Structure and Function of Mammalian SWI/SNF Complexes in Human Cancer

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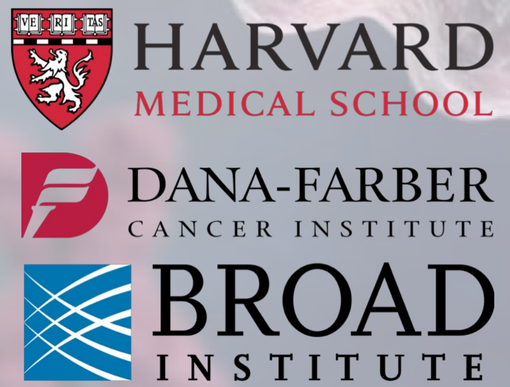


Disclosures

I have the following financial relationship to disclose:

Scientific Founder, Board of Directors,
Scientific Advisory Board, Shareholder, Consultant

Foghorn Therapeutics, Inc. (Cambridge, MA)

FOGHORN
THERAPEUTICS

I will <u>not</u> discuss any off-label use or investigational use in my presentation.

Cancer genetics unlock new biology

30+ Cancer Types

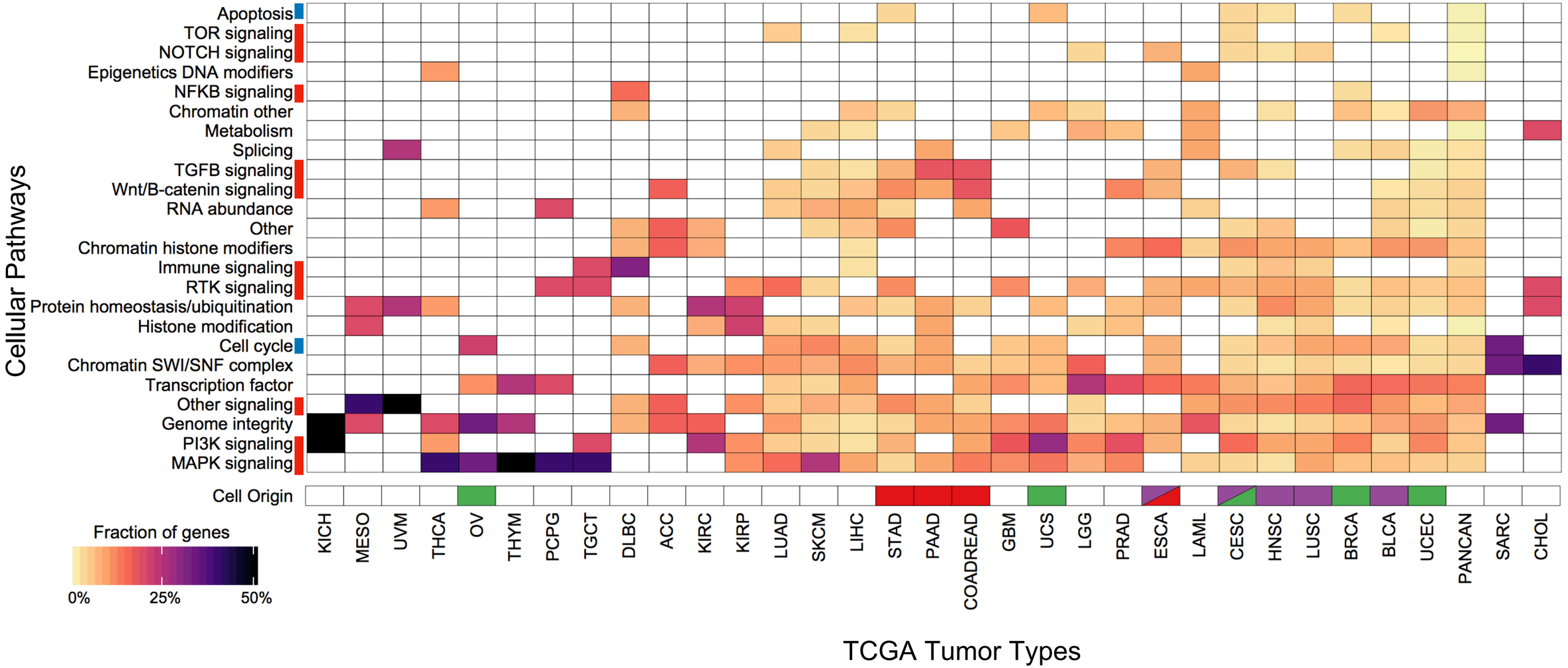
>10,000 tumor samples

~300 driver genes
>3,400 putative missense driver mutations

Bailey et al. (TCGA), ***Cell*** 2018

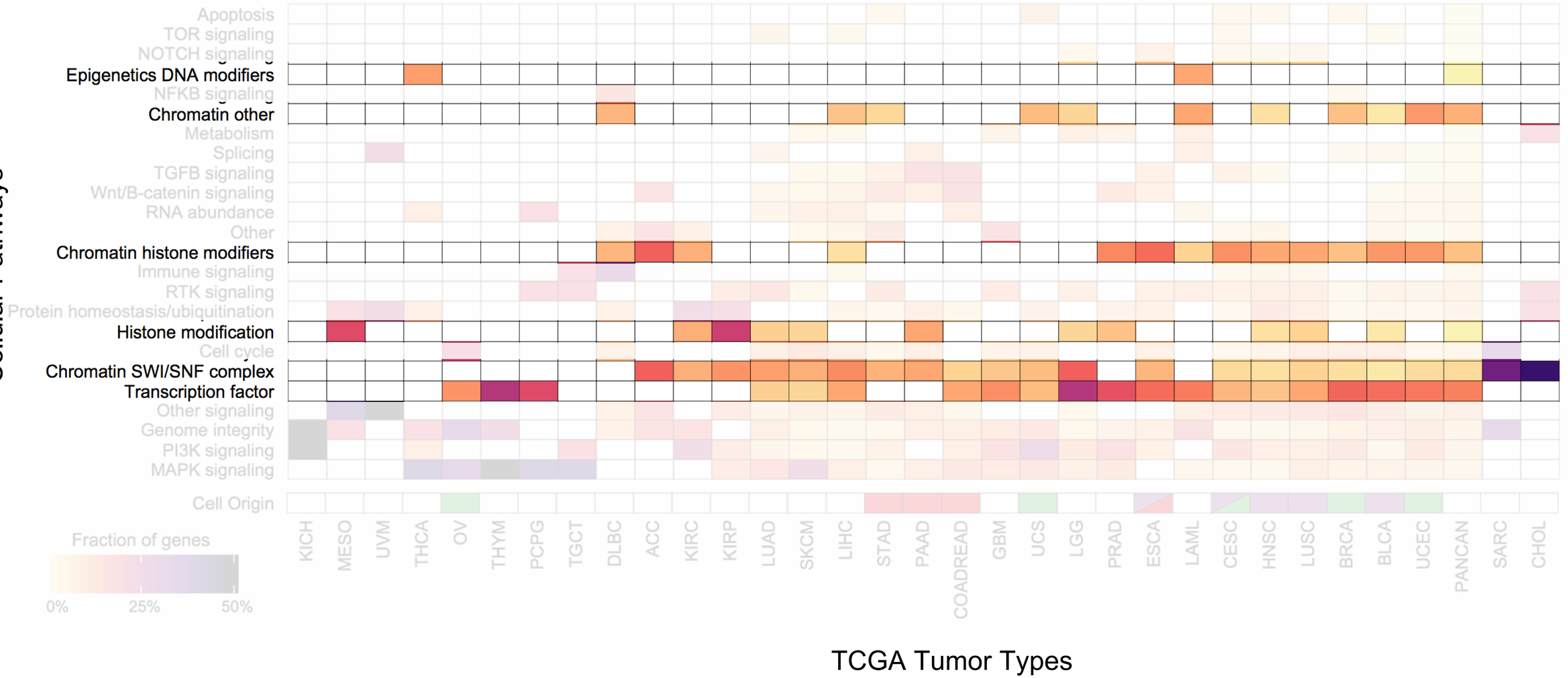
Credit: Sonya Parpart-Li

Cancer mutations span multiple cellular pathways



Adapted from Bailey et al., *Cell* 2018

Cancer mutations span multiple cellular pathways



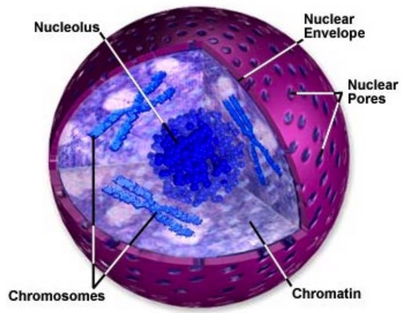
Adapted from Bailey et al., *Cell* 2018

Chromatin landscape perturbations in cancer

Histone modifiers
EZH2, MLL, KDM6B, NSD2, PRMTs, etc.

Chromatin remodeling complexes
SWI/SNF, ATRX, CHD, etc.

DNA modifiers
DNMTs, TET, etc.

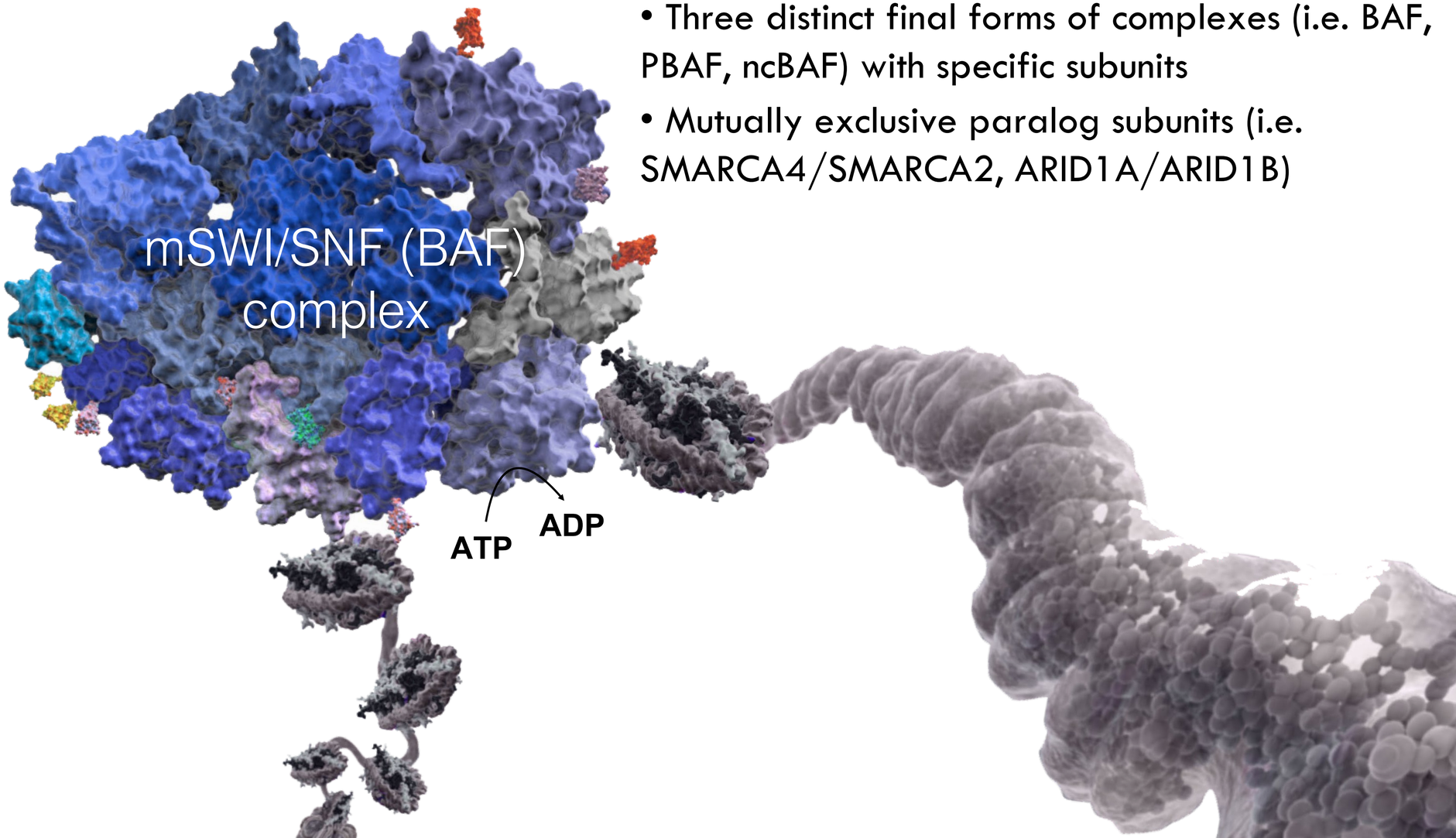


Histones
oncohistones H3.3, H3.1

Histone tails
H3K27M, H3K36M, H3G34V/R, etc.

Mammalian SWI/SNF complexes: chromatin remodeling machines

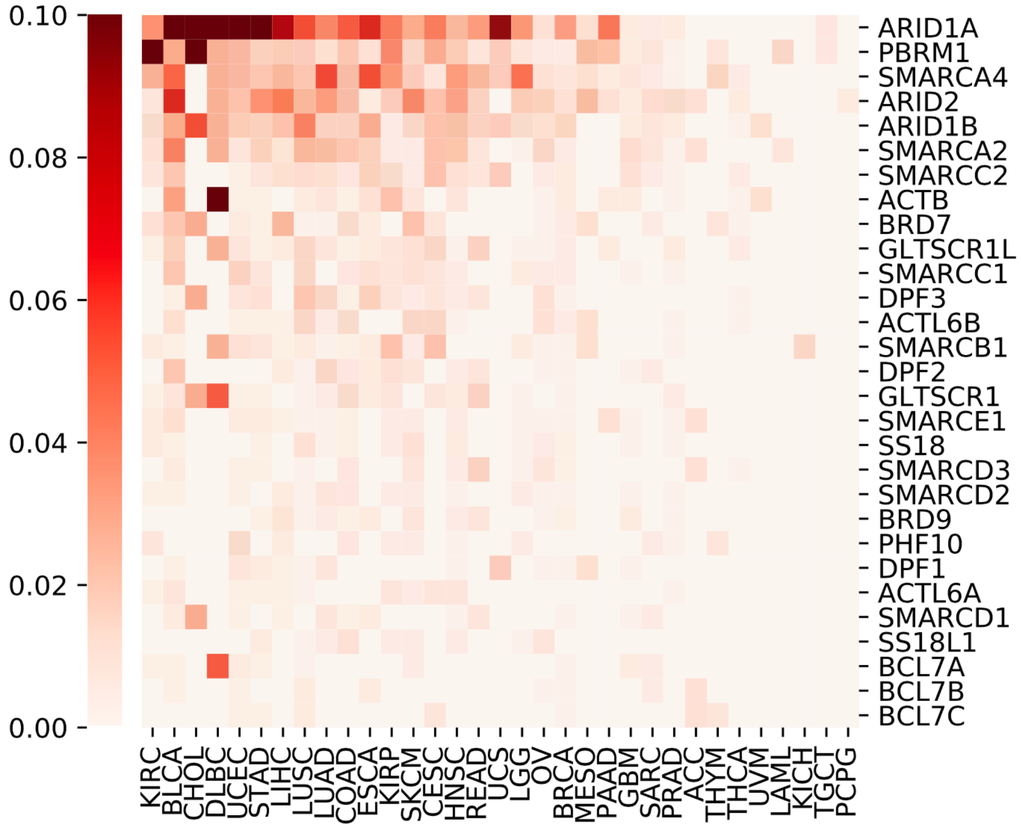
ARID1A
ARID1B
ARID2
SMARCA4
SMARCA2
SMARCC1
SMARCC2
SMARCD1
SMARCD2
SMARCD3
SMARCE1
SMARCB1
PBRM1
DPF1
DPF2
DPF3
PHF10
BRD7
BRD9
SS18
SS18L1
ACTL6A
ACTL6B
ACTB
GLTSCR1
GLTSCR1L
BCL7A
BCL7B
BCL7C



- Combinatorial assembly—> several hundred total possibilities
- Three distinct final forms of complexes (i.e. BAF, PBAF, ncBAF) with specific subunits
- Mutually exclusive paralog subunits (i.e. SMARCA4/SMARCA2, ARID1A/ARID1B)

Mutational landscape of mSWI/SNF genes in human cancer

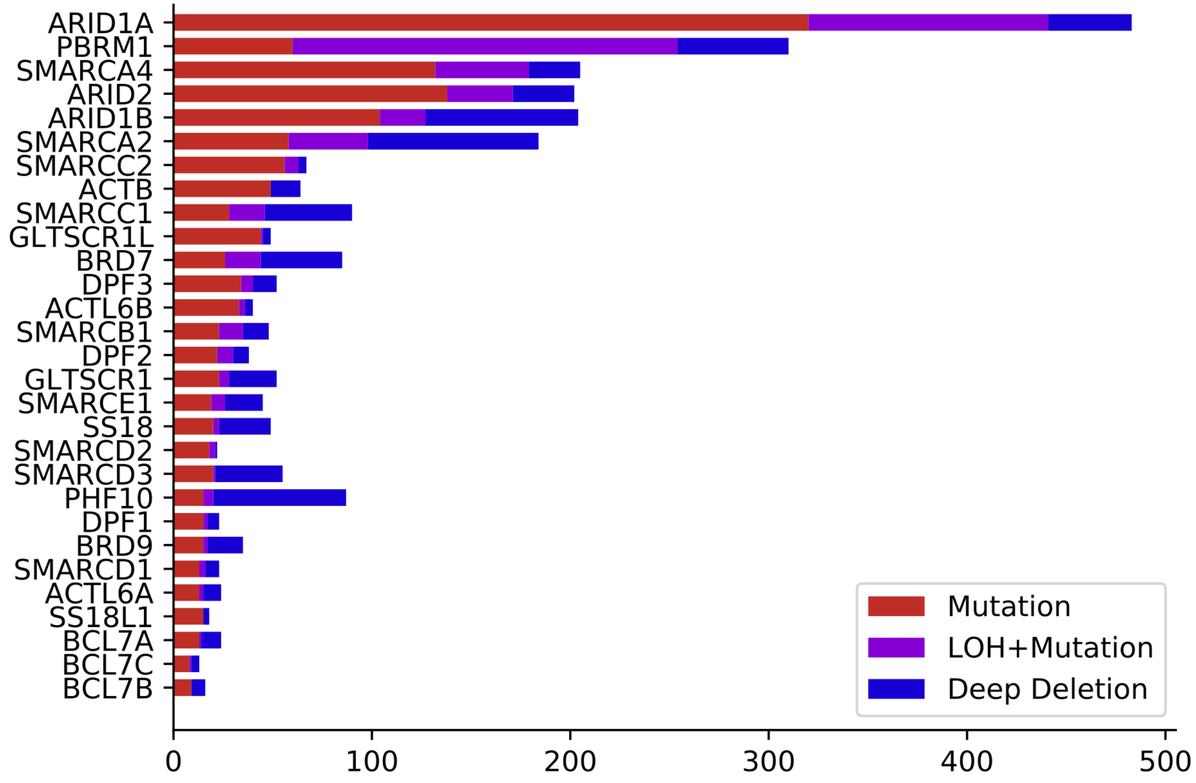
~20% of human cancers



TCGA Tumor Types

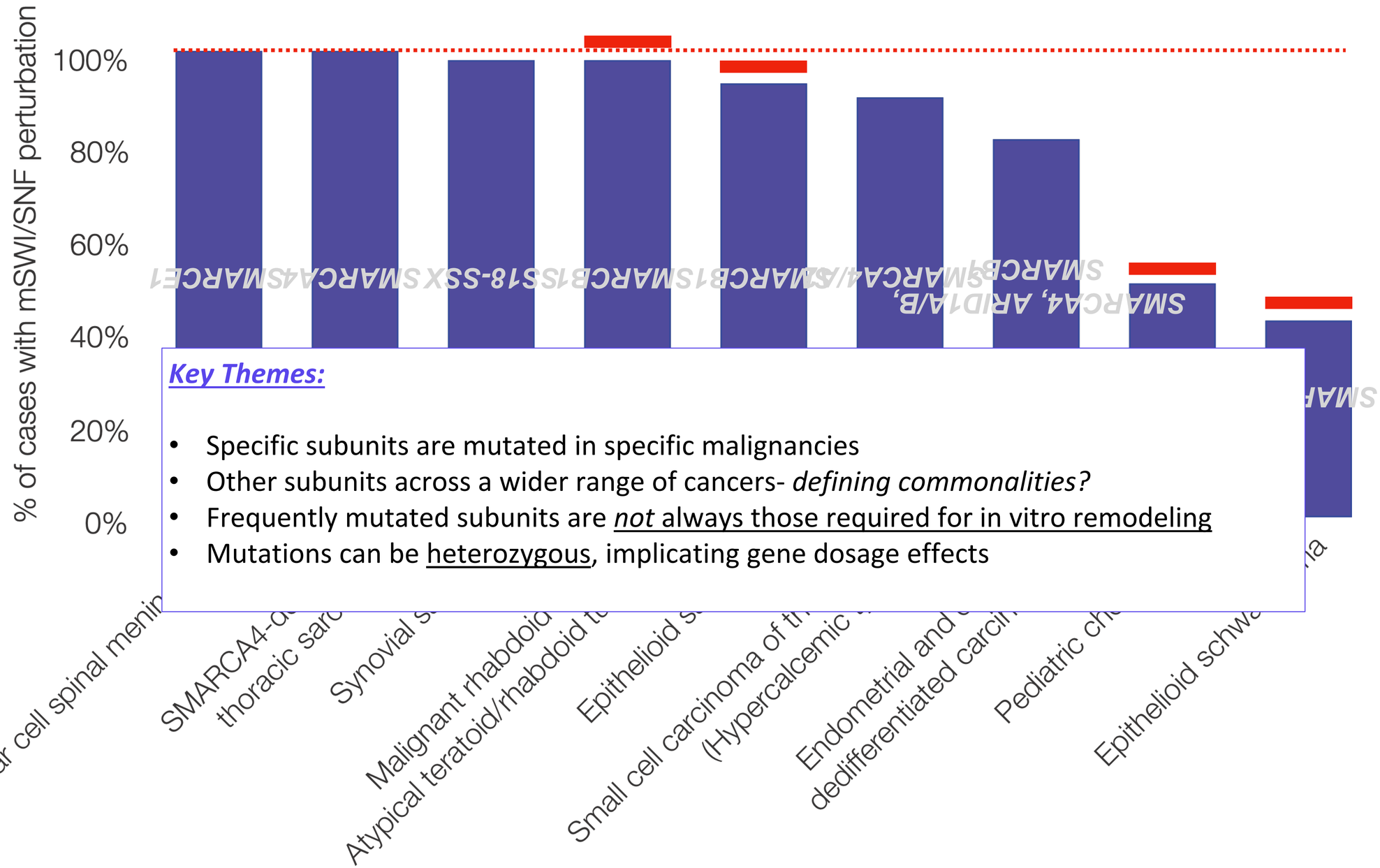
mSWI/SNF subunits

>24% of human cancers



Number of Tumors (of 8631 filtered)

Rare cancers driven by mSWI/SNF complex perturbations



Key Themes:

- Specific subunits are mutated in specific malignancies
- Other subunits across a wider range of cancers- *defining commonalities?*
- Frequently mutated subunits are *not* always those required for in vitro remodeling
- Mutations can be heterozygous, implicating gene dosage effects

Mammalian SWI/SNF complexes: Mutational landscape in human cancer

3 final-form complexes:
BAF, PBAF, and ncBAF

Mutated in >20% of cancers

Clear cell meningioma (~100%)

Malignant rhabdoid tumor (>98%)
Atypical teratoid/rhabdoid tumor (>98%)
Epithelial sarcoma (>90%)

Nakayama et al., *Nature Genetics* 2017

Valencia et al., *Cell,* 2019

Hepatocellular carcinoma

Clear cell renal cell carcinoma (>40%)

Ovarian clear cell carcinoma (68%)
Endometriod carcinoma
Neuroblastoma

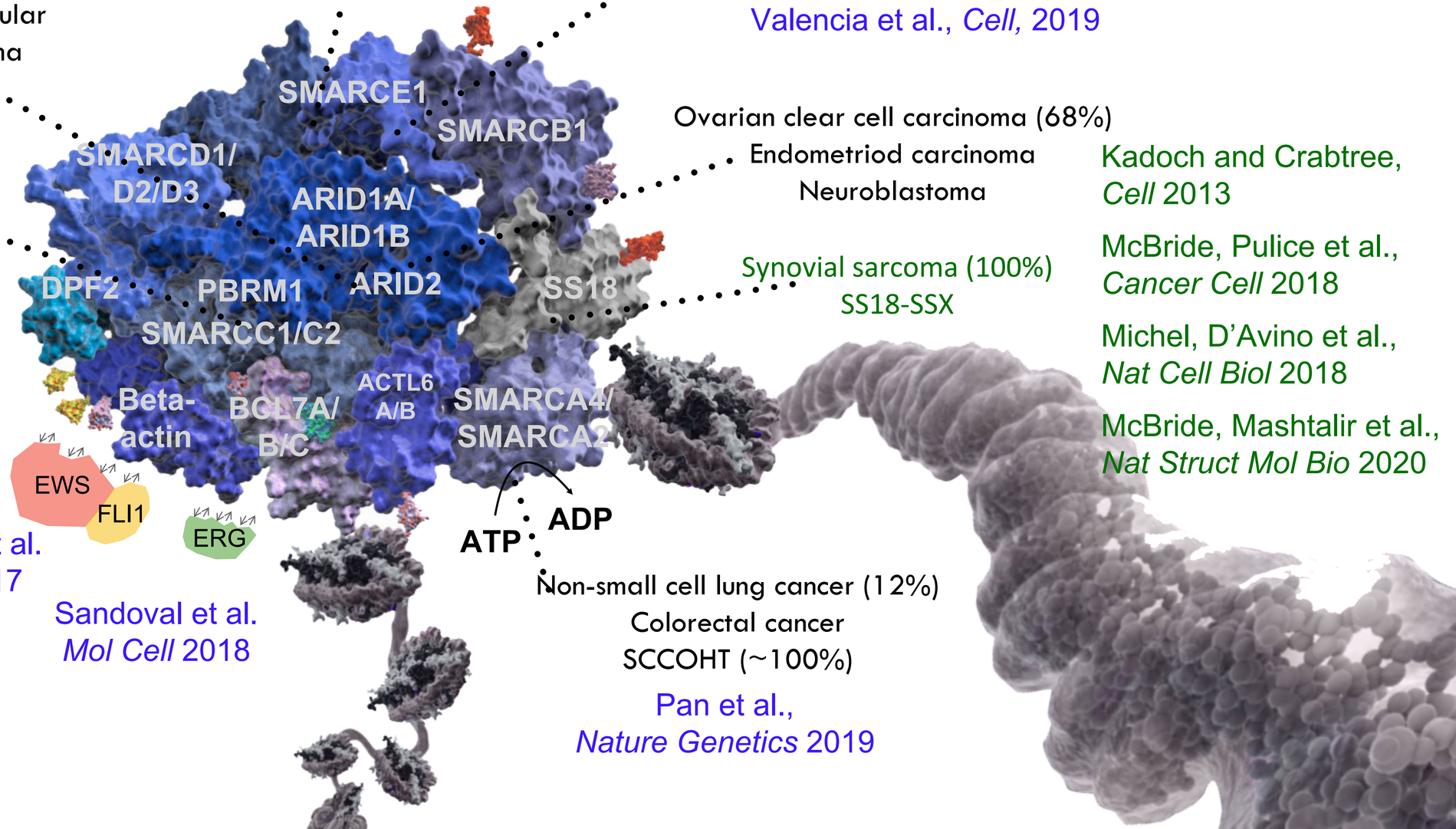
Kadoch and Crabtree, *Cell* 2013

McBride, Pulice et al., *Cancer Cell* 2018

Michel, D'Avino et al., *Nat Cell Biol* 2018

McBride, Mashtalir et al., *Nat Struct Mol Bio* 2020

Synovial sarcoma (100%)
SS18-SSX



Boulay et al.
Cell 2017

Sandoval et al.
Mol Cell 2018

Non-small cell lung cancer (12%)
Colorectal cancer
SCCOHT (~100%)

Pan et al.,
Nature Genetics 2019

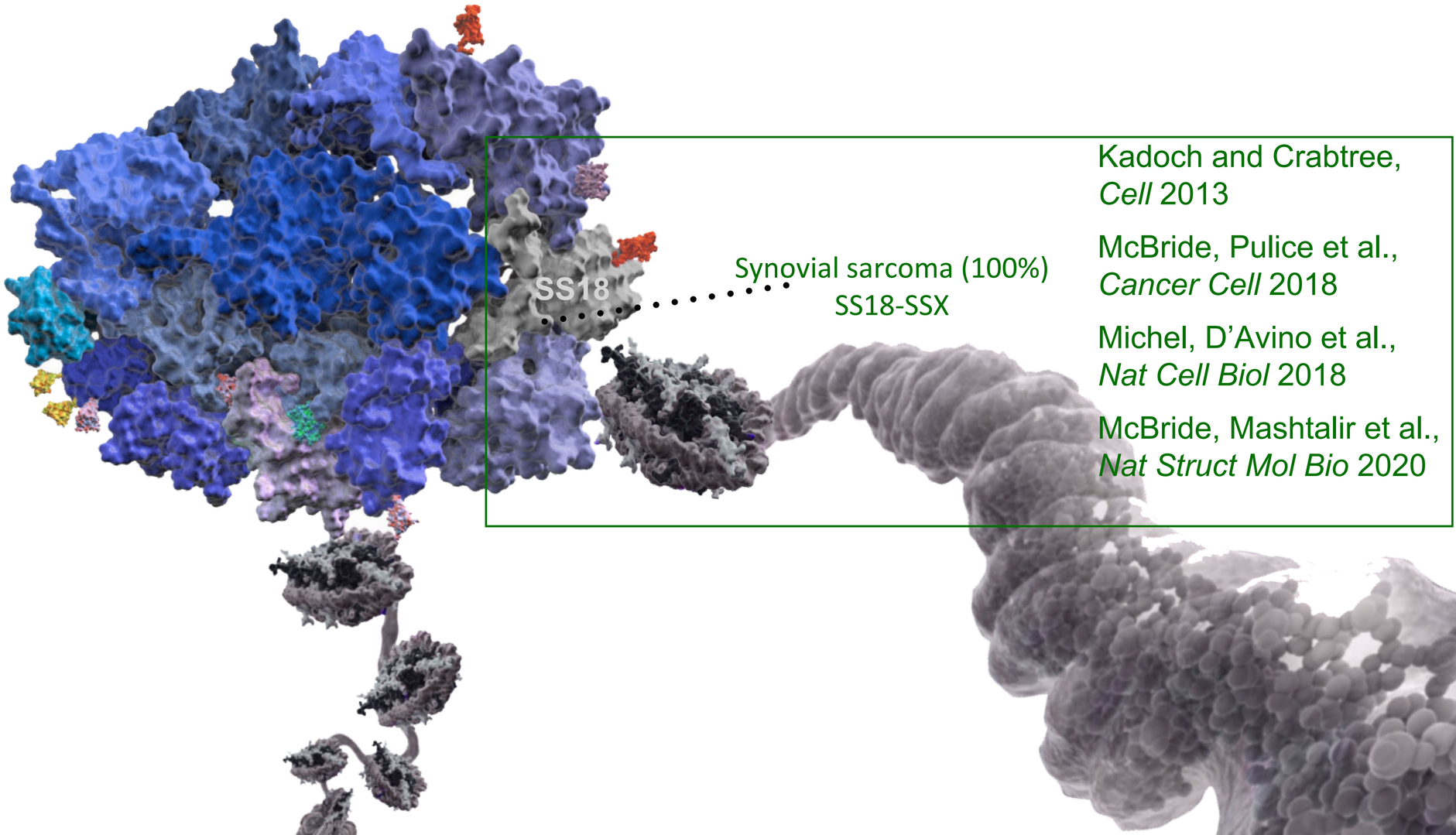
Biochemical and structural modules

Mashtalir et al., *Cell* 2018
Mashtalir et al., *Cell* 2020

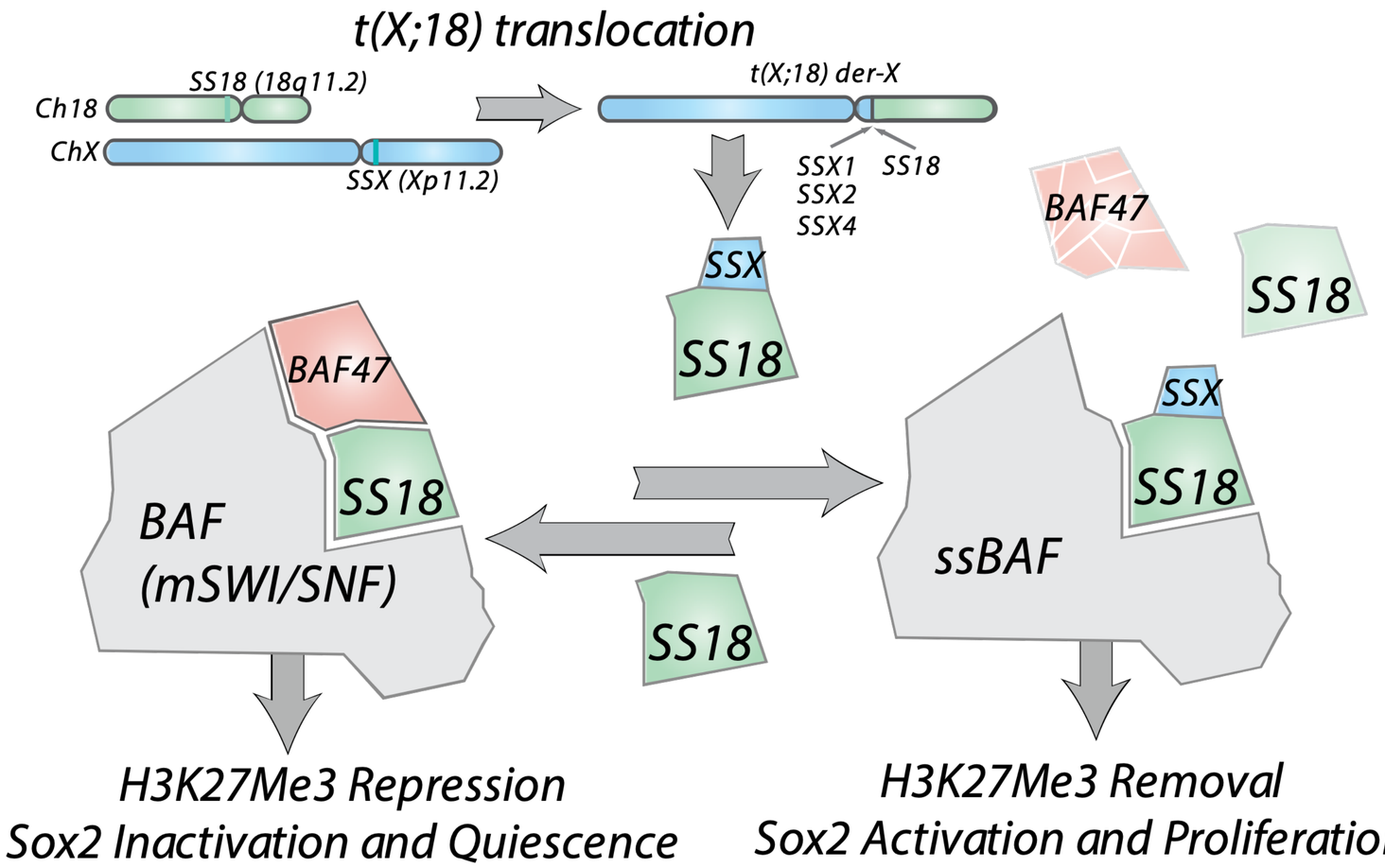
Functional modules

Pan, Meyers et al.,
Cell Systems 2018

Mammalian SWI/SNF complexes: Mutational landscape in human cancer



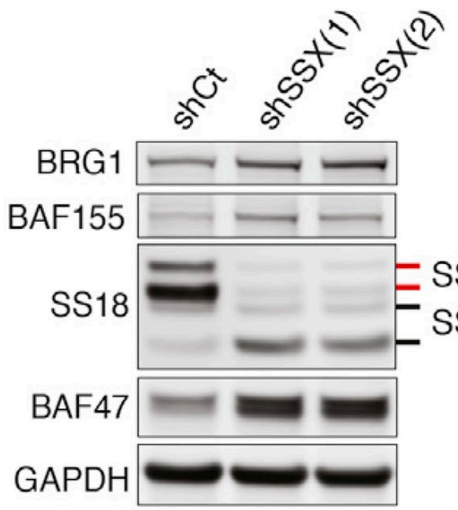
Gain-of-function mSWI/SNF complex perturbations



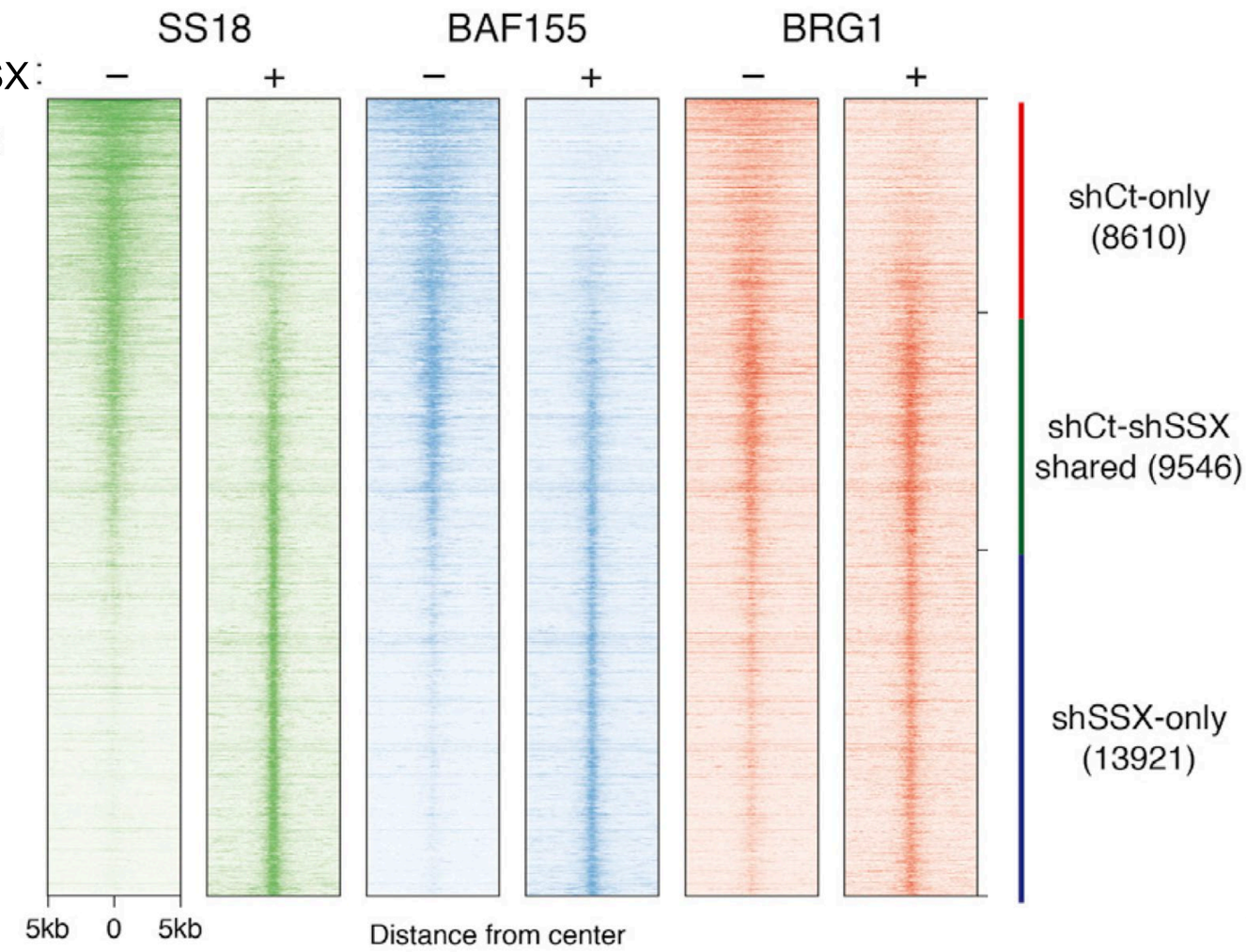
Kadoch & Crabtree, *Cell* 2013
Kadoch et al, *Nature Genetics* 2013

How does this SS18-SSX perturbation affect global targeting of BAF complexes and subsequent gene expression?

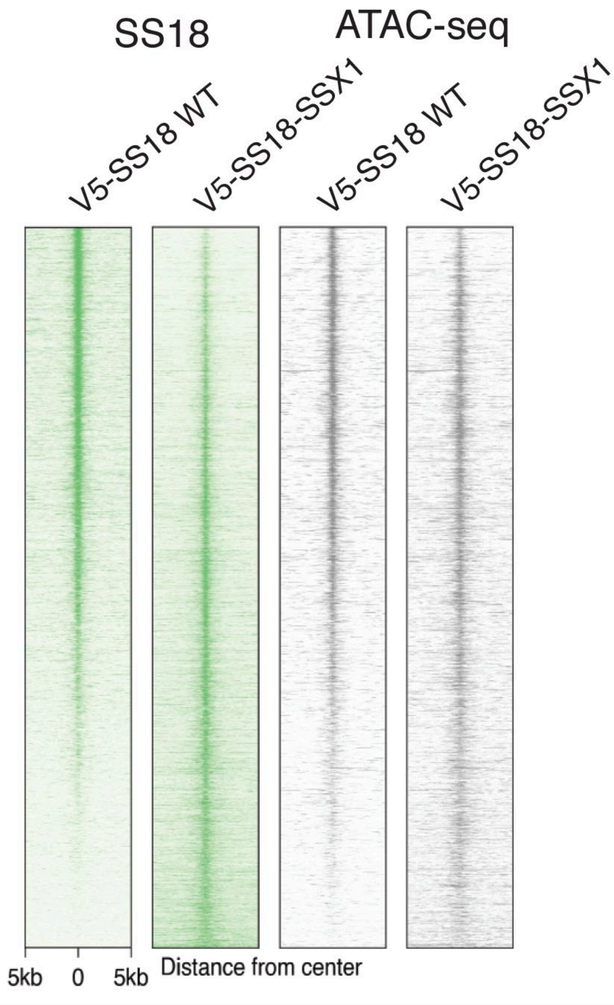
Unique targeting and localization of BAF complexes in SS



In SS cell lines (Aska-SS)



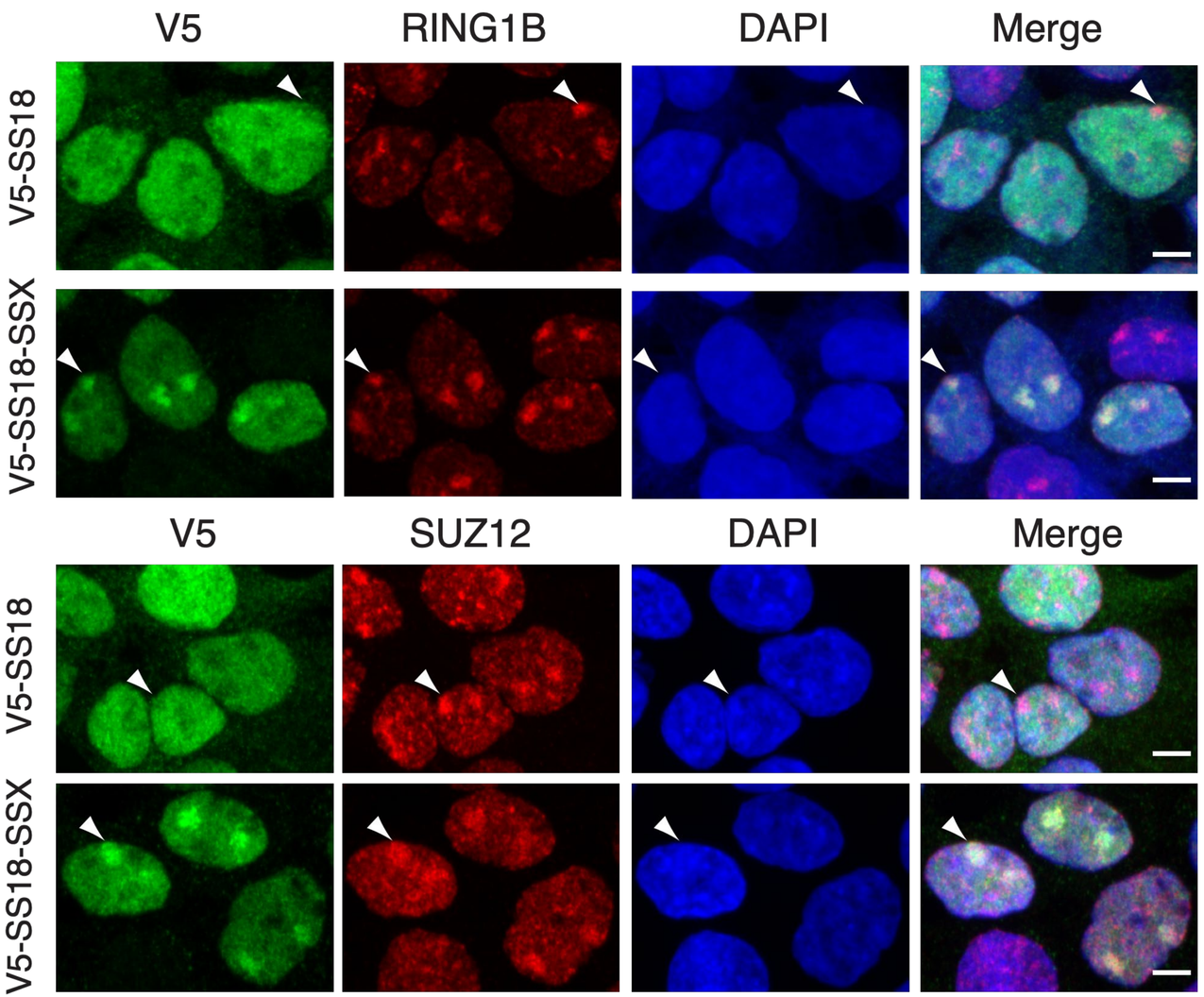
In fibroblasts (CRL7250)

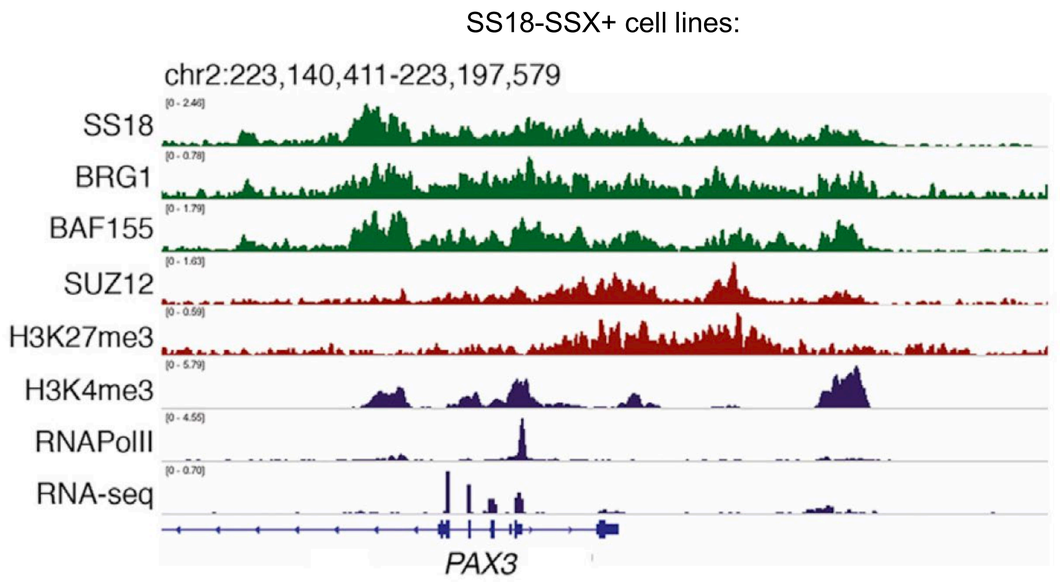


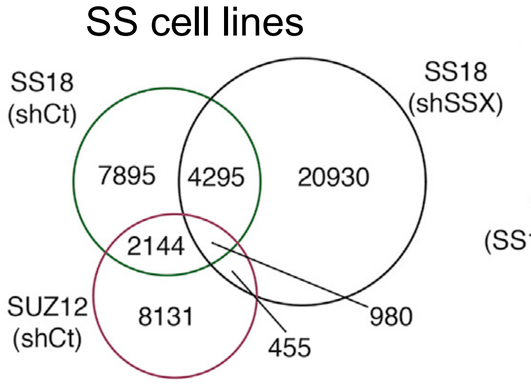
McBride et al., *Cancer Cell* 2018

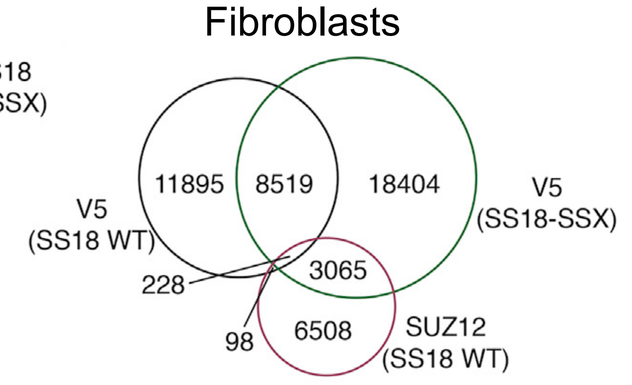
Unique targeting and localization of BAF complexes in SS

SS18-SSX-containing BAF complexes co-target with PRC2 more than WT BAF complexes









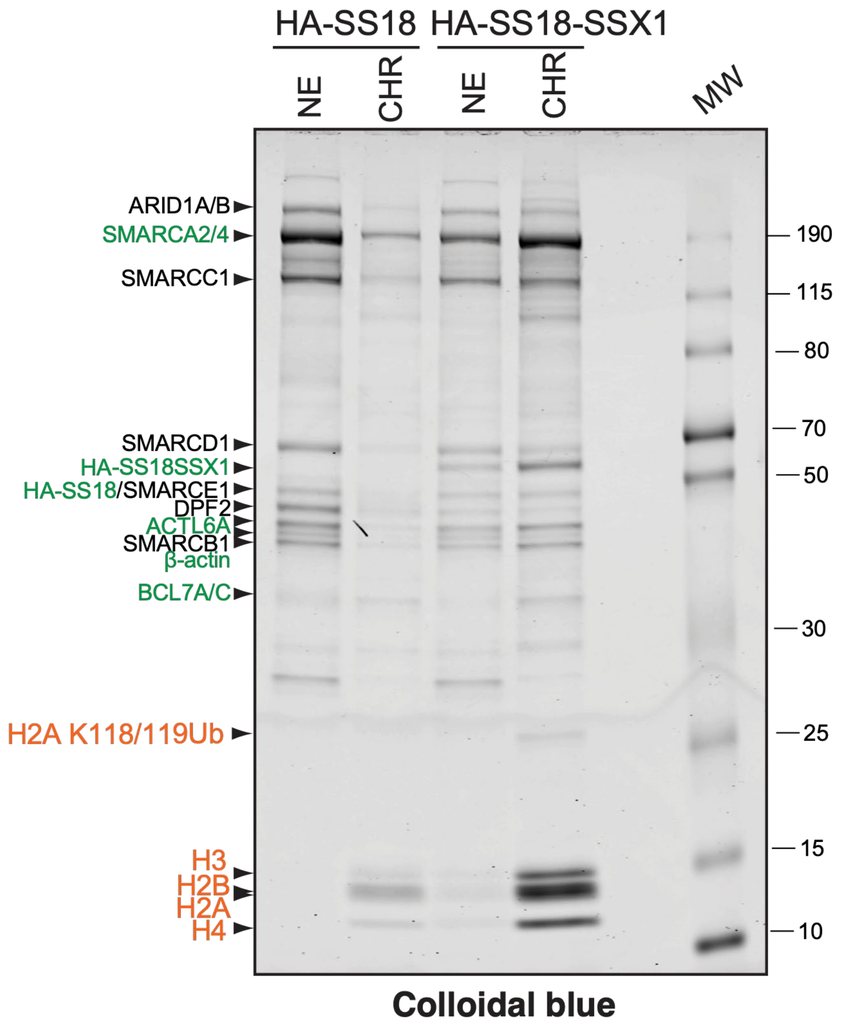
Key Question

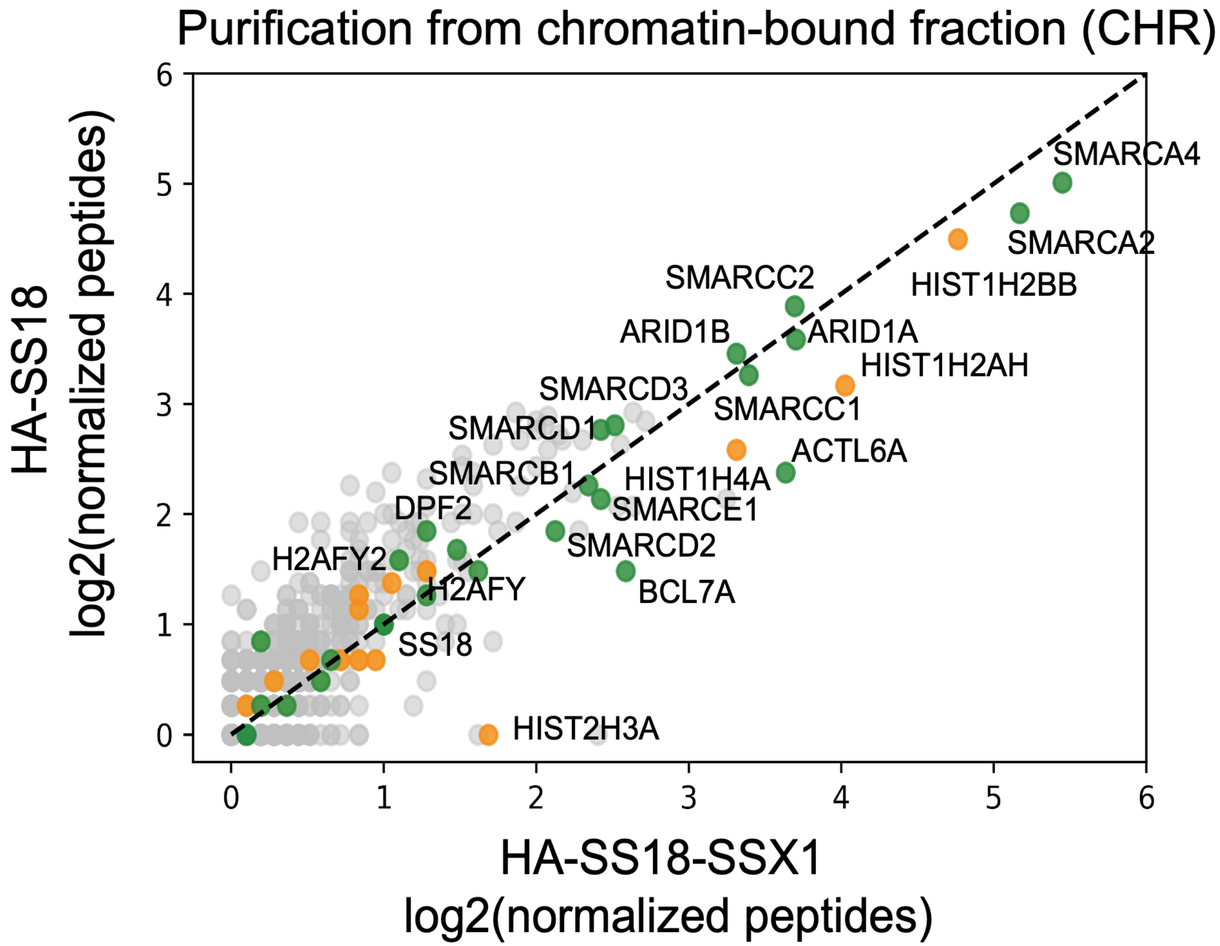
What is the mechanism that underlies *site-specific targeting* and *chromatin interaction properties* of SS complexes?

If we knew this we could:

- ***Define specific features of the interaction of SS18-SSX with chromatin***
- ***Understand cancer-specific synthetic lethal dependencies observed in SS***
- ***Inform and design new therapeutic approaches***

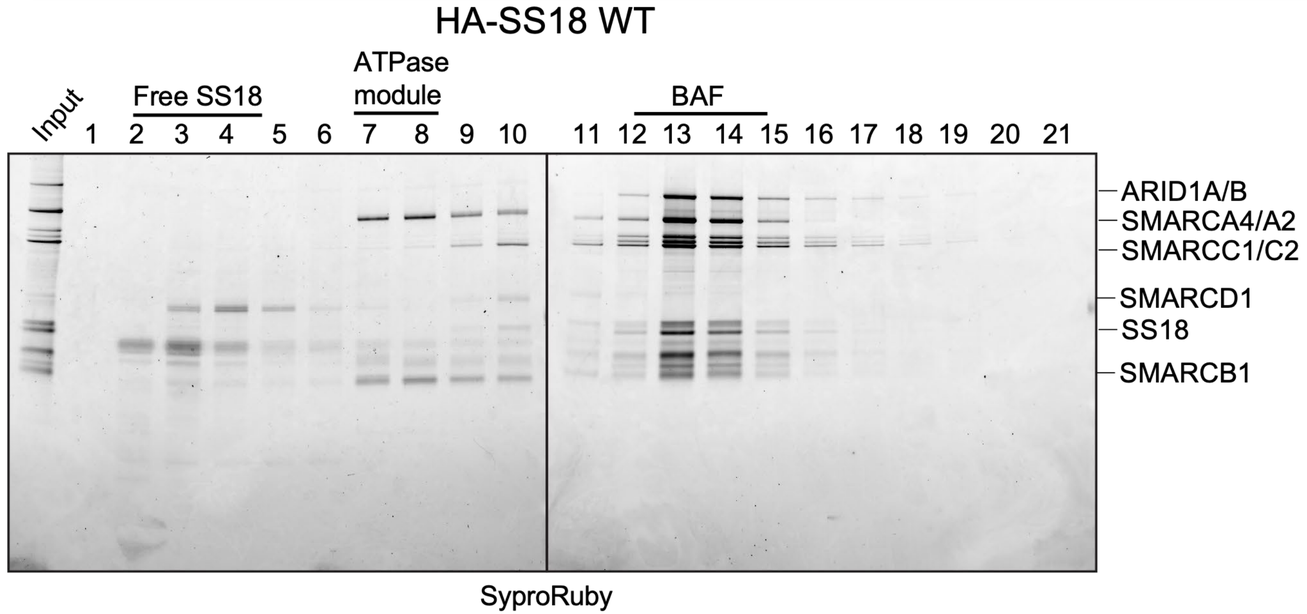
SS18-SSX-bound BAF complexes bind tightly to chromatin

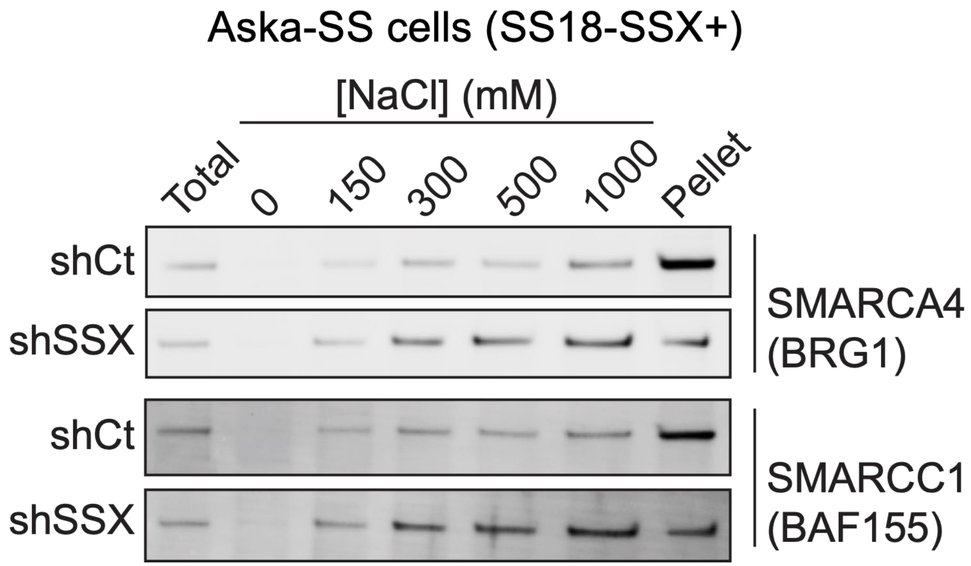


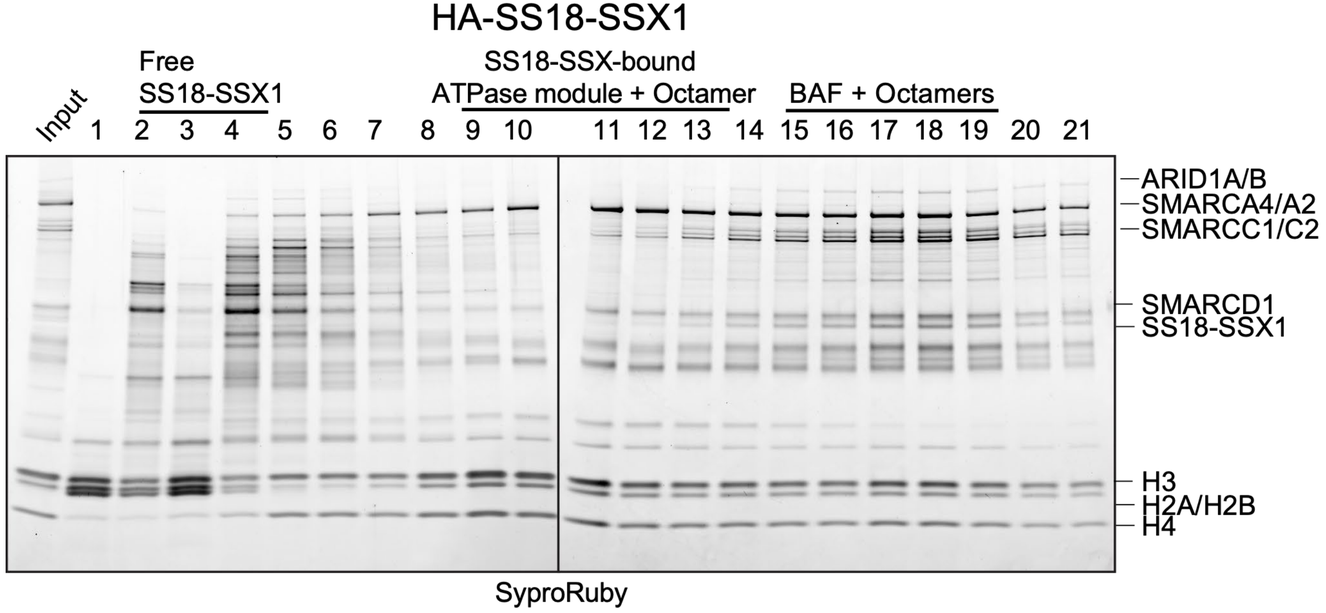


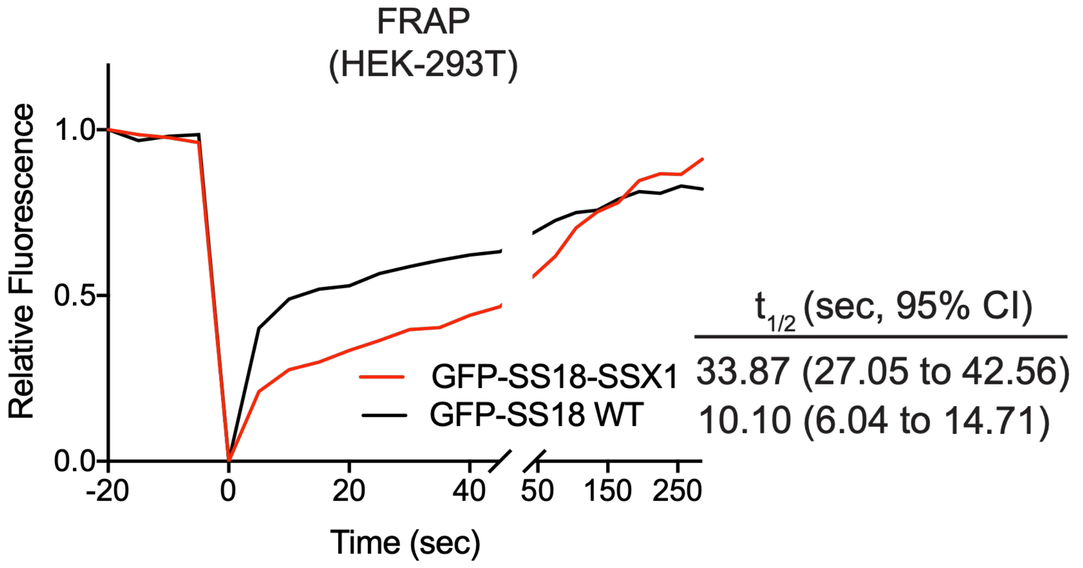
McBride, Mashtalir et al.

SS18-SSX-bound BAF complexes bind tightly to chromatin



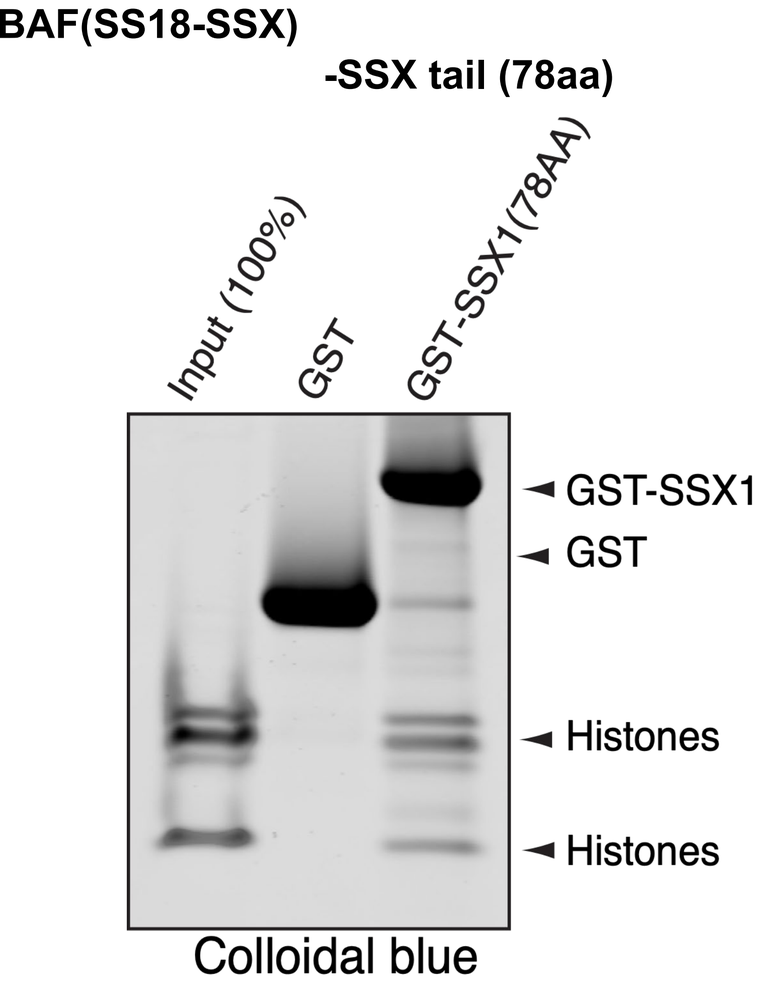






McBride, Mashtalir et al.

SSX alone is sufficient to bind nucleosomes, and targets to repressed chromatin



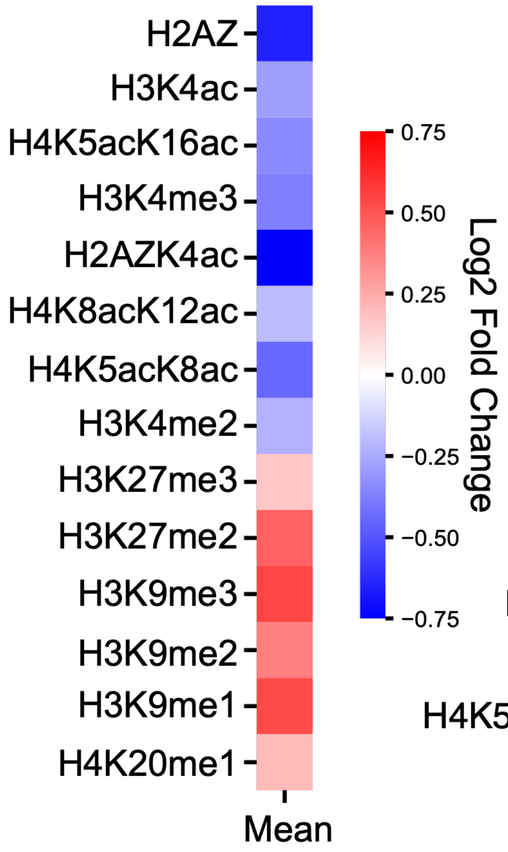
GST vs GST-SSX Protein

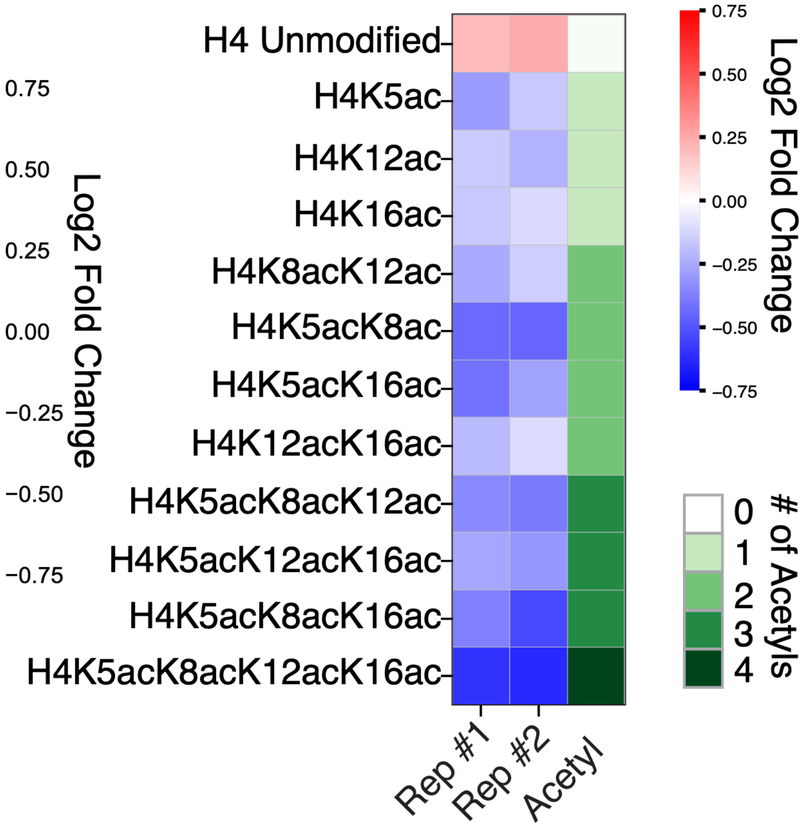
+

Mammalian Mononucleosomes (MNase-digested)

↓

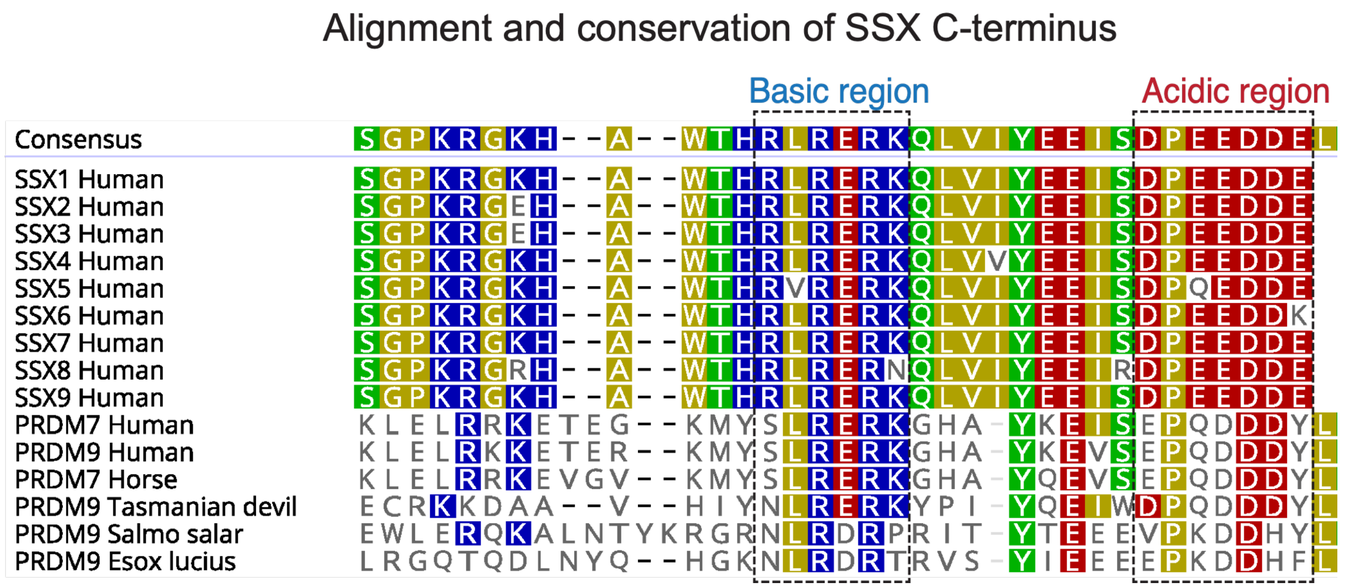
Quantitative targeted histone Mass spectrometry

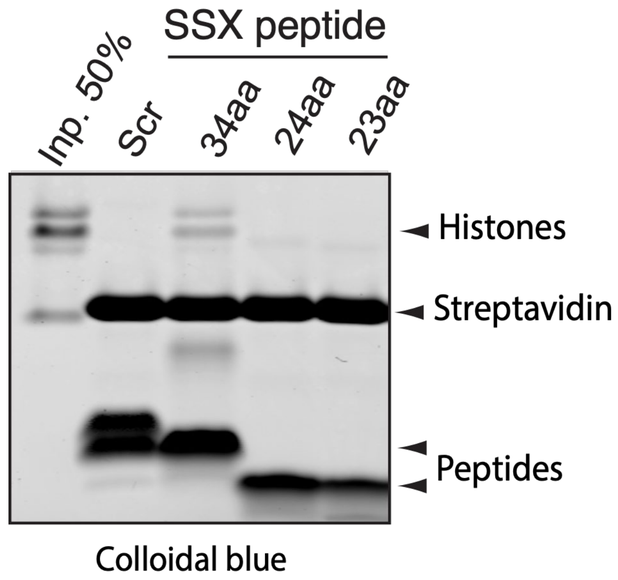


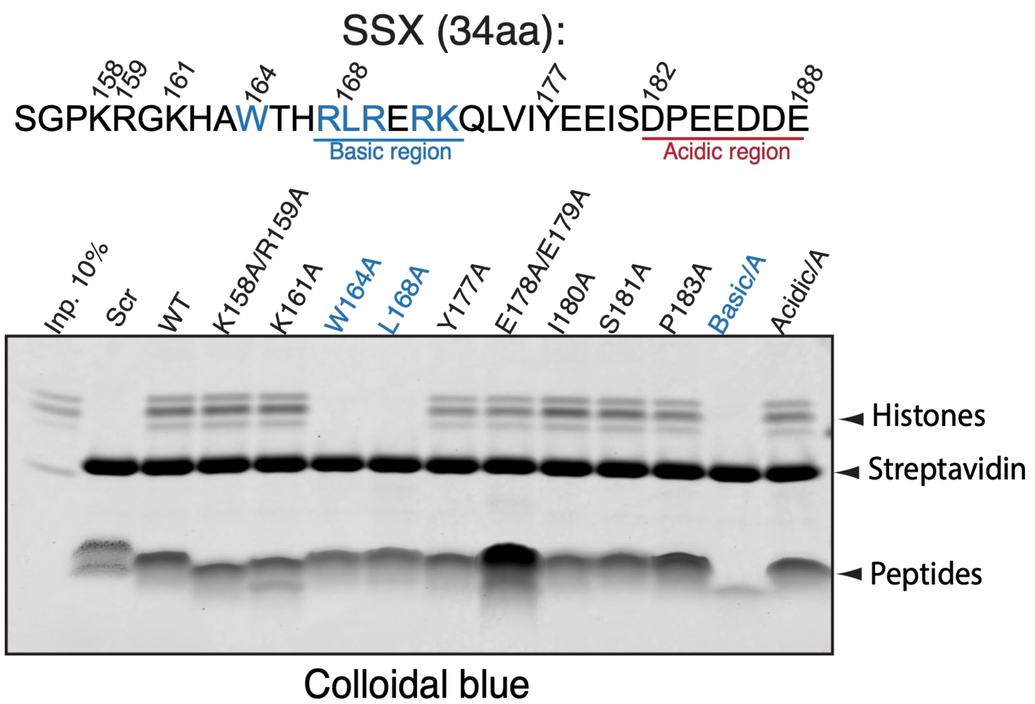


McBride, Mashtalir et al.

Two conserved regions within SSX: basic region is required for nucleosome binding



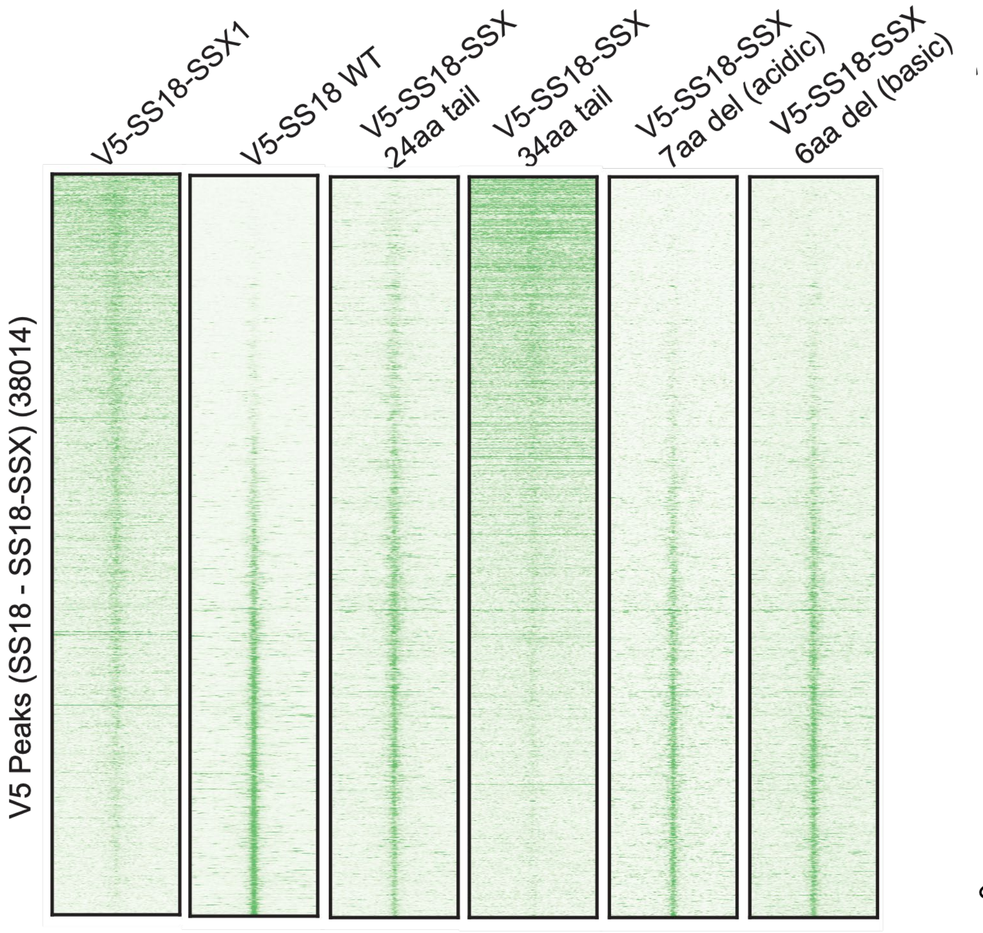




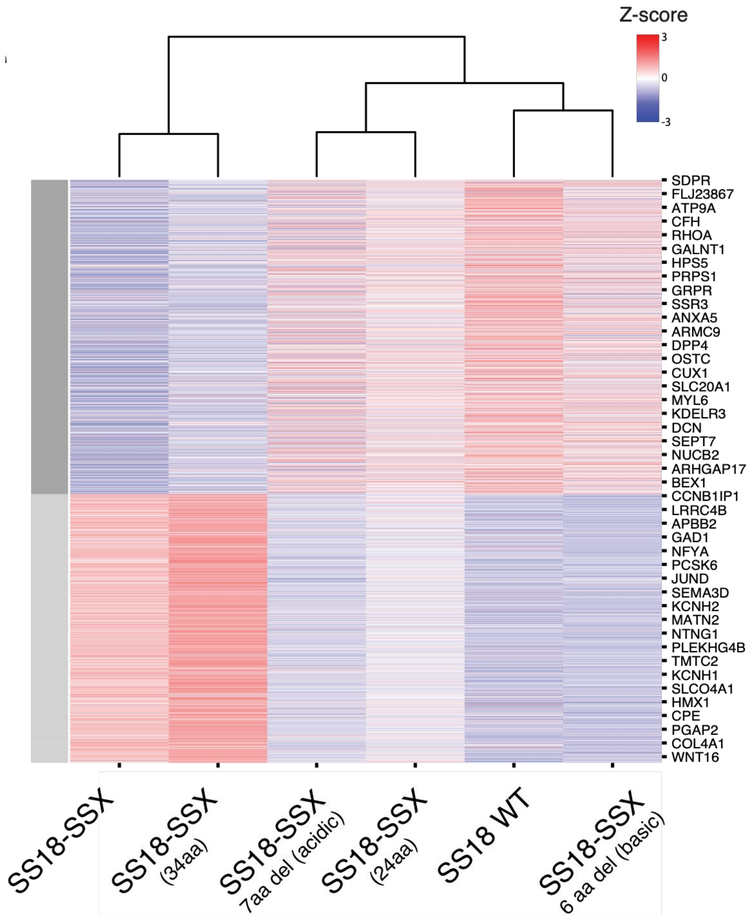
McBride, Mashtalir et al.

Both conserved regions within SSX are required for oncogenic targeting/activity

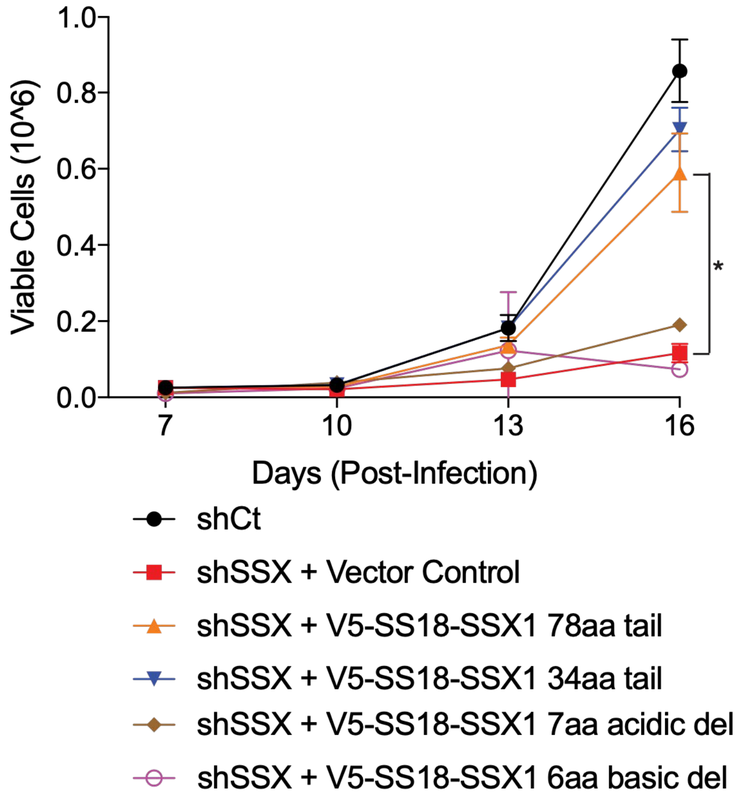
ChIP-seq (CRL7250 fibroblasts)



RNA-seq

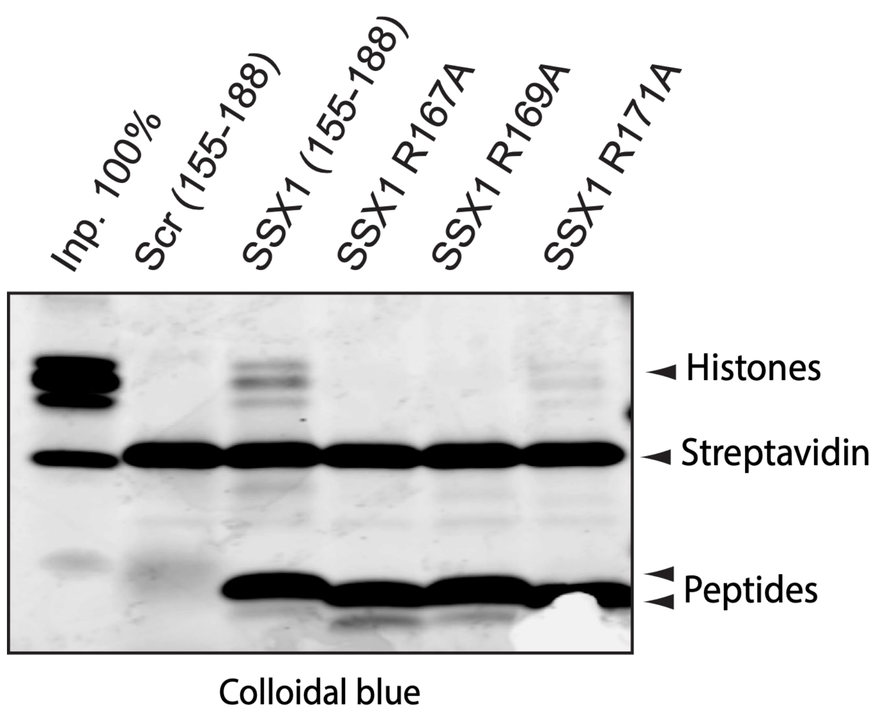


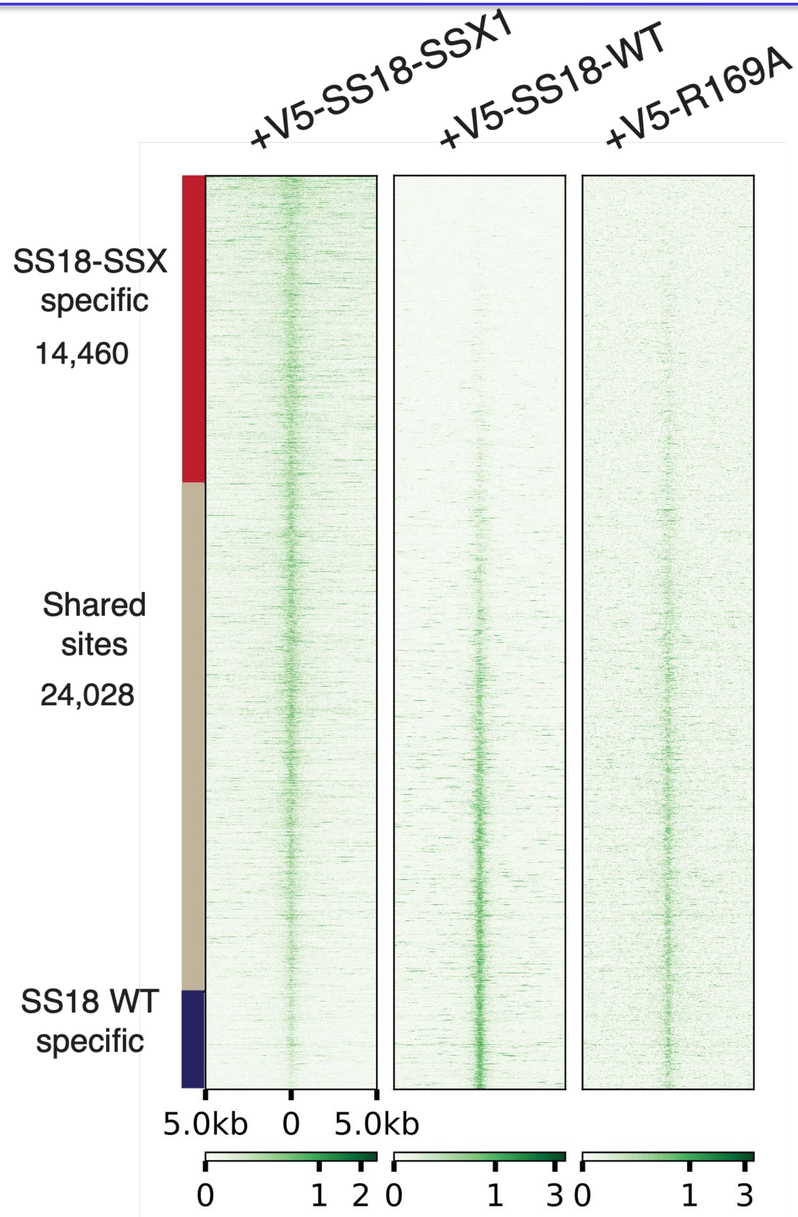
Proliferation (Aska-SS cells)



McBride, Mashtalir et al.

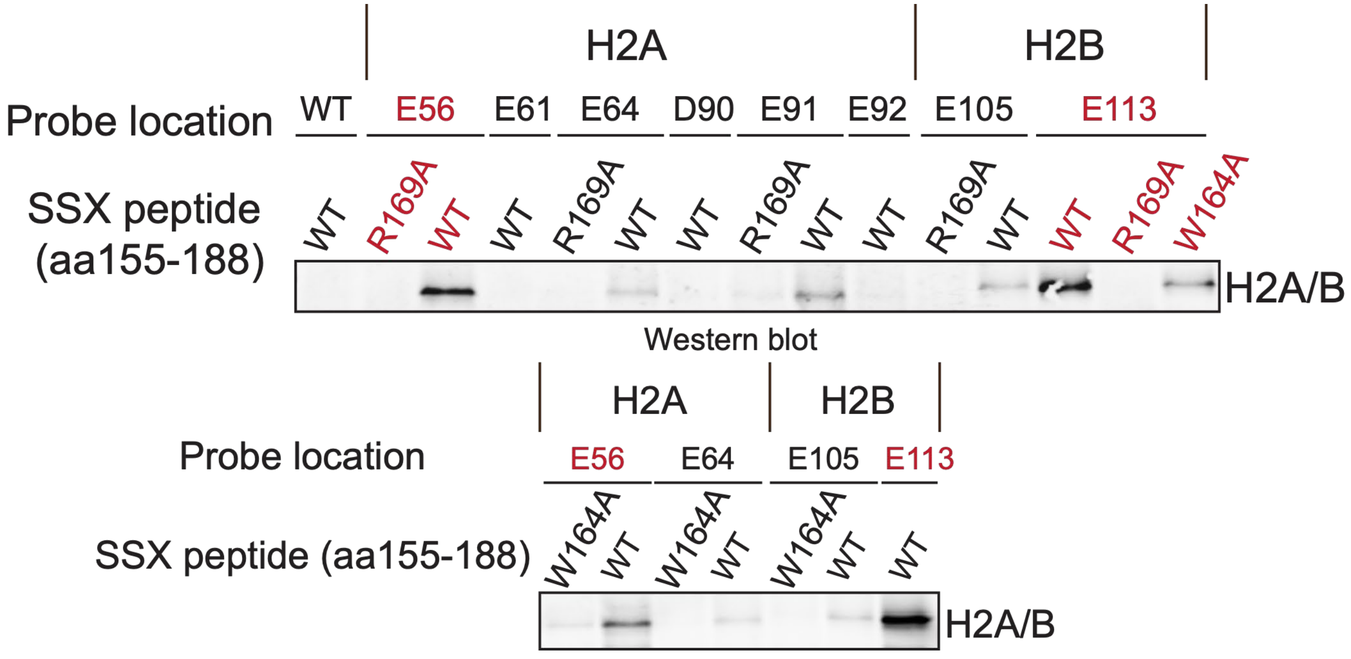
Basic region disruption breaks nucleosome binding and SSX-specific targeting

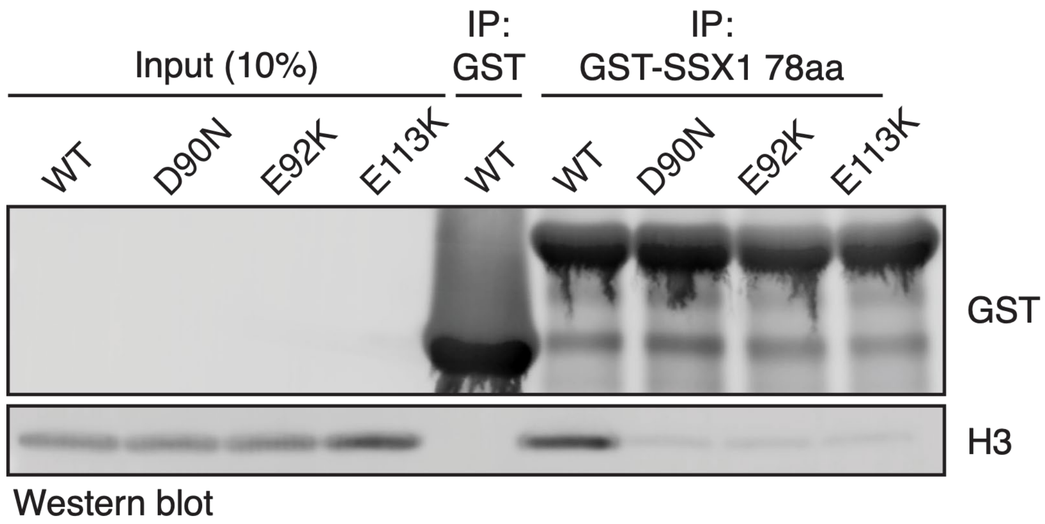


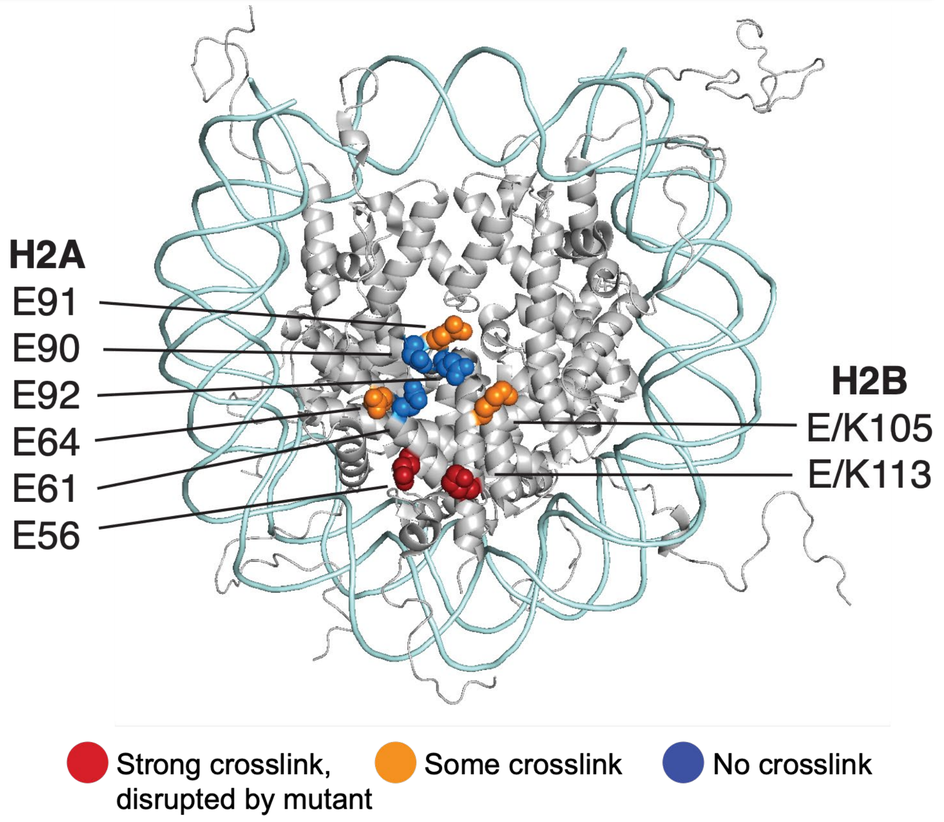


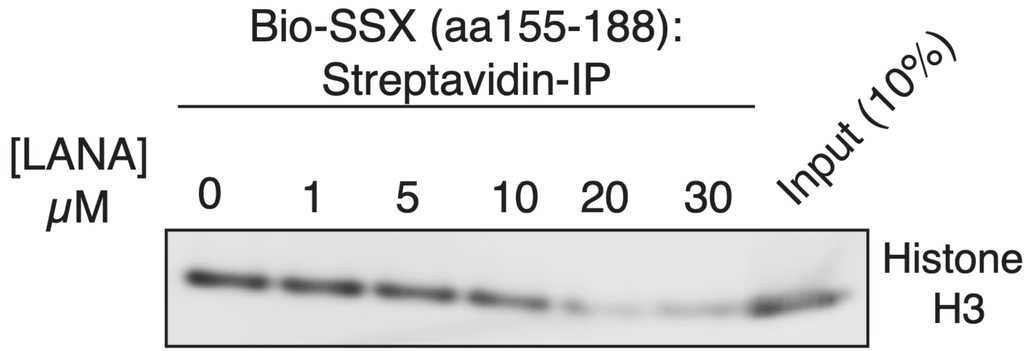
McBride, Mashtalir et al.

SSX binds directly to the nucleosome H2A/H2B acidic patch







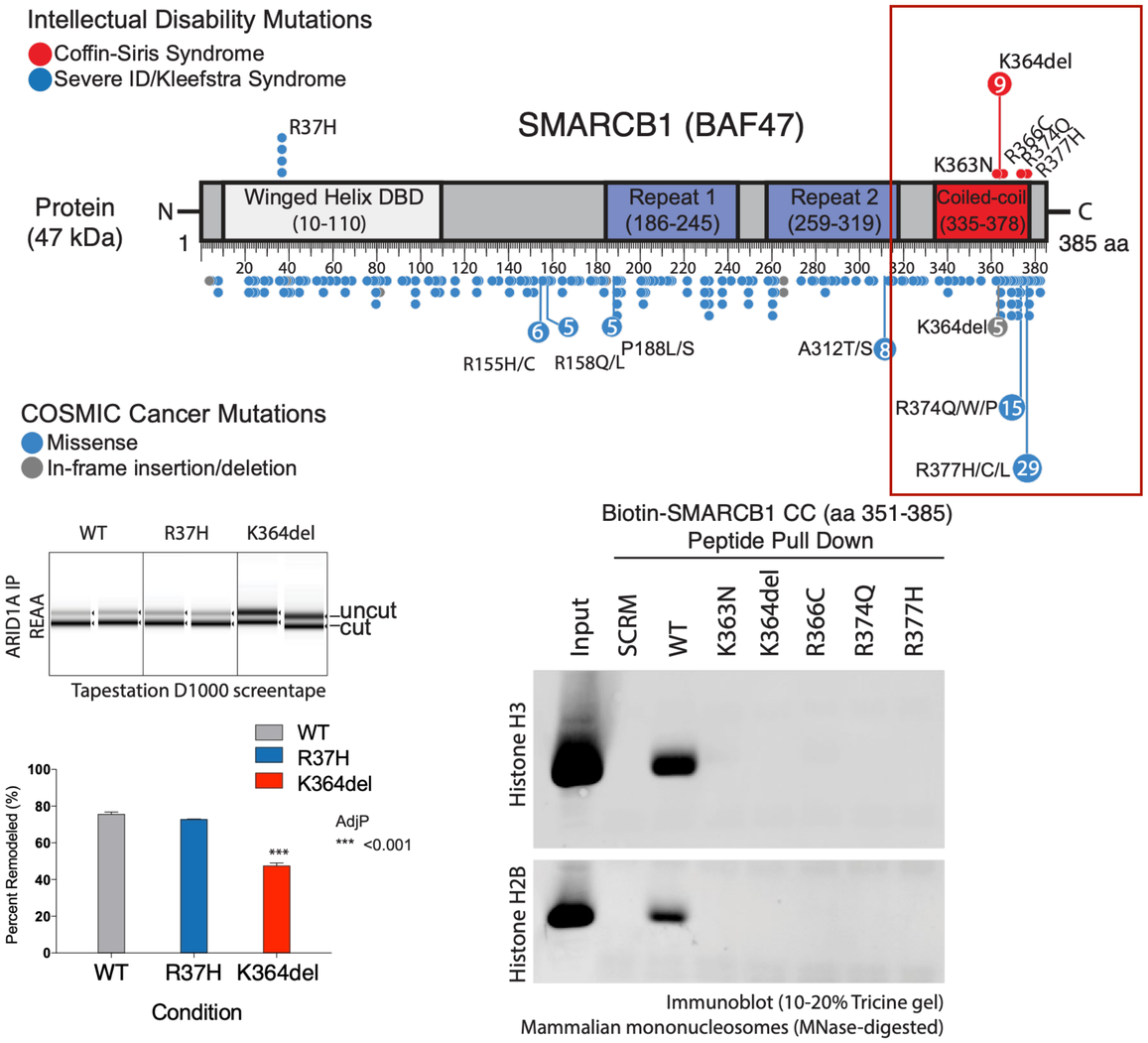


With Tom Muir, Hai Dao (Princeton University)

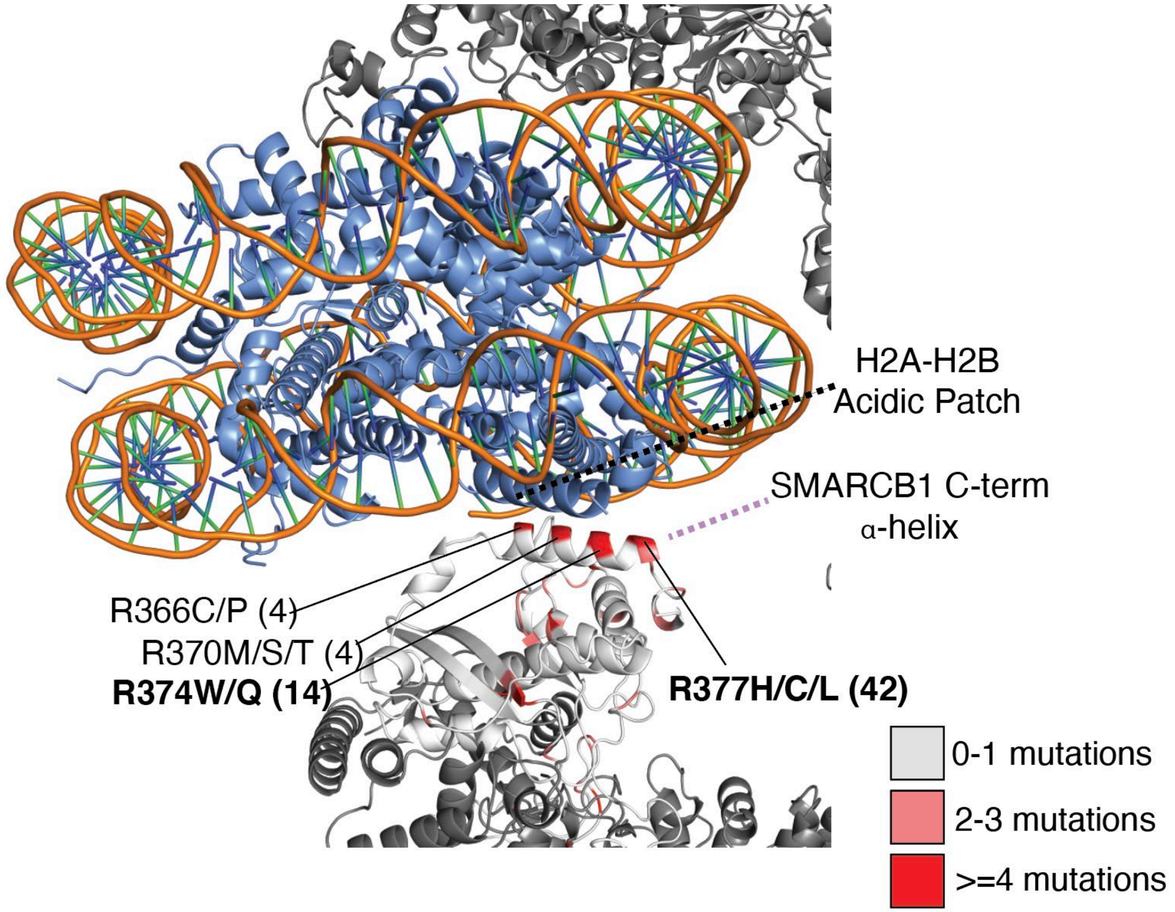
McBride, Mashtalir et al.

SSX binds directly to the nucleosome H2A/H2B acidic patch

Where studies on intellectual disability and synovial sarcoma converge….

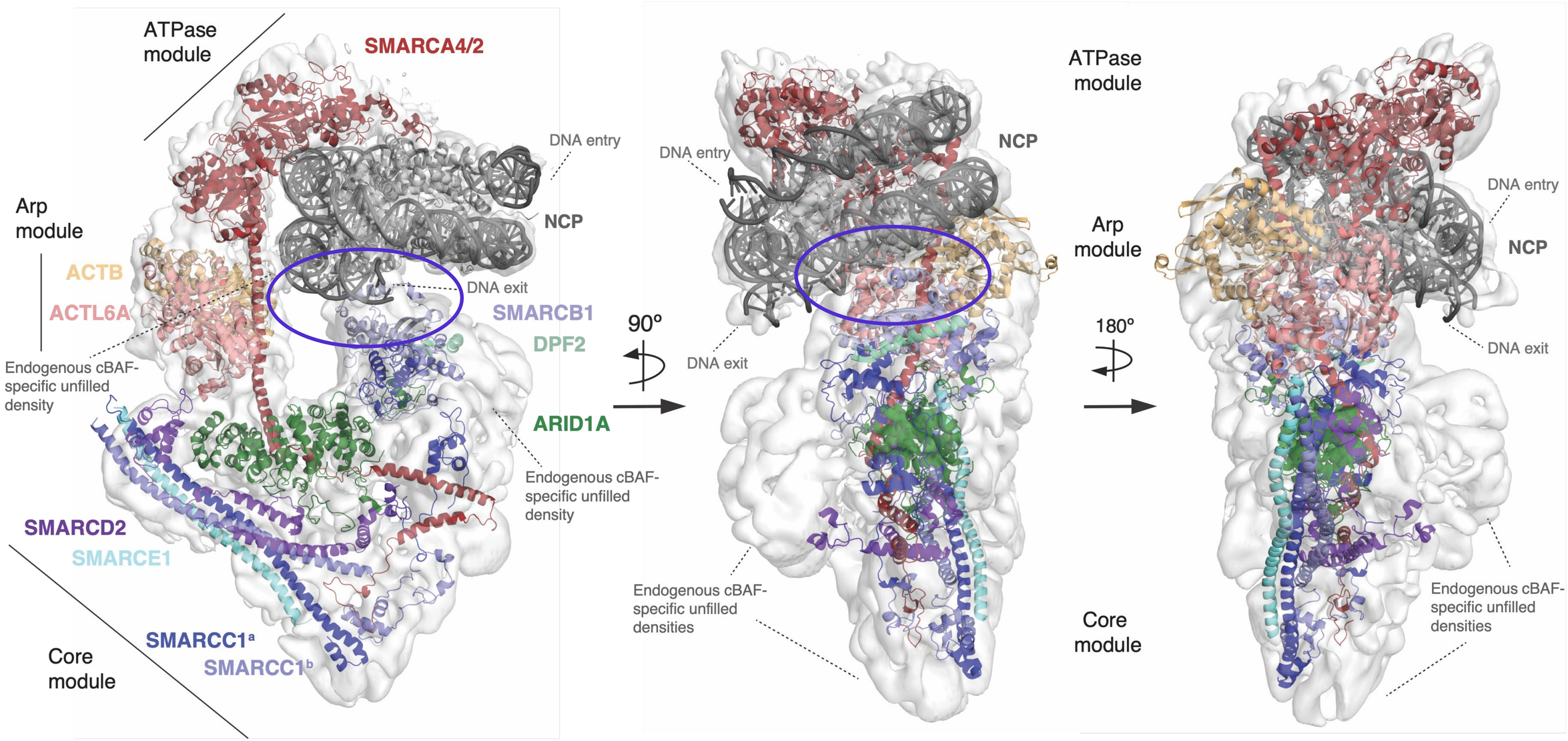


Valencia et al., *Cell*, 2019



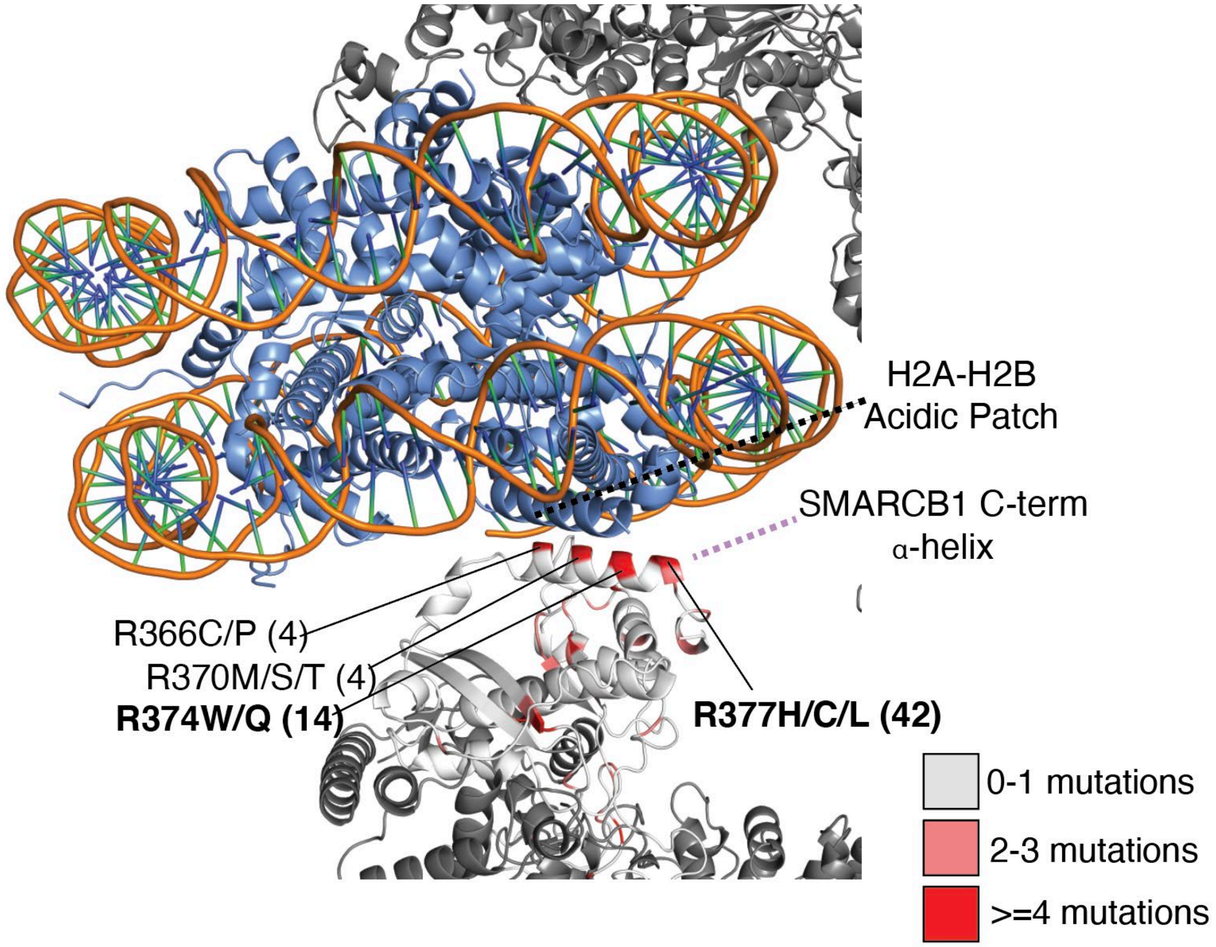
Mashtalir et al., *Cell* 2020

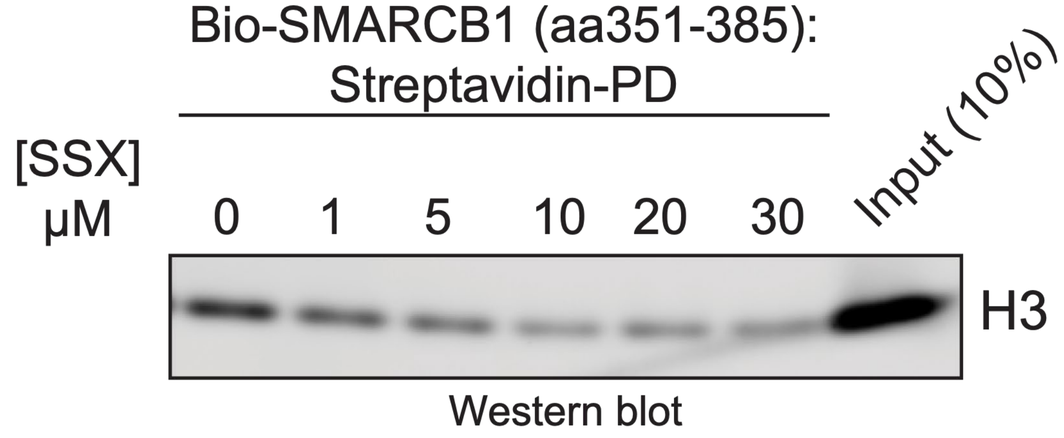
3D structural efforts of human BAF-NCP complex inform subunit function

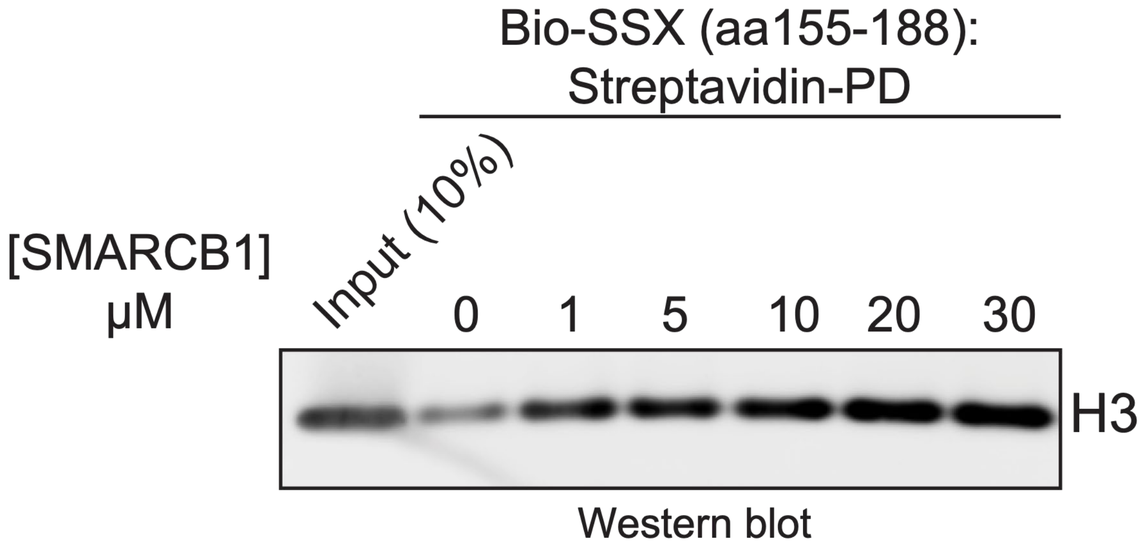


Mashtalir et al., *Cell* 2020

The SSX-acidic patch interaction displaces the SMARCB1 CTD-acidic patch interaction…..

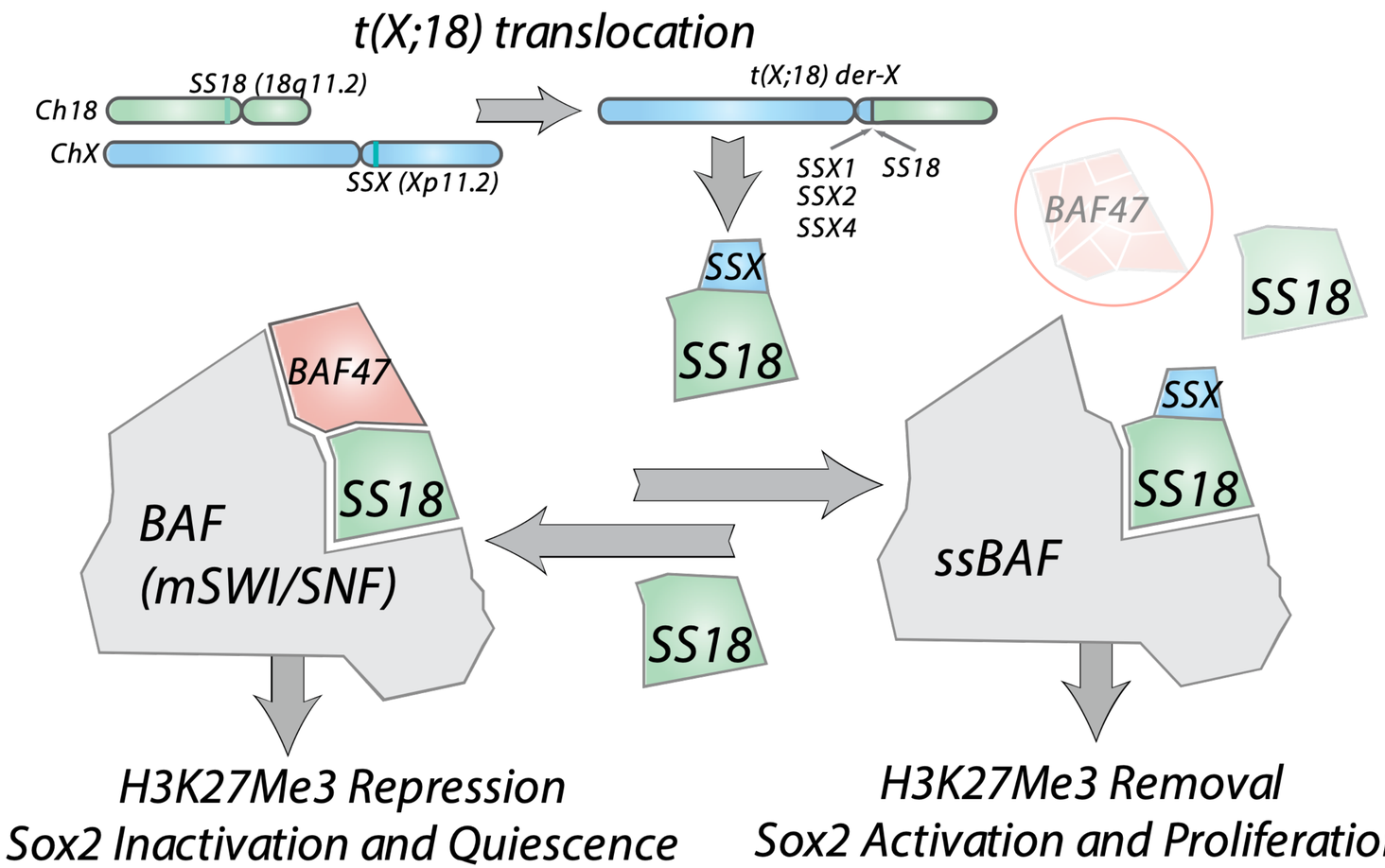


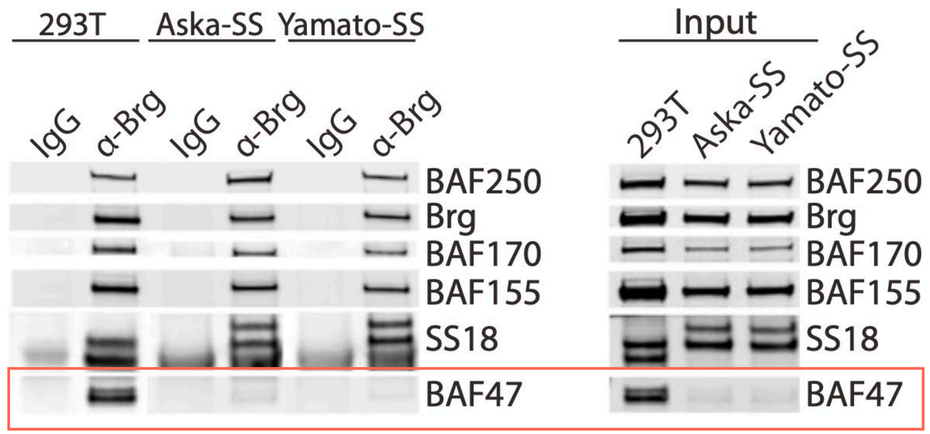




McBride, Mashtalir et al.

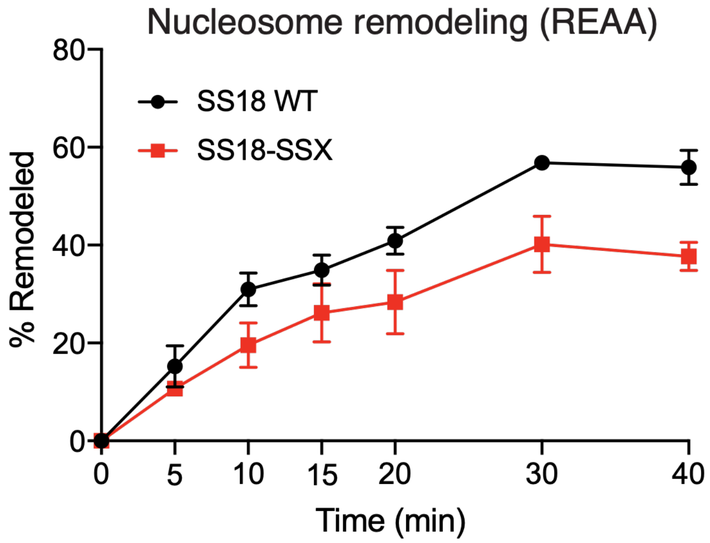
Incorporation of SS18-SSX in to BAF complexes results in destabilization of SMARCB1

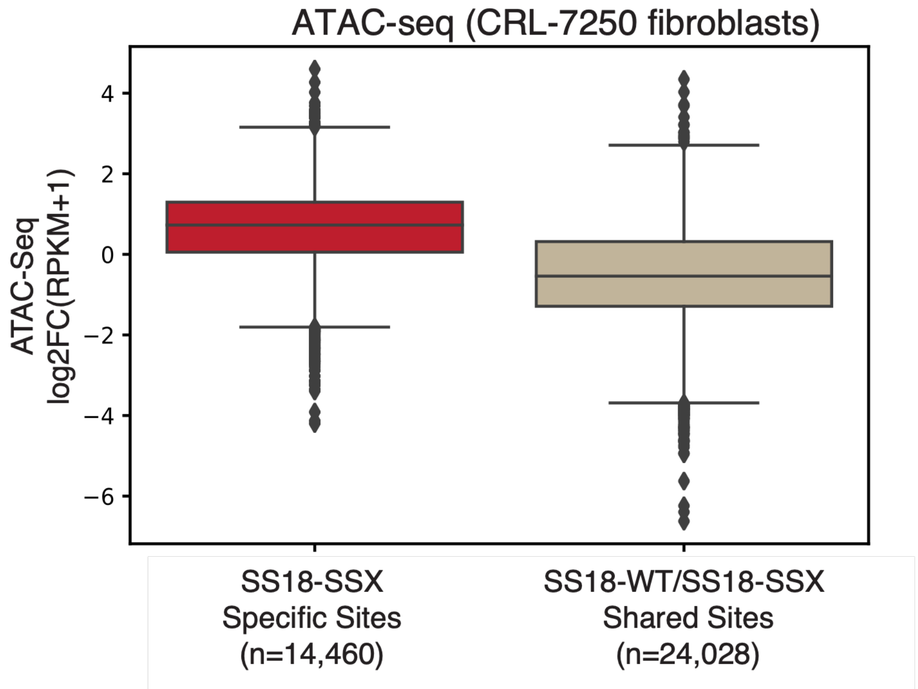


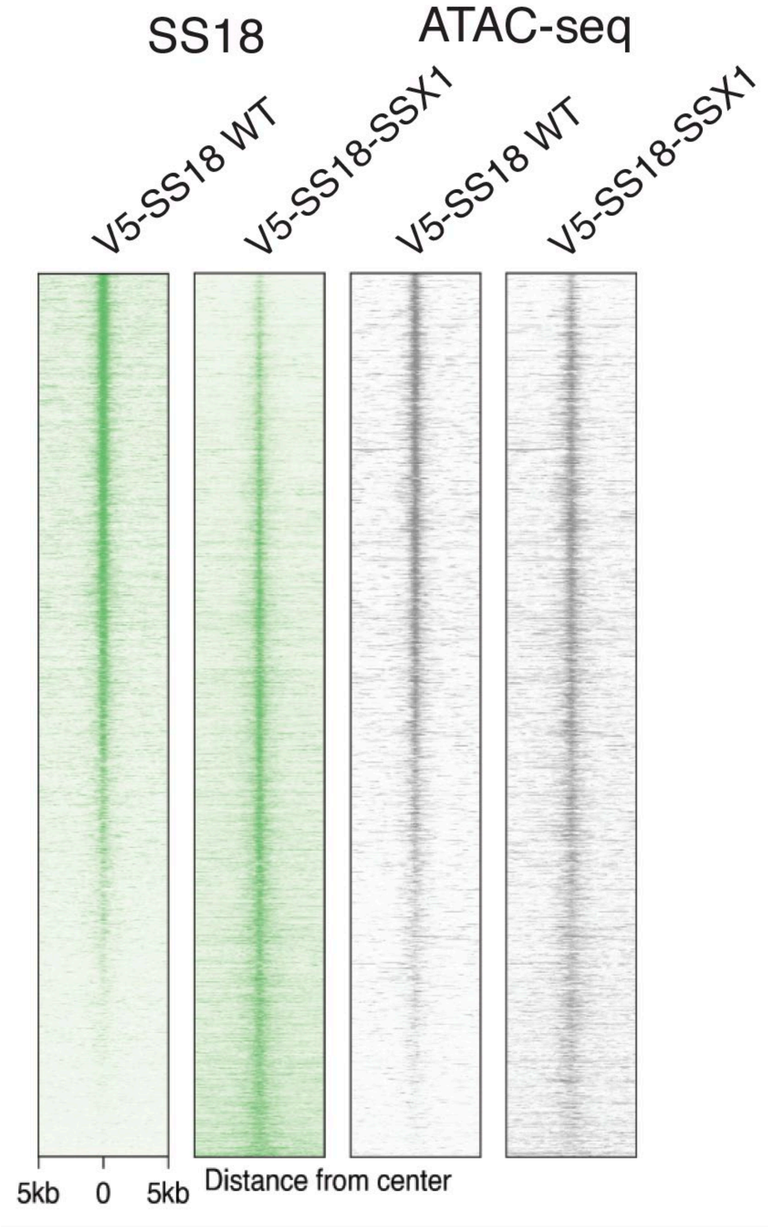


Kadoch & Crabtree, *Cell* 2013
Kadoch et al, *Nature Genetics* 2013

The swap of the SMARCB1 CTD with SSX acidic patch interaction maintains remodeling







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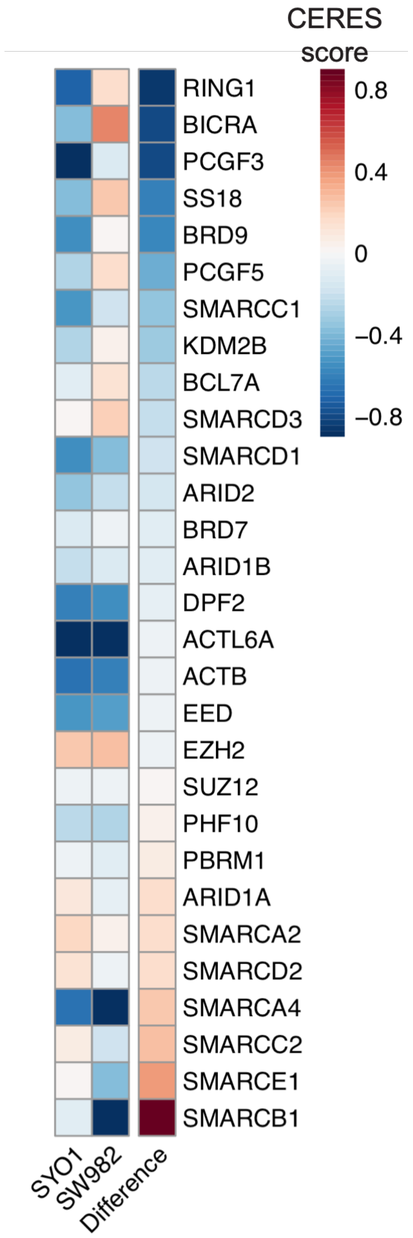
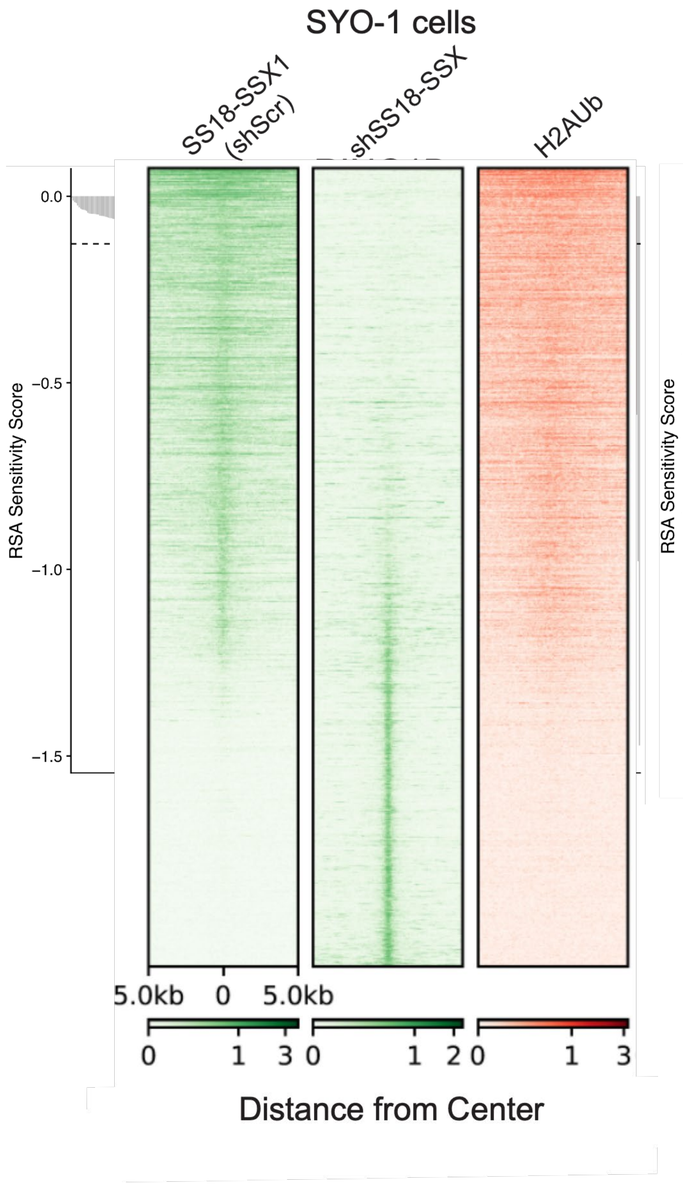
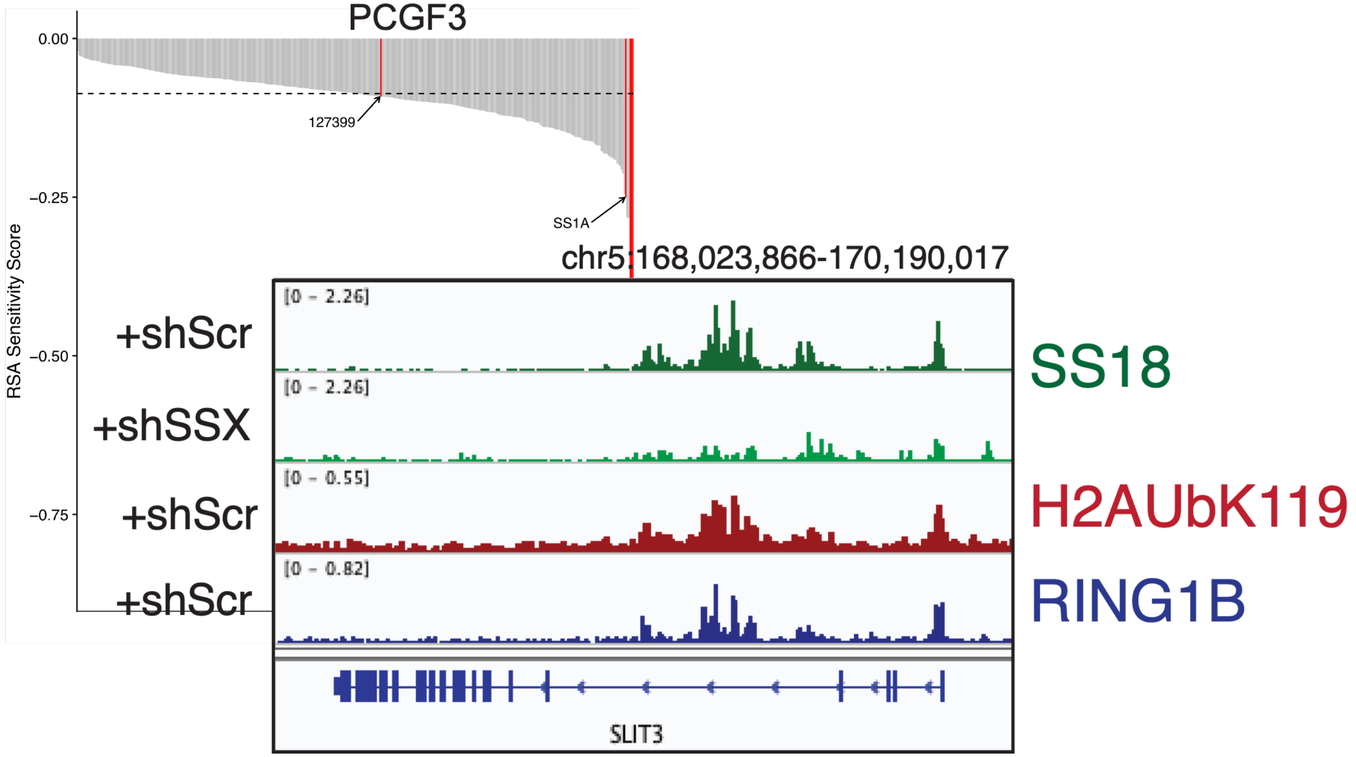
But what about targeting of SS18-SSX-bound BAF complexes to repressed regions?

Binding to the acidic patch doesn't explain this…







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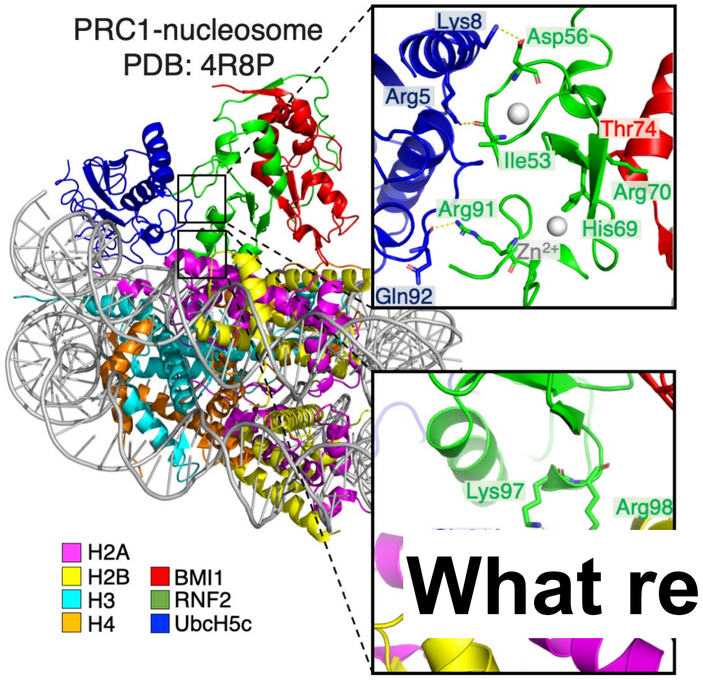
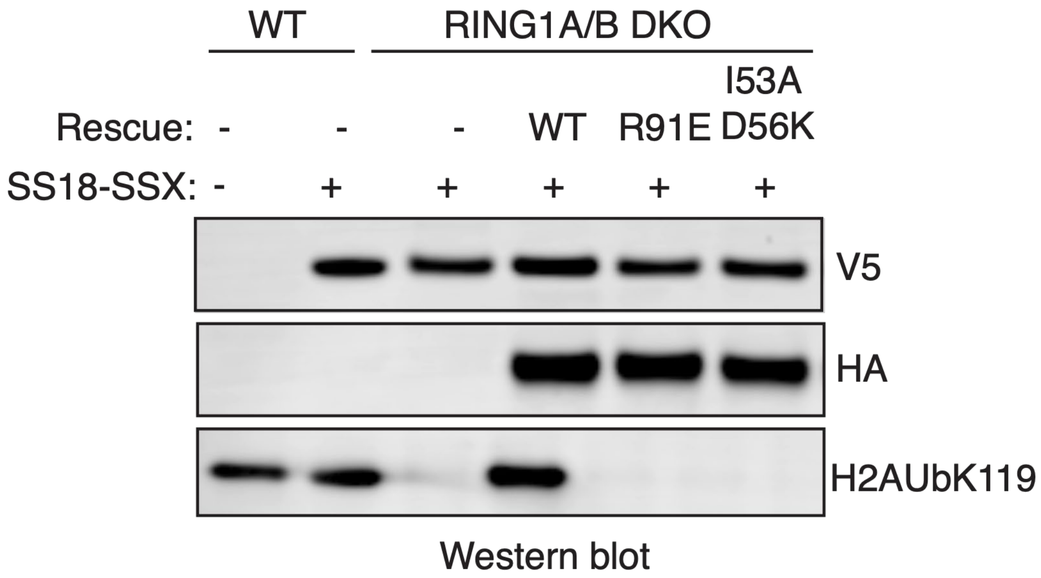
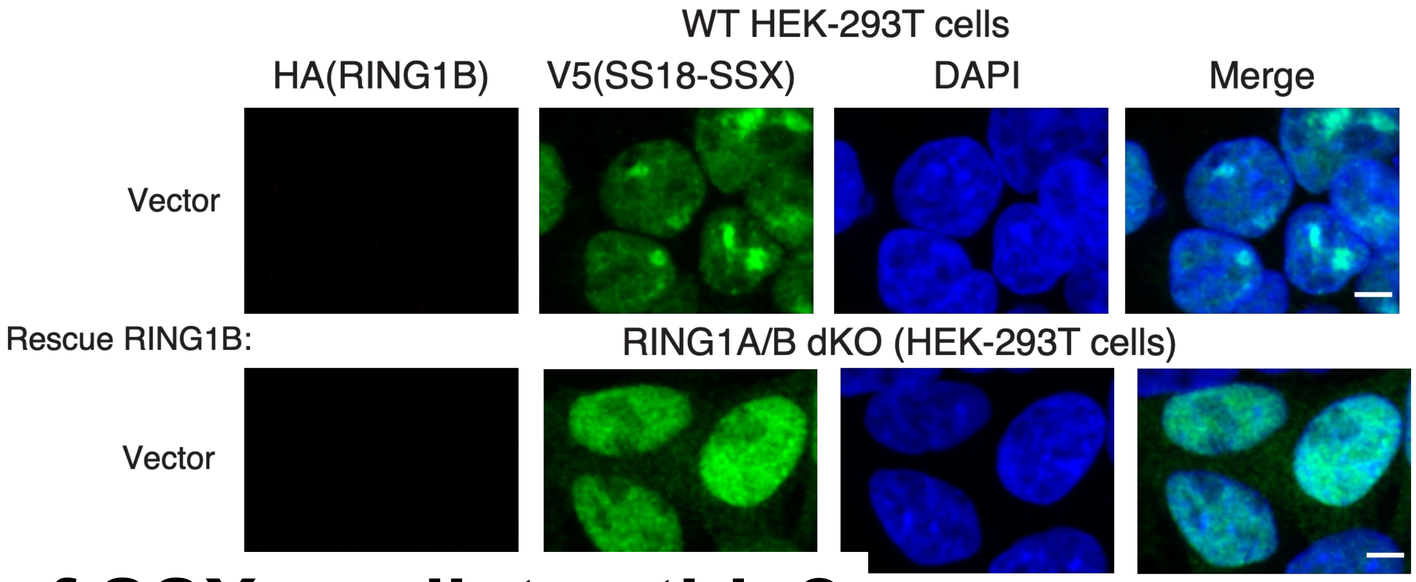
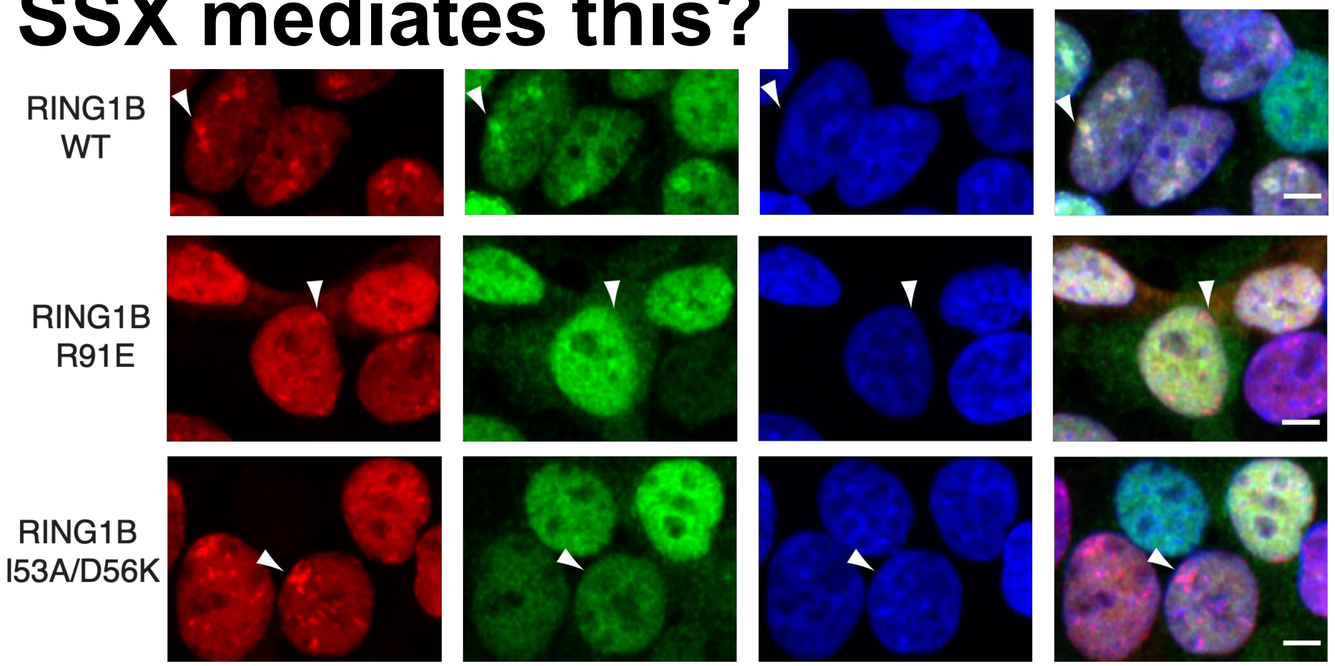
SS18-SSX targeting requires H2A K119 Ub placed by PRC1 complexes



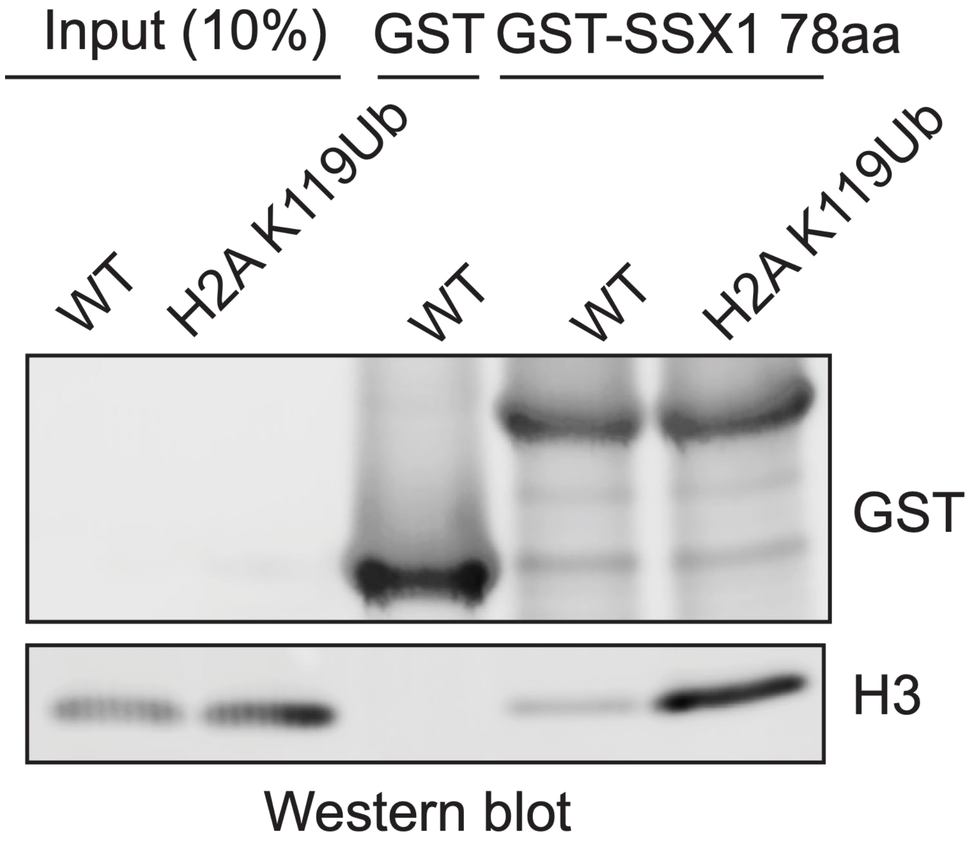




What region of SSX mediates this?



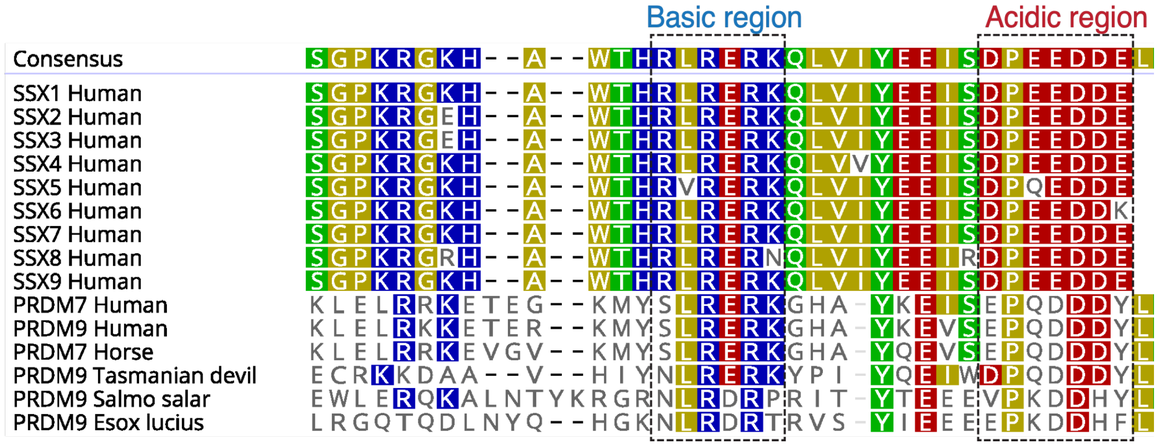
SSX preferentially binds H2A K119Ub over unmodified nucleosomes



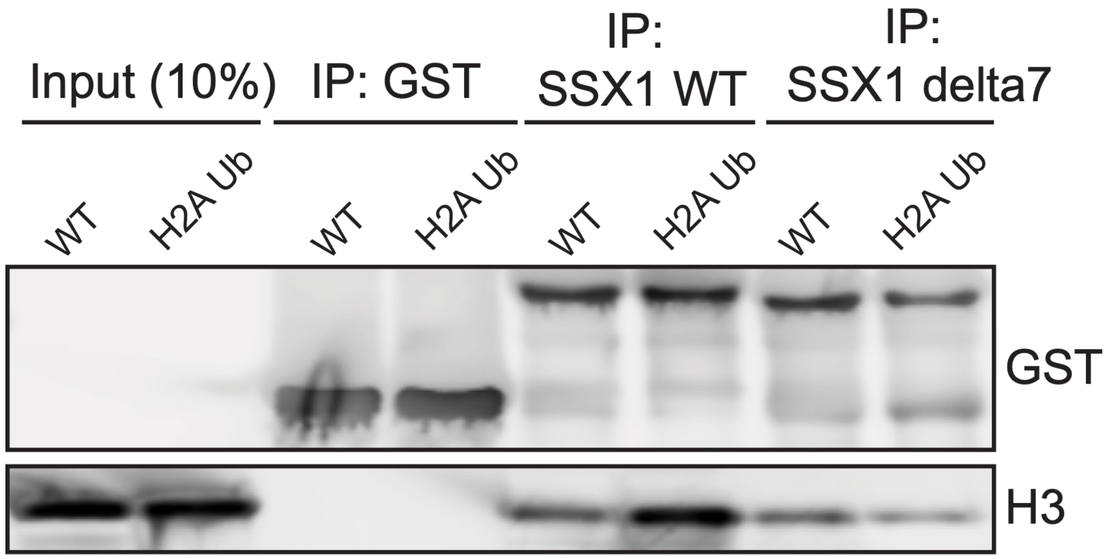
Western blot

And this is mediated by the conserved <u>acidic</u> C-terminal region of SSX

Alignment and conservation of SSX C-terminus



Acidic patch binding ?



Western blot

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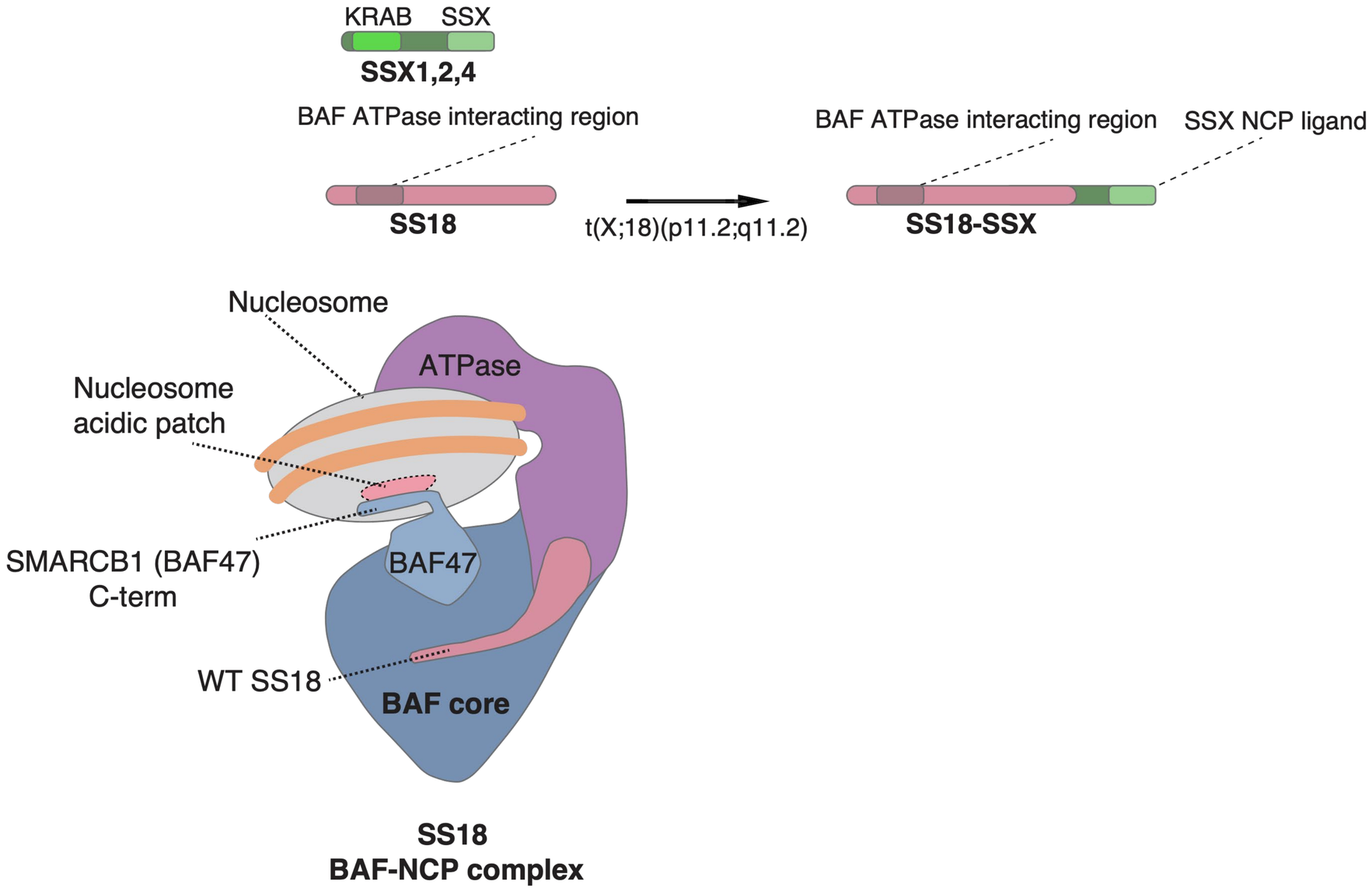
Model for SS18-SSX-containing BAF complex engagement with chromatin



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