Supporting Information

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SI Text

TIRF Assay. Rabbit skeletal actin was labeled with Bodipy TMR C5 maleimide (Invitrogen) (1). The labeling efficiency was 52%, and Bodipy TMR is conjugated at Cys-374 (2). Cover slides (FIS no. 12-545D) were cleaned thoroughly with acid–base solutions (1), washed with absolute ethanol, and dried in a plasma cleaner (Harrick Plasma) for 30 min before use. Sample cells were made by attaching an Eppendorf tube cap with a hole in the top onto a cover slide. The sample cells were incubated for 2 min with 50 μL of *N*-ethylmaleimide-inactivated myosin (1) in high-salt Tris-buffered saline [HS-TBS; 600 mM NaCl and 50 mM Tris·HCl (pH 7.5)], then washed three times with equal volumes of 1% (wt/vol) BSA in HS-TBS, followed by washing three times with 1% (wt/vol) BSA in low-salt Tris-buffered saline [50 mM NaCl and 50 mM Tris·HCl (pH 7.5)]. The sample cells were then equilibrated with the dialysis buffer before use. Monomeric actin with 14% fluorescent label at a concentration of 2.22 μM was dialyzed along with GTL and D2E2D6E6 overnight in a buffer containing 50 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM DTT, 0.2 mM ATP, 10 mM Hepes (pH 7.5), and either 0 M $CaCl_2$ or 819.7 μM $CaCl_2$. This rendered the labeled G actin to polymerize into F actin. The proteins were then in a buffer with 0 or 200 nM free Ca. The F actin was first diluted to 1.11 or 0.55 μM in dialysis buffer and immediately 54 μL was dispensed into the sample cell. The actin filaments attached to the myosin were observed while 6 μL of GTL or D2E2D6E6 was mixed into the sample cell such that the protein to actin ratio was 1:200 or 1:2. A Nikon TIRF imaging system consisting of an inverted microscope coupled with a perfect focus unit (Nikon) was used for image acquisition. A 60× objective with N.A. 1.49 (CFI Apochromat TIRF60xH; Nikon) was used. A 532-nm laser light (Compass 215M-50; Coherent) excited the Bodipy TMR. The filter cube consisted of a dual dichroic mirror (Z488-532rpc; Chroma Technology) and a dual-emission filter (Z488/532/633m; Chroma Technology). Images were recorded in an EMCCD camera (QuantEM:512SC; Photometrics). The microscope control and image acquisition were performed by using MetaMorph software (Universal Imaging). Time-lapse movies at chosen locations within the flow cell were acquired, with acquisition times ranging from 100 to 400 ms per image and time delay between images ranging from 0.3 s to tens of seconds. During the course of time-lapse movie acquisition, the perfect focus unit ensured that the focal plane of the objective was continuously parked at the glass–aqueous interface of the flow cell.

1. Kuhn JR, Pollard TD (2005) Real-time measurements of actin filament polymerization by total internal reflection fluorescence microscopy. *Biophys J* 88:1387–1402.
2. Marushchak D, Grenklo S, Johansson T, Karlsson R, Johansson LB (2007) Fluorescence depolarization studies of filamentous actin analyzed with a genetic algorithm. *Biophys J* 93:3291–3299.

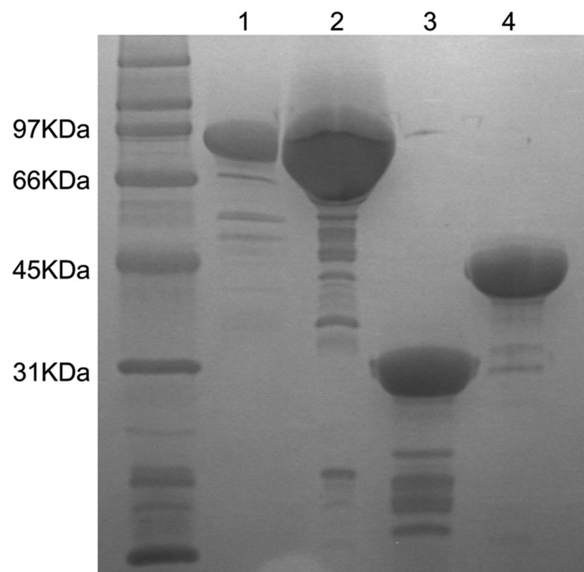


Fig. S1. SDS/PAGE analysis of prepared proteins. Lane 1, horse plasma gelsolin control, 83 kDa; lane 2, human cytoplasmic gelsolin, 81 kDa; lane 3, human cytoplasmic G2G3, 29 kDa; and lane 4, human cytoplasmic G4–G6, 37 kDa.

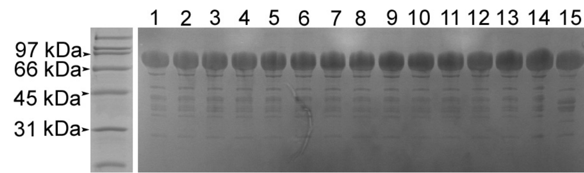


Fig. S2. SDS/PAGE analysis of mutant proteins. From left to right: GFL; gelsolin single mutants: D2 (Asp187Asn), E2 (Glu209Gln), D6 (Asp670Asn), and E6 (Glu692Gln); double mutants: D2D6 (Asp187Asn Asp670Asn), E2E6 (Glu209Gln Glu692Gln), E2D6 (Glu209Gln Asp670Asn), D2E6 (Asp187Asn Glu692Gln), D2E2 (Asp187Asn Glu209Gln), and D6E6 (Asp670Asn Glu692Gln); triple mutants: D2E2D6 (Asp187Asn Glu209Gln Asp670Asn) and D2D6E6 (Asp187Asn Asp670Asn Glu692Gln); quadruple mutant: D2E2D6E6 (Asp187Asn Glu209Gln Asp670Asn Glu692Gln); and finally GTL (residues 25–741).

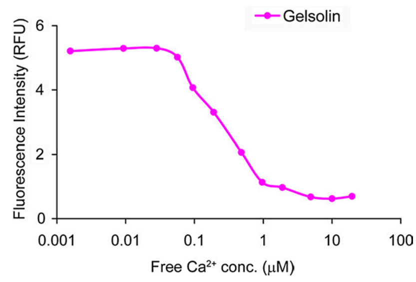
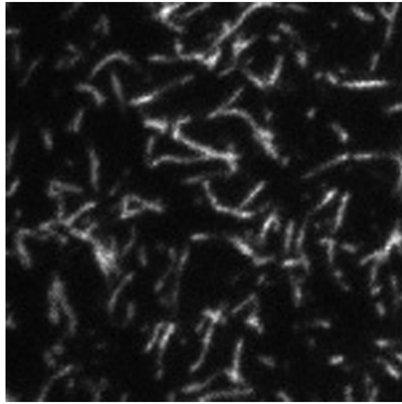


Fig. S3. GFL activity. A pyrene-actin depolymerization assay was carried out to demonstrate native activity of the recombinant gelsolin. Full-length wild-type gelsolin (6 μM) was added to 10% pyrene-labeled F actin (12 μM) at different free Ca^{2+} concentrations in the presence of 1 mM EGTA, as calculated by the webmaxchelator program (http://maxchelator.stanford.edu/).

Table S1. Data collection and refinement statistics

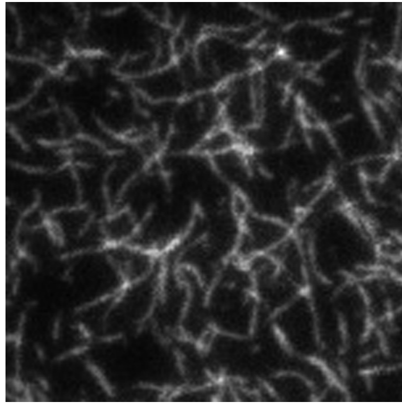
Statistic	Human gelsolin	Human G1–G3/actin
Wavelength, Å	1.00	1.00
Space group	$P42_12$	$P2_12_12_1$
Unit cell		
a, Å	170.9	102.2
b, Å	170.9	146.9
c, Å	152.2	148.3
α, °	90	90
β, °	90	90
γ, °	90	90
Resolution range, Å	30.0–3.0 (3.1–3.0)	30.0–3.0 (3.1–3.0)
Unique reflections	45,666 (4,498)	42,953 (3,119)
Redundancy	8.4 (8.1)	4.7 (4.9)
Completeness, %	100.0 (100.0)	99.7 (99.9)
Average I/I_o	27.3 (6.3)	19.2 (5.7)
R_{merge}, %	8.3 (33.8)	8.8 (28.2)
R_{factor}, %	21.8 (25.6)	20.1 (27.3)
R_{free}, %	27.0 (33.6)	27.3 (33.8)
Molecules in asymmetric unit	2	2
Gelsolin residue range	Mol 1: 21–258, 261–755; Mol 2: 21–258, 261–755	Mol 1: 28–374; Mol 2: 27–372
Actin residue range	–	Mol 1: 3–47, 49–368; Mol 2: 5–38, 50–370
Nonhydrogen atoms (waters)	11,463 (105)	11,687 (473)
Mean derived B factor (waters), Å^2	46.4 (46.6)	31.2 (31.1)
rmsd bonds, Å	0.014	0.009
rmsd angles, °	1.52	1.26

Numbers in parenthesis represent highest resolution shell.



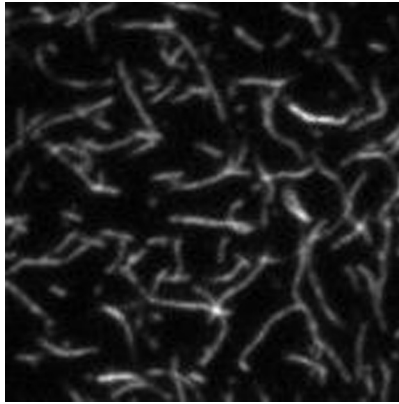
Movie S1. Actin filaments severed by GTL in EGTA visualized by TIRF microscopy. The protein concentrations are 500 nM actin and 2.5 nM GTL. The time-lapse movie was acquired with exposure time of 400 ms, with time delay between exposures ranging from an initial 0.3 s to 10 s for latter frames. The field shown is 40 $\times$ 40 μm. This movie relates to Fig. 3*E*.

Movie S1 (AVI)



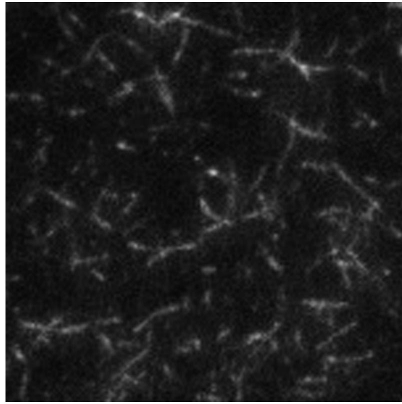
Movie S2. Actin filaments severed by the quadruple mutant (D2E2D6E6) in EGTA visualized by TIRF microscopy. The protein concentrations are 500 nM actin and 2.5 nM D2E2D6E6. The time-lapse movie was acquired with exposure time of 400 ms, with time delay between exposures ranging from an initial 0.3 s to 10 s for latter frames. The field shown is 40 $\times$ 40 μm. This movie relates to Fig. 3*F*.

Movie S2 (AVI)



Movie S3. Actin filaments severed by GFL in EGTA visualized by TIRF microscopy. The protein concentrations are 500 nM actin and 2.5 nM GFL. The time-lapse movie was acquired with exposure time of 400 ms, with time delay between exposures ranging from an initial 0.3 s to 10 s for latter frames. After the filaments were observed to be stable, $CaCl_2$ (5 mM final concentration) was added. The field shown is 40×40 μm. This movie relates to Fig. 3*G*.

Movie S3 (AVI)



Movie S4. Actin filaments severed by GFL in 200 nM free Ca visualized by TIRF microscopy. The protein ratios are the same as those for Fig. 3 *A* and *B*: 1 μM actin and 0.5 μM GFL. The time-lapse movie was acquired with exposure time of 400 ms, with time delay between exposures ranging from an initial 0.3 s to 10 s for latter frames.

Movie S4 (AVI)