SUPPORTING INFORMATION

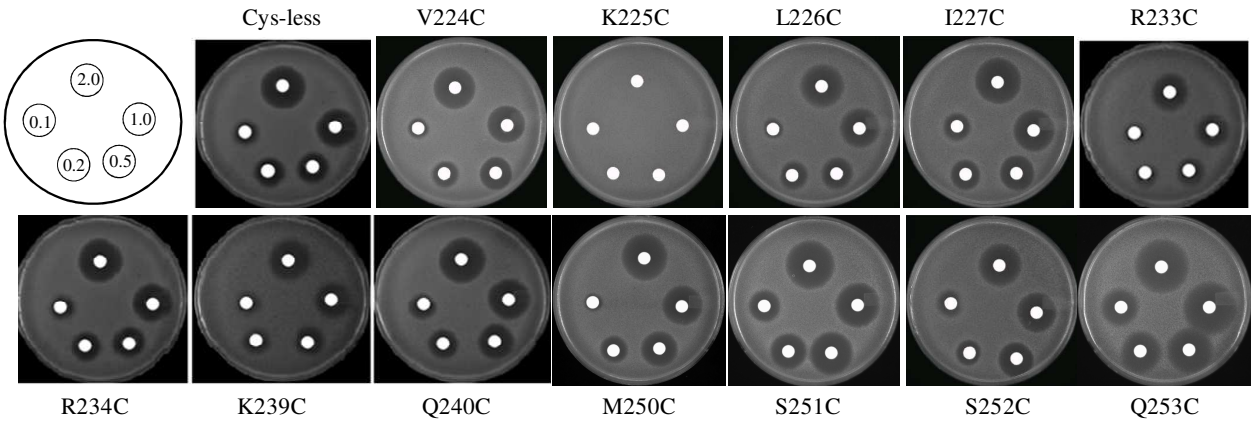
Table S1: A summary of percentage dimer foramation in receptors containing the IL3 Cys mutations. The percentage of Dimer was calculated by dividing the dimer band intensity by the total (dimer plus monomer) intensity multipled by 100%.

Ste2p IL3 Cys Mutant	Percentage of Dimer formation (%)[a]		
	No treatment	CuP treatment	α-factor and CuP
Cys-less	2.3	2.5	2.6
V224C	2.1	2.7	2.5
K225C	2.5	7.6	3.0
L226C	2.4	2.9	2.5
I227C	3.1	8.7	4.7
L228C	4.4	9.7	5.3
A229C	2.8	9.3	3.5
I230C	7.5	15.5	8.3
R231C	7.8	35.6	26.3
S232C	18.1	45.6	40.3
R233C	23.7	85.7	86.7
R234C	16.7	65.1	68.3
F235C	11.1	60.3	61.6
L236C	22.6	94.8	96.2
G237C	9.8	53.9	56.4

L238C	9.3	55.6	56.2
K239C	26.2	92.5	94.2
Q240C	8.9	55.4	54.1
F241C	12.5	22.6	18.6
D242C	11.4	15.8	14.7
S243C	6.5	11.6	9.4
F244C	5.5	11.4	12.0
H245C	5.3	23.2	17.5
I246C	7.1	30.8	29.3
L247C	6.4	22.5	7.1
L248C	2.8	11.5	2.9
I249C	3.3	12.6	3.6
M250C	4.5	15.8	5.2
S251C	3.1	21.5	6.2
S252C	2.5	3.2	2.8
Q253C	4.1	22.0	4.8

[a]Each measurement was done several times. The differences between measurements for any one receptor and any one condition were not more than 15% among duplicates or triplicates.

A



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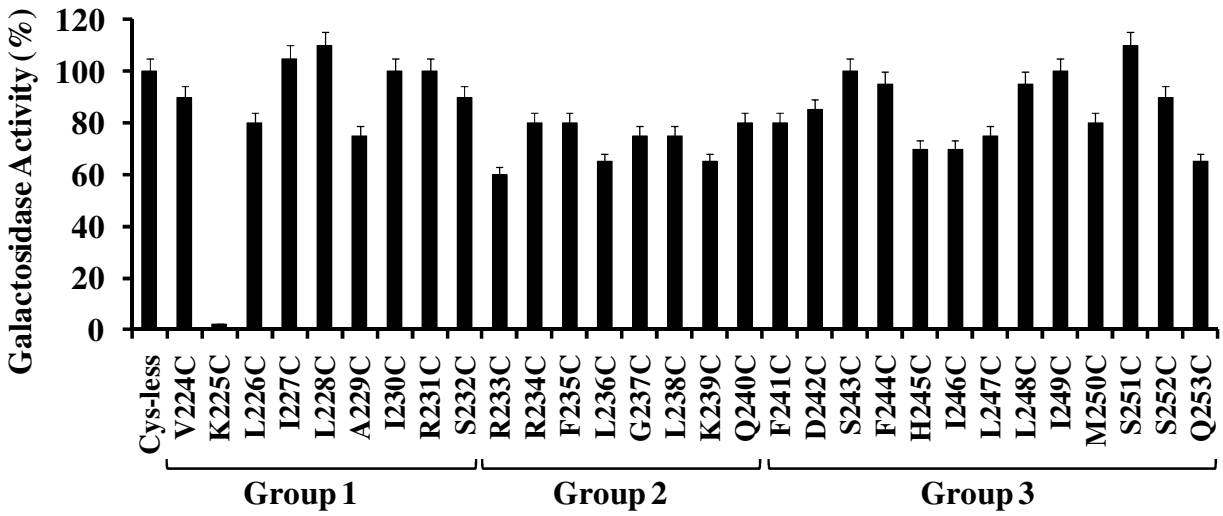


Figure S1. Phenotypes of IL3 Cys mutants. **A**: Growth arrest assay plates showing the response (halo around discs) of cells expressing the various mutants to different α-factor pheromone concentration (0.1 – 2.0 μg). Only four representatives from each group are shown. **B**: Relative (%) β-galactosidase activity of the IL3 Cys mutants in the presence of α-factor (1.0 μM).

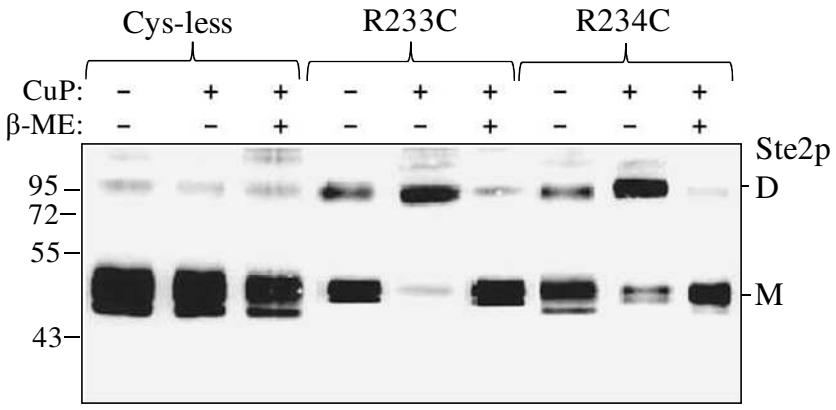
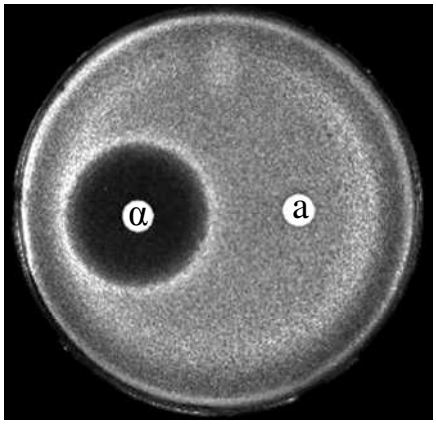


Figure S2. Dimerization of Cysless Ste2p, R233C, and R234C Ste2p mutants. Membrane samples were untreated or treated with 1.0 μM CuP reagent with or without βME (4%). M, monomer (53-55 kDa) ; D, dimer (106-110 kDa).

A



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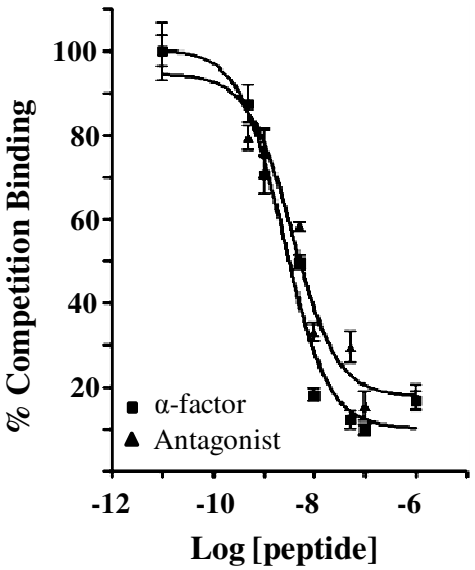

Figure S3: Growth arrest and binding activities of α-factor antagonist. **A:** Growth arrest assay plate showing the response (halo around discs) of cells expressing Ste2p to α-factor (**α**) and α-factor antagonist (**a**) at 2.0 μg concentration each. A halo is observed around the disc containing α-factor (**α**) but no halo around disc containing α-factor antagonist. **B:** Binding curves of to α-factor and α-factor antagonist. Both peptides exhibited similar binding affinity to Ste2p.