Table S1. Yeast Strains used in this study

Strain	Relevant Genotype	*iqg1* Mutations
SN001	WT background strain	WT
SN143	*cdk1-as*	WT
SN312	*IQG1, SPC42-mCherry*	WT
SN313	*iqg1-14A, SPC42-mCherry*	S7A, T30A, S49A, T225A, S268A, T299A, T315A, S317A, T338A, T348A, S354A, S365A, S399A, S404A
SN314	*iqg1-11A, SPC42-mCherry*	T225A, S268A, T299A, T315A, S317A, T338A, T348A, S354A, S365A, S399A, S404A
SN315	*iqg1-3A, SPC42-mCherry*	S7A, T30A, S49A
SN158	*iqg1-6A, SPC42-mCherry*	S919A, S956A, S961A, S1120A, S1122A, S1347A
SN320	*IQG1-eGFP, SPC42-mCherry*	WT
SN321	*iqg1-14A-eGFP, SPC42-mCherry*	S7A, T30A, S49A, T225A, S268A, T299A, T315A, S317A, T338A, T348A, S354A, S365A, S399A, S404A
SN322	*iqg1-11A-eGFP, SPC42-mCherry*	T225A, S268A, T299A, T315A, S317A, T338A, T348A, S354A, S365A, S399A, S404A
SN323	*iqg1-3A-eGFP, SPC42-mCherry*	S7A, T30A, S49A
SN324	*HOF1-eGFP, IQG1, SPC42-mCherry*	WT
SN342	*HOF1-eGFP, iqg1-14A, SPC42-mCherry*	S7A, T30A, S49A, T225A, S268A, T299A, T315A, S317A, T338A, T348A, S354A, S365A, S399A, S404A
SN326	*HOF1-eGFP, iqg1-11A, SPC42-mCherry*	T225A, S268A, T299A, T315A, S317A, T338A, T348A, S354A, S365A, S399A, S404A
SN327	*HOF1-eGFP, iqg1-3A, SPC42-mCherry*	S7A, T30A, S49A

Table S2. Plasmids used in this study

Plasmid	Source	Features
pRS306		*URA3*, *AmpR*
pSGN092	pRS306 + genomic PCR	*IQG1*, *URA3*, *AmpR*
pSGN084	Invitrogen LifeTech	*iqg1-14A* (-185 – 1464)
pSGN085	Invitrogen LifeTech	*iqg1-5A* (2635 – 3544)
pSGN094	pSGN092 + pSGN085	*iqg1-6A*, *URA3*, *AmpR*
pSGN098	pSGN094 + pSGN084	*iqg1-20A*, *URA3*, *AmpR*
pSGN109	pSGN092 + pSGN098	*iqg1-11A*, *URA3*, *AmpR*
pSGN111	pSGN092 + pSGN098	*iqg1-3A*, *URA3*, *AmpR*
pYM28	PCR Toolbox II (John Pringle)	*eGFP*, *HIS3MX6*, *AmpR*
pSGN099		*mCherry*, *kanMX*, *AmpR*

Table S3 (A) Mass Spectrometric Analysis of Band Identified as Mlc1

Rank	# Unique Peptides	% Coverage	Protein MW (kDa)	Species	Protein Name	Systematic Name (Yeast)
1	9	53.0	16.4	*S. cerevisiae*	Mlc1	YGL106W
2	5	11.3	58.8	*H. sapiens*	Keratin I	N/A
3	5	11.5	66.0	*H. sapiens*	Keratin II	N/A
4	5	42.3	14.5	*S. cerevisiae*	Rps14A	YCR031C
5	4	25.3	15.3	*S. cerevisiae*	Rps24A	YER074W
6	2	17.0	16.1	*S. cerevisiae*	Cmd1	YBR109C
7	2	13.0	24.4	*S. scrofa*	Trypsin	N/A
8	3	28.8	17.0	*S. cerevisiae*	Rps18A	YDR045W
9	1	2.0	70.9	*M. musculus*	Keratin II	N/A
10	2	14.2	14.2	*S. cerevisiae*	Rpl26A	YLR344W
11	1	5.4	22.3	*H. brasiliensis*	Rubber	N/A
12	1	8.0	14.7	*H. brasiliensis*	Rubber EF	N/A
13	1	1.8	62.1	*H. sapiens*	Keratin I	N/A
14	2	19.2	13.9	*S. cerevisiae*	Rpl35A	YDL191W

Table S3 (B) Mass Spectrometric Analysis of Band Identified as Hof1

Rank	# Unique Peptides	% Coverage	Protein MW (kDa)	Species	Protein Name	Systematic Name (Yeast)
1	25	37.7	76.2	*S. cerevisiae*	Hof1	YMR032W
2	8	10.7	99.6	*S. cerevisiae*	Pma1	YGL008C
3	7	11.6	81.4	*S. cerevisiae*	Hsp82	YPL240C
4	7	4.9	172.8	*S. cerevisiae*	Iqg1	YPL242C
5	4	8.7	69.7	*S. cerevisiae*	Ssa1	YAL005C
6	2	4.1	69.3	*B. taurus*	BSA	N/A
7	2	12.1	24.4	*S. scrofa*	Trypsin	N/A
8	1	3.4	36.9	*S. cerevisiae*	Adh1	YOL086C
9	3	4.5	77.4	*S. cerevisiae*	Sse1	YPL106C
10	1	1.8	80.8	*S. cerevisiae*	Gus1	YGL245W
11	2	3.1	92.3	*S. cerevisiae*	Ubp5	YER144C
12	1	2.4	50.6	*A. salina*	EF-1-alpha	N/A
13	1	2.7	46.8	*S. cerevisiae*	Eno1	YGR254W
14	1	1.4	78.2	*S. cerevisiae*	Grs1	YBR121C
15	1	1.2	85.7	*S. cerevisiae*	Mes1	YGR264C

Visible GelCode Blue-stained protein bands were excised for in-gel trypsin digest, HPLC fractionation, and linear ion trap – Orbitrap hybrid tandem mass spectrometry. (**A**) Analysis of the major lower MW band identified Mlc1 (P53141) as the major component (identified from 9 unique peptides, providing 53% sequence coverage). (**B**) Analysis of the higher MW band identified Hof1 (Q05080) as the major component (from 25 unique peptides, providing ~38% sequence coverage). These proteins were confirmed as unique interactors with the Iqg1-eGFP bait by comparison of the full protein content of the experimental IP with that from a control IP with a strain lacking Iqg1 (data not shown).