Regulation of the chaperone effects on a yeast prion by the cochaperone Sgt2

Denis A. Kiktev, Jesse C.Patterson, Susanne Müller, Bhawana Bariar, Tao Pan

and Yury O. Chernoff[#]

School of Biology and Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, Atlanta, GA 30332

[#]Corresponding author.

List of Supplementary materials

Table S1. Yeast strains constructed in this study

Table S2. Oligonucleotide primers used in this study

FIG. S1. Phenotypes of *get* deletions and comparisons to strains containing other aggregates

FIG. S2. Aggregation of the Get and Sgt2 proteins

Table S1. Yeast strains constructed in this study

Name	Genotypic background	Mating type	Genetic alteration	Prion composition
GT905	74-D694	*MAT*a	*get2-473*	[*PSI*$^+$ *PIN*$^+$]
GT982	74-D694	*MAT*a	*get2*Δ*:: HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1300-2D	GT81	*MAT*a	*get3*Δ*::HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1301-8C	GT81	*MATα*	*get2*Δ*::hphNT1*	[*PSI*$^+$ *PIN*$^+$]
GT1316-1B	GT81	*MAT*a	*get1*Δ*:: HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1308	GT81	*MATα*	*get4*Δ*::HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1309	GT81	*MATα*	*get5*Δ*::HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1448	GT81	*MAT*a	*sgt2*Δ*::HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1450	GT81	*MAT*a	*sgt2*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1468-14A	GT81	*MAT*a	*get4*Δ*::HIS3MX6 sgt2*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1469-2B	GT81	*MAT*a	*get5*Δ*::HIS3MX6 sgt2*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1476-1A	GT81	*MAT*a	*get1*Δ*::HIS3MX6 sgt2*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1477-1C	GT81	*MAT*a	*get3*Δ*::HIS3MX6 sgt2*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1487-4A	GT81	*MATα*	*get2*Δ*::hphNT1 sgt2*Δ*:: HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1550	GT81	*MAT*a	*SGT2-HA::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1585-2A	GT81	*MAT*a	*ssb1::HIS3 ssb2::ura3 get2::hphNT1*	[*PSI*$^+$ *PIN*$^+$]
GT1589-4A	GT81	*MAT*a	*SGT2-HA::kanMX6 get2::hphNT1*	[*PSI*$^+$ *PIN*$^+$]
GT1624	GT81	*MAT*a	*SGT2-HA::kanMX6*	[*psi*$^-$ *pin*$^-$]
GT1635	GT81	*MAT*a	*cpr7*Δ*::hphNT1*	[*PSI*$^+$ *PIN*$^+$]
GT1642	GT81	*MAT*a	*sti1*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1651-1A	GT81	*MAT*a	*ubc4*Δ*::his3MX6 get2*Δ*::hphNT1*	[*PSI*$^+$ *PIN*$^+$]
GT1657-2B	GT81	*MAT*a	*get2*Δ*::hphNT1 cpr7*Δ*::hphNT1*	[*PSI*$^+$ *PIN*$^+$]
GT1670	GT81	*MATα*	*get2*Δ*::hphNT1 sti1*Δ*::kanMX6*	[*PSI*$^+$ *PIN*$^+$]
GT1719	GT81	*MATα*	*get2*Δ*::HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1726-2A	GT81	*MATα*	*get2*Δ*::HIS3MX6 sgt2*Δ*::HIS3MX6*	[*PSI*$^+$ *PIN*$^+$]
GT1732	GT81	*MAT*a	*SGT2-HA::kanMX6*	[*psi*$^-$ *PIN*$^+$]
GT1733	GT81	*MAT*a	*SGT2-HA::kanMX6 get2*Δ*::hphNT1*	[*psi*$^-$ *PIN*$^+$]
GT1747-5A	GT81	*MAT*a	*get2Δ::HIS3MX6 sgt2Δ::kanMX6 GET4-GFP::HIS3MX*	[*PSI*$^+$ *PIN*$^+$]
GT1747-25C	GT81	*MAT*a	*get2Δ::HIS3MX6 GET4-GFP::HIS3MX*	[*PSI*$^+$ *PIN*$^+$]
GT1748-6A	GT81	*MAT*a	*get2Δ::HIS3MX6 sgt2Δ::kanMX6 GET5-GFP::HIS3MX*	[*PSI*$^+$ *PIN*$^+$]
GT1748-6D	GT81	*MATα*	*get2Δ::HIS3MX6 GET5-GFP::HIS3MX*	[*PSI*$^+$ *PIN*$^+$]
GT1929-1C	GT81	*MAT*a	*kanMX6::*P_{GAL}*-GFP-SBH1*	[*PSI*$^+$ *PIN*$^+$]
GT1939-3D	GT81	*MATα*	*sgt2*Δ*:: HIS3MX6 kanMX6::*P_{GAL}*-GFP-SBH1*	[*PSI*$^+$ *PIN*$^+$]
GT1960	GT81	*MATα*	*get2*Δ*::hphNT1 kanMX6::*P_{GAL}*-GFP-SBH1*	[*PSI*$^+$ *PIN*$^+$]
GT1961	GT81	*MATα*	*get2*Δ*::hphNT1 sgt2*Δ*:: HIS3MX6 kanMX6::*P_{GAL}*-GFP-SBH1*	[*PSI*$^+$ *PIN*$^+$]

Designations:
HIS3MX6 - *HIS5* gene of *Schizosaccharomyces pombe* (orthologous to *S. cerevisiae HIS3*)
kanMX6- bacterial gene that determines resistance to kanamycin in *E. coli* and G418 in yeast
hphNT - bacterial gene that confers resistance to hygromycin B
HA - hemagglutinin tag
GFP - green fluorescent protein

Table S2. Oligonucleotide primers used in this study

Number	Sequence 5'-3'	Gene	Direction	Application
457	GCAATCCTTGAACTACGTCTAGTTGATTGAAATAGGAGAACGGATCCCCGGGTTAATTAA	*GET1*	Forward	Deletion
458	TACATAAACATATTATATATACGTACATAATGTAATAACAGAATTCGAGCTCGTTTAAAC	*GET1*	Reverse	Deletion
459	AAACGTACGACAAGAACAAGAAGATCATCACATTGTAATTCGGATCCCCGGGTTAATTAA	*GET3*	Forward	Deletion
460	TTATATGTCGTATGTATCTATTTATGGTATTCAGGGGCTTGAATTCGAGCTCGTTTAAAC	*GET3*	Reverse	Deletion
668	TTTGCTGGGACAAAAGAACAAGAATAGGAACATGAAAAGATTTGAGAATTCGAGCTCGTTAAAGC	*GET2*	Forward	Deletion
669	GGAATAATGTCGGGTTATGAGAACAATGTATTATATTACTGAACTGCAGGTCGACGGATCCCCGG	*GET2*	Reverse	Deletion
678	AACCAAGTAAACATCATAAAGGGACATAAATAATAATAACAAGCTCGGATCCCCGGGTTAATTAA	*GET4*	Forward	Deletion
679	GCCACCGCAAACATATTTATCTATTCCTTCGCAAATATGCTCTTTGAATTCGAGCTCGTTTAAAC	*GET4*	Reverse	Deletion
680	CAGAGATAAACTAGCGAAGAATAATAACTTTATACAAAATTAATCCGGATCCCCGGGTTAATTAA	*GET5*	Forward	Deletion
681	CAACTGTGTAAAATAACAAGTATGTACGTACTAACTATACTAATCGAATTCGAGCTCGTTTAAAC	*GET5*	Reverse	Deletion
684	CACCAAGATCTTCACATTGTAATTATGG	*GET3*	Forward	Cloning
685	GGGGCCGCGGTTCCTTATCTTCTAACTC	*GET3*	Reverse	Cloning/tagging
739	CTTCTGACCAAGTGATATCTTATTAATACAAATCTACTGTACGCGGATCCCCGGGTTAATTAA	*SGT2*	Forward	Deletion
740	TATCTACATAACATGTATTGCATTAAAGGCTTATTTCAGTCCAGAATTCGAGCTCGTTTAAAC	*SGT2*	Reverse	Deletion
751	ACAAGGATCCTGTACGATGTCAGCATC	*SGT2*	Forward	Cloning
752	AGTGAGCTCCGAAAATCGACG	*SGT2*	Reverse	Cloning
753	TTCAGTCCGCGGTTGCTTGTTCTCATTGTC	*SGT2*	Reverse	Cloning/tagging
766	TAAGGATCCTCTACTGTACGATGTCAGCATC	*SGT2*	Forward	Cloning
767	ATGCTCGAGATTAAAGGCTTATTTCAGTCC	*SGT2*	Reverse	Cloning
876	TCAGCCCGGGATTGCTTGCTTGTTCTCATTGTC	*SGT2*	Reverse	Cloning
900	TCTGAAAGGTGTTCGGCAGCAACAACCTACATCCAACGCGGAATTCGAGCTCGTTAAAGC	*CPR7*	Forward	Deletion
901	GGGTTATTTAATCTCAAATTTCAGCCTTACAAGTAACTAAGCAGGTCGACGGATCCCCGG	*CPR7*	Reverse	Deletion
909	AAGCCCAAAAGTCTGCTCCC	*STI1*	Forward	Deletion
910	ATCTTCAAGTTCCGATTTCTC	*STI1*	Reverse	Deletion
938	CCTACAAGACATGATGTCGGGATTCCTGGGCGGATCGAAGCGGATCCCCGGGTTAATTAA	*GET4*	Forward	Tagging
939	GCACATACATATATATGTATATACGTCATGGTCGTCAAGCGAATTCGAGCTCGTTTAAAC	*GET4*	Reverse	Tagging
940	AGTCATGGAGCGTATACAAAAAGGCTGGTCTCTGGCCAAACGGATCCCCGGGTTAATTAA	*GET5*	Forward	Tagging
941	CGCCCAAAGGAAAGAAGAAATAGTAAGCGCAACCGTGATGAATTCGAGCTCGTTTAAAC	*GET5*	Reverse	Tagging
1042	AAAGGATCCAAATGGCTGAGGACTTGAAAATGC	*SGT2*	Forward	Cloning of the MC region
1044	GATCCCGGGATTGCTCAACCTTCTTCTTGGC	*SGT2*	Reverse	Cloning of the NM region
1045	CCACTCGAGACTATTGCTCAACCTTCTTCTTGGC	*SGT2*	Reverse	Cloning of the NM region
1057	AAGGGATCCTTATGCAATCTTTGAATCTGGAGAAAACC	*SGT2*	Forward	Cloning of the C region
1065	CTCTTCCATGTTTGTAGCATCAGCAACGTAGCTCTAGGAACGGATCCCCGGGTTAATTAA	*GET2*	Forward	Deletion
1066	CCTGAAAAGAAAGCCGGGAATAATGTCGGGTTATGAGAACGAATTCGAGCTCGTTTAAAC	*GET2*	Reverse	Deletion

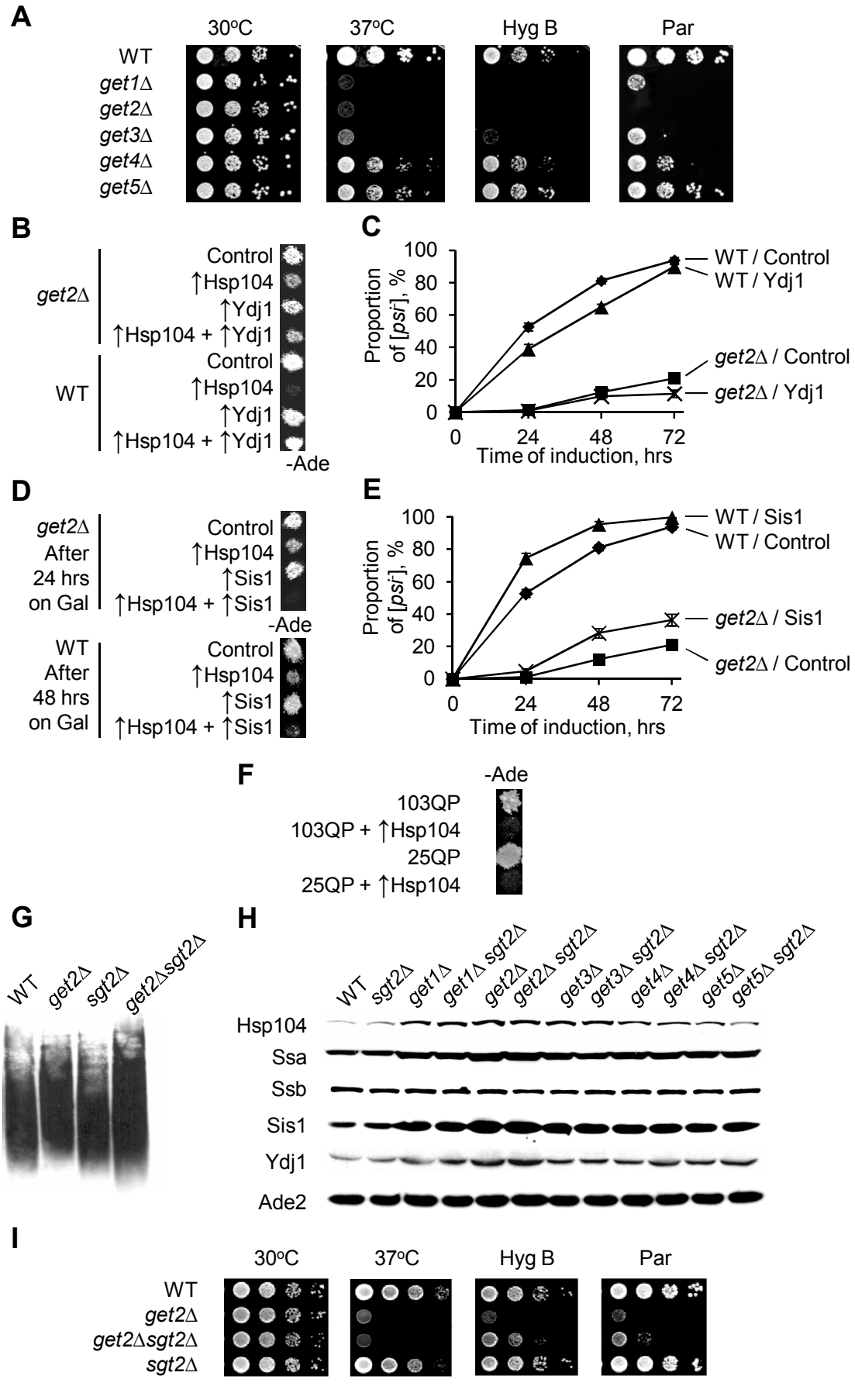
A
30°C
37°C
Hyg B
Par
WT
get1Δ
get2Δ
get3Δ
get4Δ
get5Δ
B
get2Δ
Control
↑Hsp104
↑Ydj1
↑Hsp104 + ↑Ydj1
WT
Control
↑Hsp104
↑Ydj1
↑Hsp104 + ↑Ydj1
-Ade
C
Proportion of [psi], %
100
80
60
40
20
0
0
24
48
72
Time of induction, hrs
WT / Control
WT / Ydj1
get2Δ / Control
get2Δ / Ydj1
D
get2Δ After 24 hrs on Gal
Control
↑Hsp104
↑Sis1
↑Hsp104 + ↑Sis1
-Ade
WT After 48 hrs on Gal
Control
↑Hsp104
↑Sis1
↑Hsp104 + ↑Sis1
E
Proportion of [psi], %
100
80
60
40
20
0
0
24
48
72
Time of induction, hrs
WT / Sis1
WT / Control
get2Δ / Sis1
get2Δ / Control
F
-Ade
103QP
103QP + ↑Hsp104
25QP
25QP + ↑Hsp104
G
WT
get2Δ
sgt2Δ
get2Δsgt2Δ
H
WT
sgt2Δ
get1Δ
get1Δ sgt2Δ
get2Δ
get2Δ sgt2Δ
get3Δ
get3Δ sgt2Δ
get4Δ
get4Δ sgt2Δ
get5Δ
get5Δ sgt2Δ
Hsp104
Ssa
Ssb
Sis1
Ydj1
Ade2
I
30°C
37°C
Hyg B
Par
WT
get2Δ
get2Δsgt2Δ
sgt2Δ

FIG. S1. Phenotypes of *get* deletions and comparisons to strains containing other aggregates. (**A**) *Get* deletions cause sensitivity to high temperature and to translational antibiotics hygromycin B and paromomycin. Decimal serial dilutions of mid-exponential cultures, grown in YPD, were spotted either onto YPD plates incubated at different temperatures, as specified, or onto YPD plates supplemented with either 40 μg/ml of hygromycin B (Hyg B) or 600 μg/ml of paromomycin (Par), as indicated, and incubated for 2 days at 30°C. (**B**) and (**C**) Extra copy of the *YDJ1* gene under its endogenous promoter decreases [*PSI*$^+$] curing in the wild-type (WT) strain but has little to no effect on the *get2*Δ strain, as detected in the plate assay (**B**) after 48 hrs of induction, and in the liquid assay (**C**). (**D**) and (**E**) Extra copy of the *SIS1* gene under its endogenous promoter slightly increases [*PSI*$^+$] curing in both wild-type and *get2*Δ strains in both plate (**D**) and liquid (**E**) assays. On panel **D**, *HSP104* was induced for 24 hrs in the wild type strain but for 48 hrs in the *get2*Δ strain, in order to minimize the impact of the *get2*Δ-mediated differences in the efficiency of [*PSI*$^+$] curing. In both (**C**) and (**E**), cultures were grown in liquid medium (selective for plasmids) with raffinose and galactose to induce P_{GAL}-*HSP104*. Aliquots were taken and plated onto plasmid-selective medium after specified periods of time. Presence or absence of [*PSI*$^+$] was detected after replica-plating to -Ade medium. At least three experiments were performed for each culture; standard deviations are shown by error bars. WT refers to wild-type, Control to an empty vector. (**F**) Strains expressing 103QP (fused to GFP) do not exhibit a defect of [*PSI*$^+$] curing by excess Hsp104, in contrast to *get2*Δ strains. 25QP (fused to GFP) was used as a non-aggregating control. All constructs were under P_{GAL} promoter. Image was taken after 4 days of incubation on -Ade, following three days of incubation on galactose medium. Experiment was performed in the conditions selective for 103QP (or 25QP) and *HSP104* (or control) plasmids.(**G**) *Get2* deletion slightly increases an average size of Sup35 polymers, that is ameliorated by *sgt2*Δ. Sup35 polymers in the [*PSI*$^+$] extracts were fractionated by SDD-AGE and detected by Western blotting. (**H**) Hsps levels in the cells lacking any of the Get proteins are increased, as detected by Western blotting. Ade2 protein was used as a loading control. (**I**) Temperature sensitivity of the *get2*Δ strain is not reversed by *sgt2*Δ, while sensitivities to hygromycin B and paromomycin are reversed by *sgt2*Δ. Decimal serial dilutions of mid-exponential cultures, grown in YPD, were spotted either onto YPD plated incubated at different temperatures as specified, or onto YPD plates supplemented with antibiotics as indicated on panel **A** (incubated at 30°C). Plate images were taken after 2 days.

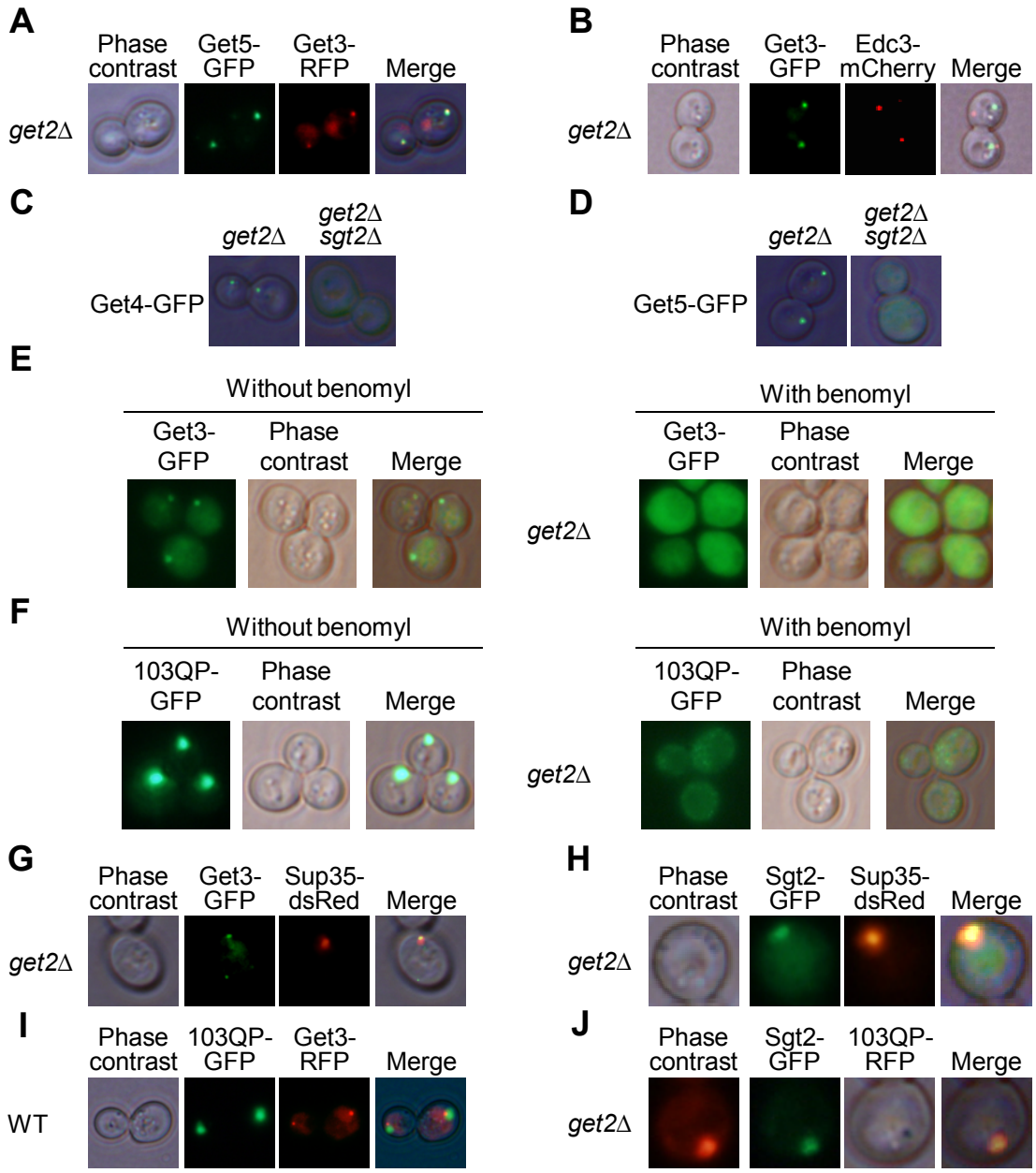

FIG. S2. Aggregation of the Get and Sgt2 proteins. (**A**) Get3 (tagged by RFP) and Get5 (tagged by GFP) are colocalized to the same aggregates in the *get2*Δ strain. (**B**) Get/TA aggregates, marked by Get3, do not colocalize with the P-body marker Edc3. (**C**) and (**D**) *Sgt2*Δ abolishes aggregation of cytosolic Get4 (**C**) and Get5 (**D**) proteins, tagged by GFP, in the *get2*Δ strains. (**E**) and (**F**) Formation of Get3-GFP foci in the *get2*Δ strain (**E**) is abolished upon addition of 10 μg/ml of benomyl. Formation of 103QP-GFP deposits, which is known to be inhibited by benomyl (see Wang *et al*. 2009 FASEB J **23:**451-63, ref. 67), is shown as a control in the same strain (**F**). Images were taken at 8 hours after the induction of tagged constructs and (where indicated) addition of benomyl. (**G**) Aggregates of Sup35 (marked with Sup35NM-dsRed) are colocalized with Get/TA aggregates (marked with Get3-GFP) in 15% of *get2*Δ [*PSI*$^+$] cells containing both types of aggregates. (**H**) Aggregates of Sup35 (marked with Sup35NM-dsRed) are colocalized with Sgt2-GFP in 12% of *get2*Δ [*PSI*$^+$] cells containing both types of aggregates. (**I**) About 8% of Get$^+$ cells bearing clumps of the GFP-tagged 103QP also contain foci of the RFP labeled Get3, which are overlapping with or juxtaposed to 103QP clumps in about 80% of the cases. (**J**) Foci of the GFP-labeled Sgt2 overlap with or are juxtaposed to the clumps of RFP-tagged 103QP in 12% of the cells bearing both types of aggregates in the *get2*Δ strain.