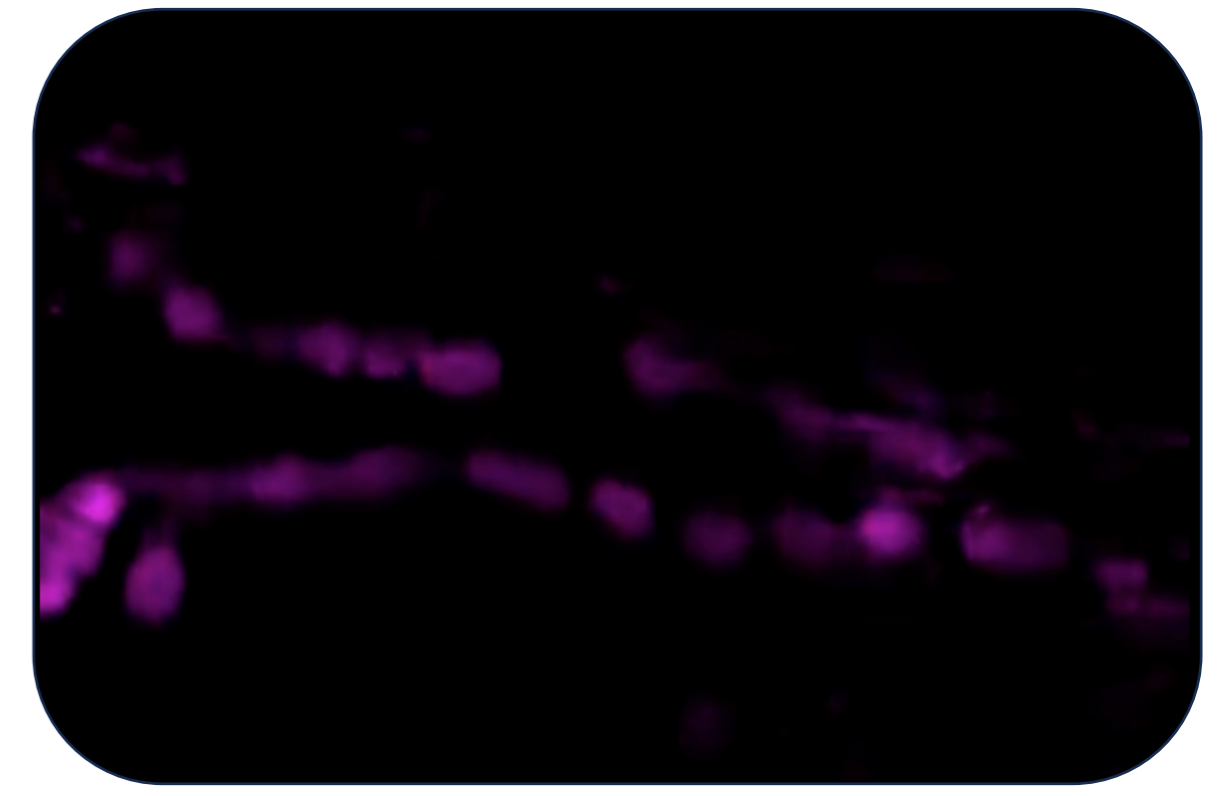


MOL 231: Cubulin: The Body's Molecular Sieve

Siril Meling Foss, Villó Balázs, Fergal O'Farrell

Background

In flies, structures in the **pericardial (PC) cells** of the heart filtrate the blood¹. Flies with less PC cells seem to be aging faster and being slower to break down misfolded proteins². To check if Cubulin, a scavenger receptor, is relevant for blood filtration of these proteins, its gene was knocked down by RNA interference (RNAi). The *Drosophila* fly was injected with a fluorescent dye tagged to a protein aggregate that will be scavenged. This made the break down process visible through a fluorescence microscope. The blood filtering capacity of the Cubulin deficient PC cells was observed at different time points and compared to wild type (wt) *Drosophila* and *Drosophila* with an induced block in dye uptake via RNAi of dynamin as positive and negative controls.



Endocytosis occurs only at membrane buds that contain both dynamin and the appropriate receptor.

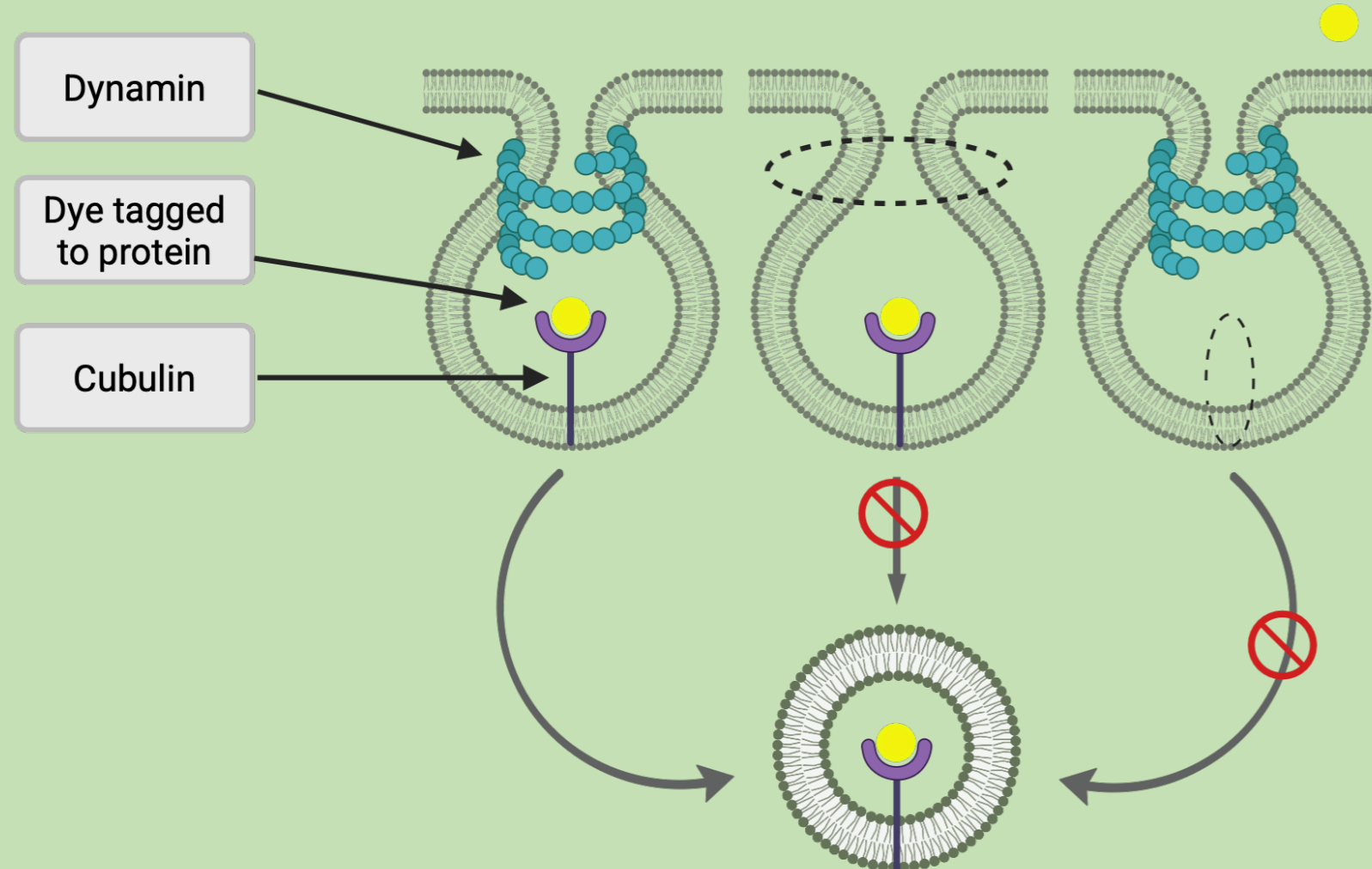


Figure 1 Endocytosis
Illustration made in Biorender

Cubulin is important to bind cargo and thought to signal to dynamin to drive membrane scission to form a vesicle. In wt PC cells this results in dye uptake and accumulation in lysosomal compartments. When dynamin is depleted but Cubulin is present, the cargo accumulates in membrane invaginations at the cell surface but cannot be internalized, as vesicle scission is impaired. In contrast, when the cubulin receptor is absent, the cargo cannot bind to the membrane and remains in the extracellular space.

Methods

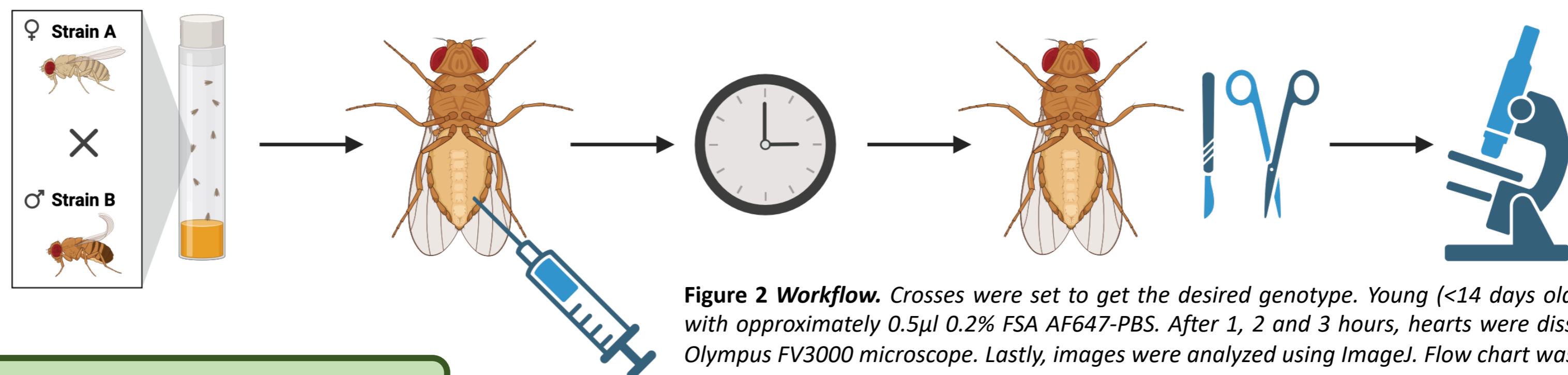
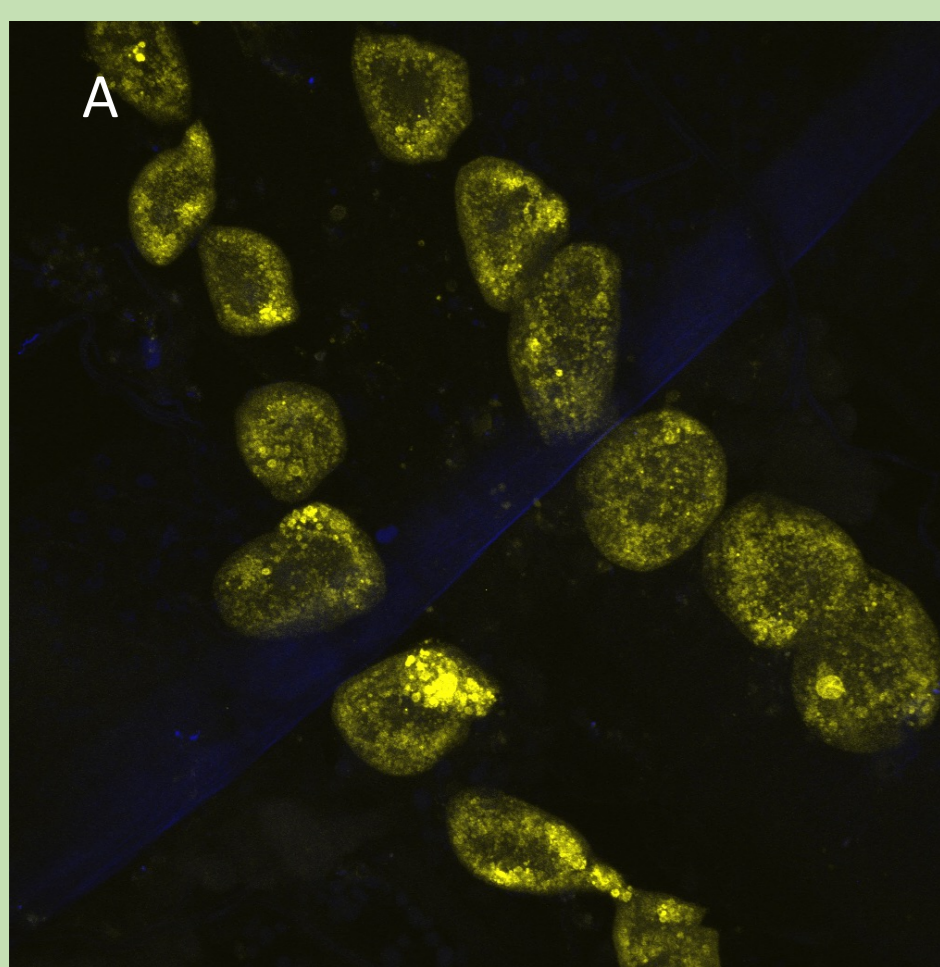


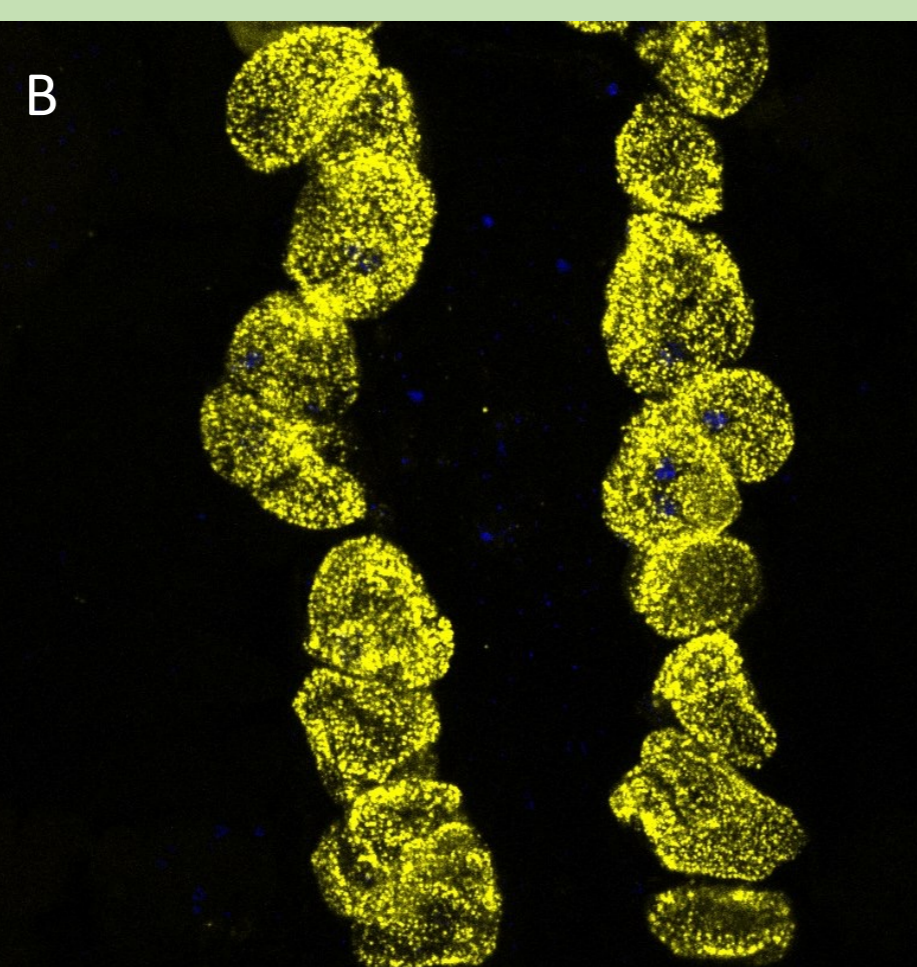
Figure 2 Workflow. Crosses were set to get the desired genotype. Young (<14 days old) female flies were injected with approximately 0.5µl 0.2% FSA AF647-PBS. After 1, 2 and 3 hours, hearts were dissected and imaged using an Olympus FV3000 microscope. Lastly, images were analyzed using ImageJ. Flow chart was made in Biorender.

Results: Protein aggregates are...

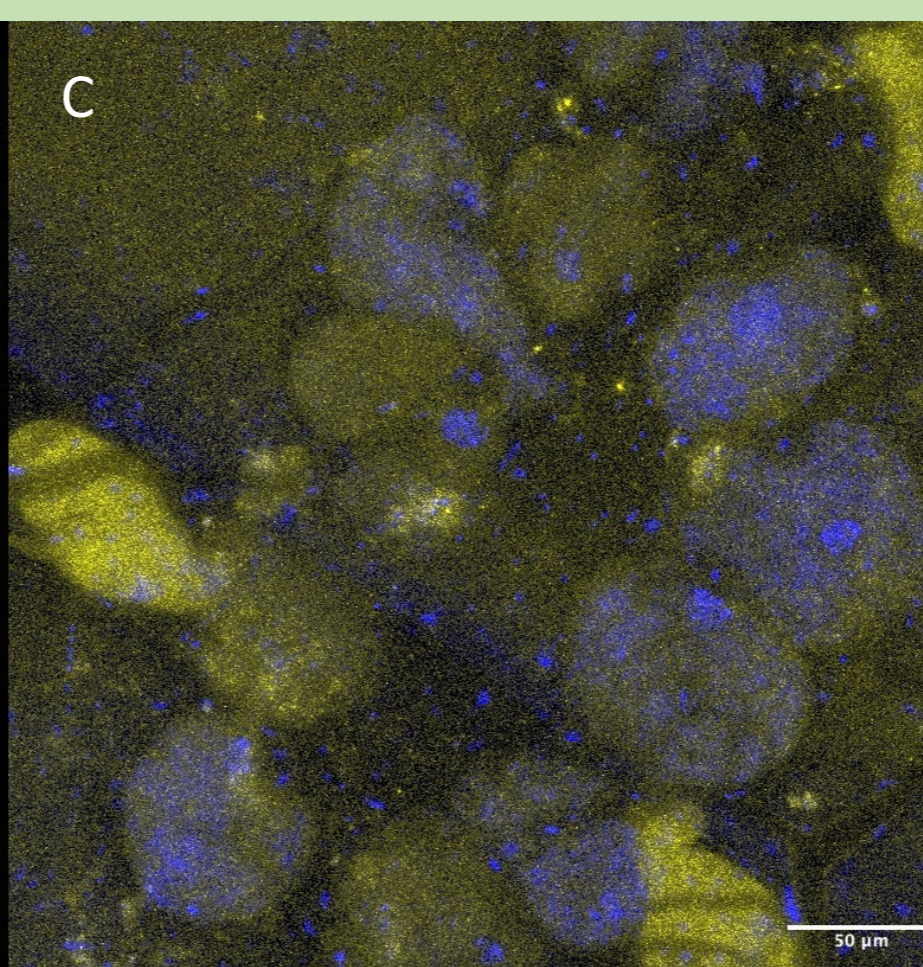
-digested by cell



-stuck on cell surface



-diffusely distributed



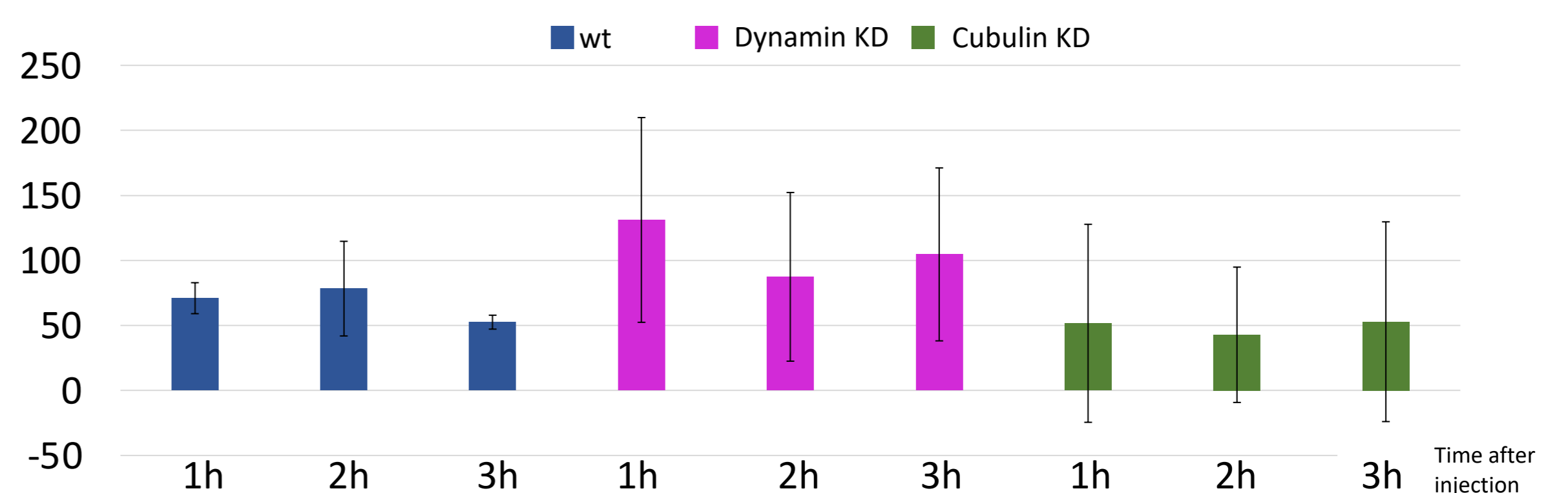
←Figure 3 Comparison of protein location 3h after injection of FSA tagged dye. Representative confocal images of PCs in A: wt, B: dynamin (*shibire*) knock down and C: cubulin knock down. Image A shows dye-tagged proteins being internalised and likely digested. Image B suggests that protein aggregates on the cell surface. Image C shows almost evenly distribution of protein aggregates in PC cells and in surrounding cells.

↓Figure 4 Particle count. PCs are analyzed for particles using ImageJ. Since the background noise was so high when Cubulin was knocked down (KD), these PCs were compared to a representative area of the background. Plot does not show a significant different in amount of particles between the genotypes. P-values for wild type (wt), Dynamin KD and Cubulin KD were found to be 0,000, 0,030 and 0,992 respectively. For all timepoints for wt, n = 45, collected from 9 flies. For the Dynamin (*Shibire*) KD 1h, n = 47 from 10 flies; 2h n = 40 from 8 flies; 3h n = 44 from 9 flies. For the Cubulin KD 1h, n = 33 from 11 flies; 2h n = 30 from 7 flies; 3h n = 43 from 10 flies. Anova test was used to find p-values and outliers to exclude them. Plot made using Microsoft Excel.

Conclusions

Although there is not a significant difference in the particle count between Cubulin KD and the controls, there is a big difference in where the dye is located, just as expected and described in figure 1. One must therefore conclude that Cubulin plays an important role in protein scavenging and endocytosis of misfolded proteins.

Particle count



References

1. Psathaki OE, Dehnen L, Hartley PS, Paululat A. *Drosophila* pericardial nephrocyte ultrastructure changes during ageing. *Mech Ageing Dev.* 2018 Jul;173:9-20. doi: 10.1016/j.mad.2018.04.006. Epub 2018 Apr 24. PMID: 29702130.
2. Das D, Aradhya R, Ashoka D, Inamdar M. Post-embryonic pericardial cells of *Drosophila* are required for overcoming toxic stress but not for cardiac function or adult development. *Cell Tissue Res.* 2008 Feb;331(2):565-70. doi: 10.1007/s00441-007-0518-z. Epub 2007 Nov 7. PMID: 17987318.

