

# Image annotation STHdh-mHTTQ97-HA cell line

Carolina K Rangel

# Recap

# Image features - Sterallis

- Magnification: 63X or higher
- Image sizes: 1024x1024 or 2048x2048 pixels

# Image analysis

- Aggregate abundancy
- Colocalization (focused on aggregates)

# What information we want to obtain

## *Aggregates*

- Total number of cells
- Number and percentage of cells with aggregates

From cells with aggregates:

- Number and percentage of cells with aggregates only in the nucleus
- Number and percentage of cells with aggregates only in the cytoplasm
- From cells with aggregates in both nucleus and aggregates

From cells with aggregates in both nucleus and aggregates:

- Number and percentage of aggregates in the nucleus
- Number and percentage of aggregates in the cytoplasm

# What information we want to obtain

## Aggregates

- Aggregates sizes and shape – limited by resolution

*STED → higher resolution, sub-groups in nm*

*Sterallis → Distinction from Inclusion body and small species*

- Considering that, what is the distribution of different aggregates sizes in the cells?
- What is the percentage distribution of each category in the nucleus and in the cytoplasm?

# What information we want to obtain

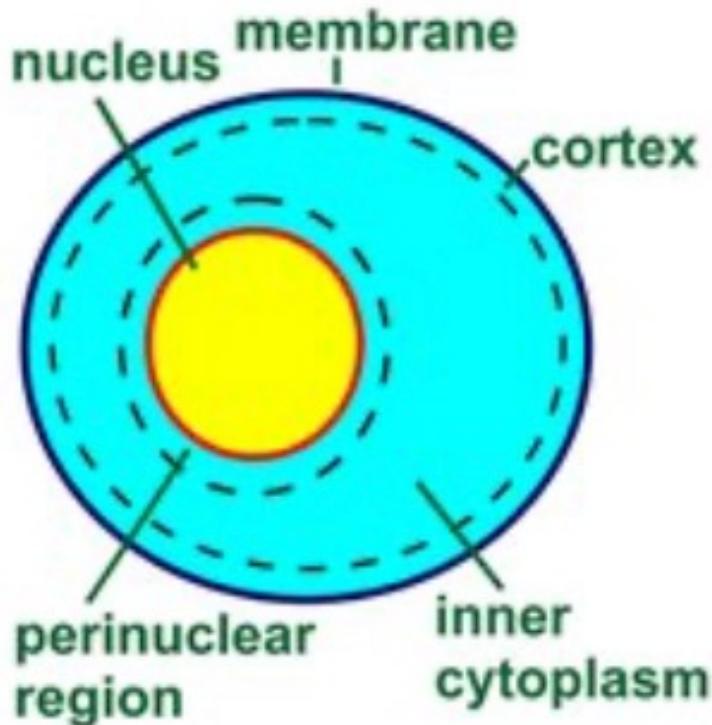
## *Co-localization*

Only in the the aggregates area:

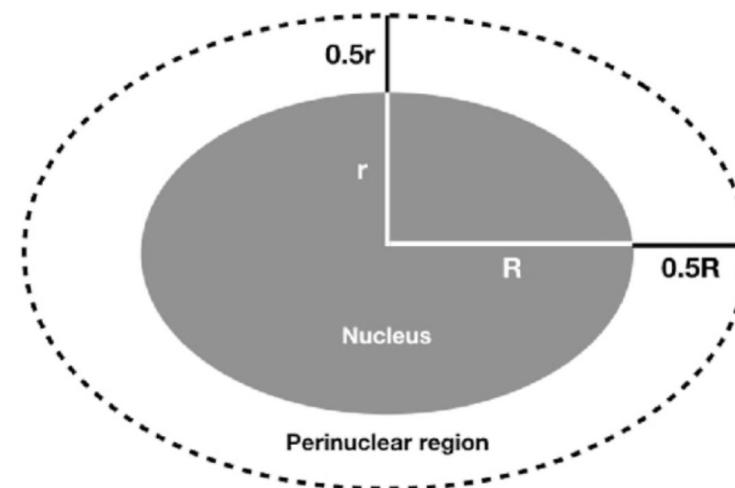
- What is the colocalization between CCT1 and HA signal  
(does it colocalize more with small or big inclusions?)
- What is the colocalization signal between CCT1 and A11
- What is the colocalization signal between CCT1 and OC

In some images we have a ring shape. Can we measure it? And the percentage of this events?

# Cell regions



## Definition of perinuclear region



**Figure**

Caption

Figure 1. Definition of the perinuclear region used to quantify fluorescence-labeled vesicles distribution around an oval-shaped nucleus.  $r$  is the nucleus short radius,  $R$  is the nucleus long radius. Perinuclear region radii are calculated starting from the nuclei center as  $r + 0.5r$  and  $R + 0.5R$  for short and large radius, respectively, to define th... [Read more](#)

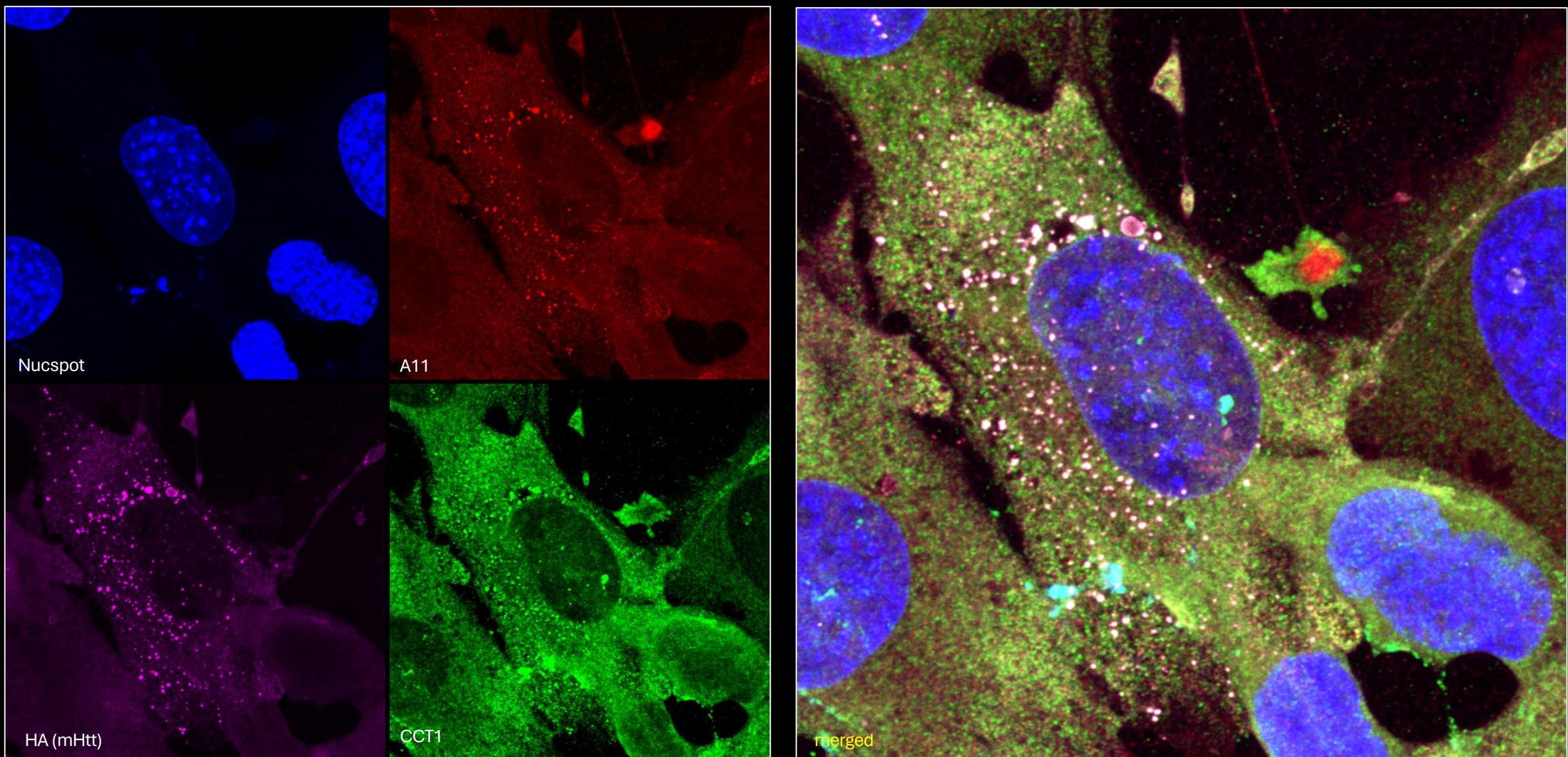
This figure was uploaded by [Natália Fernanda Do Couto](#)  
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Source: <https://bio-protocol.org/en/bpdetail?id=3703&type=0>

Ps: some tools already have this defined in their own code

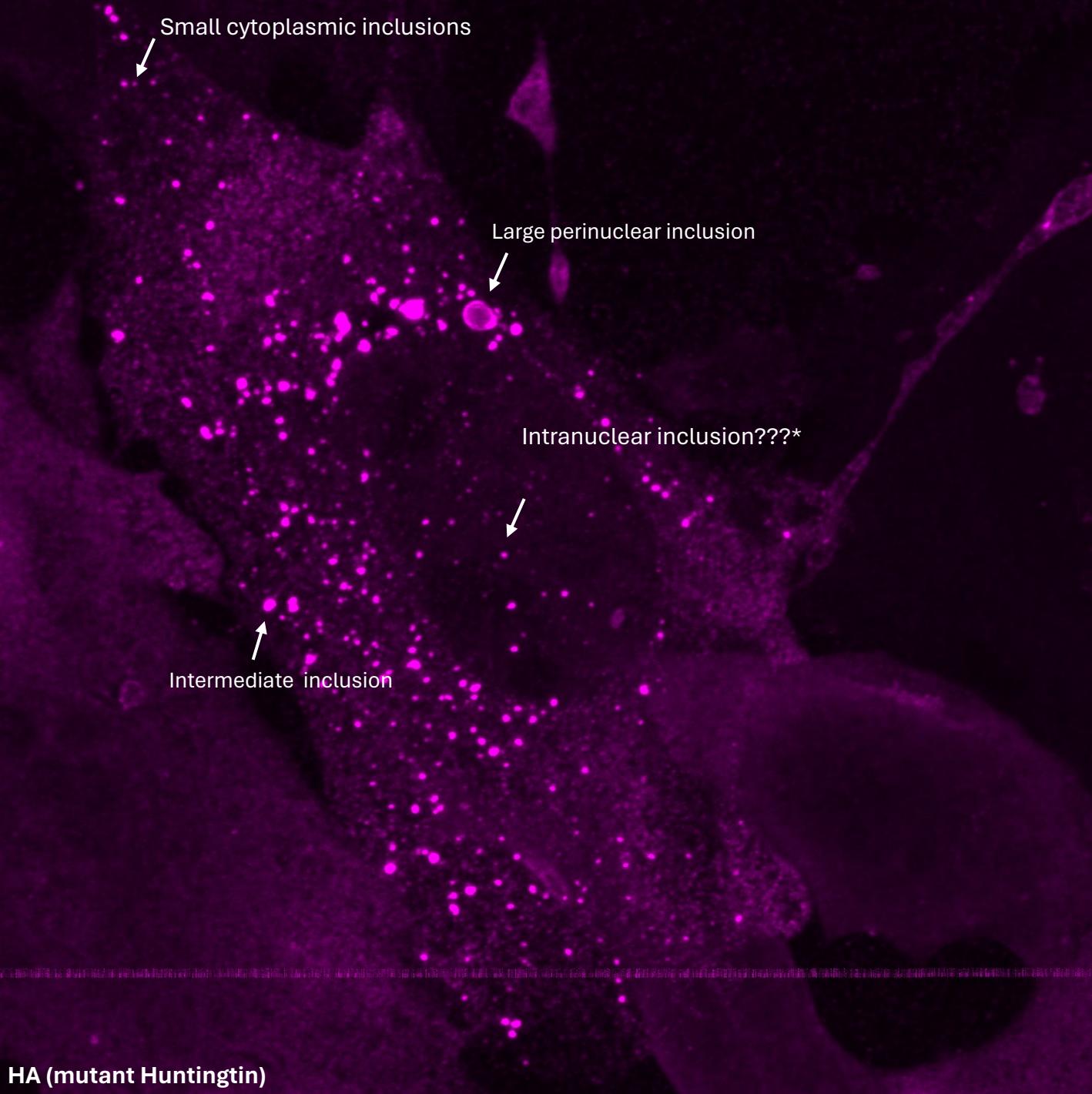
# Aggregates

Overall view of this image and channels (proteins) we stained for



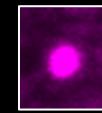
STHdh mHTT-HA Q97 cells induced for 72h

Zoomed in image to achieve higher resolution

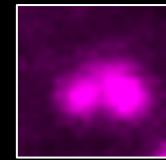


\*For Intranuclear inclusions take into account stacks.  
The aggregates are only nuclear when they colocalize with  
the blue signal (nucspot staining). Otherwise they could be only  
on top.

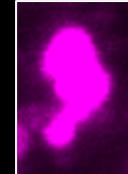
## Shape



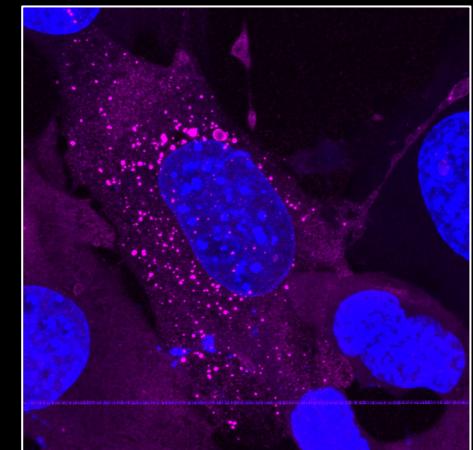
Round shape, the most common

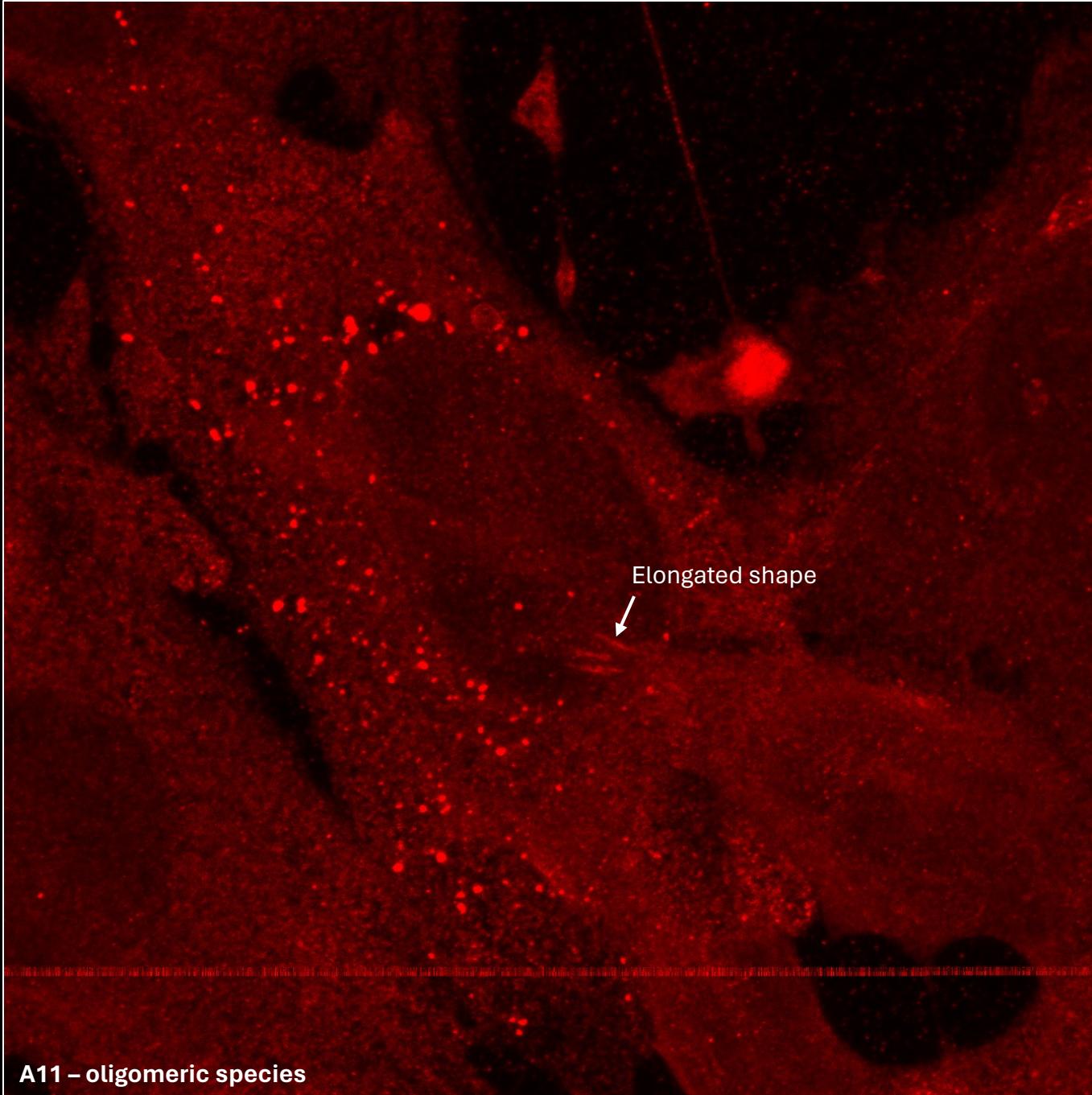


Probably 2 round aggregates getting together

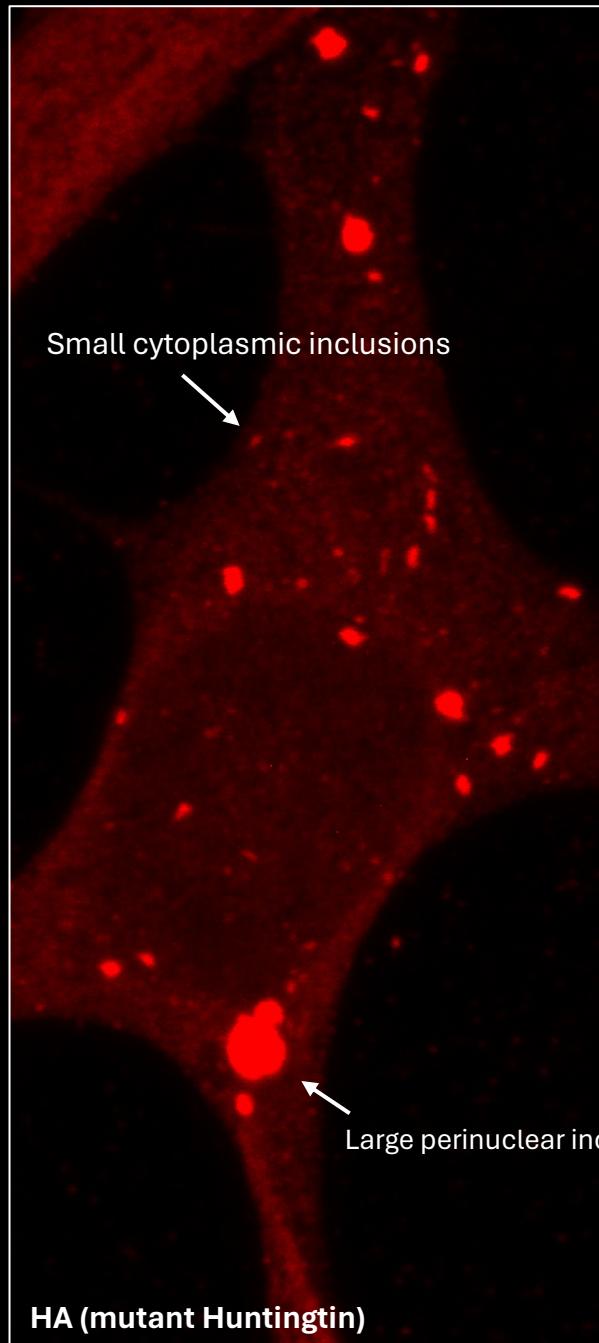


The same here, but with more aggregates, giving  
the more amorphous look

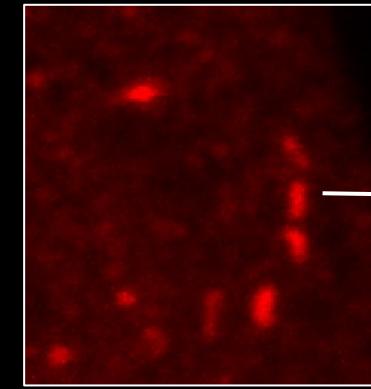




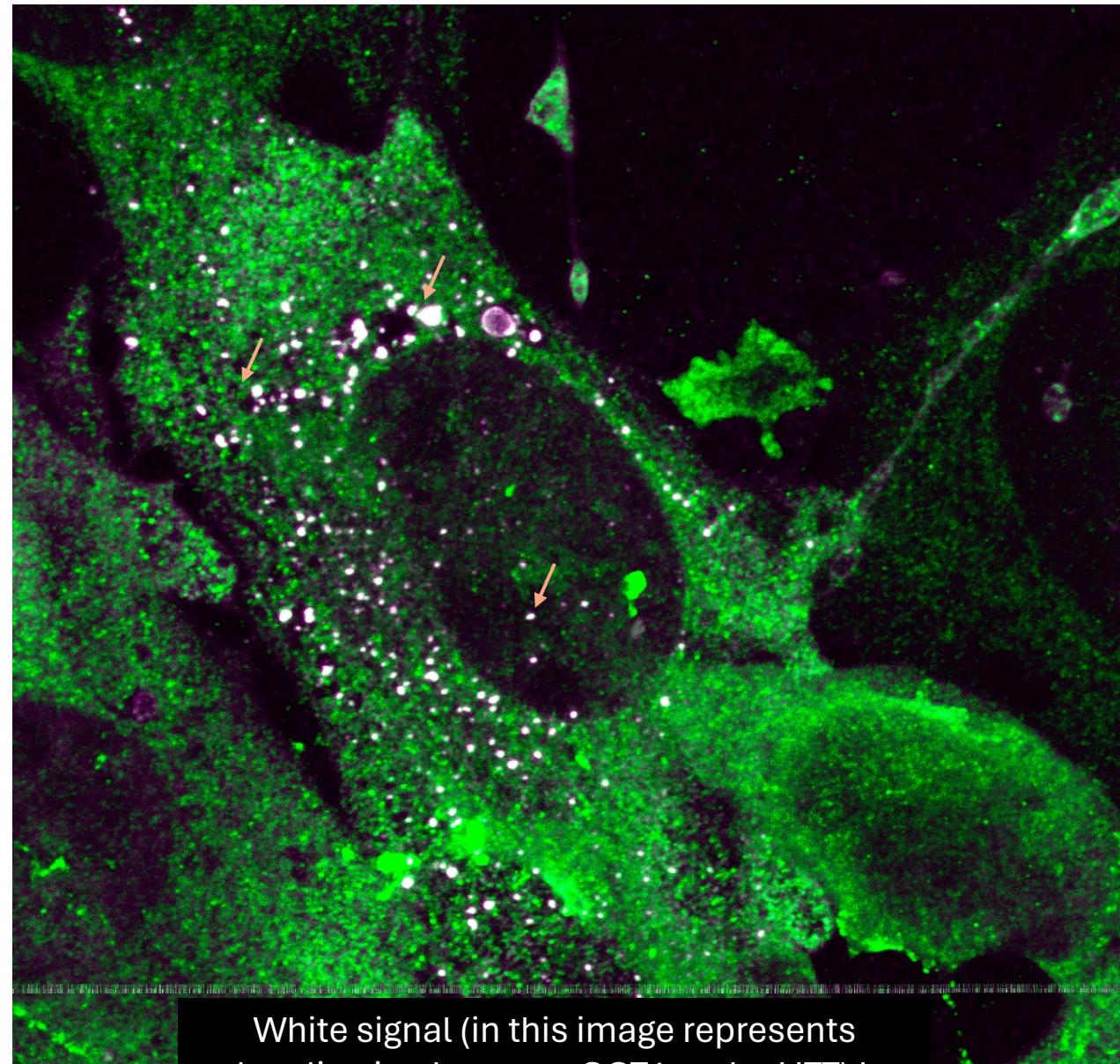
A11 – oligomeric species



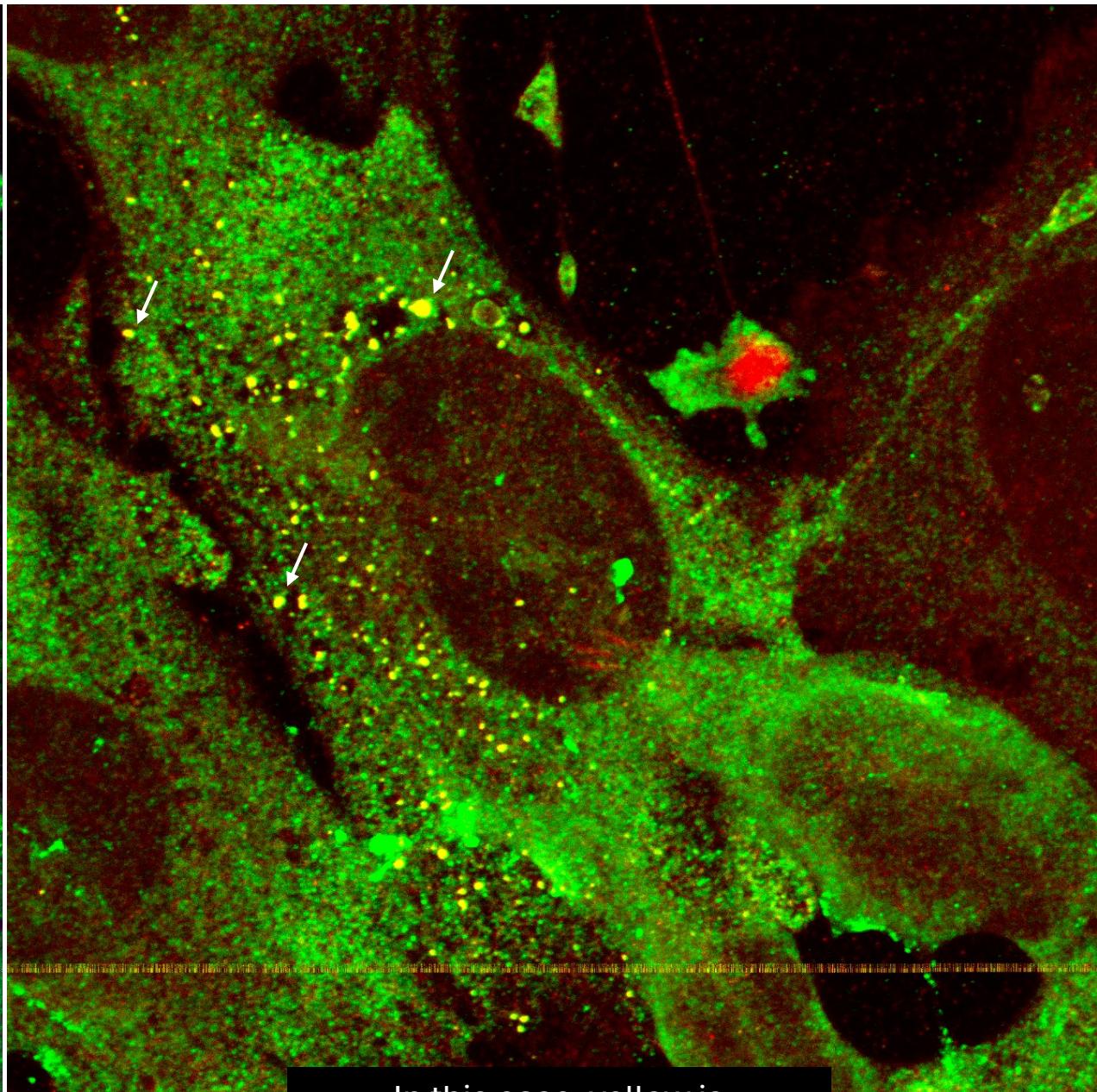
Shape



# Colocalization



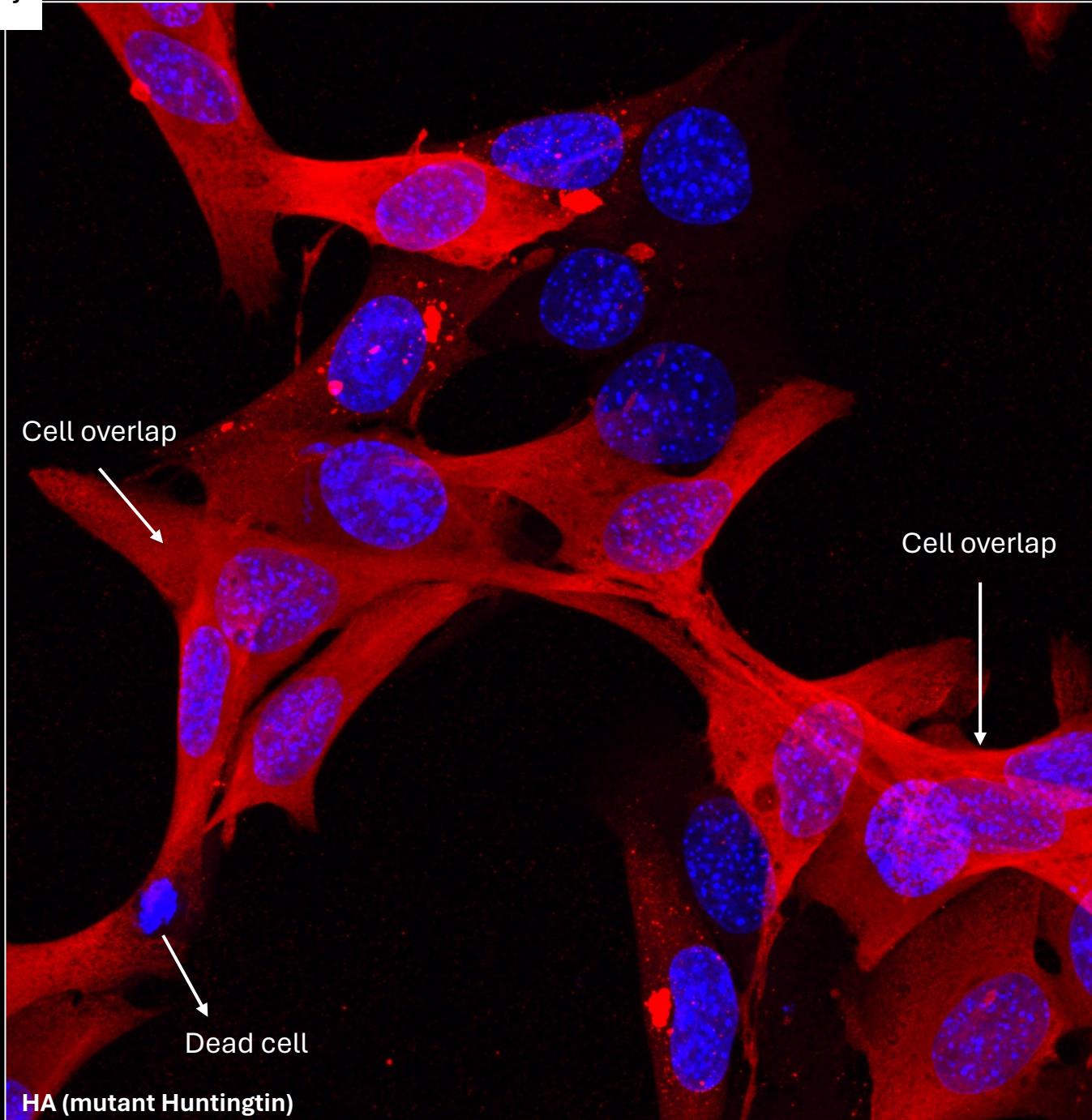
White signal (in this image represents colocalization between CCT1 and mHTT) but it depends on the stacks. Check last slides for recap.



In this case, yellow is colocalization

## Quantification by eye

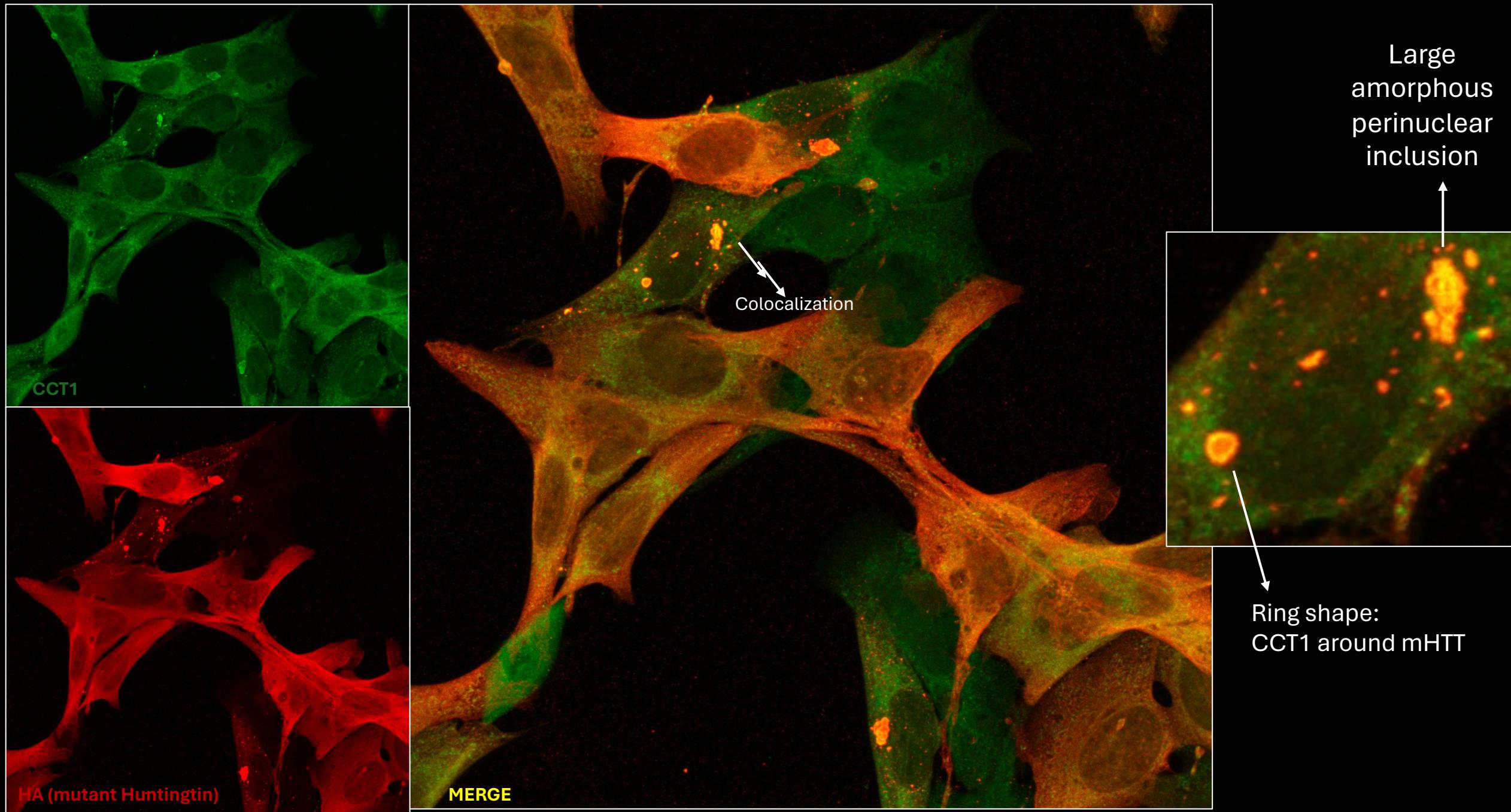
Image 1

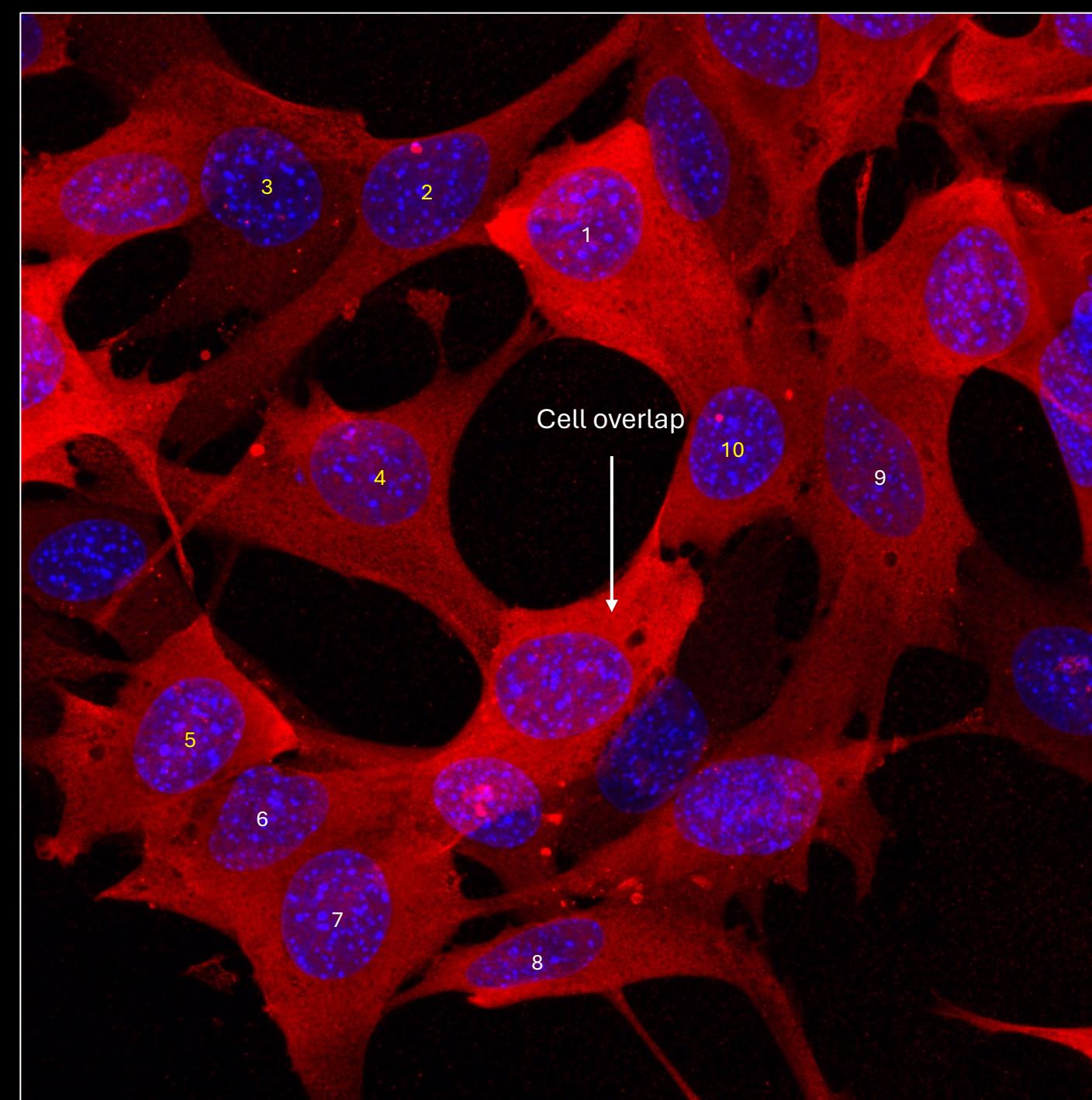


These cells really like to grow in groups so it is hard to avoid cell overlapping. But these cells should not be counted in the quantification because they will interfere with the correct result.

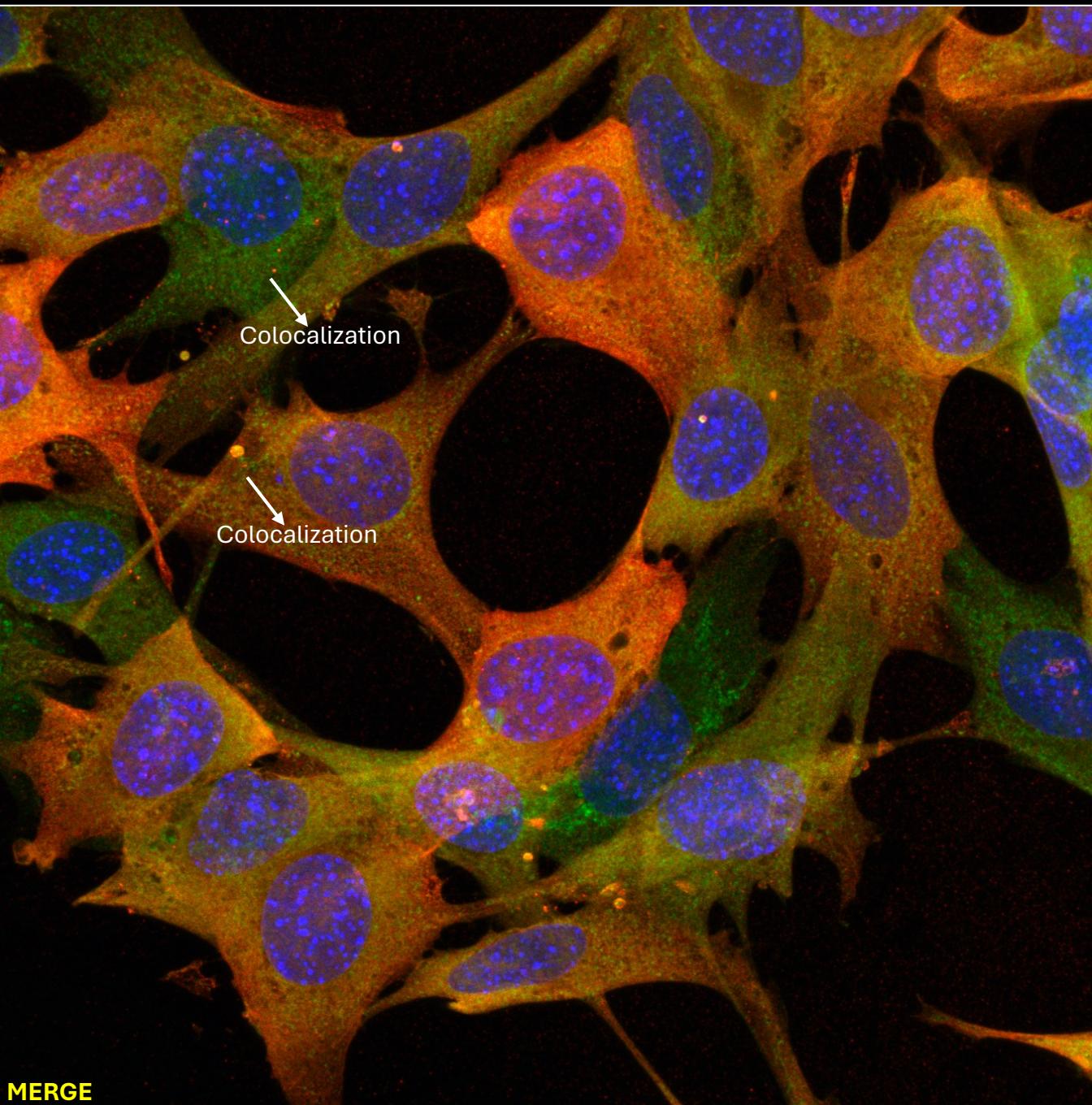
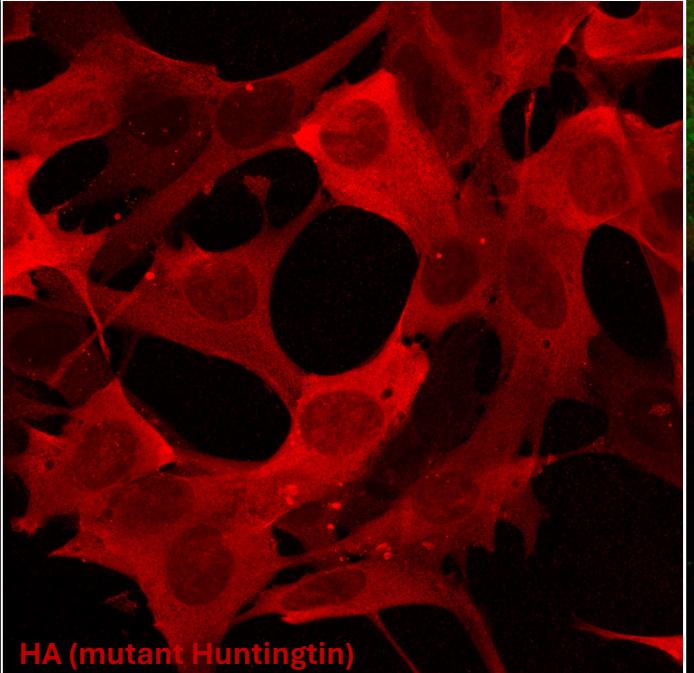
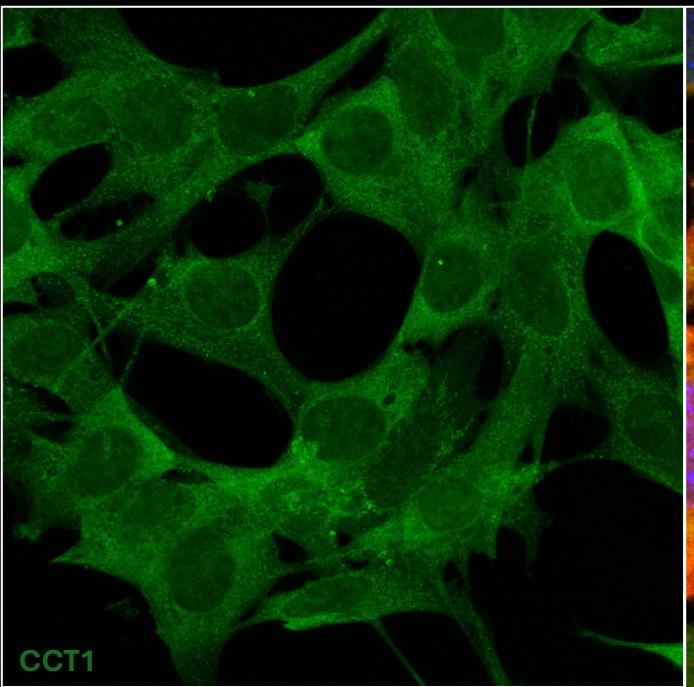
Another problem is that this cell line is polyclonal, and some cells express less mHTT than others. There is 1 cell with no red signal... so it is hard to define the cell area. So my advice is to use the TRiC/CCT1 channel to define cell area (see next slide)

Counting by eye  
(only what should be taken into consideration, also removing cells that are on the edges)  
N of cells = 9  
N of cells with aggregates = 3



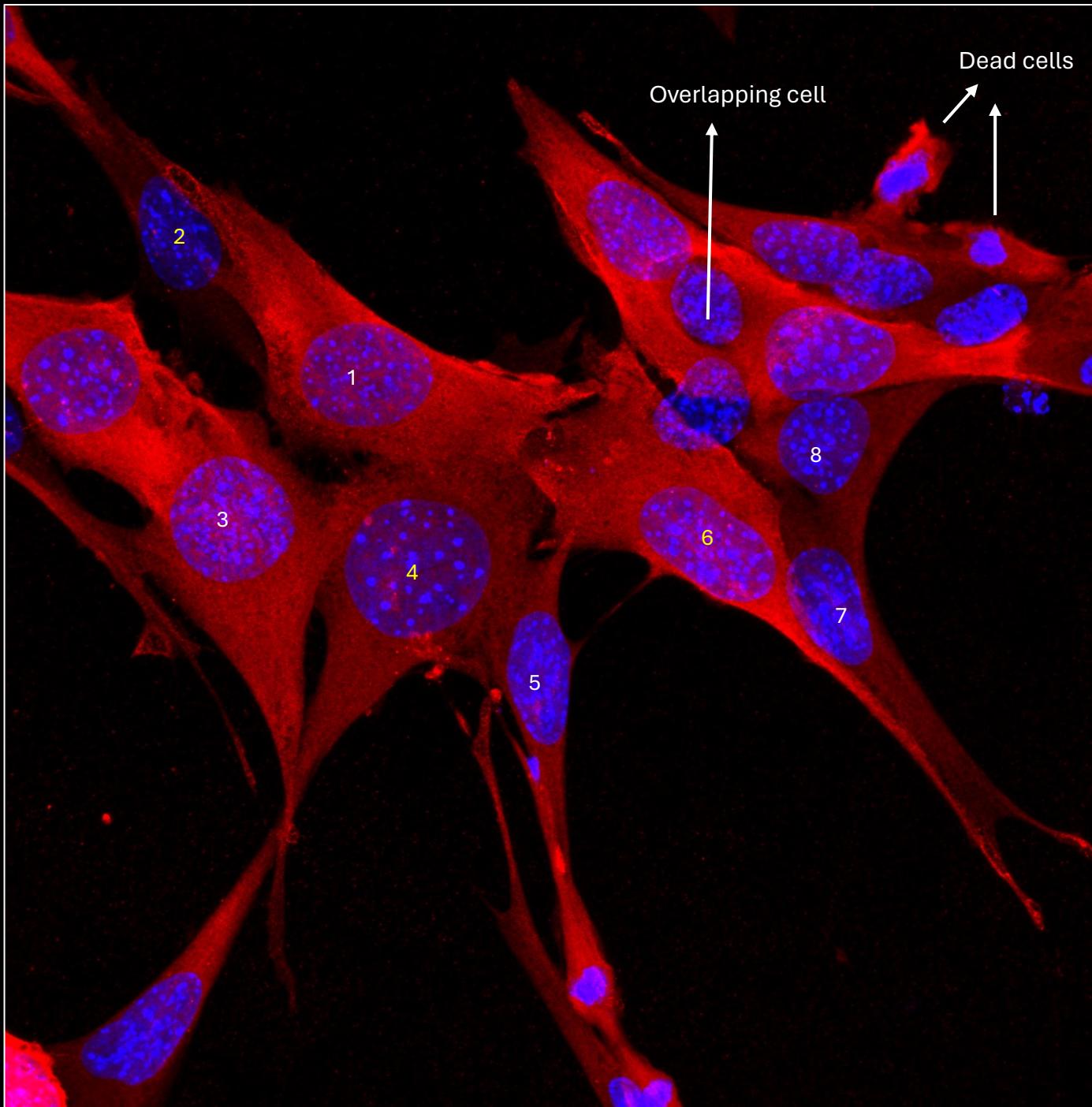


Counting by eye  
(only what should be taken into consideration,  
also removing cells that are on the edges)  
N of cells = 10  
N of cells with aggregates = 6

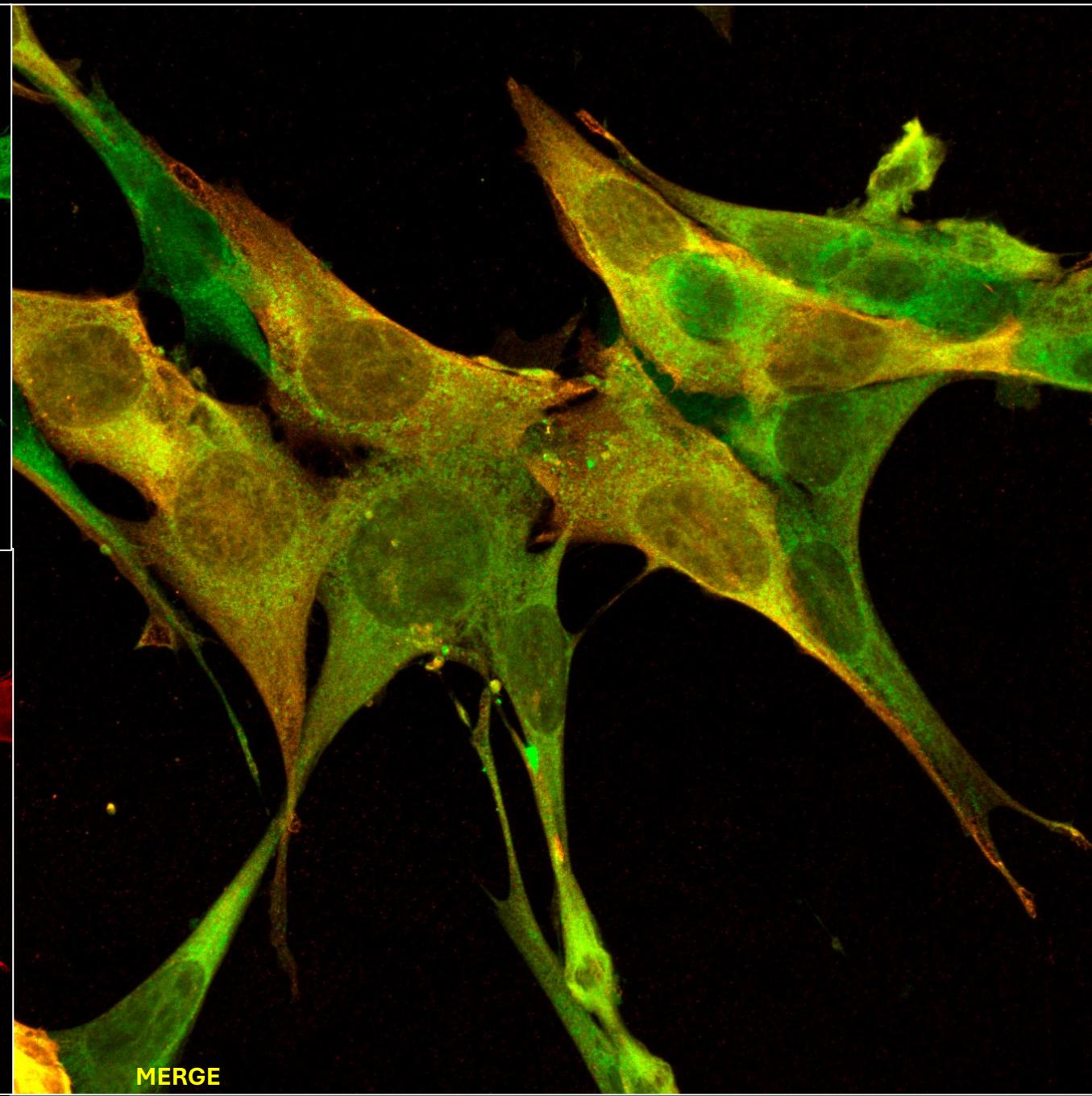
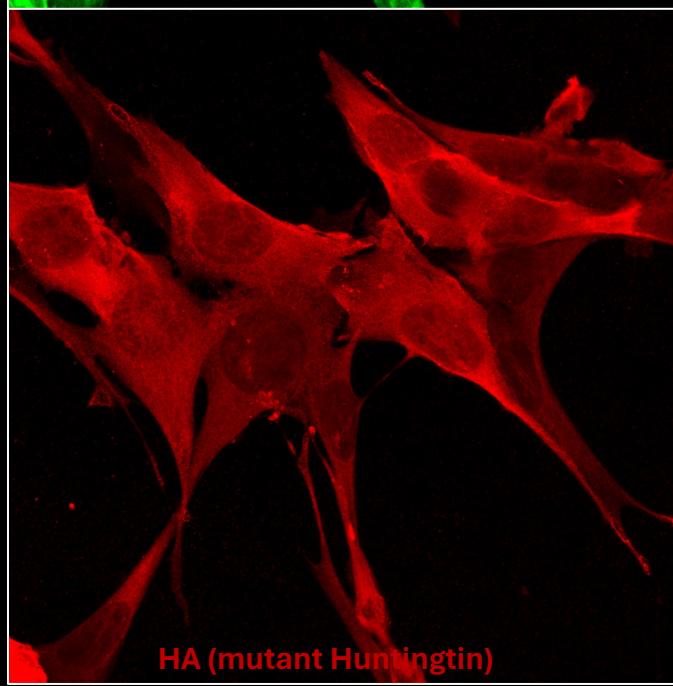
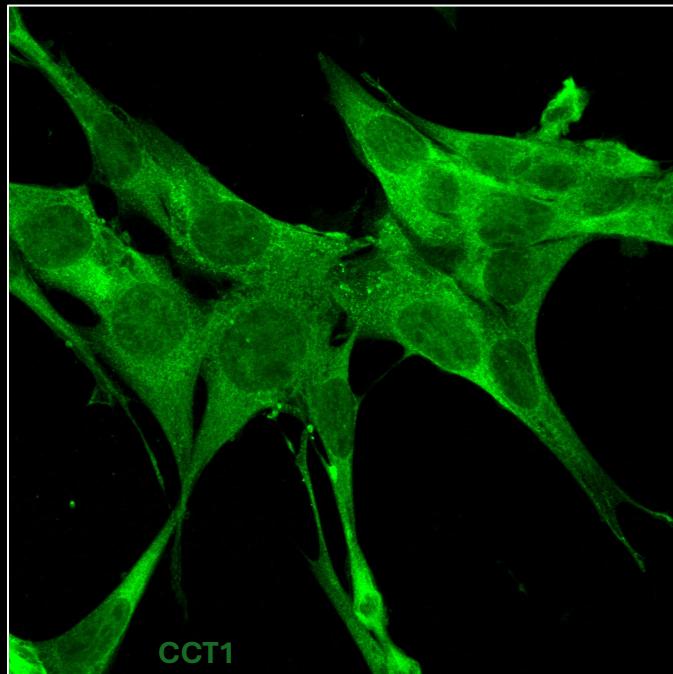


# Quantification by eye

Image 3

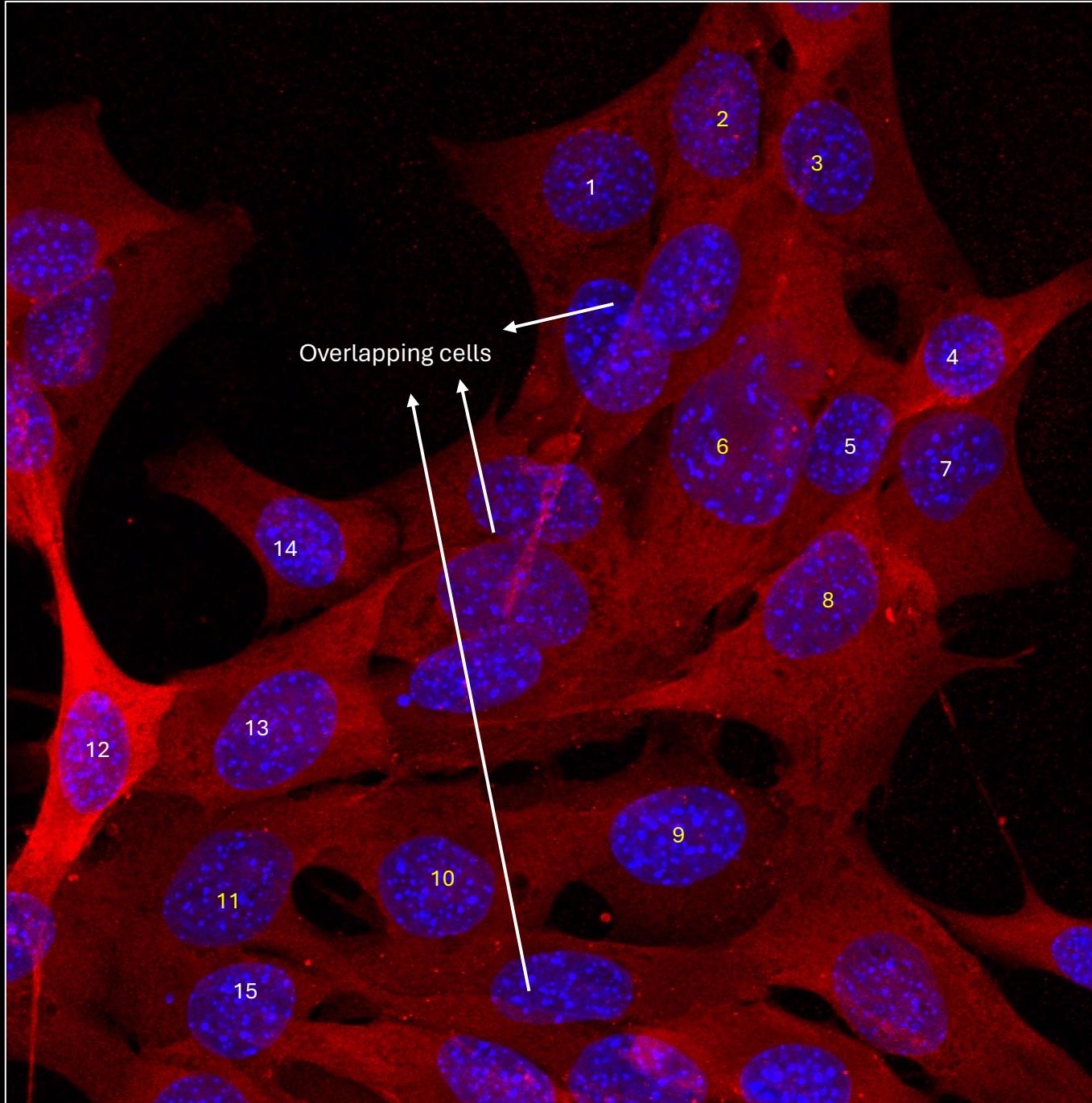


Counting by eye  
(only what should be taken into consideration,  
also removing cells that are on the edges)  
N of cells = 8  
N of cells with aggregates = 3

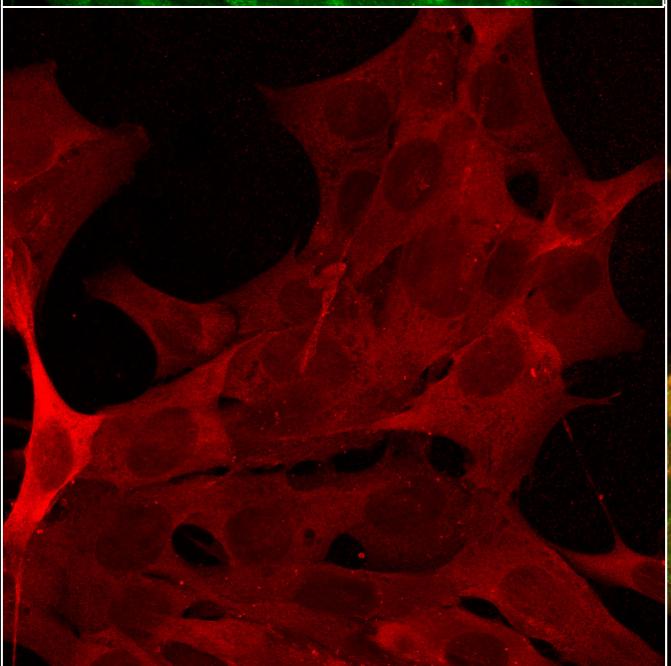
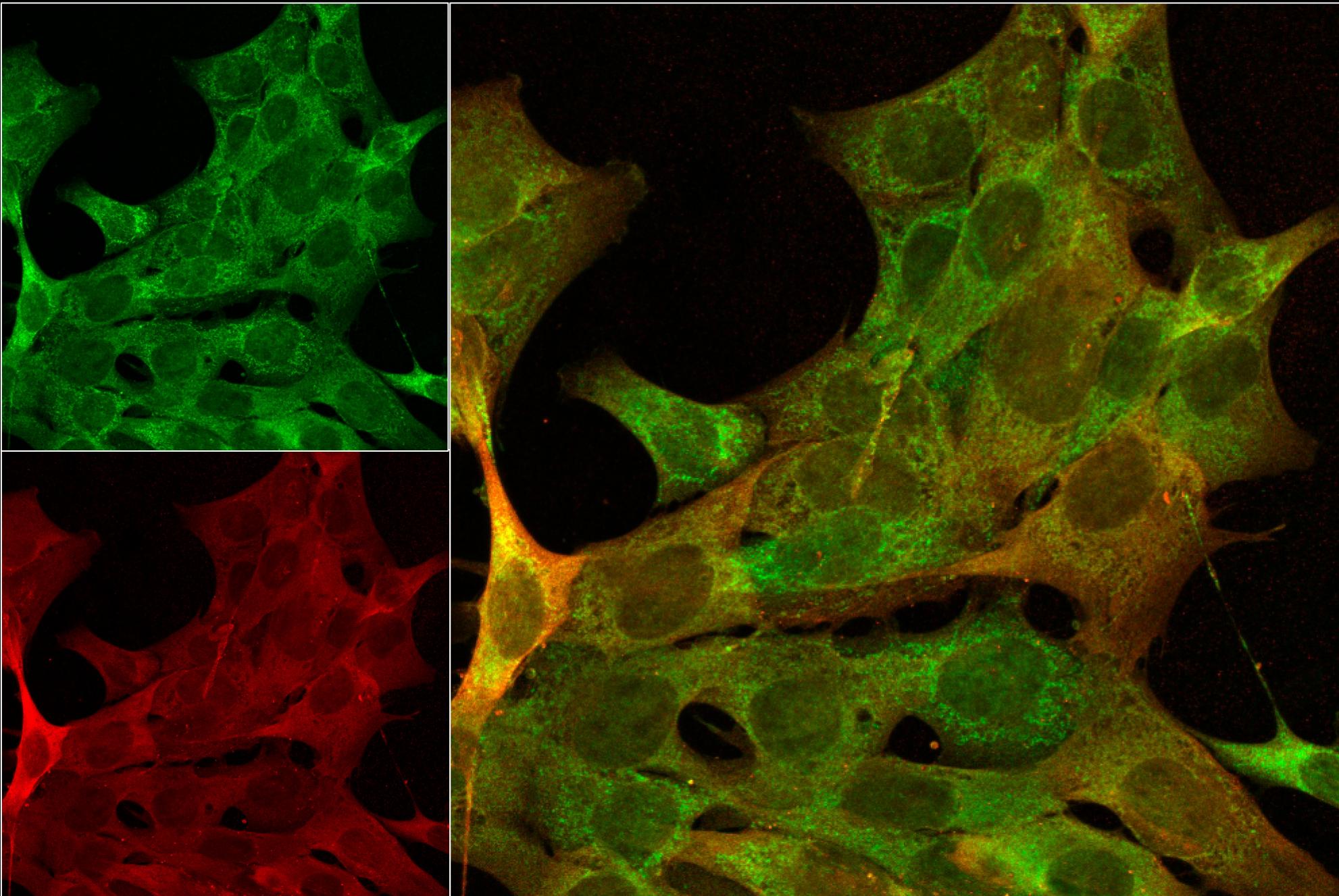


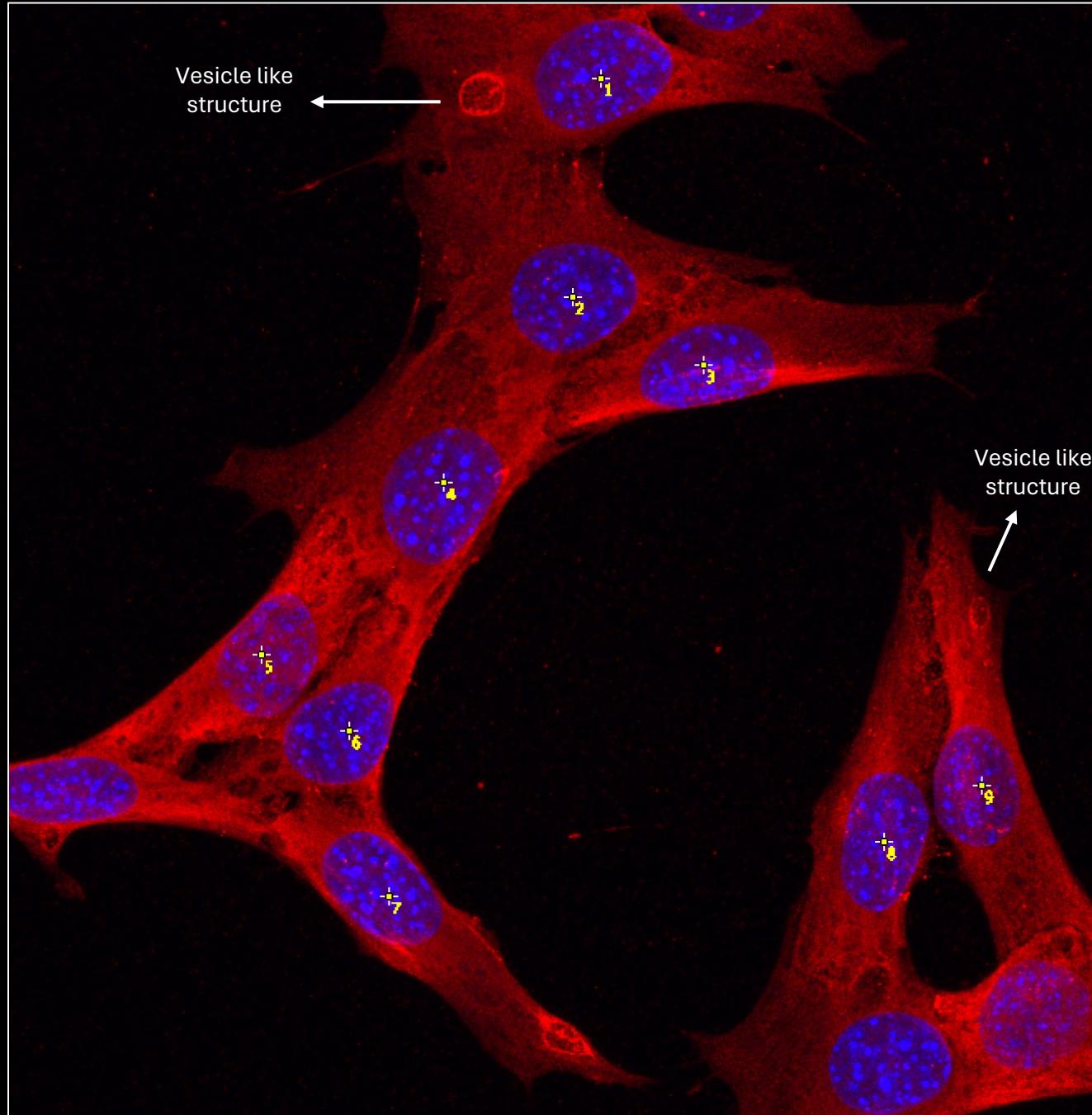
# Quantification by eye

Image 4

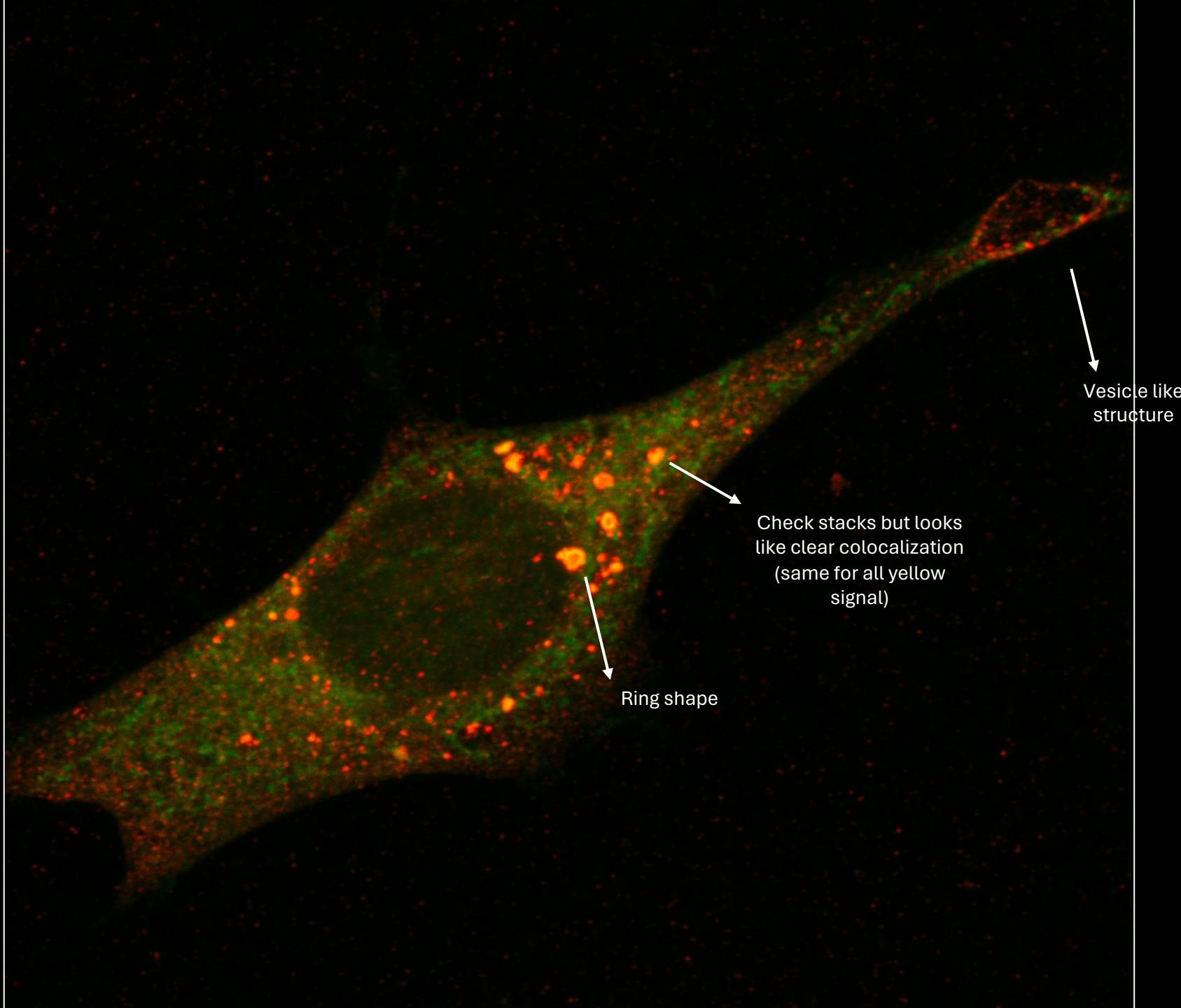


Counting by eye  
(only what should be taken into consideration,  
also removing cells that are on the edges)  
N of cells = 15  
N of cells with aggregates = 7





Counting by eye  
(only what should be taken into consideration,  
also removing cells that are on the edges)  
N of cells = 9  
**N of cells with aggregates = 6**



Check stacks but looks  
like clear colocalization  
(same for all yellow  
signal)

Ring shape

Vesicle like  
structure