**Table S1. Strains used for pooled linkage analysis and whole genome sequencing to identify *SCD1***

**A. Haploid Parents:**

|  |  |
| --- | --- |
| BJ2700 (*scd1-i*) | *MAT****a*** *leu2 ade6 his1 scd1-i* |
| BJ2738 (*scd1-v*) | *MATα ura3-52 trp1 leu2 scd1-v* |

**B. Diploid parent:** BJ2738 X 2700(*CHC1/CHC1 scd1-v/scd1-i*)

|  |  |
| --- | --- |
| BJ3068 | *MATα/****a*** *scd1-v/scd1-i ura3-52/+ trp1/+ leu2/leu2 +/ade6 +/his1 scd1-v/scd1-i* |

**C. Diploids after knockout of *CHC1* and spore segregants**

(*chc1∆:LEU2/CHC1 scd1-i/scd1-v*)

|  |  |
| --- | --- |
| **1. BJ3119** | *MATα/****a*** *ura3-52/+ trp1/+ leu2/leu2 +/ade6 +/his1 scd1-v/scd1-i chc1∆::LEU2/CHC1* |

**Spore segregants with *scd1-i* (from tetrads with 4 viable spores: 2 Chc+:2 Chc-)**

|  |  |
| --- | --- |
| BJ3240 (17a) | *MAT****a*** *CHC1 ura3-52 leu2 ade6 scd1-i* |
| BJ3243 (17d) | *MAT****a*** *CHC1 ura3-52 leu2 scd1-i* |
| BJ3244 (24a) | *MAT****a*** *CHC1 ura3-52 leu2 scd1-i* |
| BJ3246 (24c) | *MATα CHC1 ura3-52 leu2 scd1-i* |
| BJ3249 (29b) | *MAT****a*** *CHC1 ura3-52 leu2 his1 ade6 scd1-i* |
| BJ3251 (29d) | *MATα CHC1 trp1 leu2 ade6 scd1-i* |
| BJ3252 (34a) | *MAT****a*** *CHC1 ura3-52 leu2 his1 scd1-i* |
| BJ3253 (34b) | *MAT****a*** *CHC1 trp1 leu2 his1 scd1-i* |

**Spore segregants with *scd1-v* (from tetrads with 2 viable:2 dead spores)**

|  |  |
| --- | --- |
| BJ3265 (18c) | *MAT****a*** *CHC1 leu2 scd1-v scd1-v* |
| BJ3266 (18d) | *MATα CHC1 leu2 his1 ade6 scd1-v* |
| BJ3267 (14a) | *MATαCHC1 trp1 leu2 his1 ade6 scd1-v* |
| BJ3268 (14b) | *MAT****a*** *CHC1 trp1 leu2 ade6 scd1-v* |
| BJ3269 (35a) | *MAT****a*** *CHC1 trp1 leu2 his1 ade6 scd1-v* |
| BJ3270 (35d) | *MATα CHC1 ura3-52 leu2 scd1-v* |

|  |  |
| --- | --- |
| **2. BJ3120** | *MATα/****a*** *ura3-52/+ trp1/+ leu2/leu2 +/ade6 +/his1 scd1-v/scd1-i chc1∆::LEU2/CHC1* |

**Spore segregants with *scd1-i* (from tetrads with 4 viable spores: 2 Chc+:2 Chc-)**

|  |  |
| --- | --- |
| BJ3223 (2a) | *MATα CHC1 leu2 ade6 scd1-i* |
| BJ3224 (2b) | *MAT****a*** *CHC1 ura3-52 leu2 trp1 ade6 scd1-i* |

**Spore segregants with *scd1-v* (from tetrads with 2 viable:2 dead spores)**

|  |  |
| --- | --- |
| BJ3236 (4a) | *MAT****a*** *CHC1 trp1 leu2 his1 scd1-v* |
| BJ3237 (4b) | *MAT****a*** *CHC1 ura3-52 leu2 his1 ade6 scd1-v* |
| BJ3238 (10a) | *MATα CHC1 ura3-52 leu2 his1 ade6 scd1-v* |
| BJ3239 (10b) | *MATα CHC1 ura3-52 leu2 scd1-v* |

**Table S2: Yeast strains used in this study**

|  |  |
| --- | --- |
| SL Strain No. | Genotype |
| 15 | *MAT****a*** *ura3-52 trp1 GAL2 scd1-v chc1∆:LEU2 YCp50-CHC1* |
| 98 | *MAT****α*** *ura3-52 his1 ade6 GAL2 scd1-i chc1∆:LEU2 YCp50-CHC1* |
| 214 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2* |
| 350 | *MAT****a*** *GAL1:CHC1 scd1-v leu2 ura3-52 trp1 ade6 GAL2* |
| 5311 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SLA1-GFP:His3MX6 ABP1-RFP:KanMX6* |
| 5755 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v EDE1-GFP:His3MX6 ABP1-RFP:KanMX6* |
| 5806 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SYP1-GFP:His3MX6 ABP1-RFP:KanMX6* |
| 5927 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SLA2-GFP:TRP1 ABP1-RFP:KanMX6* |
| 7098 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 pal2∆:NatMX6* |
| 7099 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 pal2∆:NatMX6* |
| 7249 | *MAT****a*** *GAL1:CHC1 scd1-v leu2 ura3-52 trp1 ade6 GAL2 pal2∆:NatMX6* |
| 7251 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pal2∆:NatMX6* |
| 7255 | *MAT****α*** *leu2 ura3-52 trp1 his3-200 GAL2 scd1-v pal1∆:KanMX6* |
| 7261 | *MAT****a*** *GAL1:CHC1 scd1-v leu2 ura3-52 trp1 ade6 GAL2 pal1∆:KanMX6* |
| 7278 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pRS426* |
| 7279 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pRS426-PAL2* |
| 7280 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pal2∆:NatMX6 pRS426* |
| 7281 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pal2∆:NatMX6 pRS426-PAL2* |
| 7295 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SLA2-GFP:TRP1 ABP1-RFP:KanMX6 pal2∆:NatMX6* |
| 7298 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SYP1-GFP:His3MX6 ABP1-RFP:KanMX6 pal2∆:NatMX6* |
| 7301 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SLA1-GFP:His3MX6 ABP1-RFP:KanMX6 pal2∆:NatMX6* |
| 7330 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 lys2∆801GAL2 scd1-v EDE1-GFP:His3MX6 ABP1-RFP:KanMX6 pal2∆::NatMX6* |
| 7335 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL1-3x-GFP:KanMX6* |
| 7400 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL1-3xGFP:KanMX6 pal2∆:NatMX6* |
| 7401 | *MAT****a*** *bar1 leu2 ura3-52 his3∆200 GAL2 PAL2-GFP:NatMX6 yap1801∆:URA3 yap1802∆:KanMX6* |
| 7405 | *MAT****a*** *leu2 ura3-52 his3∆200 GAL2 scd1-v PAL2-GFP:NatMX6 syp1∆:KanMX6* |
| 7411 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6 ABP1-RFP:KanMX6* |
| 7442 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL1-GFP:NatMX6* |
| 7444 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pRS316* |
| 7447 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pal2∆:NatMX6 pRS316* |
| 7448 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pal2∆:NatMX6 pRS316-PAL2* |
| 7451 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6 chc1∆:LEU2* |
| 7455 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6* |
| 7459 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL1-3XGFP:KanMX6*  *PAL2-mCherry:His3MX6* |
| 7464 | *MAT****α*** *GAL1:CHC1 scd1-i leu2 ura3-52 trp1 ade6 GAL2 pRS316-PAL2* |
| 7468 | *MAT?? leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL1-3x-GFP:KanMX6 chc1∆:LEU2* |
| 7473 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SYP1-GFP::His3MX6 ABP1-RFP:KanMX6 pal2∆:NatMX6 pal1∆:URA3* |
| 7475 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6 pal1∆:URA3* |
| 7476 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SLA1-GFP:His3MX6 ABP1-RFP:KanMX6 pal2∆:NatMX6 pal1∆:URA3* |
| 7479 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6 SLA2-RFP:His3MX6* |
| 7483 | *MATb leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6 EDE1-mCherry:KanMX6* |
| 7487 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 PAL2-GFP:NatMX6 END3-mCherry:KanMX6* |
| 7497 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v SLA2-GFP:TRP1 ABP1-RFP:KanMX6 pal2∆:NatMX6 pal1∆:URA3* |
| 7500 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 lys2∆801GAL2 scd1-v EDE1-GFP:His3MX6 ABP1-RFP:KanMX6 pal2∆:NatMX6 pal1∆:URA3* |
| 7502 | *MAT****α*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL1-3x-GFP:KanMX6 ede1∆:His3MX6* |
| 7505 | *MAT****a*** *leu2 ura3-52 trp1 his3∆200 GAL2 scd1-v PAL2-GFP:NatMX6 ede1∆:His3MX6* |
| 7579 | *MAT****a*** *leu2 ura3-52 TRP+ his3∆200 GAL2 scd1-v PAL1-3x-GFP:KanMX6 syp1∆:KanMX6* |
| 7581 | *MAT****a*** *bar1 leu2 ura3-52 TRP+ his3∆200 GAL2 scd1-v PAL1-3x-GFP:KanMX6*  *yap1801∆:URA3 yap1802∆:KanMX6* |

**Table S3: Plasmids generated for or used in this study**

|  |  |
| --- | --- |
| Plasmid description | Source |
| YCp50-*CHC1 (CEN, URA3; CHC1)* | 1 |
| pRS316-*PAL2 (CEN, URA3; PAL2)* | This study |
| pRS426*-PAL2 (2µ, URA3; PAL2)* | This study |
| pET28c*-EDE1(EH1-3)* | Gift from B. Wendland |
| pET22c*-GFP* | Gift from D.M. Patel, F. Zhang. |

1. Lemmon, S. K. and E. W. Jones (1987). "Clathrin requirement for normal growth of yeast." Science **238**(4826): 504-509.

**Table S4: *pal1∆* is not synthetic lethal with *chc1∆***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Diploid genotype  *pal1∆* x *chc1∆*  SL7255 x SL15 | No. of tetrads at ratio of viable to dead spores | | | No. of spores at phenotype | | | |
| 4:0 | 3:1 | 2:2 | Leu-Kan± | Leu+Kan+ | Leu+Kan - | Dead |
| *pal1∆:KanMX6/PAL1*  *CHC1/chc1∆:LEU2* | 7 | 7 | 0 | 23 | 15 | 11 | 7 |

A *CHC1 scd1-v pal1∆:KanMx6* strain (SL7255) was crossed to *chc1∆:LEU2 scd1-v YCp50-CHC1* (SL15)*.* Then the *YCp50-CHC1* plasmid was dropped, and the heterozygous diploid was sporulated and subjected to tetrad dissection. Data in the table represent the number of tetrads with ratio of viable to dead spores and number of spores with different phenotypes. There was some random spore death, but inferred genotypes of these inviable spores were both *CHC1* and *chc1∆.* Also, the majority of the *chc1∆* spores survived with or without *pal1∆:KanMx6* (Leu+Kan+ or Leu+Kan-).