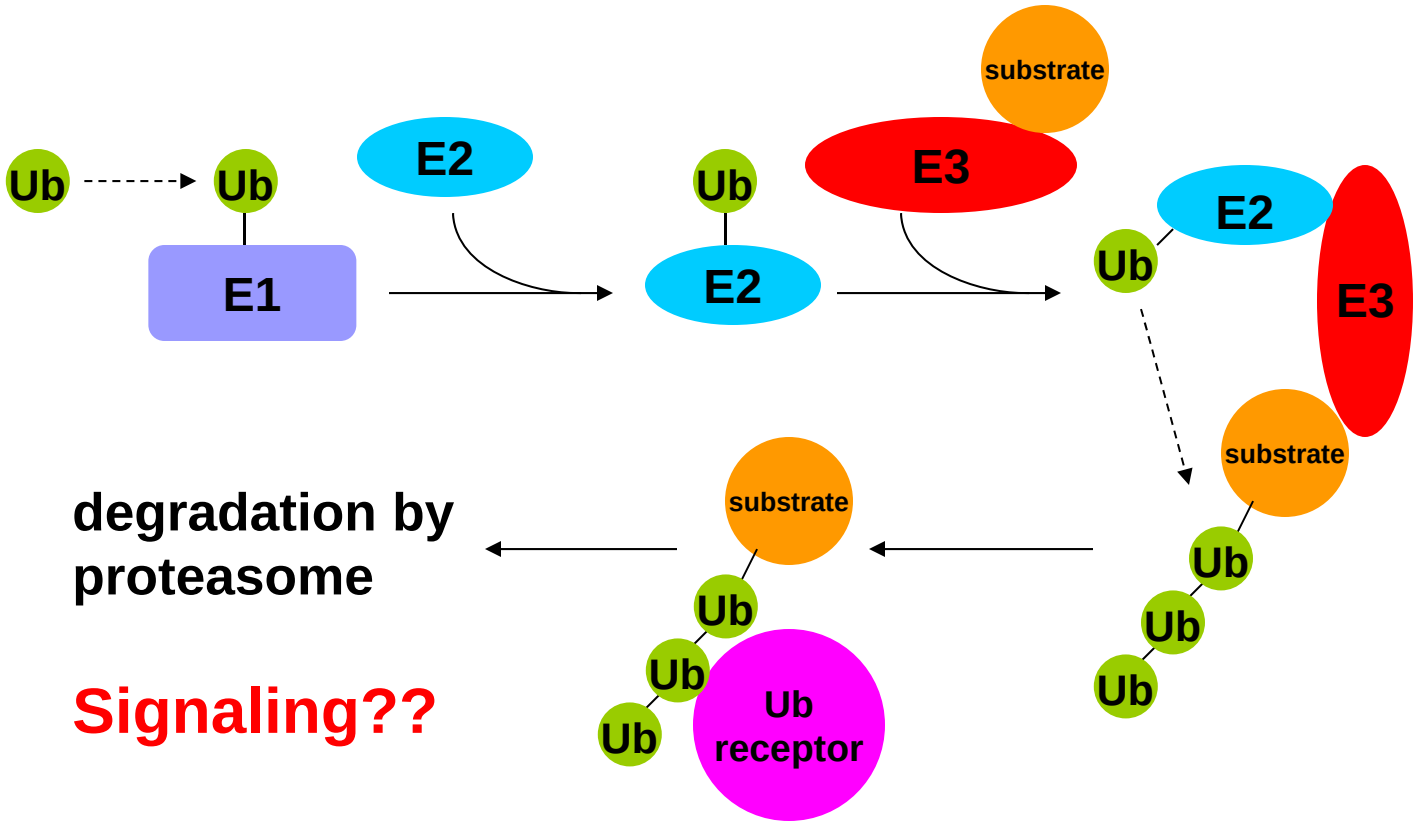
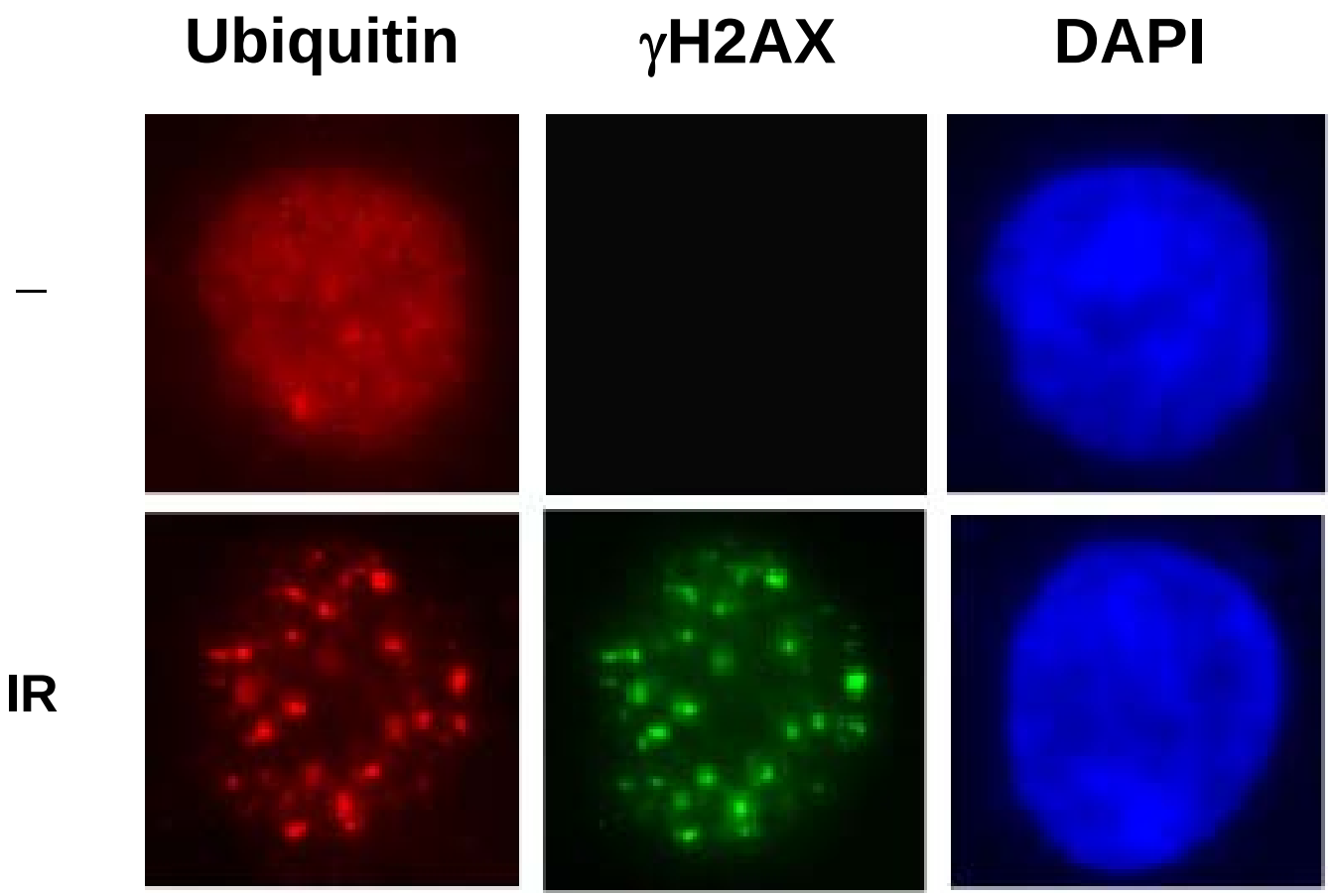
Histone ubiquitination in DNA damage response

Xiaochun Yu
University of Michigan

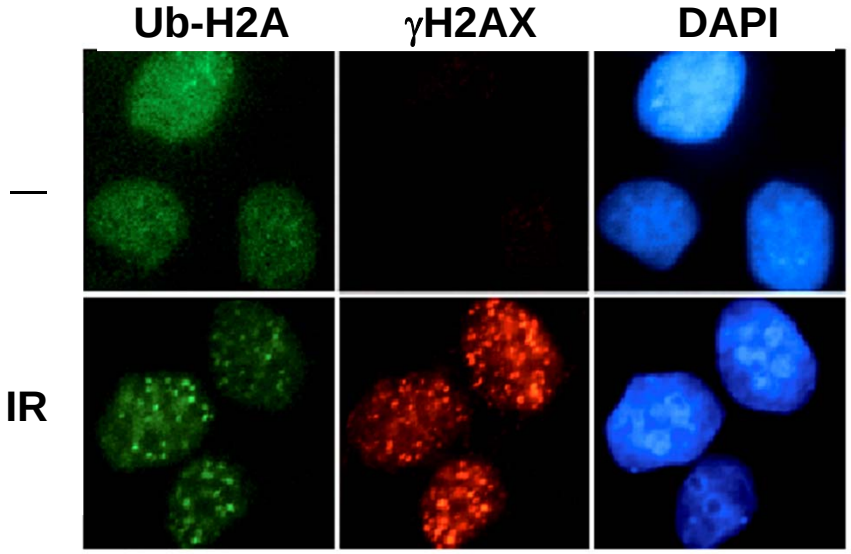
Protein ubiquitination

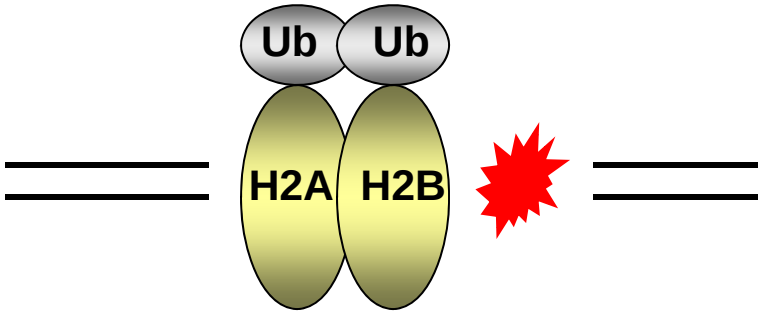


Protein ubiquitination participates in the DNA damage response



Histone H2A is ubiquitinated at the DNA damage sites





DNA damage sites

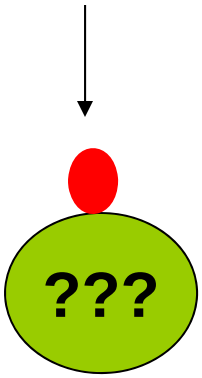
RNF8 participates in the DNA damage response

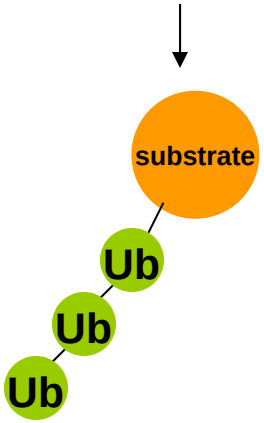
FHA

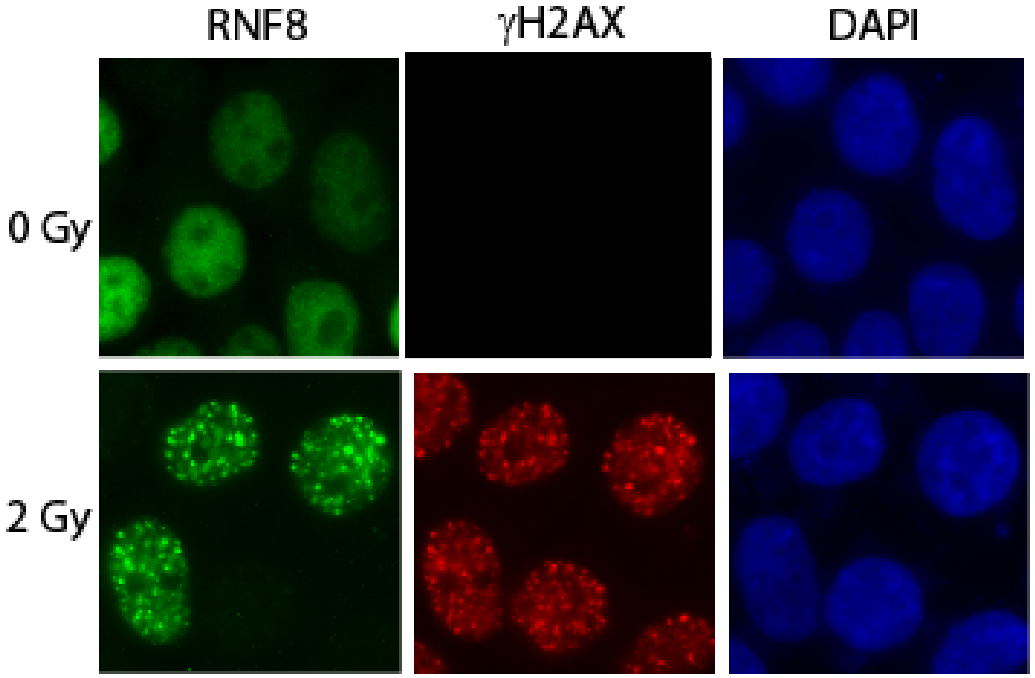
pThr binding domain

Ring

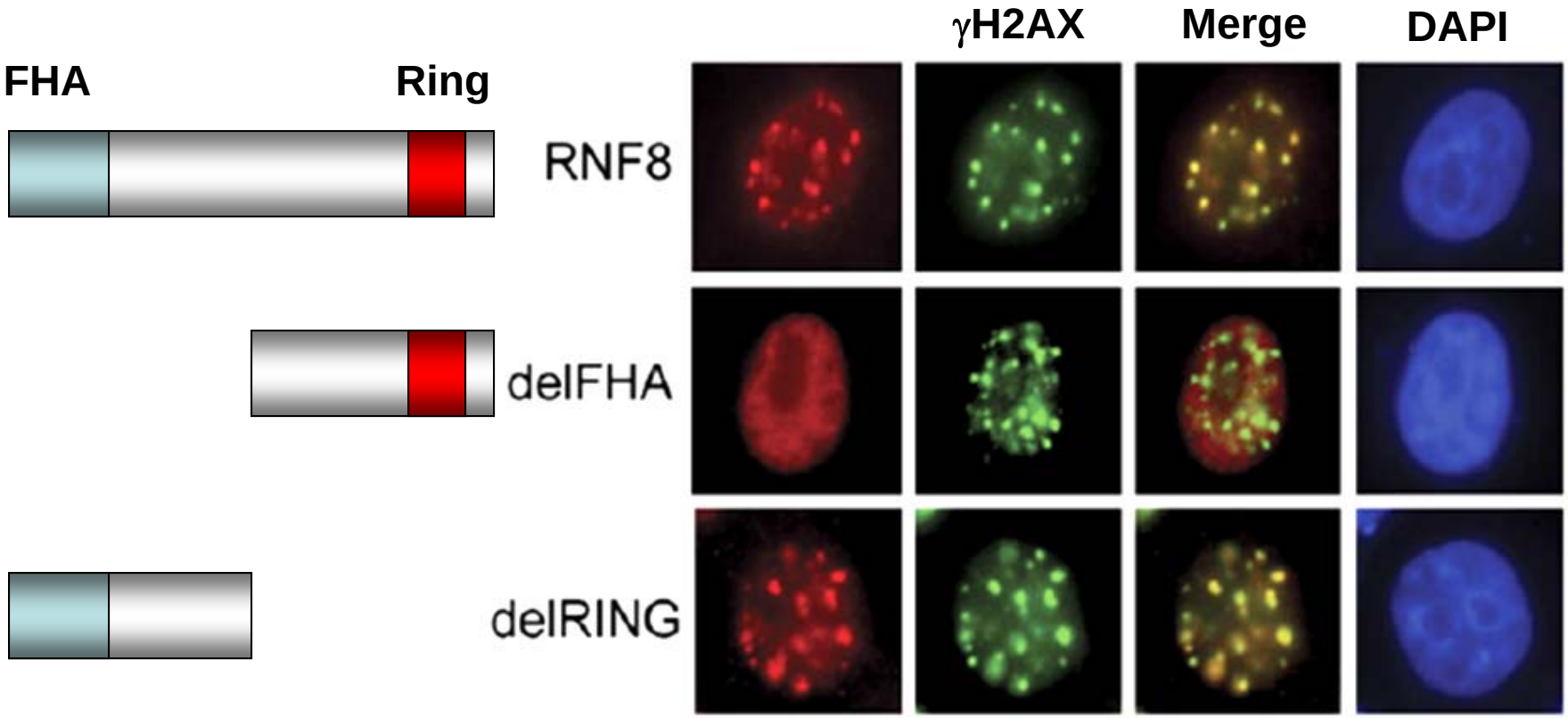
E3 ligase



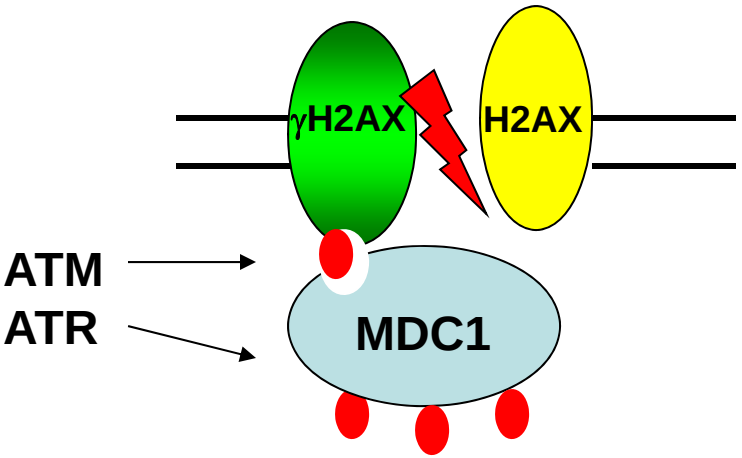




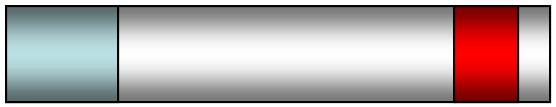
FHA domain targets RNF8 to the DNA damage sites



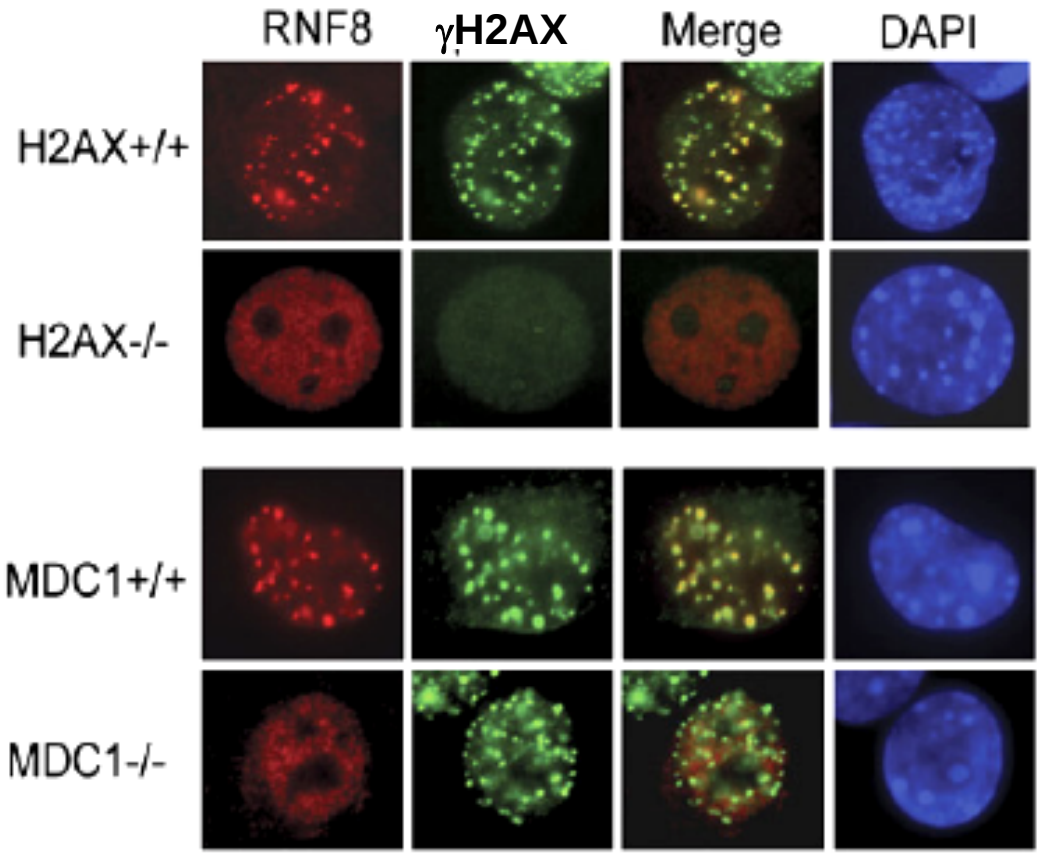
RNF8 acts downstream of H2AX and MDC1



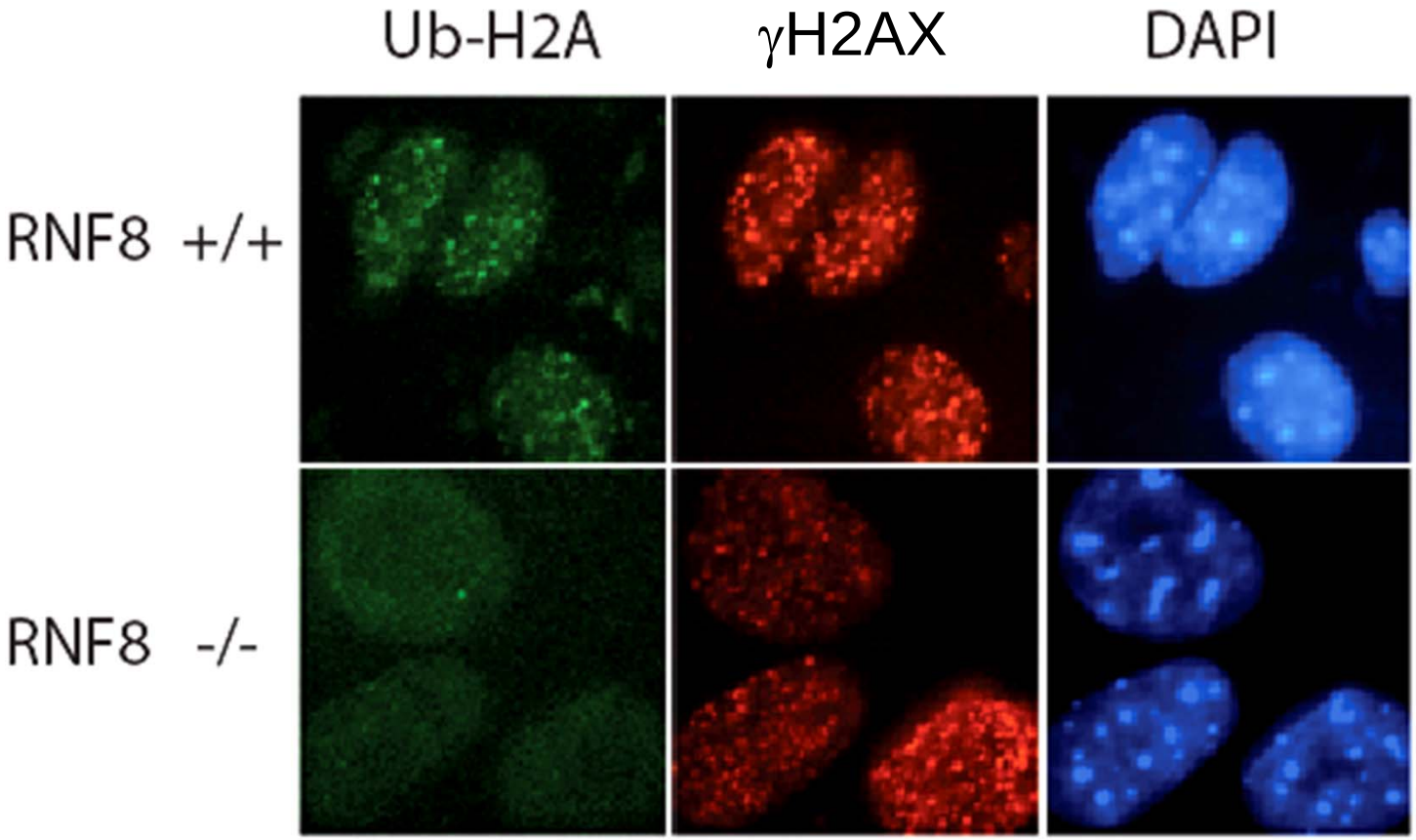
Many other factors including RNF8



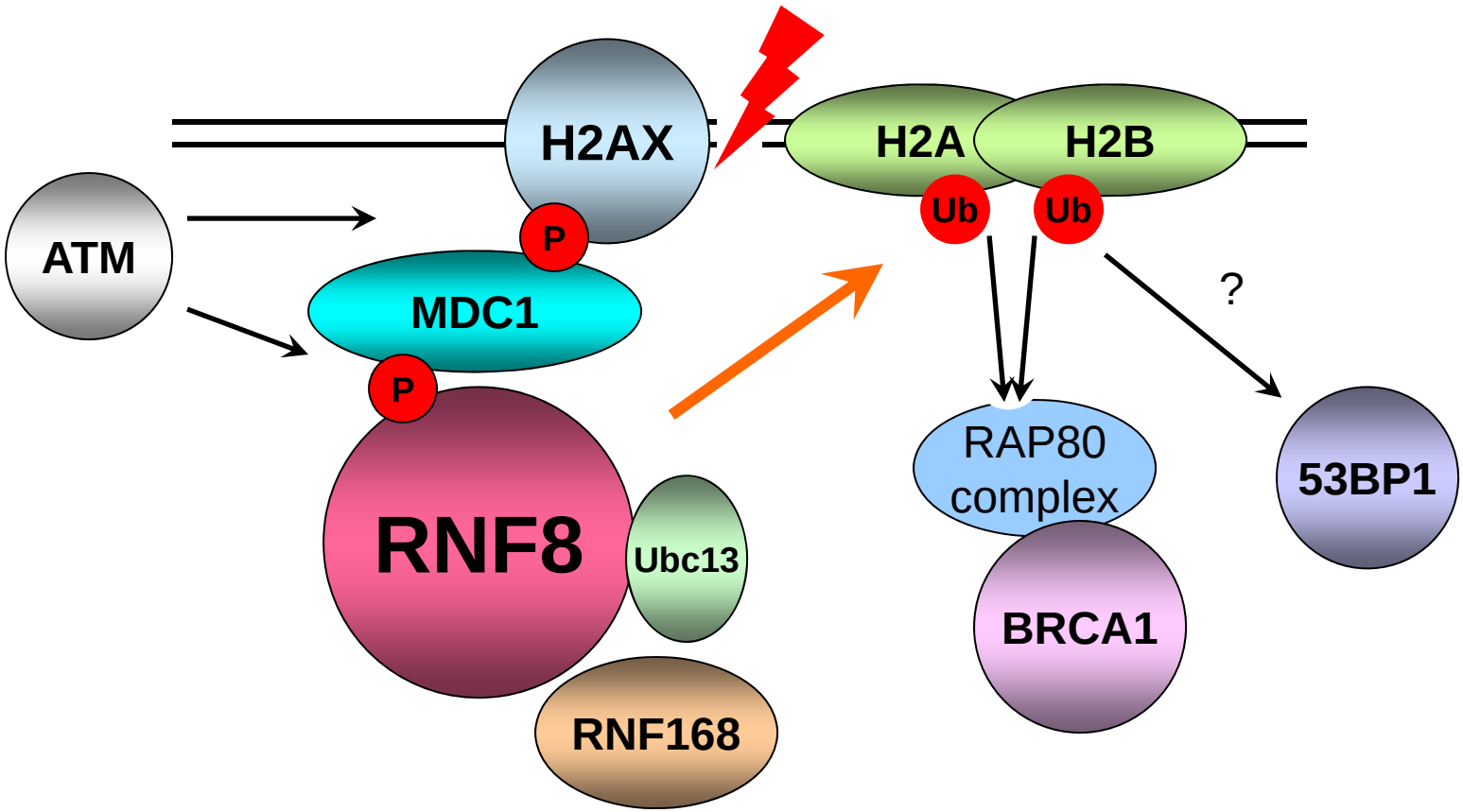
FHA



RNF8 controls Ub-H2A foci



RNF8-dependent DNA damage response



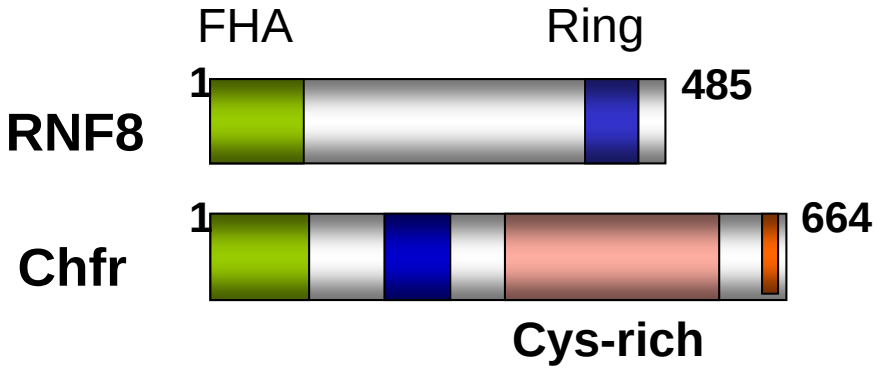
Phenotypes of RNF8 deficient mice

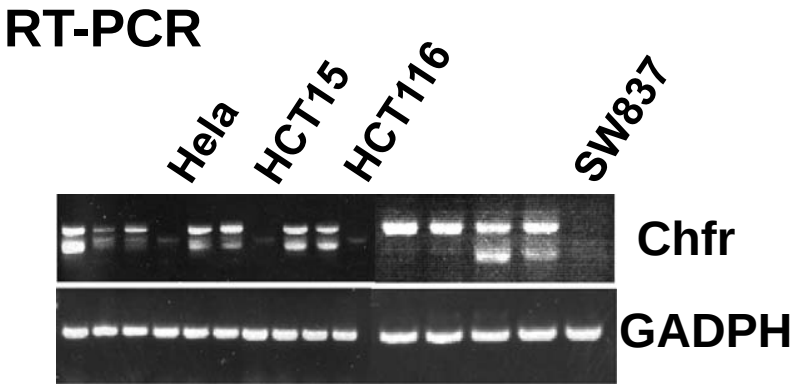
In vivo:

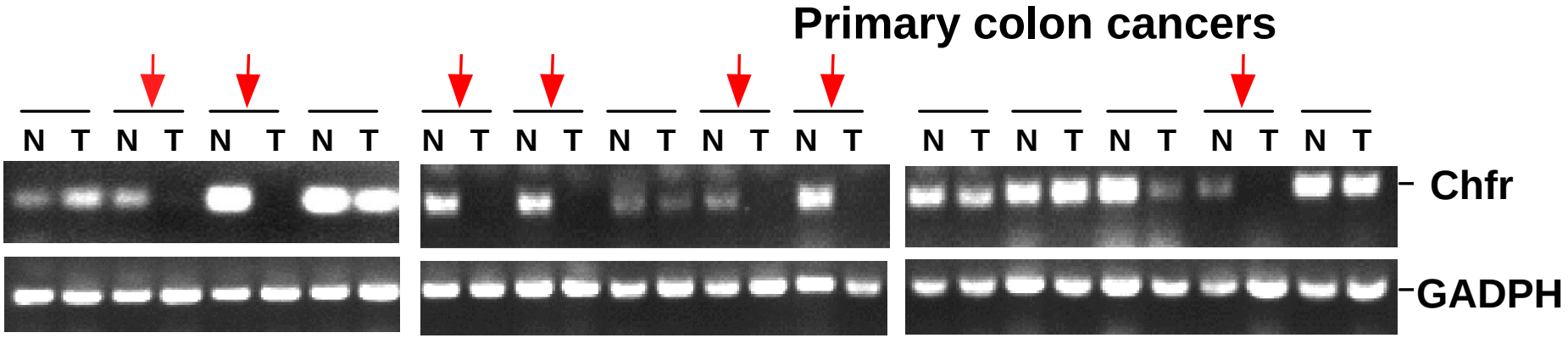
1. Few RNF8-null mice develop tumors.
2. The male RNF8-null mice are sterile.

Why??

Chfr is a potential paralog of RNF8, and is often downregulated in cancers

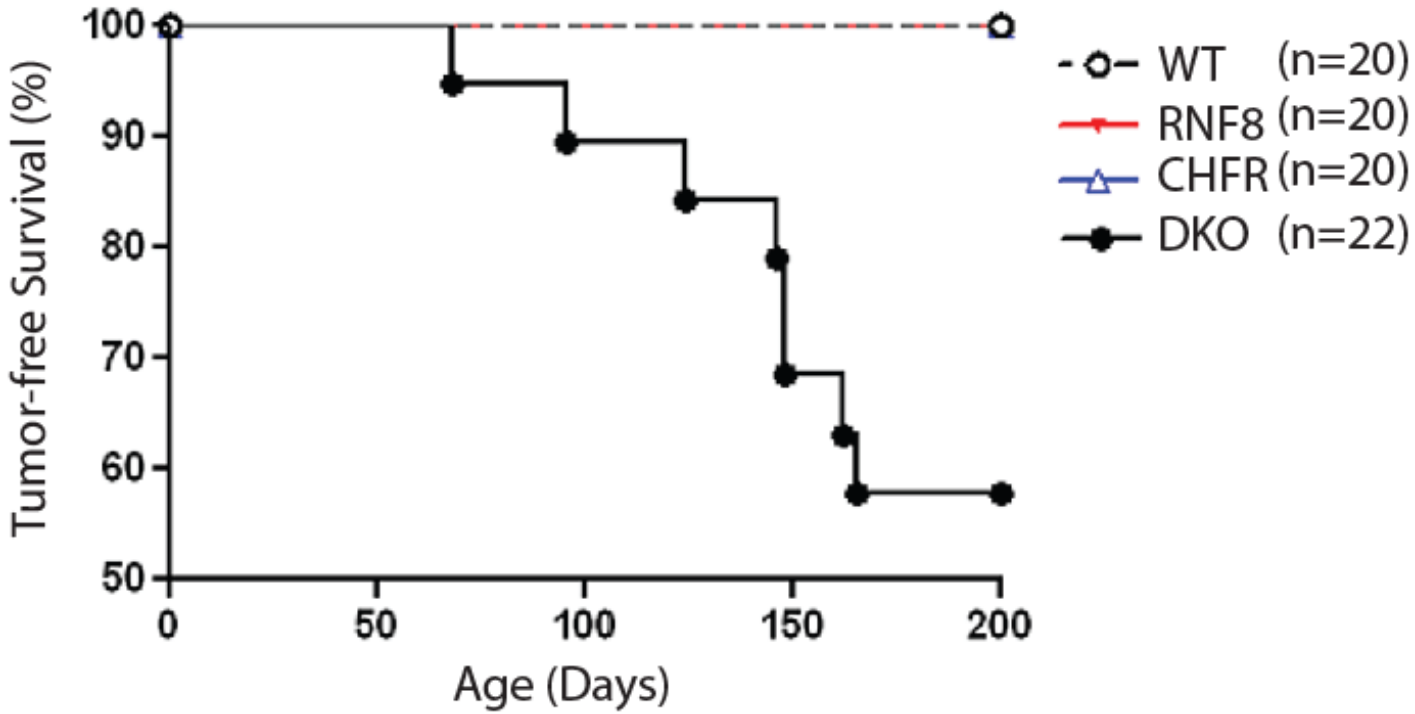


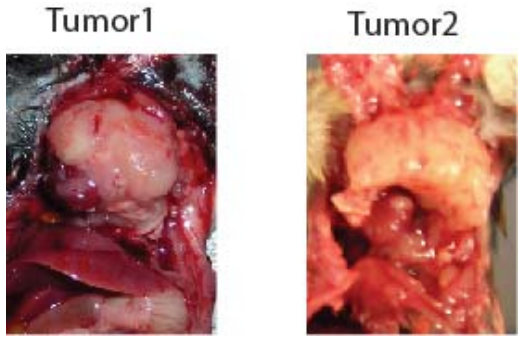


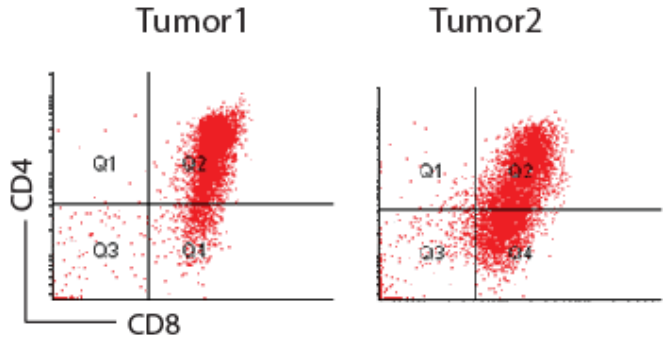


N= Normal tissue T= Tumor tissue

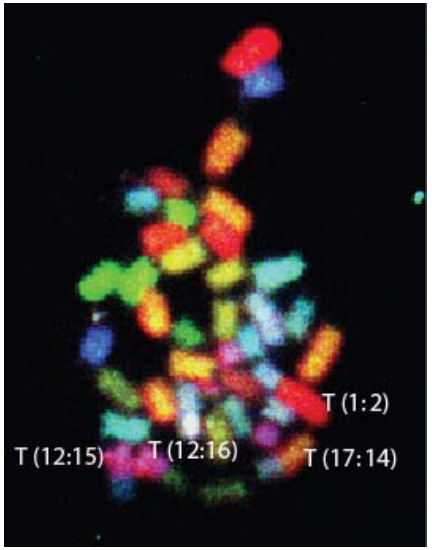
RNF8 and Chfr DKO mice develop T-cell lymphoma

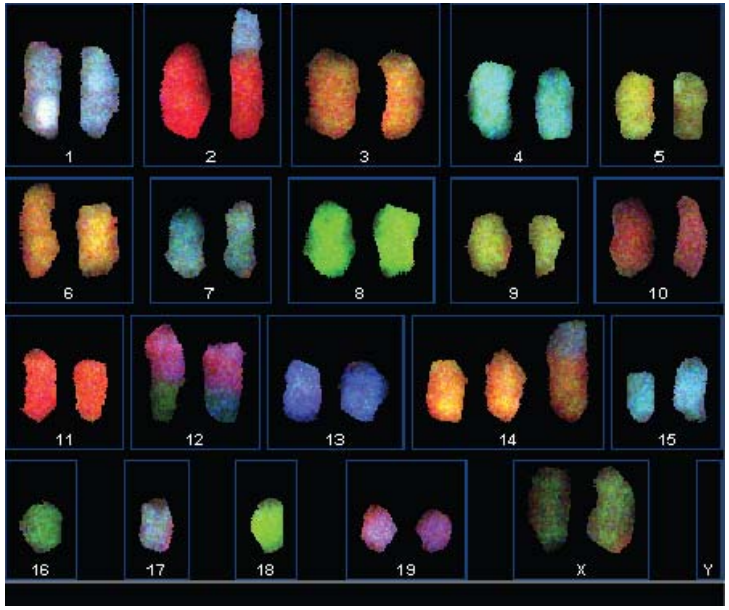






SKY analysis of T-cell lymphoma in DKO mice



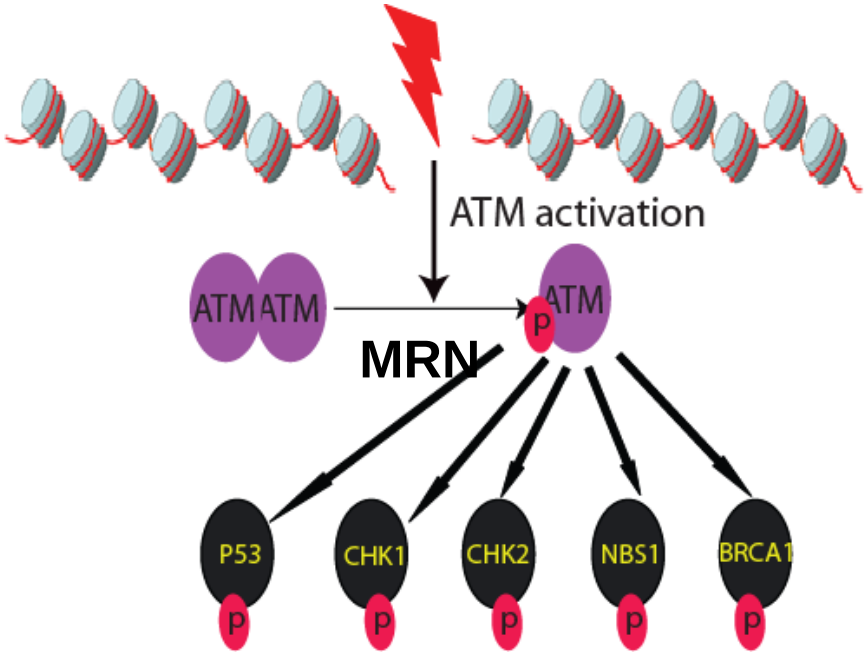


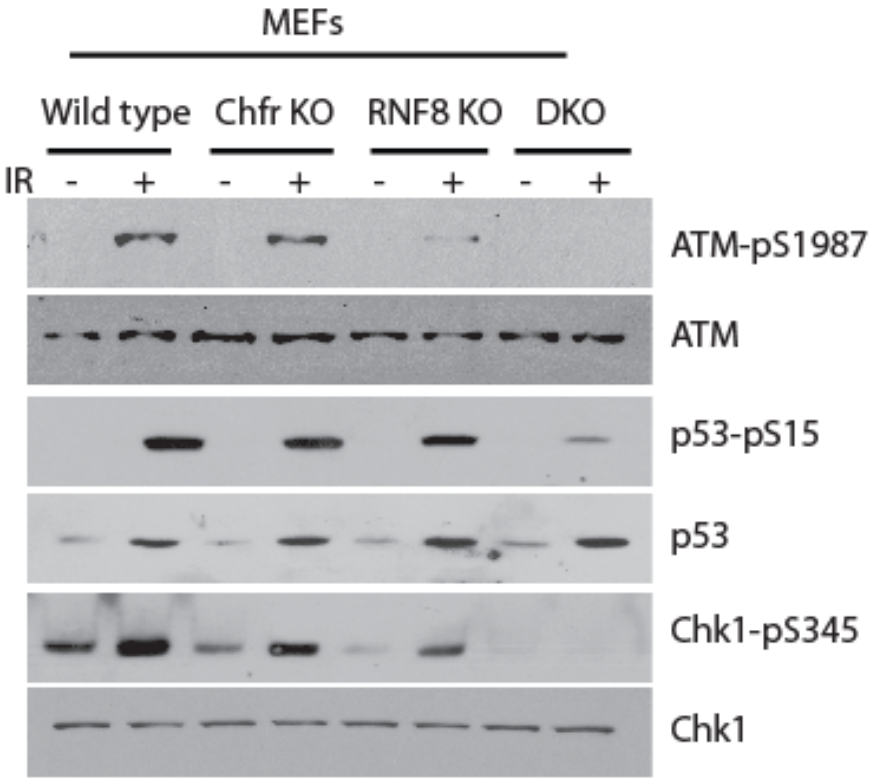
Tumor	No of chromosome	Clonal translocations
#1	38	T (2; 1) T (12; 15) T (12; 16) * T (17; 14)
#2	39	T (10; 1) T (12; 16) T (12; 1) * T (6; 7)
#3	40	T (13; 12) * T (14; 12) T (12; 1) T (3; 15)
#4	40	* T (14; 3) T (13; 12) T (19; X)
#5	39	T (2; 1) T (12; 15) T (12; 16) * T (17; 6)

Chromosome 6, 14 rearrangement

Similar to ATM -/- tumors

Loss of RNF8 and Chfr abolishes ATM-dependent DNA damage response



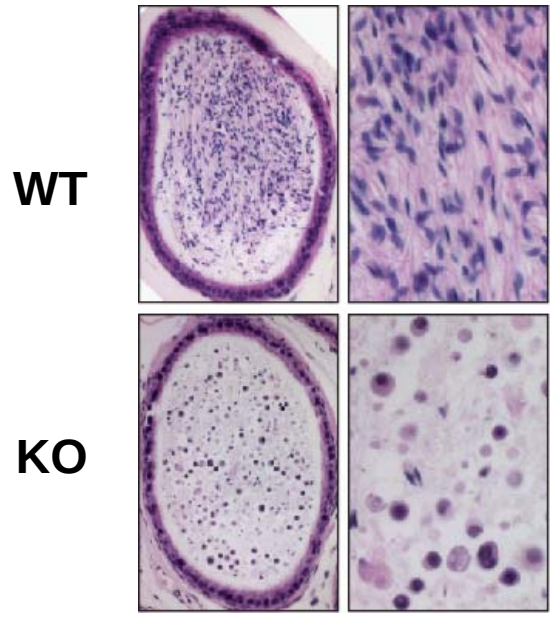


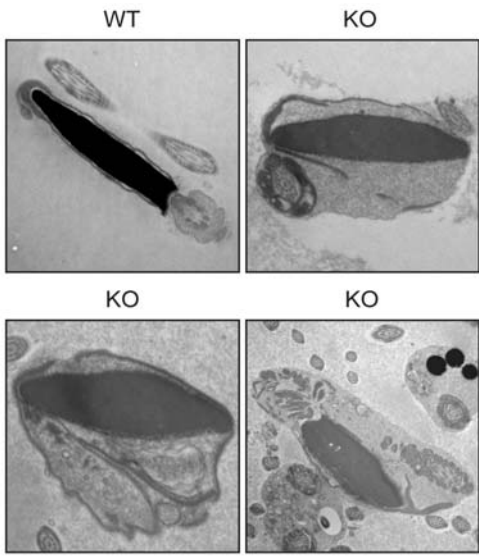
RNF8
Chfr

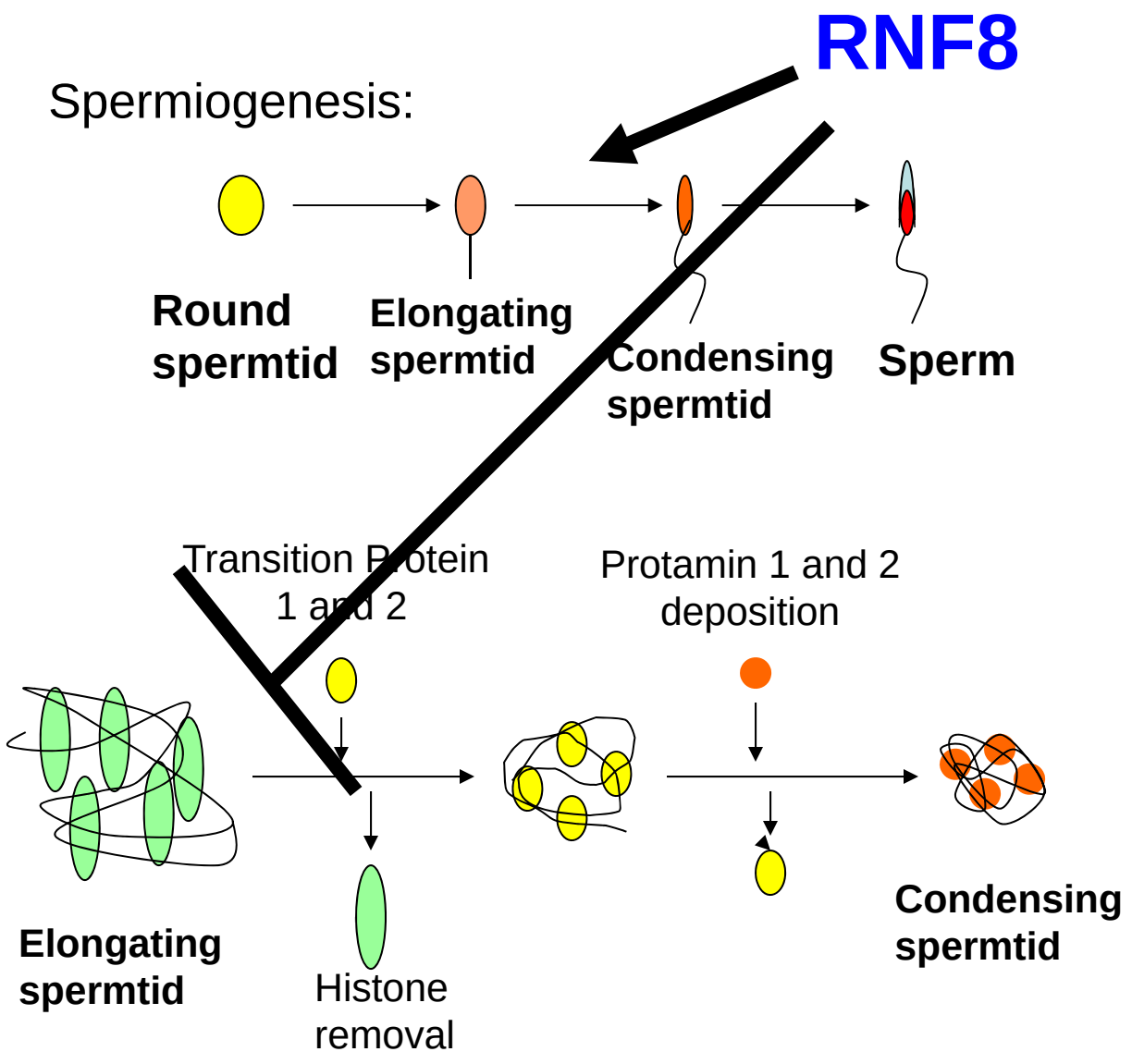
Mechanism??

ATM activation

RNF8 controls histone removal during spermiogenesis



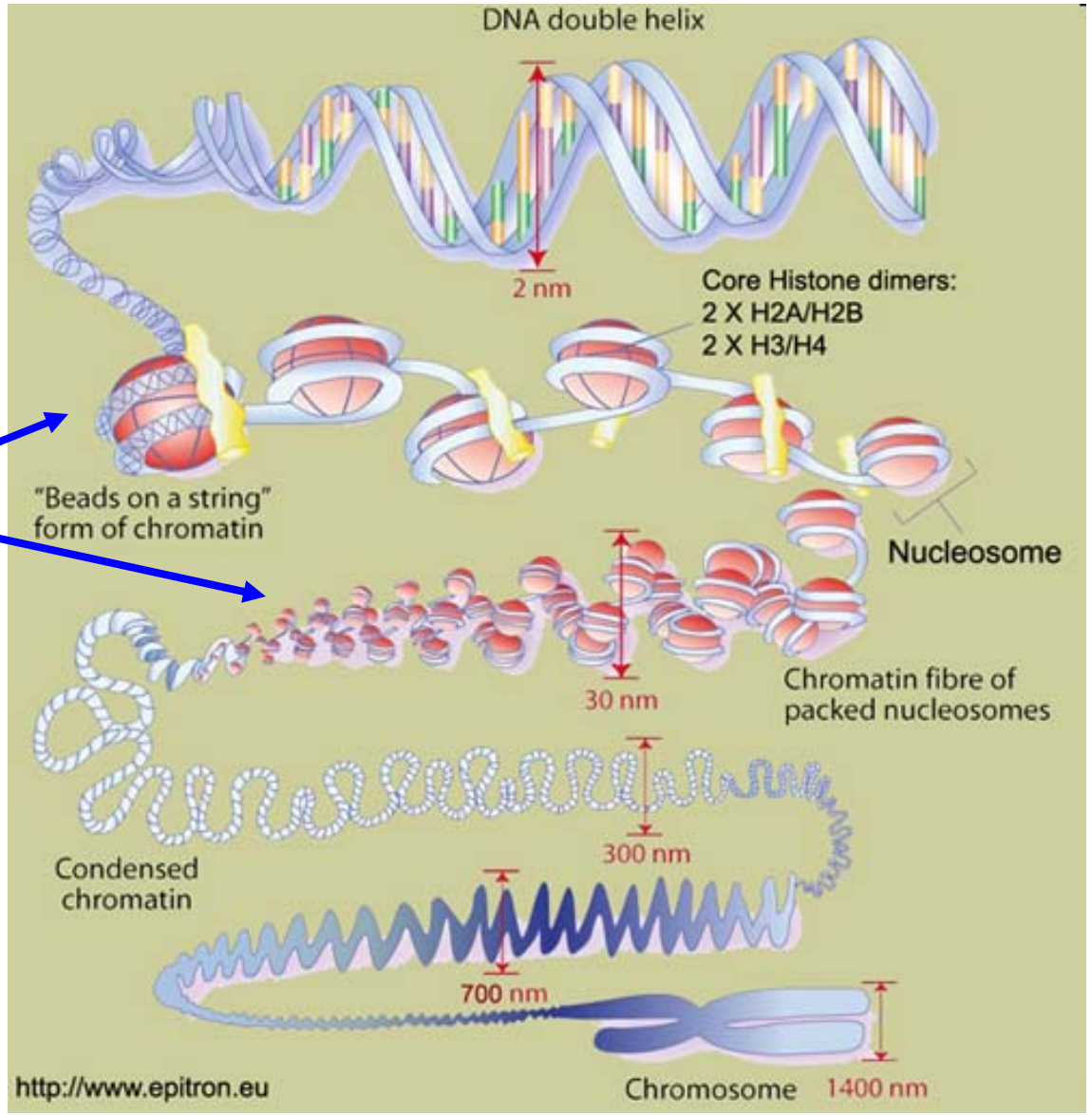




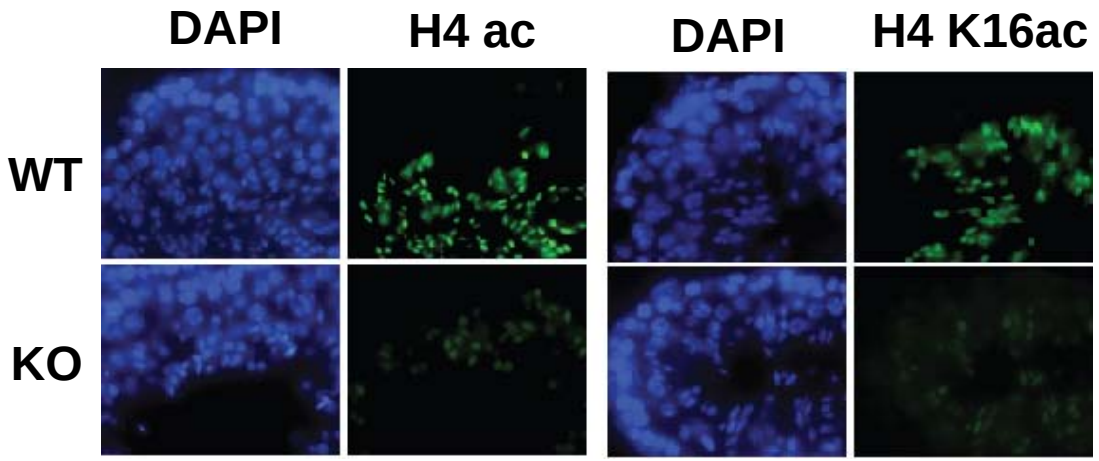
H4 acetylation controls chromatin relaxation

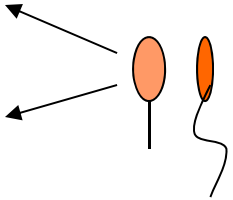
H4 ac

(H4 K16 ac)



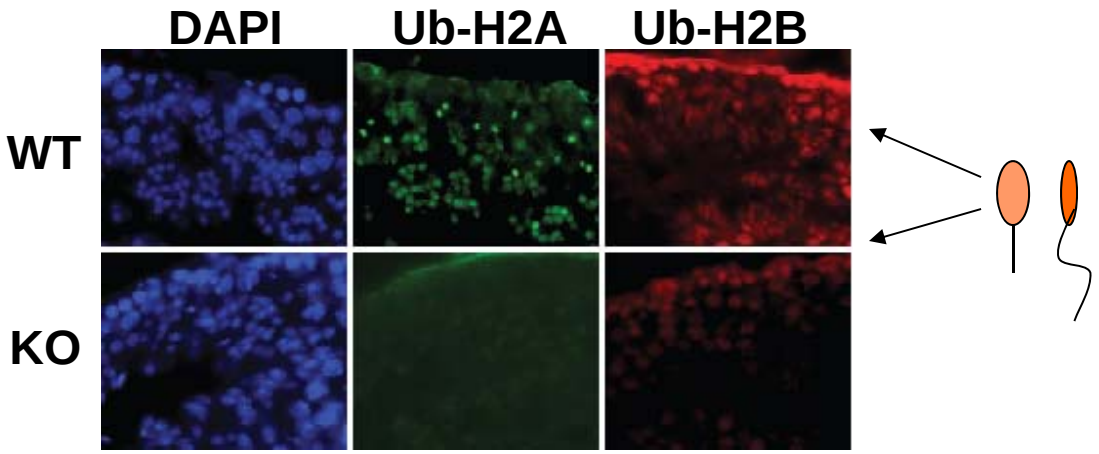
Histone ac and Ub are abolished in RNF8-deficient spermtids



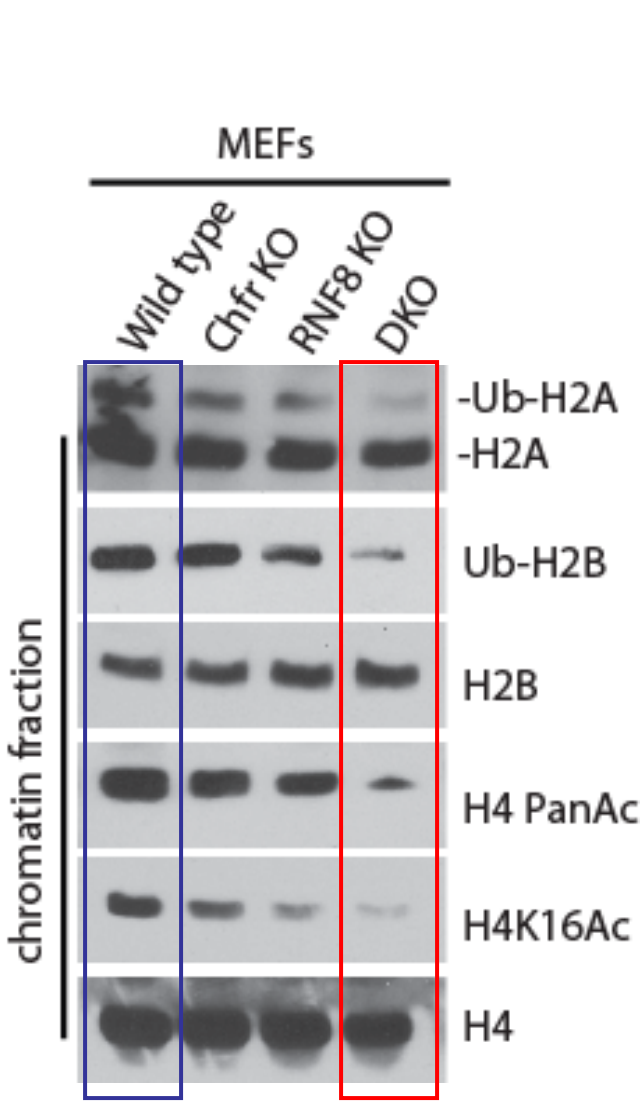


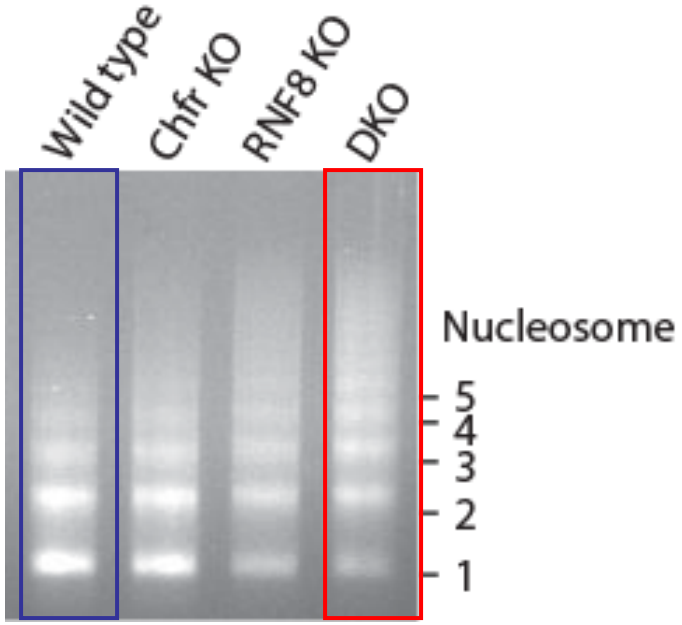
Elongating & condensing spermtid

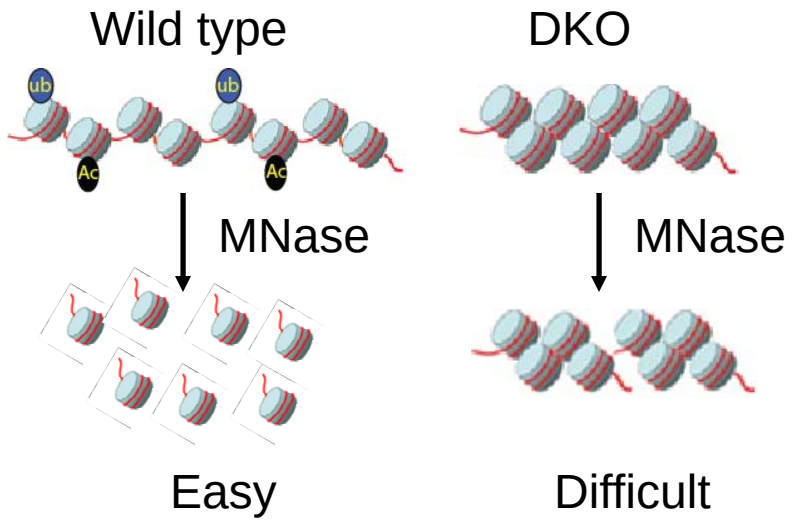
Histone H4 acetylation is the marker of elongating spermtids



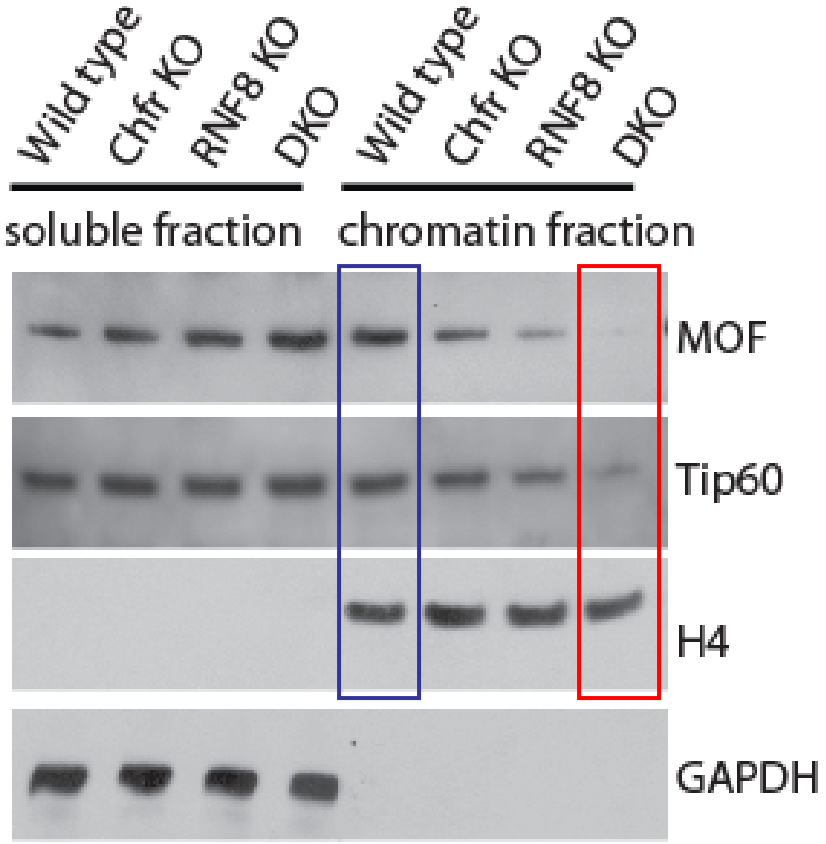
RNF8 and Chfr regulate chromatin relaxation

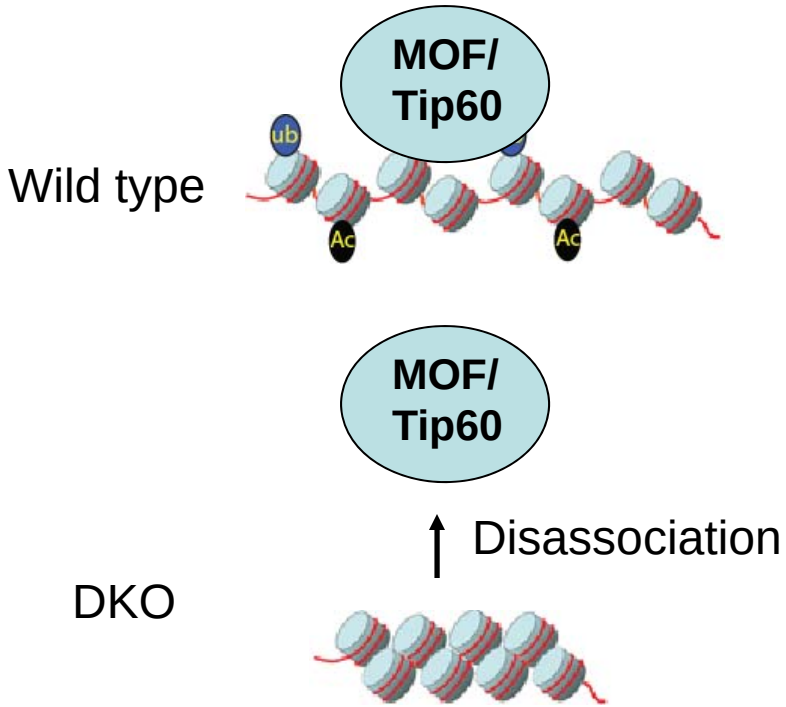






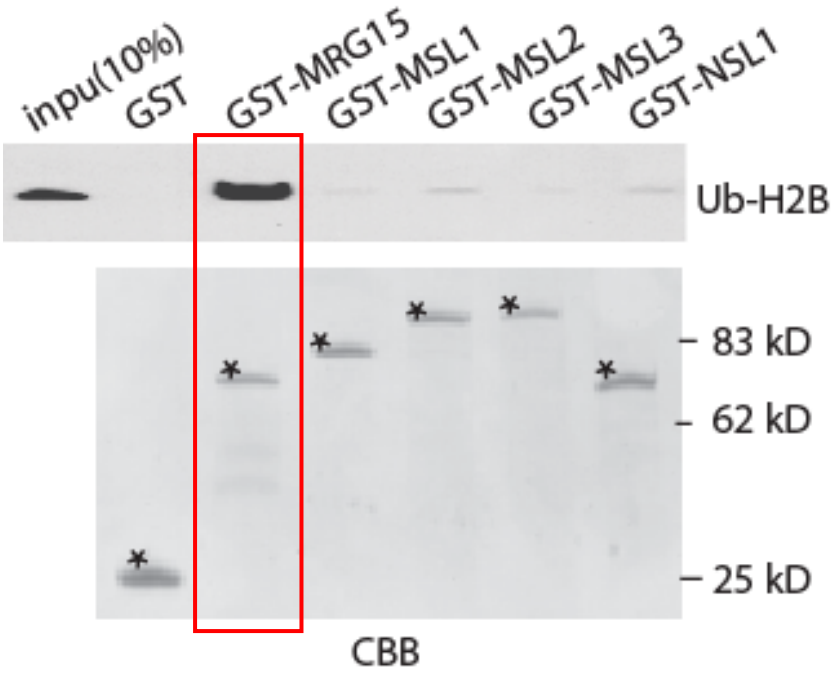
RNF8 and Chfr regulate chromatin-association of MOF and Tip60



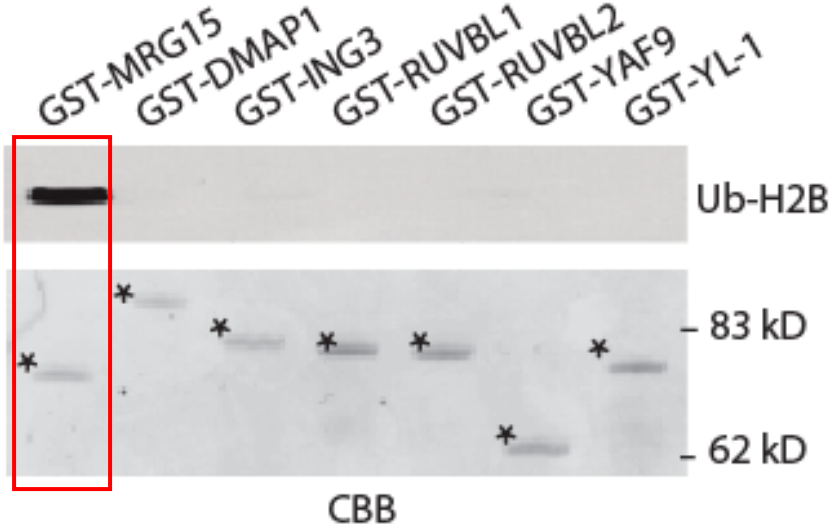


MRG15 associates with Ub-H2B

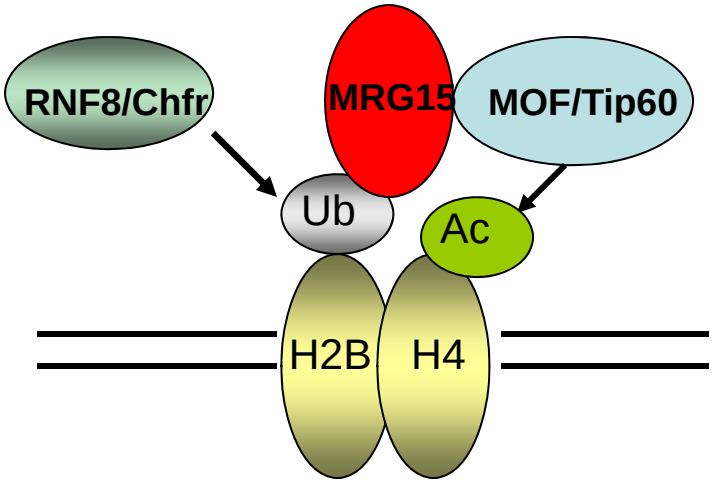
MOF complex subunits



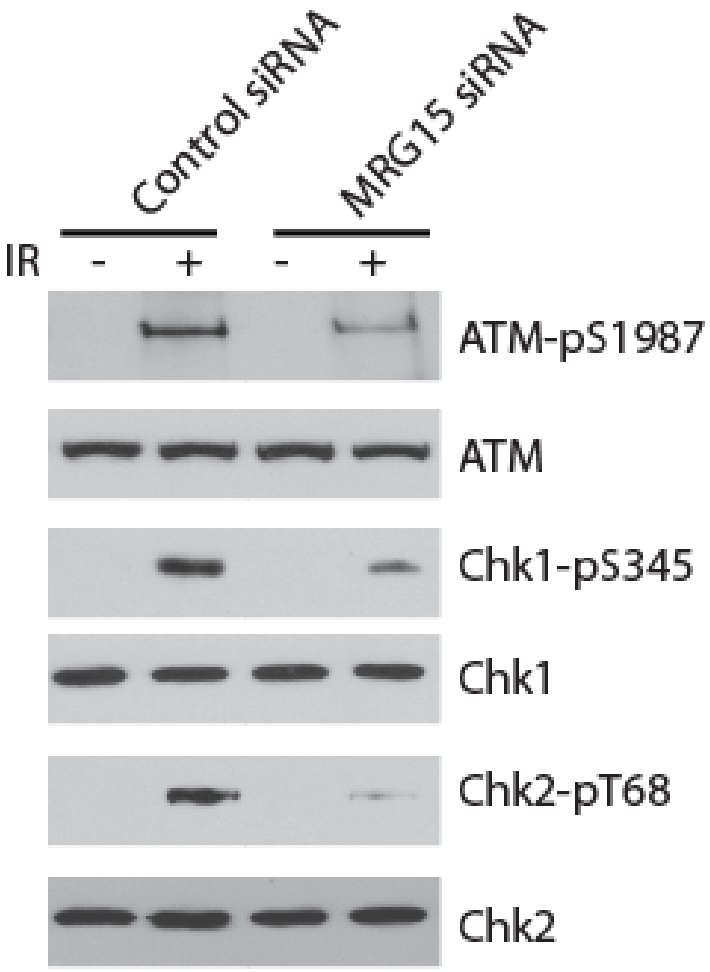
Tip60 complex subunits

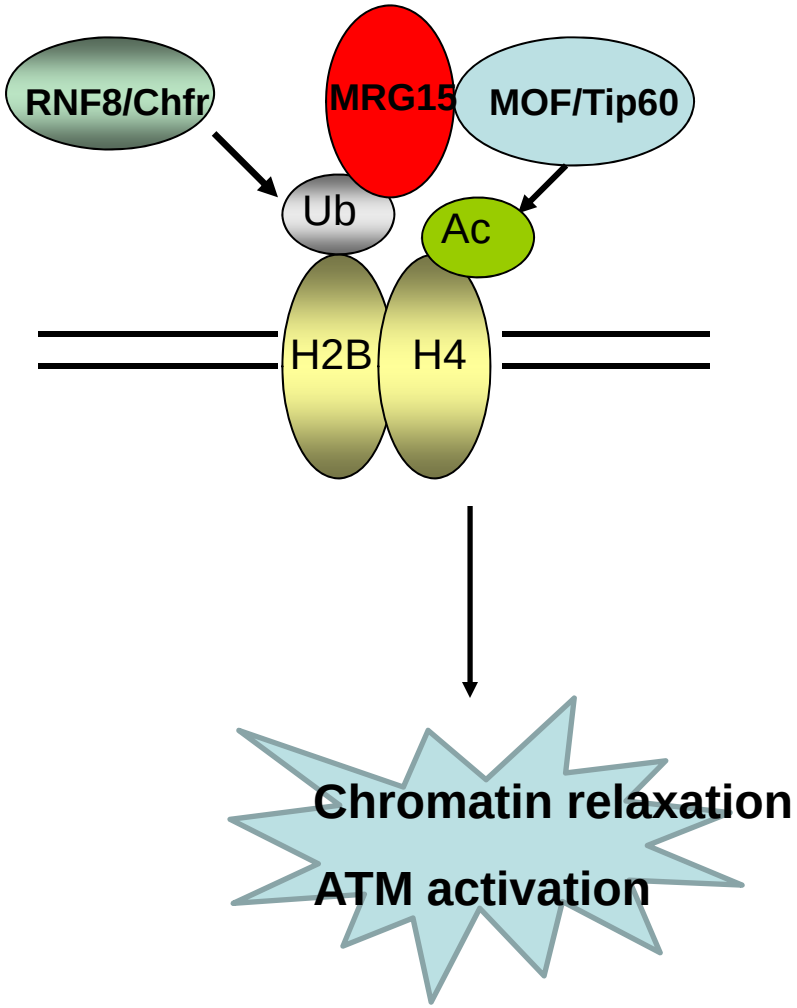


MRG15:
Common component of both
MOF and Tip60 complexes



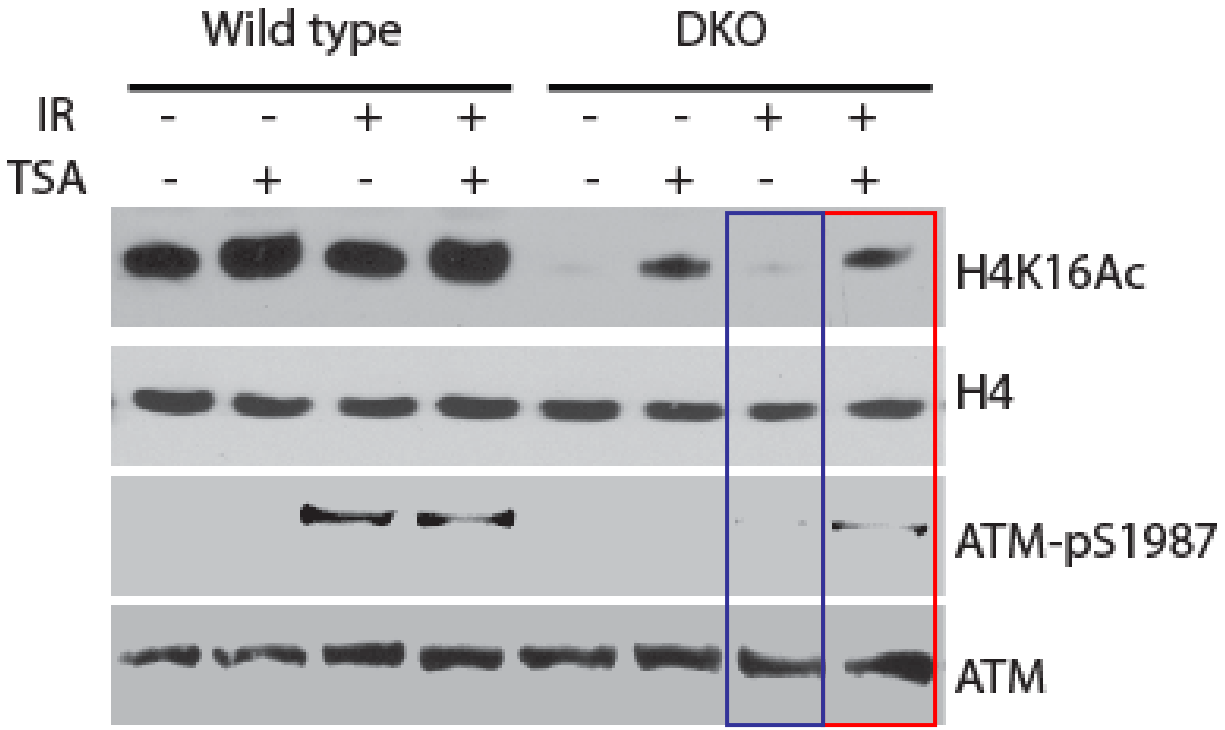
ATM-dependent DNA damage response is impaired in MRG15-depleted cells





TSA rescues ATM-dependent DNA damage response in DKO MEFs

Trichostatin A (TSA):
Class I and II histone deacetylase (HDAC) inhibitor



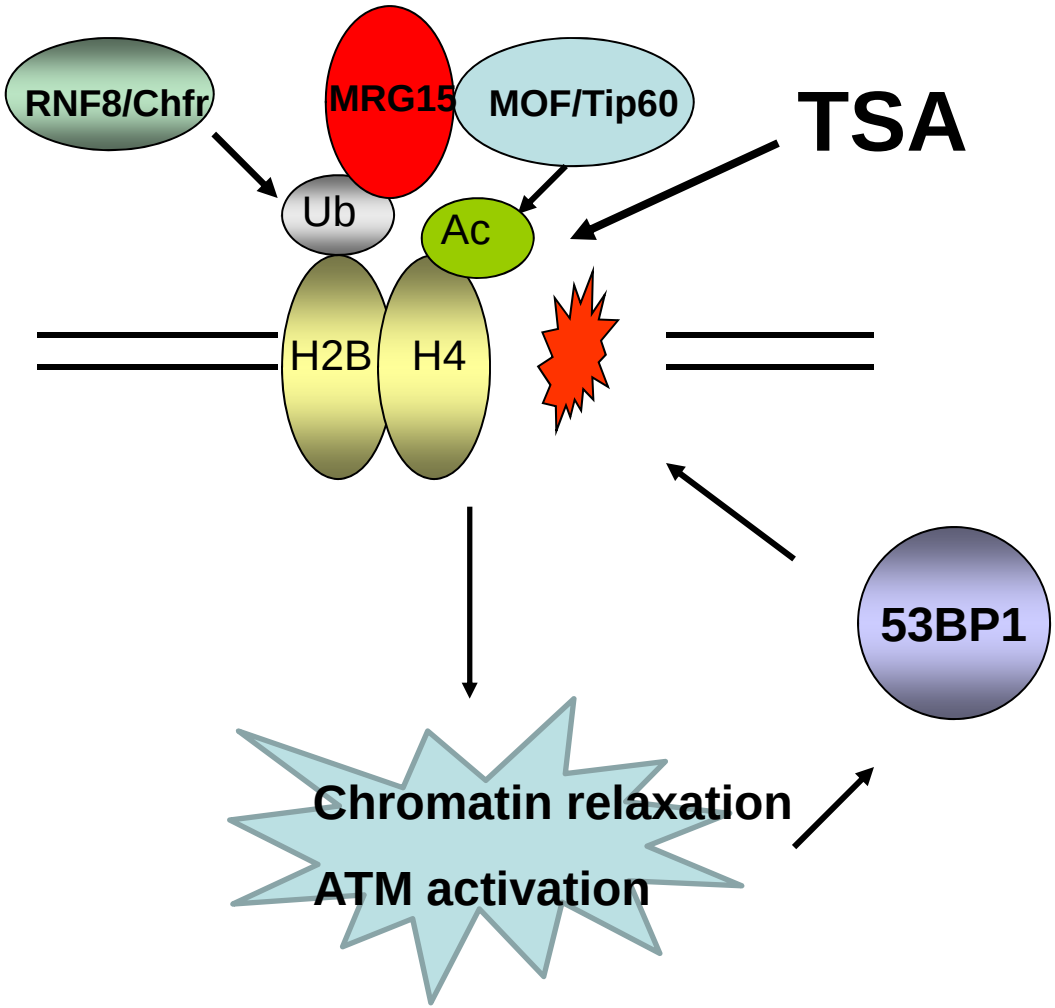
TSA

↓

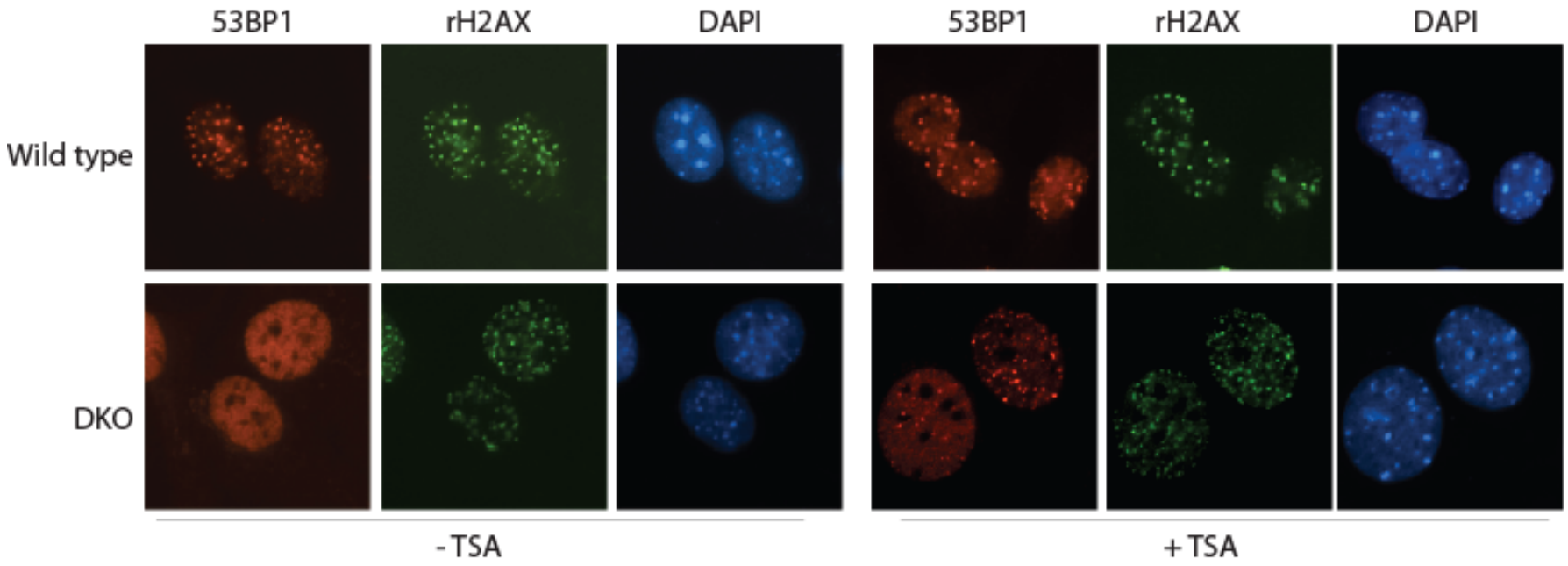
↑ H4 ac

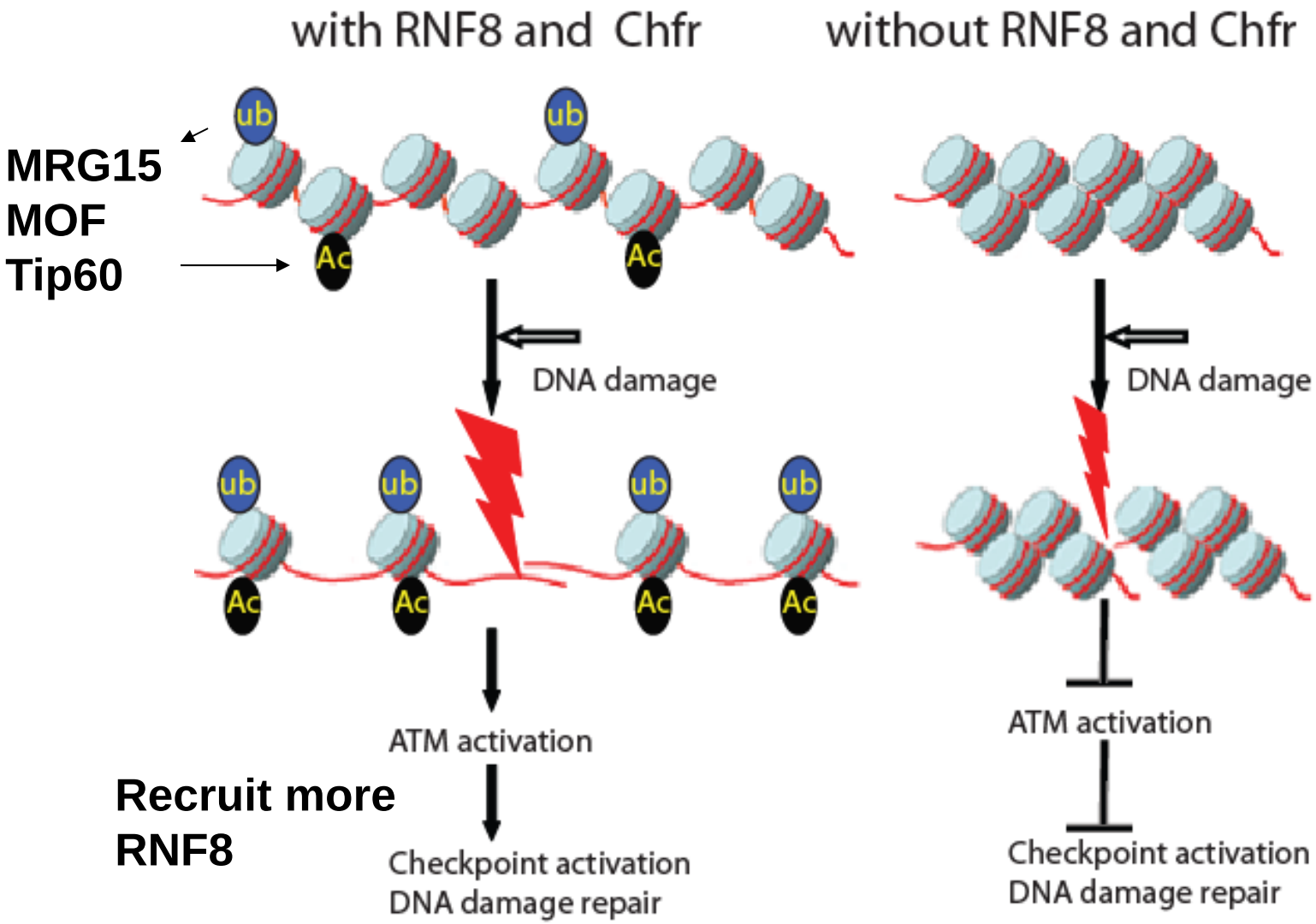
↓

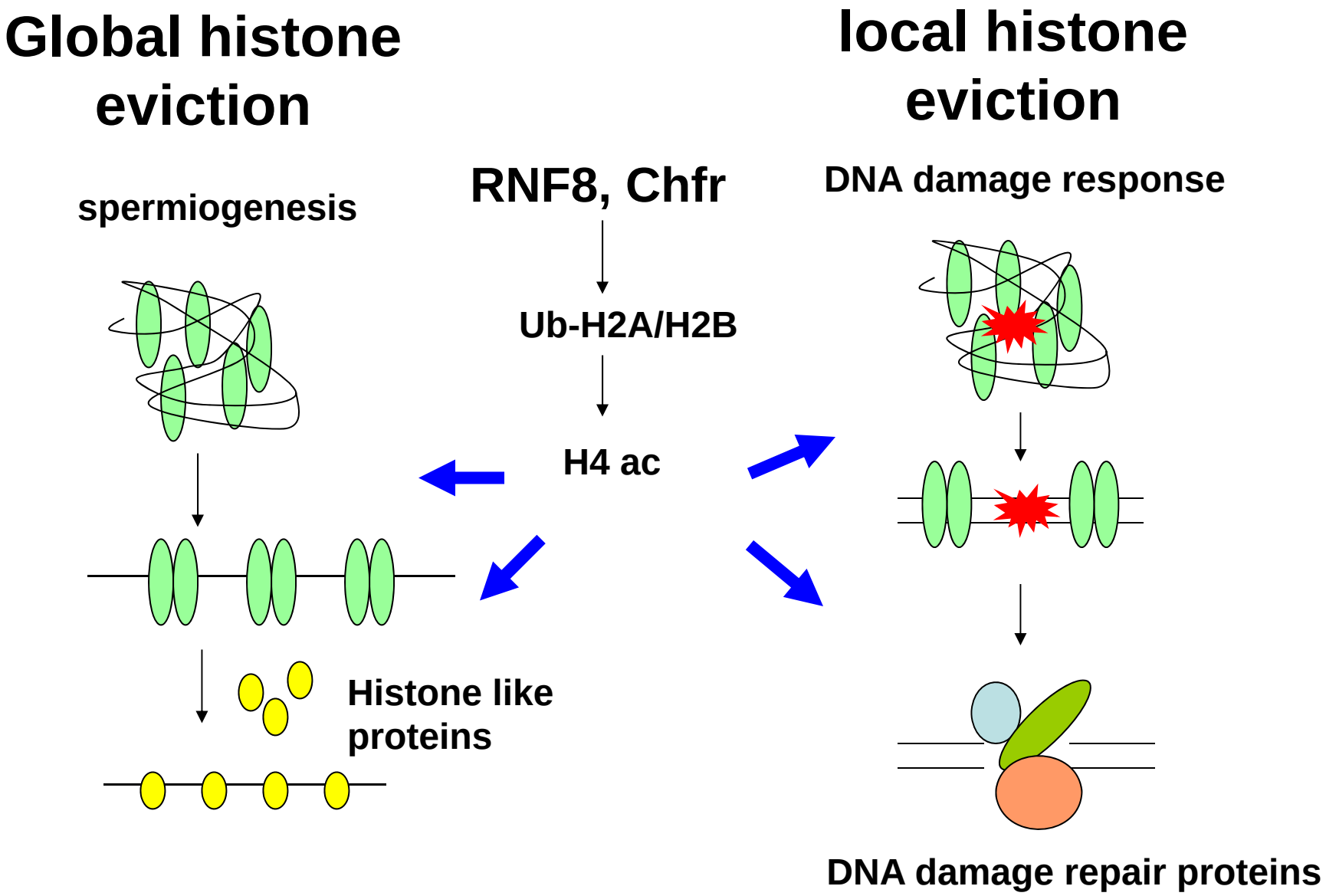
ATM activation


RNF8/Chfr
MRG15
MOF/Tip60
TSA
Ub
Ac
H2B
H4
53BP1
Chromatin relaxation
ATM activation

TSA restores 53BP foci in DKO cells




with RNF8 and Chfr
without RNF8 and Chfr
MRG15
MOF
Tip60
ub
Ac
DNA damage
ATM activation
Recruit more
RNF8
Checkpoint activation
DNA damage repair


Global histone eviction
local histone eviction
RNF8, Chfr
spermiogenesis
DNA damage response
Ub-H2A/H2B
H4 ac
Histone like proteins
DNA damage repair proteins

Acknowledgement

Lab member:

Charles Lu
Jiaxue Wu
Yibin Chen
Feng Zhang
Jennifer Keller
Teng Ma
Lin Ye
Henry Kwang
Chunjing Bian
Yi Xiong
Qiang Chen

Junjie Chen
(M.D. Anderson)

David Ferguson
(Dept. of Pathology)

Support:

NIH, DOD, ACS