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Supplemental Information

Capping Protein Insulates Arp2/3-Assembled Actin Patches from Formins

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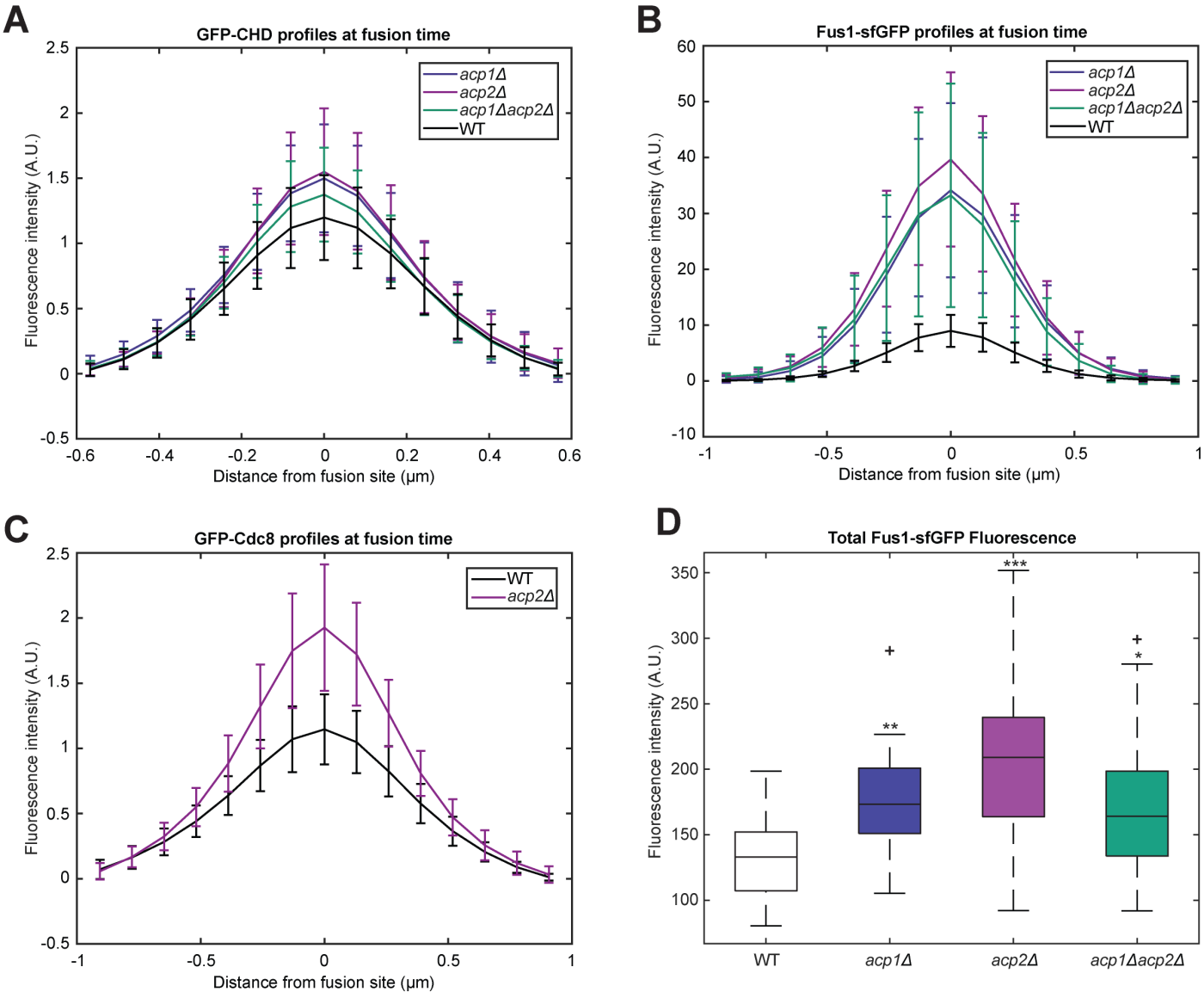

Figure S1. Quantification of the increase in actin, tropomyosin and Fus1 at the fusion focus in absence of CP. Related to Figure 2. A-C. Profiles of the bleach-corrected fluorescence intensities around the fusion focus at fusion time in the strains shown in Figure 2. The boxplot in Figure 2D shows the central points of these profiles further normalized to WT. (A) GFP-CHD profiles. p-values relative to WT are 5.4×10^{-4}, 2.9×10^{-4} and 2.5×10^{-2} (n = 40 mating pairs for each strain). (B) Fus1-sfGFP profiles. p-values relative to WT are 6.4×10^{-10}, 2.0×10^{-9} and 1.8×10^{-8} (n = 24 mating pairs for each strain). (C) GFP-Cdc8 profiles. p-values relative to WT is 1.3×10^{-8} (n = 24 mating pairs for each strain). **D.** Boxplot of total Fus1 fluorescence intensity in fusing cells, in WT and *acp2Δ*. p-value relative to WT are 3.0×10^{-6}, 1.6×10^{-8} and 8.7×10^{-4} This was measured on 32 mating pairs for each strain.

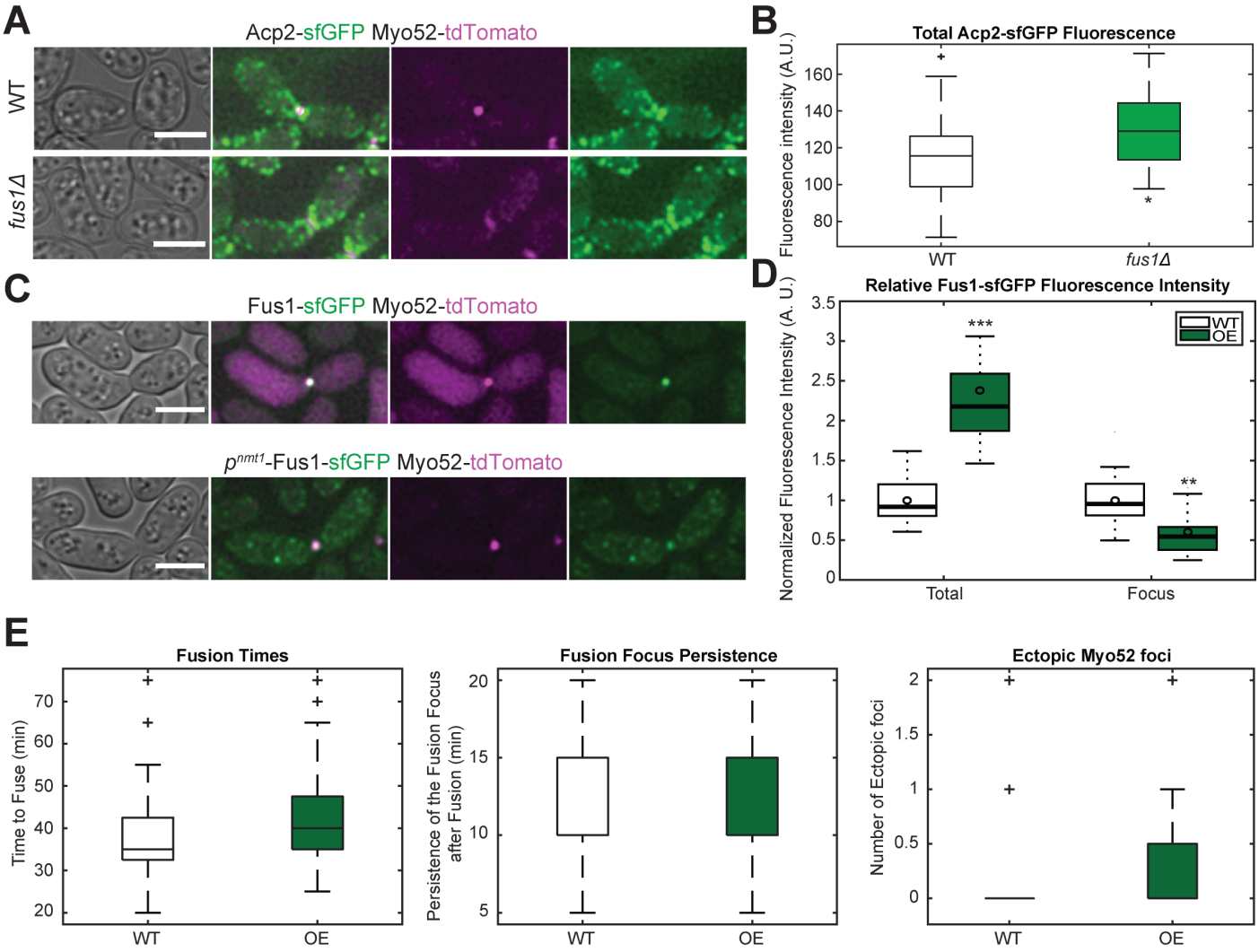

Figure S2. Weak increase in CP fluorescence in *fus1*Δ and no effect of Fus1 overexpression. **Related to Figure 2.** **A.** Myo52-tdTomato and Acp2-sfGFP in WT and *fus1*Δ at fusion time. **B.** Boxplot of total fluorescence intensities in fusing cells in strains as in (A). p-value relative to WT is 1.2×10^{-2} (n = 32 mating pairs for each strain). **C.** Myo52-tdTomato and Fus1-sfGFP at fusion time, in strains expressing Fus1 either from endogenous locus or, in addition, under the *nmt1* promoter. **D.** Boxplot of total and fusion focus fluorescence intensities normalized to WT in fusing cells in strains as in (C). p-value relative to WT is 1.3×10^{-12} for the total intensity (n = 15 and 45 mating pairs for WT and over-expressing cells) and 3.2×10^{-6} for the fusion focus intensity (n = 30 mating pairs each). Note that the quantified levels of overexpressed Fus1-sfGFP do not represent all Fus1 in the cell, as endogenous Fus1 is not tagged. **E.** Boxplots of fusion times, fusion focus persistence, and ectopic Myo52 foci in WT and Fus1-overexpressing strains. p-values relative to WT are 5.2×10^{-2} for fusion times (n = 60), 4.2×10^{-1} for persistence times (n = 60) and 3.7×10^{-1} for ectopic foci (n = 44 and 36 individual cells for WT and Fus1-overexpressing cells, respectively). Bars are 5µm.

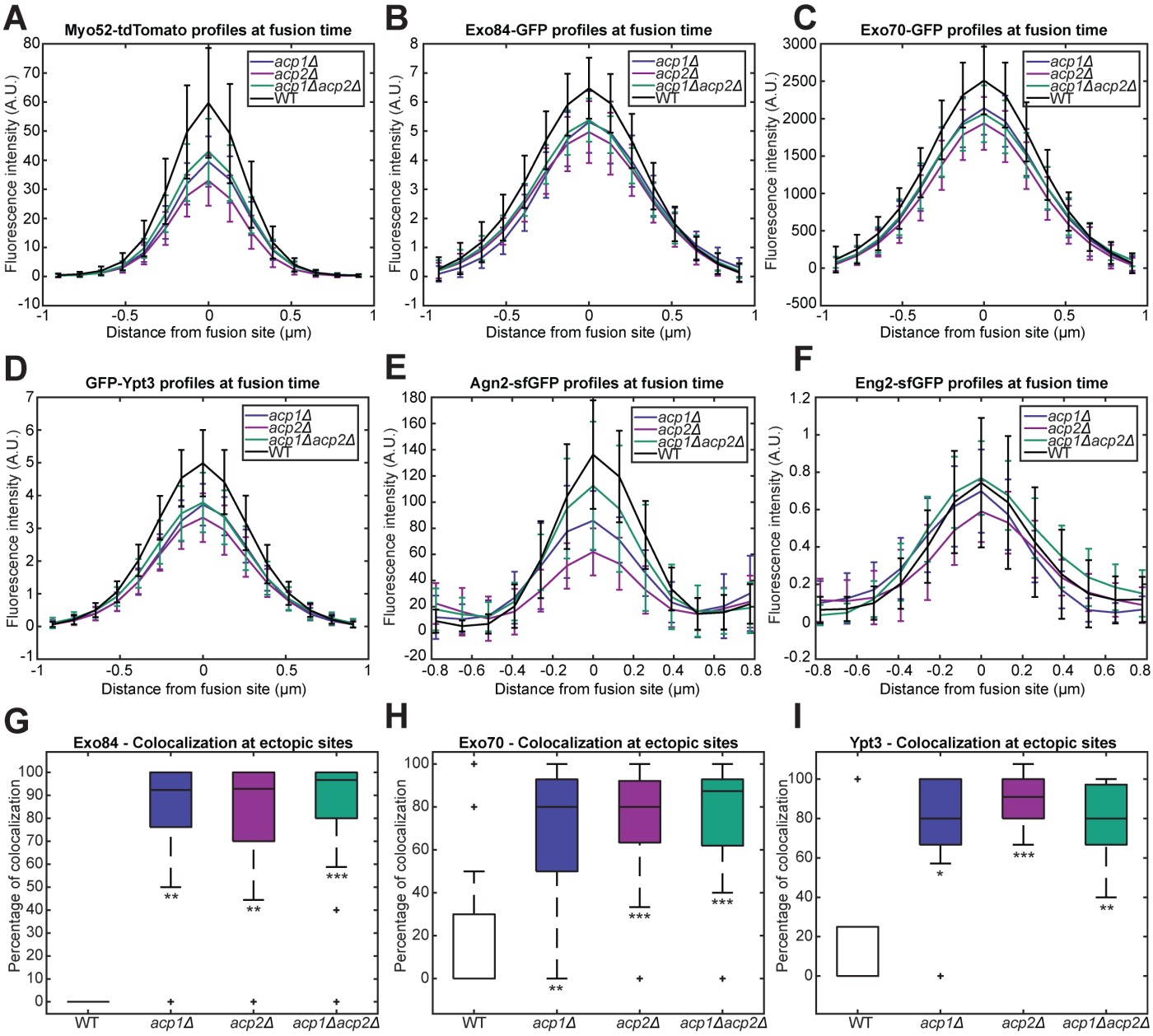

Figure S3. Quantifications of vesicular marker intensities at the fusion focus and ectopic foci in absence of CP. Related to Figure 3. A-F. Profiles of the bleach-corrected fluorescence intensities around the fusion focus at fusion time in the strains shown in Figure 3. The boxplot in Figure 3F shows the central points of these profiles further normalized to WT. (A) Myo52-tdTomato. p-values relative to WT are 3.2×10^{-8}, 3.7×10^{-11} and 1.4×10^{-5} (n = 40 for each strain). (B) Exo84-GFP. p-values relative to WT are 3.2×10^{-4}, 4.2×10^{-7} and 1.8×10^{-3} (n = 25 for each strain). (C) Exo70-GFP. p-values relative to WT are 1.1×10^{-4}, 3.4×10^{-8} and 3.0×10^{-6} (n = 40 for each strain). (D) GFP-Ypt3. p-values relative to WT are 3.1×10^{-7}, 1.2×10^{-9} and 1.1×10^{-5} (n = 30 for each strain). (E) Agn2-sfGFP. p-values relative to WT are 2.1×10^{-3}, 2.5×10^{-5} and 3.8×10^{-1} (n = 11 for each strain). (F) Eng2-sfGFP. p-values relative to WT are 7.2×10^{-1}, 1.0×10^{-1} and 8.4×10^{-1} (n = 11 for each strain). **G-I.** Boxplots of the colocalization of Myo52 with (G) Exo84, (H) Exo70, and (I) Ypt3 at ectopic sites in WT, *acp1Δ*, *acp2Δ*, and *acp1Δ acp2Δ* strains. p-values relative to WT are 1.1×10^{-7}, 8.7×10^{-7} and 6.0×10^{-9} for Exo84 (n= on 7, 16, 20, and 20 pairs respectively), 1.3×10^{-5}, 2.9×10^{-9} and 3.5×10^{-9} for Exo70 (n=28 for each strain) and 3.1×10^{-7}, 8.4×10^{-9} and 3.5×10^{-6} for Ypt3 (n=9, 29, 28, and 27 pairs respectively) for *acp1Δ*, *acp2Δ* and *acp1Δacp2Δ*, respectively.

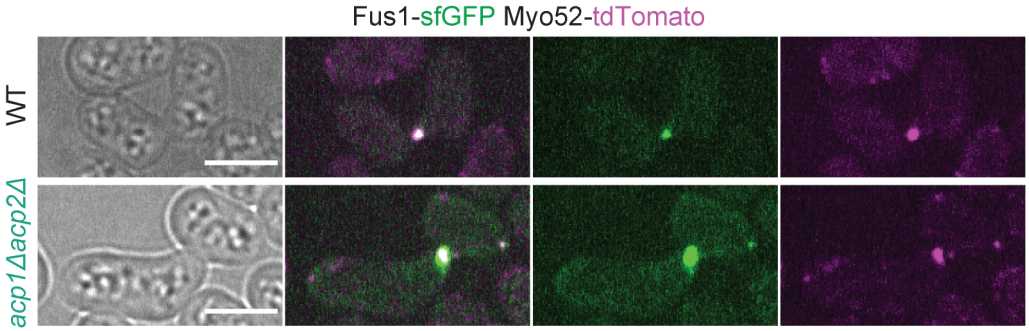

Figure S4. Fus1/Myo52 ectopic foci also form in capping protein double mutant strains. **Related to Figure 4.** Spinning-disk confocal microscopy images of Myo52-tdTomato and Fus1-sfGFP in WT and *acp1Δ acp2Δ* before fusion time. Bars are 5µm.

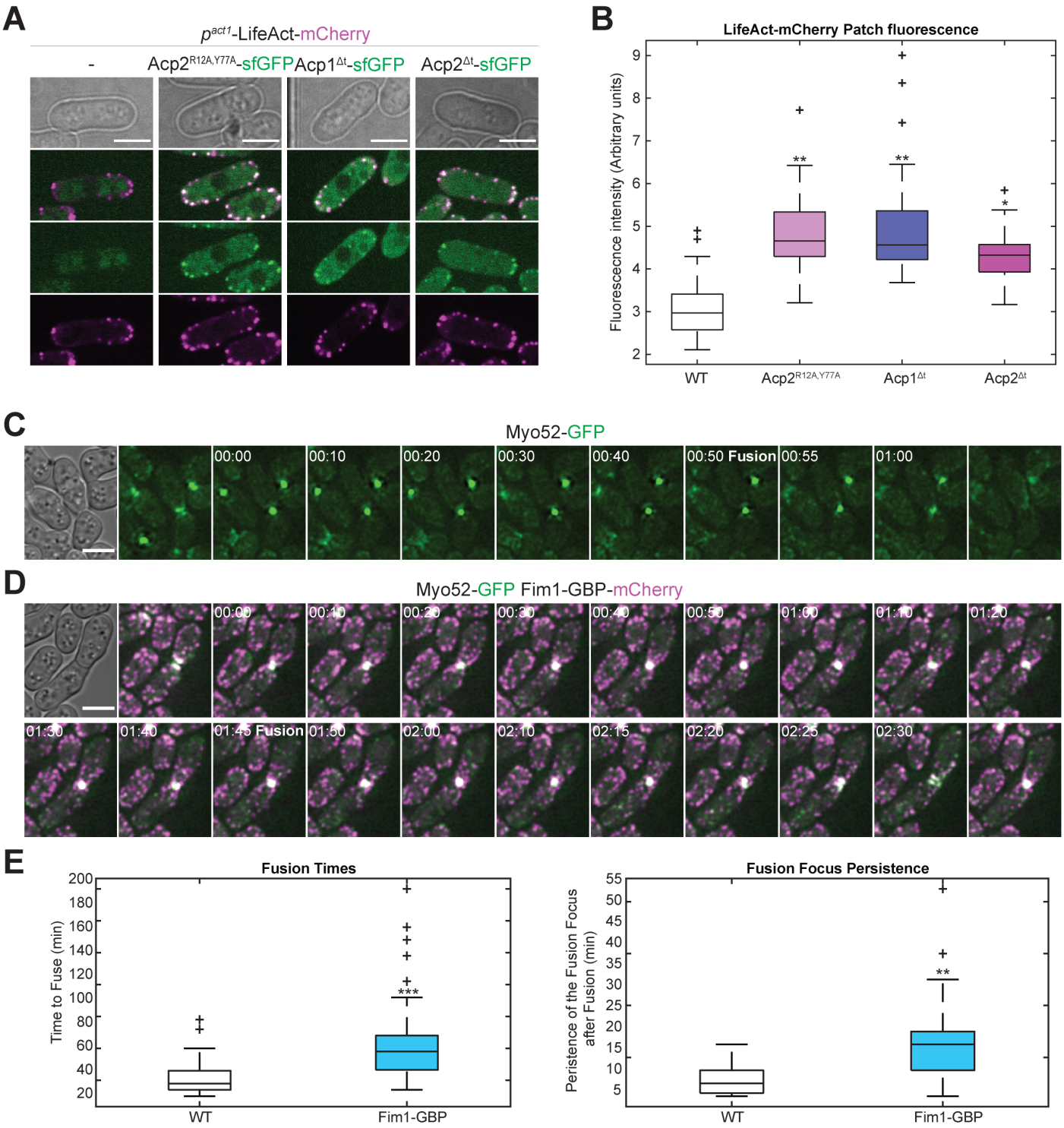

Figure S5. Actin levels are increased in *acp1* and *acp2* tentacle and CPI mutants and Forced recruitment of Myo52 to actin patches delays fusion. **Related to Figure 5. A.** Spinning-disk confocal microscopy images of LifeAct-mCherry under the actin promoter alone or in combination with Acp2^{R12A,Y77A}-sfGFP, Acp1$^{\Delta t}$-sfGFP and Acp2$^{\Delta t}$-sfGFP as described in Figure 5 in interphase cells during exponential growth. Bars are 5µm. **B.** Boxplots of LifeAct-mCherry patch fluorescence intensity in the strains shown in (A). p-values relative to WT are 1.3×10^{-7}, 1.6×10^{-6} and 1.4×10^{-4} (n = 30 cells for each strain). **C-D.** Time-lapse images of a strain expressing Myo52-GFP alone (C) or in combination with Fim1-GBP-mCherry (D) from beginning to disappearance of the fusion focus. The beginning is defined as the first formation of the focus in both cells. The fusion point is highlighted and is defined as the peak Myo52-GFP fluorescence intensity at the focus. Time in hour:min. Bars are 5µm. **E.** Boxplots of the above-mentioned strains fusion times and focus persistence times. p-values relative to WT are 2.1×10^{-8} and 9.5×10^{-8} for fusion times and persistence times, respectively (n = 55 mating pairs for each strain).

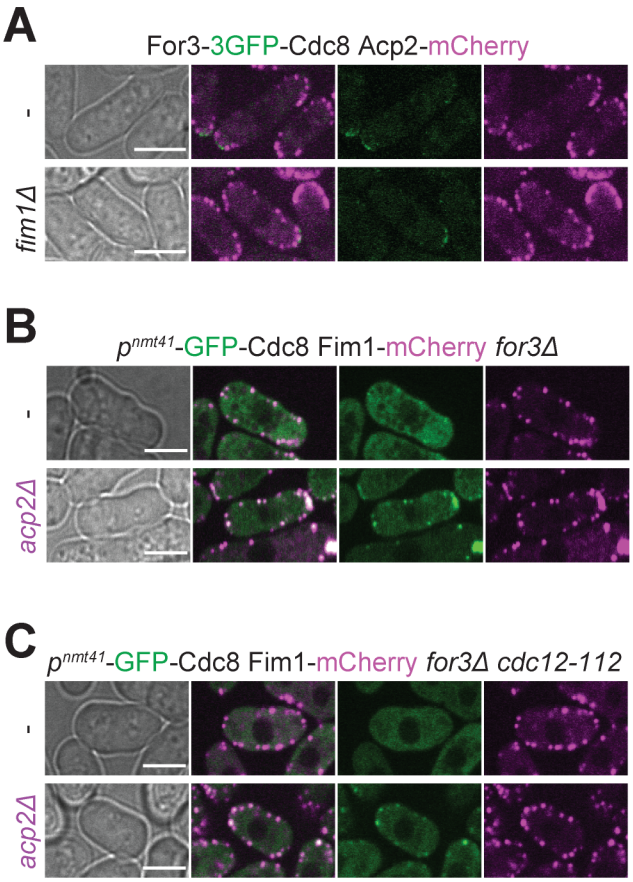

Figure S6. For3 does not localize to actin patches in *fim1Δ* cells and removing formins does not lead to the recovery of actin patch identity. Related to Figure 6. A. Spinning-disk confocal microscopy images of For3-3GFP and Acp2-mCherry in WT and *fim1Δ* cells. **B-C.** Spinning-disk confocal microscopy images of Fim1-mCherry and GFP-Cdc8 in interphase cells during exponential growth in (B) *for3Δ* and (C) *for3Δ cdc12-112* at 36°C, in otherwise WT and *acp2*Δ background. Bars are 5µm.

Oligonucleotides		
TTATCACTTGAAAATAATCATATATACGAAGAGCTTCGACTTTCAG AGTTGATAAACTTATTGGCTAAAGCTACATTACGGATCCCCGGGT TAATTAA *(Myo51 tagging)*	Sigma	osm3932
GAATATAGTATTAATGAGTACTAATATAAATTAAAAATATTGATCGG GTGTAACGTTTAATGATACTTGATAAAAAGCGAATTCGAGCTCGTT TAAAC *(Myo51 tagging)*	Sigma	osm4920
ATCTTTATTTTGCCAGAAGATATTGTGGCCGTTCGTCCTCGGTTG GTCCTTCATTTTATTGGCAGTTTAATGGCCGTACGGATCCCCGGG TTAATTAA *(Fim1 tagging)*	Sigma	osm2878
CGCAATATAAGTAATTAAATTGGGAAAAACACATGTGTTAAATCGT TTCGTTAAAAGCTATAGTTAAGTCGAAACAAAGAATTCGAGCTCGT TTAAAC *(Fim1 tagging)*	Sigma	osm2879
GAATTGCGTCGTCAACTTCCAGTCACTCGCCAGAAAATTAATTGG GAAAACGTTAGTGGCATCCGTATGAGAAATACTCGGATCCCCGG GTTAATTAA *(Acp1 tagging)*	Sigma	osm3416
AGGAAACAGCTTGAATCACCGTCTTAAAAAATATCGTCGCAAAAA AATTTCCAAAATTTATAAACACATATAAGTGTTGAATTCGAGCTCG TTTAAAC *(Acp1 tagging, full and tentacle deletion)*	Sigma	osm3417
TGCGGCATTGTCTTCACCATCCGTATTCGTTTCTTACCATTCACCA TTGTCCGCACCACATTATAGAAATTTCGAAAGCGGATCCCCGGGT TAATTAA *(Acp1 deletion)*	Sigma	osm4503
GCTCAAGTCGAAAATGGAATTCAACAGTCCTTCAACGTTGAACTT TCTTCACTTAATGACAAAAAGTTTAAAGAATTGCGGATCCCCGGG TTAATTAA *(Acp1 tentacle deletion)*	Sigma	osm5328
CATTAAGGCCTCACTTTTATTCTGAGATCGCTATCCGGTTGTATTC TTTTGTTTAAAGCATTATATCATCAACTCACCCGGATCCCCGGGTT AATTAA *(Acp2 deletion)*	Sigma	osm4504
CAATCTTTCTATGACTATTTTCGTTGAAGATGGAACGAATACTATG AGAAGATCACGGAAAGAAAACAAAAAGCAATCGAATTCGAGCTCG TTTAAAC *(Acp2 tagging, full and tentacle deletion)*	Sigma	osm4505
ACTCGTTCCATTCAACCCGTTTCCGATGCCCAACCAAATGATTCG GCTTTGCGTTCAGTTTTAAACGATCTTTCCATTCGGATCCCCGGG TTAATTAA *(Acp1 tagging)*	Sigma	osm4641
GAGGAAATGGAAACTCGGATGCGCAACTTCCTCCAGGATGTCTA CTTTGGAAAAACTAAAGATATCATCAACCAGACTCGGATCCCCGG GTTAATTAA *(Acp1 tentacle deletion)*	Sigma	osm5329
GCGGCCGCCATATGTTGTTCACTT *(Acp2 5'-ORF-sfGFP-kanMX-3' insert amplification)*	Sigma	osm5330
GCGGCCGCTTATTTCATGACCTT *(Acp2 5'-ORF-sfGFP-kanMX-3' insert amplification)*	Sigma	osm5331
GCATTAGATTTACTCGCACGATTAAACCC *(Acp2 R12A mutagenesis)*	Sigma	osm5432
GGGTTTAATCGTGCGAGTAAATCTAATGC *(Acp2 R12A mutagenesis)*	Sigma	osm5433
CCATGGAGCAATAAAGCTGATCCTCCTTTGG *(Acp2 Y77A mutagenesis)*	Sigma	osm5434
CCAAAGGAGGATCAGCTTTATTGCTCCATGG *(Acp2 Y77A mutagenesis)*	Sigma	osm5435
AAAGTCGACTGTTGCTTTGAATCATATTAC *(Fus1 5'-ORF-sfGFP-kanMX-3' insert amplification)*	Sigma	osm5449
CGTTTGATATCGATGTATTTACTGATTACTT *(Fus1 5'-ORF-sfGFP-kanMX-3' insert amplification)*	Sigma	osm5452
CTTCTAAACGGCTAGCTCAGCTTCATTGG *(Fus1 K879A mutagenesis)*	Sigma	osm5453
CAATGAAGCTGAGCTAGCCGTTTAGAAGG *(Fus1 K879A mutagenesis)*	Sigma	osm5454
CAAATGGTTAGTGCTCGTTTGCATAG *(Fus1 I951A mutagenesis)*	Sigma	osm5455
CTATGCAAACGAGCACTAACCATTTGC *(Fus1 I951A mutagenesis)*	Sigma	osm5456
GTCCTTCATATTCGTCCTTTTATGAATGATGC *(Fus1 GN1087,1088RP mutagenesis)*	Sigma	osm5457
CATCATTCATAAAAGGACGAATATGAAGGACC *(Fus1 GN1087,1088RP mutagenesis)*	Sigma	osm5458
GCTTCCATGATTGCTAATGACAAAACAG *(Fus1 K1112A mutagenesis)*	Sigma	osm5459
CCTGTTTTGTCATTAGCAATCATGGAAG *(Fus1 K1112A mutagenesis)*	Sigma	osm5460
TCCGGTACCGATCAGAAAATTATCGCC *(p^{nmt1} amplification)*	Sigma	osm3113
ACTGCGGCCGCTGATTTAACAAAGCGACTATAAGTC *(p^{nmt1} amplification)*	Sigma	osm3516
ACTGCGGCCGCATGATGACGGCTAGTTTTAAAGG *(fus1-sfGFP amplification)*	Sigma	osm3005
ACTGAGCTCTCTCTTAAGTTCATTGTTATTCTCC *(fus1-sfGFP amplification)*	Sigma	osm3006

Table S1. Oligonucleotides used in this work. Related to STAR Methods and Key Resources Table