Supplementary Materials

Molecular Biology of the Cell

Niedzialkowska *et al*.

Supplementary materials

Tip60 acetylation of histone H3K4 temporally controls Chromosome Passenger Complex localization

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Supplementary Figure 1. Superimposition of hSurvivin structures with histone H3K4 methylated peptides

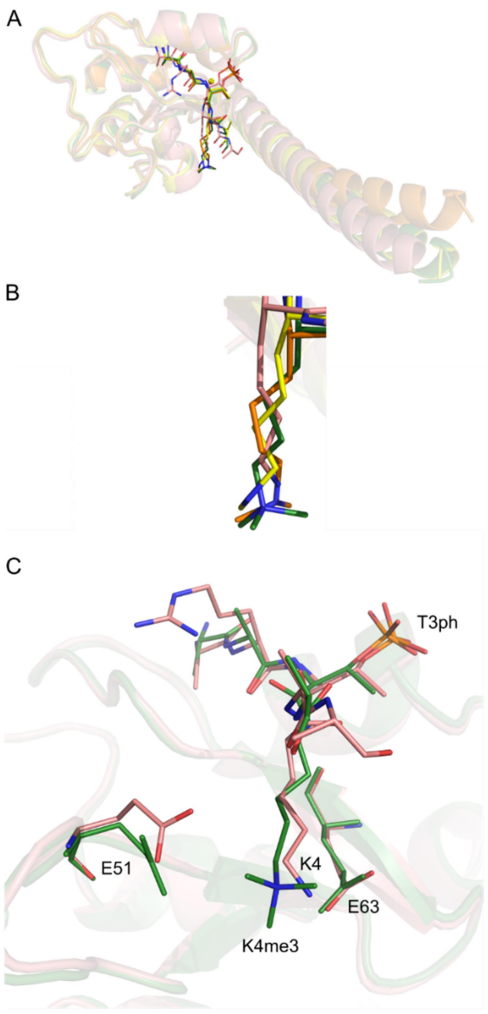
Supplementary Figure 2. Specificity of antibodies against histone H3 K4ac used in this study.

Supplementary Figure 3. Deacetylation of histone H3K4ac by HDAC3 activity is important for targeting of Aurora B to centromeres.

Table S1. Crystallization conditions

Table S2. Refinement statistics

Supplementary Figure 1.



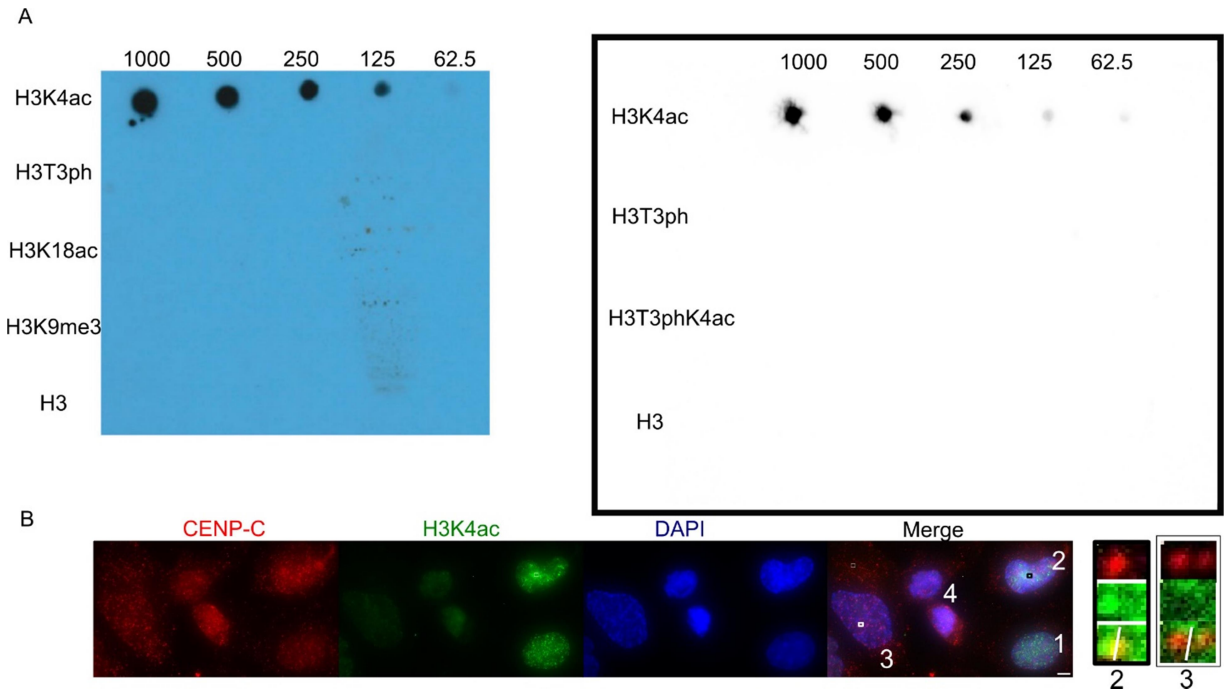
Supplementary Figure 1. Superimposition of hSurvivin structures with histone H3K4 methylated peptides

A. Overall view on the hSurvivin with bound H3K4 methylated peptides.

B. Closeup view on the binding region; hSurvivin is displayed in transparent mode, carbon atoms of unmodified histone are colored pink, carbon atoms of H3K4 monomethylated peptide are colored yellow, H3K4 dimethylated peptide are colored orange and H3K4 trimethylated peptide colored green.

C. Superimposition of hSurvivin bound to unmodified H3 (pink) and trimethylated on K4 (green).

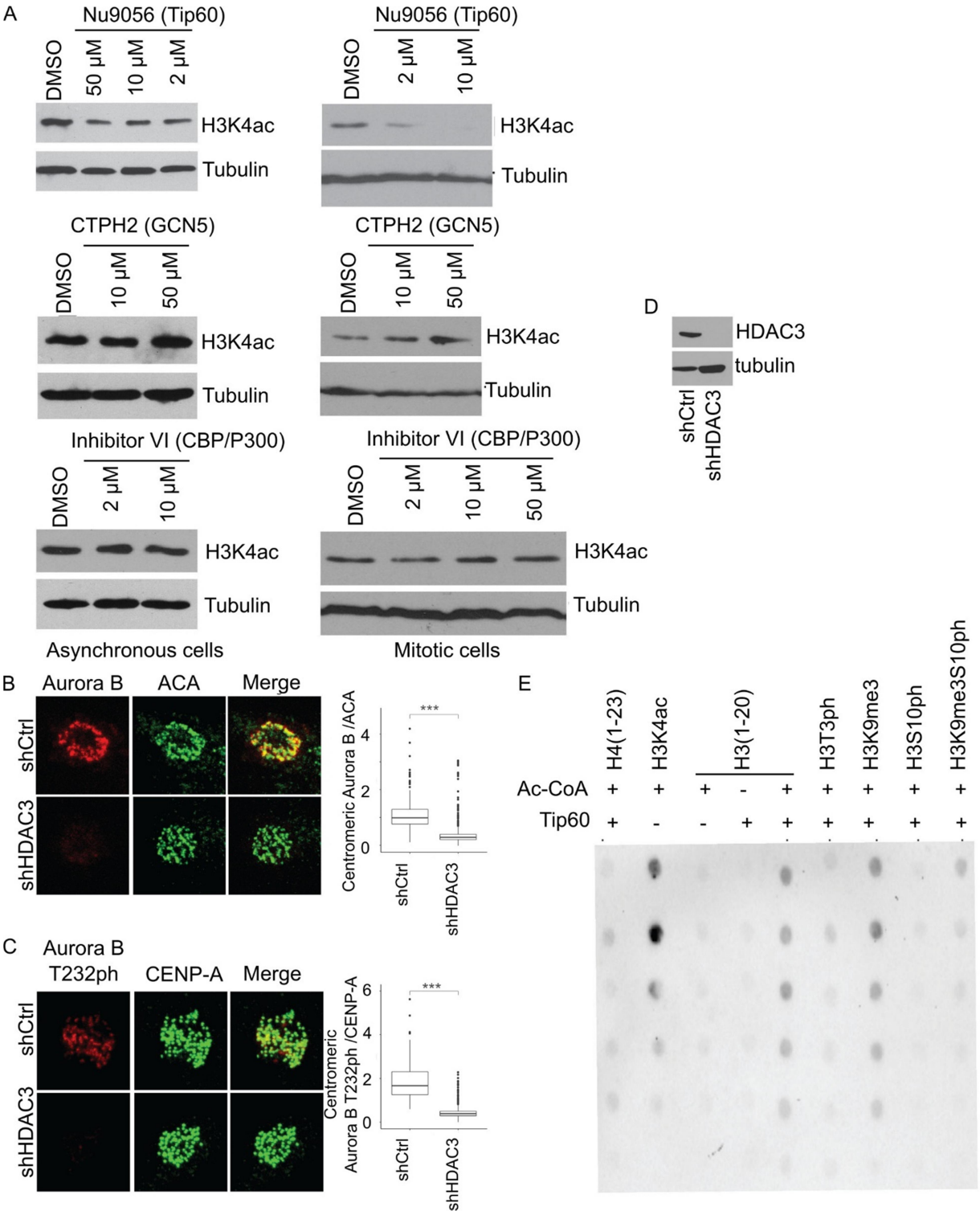
Supplementary Figure 2.



Supplementary Figure 2. Specificity of antibodies against histone H3K4ac used in this study. A. Specificity of the histone H3K4ac antibody measured by immunoblot of the indicated peptides at the specified concentrations.

B. A field of cells stained with DAPI, and H3K4ac and CENP-C antibodies. The cell 1 is in S/G2 (high H3K4ac), 2 is early prophase, 3 late prophase and 4 in telophase/G1. Insets show boxed regions. Scale bar, 5um.

Supplementary Figure 3



Supplementary Figure 3. Deacetylation of histone H3K4ac by HDAC3 activity is important for targeting of Aurora B to centromeres.

A. Comparison of inhibition of three different histone acetyltransferases suggests that Tip60 specifically acetylates histone H3 on K4. Analysis of the indicated inhibitors of acetyltransferases in asynchronous cells and cells synchronized in mitosis revealed that Nu9056, a Tip60 acetyltransferase inhibitor decreased histone H3K4 acetylation signal, but GCN5 and p300 inhibitors had little effect. The images for Nu9056 are the same as in figure 3A.

B. and C. Aurora B doesn't localize to centromeres in cells treated with shHDAC3 as shown by immunofluorescence using antibodies against Aurora B or against phosphorylated T-loop.

D. Western blot to show efficiency of knockdown of HDAC3 by siRNA targeting.

E. In vitro assay for Tip60 activity on histone H3 tail peptides with various post-translational modifications showing the full titration range of the peptides. Only the 500ng concentration of peptide is shown in figure 3C

Table S1. Crystallization conditions

Complex	**PDB code**	**Crystallization conditions**
hSurvivin wild type H3T3phK4me1(1-12)	7LBO	0.16 M potassium/sodium tartrate, 12% PEG 3350
hSurvivin wild type H3T3phK4me2(1-12)	7LBQ	2.25 mM spermine, 9 mM $MgCl_2$, 0.9 mM spermidine, 1.8 mM cobalt (III)hexamine chloride, 0.05 sodium cacodylate pH 7.0, 5% PEG 400
hSurvivin wild type H3T3phK4me3(1-12)	7LBK	0.16 M potassium/sodium tartrate, 14% PEG 3350;
hSurvivin wild type H3T3phK4ac(1-12)	7LBP	0.16 M potassium/sodium tartrate, 12% PEG 3350

Table S2. Refinement statistics

	hSurvivin wild type H3T3phK4me1 (1-12)	hSurvivin wild type H3T3phK4me2 (1-12)	hSurvivin wild type H3T3phK4me3 (1-12)	hSurvivin wild type H3T3phK4ac (1-12)
PDB code	**7LBO**	**7LBQ**	**7LBK**	**7LBP**
Data collection				
Space group	C2	I222	C2	C2
a,b,c (Å) β (°) α = γ = 90°	114.18 71.44 82.79 129.11	69.34 70.16 89.45 90	114.29 71.37 82.55 129.33	113.12 70.99 83.32 130.04
Resolution (Å)	2.50 (2.50-2.54)	2.60 (2.64 -2.60)	2.70 (2.70-2.75)	2.60 (2.60-2.64)
R_{merge}[1]	0.043 (0.329)	0.087 (0.892)	0.100 (0.764)	0.053 (0.427)
I / σ(I)[1]	38.0 (3.8)	20.7 (1.9)	28.9 (2.4)	3.0 (2.3)
Completeness (%)[1]	99.5 (100.0)	99.2 (99.7)	98.1 (93.0)	98.6 (89.3)
Redundancy[1]	3.8 (3.8)	6.6 (4.7)	14.8 (11.4)	3.7 (3.0)
Refinement				
Resolution (Å)	50.0-2.50	50-2.69	50.0-2.70	50.00-2.60
Number of reflections	17812	6097	13890	15604
R_{work} / R_{free}	20.7/26.1	24.8/26.8	21.2/25.3	21.2/24.7
Number of atoms				
Protein	2236	1054	2250	2230
Ligands/ions	2	1	2	2
Water	14	7	34	5
B factors				
Protein	90.5	53.8	40.8	105.8
Ligand/ion	70.8	25.9	22.7	91.2
Water	83.9	32.0	28.4	101.3
Structure quality				

[1] Values in parentheses correspond to the highest-resolution shell

R.m.s. Deviations				
Bond lengths (Å)	0.011	0.017	0.014	0.010
Bond angles (°)	1.5	2.1	1.7	1.4
Ramachandran Statistics				
Favored (%)	98.53	96.99	96.68	97.82
Allowed (%)	1.47	3.01	3.32	2.18