SAPIENZA
UNIVERSITÀ DI ROMA

RNAi components cooperate with SWI/SNF chromatin remodeling complex to determine nucleosome occupancy at human TSS

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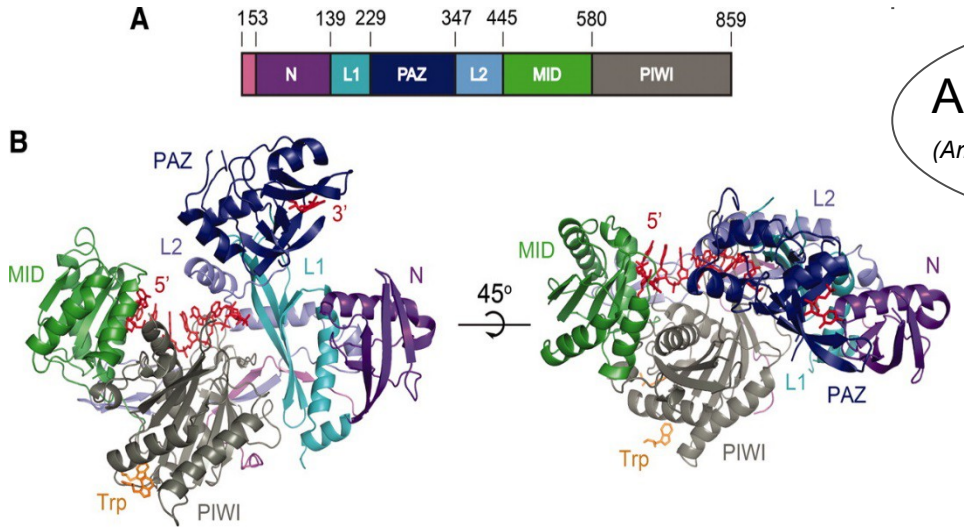
Dipartimento di Biotecnologie Cellulari ed Ematologia

Mammalian AGO2 functions

Cytoplasmic
Post-transcriptional
regulation
(miRNA,siRNA)

Transcriptional
regulation in
the nucleus
(exogenous siRNA)

(kim et al., 2006;Morris Et al., 2004, Janokwsi et al.2006),

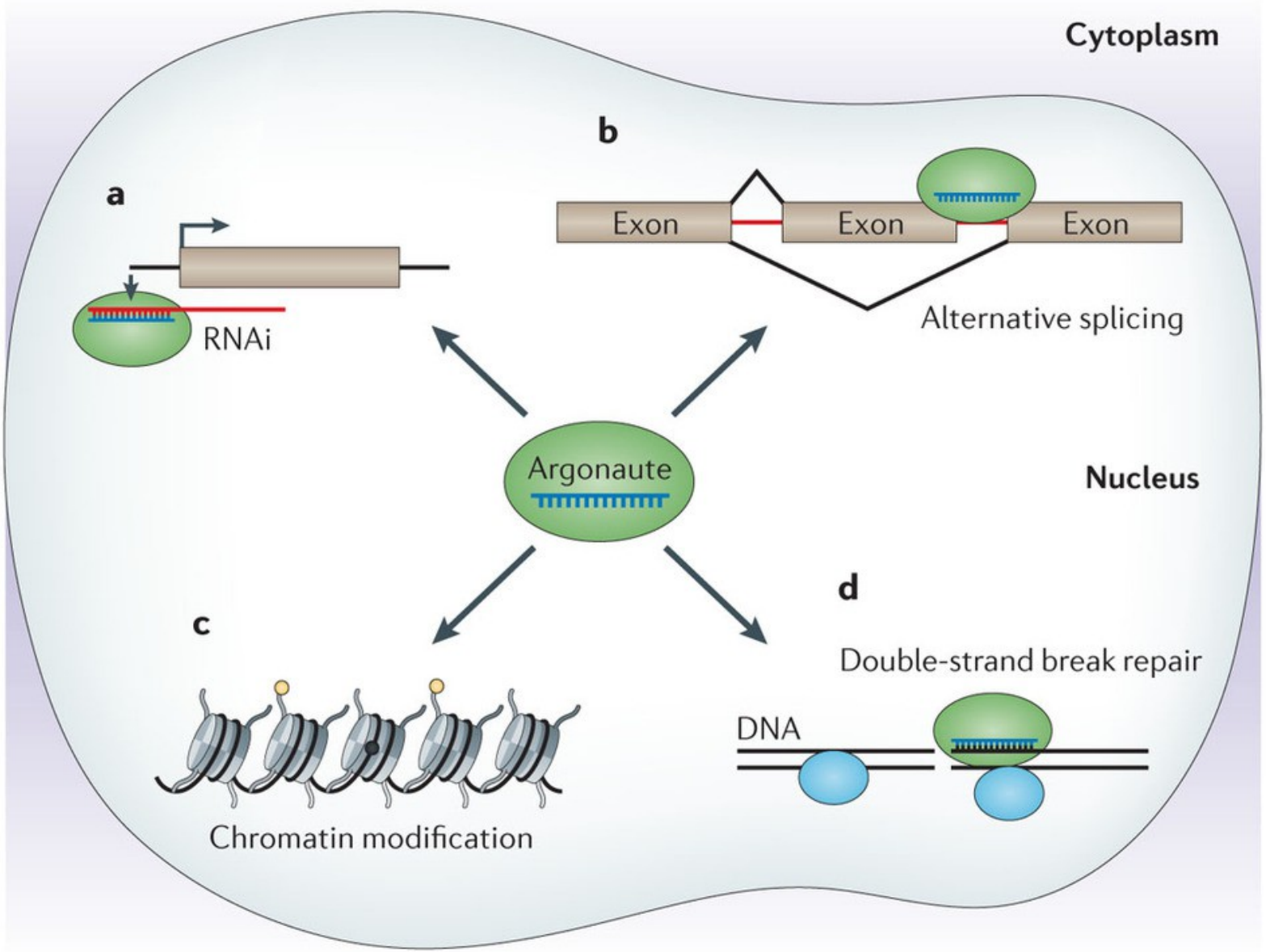


(Schirle & McRae, Science, 2012)

Alternative Splicing

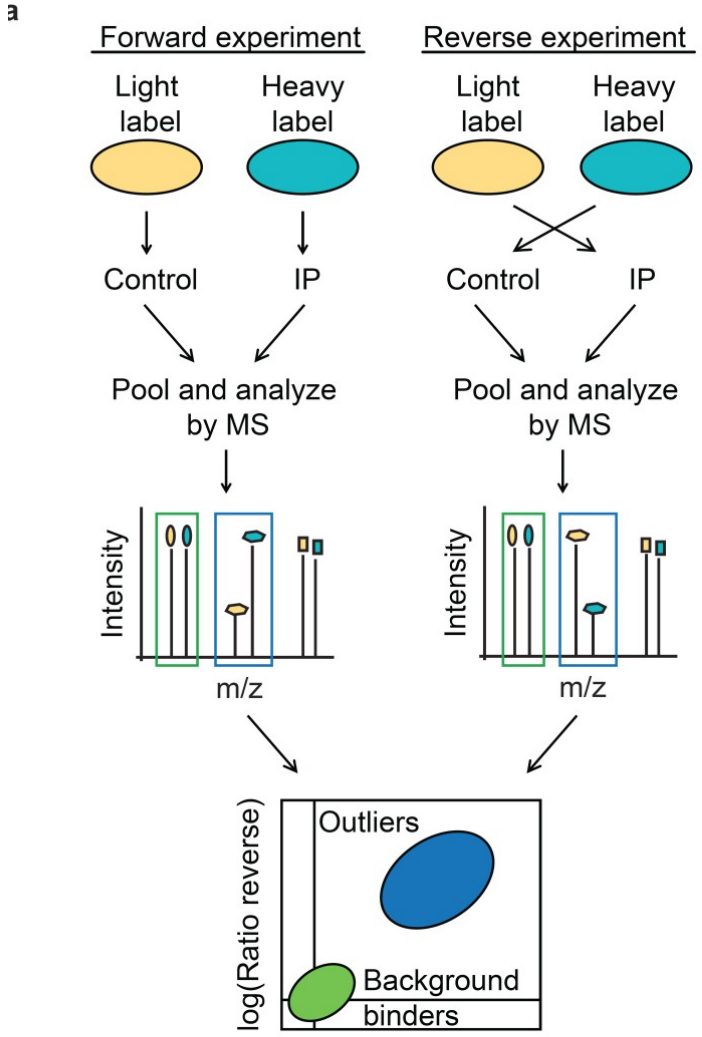
(Ameyar-Zazoua et al.,Nat.Struct.Mol.Biol.,2012)

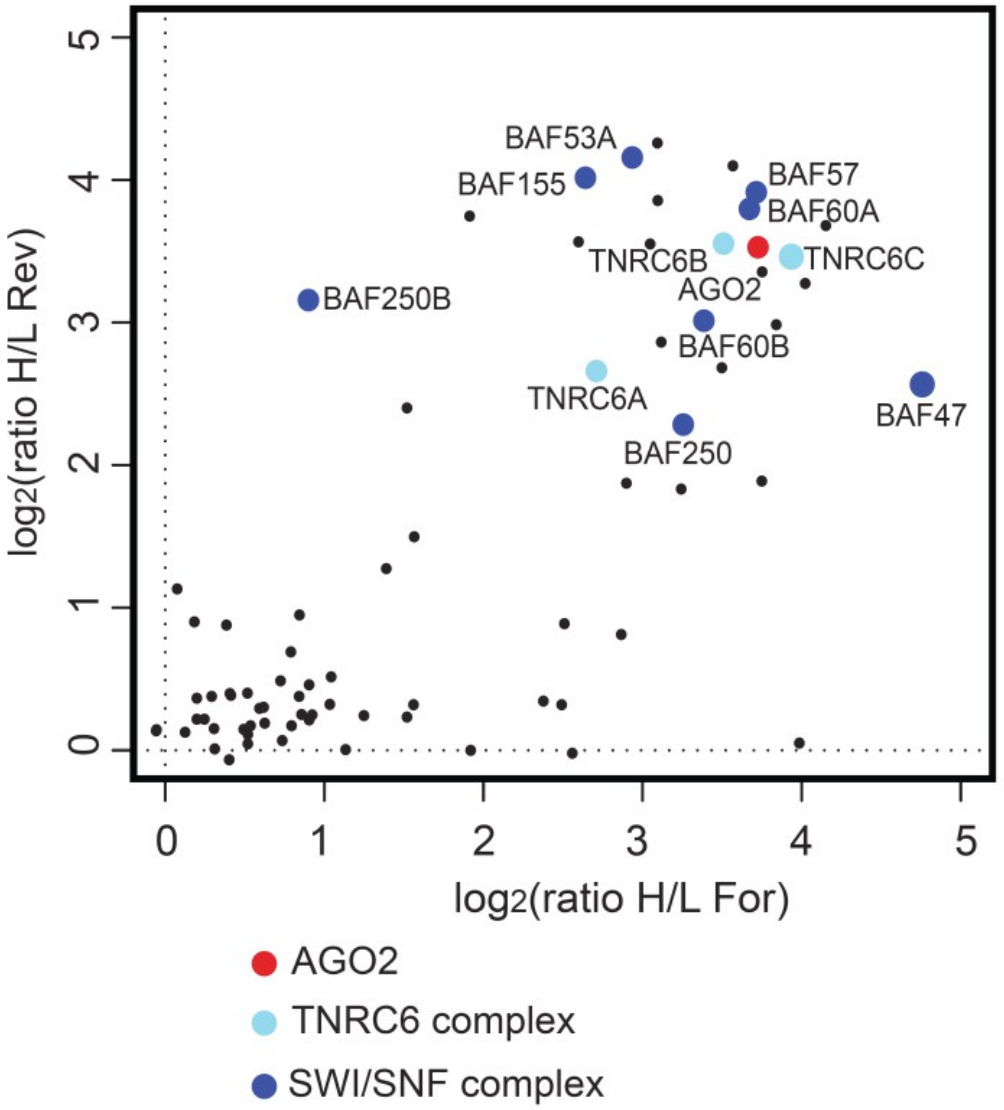
Argonaute protein functions



Nature Reviews | Genetics

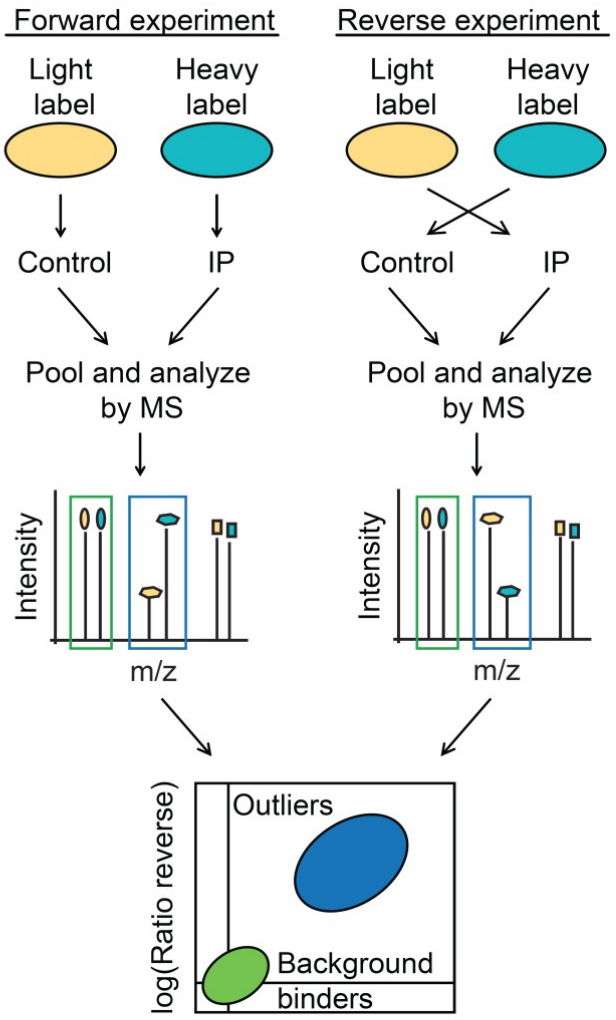
Identification of novel AGO2 partners





AGO2 interacts with SWI/SNF

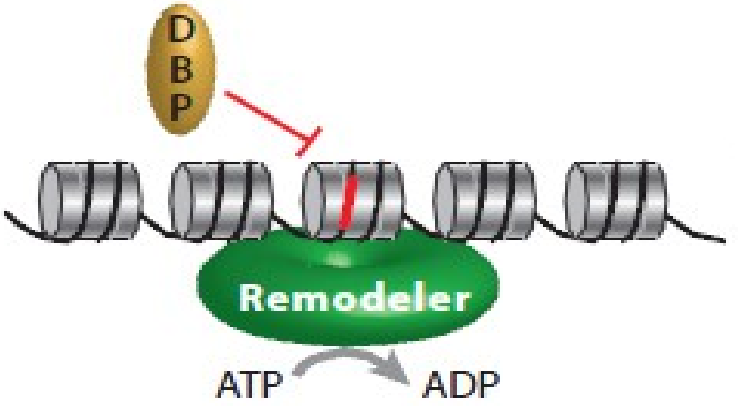
a

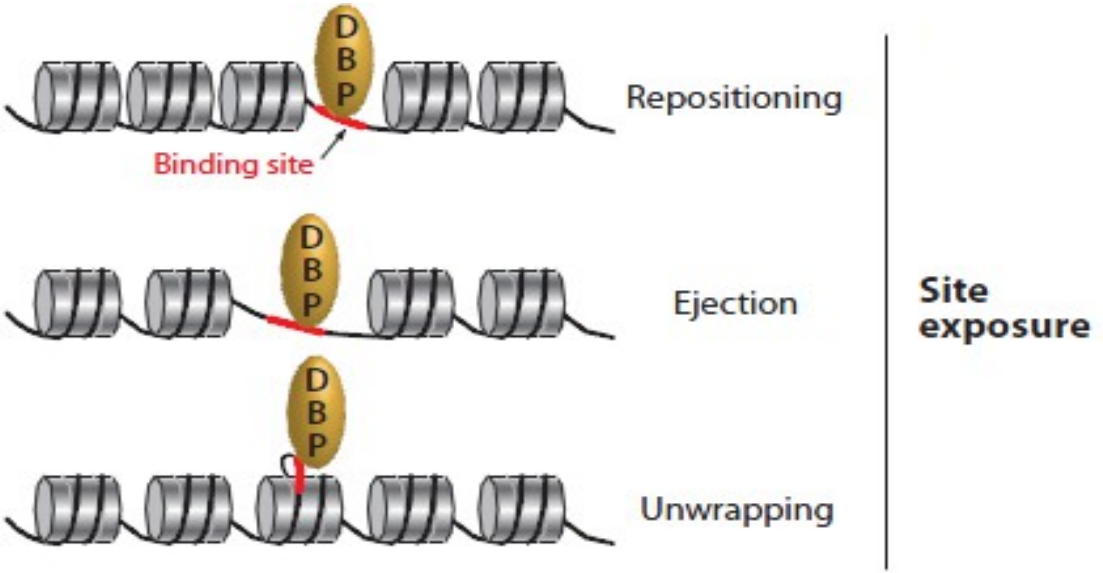


Proteins identified in top 30% In HeLaS3 and Jurkat cell lines	Complexes
EIF2C2 (AGO2)	
BAF53A BAF250 BAF47 BAF155 BAF60A BAF60B BAF57	SWI/SNF
TNRC6A TNRC6B TNRC6C	TNRC6
IGF2BP3 LSM12 MIA3 MYH9 PABC1 RBM14 TFG CSTF3 DLAT	

SWI/SNF is a conserved family of chromatin remodelers

SWI/SNF Subunits		
ATPase	Core	Accessory
BRG1	SNF5	Actin
BRM	BAF155	BAF45(a-d)
	BAF170	BAF53(a,b)
		BAF57
		BAF60(a-c)
		BAF180
		BAF200
		BAF250(a,b)

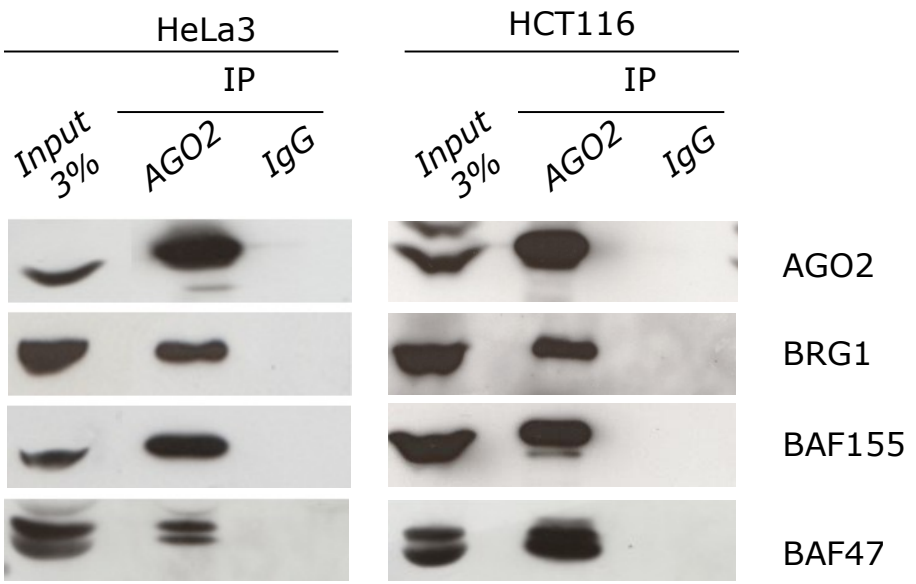




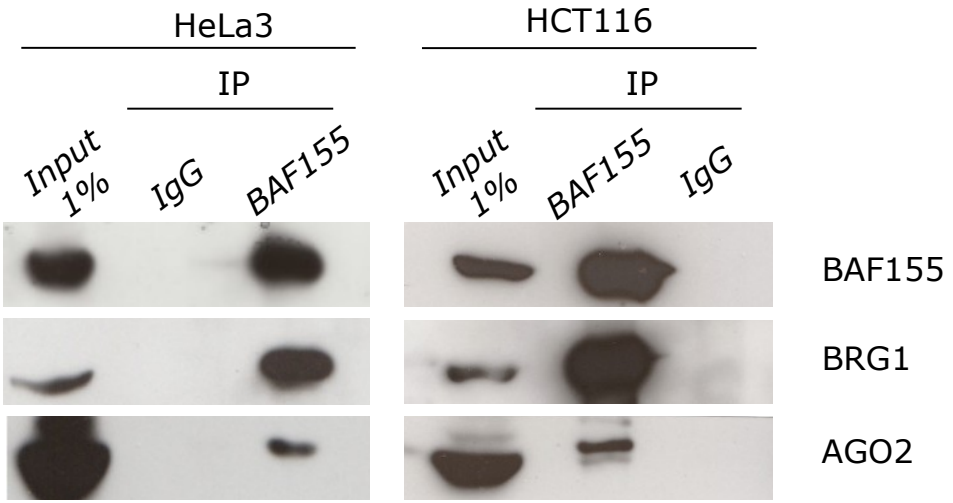
(Clapier CR et al., Annu Rev Biochem, 2009)

AGO2 (and not AGO1) interacts with SWI/SNF

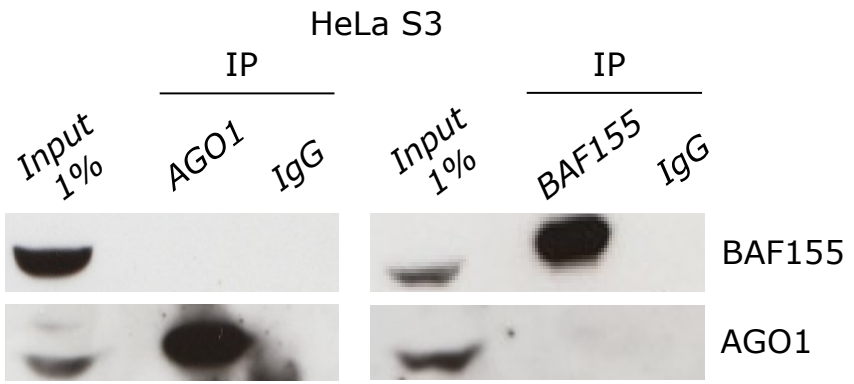
AGO2 IP pulls down SWI/SNF core components



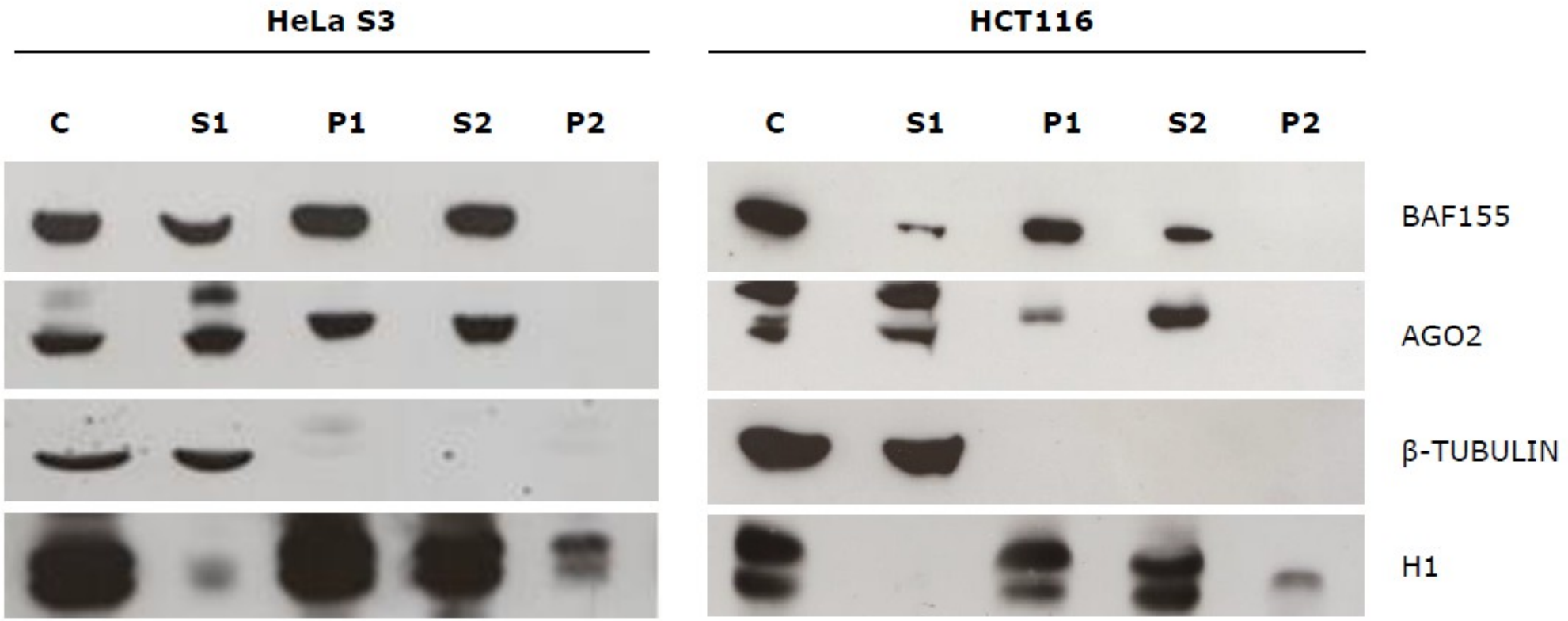
BAF155 IP pulls down AGO2



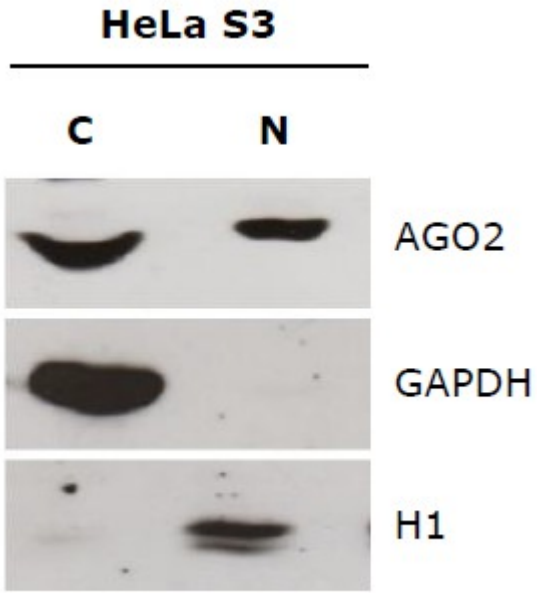
AGO1 does not interact with SWI/SNF

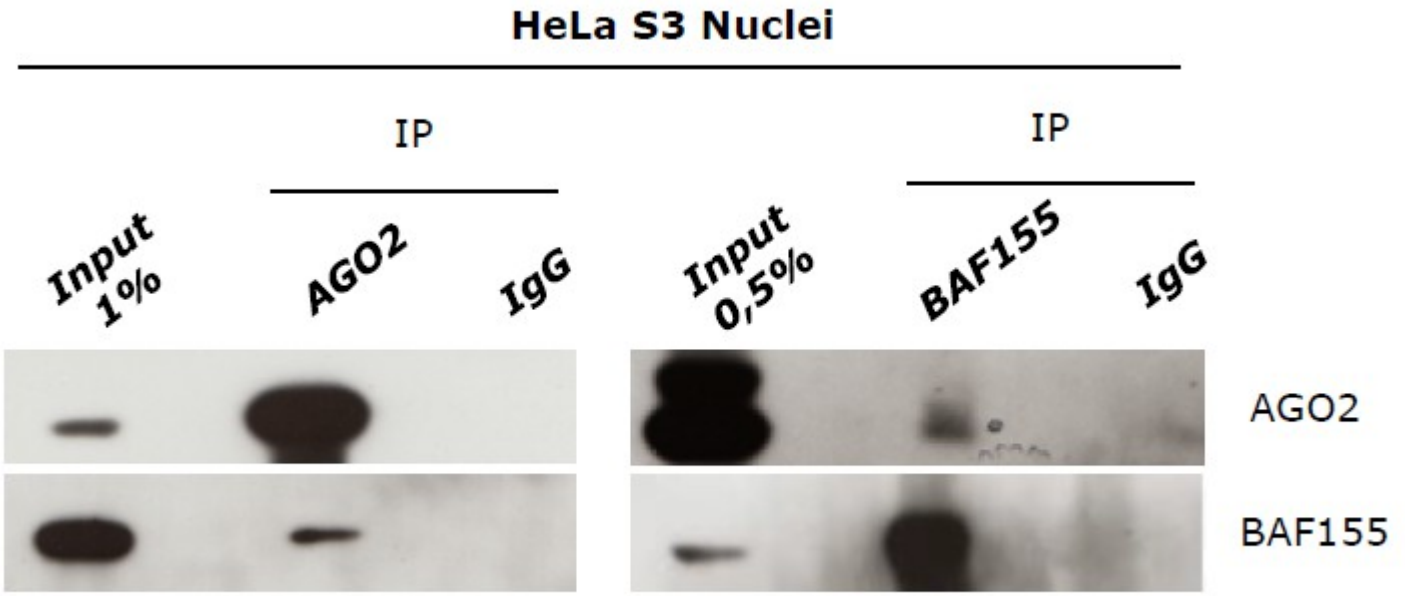


AGO2 and SWI/SNF are both associated with chromatin

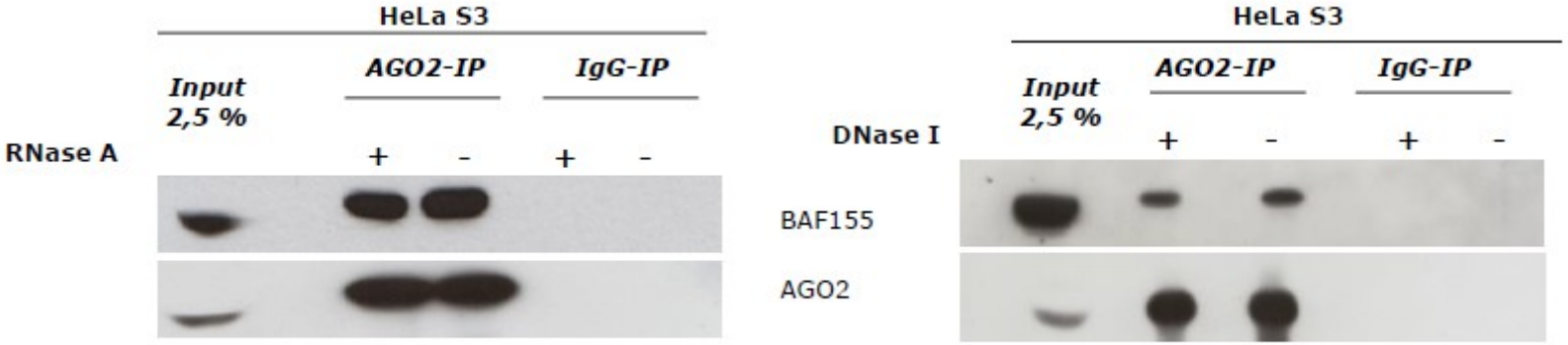


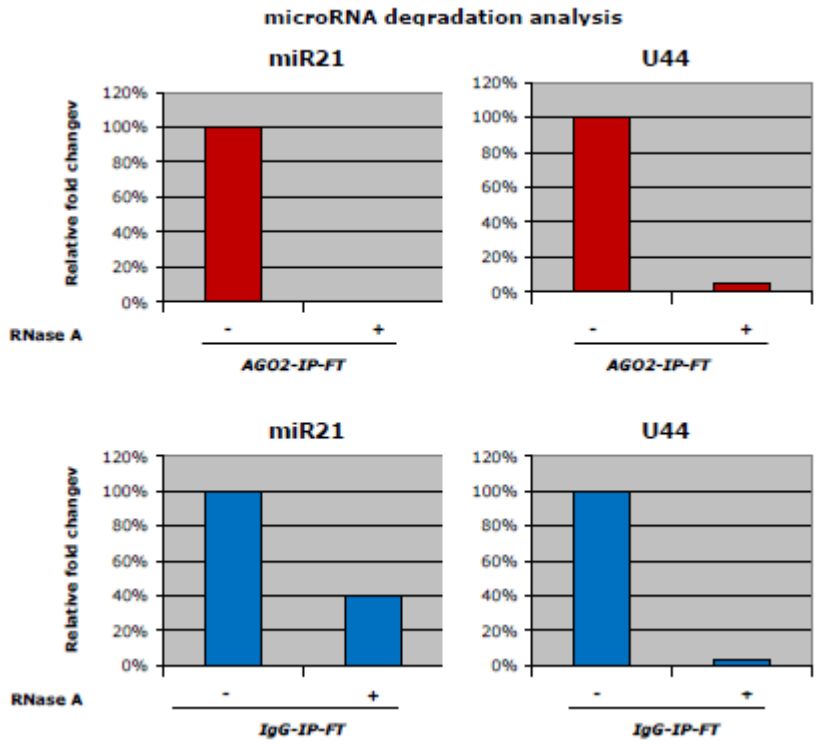
AGO2 and SWI/SNF interact in the nucleus

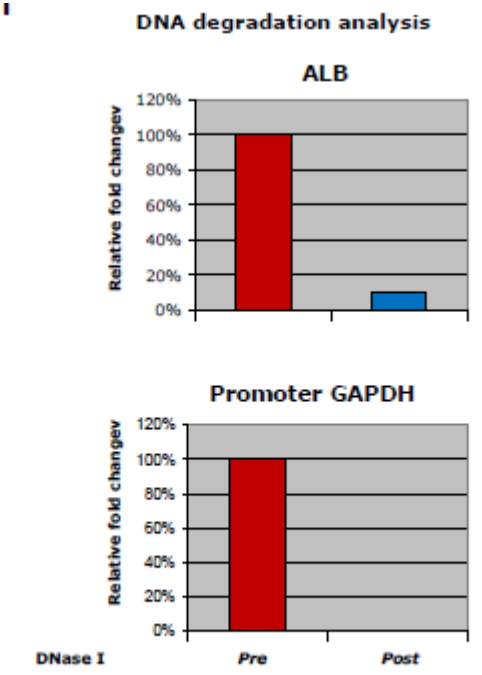




AGO2 and SWI/SNF interaction is not bridged by DNA or RNA







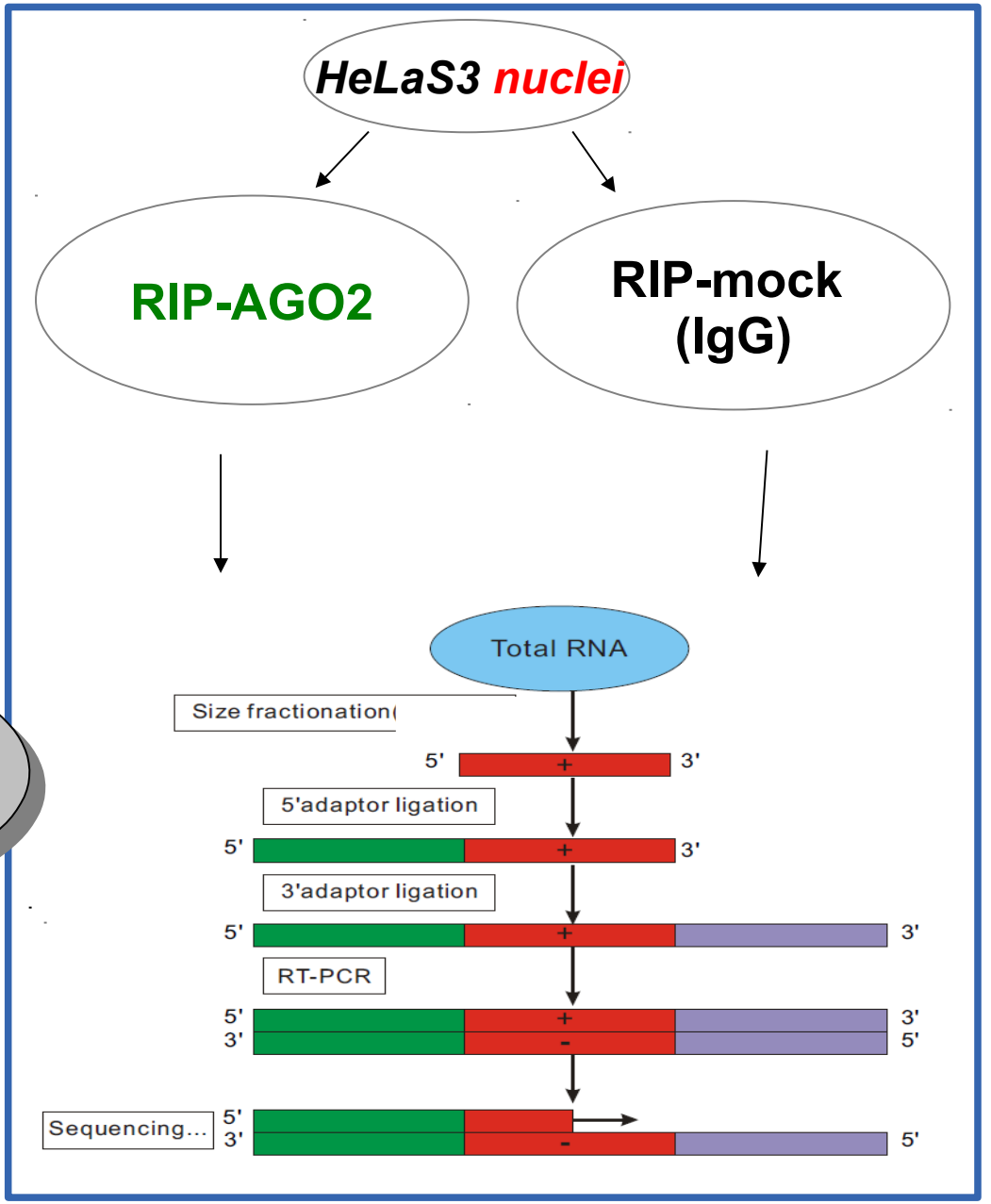
Conclusion I

- **AGO2 interacts with SWI/SNF in human cell lines (HeLaS3, Jurkat, HCT116, 293T, THP-1)**
- **AGO2-SWI/SNF interaction occurs in the nucleus**
- **AGO2-SWI/SNF interaction is not bridged by DNA or RNA**
- **AGO2 is associated with human chromatin**
- **AGO1 does not interact with SWI/SNF**

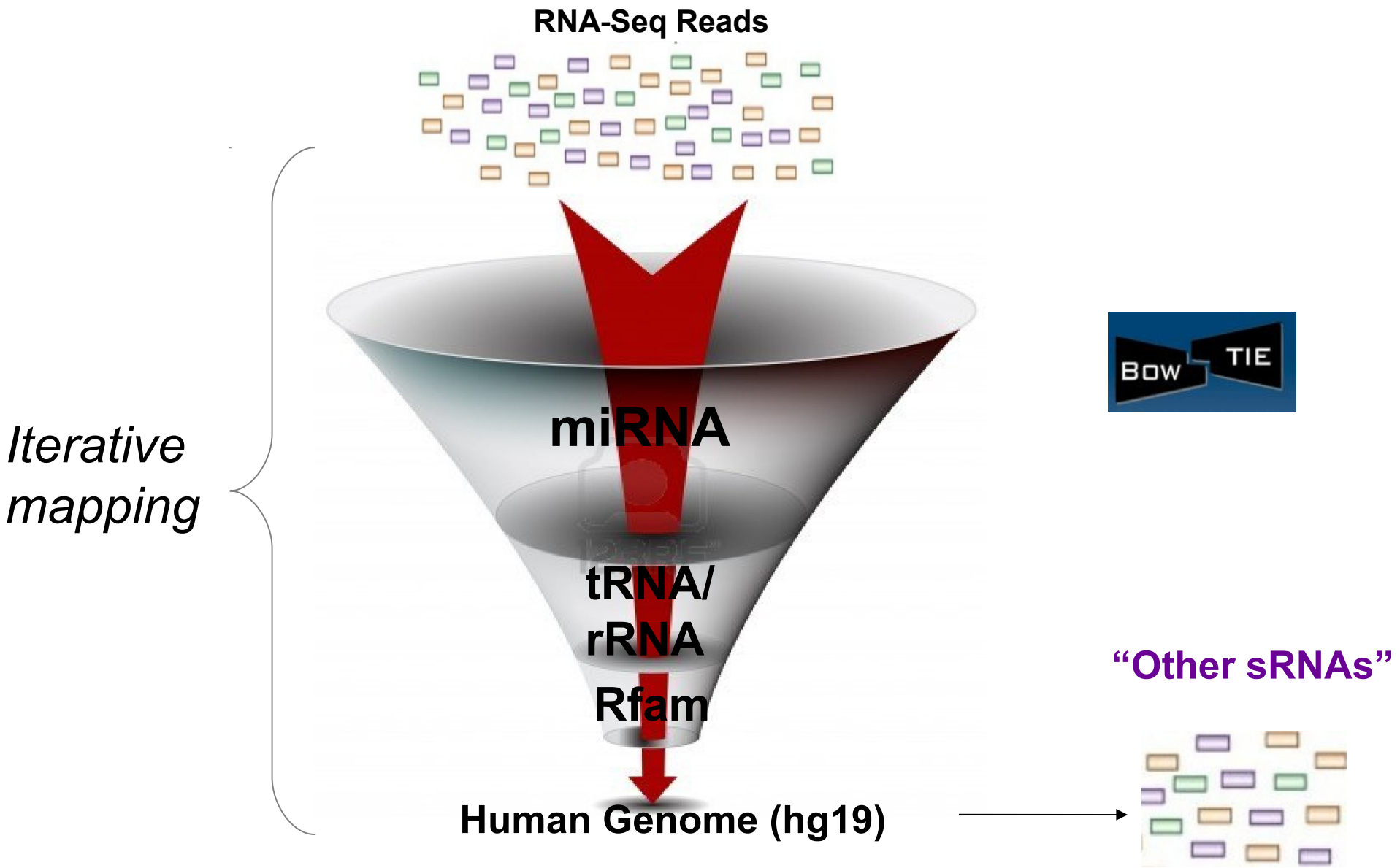
Characterization of nuclear AGO2 bound sRNAs

What is the function of AGO2 in the nucleus?

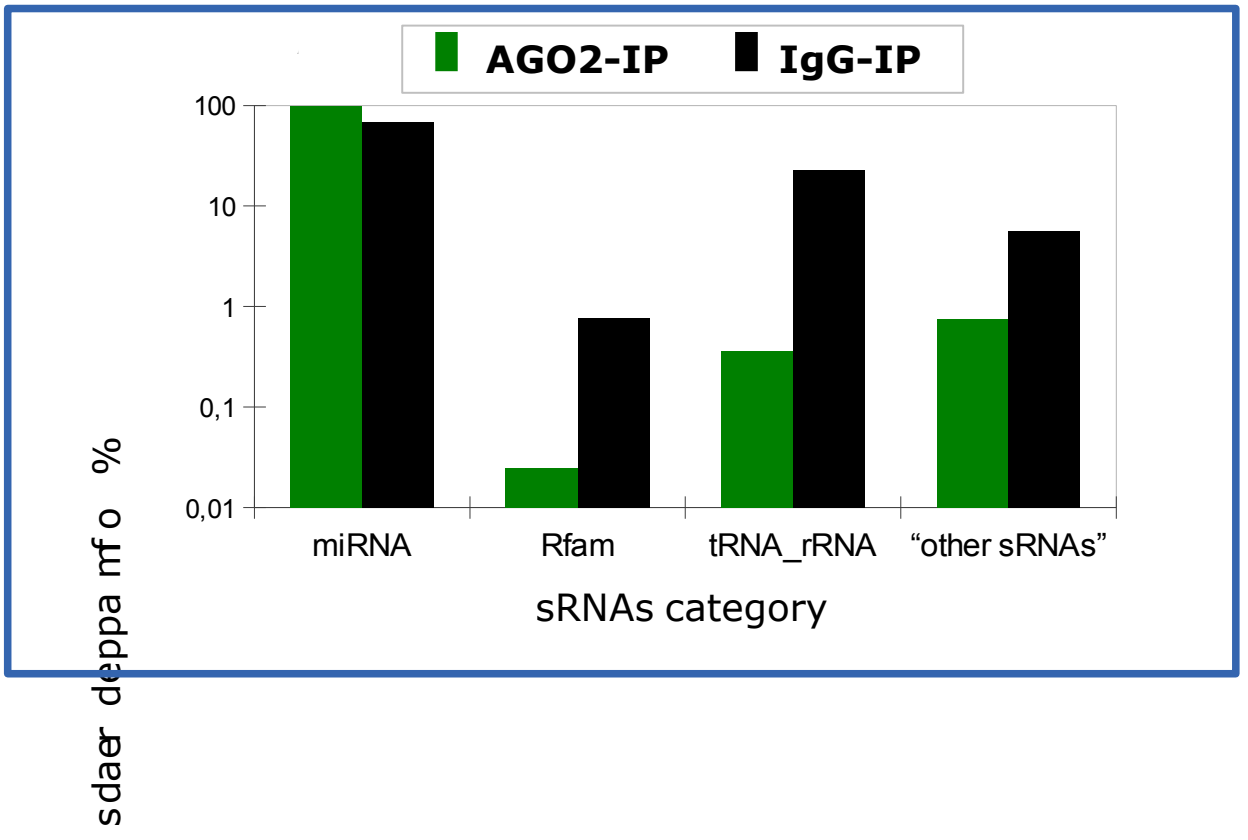
What are the small RNAs associated with nuclear AGO2?



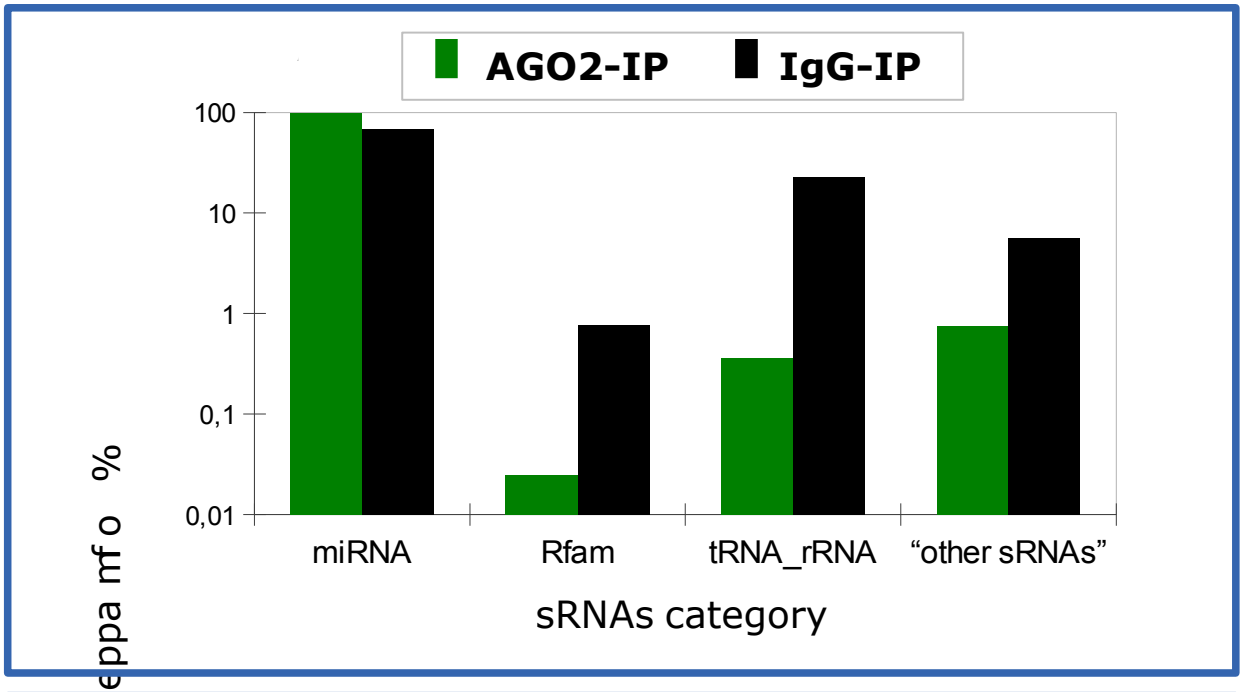
Annotation strategy to discover novel AGO2 bound sRNAs

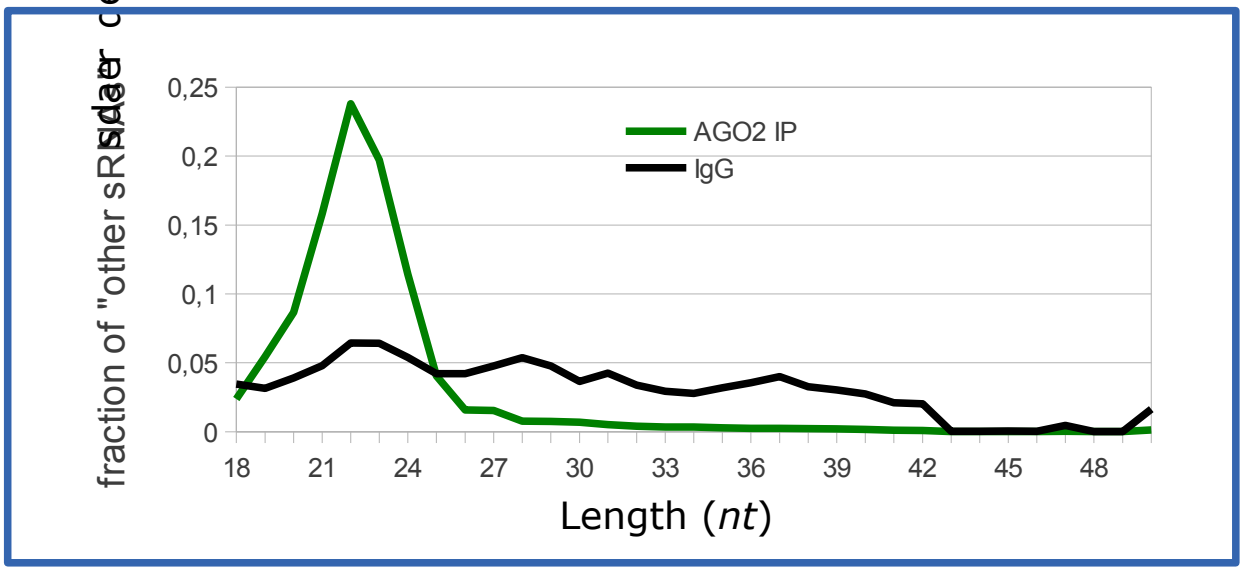


Features of nuclear AGO2 bound sRNAs



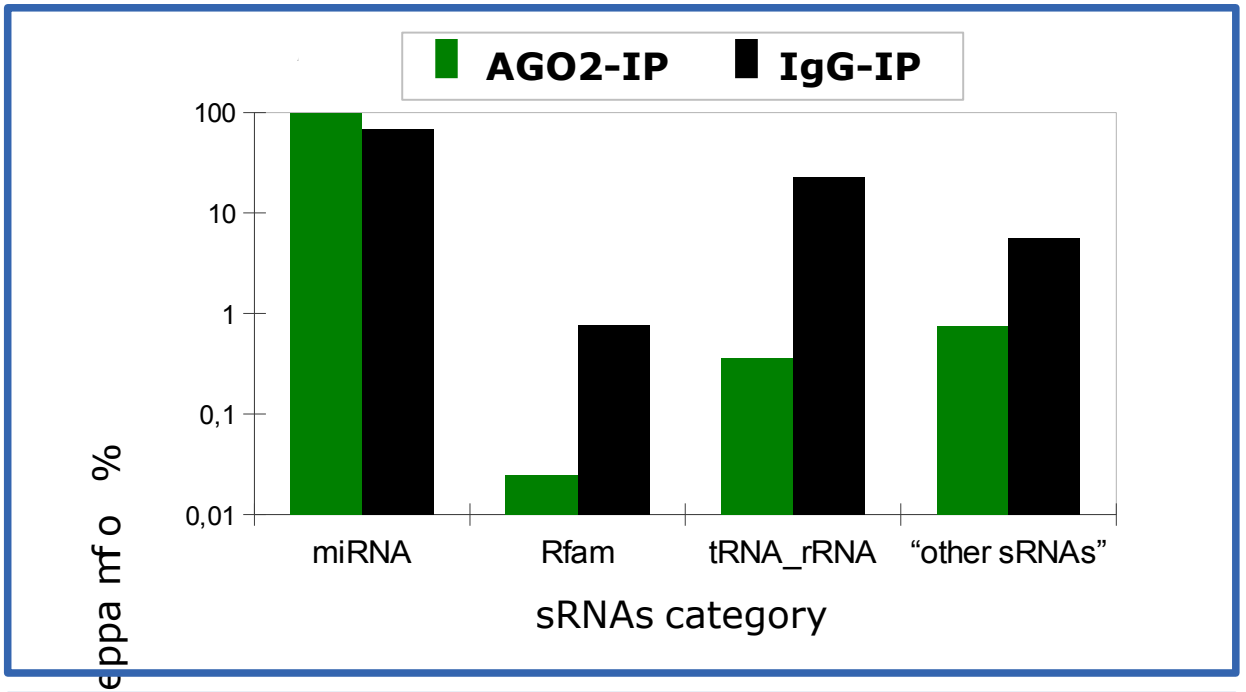
Features of nuclear AGO2 bound sRNAs

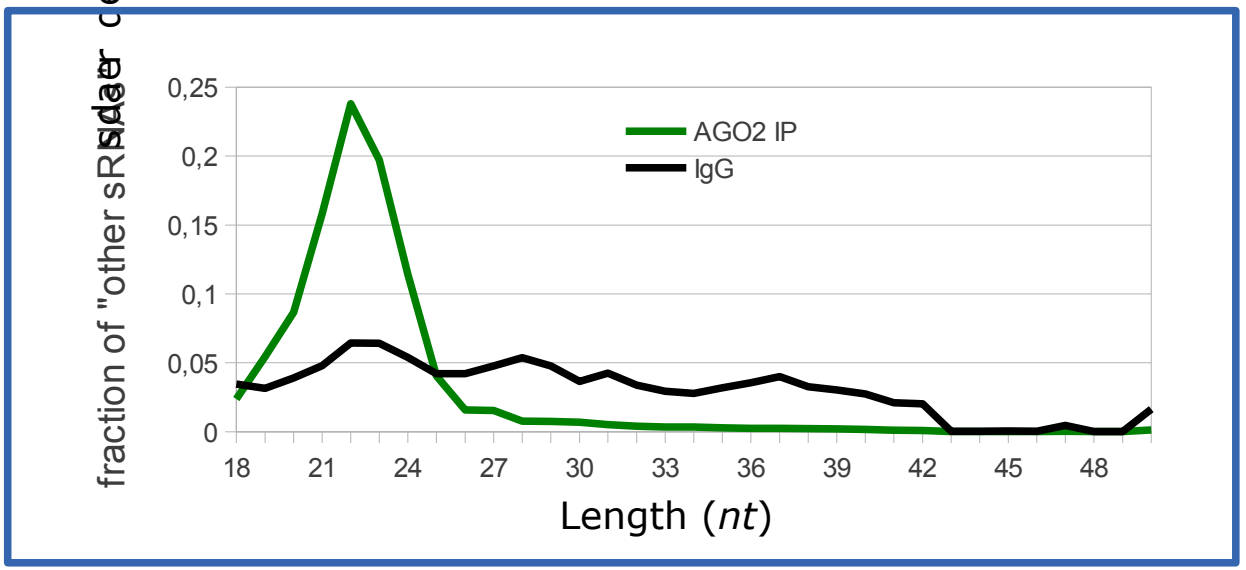


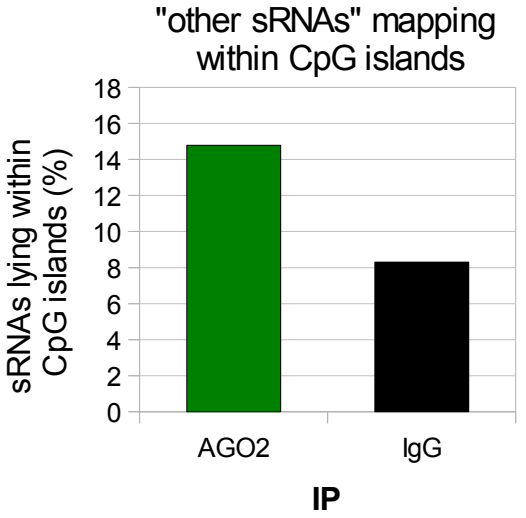


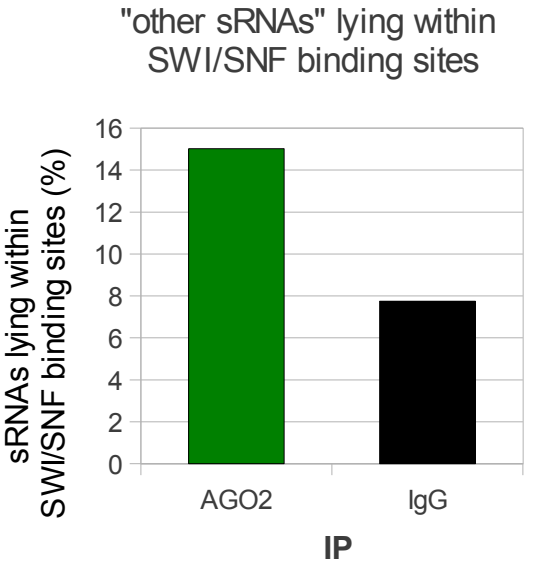
Which are the genomic features overlapping nuclear AGO2 bound sRNAs

Features of nuclear AGO2 bound sRNAs

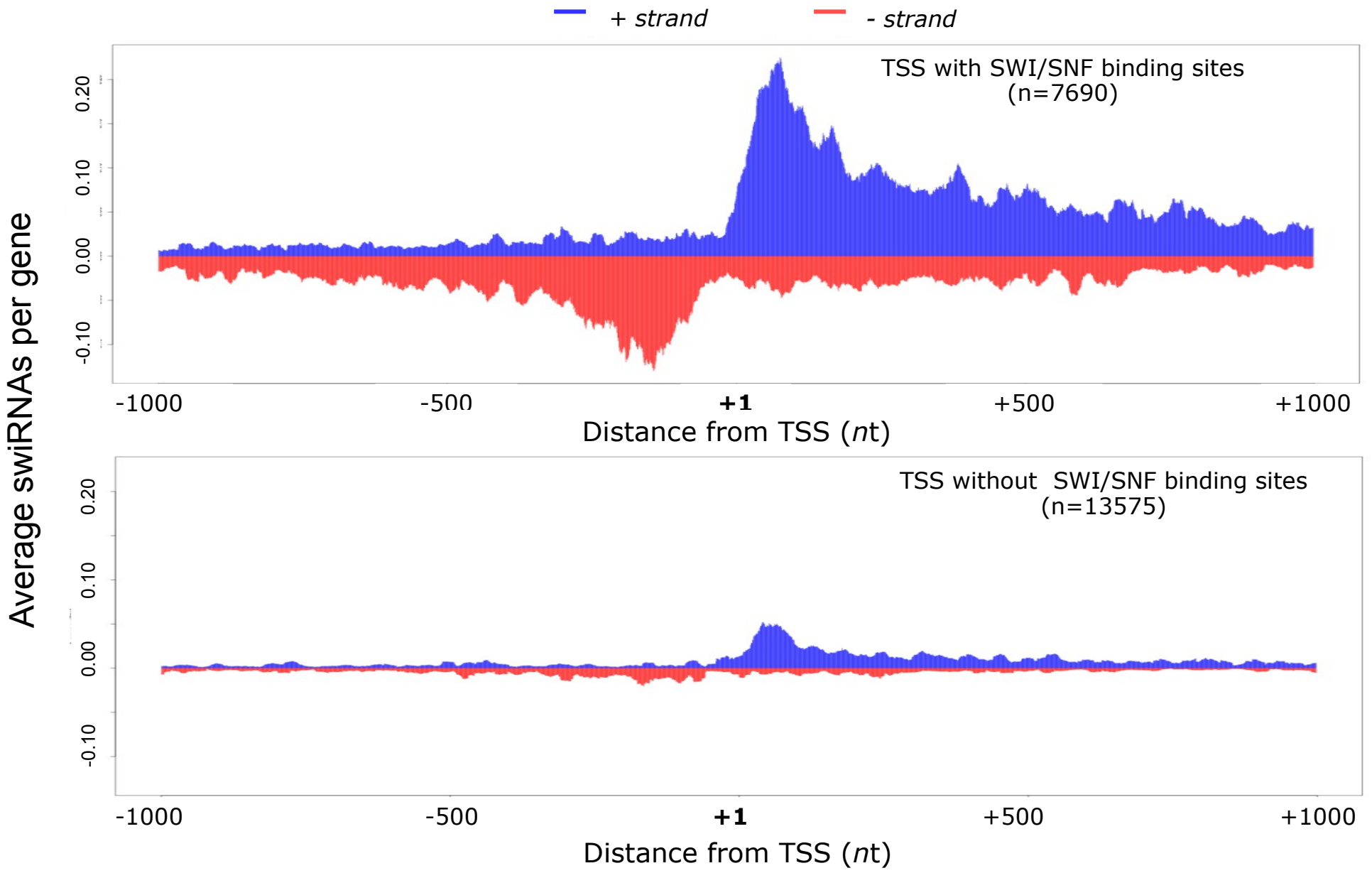








swiRNAs specifically originate from SWI/SNF bound TSS




Scale
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hg19
49,027,800 49,027,900 49,028,000 49,028,100 49,028,200 49,028,300 49,028,400 49,028,500 49,028,600 49,028,700 49,028,800 49,028,900 49,029,000 49,029,100 49,029,200 49,029,300 49,029,400 49,029,500 49,029,600 49,029,700
AGO2 associated swiRNAs
SWI/SNF ChIP-seq binding sites (ENCODE)
RefSeq Genes
PLP2
4
Placental Mammal Basewise Conservation by PhyloP
Mammal Cons
-4
Scale
chr1:
1 kb
hg19
2,343,300 2,343,400 2,343,500 2,343,600 2,343,700 2,343,800 2,343,900 2,344,000 2,344,100 2,344,200 2,344,300 2,344,400 2,344,500 2,344,600 2,344,700 2,344,800 2,344,900 2,345,000 2,345,100 2,345,200 2,345,300 2,345,400 2,345,500
AGO2 associated swiRNAs
SWI/SNF ChIP-seq binding sites (ENCODE)
RefSeq Genes
PEX10
PEX10
4
Placental Mammal Basewise Conservation by PhyloP
Mammal Cons
-4
Scale
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AGO2 associated swiRNAs
SWI/SNF ChIP-seq binding sites (ENCODE)
RefSeq Genes
ARL6IP1
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AGO2 associated swiRNAs
SWI/SNF ChIP-seq binding sites (ENCODE)
RefSeq Genes
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LSM14A
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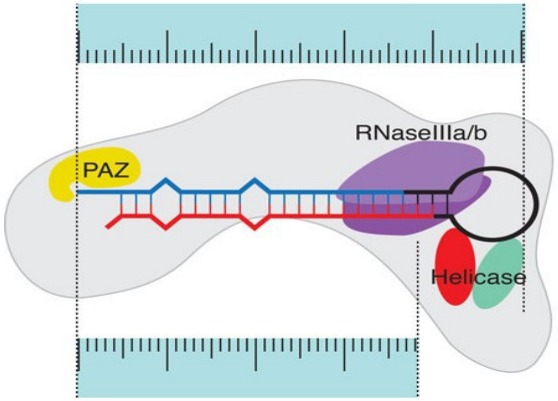
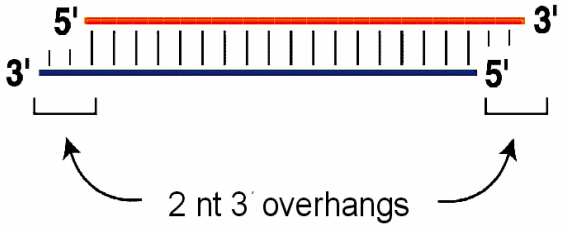
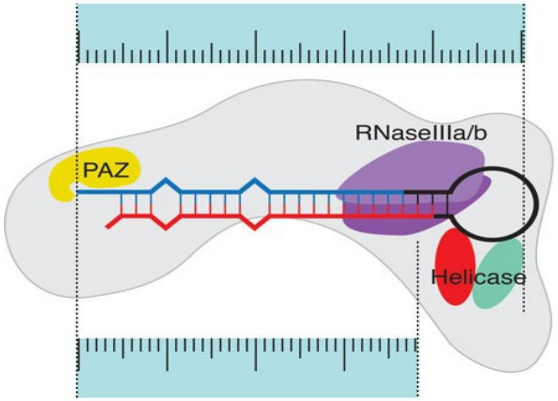
Placental Mammal Basewise Conservation by PhyloP
Mammal Cons
-4

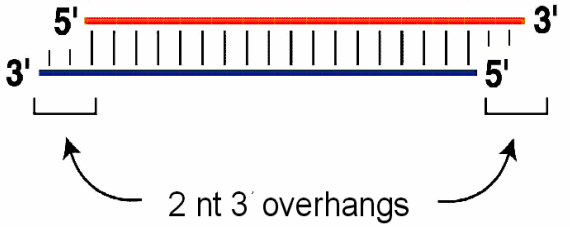
The enzymatic signature of DICER

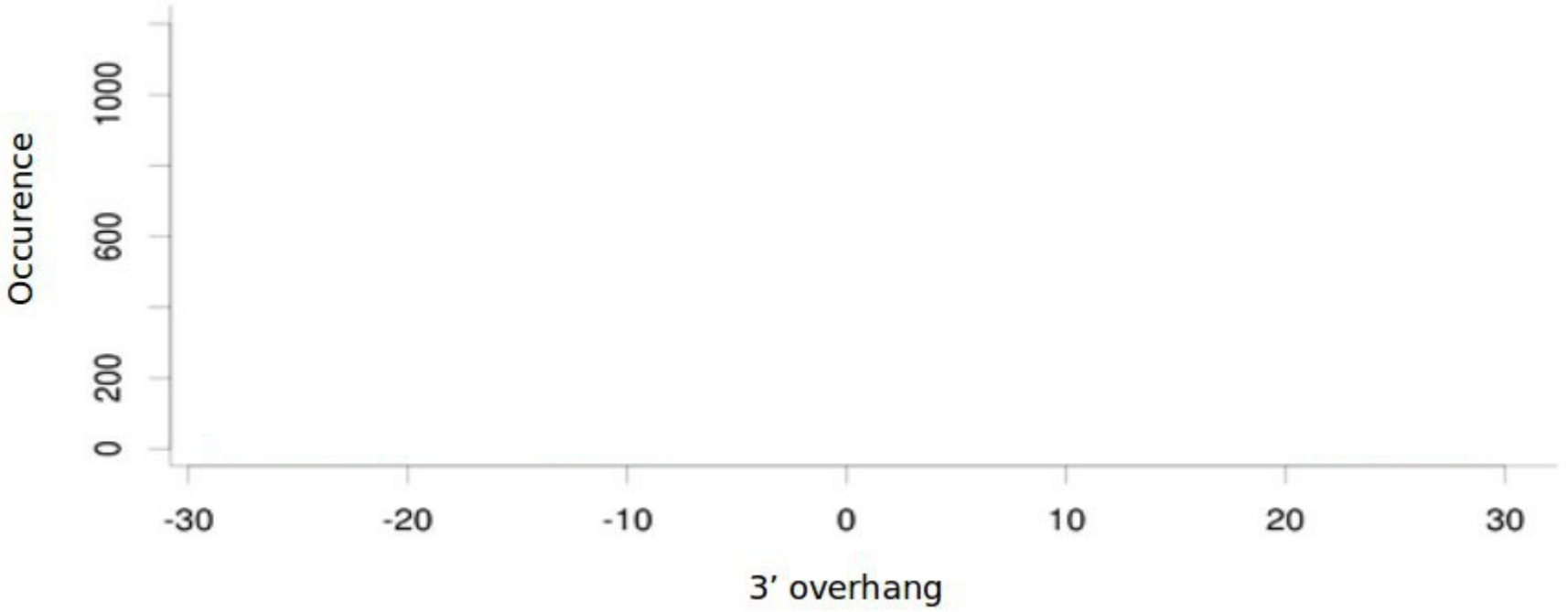




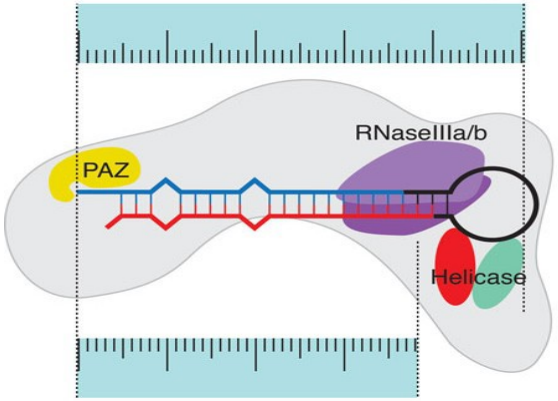
The enzymatic signature of DICER

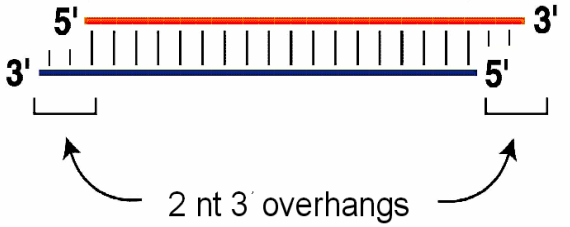


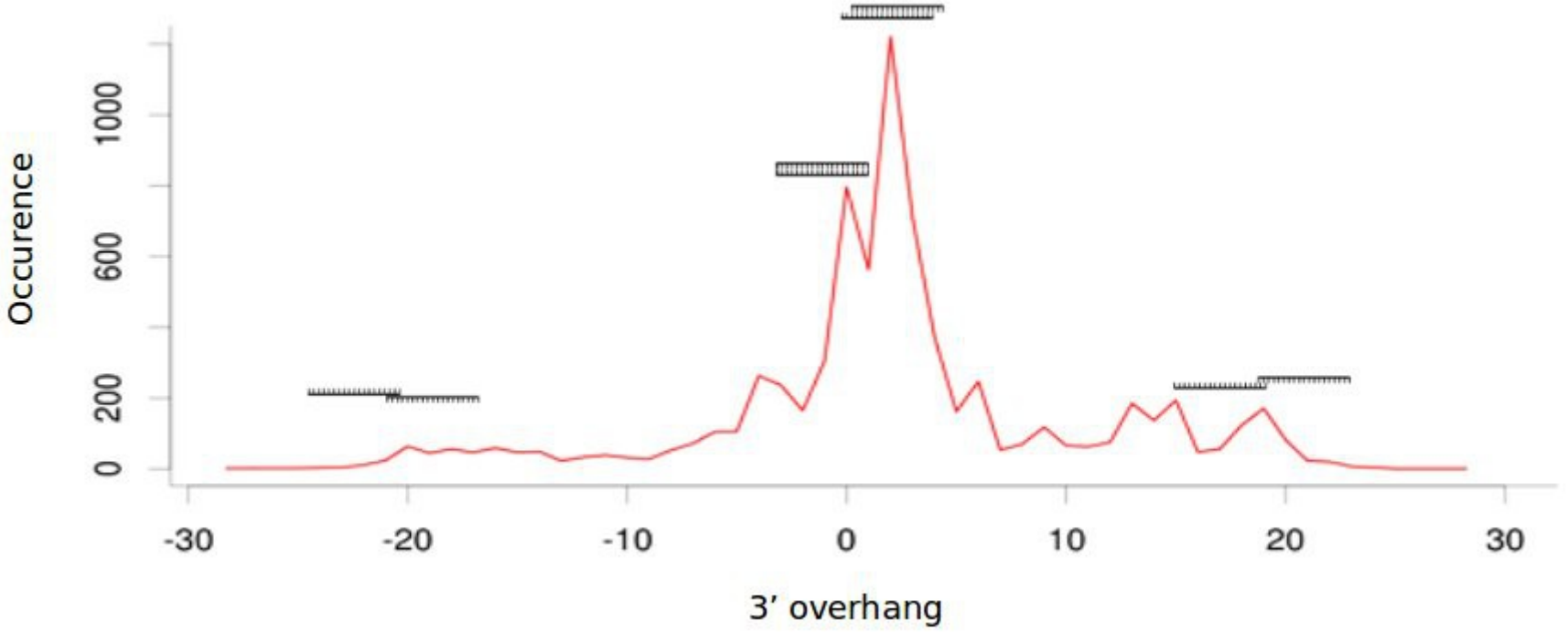




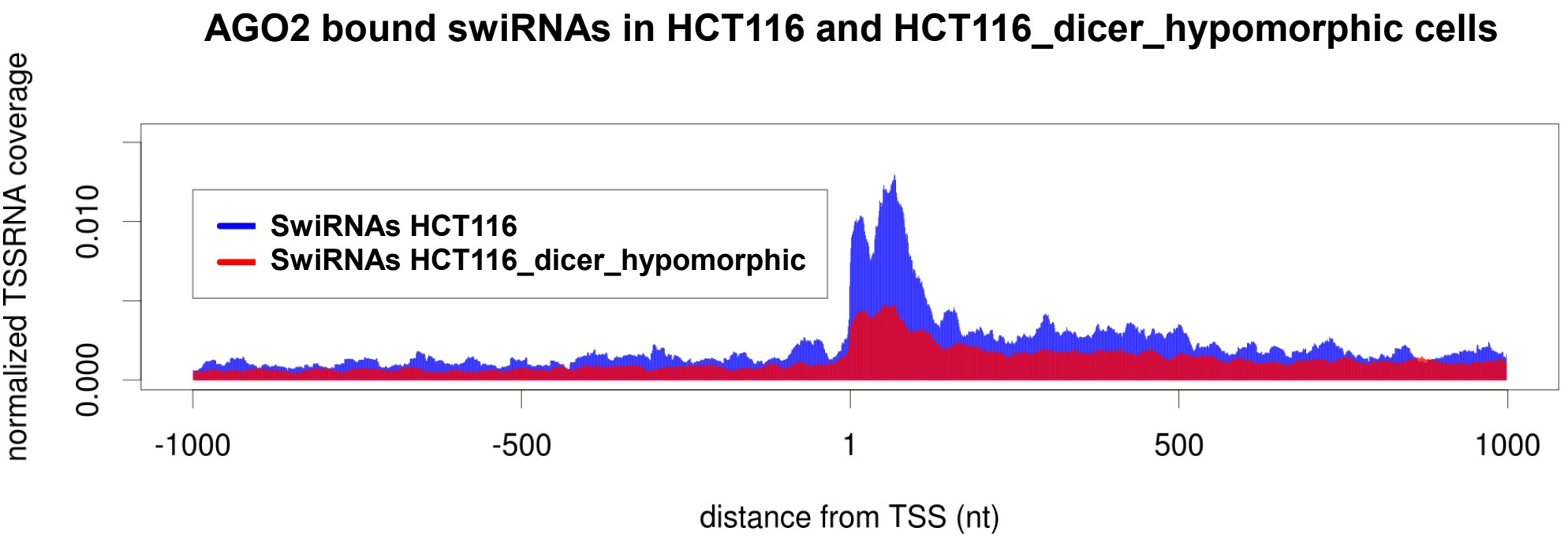
The enzymatic signature of DICER



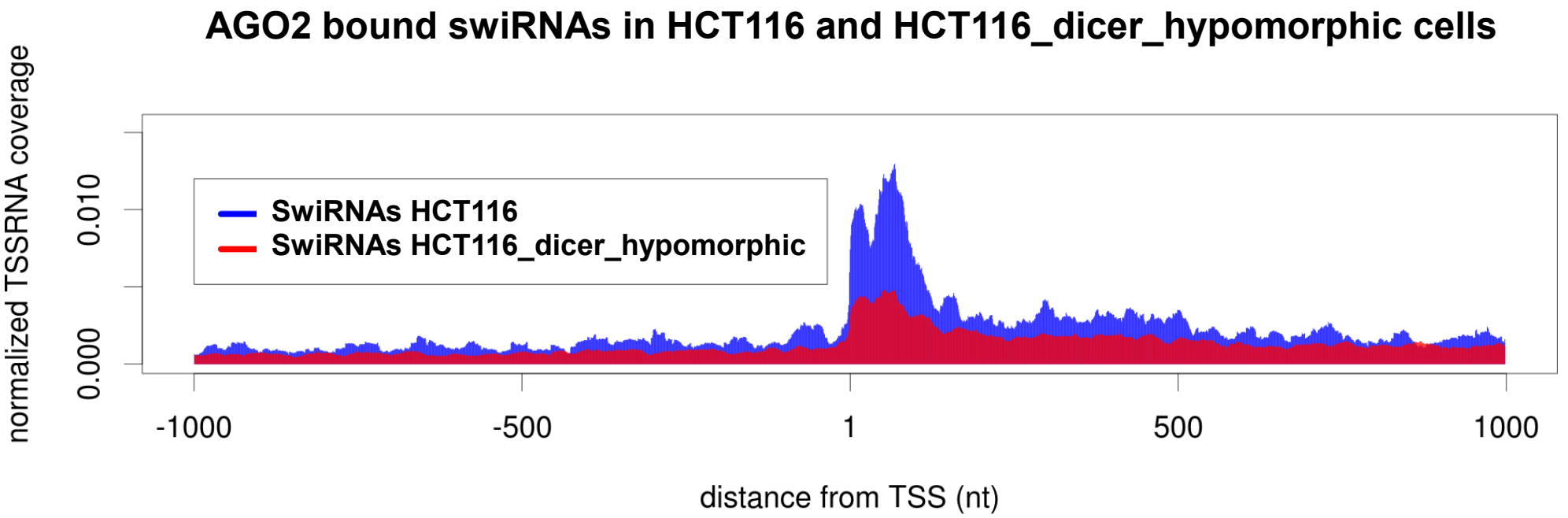


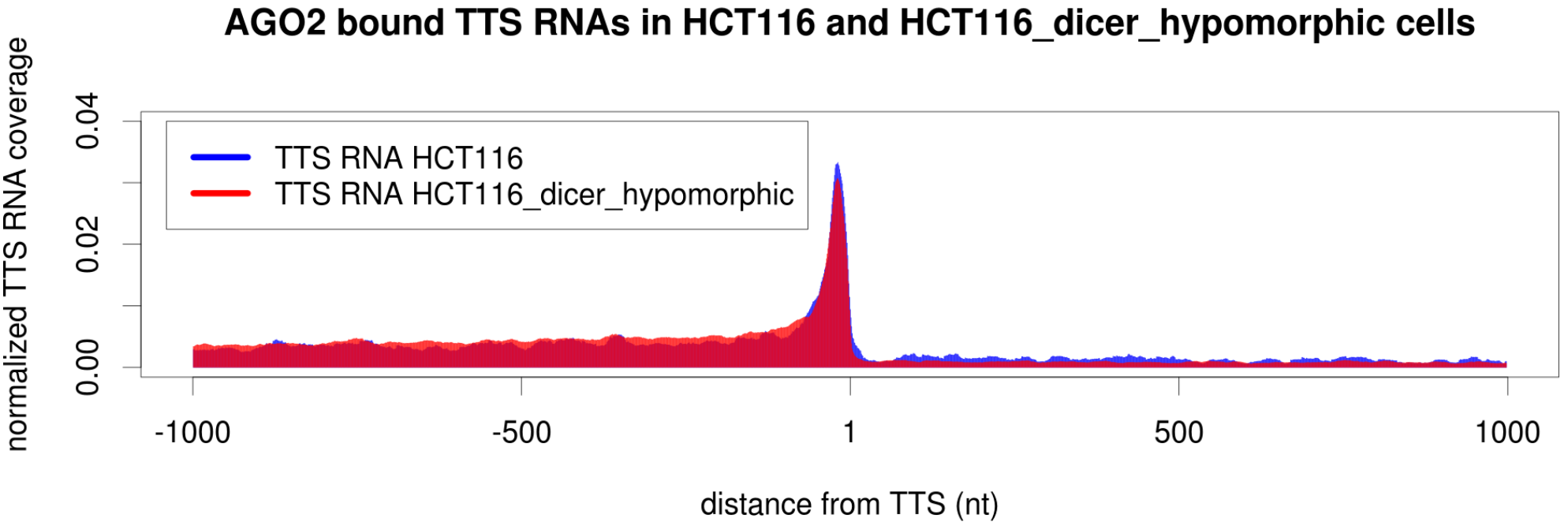


SwiRNAs are processed by DICER



SwiRNAs are processed by DICER

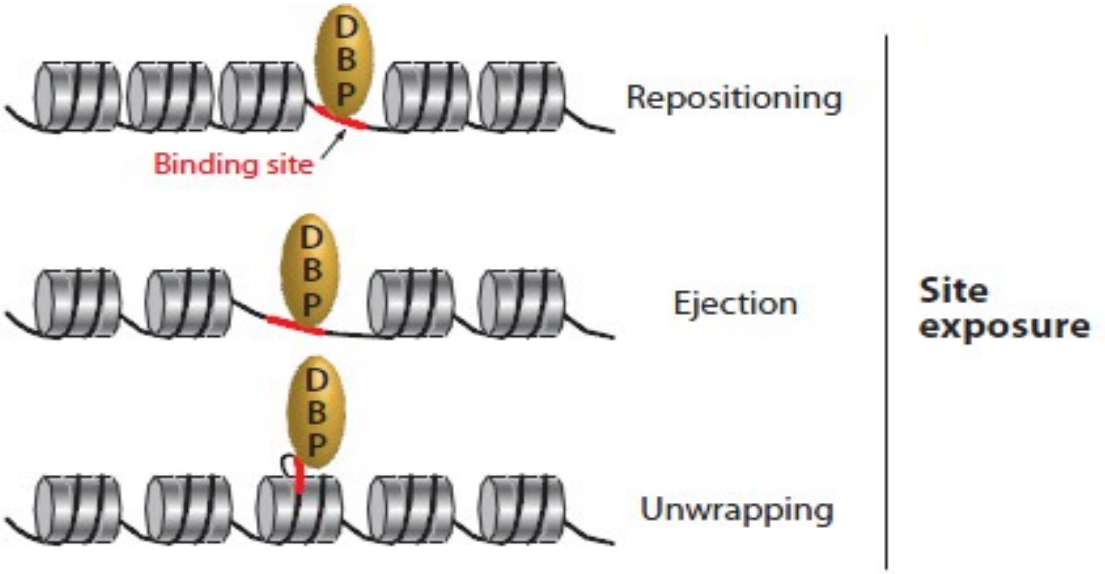


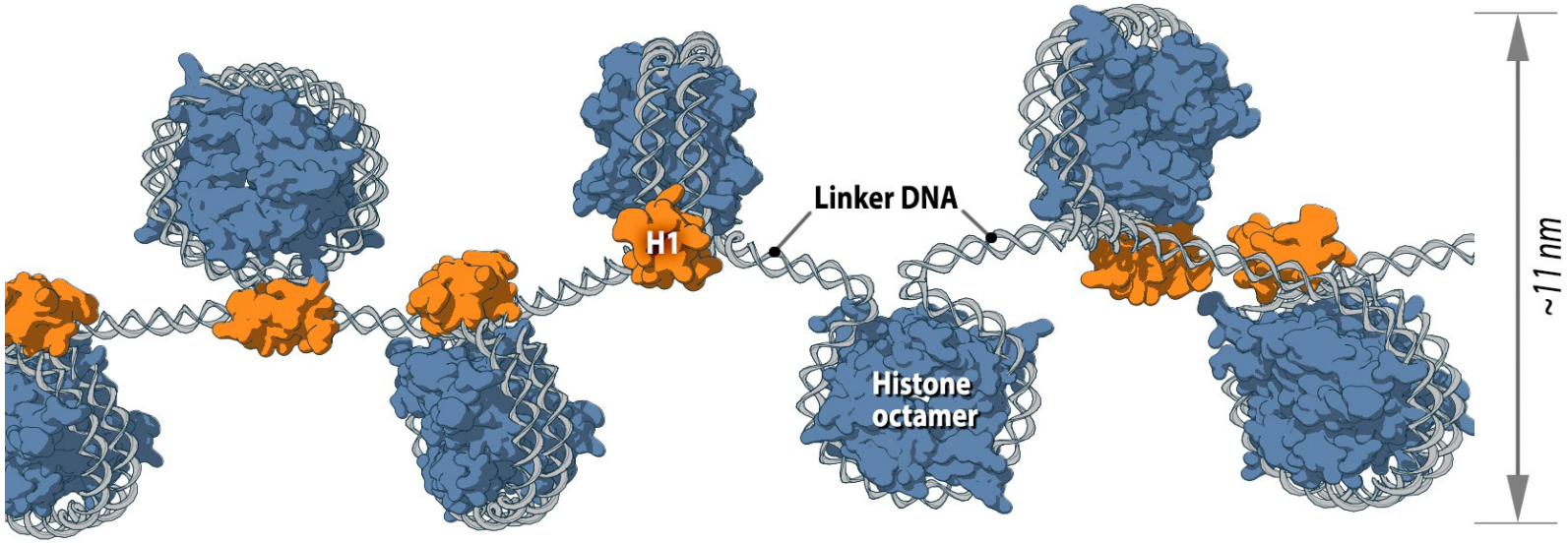


Conclusion II

- **Nuclear AGO2 is associated with a novel class of sRNAs arising from SWI/SNF bound TSS (swiRNAs)**
- **swiRNAs processing is dicer dependent**
- **swiRNAs are distinct from other classes of endogenous sRNAs mapping nearby TSS (tiRNAs, TSSa RNAs) based on their size, association with AGO2 and processing by DICER**

What is the function of AGO2-SWI/SNF complexes?



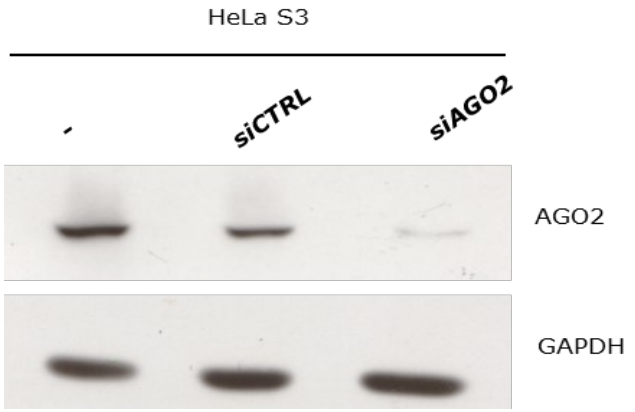


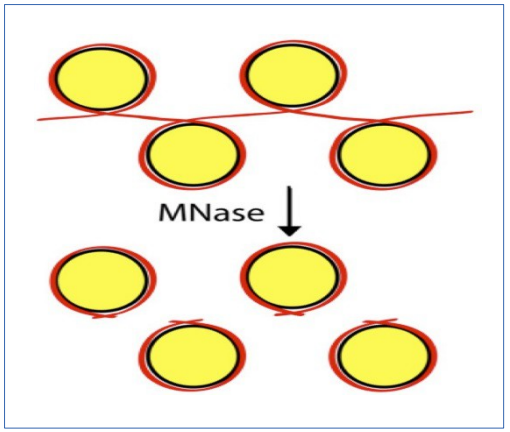
What is the function of AGO2-SWI/SNF complexes?

AGO2 knock-down in HeLaS3 cells

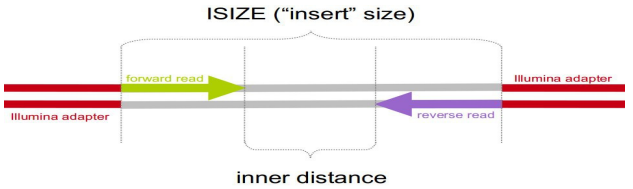
↓

Analysis of nucleosome occupancy





Paired-end sequencing



siAgo2

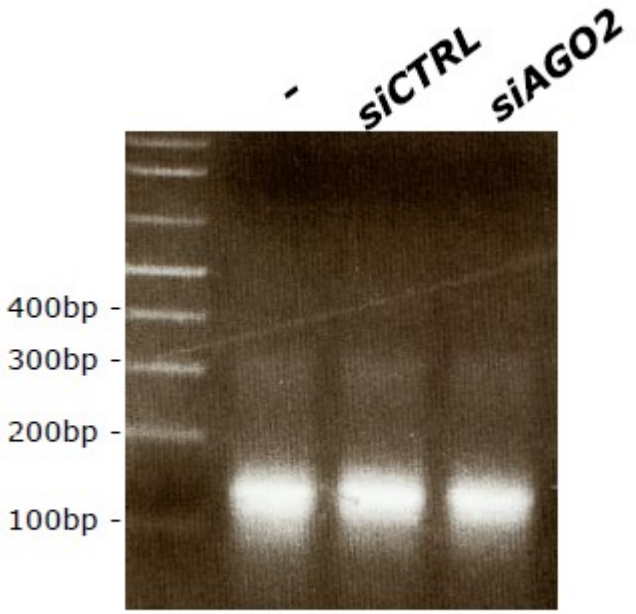
→

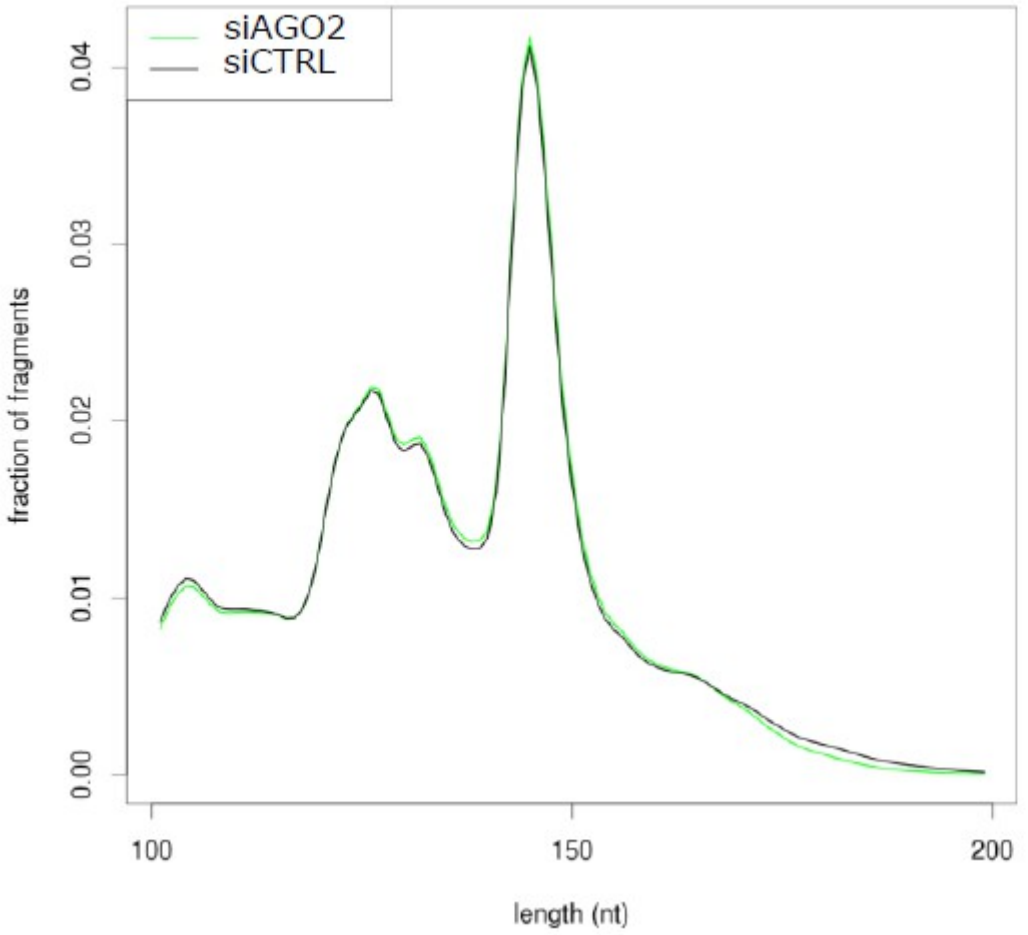
siCtrl

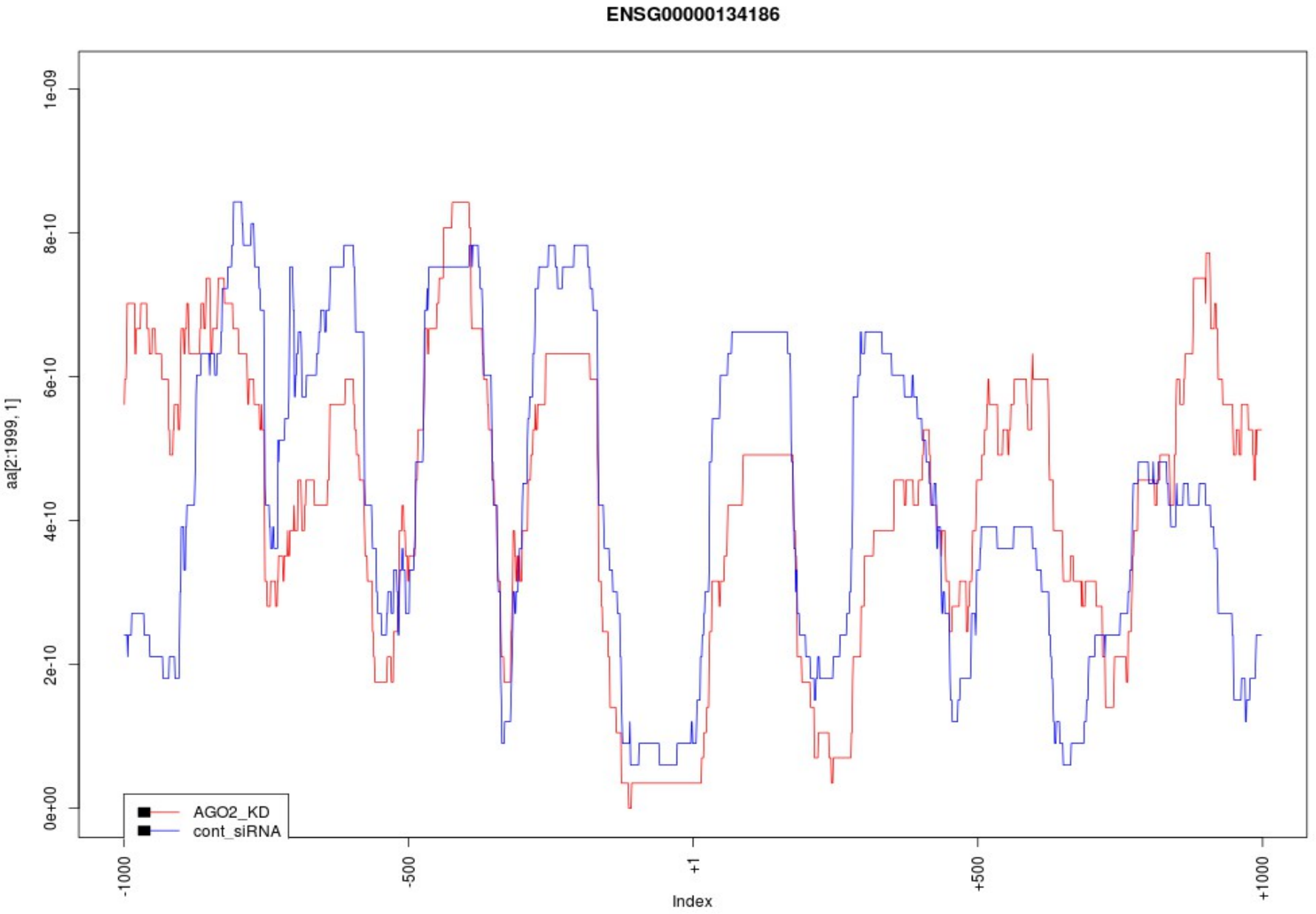
~592.000.000 Reads in AGO2 Knock-down

~711.000.000 Reads in Control sample

Mnase digestion yielded fragments of the expected size



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2e-10
4e-10
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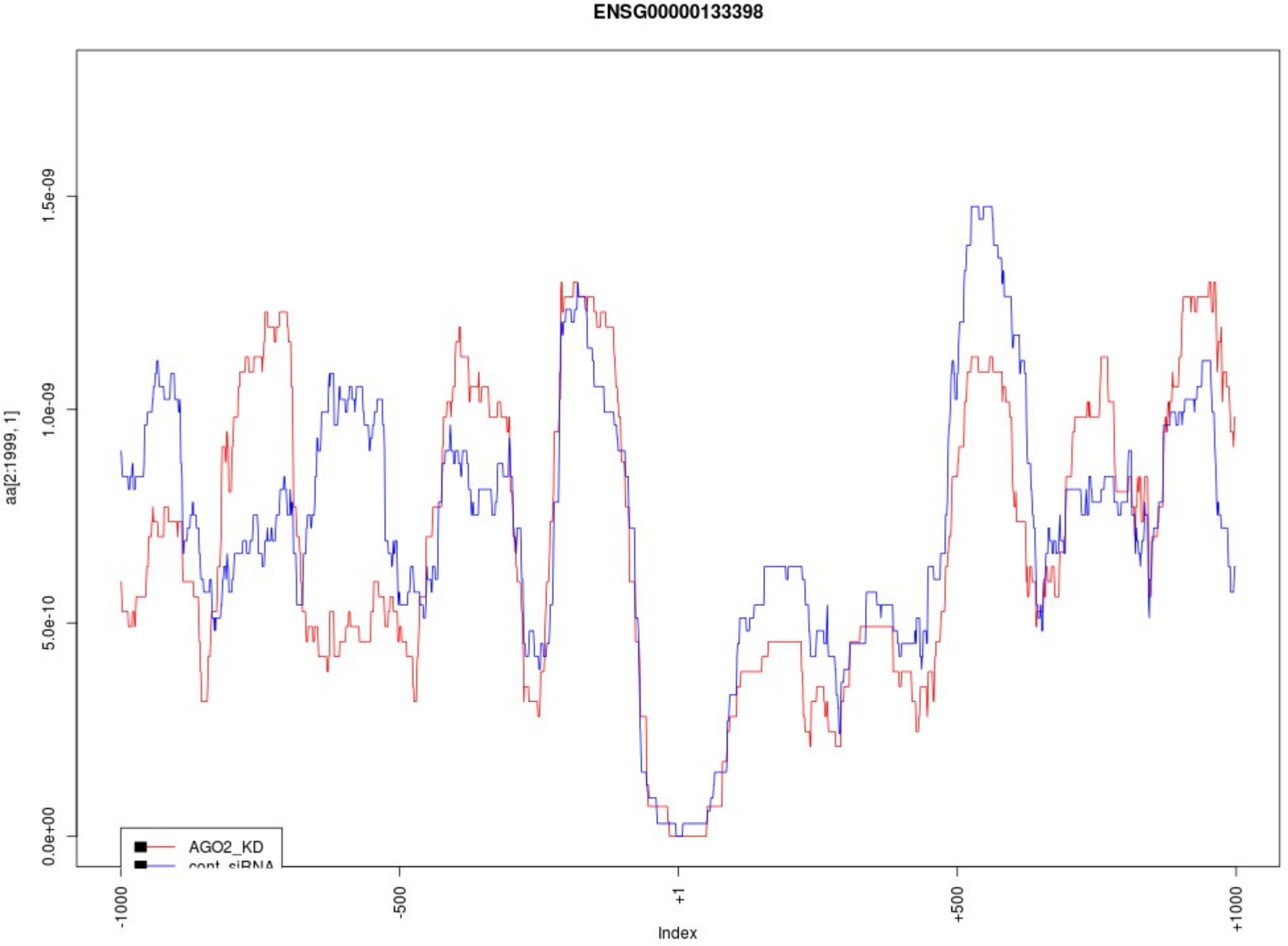
8e-10
1e-09
AGO2_KD
cont_siRNA
-1000
-500
+1
+500
+1000
Index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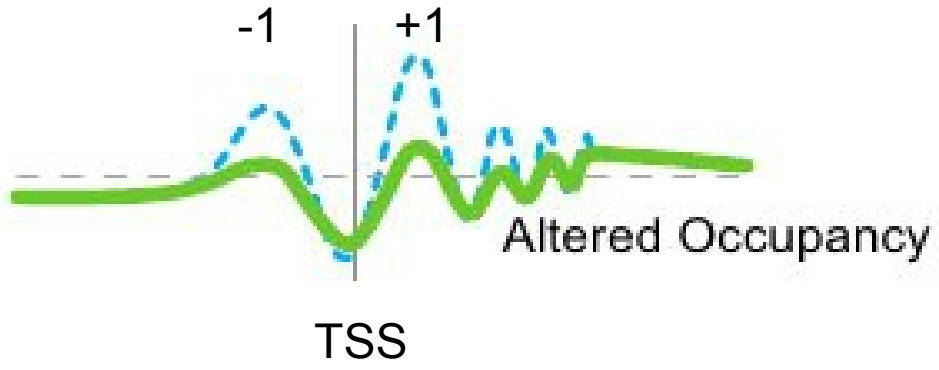
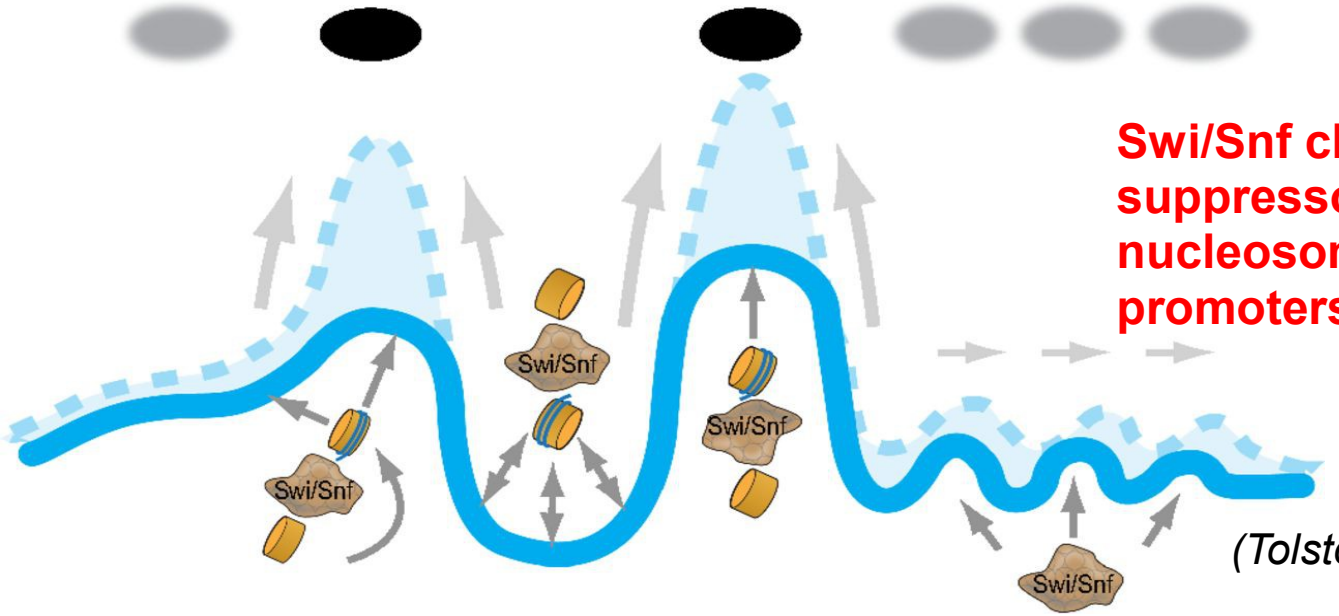
AGO2_KD
cont_siRNA
-1000
-500
+1
+500
+1000
Index

What is the function of AGO2-SWI/SNF complexes?



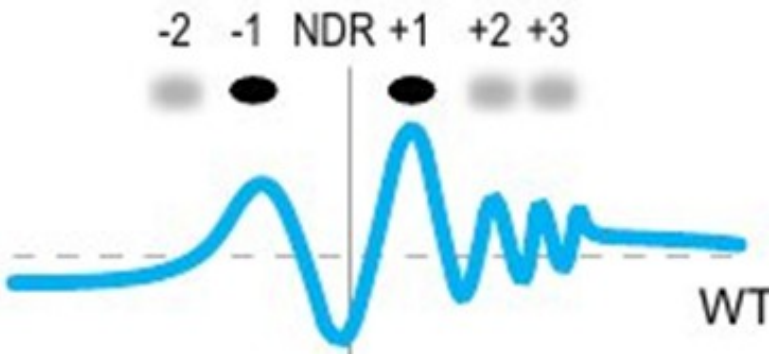
Nucleosome occupancy *= the frequency with which a nucleosome is present at a particular location within cell population.*



Swi/Snf chromatin remodeling/tumor suppressor complex establishes nucleosome occupancy at target promoters

(Tolstorukov et al., PNAS, 2013)

Does AGO2 knock-down affect nucleosome positioning?



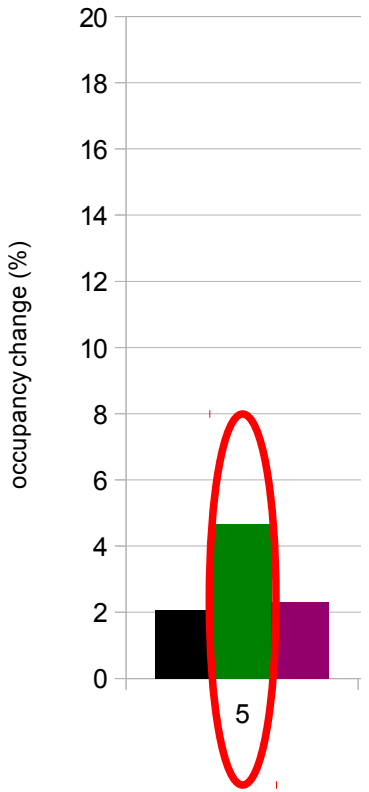
…a moderate decrease (1.98%) of occupancy (p-value = 1.5×10^{-15}) was observed when only nucleosome +1 was considered

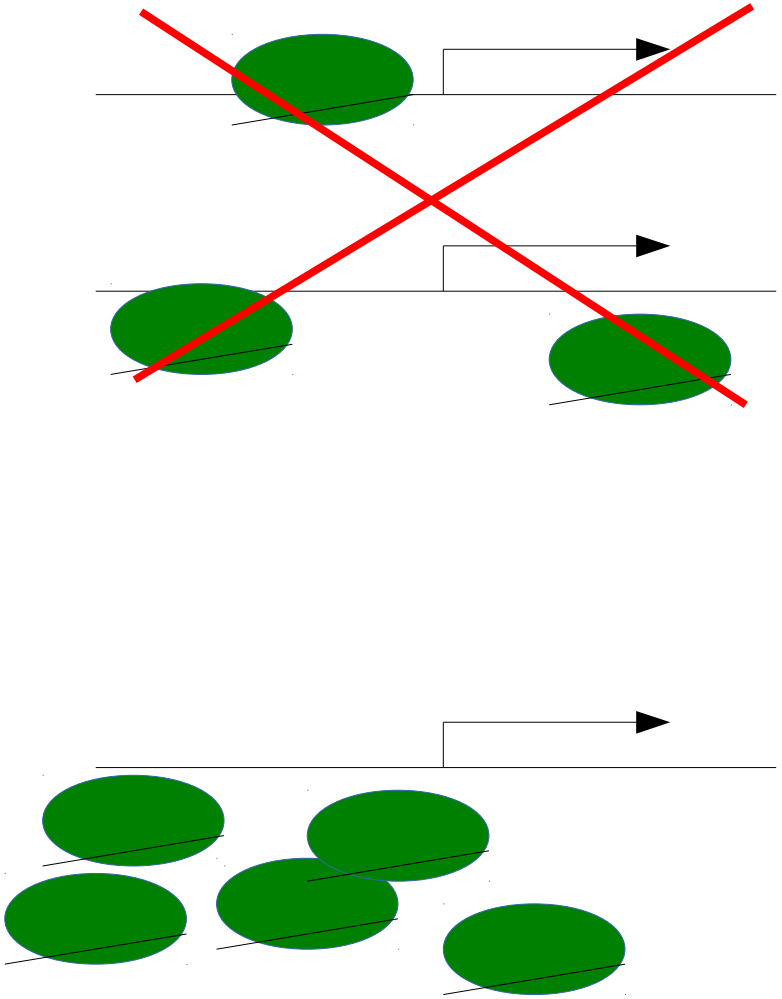
AGO2 knock-down decreases nucleosome +1 occupancy

IgG associated sRNAs

AGO2 associated sRNAs

AGO1 associated sRNAs





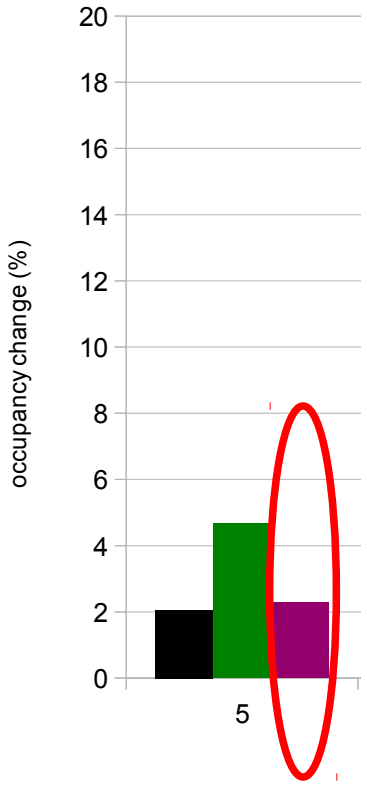
Minimum number of sRNAs overlapping each TSS

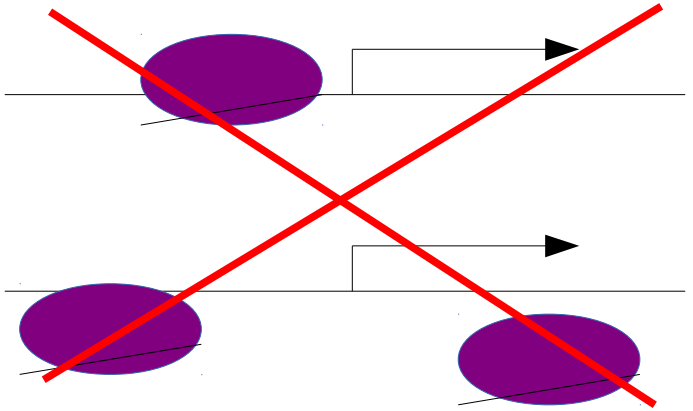
AGO2 knock-down decreases nucleosome +1 occupancy

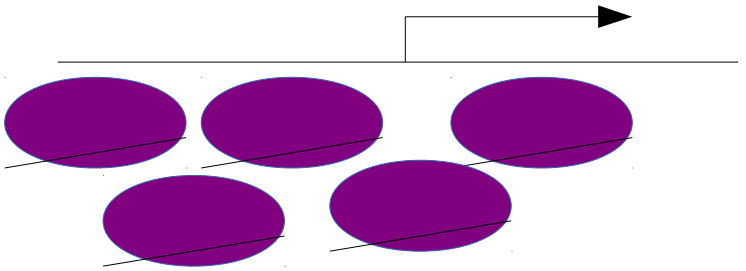
IgG associated sRNAs

AGO2 associated sRNAs

AGO1 associated sRNAs

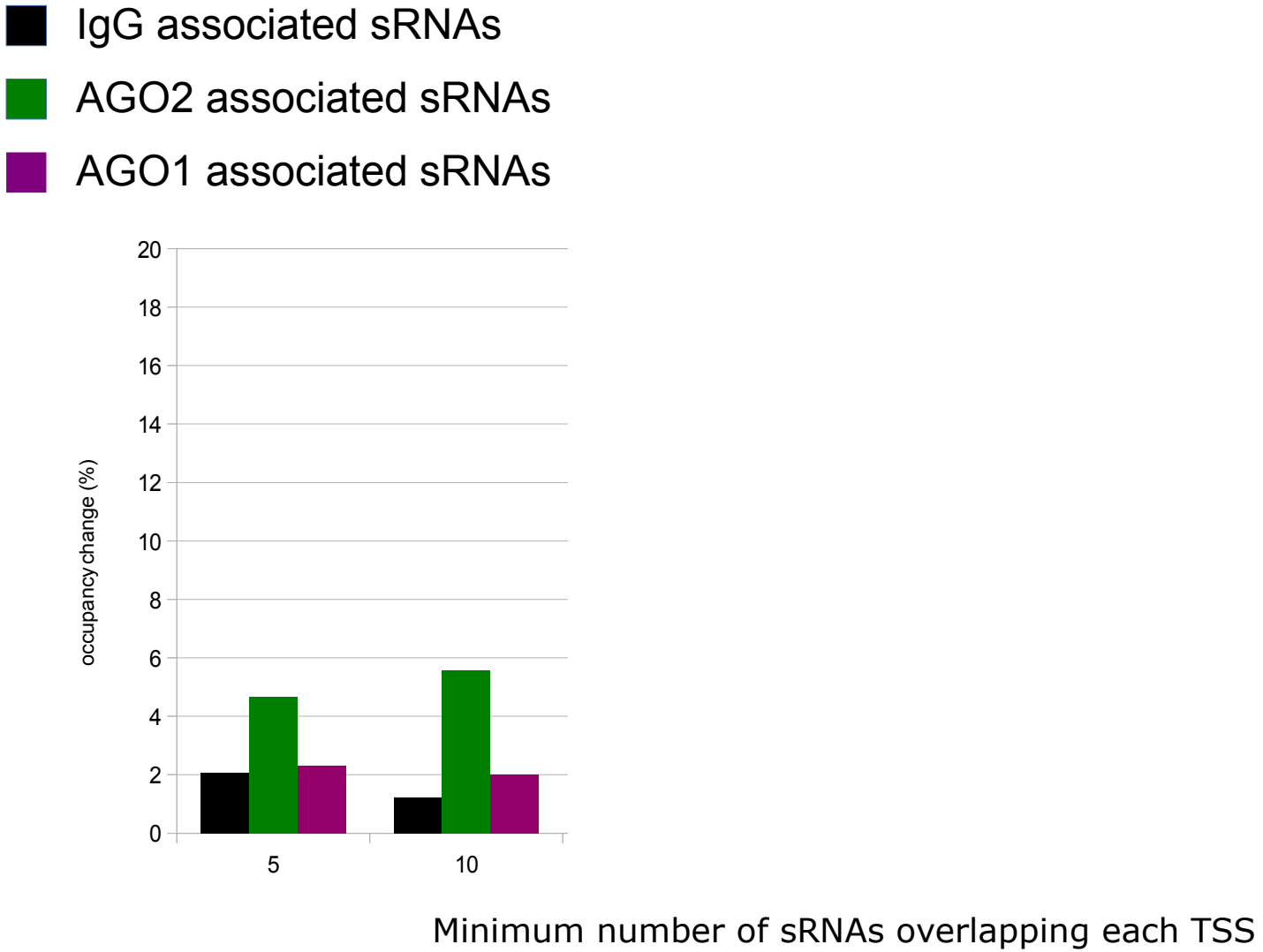




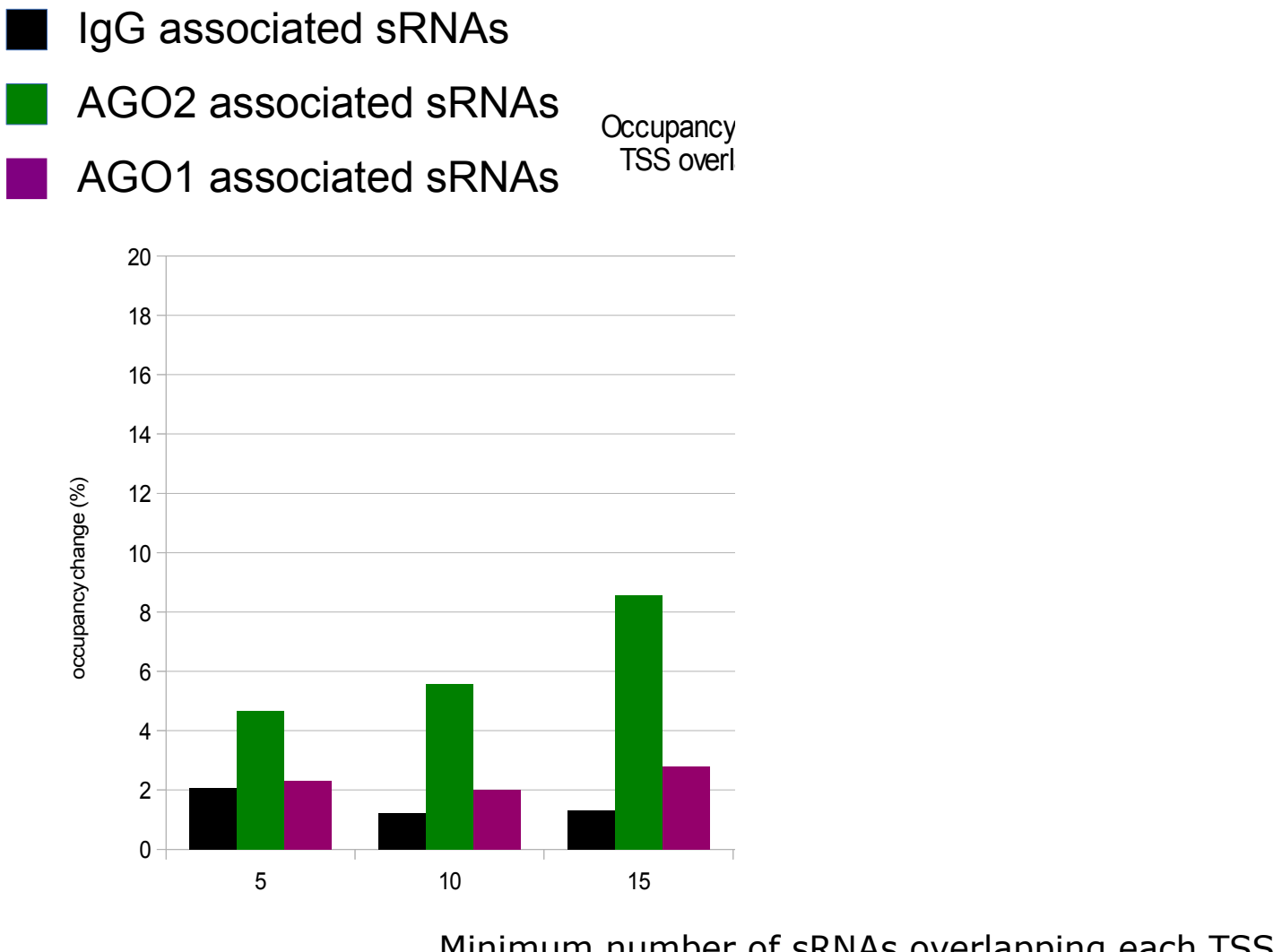


Minimum number of sRNAs overlapping each TSS

AGO2 knock-down decreases nucleosome +1 occupancy



AGO2 knock-down decreases nucleosome +1 occupancy

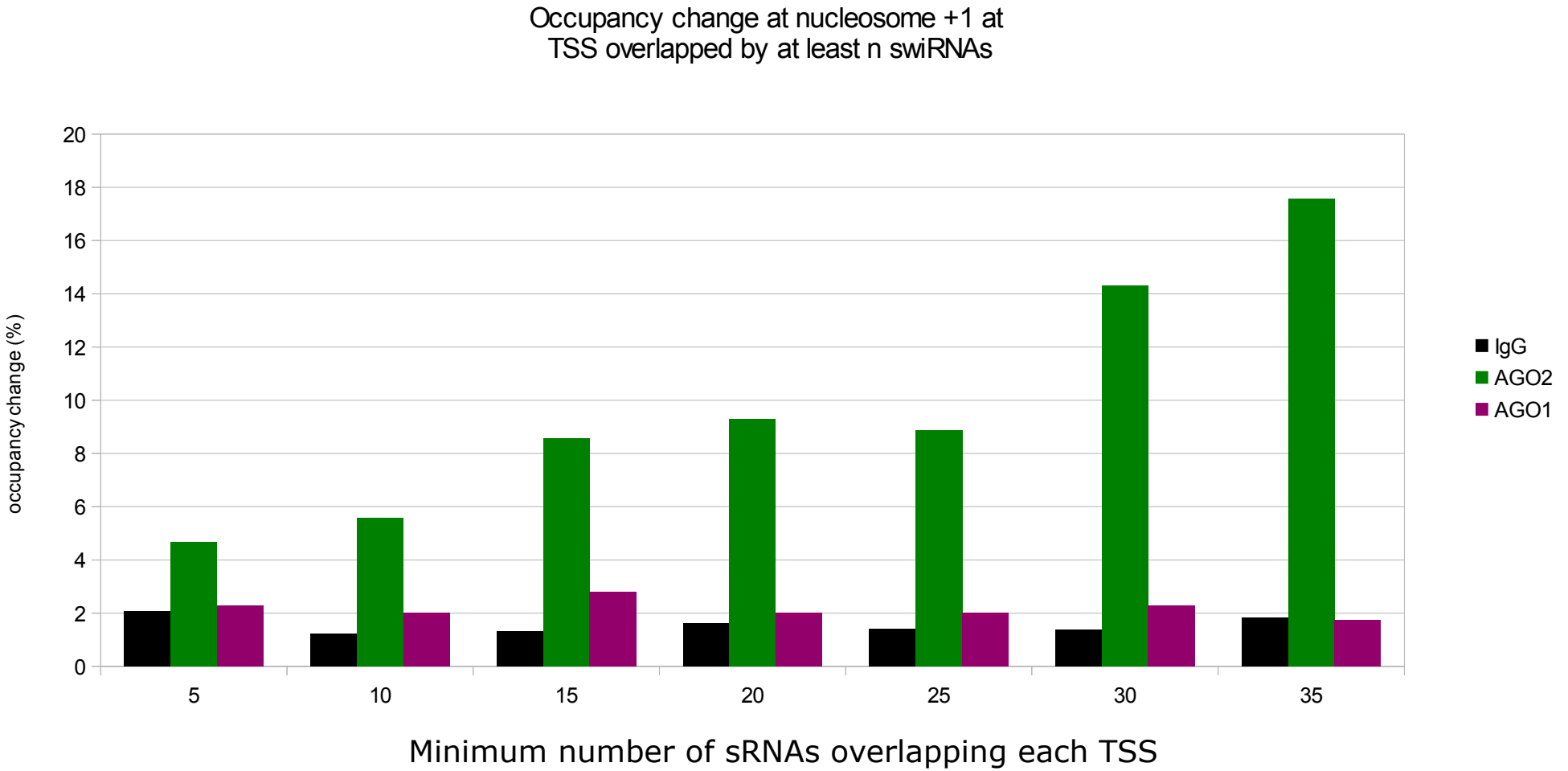


AGO2 knock-down decreases nucleosome +1 occupancy

IgG associated sRNAs

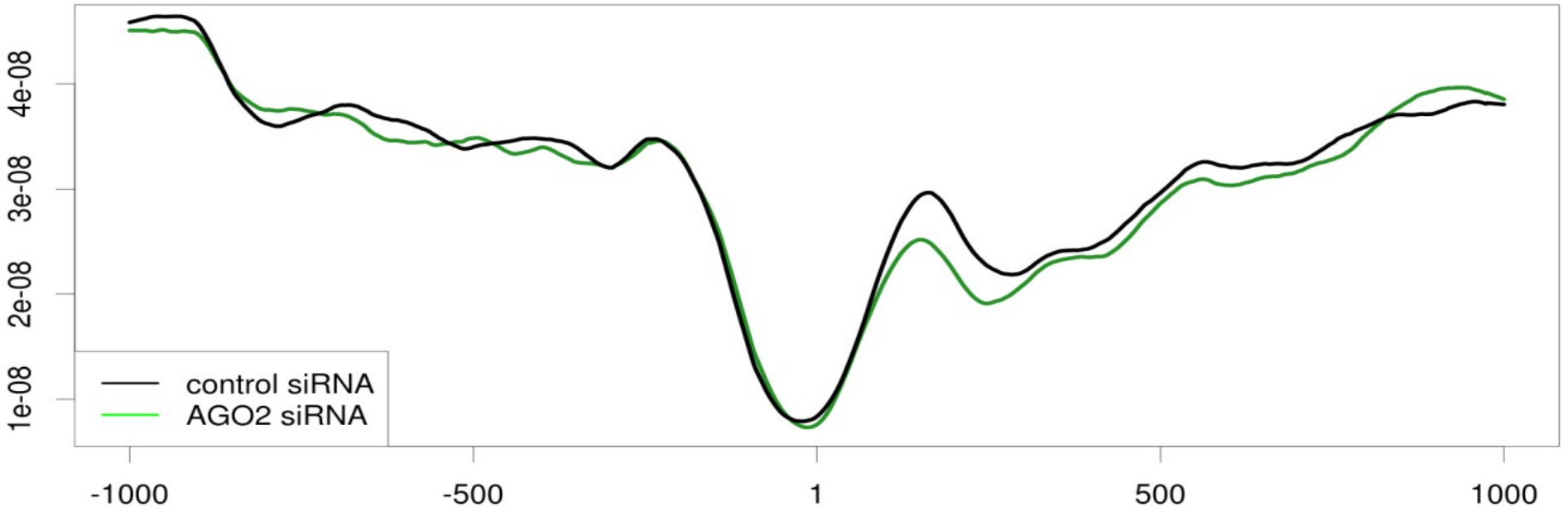
AGO2 associated sRNAs

AGO1 associated sRNAs



AGO2 knock-down decreases nucleosome +1 occupancy

*(**14% reduction**; p-value=0.0001687; FDR< 0.01)*

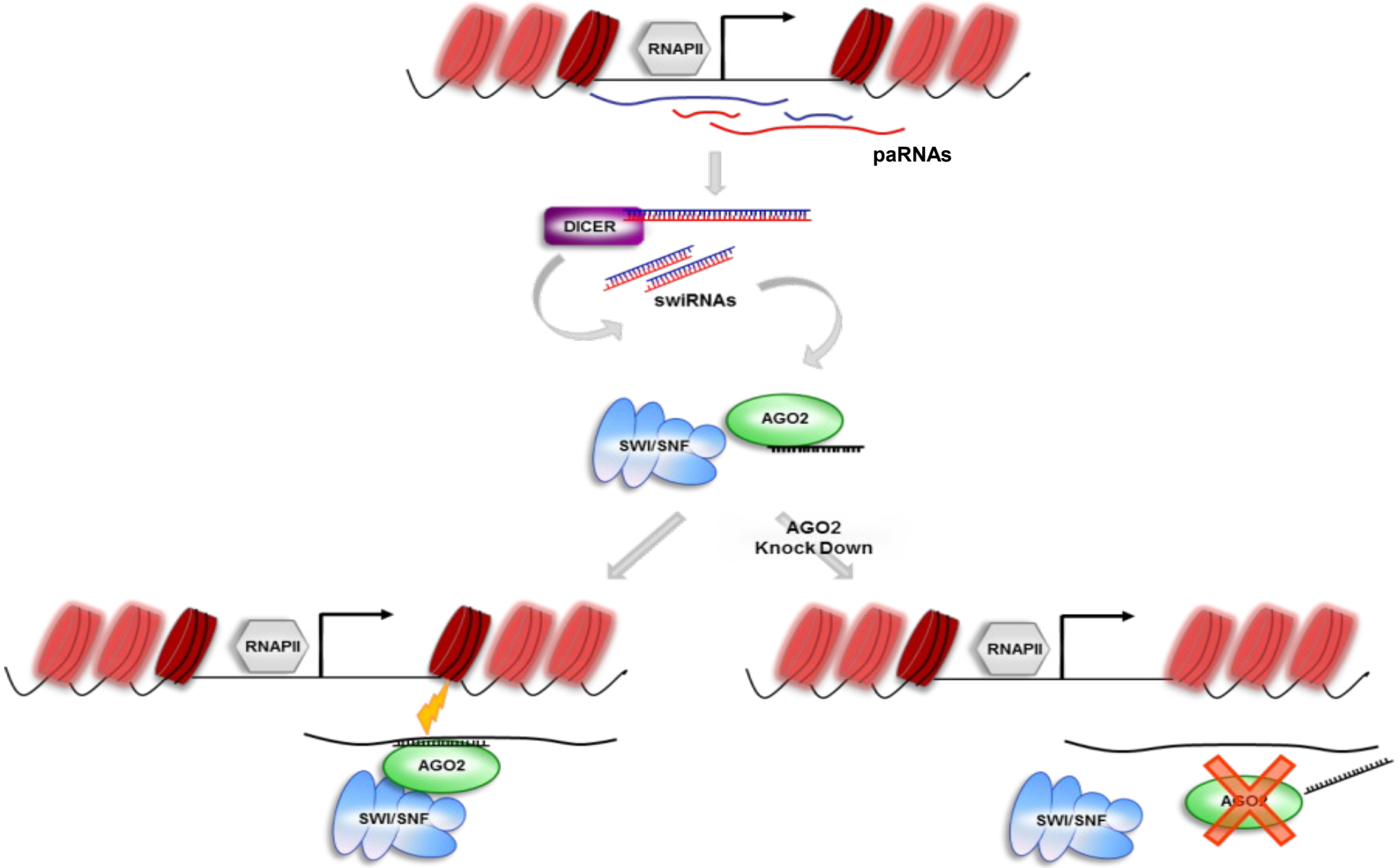


AGO2 is involved in nucleosome +1 occupancy at TSSs overlapped by swiRNAs

Conclusion III

- **AGO2 depletion results in a general yet moderate decrease of nucleosome occupancy at nucleosome +1**
- **Nucleosome occupancy decrease at nucleosome +1 is correlated with the # of swiRNAs overlapping each TSS**
- **Nuclear AGO1 bound sRNAs mapping to TSS do not correlate with occupancy decrease at nucleosome +1**

RNAi cooperates with SWI/SNF to determine occupancy at nucleosome +1



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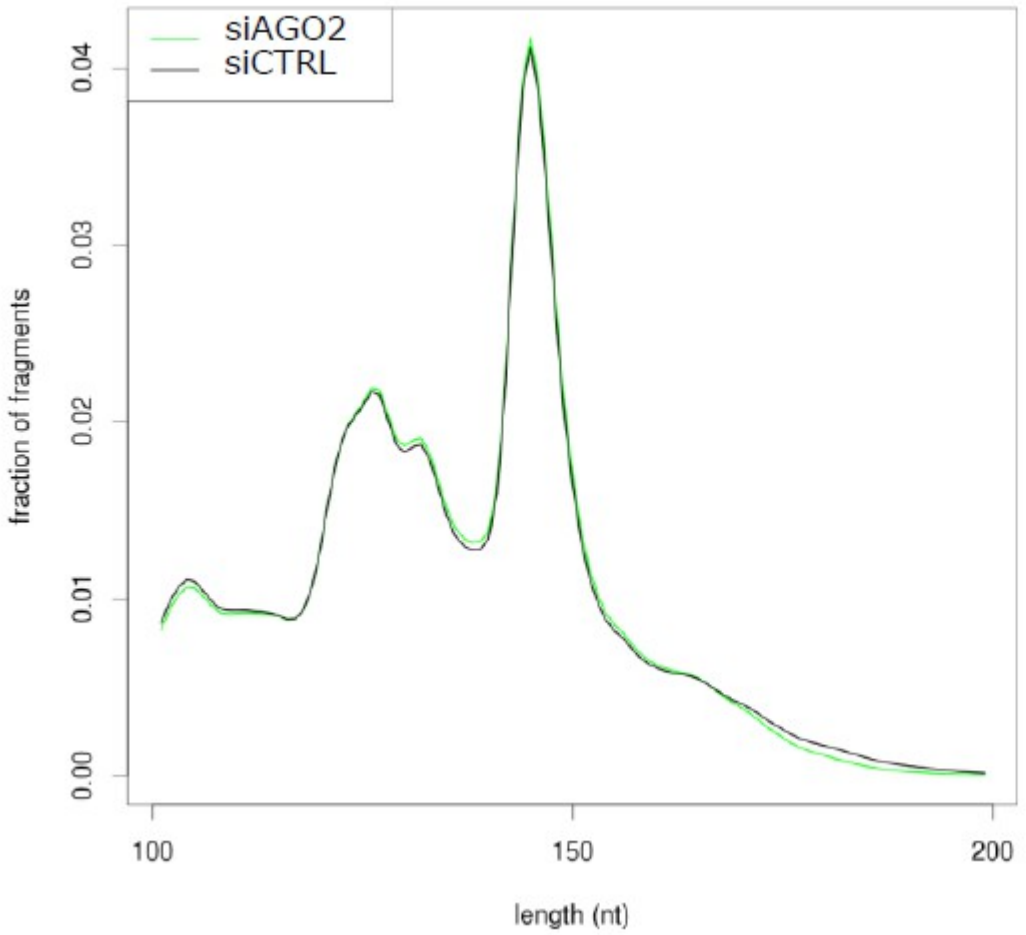
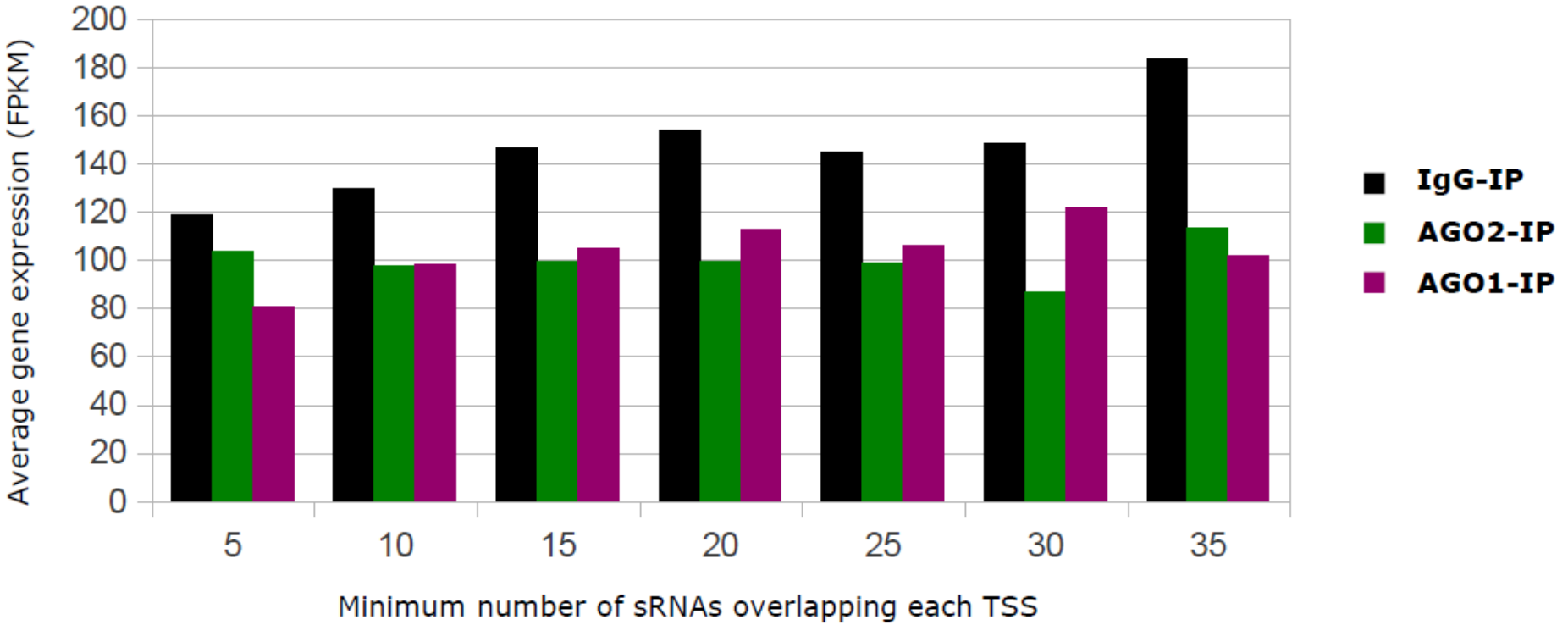

siAGO2
siCTRL
fraction of fragments
0.00
0.01
0.02
0.03
0.04
100
150
200
length (nt)


Average gene expression (FPKM)
200
180
160
140
120
100
80
60
40
20
0
5
10
15
20
25
30
35
Minimum number of sRNAs overlapping each TSS
IgG-IP
AGO2-IP
AGO1-IP