

Table S1. **Neurospora crassa strains used in this study**

Strain	Genotype (transformed plasmids are in parentheses)	Use
FGSC #3661	<i>A, alcoy, csp-2</i>	Mapping the original <i>wsc</i> mutant
FGSC #6808	<i>A, trp-1; ylo-1</i>	Mapping the original <i>wsc</i> mutant
FGSC #1208	<i>a, trp-1; ylo-1</i>	Mapping the original <i>wsc</i> mutant
FGSC #52	<i>A, ro-2</i>	Mapping the original <i>wsc</i> mutant
FGSC #4516	<i>A, pro-1</i>	Mapping the original <i>wsc</i> mutant
FGSC #3305	<i>A, ser-1</i>	Mapping the original <i>wsc</i> mutant
FGSC #5075	<i>A, sc</i>	Mapping the original <i>wsc</i> mutant
FGSC #9179	<i>a, Δmus-52::bar +</i>	Construction of deletions
FGSC #9720	<i>a, Δmus-52::bar +</i>	Construction of deletions
FGSC #6103	<i>A, his-3-</i>	Introduction of <i>his-3Δ200</i>
FGSC #465	<i>A, pan-2-</i>	Genetic screen
GSF #48	<i>a, pan-2- (POKE103::phex-GFP-PTS1)</i>	Figs. 1 A and 5 D
GSF #111	<i>A, wsc- (POKE103::phex-GFP-PTS1)</i>	Fig. 1 A
FGSC #987	<i>A</i>	Fig. 1 (B and C)
GSF #59	<i>A, wsc-</i>	Fig. 1 (B and C)
GSF #623	<i>A, pan-2- (pMF280)</i>	Fig. 1 C
GSF #626	<i>A, Δwsc, pan-2- (pMF280)</i>	Fig. 1 C
GSF #629	<i>A, Δwsc, pan-2-, his-3- (pBM60::wsc-eGFP, POKE103::phex-RFP-PTS1)</i>	Figs. 3 A, 5 A, and 8 B
GSF #411	<i>A, wsc-, (GJP#961::wsc-eGFP, POKE103::phex-RFP-PTS1)</i>	Fig. 3 B
GSF#1193	<i>A, Δmus-52::bar +, his-3- (pBM60::RFP-PTS1)</i>	Figs. 3 C and S2
GSF#1182	<i>A, Δmus-52::bar +, Δfis1::hyg +, his-3- (pBM60::RFP-PTS1)</i>	Figs. 3 C and S2
GSF#1166	<i>A, Δmus-52::bar +, Δdnm1::hyg +, his-3- (pBM60::RFP-PTS1)</i>	Figs. 3 C and S2
GSF#1164	<i>A, Δmus-52::bar +, Δvps1::hyg +, his-3- (pBM60::RFP-PTS1)</i>	Figs. 3 C and S2
GSF #404	<i>A, wsc- (GJP#961::wsc-3xHA)</i>	Fig. 4 (A and B) and Fig. 5 C
GSF #360	<i>A, wsc- (GJP#961::wsc-eGFP)</i>	Fig. 4 B
GSF #399	<i>A, wsc-, Δhex-1 (GJP#961::wsc-eGFP, POKE103::phex-RFP-PTS1)</i>	Figs. 5 A, 6 C, and 8 D
GSF #448	<i>a, Δhex-1, wsc-, pan-2- (GJP#961::wsc-3xHA, GJP#961::wsc-eGFP)</i>	Fig. 5 (B and C), Fig. 6 C, and Fig. 8 D
GSF #38	<i>A, Δhex-1, pan-2- (POKE103::phex-GFP-PTS1)</i>	Fig. 5 D
GSF #743	<i>a, wsc-, Δhex-1 (POKE103::phex-wsc-eGFP, POKE103::phex-RFP-PTS1)</i>	Fig. 6 (A, B, D, and E) and Fig. 8 E
GSF #823	<i>a, wsc-, pan-2- (OKE103::phex-RFP-PTS1, pMF272::pex14)</i>	Fig. 6 E
GSF #621	<i>A, wsc-, (GSP#961::wsc-eYFP, GSP#961::wsc-eYFP)</i>	Fig. 6 F
GJF #1732	<i>S. cerevisiae - MATα, his3D1, leu2D0, lys2D0, ura3D0 (p413GPD::rfp-pts1, p415GPD)</i>	Fig. 7 (A and B)
GJF #1758	<i>S. cerevisiae - MATα, his3D1, leu2D0, lys2D0, ura3D0 (p413GPD::rfp-pts1, p416GPD::wsc-egfp, p415GPD)</i>	Fig. 7 (A–D)
GSF #452	<i>A, Δwsc, pan-2-, his-3-</i>	Fig. 8 A
GSF #475	<i>A, Δwsc, pan-2-, his-3- (pBM60::wsc-eGFP)</i>	Fig. 8 A
GSF #506	<i>A, Δwsc, pan-2-, his-3- (pBM60::wscR102Q-eGFP)</i>	Fig. 8 A
GSF #515	<i>A, Δwsc, pan-2-, his-3- (pBM60::wscR102W-eGFP)</i>	Fig. 8 A
GSF #631	<i>A, Δwsc, pan-2-, his-3- (pBM60::wscR102Q-eGFP, POKE103::phex-RFP-PTS1)</i>	Fig. 8 B
GSF #634	<i>A, Δwsc, pan-2-, his-3- (pBM60::wscR102W-eGFP, POKE103::phex-RFP-PTS1)</i>	Fig. 8 B
GSF #562	<i>A, Δwsc, pan-2, his-3- (pBM60::wsc-3xHA)</i>	Fig. 8 C
GSF #568	<i>A, Δwsc, pan-2 his-3- (pBM60::wscR102W-3xHA)</i>	Fig. 8 C
GSF #857	<i>a, Δhex-1, wsc-, pan-2- (GJP#961::wscR102W-3xHA, GJP#961::wscR102W-eGFP)</i>	Fig. 8 D
GSF #1004	<i>a, wsc-, Δhex-1 (POKE103::phex-wscR102W-eGFP, POKE103::phex-RFP-PTS1)</i>	Fig. 8 E
GSF #302	<i>A, Δhex-1, pan-2-</i>	Fig. S1
GSF #299	<i>a, Δhex-1, wsc-, pan-2-</i>	Fig. S1
GSF #277	<i>a, wsc-, pan-2-</i>	Fig. S1
GSF #290	<i>a, pan-2-</i>	Fig. S1
GSF #459	<i>A, pex 14-, wsc-, pan-2-</i>	Fig. S1
GSF #462	<i>A, pex 14-, pan-2-</i>	Fig. S1
GSF #543	<i>A, Δwsc, pan-2-, his-3- (POKE103::phex-RFP-PTS1)</i>	Fig. S2

Table S2. **Plasmids**

Plasmid	Identity/source
pEGFP-N1	Clontech Laboratories, Inc.
pEYFP-N1	Clontech Laboratories, Inc.
pRSET-B mCherry	Shaner et al., 2004
pREP-41	Craven et al., 1998
pBM-60	Margolin et al., 1997
pMF272	Freitag et al., 2004
pMF280	Freitag et al., 2004
pBS + SK	Stratagene
pCSN43	Staben et al., 1989
p413GPD	Sikorski and Hieter, 1989
p415GPD	Sikorski and Hieter, 1989
p416GPD	Sikorski and Hieter, 1989
GJP #602	Tey et al., 2005
GJP #613	Tey et al., 2005
GJP #961	pBS + SK::hygr
GJP #1001	TOPOII::wsc
GJP #1009	TOPOII::wsc deletion cassette
GJP #1010	GJP#961::wsc
GJP #1050	GJP#961::wsc(new XbaI&SphI)
GJP #1064	GJP#961::wsc-3xha
GJP #1081	GJP#961::wsc-egfp
GJP #1245	pBM60::wsc-egfp
GJP #1302	GJP#961::wsc-eYFP1
GJP #1304	GJP#961::wsc-eYFP11
GJP #1322	pBM60::wscR102Q-egfp
GJP #1324	pBM60::wscR102W-egfp
GJP #1353	pBM60::wsc-3xha
GJP #1361	pBM60::wscR102W-3xha
GJP #1372	pBM60::wscR102Q-3xha
GJP #1406	POKE 103::phex-rfp-pts1
GJP #1500	POKE 103::phex-wsc-gfp
GJP #1530	pMF272::pex14
GJP #1561	GJP#961::wscR102W-3xha
GJP #1564	GJP#961::wscR102W-egfp
GJP #1662	p416GPD::wsc-egfp
GJP #1712	p413GPD::rfp-pts1
GJP #1821	POKE 103::phex-wscR102W-gfp
GJP #1876	pBM60::rfp-pts1

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Table S3. **Oligonucleotides**

Name of oligo	Sequence (5'-3')
BS + SK-Pacl.f	cgataccgctgacctaattaagggcccggtacc
BS + SK-Pacl.r	gggtaccggcccttaattaaggctgacggatcg
HYGr.NoI	gccggcgccgcaagatgatattgaagga
HYGr.Pacl	gccitaattaagttcggtcgcatctact
Pacl.kill.1	tgggtaccggcccttagctaagttcggtcgga
Pacl.Kill.2	tccgaccgaacttagctaaggcccggtaccca
Pacl create.1	tccagctgcaattaataatcgccaacgcgcg
Pacl create.2	cgcgctggccgattaataatgacgtggca
7842.f-2	gcgccggccgagaaatggagatcagagcagga
7842.r-2	gcgttaattaatggaaggctgcatcgaggacca
7842.r-3	gcgactagtggaggctgcatcgaggacca
7842-Seq.f-1	cacatagcagtcacgatcca
7842-Seq.f-2	gcaatgatggccatggcaaca
7842-Seq.r-1	gtaccgtaccggfaccgcta
7842-Seq.r-2	ctgacctatcgttaccctca
7842.P-1	acgcagagagccctcgcaagt
7842.P-2	ttcaatatcatctgcccgcgctcaagcctgcagaggaaggga
7842.P-3	atgccgaccgaacttaataagatggacatcgagttcgagct
7842.P-4	gltcggatgctggagggaaggt
7842.P-5	tgccttcctctgaggcttgacgcggccgcaagatgatattgaa
7842.P-6	agctcgaactcgatgtccatcctaataagttcggtcgcat
eGFP-3'-Xbal	gctctagaatgtgagcaaggcgaggagct
eGFP-3'-SphI	cgcgcatgcttactgtacagctcgctccatgc
HA-3'-Xbal	gcgtctagaatggcaccctfacgatgtt
HA-3'-SphI	gcggcatgcttaagcgtaatctggcaacgtcgtaa
7842-3'-Mut-1	ccagaacccccgtactctagaaaaatgctagctcgggccagctcaag
7842-3'-Mut-2	cttgagctggccgagctagcattttctagagtaacgggggttctgg
7842-3'-Mut-3	ctctagaaaaatgcatgctcgggccagctc
7842-3'-Mut-4	gagctggcccgagcatgcatcttctagag
NotI .pts 1 .ch.5'	gcagcgccgccatgggtgagcaaggcgca
PacI .pts 1 .ch.3'	gccttaattaaggcgggactgtacagctcgtcca
wsc5'-NotI	gcggcgccgcccgtacctctctcgcc
wsc3'-Pacl	gcgttaattaagcttactgtacagctcgtcca
EYFP-I-3'-Xbal	gcgtctagagatccaccggctgccaccat
EYFP-I-3'-SphI	gcggcatgcttactgtcggcggatagatagagct
EYFP-II-3'-Xbal	gcgtctagatcaccgccgacaagcagaagaa
EYFP-II-3'-SphI	gcggcatgcttactgtacagctcgtccat
Pex14-Spel	gcactagtatggccatccgcgaggacct
Pex14-Pacl	gccttaattaaggcggtctcgtcgcctcgt
wsc-gfp-5'-Spel	gcgactagtatgctggtaaaagaacaccaca
wsc-gfp-3'-Xhol	gcgctcaggttactgtacagctcgtccat
mCherry-5'-Spel	gcgactagtatggtgagcaaggcgaggagga
mCherry-3'-Xhol	gcgctcaggttagagctggactgtacagctcgtccatgccgcc
wsc-Mut7-f	gccatctggggacctggccgctgaagtag
wsc-Mut7-r	ctacttcacggcccagggtcccaagatggc
wsc-Mut8-f	gccatctggggaccaggcctgaagtag
wsc-Mut8-r	ctacttcacggcctgggtcccaagatggc
fis1-1	ggcggctcggcgctgatgtgaa
fis1-2	ttcaatatcatctgcccgcggtatcctgaaatagaagtaggt
fis1-3	acctactctatcaggataccggccgcaagatgatattgaa
fis1-4	tcatacctacagcgaccgtaattaagttcggtcgcat
fis1-5	atgccgaccgaacttaataacggctgcctgatggatgatga
fis1-6	ccgattaagcatgtcgcaggt
dnm1-1	ggtacacaggtcaacgtgcccgt
dnm1-2	ttcaatatcatctgcccgcggtgagctgcaagacggaa
dnm1-3	ttccgtctgctcaactccggccgcaagatgatattgaa
dnm1-4	atctgtagggtgagcctgaggtaattaagttcggtcgcat
dnm1-5	atgccgaccgaacttaataacctcaggctacacctacagat
dnm1-6	gtgtctgtgtgtgtgtgtgt
vps1-1	gacgcggcctcattcaggcaga
vps1-2	ttcaatatcatctgcccgcggtgctgctatgcagaggt
vps1-3	acctctgcatagccagcaatcgccgcccgaagatgatattgaa
vps1-4	ttctcaaacgtagtactaaccttaattaagttcggtcgcat
vps1-5	atgccgaccgaacttaattaaggttagtactacgtttgagaaga
vps1-6	ctgtcgtggagaagttgcttgg