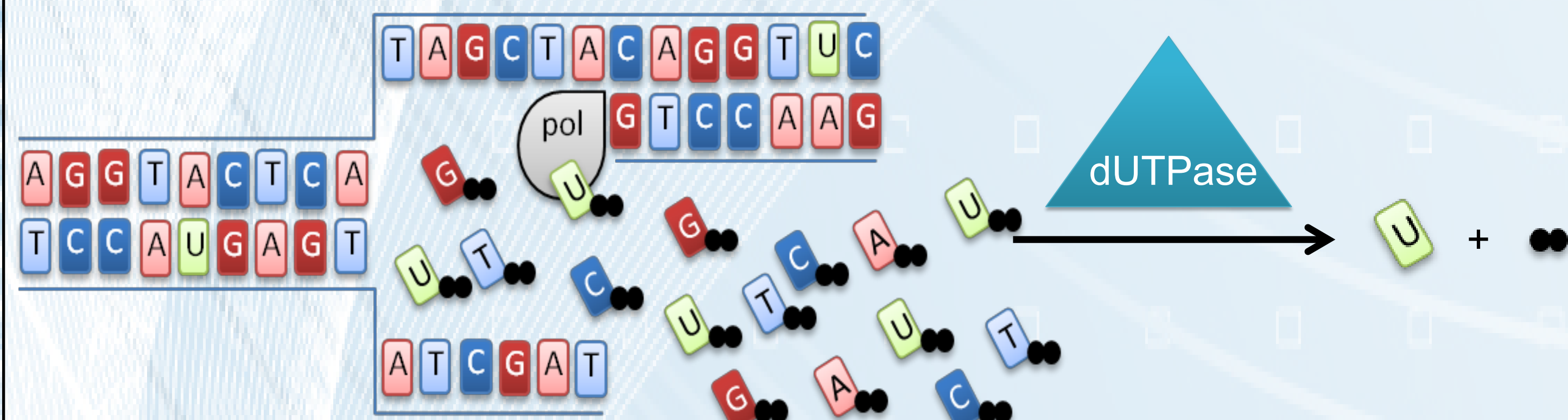


## 1. Introduction



- Faithful maintenance of genomic integrity is indispensable for life.
- dUTPase is essential in a number of organisms due to its crucial role in genome maintenance.
- Deficiency in dUTPase function results in elevated dUTP/dTTP ratio leading to misincorporation of uracil into DNA resulting in DNA fragmentation and cell death.

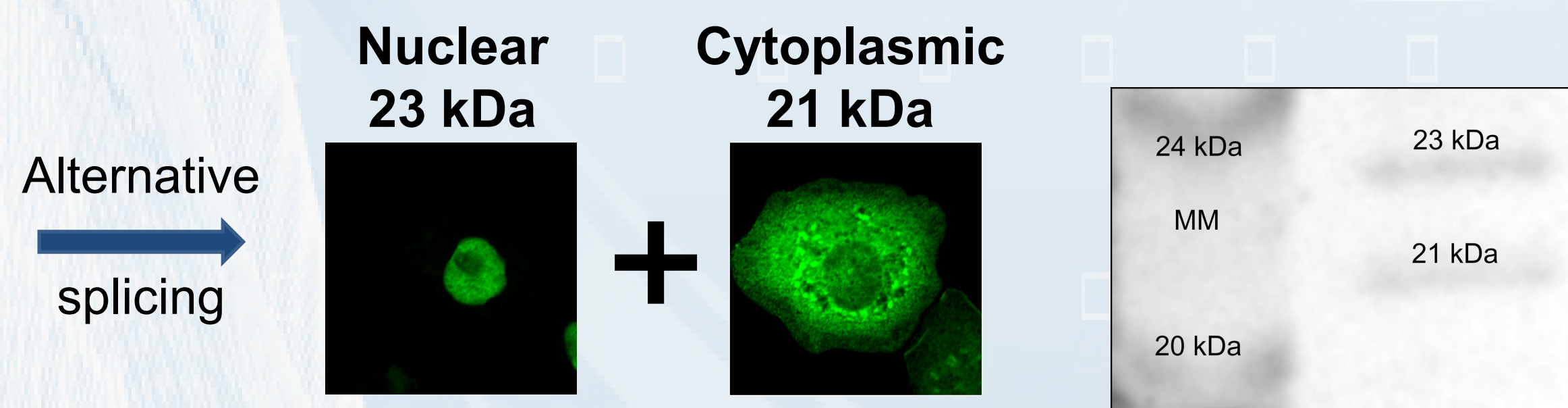
## 2. Aims

- Identify possible isoforms of dUTPase in *Drosophila virilis* that might differ in their N terminals, which contain their NLS signals, thus determining localization.
- Localization studies on *Drosophila virilis* dUTPase in comparison with *Drosophila melanogaster*.

## 3. Results – dUTPase isoforms

### Two isoforms of *D. melanogaster* dUTPase:

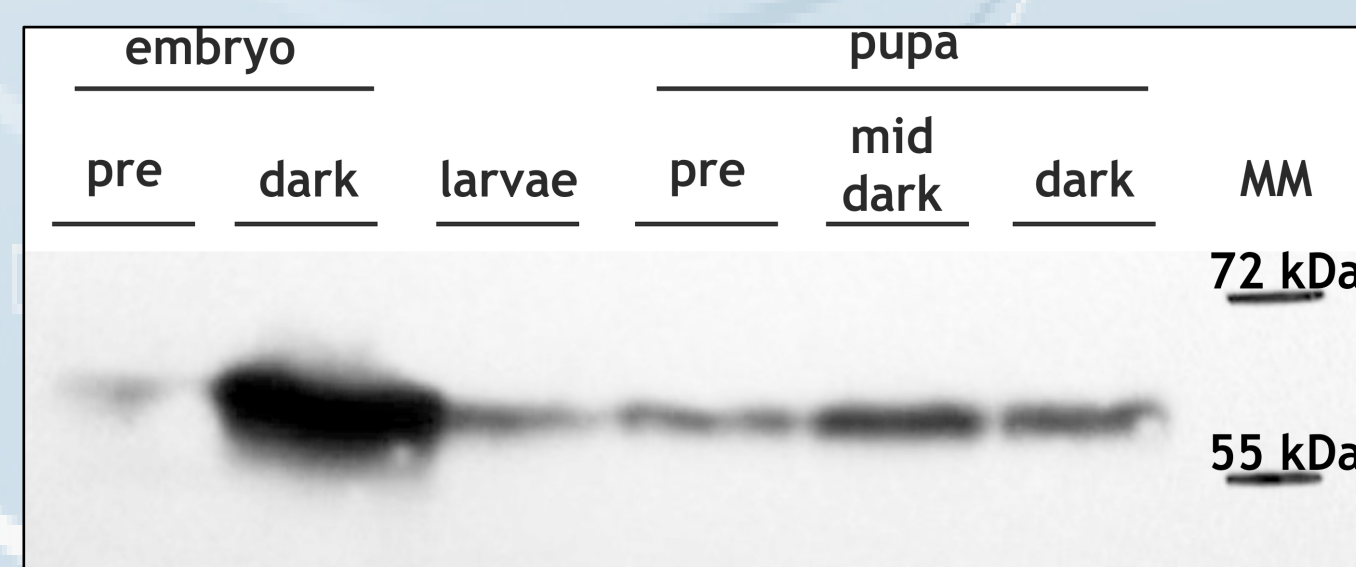
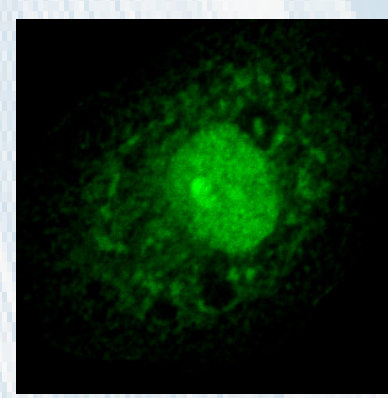
TTCCGACGCTGTGTTAAACCGCGTTATTTTCAGACCAGAATTCTGCAAGTAAGC  
TGAAAAAGTCTCTGTACTTTTCGAAGCATTCTCTGTAATAACTCAATTTGCTCCA  
AATGTCATCAACCGATTTCGCCGACATTCCAGCTGCCAAGAAGATGAAGA  
M P S T D F A D I P A A K K M K ← NLS



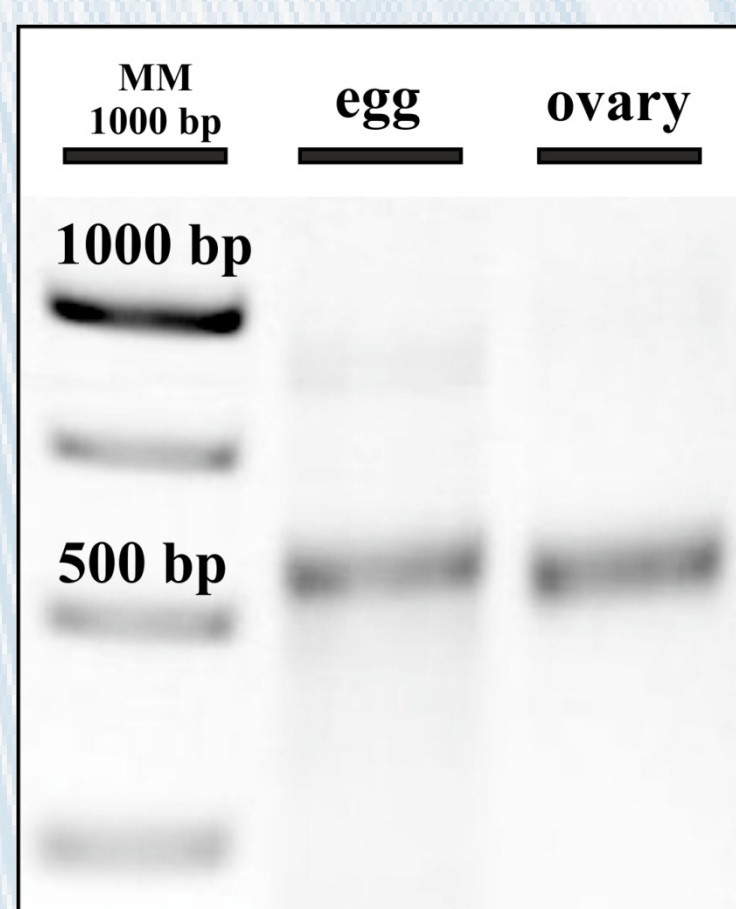
### Possible isoforms of *D. virilis* dUTPase?

AAATTCGCCAATATTTTAGCGTCGATACCAACGTCCAACCTACTTGATAGCCA  
CTTTGGGTGTTTCTCACATTTATTTTAGTGATTAATCCTCATATACACATTGCA  
GATGSCCTCGCCTGTTATTGACGATATTCAAACAGCTAAGAAATGAAAC  
M A S P V I D D I Q T A K K M K ← NLS

### Nuclear-cytoplasmic



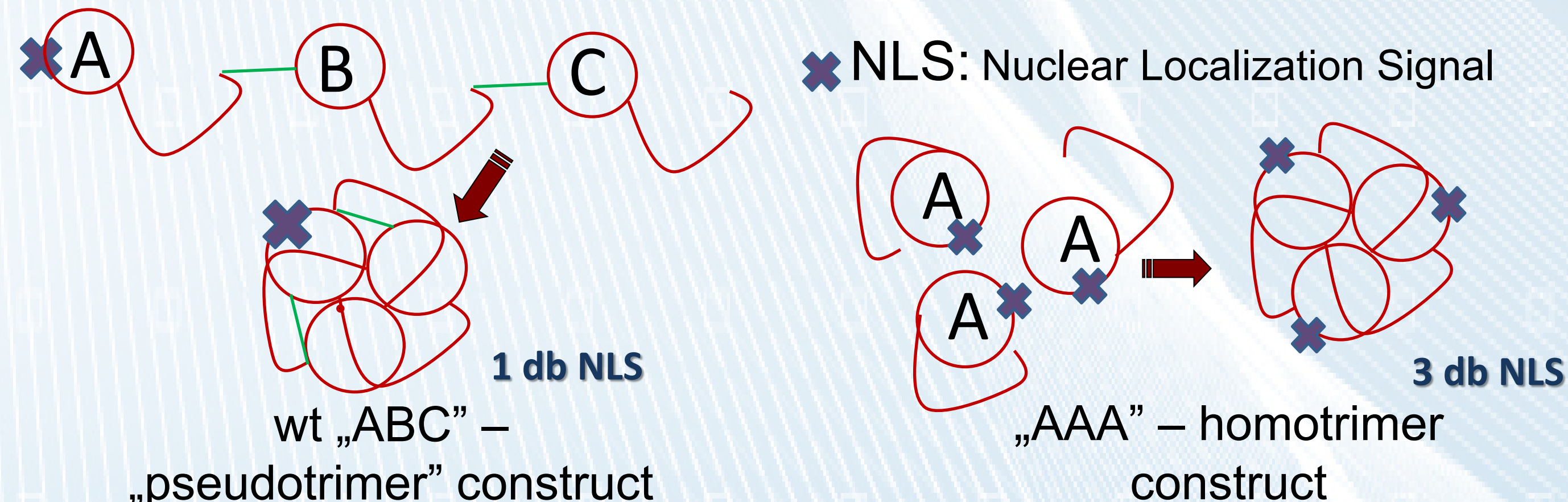
### 5' RACE analysis:



- To investigate the existence of putative isoforms, we looked for possible mRNA transcripts, differing in their 5' end using 5' RACE technique.
- 5'RACE experiments concluded that only one dUTPase isoform exists.

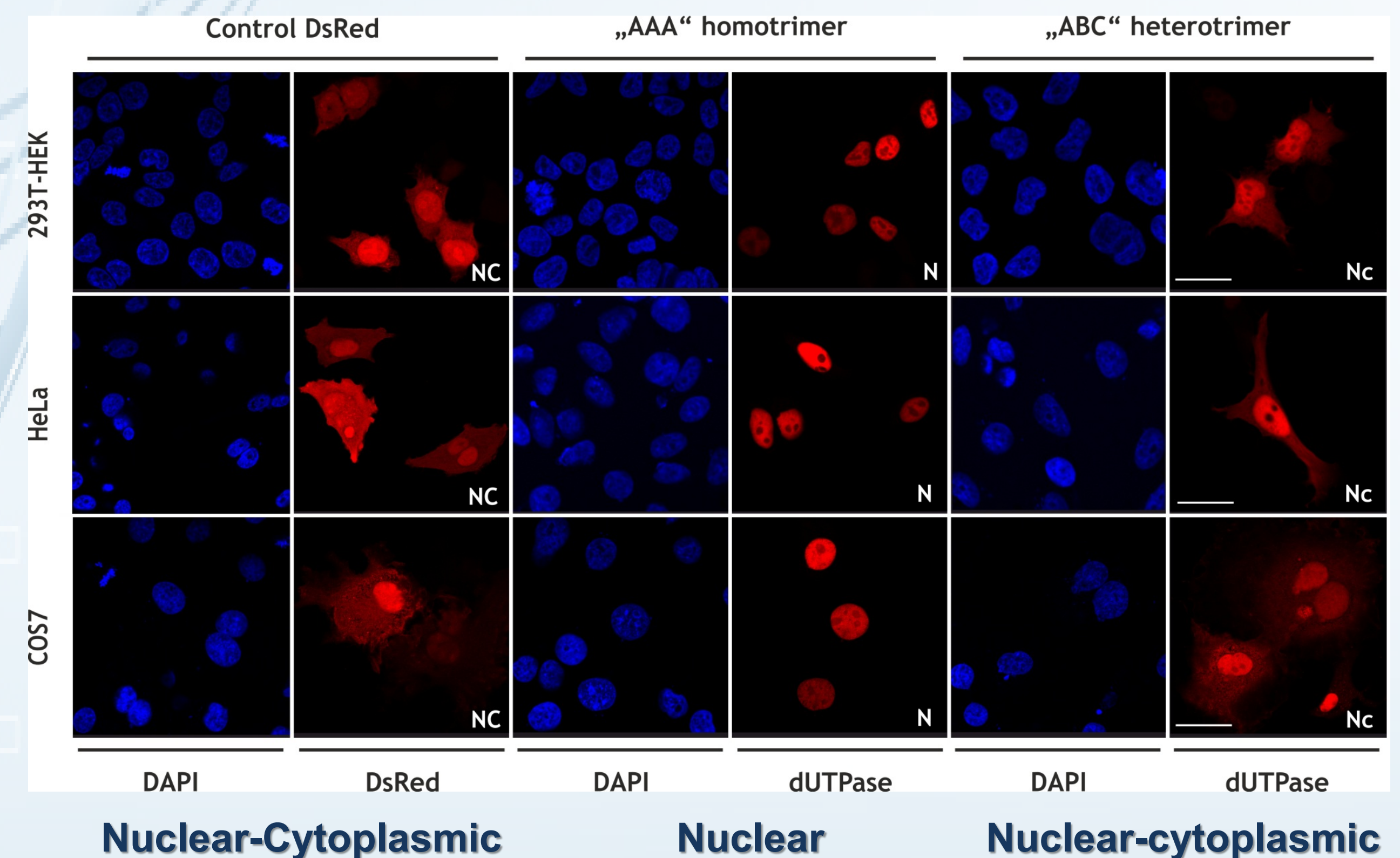
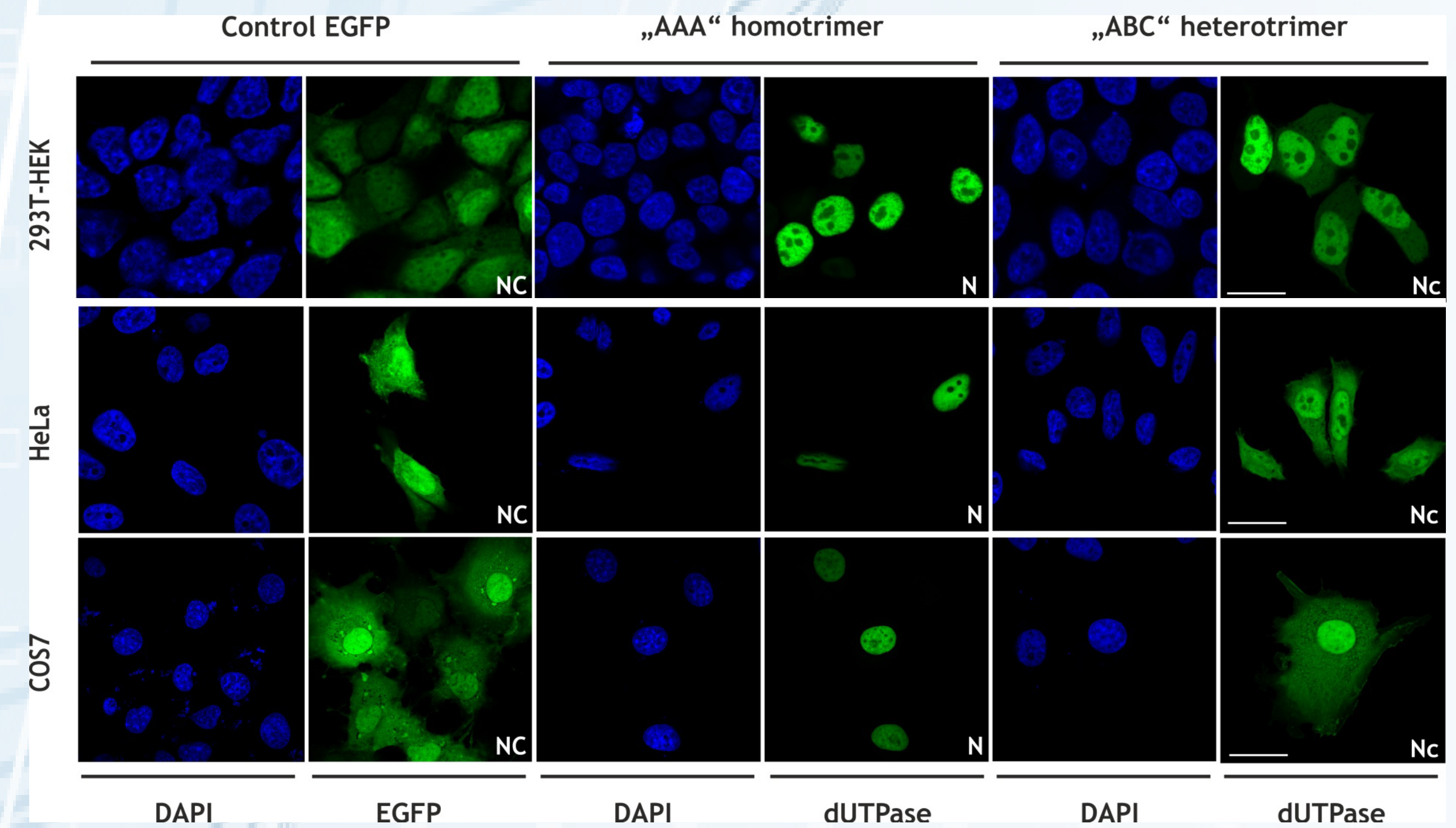
## 4. Results – dUTPase constructs

- dUTPases are usually homotrimeric enzymes harboring three cognate NLS signals, such as the enzyme of *Drosophila melanogaster*.
- Although eukaryotic organisms have more dUTPase isoforms in general, a nuclear isoform is found in the majority of the organisms.
- Drosophila virilis* encodes an unique „pseudo-heterotrimer” dUTPase consisting of three covalently linked none identical monomers.



## 5. Results – Cellular localization

### Localization in mammalian cell lines:

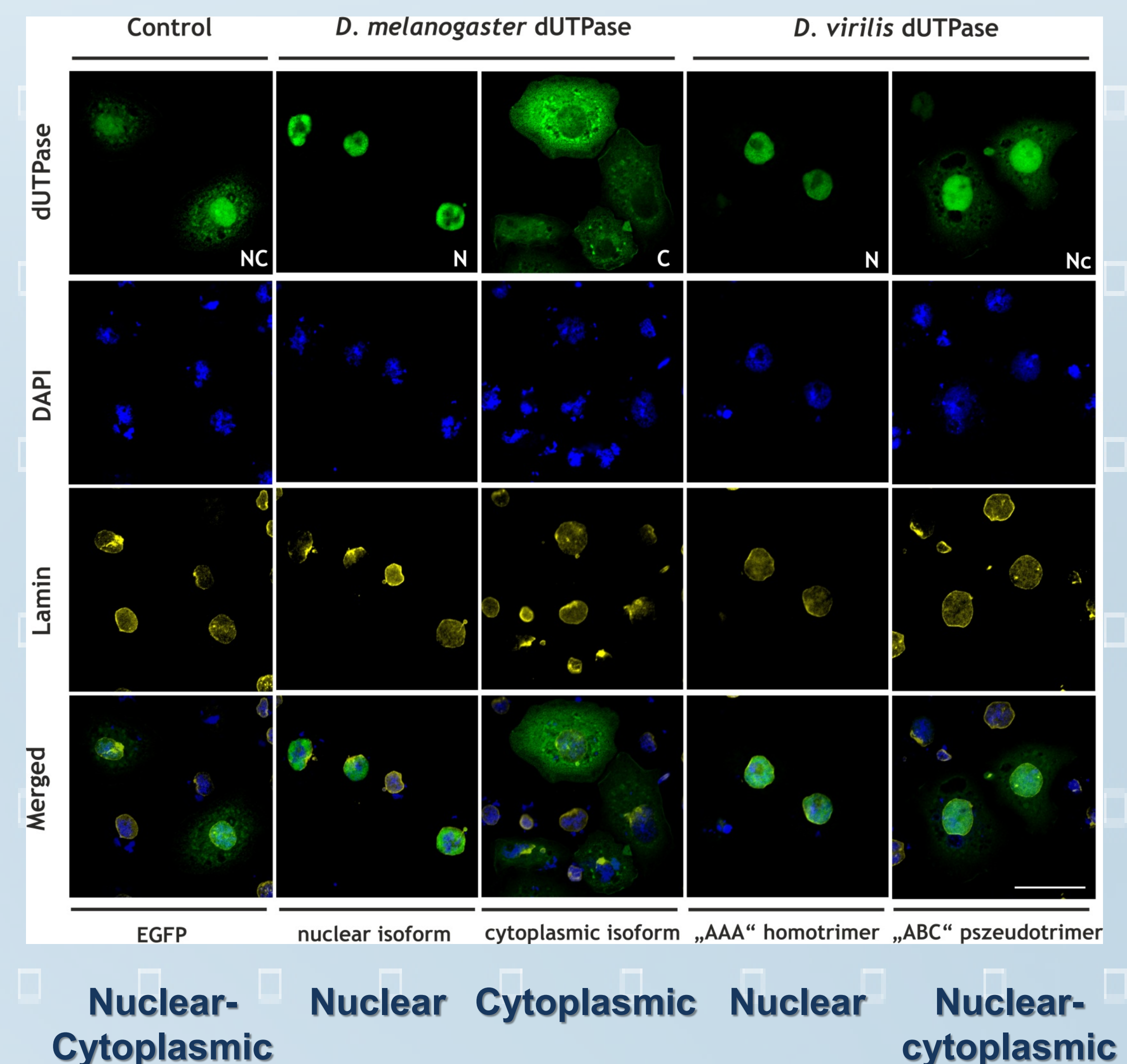


*Drosophila virilis* dUTPase („AAA”) containing 3 NLS, shows a strong nuclear accumulation while the „ABC” dUTPase with only one NLS accumulates less efficiently in the nucleus.

### Localization of fusion constructs in *Drosophila* S2 cells:

We also utilized the two isoforms of *Drosophila melanogaster* as a nuclear and cytoplasmic control.

Results resembled the localization pattern observed in the human cell lines. Namely the „AAA” form was exclusively nuclear while the „ABC” form showed less stringent nuclear localization.



## 6. Conclusion

- In silico* prediction, Western-blot experiments and 5' RACE analysis confirm one dUTPase isoform in *Drosophila virilis*, in contrast with *Drosophila melanogaster* harboring two isoforms through alternative splicing.
- The reduction of the NLS copy number in *D. virilis* dUTPase allows for both nuclear and cytoplasmic occurrence of the same protein, whereas *D. melanogaster* requires two dedicated dUTPase isoforms to accomplish such distribution pattern.
- NLS number variation due to oligomerization has an important effect on the protein compartmentalization which is indispensable to contribute their function.

### References:

Bozóky, Róna et al., Plos One, 2011; Merényi et al., The Febs Journal, 2010; Muha et al., 2009  
Affiliation: palinkas@enzim.hu, Lab. Of Genome Metabolism and Repair, Institute of Enzymology