

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# Histone H1 phosphorylation during mitosis

A dissertation presented in partial fulfilment of the  
requirements for the degree of

Doctor of Philosophy

in

Biochemistry

at Massey University, Manawatū,

New Zealand

Sarah D Bond

2016



## Abstract

Histone H1 phosphorylation is important for the regulation of high order chromosome organisation during mitosis. One of these phosphorylation sites in the linker histone subtype H1.4 is shown here to be phosphorylated by Aurora B kinase, a master regulator of mitosis. Altered phosphorylation of H1.4 on this phosphorylation site at serine 27 illustrated the significance of the timing of this phosphorylation. When serine 27 of H1.4 is mutated to prevent this phosphorylation chromosome congression to the equatorial plate during metaphase is hindered. In contrast, in the presence of the constitutive H1.4 serine 27 phosphorylation mimic, bridging and lagging chromosomes occurred, leading to a corresponding increase in the proportion of cells with a micronucleus. These phenotypes could be brought about through disruption of the Heterochromatin protein 1 family members bound to the adjacent methylated lysine. Such aberrations during mitosis can lead to genetic instability and ultimately aneuploidy, a hallmark of cancer. With the frequently reported over-expression of Aurora B in cancer this shows another mechanism in which this kinase, via histone H1.4 phosphorylation, can push a cell toward malignancy.

Another important mitotic kinase, Cyclin dependent kinase 1 together with cyclin B, is responsible for the hyperphosphorylation of histone H1.4 during mitosis; which is required for condensing the cells genetic information into highly compact metaphase chromosomes. This vital mitotic event ensures the faithful transmission of the duplicated DNA into the dividing daughter cells. The mechanisms through which histone H1 hyperphosphorylation contribute to chromosome condensation are poorly understood. One mechanism through which this may occur is via the recruitment of condensation factors such as the condensins or Topoisomerase II. Here the interaction between the Condensin I subunit, CAPD2, and histone H1.4 is explored. CAPD2 interacts with the two most prominent linker histone subtypes, H1.4 and H1.2, through their C-terminal tails. H1.4 and CAPD2 can interact *in vitro* whilst each is phosphorylated by cyclin dependent kinase as they are during mitosis, in a manner dependent on RNA.

Overall, these results indicate that histone H1.4 is a vital component of higher order chromatin and its phosphorylation is essential for the normal progression through mitosis.



## Acknowledgements

First and foremost, I would like to thank my supervisor Dr Tracy Hale. I am immensely grateful for her guidance, support and encouragement throughout my doctoral studies. Tracy has been there for me through challenging times and is an inspiration, and I will be forever grateful for all she has taught me. Our regular meetings have led to many helpful discussions and I appreciate this time Tracy has generously spent with me. I wish Tracy all the best in her future endeavours.

My co-supervisors Dr Helen Fitzsimons and Professor Kathryn Stowell have provided helpful advice and support. I am thankful for this and their guidance and constructive discussions. I am also grateful to Helen for her generosity in sharing her lab space and equipment with our group. Kathryn kindly allowed our group to join in with shared lab meetings which has been platform for friendly discussions, advice and constructive criticism.

I have really enjoyed working in the laboratory thanks to the supportive environment created by colleagues. Both present and past lab members of the Chromatin Research Group and the Molecular Neurogenetics Group have assisted me through their advice and sharing of lab reagents, equipment and protocols, thank you to you all.

There are many colleagues within the Institute of Fundamental Sciences that have generously given their time for training me to use specialised equipment and kindly shared reagents; I appreciate all of this assistance. Thank you to those of you who maintain equipment, assist in getting the reagents to the right place and for the friendly environment within the institute.

I would also like to thank the staff within the Manawatu Microscopy and Imaging Centre for providing training, advice and assistance with microscopy.

In the wider university campus colleagues have allowed me to visit their institute and have assisted using equipment, thank you to Dr Fran Wolber for her flow cytometry expertise, and to Dr Matthew Perrott and his staff, for allowing me to use their immunohistochemistry facilities. I would also like to thank pathologists Dr Bruce

Lockett from Medlab Central, and Assistant Professor Alejandro Contreras from The MD Anderson Cancer centre, for their assistance analysing tumour samples.

I have been fortunate to receive financial support for my doctoral studies, thank you to Massey providing support through the Vice Chancellors Doctoral Scholarship. I am immensely grateful to the Institute of Fundamental Sciences for their support and for funding me for an additional six months to allow me to complete my studies. Thank you to the Graduate Women Manawatu Trust and Zonta for the Zonta Manawatu Women in Science Award which allowed me to travel to Boston, Massachusetts for the Gordon Research Conference on Chromatin Structure and Function in 2014.

Last, but not least I would like to thank family and friends for their support and for always being there for me.

# Table of Contents

Abstract.....	i
Acknowledgements.....	iii
List of Figures and Tables.....	xi
Abbreviations.....	xiii
<b>Chapter One</b> <b>Introduction.....</b>	<b>1</b>
<b>1.1</b> <b>The cell cycle.....</b>	<b>3</b>
<b>1.2</b> <b>Organisation of the genome.....</b>	<b>4</b>
<b>1.3</b> <b>The hierarchy of chromatin folding.....</b>	<b>5</b>
<b>1.3.1</b> The nucleosome.....	5
<b>1.3.2</b> The chromatosome.....	6
<b>1.3.3</b> The 30 nm fibre.....	9
<b>1.3.4</b> Chromosome structure during mitosis.....	10
<b>1.4</b> <b>Histone H1, the linker histone.....</b>	<b>11</b>
<b>1.4.1</b> The structure of histone H1.....	12
<b>1.4.2</b> Histone H1 subtypes.....	13
<b>1.4.3</b> The function of histone H1.....	16
<b>1.5</b> <b>Histone phosphorylation regulates chromatin structure during the cell cycle.....</b>	<b>17</b>
<b>1.5.1</b> Histone phosphorylation and mitotic chromosome condensation.....	18
<b>1.5.2</b> CDK-mediated histone H1 phosphorylation.....	19
<b>1.5.3</b> Aberrant phosphorylation of histone H1 in cancer.....	23
<b>1.5.4</b> Non-CDK phosphorylation of H1.4.....	24
<b>1.5.4.1</b> The similarities between the 'ARKS' motifs at H1.4K26S27 and H3K9S10.....	24
<b>1.6</b> <b>The interacting partners of Histone H1.4.....</b>	<b>28</b>
<b>1.6.1</b> Heterochromatin protein 1.....	28
<b>1.6.2</b> CAPD2, a component of Condensin I, interacts with histone H1.....	29
<b>1.7</b> <b>Research Aims.....</b>	<b>31</b>



<b>Chapter Two</b>	<b>Materials and Methods .....</b>	<b>33</b>
<b>2.1</b>	<b>Materials.....</b>	<b>35</b>
<b>2.2</b>	<b>DNA and RNA manipulations .....</b>	<b>35</b>
2.2.1	Determining the concentration of DNA and RNA .....	35
2.2.2	Agarose gel electrophoresis .....	35
2.2.3	RNA extraction .....	36
2.2.4	Complementary DNA synthesis .....	36
2.2.5	Polymerase chain reaction.....	36
2.2.5.1	Primers used in this study .....	36
2.2.6	Site-directed mutagenesis.....	37
2.2.7	Cloning.....	37
2.2.8	Transformation of competent <i>Escherichia coli</i> cells.....	38
2.2.9	Plasmid purification.....	38
2.2.10	DNA sequencing.....	38
<b>2.3</b>	<b>Protein manipulations .....</b>	<b>40</b>
2.3.1	Protein quantification.....	40
2.3.2	SDS-PAGE .....	40
2.3.3	Autoradiography .....	41
2.3.4	Western blotting.....	41
2.3.5	Expression of mammalian proteins in bacteria .....	43
2.3.6	Harvest of bacterially expressed protein.....	43
2.3.7	Acid extraction from bacterial cell pellets.....	43
2.3.8	Protein purification .....	44
2.3.9	<i>In vitro</i> methylation assays .....	44
2.3.10	<i>In vitro</i> kinase assays .....	45
2.3.11	Radioactive <i>in vitro</i> kinase assays.....	45
2.3.12	GST pull-down assays .....	45
<b>2.4</b>	<b>Tissue analysis .....</b>	<b>46</b>
2.4.1	Immunohistochemistry.....	46
<b>2.5</b>	<b>Cell culture and techniques.....</b>	<b>47</b>
2.5.1	Cell lines.....	47
2.5.2	Maintenance of cells .....	47
2.5.3	Cell synchronisation.....	48
2.5.3.1	Mitotic arrest .....	48
2.5.3.2	The double thymidine block .....	48
2.5.3.3	Mitotic shake-off .....	49
2.5.4	Cell lysis .....	49
2.5.5	Acid extraction from eukaryotic cells.....	49
2.5.6	Acid etch and poly-D-lysine coating of coverslips .....	50
2.5.7	Immunofluorescence .....	50
2.5.8	Metaphase spread immunofluorescence.....	51
2.5.9	Transient transfection of siRNA targeting Aurora A and B kinases.....	52

2.5.10	Inhibition of PP1 with calyculin A .....	52
2.5.11	Creation of stable H1.4-FLAG phosphorylation mutant inducible cell lines .....	52
2.5.11.1	Determining the effective concentration of hygromycin B for selection .....	53
2.5.11.2	Transfection of the H1.4-FLAG constructs .....	53
2.5.11.3	Selection and maintenance of stable H1.4-FLAG cell lines .....	53
2.5.11.4	Induction of the H1.4-FLAG proteins .....	54
2.5.12	Quantitative analysis of mitotic defects and micronuclei .....	54
2.5.13	Cell proliferation assay .....	55
2.5.14	Flow cytometry .....	55
2.5.15	Subcellular fractionation .....	56
2.5.16	Preparing soluble chromatin arrays for immunoprecipitation .....	56
2.5.16.1	Nucleosome ladders .....	57
2.5.16.2	Immunoprecipitation .....	57

<b>Chapter Three</b>	<b>Post-translational modification of the 'ARKS' motif within the N-terminus of histone H1.4.....</b>	<b>59</b>
3.1	<b>Introduction .....</b>	<b>61</b>
3.2	<b>Results.....</b>	<b>64</b>
3.2.1	<b>H1.4S27 phosphorylation is cell cycle regulated.....</b>	<b>64</b>
3.2.1.1	The localisation of phosphorylated H1.4S27 during mitosis..	66
3.2.1.2	H1.4S27 phosphorylation is a potential marker of mitosis.....	70
3.2.2	<b>Enzymes responsible for the mitotic phosphorylation of H1.4S27 .....</b>	<b>70</b>
3.2.2.1	Histone H1.4S27 is phosphorylated by the Aurora kinases ...	72
3.2.2.2	Aurora B kinase phosphorylates histone H1.4S27 <i>in vivo</i> .....	72
3.2.2.3	The localisation of Aurora B kinase overlaps with phosphorylated H1.4S27 .....	73
3.2.2.4	Protein phosphatase 1 dephosphorylates H1.4 at serine 27 ...	77
3.2.3	<b>Aurora B phosphorylation of H1.4S27 regulates the interaction with HP1.....</b>	<b>77</b>
3.2.3.1	Preparation of histone H1.4-FLAG and GST-HP1 $\beta$ .....	79
3.2.3.2	<i>In vitro</i> post-translational modification of H1.4.....	82
3.2.3.3	H1.4S27 phosphorylation disrupts the interaction between methylated H1.4 and HP1 $\beta$ .....	82
3.3	<b>Discussion .....</b>	<b>85</b>

<b>Chapter Four</b>	<b>Functional analysis of H1.4S27 phosphorylation during mitosis</b> .....	<b>89</b>
<b>4.1</b>	<b>Introduction</b> .....	<b>91</b>
<b>4.2</b>	<b>Results</b> .....	<b>93</b>
4.2.1	<b>Construction of cell lines to explore H1.4S27 phosphorylation</b> .....	<b>93</b>
4.2.1.1	Selection of inducible H1.4S27 phosphorylation mutant cell lines.....	93
4.2.2	<b>Characterisation of histone H1.4-FLAG phosphorylation mutant cell lines</b> .....	<b>96</b>
4.2.2.1	Localisation of the H1.4-FLAG proteins.....	96
4.2.2.2	Mitotic defects occur when H1.4S27 phosphorylation is aberrant .....	100
4.2.2.3	Expression of the H1.4S27 phosphorylation mutants does not alter cell proliferation or viability .....	104
4.2.2.4	Characterisation of endogenous histone H1 protein levels and phosphorylation in the H1.4-FLAG cell lines.....	107
4.2.2.5	Localisation of the H1.4-FLAG proteins on metaphase chromosomes .....	111
4.2.3	<b>Does mutation of H1.4S27 alter the interaction with HP1<math>\beta</math> <i>in vitro</i>?</b> .....	<b>111</b>
4.2.3.1	Mutation of H1.4S27 reduces K26 methylation by G9a <i>in vitro</i> .....	114
4.2.3.2	Decreased methylation of the H1.4S27 phosphorylation mutants reduces the interaction with HP1 $\beta$ .....	114
4.2.4	<b>The effect of H1.4S27A or H1.4S27E expression on HP1 localisation <i>in vivo</i></b> .....	<b>116</b>
4.2.4.1	HP1 $\alpha$ localisation is not affected by the presence of H1.4S27A or H1.4S27E .....	116
4.2.4.2	Investigating the interaction between HP1 and chromatin in the presence of H1.4S27A or H1.4S27E.....	119
4.2.4.2.1	Preparation of chromatin arrays for immunoprecipitation of the H1.4S27 phosphorylation mutants .....	121
4.2.4.2.2	Optimising the immunoprecipitation reaction .....	124
4.2.4.3	Exploring the interaction between HP1 $\beta$ and chromatin in asynchronous cells .....	127
4.2.4.4	The mitotic chromatin landscape with H1.4S27A and H1.4S27E expression.....	131
<b>4.3</b>	<b>Discussion</b> .....	<b>135</b>

<b>Chapter Five</b>	<b>Investigating the contribution of CDK phosphorylation of H1.4 on the interaction with HP1<math>\beta</math> and CAPD2 during mitosis .....</b>	<b>139</b>
<b>5.1</b>	<b>Introduction .....</b>	<b>141</b>
<b>5.2</b>	<b>Results.....</b>	<b>143</b>
	<b>5.2.1 Total mitotic phosphorylation of H1.4 abolishes the interaction with HP1<math>\beta</math>.....</b>	<b>143</b>
	<b>5.2.1.2 CDK phosphorylation of H1.4T18 disrupts the interaction with HP1<math>\beta</math> .....</b>	<b>145</b>
	<b>5.2.2 The interaction between the Condensin I subunit, CAPD2, and H1.4.....</b>	<b>145</b>
	<b>5.2.2.1 H1.4 interacts with the C-terminal domain of CAPD2.....</b>	<b>147</b>
	<b>5.2.2.2 The C-terminal tail in H1.4 mediates the interaction with CAPD2.....</b>	<b>151</b>
	<b>5.2.2.3 H1.4 is not the only linker histone subtype that can interact with CAPD2.....</b>	<b>151</b>
	<b>5.2.2.4 H1.4 and CAPD2 interact when phosphorylated by CDK ...</b>	<b>154</b>
	<b>5.2.2.5 RNA is required for the interaction between H1.4 and CAPD2.....</b>	<b>157</b>
<b>5.3</b>	<b>Discussion .....</b>	<b>159</b>
<b>Chapter Six</b>	<b>Discussion.....</b>	<b>163</b>
<b>6.1</b>	<b>Conclusions.....</b>	<b>175</b>
<b>References</b>	<b>.....</b>	<b>177</b>
<b>Appendix One</b>	<b>Full western blots .....</b>	<b>197</b>
<b>Appendix Two</b>	<b>Quantitation of selected western blots.....</b>	<b>229</b>



## List of Figures and Tables

<b>Figure 1.1.</b>	The hierarchy of chromatin folding. ....	7
<b>Figure 1.2.</b>	The structure of the nucleosome core. ....	8
<b>Figure 1.3.</b>	The chromatosome. ....	8
<b>Figure 1.4.</b>	Sequence alignment of the somatic main H1 subtypes. ....	15
<b>Figure 1.5.</b>	A diagram of H1.4 and its key phosphorylation sites. ....	21
<b>Figure 1.6.</b>	The mechanism of HP1 dislodgement from H1.4. ....	26
<b>Figure 3.1.</b>	Post-translational modification of H1.4 during the cell cycle. ....	65
<b>Figure 3.2.</b>	H1.4 is phosphorylated on serine 27 within the mitotic chromosomes. ....	67
<b>Figure 3.3.</b>	H1.4 serine 27 phosphorylation is enriched at the centromere. ....	69
<b>Figure 3.4.</b>	H1.4S27 phosphorylation as a mitotic marker in breast tumour tissue. ....	71
<b>Figure 3.5.</b>	Aurora B kinase phosphorylates H1.4 at serine 27. ....	74
<b>Figure 3.6.</b>	Aurora B localisation during mitosis. ....	76
<b>Figure 3.7.</b>	PP1 inhibition increases H1.4 serine 27 phosphorylation. ....	78
<b>Figure 3.8.</b>	Expression and purification of H1.4-FLAG and GST-HP1 $\beta$ . ....	80
<b>Figure 3.9.</b>	H1.4S27 phosphorylation reduces the interaction with HP1 $\beta$ . ....	83
<b>Figure 4.1</b>	Comparing H1.4S27 phosphorylation and the mutations that prevent or mimic this phosphorylation. ....	94
<b>Figure 4.2.</b>	Screening the H1.4S27 phosphorylation mutant cell lines. ....	95
<b>Figure 4.3.</b>	H1.4-FLAG colocalises with endogenous H1.4. ....	97
<b>Figure 4.4.</b>	Exogenous H1.4-FLAG, H1.4S27A and H1.4S27E localise to the nucleus. ....	98
<b>Figure 4.5.</b>	Mitotic defects occur with expression of H1.4S27A or H1.4S27E. ....	101
<b>Figure 4.6.</b>	Cells that express H1.4S27E have an increased incidence of micronuclei. ....	103
<b>Figure 4.7.</b>	Expression of the H1.4S27 phosphorylation mutants does not alter cellular proliferation. ....	105
<b>Figure 4.8.</b>	Expression of the H1.4S27 phosphorylation mutants does not affect programmed cell death. ....	106
<b>Figure 4.9.</b>	Reduced K26 methylation of the H1.4S27 phosphorylation mutants in asynchronously grown cells. ....	108
<b>Figure 4.10.</b>	H1 protein levels are not altered by expression of the H1.4S27 phosphorylation mutants in mitotic cells. ....	110

<b>Figure 4.11.</b>	Expression of the H1.4S27 phosphorylation mutants does not affect metaphase chromosome structure. ....	112
<b>Figure 4.12.</b>	Reduced H1.4K26me in the H1.4S27 phosphorylation mutants led to a concomitant reduction in the interaction with HP1 $\beta$ . ....	115
<b>Figure 4.13.</b>	Cells in G <sub>2</sub> /M peak 6.5 hours after release from the double thymidine block. ....	117
<b>Figure 4.14.</b>	Expression of H1.4S27A or H1.4S27E does not change HP1 $\alpha$ localisation. ....	120
<b>Figure 4.15.</b>	Obtaining chromatin arrays for immunoprecipitation. ....	122
<b>Figure 4.16.</b>	Optimisation of fixation for immunoprecipitation. ....	125
<b>Figure 4.17.</b>	Non-specific binding in the immunoprecipitation reaction. ....	126
<b>Figure 4.18.</b>	Triton X-100 in the immunoprecipitation reaction reduced non-specific binding. ....	128
<b>Figure 4.19.</b>	Nucleosome ladders for fixed immunoprecipitation. ....	130
<b>Figure 4.20.</b>	HP1 $\beta$ interacts with chromatin containing the H1.4S27 phosphorylation mutants from asynchronous cells. ....	133
<b>Figure 4.21.</b>	The chromatin landscape in mitotic cells expressing the H1.4S27 phosphorylation mutants. ....	134
<b>Figure 5.1.</b>	CDK phosphorylation of H1.4 abolishes the interaction with HP1 $\beta$ . ..	144
<b>Figure 5.2.</b>	H1.4T18 phosphorylation contributes to the loss of HP1 $\beta$ binding. ....	146
<b>Figure 5.3.</b>	Expression and purification of GST-CAPD2 and GST-CTD. ....	148
<b>Figure 5.4.</b>	H1.4 interacts with CAPD2. ....	150
<b>Figure 5.5.</b>	The C-terminus of H1.4 interacts with CAPD2. ....	152
<b>Figure 5.6.</b>	The C-terminus of H1.2 interacts with CAPD2. ....	153
<b>Figure 5.7.</b>	H1.4 and the CDK phosphorylation mimic E1.5 interact with CAPD2. ....	155
<b>Figure 5.8.</b>	H1.4 and CAPD2 still interact when phosphorylated by CDK. ....	156
<b>Figure 5.9.</b>	RNA is required to mediate the interaction between H1.4 and CAPD2. ....	158
<b>Figure 6.1.</b>	Model for the interactions of H1.4 during mitosis .....	173
<b>Table 1.1.</b>	H1 subtypes and their properties .....	15
<b>Table 2.1.</b>	Plasmids used in this study .....	39
<b>Table 2.2.</b>	Primary antibodies used in this study .....	42
<b>Table 2.3.</b>	Secondary antibodies used in this study .....	42

## Abbreviations

2N	Diploid
4N	Tetraploid
A	Adenine
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
BD	Becton Dickinson
bp	Base pairs
BSA	Bovine serum albumin
C	Cytosine
C-terminus	Carboxyl-terminus
CAPD2	Condensin complex subunit 1
CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid
CDK	Cyclin dependent kinase
cDNA	Complementary DNA
CENPA	Centromere protein A
CRG	Chromatin Research Group
CTD	C-terminal domain of CAPD2 (final 113 amino acids)
DAB+	3,3'-Diaminobenzidine
DAPI	4',6-Diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
Dox	Doxycycline
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
EZH2	Enhancer of zeste homolog 2
FBS	Fetal bovine serum
FFPE	Formalin fixed paraffin embedded
FRAP	Fluorescence recovery after photobleaching
g	Grams
G	Guanine
G <sub>1</sub> phase	Gap 1 phase
G <sub>2</sub> phase	Gap 2 phase
GFP	Green fluorescent protein
GST	Glutathione S-transferase
GST-CAPD2	Wild-type CAPD2 with an N-terminal GST-tag
GST-CTD	CTD of CAPD2 with an N-terminal GST-tag
GST-HP1 $\beta$	HP1 $\beta$ with an N-terminal GST-tag



H1	Histone H1
H1.4-FLAG	Wild-type histone H1.4 with a C-terminal FLAG-tag
H1.4K26	Histone H1.4 lysine 26
H1.4K26A	H1.4-FLAG where lysine at position 26 was substituted with alanine to prevent K26 methylation
H1.4K26me3	Histone H1.4 trimethylated on lysine 26
H1.4S27	Histone H1.4 serine 27
H1.4S27A	H1.4-FLAG where serine at position 27 was substituted with alanine to prevent S27 phosphorylation
H1.4S27E	H1.4-FLAG where serine at position 27 was substituted with glutamic acid to mimic constitutive phosphorylation
H1.4S27p	H1.4 phosphorylated on serine 27
H3	Histone H3
H3K9	Histone H3 lysine 9
H3K9me3	Histone H3 trimethylated on lysine 9
H3S10	Histone H3 serine 10
H3S10A	H3 where serine at position 10 was substituted with alanine to prevent S10 phosphorylation
H3S10E	H3 where serine at position 10 was substituted with glutamic acid to mimic constitutive S10 phosphorylation
H3S10p	H3 phosphorylated on serine 10
HAT	Histone acetyltransferase
HEPES	2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HMT	Histone methyltransferase
HP1	Heterochromatin protein 1
HRP	Horseradish peroxidase
IDT	Integrated DNA Technologies
IF	Immunofluorescence
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IP	Immunoprecipitation
IPTG	Isopropyl $\beta$ -D-1-thiogalactopyranoside
K9	Lysine 9
K26	Lysine 26
Kb	Kilobase
KCM buffer	Potassium chromosome medium buffer
$K_d$	Dissociation constant
kDa	Kilodalton
L	Litre
LB	Lysogeny broth
M phase	Mitotic phase
Min	Minutes

MGS	Massey Genome Service
MMIC	Massey Microscopy and Imaging Centre
MNase	Micrococcal nuclease
MSK1	Mitogen- and stress-activated kinase 1
N-terminus	Amino-terminus
NEB	New England BioLabs
NFM	Non-fat milk
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PI	Propidium iodide
PP1	Protein phosphatase 1
PP2A	Protein phosphatase 2A
PRC2	Polycomb repressive complex 2
PTM	Post-translational modification
RIPA	Radioimmunoprecipitation assay buffer
RNA	Ribonucleic acid
RNase A	Ribonuclease A
rpm	Revolutions per minute
RT	Room temperature
S phase	Synthesis phase
S10	Serine 10
S27	Serine 27
SDM	Site-directed mutagenesis
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SMC	Structural maintenance of chromosomes
S.O.C	Super optimal broth with catabolite repression
Supplemented DMEM	DMEM supplemented with 10% FBS and 1% penicillin/ streptomycin
SUV39H1	Suppressor of variegation 3-9 homolog 1
SWI/SNF	Switch/Sucrose non-fermentable
T	Thymine
TAE	Tris-acetate-EDTA
TBS	Tris-buffered saline
TBST	TBS with 0.1% Tween 20
TCA	Trichloroacetic acid
TGS	Tris-glycine-SDS
U	Unit
UV	Ultraviolet
WB	Western blotting

