SUPPLEMENTARY DATA

Fig. S1. HSQC spectra of Scar1 VCA truncations with and without actin.

A, Overlay of the ^{1}H-^{15}N TROSY-HSQC spectrum of [^{15}N,^{2}H]-Scar1 VC bound to Ca^{2+}-ATP-actin (shown in red) with ^{1}H-^{15}N HSQC spectra of [^{15}N,^{2}H]-Scar1 VCA free (shown in gray) and bound to Ca^{2+}-ATP-actin (shown in green), at 25.0°C. Circles denote residues Gly 508 and Trp 557. Gly 508 shifts upon actin-binidng (arrow) whereas Trp 557 in the A domain does not. Conditions were the same as described in "Materials and Methods". *B*, Overlay of the ^{1}H-^{15}N HSQC spectra of 200 μM [^{15}N,^{1}H]-Scar1 C free (shown in black) and bound to 200μM Ca-ATP-actin (shown in red) at 25.0 °C in "buffer A" as defined in "Materials and Methods". Circles denote resonances that significantly broaden upon addition of actin and arrows denote resonances that shift upon addition of actin.

Fig. S2. Normalized ^{15}N,^{1}H HSQC peak intensities of residue-labeled Scar1 VCA peptides in the presence of actin. Bars represent intensities of residue-labeled Scar VCA peaks measured in the presence of actin. The intensity of each peak is divided by the intensity of a well-separated reference peak in the same spectrum that did not shift or change intensity in the presence of actin. Red denotes assigned peaks with chemical shifts that changed position in the presence of actin. Linewidths of the actin-perturbed chemical shifts were 2-3 fold higher than those that did not change upon addition of actin (40-60 Hz versus 20 Hz).

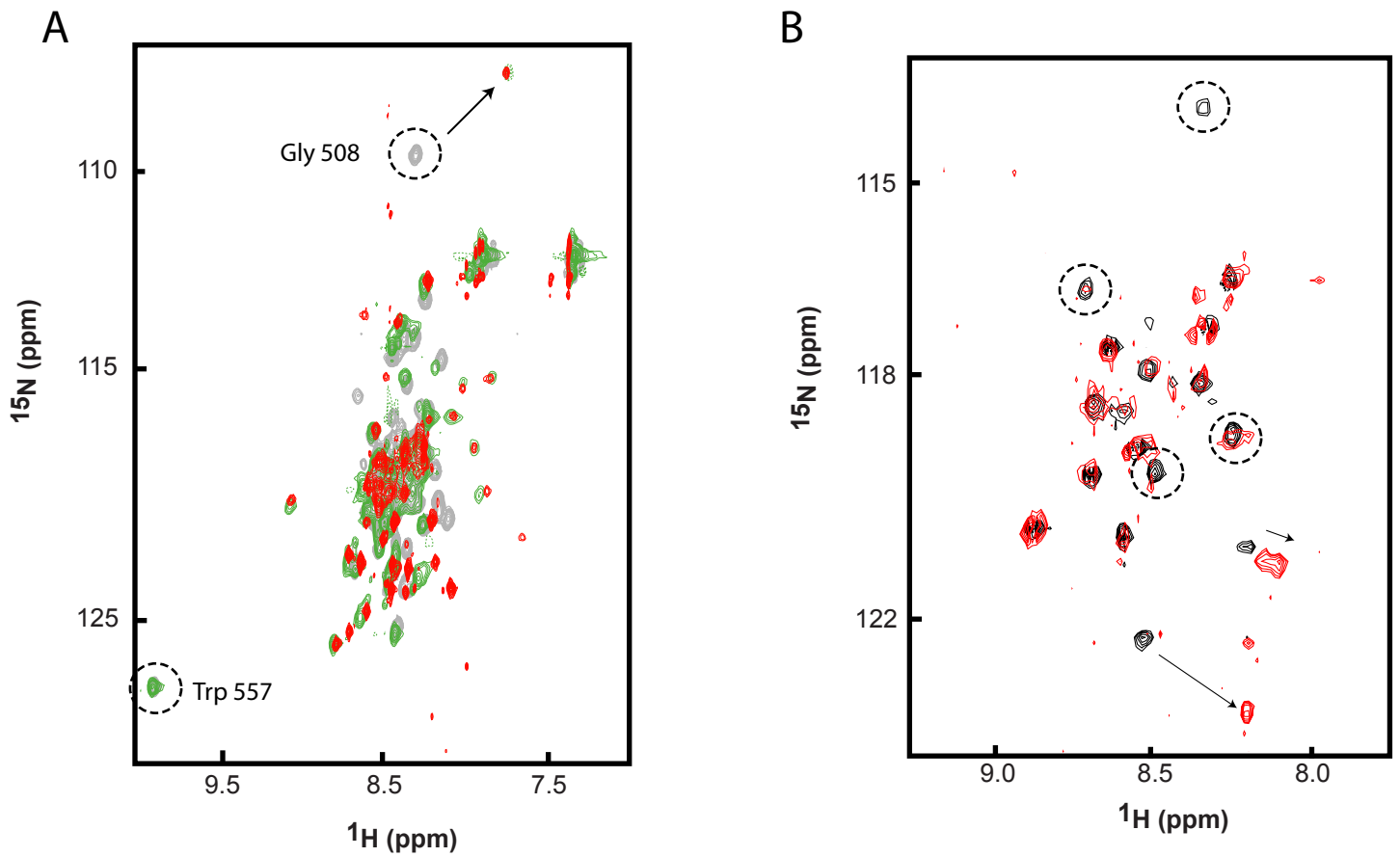
Fig. S3. Crosslinking of the C domain to the Arp2/3 complex in the presence of actin and F-actin. EDC/NHS crosslinking of 8 μM Alexa-488 labeled C domain (522-543) to

5 μM Arp2/3 complex in the presence of 20 μM monomeric actin labeled with Alexa-648 (Molecular Probes) and/or 8 μM unlabeled phalloidin-stabilized F-actin. The coomassie, Alexa-648 fluorescence (a648 actin) and Alexa-488 fluorescence (a488 C) signals for each condition are shown. All lanes shown are after completion of crosslinking except for the 1st lane of the group "Arp2/3", showing 5 μM Arp2/3 before crosslinking. The positions of the C domain, actin, Arp2/3, actin-C crosslink, and p40-C crosslink are shown. No Actin-C-Arp2/3 crosslinks were observed. Crosslinking conditions were the same as in "Materials and Methods".

Fig. S4. Binding of Scar1 VCA mutants to actin and the Arp2/3 complex.

A, Competition between unlabeled Scar1 VCA (green squares), V531D VCA (black circles) and L535A VCA (blue squares) peptides with 0.02 μM Alexa-488 N-terminal labeled Scar1 VCA for binding to 0.4 μM monomeric actin as measured by fluorescence anisotropy. Black lines are best fits to a full solution of the equilibrium competition binding equation using KaleidaGraph (1). Conditions were as described in "Materials and Methods". *B*, Competition between unlabeled Scar1 VCA (green triangles), V531D VCA (black circles) and L535A VCA (blue squares) peptides with 0.02 μM Alexa-488 C-terminal labeled Scar1 CA for binding to 1 μM Arp2/3 complex as measured by fluorescence anisotropy. Black lines are best fits to a full solution of the equilibrium competition binding equation using KaleidaGraph (1). Conditions were as described in "Materials and Methods".

1. Marchand, J. B., Kaiser, D. A., Pollard, T. D., and Higgs, H. N. (2001) *Nat Cell Biol* **3**(1), 76-82


A
Gly 508
Trp 557
110
115
125
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8.5
7.5
15N (ppm)
1H (ppm)
B
115
118
122
9.0
8.5
8.0
15N (ppm)
1H (ppm)

Figure S1

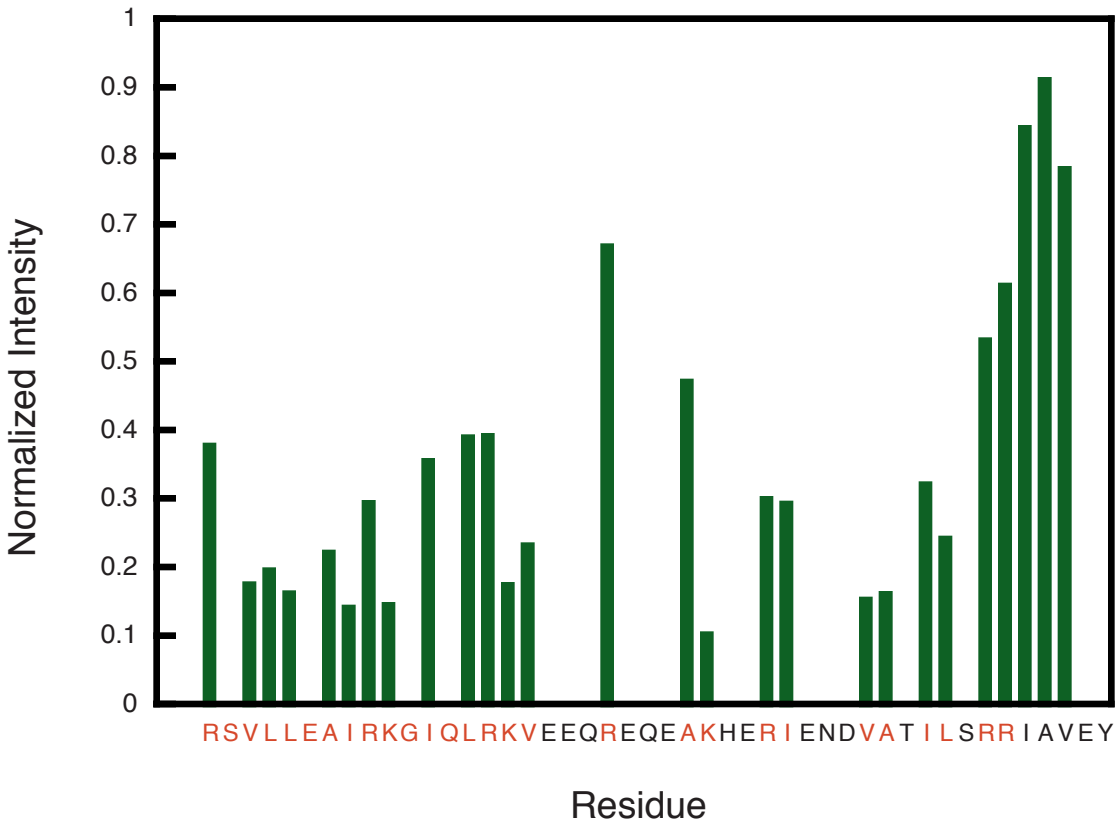

Normalized Intensity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
RSVLLEAIRKGIQLRKVEEQREQEAKHERIENDVATILSRRIAVEY
Residue

Figure S2

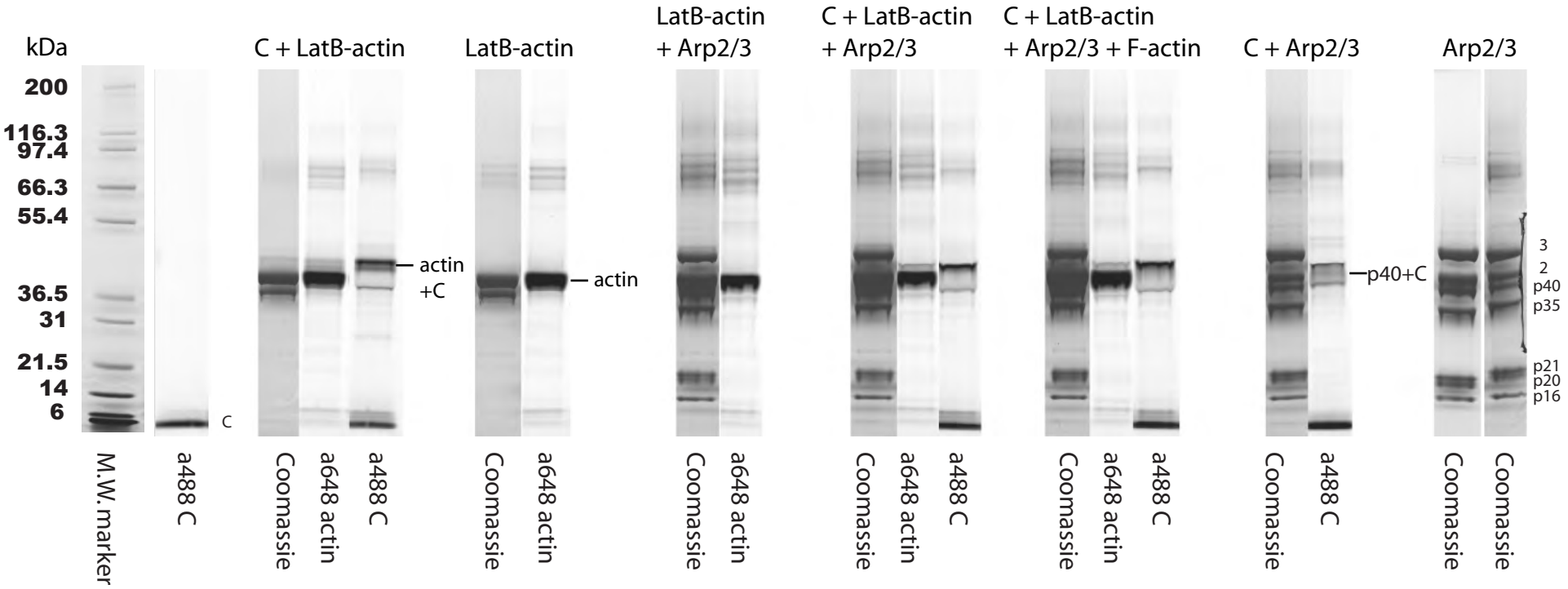
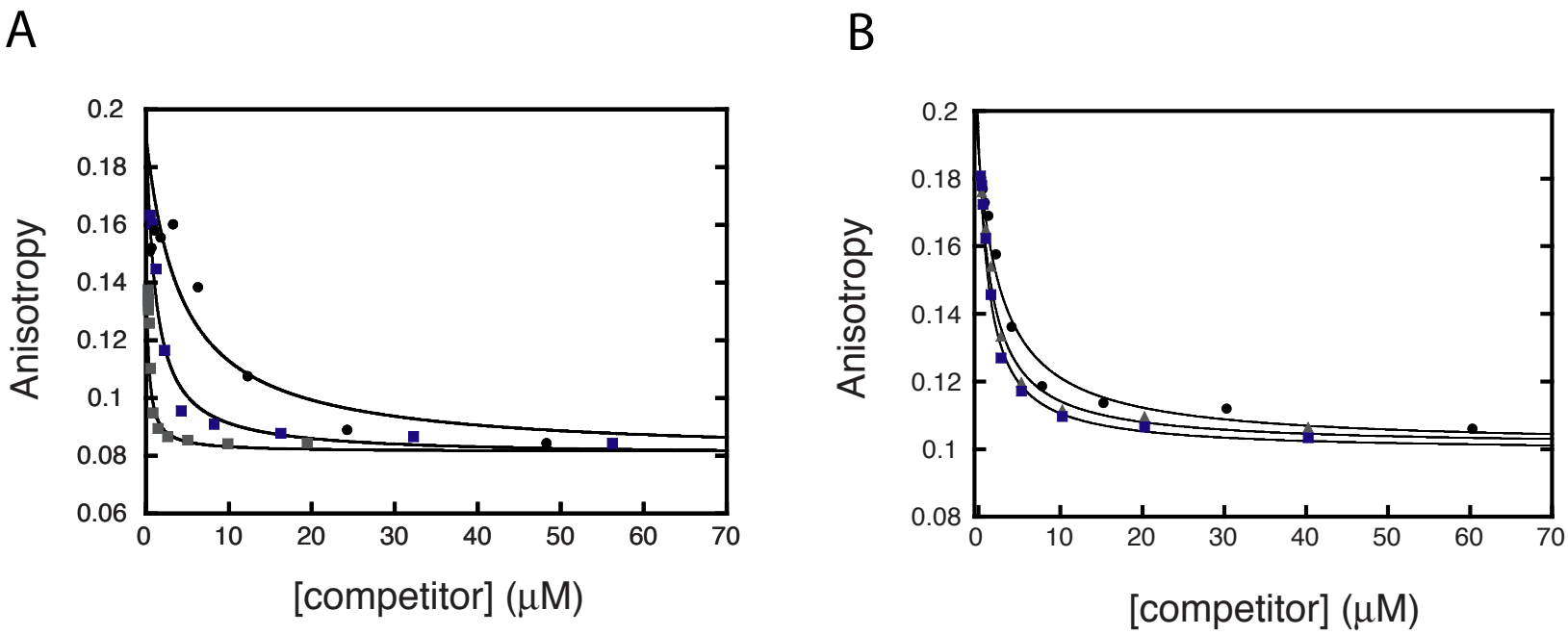

kDa
200
116.3
97.4
66.3
55.4
36.5
31
21.5
14
6
M.W. marker
a488 C
C
C + LatB-actin
Coomassie
a648 actin
a488 C
actin
+C
LatB-actin
Coomassie
a648 actin
actin
LatB-actin
+ Arp2/3
Coomassie
a648 actin
C + LatB-actin
+ Arp2/3
Coomassie
a648 actin
a488 C
C + LatB-actin
+ Arp2/3 + F-actin
Coomassie
a648 actin
a488 C
C + Arp2/3
Coomassie
a488 C
p40+C
Arp2/3
Coomassie
Coomassie
3
2
p40
p35
p21
p20
p16

Figure S3


A
B
Anisotropy
[competitor] (μM)
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0.18
0.16
0.14
0.12
0.1
0.08
0.06
0
10
20
30
40
50
60
70

Figure S4