

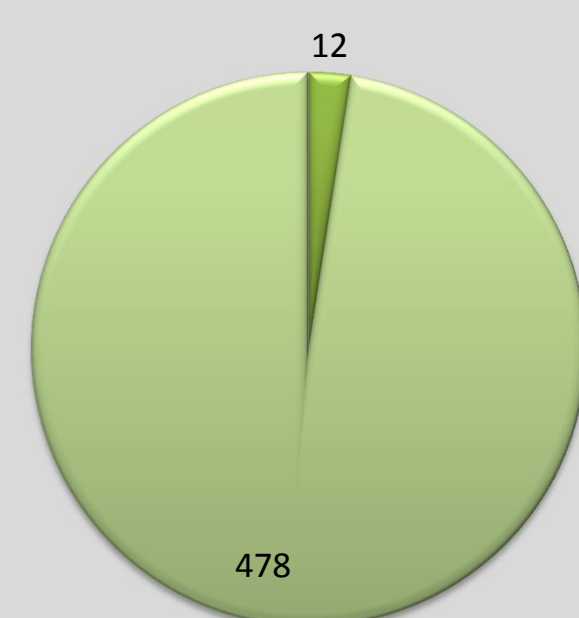
## Screening of the publicly available homozygous viable *Drosophila Minos* and *piggyBac* element insertion collections for sterility

The basic principles of sperm structure and the differentiation of the individual spermatid components are conserved across the whole animal kingdom including *Drosophila melanogaster*. Our aim was to identify new genes involved in the early (gonial mitosis) and late stages of spermatogenesis (meiotic division, spermatid elongation and individualization), and describe the precise molecular function of these proteins. The advent of collections of precisely mapped transposon element insertion lines, which are available for almost each gene, allows us to identify male sterile mutations without new mutagenesis experiments. The „semi-lethal” lines of the collections provide an enriched source of sterile mutations. We used P element, *piggyBac* and *Minos* element insertion lines, and categorized them based on their insertion site (exon, intron) and the mRNA expression profile. Fertility tests were

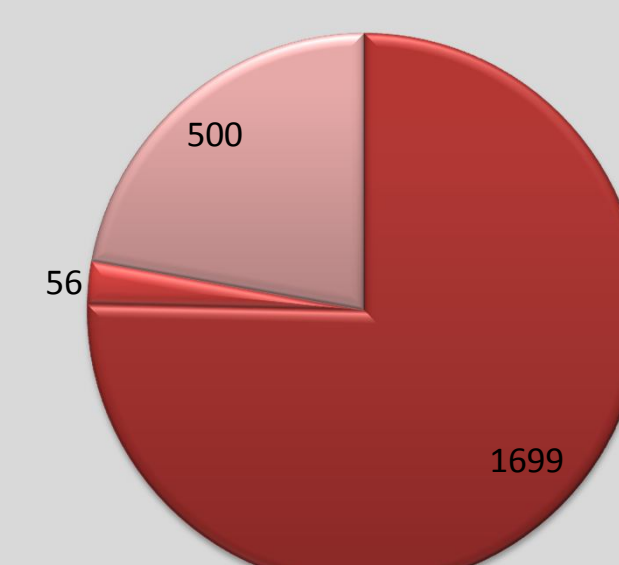
started on homozygote males and females of exonic insertions in genes which have high expression in testis or ovary. We tested approximately 1000 lines and found 26 male or female sterile lines.

### Preliminary experiment

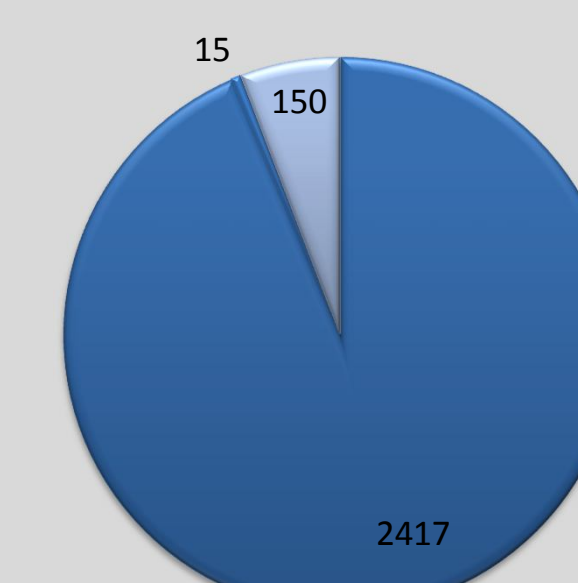
3rd chromosome P element collection fertility screen (Department of Genetics, University of Szeged).



P-element sterile  
P-element fertile



piggyBac lines viable  
piggyBac lines semi-lethal  
piggyBac lines lethal

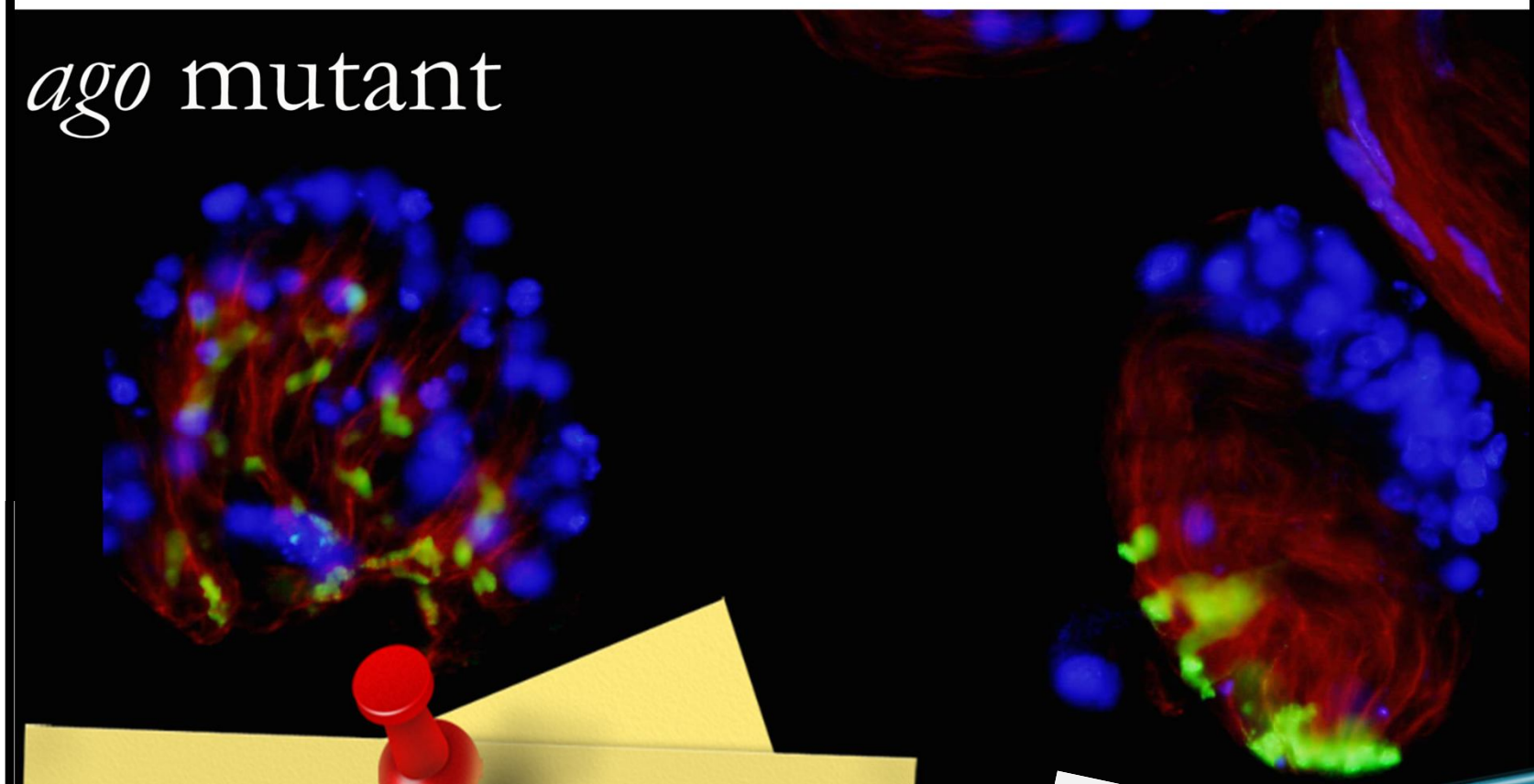
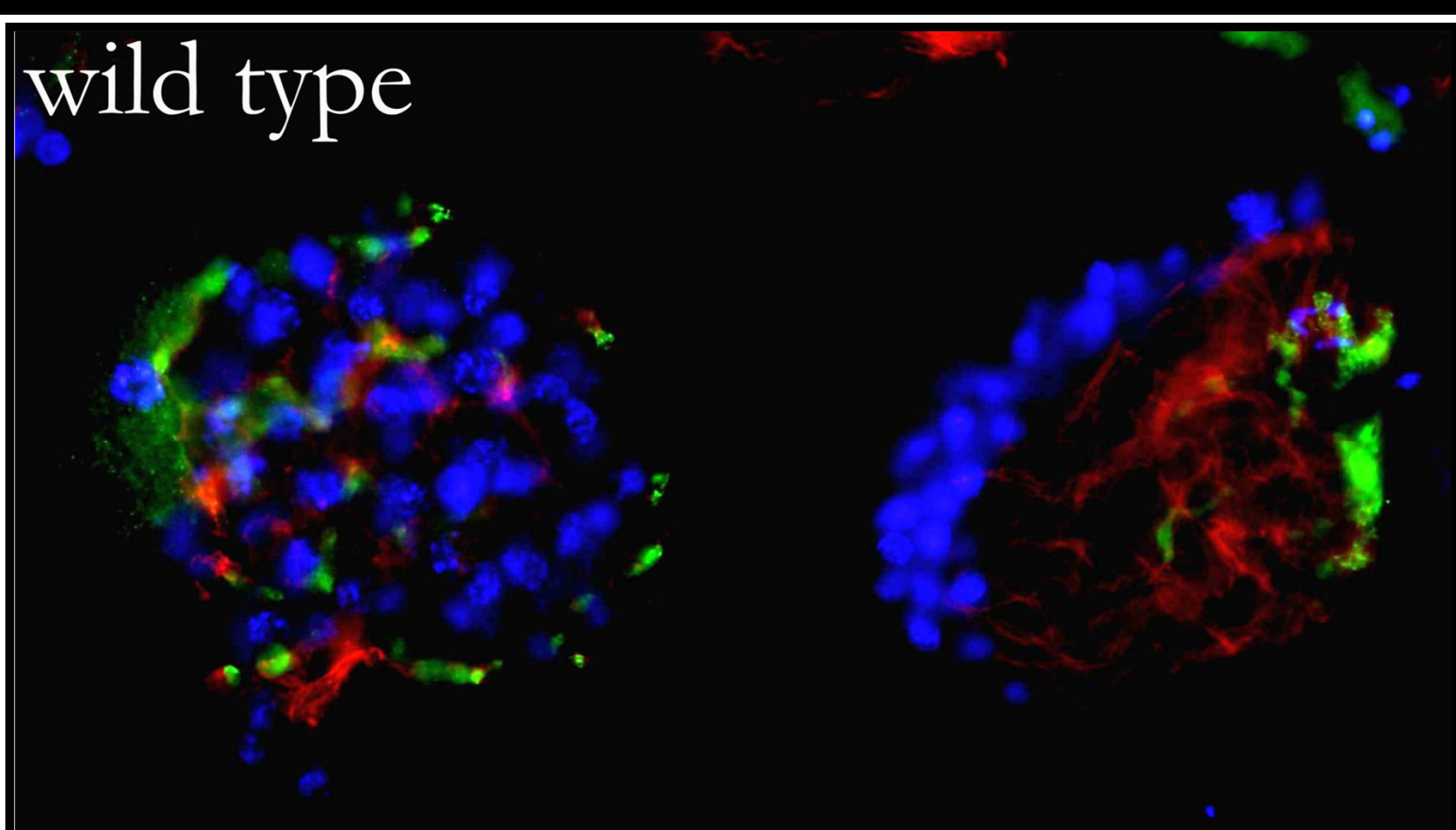


Minos lines viable  
Minos lines semi-lethal  
Minos lines lethal

### Bloomington Stock

*In silico* screen for:

- Viable mutants, segregating over balancer chromosome
- Insertion in exon (or UTR)
- High expression profile in testes or ovaries



### Early stages

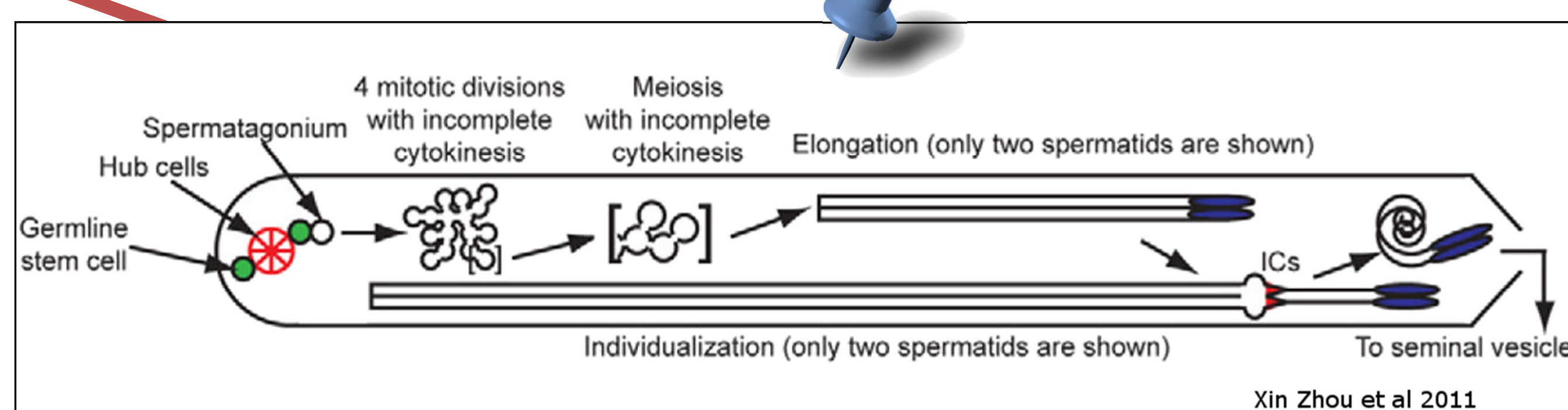
The early stages of spermatogenesis are not affected in the candidates.

Spectrin – ring canals in early stage, proximal end of elongating spermatids  
Phalloidin – actin  
DAPI – nuclei

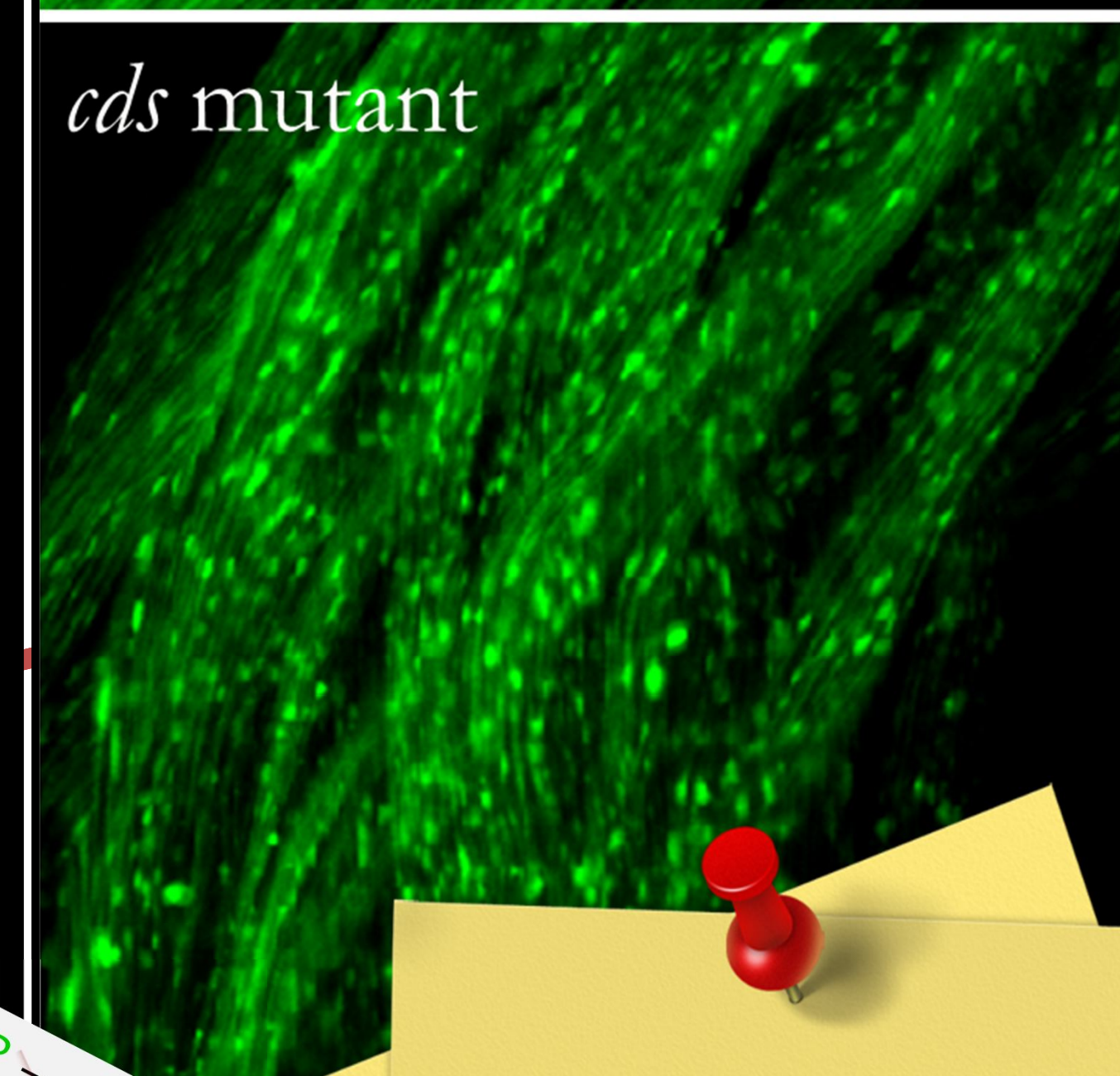
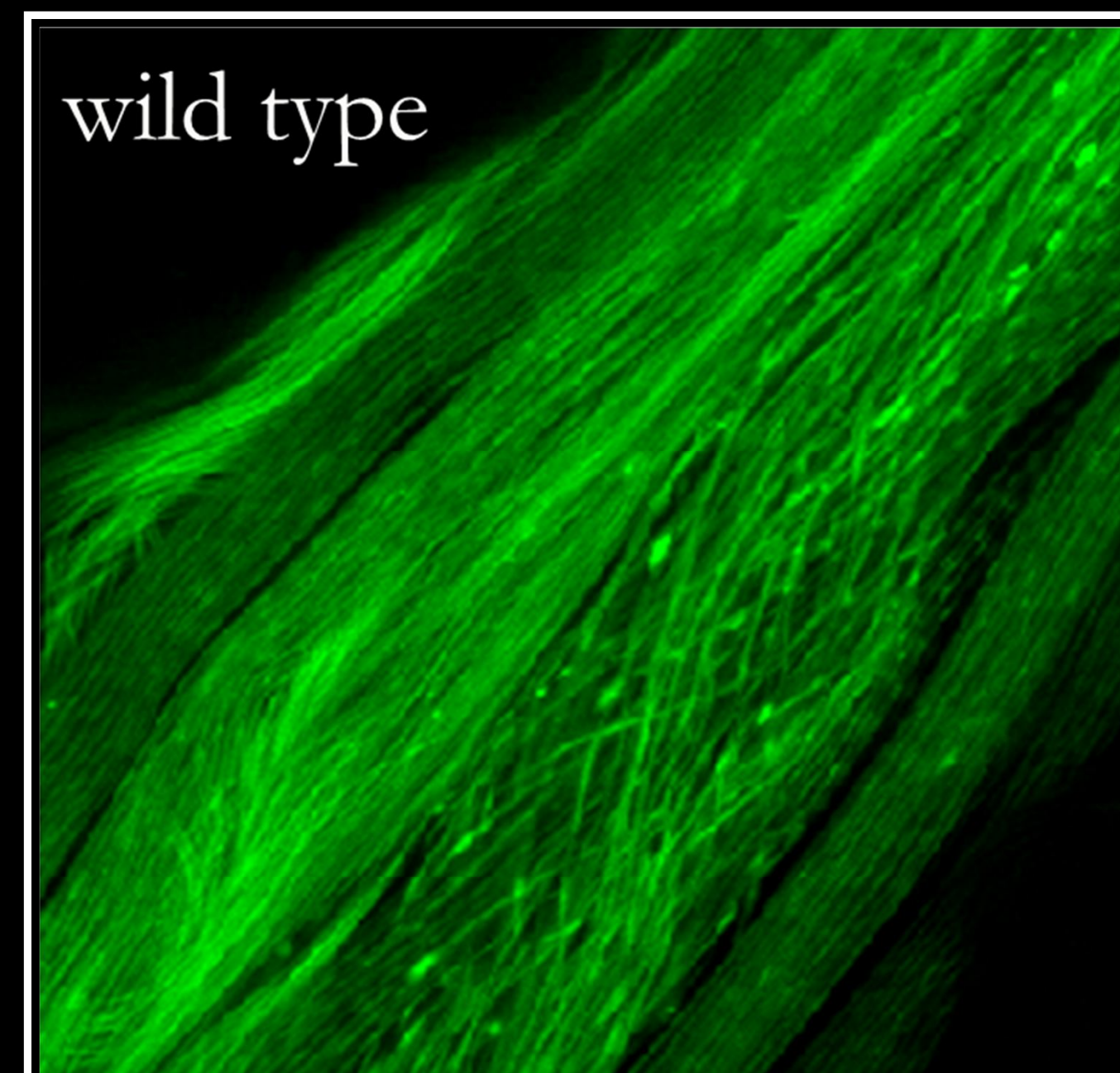
## Candidates

Gene	Function	Male	Female	hemizygote ♂	hemizygote ♀
CG7052	peptidase inhibitor activity	sterile	fertile	sterile	fertile
S-Lap3	sperm-leucylaminopeptidase 3	sterile	fertile	sterile	fertile
Caliban	unknown	sterile	fertile	sterile	fertile
CG3581	unknown	sterile	fertile	sterile	fertile
CG4434	glutamate dehydrogenase activity	sterile	fertile	sterile	fertile
cdsA	phosphatidate cytidyltransferase	sterile	fertile	sterile	fertile
ago	E3 ubiquitin ligase	sterile	fertile	sterile	fertile
CG32436	unknown	sterile	fertile	sterile	fertile
CG6752	zinc ion binding	sterile	fertile	sterile	fertile
CG2046	unknown	sterile	fertile	sterile	fertile
CG17118	DNA binding transcription factor activity	sterile	fertile	sterile	fertile
CG31752	unknown	sterile	fertile	sterile	fertile
Dys	neuromuscular synaptic transmission	sterile	fertile	sterile	fertile
CG6332	unknown	sterile	fertile	sterile	fertile
Pi4K1α	1-phosphatidylinositol 4-kinase	sterile	fertile	sterile	fertile
CG6931	unknown	sterile	fertile	sterile	fertile
CG4329	unknown	sterile	fertile	sterile	sterile
CG7716	structural constituent of cytoskeleton	sterile	fertile	sterile	fertile
CG5342	GPI anchor biosynthetic process	fertile	sterile	fertile	sterile
CG12984	unknown	fertile	sterile	fertile	sterile
Mig-2-like	GTPase activity	fertile	sterile	fertile	sterile
CG14238	unknown	fertile	sterile	fertile	sterile
CG42575	sodium-dependent phosphate transporter	fertile	semi-sterile	fertile	sterile
CG9706	acetyl-CoA transporter activity	fertile	sterile	fertile	sterile

Male sterile  
Male Sterile - malfunction in individualisation  
Female sterile



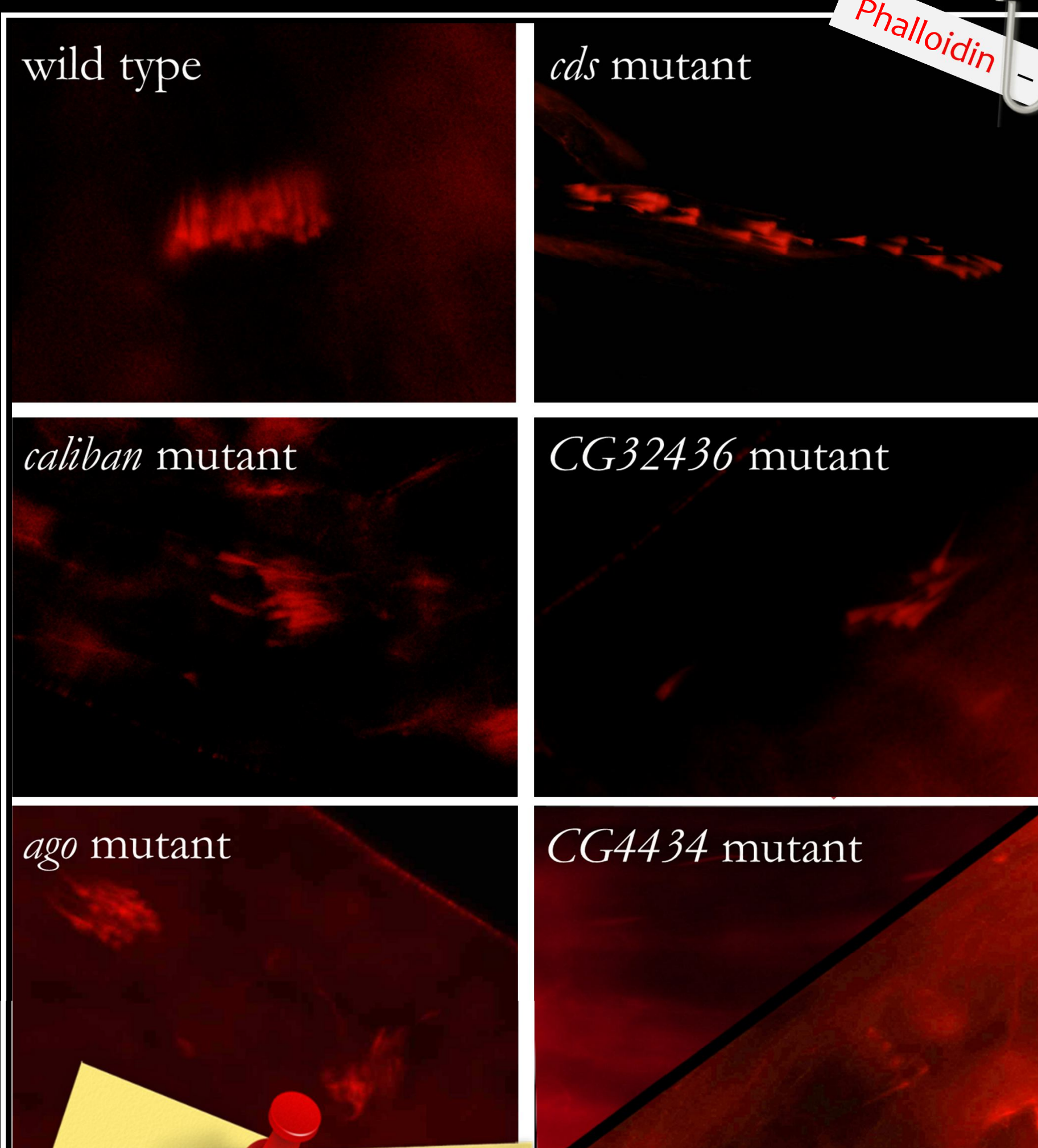
Xin Zhou et al 2011



Don Juan-GFP – elongated mitochondria

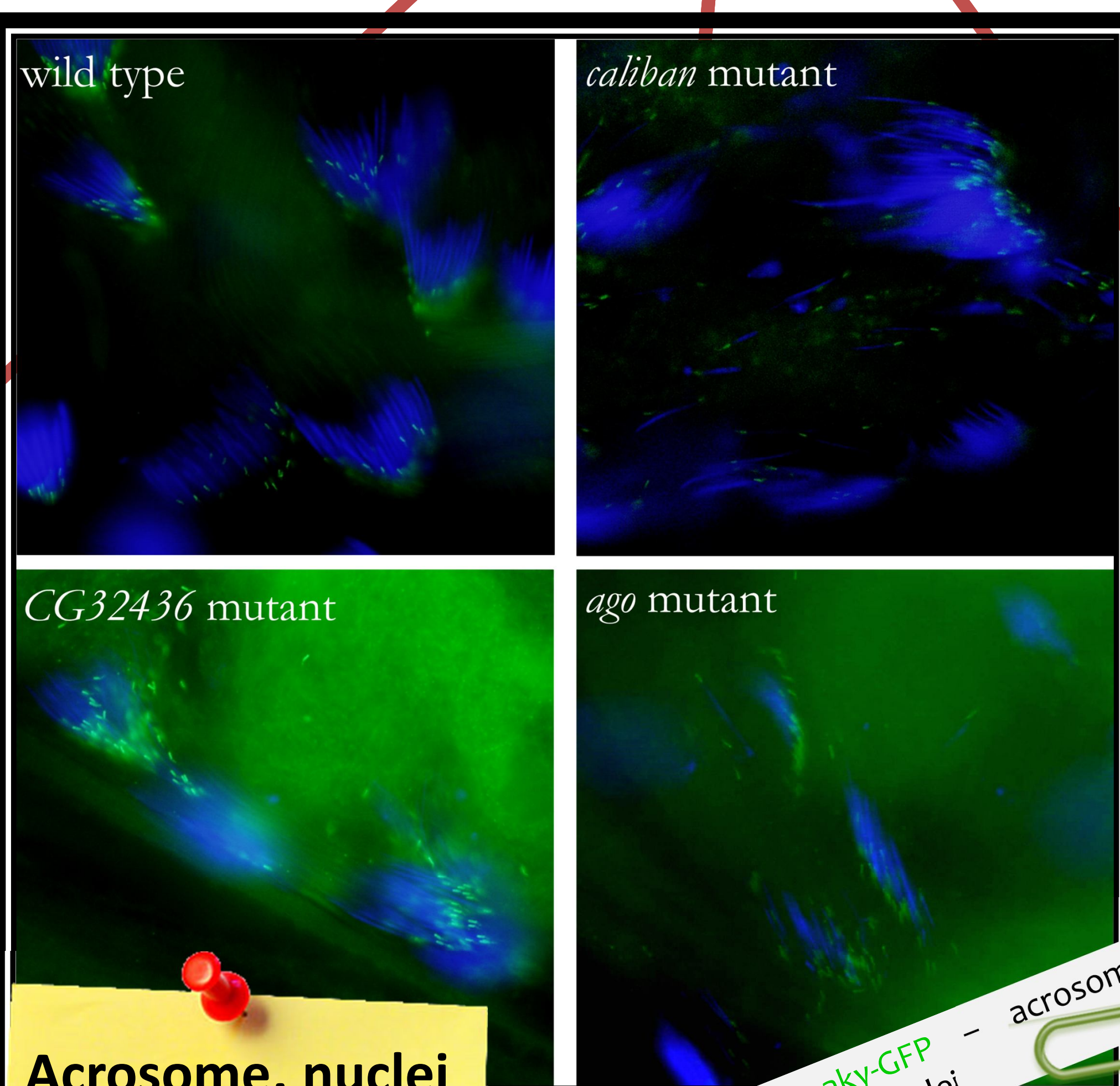
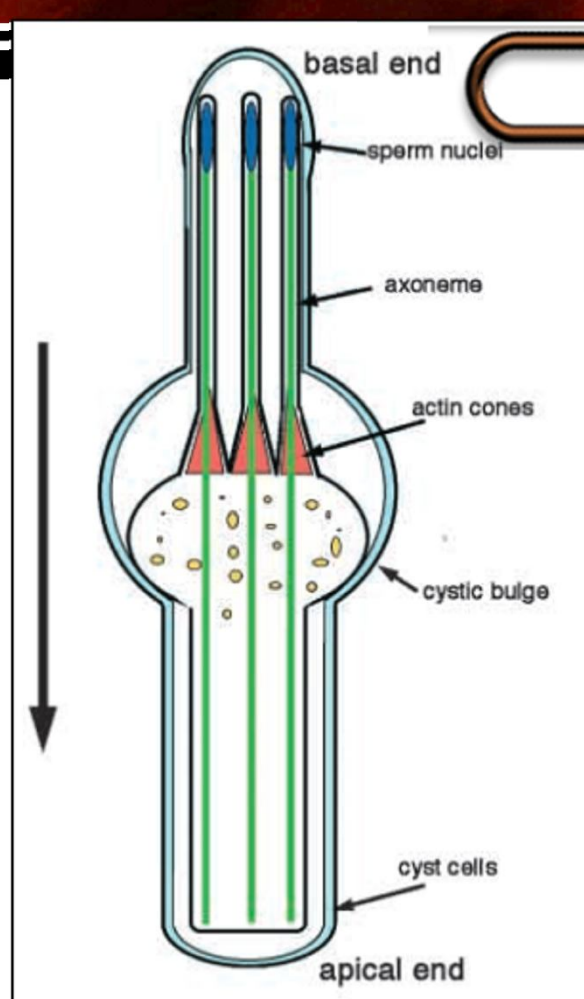
### Mitochondria

With Don Juan-GFP we can visualize the mitochondria of the elongated sperm.



### Actin Cones

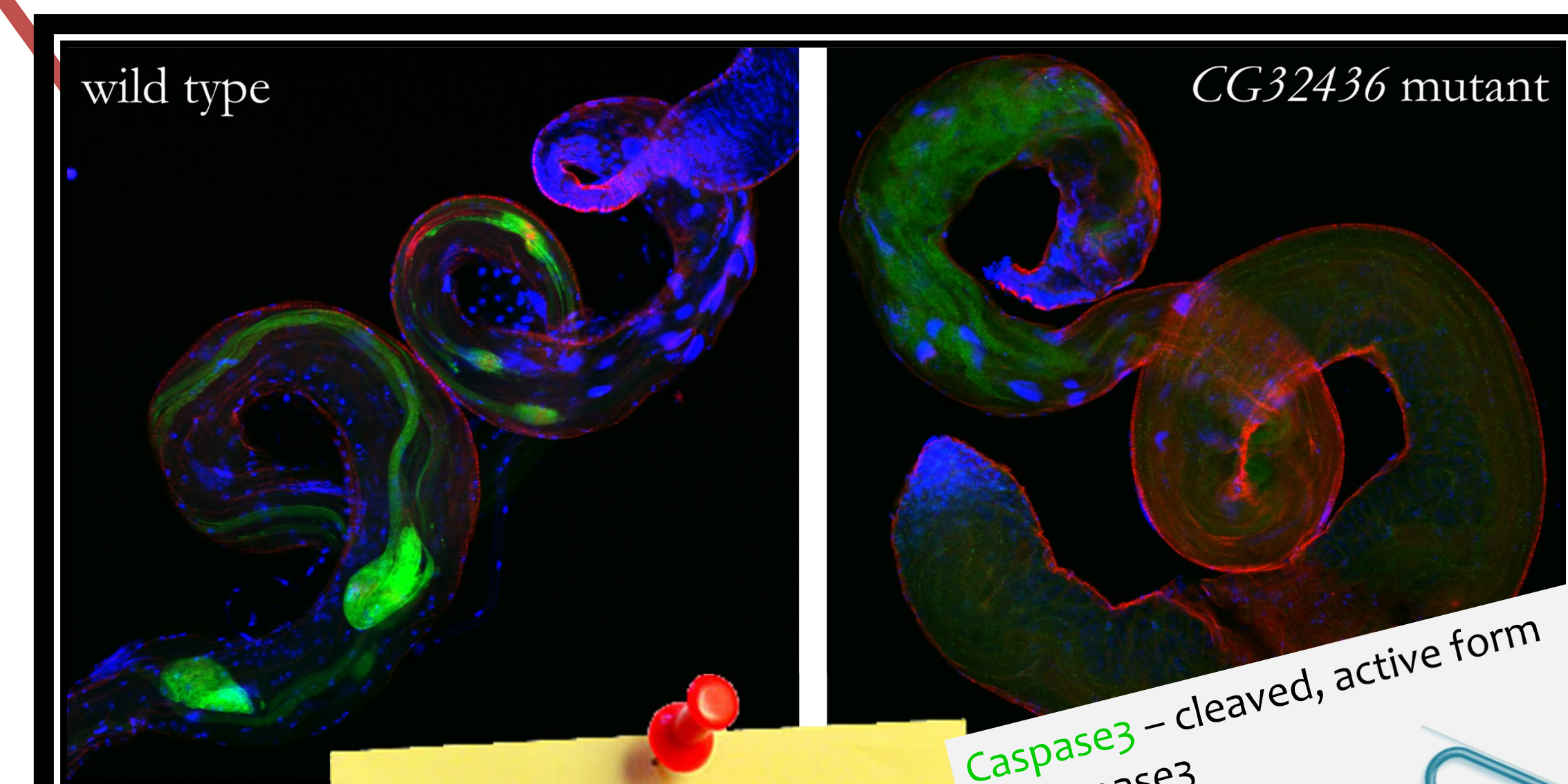
Many of our candidates showed disruption of investment cone, which suggests that the process of individualization is affected.



### Acrosome, nuclei

The position of the nuclei shows if the cyst is disoriented. Sneaky-GFP transgene shows that the acrosomes are formed.

Sneaky-GFP – acrosome  
DAPI – nuclei



### Caspase3

Active Caspase3 pathway plays role in the process of individualization. It is independent from actin cone formation.

Caspase3 – cleaved, active form of Caspase3  
Phalloidin – actin  
DAPI – nuclei

## Summary

We identified 26 male or female sterile transposon insertion mutants. Nine lines showed malfunctions at the later stages of spermatogenesis, spermatids are elongated, but not individualized. After the phenotypical characterization of the candidates we plan a detailed cellular and molecular analysis.