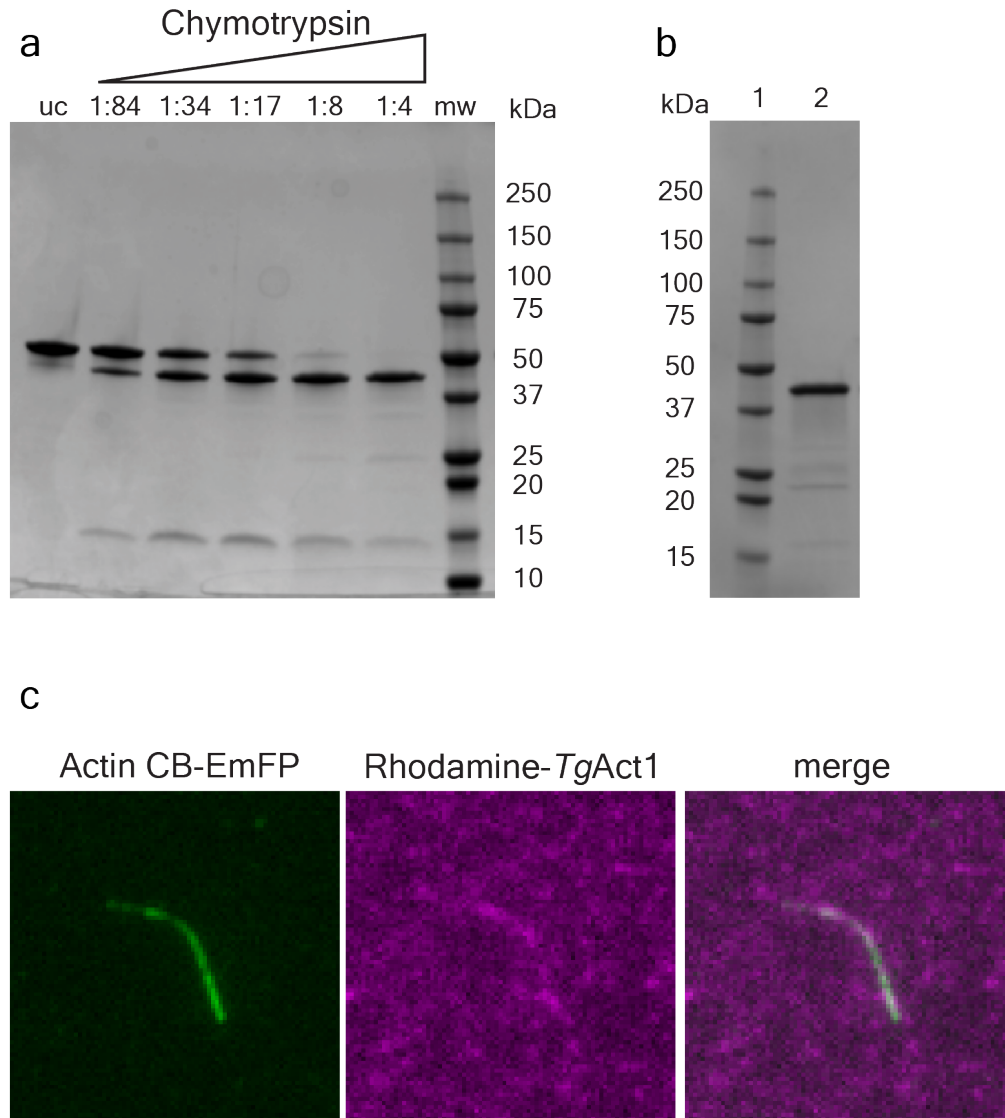
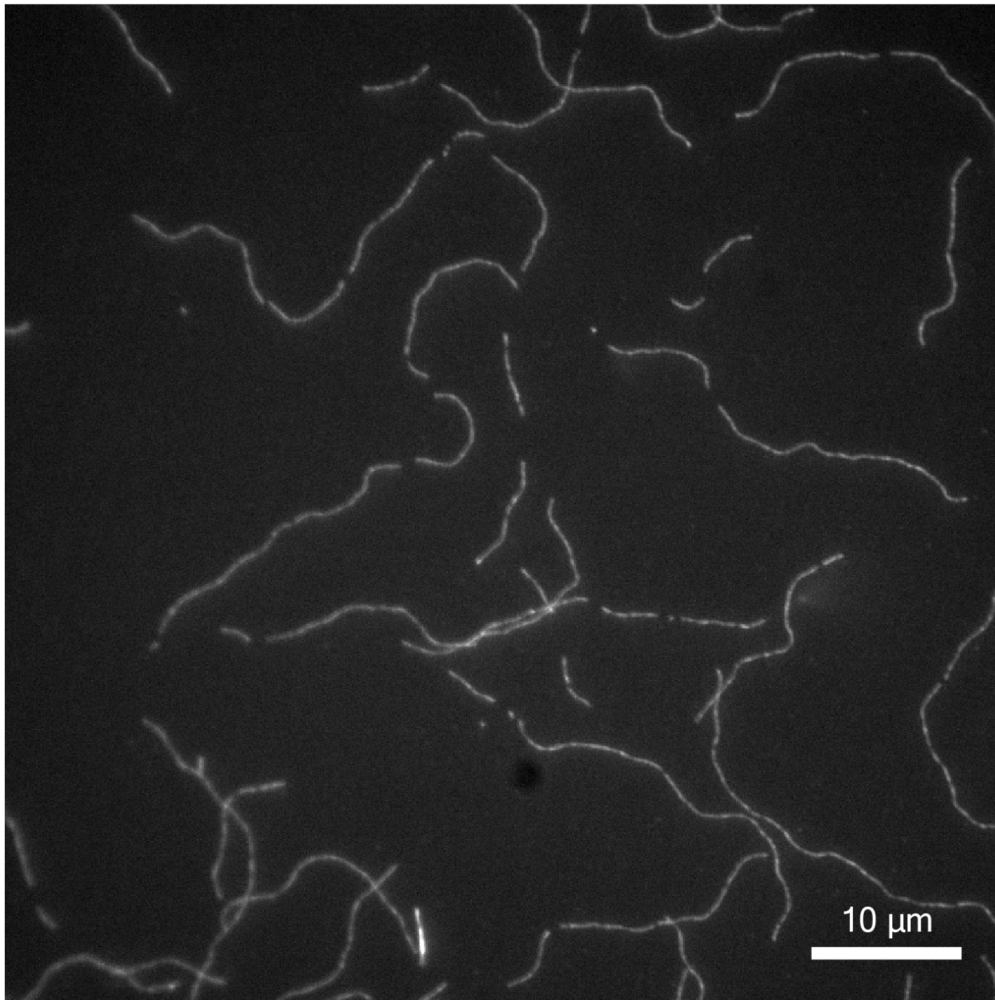
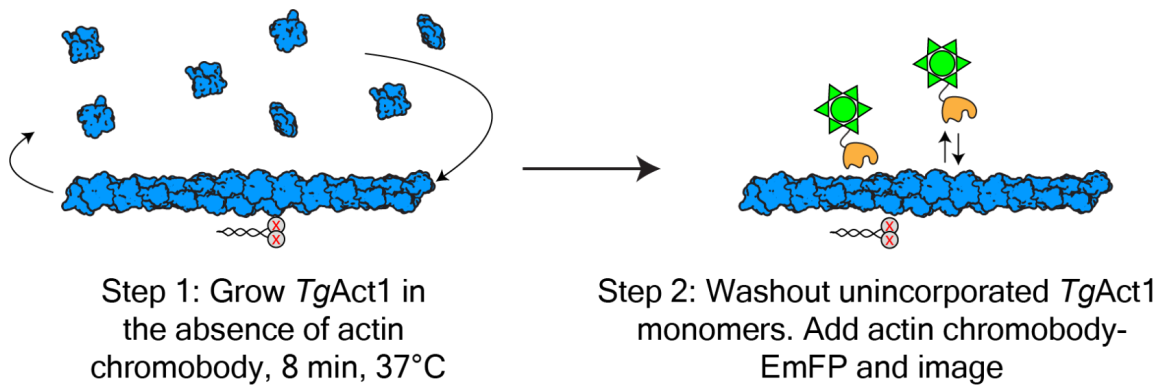


Supplementary Figures



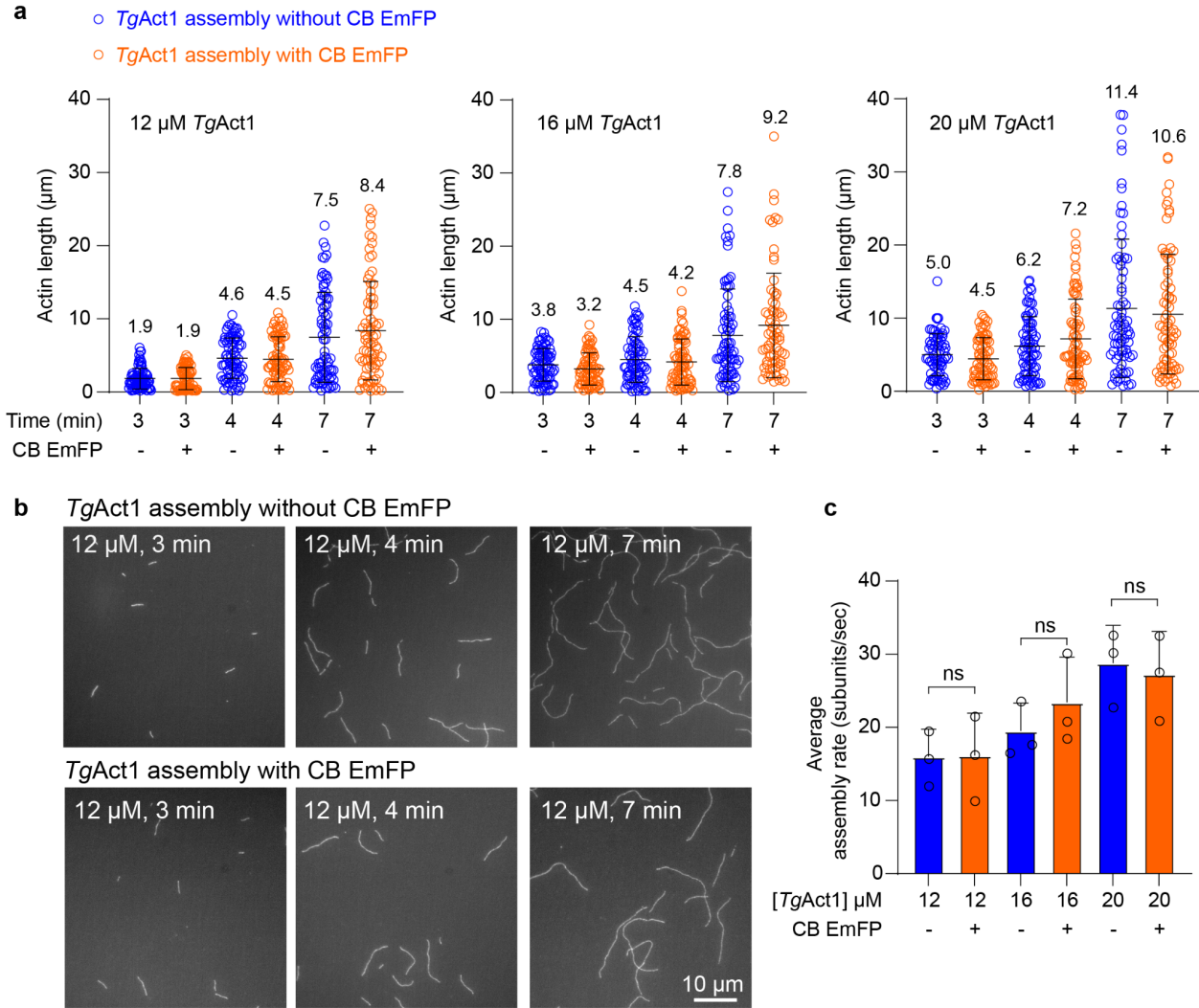
Supplementary Figure 1: Protein preparations and growth of *TgAct1* in the presence of labeled Rhodamine actin.

a, Coomassie stained SDS-PAGE showing *TgAct1*– β -thymosin–HIS incubated with different molar ratios of chymotrypsin:actin for 15 min at 27°C, to determine optimal conditions for removal of β -thymosin–HIS tag. uc: uncut; mw: protein molecular weight marker. b, Coomassie stained SDS-PAGE gel showing (lane 1) protein molecular weight marker and (lane 2) 2 μ g purified actin chromobody-EmeraldFP. c, Unlabeled *TgAct1* was mixed with Rhodamine-labeled *TgAct1* at a ratio of 20:1 (5%) and induced for polymerization for 15 minutes. The resulting filaments were adhered to a flow chamber, washed with an imaging buffer containing actin chromobody-EmeraldFP and imaged. The average percent incorporation ($0.5 \pm 0.07\%$, $n = 4$ filaments) was determined by visualizing the number of Rhodamine positive monomers per filament length. Source data are provided as a Source Data file.



Supplementary Figure 2: Imaging *TgAct1* polymerized in the absence of chromobody.

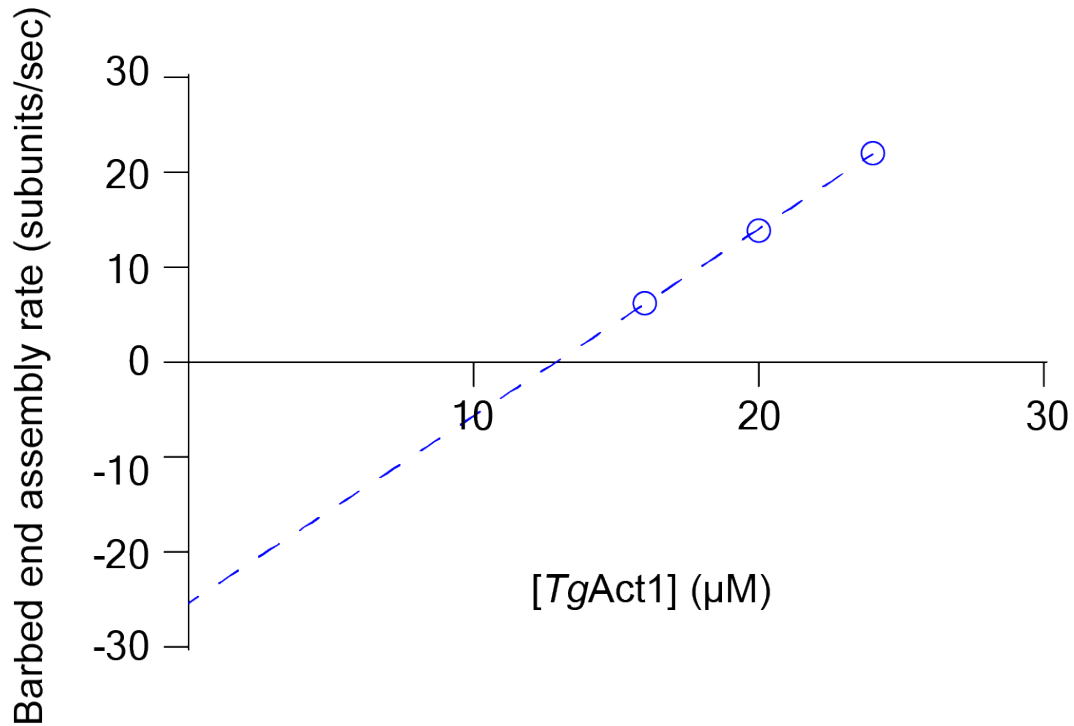
TgAct1 was induced for polymerization in a flow chamber in the absence of actin chromobody for 10 min at 37°C. Then, unincorporated *TgAct1* monomers were washed out and replaced with a solution containing 50 nM actin chromobody-EmeraldFP and imaged before filaments depolymerized. Polymerization conditions: 25 mM imidazole, pH 7.4, 50 mM KCl, 2.5 mM MgCl₂, 1 mM EGTA, 2.5 mM MgATP, 10 mM DTT, 0.25% methylcellulose, 2.5 mg/mL BSA, 0.5% Pluronic F-127, oxygen scavenging system (0.13 mg/mL glucose oxidase, 50 μg/mL catalase, and 3 mg/mL glucose), 37°C.



Supplementary Figure 3: The actin chromobody EmFP has no effect on the average assembly rate of *TgAct1* filaments.

TgAct1 was induced for polymerization in a flow chamber in the absence or presence of 50 nM actin chromobody (actin CB EmFP). After the indicated time interval, actin monomers were removed to stop further assembly and 50 nM actin chromobody was added to visualize the resulting filaments. a, Comparison of actin length distributions assembled in the absence (blue) or presence of actin CB EmFP (orange) for 3, 4, and 7 minutes at 37°C. Descriptive statistics for actin distributions are shown in Ext. Data Table 1. b, Montage of images showing the assembly of 12 μM *TgAct1* in the absence (top) and presence (bottom) of actin CB EmFP over time. c, The average rates of *TgAct1* assembly in the absence vs. presence of CB EmFP were determined by comparing the maximum actin lengths over time for each actin concentration. No significant differences in the average assembly rate ± actin CB EmFP were detected by an unpaired two-tailed parametric t-test (12 μM ± actin CB EmFP, $p = 0.969$; 16 μM ± actin CB EmFP, $p = 0.4033$; 20 μM ± actin CB EmFP, $p = 0.752$). Maximum values at each time interval were averaged to generate mean ± SD which was converted to subunits/sec. Mean ± SD; n (12 μM: 16.0 ± 3.8; $n=3$, 12 μM + actin CB EmFP: 16.2 ± 5.8; $n=3$, 16 μM: 19.5 ± 3.8; $n=3$, 16 μM + actin CB EmFP: 23.4 ± 6.2; $n=3$, 20 μM: 28.8 ± 5.1; $n=3$, 20 μM + actin CB EmFP: 27.3 ± 5.9;

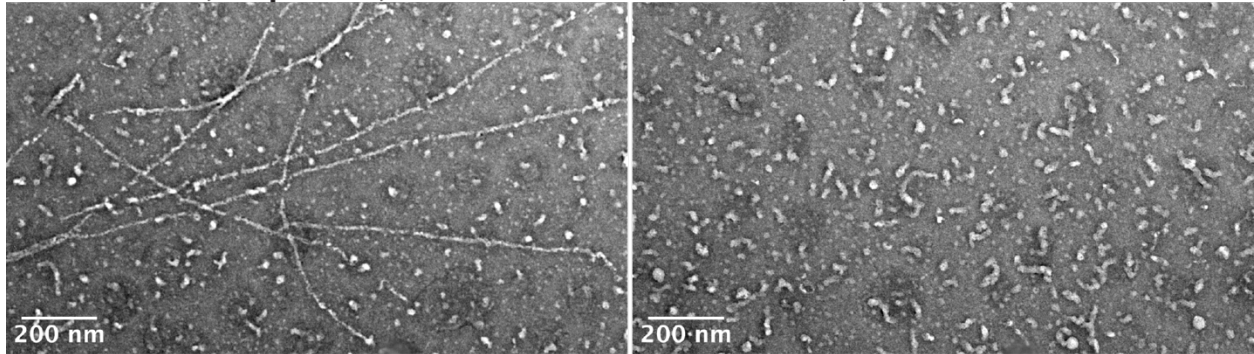
n=3). Polymerization conditions match those in Figure 1/Table 1. Source data are provided as a Source Data file.



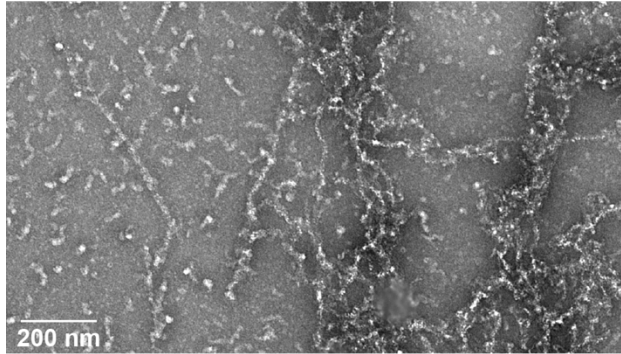
Supplementary Figure 4: Plot of the rate of barbed-end growth in subunits/sec, per actin concentration for *TgAct1* in the presence of 0.1 mM AMPPNP.

The critical concentration (C_c) (x-intercept of the fitted line) is 12.9 μM . The dissociation constant (k_-) (y-intercept) is 25.4 sec^{-1} and the association constant (k_+) (slope) is 2.0 $\text{sec}^{-1} \mu\text{M}^{-1}$. Source data are provided as a Source Data file.

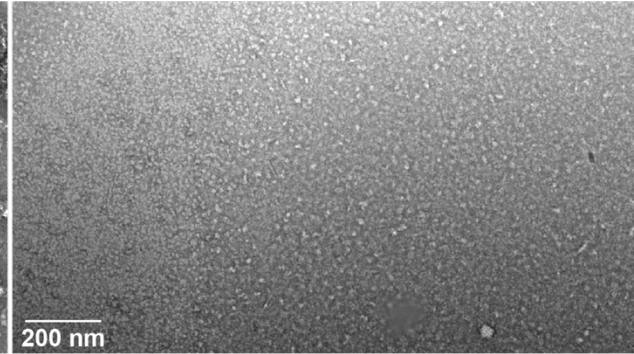
a 0.1 mM ATP, 33 μ M Jas, 60 min. at 37°C **b** 0.1 mM ATP, 60 min at 37°C



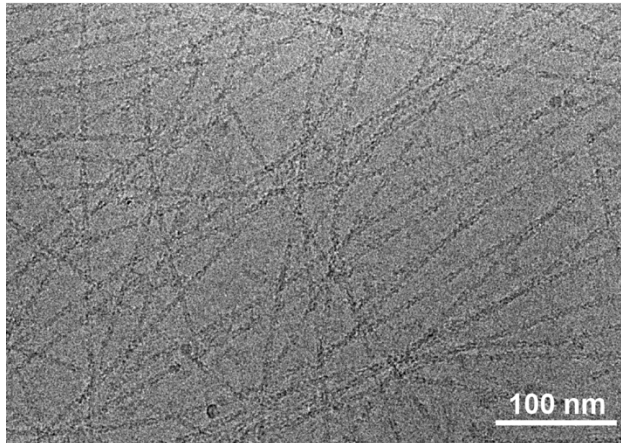
c 0.1 mM AMPPNP, 45 min. at 37°C



d 0.1 mM ATP, 20 min. at 25°C



e



Supplementary Figure 5: Negative stain and cryo electron microscopy of *TgAct1* filaments.

a, After 1 hour at 37°C in polymerization buffer + jasplakinolide, filaments are observed. b, Under the same conditions but without jasplakinolide, no filaments are observed. c, Substitution of AMPPNP for Mg-ATP produces observable filaments. d, No filaments were observed by shortening the incubation time and a decrease in the temperature. For panels a-d, one grid was imaged at a minimum of two separate locations. e, Representative cryo-micrograph from the *TgAct1* + jasplakinolide dataset; micrograph count found in Table 2.

Sample Preparation

C-flat 2/2 holey-carbon
400 mesh, Manual plunging

Data Collection

Appion/Leginon
0.842 Å/px

Relion

Motion Correction
Relion's Implementation

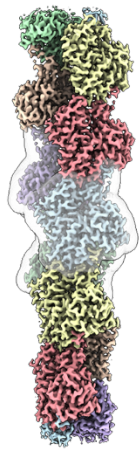
CTF Estimation
CTF Find 4

Micrographs

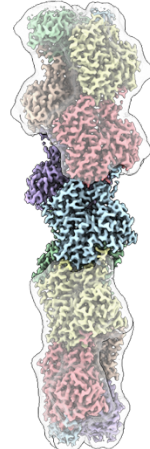
Dynamic
Filament Mask



Static
Trimer Mask

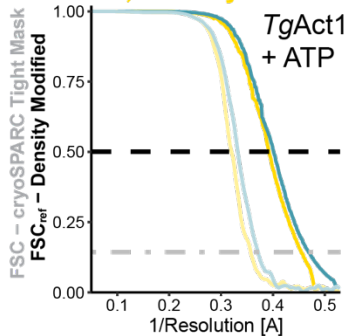


Static
Subtraction Mask

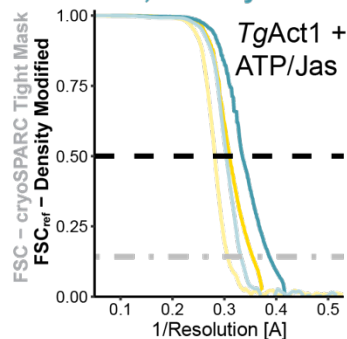


FSC Curves:

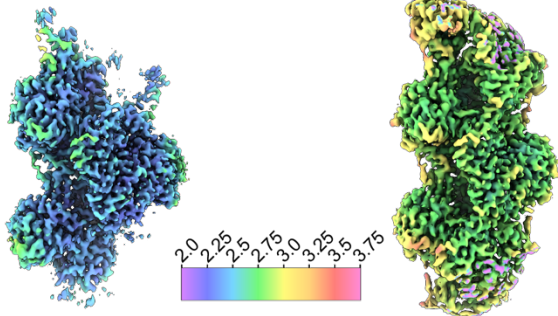
Filament, cryoSPARC
Filament, Density Mod.



3 Protomer, cryoSPARC
3 Protomer, Density Mod.



Local Resolution Estimation



cryoSPARC

CTF Estimation
Patch

Particle Picking
Filament Tracer
Sep. Distance: 40 Å

Extract
Bin 2x, 2.8 million particles

2D Classification
2x

3D Ab-Initio
C1 symmetry

3D Homogenous Refinement
C1 symmetry

Extract
No binning, 2.2 million particles

Helix Refinement
Est. Twist: -167°; Rise: 28 Å
Dynamic Mask

Global CTF Refinement
Tilt, Trefoil; 1x Iteration

Global CTF Refinement
Spher. Ab., Tetrafoil,
Aniso. Mag; 2x Iteration

Helix Refinement
Dynamic Mask

Local CTF Refinement

3D Local Refinement
C1; Static Trimer Mask

3D Classification
Filament Solvent Mask
Trimer Focus Mask
K = 5

3D Local Refinement
C1; Static Trimer Mask

Particle Subtraction

3D Local Refinement
C1; Static Trimer Mask

Unsharp.
Half-maps

Phenix

Density Modification

Model Refinement

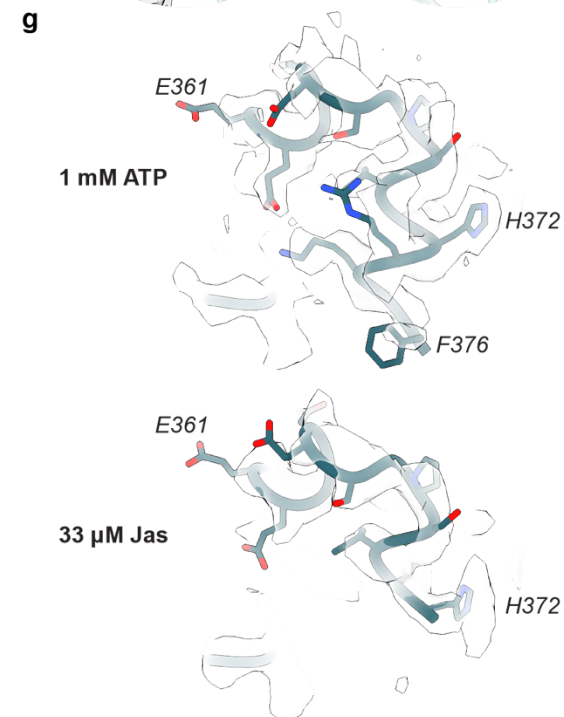
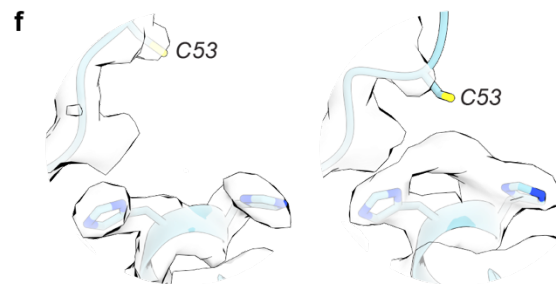
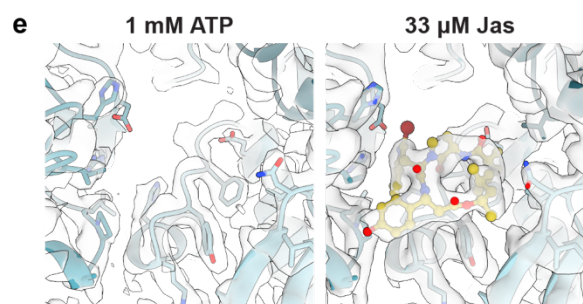
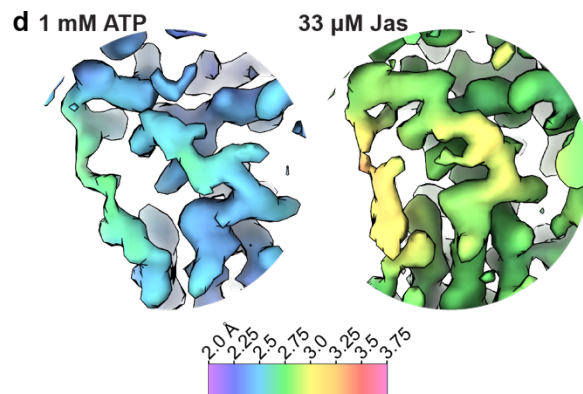
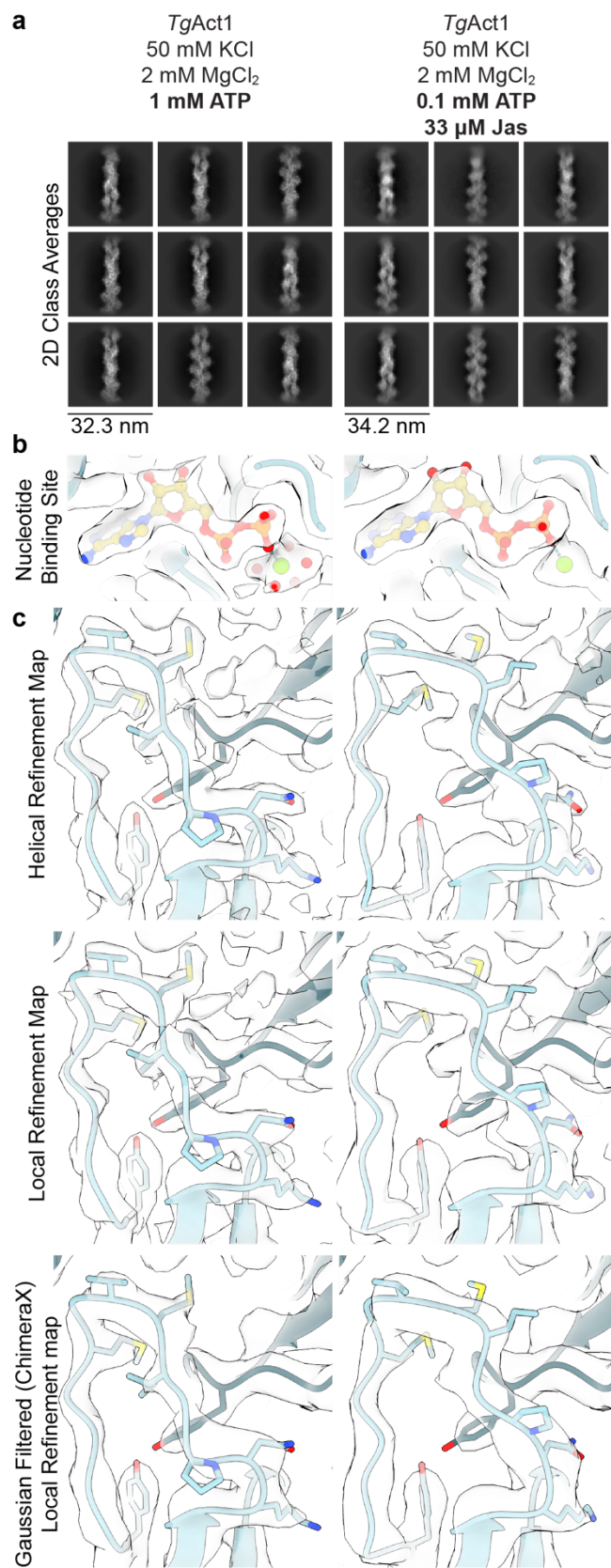
ChimeraX/Isolde

Coot

Model Refinement

Supplementary Figure 6: Data processing scheme and statistics for cryo electron microscopy datasets.

The flowchart shows the data processing scheme for *TgAct1* + 1 mM ATP; a similar scheme without particle subtraction was used for *TgAct1* + jasplakinolide. The masks shown were used as described in the flowchart; volume protomers shown in rainbow colors. Fourier shell correlation curves for the helical reconstructions are shown in yellow and curves for the locally-refined, three-protomer volume are shown in blue, shaded based on the software in which they were calculated (cryoSPARC, light colors; Phenix Density Modification, dark colors). Local resolutions estimated in cryoSPARC were mapped onto volumes locally filtered in cryoSPARC; colors represent estimated resolution in scale.

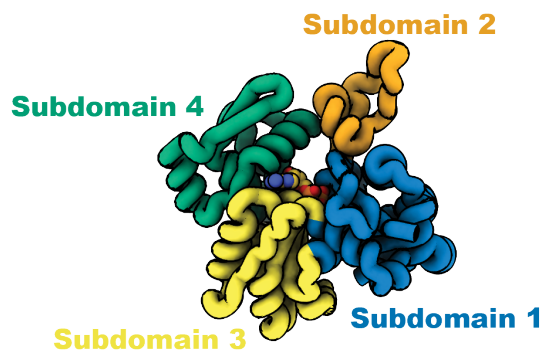


Supplementary Figure 7: Exploration of *TgAct1* filament data, maps, and models.

a, Representative two-dimensional class averages from the data sets. b, Map and model from each dataset showing the nucleotide binding site, with the protein in blue and the nucleotide (ADP in both structures) in yellow. c, Volumes from helical refinement (top) and local refinement (middle) maps. The bottom panels show the local refinement map after Gaussian filtering in ChimeraX. All models are the final model for the dataset indicated. d, Local resolution maps of the D-loop for each filament (unstabilized *TgAct*, left and *TgAct1* + jasplakinolide, right). Colors represent estimated resolution in scale. e, Map and model from each dataset showing the jasplakinolide binding site (unstabilized *TgAct*, left and *TgAct1* + jasplakinolide, right). f, View of Cys53 from each dataset showing volume that suggests the cysteine has been oxidized. g, Volumes and models of the C-termini (unstabilized *TgAct*, top and *TgAct1* + jasplakinolide, bottom).

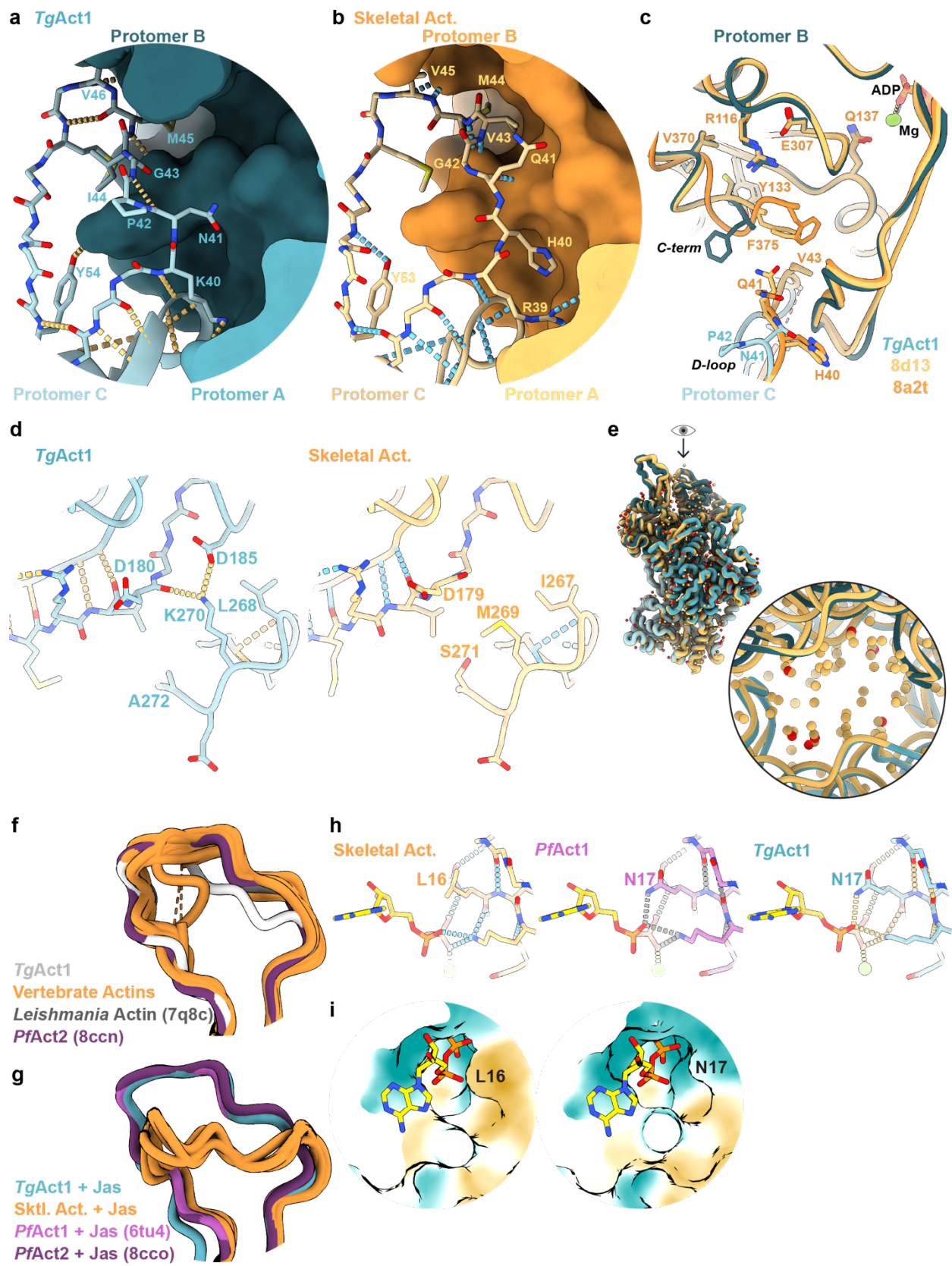
Toxo. Act1	-MADEEVQAL	VVDNGSGNVK	AGVAGDDAPR	AVFPSIVGKP	KNPGIMVGME	EKDCYVGDEA	59
Plasmo. Act1	-MGEEDVQAL	VVDNGSGNVK	AGVAGDDAPR	SVFPSIVGRP	KNPGIMVGME	EKDAFVGDEA	59
Chicken Act1	MCDEDETTAL	VCDNGSGIVK	AGFAGDDAPR	AVFPSIVGRP	RHQGVVMVGMG	QKDSYVGDEA	60 (58)
Plasmo. Act2	--MSEEAVAL	VVDNGSGMVK	SGLAGDDAPK	CVFPSIVGRP	KMPNIMIGME	QKECYVGDEA	58
	... ** * ***** ** :*.*****. .*****.* . .:*** :*.:*****						
Toxo. Act1	QSKRGILTLK	YPIEHGIVTN	WDDMEKIWHH	TFYNELRVAP	EEHPVLLTEA	PLNPKANRER	119
Plasmo. Act1	QTKRGILTLK	YPIEHGIVTN	WDDMEKIWHH	TFYNELRAAP	EEHPVLLTEA	PLNPKGNRER	119
Chicken Act1	QSKRGILTLK	YPIEHGIITN	WDDMEKIWHH	TFYNELRVAP	EEHPTLLTEA	PLNPKANREK	120 (118)
Plasmo. Act2	QNKRGILTLK	YPIEHGIVTN	WDDMEKIWHH	TFYNELRVSP	EEHPVLLTEA	PLNPKTNREK	118
	*.***** *****:** ***** ***** .:* ***** ***** **.						
Toxo. Act1	MTQIMFETFN	VPAMYVAIQ	VLSLYSSGRT	TGIVLDSGDG	VSHTVPIYEG	YALPHAIMRL	179
Plasmo. Act1	MTQIMFESFN	VPAMYVAIQ	VLSLYSSGRT	TGIVLDSGDG	VSHTVPIYEG	YALPHAIMRL	179
Chicken Act1	MTQIMFETFN	VPAMYVAIQ	VLSLYASGRT	TGIVLDSGDG	VTHNVPIYEG	YALPHAIMRL	180 (178)
Plasmo. Act2	MTQIMFETFD	VPAMYVSIQA	ILSLYASGRT	TGIVLDSGDG	VSHTVPIYEG	YVLPCHAINRI	178
	*****:** *****:** :***** ***** ***** *.* ***** *.***** *:						
Toxo. Act1	DLAGRDLTEY	MMKILHERGY	GFTTSAEKEI	VRDIKEKLCY	IALDFDEEMK	AAEDSSD-IE	238
Plasmo. Act1	DLAGRDLTEY	LMKILHERGY	GFSTSAEKEI	VRDIKEKLCY	IALNFDEEMK	TSEQSSD-IE	238
Chicken Act1	DLAGRDLTDY	LMKILTERGY	SFVTTAEREI	VRDIKEKLCY	VALDFENEMA	TAASSSS-LE	239 (237)
Plasmo. Act2	DMAGRDLTYH	MMKLFTERGH	TFTTTAEREI	VRDIKEKLCY	IAMDYDEELK	RSEHSDEIE	238
	*.***** : :***: ***: * *:*.* ***** :*****: : . * . :*						
Toxo. Act1	KSYELPDGNI	ITVGNERFRC	PEALFQPSFL	GKEAAGVHRT	TFDSIMKCDV	DIRKDLYGNV	298
Plasmo. Act1	KSYELPDGNI	ITVGNERFRC	PEALFQPSFL	GKEAAGIHTT	TFNSIKKCDV	DIRKDLYGNI	298
Chicken Act1	KSYELPDGQV	ITIGNERFRC	PETLFQPSFI	GMEAGIHET	TYNSIMKCDI	DIRKDLYANN	299 (297)
Plasmo. Act2	EIYELPDGNI	ITVGSEFRFC	PEALFNPTLI	GRECPGLHIT	AYQSIMKCDI	DIRKELYNNI	298
	: *****: **:* ***** **:*:*:*: * *..* * :*:** ***: ******* *						
Toxo. Act1	VLSGGTTMYE	GIGERLTKEI	TSLAPSTMKI	KVVAPPERKY	SVWIGGSILS	SLSTFQQMWI	358
Plasmo. Act1	VLSGGTTMYE	GIGERLTRDI	TTLAPSTMKI	KVVAPPERKY	SVWIGGSILS	SLSTFQQMWI	358
Chicken Act1	VMSGGTTMYP	GIADRMQKEI	TALAPSTMKI	KIIAPPERKY	SVWIGGSILA	SLSTFQQMWI	359 (357)
Plasmo. Act2	VLSGGTTMYN	NIGERLTKEI	TNLAPSSMKI	KVIAPPERKY	SVWIGGSILS	SLSTFQQMWI	358
	*.***** .*.:*.: :* * ***** *.:***** *****: *****						
Toxo. Act1	TKEEYDESGP	SIVHRKCF					376
Plasmo. Act1	TKEEYDESGP	SIVHRKCF					376
Chicken Act1	TKQEYDEAGP	SIVHRKCF					377 (375)
Plasmo. Act2	TKEEYEDSGP	SIVHRKCF					376
	:*:*:*: ***						

- TgAct1 Asn17
- DNase Loop
- TgAct1 Tyr54
- Hydrophobic Plug
- DNase Loop Pocket



Supplementary Figure 8: Sequence alignment.

Sequence alignment of TgAct1, skeletal actin, *P. falciparum* Act1, and *P. falciparum* Act2. Sequences retrieved from Uniprot (Uniprot IDs: Q8ILW9, P68139, Q8I4X0, P53476) and aligned with the EMBL-EBI portal for MAFFT (v7, <https://www.ebi.ac.uk/Tools/msa/mafft/>).



Supplementary Figure 9: Actin filament comparisons.

a/b, Hydrogen bonding network of D-loop from unstabilized *TgAct1* actin filaments (protein in blue, hydrogen bonds in orange) and skeletal actin filaments (protein in orange, hydrogen bonds in blue). Side chains of amino acids 49-53 (skeletal actin 48-52) have been omitted for clarity. c, Nucleotide sensing and transmission residues from unstabilized *TgAct1* (teals) and Mg-ADP skeletal actins (8d13, light orange; 8a2t, dark orange). For clarity, only residue numbers from skeletal actin are shown for Protomer B. d, Hydrogen bonding network of the hydrophobic plug region of unstabilized *TgAct1* actin filaments (protein in blue, hydrogen bonds in orange) and skeletal actin filaments (protein in orange, hydrogen bonds in blue). e, Ribbon diagram of three-protomer filament of unstabilized *TgAct1* (teals) and skeletal actin (8d13, orange). Waters from *TgAct1* shown in red and waters from skeletal actin shown in orange. f, The D-loops from unstabilized *TgAct1* (gray), several vertebrate actins (orange, PDB IDs 7bt7, 7r8v, 8a2r, 8a2t, 8d13, 8dmx, 8dmy, 8dnf, 8dnh), *Leishmania* actin (dark gray, PDB ID 7q8c), and *P. falciparum* Act2 (purple, PDB ID 8ccn). g, The D-loops from *TgAct1* + jasplakinolide (blue), skeletal actin + jasplakinolide (orange, PDB IDs 5ooc, 5ood, 6t24, 7pm3), *P. falciparum* Act1 + jasplakinolide (magenta, PDB ID 6tu4), and *P. falciparum* Act2 + jasplakinolide (purple, PDB ID 8cco). h, View of Leu16 or Asn17 from the nucleotide binding pocket of skeletal actin filaments (orange, hydrogen bonds in blue), *P. falciparum* Act1 filaments (magenta, hydrogen bonds in grey), and unstabilized *TgAct1* (blue, hydrogen bonds in orange). i, Surface representation of the nucleotide binding pocket of skeletal G-actin (PDB ID 1eqy) and *P. falciparum* Act1 G-actin (PDB ID 6i4e) with the nucleotide shown as sticks. Surface colors represent hydrophobicity, with oranges being the most hydrophobic and teals being the most hydrophilic.

Supplementary Data Tables

Condition	Mean $\pm$ SD	Max	n
12 μ M, 3 min	1.9 $\pm$ 1.4	6	78
12 μ M, 3 min + actin CB EmFP	1.9 $\pm$ 1.5	5	75
12 μ M, 4 min	4.6 $\pm$ 2.8	10.5	77
12 μ M, 4 min + actin CB EmFP	4.5 $\pm$ 3.1	10.9	75
12 μ M, 7 min	7.5 $\pm$ 6.1	22.7	76
12 μ M, 7 min + actin CB EmFP	8.4 $\pm$ 6.7	25.1	75
16 μ M, 3 min	3.8 $\pm$ 2.2	8.3	77
16 μ M, 3 min + actin CB EmFP	3.2 $\pm$ 2.2	9.2	70
16 μ M, 4 min	4.5 $\pm$ 3.1	11.8	75
16 μ M, 4 min + actin CB EmFP	4.2 $\pm$ 3.2	13.9	74
16 μ M, 7 min	7.8 $\pm$ 6.3	27.4	76
16 μ M, 7 min + actin CB EmFP	9.2 $\pm$ 7.1	35	75
20 μ M, 3 min	5.0 $\pm$ 2.9	15	69
20 μ M, 3 min + actin CB EmFP	4.5 $\pm$ 2.9	10.4	75
20 μ M, 4 min	6.2 $\pm$ 4.0	15.1	75
20 μ M, 4 min + actin CB EmFP	7.2 $\pm$ 5.4	21.6	75
20 μ M, 7 min	11.4 $\pm$ 9.5	37.8	76
20 μ M, 7 min + actin CB EmFP	10.6 $\pm$ 8.2	32	75

Supplementary Table 1: Descriptive statistics for Ext. Data Figure 3a.

Mean, maximum and number of values for actin length distributions assembled in the presence or absence of chromobody (CB-EmFP) for the indicated time period. Source data are provided as a Source Data file.