

## **Supplemental Materials**

### **Supplemental Figures 1-7**

## Supplemental figure 1: Summary of the image processing of native wild type fibrils.

Initial 2D classification revealed three distinct groups of particles: 1-start/thin filaments (red squares), 2-start/thick filaments (blue squares), and higher-order filament bundles (yellow squares).

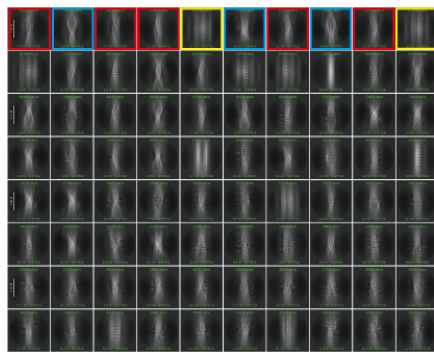
Native WT: 16,081 micrographs

CryoSPARC filament tracer: 30,430,102 particles

CryoSPARC 2D classification

1-start: 6,147,984

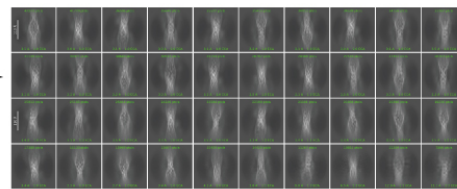
2-start: 4,966,572



CryoSPARC 2D classification

Polymorph 1: 1,041,193 particles

Polymorph 2: 1,046,977 particles



CryoSPARC

Helical refinement

Local CTF refinement

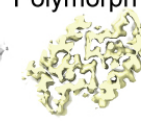
Reference-based motion correction

3D classification

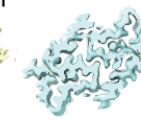
Polymorph 1



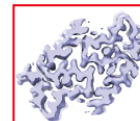
218,876



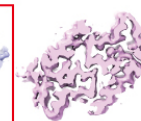
83,727



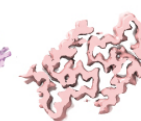
177,735



286,491

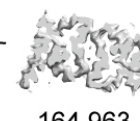


83,918



190,254

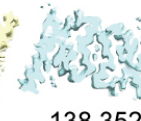
Polymorph 2



164,963



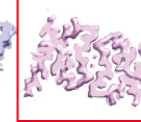
145,876



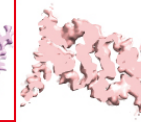
138,352



151,770

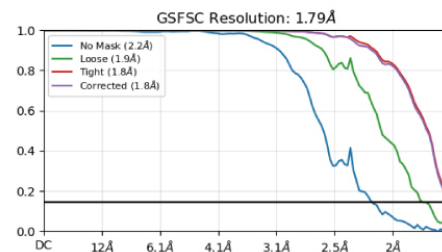
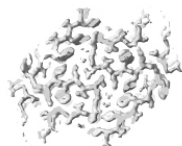


352,878

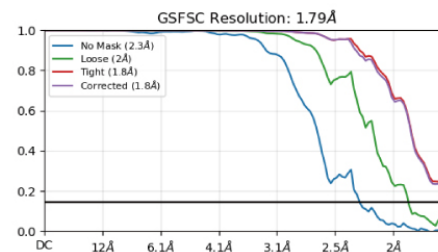
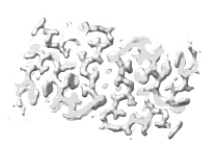


93,138

Polymorph 1



Polymorph 2



## Supplemental figure 2: Summary of the image processing of native G175S fibrils

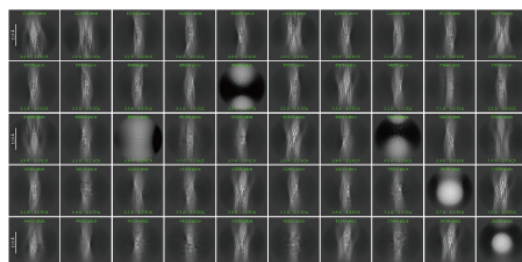
Native G175S: 7,280 micrographs

CryoSPARC filament tracer: 6,395,243 particles

CryoSPARC 2D classification

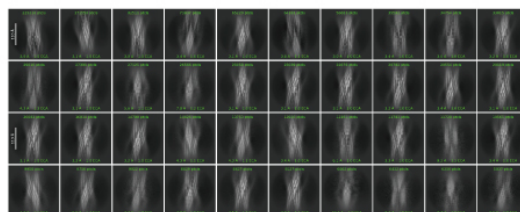
1-start: 1,907,751 particles

2-start: 1,238,734 particles



CryoSPARC 2D classification

2-start: 1,195,902 particles



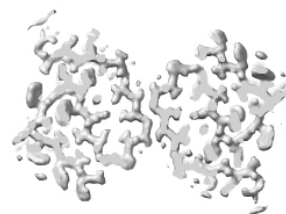
CryoSPARC

Helical refinement

Local CTF refinement

Reference-based motion correction

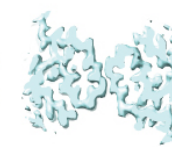
3D classification



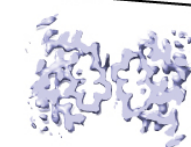
289,758



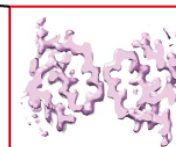
100,509



227,637



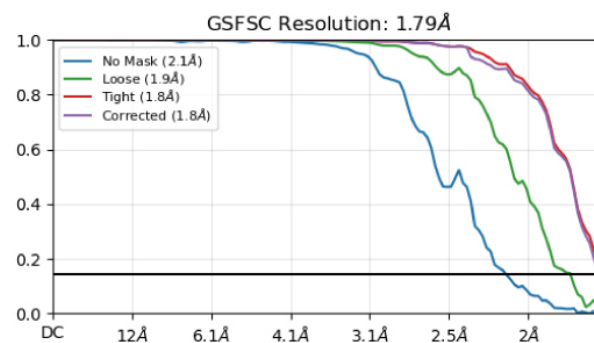
147,413



287,239



142,605

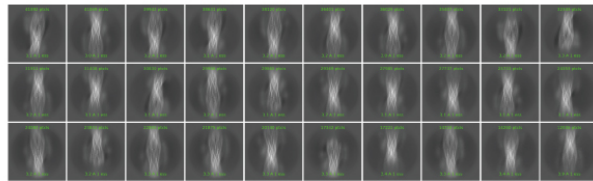
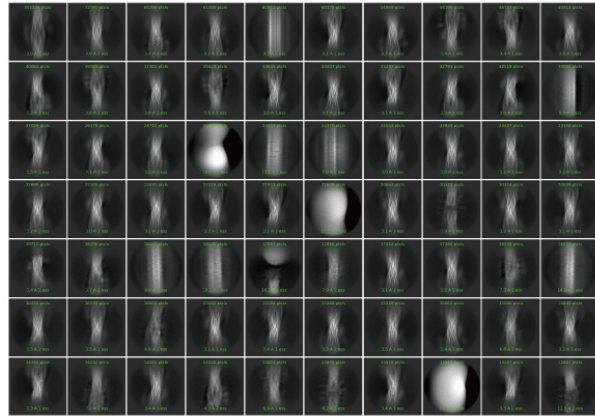


### Supplemental figure 3: Summary of the image processing of in vitro-polymerized wild-type PMEL CAF fibrils

In vitro wild-type: 4,331 micrographs

CryoSPARC filament tracer: 3,826,618 particles

CryoSPARC 2D classification: 754,897 particles

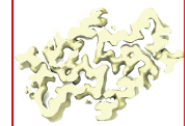


CryoSPARC

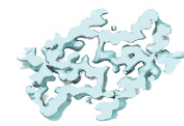
Helical refinement  
Global CTF refinement  
Local CTF refinement  
3D classification



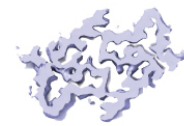
155,900



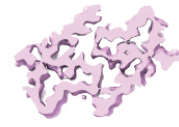
147,735



109,524



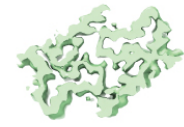
75,003



70,690



69,419



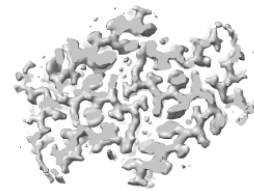
69,275



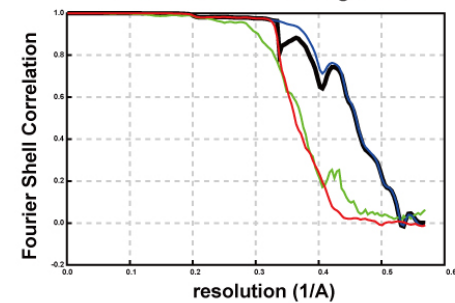
57,351

Relion

3D refinement  
CTF refinement  
3D refinement  
Bayesian Polishing  
3D refinement  
CTF refinement  
3D refinement



Final resolution = 1.9 Angstroms



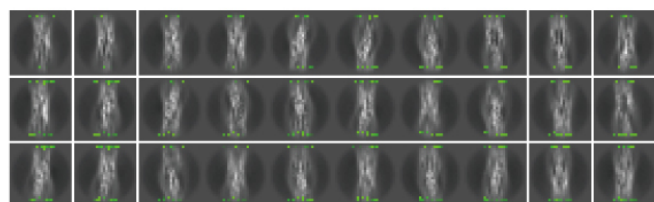
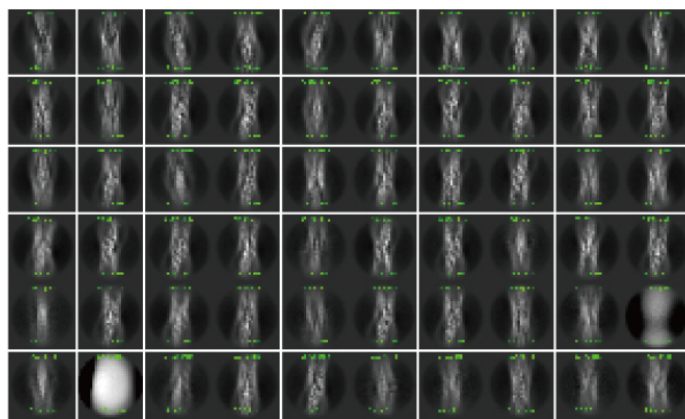
■ rlnFourierShellCorrelationCorrected  
■ rlnFourierShellCorrelationUnmaskedMaps  
■ rlnFourierShellCorrelationMaskedMaps  
■ rlnCorrectedFourierShellCorrelationPhaseRandomizedMaskedMaps

# Supplemental figure 4: Summary of the image processing of in vitro-polymerized G175S PMEL CAF fibrils

In vitro G175S: 5,020 micrographs

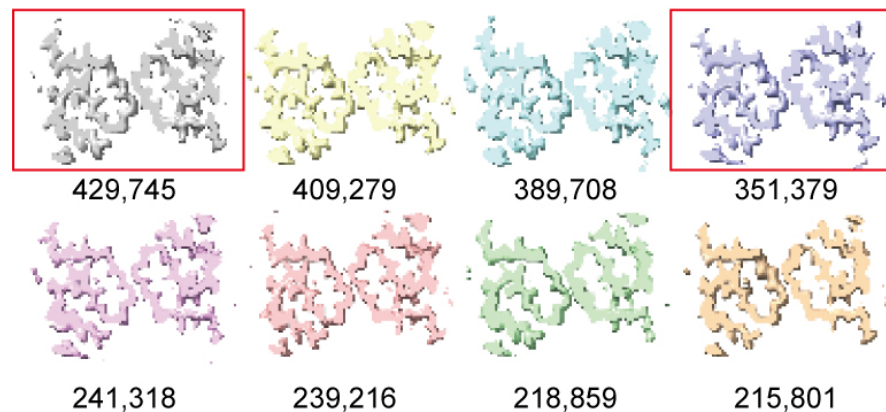
CryoSPARC filament tracer: 3,606,507 particles

CryoSPARC 2D classification: 2,495,305 particles



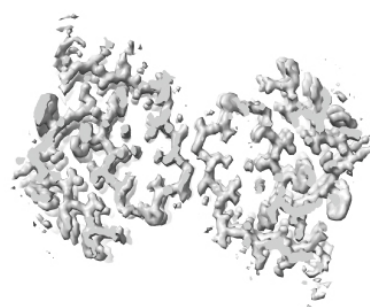
CryoSPARC

Helical refinement  
Global CTF refinement  
Local CTF refinement  
3D classification

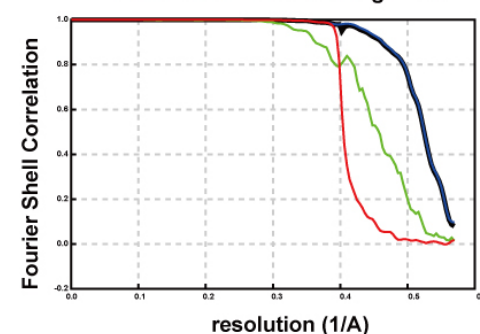


Relion

CTF refinement  
Bayesian Polishing  
3D refinement



Final resolution = 1.9 Angstroms



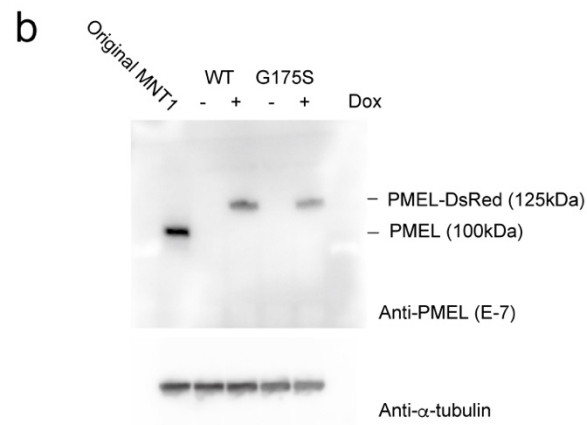
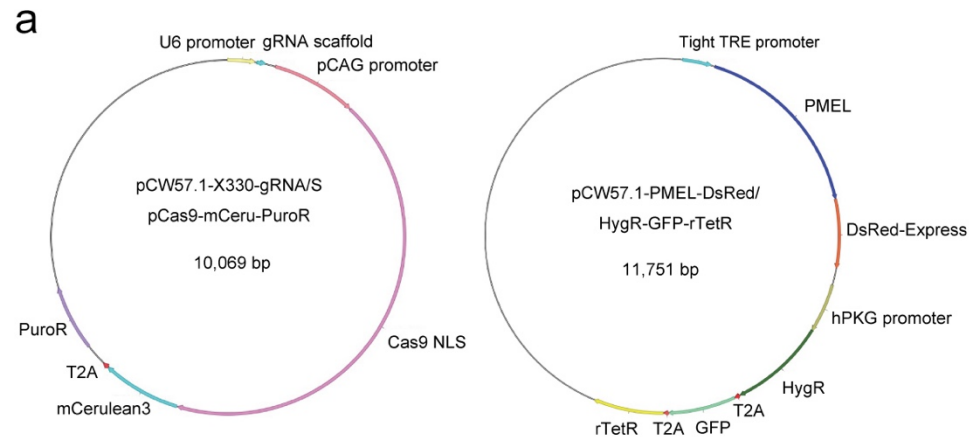
■ rlnFourierShellCorrelationCorrected  
■ rlnFourierShellCorrelationUnmaskedMaps  
■ rlnFourierShellCorrelationMaskedMaps  
■ rlnCorrectedFourierShellCorrelationPhaseRandomizedMaskedMaps



### Supplemental figure 5: Generation of Dox-inducible PMEL WT/G175S Cell Lines

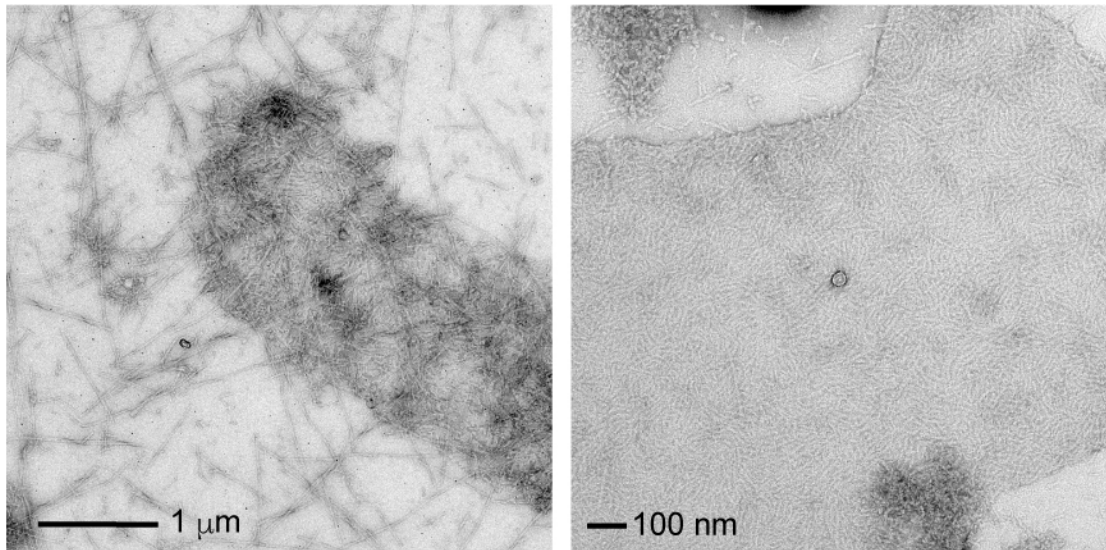
(a) Vector maps of pCW57.1-X330-gRNA/SpCas9-mCerulean-PuroR and pCW57.1-PMEL-DsRed-HygR-GFP-rTetR. The pCW57.1-X330-gRNA/SpCas9-mCerulean-PuroR plasmid enables the knockout of the native PMEL gene, while pCW57.1-PMEL-DsRed-HygR-GFP-rTetR allows for doxycycline-inducible expression of the PMEL WT or G175S mutant fused to DsRed.

(b) Western blot analysis of MNT1 cells confirming the knockout of the native PMEL gene (WT/G175S Dox (-)) and the doxycycline-dependent expression of PMEL-DsRed (WT/G175S Dox (+)). Anti-PMEL (E-7) was used to detect both the native PMEL (100 kDa) and the PMEL-DsRed fusion protein (125 kDa). Anti- $\alpha$ -tubulin antibody (B512, Thermo Fisher Scientific) was used as a loading control.



**Supplemental Figure 6: Negative-stain EM images of disintegrated melanin granules for PMEL amyloid extraction.**

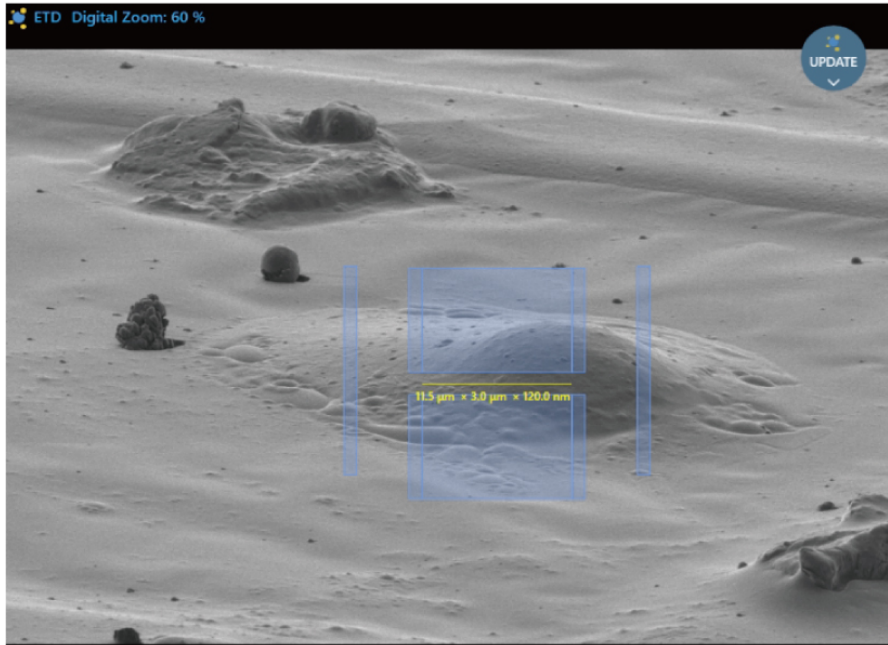
Negatively-stained EM images show the fibrillar structures observed after disintegration of melanin granules, highlighting the presence of entangled amyloid fibrils within the lamellar structures.



**Supplemental figure 7: Representative images of cryo-FIB-SEM tomography.**

(a) Scanning electron microscopy (SEM) image of an MNT1 cell during lamella targeting, with the targeted area outlined. The dimensions of the patterns are indicated. (b) Transmission electron microscopy (TEM) image of the prepared lamella, showing the dark electron-dense melanosomes.

a



b

