Deciphering the Nucleosome Interactome

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Abstract

Nuclear proteins bind chromatin to execute and regulate genome-templated processes. While studies of individual nucleosome interactions have suggested that an acidic patch on the nucleosome disk may be a common site for recruitment to chromatin, the pervasiveness of acidic patch binding and whether other nucleosome binding hot-spots exist remain unclear. Here we report a comprehensive nucleosome affinity proteomics screen to define the nucleosome interactome and to establish universal principles for nucleosome recognition. Overall, our interactome screen illuminates a highly competitive nucleosome binding hub and pairs about 300 proteins engaged in diverse nuclear processes with specific nucleosome surface requirements.

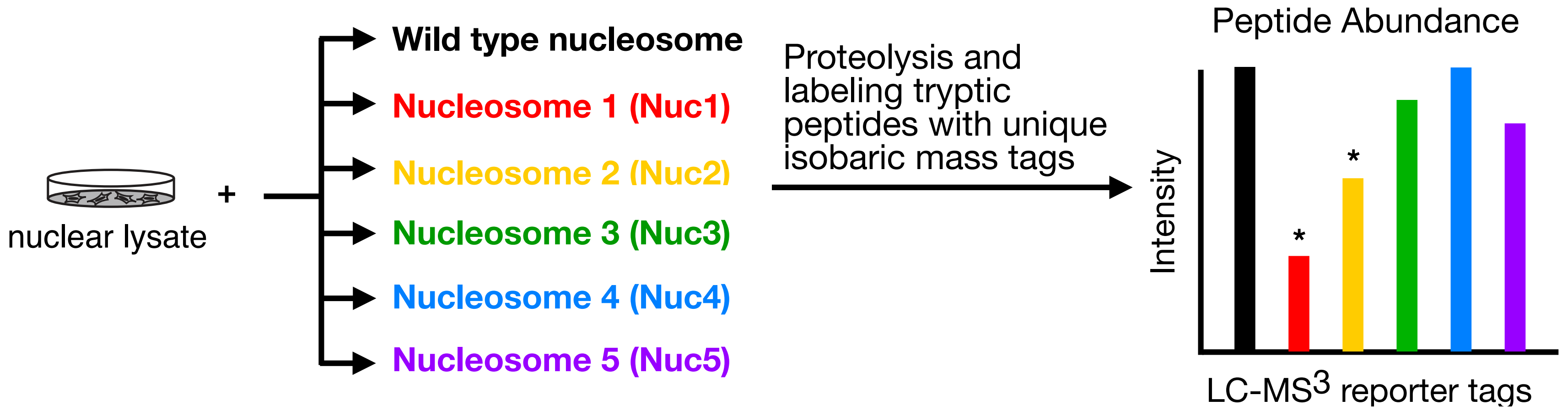
Objectives

I. Determine if the acidic patch is a hot spot for nucleosome binding.
II. Identify if other hot-spots exist.
III. Characterize novel nucleosome interactions.

Experimental design

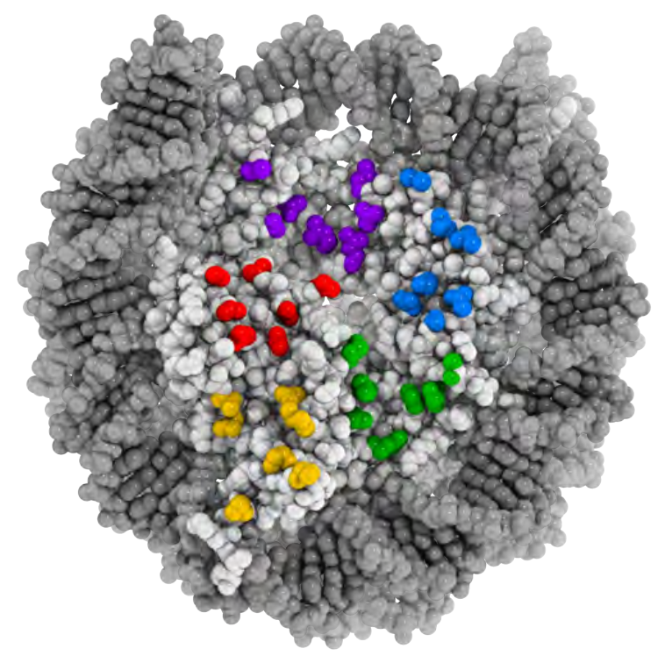
For the nucleosome interactome screen, a biotinylated nucleosome library, bearing mutations collectively disrupting the entire nucleosome disk surface, was used as bait in pulldowns from mouse embryonic stem cells nuclear lysates. Nucleosome binding proteins were trypsinized and labeled with isobaric mass tags for LCMS analysis.

Nucleosome affinity proteomics



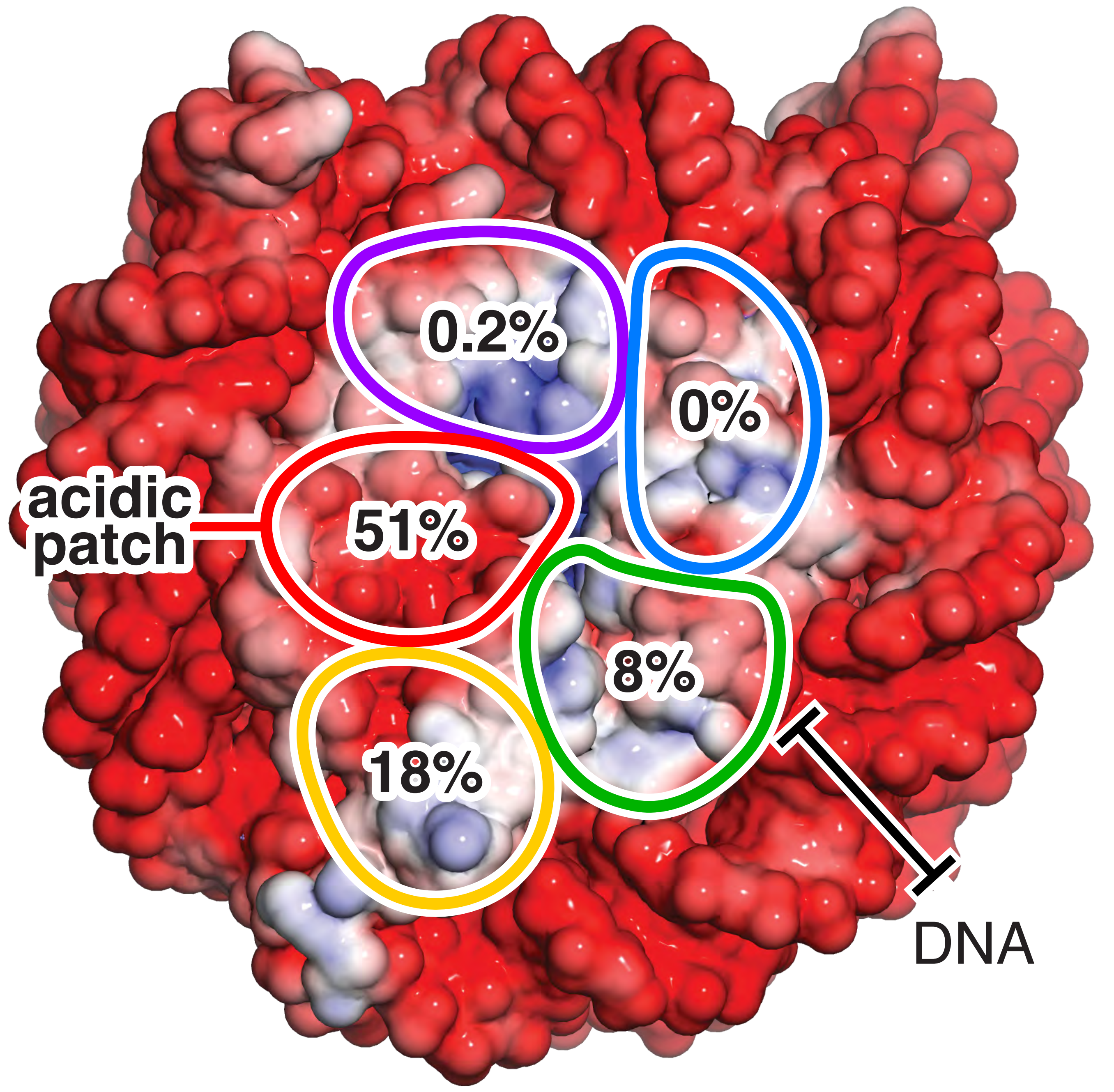
Biotinylated Nucleosome library

	Histone	Mutations
Nuc1 acidic patch	H2A	E61A E64S N68A D72S N89A D90A E91S
Nuc2	H2A	K15A Q24A
	H2B	Q47S E113A K116S K120S K125A
Nuc3	H2B	R92S Q95A R99S K108A
	H3	K79S T80A
	H4	E74A
Nuc5	H2A	N110A Q112S L116A
	H3	K122S Q125A R129A R134S
Nuc4	H3	E73S D77A
	H4	Q27A L49A E52S K59S E63A



Conclusions

Nucleosome interaction hot-spots



Percentages of proteins binding to outlined nucleosome disk surface patches identified in our nucleosome interactome screen

- **over 50% of nucleosome binding proteins requires the acidic patch**
- **two acidic patch adjacent patches (yellow and green) contribute to nucleosome recognition but largely in acidic patch dependent manner**

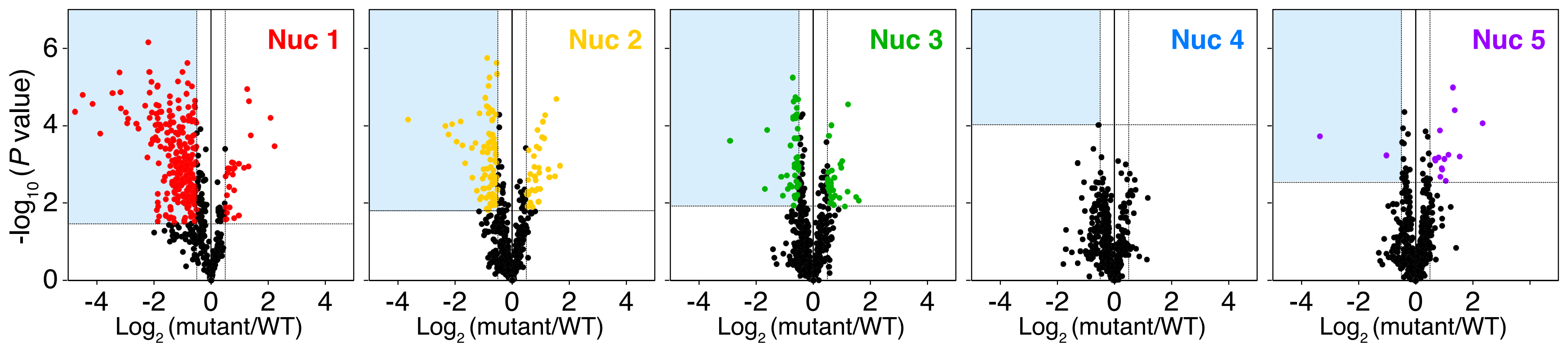
Learn more

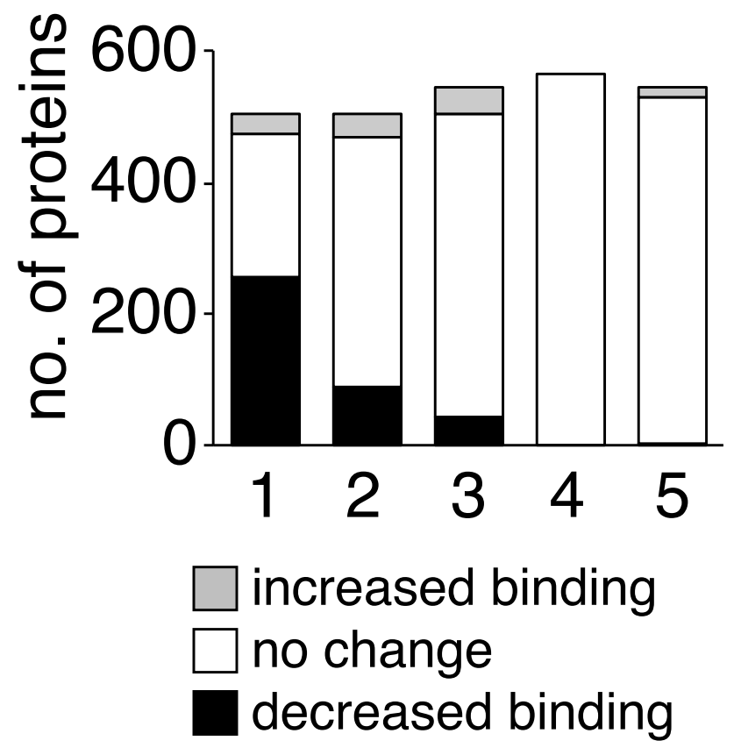
https://doi.org/10.1093/nar/gkaa544

https://youtu.be/3PRcMTWwscY

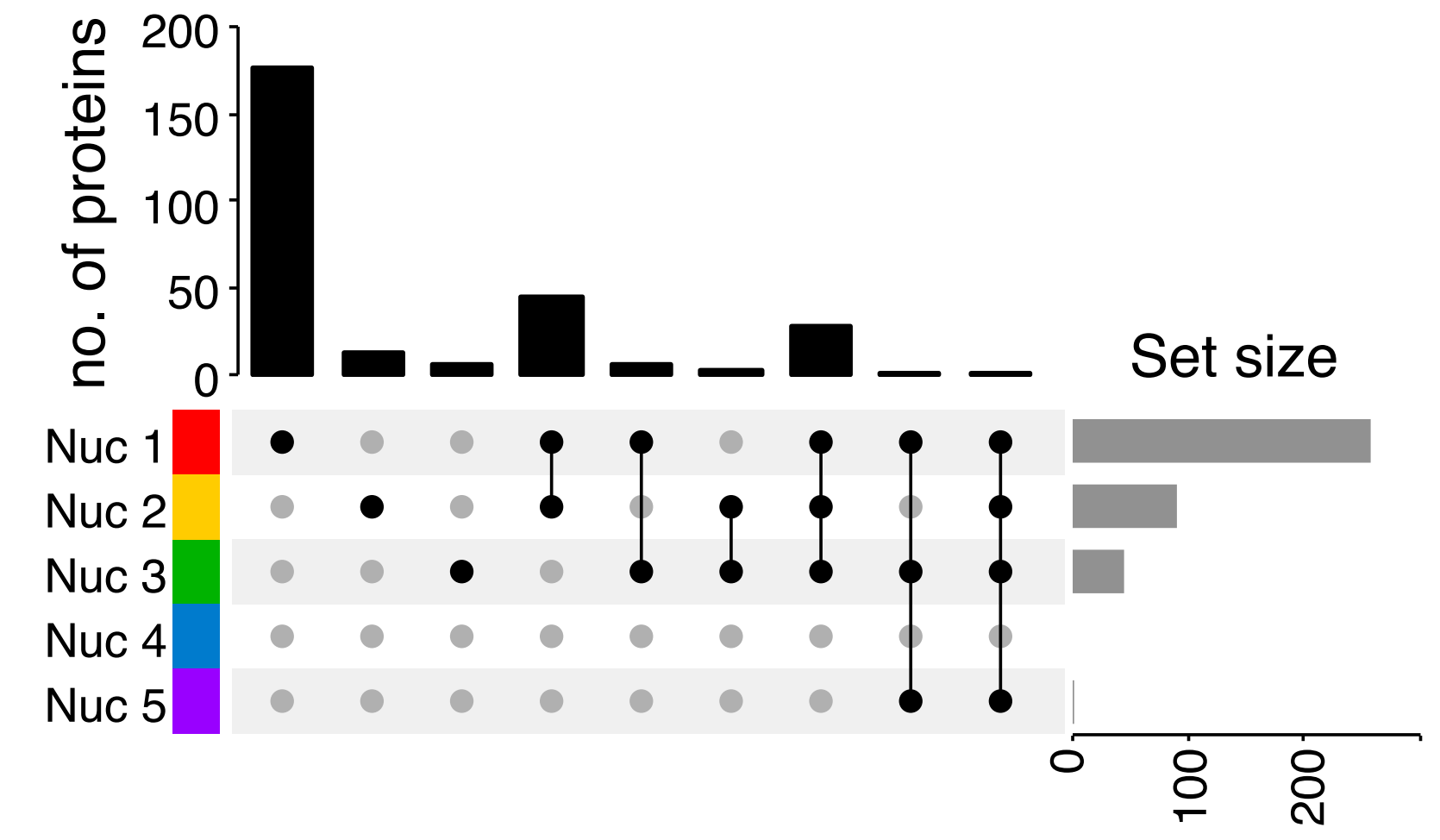
Selected results

Pervasiveness of acidic patch binding



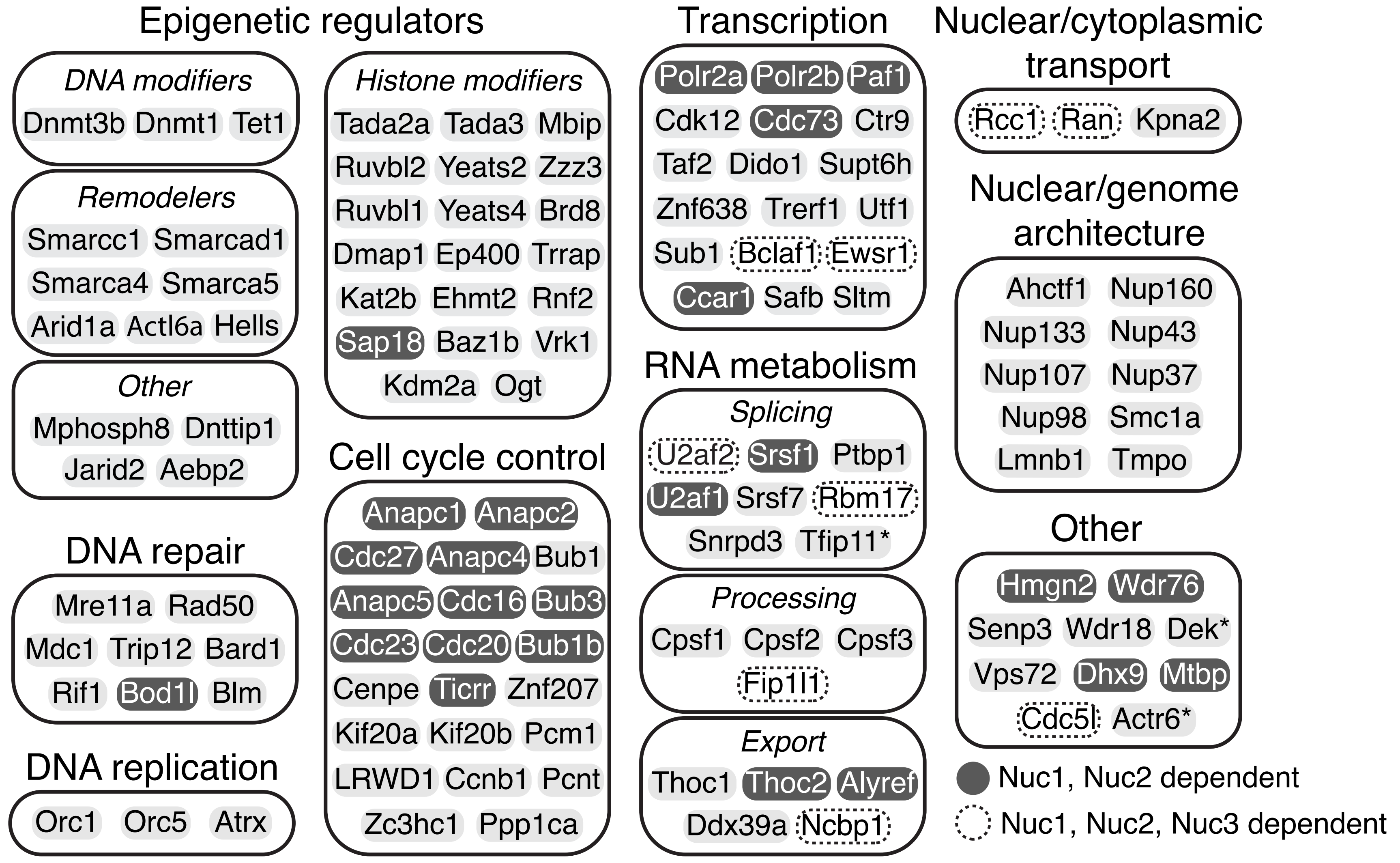


Volcano plots show nucleosome binding changes in each disk patch mutant nucleosome relative to WT nucleosomes. Horizontal and vertical lines designate 5% FDR and 1.4-fold change significance thresholds, respectively. Protein hits with significantly decreased binding are highlighted with blue background. Graph on the right summarizes changes in binding to mutant nucleosomes.



Upset plot showing number of significantly decreased proteins in subsets of mutant nucleosomes, listed below, demonstrates that ~80% of proteins that recognize patches 2 and 3 (yellow and green) are also dependent on the acidic patch.

Selected acidic-patch binding protein hits



Epigenetic regulators

DNA modifiers
Dnmt3b Dnmt1 Tet1

Remodelers
Smarcc1 Smarcad1
Smarca4 Smarca5
Arid1a Actl6a Hells

Other
Mphosph8 Dnttip1
Jarid2 Aebp2

Histone modifiers
Tada2a Tada3 Mbip
Ruvbl2 Yeats2 Zzz3
Ruvbl1 Yeats4 Brd8
Dmap1 Ep400 Trrap
Kat2b Ehmt2 Rnf2
Sap18 Baz1b Vrk1
Kdm2a Ogt

DNA repair
Mre11a Rad50
Mdc1 Trip12 Bard1
Rif1 Bod1l Blm

DNA replication
Orc1 Orc5 Atrx

Cell cycle control
Anapc1 Anapc2
Cdc27 Anapc4 Bub1
Anapc5 Cdc16 Bub3
Cdc23 Cdc20 Bub1b
Cenpe Ticrr Znf207
Kif20a Kif20b Pcm1
LRWD1 Ccnb1 Pcnt
Zc3hc1 Ppp1ca

Transcription
Polr2a Polr2b Paf1
Cdk12 Cdc73 Ctr9
Taf2 Dido1 Supt6h
Znf638 Trerf1 Utf1
Sub1 Bclaf1 Ewsr1
Ccar1 Safb Sltm

RNA metabolism

Splicing
U2af2 Srsf1 Ptbp1
U2af1 Srsf7 Rbm17
Snrpd3 Tfip11*

Processing
Cpsf1 Cpsf2 Cpsf3
Fip1l1

Export
Thoc1 Thoc2 Alyref
Ddx39a Ncbp1

Nuclear/cytoplasmic transport
Rcc1 Ran Kpna2

Nuclear/genome architecture
Ahctf1 Nup160
Nup133 Nup43
Nup107 Nup37
Nup98 Smc1a
Lmnb1 Tmpo

Other
Hmgn2 Wdr76
Senp3 Wdr18 Dek*
Vps72 Dhx9 Mtbp
Cdc5l Actr6*

● Nuc1, Nuc2 dependent
○ Nuc1, Nuc2, Nuc3 dependent

*DEK is dependent on all but Nuc4 and Tfip11 and Actr6 are dependent on Nuc1 and Nuc3.

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