}
PRPF8	PRPF8/RP13/p2	PRPF8/DBF3	Spp42/Cwf6/	PRP8/USA2/
	20		SPAC4F8.12c*	YHR165C*
HSP7C	HSP7C/HSC70/	HSP7C/HSPA8	multiple homologues	multiple
	HSPA8/HSP73			homologues

Official nomenclature is indicated first. * Known essential and ^{NE} known non-essential proteins. ^{EL} indicates reported mouse embryonic lethality

Supplementary Figure Legends

Figure S1. Mutants of CTNNBL1 retain their interaction with N-terminal portion of CDC5L. CDC5L[1-195] MBP fusion purified on amylose resin in the presence of wild type or mutant His-CTNNBL1[77-563] (as described in Ganesh, et al. 2014) separated by SDS-PAGE. WT, CTNNBL1[77-563]; mutant 1 [77-520], mutant 2 [D153A, N154A, T155A, D156A], mutant 3 [L321A, M322A, H359A, I362A], mutant 4 [D170A, D172A], mutant 5 [H216A, N217A], mutant 6 [Q166A], mutant 7 [E223A, N224A, Y261A], mutant 8 [E264A, I268A], mutant 9 [D315A, S319A, H359A], WT, GST control. Asterisks indicate bait of pull downs. Input proteins (lower panel) and size markers (kDa) are shown.

Figure S2. The NLS3 peptide of CDC5L is not sufficient to bind CWC15.

Pull down of recombinant human CWC15[80-229] (lanes 1 and 3) or His-CTNNBL1[77-563] (lanes 2 and 4) by GST-NLS3 (lane 1-2) or GST only as a control (lane 3-4) on glutathione sepharose 4B beads. Asterisks indicate bait of the pull down assays. Input proteins (lower panel) and size markers (kDa) are indicated.

Figure S3. Inter-protein crosslinks of CTNNBL1, CDC5L and CWC15 complexes in solution. Mobility of protein complexes separated by SDS-PAGE after crosslinking with Bis(sulfosuccinimidyl) suberate deuterated and non-deuterated crosslinking reagent mix (BS3-d4/d0) for the indicated times. The human recombinant proteins used as input are indicated on the top, while the resulting complexes are denoted on the right of each panel. Size markers (kDa) are indicated. Cross-links for: A) each protein on its own; B) a 1 to 1 molar mix of two of the proteins at a time and C) an equimolar mix of the three proteins. A faint band corresponding to the molecular weight of a dimer of the CDC5L fragment is indicated in panel A.

Figure S4. Chemical crosslinking identifies interaction sites between CTNNBL1, CDC5L and CWC15.

Graphical representation of the crosslinks found between A) recombinant human CTNNBL1 and CWC15, B) CTNNBL1 and CDC5L, and C) CWC15 and CDC5L. Crosslinks between peptides within the same protein are visualised with grey lines. Increased line width denotes the frequency in which the crosslink was detected. A surface model of CTNNBL1 is shown in gray with surface lysines coloured in yellow. Linear sequences of the CWC15[80-229] and CDC5L[1-195] fragments analysed are shown, with the aa numbering and the position of lysines indicated. Secondary structure predictions are shown, with helices and beta sheets marked with H and S in red and yellow blocks respectively. D) Coverage of the un-crosslinked peptides for each protein, with the frequency (y-axis) of each amino acid along the protein (aa) detected in the recovered peptides.

Figure. S5. Global hydrogen deuterium exchange levels over time of the different proteins and complexes in solution.

Exchange levels for peptides from A) His-CTNNBL1[77-563], B) CWC15[80-229], and C) CDC5L[1-195] alone or as part of the indicated complex. The residue start number (Start), residue end number (End), the charge state (CS), number of potential residues to be deuterated (#D), as well as the retention time (RT) are displayed for every peptide. The time points are labelled for the conditions tested. The relative level of HDX (%HDX, see SI methods) is coloured according to the gradient indicated. The data listed are the average of three independent experiments performed on the same day, with standard deviations (STDEVS) given in the columns on the right.

Figure. S6. Hydrogen deuterium exchanges over time in CTNNBL1 peptides.

Plots presenting the relative deuterium exchange (%HDX, see SI Methods) over time for CTNNBL1[77-563] peptides where the difference was considered significant. The blue curves represent the uncomplexed CTNNBL1, red are CTNNBL1 peptides from the complex of CWC15[80-229] and His-CTNNBL1[77-563], while the green curve is the exchange for the same peptides in the His-CTNNBL1[77-563]/CWC15[80-229]/CDC5L[1-195] complex. The surface model of CTNNBL1 shows the regions corresponding to the peptides. Individual colour represents non-overlapping peptides. Overlapping peptides are grouped and their position linked by the black lines

Figure S7. CTNNBL1 self-associates into a homo-trimer.

SEC-MALS analysis of full length recombinant human His-CTNNBL1[1-563] (red curve), His-CTNNBL[77-563] (green curve), His-CTNNBL[77-563] in 500 mM NaCl (black curve), and the His-CTNNBL[77-563] in complex CWC15[80-229] (blue curve), with excess CWC15 in the second smaller blue peak. Elution volume is depicted on the x-axis, the calculated molar mass in gram/mol on the left y-axis, and the refractive index in arbitrary units (a.u.) on the right y-axis.

Figure S8. CDC5L abundance is reduced but localisation is normal in CTNNBL1 deficient mouse cells.

A) Immunofluorescence for CDC5L and DAPI staining in 4% paraformaldehyde fixed *CD19-Cre Ctnnbl1*^{+/flx} controls (+/-) and *CD19-Cre Ctnnbl1*^{-/flx} (-/-) resting B cells. The signal in the enlarged box showing a single nucleus has been linearly enhanced in the (-/-) pictures to allow visualisation of the characteristic speckled appearance. B) Two independent experiments show reduced levels of CDC5L and PRP19 protein in asynchronous control (+) and Ctnnbl1 deficient (-) ES cell cultures, compared to β -tubulin. Size markers (kDa) are indicated.

Figure S9. Protein disorder predictions for full length human CWC15, CDC5L and CTNNBL1.

For each region of the protein (aa position) disorder disposition is indicated in the y-axis, with values above 0.5 predicting a disordered secondary protein structure and below structured regions as

predicted by three methods, VSL2b (5) (blue curve), VL3 (6)(red curve), VLXT (7) (grey curve) (http://www.disprot.org/).

Fig. S1





CWC15[90-229]

Fig. S3





		Global HDX levels CTNNBL1	CTNNBL1/CWC15	CTNNBL1/CWC15/CDC5L	STDEVS CTNNBL1	CTNNBL1/CWC15	CTNNBL1/CWC15/CDC5L
Start End C	S #D RT	3s/4C 3s 30s 300s 3000s	3s/4C 3s 30s 300s 3000s	3s/4C 3s 30s 300s 3000s	3s/4C 3s 30s 300s 3000s	3s/4C 3s 30s 300s 3000s	3s/4C 3s 30s 300s 3000s
69 87 3 69 87 4	3 17 6.36 - 6.40 1 17 6.27 - 6.43	6% 9% 15% 24% 30% 7% 9% 15% 24% 31%	6% 9% 16% 25% 28% 6% 9% 14% 24% 26%	7% 11% 18% 26% 29% 6% 10% 17% 24% 28%			
69 87 5	5 17 6.24 - 6.55	6% 10% 16% 24% 30%	6% 9% 15% 24% 28%	6% 10% 17% 25% 28%	2 1 1 1 2	1 0 0 1 0	0 1 0 0 2
69 88 3 69 88 4	3 18 6.04 - 6.28 1 18 6.08 - 6.27	7% 9% 15% 25% 33% 7% 10% 16% 26% 34%	6% 9% 14% 24% 30% 6% 9% 15% 25% 31%	6% 10% 17% 26% 32% 6% 10% 17% 26% 32%		1 0 0 1 1	0 1 0 0 2
69 88 5	5 18 6.11 - 6.28	6% 9% 15% 25% 34%	6% 9% 15% 25% 31%	6% 11% 18% 26% 33%	1 1 1 1 1	0 0 0 1 0	0 1 0 0 2
88 98 2	2 9 3.76 - 3.96	1% 2% 10% 32% 47%	2% 2% 3% 6% 22%	5% 2% 3% 5% 19%	0 0 1 1 3	1 0 0 0 1	1 0 1 0 2
89 99 2	s 9 3.76 - 3.96 2 9 4.75 - 4.93	1% 2% 10% 33% 48%	1% 2% 2% 5% 18%	2% 2% 2% 4% 15%	0 0 1 1 3	0 0 0 0 0	0 0 1 1 2 0 0 1 0 n/a
99 110 3	8 8.35 - 8.55	9% 15% 25% 50% 56%	3% 8% 15% 17% 25%	3% 8% 15% 16% 21%	3 1 1 1 0	0 0 0 1 1	0 1 1 0 2
99 114 3 100 110 2	3 12 10.00 - 10.09 2 7 7.59 - 7.79	20% 26% 39% 62% 68% 10% 18% 30% 57% 62%	14% 19% 26% 32% 39% 3% 10% 18% 22% 31%	14% 18% 26% 31% 36% 3% 10% 19% 20% 27%	3 1 1 1 2 3 1 1 1 2		
100 110 3	3 7 7.61 - 7.74	11% 19% 31% 58% 63%	4% 10% 19% 23% 31%	3% 10% 19% 21% 28%	4 1 1 1 3	0 0 0 1 1	0 0 1 0 2
100 115 2	2 12 9.84 - 10.07	20% 26% 39% 62% 69%	14% 19% 26% 32% 39% 14% 19% 26% 32% 38%	14% 18% 26% 31% 36% 14% 18% 26% 30% 36%	3 1 1 1 2	1 0 0 0 0	
118 124 1	1 5 9.37 - 9.59	1% 2% 2% 2% 15%	1% 2% 3% 2% 2%	0% 3% 2% 2% 3%	1 1 0 0 2	0 1 1 0 0	0 1 1 1 1
118 133 3	3 13 11.09 - 11.38	2% 3% 10% 13% 31%	2% 3% 10% 16% 29%	2% 6% 15% 21% 31%	2 0 1 0 1	0 0 0 0 1	0 0 0 1 1
120 124 1	1 3 7.18 - 7.44 2 11 9.91 - 10.09	0% 1% 1% 2% 13% 2% 3% 10% 14% 31%	0% 1% 3% 3% 3% 2% 2% 10% 18% 34%	0% 1% 2% 2% 5% 3% 6% 17% 22% 37%	2 0 0 0 1	1 0 1 1 2	1 0 0 1 1 1 1 1 0 1
125 133 2	2 6 8.49 - 8.78	2% 3% 5% 8% 26%	2% 2% 5% 14% 29%	2% 4% 12% 18% 36%	1 0 0 1 2	0 0 0 0 1	0 0 0 0 1
125 140 3	3 13 11.63 - 11.89 8 11.79 - 11.95	3% 3% 5% 13% 34% 7% 3% 4% 15% 36%	3% 3% 5% 15% 36%	3% 3% 8% 20% 40% 5% 5% 9% 21% 40%			0 0 1 2 2
141 160 2	2 18 11.50 - 11.66	4% 8% 18% 24% 29%	2% 9% 19% 23% 28%	2% 9% 20% 23% 30%	4 0 1 1 1	0 0 0 1 0	0 0 0 0 1
147 158 2	2 10 7.88 - 8.00	2% 3% 8% 10% 14%	2% 4% 8% 9% 12%	1% 3% 8% 8% 11%	2 0 1 1 2	0 0 0 0	0 0 1 0 1
147 160 2	2 12 8.95 - 9.11 2 13 9.53 - 9.71	3% 4% 11% 15% 19%	2% 5% 11% 12% 15%	2% 6% 11% 12% 15%	3 0 0 0 1	1 0 0 0 0	0 2 0 0 1
148 159 2	2 10 7.89 - 8.00	2% 3% 8% 10% 14%	2% 4% 8% 9% 12%	1% 3% 8% 8% 11%	2 0 1 1 2	0 0 0 0 0	0 0 1 0 1
160 164 1	1 3 8.38 - 8.63 7 14 11 38 - 11 51	2% 3% 4% 10% 15% 2% 10% 23% 30% 37%	4% 6% 6% 8% 15% 2% 10% 24% 30% 35%	6% 6% 6% 6% 12% 2% 11% 24% 29% 37%			1 1 3 2 0
165 170 1	4 6.27 - 6.55	0% 1% 1% 0% 0%	0% 0% 1% 1% 1%	1% 0% 1% 0% 1%	0 0 0 0 1	0 0 0 2 1	1 0 0 0 1
165 173 1	L 7 8.18 - 8.45	3% 2% 2% 8% 24%	3% 3% 13% 18% 27% 3% 7% 15% 23% 25%	2% 8% 17% 19% 32% 4% 11% 21% 21% 27%	4 1 1 1 1		1 1 3 2 1
171 184 2	2 12 8.30 - 8.45	3% 9% 15% 24% 40%	5% 13% 24% 36% 42%	9% 22% 36% 43% 50%	0 1 1 1 2	0 2 2 2 3	0 1 0 0 4
183 196 2	2 12 11.28 - 11.44	1% 1% 2% 6% 19%	1% 1% 2% 7% 22%	0% 1% 5% 11% 28%	0 0 0 0	0 0 0 0	0 1 1 0 2
185 196 2	2 10 9.96 - 10.20	0% 0% 1% 4% 21%	0% 1% 1% 6% 22%	-1% 1% 2% 8% 27%	0 0 0 0 0	0 0 0 0 0	0 0 0 0 1
197 206 2	2 8 7.64 - 7.81	1% 1% 4% 10% 26%	2% 2% 4% 11% 27%	2% 2% 4% 8% 26%	1 0 0 1 1	0 1 1 0 1	0 1 1 1 3
197 220 3	3 22 9.16 - 9.30 1 3 4.19 - 4.45	7% 9% 18% 34% 48% 2% 3% 6% 12% 19%	4% 5% 16% 28% 44%	5% 8% 15% 23% 42% 7% 7% 9% 6% 12%	2 0 1 0 1	1 2 0 0 1	1 1 4 0 2
202 219 3	3 16 7.47 - 7.64	9% 12% 22% 39% 48%	7% 11% 19% 31% 43%	7% 10% 18% 26% 40%	3 0 1 0 1	0 0 0 0 0	0 1 0 0 2
202 220 3	3 17 7.42 - 7.61 11 5.36 - 5.89	9% 11% 21% 38% 48% 10% 13% 20% 34% 46%	7% 10% 18% 30% 42% 9% 12% 17% 25% 38%	7% 10% 17% 24% 39% 8% 11% 17% 20% 34%	2 0 0 1 1		
207 219 3	3 11 5.37 - 5.92	10% 13% 20% 34% 46%	9% 12% 17% 26% 38%	9% 12% 18% 21% 35%	2 0 1 1 2	1 0 1 1 0	0 0 1 1 2
221 225 1	1 3 6.04 - 6.38	1% 1% 2% 0% -1%	2% 3% 2% 2% 2%	2% 1% 2% 1% 0%	2 1 1 1 1	2 2 0 1 2	2 0 1 1 1
221 22/ 1 226 232 1	L 5 5.70 - 6.06 L 4 7.69 - 7.82	1% 2% 2% 2% 3% 12% 20% 25% 36% 44%	2% 2% 2% 4% 7% 8% 19% 26% 43% 48%	1% 2% 2% 2% 5% 8% 20% 28% 38% 49%	7 1 1 1 2	1 0 0 1 1 1 1 1 4 1	0 0 1 1 0
226 232 2	2 4 7.68 - 7.82	14% 20% 25% 38% 45%	10% 21% 27% 41% 47%	10% 21% 29% 40% 49%	7 1 1 1 2	0 0 1 1 1	1 1 2 0 5
227 232 1	1 3 7.65 - 7.88	19% 27% 35% 44% 52% 11% 28% 34% 49% 50%	12% 24% 34% 47% 54% 17% 28% 35% 54% 61%	14% 25% 34% 48% 57% 10% 27% 34% 53% 57%	10 3 1 5 3		
233 260 3	3 25 10.87 - 11.03	11% 15% 24% 33% 41%	10% 14% 25% 35% 39%	9% 14% 25% 32% 40%	3 1 2 1 3	1 1 0 2 1	1 1 1 0 2
233 260 4	25 10.86 - 11.12	11% 15% 24% 33% 41%	9% 14% 23% 34% 38%	9% 14% 22% 29% 38%	3 1 2 1 3	0 0 1 1 1	0 0 1 0 2
261 266 1	4 12 0.04 - 0.81 L 4 8.55 - 8.80	3% 2% 3% 3% 6%	18% 23% 29% 37% 40% 5% 3% 4% 6% 7%	5% 4% 5% 2% 8%	2 2 2 1 2 2 1 2 2 1 0 0 1	0 1 2 4 2	n/a 0 2 2 n/a
268 280 2	2 11 9.27 - 9.46	2% 3% 3% 6% 12%	2% 4% 6% 10% 14%	2% 3% 4% 7% 11%	0 0 0 1 1	1 1 0 1 1	0 1 1 2 1
268 283 2	2 14 10.30 - 10.50 1 4 3.92 - 4.15	1% 2% 3% 6% 13% 30% 41% 53% 54% 58%	1% 1% 3% 7% 14% 24% 39% 49% 56% 54%	1% 1% 3% 5% 11% 23% 36% 50% 53% 56%	9 3 3 1 7		2 2 2 2 6
270 280 2	2 9 7.97 - 8.13	2% 3% 4% 7% 9%	2% 3% 4% 8% 9%	1% 2% 4% 7% 9%	1 1 1 1 1	1 1 0 1 1	0 0 1 0 1
270 283 2	2 12 9.61 - 9.84 2 16 9.79 - 10.27	2% 1% 3% 5% 10% 1% 1% 4% 12% 19%	2% 1% 2% 6% 12%	2% 2% 2% 5% 10% 1% 1% 4% 12% 18%			
270 289 2	2 18 11.14 - 11.33	1% 1% 2% 8% 16%	1% 1% 3% 10% 16%	0% 1% 3% 9% 16%	0 0 0 0 2	1 0 0 0 1	0 0 0 0 2
276 283 2	2 6 9.34 - 9.49	2% 2% 2% 2% 9%	2% 2% 2% 3% 12%	1% 1% 2% 2% 11%		2 1 1 0 1	
284 289 1 288 295 1	1 4 9.63 - 9.80 1 6 9.82 - 10.05	1% 2% 11% 17% 25%	1% 2% 13% 19% 26%	0% 4% 15% 17% 27%	1 0 0 0 1		
288 313 4	4 23 9.84 - 10.12	5% 7% 11% 15% 23%	5% 8% 14% 18% 26%	3% 6% 13% 15% 19%	1 1 1 1 2	1 1 1 0 1	0 0 1 1 1
290 309 3 290 313 4	3 17 7.21 - 7.54 1 21 9.06 - 9.29	8% 12% 20% 25% 30% 5% 7% 11% 14% 21%	7% 13% 22% 26% 32% 8% 10% 15% 18% 27%	6% 9% 21% 23% 26% 6% 8% 13% 15% 20%	2 1 1 1 2		1 1 1 1 2 0 1 1 1 n/a
296 306 2	2 8 3.39 - 3.70	22% 27% 31% 33% 38%	21% 26% 32% 34% 38%	19% 25% 32% 31% 34%	3 0 1 1 2	1 1 1 1 0	1 1 1 0 3
321 338 3	3 16 8.95 - 9.06	6% 8% 14% 24% 32%	6% 8% 16% 27% 34%	5% 8% 16% 24% 33%	1 0 1 1 3	0 0 1 1 2	0 0 1 1 3
324 338 3	3 13 8.06 - 8.38	7% 10% 17% 31% 42%	7% 10% 22% 35% 43%	7% 10% 21% 31% 45%	1 1 1 2 2	0 0 1 1 1	0 1 0 1 n/a
342 353 3	3 10 3.56 - 3.75	19% 25% 30% 36% 40%	16% 24% 29% 38% 37%	8% 21% 27% 32% 39%	4 1 2 1 3	1 1 1 2 2	1 2 1 1 4
342 354 2	2 11 4.48 - 4.63 3 11 4.49 - 4.62	11% 15% 19% 24% 32%	10% 14% 18% 26% 30%	5% 13% 17% 20% 27%	2 1 2 1 3	1 1 1 1 1 1	0 1 1 1 3
342 378 5	5 34 8.76 - 8.92	3% 5% 8% 10% 16%	4% 5% 7% 10% 15%	2% 5% 7% 8% 13%	1 1 1 0 2	0 0 0 0 1	0 0 1 0 1
342 379 5	5 35 9.47 - 9.74 5 35 9.55 - 9.76	4% 7% 10% 12% 18% 4% 6% 9% 12% 17%	3% 5% 9% 11% 16%	2% 4% 7% 9% 14% 2% 4% 7% 9% 14%	1 1 1 0 2		
343 353 3	3 9 3.63 - 3.81	17% 24% 27% 31% 37%	15% 22% 25% 32% 37%	7% 18% 22% 25% 33%	2 2 2 2 2	2 1 1 1 1	0 1 1 2 5
343 354 3	3 10 3.92 - 4.50	13% 19% 22% 26% 33%	12% 15% 21% 27% 31%	6% 15% 17% 20% 28%	3 2 2 1 2	1 1 1 1 1	0 2 1 1 2
343 379 0	5 34 9.54 - 9.78 2 9 3.65 - 3.82	17% 23% 26% 30% 37%	476 576 876 1176 1676 15% 21% 23% 32% 36%	7% 17% 21% 24% 29%	2 2 2 1 2	2 1 1 1 1	0 2 2 3 3
344 354 3	3 9 3.63 - 3.82	17% 24% 27% 31% 38%	15% 22% 25% 33% 36%	7% 18% 22% 25% 33%	2 2 2 2 2 2	2 1 1 1 1	0 1 1 2 5
354 379 3	3 23 10.33 - 10.62 1 23 10.35 - 10.59	3% 5% 8% 10% 14% 3% 5% 8% 11% 15%	2% 3% 7% 9% 13% 2% 4% 7% 10% 14%	1% 3% 6% 8% 13% 1% 3% 6% 8% 13%	1 0 1 0 1		
355 373 3	3 16 7.41 - 7.61	9% 12% 22% 40% 51%	8% 11% 19% 32% 45%	7% 10% 18% 26% 42%	2 0 0 1 2	0 0 0 0 0	0 1 1 0 2
355 377 4	4 20 9.11 - 9.24 1 21 8.95 - 9.07	2% 4% 7% 8% 13% 3% 4% 7% 10% 21%	3% 4% 6% 8% 13% 2% 3% 7% 11% 13%	2% 4% 6% 7% 13% 1% 3% 7% 10% 13%			
355 379 2	2 22 10.16 - 10.61	3% 6% 10% 12% 17%	2% 4% 8% 11% 16%	2% 3% 7% 10% 15%	1 0 1 0 1	0 0 0 0 0	0 0 1 0 1
355 379 3	3 22 10.18 - 10.42 1 22 10.16 - 10.41	3% 6% 9% 12% 16%	2% 4% 8% 10% 15%	2% 3% 7% 9% 15% 7% 3% 7% 10% 15%	1 0 1 0 1		
371 379 2	2 7 10.30 - 10.41	4% 8% 13% 19% 27%	2% 5% 12% 16% 26%	2% 3% 6% 13% 22%	2 0 0 1 1	1 0 1 0 1	0 0 1 0 1
378 386 2	2 6 11.77 - 11.97	2% 3% 7% 11% 30% 1% 1% 2% 5% 32%	1% 2% 5% 10% 19% 1% 1% 1% 2% 0%	1% 1% 3% 6% 13%			
380 386 1	L 4 11.37 - 11.58	-1% 1% 1% 4% 22%	-1% 0% 1% 0% 5%	-1% 0% 1% 0% 1%	0 0 0 0 1	0 0 0 0 0	0 0 1 0 1
380 386 2	2 4 11.41 - 11.62	1% 1% 1% 5% 23%	1% 1% 1% 2% 7%	1% 1% 1% 1% 2%	0 0 0 0 1	0 0 0 0 0	0 0 1 0 0
387 411 5	3 22 4.92 - 5.23	14% 16% 17% 18% 22%	14% 15% 17% 19% 20%	10% 14% 16% 16% 17%	1 1 2 0 1	1 0 1 1 1	1 1 1 1 1
387 411 4	4 22 4.92 - 5.47	13% 17% 18% 17% 22%	14% 16% 17% 19% 21%	10% 14% 17% 17% 18%	1 2 1 1 3		0 1 1 0 2
30/ 411 5 415 426 2	, 22 4.92 - 5.37 2 10 4.21 - 4.38	12% 17% 27% 31% 34%	11% 16% 24% 30% 33%	10% 15% 10% 10% 17% 11% 17% 25% 28% 35%	2 1 1 1 0		1 1 0 1 1 1 1 0 1 3
415 441 4	4 25 6.39 - 6.69	2% 4% 9% 15% 20%	2% 3% 7% 14% 18%	2% 3% 8% 13% 19%	1 0 1 1 2	1 0 0 1 0	0 0 0 1 1
415 441 5	5 25 6.47 - 6.57 5 25 6.49 - 6.71	2% 3% 9% 15% 20% 2% 3% 9% 15% 19%	2% 3% 7% 15% 17% 2% 3% 7% 14% 17%	2% 3% 8% 14% 18%	1 0 1 1 3		
427 436 2	2 8 4.95 - 5.47	11% 11% 23% 37% 43%	9% 9% 18% 35% 37%	8% 10% 19% 35% 40%	2 1 3 1 5	1 0 2 1 1	0 1 1 1 5
427 441 2	2 13 6.44 - 6.78 3 13 6.44 - 6.83	2% 3% 9% 20% 23% 2% 3% 9% 20% 23%	4% 5% 9% 22% 27% 4% 4% 9% 27% 27%	3% 4% 10% 19% 25% 3% 4% 9% 19% 25%		1 U O 1 O 1 O O 1 1	
427 441 4	4 13 6.47 - 6.77	2% 3% 9% 20% 24%	4% 5% 9% 22% 27%	3% 4% 10% 20% 25%	1 0 0 1 1	1 0 0 1 0	0 0 0 0 1
428 441 3	3 12 6.44 - 6.59 9 5 70 - 5 74	2% 3% 10% 21% 24% 2% 3% 10% 21% 25%	5% 5% 9% 23% 28% 3% 4% 7% 17% 33%	4% 4% 10% 21% 28% 3% 3% 7% 14% 33%			0 0 0 0 2
442 451 2	2 8 9.13 - 9.29	2% 4% 13% 16% 16%	2% 4% 14% 16% 16%	n/a 4% 14% 17% 19%	0 0 0 0 1	0 0 0 1 0	n/a 0 0 1 1
442 481 5	5 38 8.93 - 9.07	9% 36% 56% 55% 58%	6% 35% 55% 56% 56%	6% 22% 50% 54% 57%	4 0 1 1 2		0 1 1 0 3
452 480 3	3 27 5.87 - 6.12	8% 39% 55% 54% 55%	7% 38% 53% 55% 54%	8% 23% 51% 54% 56%	0 1 2 0 3	1 0 1 1 0	0 1 1 1 4
452 481 3	3 28 7.18 - 7.34	8% 41% 58% 57% 58%	8% 39% 57% 58% 57%	8% 25% 53% 57% 59%	0 2 1 1 3	1 0 0 1 1	0 1 2 1 2
453 481 3 481 490 7	2 / /.u3 - /.26 2 8 9.02 - 9.32	2% 14% 42% 49% 52%	1% 13% 42% 50% 49%	4% 6% 17% 34% 54%			0 1 1 0 4 1 2 1 1 n/a
481 490 3	8 9.17 - 9.20	1% 15% 45% 52% 54%	1% 14% 44% 53% 51%	1% 3% 16% 33% 52%	1 1 3 2 5	0 0 2 3 2	0 0 0 1 5
482 490 2 483 490 7	2 7 7.54 - 7.70 2 6 9.12 - 9.34	1% 17% 46% 52% 54% 1% 22% 54% 60% 63%	1% 16% 44% 52% 49% 2% 19% 52% 61% 60%	1% 4% 19% 34% 49% 2% 4% 23% 43% 63%	1 1 2 2 4 1 2 3 7 5	0 0 1 2 2 1 1 2 7 7	0 0 1 1 5 0 0 0 7 3
491 500 2	2 8 11.25 - 11.45	0% 0% 0% 0% 2%	0% 0% 0% 0% 2%	0% 0% 0% 0% 0%	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
493 498 1	1 4 9.66 - 9.95 1 4 7.67 - 7.84	0% -1% 0% -1% 1% 8% 14% 18% 25% 20%	0% -1% 0% 0% 1% 10% 14% 19% 27% 22%	1% 0% 0% -1% -1% 6% 13% 19% 26% 20%			
499 521 3	3 20 10.23 - 10.41	10% 15% 25% 36% 48%	9% 15% 26% 37% 47%	9% 15% 23% 32% 45%	2 1 1 0 2	1 0 0 0 1	0 1 1 1 3
503 519 3	3 14 7.81 - 8.24 16 9.40 0.50	9% 13% 19% 29% 41% 9% 13% 25% 27% 50%	10% 13% 19% 32% 40%	11% 13% 19% 27% 38%	1 1 1 2 2	0 1 1 1 2	
505 521 3	2 14 8.98 - 9.10	7% 11% 24% 36% 52%	6% 12% 25% 38% 52%	5% 11% 24% 36% 51%	2 1 1 2 4		
522 535 4	4 12 4.75 - 5.02	16% 20% 27% 53% 63%	15% 20% 30% 57% 60%	14% 18% 22% 36% 60%	1 0 1 1 3	1 0 0 1 1	1 1 0 1 4
522 535 5 522 539 3	5 12 4.78 - 4.97 3 16 7.11 - 7.30	16% 20% 28% 52% 62% 12% 17% 30% 61% 71%	15% 20% 30% 56% 59% 12% 17% 34% 66% 59%	15% 18% 22% 35% 60% 11% 15% 21% 38% 65%	2 0 1 0 2 2 1 1 0 4	1 1 1 2 1 1 0 1 1 1	
522 563 6	5 39 10.09 - 10.37	13% 20% 38% 58% 64%	12% 19% 40% 59% 60%	11% 16% 29% 47% 63%	2 1 2 0 3	1 1 1 1 1	1 1 1 0 3
526 535 2 526 535 3	2 8 4.59 - 4.75 3 8 4.64 - 4.65	2% 4% 10% 30% 41% 2% 4% 10% 30% 41%	2% 3% 12% 35% 39% 2% 3% 12% 35% 39%	2% 3% 5% 17% 40% 1% 2% 4% 16% 38%		1 0 0 2 1 0 0 0 1 1	
526 548 3	3 20 8.32 - 8.55	17% 22% 36% 57% 64%	15% 20% 36% 59% 61%	12% 15% 24% 41% 60%	2 1 1 1 3	1 1 1 0 1	0 0 1 1 3
536 548 2	2 10 8.12 - 8.35	27% 33% 41% 41% 42%	26% 31% 39% 42% 40%	22% 25% 33% 38% 42%	3 2 2 1 3	2 1 2 2 1	2 2 1 1 4
540 548 2	2 6 7.47 - 7.64	34% 42% 44% 42% 43%	32% 38% 40% 41% 40%	32% 39% 41% 40% 42%	2 2 2 1 2	2 1 1 1 1	1 2 1 1 3
540 559 4	17 7.81 - 8.15	7% 11% 26% 42% 44%	6% 10% 27% 44% 43%	7% 12% 21% 36% 38%	1 1 2 1 3		1 1 3 1 3
549 559 3	3 9 7.25 - 7.43	2% 5% 31% 60% 64%	2% 5% 33% 60% 61%	2% 3% 19% 51% 65%	1 0 1 2 4	0 0 1 1 2	0 0 0 2 4
549 560 3	3 10 8.84 - 9.12	3% 6% 35% 65% 68%	2% 6% 40% 67% 67%	1% 4% 25% 58% 71%	1 1 2 0 3	1 1 3 1 0	0 1 1 1 4
349 563 3	3 13 10.45 - 10.69 3 13 10.45 - 10.70	11% 18% 48% 72% 75%	8% 18% 52% 74% 74%	9% 16% 42% 68% 77%		1 0 1 0 0	

Relative deuteration % 10% 20% 30% 40% 50% 60%

Fig. S5-A

Fig. S5-B

	Global HDX levels				STDEVS					
	CWC15	CWC15/CTNNBL1	CWC15/CDC5L	CWC15/CDC5L/CTNNBL1	CWC15	CWC15/CTNNBL1	CWC15/CDC5L	CWC15/CDC5L/CTNNBL1		
Start End CS #D RT	3s/4C 3s 30s 300s 3000s	3s/4C 3s 30s 300s 300os	3s/4C 3s 30s 300s 3000s							
75 95 3 18 4.36 - 4.60	30% 29% 30% 28% 29%	30% 27% 27% 28% 26%	27% 28% 27% 27% 28%	27% 26% 26% 26% 27%	1% 1% 1% 1% 1%	3% 1% 1% 1% 1%	0% 0% 1% 0% 1%	1% 1% 1% 1% 2%		
75 95 4 18 4.33 - 4.64	30% 29% 30% 28% 29%	30% 27% 27% 28% 26%	27% 28% 28% 28% 28%	27% 27% 27% 26% 27%	1% 1% 0% 1% 1%	3% 1% 1% 2% 1%	0% 0% 1% 0% 1%	1% 2% 1% 1% 2%		
75 95 5 18 4.32 - 4.65	30% 29% 30% 28% 29%	30% 28% 27% 29% 27%	28% 28% 28% 28% 28%	27% 26% 27% 26% 27%	1% 2% 1% 1% 1%	4% 1% 1% 2% 1%	0% 1% 1% 0% 1%	1% 1% 1% 1% 2%		
78 95 4 15 4.31 - 4.48	34% 34% 34% 33% 34%	34% 32% 32% 32% 32%	31% 32% 32% 32% 32%	33% 32% 31% 31% 31%	1% 2% 1% 1% 2%	3% 2% 1% 1% 1%	1% 0% 1% 0% 1%	2% 1% 1% 1% 3%		
96 103 1 5 7.80 - 7.99	69% 70% 72% 70% 72%	69% 71% 70% 70% 67%	70% 74% 74% 74% 74%	68% 68% 69% 69% 75%	1% 1% 1% 1% 1%	1% 1% 3% 1% 2%	2% 1% 0% 1% 1%	3% 1% 0% 1% 1%		
104 117 2 11 9.82 - 10.37	23% 31% 32% 30% 31%	24% 29% 29% 30% 28%	26% 32% 31% 31% 32%	24% 30% 30% 28% 30%	1% 2% 1% 1% 2%	1% 1% 1% 1% 1%	1% 1% 1% 1% 1%	2% 1% 1% 1% 3%		
118 129 2 10 6.80 - 7.02	24% 32% 33% 31% 32%	24% 30% 30% 31% 29%	25% 32% 33% 34% 34%	19% 28% 32% 32% 33%	1% 1% 1% 1% 1%	2% 1% 1% 1% 1%	1% 0% 1% 1% 1%	1% 1% 1% 1% 4%		
130 155 3 24 4.59 - 4.70	21% 75% 79% 78% 79%	19% 75% 77% 79% 78%	16% 72% 78% 78% 78%	10% 56% 76% 77% 80%	1% 2% 1% 1% 1%	0% 2% 1% 1% 0%	1% 1% 1% 1% 1%	1% 2% 1% 1% 5%		
130 160 4 29 5.22 - 5.91	22% 82% 87% 86% 87%	19% 83% 85% 86% 86%	17% 81% 86% 86% 87%	12% 65% 85% 86% 89%	0% 2% 0% 0% 1%	0% 1% 1% 2% 0%	1% 1% 0% 0% 0%	1% 2% 0% 1% 4%		
156 172 2 14 11.08 - 11.34	48% 60% 62% 60% 61%	45% 57% 57% 59% 57%	45% 59% 60% 59% 60%	41% 54% 57% 56% 59%	1% 2% 1% 1% 2%	3% 1% 1% 2% 1%	2% 0% 1% 1% 1%	2% 2% 1% 1% 3%		
156 182 3 22 10.89 - 11.05	52% 60% 61% 59% 60%	48% 55% 55% 57% 54%	49% 59% 59% 60% 59%	43% 50% 51% 52% 55%	1% 2% 2% 1% 2%	2% 2% 2% 2% 1%	1% 1% 1% 1% 2%	3% 2% 1% 0% 3%		
161 172 2 9 11.59 - 11.86	55% 60% 62% 60% 61%	52% 57% 57% 59% 57%	53% 61% 61% 61% 61%	48% 55% 57% 56% 58%	3% 2% 1% 2% 1%	2% 2% 1% 2% 1%	2% 1% 1% 1% 1%	2% 2% 1% 1% 4%		
161 182 2 17 11.18 - 11.39	56% 59% 61% 58% 60%	53% 54% 54% 55% 52%	55% 60% 60% 60% 61%	47% 51% 52% 51% 53%	1% 2% 2% 1% 2%	3% 2% 2% 2% 2%	2% 1% 1% 1% 1%	2% 2% 2% 2% 5%		
161 182 3 17 11.20 - 11.42	57% 60% 61% 59% 60%	53% 54% 53% 55% 52%	55% 61% 60% 60% 61%	47% 51% 52% 50% 53%	1% 2% 2% 1% 2%	4% 2% 2% 2% 2%	2% 1% 1% 1% 1%	2% 3% 2% 2% 5%		
161 194 4 29 10.47 - 10.68	49% 52% 53% 51% 52%	44% 46% 46% 48% 45%	46% 51% 51% 52% 52%	41% 44% 46% 46% 48%	1% 2% 1% 1% 2%	2% 2% 1% 2% 2%	2% 1% 1% 1% 1%	2% 2% 2% 1% 3%		
182 194 3 11 8.24 - 8.46	40% 41% 42% 41% 42%	37% 38% 37% 39% 37%	40% 45% 44% 42% 42%	39% 40% 40% 38% 39%	0% 1% 1% 0% 1%	2% 1% 1% 1% 1%	1% 2% 2% 1% 1%	3% 1% 1% 1% 3%		
183 194 2 10 7.86 - 8.12	32% 34% 35% 34% 35%	30% 33% 32% 34% 32%	31% 35% 34% 35% 35%	29% 32% 33% 33% 33%	1% 1% 1% 1% 1%	2% 1% 1% 1% 0%	1% 1% 1% 1% 1%	1% 2% 1% 1% 3%		
183 194 3 10 7.88 - 8.11	32% 34% 35% 34% 35%	30% 33% 33% 34% 32%	32% 36% 35% 35% 35%	29% 33% 34% 33% 35%	1% 1% 1% 1% 1%	2% 1% 1% 1% 1%	1% 1% 1% 1% 1%	1% 2% 1% 1% 2%		
195 213 3 17 3.73 - 3.85	46% 45% 46% 44% 46%	46% 44% 44% 45% 43%	45% 47% 46% 46% 46%	44% 44% 45% 44% 45%	1% 2% 1% 1% 1%	3% 2% 1% 1% 1%	1% 1% 1% 1% 1%	1% 2% 1% 1% 4%		
195 213 4 17 3.69 - 3.99	45% 45% 45% 44% 45%	45% 44% 43% 45% 42%	44% 46% 45% 45% 45%	44% 43% 44% 43% 45%	1% 2% 1% 1% 1%	3% 2% 1% 2% 1%	1% 1% 1% 1% 1%	1% 2% 1% 1% 4%		
195 215 2 19 4.81 - 5.01	48% 49% 49% 47% 49%	47% 46% 46% 47% 45%	47% 49% 48% 48% 49%	46% 45% 47% 46% 47%	1% 2% 2% 1% 2%	1% 2% 1% 1% 2%	2% 1% 1% 1% 2%	1% 2% 2% 1% 4%		
195 215 3 19 5.02 - 5.11	49% 48% 49% 48% 49%	47% 47% 46% 48% 46%	47% 49% 48% 48% 49%	46% 46% 47% 46% 48%	1% 2% 1% 1% 1%	2% 1% 1% 2% 1%	1% 0% 1% 1% 1%	1% 2% 1% 1% 4%		
195 215 4 19 4.81 - 5.18	48% 50% 49% 47% 48%	48% 47% 47% 48% 46%	47% 48% 48% 48% 48%	47% 47% 48% 46% 47%	1% 1% 1% 1% 2%	2% 1% 1% 1% 1%	2% 1% 1% 1% 1%	1% 2% 1% 1% 5%		
195 219 4 23 6.53 - 6.82	56% 55% 56% 56% 55%	52% 54% 54% 55% 54%	54% 55% 55% 55% 55%	50% 52% 54% 53% 55%	0% 2% 1% 0% 1%	1% 1% 1% 1% 0%	1% 1% 0% 0% 1%	1% 2% 0% 1% 4%		
195 219 5 23 6.49 - 6.66	56% 56% 57% 56% 57%	53% 55% 54% 55% 54%	55% 56% 56% 56% 57%	51% 53% 54% 54% 56%	0% 1% 1% 0% 1%	1% 1% 1% 1% 1%	1% 1% 1% 0% 1%	1% 2% 0% 1% 4%		
214 229 4 14 6.81 - 6.99	46% 50% 53% 51% 52%	26% 43% 53% 53% 51%	44% 48% 50% 50% 52%	21% 31% 49% 50% 51%	1% 2% 1% 1% 1%	1% 0% 2% 1% 1%	1% 1% 1% 1% 1%	0% 1% 1% 2% 3%		
216 229 3 12 4.92 - 5.42	34% 37% 41% 40% 41%	12% 28% 39% 40% 39%	33% 38% 40% 40% 40%	9% 16% 36% 39% 40%	0% 1% 1% 0% 1%	2% 1% 1% 1% 1%	1% 0% 1% 1% 0%	0% 1% 0% 1% 3%		
216 229 4 12 4.92 - 5.34	34% 38% 41% 40% 41%	13% 26% 39% 40% 39%	33% 38% 40% 40% 40%	9% 16% 36% 39% 40%	1% 1% 1% 0% 1%	1% 1% 1% 1% 0%	1% 0% 1% 0% 0%	2% 0% 0% 1% 2%		
216 229 5 12 4.93 - 5.34	35% 37% 41% 40% 41%	13% 29% 39% 40% 38%	32% 38% 40% 40% 40%	10% 16% 37% 39% 40%	0% 0% 1% 0% 2%	2% 1% 1% 1% 2%	2% 1% 1% 1% 1%	2% 1% 0% 1% 2%		
220 229 2 8 4.58 - 4.81	38% 43% 47% 45% 47%	15% 33% 44% 46% 45%	38% 44% 47% 48% 48%	11% 19% 42% 45% 47%	1% 2% 1% 1% 1%	1% 1% 1% 2% 1%	1% 0% 1% 1% 1%	0% 1% 1% 1% 4%		
220 229 4 8 4.53 - 4.81	39% 44% 47% 46% 47%	16% 33% 45% 47% 45%	38% 45% 48% 48% 49%	12% 18% 43% 45% 47%	1% 2% 1% 1% 1%	1% 1% 1% 1% 1%	1% 1% 1% 1% 1%	1% 1% 1% 1% 3%		

Relative deuteration % 10% 20% 30% 40% 50% 60% 70% 80%

Fig. S5-C

				Globa	I HDX	levels														STDEV	STDEVS																	
					CDC5L			CDC5L					CDC5L/CWC15					C5L/CV	VC15,	CTNN/	BL1		CDC5L	-				CDC5L	/cwc	15			CDC5L/CWC15/CTNNBL1					
Start	End	CS	#D	R	г	3s/4C	3s	30s	300s	3000s		3s/4C	3s	30s	3009	3000s	3s/	4C 3	3s	30s	300s	3000s	3s/4C	3s	30s	3009	s 3000s	3s/4C	3s	30s	300s	3000s	3s/4C	3s	30s	300s	3000s	
11	19	2	7	8.83 -	9.10	3%	9%	24%	35%	37%		4%	9%	22%	37%	39%	6	% 8	%	17%	29%	37%	0	1	1	1	1	0	0	2	1	1	0	1	1	1	4	
18	24	2	5	6.68 -	6.91	0%	9%	62%	80%	82%		1%	6%	45%	82%	83%	3	% θ	%	25%	63%	71%	0	1	1	1	2	1	1	3	2	1	1	2	3	0	n/a	
20	37	4	16	7.72 -	8.05	19%	31%	58%	61%	62%		18%	28%	50%	63%	65%	13	% 2	4%	41%	59%	66%	1	2	1	2	1	0	1	2	1	0	0	2	1	1	4	
20	38	4	17	9.42 -	9.75	19%	30%	59%	64%	65%		17%	26%	49%	65%	66%	12	% 2	2%	40%	60%	68%	0	1	1	0	0	1	0	1	0	1	0	1	1	1	4	
22	37	3	14	8 32 -	8.63	20%	31%	56%	59%	60%		18%	28%	49%	62%	62%	13	% 2	3%	40%	57%	63%	1	2	1	1	1	1	ñ	2	ő	1	0	1	1	1	4	
22	37	4	14	834 -	8.65	20%	31%	56%	59%	60%		18%	27%	49%	61%	62%	13	% 2	3%	40%	57%	63%	1	1	2	1	2	1	ő	2	1	1	0	1	1	1	4	
22	38	2	15	9.93	10.20	20%	30%	58%	61%	63%		17%	26%	49%	63%	64%	12	% 2	2%	38%	57%	61%	1	2	1	1	1	1	0	2	0	1	1	1	1	1	1	
22	50	6	27	7.61	7 70	1.0%	17%	26%	27%	20%		17%	17%	2.4%	/2%	4.4%		/0 2.	2%	25%	27%	42%	0	1	1	0	1	1	1	1	0	1	0	1	-	1	4	
22	27	2	12	0.00 -	7.75	2.29/	2.49/	6.0%	6.29/	55%		209/	20%	54/0	67%	6.7%	1/	a/ 21	2 /0	439/	6.7%	42/0	1	2	1	0	1	1	1	2	0	1	0	1	0	1	4	
24	37	2	12	0.20 -	0.39	2270	34%	E 20/	EE0/	E 70/		20%	30%	33%	E 99/	E 09/	14	70 Z:	570 50/	42%	C3%	E 00/	1	2	1	1	2	1	1	2	1	1	0	1	1	1	4	
25	37	2	11	7.87 -	8.23	20%	29%	53%	55%	57%		20%	29%	45%	58%	59%	13	% Z:	5% 20/	35%	52%	58%	1	1	1	1	2	1	0	1	1	0	0	1	1	1	4	
25	37	3	11	7.93 -	8.19	20%	29%	52%	54%	50%		18%	21%	43%	50%	56%	15	% Z.	2%	34%	51%	57%	1	1	1	1	0	1	0	2	1	0	0	1	0	1	3	
38	50	2	11	3.48 -	3.74	2%	11%	26%	26%	27%		2%	8%	24%	27%	28%	1	% <u> </u>	%	15%	25%	27%	0	1	0	0	0	0	0	1	0	1	0	0	0	1	2	
38	50	3	11	3.48 -	3.70	2%	11%	26%	26%	27%		2%	8%	24%	28%	27%	1	6 З	%	15%	25%	27%	0	1	0	0	0	0	0	1	0	1	0	0	1	1	2	
38	50	4	11	3.49 -	3.77	2%	11%	26%	26%	27%		2%	8%	25%	28%	28%	2	% З	%	15%	26%	27%	0	1	0	0	0	0	0	1	1	1	0	0	0	1	2	
38	50	5	11	3.56 -	3.73	2%	12%	26%	26%	27%		2%	8%	25%	28%	28%	1	% 3	%	15%	26%	27%	0	1	0	0	1	0	0	1	0	1	0	0	1	1	2	
38	71	6	31	8.08 -	8.33	11%	19%	37%	41%	42%		11%	19%	34%	44%	45%	10	% 1	5%	28%	40%	43%	1	1	1	1	1	0	0	2	1	0	1	1	1	1	3	
39	50	4	10	3.48 -	3.72	2%	12%	28%	28%	29%		2%	8%	26%	30%	30%	2	% 3	%	16%	28%	29%	0	1	1	0	0	0	0	1	1	1	0	1	0	1	3	
51	66	4	13	8.62 -	8.82	19%	26%	34%	34%	35%		20%	29%	37%	40%	40%	17	% 2	5%	33%	35%	37%	1	2	1	0	1	1	1	1	1	1	1	2	1	1	3	
51	71	4	18	8.59 -	8.83	12%	20%	35%	40%	41%		13%	22%	35%	46%	46%	12	% 1	9%	32%	42%	45%	1	1	1	1	1	0	0	2	1	1	1	1	1	1	3	
51	74	4	21	9.42 -	9.73	12%	21%	44%	52%	53%		13%	22%	41%	55%	56%	12	% 1	9%	36%	51%	54%	1	2	1	1	1	1	0	3	1	1	1	1	1	1	3	
51	86	6	32	10.07 -	10.26	16%	29%	56%	62%	63%		15%	27%	52%	65%	65%	13	% 2	2%	46%	61%	65%	1	2	1	1	1	1	0	2	1	1	1	1	1	1	3	
51	96	6	41	10.32 -	10.54	18%	39%	65%	71%	72%		17%	35%	61%	71%	72%	15	% 3	0%	57%	69%	73%	1	2	0	1	1	1	0	2	1	1	1	1	1	0	3	
51	98	6	43	10.78 -	11.02	17%	38%	66%	71%	73%		15%	34%	62%	73%	73%	13	% 3	0%	59%	71%	74%	1	2	0	1	1	1	0	2	1	1	1	1	1	0	3	
72	86	3	12	8.88 -	9.15	17%	34%	55%	54%	56%		18%	33%	56%	60%	61%	13	% 2	5%	51%	56%	58%	1	2	1	1	1	1	0	2	1	1	0	2	1	1	4	
75	86	3	9	8.16 -	8.45	23%	42%	58%	58%	61%		34%	46%	60%	63%	64%	24	% 3	5%	55%	57%	60%	1	2	0	1	3	1	1	2	1	2	1	1	2	1	2	
75	96	3	18	9.03 -	9.26	21%	53%	72%	71%	73%		19%	46%	73%	75%	76%	17	% 4	1%	70%	73%	75%	0	2	0	1	1	1	0	1	1	1	1	2	0	1	4	
87	96	2	8	5.80 -	6.23	23%	60%	75%	74%	76%		20%	53%	73%	75%	77%	18	% 4	5%	70%	71%	73%	0	2	1	1	1	1	1	2	1	1	1	2	1	1	5	
87	98	2	10	797 -	8 26	16%	45%	64%	63%	66%		15%	40%	66%	67%	69%	1/	% 3	9%	67%	67%	69%	0	3	2	2	1	1	0	2	1	1	1	2	1	1	1	
96	103	2	6	10.20 -	10.47	1%	8%	29%	30%	30%		1%	6%	28%	33%	33%	2	% F	%	29%	31%	32%	0	1	1	1	1	0	0	1	0	1	0	1	1	1	3	
07	103	2	4	0.20	0 00	1%	1.2%	42%	/ 30%	/2%		19/	0%	20%	A 49/	1.4%	2		10/	27%	20%	40%	0	1	0	1	1	0	0	2	1	0	0	1	1	1	4	
07	102	2	5	10.00	10.20	2%	20%	20%	20%	21%		19/	6%	20%	22%	2/10/	2	× 6	.0/	26%	20%	20%	0	1	0	0	1	0	0	1	1	1	0	1	1	1	2	
00	103	2	5	10.00 -	10.29	2 /0	0/0	30%	30%	210/		10/	69/	20/0	33%	34/0	2		0/	20%	20%	20%	0	1	1	1	1	0	0	1	1	1	0	1	1	1	2	
90	104	2	10	7 80	10.29	2.0%	0%	30%	40%	51%		1.00/	2.49/	20%	55%	33%	12	% C	0.00/	20%	2070	29%	0	1	1	1	1	1	0	2	1	1	0	1	1	1	1	
102	115	2	10	7.60 -	6.14	20%	20%	40%	49%	070/		10%	2470	59%	50%	49%	12	70 Z	J76	50%	45%	50%	1	1	0	1	1	1	0	2	0	1	0	1	0	1	1	
103	111	2		5.85 -	6.19	27%	67%	85%	84%	87%		23%	61%	84%	80%	88%	21	% 5. ×	5%	80%	81%	84%	1	0	1	2	2	1	0	2	1	1	1	2	2	1		
103	113	2	9	4.04 -	4.21	51%	57%	58%	57%	58%		48%	58%	58%	58%	58%	45	% 5	4%	56%	55%	57%	2	2	1	1	1	2	0	1	1	1	1	2	1	2	5	
103	113	3	9	4.04 -	4.26	52%	59%	59%	58%	59%		49%	59%	59%	59%	60%	46	% 5	5%	57%	56%	58%	2	2	1	1	1	2	1	1	1	1	1	2	1	1	4	
103	144	5	33	6.69 -	6.77	38%	41%	41%	39%	41%		40%	45%	45%	45%	45%	36	% 4	0%	41%	40%	42%	2	2	1	1	1	1	1	1	1	1	1	2	1	1	4	
103	144	6	33	6.66 -	7.04	38%	41%	40%	39%	40%		40%	45%	44%	44%	44%	36	% 4	0%	41%	40%	42%	2	1	1	0	1	1	1	1	1	1	1	2	1	1	4	
103	146	6	35	6.67 -	6.81	34%	37%	37%	36%	37%		37%	42%	41%	41%	41%	33	% 3	7%	38%	37%	39%	2	2	1	1	1	1	0	1	1	1	1	2	1	1	4	
104	113	2	8	4.03 -	4.21	54%	61%	61%	60%	62%		50%	61%	61%	61%	61%	48	% 5	8%	59%	58%	60%	2	2	1	1	1	2	1	2	1	1	1	2	1	1	5	
104	144	5	32	6.54 -	6.76	37%	40%	40%	39%	40%		40%	45%	45%	45%	45%	35	% 3	9%	40%	39%	41%	2	2	0	1	0	1	1	1	1	1	1	2	1	1	4	
105	113	2	7	4.03 -	4.21	59%	67%	68%	66%	68%		56%	68%	68%	68%	69%	53	% 6	3%	65%	64%	66%	2	2	1	1	1	2	1	1	1	1	1	3	1	2	5	
114	143	4	21	6.01 -	6.30	43%	45%	46%	45%	46%		46%	50%	50%	50%	50%	41	% 4	4%	45%	45%	47%	2	1	0	0	0	1	1	1	0	1	2	2	0	1	4	
114	144	3	22	6.91 -	6.99	41%	44%	44%	42%	44%		44%	48%	48%	48%	48%	38	% 4	2%	43%	43%	45%	2	2	1	1	1	1	1	1	1	1	1	2	1	1	4	
114	144	5	22	6.86 -	7.02	42%	44%	44%	43%	44%		44%	48%	48%	47%	48%	39	% 4	3%	44%	43%	45%	2	2	0	0	1	1	1	1	1	1	1	2	1	1	4	
150	195	6	44	4.96 -	5.62	47%	58%	58%	58%	60%		36%	59%	59%	60%	60%	14	% 24	4%	50%	58%	60%	0	1	0	0	1	1	1	1	1	1	1	0	1	1	4	
179	195	4	15	3.96 -	4.18	41%	50%	50%	49%	51%		40%	52%	51%	51%	52%	27	% 3	4%	45%	48%	50%	1	2	1	1	1	2	1	1	1	1	1	1	1	1	4	
								0.070							0 2 / 0	02/0							-	-	-	-	-	-	-	-	-	-	-	-	-	-		

 Relative deuteration %
 10%
 20%
 30%
 40%
 50%
 60%
 70%
 80%

Fig. S6



Time (s)





Time (s)









Fig. S9

