Supplementary Tables and Figures

Supplementary Table S1. List of strains and plasmids for expressing temperature sensitive *ste2* alleles

	Full-Length Multicopy		Truncated Multicopy		Full-Length-*CEN*	
Mutation	**Plasmid**	**Strain (A232)**	**Plasmid**	**Strain**	**Plasmid**	**Strain**
Wild-Type	pMD1483	A3636	pMD1422	A3365	pMD149	A3923
Vector Only	pMD1383	A3637			pMD46	A3924
A52T	pMD1384	A3638	pMD1592	A3668	pMD1725	A3950
S95Y	pMD1580	A3648	pMD1586	A3662	pMD1726	A3951
I260K	pMD1581	A3649	pMD1587	A3663	pMD1727	A3952
H94Y	pMD1582	A3650	pMD1588	A3664	pMD1728	A3953
S104Y	pMD1583	A3651	pMD1589	A3665	pMD1729	A3954
V49D	pMD1584	A3652	pMD1590	A3666	pMD1730	A3955
L211R	pMD1585	A3653	pMD1591	A3667	pMD1731	A3956
G31R	pMD1522	A3676			pMD1701	A3926
V45A	pMD1523	A3677			pMD1702	A3927
Q51P	pMD1524	A3678			pMD1703	A3928
I53V	pMD1525	A3679			pMD1704	A3929
F55L	pMD1526	A3680			pMD1705	A3930
M69K	pMD1529	A3681			pMD1708	A3933
L113R	pMD1531	A3682			pMD1710	A3935
F116S	pMD1532	A3683			pMD1711	A3936
Q135H	pMD1533	A3684			pMD1712	A3937
V152L	pMD1534	A3685			pMD1713	A3938
N158D	pMD1535	A3686			pMD1714	A3939
L164P	pMD1536	A3687			pMD1715	A3940
G174W	pMD1538	A3688			pMD1717	A3942
T179I	pMD1539	A3689			pMD1718	A3943
S207I	pMD1540	A3690			pMD1719	A3944
S213F	pMD1541	A3691			pMD1720	A3945
G237S	pMD1544	A3692			pMD1723	A3948
Q253K	pMD1545	A3693			pMD1724	A3949
F55L, M69K	pMD1657	A3848				
S207I, L113R	pMD1659	A3850				
S207I, F55L	pMD1661	A3852				
S207I, M69K	pMD1662	A3853				
S207I, G174W	pMD1663	A3854				
S213F, L113R	pMD1664	A3855				
S213F, F55L	pMD1665	A3856				
S213F, M69K	pMD1666	A3857				
S213F, G174W	pMD1667	A3858				
M69K, N158D	pMD1668	A3859				
Q51P, S207I	pMD1677	A3862				
Q135H, G174W	pMD1678	A3863				
Q135H, S207I	pMD1679	A3864				
Q51P, Q135H	pMD 1680	A3865				

Supplementary Table S2. Reconstructed plasmids containing temperature sensitive and suppressor mutations of *STE2*

Mutation		None	F38Y	V49D	A52T	H94Y	S95Y	S104Y	I260K
	Oligonucleotide		ON1472	ON1209	ON861	ON1207	ON1205	ON1208	ON1206
None		pMD1422	pMD2440	pMD1590	pMD1592	pMD1588	pMD1586	pMD1589	pMD1587
R58A	ON1454	pMD1978	pMD2087	pMD2085	pMD2086	pMD2083	pMD2081	pMD2084	pMD2082
R58G	ON1444	pMD1957	pMD2024	pMD2022	pMD2023	pMD2020	pMD2018	pMD2021	pMD2019
R58S	ON1443	pMD1956	pMD2017	pMD2015	pMD2016	pMD2013	pMD2011	pMD2014	pMD2012
A112T	ON1448	pMD1972	pMD2045	pMD2043	pMD2044	pMD2041	pMD2039	pMD2042	pMD2040
L113I	ON1450	pMD1974	pMD2059	pMD2057	pMD2058	pMD2055	pMD2053	pMD2056	pMD2054
F119L	ON1447	pMD1971	pMD2028	pMD2036	pMD2037	pMD2034	pMD2032	pMD2035	pMD2033
F119S	ON1446	pMD1970	pMD2031	pMD2029	pMD2030	pMD2027	pMD2025	pMD2028	pMD2026
L164M	ON1441	pMD1954	pMD2003	pMD2001	pMD2002	pMD1999	pMD1997	pMD2000	pMD1998
K187N	ON1451	pMD1975	pMD2066	pMD2064	pMD2065	pMD2062	pMD2060	pMD2063	pMD2061
Q200L	ON1452	pMD1976	pMD2073	pMD2071	pMD2072	pMD2069	pMD2067	pMD2070	pMD2068
M218T	ON1453	pMD1977	pMD2080	pMD2078	pMD2079	pMD2076	pMD2074	pMD2077	pMD2075
S243R	ON1470	pMD1981	pMD2108	pMD2106	pMD2107	pMD2104	pMD2102	pMD2105	pMD2103
I246N	ON1468	pMD1979	pMD2094	pMD2092	pMD2093	pMD2090	pMD2088	pMD2091	pMD2089
L248R	ON1442	pMD1955	pMD2010	pMD2008	pMD2009	pMD2006	pMD2004	pMD2007	pMD2005
P258A	ON1471	pMD1982	pMD2115	pMD2113	pMD2114	pMD2111	pMD2109	pMD2112	pMD2110
P258L	ON99	pMD2139	pMD2146	pMD1214	pMD2145	pMD2142	pMD12140	pMD2143	pMD2141
I261M	ON1449	pMD1973	pMD2052	pMD2050	pMD2051	pMD2048	pMD2046	pMD2049	pMD2047
G273D	ON1438	pMD1952	pMD1989	pMD1987	pMD1988	pMD1985	pMD1983	pMD1986	pMD1984
T279A	ON1469	pMD1980	pMD2101	pMD2099	pMD2100	pMD2097	pMD2095	pMD2098	pMD2096
T279N	ON1439	pMD1953	pMD1996	pMD1994	pMD1995	pMD1992	pMD1990	pMD1993	pMD1991

Supplementary Table S3. Reconstructed yeast strains expressing *STE2* mutations

Mutation		Strain	F38Y	V49D	A52T	H94Y	S95Y	S104Y	I260K
	Oligonucleotide		ON1472	ON1209	ON861	ON1207	ON1205	ON1208	ON1206
None		A3365	A4783	A3666	A3668	A3664	A3662	A3665	A3663
R58A	ON1454	A4372	A4512	A4472	A4492	A4432	A4392	A4452	A4412
R58G	ON1444	A4363	A4503	A4463	A4483	A4423	A4383	A4443	A4403
R58S	ON1443	A4362	A4502	A4462	A4482	A4422	A4382	A4442	A4402
A112T	ON1448	A4366	A4506	A4466	A4486	A4426	A4386	A4446	A4406
L113I	ON1450	A4368	A4508	A4468	A4488	A4428	A4388	A4448	A4408
F119L	ON1447	A4365	A4505	A4465	A4485	A4425	A4385	A4445	A4405
F119S	ON1446	A4364	A4504	A4464	A4484	A4424	A4384	A4444	A4404
L164M	ON1441	A4360	A4500	A4460	A4480	A4420	A4380	A4440	A4400
K187N	ON1451	A4369	A4509	A4469	A4489	A4429	A4389	A4449	A4409
Q200L	ON1452	A4370	A4510	A4470	A4490	A4430	A4390	A4450	A4410
M218T	ON1453	A4371	A4511	A4471	A4491	A4431	A4391	A4451	A4411
S243R	ON1470	A4375	A4515	A4475	A4495	A4435	A4395	A4455	A4415
I246N	ON1468	A4373	A4513	A4473	A4493	A4433	A4393	A4453	A4413
L248R	ON1442	A4361	A4501	A4461	A4481	A4421	A4381	A4441	A4401
P258A	ON1471	A4376	A4516	A4476	A4496	A4436	A4396	A4456	A4416
P258L	ON99	A4377	A4517	A4477	A4497	A4437	A4397	A4457	A4417
I261M	ON1449	A4367	A4507	A4467	A4487	A4427	A4387	A4447	A4407
G273D	ON1438	A4358	A4498	A4458	A4478	A4418	A4378	A4438	A4398
T279A	ON1469	A4374	A4514	A4474	A4494	A4434	A4394	A4454	A4414
T279N	ON1439	A4359	A4499	A4459	A4479	A4419	A4379	A4439	A4399

Supplementary Table S4. Oligonucleotides and plasmids for testing suppression by site-directed substitutions at R58.

Oligonucleotide			ON1472	ON861	ON1205	ON1208
	Mutation	None	F38Y	A52T	S95Y	S104Y
ON1621	R58K	pMD2495	pMD2511	pMD2507	pMD2499	pMD2503
ON1623	R58L	pMD2497	pMD2513	pMD2509	pMD2501	pMD2505
ON1622	R58Q	pMD2496	pMD2512	pMD2508	pMD2500	pMD2504
ON1620	R58W	pMD2494	pMD2510	pMD2506	pMD2498	pMD2502

Supplementary Table S5. Yeast strains for testing suppression by site-directed substitutions at R58.

Mutation	None	F38Y	A52T	S95Y	S104Y
R58K	A4813	A4829	A4825	A4817	A4821
R58L	A4815	A4831	A4827	A4819	A4823
R58Q	A4814	A4830	A4826	A4818	A4822
R58W	A4812	A4828	A4824	A4816	A4820

Supplementary Table S6. List of oligonucleotides.

Oligo	Mutation	Sequence
ON861	A52T	CACCAAACATAATGGTCTGAGTAACAGTAC
ON1120	I29K	GATCCATTCCCATATTTGGAAGTGTAGTTAA
ON1121	G31R	TGGTAGATCCATTCCTATATATGGAAGTGTA
ON1122	V45A	GTAACAGTACTGTTTGCTAAACCTTGCAACT
ON1123	Q51P	CCAAACATAATGGCCGGAGTAACAGTACTGT
ON1124	I53V	CTGACACCAAACATTACGGCCTGAGTAACAG
ON1125	F55L	CCACATCTGACACCCAGCATAATGGCCTGAG
ON1126	G60S	GTCAAAGCAGCTGCTGAACATCTGACACCAA
ON1127	T65A	CACATGACAATCAATGCCAAAGCAGCTGCAC
ON1128	M69K	CTCGATGTCATCCATTTGACAATCAAAGTCA
ON1129	R74S	GCGTTTTTCTGCTTGACGATGTCATCCACAT
ON1130	L113R	TGAGGAAATCCGGTCCTAGCGTAAGTCACTG
ON1131	F116S	GAGATGAACTGAGGTGATCCGGTGAGAGCGT
ON1132	Q135H	AGCCACAAGAAGGACATGAATTATATTTGTA
ON1133	V152L	TCGCCTGTGAAAATCAGTTTTATCTGAAACA
ON1134	N158D	CCTATCCTTTTGAAATCGTCGCCTGTGAAAA
ON1135	L164P	ATCGACGTCAGCATCGGGCCTATCCTTTTGA
ON1136	T172N	GTAGCAATCCCTAAATTGAAAGATATCGACG
ON1137	G174W	TAACTGTAGCAATCCATAAAGTGAAAGATAT
ON1138	T179I	CTTACAAAATACATTATAACTGTAGCAATCC
ON1139	S207I	GCAAGTAAAATTGTTATAGCATTGAAGTATT
ON1140	S213F	ATAAAGTTTATTGAAAATGCAAGTAAAATTG
ON1141	K225T	ATAGCTAAAATCAATGTAACTACCAGGACAA
ON1142	I227S	GATCTAATAGCTAATGACAATTTAACTACCA
ON1143	G237S	TCGAACTGCTTGAGTGAAAGGAATCTTCTTG
ON1144	Q253K	GAACCAACAAAGATTTACATGACATTATGAG
ON1205	S95Y	TTAAAATAGAGTGCATAATGCAAAATGATTA
ON1206	I260K	GCGAGGATGAATATTTTCGATGGAACCAACA
ON1207	H94Y	AATAGAGTGCAGAATACAAAATGATTAAAAA
ON1208	S104Y	ACTGAAGAGTAATTATACAGTAAATATTTAA
ON1209	V49D	ATAATGGCCTGAGTATCAGTACTGTTAACTA
ON1210	L211R	TTTATTGAGGATGCACGTAAAATTGTGCTAG
ON1436	V152A	GTCGCCTGTGAAAATAGCTTTTATCTGAAAC
ON1437	M54V	CATCTGACACCAAACACAATGGCCTGAGTAT
ON1438	G273D	CAAGACATCGGTATCCTGGTTTGGTTTCAAA
ON1439	T279N	TAATGTTGCAACATTAGTCAAGACATCGGTA
ON1440	K160I	ATCAGGCCTATCCTTATGAAGTTGTCGCCTG
ON1441	L164M	ATCGACGTCAGCATCATGCCTATCCTTTTGA
ON1442	L248R	ACATGACATTATGCGTAAAATATGGAAACTA

Supplementary Table S6. List of oligonucleotides (continued).

Oligo	Mutation	Sequence
ON1443	R58S	GCAGCTGCACCACAACTGACACCAAACATAA
ON1444	R58G	GCAGCTGCACCACATCCGACACCAAACATAA
ON1445	F119I	TCACCTCTAGAGATGATCTGAGGAAATCCGG
ON1446	F119S	TCACCTCTAGAGATGGACTGAGGAAATCCGG
ON1447	F119L	TCACCTCTAGAGATGAGCTGAGGAAATCCGG
ON1448	A112T	GGAAATCCGGTGAGAGTGTAAGTCACTGAAG
ON1449	I261M	TGCGAGGATGAACATTATCGATGGAACCAAC
ON1450	L113I	TGAGGAAATCCGGTGATAGCGTAAGTCACTG
ON1451	K187N	GTCACAATCATACCGTTAACAGCGCTTACAA
ON1452	Q200L	TTGAAGTATTTATCTAGGGTGGCACTAACAT
ON1453	M218T	ACCAGGACAAATGACGTAAAGTTTATTGAGG
ON1454	R58A	GCAGCTGCACCACAGGCGACACCAAACATAA
ON1468	I246N	GACATTATGAGTAAATTATGGAAACTATCGA
ON1469	T279A	GTAATGTTGCAACAGCAGTCAAGACATCGGT
ON1470	S243R	GAGTAAAATATGGAATCTATCGAACTGCTTG
ON1471	P258A	TGAATATTATCGATGCAACCAACAAAGATGG
ON1472	F38Y	CCTTGCAACTCATCGTAAGTGATGGTAGATC
ON1473	R58G and A52T	TGCACCACATCCGACACCAAACATAATGGTCTGAGTAAC
ON1474	R58S and A52T	TGCACCACAACTGACACCAAACATAATGGTCTGAGTAAC
ON1475	R58A and A52T	TGCACCACAAGCGACACCAAACATAATGGTCTGAGTAAC
ON1477	I261M and I260K	GTATGCGAGGATGAACATTTTCGATGGAACCAACAA
ON1478	L113I and S104Y	AAATCCGGTGATAGCGTAAGTCACTGAAGAGTAATTATACAGTAAATA
ON1620	R58W	AGCAGCTGCACCACACCAGACACCAAACATAAT
ON1621	R58K	AGCAGCTGCACCACATTTGACACCAAACATAAT
ON1622	R58Q	AGCAGCTGCACCACATTGGACACCAAACATAAT
ON1623	R58L	AGCAGCTGCACCACATAAGACACCAAACATAAT
ON1628	A52T and R58W	TGCACCACACCAGACACCAAACATAATGGTCTAAGTAAC
ON1629	A52T and R58K	TGCACCACATTTGACACCAAACATAATGGTCTGAGTAAC
ON1630	A52T and R58Q	TGCACCACATTGGACACCAAACATAATGGTCTGAGTAAC
ON1631	A52T and R58L	TGCACCACATAAGACACCAAACATAATGGTCTGAGTAAC
ON915	Forward PCR Primer	GCGGCTCCTTCATTGAGCAATCTATTTTATGATCCAACGT ATAATCCTGGTCAAAGCAC
ON918	Reverse PCR Primer	GTCTGAAGTAATTGTGTTTGTTTTGGATGCATTATTAGCA GCCGTGGCCCACATTG

Supplementary Table S7. Summary of individual mutations with temperature-sensitive phenotypes.

		Full-Length Multicopy			Truncated Multicopy		
Mutation	**Type of Screen[a]**	**Growth[b]**	**Binding[c]**	**Expression[d4]**	**Growth[b]**	**Binding[c]**	**Expression[d]**
F38Y	B				F	88	+++
V49D	G, B	pTS	80	+	TS	23	ND
H94Y	G	pTS	30	+++	F	29	++
S95Y	G	TS	<5	ND	NF	<5	+
S104Y	G	TS	26	+	NF	5.3	ND
L211R	G	TS	10	++	TS	<5	+++
I260K	G	TS	8.4	+	F	<5	ND

[a] B, Two Stage Binding Screen. G, Growth Arrest Screen

[b] F, Functional. TS, Temperature-Sensitive. pTS, Partial Temperature-Sensitive. NF, Non-Functional. CS, Cold Sensitive.

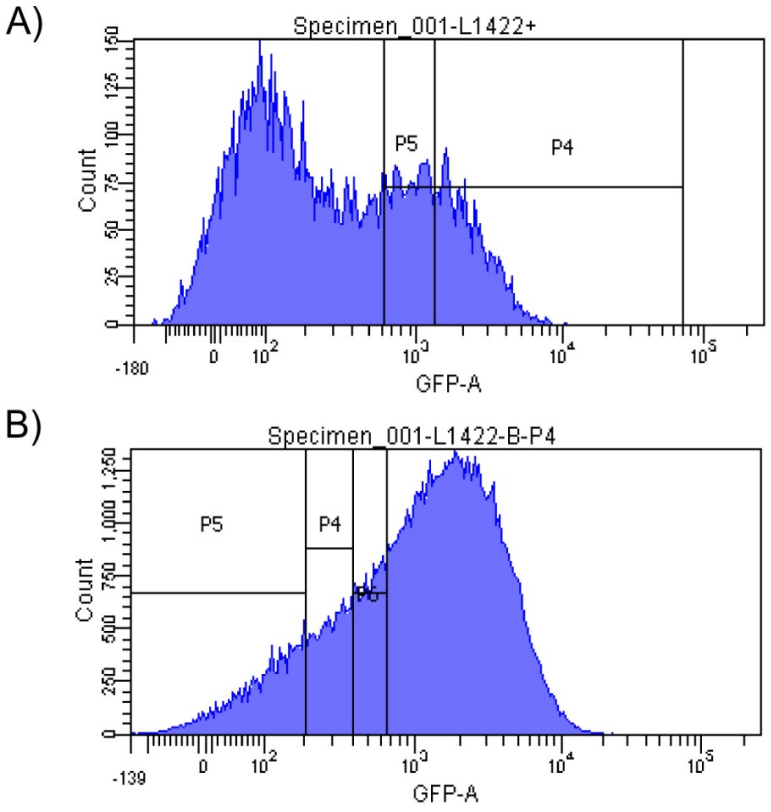
[c] Percent of Wild-type Binding Capacity (B_{maxTS}/B_{maxWT}) after growth at 24 °C.

[d] Based on immunoblotting of whole-cell extracts. ND, Not Detected. +, weak expression. ++, moderate expression, +++, good expression

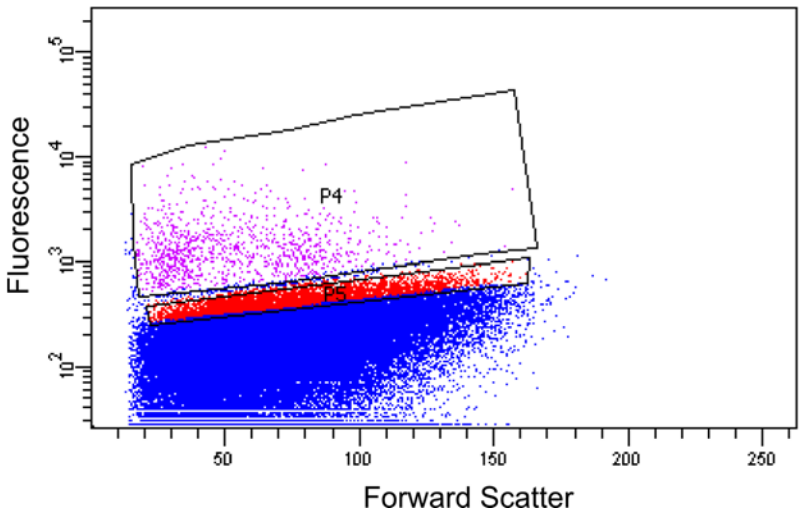
Supplementary Table S8. Mutations recovered from the suppressor library

Starting Mutation	Suppressor Mutations[a]
V49D	A176A, N300T
V49D	A212A, S254S, K304R
V49D	C59S
V49D	D242N
V49D	**F119L**
V49D	I190I, **Q200L**
V49D	K187N
V49D	K269E
V49D	L103L, Q310L
V49D	L113I
V49D	L93L, **A112T**, S145S, **I261M**
V49D	T297M, G305G
V49D	Y203Y, **M218T**, T274T
S95Y	**R58G**
S95Y	**R58G**, G129C
S95Y	**R58G**, T274T
S95Y	**R58G**, V136Y
S95Y	**R58S**, A176A
S95Y	**R58S**, G129G, T208A
S95Y	**R58S**, K77I
S95Y	S47T, **R58G**, N158N
S95Y	T50I, **R58G**
S95Y	Y13N, **R58G**, G123G, L164P, R233S, H245N
S104Y	L103L, V152A
S104Y	M54V
S104Y	R76K, **F119I**
I260K	A62A, V257V, **T279N**, T297T
I260K	G163G, R234K, **G273D**
I260K	**G273D**
I260K	I249K
I260K	L248P
I260K	**L248R**
I260K	M250L, S267C, K304E
I260K	**P258L**
I260K	**P258L**, S259S
I260K	**R58S**
I260K	**R58S**, F241F
I260K	R76R, K160I, **L164M**, S293S
I260K	S259A, S308P
I260K	T179I, **G273D**
I260K	**T279N**
I260K	**T279N**, G306D

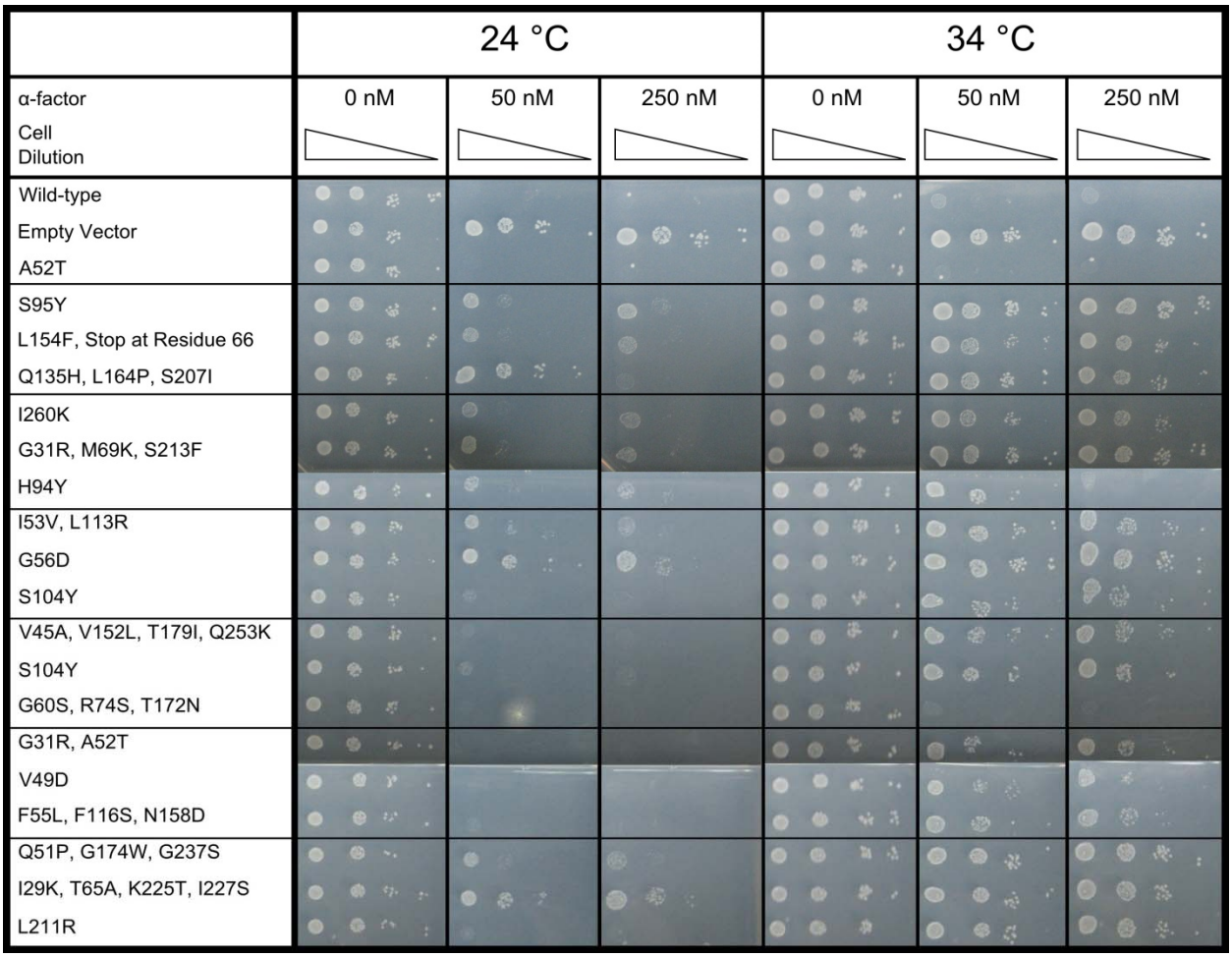
[a]Silent mutations, which are useful for determining the number of times a suppressing mutation was independently recovered, are indicated in gray, while the mutations selected for further study as potential suppressors are highlighted in bold.



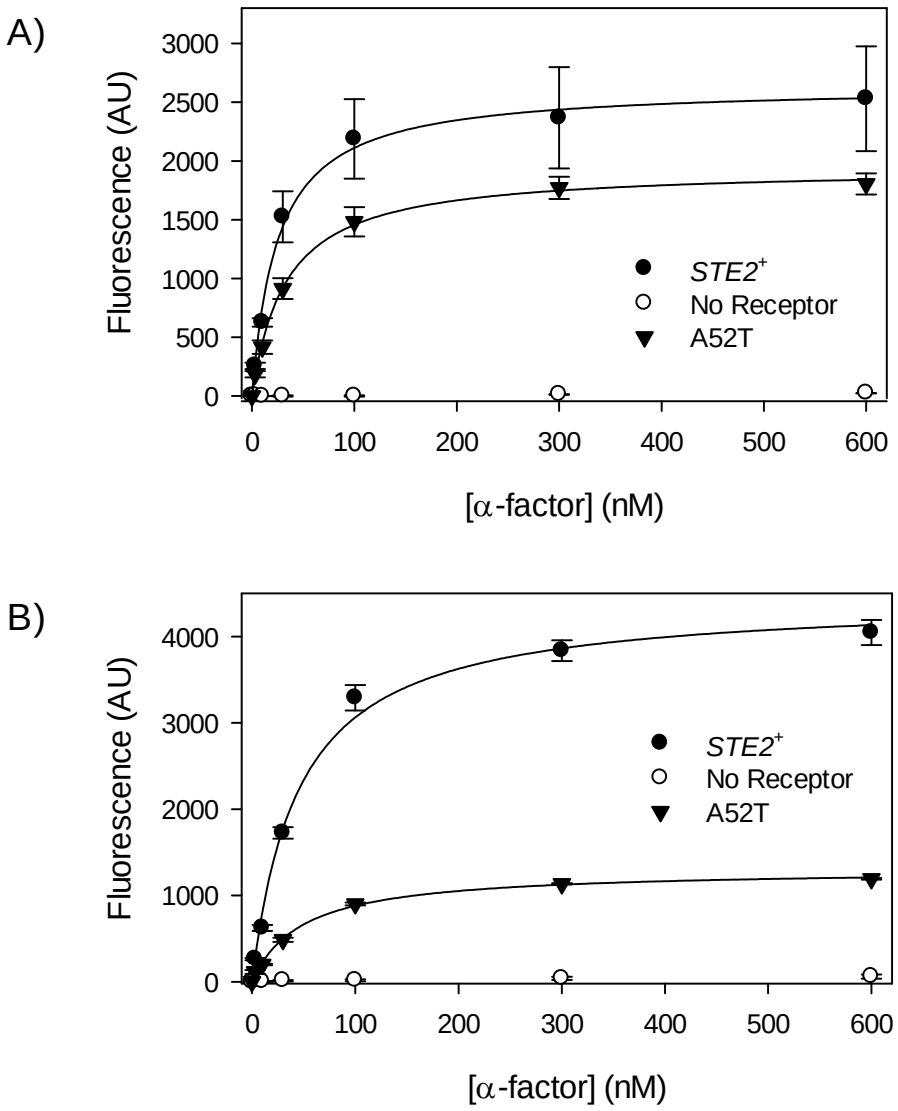
Supplementary Figure S1. Sorting gates used in the two stage screen for temperature-sensitive receptors. A) A library expressing randomly mutagenized truncated Ste2p was cultured overnight at 24 °C and then incubated with 300 nM [K^7(NBD),Nle^{12}] α-factor. The P4 gate collected cells in the top 15% of the fluorescent population and the P5 gate collected those in the 30-15% range. B) Pools of clones collected by the gates shown in (A) were either i) cultured overnight at 24 °C and then heat treated at 48 °C for 30 minutes or ii) cultured overnight at 34 °C, followed by incubation with 300 nM [K^7(NBD),Nle^{12}] α-factor. The P5 gate collected the 10% of the clones with the lowest level of fluorescence, the P4 gate collected next 10% least fluorescent clones, and the P6 gate collected another 10%.



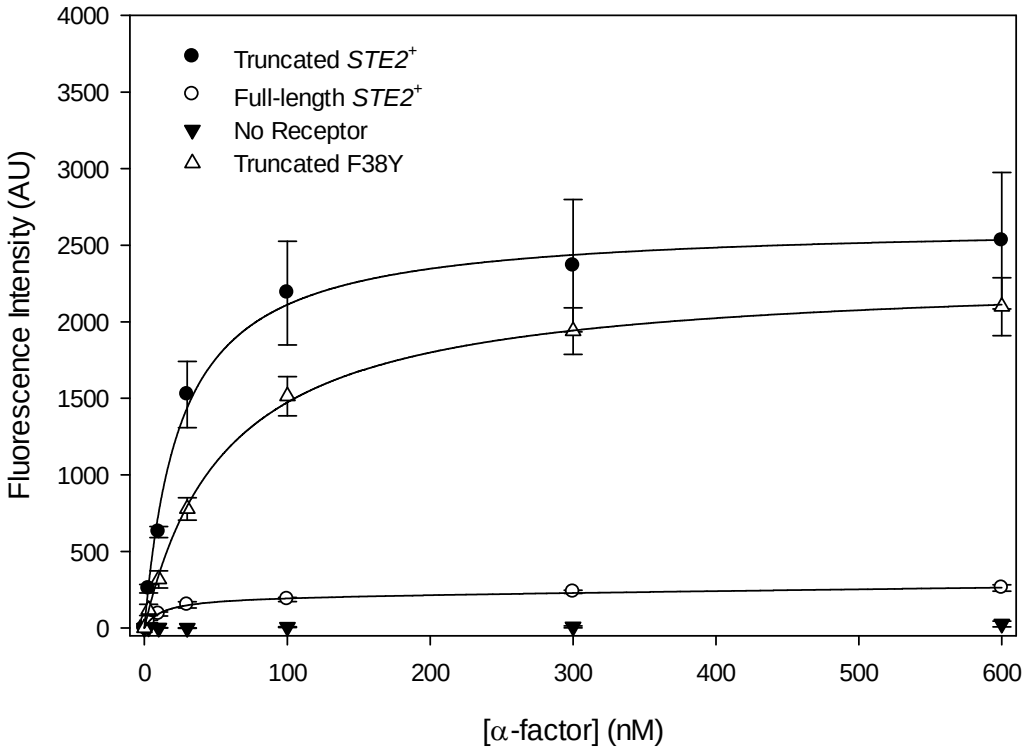
Supplementary Figure S2. Representative plot of the gates used to screen the truncated libraries by FACS. The fluorescent signal from the bound ligand is on the y-axis, while forward scatter, which correlates to cell size, is shown on the x-axis. The P4 gate (purple events) represents the stringent gate used for sorting. Cells were also collected in the less stringent gate P5, but were not subjected to further analysis.



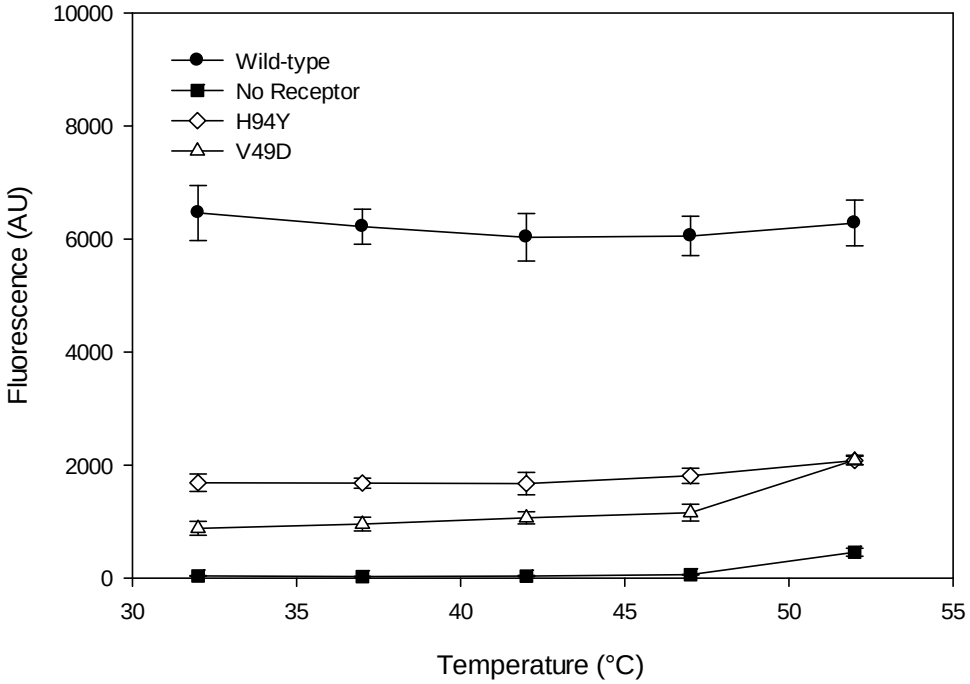
Supplementary Figure S3. Identification of temperature-sensitive *STE2* alleles based on temperature dependent inhibition of α-factor-induced growth arrest. Plasmids encoding temperature-sensitive Ste2p receptors identified in a preliminary growth arrest screen were retransformed into a fresh yeast host. Cells were spotted in a 10x dilution series onto plates with varying concentrations of α-factor and then incubated at either 24 °C or 34 °C. Cells expressing functional Ste2p will not grow in the presence of α-factor. Cells expressing temperature-sensitive Ste2p will not grow in the presence of α-factor at 24 °C, but will grow at 34 °C. (Note that some of the alleles presented in the figure were not considered to be temperature-sensitive and were not studied further: the allele containing the G56D substitution exhibited only minimal growth arrest at 24 ºC and the allele containing the G60S, R74S, and T172N substitutions exhibited robust growth arrest at 34 ºC.)



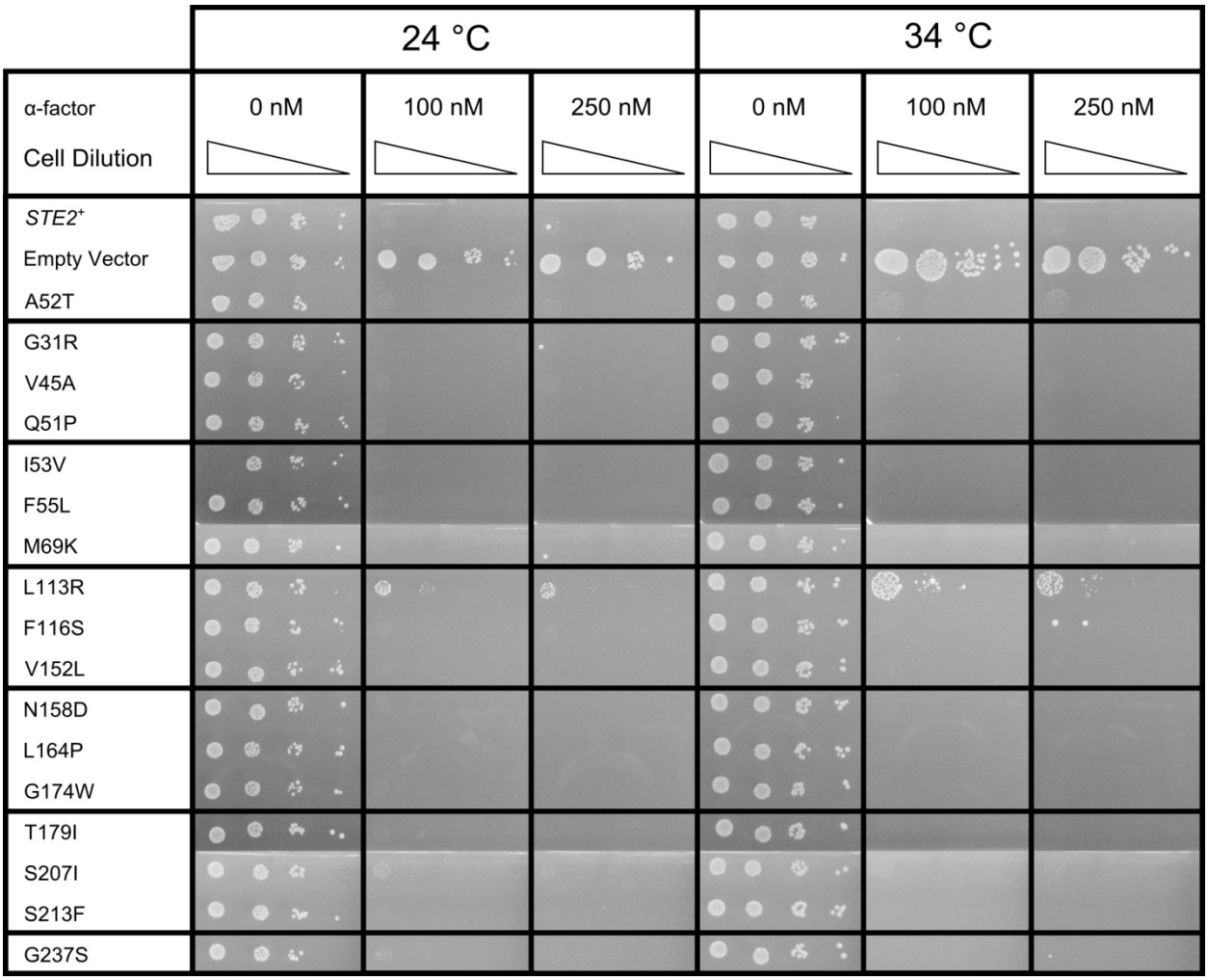
Supplementary Figure S4. Ligand binding to A52T Ste2p variants at different temperatures. Strains expressing C-terminally truncated Ste2p were cultured overnight at either 24 °C (A) or 34 °C (B), and then assayed for fluorescent ligand binding in the presence of the indicated concentrations of [K^7(NBD),Nle^{12}] α-factor as described in Materials and Methods.



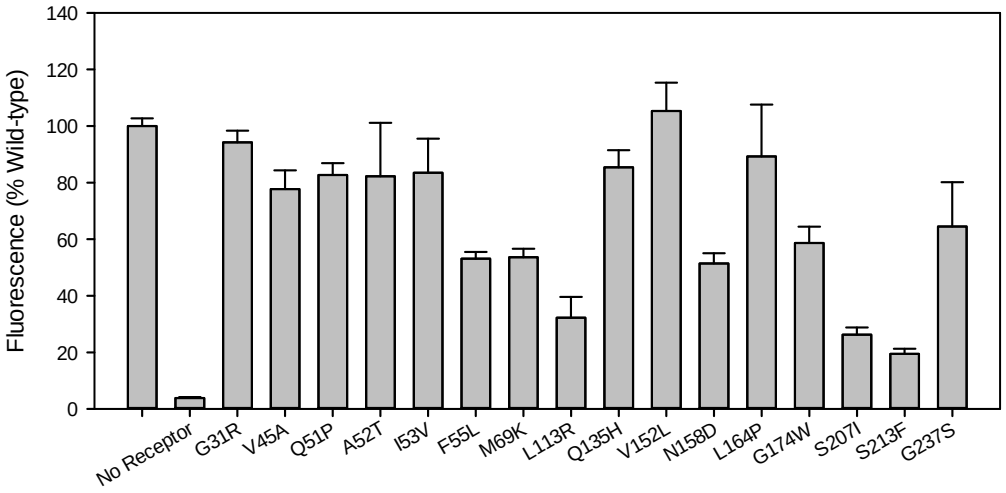
Supplementary Figure S5. Comparison of ligand binding to C-terminally truncated vs. full-length Ste2p. Strains expressing either truncated or full-length Ste2p variants were cultured overnight at 24 °C and then assayed for fluorescent ligand binding in the presence of the indicated concentrations of [K^7(NBD),Nle^{12}] α-factor as described in Materials and Methods. The error bars represent the standard error based on assay of binding to three independent isolates of each strain.



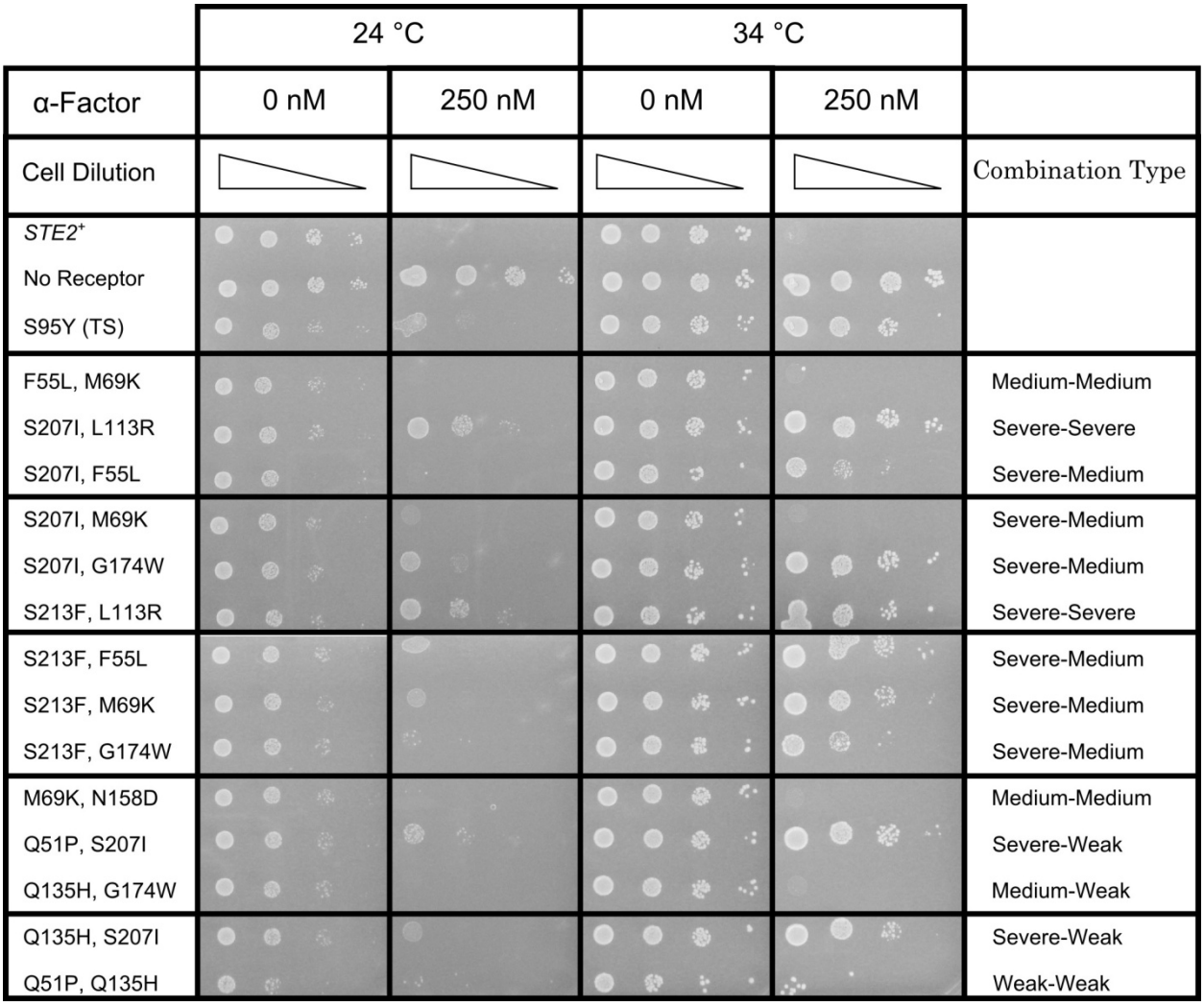
Supplementary Figure S6. Ligand binding to C-terminally truncated temperature sensitive Ste2p variants following short-term shift to high temperature. Strains expressing different Ste2p variants were cultured overnight at 24 °C, treated for 30 minutes at the indicated temperatures, then assayed for binding of [K^7(NBD),Nle^{12}] α-factor . The values charted in the figure represent the average specific fluorescence (difference in fluorescence between a sample that had been assayed in the presence of 300nM fluorescent ligand and one that had been assayed in the absence of fluorescent ligand) for three genetically independent isolates. The error bars indicate standard error calculated from measurements on three independent isolates.



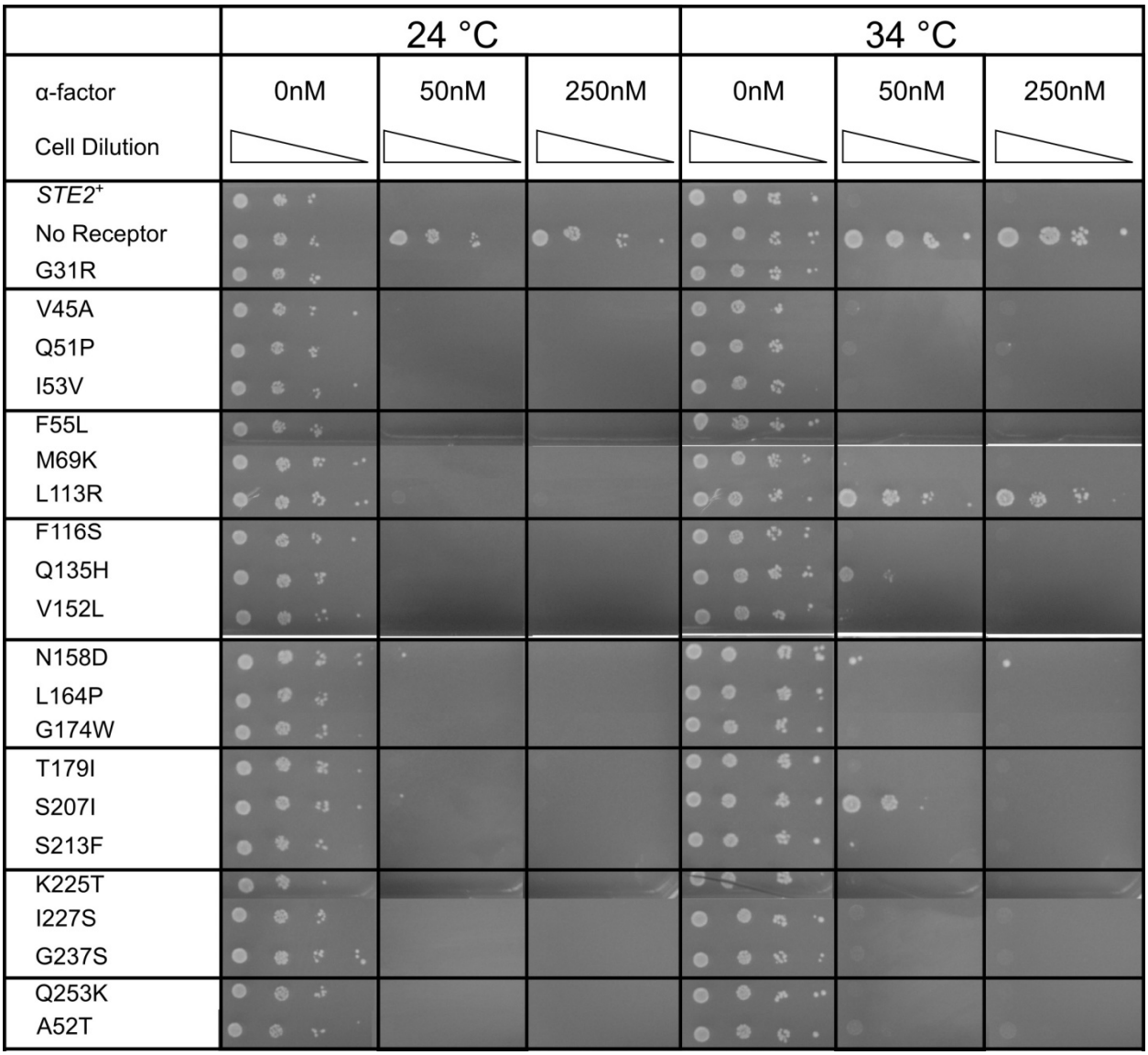
Supplementary Figure S7. Individual mutations from the multiply-substituted temperature-sensitive alleles have no phenotype when tested alone. The individual mutations were re-created as the only substitutions in full-length Ste2p by site-directed mutagenesis and assayed for α-factor-dependent growth arrest.



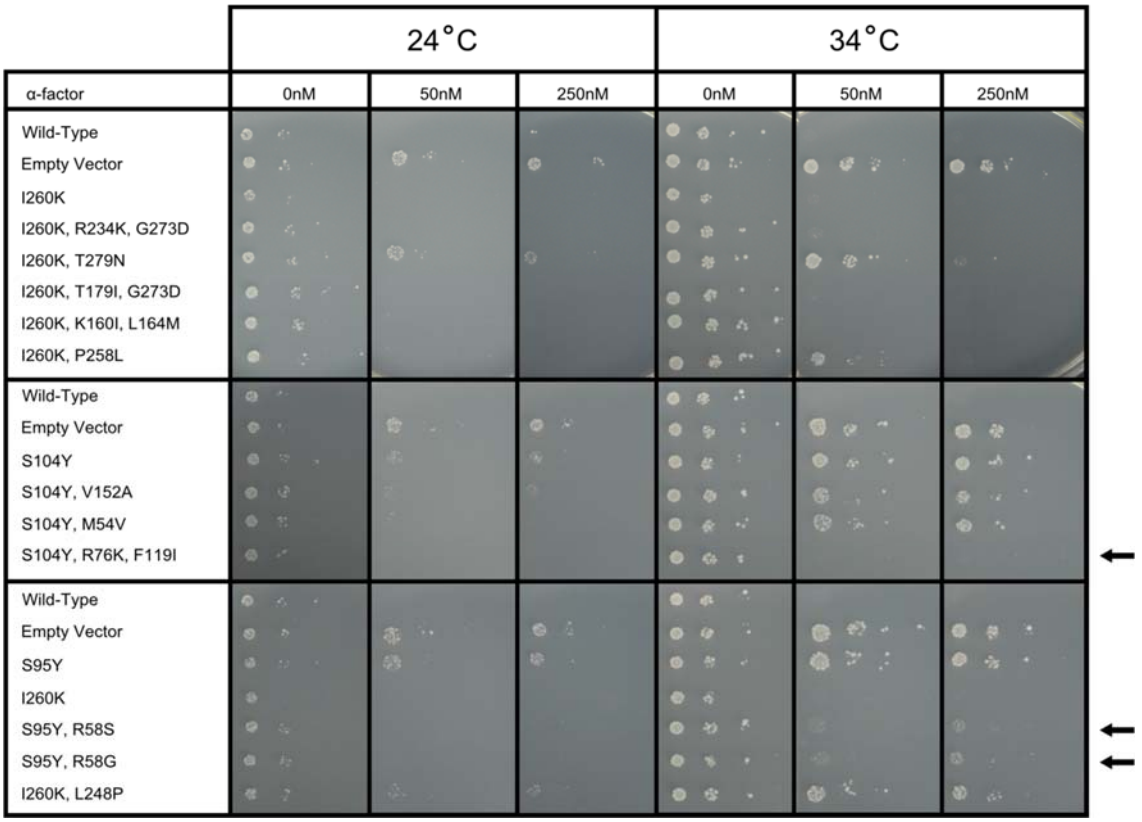
Supplementary Figure S8. Single amino acid substitutions derived from the multiply-substituted temperature-sensitive alleles have a range of effects on ligand binding when tested individually. Strains expressing full-length receptors were grown in liquid cultures at 24 °C. The error bars represent the standard error of three independent isolates of each strain. Fluorescent ligand binding was measured in the presence of 300 nM [K^{7}(NBD),Nle^{12}] α-factor. Based on these results the substitutions were classified as follows: Severe- L113R, S207I, and S213F; Medium- F55L, M69K, N158D, G174W, and G237S; Weak- G31R, V45A, Q51P, I53V, Q135H, V152L, and L164P.



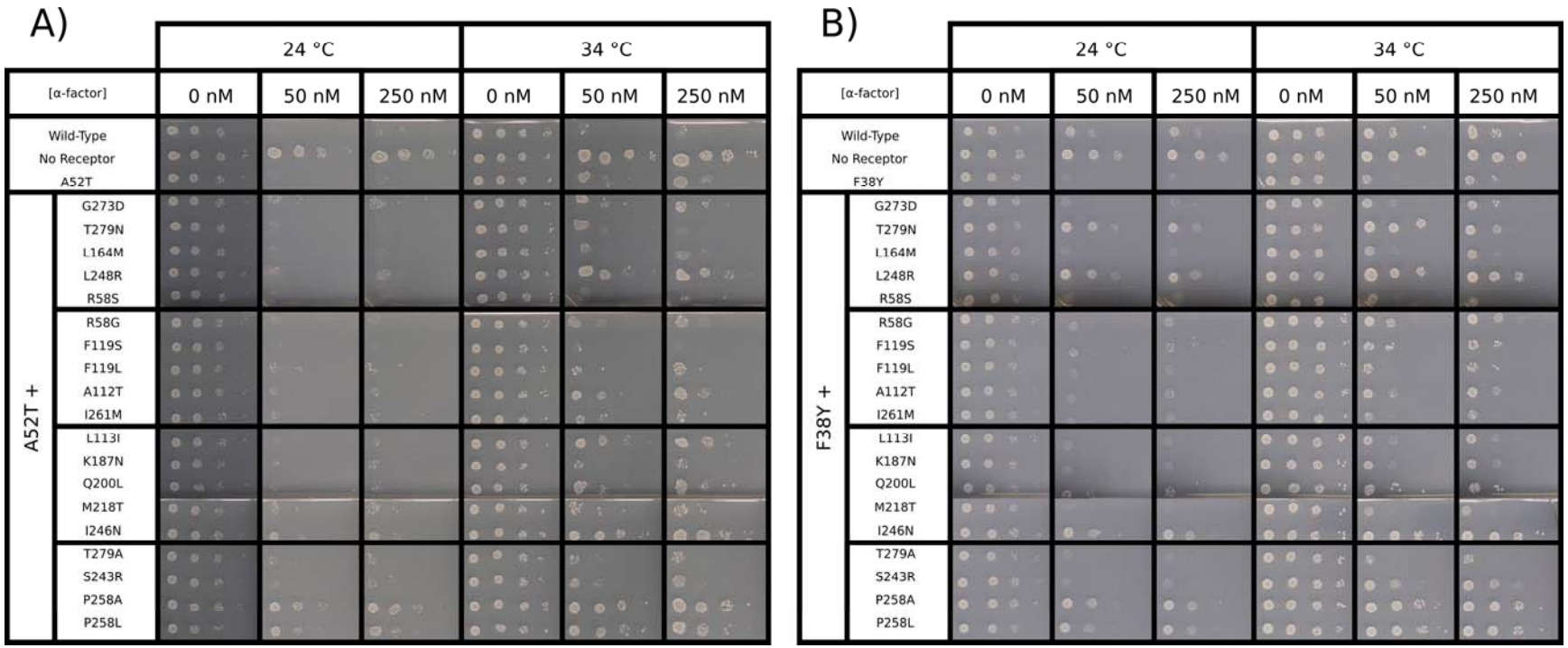
Supplementary Figure S9. Combinations of temperature-sensitive mutations exhibit additive effects. Mutations that did not exhibit temperature-dependent growth arrest phenotypes when tested individually were combined into doubly substituted alleles and strains expressing these full-length alleles were assayed for α-factor-dependent growth arrest.



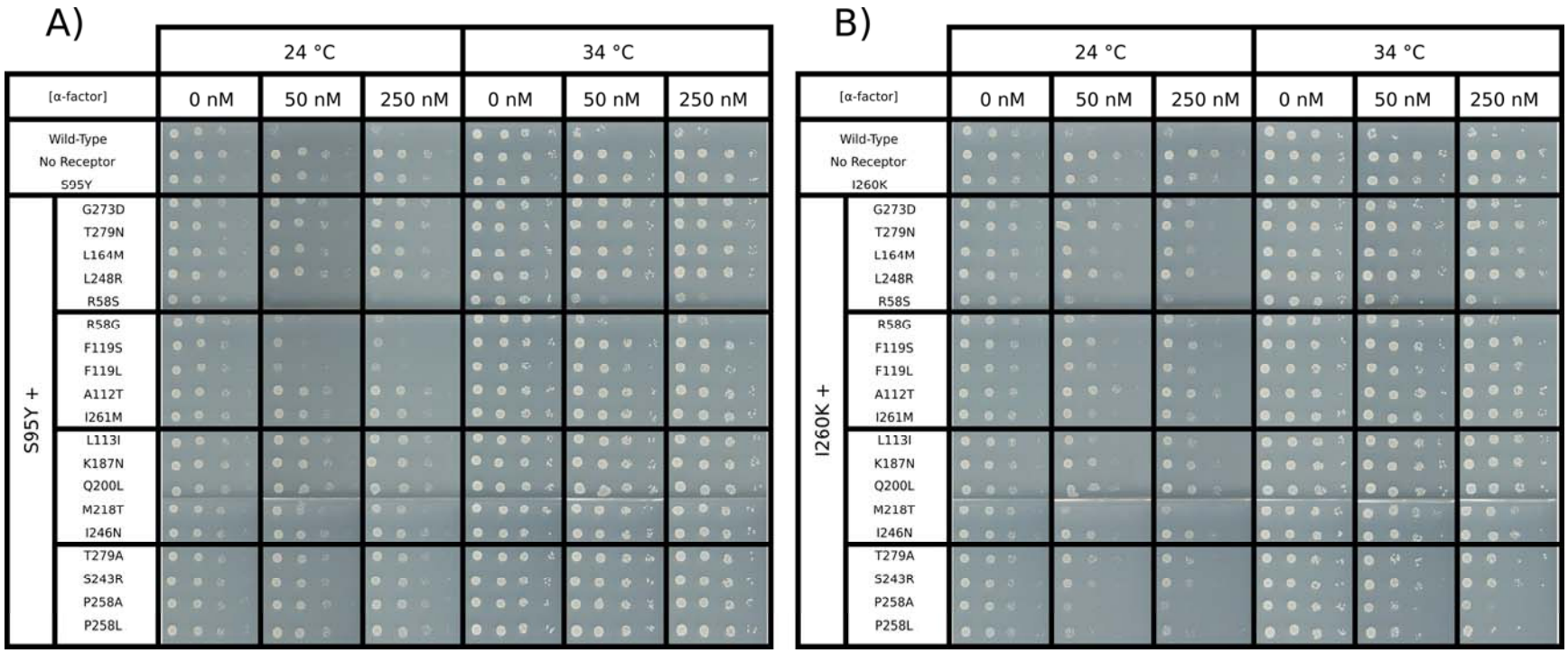
Supplementary Figure S10. Few of the mutations from the multi-mutant alleles have a phenotype when expressed from *CEN* plasmids. Cells expressing various mutant variants of full-length Ste2p from *CEN* plasmids were tested by growth arrest assay.



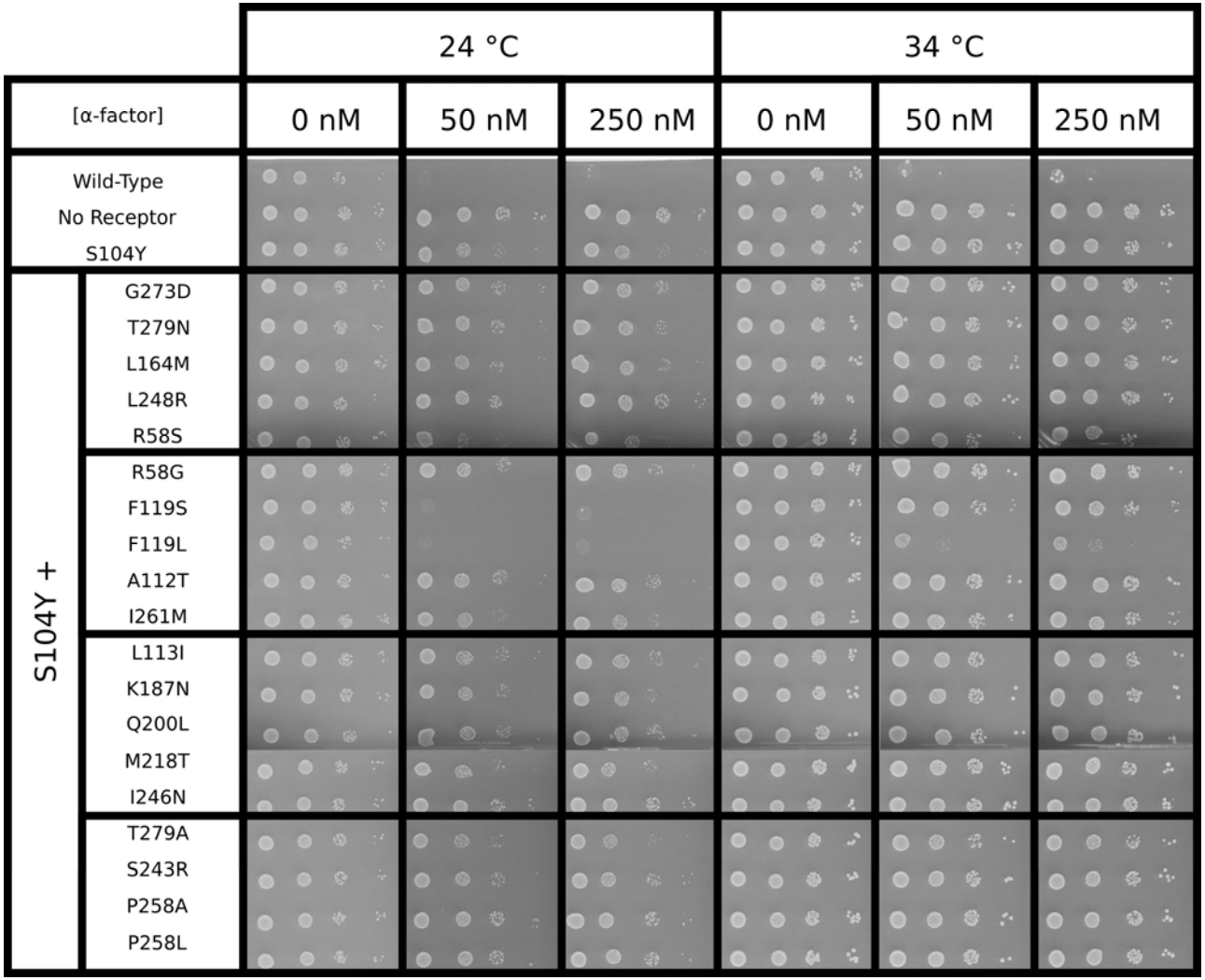
Supplementary Figure S11. Pheromone-dependent growth arrest of clones isolated from the suppressor library. Plasmids were isolated from library-derived clones, retransformed into a fresh yeast host, and tested using the growth arrest assay in which cells responsive to α-factor undergo cell cycle arrest. Several clones exhibit enhanced growth arrest relative to control cells expressing receptors with temperature sensitive mutations alone (indicated by arrows).



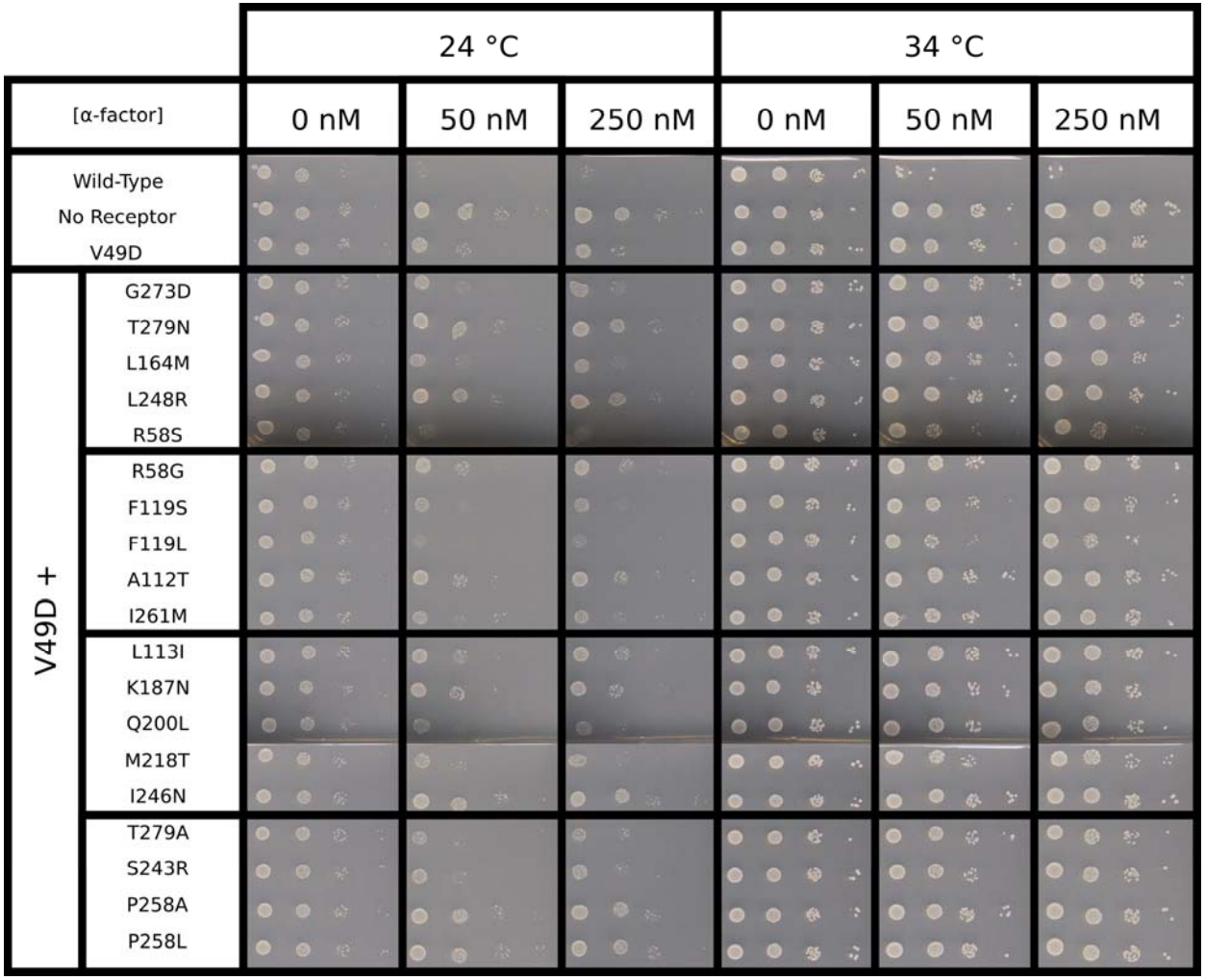
Supplementary Figure S12. Growth arrest assays performed on the double mutant array for temperature sensitive mutations A52T and F38Y. Strains from the double mutant array were cultured at 30 °C and spotted in ten-fold dilutions onto plates containing the indicated concentrations of α-factor (as described in Materials and Methods). Strains expressing functional receptor variants will not grow in the presence of α-factor, while non-functional receptor variants will.



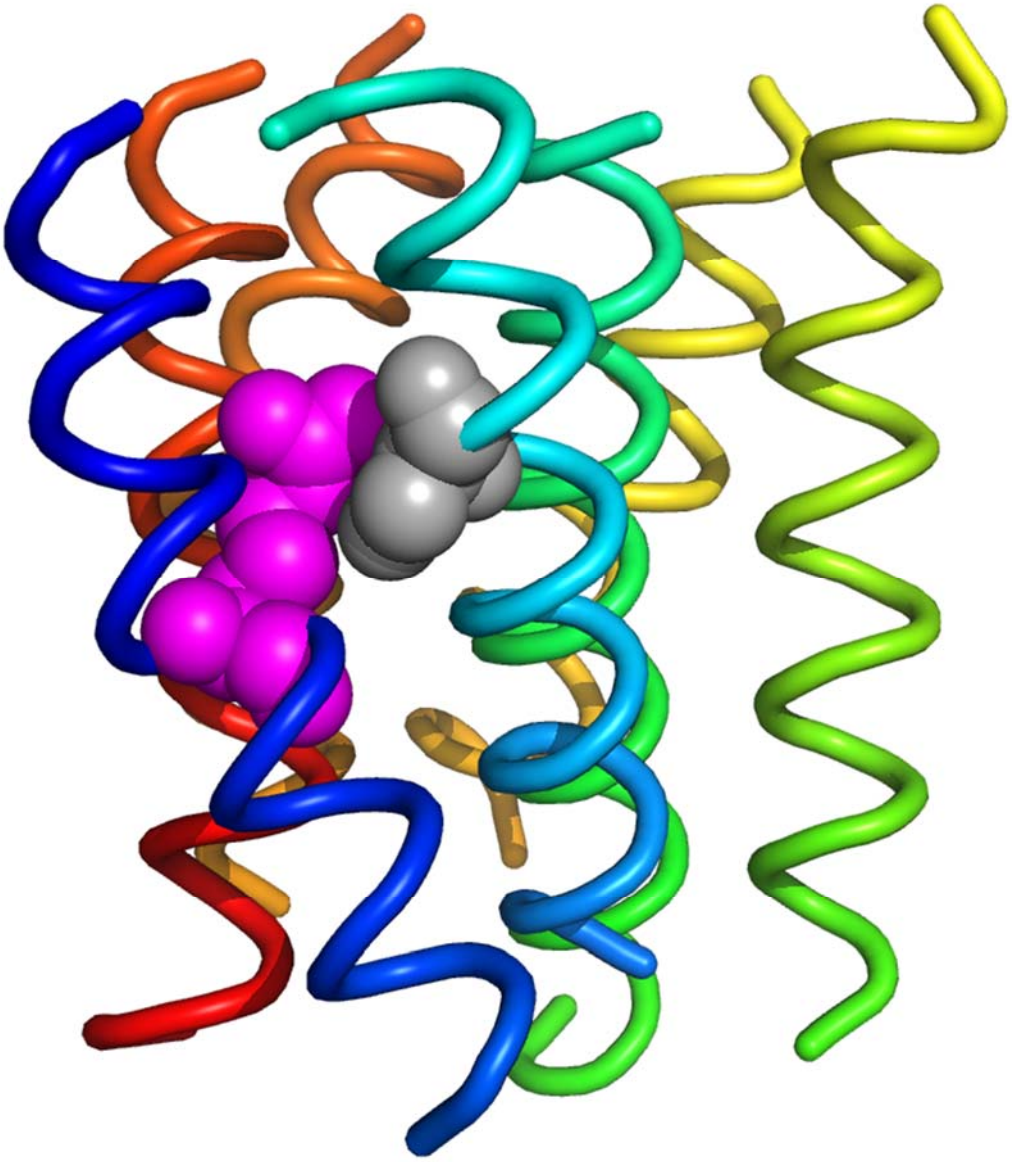
Supplementary Figure S13. Growth arrest assays performed on the double mutant array for temperature sensitive mutations S95Y and I260K.



Supplementary Figure S14. Growth arrest assays performed on the double mutant array for the temperature sensitive mutation S104Y.

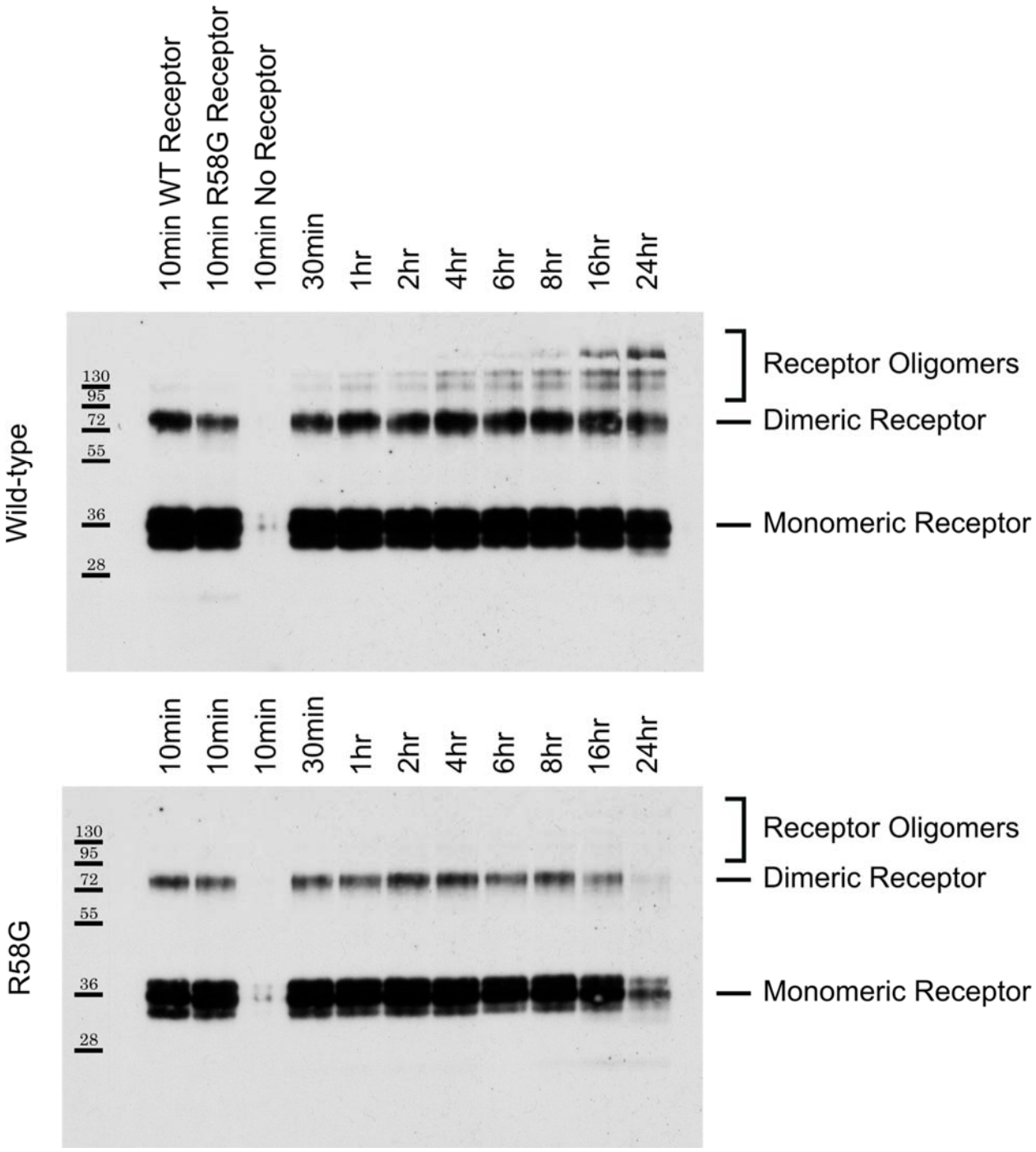


Supplementary Figure S15. Growth arrest assays performed on the double mutant array, for the temperature sensitive mutation V49D.



Supplementary Figure S16. Potential interaction between the R58 and S95 residues.

Based on a homology model of the seven transmembrane helices of Ste2p similar to that based on the crystal structure of rhodopsin [1], the residues R58 (highlighted in magenta) and S95 (highlighted in grey) appear to be optimally oriented to participate in interactions. The helices are colored as a gradient starting from the N-terminal end (blue) to the C-terminal end (red).



Supplementary Figure S17. Determination of the lifetime of solubilized Ste2p in cell lysates. Solubilized Ste2p was diluted to 0.1% DDM and incubated at room temperature. At different time points, aliquots were mixed with gel loading buffer and frozen for subsequent analysis by immunoblotting with anti-c-myc antibodies.

References

(1) Eilers, M., Hornak, V., Smith, S. O., and Konopka, J. B. (2005) Comparison of Class A and D G Protein-Coupled Receptors: Common Features in Structure and Activation†, *Biochemistry 44*, 8959-8975.