PROTEIN LOCALIZATION IN MAMMALIAN CELLS

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OBJECTIVE

- Compare the localization of proteins in mammalian cells
- Cells used:
  - Human embryonic kidney cells and mouse embryonic fibroblast cells
- Proteins used:
  - ZFP568 and GALT

OBJECTIVE CONT.

- Proteins are chains of amino acids determined by DNA
- Significance→ the specific locations of proteins are essential to an organism's proper function
- ZFP568→ basic development
- GALT→ sugar metabolism that affects the development of organs

PREVIOUS KNOWLEDGE

- We knew what type of cells we worked with
- We knew where the proteins were supposed to be located
  - ZFP568→ nucleus
  - GALT→ Golgi body
- We did not know which proteins we worked with specifically

UNKNOWN KNOWLEDGE

- Protein A and Protein B
    - PLAN TO COMPLETE GOAL
- Once placing the proteins into the cells
    - Upon analyzing the cells→ based on the location of the proteins
    - the specific proteins can be identified

MODEL CELL

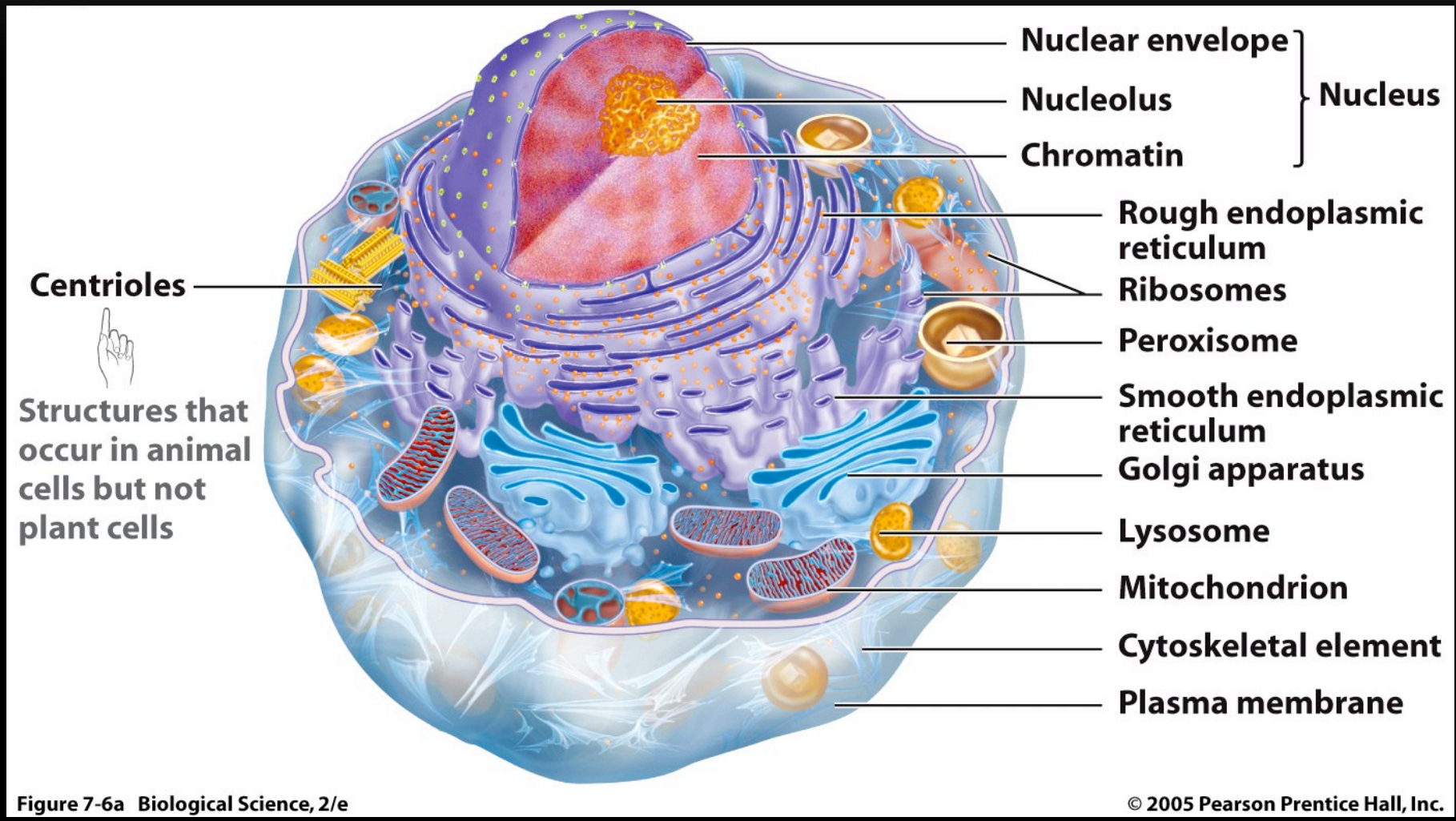

Figure 7-6a Biological Science, 2/e

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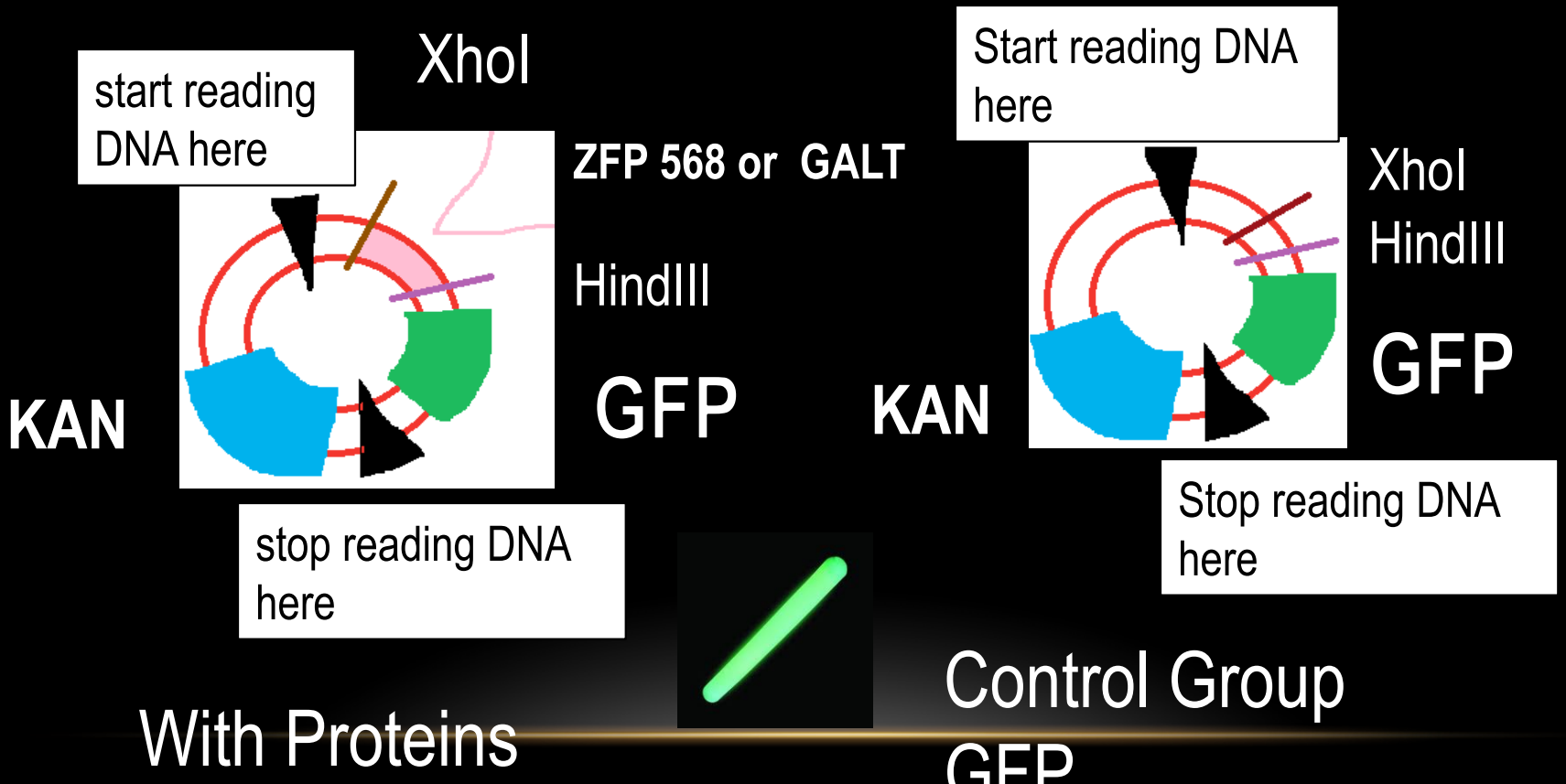
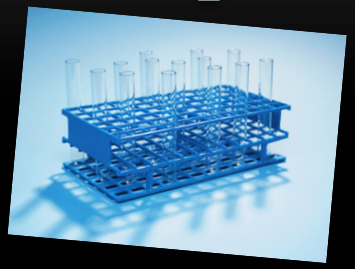
PLASMIDS GALORE

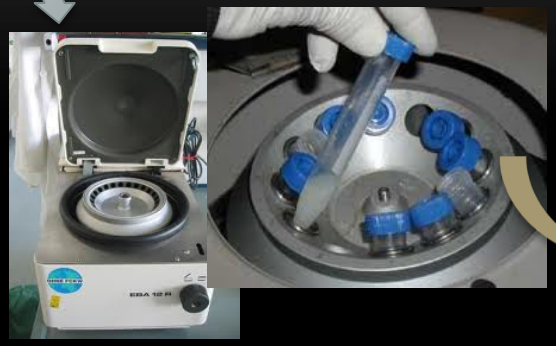
- Circular DNA that contain genes for survival



Extracting and Purifying DNA

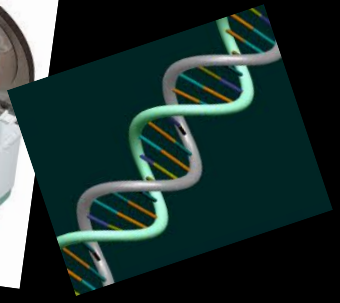


Start

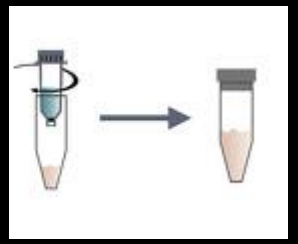


Centrifuge

Lysed



Separate



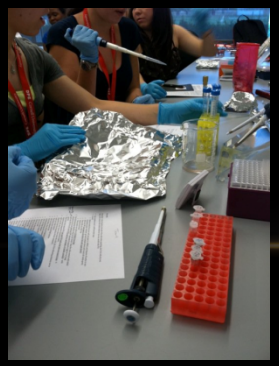
Purify

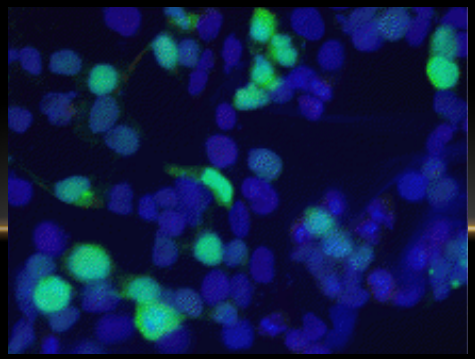
Transfection in Mouse and Human Kidney Cells

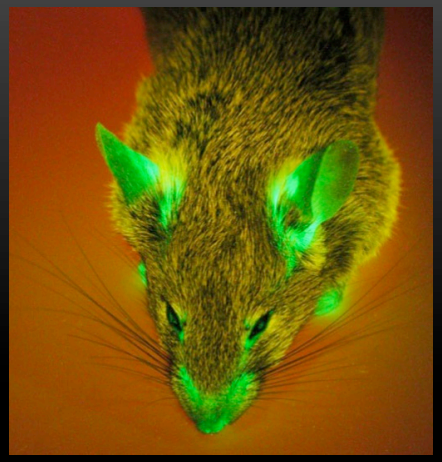
- Transfection: Putting DNA into mammalian cells
- Mix DNA in media with Lipofectamine
  - Allows DNA transportation in a liposome
    - A vesicle made of a phospholipid bilayer
- Cells take up DNA and express the proteins
  - GFP-alone
  - GFP-ZFP568
  - GFP-GALT

WHY NIH3T3 AND HEK293T?

- NIH3T3 Cells (Mouse Embryonic Fibroblasts)
  - Have more cytoplasm
- HEK293T Cells (Human Embryonic Kidney)
  - Good for transfection
- Commonly used by scientists; easy to transfect
- Mislocalization of proteins commonly signify disease
  - Ex: Cystic Fibrosis (CF)



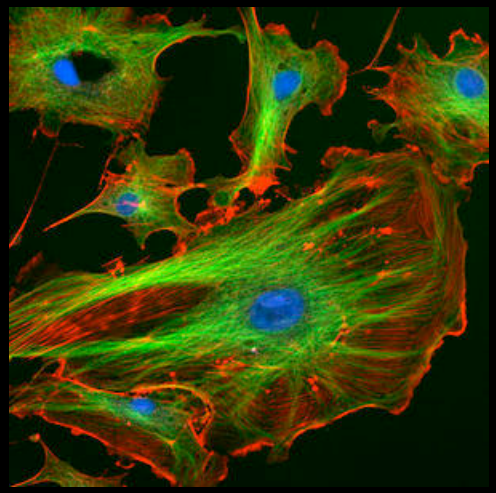




THE MAGIC OF STAINING

We stained each sample with three different stains:

1. GFP (Green Fluorescent Protein)
2. DAPI (Blue, stains nuclei)
3. Phalloidin (Red, stains cytoskeleton)

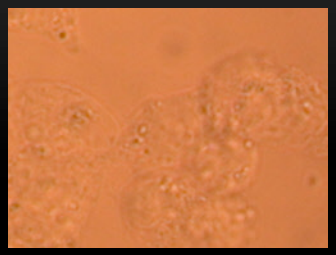
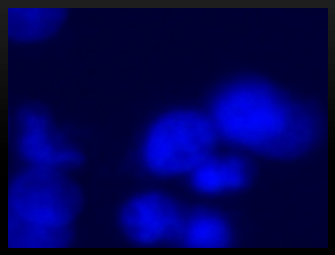
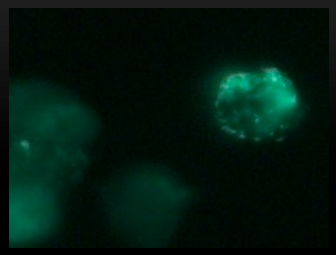
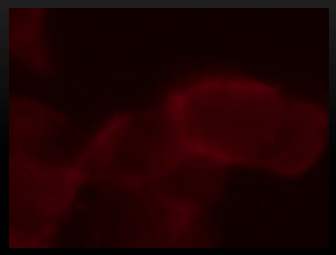
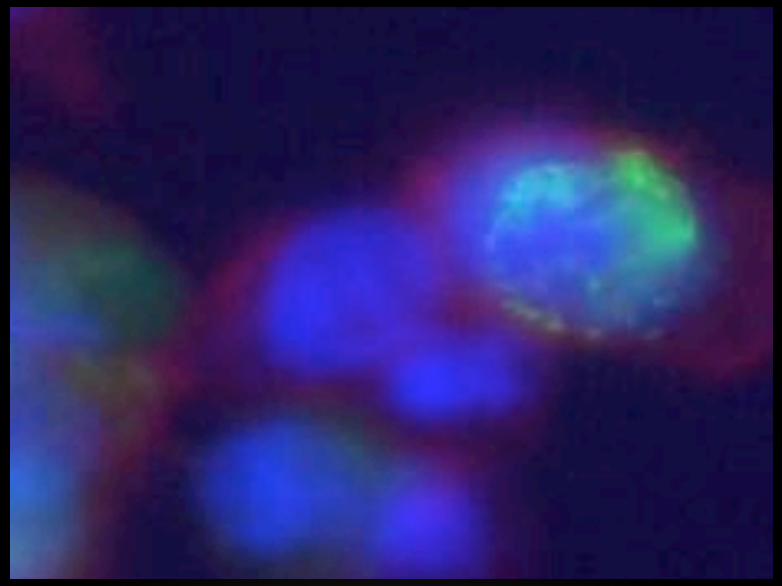


HOW DID WE DO IT?

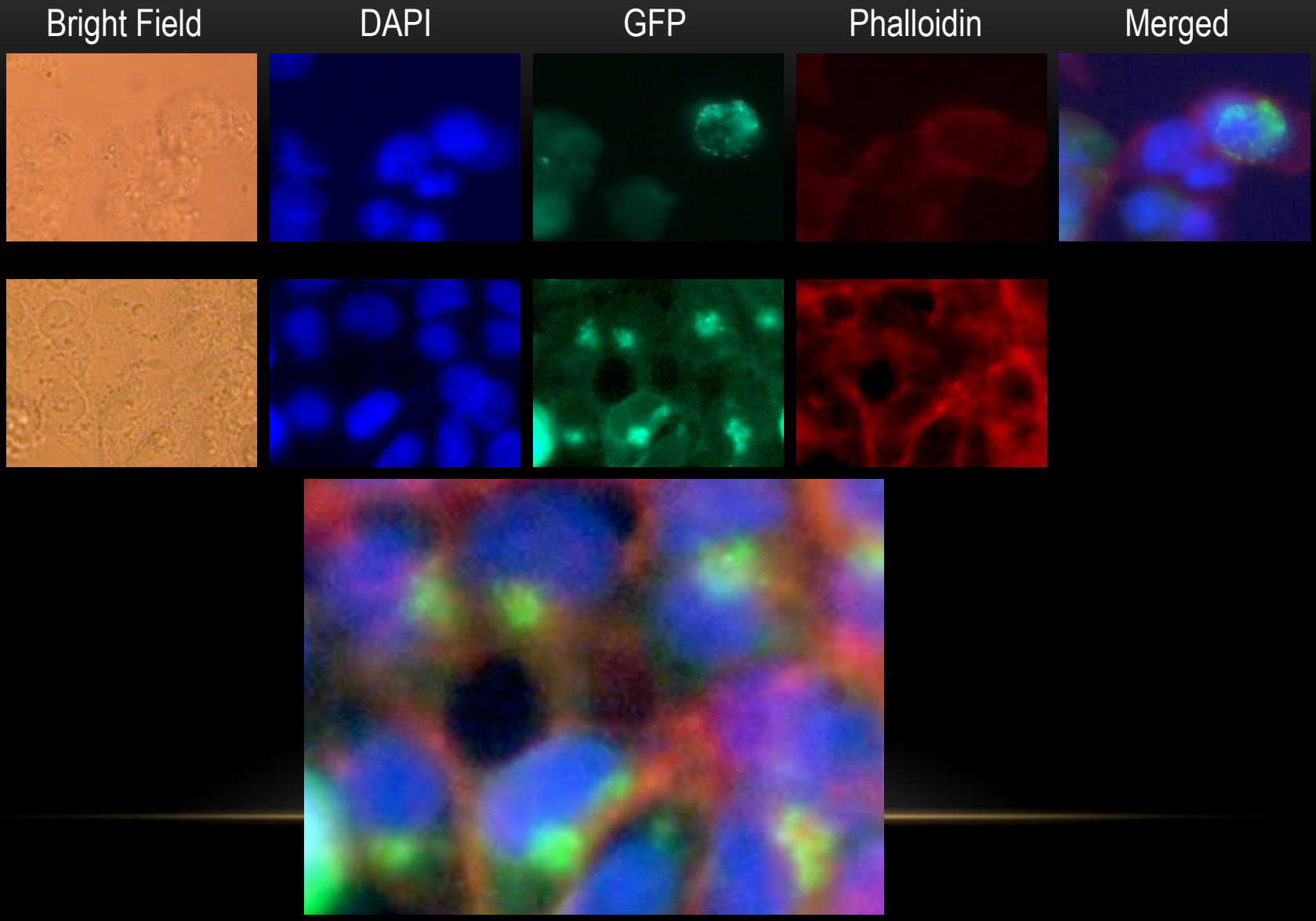
- We used special pipetting techniques to stain cell structures

- Kept cells covered from light and exposure to air
- Secured with cover slip

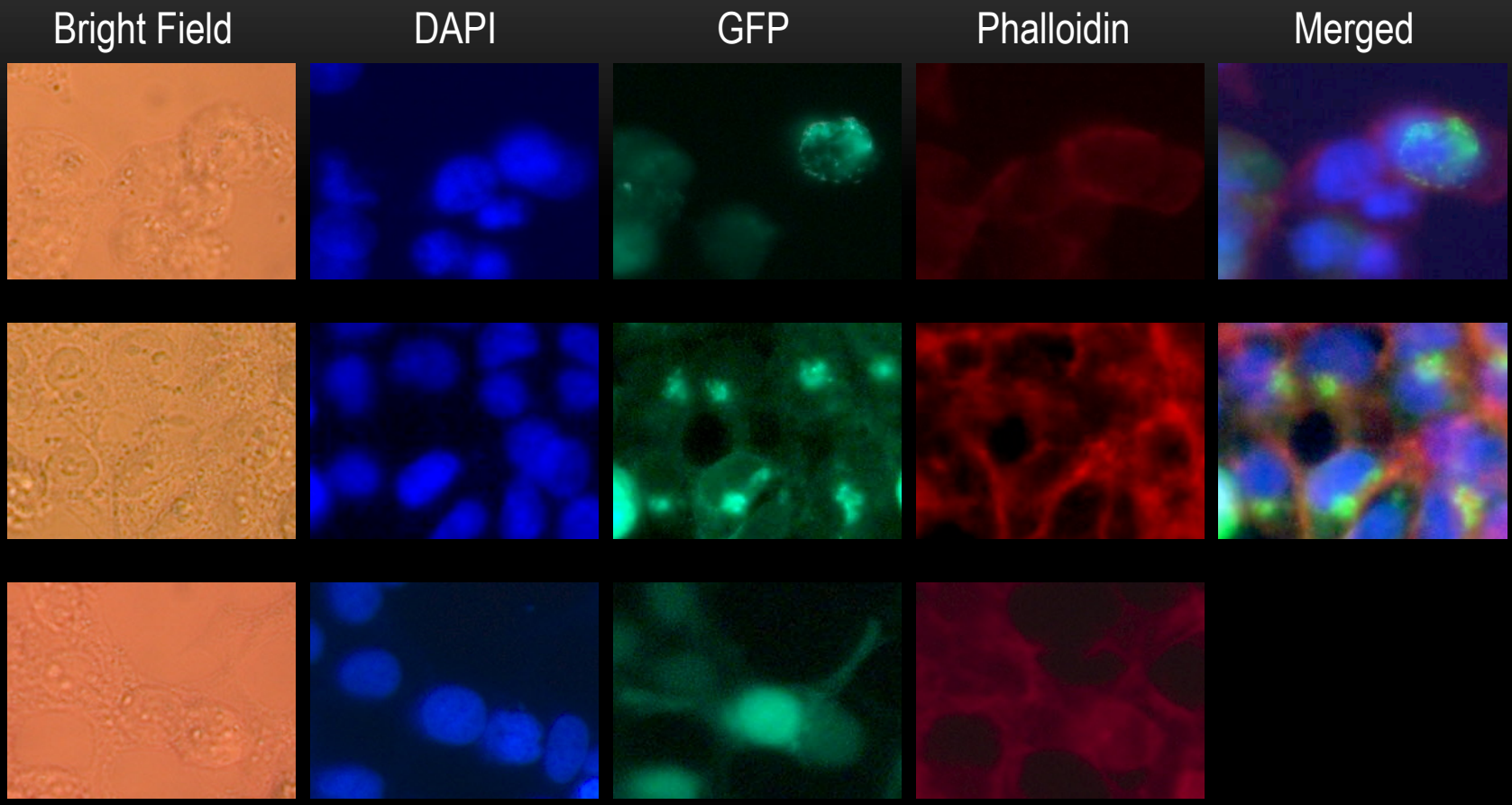
HEK293T Cells

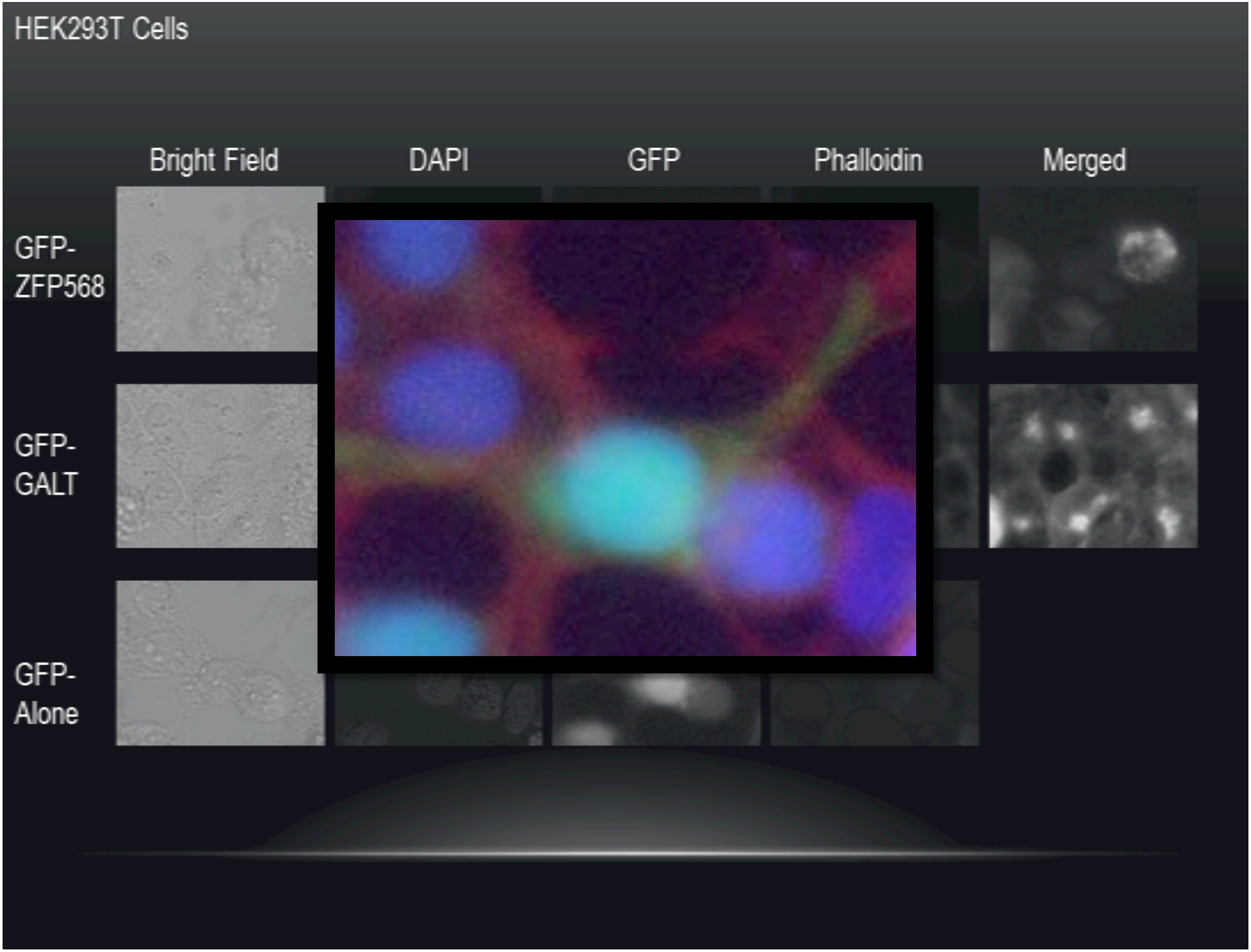
	Bright Field	DAPI	GFP	Phalloidin	Merged
GFP-ZFP568					

HEK293T Cells

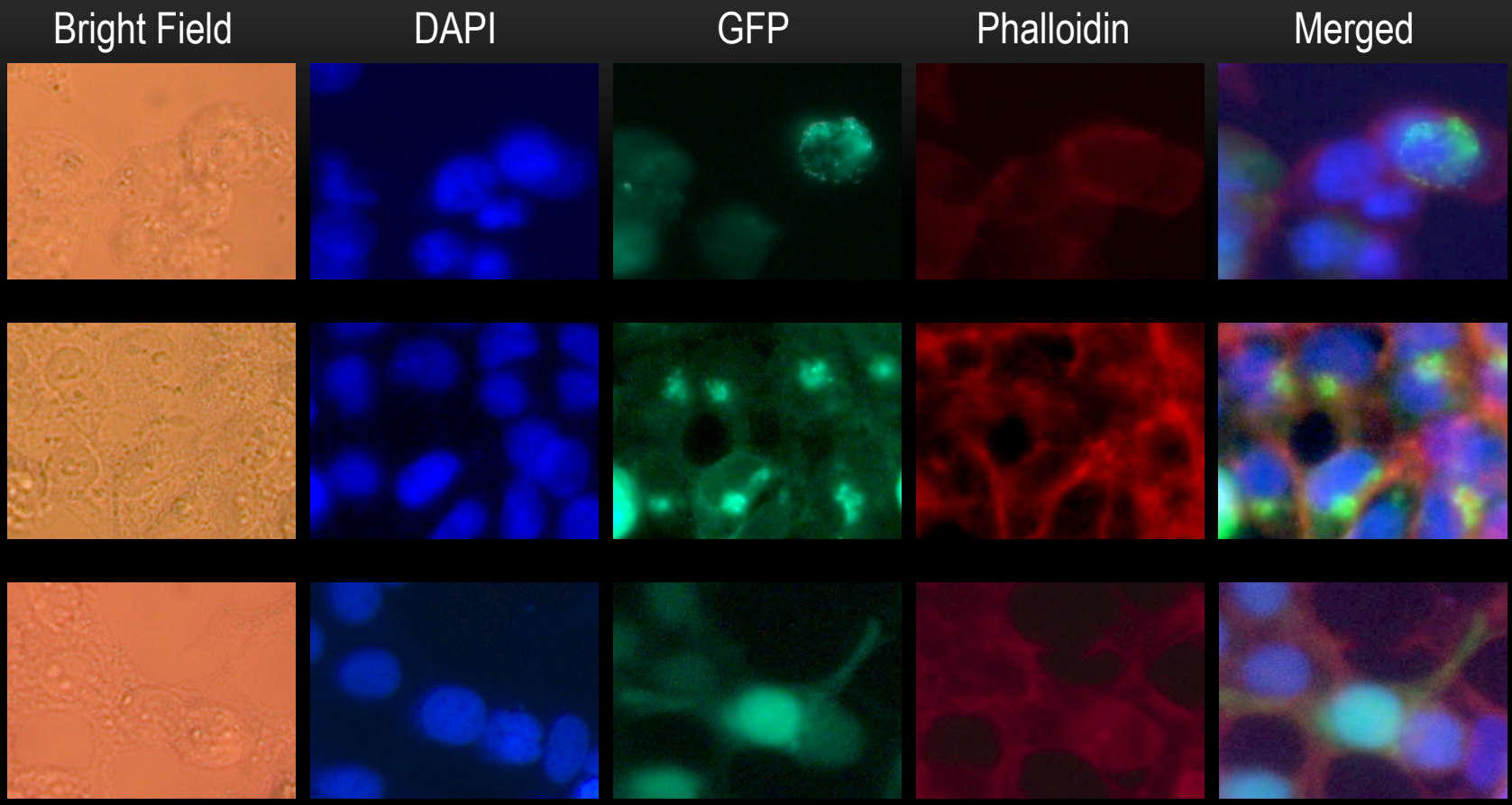


HEK293T Cells

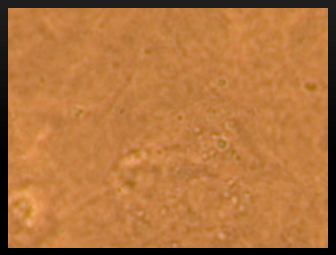
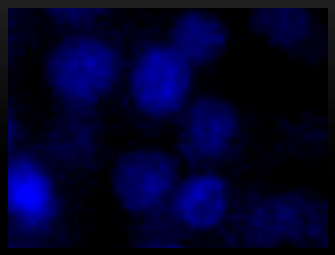
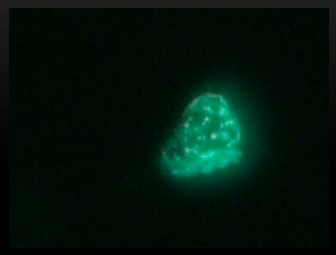
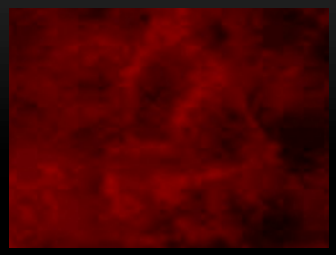
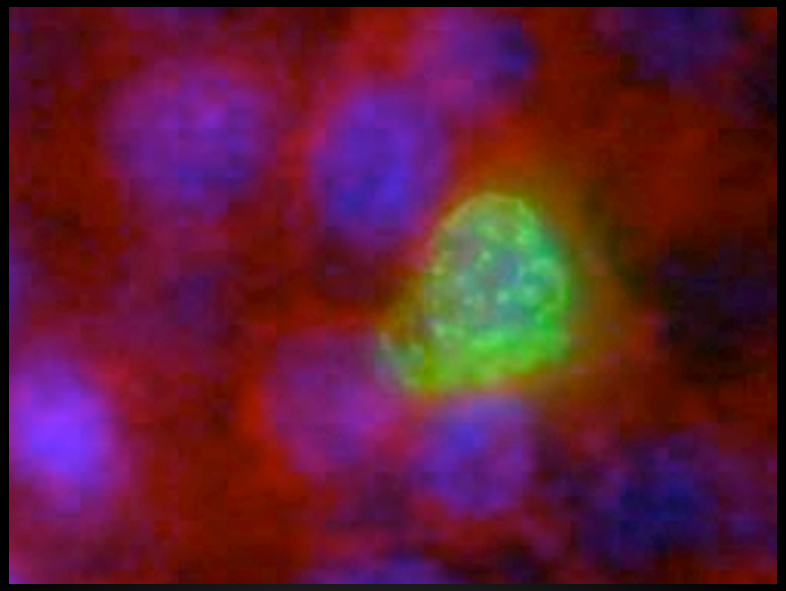



HEK293T Cells
Bright Field
DAPI
GFP
Phalloidin
Merged
GFP-
ZFP568
GFP-
GALT
GFP-
Alone

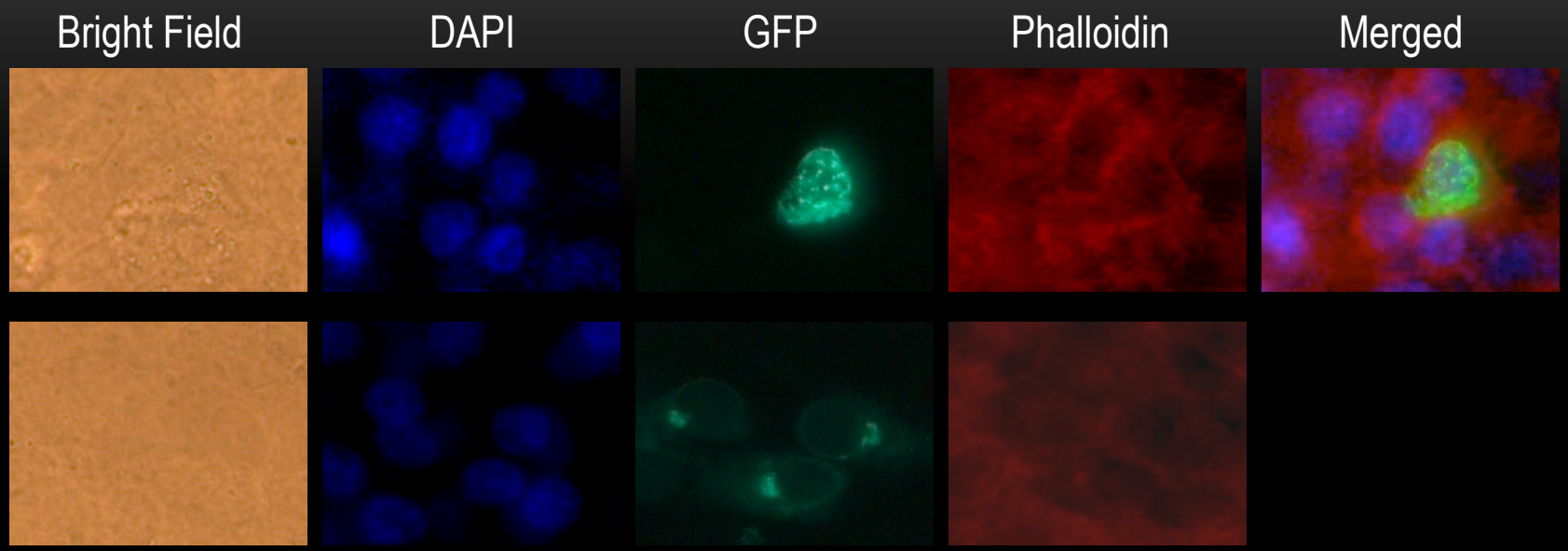
HEK293T Cells

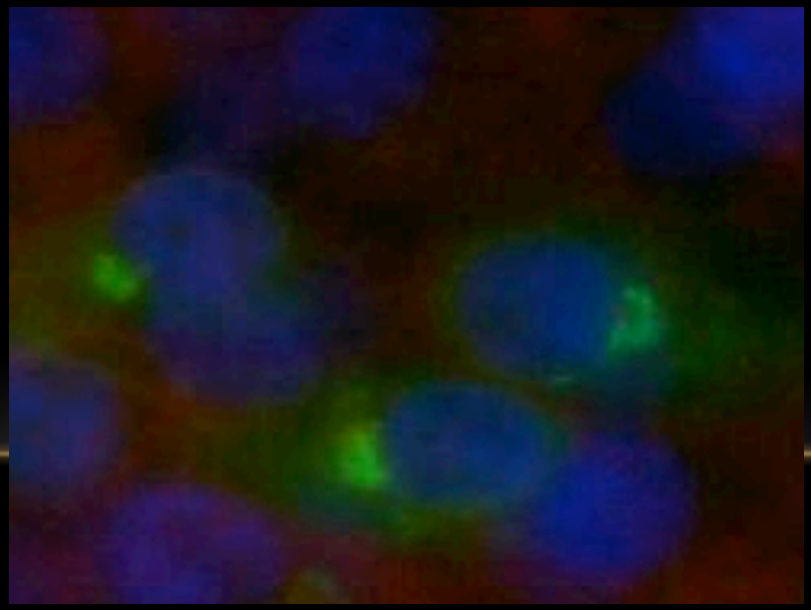


NIH3T3 Cells

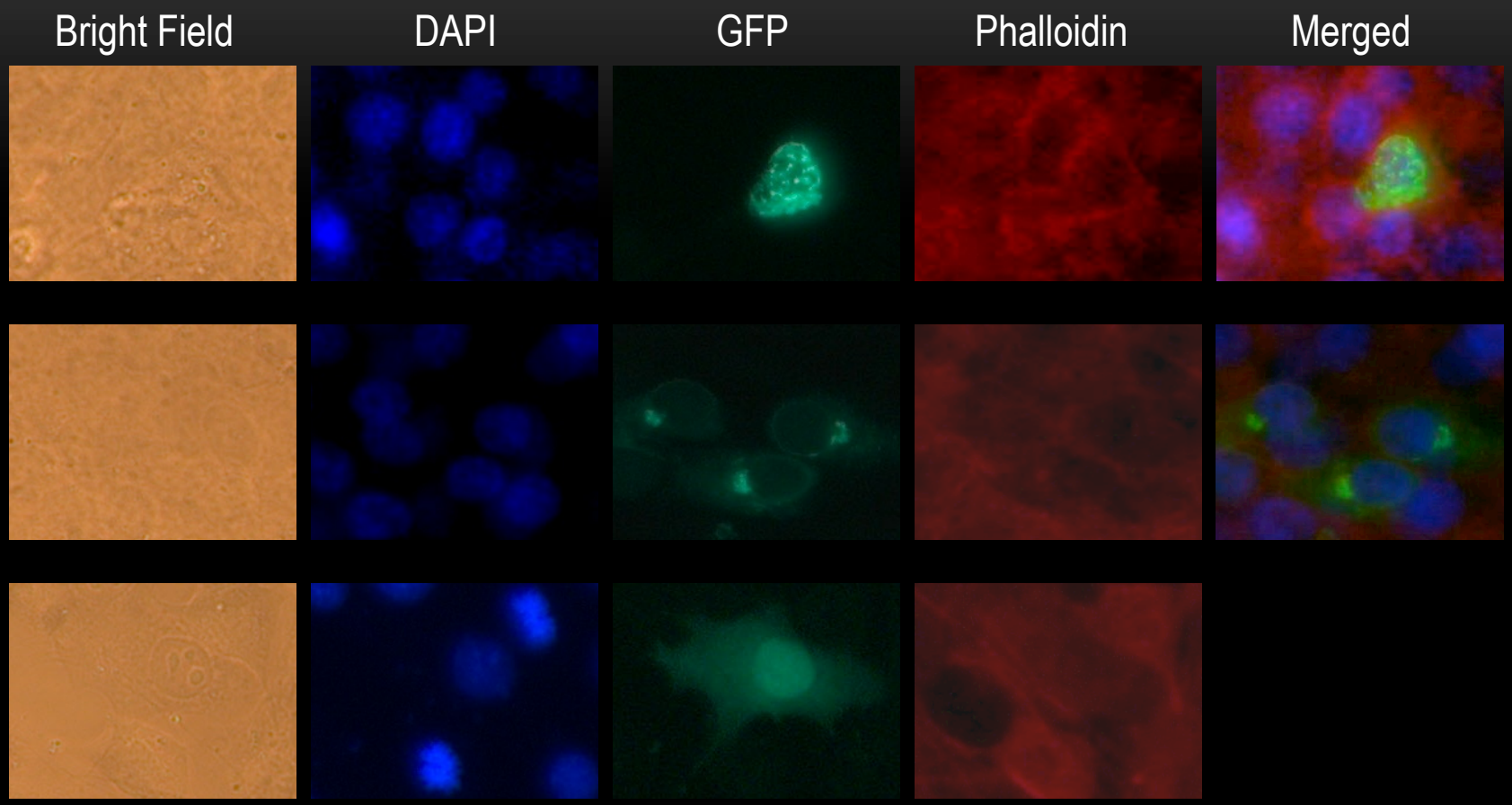
	Bright Field	DAPI	GFP	Phalloidin	Merged
GFP-ZFP568					

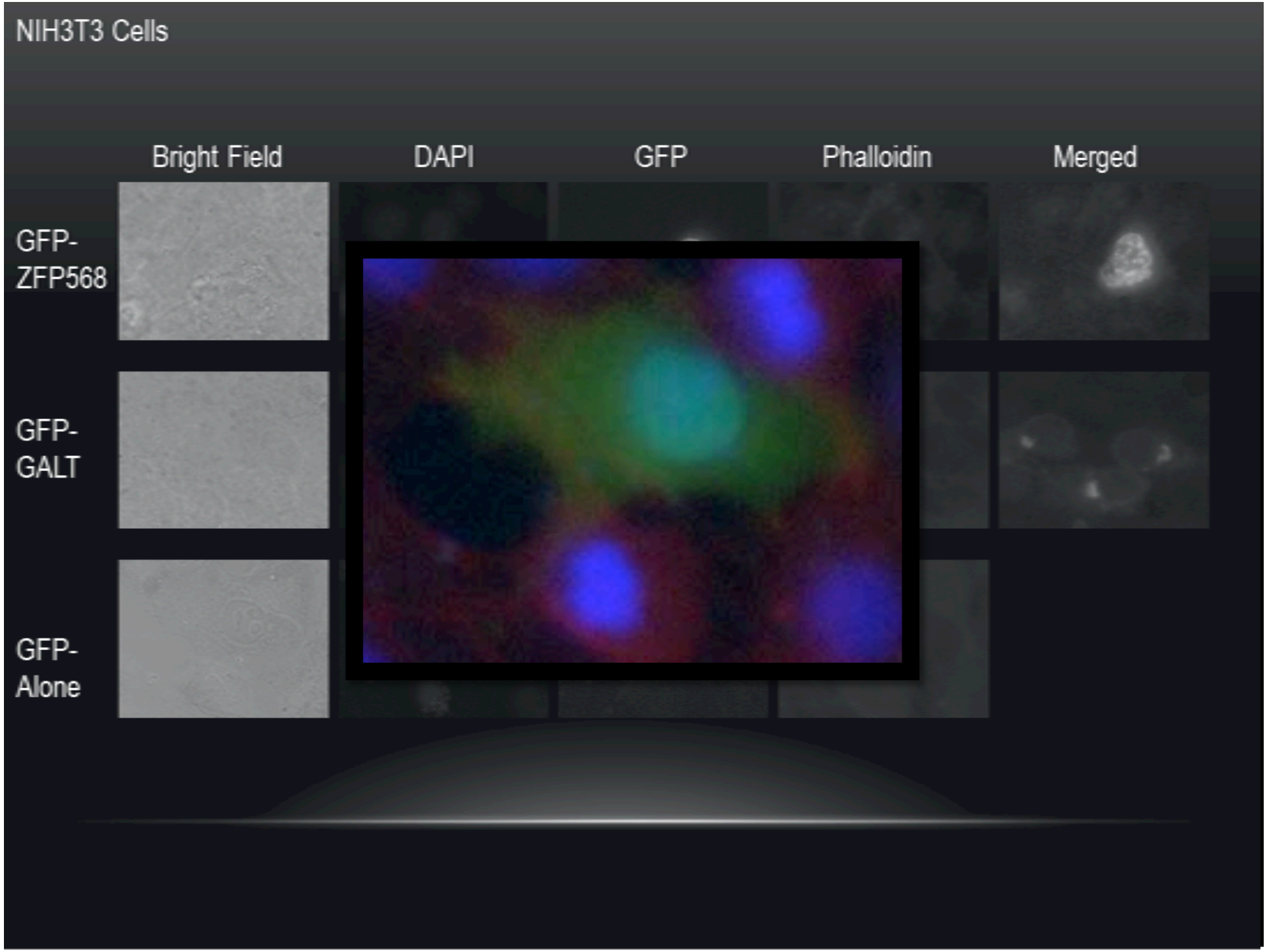
NIH3T3 Cells





NIH3T3 Cells

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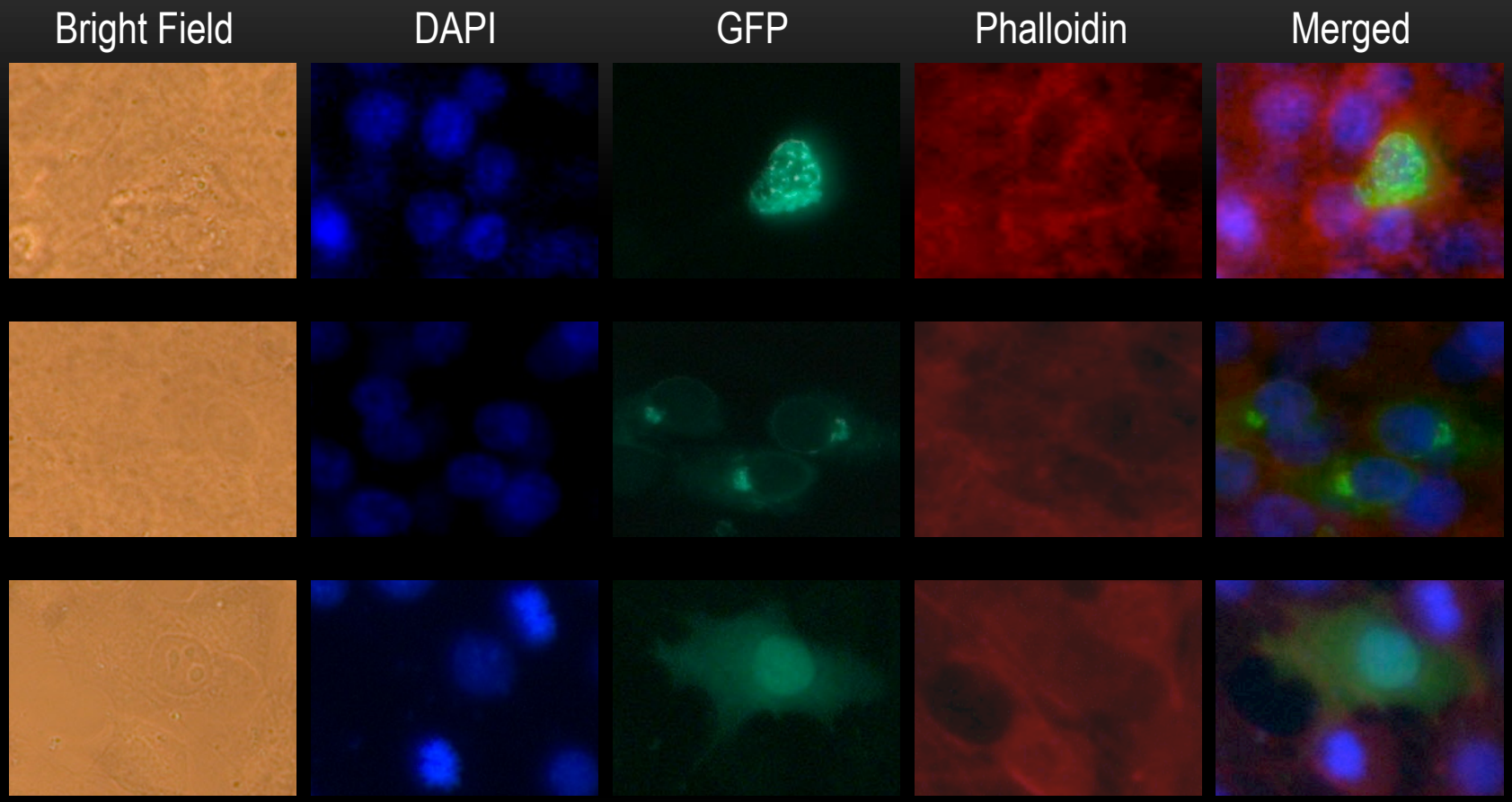
NIH3T3 Cells



SIGNIFICANCE

- The Zinc Finger Protein 568 affects the transcription of DNA in the nucleus

- Galt affects the sugar metabolism and the Golgi in the cell

So why is it important?

- Mislocalization of protein leads to diseases and its important for scientific understanding

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