

## Supplementary Material

1 Figure and 3 Tables

### Figure Legend

Supplementary Figure 1 Rhodamine-phalloidin staining of actin structures in actin alleles. Polarised cortical actin patches (arrowhead) and actin cables (arrow) are visible in strains expressing wild-type actin (A), *act1-102* (B), *act1-157* (C). Depolarised cortical actin patches with few or no actin cables are observed in cells expressing *act1-115* (D). Visualisation of the cells was performed using an Olympus BX60 fluorescence microscope. (Bar: 10  $\mu\text{m}$ , Exposure time: 500 msec)

**Supplementary Table 1. Yeast strains**

<b>Strain</b>	<b>Genotype</b>	<b>Origin/ Reference</b>
<b>KAY 141</b>	MATa <i>his3Δ200 leu2-3.112 ura3-52 cry1 can1-1 tub2-201 (benR) act1-133::HIS3</i>	DDY336 (D.Drubin)
<b>KAY 143</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 cry1 can1-1 tub2-201 act1-101::HIS3</i>	DDY338(D.Drubin)
<b>KAY 166</b>	MATα <i>ura3-52 leu2-3.112 his3Δ200 can1-1 ade4 cry1 tub2-201 act1-102:: HIS3</i>	DDY339 (D.Drubin)
<b>KAY 147</b>	MATα <i>ura3 leu2 his3 can1-1 tub2-201 act1-113::HIS3</i>	DDY342 (D.Drubin)
<b>KAY 172</b>	MATα <i>ura3-52 leu2 his3 can1-1 tub2-201 act1-115::HIS3</i>	DDY343 (D.Drubin)
<b>KAY 151</b>	MATa <i>ura3 leu2 his3 ade2 cry1 bry2-201 act1-119::HIS3</i>	DDY346 (D.Drubin)
<b>KAY 152</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 cry1 tub2-201 can1-1 act1-120::HIS3</i>	DDY347 (D.Drubin)
<b>KAY 156</b>	MATα <i>ura3 leu2 his3 tub2-201 can1-1 act1-129::HIS3 (R177A, D179A)</i>	DDY351 (D.Drubin)
<b>KAY159</b>	MATa <i>ura3 leu2 his3 cry1 tub-201 ACT1::HIS3</i>	DDY354 (D.Drubin)
<b>KAY 207</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: HIS3</i>	DBY7062 (D.Botstein)
<b>KAY 208</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-45 HIS3 (I341A)</i>	DBY7063 (D.Botstein)
<b>KAY 209</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-53 HIS3 (I345A)</i>	DBY7064 (D.Botstein)
<b>KAY 210</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-54 HIS3 (Y166T)</i>	DBY7067 (D.Botstein)
<b>KAY 211</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-57 HIS3 (I341K)</i>	DBY7072 (D.Botstein)
<b>KAY 212</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-61 HIS3 (Y166A)</i>	DBY7075 (D.Botstein)
<b>KAY 213</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-62 HIS3 (F169A)</i>	DBY7076 (D.Botstein)
<b>KAY 214</b>	MATa <i>ura3-52 leu2-3.112 his3Δ200 ade2-101 (am) can1 cry1 + CEN ACT1:: act1-63 HIS3 (Y166A, F169A)</i>	DBY7078 (D.Botstein)
<b>KAY 1199</b>	MATα <i>tub2-201 his3Δ200 leu2-3.112 ura3-52 act1-157::HIS3</i>	This study
<b>KAY 446</b>	MATa, <i>his3Δ1 leu2Δmet15Δura3Δ</i>	Invitrogen BY4741
<b>KAY 448</b>	MATa <i>Δscp1::KanMx his3Δ leu2Δ met15Δ ura3Δ</i>	Invitrogen
<b>KAY 593</b>	MATa <i>Δsac1::KanMx his3Δ leu2Δ met15Δ ura3Δ</i>	Invitrogen
<b>KAY 1242</b>	MATa <i>Δscp1::KanMx his3Δ leu2Δ met15Δ ura3Δ, sac6Δ:: LEU2</i>	This study
<b>KAY 684</b>	MATα <i>Sac6-RFP::KanMx his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Huh et al., 2003
<b>KAY1178</b>	MATa, <i>his3Δ1 leu2Δmet15Δura3Δ, SCP1-GFP</i>	Invitrogen
<b>KAY1199</b>	MATα <i>his3Δ200, leu2-3,112,ura3-52, act1-157::HIS3</i>	This study
<b>KAY1304</b>	As KAY159 + Abp1mRFP	This study
<b>KAY1305</b>	As KAY166 + Abp1mRFP	This study
<b>KAY1306</b>	As KAY172 + Abp1mRFP	This study

<b>KAY1307</b>	As KAY1199 + Abp1mRFP	This study
<b>DGY157</b>	BY4741 <i>Scp1</i> (1-154):: <i>HIS5</i>	This study
<b>DGY159</b>	BY4741 <i>Scp1</i> (1-172):: <i>HIS5</i>	This study

**Supplementary Table 2. Plasmids**

<b>Plasmid name</b>	<b>Description</b>	<b>Origin/ Reference</b>
<b>pKA 88</b>	GFP- <i>ABP1</i> under the control of the <i>ACT1</i> promoter and terminator	Gift-D. Botstein, Princeton
<b>pKA 211</b>	Scp1 cloned between NdeI and SalI sites on the pSJW1 vector (2585 bp, AmpR); used for Scp1p purification	(5)
<b>pKA 148</b>	PCR template used to create <i>SCPI</i> deletions and truncations using the <i>HIS 3</i> cassette	(12)
<b>pKA280</b>	p416 CEN URA MET 25 promoter - <i>SCPI</i>	(5)
<b>pKA281</b>	p426 2 $\mu$ URA MET 25 promoter - <i>SCPI</i> 2 $\mu$	(5)
<b>B4189</b>	GFP-Scp1 expressed under the control of the <i>SCPI</i> native promoter (contains two polymorphisms at positions 394 and 420)	Gift-G. Fink, Whitehead Inst.
<b>pKA 491</b>	pET14b vector containing Scp1 $\Delta$ 180 (expresses 6 x His-Scp1 $\Delta$ 180)	This study
<b>pKA 501</b>	pET14b vector containing Scp1 full length cloned between the NdeI and the Sal I sites (6 x His- Scp1)	This study
<b>pKA 504</b>	A point mutation was introduced at position 172 in Scp1 in B4189	This study
<b>pKA 505</b>	A point mutation was introduced at position 180 in Scp1 in B4189	This study
<b>pKA 530</b>	A point mutation was introduced at position 154 in Scp1 in B4189	This study
<b>pKA542</b>	A point mutation was introduced to generate S185A in Scp1 in B4189	This study
<b>pKA543</b>	A point mutation was introduced to generate S185D in Scp1 in B4189	This study
<b>pKA474</b>	PCR template plasmid used to create <i>SAC6</i> deletions <i>LEU2</i>	Gift-D.Drubin

### Supplementary Table 3 Oligonucleotides used in this study

OLIGO	SEQUENCE	Notes
<b>oKA313</b>	CTAGGACCACAACCTGTCAATCTAGAAGCCAAGACCCCCGGTT	IVM for Sep1Δ154
<b>oKA314</b>	AACCGGGGGTCTTGGCTTCTAGATTGACAGTTGTGGTCCCTAG	IVM for Sep1Δ154
<b>oKA 315</b>	CATCTACAAGATGGTACTGTCTAGAGTACTTTTGAATACGGT	IVM for Sep1Δ172
<b>oKA 316</b>	ACCGTATTCAAAAAGTGCTCTAGACAGTACCATCTTGTAGATG	IVM for Sep1Δ172
<b>oKA 317</b>	TGGAGCACTTTTGAATACGTCTAGATGAAAGGTGCATCTCAG	IVM for Sep1Δ180
<b>oKA 318</b>	CTGAGATGCACCTTTCATCTAGACGTATTCAAAAAGTGCTCCA	IVM for Sep1Δ180
<b>oKA 319</b>	GGTTATATGAAAGGTGCAGCTCAGGCTACTGAAGGAGTG	IVM for Sep1S185A
<b>oKA 320</b>	CACTCCTTCAGTAGCCTGAGCTGCACCTTTCATATAACC	IVM for Sep1S185A
<b>oKA 321</b>	GGTTATATGAAAGGTGCAGATCAGGCTACTGAAGGAGTG	IVM for Sep1S185D
<b>oKA 322</b>	CACTCCTTCAGTAGCCTGATCTGCACCTTTCATATAACC	IVM for Sep1S185D
<b>oKA 690</b>	TATCAGAGAAGAAGCTGATATATTAGCCCTAAGGAGTACACCAAAACACAT CGAGGTCGACGGTATC	Δsac6 deletion 5'
<b>oKA 691</b>	TGGAACAAGAAAGCTGAGTAGAAAACAGGTTACGAAAGTTGTTTGTGGCC GCTCTAGAAGTAGTGGATC	Δsac6 deletion 3'
<b>oKA 692</b>	GTTCCCGATGGCCTTCATGTG	Δsac6 deletion check
<b>oKA 693</b>	GGAATCATTTGAGCGTTGGAGGC	Δsac6 deletion check

Supplementary Figure 1

