Viral Epitranscriptomics

Bryan R. Cullen

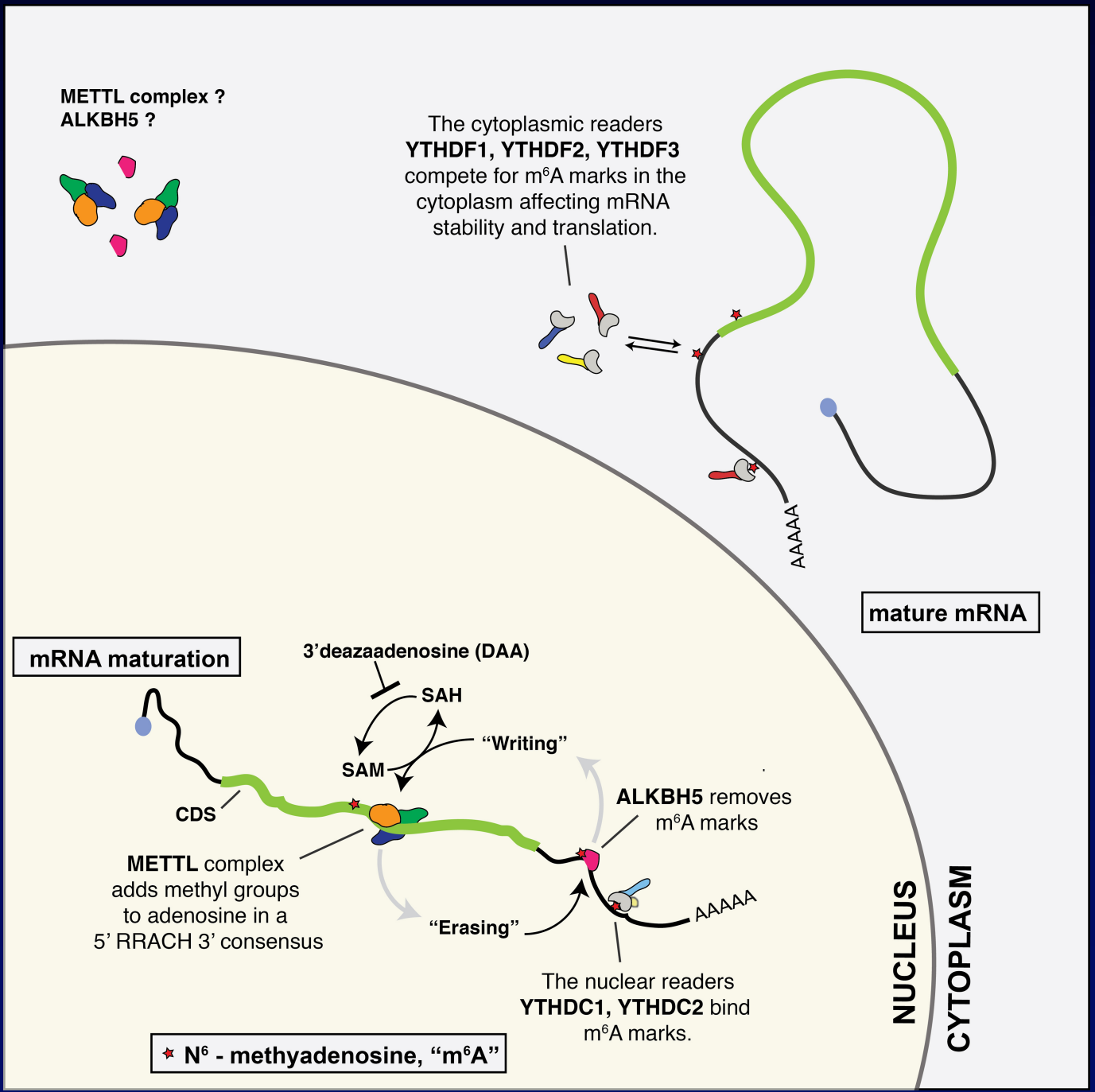
Department of Molecular Genetics and Microbiology and Center for Virology
Duke University Medical Center, Durham, NC

Viral and cellular mRNAs are subject to post-transcriptional modifications

- Several covalent modifications of individual nucletides in eukaryotic mRNAs have been reported at levels >0.1%.
- Of these, the most common is the addition of a methyl group to the N6 position of adenosine, called m^6A.
- The presence of high level of m^6A in viral mRNAs was first reported for influenza A virus by Krug et al in 1976 and subsequently for a range of viruses, including avian retroviruses (Kane and Beemon, 1985), but the function of m^6A has remained unclear.

Viruses encoding RNAs with reported m^{6}A residues

Virus	No. of m^{6}A residues
RNA viruses	
Influenza A virus	~24
Avian sarcoma virus	13-15
Rous sarcoma virus	10-12
Feline leukemia virus	NA
HIV-1	10-14
Hepatitis C virus	~16
Flaviviruses*	5-12
DNA viruses	
Adenovirus	NA
SV40	NA
Herpes simplex virus 1	NA
KSHV	NA

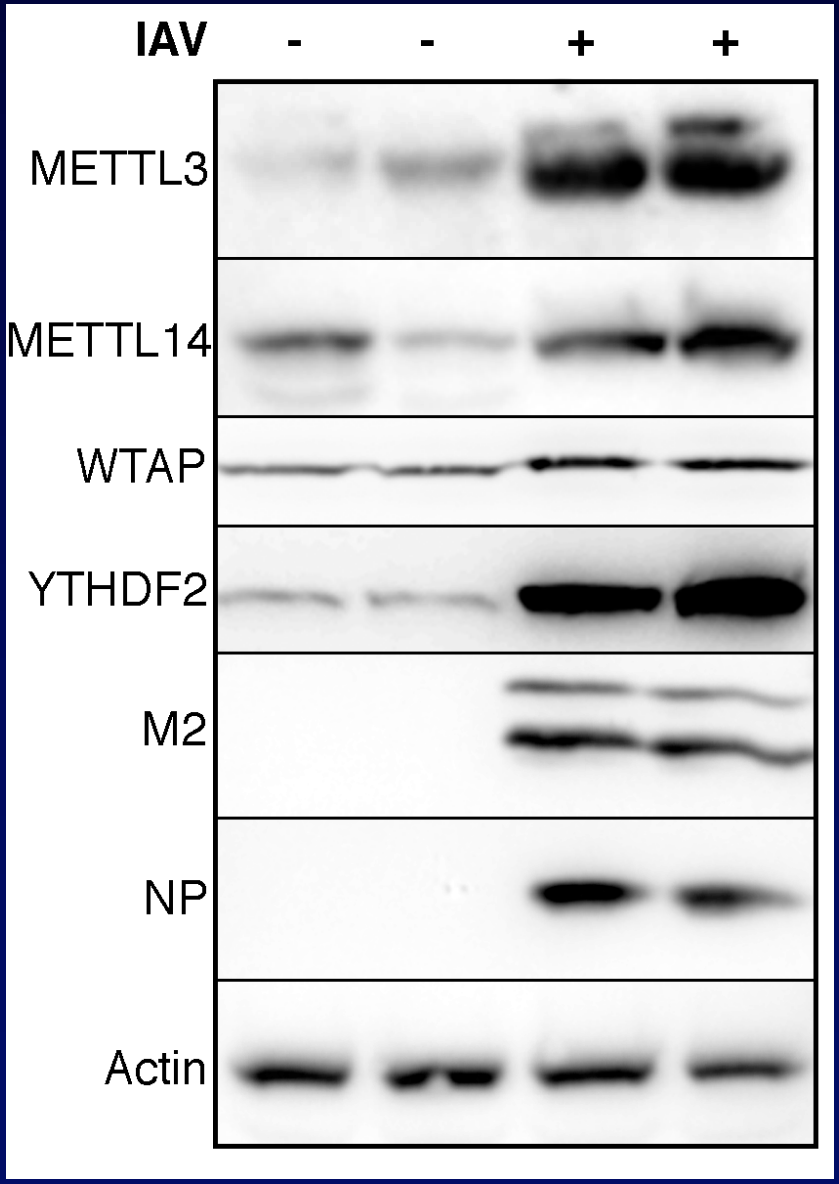

METTL complex ?
ALKBH5 ?
The cytoplasmic readers
YTHDF1, YTHDF2, YTHDF3
compete for m6A marks in the
cytoplasm affecting mRNA
stability and translation.
AAAAA
mature mRNA
mRNA maturation
3'deazaadenosine (DAA)
SAH
SAM
"Writing"
CDS
ALKBH5 removes
m6A marks
METTL complex
adds methyl groups
to adenosine in a
5' RRACH 3' consensus
"Erasing"
AAAAA
The nuclear readers
YTHDC1, YTHDC2 bind
m6A marks.
N6 - methyadenosine, "m6A"
NUCLEUS
CYTOPLASM

Question:

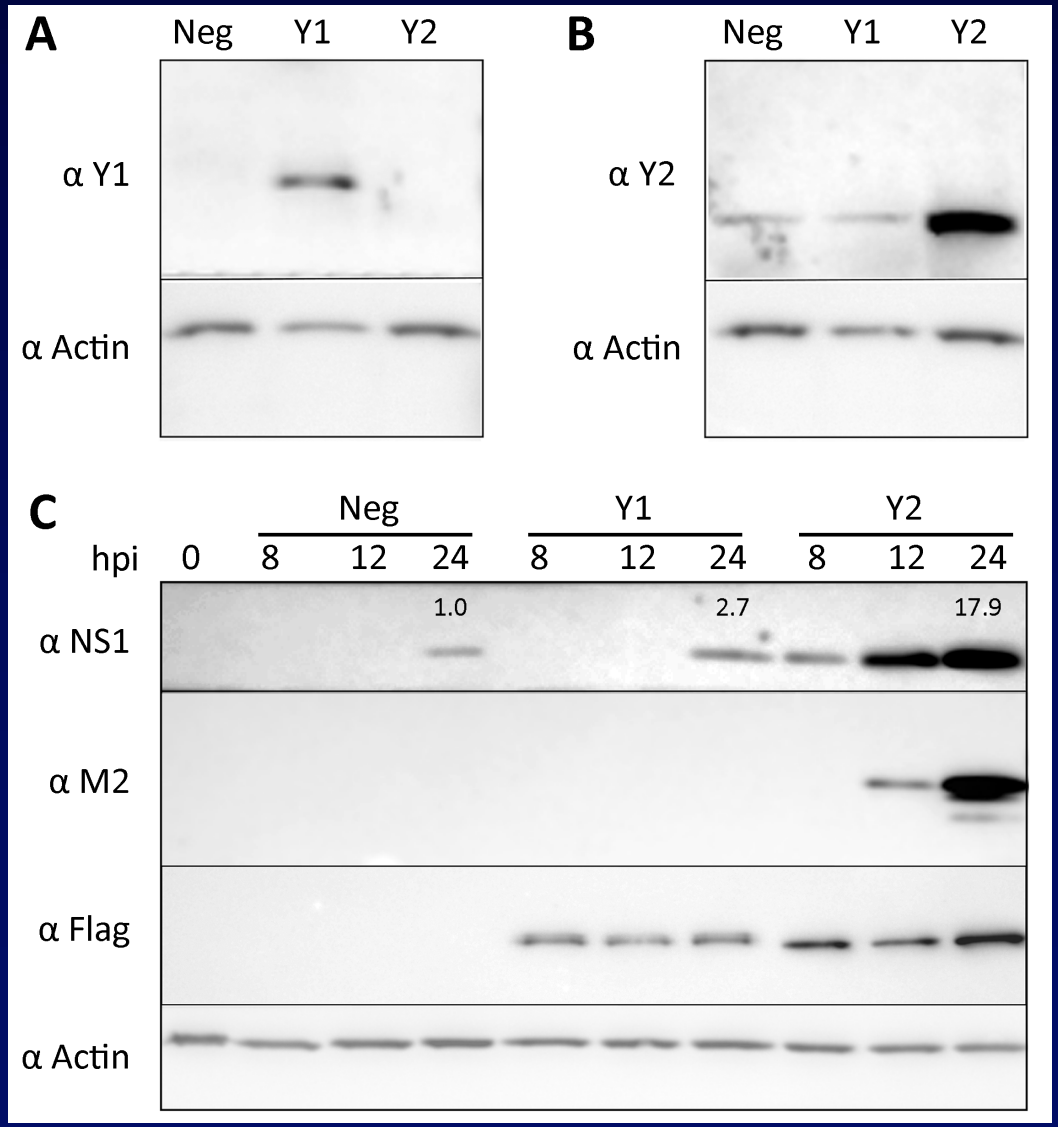
1) Influenza A virus (IAV) was reported 40 years ago
(Krug et al, 1976) to express RNAs that are highly m^6A modified.

2) Does the cellular m^6A machinery also affect IAV replication?
In particular, does m^6A addition boost IAV replication in the
A549 lung epithelial cell line?

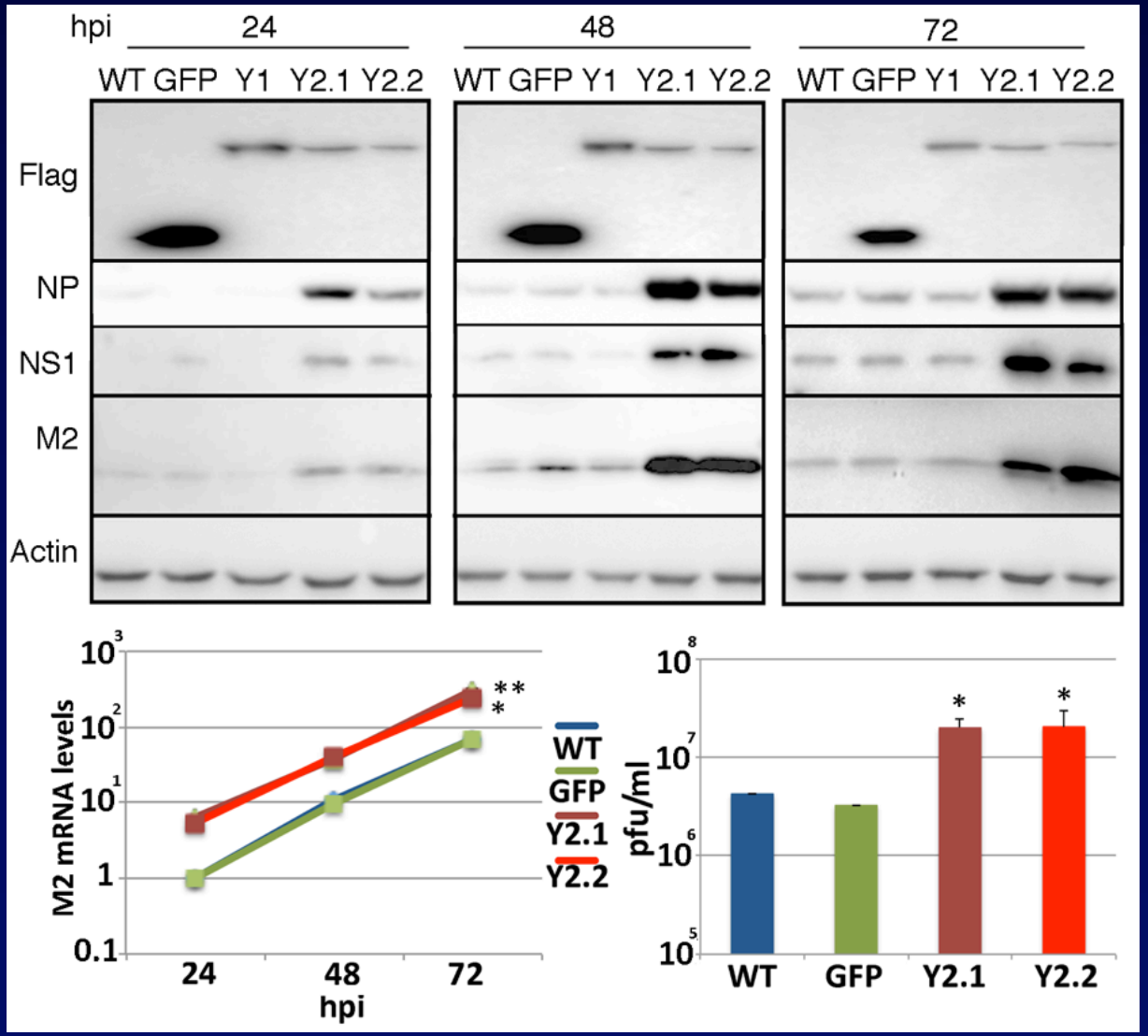
IAV infection induces factors that mediate m^{6}A addition and detection



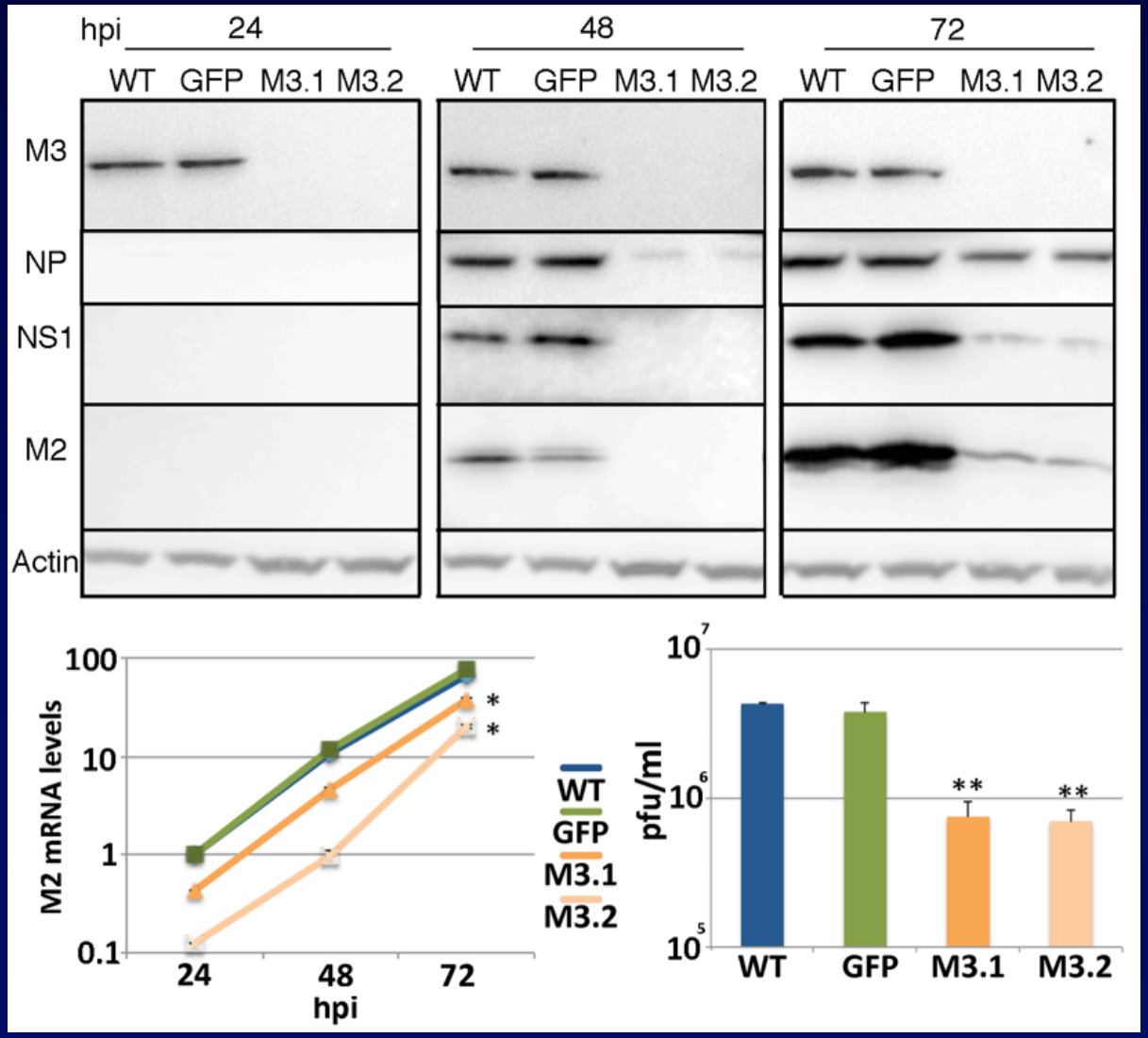
Overexpression of YTHDF2 strongly enhances IAV replication under non-spreading conditions



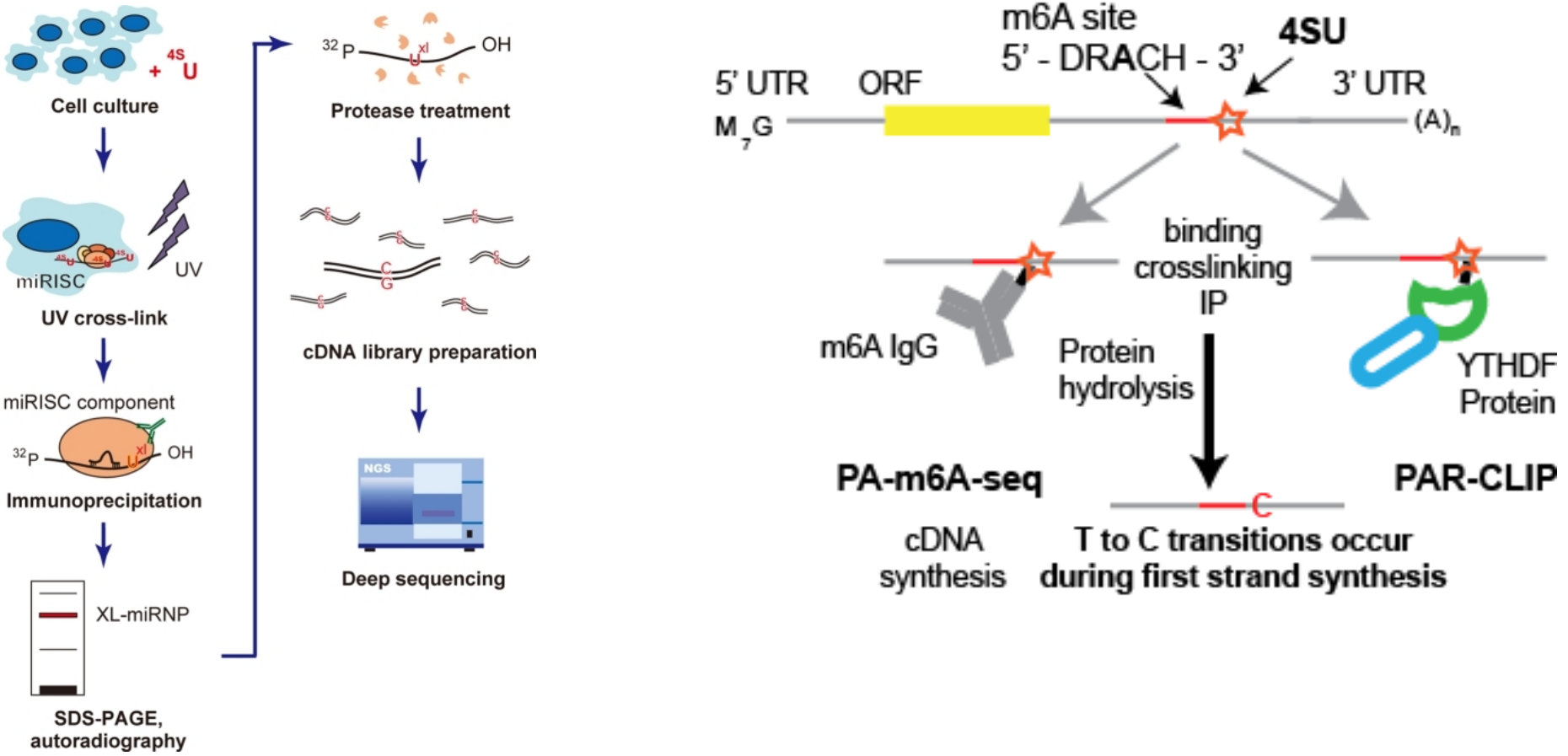
YTHDF2, but not YTHDF1, overexpression also increases IAV replication under spreading conditions



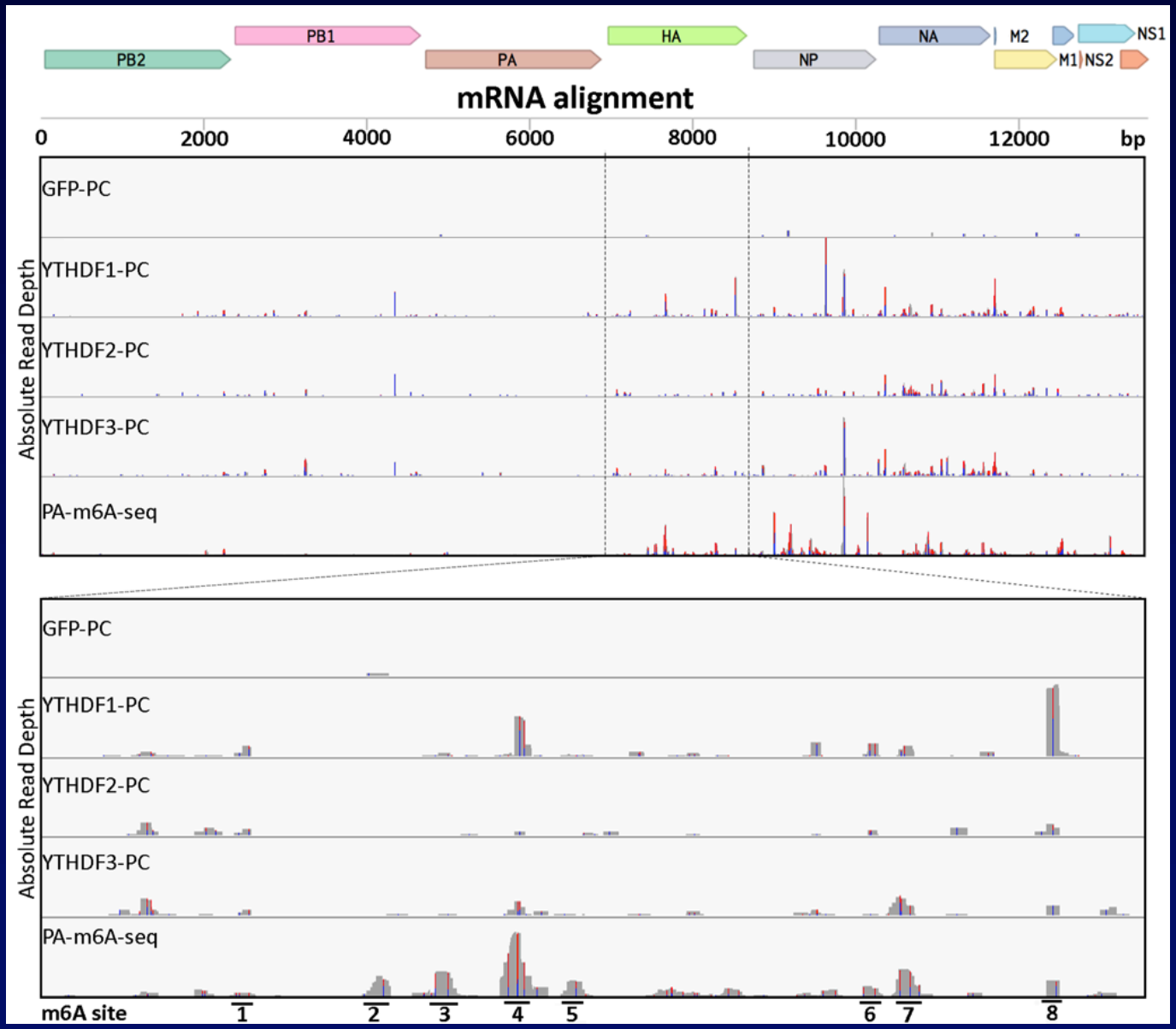
Loss of the m^{6}A methyltransferase METTL3 strongly inhibits IAV replication



Mapping m^{6}A sites on viral RNAs

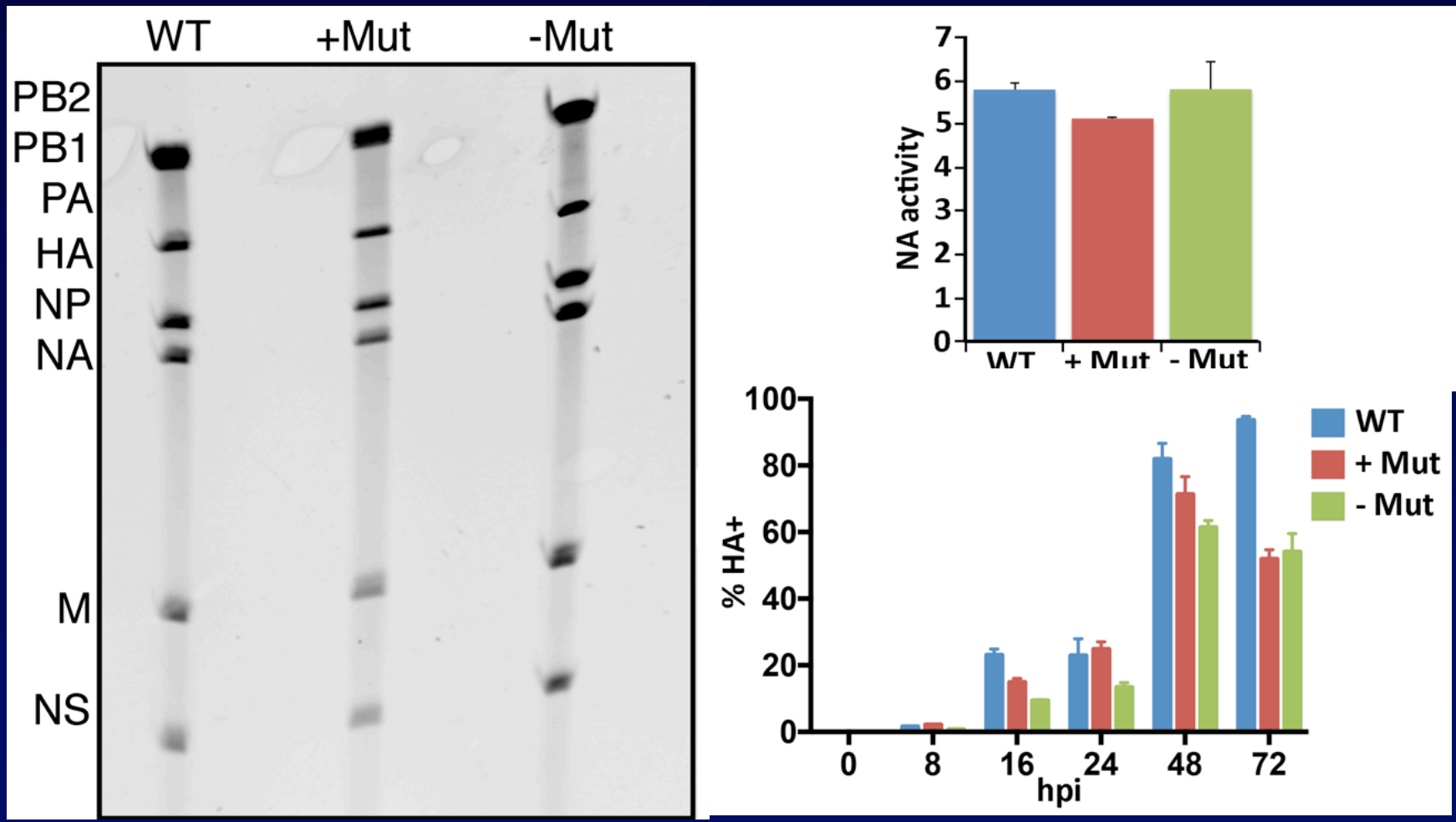


Mapping m^{6}A sites on IAV mRNAs by PAR-CLIP

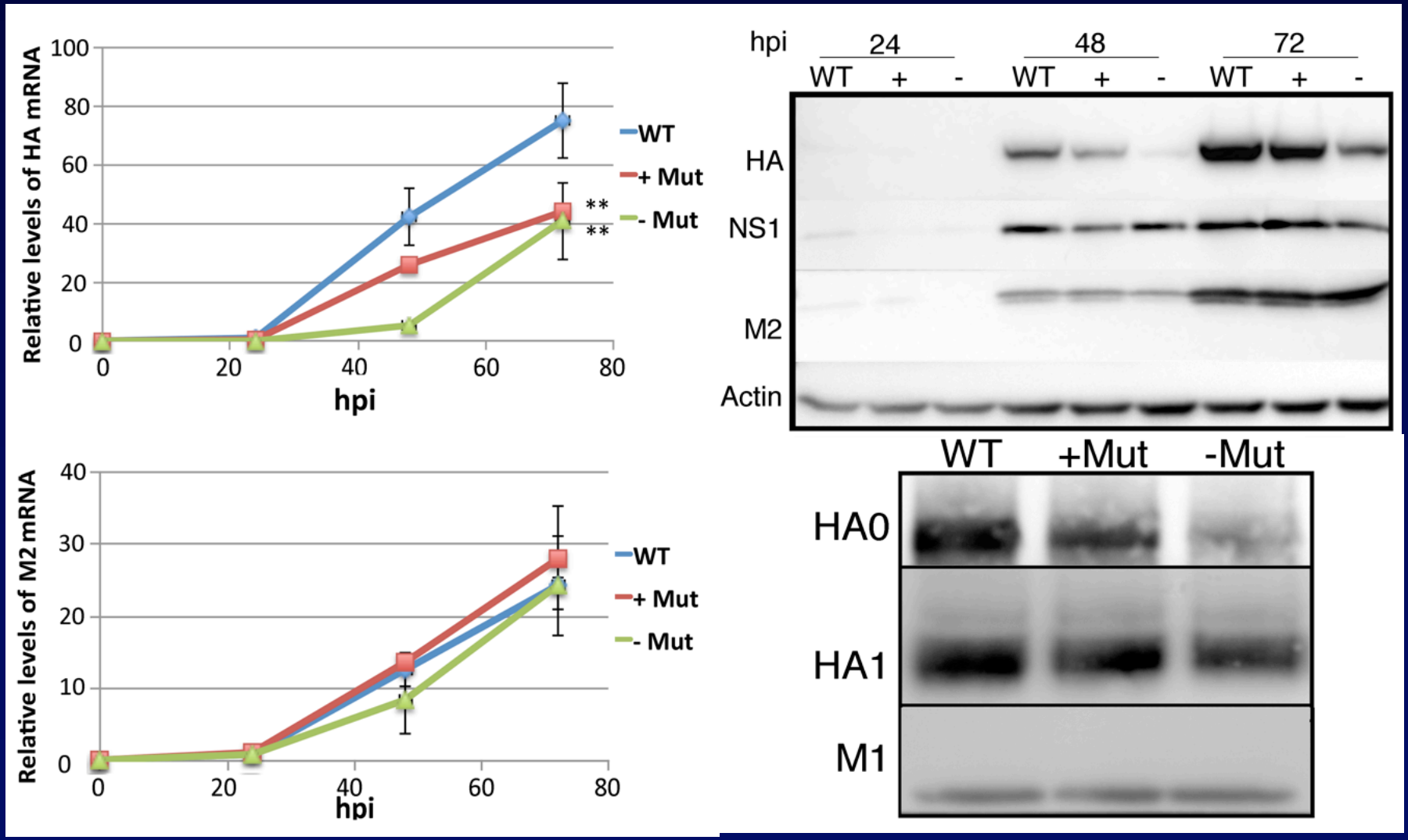


Mapping m^{6}A sites on IAV vRNAs by PAR-CLIP

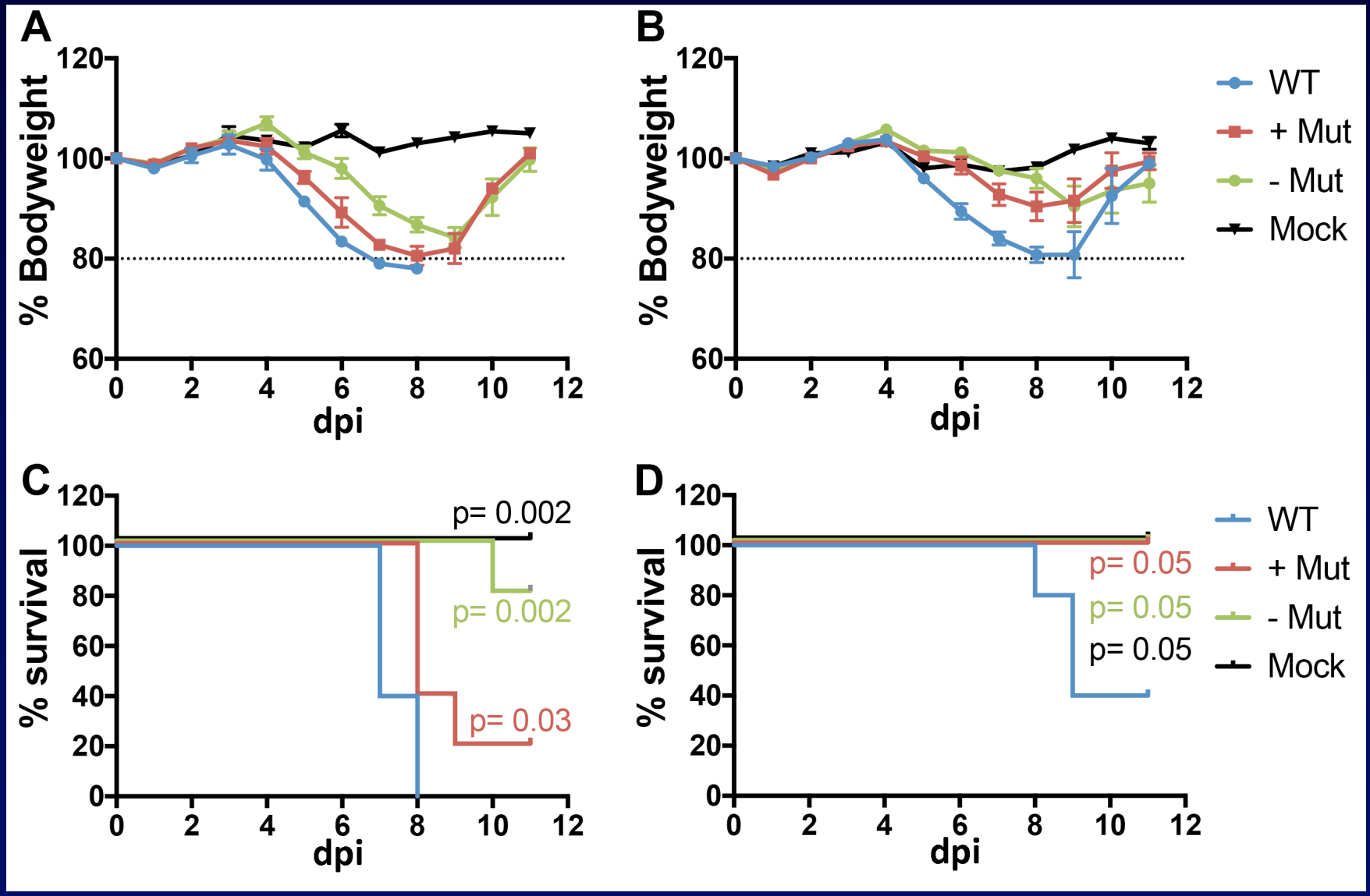
Loss of m^{6}A sites on the IAV HA segment only modestly affects viral spread

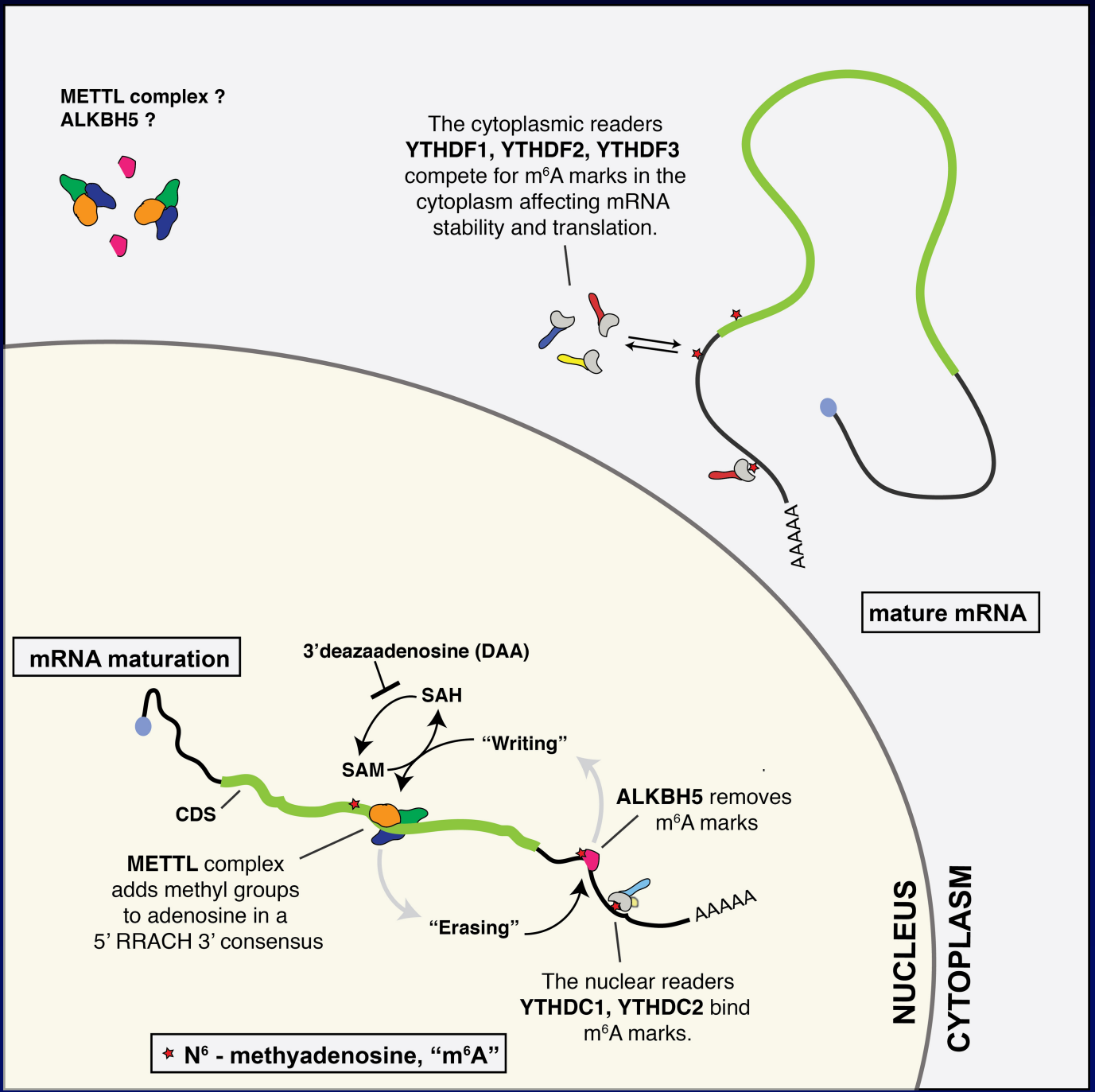


m^6A sites on the IAV HA segment enhance HA mRNA and protein expression

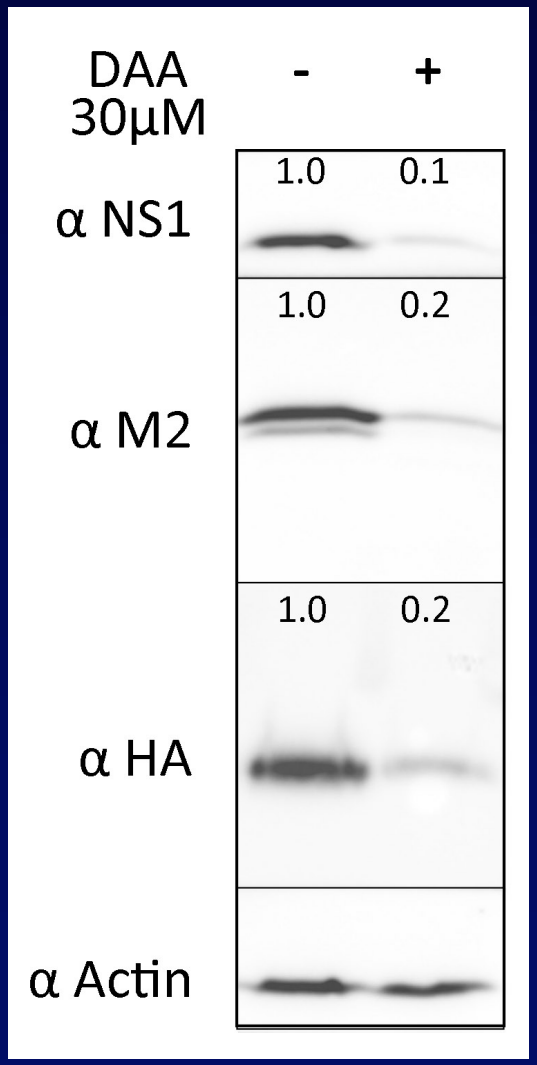


Loss of m^{6}A sites on the HA segment reduces IAV pathogenicity *in vivo*




METTL complex ?
ALKBH5 ?
The cytoplasmic readers
YTHDF1, YTHDF2, YTHDF3
compete for m6A marks in the
cytoplasm affecting mRNA
stability and translation.
AAAAA
mature mRNA
mRNA maturation
3'deazaadenosine (DAA)
SAH
SAM
"Writing"
CDS
ALKBH5 removes
m6A marks
METTL complex
adds methyl groups
to adenosine in a
5' RRACH 3' consensus
"Erasing"
AAAAA
The nuclear readers
YTHDC1, YTHDC2 bind
m6A marks.
N6 - methyadenosine, "m6A"
NUCLEUS
CYTOPLASM

DAA potently inhibits IAV replication in A549 cells



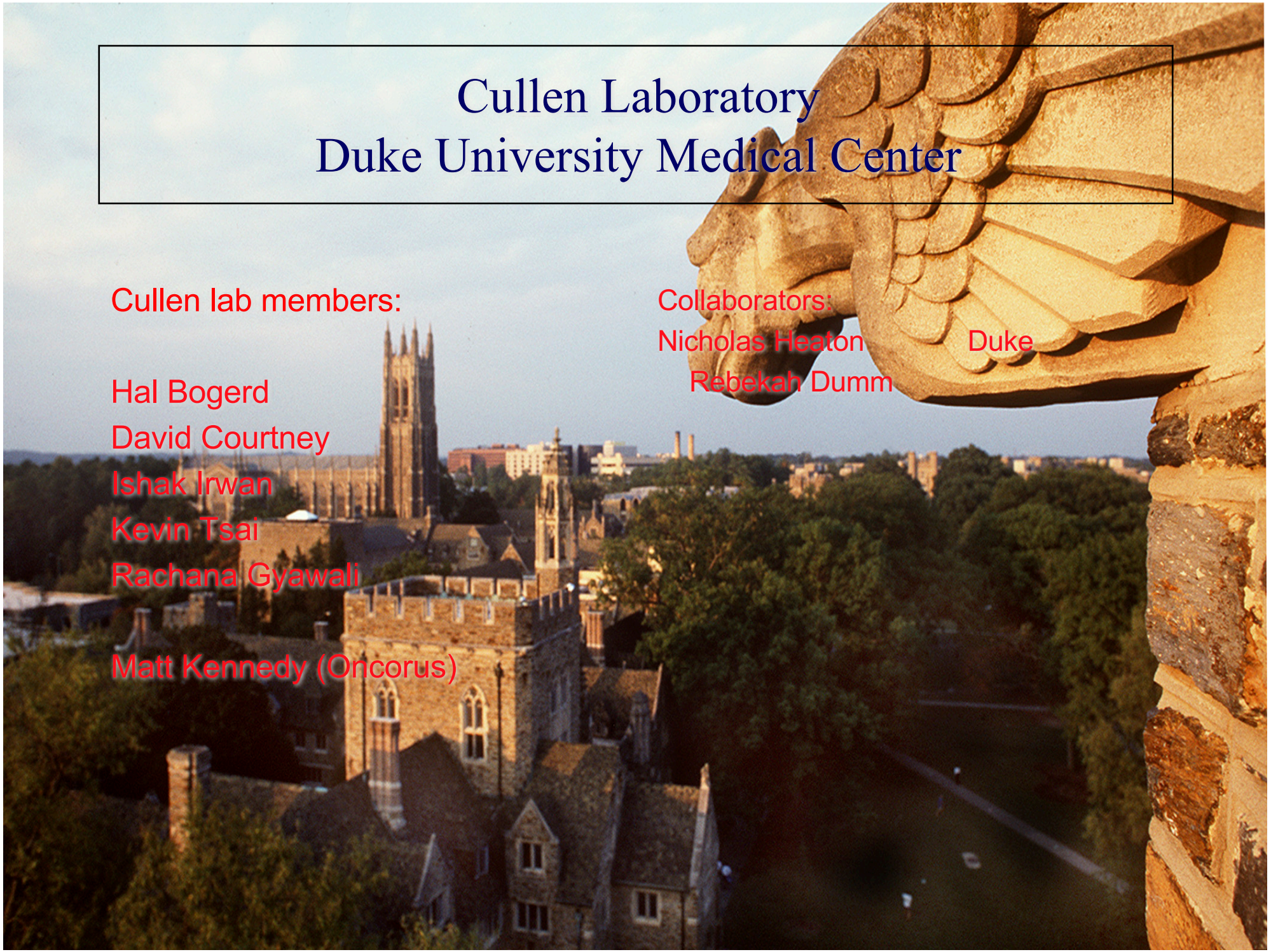
Conclusions

1) Influenza A virus (IAV) transcripts are extensively m^{6}A modified.

2) Inhibition of m^{6}A addition by mutational inactivation of METTL3, by mutagenesis of m^{6}A addition sites or using the drug DAA inhibits IAV replication and pathogenesis.

3) Conversely, overexpression of the m^{6}A reader protein YTHDF2 strongly enhances IAV replication.

4) We have also observed that m^{6}A sites present on viral transcripts strongly enhance the replication of HIV-1 and SV40.



Cullen Laboratory
Duke University Medical Center

Cullen lab members:

Hal Bogerd
David Courtney
Ishak Irwan
Kevin Tsai
Rachana Gyawali

Matt Kennedy (Oncorus)

Collaborators:
Nicholas Heaton Duke
Rebekah Dumm