

**Supplementary information**

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**Structural basis of actin filament assembly and aging**

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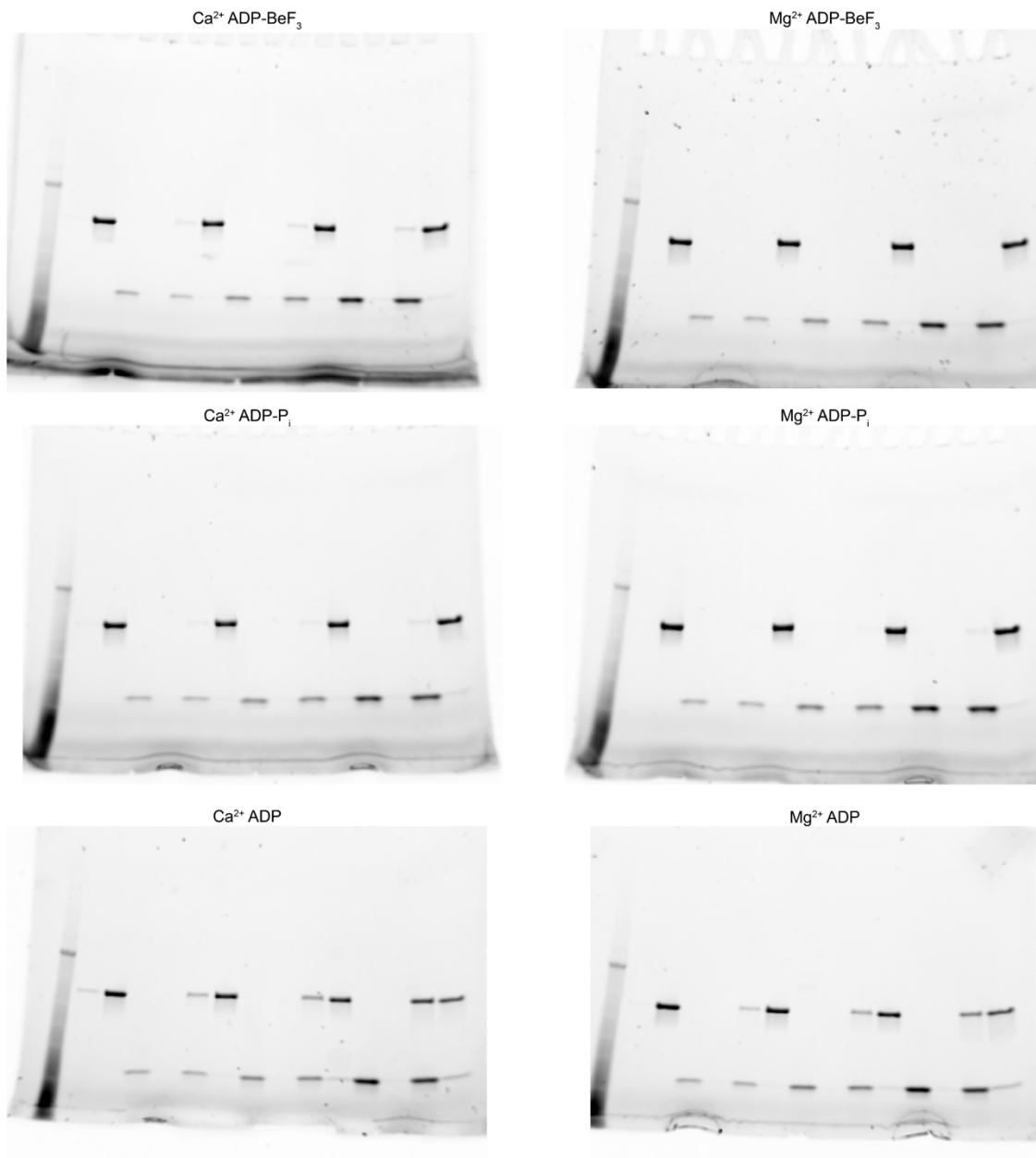
In the format provided by the authors and unedited

**Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics for Mg<sup>2+</sup>-F-actin datasets.**

<b>F-actin state</b>	<b>Mg<sup>2+</sup>-ADP-BeF<sub>3</sub><sup>-</sup></b> EMD-15104 PDB 8A2R	<b>Mg<sup>2+</sup>-ADP-P<sub>i</sub></b> EMD-15105 PDB 8A2S	<b>Mg<sup>2+</sup>-ADP</b> EMD-15106 PDB 8A2T
<b>Data collection and processing</b>			
Magnification	130,000	130,000	130,000
Voltage (kV)	300	300	300
Electron exposure (e <sup>-</sup> /Å <sup>2</sup> )	78.9	88.8	76.4
Defocus range (μm)	-0.7 to -2.0	-0.7 to -2.0	-0.7 to -2.0
Pixel size (Å)	0.695	0.695	0.695
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	2,897,679	2,349,979	1,296,776
Final particle images (no.)	2,228,553	1,808,554	1,114,051
Map resolution (Å)	2.17	2.22	2.24
0.143 FSC threshold			
Map resolution range (Å)	2.1 – 3.2	2.2 – 3.2	2.2 – 3.4
Measured helical symmetry			
Helical rise (Å)	27.47±0.05	27.50±0.02	27.50±0.07
Helical twist (°)	166.6±0.2	166.5±0.4	166.7±0.1
<b>Refinement</b>			
Initial model used (PDB code)	8A2Z	8A2Z	8A2Z
Model resolution (Å)	2.20	2.28	2.35
0.5 FSC threshold			
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-50	-64	-65
Model composition			
Non-hydrogen atoms	14,990	15,141	15,046
Protein residues	1,830	1,835	1,830
Waters	480	591	556
Ligands	15	15	10
<i>B</i> factors (Å <sup>2</sup> )			
Protein	31.53	34.56	38.07
Waters	31.82	23.19	31.98
Ligand	22.89	31.58	31.71
R.m.s. deviations			
Bond lengths (Å)	0.004	0.008	0.003
Bond angles (°)	0.592	0.669	0.549
Validation			
EM-ringer score	5.94	6.45	5.89
MolProbity score	1.17	1.12	1.03
Clashscore	2.75	3.33	2.50
Poor rotamers (%)	0.65	0.64	0.32
Ramachandran plot			
Favored (%)	97.49	98.06	98.05
Allowed (%)	2.51	1.94	1.95
Disallowed (%)	0	0	0

**Supplementary Table 2. Cryo-EM data collection, refinement and validation statistics for Ca<sup>2+</sup>-F-actin datasets.**

<b>F-actin state</b>	<b>Ca<sup>2+</sup>-ADP-BeF<sub>3</sub><sup>-</sup></b> EMD-15107 PDB 8A2U	<b>Ca<sup>2+</sup>-ADP-P<sub>i</sub></b> EMD-15108 PDB 8A2Y	<b>Ca<sup>2+</sup>-ADP</b> EMD-15109 PDB 8A2Z
<b>Data collection and processing</b>			
Magnification	130,000	130,000	130,000
Voltage (kV)	300	300	300
Electron exposure (e <sup>-</sup> /Å <sup>2</sup> )	84.6	88.8	75.6
Defocus range (μm)	-0.7 to -2.0	-0.7 to -2.0	-0.7 to -2.0
Pixel size (Å)	0.695	0.695	0.695
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	2,246,622	3,031,270	1,873,773
Final particle images (no.)	1,719,432	2,171,987	1,073,455
Map resolution (Å)	2.21	2.15	2.15
0.143 FSC threshold			
Map resolution range (Å)	2.15 – 3.2	2.1 – 3.1	2.1 – 3.5
Measured helical symmetry			
Helical rise (Å)	27.46±0.05	27.47±0.08	27.47±0.05
Helical twist (°)	166.7±0.1	166.6±0.1	166.5±0.1
<b>Refinement</b>			
Initial model used (PDB code)	8A2Z	8A2Z	7AHN
Model resolution (Å)	2.23	2.16	2.17
0.5 FSC threshold			
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-55	-55	-55
Model composition			
Non-hydrogen atoms	15,022	15,078	15,060
Protein residues	1,830	1,835	1,830
Waters	512	528	570
Ligands	15	15	10
<i>B</i> factors (Å <sup>2</sup> )			
Protein	24.67	21.12	26.49
Waters	23.66	23.30	28.02
Ligand	16.14	15.86	20.53
R.m.s. deviations			
Bond lengths (Å)	0.003	0.004	0.003
Bond angles (°)	0.542	0.596	0.568
Validation			
EM-ringer score	6.31	6.39	6.56
MolProbity score	1.17	1.02	0.99
Clashscore	2.75	2.39	1.81
Poor rotamers (%)	0.65	0.64	0.00
Ramachandran plot			
Favored (%)	97.49	98.06	97.77
Allowed (%)	2.51	1.94	1.95
Disallowed (%)	0	0	0.28



**Supplementary Figure 1. Uncropped gel images.**

This figure depicts uncropped versions of the gels shown in Extended Data Fig. 10a.