

Table S1. The strains and plasmids used in this study.

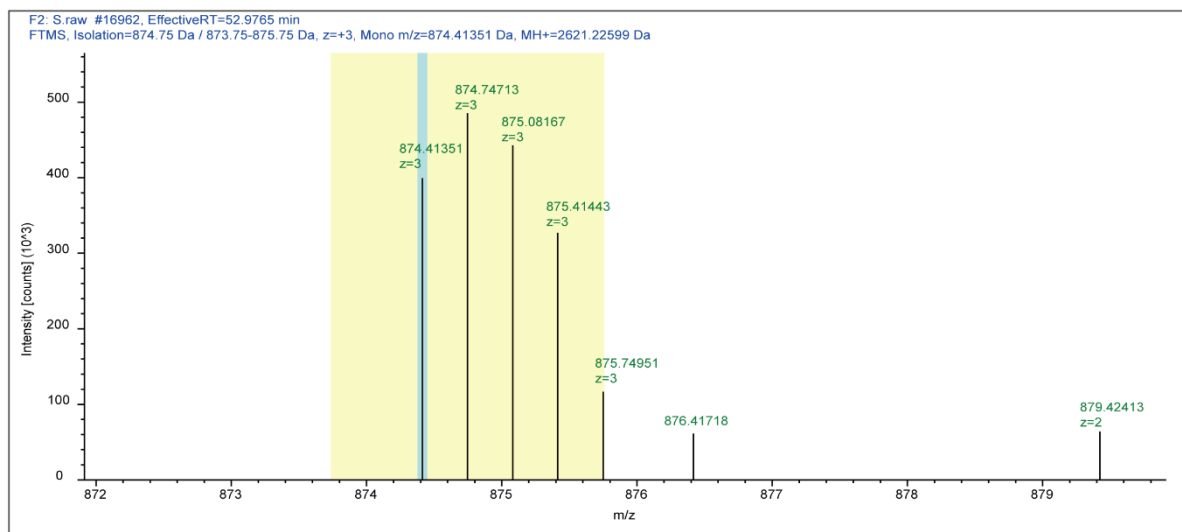
Strain/plasmid	Relevant characteristic(s)	source
<i>S. cerevisiae</i>		
BY4742	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Lab stock
BY4742 <i>Δcys3</i>	<i>Δcys3::loxp</i>	This study
BY4742 <i>Δcys3 Δyca1</i>	<i>Δcys3::loxp Δyca1::loxp</i>	
BY4742 <i>BIR1-GFP</i>	<i>BIR1-GFP(S65T)-HIS3</i>	This study
BY4742 <i>Δcys3 BIR1-GFP</i>	<i>Δcys3::loxp BIR1-GFP(S65T)-HIS3</i>	This study
<i>E. coli</i>		
DH5α	<i>supE44 ΔlacU169(Φ80dlacZΔM15) hsdR17 recA1 endA1</i> <i>gyrA96 thi-1 relA1</i>	Lab stock
BL21(DE3)	<i>F-ompT hsdSB (rB-mB-) gal (λ1857 ind1 Sam7 nin5 lacUV5</i> <i>T7gene1) dcm.</i>	Lab stock
Plasmid		
pUG6	Template plasmid	Lab stock
pFA6a-GFP(S65T)-His3MX6	Template plasmid	Lab stock
pET21b	Expression plasmid in <i>E. coli</i>	Lab stock
pET15b	Expression plasmid in <i>E. coli</i>	Lab stock
pET21b- <i>YCA1</i>	<i>YCA1</i> in pET21b, control by IPTG-induced lac promoter	This study
pET15b- <i>BIR1</i>	<i>BIR1</i> in pET15b, control by IPTG-induced lac promoter	This study

Table S2. List of gene with significant changes in transcription levels.

	gene	Log ₂ (Fold change)	p-value
	<i>spl2</i>	2.5752	1.72E-62
	<i>pho84</i>	2.5095	1.56 E-12
	<i>ydr316w-b</i>	2.473	0.0023443
	<i>met17</i>	2.3628	3.46 E-16
	<i>met14</i>	2.3466	2.55 E-10
	<i>pho12</i>	2.2886	2.86E-141
	<i>ddr2</i>	2.2731	6.47E-45
	<i>met3</i>	2.1144	4.20E-286
	<i>ylr466c-b</i>	2.0996	2.46E-20
	<i>pho89</i>	2.057	3.59E-94
	<i>sul2</i>	1.9295	9.64E-210
	<i>tsl1</i>	1.9015	1.36E-133
	<i>dpi8</i>	1.8684	3.06E-26
	<i>met5</i>	1.7585	4.25E-255
	<i>yfl064c</i>	1.7105	8.39E-06
	<i>vtc3</i>	1.6632	1.74E-287
	<i>hsp82</i>	1.6142	5.54E-22
	<i>hsp42</i>	1.5641	2.99E-53
	<i>pho11</i>	1.5456	1.14E-27
Up-regulated	<i>met10</i>	1.5018	5.01E-178
	<i>pho5</i>	1.4952	2.32E-106
	<i>mht1</i>	1.4498	2.87E-47
	<i>yfl065c</i>	1.4458	0.00063113
	<i>str3</i>	1.3663	3.57E-28
	<i>ylr108c</i>	1.3193	6.33E-67
	<i>glk1</i>	1.3106	1.69E-122
	<i>gsy1</i>	1.275	1.92E-42
	<i>met16</i>	1.2458	1.21E-44
	<i>hsp12</i>	1.2345	0.00013133
	<i>yrf1-1</i>	1.2283	1.59E-09
	<i>hsp78</i>	1.2188	9.10E-47
	<i>yol162w</i>	1.215	0.0029863
	<i>phm6</i>	1.2129	7.08E-16
	<i>yll066c</i>	1.2091	4.59E-44
	<i>whi2</i>	1.1887	7.20E-136
	<i>mmp1</i>	1.1862	1.81E-87
	<i>mup1</i>	1.182	3.47E-167
	<i>ypr204w</i>	1.1734	1.07E-18
	<i>btn2</i>	1.1629	1.93E-29
	<i>cyc7</i>	1.1489	1.85E-05
	<i>hsp104</i>	1.1481	4.13E-64

	<i>opt1</i>	1.1402	4.73E-127
	<i>yfl067w</i>	1.1321	1.20E-12
	<i>hsp26</i>	1.1245	1.19E-39
	<i>hxx1</i>	1.1108	9.82E-52
	<i>yrf1-5</i>	1.107	1.52E-11
	<i>gad1</i>	1.0961	4.80E-50
	<i>cwp1</i>	1.0955	5.26E-136
	<i>gpm2</i>	1.0708	4.44E-08
Up-regulated	<i>pdh6</i>	1.0708	0.0037978
	<i>sfc1</i>	1.0643	0.0011762
	<i>cur1</i>	1.0489	4.74E-14
	<i>yol163w</i>	1.0487	0.0012575
	<i>seol</i>	1.0376	1.97E-05
	<i>cit1</i>	1.0268	2.15E-100
	<i>met1</i>	1.0125	1.47E-47
	<i>yfl066c</i>	1.0106	2.29E-08
	<i>sam3</i>	1.0062	6.86E-109
	<i>tis11</i>	1.0059	9.67E-14
	<i>snr189</i>	-1.0043	0.0026016
	<i>mael</i>	-1.0343	6.90E-117
	<i>aro5</i>	-1.0689	6.64E-27
	<i>arg4</i>	-1.0777	1.12E-19
	<i>ypr145c-a</i>	-1.0788	2.18E-42
	<i>ural</i>	-1.1201	2.37E-107
	<i>pbi1</i>	-1.1527	1.52E-129
	<i>bsc1</i>	-1.1614	1.43E-54
Down-regulated	<i>prm7</i>	-1.2831	4.82E-30
	<i>snr35</i>	-1.3268	9.56E-06
	<i>cpa2</i>	-1.5119	6.04E-22
	<i>arg3</i>	-1.5172	8.96E-25
	<i>arg1</i>	-1.553	1.62E-28
	<i>srd1</i>	-1.6645	9.09E-60
	<i>tf(gaa)h1</i>	-1.8982	0.010531
	<i>pau9</i>	-2.0243	0.00029412
	<i>ybl107w-a</i>	-3.6909	0.0035597
	<i>tk(cuu)f</i>	-4.2844	0.013111

A



B

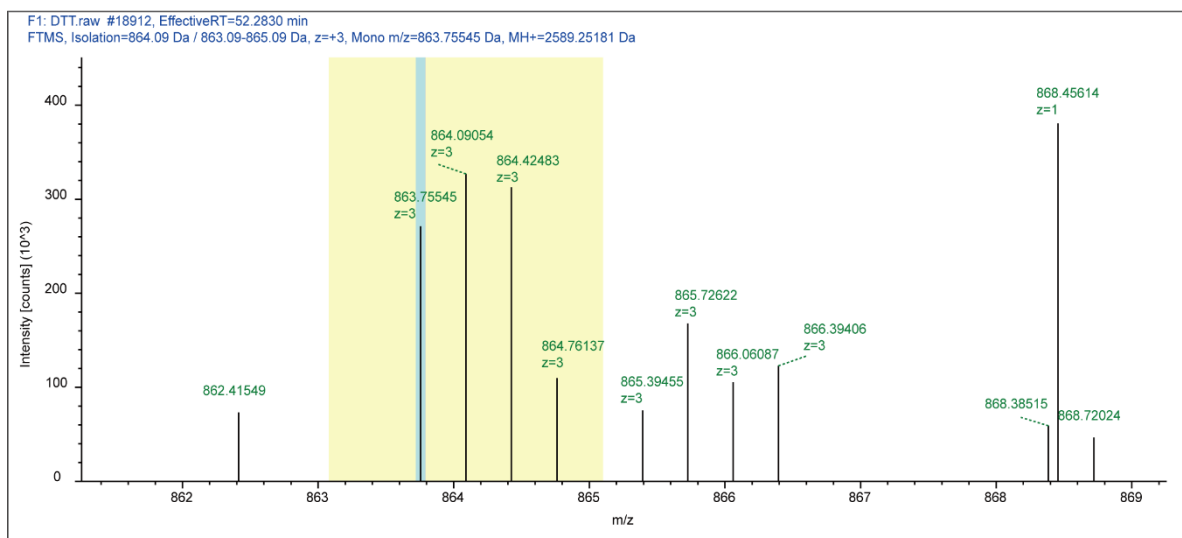


Figure S1. MS spectra of the Cys₂₇₆ containing peptide from YCA1. (A) The peptide from HS_nH-reacted YCA1. (B) The peptide from DTT-reacted YCA1. “Z” represents the charge carried by the detected peptide segment. The molecular weights marked in blue indicate the detected values. The actual molecular weight is obtained by multiplying the detected molecular weight by the charge number.

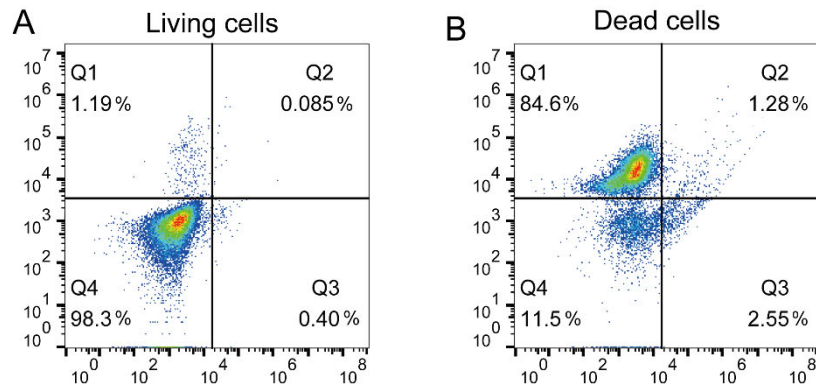


Figure S2. Flow cytometry analysis of BY4742 living and dead cells. A total of 20,000 stained cells were analyzed using flow cytometry to classify them into four quadrants. The cells underwent V-FITC and PI double staining. Viable cells (A) consisted of logarithmically growing yeast cells, while dead cells (B) were yeast cells killed by high temperature.