Real-time single molecule kinetics analyses of actin filament severing by cofilin AIP1.

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Supporting Information

Figure S1. A schematic drawing of an actin filament tethered to the surface of a coverslip via biotin and anti-biotin antibody. Both AIP1 (100 nM) and cofilin (20 μM) in the pipette solution were ejected to an actin filament through a fine glass capillary.

Figure S2. The intensity profile of AIP1 fluorescence was fitted with two Gaussian distributions (basal noise and single AIP1 bindings, respectively), indicating that bindings of single AIP1 molecules conjugated with fluorophores were detected. The arrow shows the mean intensity of a single AIP1 binding, and the arrowhead shows the mean noise level. The inset shows the typical examples of transient increases in AIP1 fluorescence. The duration of the increase was measured at the half-maximum intensity level (dotted lines).

Figure S3. The number of Alexa-488 molecules labeling a GST-AIP1. A, a typical single step decrease in fluorescence intensity of a GST-AIP1 fluorescent spot (i.e., Alexa-AIP1 in the main text). B, a typical multi-step (three steps) decrease in fluorescence intensity is shown. The intensity levels of steps and the background level are shown by arrows. C, distribution of number of step decreases in the fluorescence intensity of a GST-AIP1 fluorescent spot. Inset shows a fluorescence image of GST-AIP1 fluorescent spots. Laser power 25% (12.5 mW) and exposure time 100 ms. Bar: 10 μm. The unit step size is ca. 8 counts. Gray spots and lines show a binomial function with 5 labeling sites and probability=0.3 for labeling each site.

Figure S4. The time dependent decline of the chance to detect AIP1 dissociated from the actin filament before severing. AIP1 stayed on the filament in 79% of cases, however, in 19 out of 90 severing cases AIP1 dissociated from the actin filament 0-30 ms before the severing. Panel A shows the typical examples of duration of AIP1 binding (gray) and the period between the dissociation of the AIP1 to the severing (red, named Tp) of these 19 cases. Bar denotes 100 ms. Panel B shows the histogram of the ensemble of these 19 Tps. The red line denotes the single exponential function fitting ($\tau = 42 \pm 4$ ms), showing the close relationship between AIP1 binding and severing.

Movie S1. Typical time lapse images of GST-AIP1 binding to an actin filament. AIP1 is colored green and the actin filament is colored red. The length of this movie is 4 s (images were taken with 30 ms/frame), and the area is 1.3 × 2.6 μm (width × height).

Movie S2. Typical time lapse images of monomeric AIP1 binding to an actin filament. AIP1 is colored

green and the actin filament is colored red. The length of this movie is 8 s (original images were taken with 66 ms/frame and slowed down 1.5 times), and the area is 3.4 × 1.7 μm (width × height).

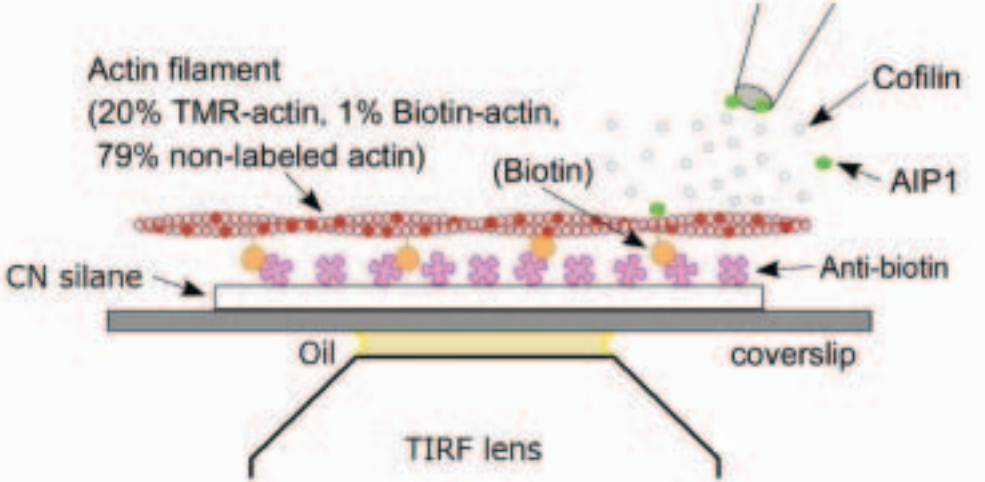
Actin filament
(20% TMR-actin, 1% Biotin-actin,
79% non-labeled actin)
(Biotin)
Cofilin
AIP1
CN silane
Anti-biotin
Oil
coverslip
TIRF lens

Fig S1

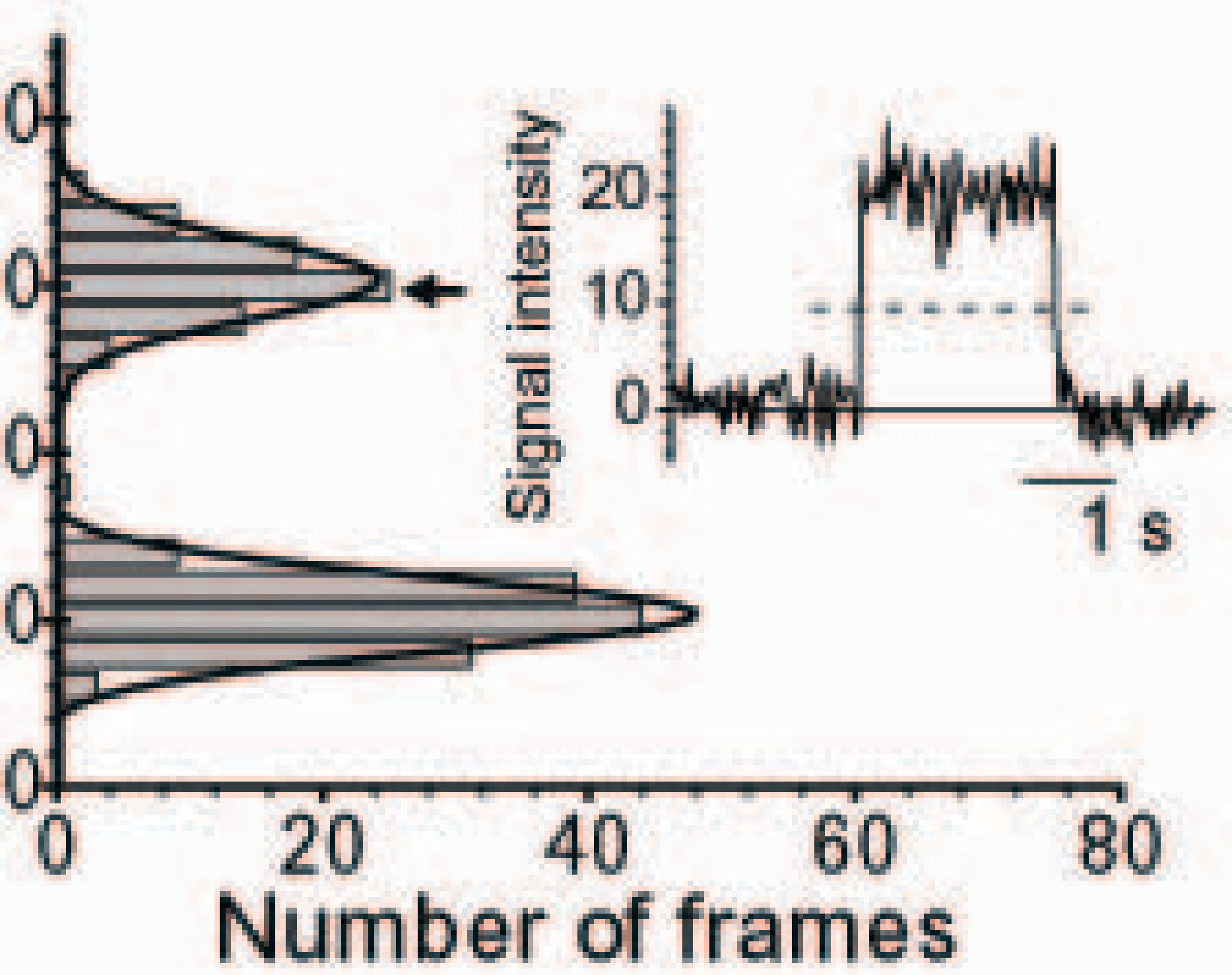
signal intensity
20
10
0
1 s
0
0
0
0
0
0
20
40
60
80
Number of frames

Fig S2

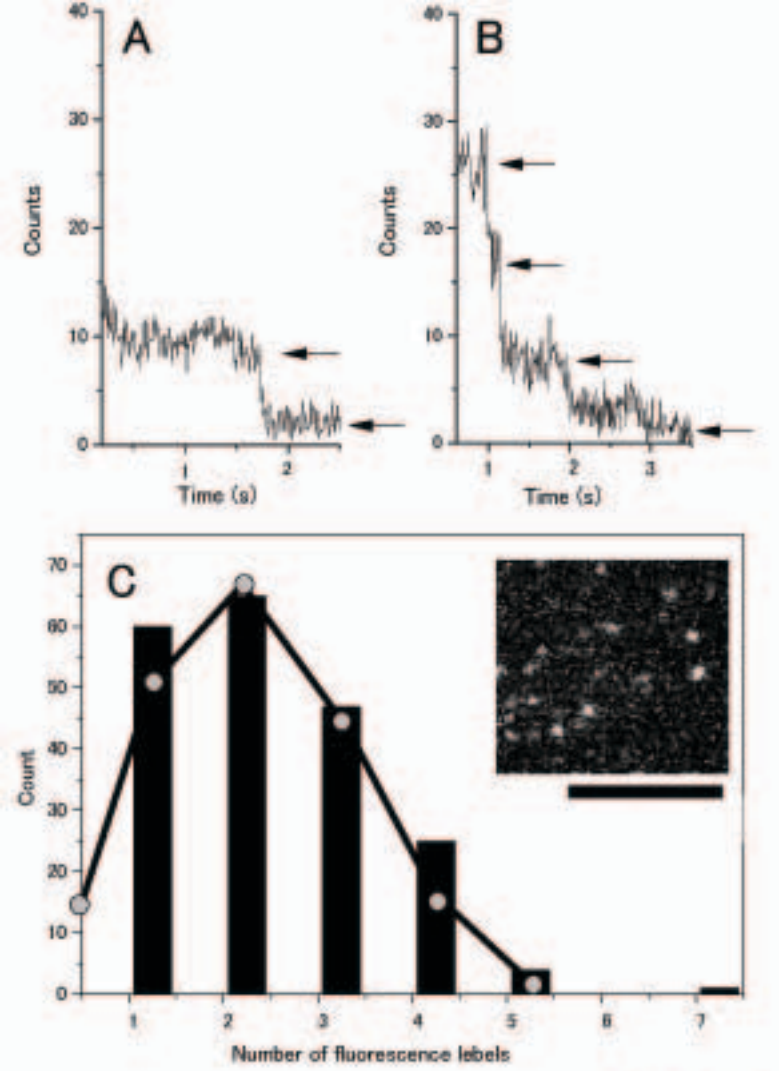

Fig S3

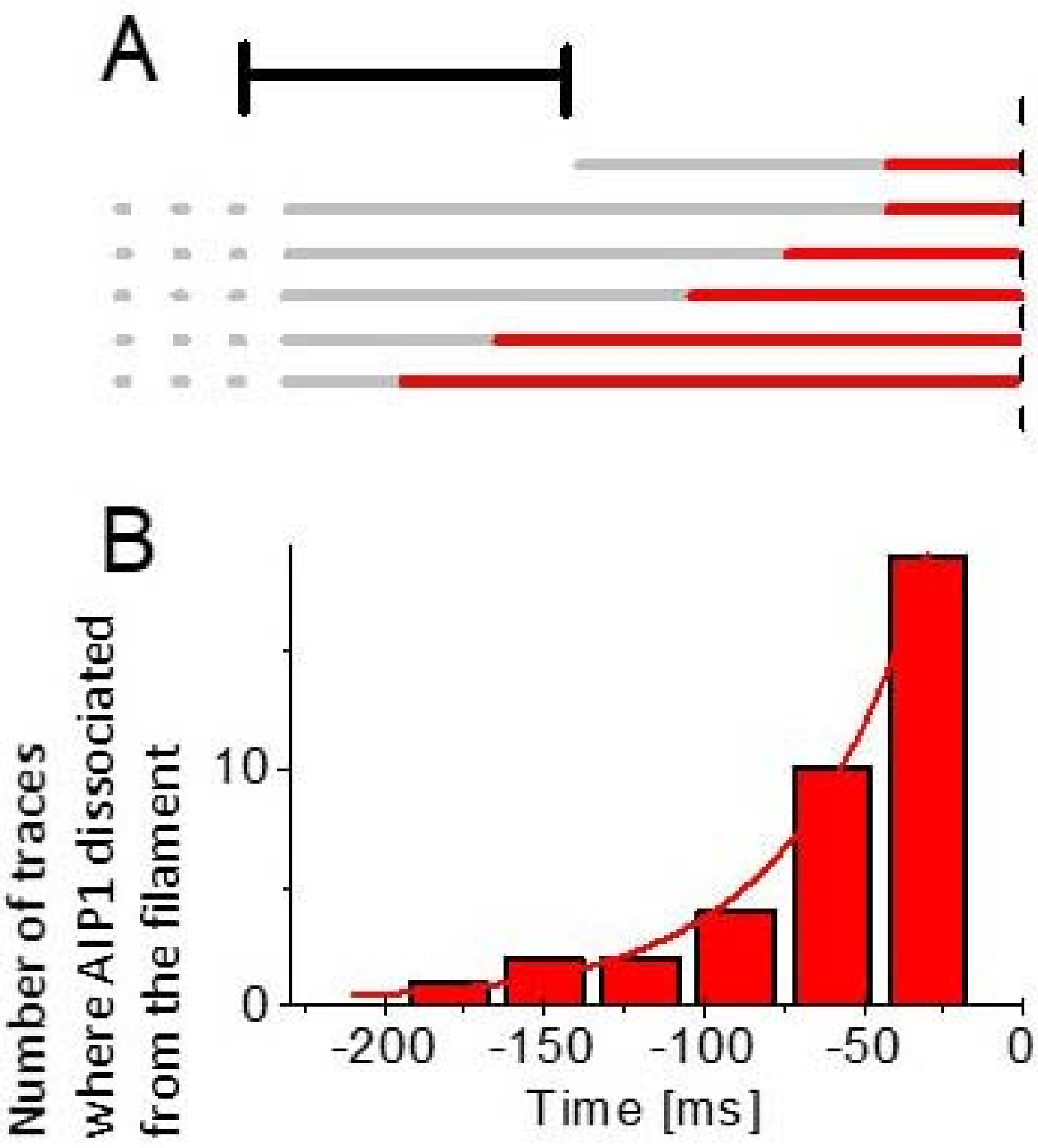
A
B
Number of traces where AIP1 dissociated from the filament
10
0
-200
-150
-100
-50
0
Time [ms]

Fig S4