Supplemental Materials

Molecular Biology of the Cell

Hatch et al.

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Figure S1: Quantification of Drp1 and actin in U2OS cells. (A) Western blot of Drp1 from known amounts of U2OS cell extract (0.40 ± 0.015 ng protein/cell). Lanes to the left contain varying cell extract. Lanes to the right contain 5 µg cell extract with varying ng amounts of purified Drp1. (B) Graph of anti-Drp1 signal versus U2OS extract amount, quantified from blots similar to that in A. (C) Graph of anti-Drp1 signal versus purified Drp1 amount, in a background of 5 µg U2OS extract. For quantification, combined density of all three Drp1 bands was analyzed. See Methods for details. (D) Coomassie-stained SDS-PAGE of actin from known amounts of U2OS cell extract. Lanes to left contain varying cell extract. Lanes to right contain varying µg amounts of purified actin. (E) Graph of µg of actin versus µg of U2OS extract, using a standard curve generated by varying purified actin.

Figure S2: Drp1/actin interaction by co-sedimentation assay. (A) Dot plots showing the range of K_d values (top) and % Drp1 bound at saturation (bottom) from six independent Drp1/actin binding assays containing 1.3 µM Drp1, 65 mM NaCl, and no nucleotide. (B) Coomassie-stained SDS-PAGE of Drp1/actin co-sedimentation assay similar to graph in Figure 1A. Standards of known µM amounts shown on left, pellets shown on right. (C) Coomassie-stained SDS-PAGE of Drp1/actin co-sedimentation assay in which Drp1 concentration and incubation time is varied. Pellets shown. Note that actin levels in the pellet remain constant, suggesting that Drp1 does not alter the polymerization equilibrium of actin. All lanes from the same gel, cropped to remove intervening lanes. (D) Coomassie-stained SDS-PAGE of sequential pelleting assay depicted in Figure 1C. Standards shown on left, pellets on right. (E) Coomassie-stained SDS-PAGE of co-sedimentation assay showing effect of added phosphate (10 mM) on Drp1 binding to actin filaments. 10 µM actin, 1.3 µM Drp1. Pellets shown. All lanes from the same gel, cropped to remove intervening lanes. (F) Graph showing the effect of TRITC-phalloidin on Drp1 binding to actin filaments (by co-sedimentation assay). 1 µM actin. (G) Graph showing binding of GFP-Drp1 or unlabeled Drp1 to actin filaments (by co-sedimentation assay). 1.3 µM Drp1.

Supplemental Figure S3: Effect of non-hydrolyzable GTP analogue (GMP-PCP, 500 µM) on oligomerization of wild-type and dimer mutant Drp1, assessed by sedimentation. S = supernatant, P = pellet. WT and dimer mutant run on separate gels.

Supplemental Figure S4: Actin filament bundling by Drp1. (A) Graphs of low-speed co-sedimentation assays, showing actin and Drp1 in the pellet as a function of varying Drp1 (left, 0.5 µM actin) or actin (right, 1.5 µM Drp1). (B) Coomassie-stained SDS-PAGE of low-speed co-sedimentation assays from A (varying Drp1 on left, varying actin on right). In both cases, gels have been cropped from one original gel to remove intervening lanes. Note absence of pelleted actin in actin alone controls in both cases. (C and D) Graph of aspect ratios (ratio of orthogonal diameters) and diameters for 18 circular bundles.

Supplemental Figure S5: Effects of free Drp1 and nucleotide on GFP-Drp1 dissociation kinetics from actin bundles. (A) Graph of GFP-Drp1 fluorescence decay from actin bundles when free GFP-Drp1 is washed-out with buffer alone (black curve), buffer containing 2.5 µM unlabeled Drp1 (blue), 500 µM GTP (red), or 500 µM GDP (green). Error bars removed for clarity. N = 14, 11, 10 and 9 filaments, respectively. (B) Representative images of GFP-Drp1 dissociation from actin bundles. Scale bar, 2 µm. (C) Graph of GFP-Drp1 $T_{1/2}$ values from bundles.

Supplemental Figure S6: Dissociation kinetics of GFP-Drp1 from actin after pre-charging with GTP. (A) Graph of GFP-Drp1 fluorescence decay from single actin filaments when free

GFP-Drp1 and GTP is washed-out with buffer alone (black curve), buffer containing 2.5 μM unlabeled Drp1 (blue), buffer containing 500 μM GTP (red), buffer containing 2.5 μM unlabeled Drp1 + 500 μM GTP (orange), buffer containing 500 μM GDP (light green), or with buffer containing 2.5 μM unlabeled Drp1 + 500 μM GDP (dark green). N = 19, 12, 11, 8, 5, and 9 filaments, respectively. (B) Graph of GFP-Drp1 $T_{1/2}$ values. (C) Representative images of GFP-Drp1 dissociation from single filaments. Scale bar, 2 μm. (D) Graph of FRAP on single actin filaments saturated with GFP-Drp1 (2.5 μM GFP-Drp1, 0.2 μM actin) in the presence or absence of 500 μM GTP in solution with GFP-Drp1. N = 5 - 6 filaments for each condition. The Drp1 curve in the absence of GTP is the same curve shown in Figure 5C. (E) Representative images of each FRAP condition before photo-bleaching, immediately after bleaching, or at 120 sec after photo-bleaching. Scale bar, 2 μm.

Supplemental Figure S7: GFP-Drp1 binds actin bundles at 150 mM KCl in presence of GTP. (A) TIRF microscopy images of 0.2 μM TRITC-phalloidin-stabilized actin filaments with 2.5 μM GFP-Drp1 in buffer containing 150 mM KCl in the presence of 500 μM GTP. Scale bar, 5 μm. Actin is thresholded to reveal individual filaments.

Figure S1

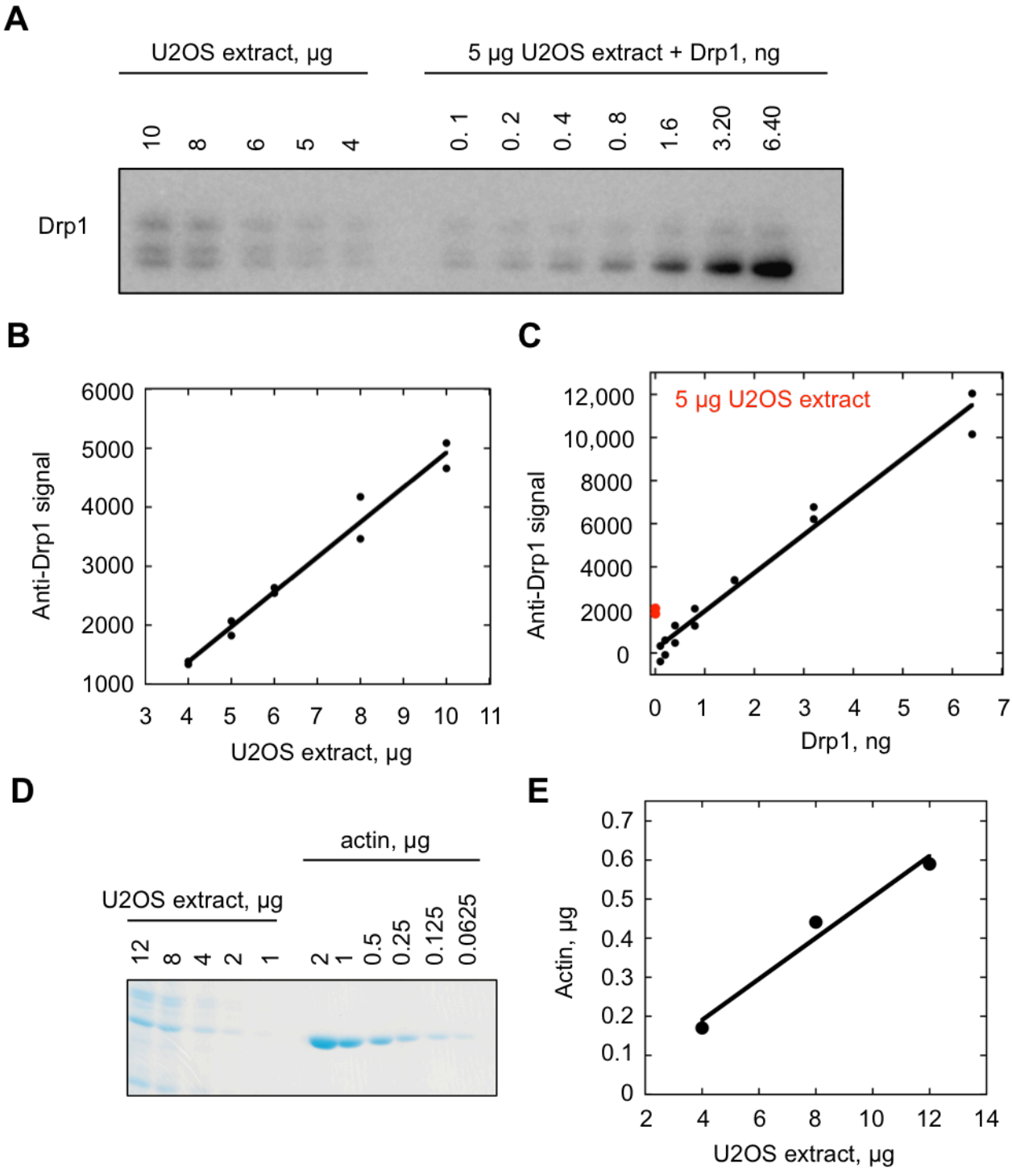
A
U2OS extract, µg
5 µg U2OS extract + Drp1, ng
10
8
6
5
4
0. 1
0. 2
0. 4
0. 8
1.6
3.20
6.40
Drp1
B
Anti-Drp1 signal
6000
5000
4000
3000
2000
1000
3
4
5
6
7
8
9
10
11
U2OS extract, µg
C
5 µg U2OS extract
Anti-Drp1 signal
12,000
10,000
8000
6000
4000
2000
0
0
1
2
3
4
5
6
7
Drp1, ng
D
actin, µg
U2OS extract, µg
12
8
4
2
1
2
1
0.5
0.25
0.125
0.0625
E
Actin, µg
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
8
10
12
14
U2OS extract, µg

Figure S2

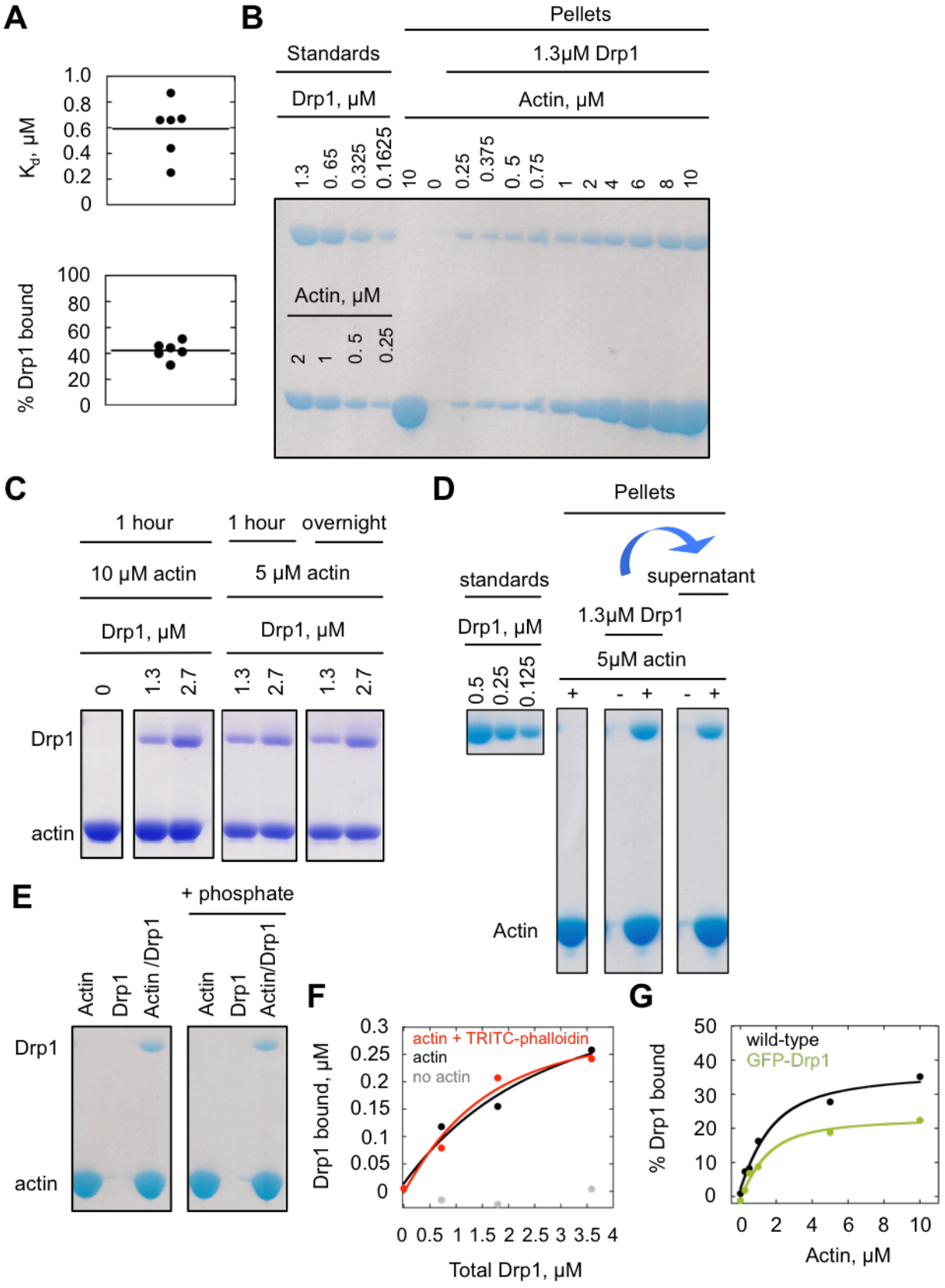
A
B
Pellets
Standards
1.3μM Drp1
Drp1, μM
Actin, μM
1.3
0. 65
0.325
0.1625
10
0
0.25
0.375
0. 5
0.75
1
2
4
6
8
10
Actin, μM
2
1
0. 5
0.25
K_d, μM
% Drp1 bound
C
1 hour
1 hour
overnight
10 μM actin
5 μM actin
Drp1, μM
Drp1, μM
0
1.3
2.7
1.3
2.7
1.3
2.7
Drp1
actin
D
Pellets
standards
supernatant
Drp1, μM
1.3μM Drp1
5μM actin
0.5
0.25
0.125
+
-
+
-
+
Actin
E
+ phosphate
Actin
Drp1
Actin /Drp1
Actin
Drp1
Actin/Drp1
Drp1
actin
F
actin + TRITC-phalloidin
actin
no actin
Drp1 bound, μM
0 0.5 1 1.5 2 2.5 3 3.5 4
Total Drp1, μM
G
wild-type
GFP-Drp1
% Drp1 bound
0 2 4 6 8 10
Actin, μM

Figure S3

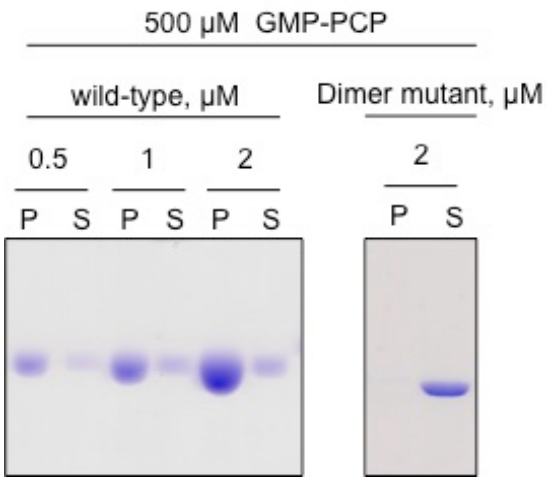

Figure S4

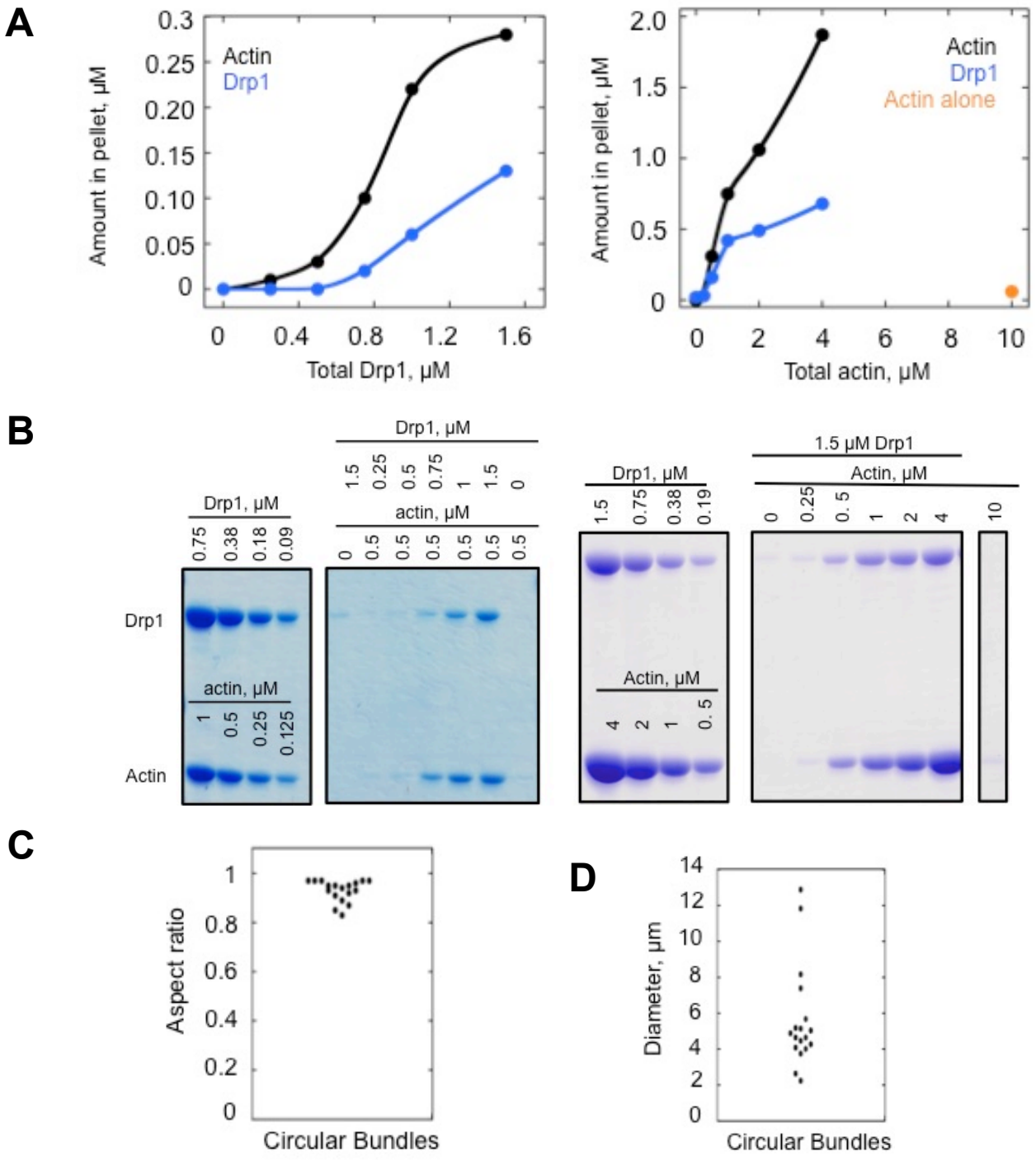
A
Amount in pellet, μM
Actin
Drp1
Total Drp1, μM
Actin
Drp1
Actin alone
Total actin, μM
B
Drp1, μM
actin, μM
Drp1
Actin
1.5 μM Drp1
Actin, μM
C
Aspect ratio
Circular Bundles
D
Diameter, μm
Circular Bundles

Figure S5

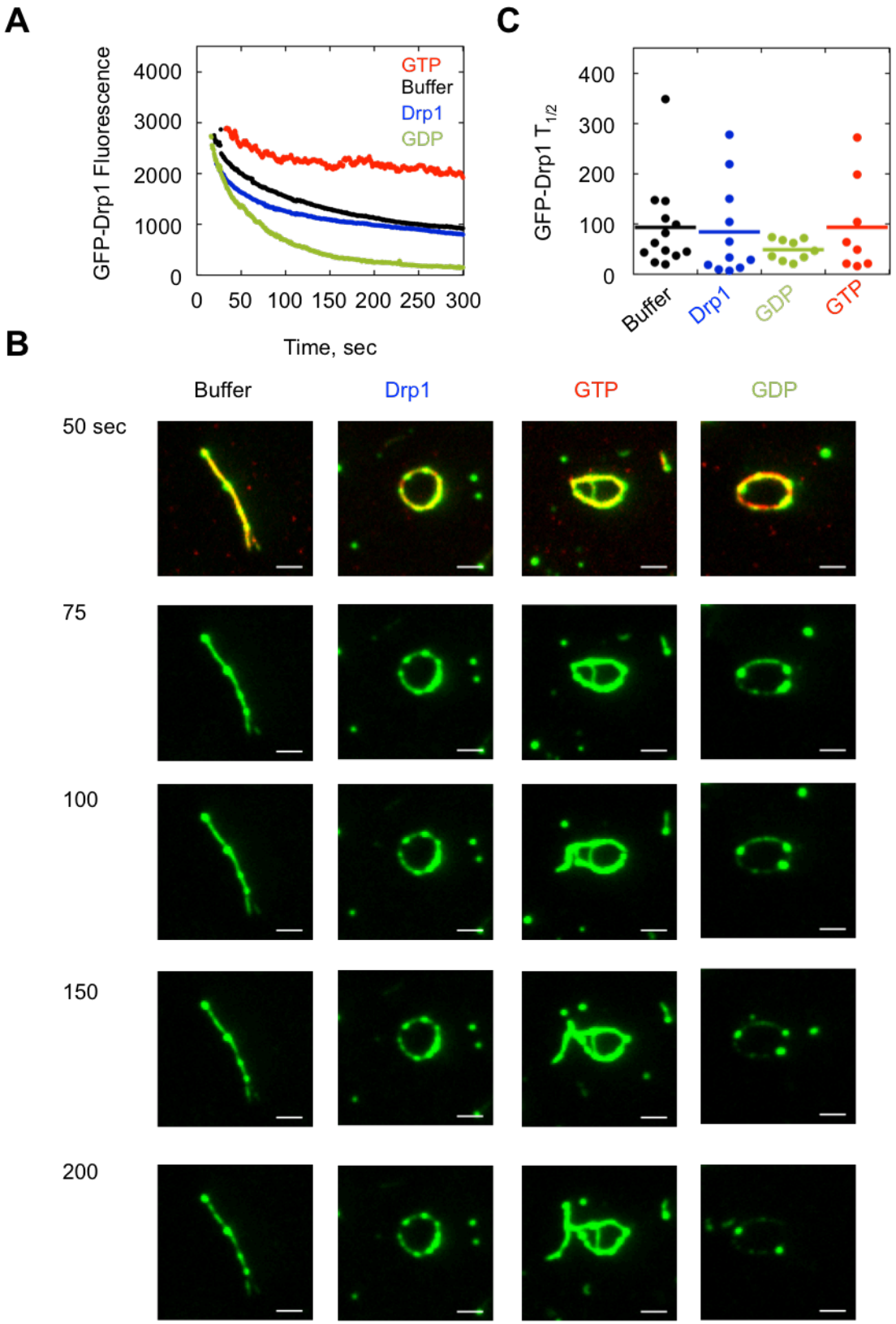
A
GFP-Drp1 Fluorescence
4000
3000
2000
1000
0
0 50 100 150 200 250 300
Time, sec
GTP
Buffer
Drp1
GDP
C
GFP-Drp1 T1/2
400
300
200
100
0
Buffer
Drp1
GDP
GTP
B
Buffer
Drp1
GTP
GDP
50 sec
75
100
150
200

Figure S6

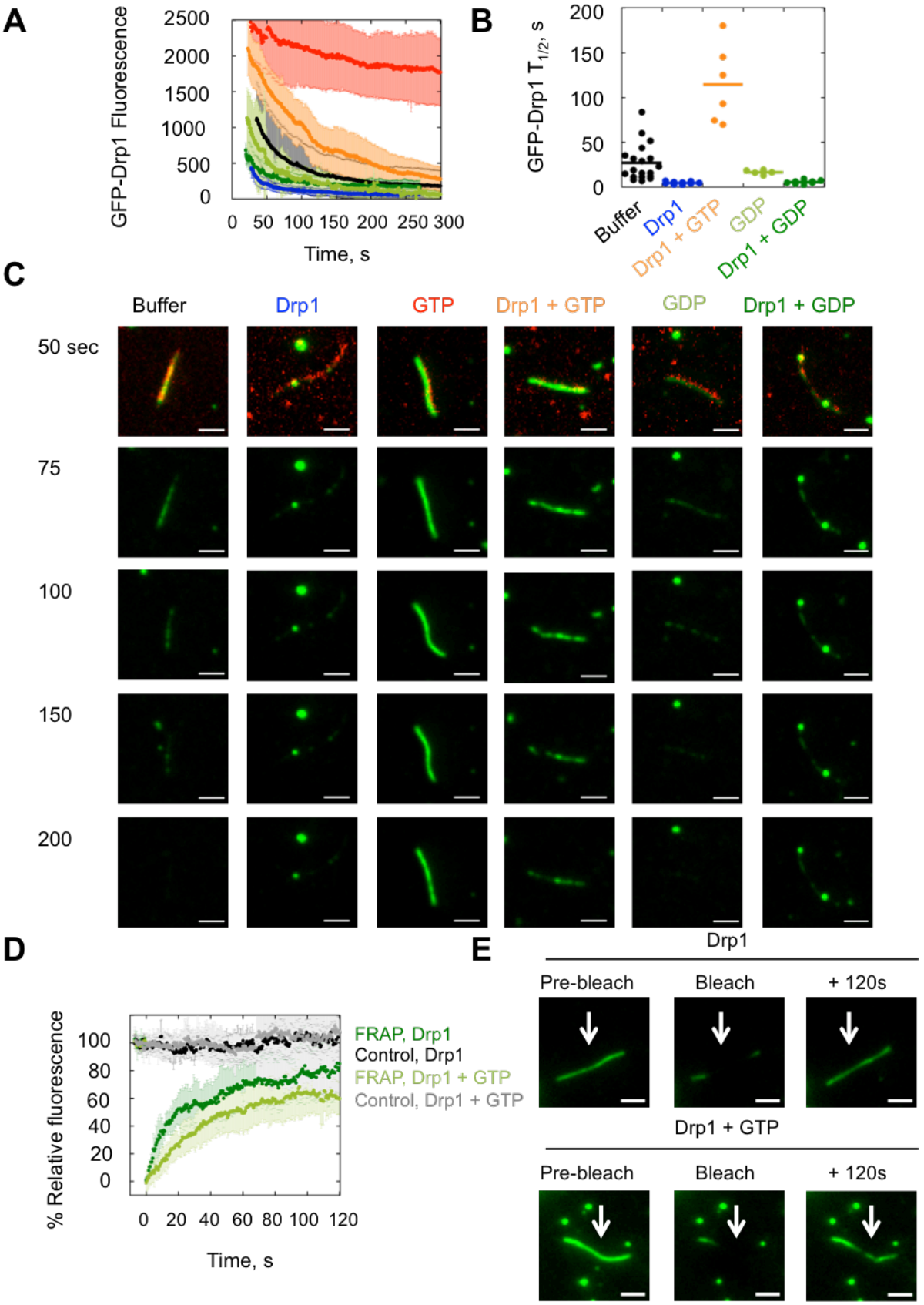
A
GFP-Drp1 Fluorescence
2500
2000
1500
1000
500
0
0 50 100 150 200 250 300
Time, s
B
GFP-Drp1 T1/2, s
200
150
100
50
0
Buffer
Drp1
Drp1 + GTP
GDP
Drp1 + GDP
C
Buffer
Drp1
GTP
Drp1 + GTP
GDP
Drp1 + GDP
50 sec
75
100
150
200
D
% Relative fluorescence
100
80
60
40
20
0
0 20 40 60 80 100 120
Time, s
FRAP, Drp1
Control, Drp1
FRAP, Drp1 + GTP
Control, Drp1 + GTP
E
Drp1
Pre-bleach
Bleach
+ 120s
Drp1 + GTP
Pre-bleach
Bleach
+ 120s

Figure S7