

Supplementary Materials for

Genetic regulation of mitotic competence in G₀ quiescent cells

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Fig. S1. Fluorescence images of nitric oxide (green, DAF-FM DA) and vacuoles (red, FM4-64) and merged images of indicated strains.

Fig. S2. A low-mobility band of Ned1-FLAG is phosphorylated.

Fig. S3. Electron micrograph of $\Delta SPBC902.03$ at 24 hours after -N.

Fig. S4. *ned1-264* showed MC loss and deformed nuclei, which were rescued by deletions of autophagy genes.

Fig. S5. Scheme of two screening procedures.

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/8/eaat5685/DC1)

Table S1 (Microsoft Excel format). Table of 85 GZE genes.

Table S2 (Microsoft Excel format). List of cancer-related references for 85 GZE genes.

Table S3 (Microsoft Excel format). Strains that failed to backcross or grow.

Fig. S1

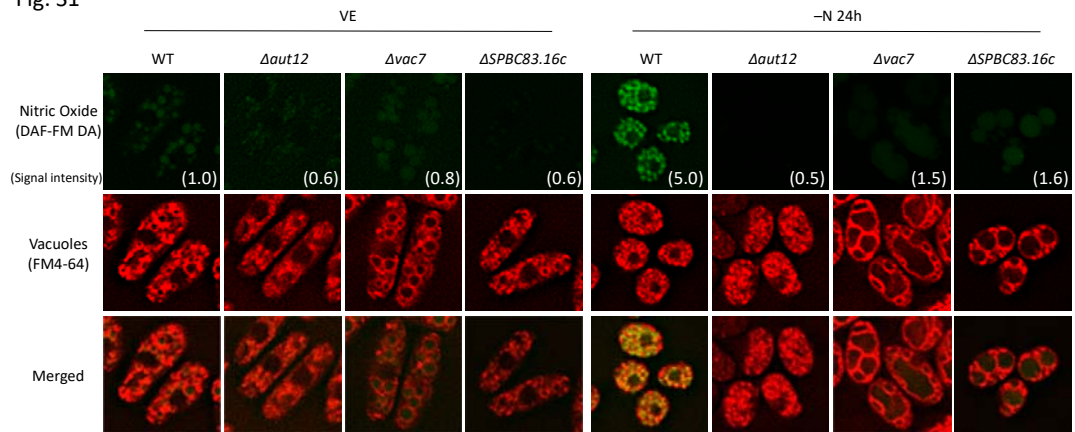


Fig. S1. Fluorescence images of nitric oxide (green, DAF-FM DA) and vacuoles (red, FM4-64) and merged images of indicated strains. Left columns show VE cells, and right columns show cells 24 hours after -N. Signal intensity of DAF-FM DA was quantified and shown in the images, proportional to WT VE cells.

Fig. S2

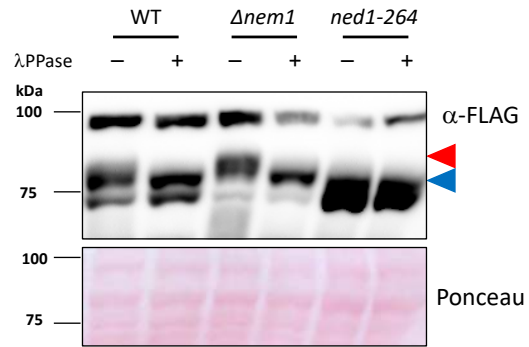


Fig. S2. A low-mobility band of Ned1-FLAG is phosphorylated. Western blot analysis of Ned1-FLAG-tagged strains of WT, $\Delta nem1$ and $ned1-264$ was performed in a 6% Phos-tag gel. Samples were prepared from VE cells, treated with (+) or without (–) lambda protein phosphatase (λ PPase). A low-mobility band (red arrowhead) increased its electrophoretic mobility (blue arrowhead) after λ PPase treatment.

Fig. S3

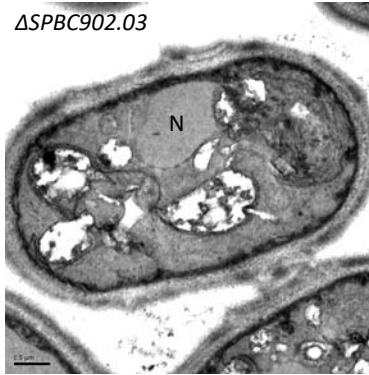


Fig. S3. Electron micrograph of *ΔSPBC902.03* at 24 hours after -N. N, nucleus.

Fig. S4

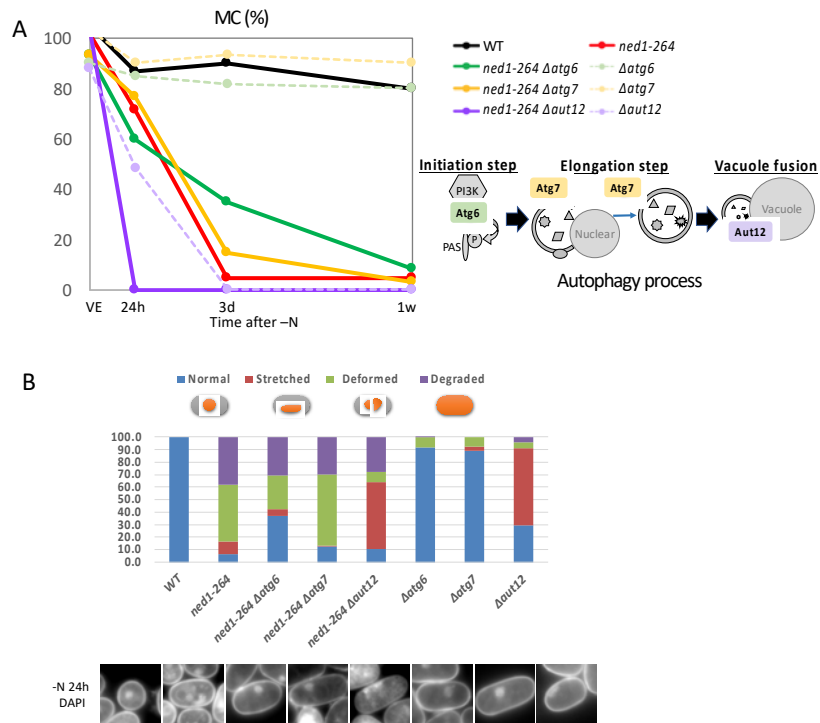


Fig. S4. *ned1-264* showed MC loss and deformed nuclei, which were rescued by deletions of autophagy genes. (A) MC graphs of the indicated strains. Diagram showing autophagy processes associated with these strains. (B) Percentages of DAPI-stained nuclear shapes found in indicated strains and DAPI images 24 hours after -N. 200 cells for each strain were counted.

Fig. S5

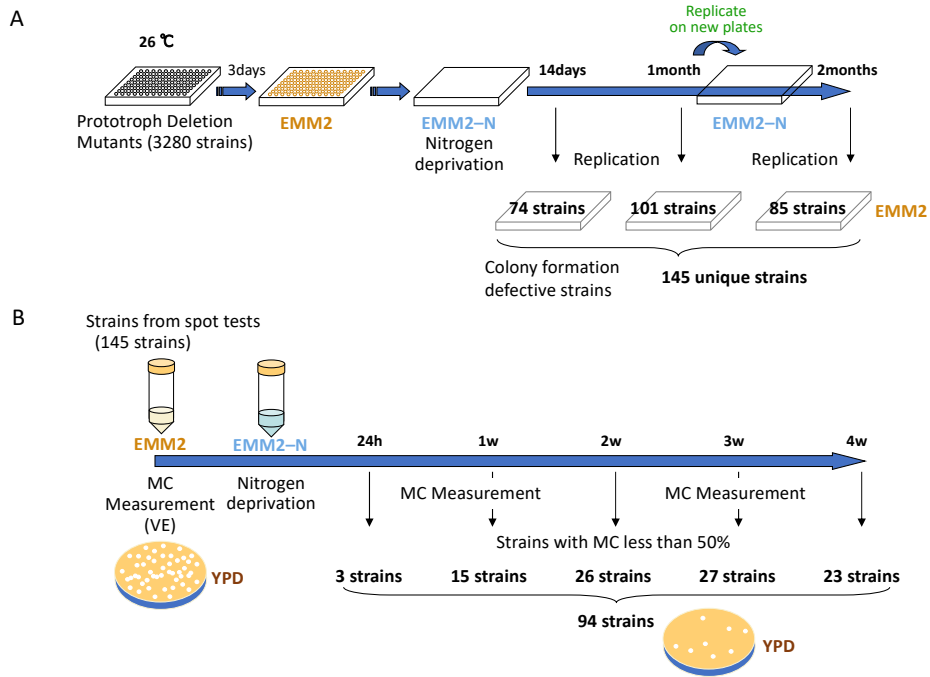
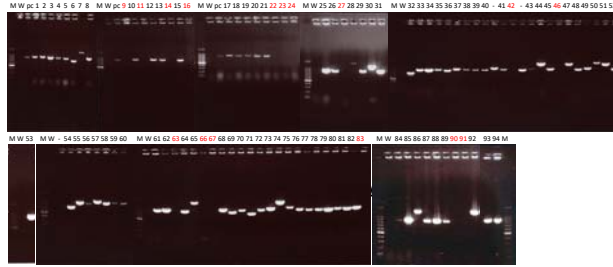


Fig. S5. Scheme of two screening procedures. (A) Spot tests of 3280 prototrophic deletion strains. Colony formation ability was tested after -N on agar plates for a maximum of 2 months. (B) MC measurements of selected strains from spot tests. MC was measured after -N using liquid medium for a maximum of 4 weeks.

Fig. S6

A 5' end check
cp5 – CPN1

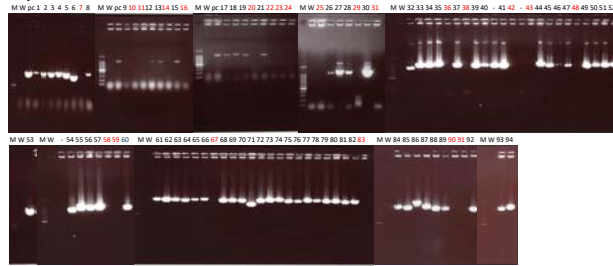


cp5 – CPN1



PCR No.	Gene ID	Gene Name	PCR No.	Gene ID	Gene Name	PCR No.	Gene ID	Gene Name	PCR No.	Gene ID	Gene Name
1	SPAC10F6.16	msg134	25	SPAC13G5.04	lys2	49	SPBC18E5.04	rgt1001	73	SPBC647.15c	ndc1
2	SPAC11D3.15	SPAC11D3.15	26	SPAC3A12.13c	hxt1	50	SPBC19H10.01	vac4	74	SPBC687.13	vac7
3	SPAC11H11.01	ssg1	27	SPAC3E10.17	hvt1	51	SPBC215.14c	vps20	75	SPBC687.16c	dap2
4	SPAC144.02	scf1	28	SPAC4F10.04	yca1	52	SPBC21C3.02c	dep1	76	SPCC11E10.06c	epd4
5	SPAC144.06c	spc1	29	SPAC4F10.07c	spg13	53	SPBC12C3.03	SPBC12C3.03	77	SPCC11E10.08	rk1
6	SPAC148B.01	spc2	30	SPAC4F8.01	dtd4	54	SPBC21D16.16	mhf1	78	SPCC1393.08	SPCC1393.08
7	SPAC17A5.14	exo2	31	SPAC4G9.13c	vps26	55	SPBC23G2.03c	hst1	79	SPCC1612.06c	asp1
8	SPAC17G8.06c	hst1	32	SPAC47A15.14	spg11	56	SPBC365.16	SPBC365.16	80	SPCC16C4.20c	hsc2
9	SPAC17G8.05	mes20	33	SPAC47A12	ssr1	57	SPBC388.10c	nem1	81	SPCC1739.07	chl1
10	SPAC17G8.06c	SPAC17G8.06c	34	SPAC48B.02	spg18	58	SPBC389.06c	als3	82	SPCC188.02	par1
11	SPAC18G2.08c	SPAC18G2.08c	35	SPAC49.05	hsp7	59	SPBC389.11c	chl1	83	SPCC248H10.06	SPCC248H10.06
12	SPAC183.16c	vht1	36	SPAC49.09	SPAC49.09	60	SPBC389.13c	ppg102	84	SPCC248H10.11c	hsp7
13	SPAC184.03c	aur12	37	SPAC49.04c	SPAC49.04c	61	SPBC428.04	spg12	85	SPCC264.05	vps3
14	SPAC184.02c	shu1	38	SPAC49.11.02c	dph3	62	SPBC428.10c	atg5	86	SPCC263.04c	occp
15	SPAC20G8.10c	atg6	39	SPAC49.13c	zwf16	63	SPBC446.06	ket1	87	SPCC262.12c	gph1
16	SPAC22F8.12c	spg1	40	SPAC49.14.01.14	spg15	64	SPBC462.11	meb6	88	SPCC263.14c	tpg63
17	SPAC22H12.02	flg3	41	SPAP4691.02	SPAP4691.02	65	SPBC477.04	SPBC477.04	89	SPCC274.02c	pen1
18	SPAC23D3.09	spg2	42	SPBC1347.08c	SPBC1347.08c	66	SPBC484.13	spg23	90	SPCC283.09	mgp4
19	SPAC23A8.01c	hls	43	SPBC13G1.09c	als3	67	SPBC484.15c	spg16	91	SPCC172.09c	rcf1
20	SPAC23A8.02	atg14	44	SPBC13G1.12	dtd2	68	SPBC481.05c	atg7	92	SPCC177.13	vps35
21	SPAC23A3.04	rgp2002	45	SPBC16B.01	pmg1	69	SPBC725.01	SPBC725.01	93	SPCC270.05	rgp561
22	SPAC23A3.07c	rgp1101	46	SPBC16G8.11	us3201	70	SPBC83.16c	SPBC83.16c	94	SPCPIE11.05c	are2
23	SPAC23A4.18	prn1	47	SPBC1711.04	msd1	71	SPBC83.19c	SPBC83.19c			
24	SPAC31F12.01	zst1	48	SPBC1718.03	ket1	72	SPBC902.03	SPBC902.03			

B 3' end check
cp3 – CPC3



cp3 – CPC1



PCR No.	Gene ID	Gene Name	PCR No.	Gene ID	Gene Name	PCR No.	Gene ID	Gene Name
1	SPAC10F6.16	msg134	33	SPAC57A7.12	ssr1	65	SPBC577.04	SPBC577.04
2	SPAC11D3.15	SPAC11D3.15	34	SPAC58B.07c	atg1801	66	SPBC586.13	spg23
3	SPAC11H11.01	ssg1	35	SPAC59.05	zsp7	67	SPBC648.15c	spg16
4	SPAC144.02	scf1	36	SPAC59.09	SPAC59.09	68	SPBC681.05c	atg7
5	SPAC144.06c	spc1	37	SPAC59M.04c	SPAC59M.04c	69	SPBC725.01	SPBC725.01
6	SPAC148B.01	spc2	38	SPAC6E11.02c	spg3	70	SPBC81.19c	SPBC81.19c
7	SPAC17A5.14	exo2	39	SPAC9.13c	zwf16	71	SPBC83.19c	SPBC83.19c
8	SPAC17G8.06c	hst1	40	SPAP1A10.14	spg15	72	SPBC92.03	SPBC92.03
9	SPAC17G8.05	mes20	41	SPAP4691.02	SPAP4691.02	73	SPBC47.15c	msg1
10	SPAC17G8.06c	SPAC17G8.06c	42	SPBC1347.08c	SPBC1347.08c	74	SPBC687.13	vac7
11	SPAC18G2.08c	SPAC18G2.08c	43	SPBC13G1.09c	als3	75	SPBC687.16c	dap2
12	SPAC183.16c	vht1	44	SPBC13G1.12	dtd2	76	SPCC11E10.06c	sp4
13	SPAC184.03c	aur12	45	SPBC16B5.01	pmg1	77	SPCC11E10.08	rk1
14	SPAC184.02c	shu1	46	SPBC16G8.11	us3201	78	SPCC1393.08	SPCC1393.08
15	SPAC20G8.10c	atg6	47	SPBC1711.04	msd1	79	SPCC1672.06c	asp1
16	SPAC22F8.12c	spg1	48	SPBC1718.03	ket1	80	SPCC16C4.20c	hsc2
17	SPAC22H12.02	flg3	49	SPBC18E5.04	rgt1001	81	SPCC1739.07	chl1
18	SPAC23D3.09	spg2	50	SPBC18H10.07	atp2	82	SPCC188.02	par1
19	SPAC23A8.01c	hls	51	SPBC215.14c	vps20	83	SPCC248H10.06	SPCC248H10.06
20	SPAC23A8.02	atg14	52	SPBC21C3.02c	dep1	84	SPCC248H10.11c	hsp7
21	SPAC23A3.04	rgp2002	53	SPBC21C3.03	SPBC21C3.03	85	SPCC264.05	vps3
22	SPAC23A3.07c	rgp1101	54	SPBC21D16.16	mhf1	86	SPCC263.04c	occp
23	SPAC23A4.18	prn1	55	SPBC22D.03c	hst1	87	SPCC262.12c	gph1
24	SPAC31F12.01	zst1	56	SPBC365.16	SPBC365.16	88	SPCC263.14c	tpg63
25	SPAC31F12.01	zst1	57	SPBC388.10c	nem1	89	SPCC274.02c	pen1
26	SPAC31A12.13c	hxt1	58	SPBC389.06c	als3	90	SPCC274.09	mgp4
27	SPAC3F10.17	hvt1	59	SPBC389.11c	chl1	91	SPCC275.09c	rcf1
28	SPAC4E10.04	spg1	60	SPBC389.13c	ppg102	92	SPCC177.13	vps35
29	SPAC4F10.07c	spg13	61	SPBC428.04	spg12	93	SPCC270.05	rgp561
30	SPAC4F8.01	dtd4	62	SPBC428.10c	atg5	94	SPCPIE11.05c	are2
31	SPAC49.13c	spg15	63	SPBC446.06	ket1			
32	SPAC57A15.14	spg11	64	SPBC462.11	meb6			

Fig. S6. PCR confirmation of gene deletion. M: Molecular weight DNA ladder. W: wild-type control. pc: positive control. **(A)** Deletion check of the 5' end. Strains that failed to amplify with the first primer set (cp5-CPN10) were also tested with the second primer set (cp5-CPN1). Nine strains (11, 14, 22, 23, 27, 42, 67, 83, 90) failed to amplify with both primer sets, and they were removed from the list. **(B)** Deletion check of the 3' end. Strains that failed to amplify with the first primer set (cp3-CPC3) were also tested with the second primer set (cp3-CPC1). There were eight strains (11, 14, 22, 23, 42, 67, 83, 90) that failed to amplify with both primer sets.

Table S1. Table of 85 GZE genes. Columns show classes, functional groups, strain names, human and budding yeast orthologs, MC (%) at the indicated time points, average cell length 24 hours after –N, cell number increment 24 hours after –N and major DNA peaks in VE and 24 hours after –N measured by FACS.

Table S2. List of cancer-related references for 85 GZE genes. More than 40% (36 of 85) of these genes are implicated in the etiology of various cancers.

Table S3. Strains that failed to backcross or grow. There were 175 unique strains excluded for viability failure (179 strains were listed, but 4 strains were duplicates of other versions), which is about 5% of all 3280 strains.