Molecular Analysis of Arp2/3 Complex Activation in Cells

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Arp2/3 Activation in Cells

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Supplementary Information

Figure S1. Histograms of the rates and durations of the linear phases of assembly and disassembly of Arc35 and Cap1 in wild-type, *las17∆acidic myo5∆acidic* and *las17∆acidic myo3∆acidic myo5∆acidic* cells. These plots were generated from the data in Figures 2 and 3.

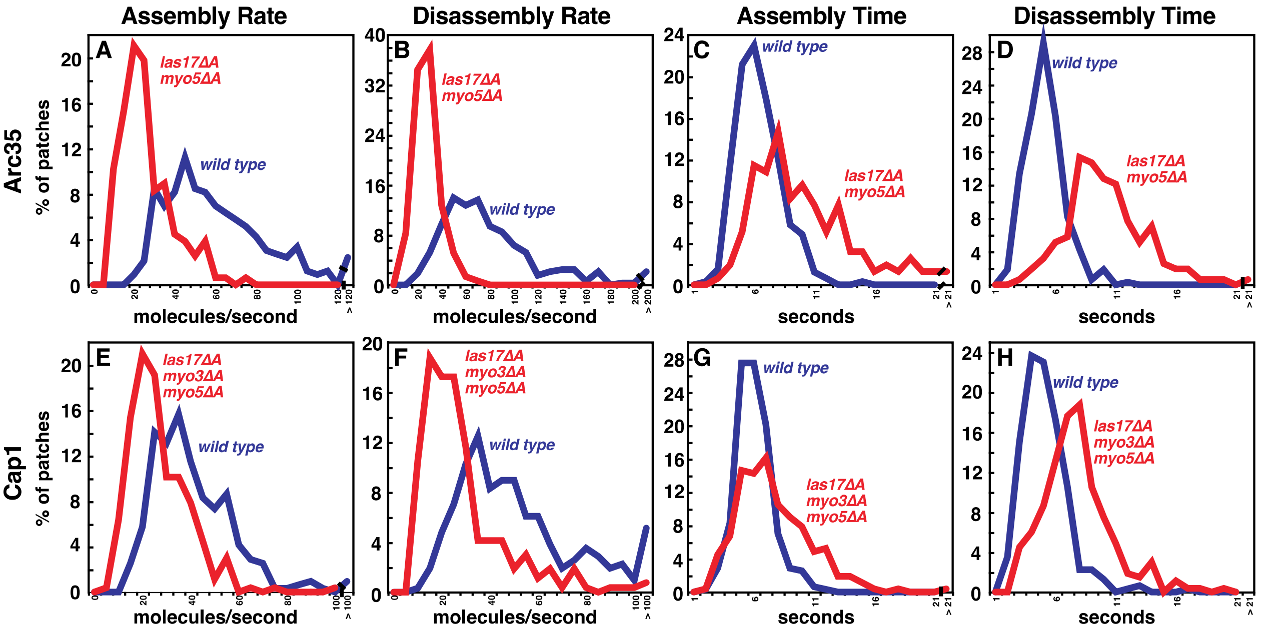

Figure S2. Histograms of the rates and durations of the linear phases of assembly and disassembly of Arc35, Cap1 and Abp1 in wild type and *las17Δacidic* cells. These plots were generated from the data in Figure 4.

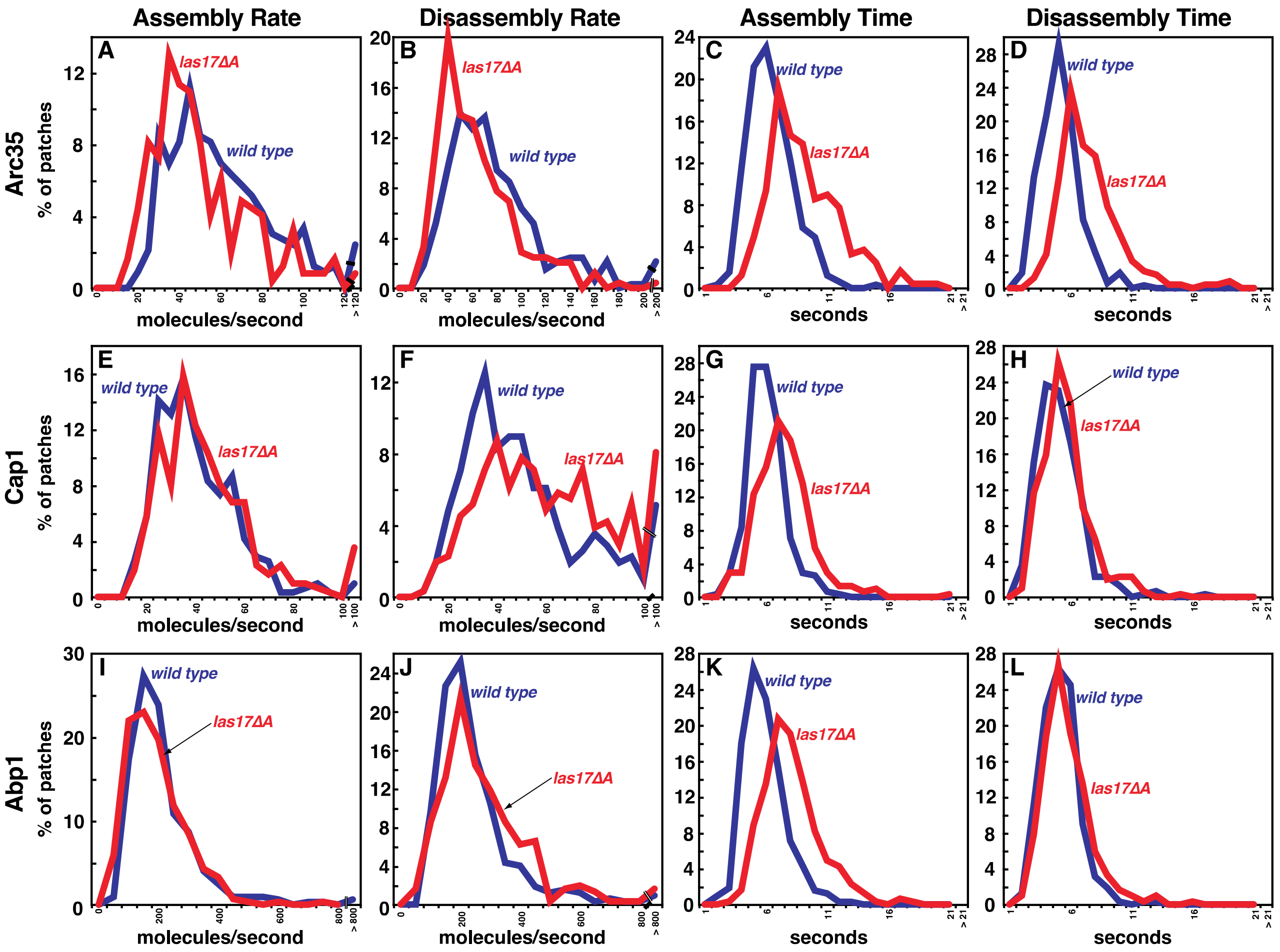

Figure S3. Histograms of the rates and durations of the linear phases of assembly and disassembly of Arc35, Cap1 and Abp1 in wild type and *myo3∆acidic myo5∆acidic* cells. These plots were generated from the data in Figure 5.

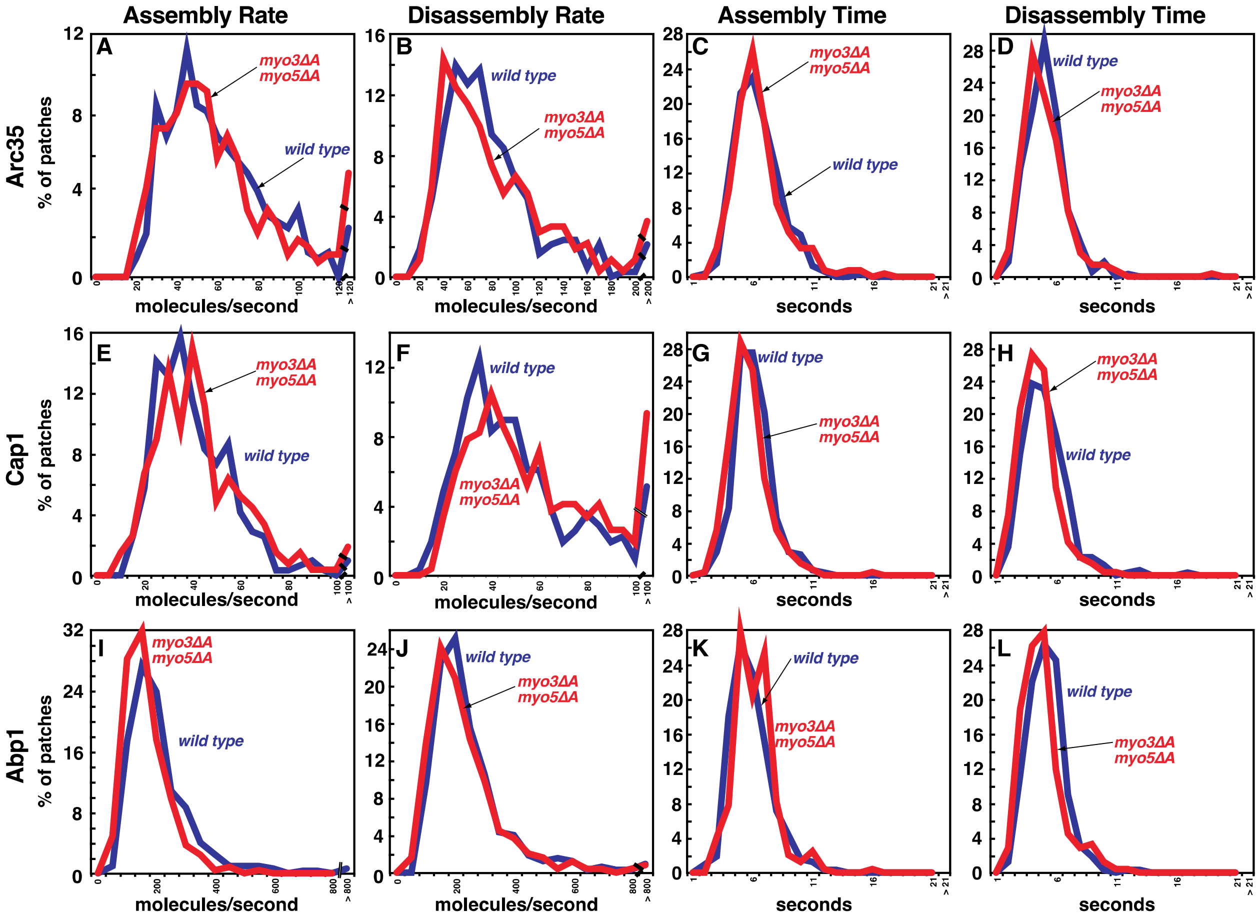

Figure S4. Assembly of Arp2/3 complex as GFP-Arc35 (A-F), Capping protein as GFP-Cap1 (G-L) and F-actin as GFP-Abp1 (M-R) in wild type (blue) and *pan1∆acidic* (red) cells. Data are from 3 segregants of each genotype. Panels as follows: (A, G, M) The number of molecules of the GFP-labeled protein per patch versus time. Plots were generated from data aligned at the maximal (peak) value for each patch and the mode of the distribution at each time point is presented. Error bars are ± standard error of the mode. (B, H, N) Histograms of the peak number of molecules per patch. (C, I, O) Histograms of the rates of assembly. (D, J, P) Histograms of the rates of disassembly. (E, K, Q) Histograms of the duration of the assembly phase. (F, L, R) Histograms of the duration of the disassembly phase. Strain numbers and numbers of patches analyzed were as follows. Wild type data are from Figures 2 and 3. Arc35-GFP: *pan1∆acidic*, YJC7102-3, N = 121, 123. Cap1-GFP: *pan1∆acidic*, YJC7024-6, N = 83, 82, 90. Abp1-GFP: *pan1∆acidic*, YJC6958-60, N = 115, 103, 152. Cse4-GFP: YJC6725-6.

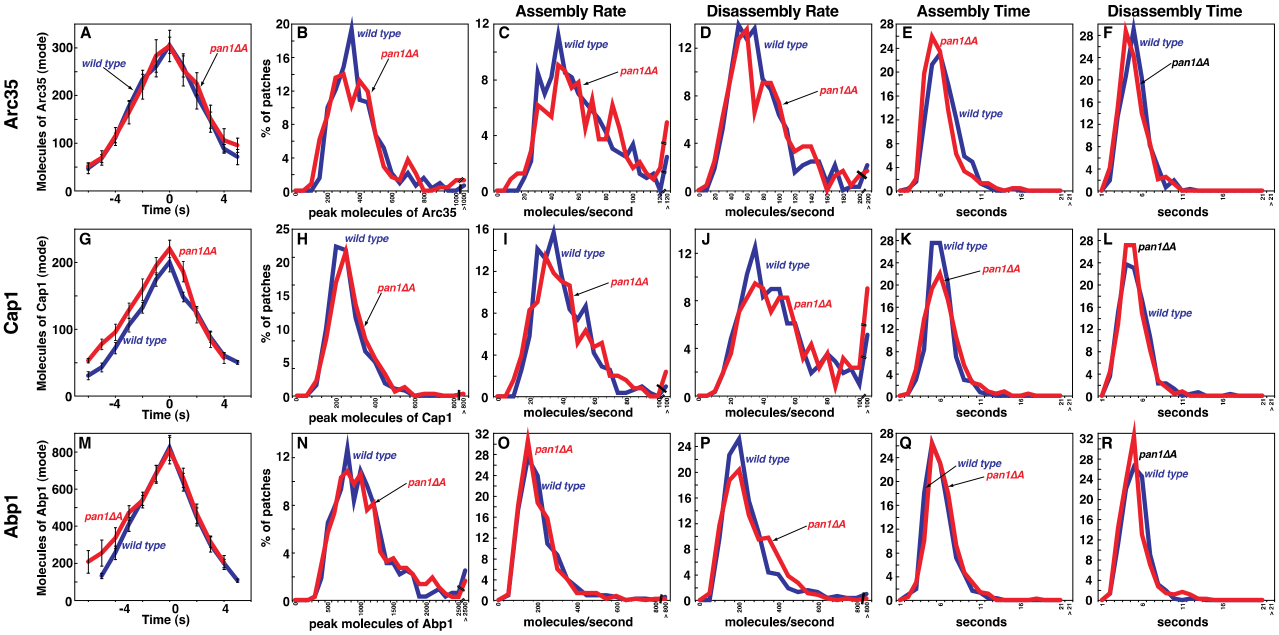

Figure S5. Histograms of the rates and durations of the linear phases of assembly and disassembly of Arc35, Cap1and Abp1 in wild type and *las17∆acidic pan1∆acidic* cells. These plots were generated from the data in Figure 6.

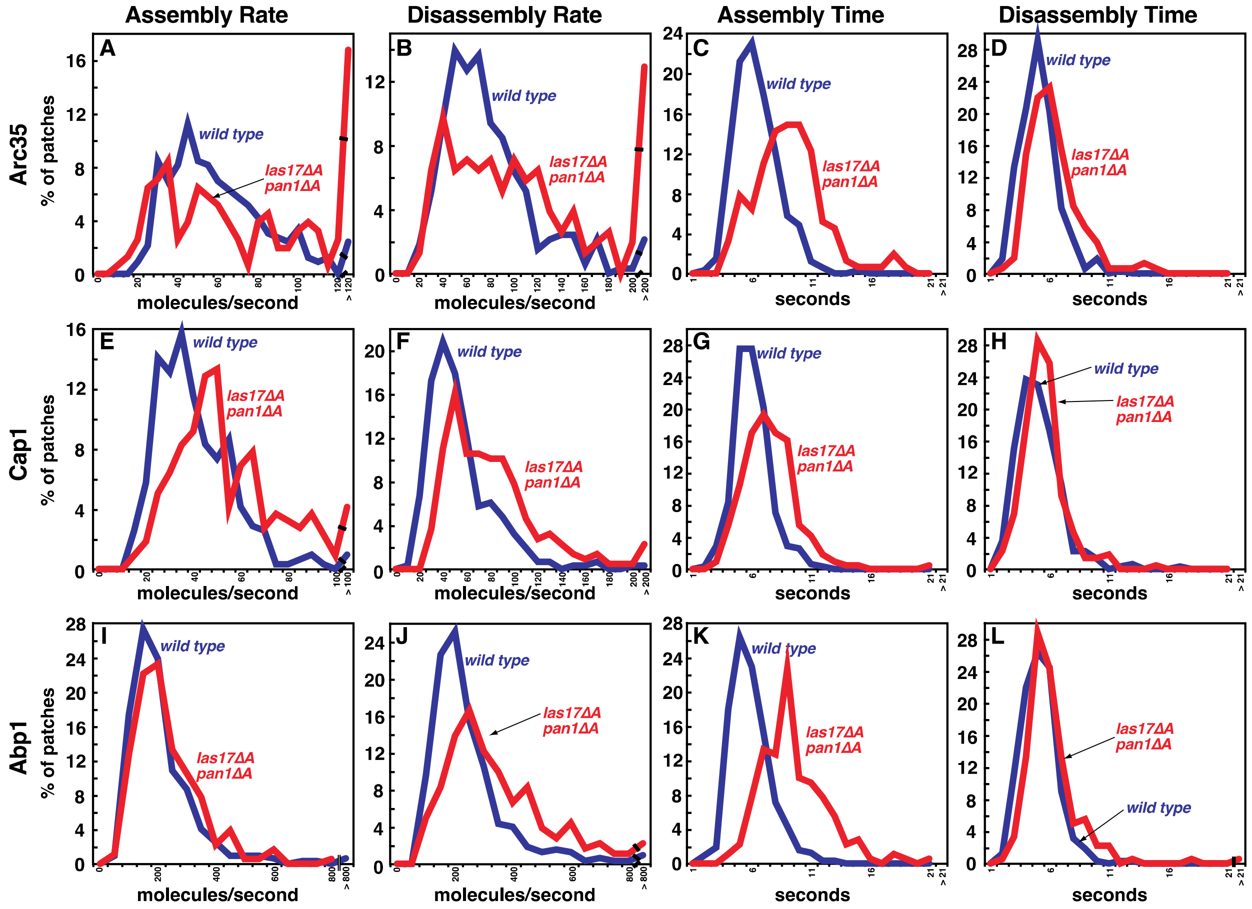

Figure S6. Histograms of the rates and durations of the linear phases of assembly and disassembly of Arc35, Cap1 and Abp1 in wild type and *myo3Δacidic myo5Δacidic pan1Δacidic* cells. These plots were generated from the data in Figure 7.

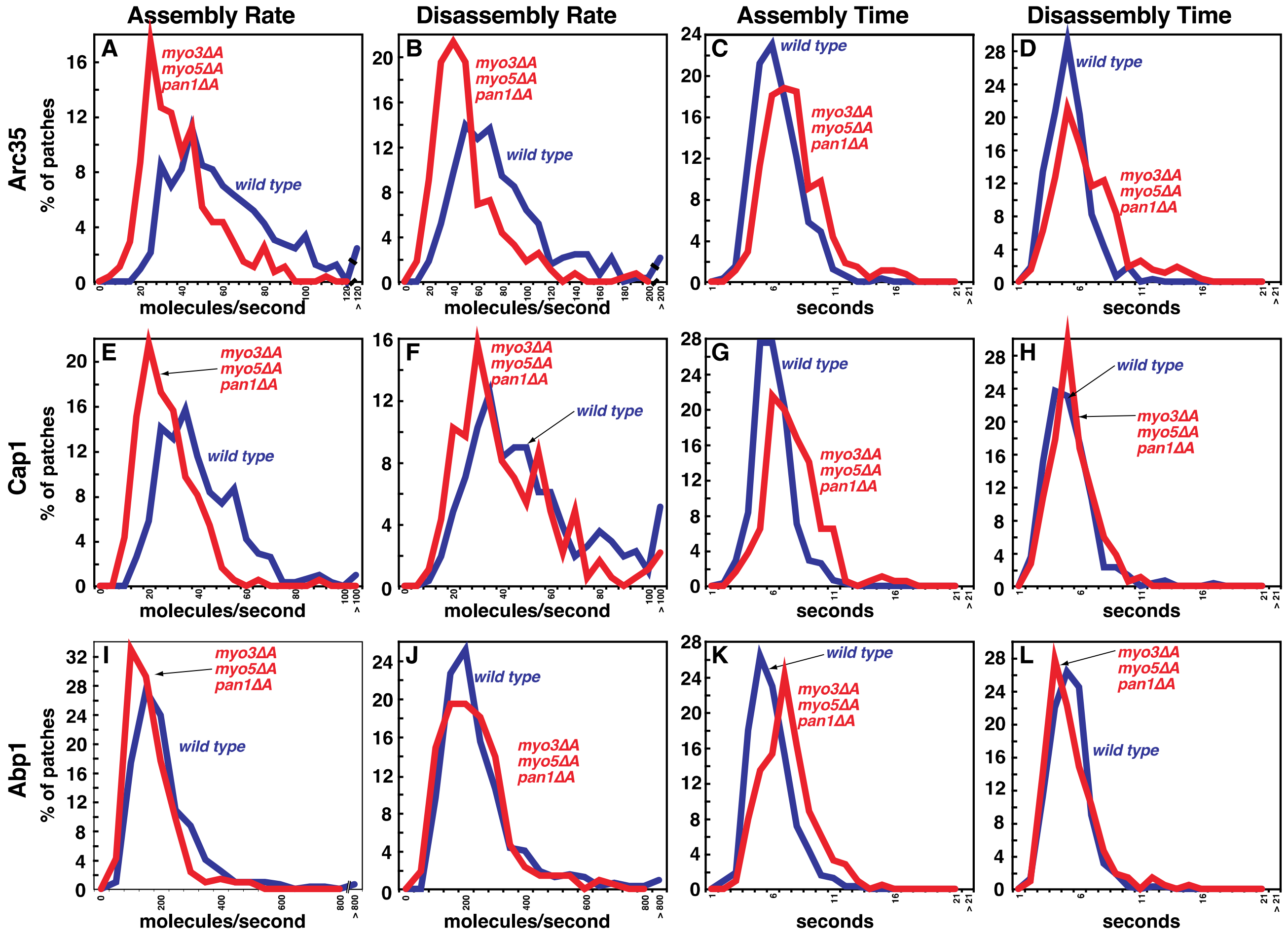

Figure S7. Quantitative analysis of actin patch motility in cells lacking the acidic Arp2/3-binding regions of Myo3, Myo5 and Pan1. Cells express Abp1-GFP. (A) MSD plots for wild-type (blue) and mutant patches (red) aligned at the start (left) or end (right) of their lifetimes. The curves on the left are truncated at the median lifetime. (B) Percentage of patches that leave the origin. Mean of values for three segregants is shown. (C) Average time at the origin, from the appearance of a patch until it moved away or disappeared. (D) Phase III movement only. For each patch, data prior to the time the patch traveled 200 nm were removed. Only patches that moved more than 200 nm from the origin were included. Wild type data are replotted from (23). Strain numbers and numbers of patches: *myo3Δacidic myo5Δacidic pan1Δacidic*, YJC7310-2, N = 68, 59, 109. Error bars are ± one standard error.

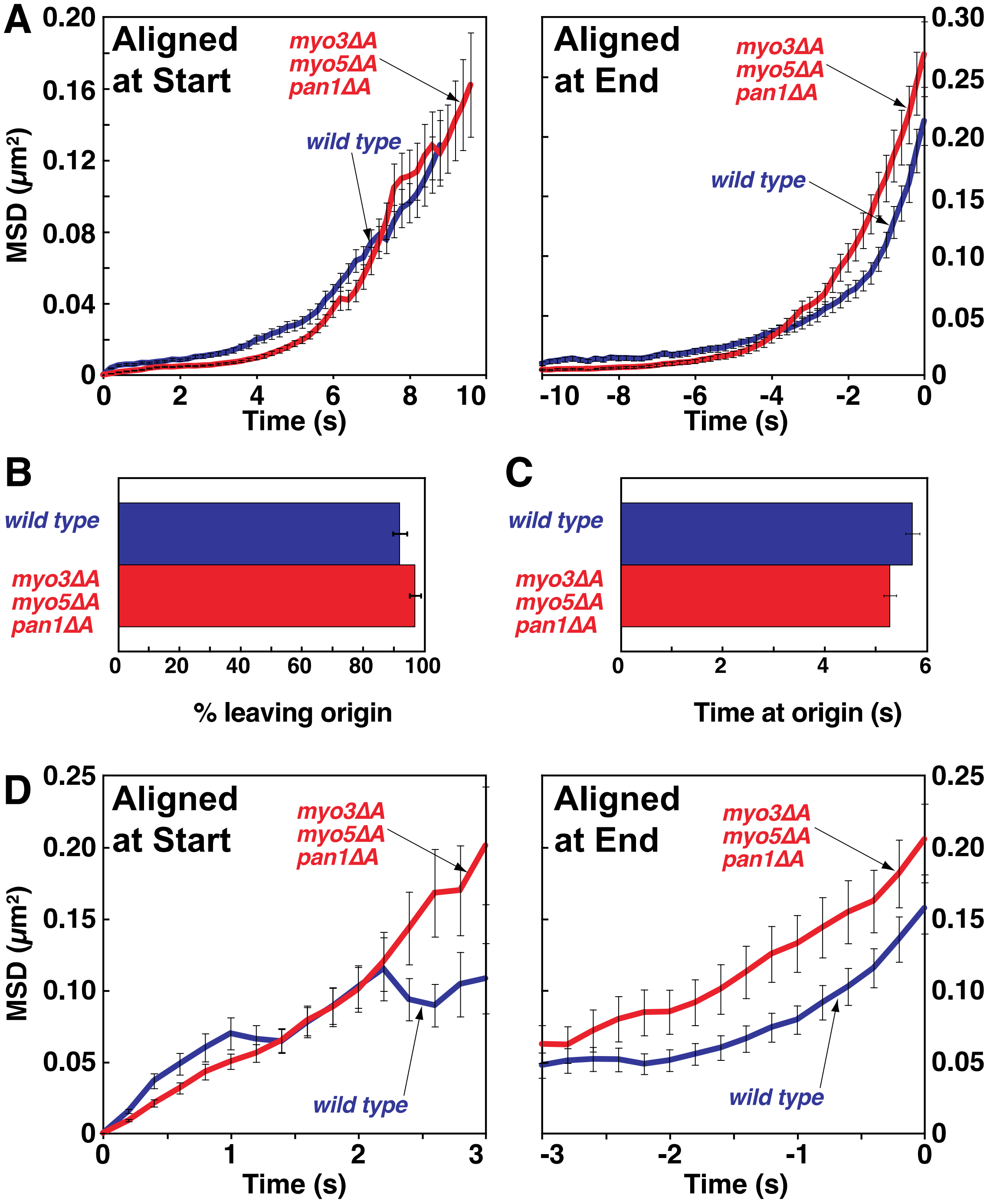

Figure S8. Localization of acidic domain mutants to patches. Representative micrographs of cells expressing GFP fusions of the Arp2/3 regulator mutants used in this study. GFP fusion proteins are expressed from their endogenous locus. (A) Las17∆acidic-GFP (YJC7440) (B) Myo3∆acidic-GFP (YJC7453) (C) Myo5∆acidic-GFP (YJC7458) (D) Pan1∆acidic-GFP (YJC7430). Scale bar = 5 µm.

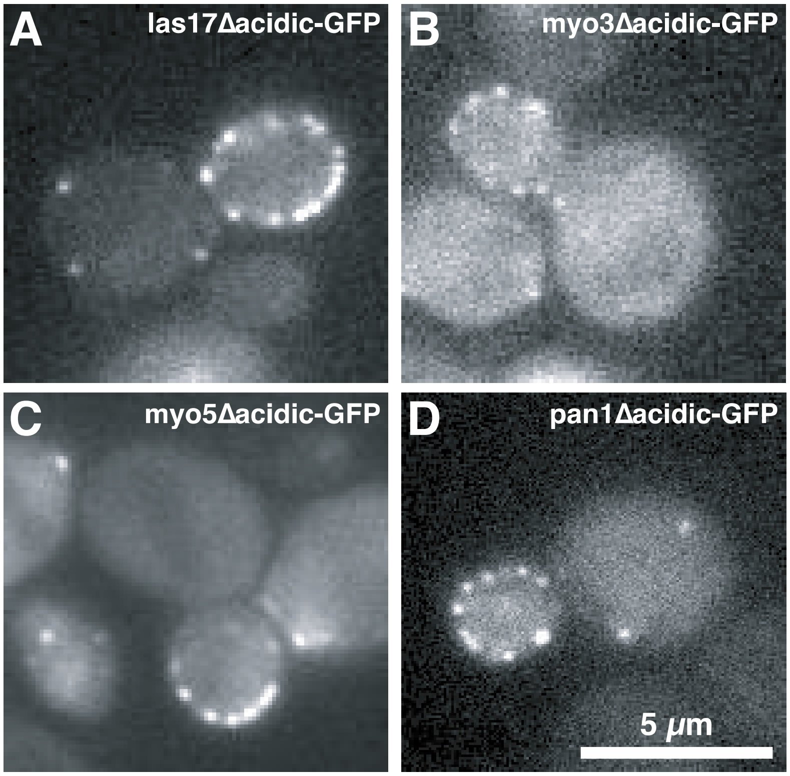

Figure S9. A summary of the changes in actin patch composition and behavior in Arp2/3 regulator mutants reported in this study. (A) Number of Arp2/3 complexes, assayed by Arc35-GFP, vs. time. (B) Number of CP, assayed by Cap1-GFP, vs. time. (C) Amount of F-actin, assayed by the number of Abp1-GFP molecules, vs. time. (A-C) Aligned at peak number of molecules. (D, E) MSD plots of Abp1-GFP movement, aligned at the beginning of patch lifetimes (D) or the end of patch lifetime (E). Error bars have been removed for ease of viewing. Data in panel D and E are reproduced from our previous publication (1).

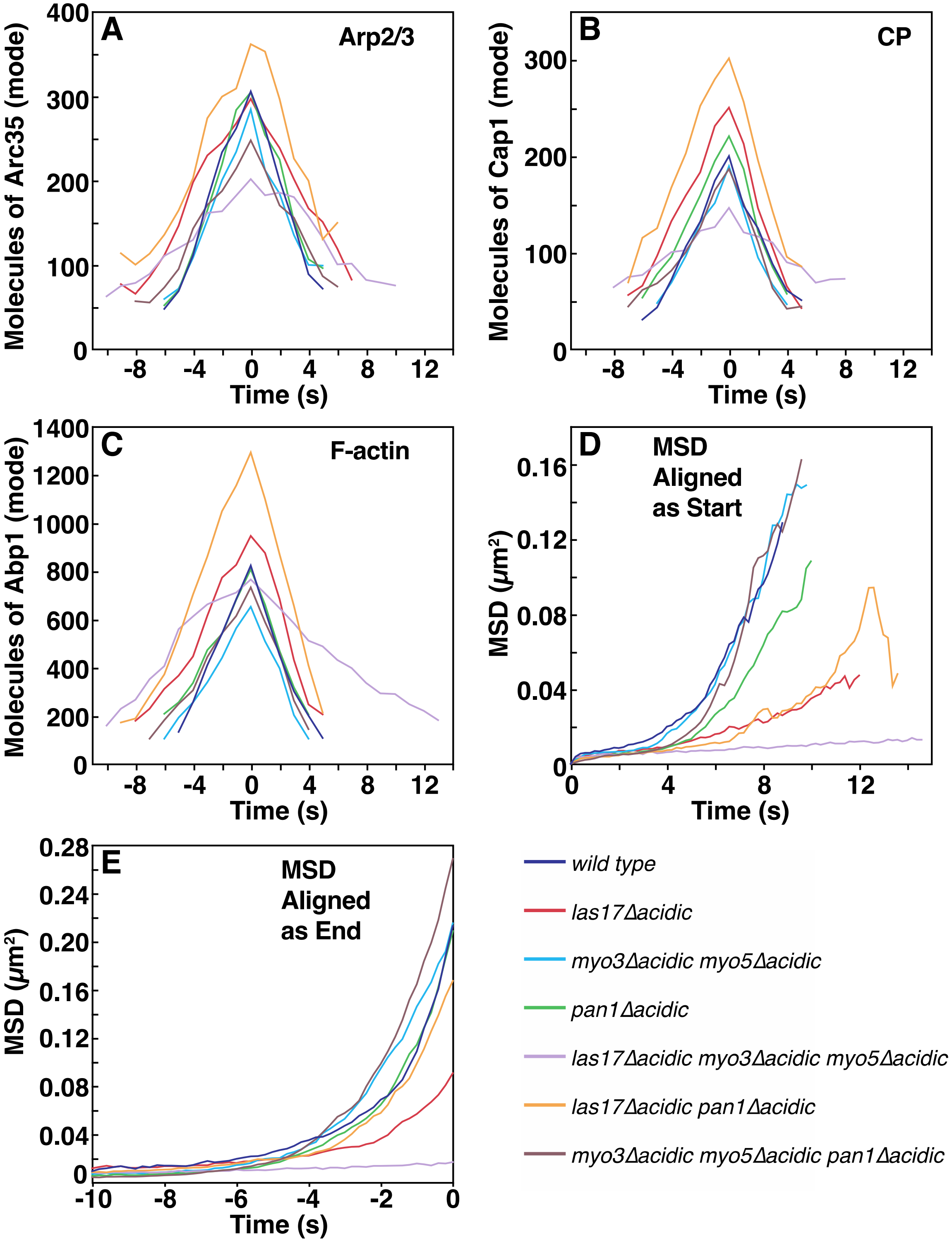

Table S1. Yeast strains used in this study.

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(2)
YJC6494	*MATα/MATa his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0*	This study
YJC6718	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6719	*MATα Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6720	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6721	*MATα Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6722	*MATα Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6723	*MATa Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6725	*MATa Cse4-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6726	*MATα Cse4-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6756	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6757	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6758	*MATα Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6759	*MATa Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6760	*MATa Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6761	*MATα Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6804	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6805	*MATα Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6806	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6807	*MATa Cap1-GFP-SpHIS5 las17Δacidic-CaURA3MX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6808	*MATa Cap1-GFP-SpHIS5 las17Δacidic-CaURA3MX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6809	*MATα Cap1-GFP-SpHIS5 las17Δacidic-CaURA3MX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
YJC6958	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC6959	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC6960	*MATα Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC6961	*MATa Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC6962	*MATa Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC6963	*MATα Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7024	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7025	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7026	*MATα Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7027	*MATa Cap1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7028	*MATa Cap1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7029	*MATα Cap1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7102	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7103	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7104	*MATa Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7105	*MATα Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6*	This study

Strain	Genotype	Source
	ura3Δ0	
YJC7106	*MATα Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7123	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7124	*MATα Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7125	*MATα Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7126	*MATa Cap1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7127	*MATa Cap1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7128	*MATα Cap1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7148	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7149	*MATα Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7150	*MATα Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7151	*MATα Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7152	*MATα Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7153	*MATα Abp1-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7167	*MATa Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7168	*MATa Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7169	*MATα Arc35-GFP-SpHIS5 his3Δ1 las17Δacidic-CaURA3MX4 leu2Δ0 met15Δ0 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7173	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7174	*MATα Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7175	*MATα Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-kanMX6 ura3Δ0*	This study
YJC7310	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7311	*MATa Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7312	*MATα Abp1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7344	*MATα Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7345	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7346	*MATa Cap1-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7348	*MATα Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7349	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4 pan1Δacidic-kanMX6 ura3Δ0*	This study
YJC7350	*MATa Arc35-GFP-SpHIS5 his3Δ1 leu2Δ0 met15Δ0 myo3Δacidic-hphMX4 myo5Δacidic-natMX4*	This study

Strain	Genotype	Source
	pan1Δacidic-kanMX6 ura3Δ0	

Table S2. Peak number of molecules. The mode ± standard error of the mode, median, mean and 90% confidence interval (C.I.) are listed. N is the number of actin patches analyzed.

Genotype	Arc35					Cap1					Abp1				
	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N
wild type	305 ± 17	330	360	346-374	331	200 ± 14	215	233	224-242	313	822 ± 67	933	1026	980-1073	323
las17Δa	296 ± 13	360	458	430-486	247	250 ± 18	289	314	302-326	310	945 ± 66	1121	1287	1225-1349	305
myo3Δa myo5Δa	284 ± 17	336	366	349-383	273	190 ± 11	211	229	220-238	268	650 ± 39	740	844	800-887	245
pan1Δa	303 ± 33	334	363	342-382	244	220 ± 13	232	249	238-260	255	807 ± 71	926	1034	992-1077	370
las17Δa pan1Δa	361 ± 53	560	678	622-735	155	301 ± 38	366	401	382-421	218	1292 ± 113	1574	1774	1658-1890	181
myo3Δa myo5Δa pan1Δa	247 ± 8	257	287	275-300	277	187 ± 10	190	198	189-206	186	731 ± 80	838	933	881-986	216
las17Δa myo3Δa myo5Δa [a]	201 ± 19	233	259	244-274	157	146 ± 12	190	219	208-230	267	765 ± 61	1040	1195	1113-1278	184

[a] For Arc35 measurements, *las17Δacidic myo5Δacidic* cells were used.

Table S3. Ratios of peak protein levels, based on mode of numbers of molecules.

Genotype	Cap1/Arc35	Abp1/Arc35	Abp1/CP
wild type	0.66	2.7	4.1
las17Δacidic	0.84	3.2	3.8
myo3Δacidic myo5Δacidic	0.67	2.3	3.4
pan1Δacidic	0.73	2.7	3.7
las17Δacidic pan1Δacidic	0.83	3.6	4.3
myo3Δacidic myo5Δacidic pan1Δacidic	0.75	3.0	3.9
las17Δacidic myo3Δacidic myo5Δacidic [a]	0.72	3.8	5.2

[a] For Arc35 measurements, *las17Δacidic myo5Δacidic* cells were used.

Table S4. Rate of Assembly (molecules/second). The mode and one standard error of the mode are listed. N is the number of actin patches analyzed.

Genotype	Arc35					Cap1					Abp1				
	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N
wild type	44.0 ± 3.2	51.6	57.6	55.2-59.9	331	31.0 ± 1.9	34.7	38.4	36.8-39.9	313	129 ± 14	160	188	176-199	323
las17Δa	36.6 ± 2.5	41.9	48.3	45.7-50.9	247	33.8 ± 2.0	37.7	42.3	40.3-44.4	310	114 ± 14	146	166	157-175	305
myo3Δa myo5Δa	43.9 ± 3.7	50.7	57.6	54.7-60.1	273	33.9 ± 2.7	37.2	40.8	38.7-42.8	268	101 ± 6	123	142	134-150	245
pan1Δa	48.9 ± 2.9	56.2	62.0	58.6-65.3	244	32.4 ± 2.2	35.5	40.0	37.8-42.1	255	130 ± 5	151	178	169-187	370
las17Δa pan1Δa	37.5 ± 6.5	59.5	74.7	68.1-81.2	155	43.2 ± 2.4	47.2	52.6	50.0-55.2	218	158 ± 18	182	206	192-220	181
myo3Δa myo5Δa pan1Δa	25.8 ± 2.9	33.4	36.5	34.8-38.2	277	19.0 ± 2.0	22.6	24.5	23.1-25.8	186	96 ± 8	117	137	128-146	216
las17Δa myo3Δa myo5Δa [a]	18.1 ± 1.7	20.8	23.8	22.1-25.4	157	17.3 ± 1.6	22.0	24.4	23.2-25.6	267	85 ± 18	113	118	111-124	184

[a] For Arc35 measurements, *las17Δacidic myo5Δacidic* cells were used.

Table S5. Rate of Disassembly (molecules/second). The mode and one standard error of the mode are listed. N is the number of actin patches analyzed.

Genotype	Arc35					Cap1					Abp1				
	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N
wild type	56.9 ± 4.0	63.6	74.2	70.4-78.0	331	34.3 ± 3.2	42.3	49.9	47.0-52.7	313	152 ± 9	182	219	207-231	323
las17Δa	37.7 ± 3.4	51.5	59.1	55.7-62.5	247	44.7 ± 5.0	54.5	60.9	57.7-64.2	310	179 ± 14	220	254	239-268	305
myo3Δa *myo5Δa*	44.5 ± 4.3	65.7	79.4	74.4-84.5	273	37.3 ± 2.8	48.3	57.1	53.8-60.4	268	147 ± 14	171	204	191-219	245
pan1Δa	53.8 ± 3.8	64.0	77.2	72.4-82.0	244	38.4 ± 4.8	46.9	54.5	51.1-57.9	255	158 ± 17	196	226	216-237	370
las17Δa *pan1Δa*	60.1 ± 13.2	90.2	112	100-124	155	51.5 ± 6.8	67.9	76.6	71.9-81.3	218	222 ± 22	273	324	300-349	181
myo3Δa *myo5Δa* *pan1Δa*	32.9 ± 3.6	39.0	45.6	42.8-48.4	277	28.4 ± 2.2	33.6	39.0	36.5-41.5	186	166 ± 23	187	202	190-215	216
las17Δa *myo3Δa* *myo5Δa*[a]	20.7 ± 1.1	21.4	22.7	21.3-24.1	157	16.4 ± 2.3	21.1	26.0	24.1-27.9	267	69 ± 6	78	99	90-108	184

[a] For Arc35 measurements, *las17Δacidic myo5Δacidic* cells were used.

Table S6. Time of Assembly (seconds). The mode and one standard error of the mode are listed. N is the number of actin patches analyzed.

Genotype	Arc35					Cap1					Abp1				
	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N
wild type	5.8 ± 0.3	6	6.4	6.2-6.6	331	5.6 ± 0.3	6	6.0	5.9-6.2	313	5.2 ± 0.3	6	5.9	5.7-6.0	323
las17Δa	7.4 ± 0.3	9	9.1	8.8-9.4	247	7.2 ± 0.3	7	7.5	7.2-7.7	310	7.4 ± 0.3	8	8.1	7.9-8.3	305
myo3Δa *myo5Δa*	5.9 ± 0.3	6	6.5	6.3-6.8	273	5.3 ± 0.2	5	5.6	5.5-5.8	268	5.7 ± 0.5	6	6.2	6.0-6.3	245
pan1Δa	5.2 ± 0.3	6	6.0	5.8-6.2	244	5.9 ± 0.3	6	6.3	6.1-6.5	255	5.3 ± 0.3	6	6.2	6.1-6.4	370
las17Δa *pan1Δa*	9.1 ± 0.5	9	9.1	8.8-9.5	155	7.4 ± 0.5	7	7.5	7.3-7.8	218	8.9 ± 0.2	9	9.5	9.1-9.9	181
myo3Δa *myo5Δa* *pan1Δa*	7.1 ± 0.5	7	7.8	7.5-8.0	277	6.7 ± 0.4	7	7.6	7.4-7.9	186	7.0 ± 0.2	7	7.2	6.9-7.4	216
las17Δa *myo3Δa* *myo5Δa*[a]	7.8 ± 0.6	9	10.1	9.6-10.6	157	6.3 ± 0.5	7	7.6	7.3-7.9	267	9.1 ± 0.6	9	9.5	9.1-10.0	184

[a] For Arc35 measurements, *las17Δacidic myo5Δacidic* cells were used.

Table S7. Time of Disassembly (seconds). The mode and one standard error of the mode are listed. N is the number of actin patches analyzed.

Genotype	Arc35		Arc35			Cap1					Abp1				
	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N	Mode ± SEM	Median	Mean	90% C.I.	N
wild type	5.0 ± 0.1	5	5.1	5.0-5.2	331	4.5 ± 0.3	5	5.1	4.9-5.3	313	5.2 ± 0.4	6	5.2	5.0-5.3	323
las17Δa	6.2 ± 0.2	7	7.5	7.2-7.7	247	5.2 ± 0.2	5	5.6	5.4-5.8	310	5.0 ± 0.2	5	5.7	5.5-5.9	305
myo3Δa *myo5Δa*	4.2 ± 0.3	5	5.0	4.8-5.1	273	4.3 ± 0.3	4	4.5	4.3-4.6	268	4.6 ± 0.3	5	4.8	4.6-5.0	245
pan1Δa	4.2 ± 0.2	5	4.8	4.7-5.0	244	4.5 ± 0.3	5	4.9	4.7-5.0	255	4.9 ± 0.1	5	5.1	5.0-5.3	370
las17Δa *pan1Δa*	5.6 ± 0.3	6	6.3	6.0-6.6	155	5.3 ± 0.3	5	5.5	5.3-5.7	218	5.3 ± 0.3	6	6.2	5.8-6.4	181
myo3Δa *myo5Δa* *pan1Δa*	5.2 ± 0.3	6	6.5	6.3-6.8	277	5.0 ± 0.1	5	5.3	5.1-5.5	186	4.2 ± 0.2	5	5.2	5.0-5.5	216
las17Δa *myo3Δa* *myo5Δa*[a]	9.1 ± 0.6	10	10.2	9.8-10.6	157	7.5 ± 0.3	8	7.8	7.5-8.1	267	10.0 ± 0.7	11	11.9	11.3-12.4	184

[a] For Arc35 measurements, *las17Δacidic myo5Δacidic* cells were used.

Supporting References

1. Galletta, B.J., D.Y. Chuang, and J.A. Cooper. 2008. Distinct Roles for Arp2/3 Regulators in Actin Assembly and Endocytosis. PLoS Biol. 6:e1.

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