Visualizing the (ultra-) structures driving cell migration by cryo-electron tomography and subtomogram averaging

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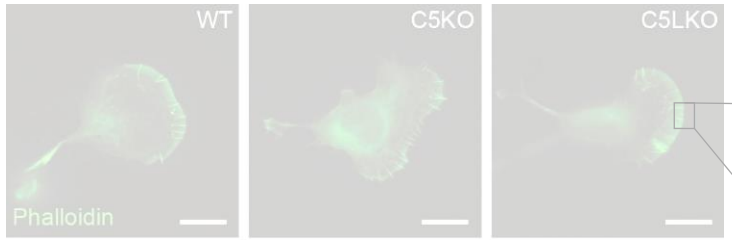
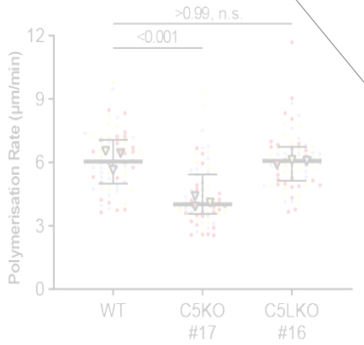
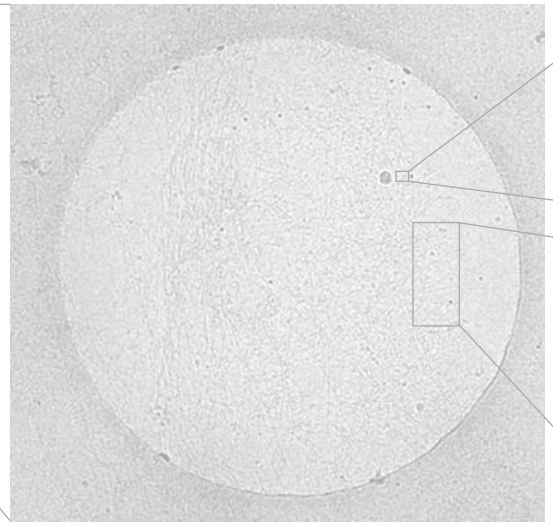
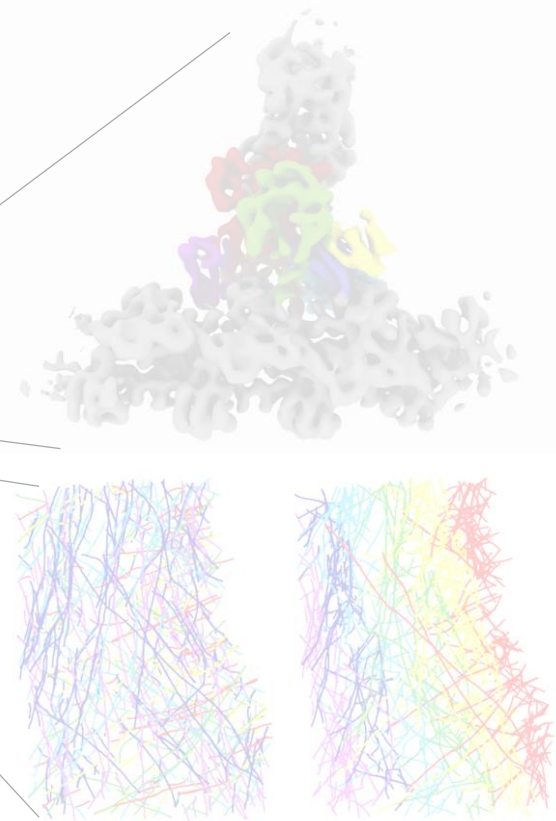
13ème Forum du Cancéropôle Est





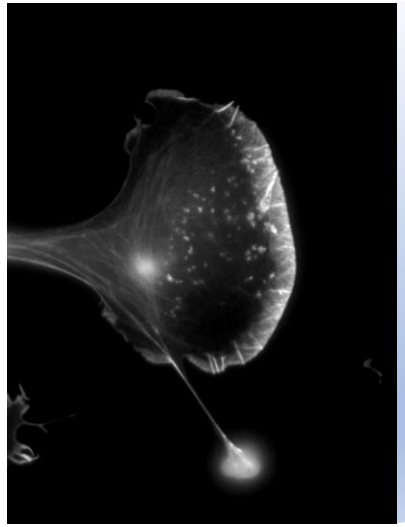




The difference in scales between cell and structural biology

10^5 -10^2 nm

Cell morphology and dynamics



Light microscopy

10^2- 10^0 nm

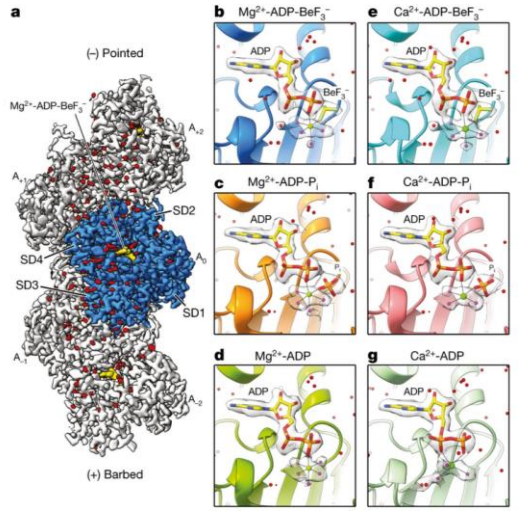
Cryo-electron tomography

Ultrastructural analysis

Subtomogram averaging

10^0- 10^{-1} nm

High resolution structure



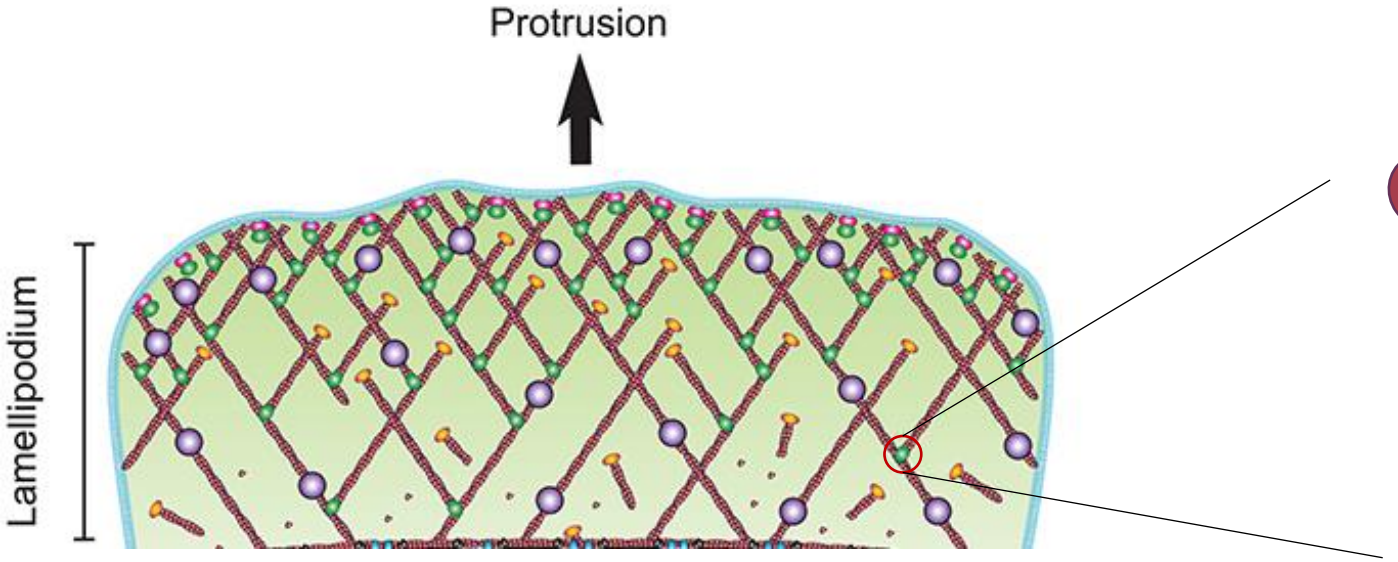
Crystallography, Cryo-EM, NMR

Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

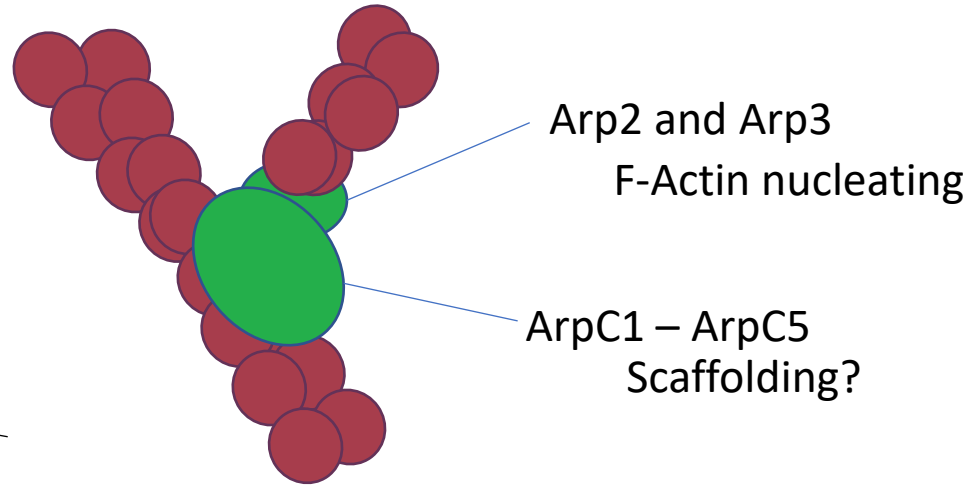
Adapted from Oosterheert et al., 2022, doi.org/10.1038/s41586-022-05241-8

The Arp2/3 complex in branched actin networks

- 7 Subunits; initiates branching in the actin cytoskeleton
  - Actin-related proteins 2 and 3 were name-giving
- Actin polymerization in resulting networks generates forces
- Driving cell motility, trafficking and cell division

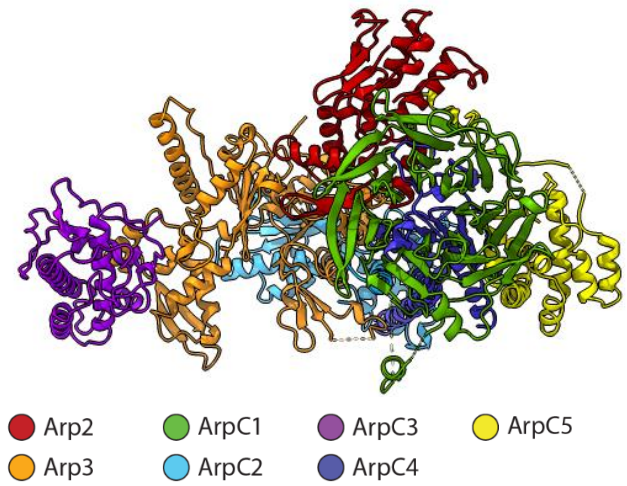


Adapted from Jaumouille and Waterman, 2020, doi.org/10.3389/fimmu.2020.01097



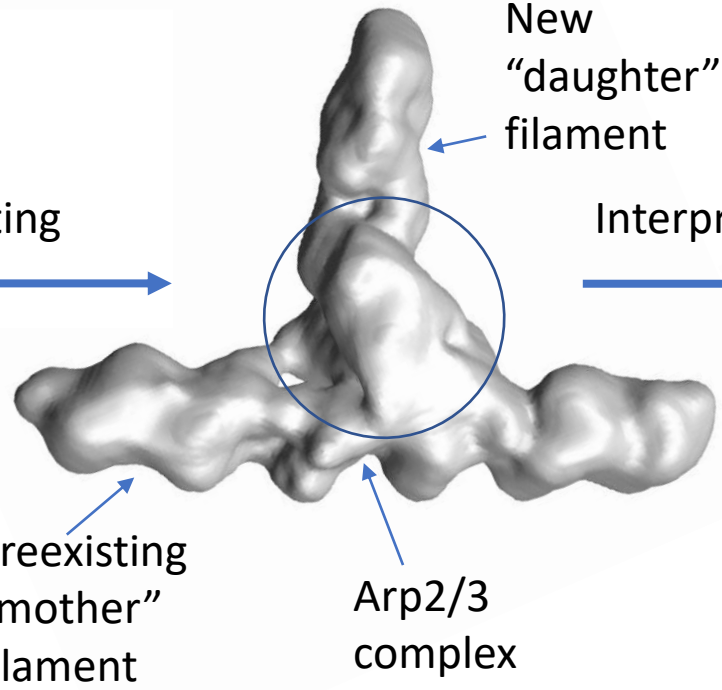
Available structure data prior to 2020

Model derived from crystal structure, inactive, ~2-3 Å resolution



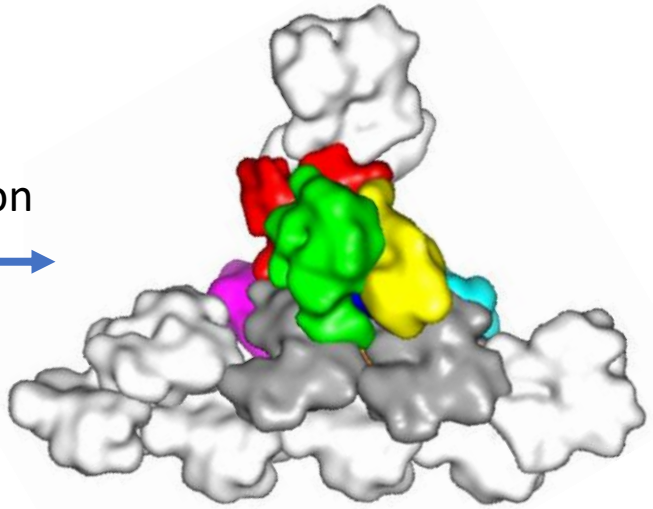
Fitting

ET density map, *in vitro* branch junction, ~30 Å resolution



Interpretation

Ambiguous fit



Adapted from Robinson et al., 2001, doi.org/ 10.1126/science.1066333

Adapted from Rouiller et al., 2008, 10.1083/jcb.200709092

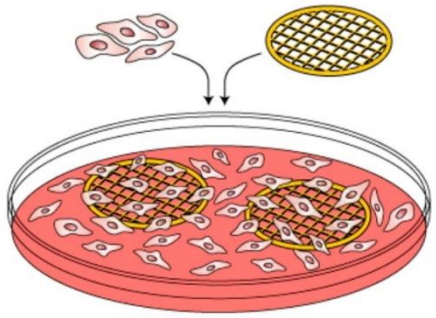
Improve the previously proposed branch junction model

- Needed: “high” resolution structure of the branch junction
  - Achieve sufficient resolution to fit existing models unambiguously
  - Describe structural changes between inactive Arp2/3 and the complex in its branch junction state

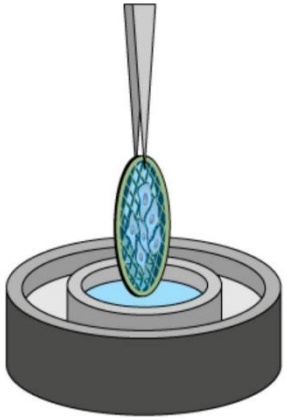
- Approach: Cryo-ET and subtomogram averaging of branch junctions in lamellipodia
  - Lamellipodia are thin enough to be accessibly to Cryo-ET
  - Lamellipodia are easy to identify and high in branch junction content

Cryo-electron tomography of lamellipodia

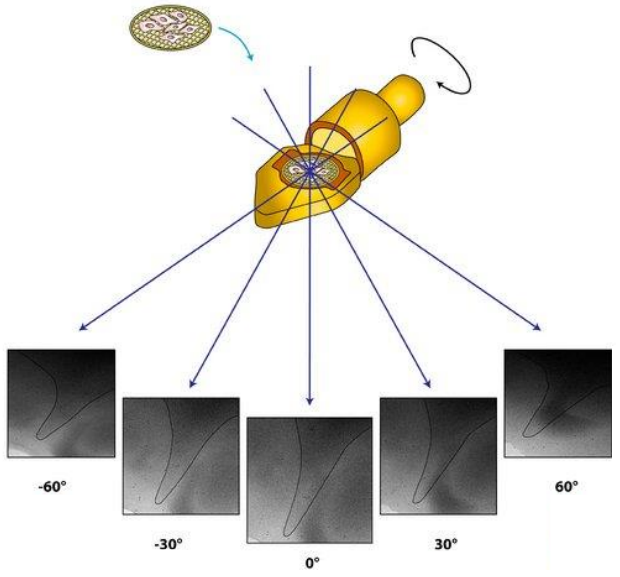
Seed cells on electron microscopy grids



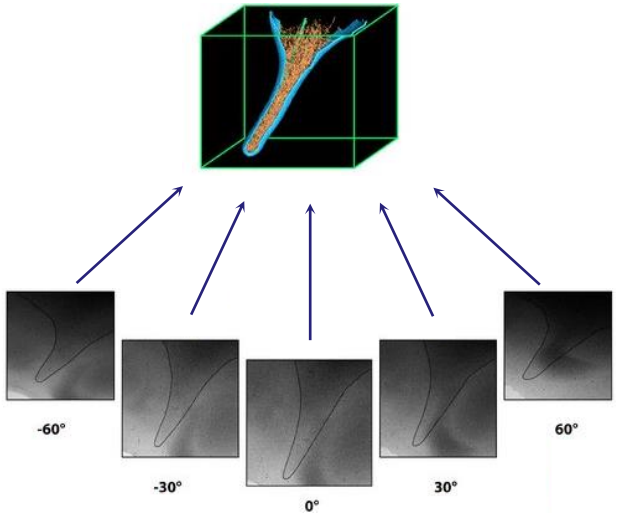
Vitrify cells by plunge freezing in liquid ethane



Acquire projection images of lamellipodia at different angles

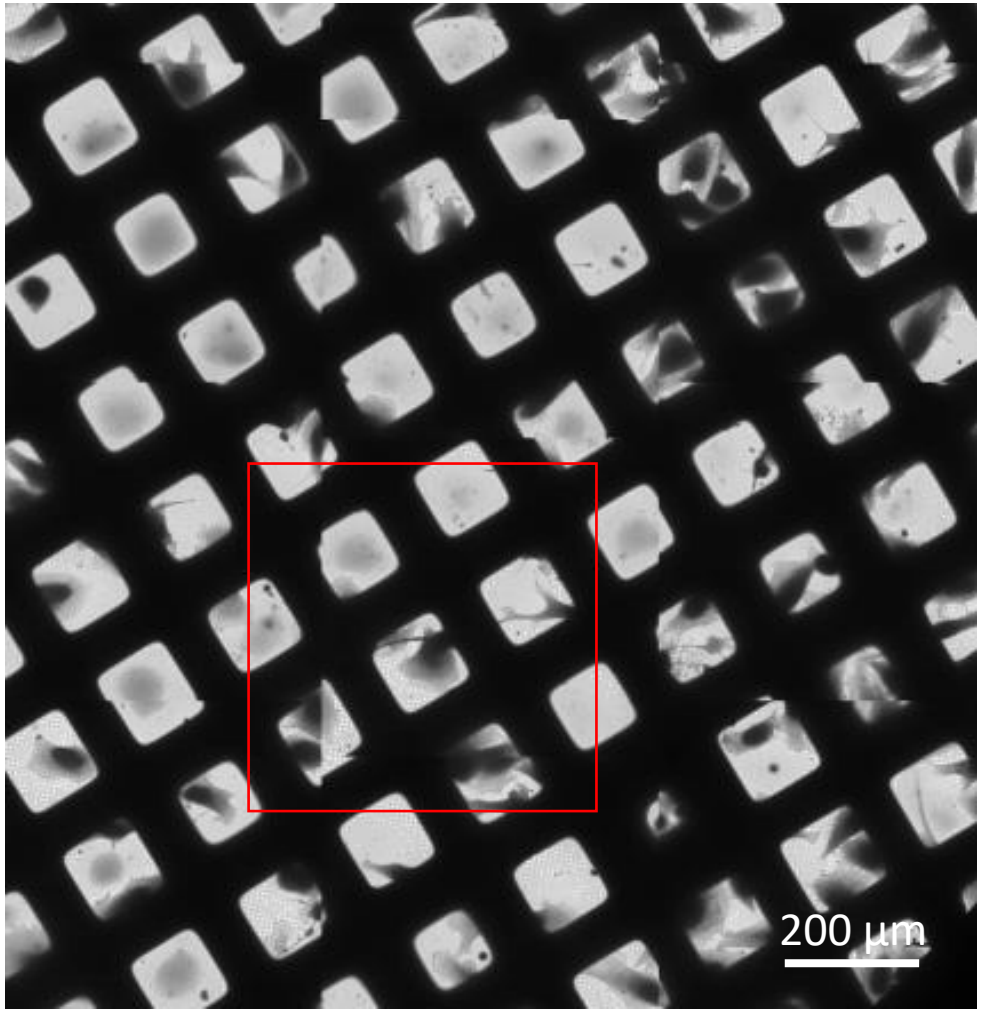


Reconstruct 3D volume (tomogram) from projections



Adapted from Weber et al., 2019, doi.org/10.3390/cells8010057

Cryo-ET – identifying a target site



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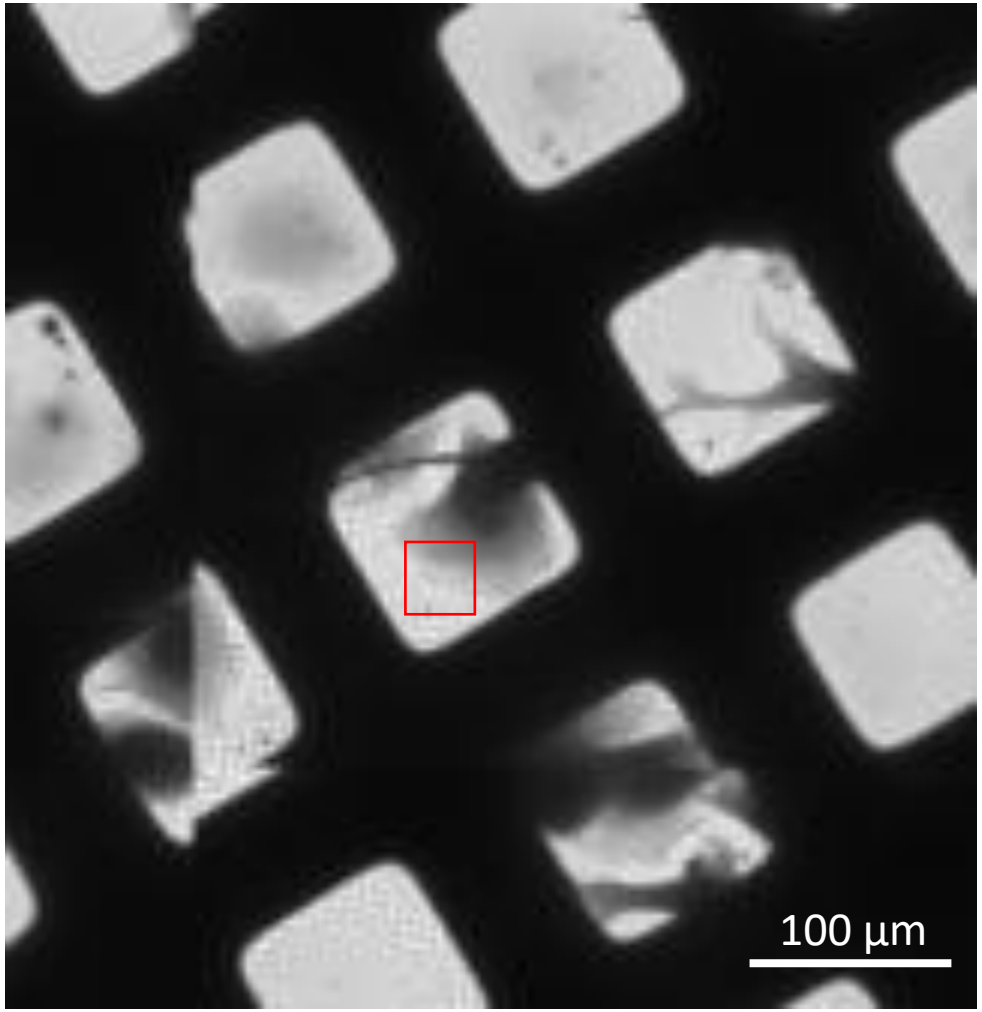
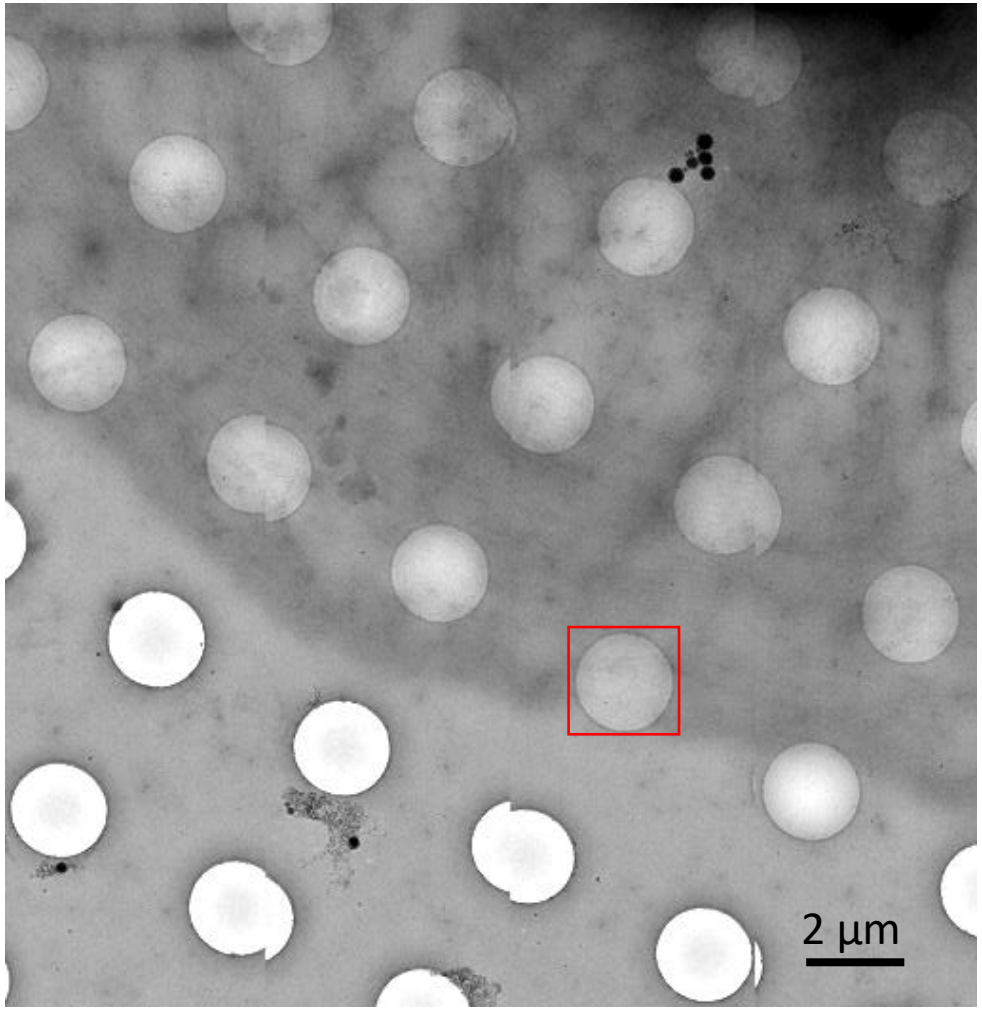
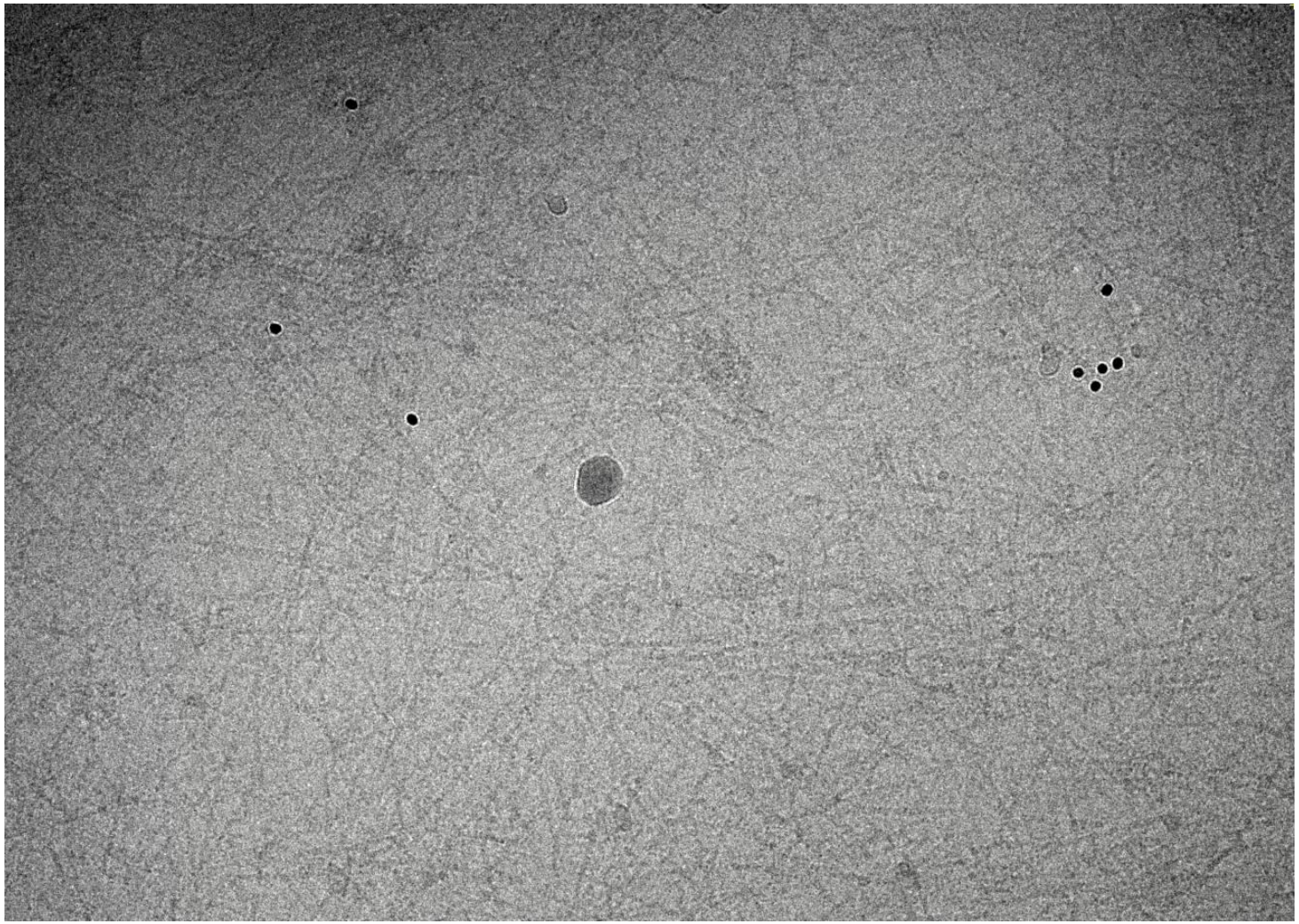


Cryo-ET – identifying a target site



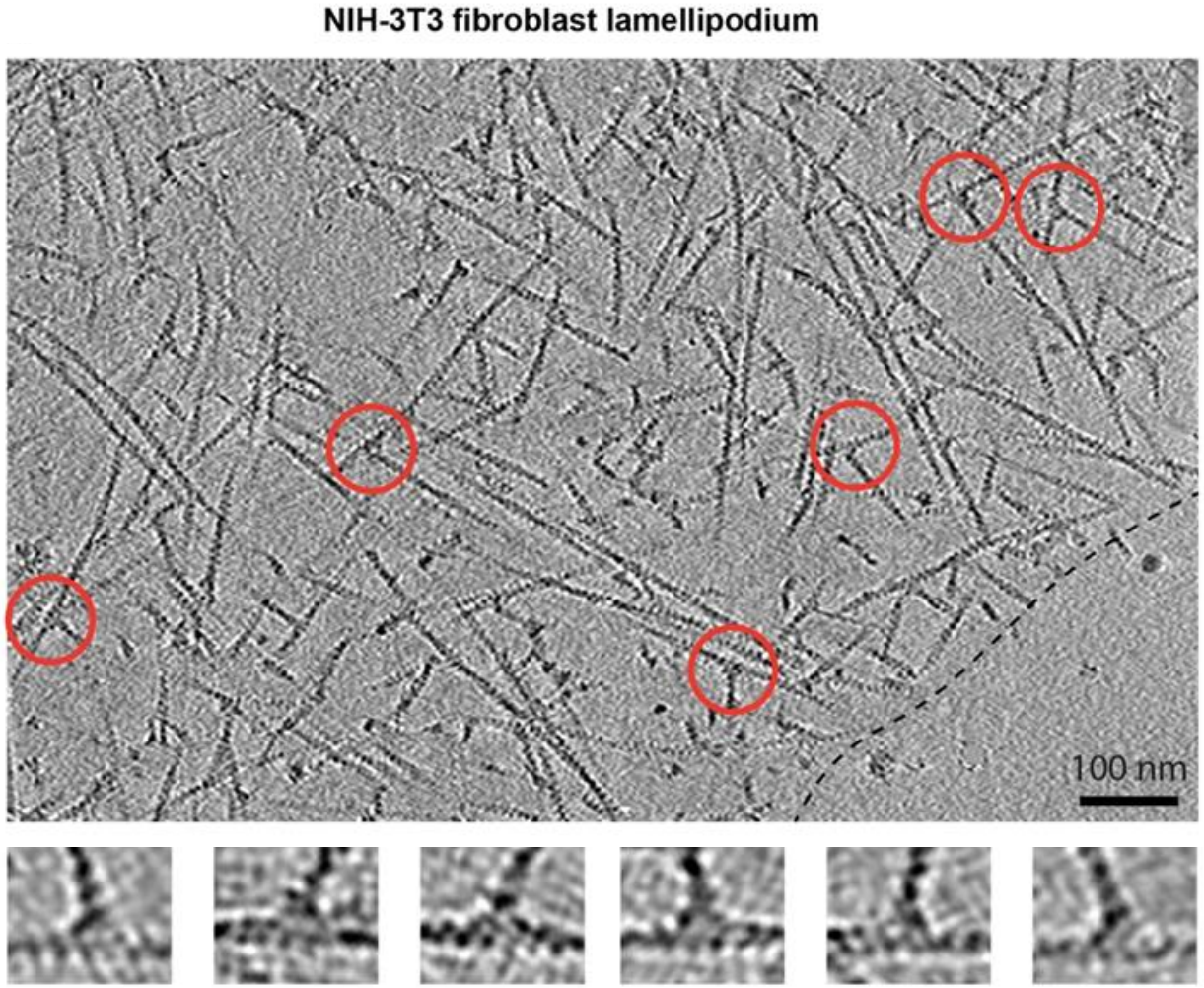
Cryo-ET – a tilt-series of a lamellipodium



Cryo-ET – a tomogram of a lamellipodium

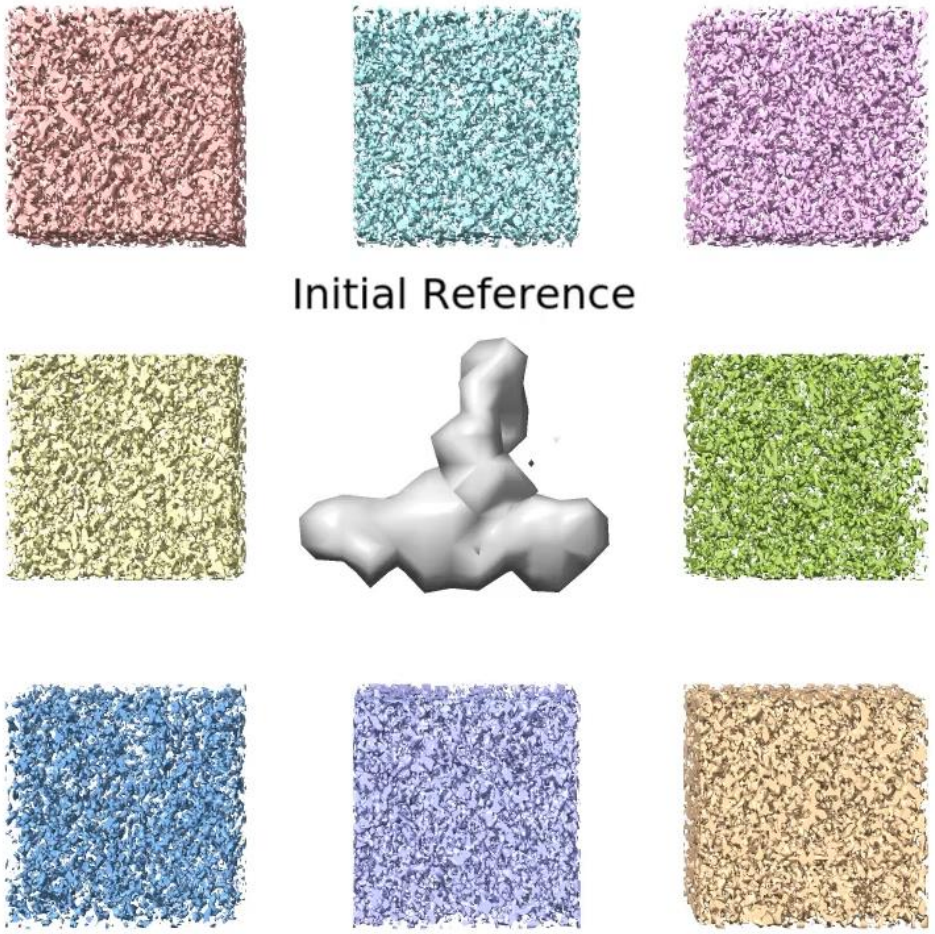
Subtomogram averaging (STA) – general principle

- Cryo-ET data contains information on protein structure but is quite noisy
- If multiple instances of a protein are found within a data set, they can be aligned and averaged
- Averaging improves the signal-to-noise ratio and allows for structure determination

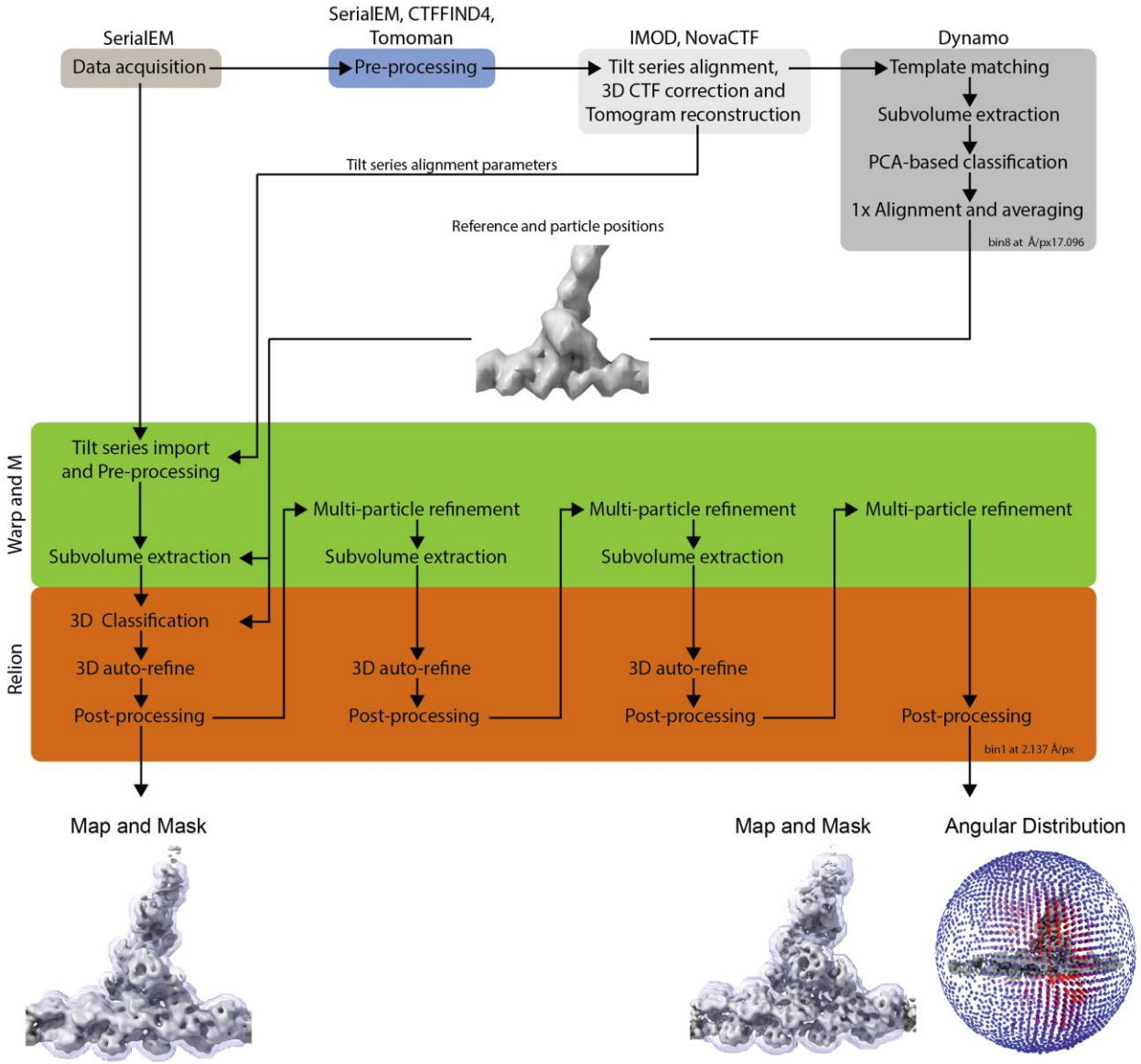


Adapted from Fäßler et al., 2020, doi.org/10.1038/s41467-020-20286-x

Subtomogram averaging (STA) – general principle



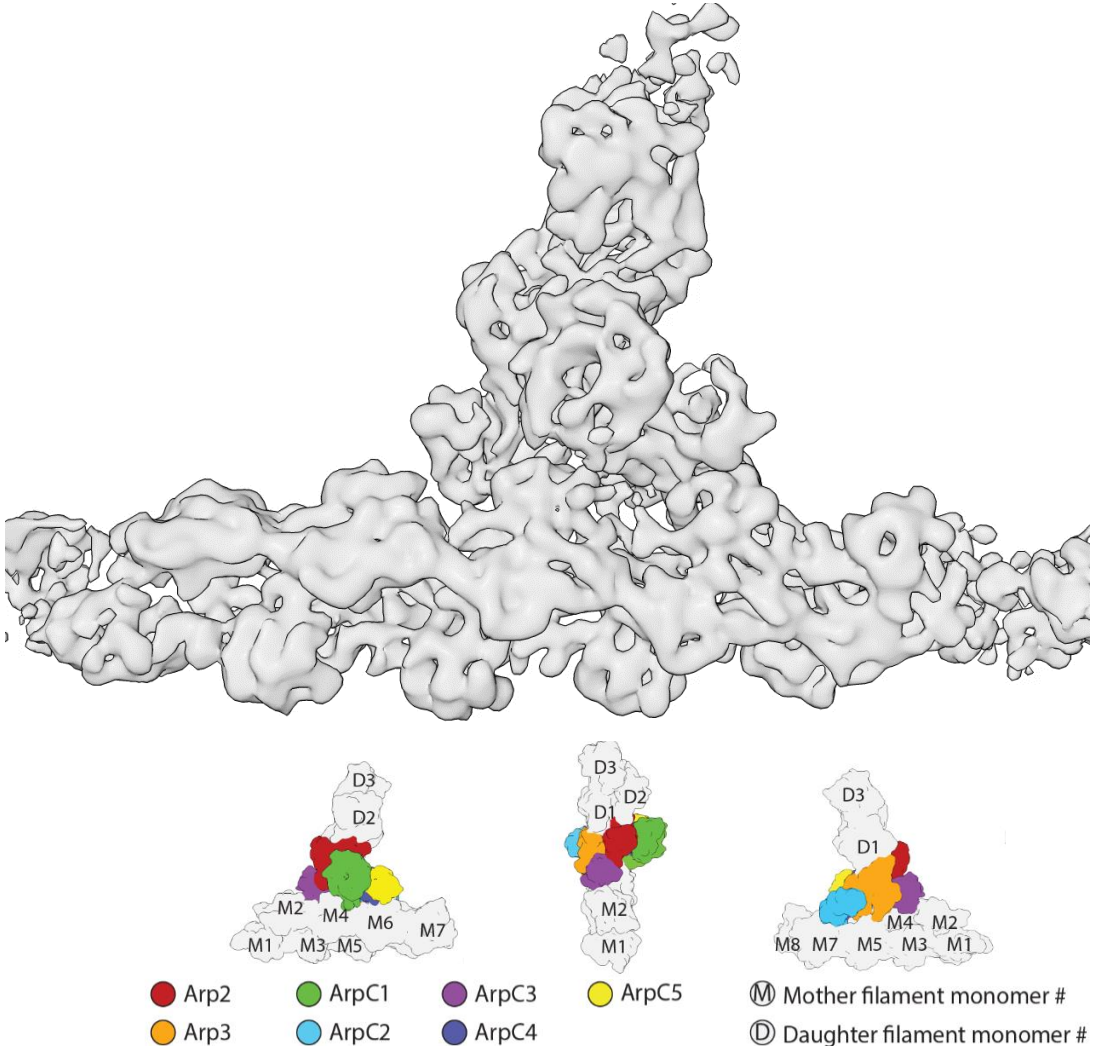
Actual pipeline



Adapted from Fäßler et al., 2020, doi.org/10.1038/s41467-020-20286-x

9Å resolution in-cell structure of the branch junction

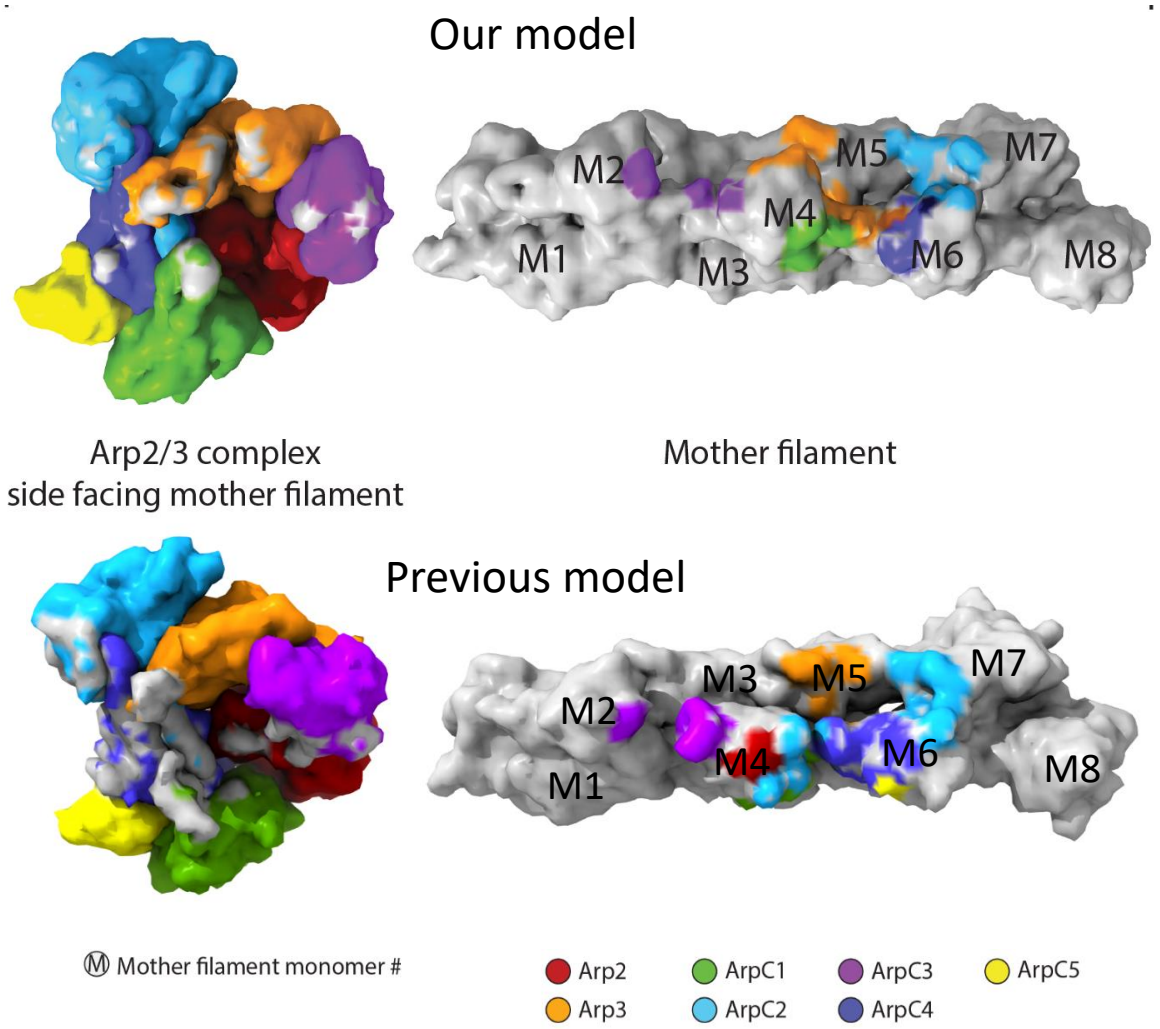
- Visibility of α-helices confirms sub-nanometer resolution
- Structure is featured enough for fitting molecular models



Adapted from Fäßler et al., 2020, doi.org/10.1038/s41467-020-20286-x

Interactions between Arp2/3 complex and the mother filament

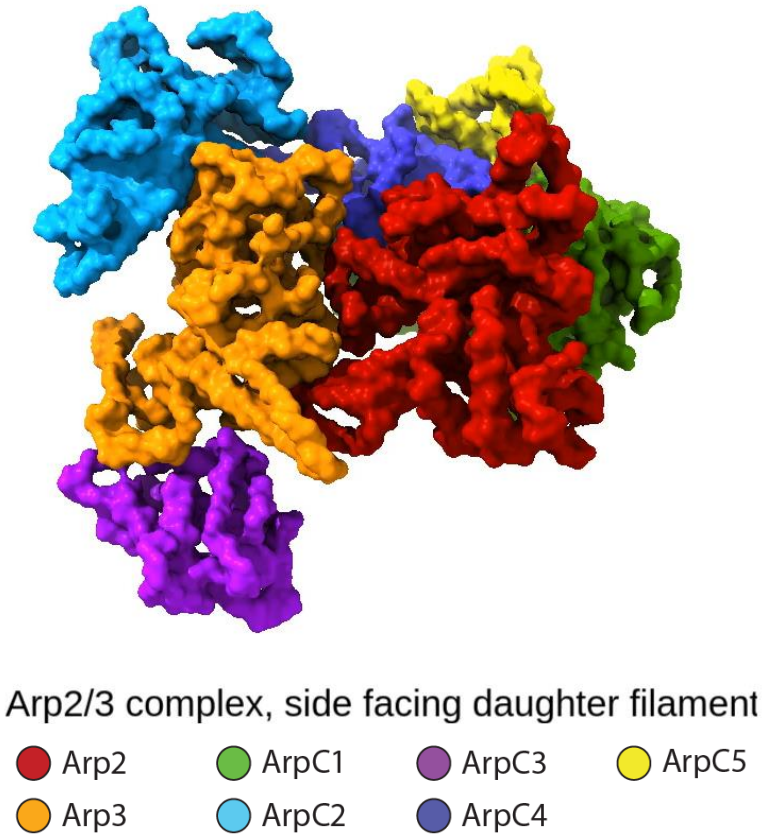
- Not all subunits bind the mother filament
- The interaction surface is smaller than previously postulated



Adapted from Fäßler et al., 2020, doi.org/10.1038/s41467-020-20286-x

Conformational differences to the inactive complex

- 2 subcomplexes rotate against each other:
- Arp2 is relocated to the side of Arp3
- ArpC3 moves towards Arp2 and contacts it

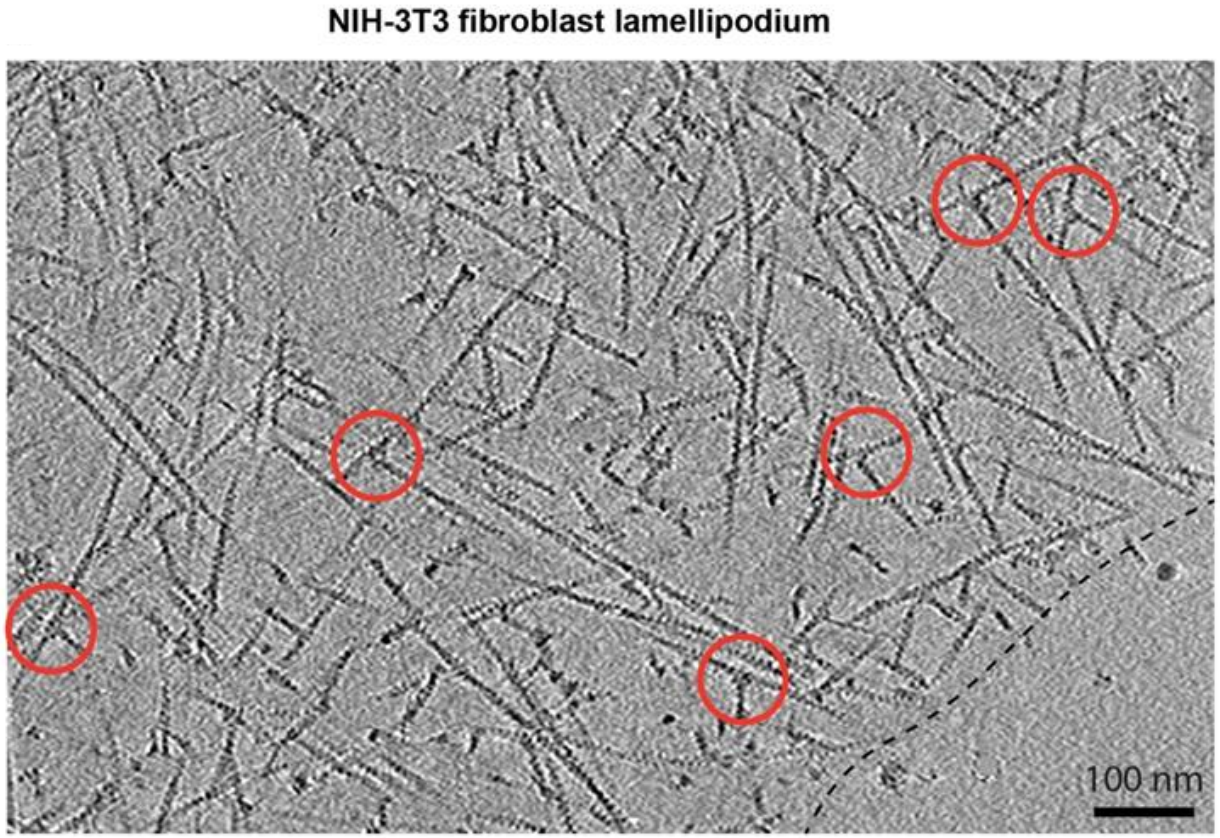


Adapted from Fäßler et al., 2020, doi.org/10.1038/s41467-020-20286-x

Cryo-ET captures vast amounts of contextual information

- Next to studying structures by subtomogram averaging we can characterize the occurring ultrastructural assemblies

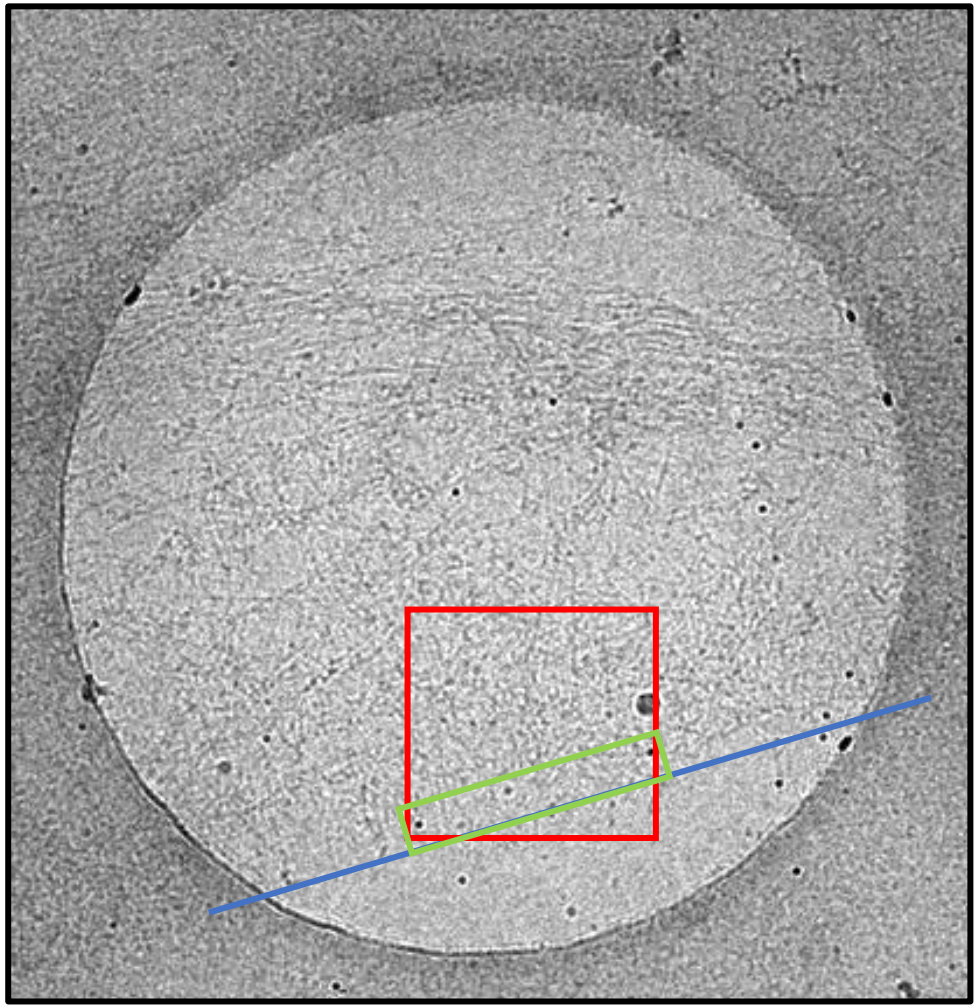
- Here: The actin filament meshwork



Ultrastructural analysis of filament networks

- Filament position and orientation is crucial for function

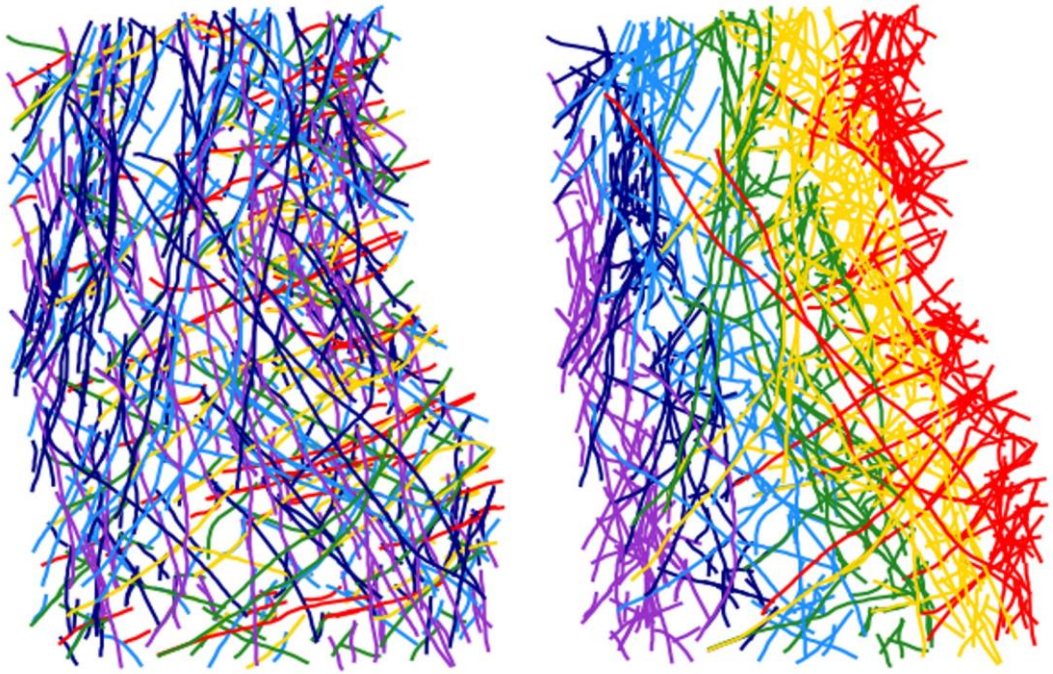
- Coordinates of filaments and reference structures, e.g., leading edge, need to be determined



Vectorization of filaments in tomograms

- Deriving vector-based representations from graphical representations via automated tracking

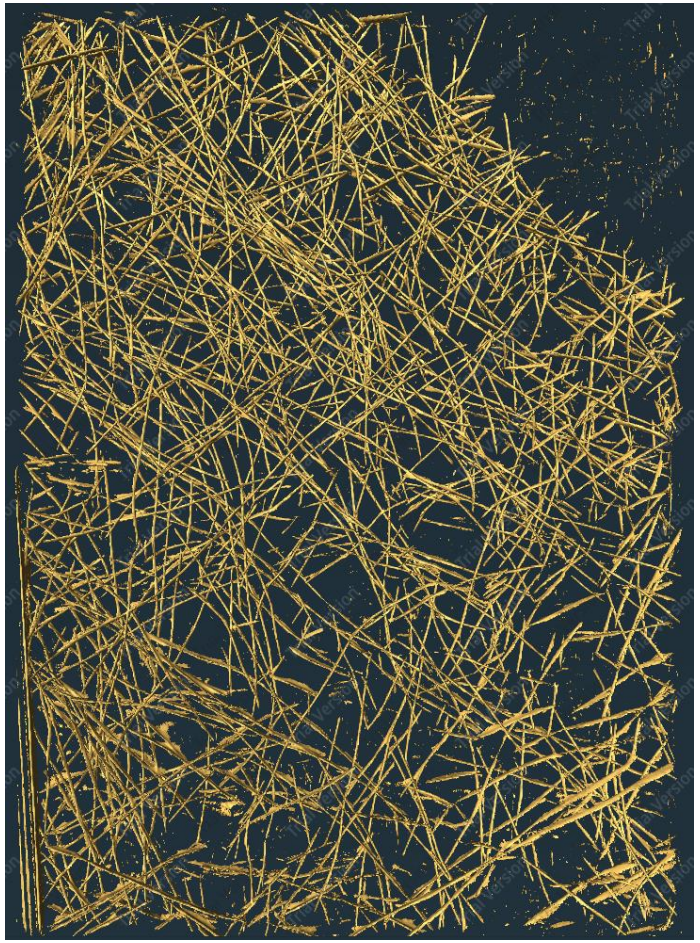
- Automated quantitative analysis of complete lamellipodia and individual filament traits



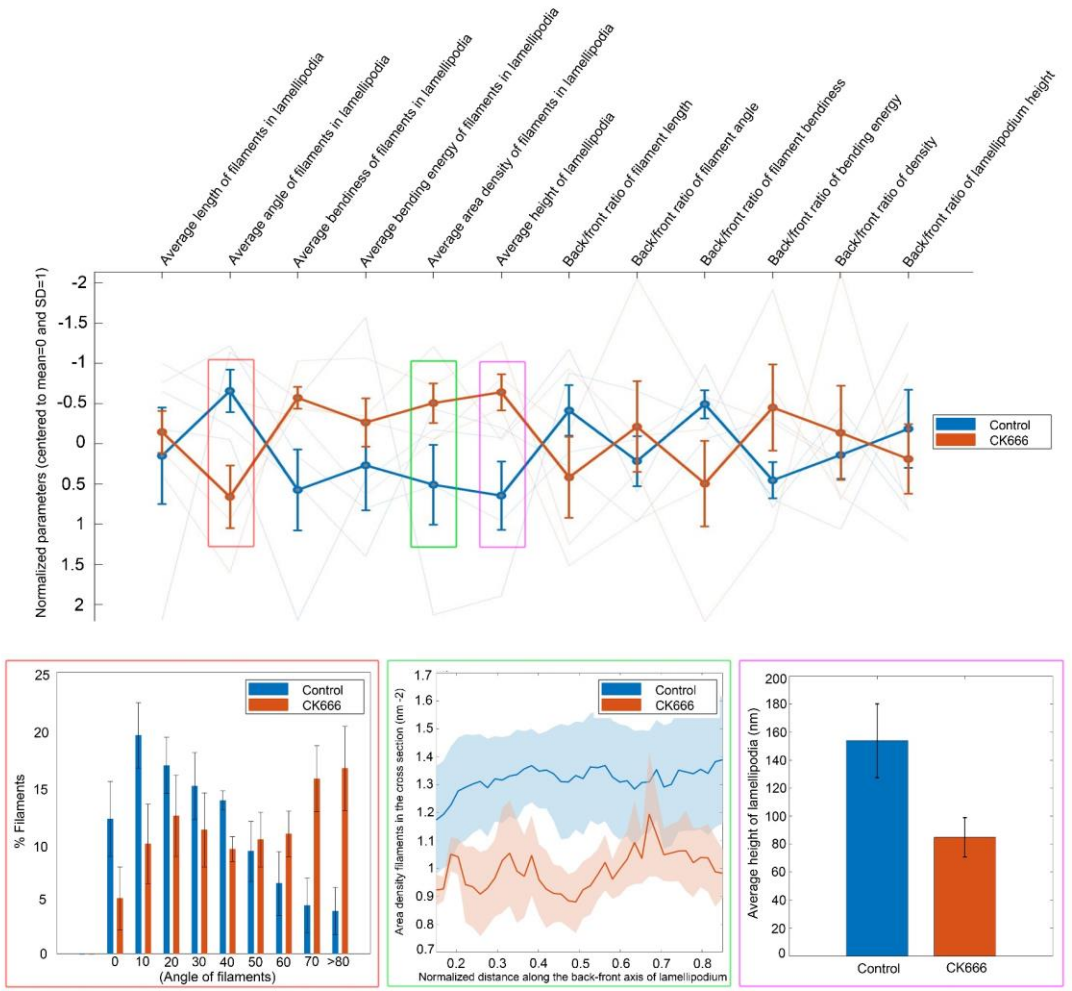
angle
to edge

distance
to edge

Quantification of vectorized filaments



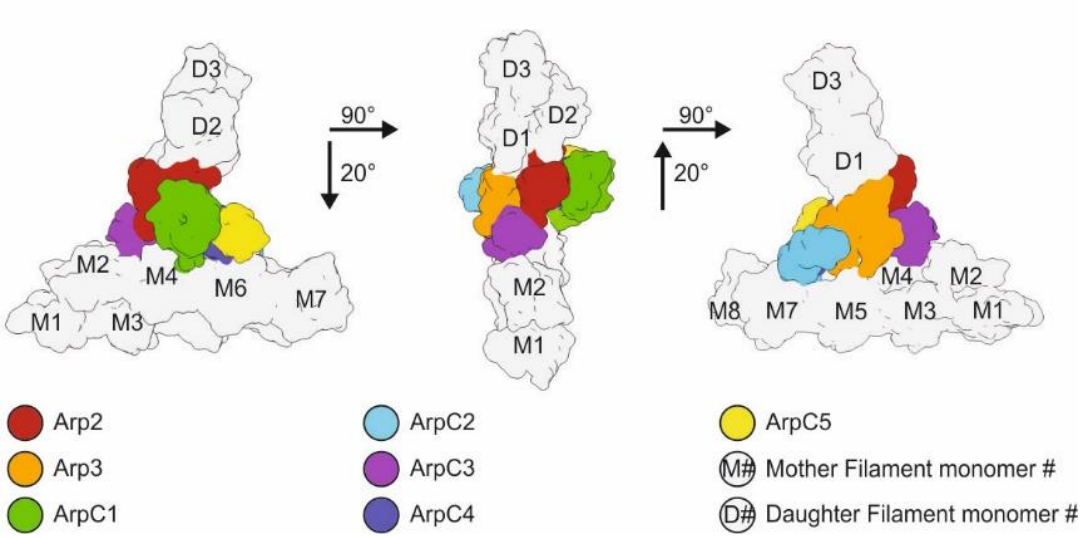
Template matching based tracking in Amira



Adapted from Dimchev et al., 2021, doi.org/10.1016/j.jsb.2021.107808

Differential behavior of Arp2/3 subunit isoforms

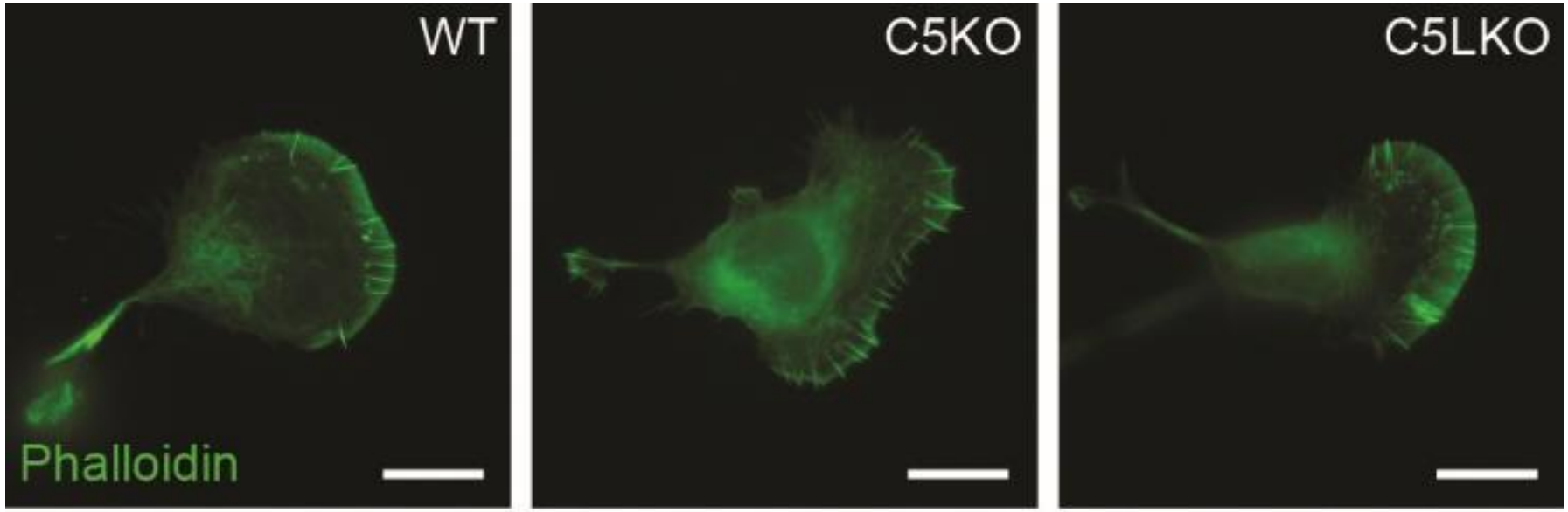
- Two different ArpC5 subunit isoforms: ArpC5 and ArpC5L
- Specifically, ArpC5 is associated with more metastasis and worse outcome in cancer



Adapted from Fäßler et al., 2020, doi.org/10.1038/s41467-020-20286-x

Morphology of isoform-specific knockout cells

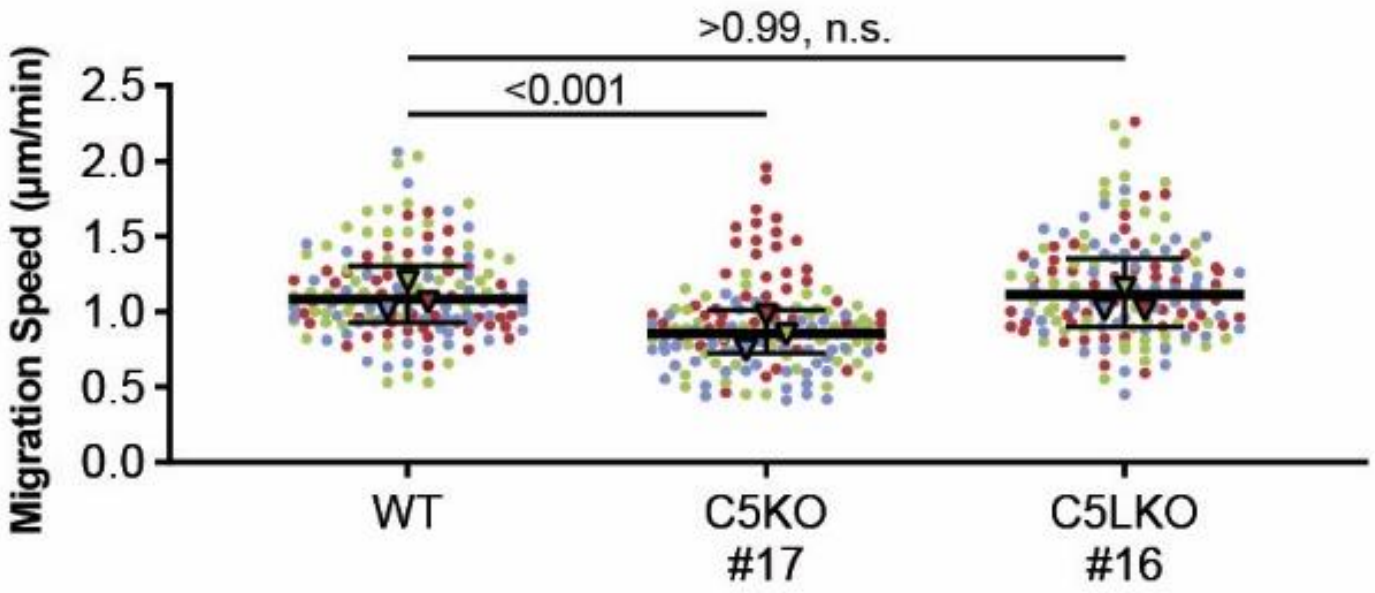
- ArpC5 knockout cells (C5KO) exhibit narrower lamellipodia
- ArpC5L knockout cells (C5LKO) exhibit wider lamellipodia



Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

Random migration of isoform-specific knockout cells

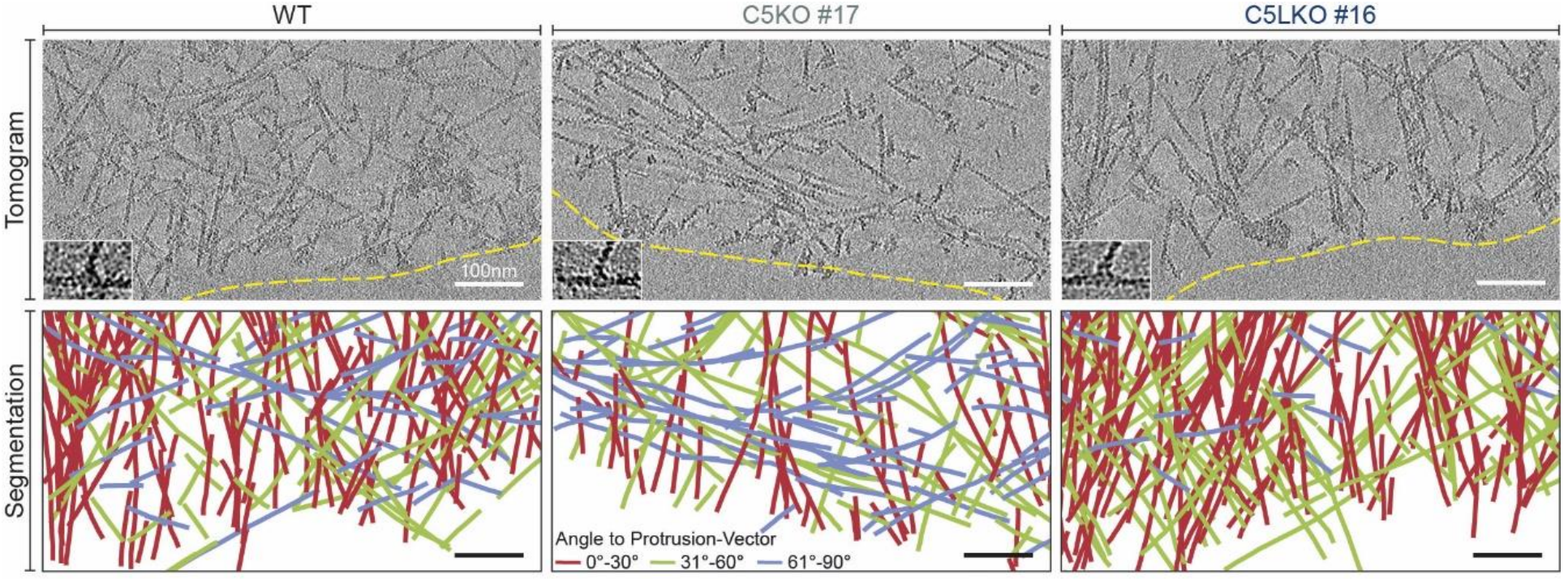
- Random migration speed of C5KO cells is reduced
- Random migration speed of C5LKO cells is comparable to WT



Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

Actin architecture of isoform-specific knockout cells

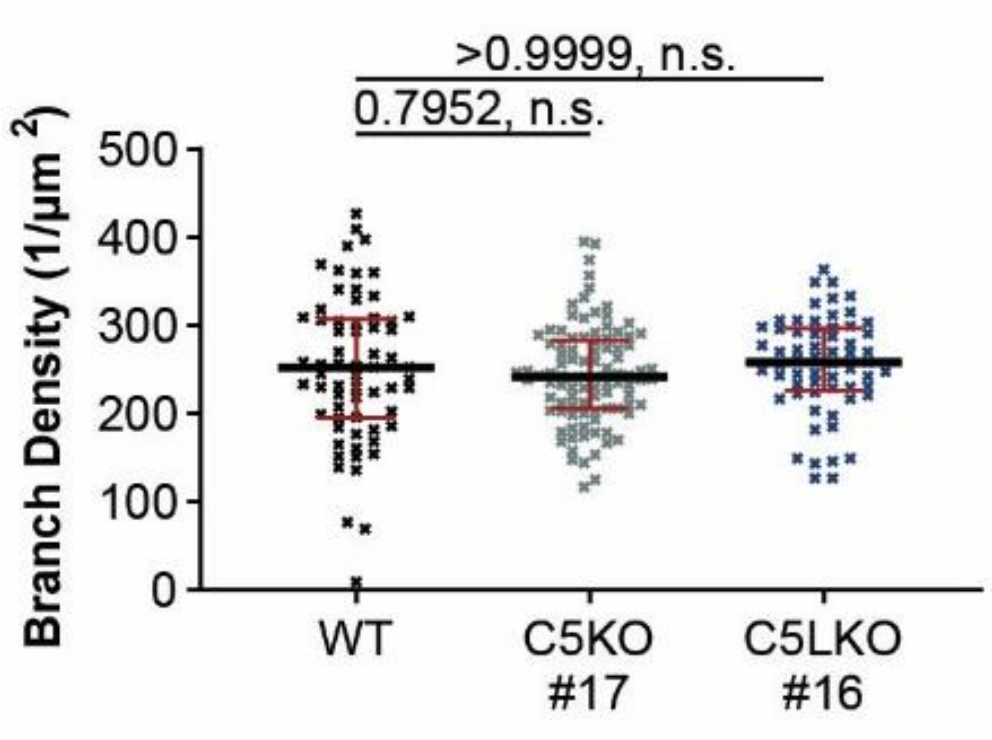
- Actin filaments in lamellipodia of C5KO run rather perpendicular to the protrusion-vector
- Actin filaments in lamellipodia of C5LKO run rather parallel to the protrusion-vector



Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

Branch junction density is unaltered in both KO genotypes

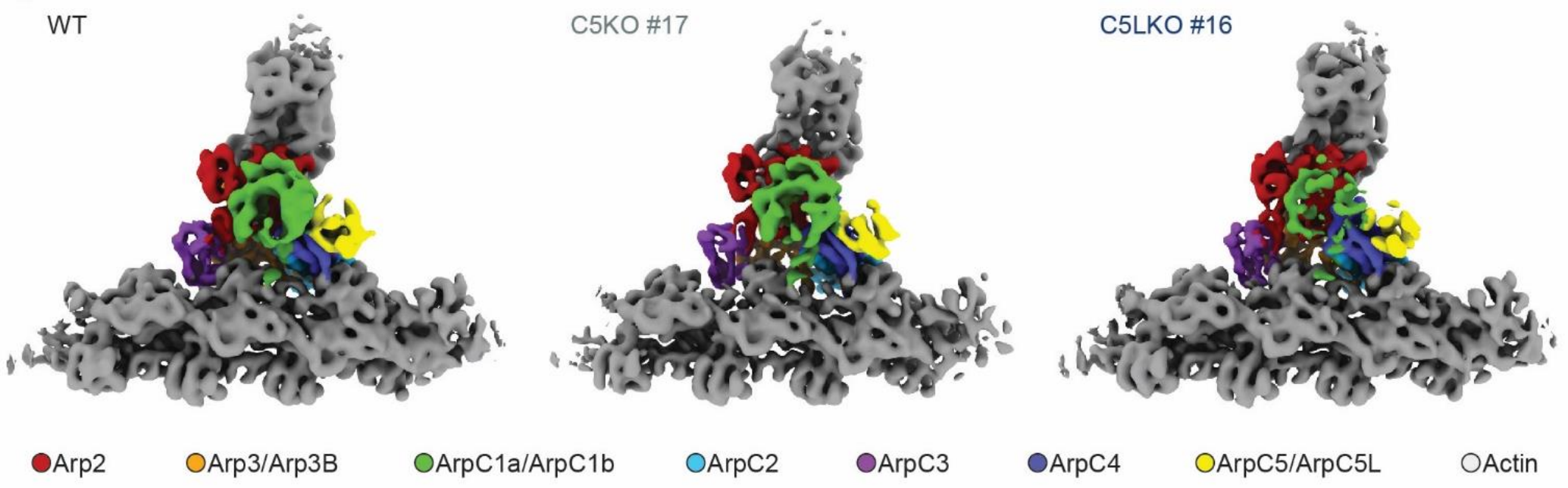
- Branch junction number cannot be the cause of altered phenotypes
- There are sufficient branches in the KO lines to probe for structural differences



Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

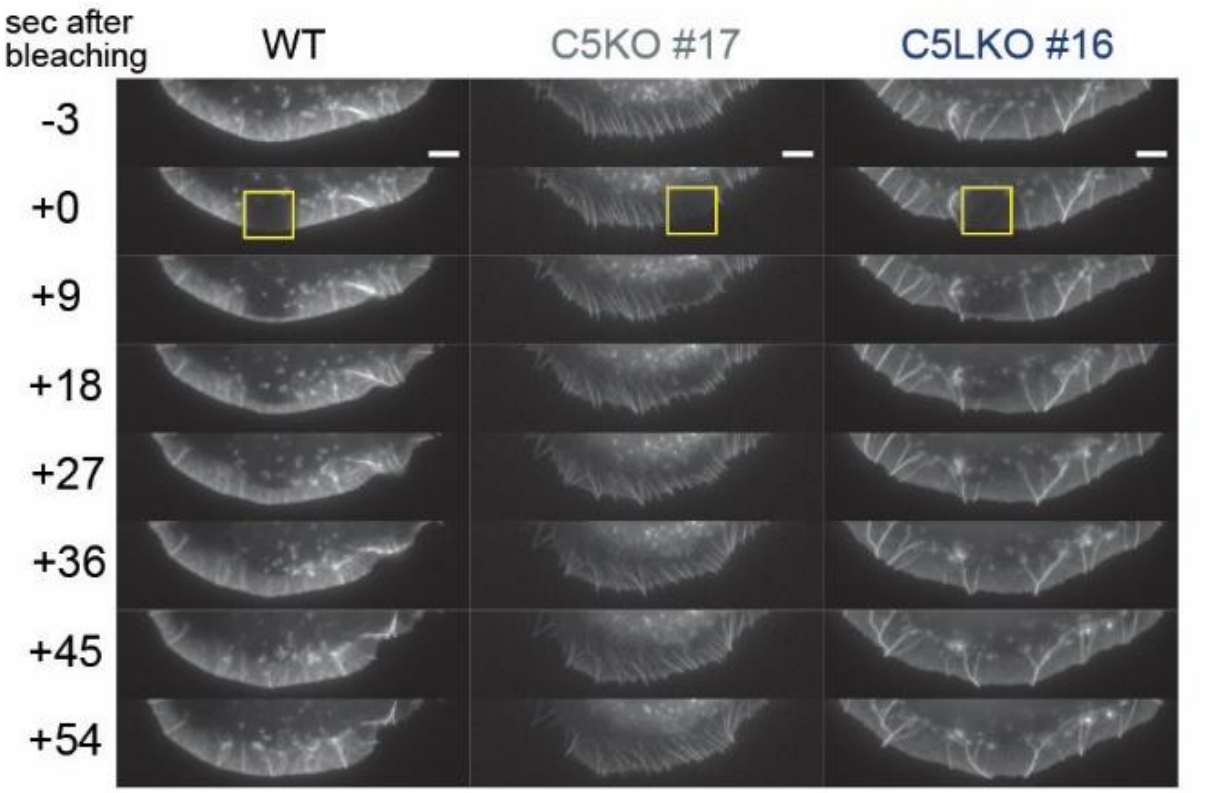
Isoform-specific branch junction structures

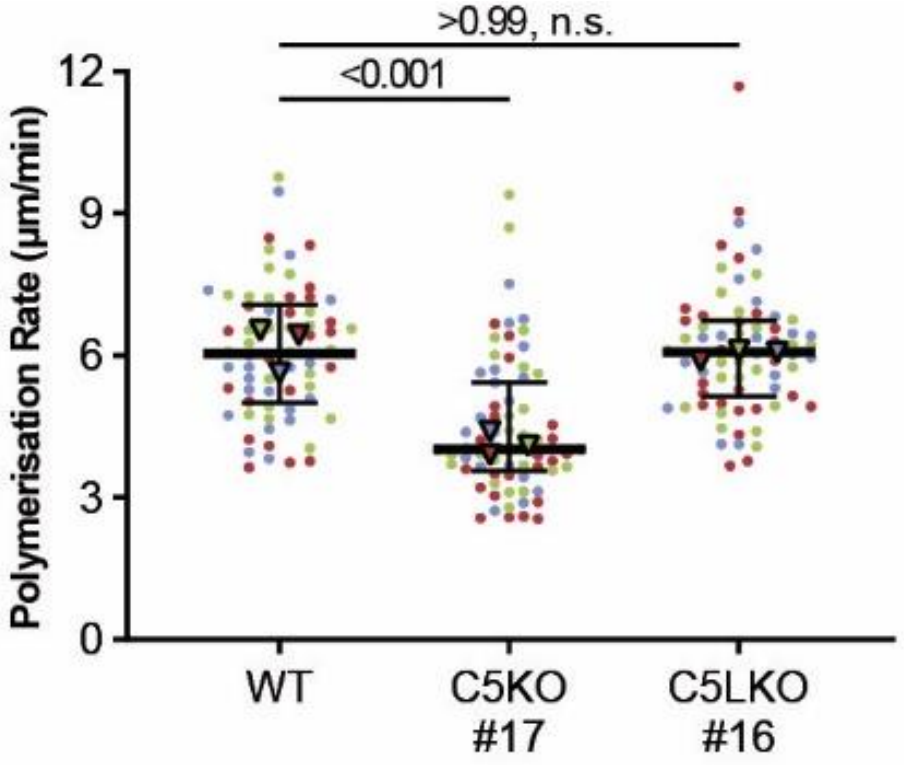
- ArpC1 appears more stable in C5KO branch junctions
- ArpC1 appears less stable in C5LKO branch junctions
- ArpC1 mediates interactions between the complex and other actin organizers



Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

Actin polymerization is reduced in lamellipodia of C5KO cells

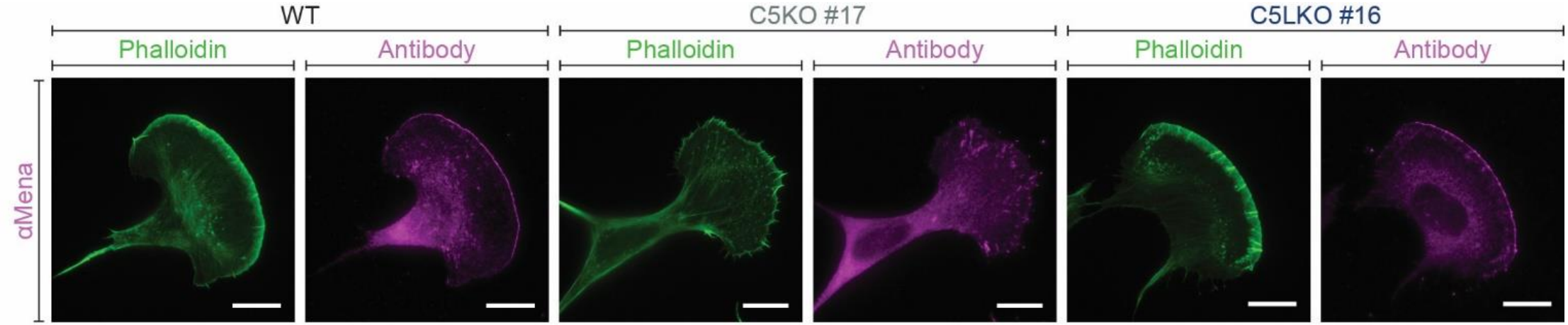




Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

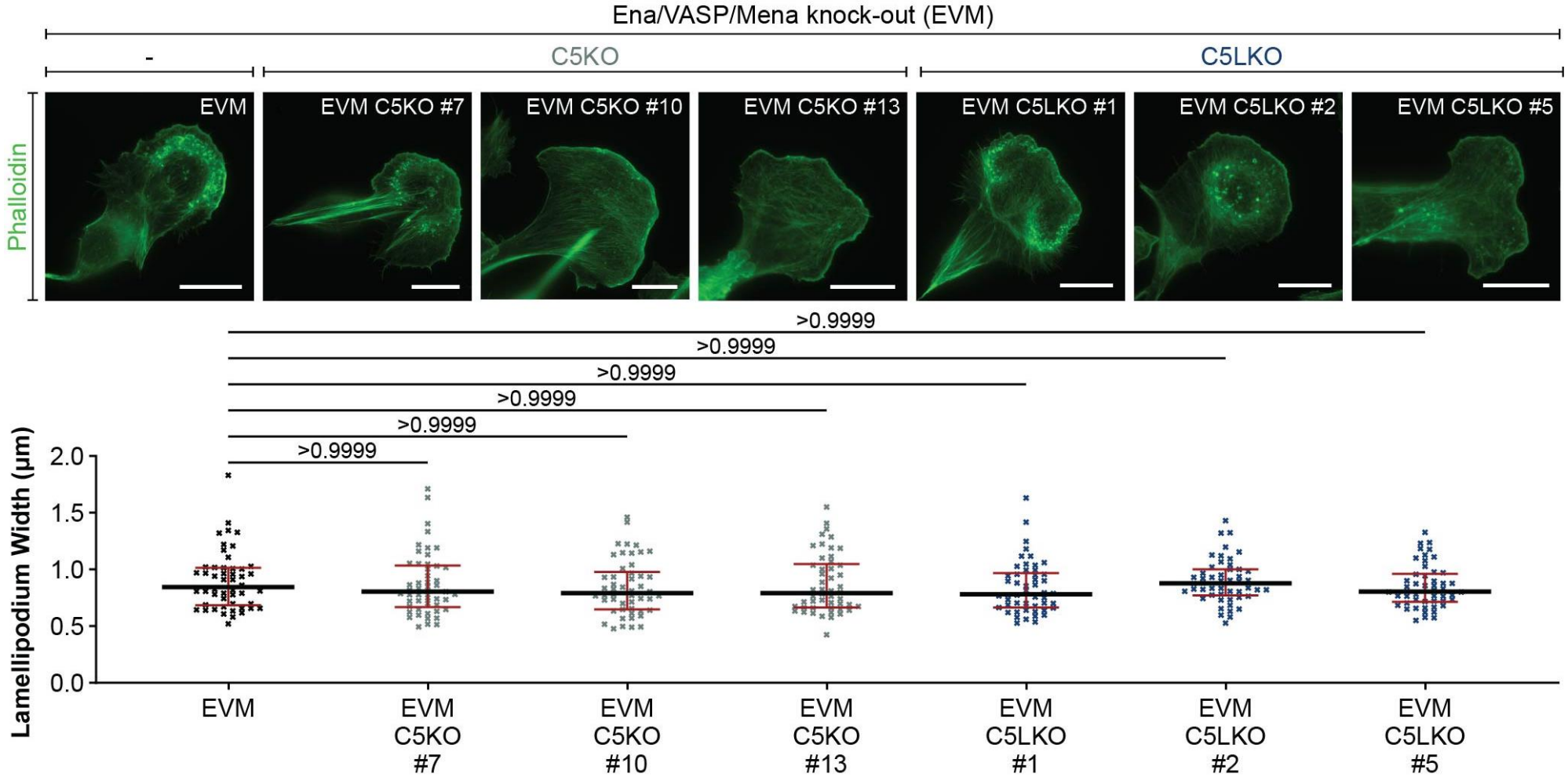
Isoform-specific recruitment of actin filament elongators

- Ena/VASP family members are depleted at the leading edge in C5KO cells



Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

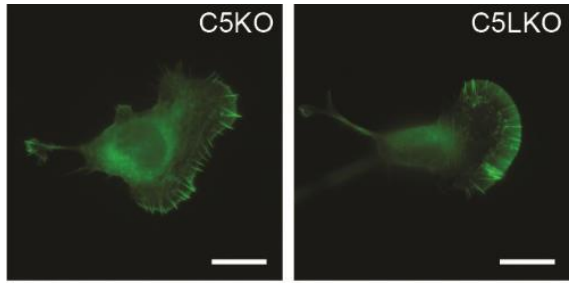
C5KO and C5LKO phenotypes depend on filament elongators



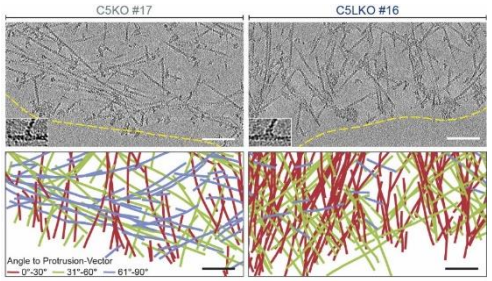
Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

ArpC5 isoforms affect lamellipodia and cell migration across scales

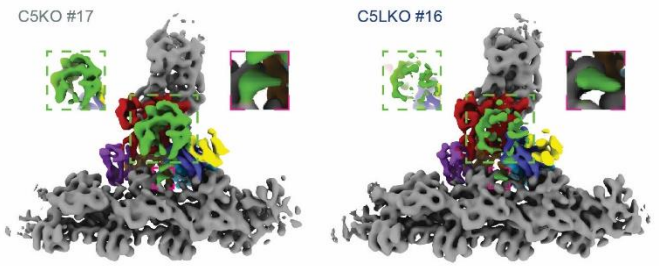
Cell Morphology



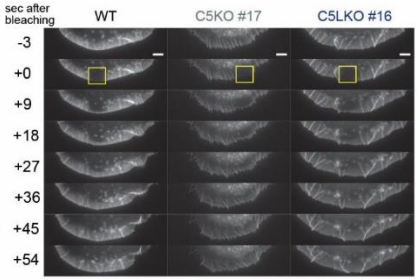
Lamellipodium Ultrastructure



Branch Junction Structure



Actin Polymerization



- Isoform differ in distinct recruitment of filament elongators, which then cause the observed phenotypes

Adapted from Fäßler et al., 2023, doi.org/10.1126/sciadv.add6495

Acknowledgements I

Schur group

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Darya Chernikova

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Victor-Valentin Hodirnau

Lab Support Facility
IOF Facility
Scientific Computing

HZI

Klemens Rottner
Hermann Döring

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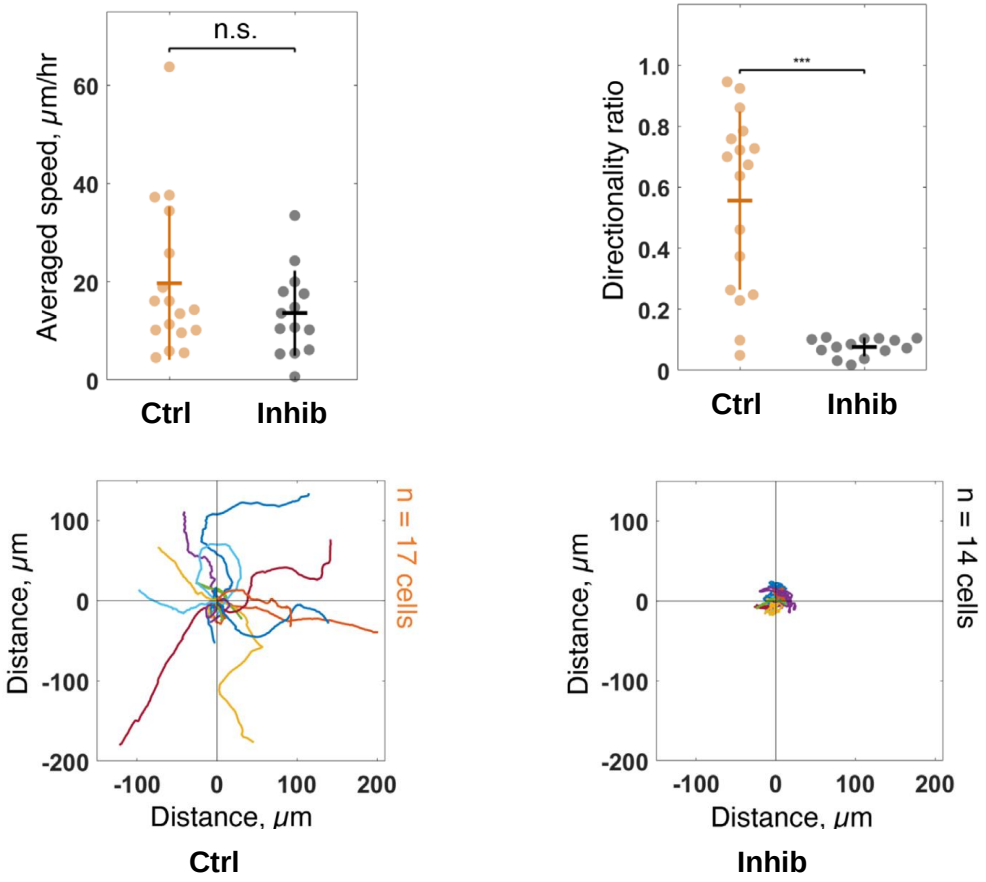
ISTA Institute of Science and Technology Austria

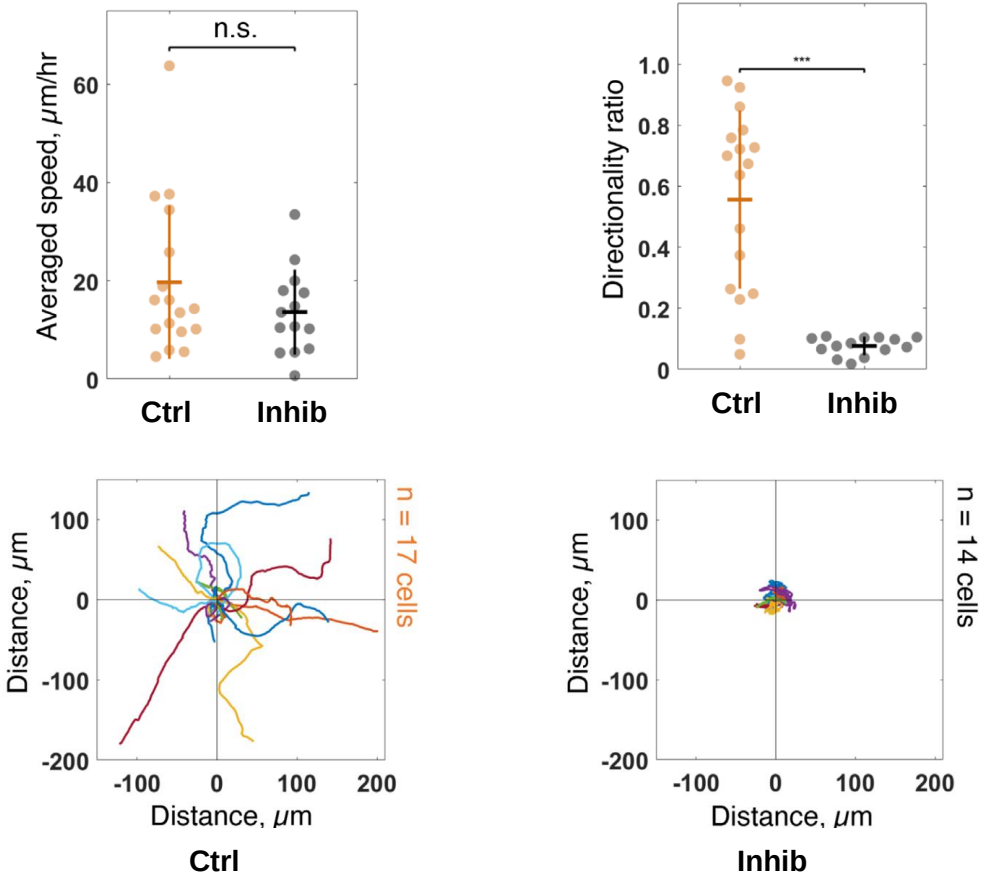
FWF Der Wissenschaftsfonds.

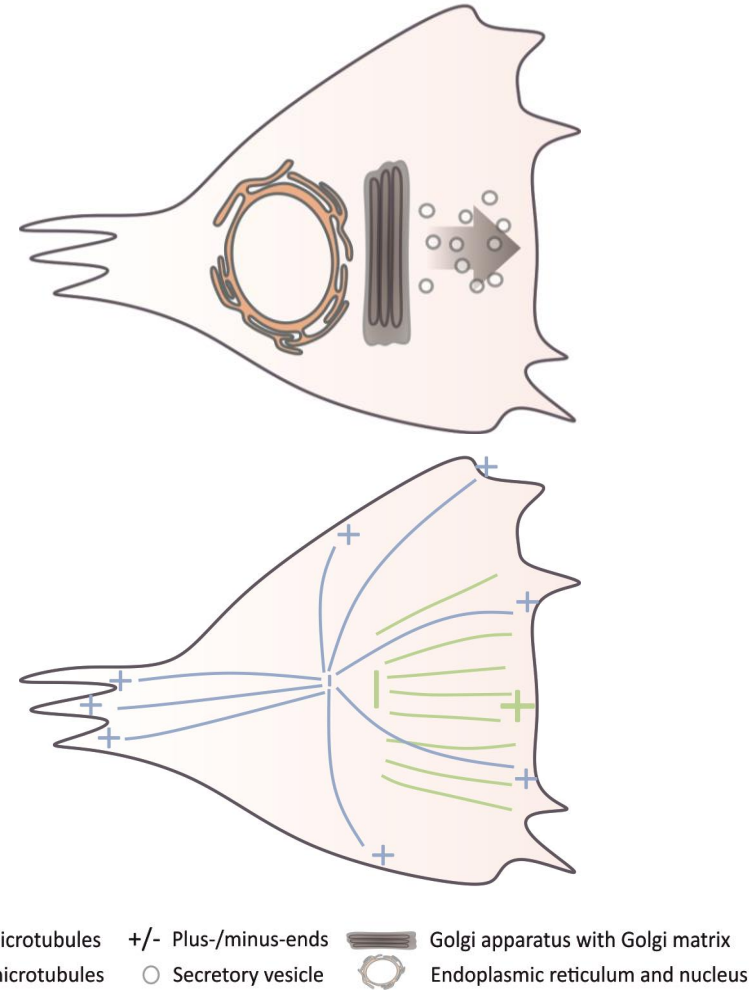
Directional Microtubule arrays are central for persistent directional cell migration



Adapted from Vaidžiulytė et al., 2022, doi.org/10.7554/eLife.69229

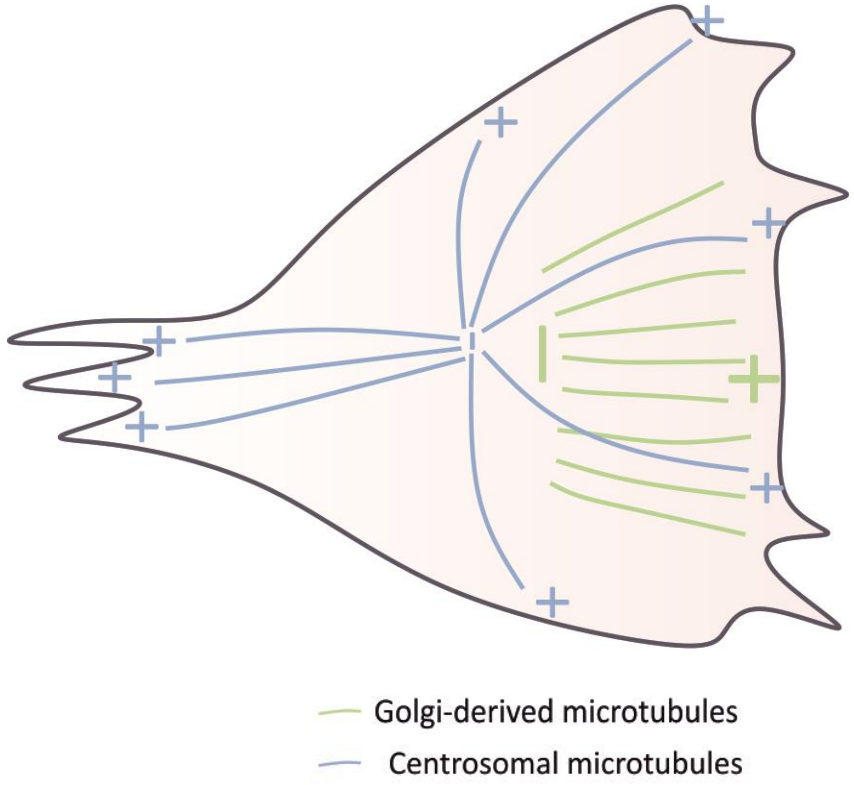
Directional Golgi-derived Microtubule arrays are central for persistent directional cell migration



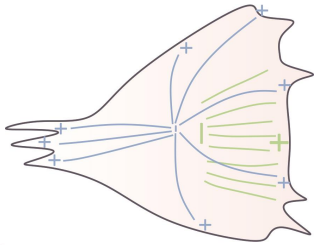


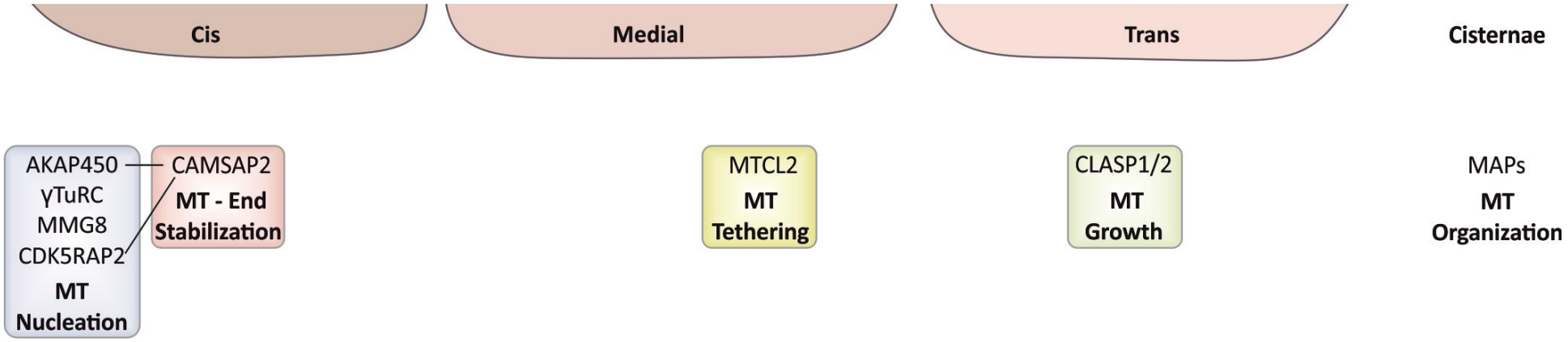
Adapted from Vaidžiulytė et al., 2022, doi.org/10.7554/eLife.69229

How are directional Microtubule arrays organized at the Golgi?

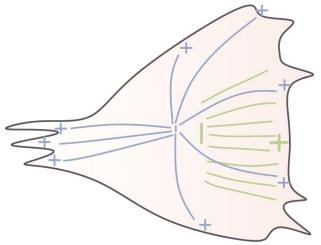


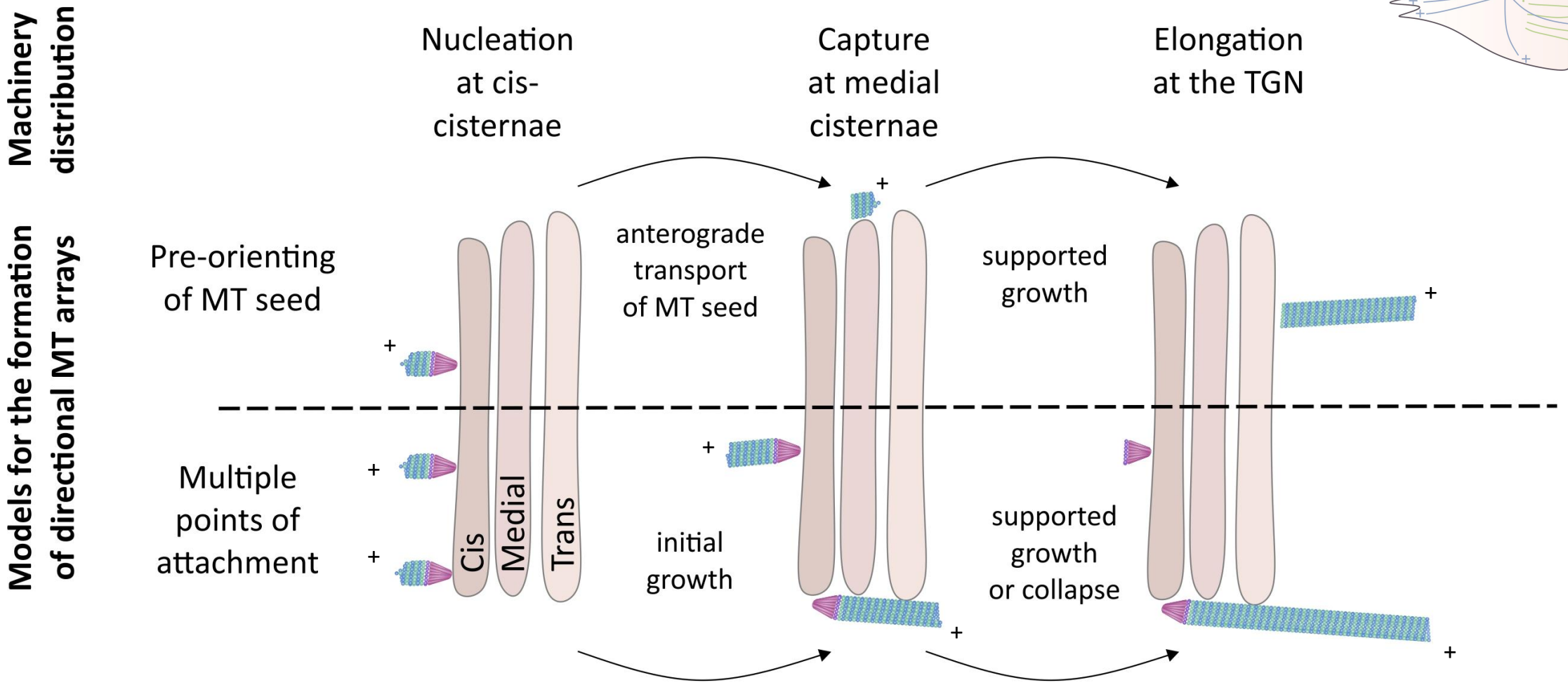
Observation: Microtubule nucleation and elongation at the Golgi is spatially separated





How could spatial separation of nucleation and elongation support direction Microtubule growth?





What do we need to understand array formation at the Golgi?

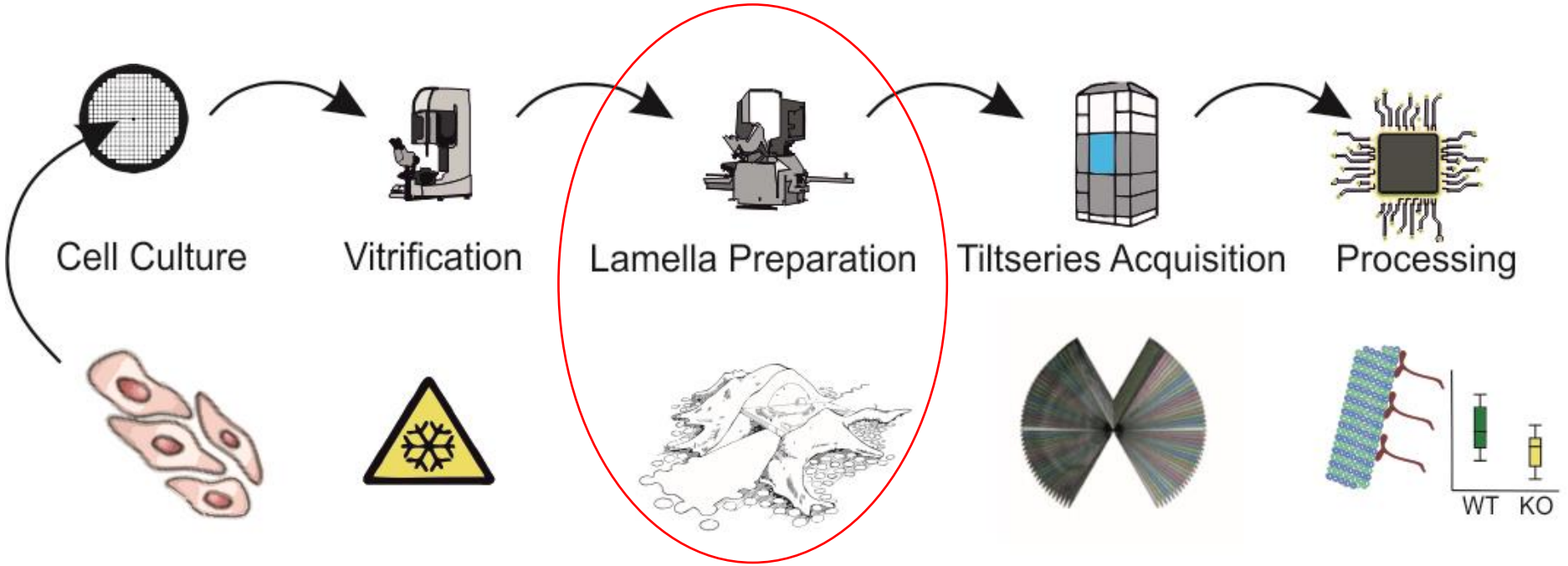
- Microtubule length and positioning at different states during their alignment

  -> Cryo-ET and ultrastructural analysis

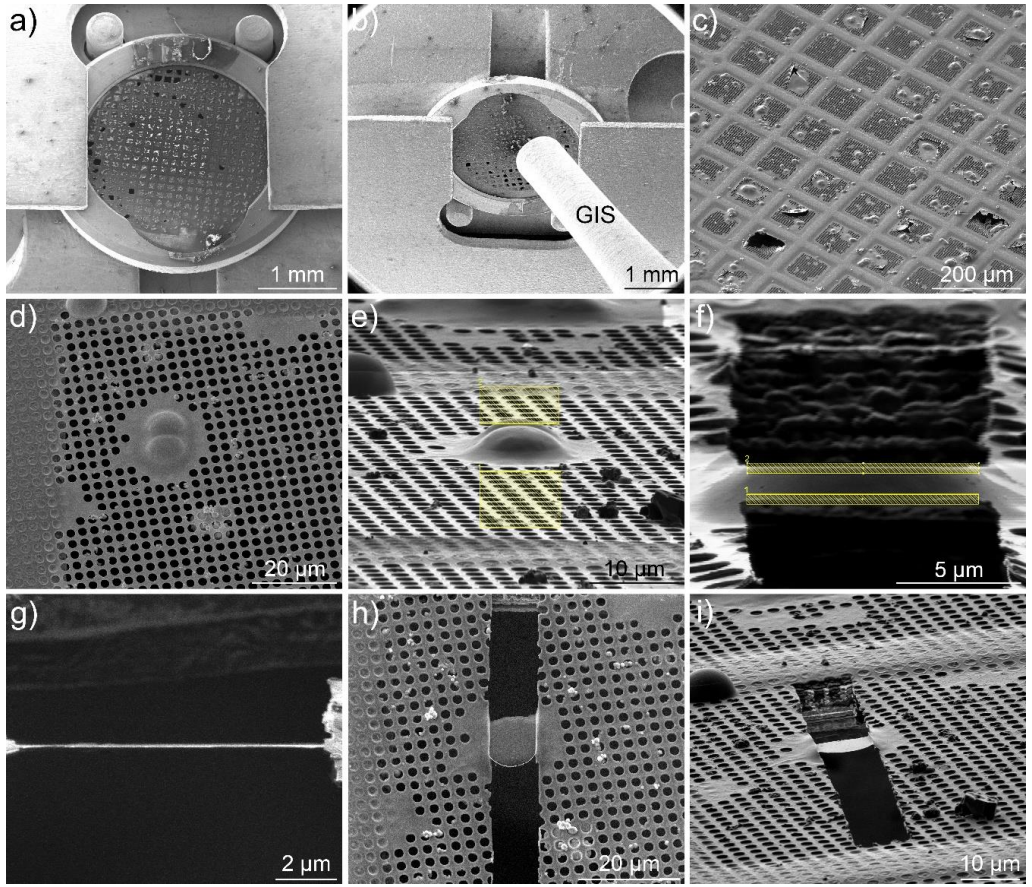
- MAP quantity and distribution on the Microtubules during the different states of alignment

  -> Subtomogram averaging for identification of MAPs

Microtubule organization at the Golgi: General approach



Lamella preparation by focused ion beam milling (FIB)



Adapted from Schaffer et al., 2015, doi/10.21769/bioprotoc.1575

Acknowledgements II

Team Cellular Architecture

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BSI Department

Platforms & Services
Integrated structural biology
Cell culture
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IT