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REVEALING PALLADIN'S ROLE IN METASTASIS BY DIRECTLY OBSERVING ITS INFLUENCE ON ACTIN POLYMERIZATION

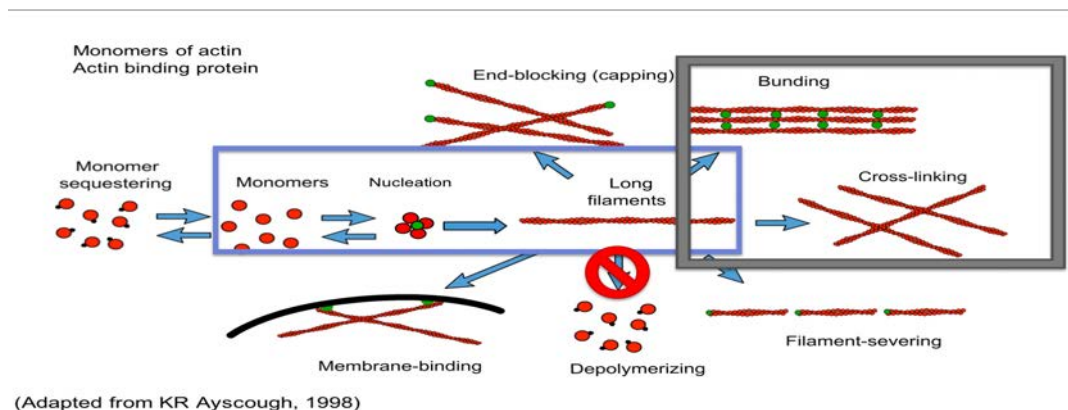


Derek Baldwin, Sharifah Albraiki, Moriah R. Beck

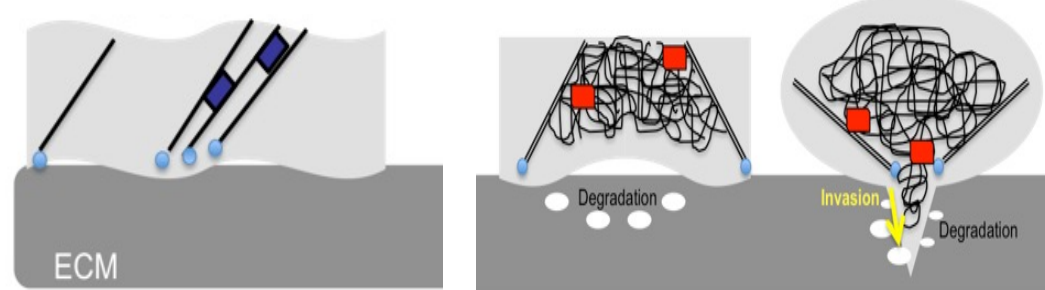
Department of Chemistry, Wichita State University

Introduction

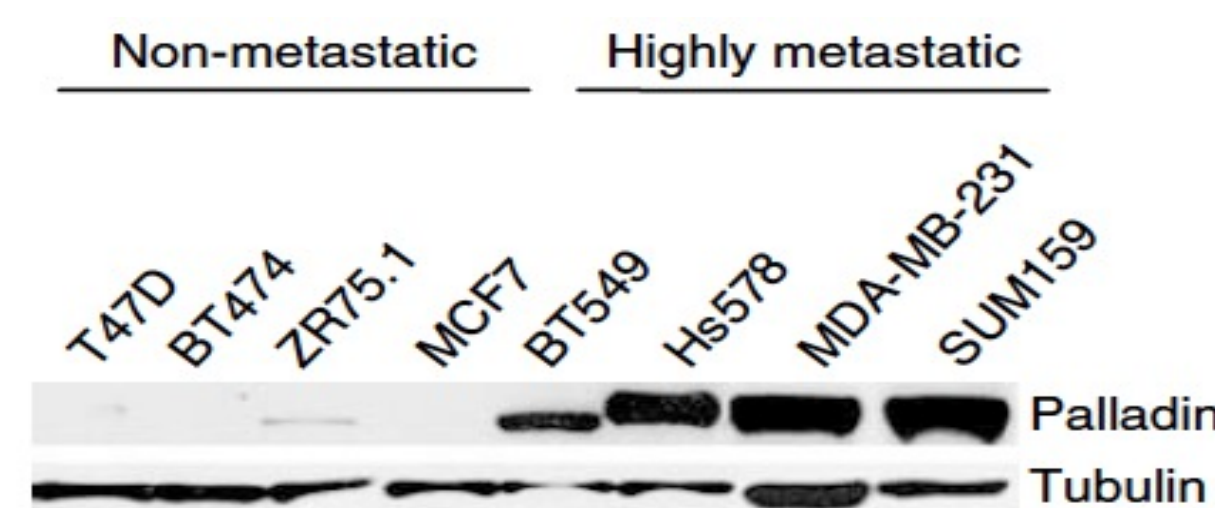
Palladin (pictured in green) is an actin binding protein that is responsible for regulating cytoskeletal structure, cell adhesion, cell motility, and muscle movement.



Actin binding proteins (below) can organize actin filaments within cells to encourage either normal motility (left) or invasive motility (right)



Immunoblot analysis (pictured below) shows that palladin is expressed much more frequently in cancer cell lines that are metastatic.



Background

Figure 1. (Shown below) The Beck lab studies a variety of Palladin isoforms. Ig3 (red) is the minimum actin binding domain of Palladin. 90 kDa Palladin (isoform #4) (pictured in blue) is the most widely expressed form of palladin in all tissue types.

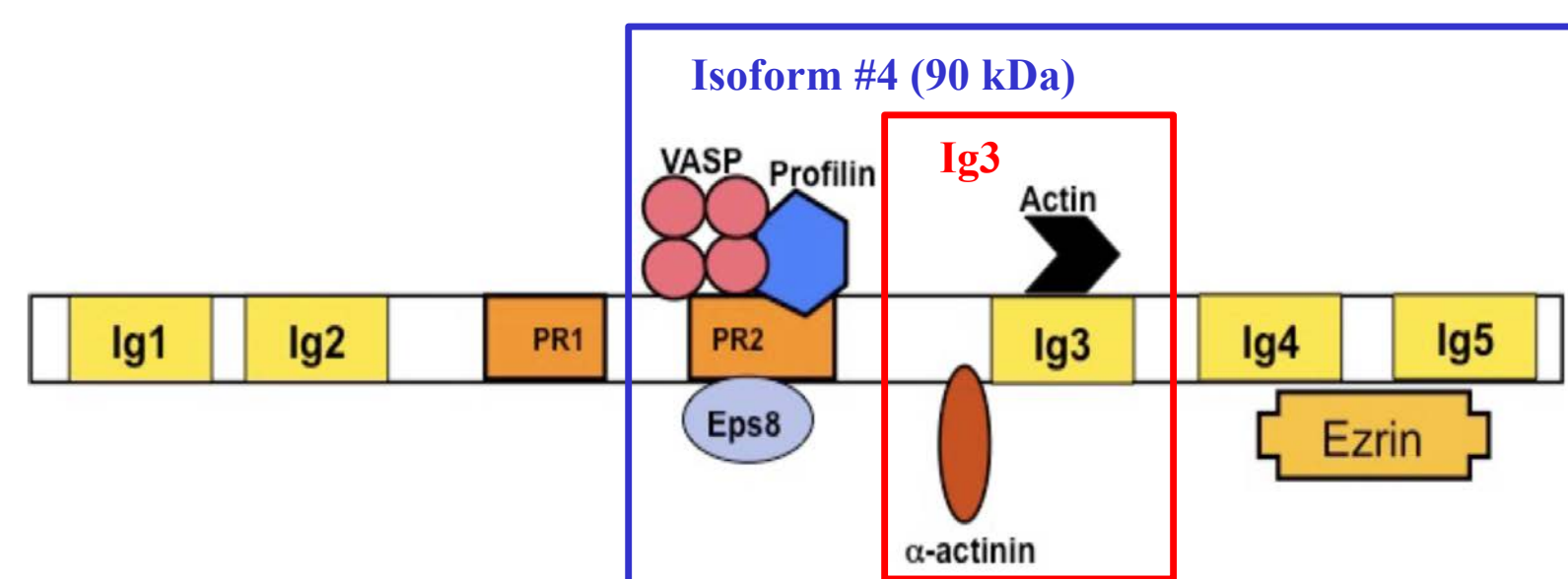
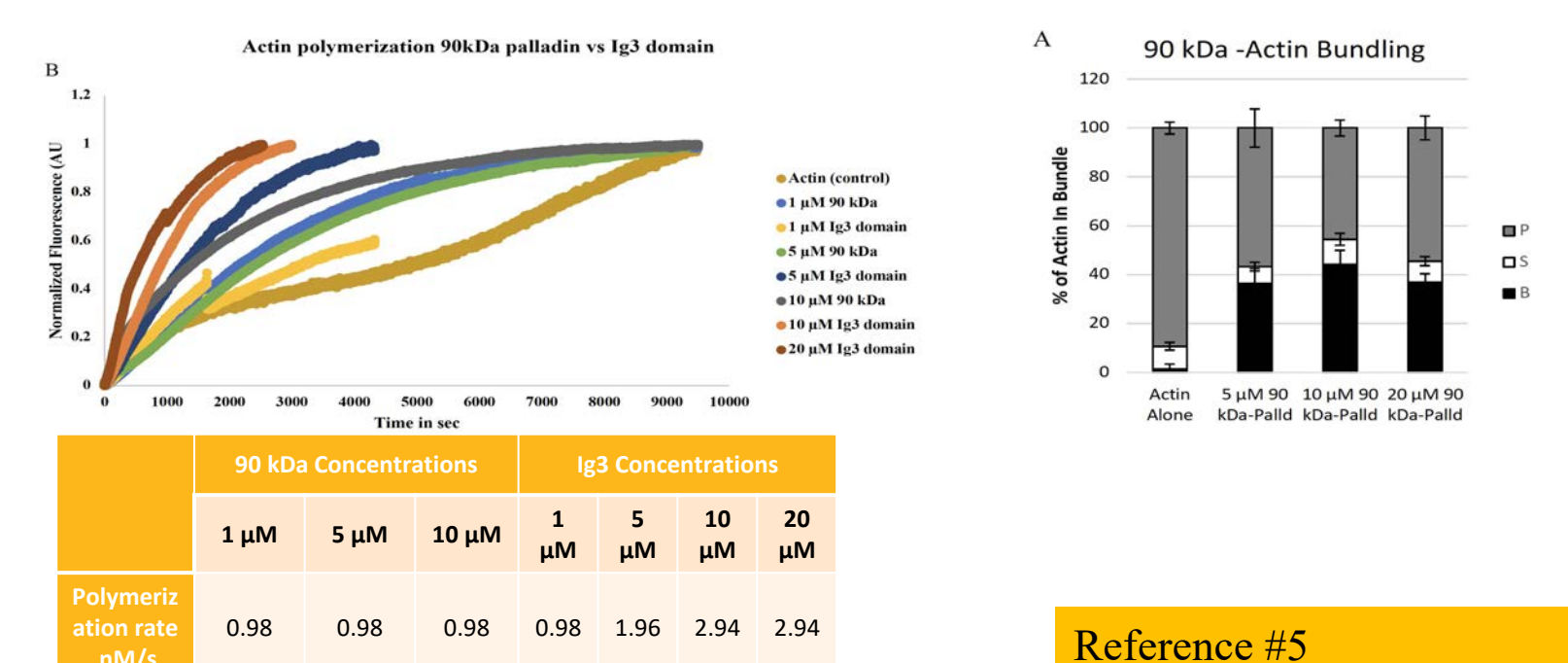


Figure 2. (Below) Pyrene assays (left) show that Ig3 increases the polymerization rate of monomeric actin. Co-sedimentation assays (right) 90 kDa palladin increases the bundling of polymerized actin.



Reference #5

Background (Cont.)

Filament Dynamics in Filament Topology

- Observing filament topology may help explain polymerization metrics found in previous assays.
- Negative Stain EM/ fluorescence CM helps document final fully polymerized actin product, despite containing a lot of background noise

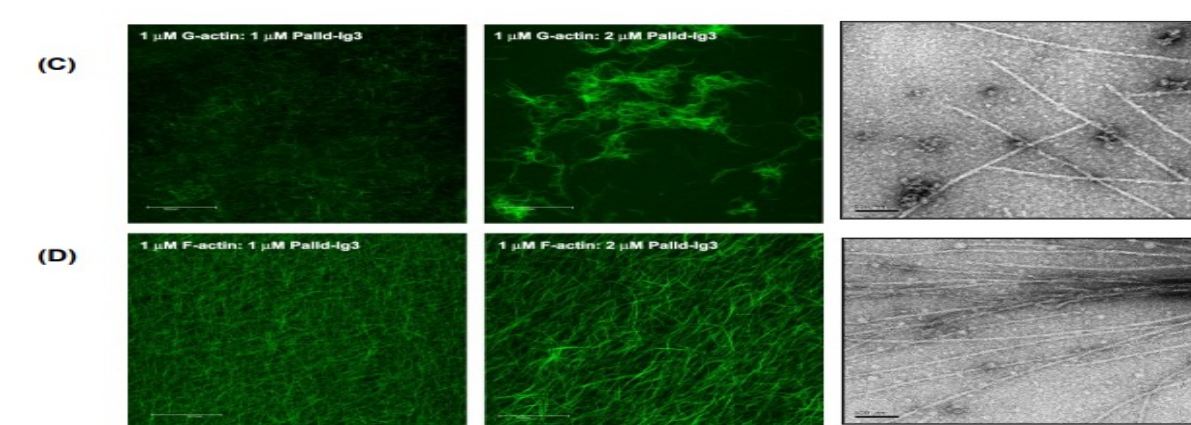


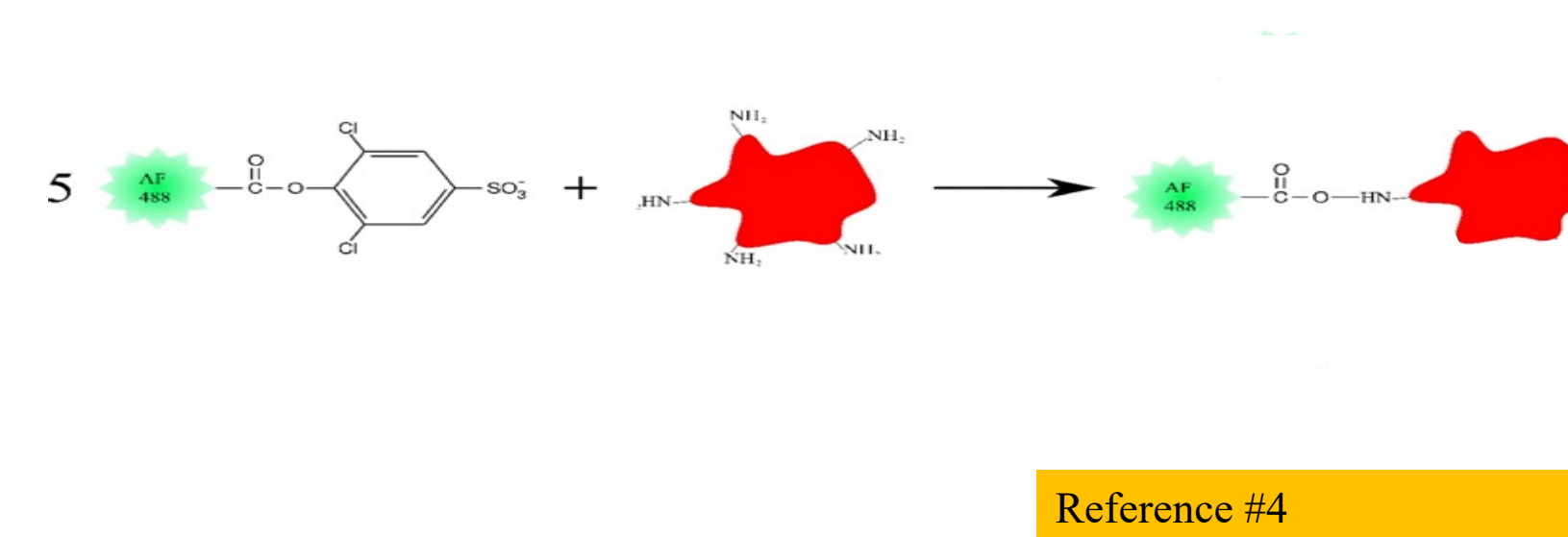
Figure 3. Micrographs of polymerized actin collected using of Negative Stain Electron Microscopy (green) and fluorescence-labeling confocal microscopy (white)

Aim

The Beck Lab hypothesizes that palladin plays a major part in promoting invasive cell motility. TIRF microscopy should provide insight into palladin's affect on actin.

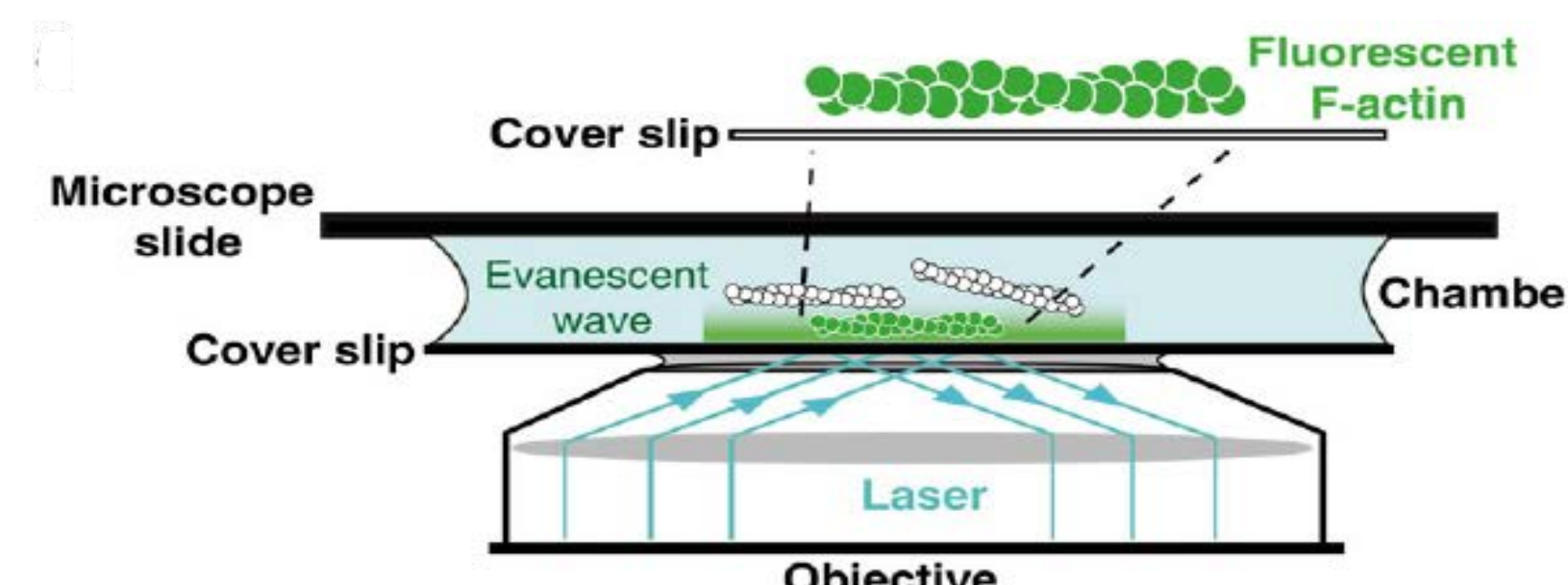
Method

Figure 4. Fluorescently labelled actin is created AlexaFluor 488 labelling dye (shown below). The Alexa Fluor fluorophore (shown in green) couples with a lysin group on our actin monomers (red), resulting in light-sensitive fluorescent actin.⁴



Reference #4

Figure 5. The schematic pictured below shows through-the-objective TIRF microscopy imaging of fluorescently labeled actin filaments. At its critical angle, incoming light is completely reflected. Only fluorophores close to the surface (i.e., 50-200 nm) are excited, which enables imaging with high signal-to-noise ratio³.



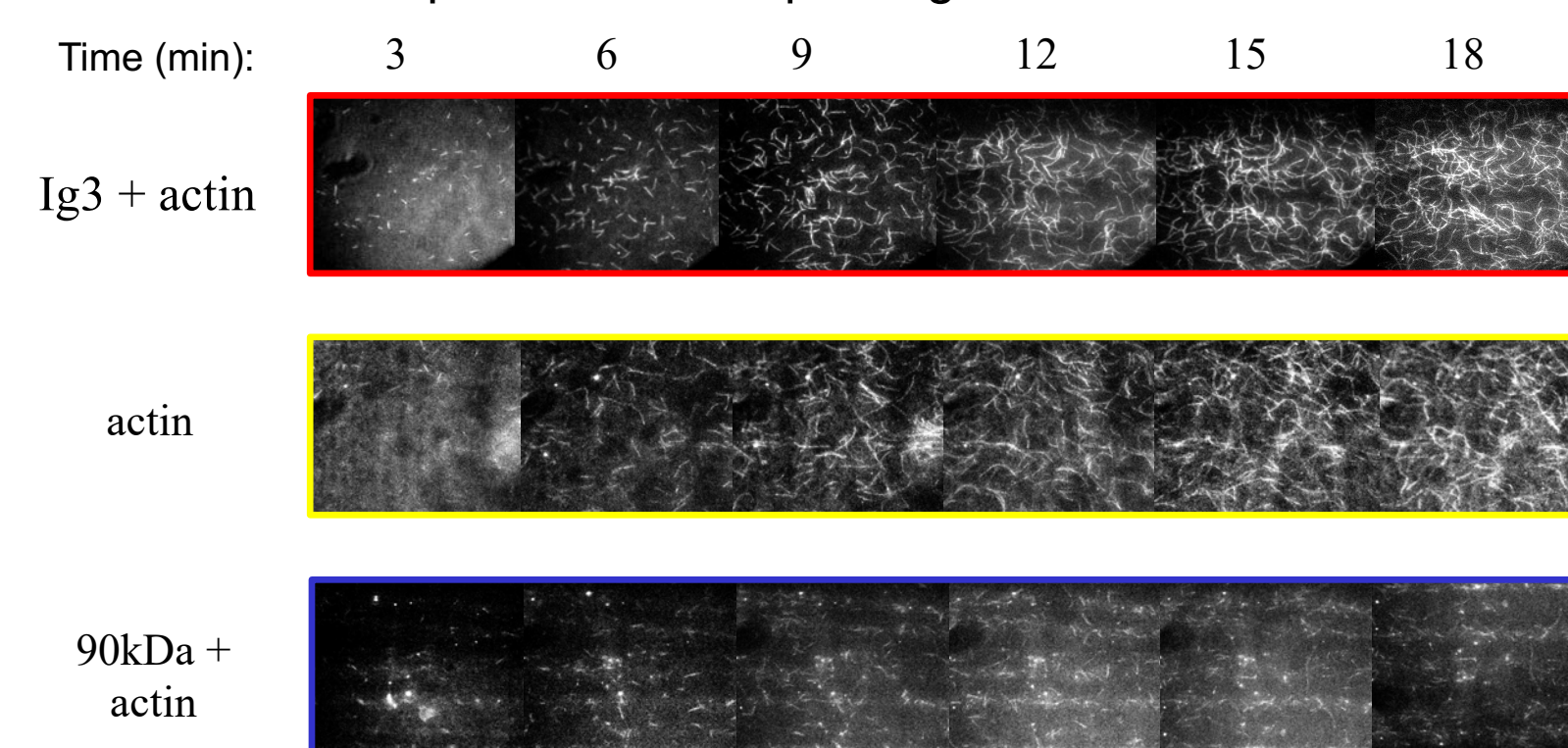
Reference #3

Results

Using TIRF microscopy we can now generate visual data to compare with previous assay data. Reviewing the data collected thus far has helped us detect two separate trends.

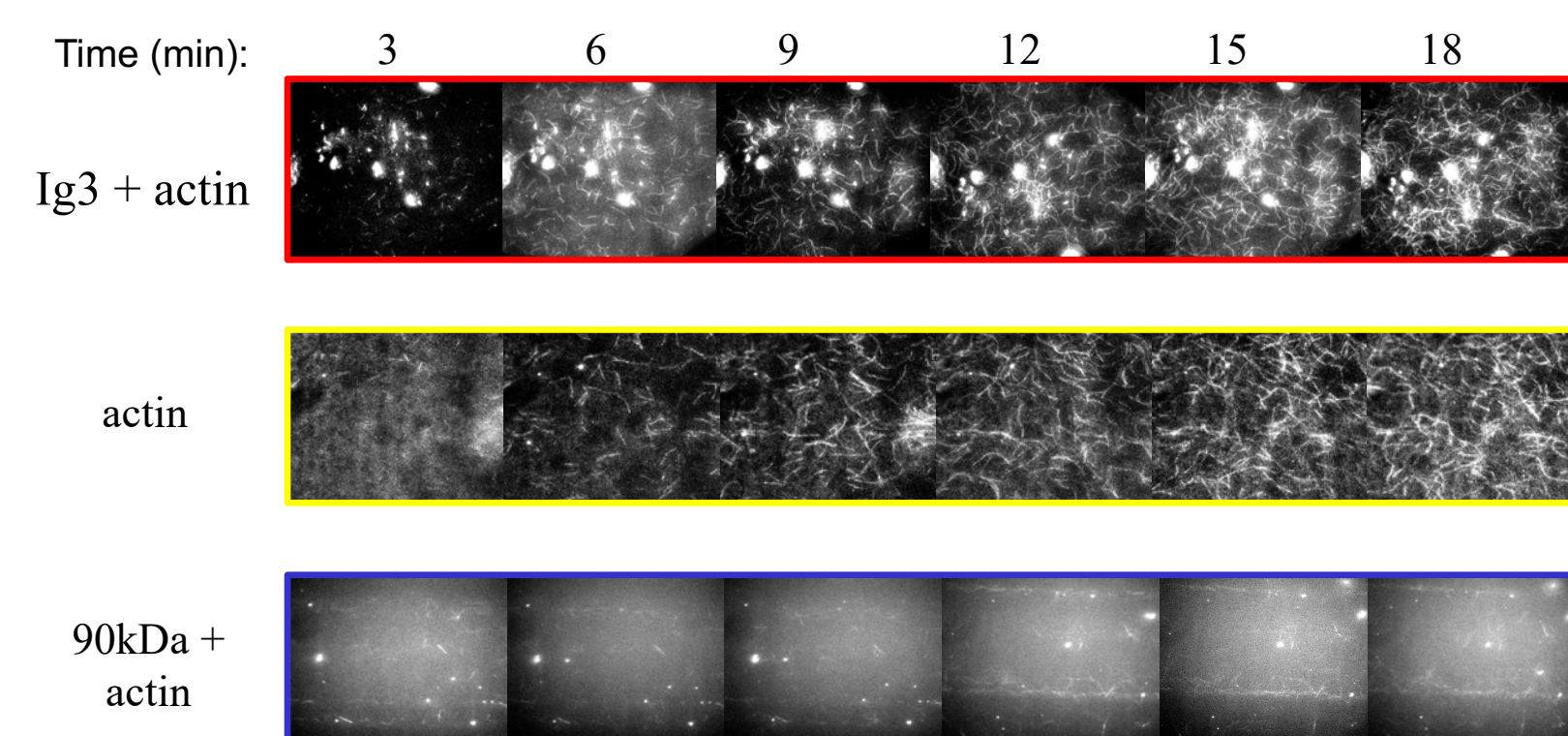
Palladin's influence on actin polymerization

Figure 6. Actin monomers (9 mM 10% Alexa 488-labeled) were combined with palladin and TIRF solutions in a flow cell. Shown below are frames representing the time-course of polymerization for G-actin upon addition of 45 uM Ig3 and priming solution (red), priming solution by itself (yellow) and 30 uM 90kDa palladin with priming solution (blue). The time indicates the time in minutes after addition of palladin and/or priming solution.



Polymerization vs. Nucleation

- Visual data supports previous assay data
- Ig3 induced a significant increase in actin's polymerization rate
- 90kDa palladin caused a significant drop in actin's rate of polymerization
- Visual data suggests new trends
- 90kDa-bound influenced actin monomers to nucleate much more rapidly than normal

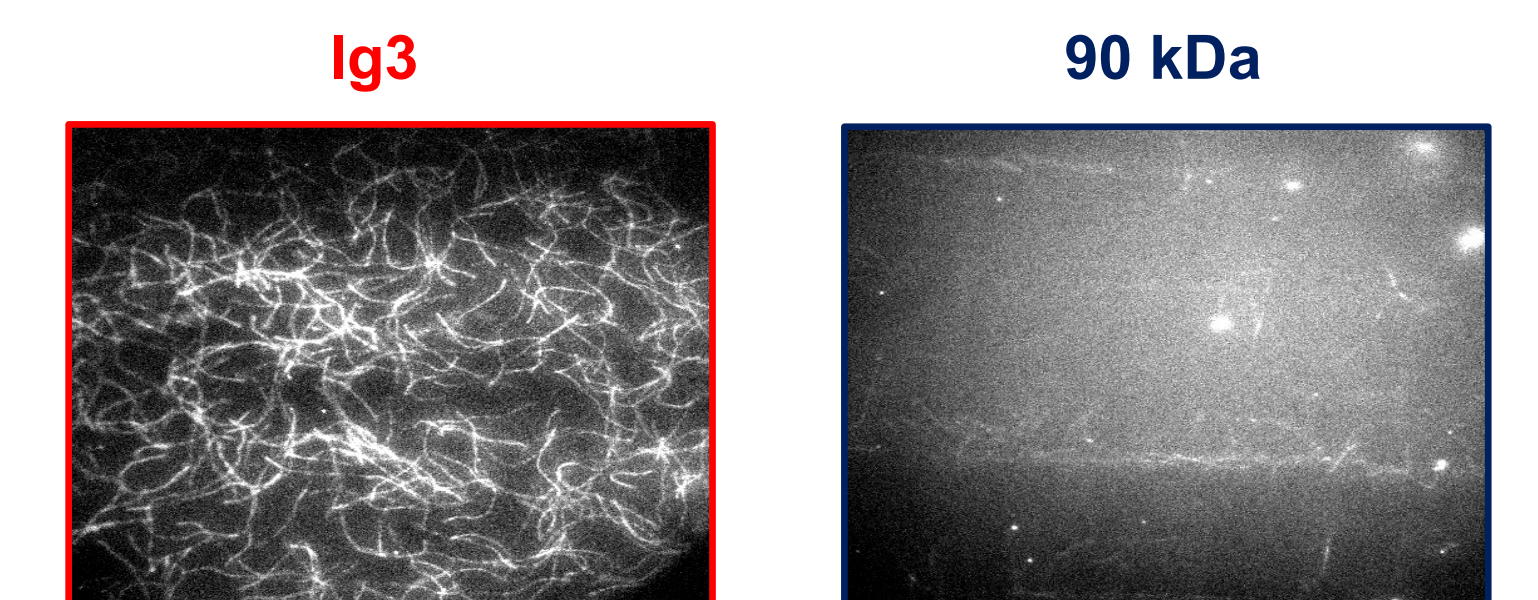


Filament Organization

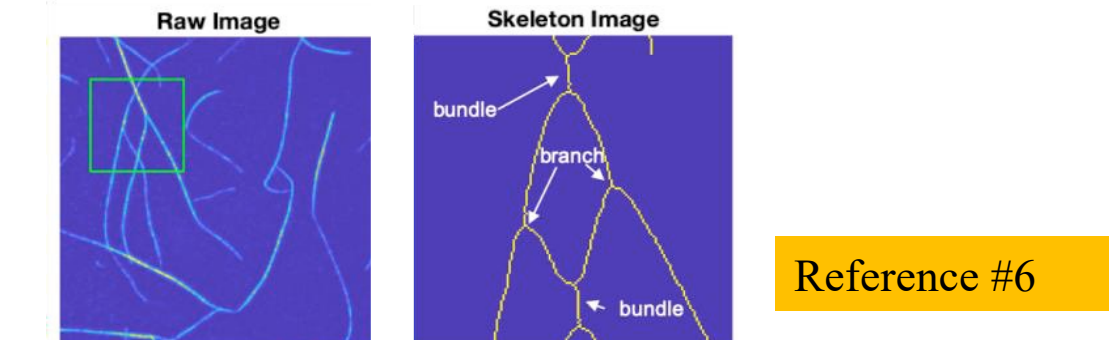
Figure 7. Increasing the amount of palladin added to actin samples (pictured above) resulted in the protein having a more extreme effect on filaments. For Ig3-bound filaments (pictured in red) this meant a higher rate of overall polymerization. In the case of 90kDa-bound samples (blue) this induced the formation of organized bridge-like structures.

Conclusions & Future Work

- 90kDa palladin has thus far been found to induce a high rate of nucleation in monomeric actin, despite its inhibiting of polymerization in mature filaments. This leads to further questions seeking to define the domain(s) responsible for this behavior
- Highly organized, bridge-like structures in 90kDa-bound actin filaments help to define palladin's capacity for filament organization and supports our general hypothesis for its involvement in metastasis.



- Organized structures in 90kDa-bound filaments and highly branched structures in Ig3-bound filaments suggest structural evidence of the occurrence of cross-linking between filaments.



- Application of Vince Rossi's "filament skeletonization" will allow us to define filament behavior to a more precise degree.

References

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- 6 Beck, Moriah. Proposal for Flossie West Memorial Trust grant. March 1, 2021.

Acknowledgements

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