Specialization of actin isoforms derived from the loss of key interactions with regulatory factors

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Appendix Figures legends

Appendix Figure S1, related to Figure 1. Selection of actins strategy.

A Complete phylogenetic tree that was used as input for FastML ancestral reconstruction analysis (Ashkenazy *et al*, 2012).

B Posterior probability for the ancestral sequences used in this study, showing high confidence in the predicted sequences.

C (Top) Multiple sequence alignment for all actin sequences used in this study. (Bottom) Schematic representations of actin 3D structure (1YAG, (Vorobiev *et al*, 2003)), with position of amino acid differences shown with colored dots for each actin.

D Schematic representation of mutagenesis strategy by homologous recombination used in this study (see also Methods).

Appendix Figure S2, related to Figure 2. Effect of removing *S. cerevisiae*'s Act1 intron and of silent mutations in the actin gene.

In this figure, the shape of the dots allows to identify the strains on the different graphs (circles for Sc, pentagons for ScI, squares for ScNI, triangles for Sc[Ca], inversed triangles for Sc[Sp] and diamonds for Sc[At]). The color of the dots indicates the percentage of identity of the nucleotide sequences to the actin gene of S. cerevisiae, ranging from 100% (blue) to 76% (orange).

A Growth phenotypes, evaluated by 3-fold serial dilutions of different yeast strains cultures grown at 25ºC for 2 days on a YPD plate. Abbreviations: Sc - wild-type *S. cerevisiae* cells, ScI - *S. cerevisiae* cells where the actin gene has been replaced with the full construct carrying the wild-type gene, ScNI - *S. cerevisiae* cells where the actin gene has been replaced with the wild-type gene but without the intron.

B Quantification of (A) by measurement of colony area. Data are presented as mean +/- SD (n = 31 for Sc, n = 32 for ScI, and n = 35 for ScNI). (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

C Actin expression levels shown by western blotting, with tubulin (Tub1p) as a loading control.

D Quantification of actin expression levels. Data are presented as mean +/- SD (n = 2 for all conditions, biological replicates). (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

E Phalloidin stain depicting F-actin organization. Images are maximum intensity projections of 3D stacks. Micrographs of Sc and ScNI cells are reproduced from Figure 2E. Scale bar: 3 µm.

F *In vivo* actin network deviation indexes, defined to evaluate the patch-cable balance compared to *S. cerevisiae* haploid cells (value is 0 in *S. cerevisiae*'s cells, 1 when cells contain only actin patches and -1 when cells contain only cables). Data are presented as mean +/- SD (n = 10 for all conditions). (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

G Polarity indexes. Data are presented as mean +/- SD (n = 10 for all conditions). (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

H Multiple sequence alignment of the beginning of the nucleotide sequence (top) and the beginning of the amino acid sequence (bottom), as an example of how we used coding sequences from other organisms that we modified minimally so that the final product remained *S. cerevisiae* actin.

I Growth phenotypes, evaluated by 3-fold serial dilutions of different yeast strains cultures grown at 25ºC for 2 days on a YPD plate, showing the effect of silent mutations on the actin gene.

J Colony area as a function of nucleotide identity, showing a threshold of nucleotide conservation (78%<id<82%) below which growth rates drastically reduce. Data are presented as mean +/- SD (n = 17 for Sc, n = 11 for ScNI, n = 27 for Sc[Ca], n = 23 for Sc[Sp], n = 48 for Sc[At]). ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

K Organization of the cytoskeleton, assessed by quantification of total patch and cable intensities of phalloidin-stained cells. Data are presented as mean +/- SD (n = 30 for all conditions). **P<0.01, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

L Organization of the cytoskeleton, assessed by quantification of the number of visible patch and cables of phalloidin-stained cells. Data are presented as mean +/- SD (n = 30 for all conditions). *P<0.05, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

Appendix Figure S3, related to Figure 3. Effects of swapping actin for different variants.

In this figure, the shape of the dots allows to identify the strains on the different graphs (closed circles for Sc, closed squares for ScNI, closed triangles for N1, inversed closed triangles for Kl, closed diamonds for N2, closed hexagons for Op, stars for Ca, half-open inversed triangles for Hs). The color of the dots indicates the percentage of identity of the amino acid sequences to S. cerevisiae's actin, ranging from 100% (green) to 84% (magenta).

A Characterization of the C4 antibody: The binding site of the C4 antibody, indicated as "C4_Epitope", is found on Act_Hs and on rabbit muscle actin. In all other actin variants used in this study, the sequence varies of one amino acid (called here "Mutated_Epitope") but is recognized by C4 antibody.

B Western blot with equivalent amounts of purified yeast actin and rabbit actin. The amount of protein was revealed by two methods: Ponceau staining and chemiluminescence. The chemiluminescence signal corresponds to the one produced by the secondary antibody after incubation with a primary antibody anti-actin C4 and a secondary antibody conjugated with HRP.

C C4 actin antibody has a higher affinity for rabbit muscle actin than for *S. cerevisiae* actin: Quantification of (B) indicates that immunolabeling of rabbit muscle actin with C4 antibody leads to a 1.48-fold more intense signal than immunolabeling of *S. cerevisiae* actin. Data are presented as mean +/- SD (n = 12 for both conditions). **P<0.01 (Unpaired t test with Welch's correction).

D Growth phenotypes, evaluated by 3-fold serial dilutions of different yeast strains cultures grown at 25ºC for 2 days on a YPD plate, showing the effect of swapping actin for different variants.

E Quantification of (D) by measurement of colony area. Data are presented as mean +/- SD (n = 23 for Sc, n = 18 for ScNI, n = 20 for N1, n = 23 for Kl, n = 45 for N2, n = 45 for Op, n = 31 for Ca, n = 51 for Nc, n = 31 for Yl, n = 28 for Hs) **P<0.01, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

F Organization of the cytoskeleton, assessed by quantification of total patch and cable intensities of phalloidin-stained cells. Please note that these results, provided for information, should be interpreted with caution as all actin variants except Act_N1 have different phalloidin binding sites from Act_Sc; this quantification is absent for Act_Nc and Act_Yl which cannot be phalloidin-stained. Data are presented as mean +/- SD (n = 30 for all conditions). *P<0.05, **P<0.01, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

G Organization of the cytoskeleton, assessed by quantification of the number of visible patch and cables of phalloidin-stained cells. Data are presented as mean +/- SD (n = 30 for all conditions). *P<0.05, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

H Phalloidin-binding sites of the actin variants expressed homozygously.

I Effect of CK-666 (DMSO control, 150 µM and 300 µM) on the organization of the actin cytoskeleton. Cells were stained with phalloidin after 30 min incubation with CK-666. Images are maximum intensity projections of 3D stacks.

J Quantification of actin patch resistance to CK-666 treatment. Bar graphs represent the percentage of cells with a given number of visible actin patches after CK-666 treatment. (DMSO, n = 27 for ScNI, n = 96 for N2, n = 47 for Ca, n = 24 for Op, n = 47 for Hs) (150 µM, n = 53 for ScNI, n = 45 for N2, n

= 37 for Ca, n = 49 for Op, n = 33 for Hs) (300 µM, n = 61 for ScNI, n = 42 for N2, n = 43 for Ca, n = 47 for Op, n = 49 for Hs).

K Western-blot control of similar Arp2 expression in cells expressing wild-type or Act_N2 actins.

L Snapshots of cells expressing Arc15-GFP and wild-type or Act_N2 actins.

M Quantification of the Arc15-GFP intensity in the patches normalized by the mean actin intensity of the patches for cells expressing Act_Sc and Act_N2. Data are presented as mean +/- SD (left n = 30 for all conditions, right, n = 114 for Sc and n = 176 for N2). ***P<0.001 (Unpaired t test with Welch's correction and Kolmogorov-Smirnov test).

Appendix Figure S4, related to Figure 4.

A Single actin filaments assembled from 3 µM of purified Act_Sc, Act_N2 or Act_Ca G-actins, in the presence of 1% of Alexa-568 labeled rabbit muscle actin, and stabilized with phalloidin (unlabeled).

B Quantification of the fluorescence intensity along the actin filaments, showing similar degrees of integration of the fluorescent actin monomers. Data are presented as mean +/- SD (n = 20 for Act_Sc, n = 28 for Act_N2 and n = 18 for Act_Ca). (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

Appendix Figure S5, related to Figure 6. Effect of a dual expression of actins.

A Growth phenotypes, evaluated by 3-fold serial dilutions of different yeast strains cultures grown at 25ºC for 2 days on a YPD plate, showing the effect of swapping actin for different variants.

B Quantification of (A) by measurement of colony area. Data are presented as mean +/- SD (n = 21 for Sc/Sc, n = 50 for N2/N2, n = 27 for Ca/Ca, n = 21 for N2/Ca, n = 22 for Ca/N2). ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

C Quantification of total patch and cable intensities of phalloidin-stained cells. Data are presented as mean +/- SD (n = 30 for all conditions). *P<0.05, **P<0.01, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

D Quantification of number of visible patch and cables of phalloidin-stained cells. Data are presented as mean +/- SD (n = 30 for all conditions). *P<0.05, **P<0.01, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

E *In vivo* actin network deviation indexes of cells treated with DMSO or 35 µM, 75 µM or 150 µM CK-666. Data are presented as mean +/- SD (n = 10 for all conditions). *P<0.05, **P<0.01, ***P<0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

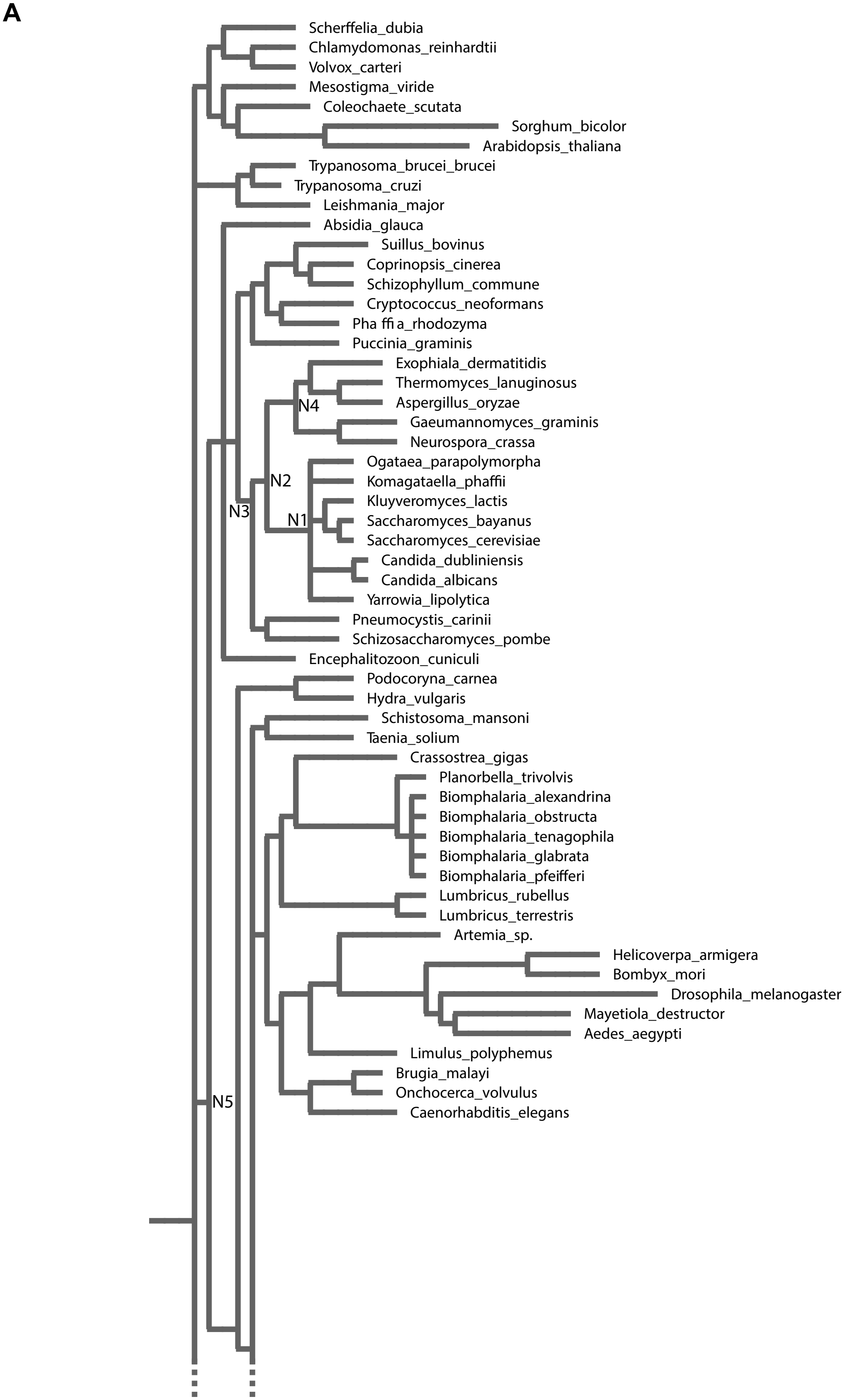
Appendix Tables legends

Appendix Table S1. Complete list of all actins used for the ancestral sequence reconstruction using FastML.

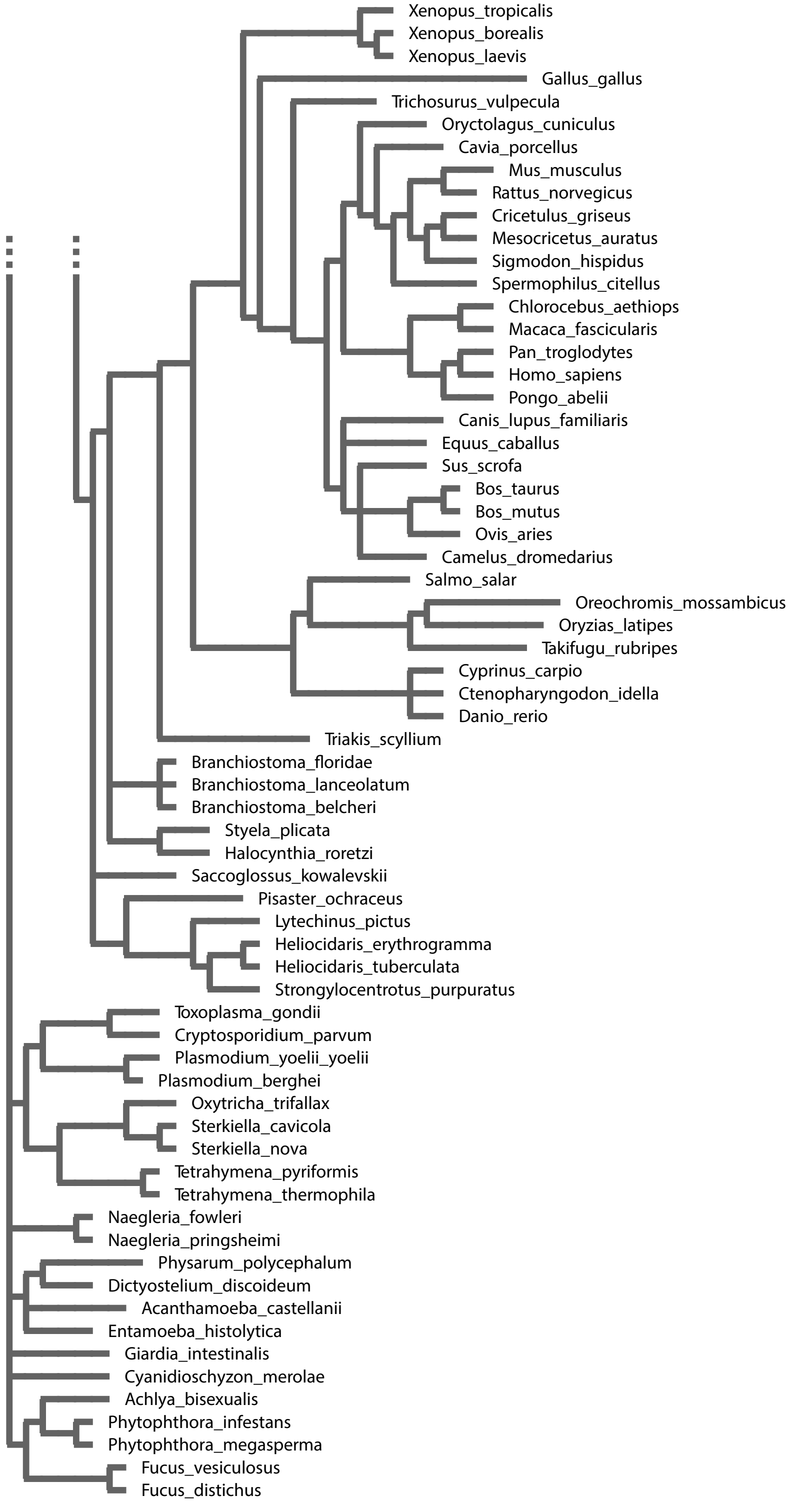
Appendix Table S2. List of plasmids used in this study. All plasmids were done in a pGEX-4T1 backbone.

Appendix Table S3. List of yeast strains in this study.

Appendix Figure S1



Appendix Figure S1



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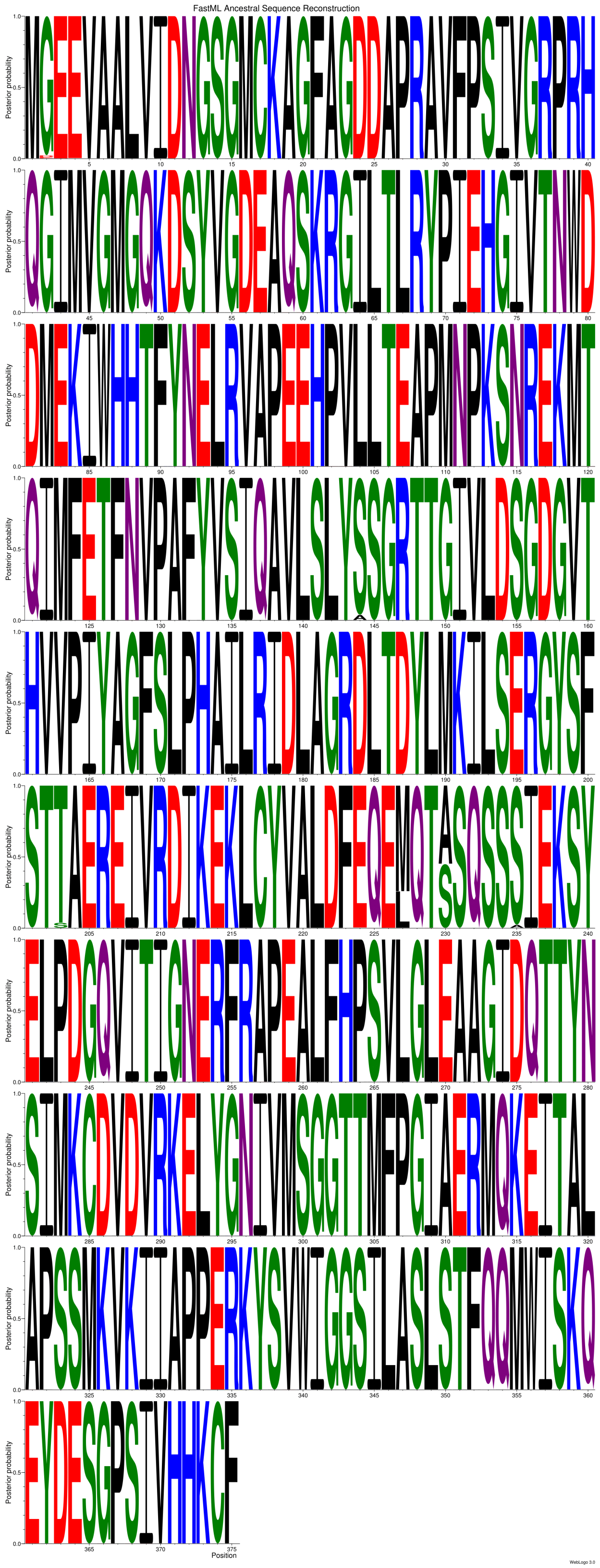
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Appendix Figure S1

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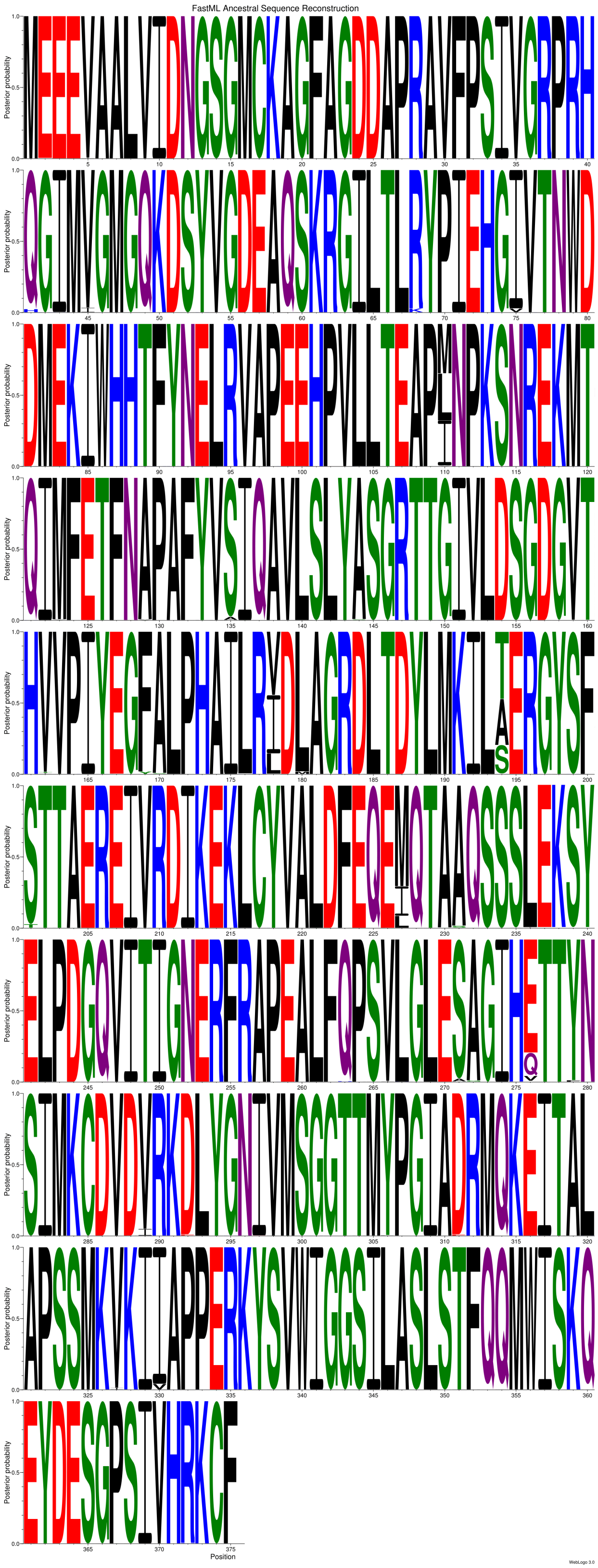

Appendix Figure S1

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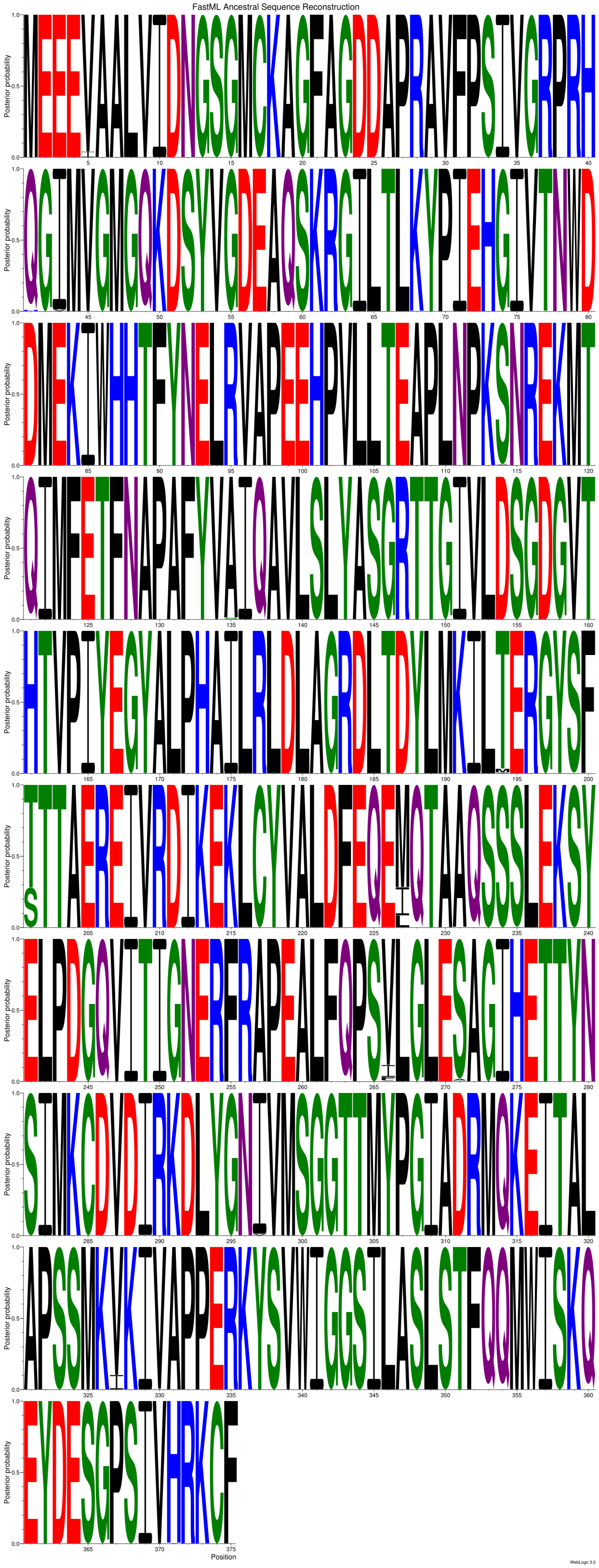

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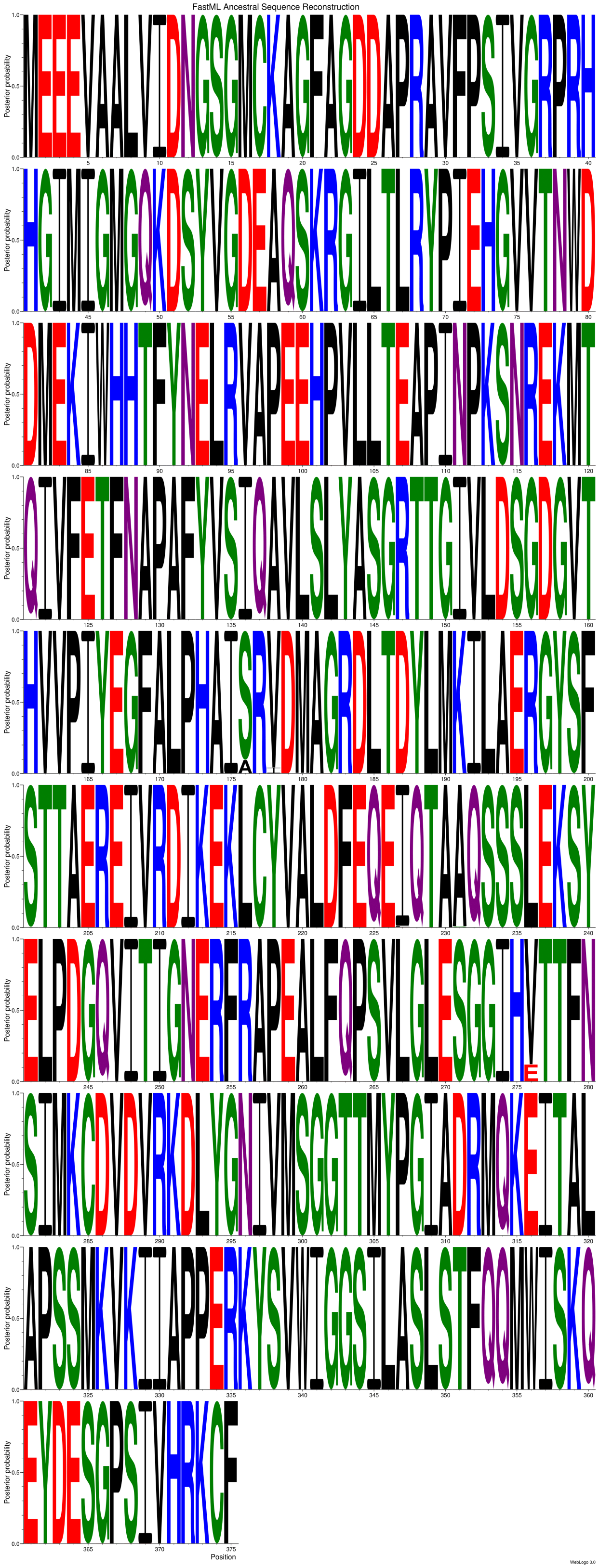

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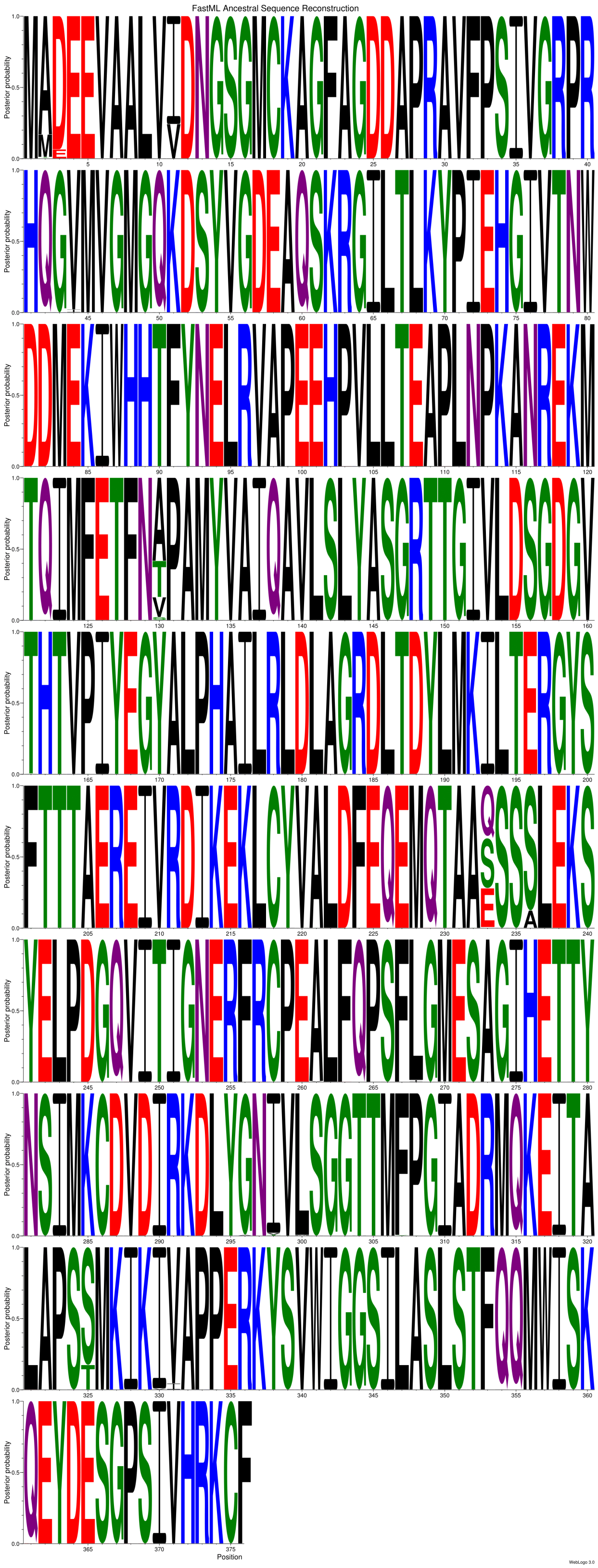
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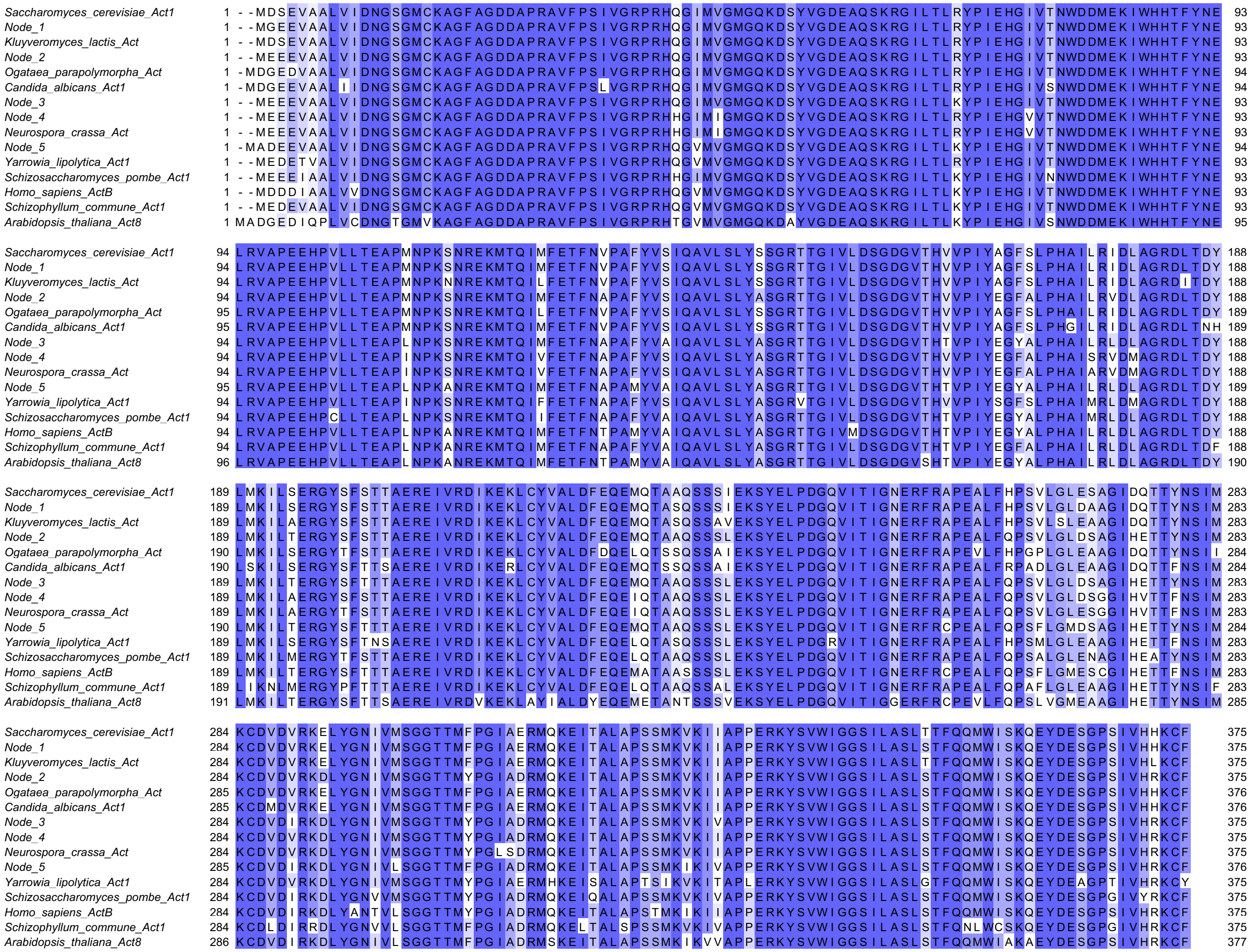
Posterior probability
Position
WebLogo 3.0

C

Appendix Figure S1



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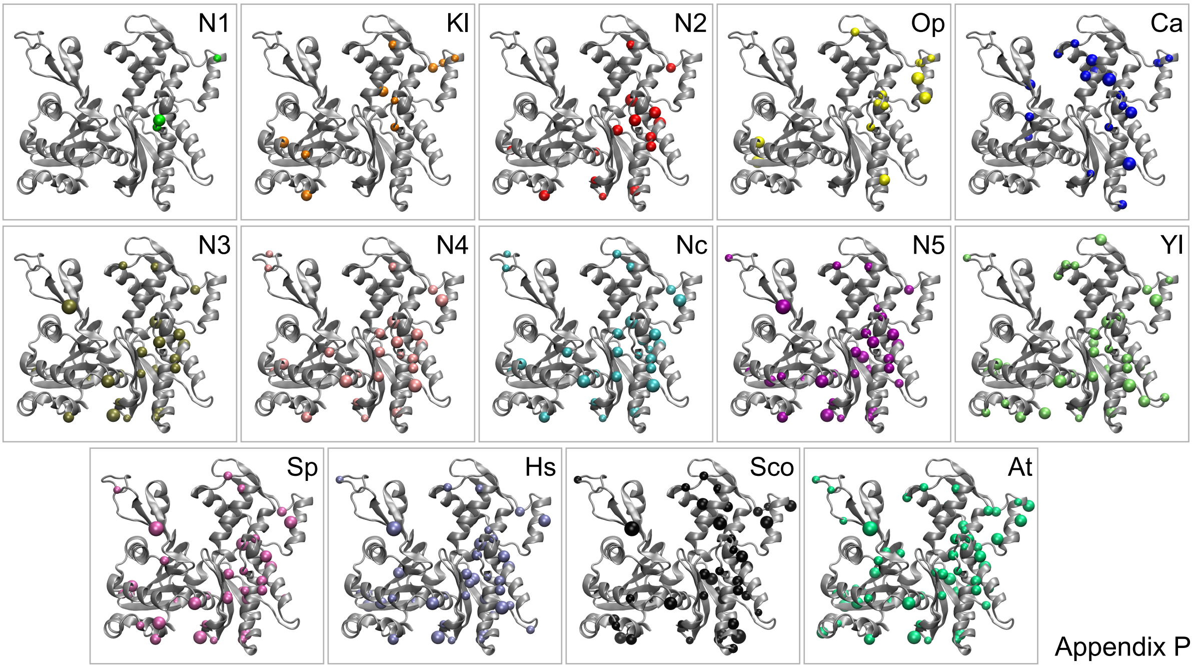

Appendix Figure S1

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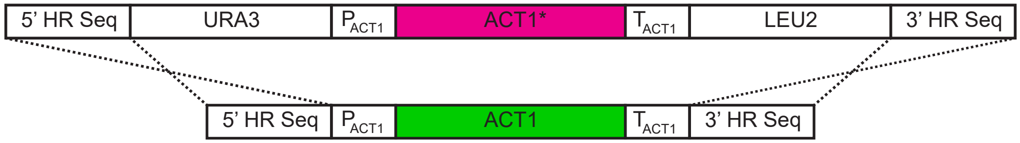
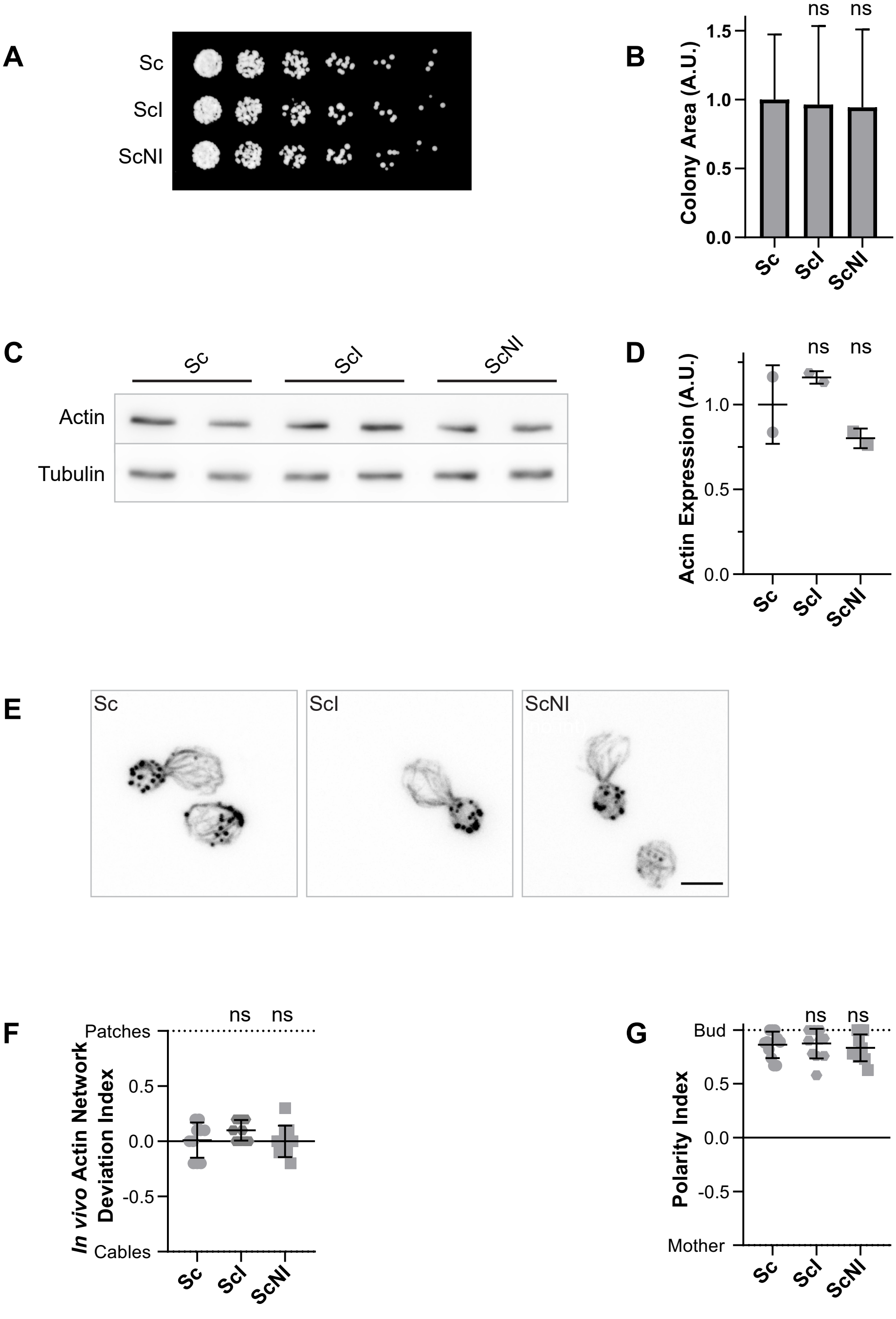
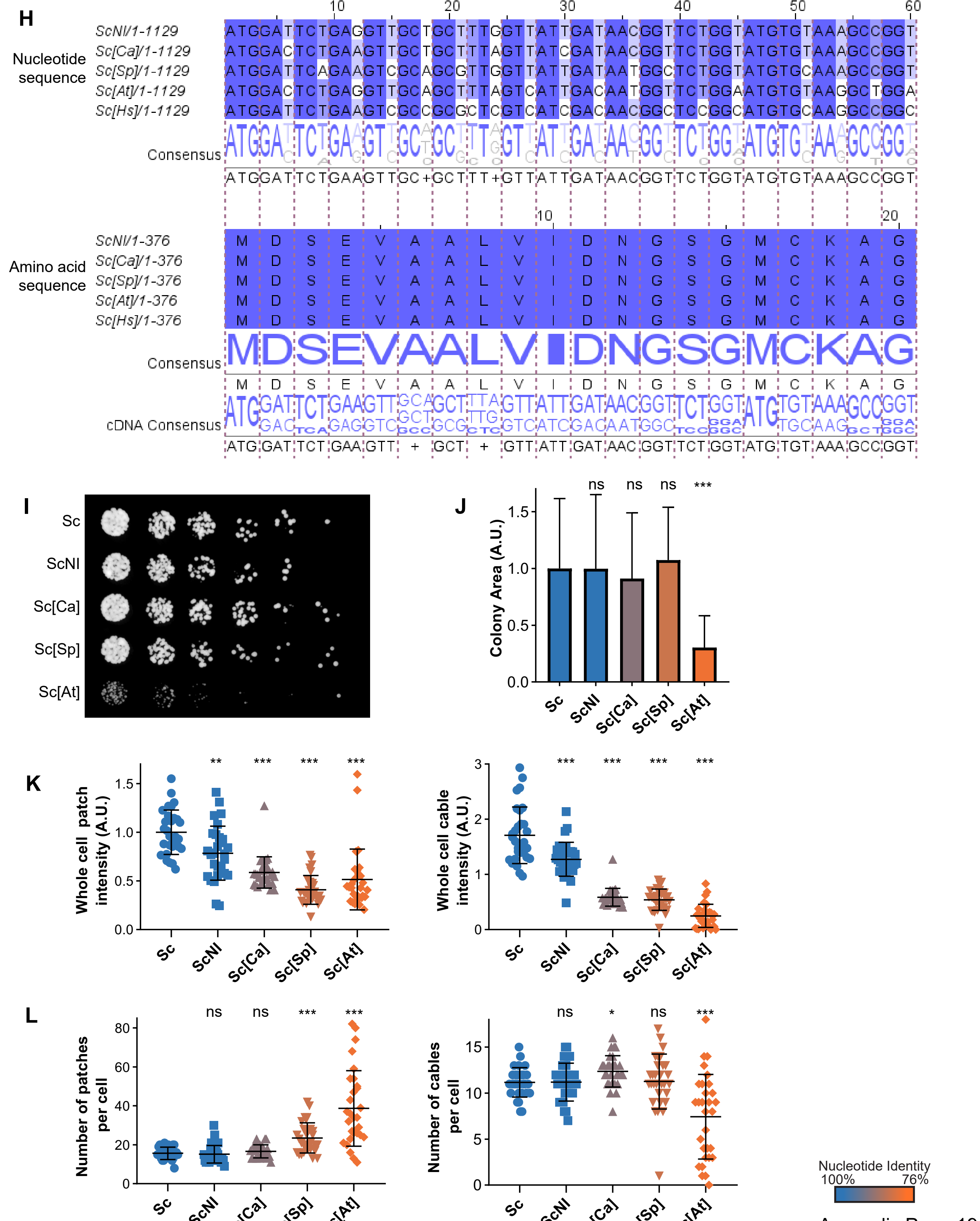


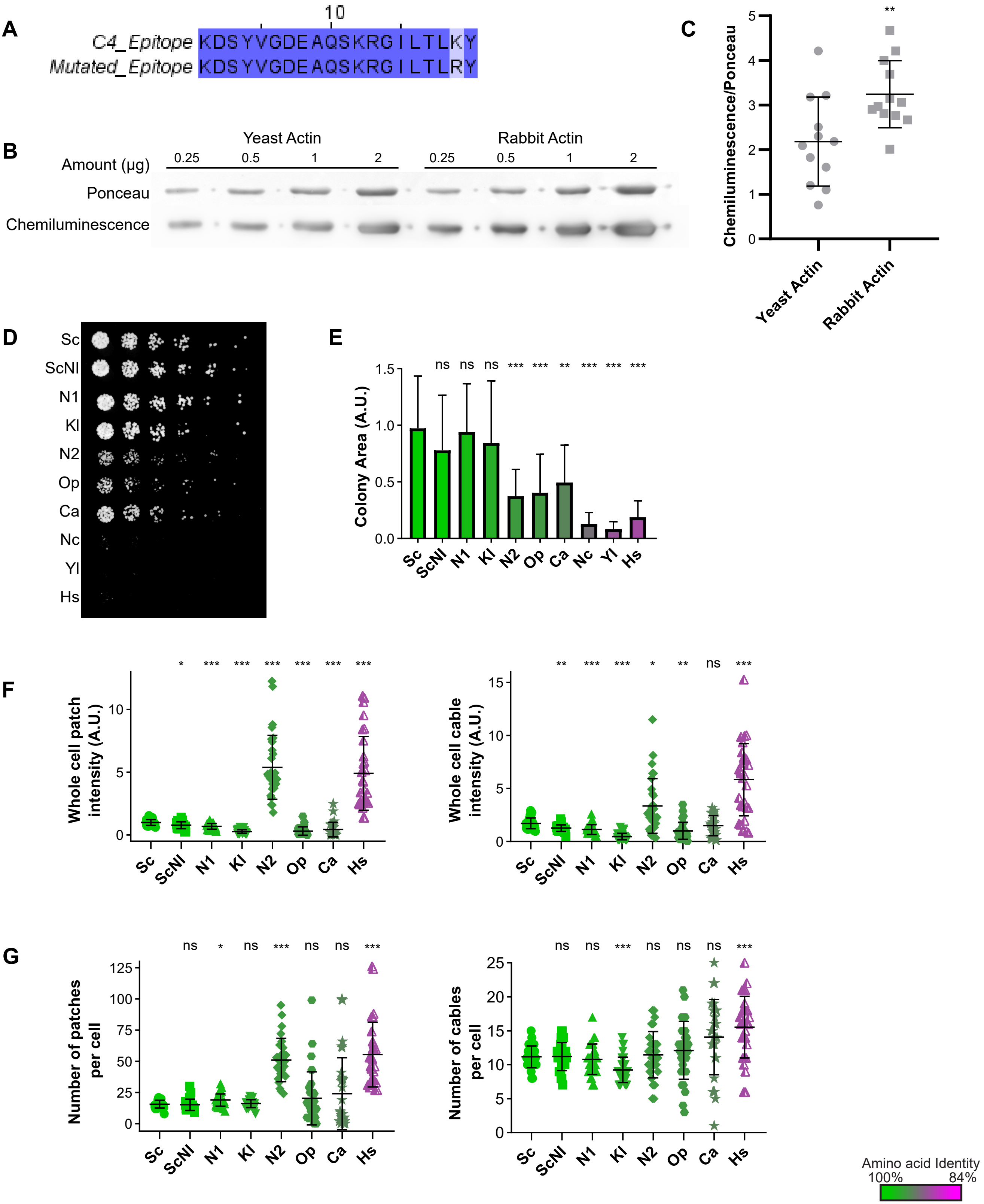
Appendix Figure S2



Appendix Figure S2



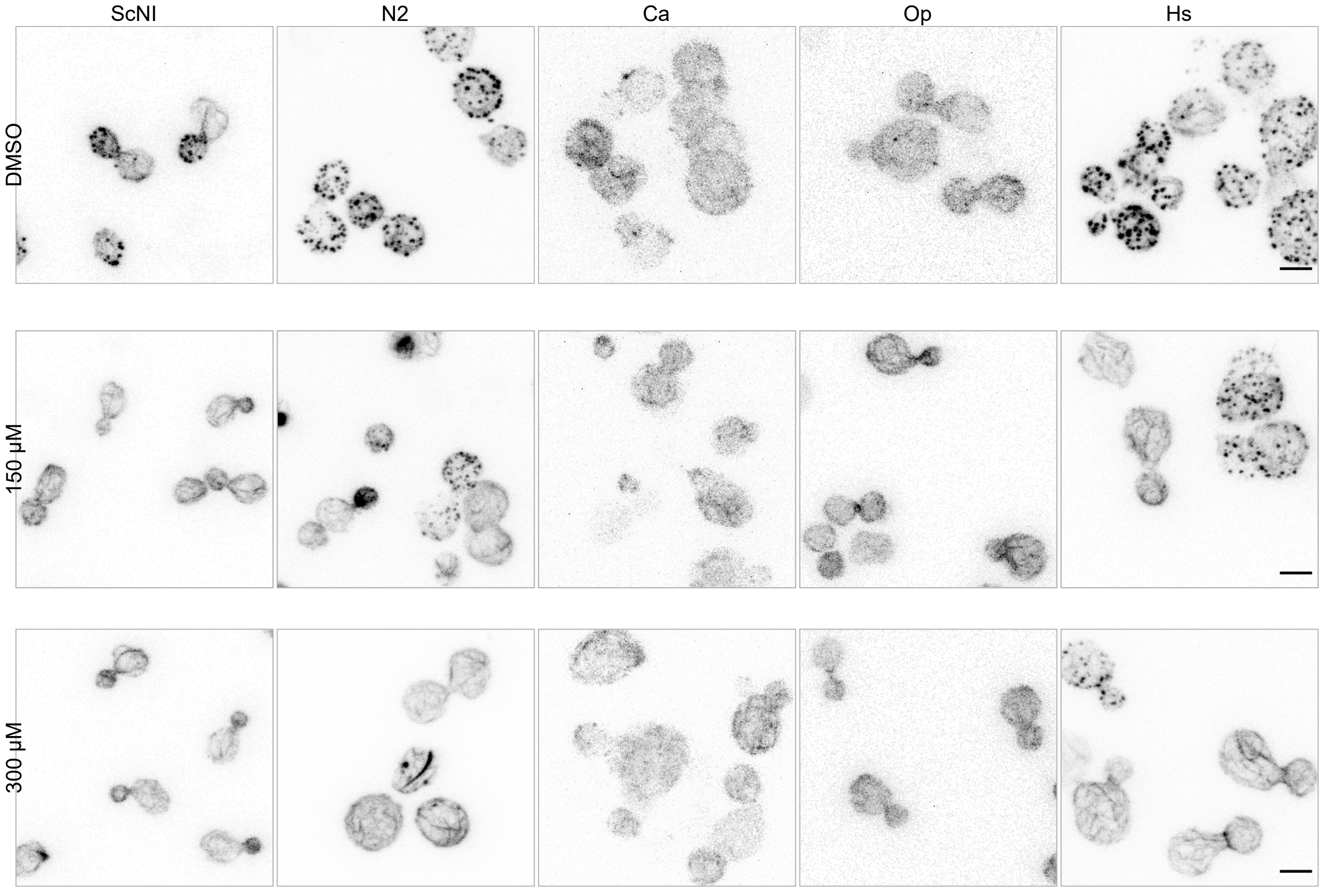
Appendix Figure S3



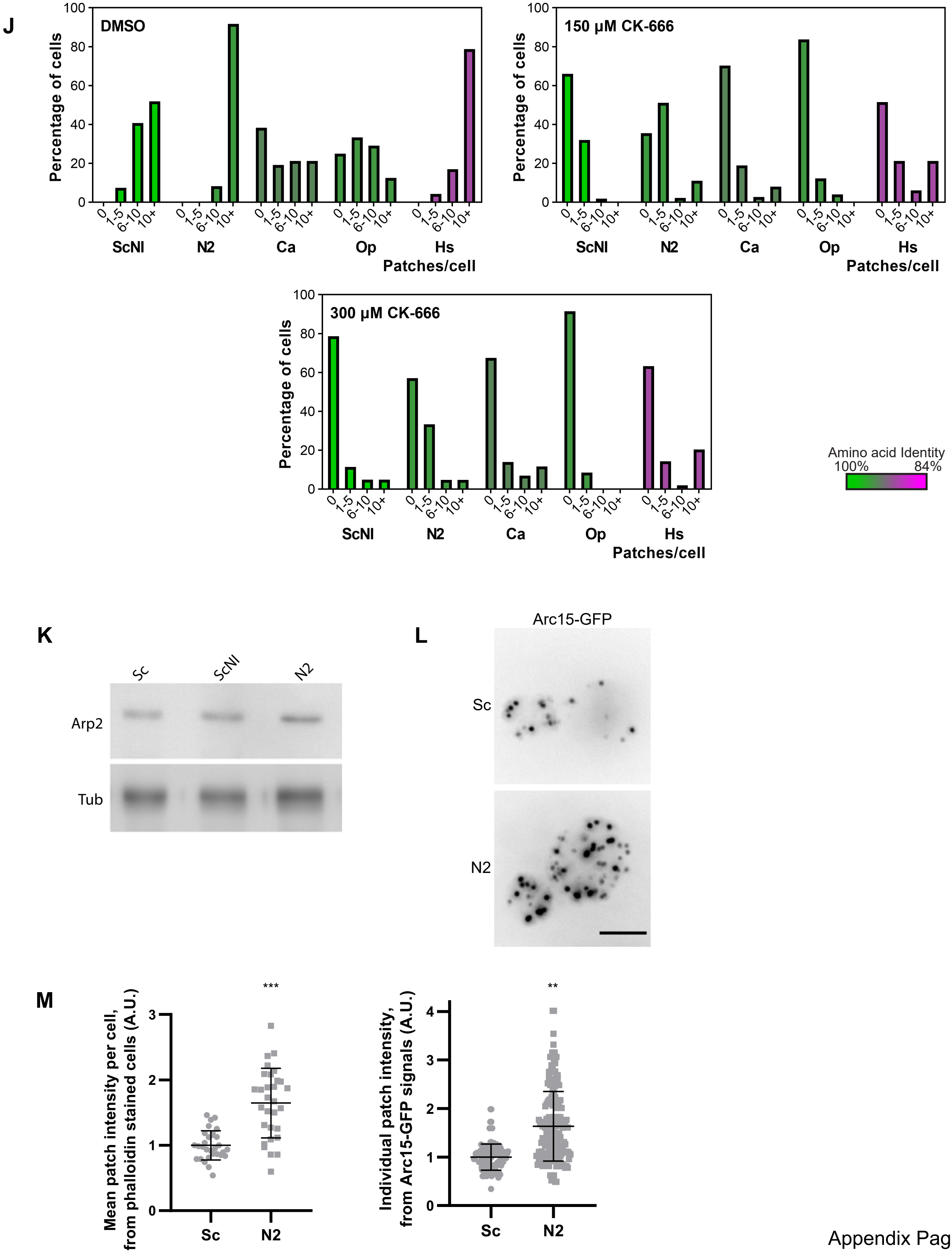
H

	72	73	75	77	110	111	112	177	179	194	197	198	199	200	205	242	287
Act_Sc	H	H	I	T	M	N	P	R	D	S	G	Y	S	F	E	L	V
Act_N1	H	H	I	T	M	N	P	R	D	S	G	Y	S	F	E	L	V
Act_Kl	H	H	I	T	M	N	P	R	D	A	G	Y	S	F	E	L	V
Act_N2	H	H	I	T	M	N	P	R	D	S	G	Y	S	F	E	L	V
Act_Op	H	H	I	T	M	N	P	R	D	S	G	Y	T	F	E	L	V
Act_Ca	H	H	I	S	M	N	P	R	D	S	G	Y	S	F	E	L	M
Act_Nc	H	H	V	T	I	N	P	R	D	A	G	Y	T	F	E	L	V
Act_Yl	H	H	I	T	I	N	P	R	D	S	G	Y	S	F	E	L	V
Act_Hs	H	H	I	T	L	N	P	R	D	T	G	Y	S	F	E	L	V

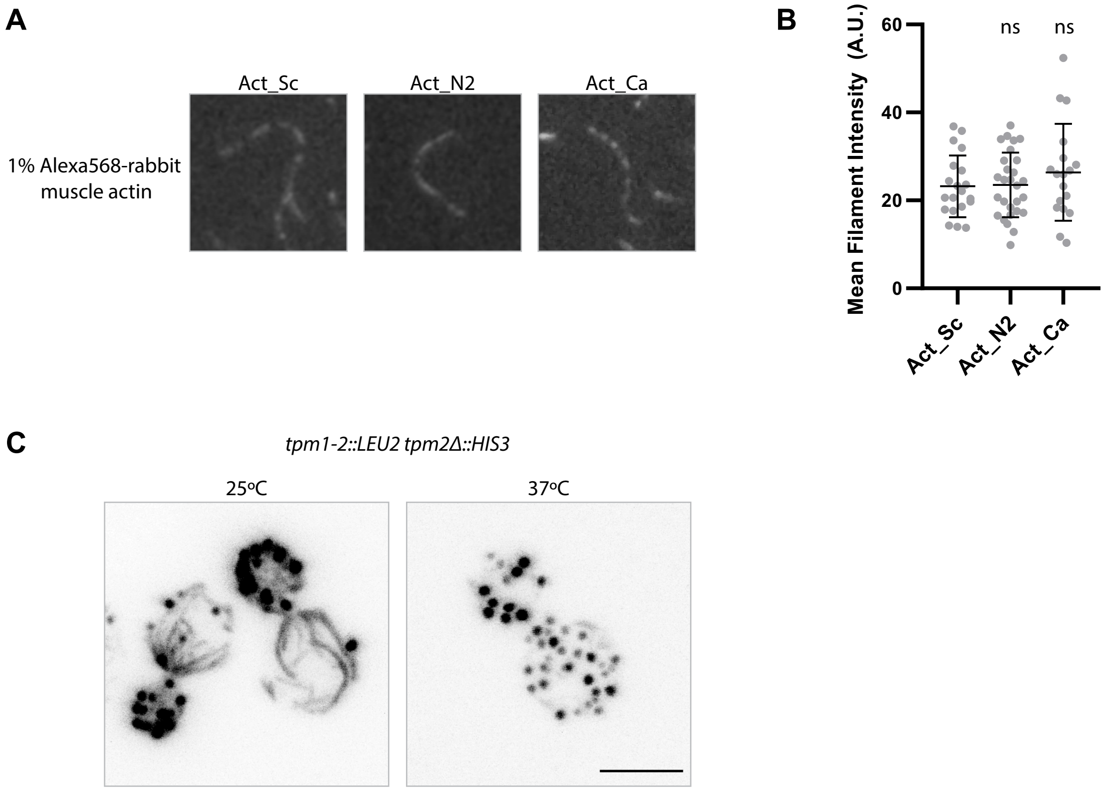
I



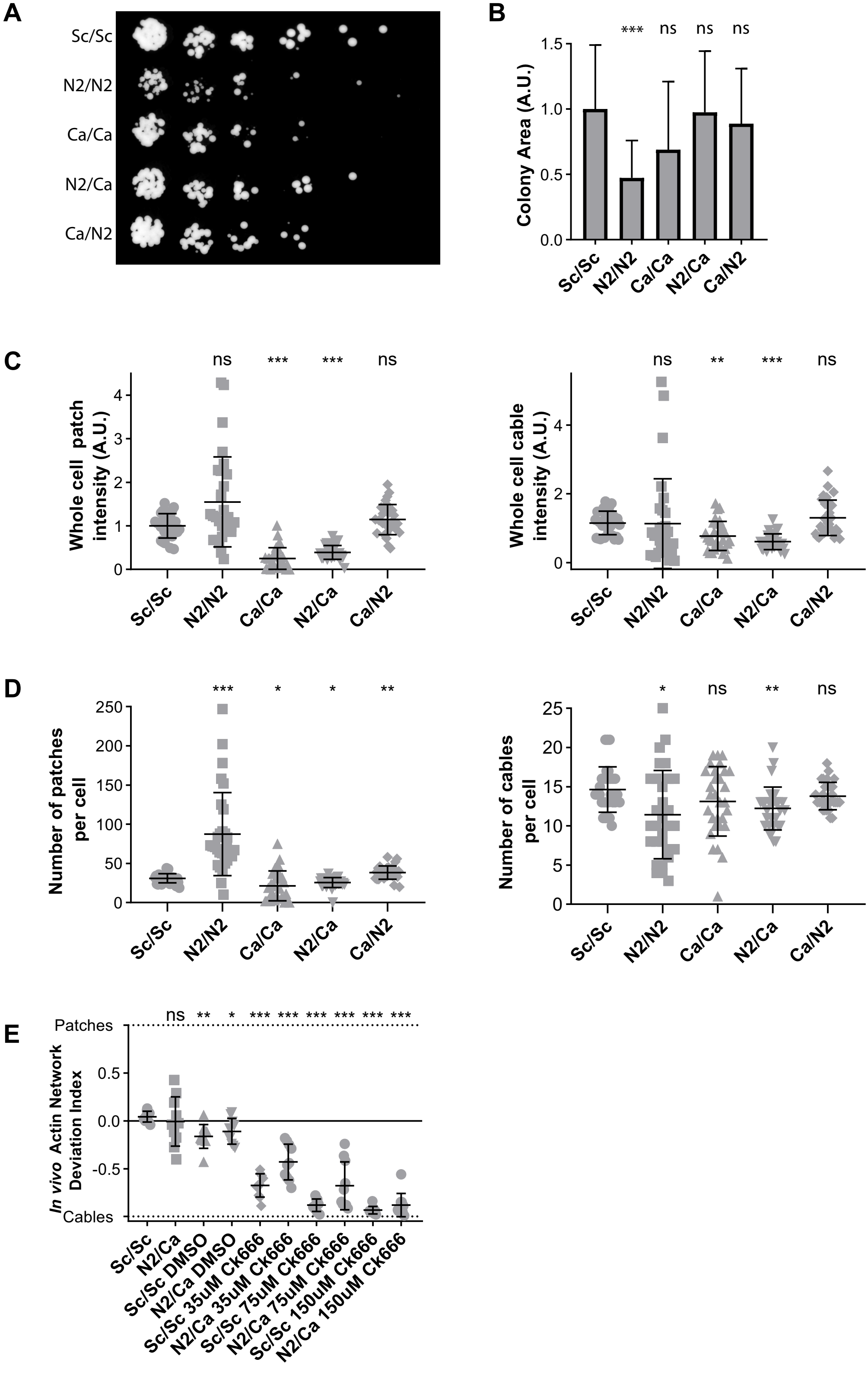
Appendix Figure S3



Appendix Figure S4



Appendix Figure S5



Appendix Table S1.

Species	**UniProt Entry**
Absidia glauca	P26197
Acanthamoeba castellanii	P02578
Achlya bisexualis	P26182
Aedes aegypti	P49128
Arabidopsis thaliana	P53496
Artemia sp.	P18600
Aspergillus oryzae	Q2U7A3
Biomphalaria alexandrina	Q964E3
Biomphalaria glabrata	P92179
Biomphalaria obstructa	Q964E1
Biomphalaria pfeifferi	Q964E2
Biomphalaria tenagophila	Q964E0
Bombyx mori	P84183
Bos mutus grunniens	Q0PGG4
Bos taurus	P60712
Branchiostoma belcheri	Q93129
Branchiostoma floridae	Q93131
Branchiostoma lanceolatum	O17503
Brugia malayi	P90689
Caenorhabditis elegans	P10984
Camelus dromedarius	P84336
Candida albicans	P14235
Candida dubliniensis	Q9UVZ8
Canis lupus familiaris	O18840
Cavia porcellus	Q71FK5
Chlamydomonas reinhardtii	P53498
Chlorocebus aethiops	Q76N69
Coleochaete scutata	O65315
Coprinopsis cinerea	Q9UVX4
Crassostrea gigas	O17320
Cricetulus griseus	P48975
Cryptococcus neoformans	P48465
Cryptosporidium parvum	P26183
Ctenopharyngodon idella	P83751
Cyanidioschyzon merolae	P53500
Cyprinus carpio	P83750
Danio rerio	Q7ZVF9
Dictyostelium discoideum	P07830
Drosophila melanogaster	P10981

Encephalitozoon cuniculi	Q8SWN8
Entamoeba histolytica	P11426
Equus caballus	P60708
Exophiala dermatitidis	Q8X119
Fucus distichus	P53502
Fucus vesiculosus	Q39758
Gaeumannomyces graminis	Q6TCF2
Gallus gallus	P60706
Giardia intestinalis	P51775
Halocynthia roretzi	P53461
Helicoverpa armigera	P84184
Heliocidaris erythrogramma	P69002
Heliocidaris tuberculata	P69003
Homo sapiens	P60709
Hydra vulgaris	P17126
Kluyveromyces lactis	P17128
Komagataella phaffii	Q9P4D1
Leishmania major	P45520
Limulus polyphemus	P41340
Lumbricus rubellus	P91754
Lumbricus terrestris	P92182
Lytechinus pictus	P53465
Macaca fascicularis	Q4R561
Mayetiola destructor	O16808
Mesocricetus auratus	Q711N9
Mesostigma viride	O65316
Mus musculus	P60710
Naegleria fowleri	P27131
Naegleria pringsheimi	Q9NJV4
Neurospora crassa	P78711
Ogataea parapolymorpha	O74258
Onchocerca volvulus	P30163
Oreochromis mossambicus	P68143
Oryctolagus cuniculus	P29751
Oryzias latipes	P79818
Ovis aries	P60713
Oxytricha trifallax	P53468
Pan troglodytes	Q5R1X3
Phaffia rhodozyma	P53689
Physarum polycephalum	P02576
Phytophthora infestans	P22131
Phytophthora megasperma	P13363

Pisaster ochraceus	P12716
Planorbella trivolvis	Q964D9
Plasmodium berghei	Q4Z1L3
Plasmodium yoelii yoelii	Q7RME1
Pneumocystis carinii	P43239
Podocoryna carnea	P41112
Pongo abelii	Q5R6G0
Puccinia graminis	P50138
Rattus norvegicus	P60711
Saccharomyces bayanus	P60011
Saccharomyces cerevisiae	P60010
Saccoglossus kowalevskii	O18499
Salmo salar	O42161
Scherffelia dubia	O65314
Schistosoma mansoni	P53471
Schizophyllum commune	Q9Y702
Schizosaccharomyces pombe	P10989
Sigmodon hispidus	Q91ZK5
Sorghum bicolor	P53504
Spermophilus citellus	Q4L0Y2
Sterkiella cavicola	O00937
Sterkiella nova	P12715
Strongylocentrotus purpuratus	P12431
Styela plicata	Q00215
Suillus bovinus	Q9Y707
Sus scrofa	Q6QAQ1
Taenia solium	P68555
Takifugu rubripes	P68142
Tetrahymena pyriformis	P10993
Tetrahymena thermophila	P10992
Thermomyces lanuginosus	P10365
Toxoplasma gondii	P53476
Triakis scyllium	Q8JJB8
Trichosurus vulpecula	P60707
Trypanosoma brucei brucei	P12432
Trypanosoma cruzi	P53477
Volvox carteri	P20904
Xenopus borealis	P15475
Xenopus laevis	O93400
Xenopus tropicalis	Q6NVA9
Yarrowia lipolytica	Q9UVF3

Appendix Table S2.

Plasmid Name	**Insert**	**Markers**	**Full description**
pMA253	Sc	LEU2/URA3	Base plasmid LEU2/URA3. pGEX-4T1 replaced in between AatII and Bsu36I. Full insert: AatII/5'RS/BamHI/URA3/SalI/pAct1/Act1 Gene with intron/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA254	Sc	HIS3/KanMX3	Base plasmid His/Kan. pGEX-4T1 replaced in between AatII and Bsu36I. Full insert: AatII/5'RS/BamHI/HIS3/SalI/pAct1/Act1 Gene with intron/tAct1/NotI/KanMX3/SacI/3'RS/Bsu36I
pMA255	ScNI	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/Act1 Gene without intron/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA256	Act_Sc[Ca]	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Sc[Ca]/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA257	Act_Sc[Sp]	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Sc[Sp]/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA258	Act_Sc[At]	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Sc[At]/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA259	Act_Sc[Hs]	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Sc[Hs]/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA260	Act_N1	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_N1/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA261	Act_Kl	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Kl/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA262	Act_N2	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_N2/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA263	Act_Op	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Op/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA264	Act_Ca	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Ca/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA265	Act_N3	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_N3/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA266	Act_N4	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_N4/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA267	Act_Nc	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Nc/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA268	Act_N5	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_N5/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA269	Act_Yl	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Yl/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA270	Act_Sp	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Sp/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA271	Act_Hs	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Hs/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA272	Act_Sco	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_Sco/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA273	Act_At	LEU2/URA3	AatII/5'RS/BamHI/URA3/SalI/pAct1/PacI/Act_At/XbaI/tAct1/NotI/LEU2/SacI/3'RS/Bsu36I
pMA274	Act_N2	HIS3/KanMX3	AatII/5'RS/BamHI/HIS3/SalI/pAct1/PacI/Act_N2/XbaI/tAct1/NotI/LEU2/KanMX3/3'RS/Bsu36I
pMA275	Act_Ca	HIS3/KanMX3	AatII/5'RS/BamHI/HIS3/SalI/pAct1/PacI/Act_Ca/XbaI/tAct1/NotI/LEU2/KanMX3/3'RS/Bsu36I

Appendix Table S3.

Yeast Strain Name	**Mating type**	**Actin**	**Genotype**
MAY002	a	Sc	MATa his3-Δ200 ura3-52 leu2-3,112
MAY003	α	Sc	MATα his3-Δ200 ura3-52 leu2-3,112
MAY258	α	ScI	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-ACT1-LEU2
MAY259	α	ScNI	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Δintron-LEU2
MAY260	α	N2	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-N2-LEU2
MAY261	α	Ca	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Ca-LEU2
MAY262	N/A	Nc	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Nc-LEU2
MAY263	α	Op	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Op-LEU2
MAY264	α	N1	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-N1-LEU2
MAY265	α	Sc[Ca]	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Sc[Ca]-LEU2
MAY266	α	Hs	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Hs-LEU2
MAY267	N/A	Yl	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Yl-LEU2
MAY268	α	Kl	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Kl-LEU2
MAY269	α	Sc[At]	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Sc[At]-LEU2
MAY270	α	Sc[Sp]	MATα his3-Δ200 ura3-52 leu2-3,112 act1Δ::URA3-act1-Sc[Sp]-LEU2
MAY271	a/α	Sc/Sc	MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2-3,112/leu2-3,112
MAY272	a/α	N2/N2	MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 act1Δ::URA3-act1-N2-LEU2/act1Δ::HIS3-act1-N2-KanMX3
MAY273	a/α	N2/Ca	MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 act1Δ::URA3-act1-N2-LEU2/act1Δ::HIS3-act1-Ca-KanMX3
MAY274	a/α	Ca/N2	MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 act1Δ::URA3-act1-Ca-LEU2/act1Δ::HIS3-act1-N2-KanMX3
MAY275	a/α	Ca/Ca	MATa/MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 act1Δ::URA3-act1-Ca-LEU2/act1Δ::HIS3-act1-Ca-KanMX3

Supplementary Methods: Equipment and settings

Yeast cell phalloidin staining and imaging

Imaging conditions:

MicroscopeName: Leica TCS SP8 X

ImageSize: 14.56x14.56 microns (512x512 pixels) (single cells), 116.33x116.33 microns (1392x1392 pixels) (full fields)

BitsPerPixel: 16

Step size: 30

Detector: PMT

TimeGatePulseEnd: 6000

TimeGatePulseStart: 500

TimeGateWavelength: 578

LaserName: White Light Laser

OutPutPowerPercentage: 0.7

LineAverage: 3

DyeName: Alexa-568

TargetWaveLengthBegin: 794

TargetWaveLengthEnd: 799

ObjectiveName: HC PL APO CS2 100x/1.40 OIL

ScanSpeed: 400

Zoom: 8 (single cells), 1 (full fields)

Image processing: Maximum intensity z-projection

Imaging media: 70% glycerol in PBS

Imaging temperature: 20-25 degrees

Image acquisition for branched and linear network reconstitution

Imaging conditions:

MicroscopeName: Axio Observer.Z1 / 7

ImageSize: 133.12x133.12 microns (2048x2048 pixels)

BitsPerPixel: 16

SelectedDetector: MTBCamera_MTBSideportChanger_Left.HDCamC11440-42U

SelectedLighthouse: HXP 120 V

Intensity: 20%

ExposureTime: 200 ms

DyeName: Alexa-568

ObjectiveName: Plan-Apochromat 100x/1.40 Oil Ph 3 M27

ChannelFilter: Texas Red

Imaging media: Motility buffer (50 mM KCl, 5 mM Hepes, 2.4 mM $MgCl_2$; 4 mM DTT; 1 mM ATP; 0.36% methylcellulose 1500 cP and 1.5% BSA)

Imaging temperature: 20-25 degrees

Settings for bead images showed in figure 4.

Actin alone, branched

Minimum displayed value: 140

Maximum displayed value: 12700

Actin alone, linear

Minimum displayed value: 120

Maximum displayed value: 2000

Actin/Tpm, branched

Minimum displayed value: 150/250

Maximum displayed value: 17800/34452

Actin/Tpm, linear

Minimum displayed value: 130/310

Maximum displayed value: 3600/5800

Actin/Cof, branched

Minimum displayed value: 130/30

Maximum displayed value: 17500/6922

Actin/Cof, linear

Minimum displayed value: 120/50

Maximum displayed value: 6700/2650