An Hsp90 co-chaperone links protein folding and degradation and is part of a conserved protein quality control

Highlights

- Sgt1 is required for efficient proteasomal degradation of misfolded proteins
- Hul5 partially rescues defects caused by Sgt1 deficiency
- Sgt1 is involved in the merging and subsequent resolution of protein aggregates
- Efficient proteostasis is dependent on Sgt1 and Hsp82 localizing to Q-bodies

In brief

Eisele et al. demonstrate that the Hsp90 co-chaperone Sgt1 has a widespread role in the folding and degradation of misfolded proteins. Furthermore, Sgt1 localizes upon heat stress to early quality-control sites in an Hsp90- and proteasome-dependent manner, a process that is necessary for efficient resolution of protein inclusions.

Authors

Frederik Eisele, Anna Maria Eisele-Bürger, Xinxin Hao, Lisa Larsson Berglund, Johanna L. Höög, Beidong Liu, Thomas Nyström

Correspondence
friderik.eisele@gmail.com (F.E.), thomas.nystrom@gu.se (T.N.)
An Hsp90 co-chaperone links protein folding and degradation and is part of a conserved protein quality control

Frederik Eisele,1,4,* Anna Maria Eisele-Bürger,1,3,4 Xinxin Hao,1 Lisa Larsson Berglund,1,2 Johanna L. Höög,2 Beidong Liu,2 and Thomas Nyström1,5,*

1Institute for Biomedicine, Sahlgrenska Academy, Centre for Ageing and Health – AgeCap, University of Gothenburg, Medicinaregatan 7A, 413 90 Gothenburg, Sweden
2Department of Chemistry & Molecular Biology, University of Gothenburg, Medicinaregatan 9 C, 413 90 Gothenburg, Sweden
3Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, PO Box 7015, 75007 Uppsala, Sweden
4These authors contributed equally
5Lead contact
*Correspondence: frederik.eisele@gmail.com (F.E.), thomas.nystrom@gu.se (T.N.)
https://doi.org/10.1016/j.celrep.2021.109328

SUMMARY

In this paper, we show that the essential Hsp90 co-chaperone Sgt1 is a member of a general protein quality control network that links folding and degradation through its participation in the degradation of misfolded proteins both in the cytosol and the endoplasmic reticulum (ER). Sgt1-dependent protein degradation acts in a parallel pathway to the ubiquitin ligase (E3) and ubiquitin chain elongase (E4), Hul5, and overproduction of Hul5 partly suppresses defects in cells with reduced Sgt1 activity. Upon proteostatic stress, Sgt1 accumulates transiently, in an Hsp90- and proteasome-dependent manner, with quality control sites (Q-bodies) of both yeast and human cells that co-localize with Vps13, a protein that creates organelle contact sites. Misfolding disease proteins, such as synphilin-1 involved in Parkinson’s disease, are also sequestered to these compartments and require Sgt1 for their clearance.

INTRODUCTION

A balanced interplay between the systems involved in protein folding and protein degradation is key for cellular fitness, longevity, and prevention of neurodegenerative diseases. These two processes are often collectively referred to as protein homeostasis or proteostasis (Labbadia and Morimoto, 2015). The folding process is dependent on a network of molecular chaperones (Hartl et al., 2011), whereas the degradation process relies on the ubiquitin proteasome system (UPS) (Ammer et al., 2014). A second line of defense in proteostasis, called spatial Protein Quality Control (spatial PQC), sequesters oligomers and aggregated proteins into large inclusions at certain protective locations within the cell (Sontag et al., 2017), a process limiting the toxicity of aberrant proteins. Specifically, upon proteostatic stress of yeast cells, the misfolded proteins initially accumulate at multiple sites, called CytoQs (Miller et al., 2015b), stress foci (Spokoin et al., 2012), or Q-bodies (Escusa-Toret et al., 2013), which later coalesce into larger inclusions at, at least, four distinct spatial quality control sites: the juxtanuclear quality control site (JUNQ); the intranuclear quality control site (INQ); the peripheral, vacuole-associated Insoluble Protein Deposit (IPOD) (Kaganovich et al., 2008; Miller et al., 2015a); and a site adjacent to mitochondria (Braun and Westermann, 2017).

The Hsp90 chaperones represent a major class of chaperones that work together with a variety of co-chaperones (Sahasrabudhe et al., 2017), many of which show high specificity for a small set of client proteins. The Hsp90 co-chaperone Sgt1 (Catlett and Kaplan, 2006) is known to possess such a specific role as an adaptor protein linking the Skp, Cullin, F-box (SCF) ubiquitin ligase complex to the Hsp90 system and to aid in the assembly of the kinetochore complex (Bansal et al., 2009; Kadota et al., 2010; Kitagawa et al., 1999). Recently, Sgt1 was shown to affect a relatively broad range of client proteins (Sahasrabudhe et al., 2017) indicating a more widespread role in Hsp90 folding processes.

Following two genome-wide screens, we here report that the Sgt1 co-chaperone is a much more general player in cellular PQC than previously anticipated and acts as an important linker, together with Hsp90, between the proteolytic and the folding branches of the proteostatic network.

RESULTS

Genome-wide identification of genes required for the degradation of misfolded proteins in the cytosol

To identify components of the PQC system required for the degradation of cytosolic misfolded proteins, we performed a systematic genome-wide screen following the SGA (Synthetic
Genetic Array methodology in the complete EUROSCARF deletion library and a collection containing temperature-sensitive (ts) alleles of most essential genes. In both collections, we expressed the misfolding cytosolic mutant of the yeast carboxypeptidase Y (DssCPY*) fused to Leu2, an often-used prototrophic marker in Saccharomyces cerevisiae laboratory strains, tagged with a C-terminal myc tag (DssCL*). For a second screen, we introduced Von Hippel-Lindau (VHL)-LEU2, encoding the misfolding mammalian VHL tumor suppressor protein (McClellan et al., 2005) fused to Leu2. Due to the misfolding of DssCL* and VHL-LEU2, cells degrade these proteins rapidly (Eisele and Wolf, 2008; McClellan et al., 2005). Thus, mutants in which the misfolded proteins are stabilized can easily be scored for, because they grow better than wild-type cells on media lacking leucine.

Spatial Analysis of Functional Enrichment (SAFE) (Baryshnikova, 2016; Usaj et al., 2017) of common hits of the two screens showed that four major functional groups among the essential genes were required for proper degradation of the two misfolded proteins. Among the non-essential genes, those linked to the functional groups’ transcription, chromatin, and protein turnover were markedly enriched (Figure 1A; Table S1). In contrast, cells with mutations in single genes, essential or non-essential, functioning as chaperones and/or co-chaperones did not display

**Figure 1.** Identification of the Hsp90 co-chaperone Sgt1 as an essential protein required for the degradation of misfolded cytosolic proteins

(A) SAFE analysis performed of common hits from two SGA screens of strains expressing one of the chimeric cytosolic substrates, DssCL* or VHL-LEU2, of the 26S proteasome. A cut-off value of $\kappa^2 = 2$ was chosen for visualization at https://thecellmap.org.

(B) Spot test of different strains expressing DssCL*myc on media lacking leucine and uracil (-Leu -Ura) or uracil (-Ura) only as a control.

(C and D) Analysis of DssCL* and DssCG* degradation during a cycloheximide chase at 30°C. Pgk1 served as a loading control. (C) Band 1 indicates full-length and bands 2 and 3 indicate C-terminal partial degradation products of DssCL* detected with antibodies specific for the C-terminal myc tag. (D) DssCG* was detected with antibodies specific for its C-terminal GFP tag. (C and D) Average of three biological replicates was used for quantification. Error bars indicate standard error of the mean (SEM).

See also Figure S1 and Table S1.
defects in the degradation of misfolded cytosolic proteins, suggesting that the chaperone/co-chaperone network of the cell is genetically well buffered. However, there was one noteworthy exception: the Hsp90 co-chaperone Sgt1.

Cells with reduced activity of Sgt1 displayed enhanced growth on plates lacking leucine and grew as well as cells lacking the ubiquitin ligase Ubr1, which has previously been shown to be a key factor in tagging misfolded ΔssCPY⁺ for degradation (Eisele and Wolf, 2008; Heck et al., 2010). Combining the sgt1-3 mutation with an UBR1 deletion did not result in additive effects, demonstrating that they act in common pathways (Figure 1B). The sgt1-3-dependent stabilization of ΔssCL⁺ could be complemented by extra-chromosomal SGT1 expression, and we confirmed that the Hsp90 system and Sgt1 are not involved in the folding or degradation of the Leu2-myc domain alone (Figures S1A and S1B).

SGT1 mutants displayed an enhanced sensitivity to the proline analog, AZC, which causes severe and general protein misfolding (Trotter et al., 2001) (Figure S1C). The data suggest that Sgt1 might be a more general player in cytosolic quality control than previously anticipated with a functional link to degradation of misfolded cytosolic proteins. Further, we observed that the sgt1-3 ubr1Δ double mutant showed a reduced heat sensitivity compared with the sgt1-3 mutant alone (Figure S1D). Ubr1 is known to have a role in degradation of several ts mutants (Khosrow-Khavar et al., 2012), and we could demonstrate that Sgt1 mutant protein also is a substrate of this E3 ligase (Figure S1E).

The Hsp90 co-chaperone Sgt1 physically interacts with cytosolic misfolded proteins and is required for their degradation

Cycloheximide chase experiments demonstrated that the rate of degradation of ΔssCL⁺ was delayed in cells with reduced Sgt1 activity to almost the same extent as in cells lacking Ubr1 (Figure 1C). However, degradation of ΔssCL⁺ was not much altered in a mutant of CDC34, the ubiquitin-conjugating enzyme (E2) of the SCF complex, demonstrating a Sgt1 role independent of this complex (Figure S1F). Moreover, Sgt1 and ΔssCL⁺ interacted physically, indicating a direct role for Sgt1 in modulating the stability of this misfolded substrate (Figure S1G). Degradation of VHL in the sgt1-3 mutant was also retarded (Figure S1H). Previously, VHL degradation was shown to be dependent on Hsp90s (McClellan et al., 2005); however, cycloheximide chase experiments of ΔssCL⁺ in the presence of Hsp90 inhibitor geldanamycin (GA) in hsc82Δ or sgt1-3 strains did not support a role for Hsp90 in degradation of this substrate (Figure S1I). Also, the physical interaction between Sgt1 and ΔssCL⁺ was not altered when Hsp90 activity is inhibited, neither by deletion of Hsc82 nor by addition of GA (Figure S1G).

A GFP-tagged version of misfolded cytosolic CPY⁺ (ΔssCG⁺) has previously been shown to be dependent on both the nuclear localized E3 ligase San1 and the cytoplasmic Ubr1 for its proteasomal degradation (Heck et al., 2010); if one of the two ligases was deleted, degradation of ΔssCG⁺ was only slightly delayed. However, double deletions of UBR1 and SANT1 led to strong stabilization of this substrate (Heck et al., 2010) (Figure 2C). We found that sgt1-3 mutation stabilized ΔssCG⁺ more than either one of the single ubr1Δ and san1Δ mutations (Figure 1D). Combining sgt1-3 with single mutants in UBR1 or SANT1 did not result in additive effects, suggesting that Sgt1 is a key co-factor in the Ubr1/San1-dependent degradation of ΔssCG⁺. A summary of factors involved in quality control of established fusion model substrates used in this study can be found in Table 1.

Sgt1 is required for endoplasmic reticulum (ER)-associated degradation (ERAD) in parallel with the multi-ubiquitin chain assembly factor Hul5

We noticed that fragments derived from ΔssCL⁺ degradation accumulated in sgt1-3 cells with sizes around 100 and 75 kDa (bands 2 and 3, respectively; Figure 1C). A similar accumulation of fragments of the ERAD substrate CTL⁺, a model substrate consisting of the ER luminal ERAD substrate CPY⁺ fused to a transmembrane domain and Leu2 (Medicherla et al., 2004), has been reported previously in cells lacking Hul5 (Kohlmann et al., 2008), a ubiquitin ligase (E3)/ubiquitin chain elongating enzyme (E4) (Crosas et al., 2006). We found that similar fragments of ΔssCL⁺ accumulated in hul5Δ cells (Figures 2A and S2A). Like for CTL⁺ (Kohlmann et al., 2008), deletion of HUL5 had no effect on degradation of full-length ΔssCL⁺. Deleting HUL5 in the sgt1-3 mutant generated an additive effect on the stabilization of fragments, and overproduction of Hul5 in sgt1-3 cells suppressed such accumulation of fragments (Figures 2A and S2A). The accumulation of ΔssCL⁺ fragments containing Leu2 in sgt1-3 and hul5Δ strains explains their enhanced growth on media lacking leucine (Figure S2B). We discovered more ubiquitylated ΔssCL⁺ was accumulating in the sgt1-3 mutant. Overexpression of HUL5 led to a wild-type-like ubiquitylation state and to suppression of the enhanced ubiquitylation phenotype of the sgt1-3 strain. A HUL5 deletion led to comparably lower ubiquitylation levels than the sgt1-3 mutant. As expected, the UBR1 deletion strain displayed a markedly reduced ubiquitylation of this substrate (Figure S2C).

Because Hul5 has been shown to be required for the degradation of some ERAD substrates (Kohlmann et al., 2008), we tested if this was true also for Sgt1. Indeed, CTL⁺ was stabilized in sgt1-3 cells, and two fragments, corresponding to those accumulating in hul5Δ cells (Kohlmann et al., 2008), were found to accumulate also in sgt1-3 cells (bands 2 and 3; Figure 2B). Again, stabilization of CTL⁺ fragment accumulation and enhanced growth on plates lacking leucine of sgt1-3 cells could be suppressed by elevating Hul5 levels (Figures 2B and S2D). The ER luminal misfolded ERAD substrate CPY⁺-HA (Taxis et al., 2002) was also dependent on proper SGT1 functionality for its degradation, and HUL5 overexpression improved the degradation of this substrate in the sgt1-3 mutant (Figure 2C). In contrast, the previously described cytosolic Hsp70-dependent degradation of the ERAD substrate CTG⁺ (Taxis et al., 2003) was not dependent on Sgt1 (Figure S2E), ruling out the possibility that mutation in SGT1 leads to a general inhibition of the 26S proteasome. Taken together, the data suggest that Sgt1 and Hul5 act in parallel pathways and display similar mechanisms involved in protein degradation.

Overproduction of Hul5 increases the fitness and longevity of cells mutated in SGT1

Mutation in SGT1 caused severe fitness defects, but overexpression of HUL5 could partially compensate for reduced Sgt1 activity.
up to 33°C (Figure 2D). Similarly, the rate of replicative aging was drastically accelerated in sgt1-3 cells, and overproduction of Hul5 could retard such aging to a certain degree (Figure 2E). In contrast, Hul5 overproduction alone accelerated replicative aging (Figure 2F). Deletion of HUL5 had no effect, while overexpression of SGT1 caused a modest decrease in lifespan (Figure S2F).

**Sgt1 is required for aggregate coalescence and clearance**

Using a GFP-tagged version of the yeast disaggregase Hsp104, which specifically binds to aggregated proteins (Erjavec et al., 2007; Spokoini et al., 2012), we found that a larger fraction of sgt1-3 cells growing at 30°C contained aggregates compared with wild-type cells (Figure 3A). Upon heat shock at 38°C for 90 min, sgt1-3 cells failed to form the typical one to two protein inclusions seen in wild-type cells, and instead exhibited multiple (three or more; type 3 cells) aggregates in the cytoplasm (Figures 3A and S3A). Moreover, when cells were incubated at 42°C for 30 min and then allowed to recover at 30°C for 90 min, the rate of clearance of aggregates was markedly delayed in sgt1-3 cells (Figure S3B). This delay of recovery did not correlate with increased cell death in the ts sgt1-3 mutant (Figure S3C). The disaggregation of Ubc9ts-GFP, a fusion protein of a ts allele of UBC9 that misfolds upon a 38°C heat shock (Kaganovich et al., 2008), was also delayed in cells with reduced Sgt1 activity (Figures S3D and S3E).
Sgt1 was found to be crucial also for alleviating accumulation of aggregates during aging (Figure S3F). Moreover, although the “middle-aged” (10–15 generations old) wild-type cells displayed mainly one or two inclusions, middle-aged sgt1-3 cells contained multiple aggregates (Figure S3F).

**Sgt1 clusters in a quality control compartment dependent on Hsp90 and proteasome activity**

Spatial quality control sites in yeast include JUNQ, INQ, IPOD, and CytoQs (Escusa-Toret et al., 2013; Kaganovich et al., 2008; Miller et al., 2015a, 2015b; Spokoini et al., 2012). We used a GFP-tagged version of SGT1 to elucidate if Sgt1 accumulates at any such sites. By crossing GFP-SGT1 with a sgt1-3 strain, we found that the sgt1-3 allele was fully complemented demonstrating the functionality of the tagged GFP-Sgt1 protein (Figure S3G). We found that GFP-Sgt1 normally displayed a uniform distribution in the cytosol and nucleus. However, when cells were subjected to a heat shock at 42°C, the fusion protein formed multiple small foci (Figure 3B). Because Sgt1 is a known co-chaperone of Hsp90s (Hsp82 and Hsp82), we tested if Hsp82-GFP accumulated in similar foci (Figure 3B) and found overlapping GFP-Sgt1 and Hsp82-Ruby foci (Figure S3H). Deleting the constitutive Hsp90 gene, HSC82, drastically reduced formation of the GFP-Sgt1 foci upon heat shock (Figure 3C), and inhibiting Hsp90 activity with GA prevented both Sgt1 and Hsp82 foci formation (Figure 3D). In addition, Sgt1 and Hsp82 foci formation required the Hsp70s Ssa1 and Ssa2 and the disaggregase Hsp104 (Figure 3C), but not the small heat shock protein, Hsp42, which is an essential factor for sequestration of misfolded proteins to peripheral aggregates and Q-bodies (Escusa-Toret et al., 2013; Specht et al., 2011) (Figure S3I). By co-expressing Hsp42-Ruby with GFP-Sgt1, we found that both foci partially overlap after a heat shock at 42°C, but after recovery at 30°C, Sgt1 foci resolve quickly, while Hsp42 foci remain and coalesce into few inclusions free of Sgt1 (Figure S3J). We also found that proteasome activity was required for Sgt1 and Hsp90 foci formation (Figure 3E). Therefore, we refer to these foci as Hsp70/90/104 and proteasome-dependent heat-induced inclusions (HAPIs) that are formed transiently during proteostatic stress and that do

<table>
<thead>
<tr>
<th>Fusion model substrate proteins</th>
<th>Examples of previously shown factors involved in protein quality control</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔssCL*</td>
<td>Ubr1; Ubp3; Ssa1/2/4; Tsa1; Hsp31/32/33</td>
<td>Eisele and Wolf (2008)</td>
</tr>
<tr>
<td></td>
<td>Proteasome; Ubc4/5; JUNQ/INQ, IPOD, Sti1; Fes1; Hsp104; Sis1; Bln2; Hsp42</td>
<td>Kaganovich et al. (2008) Malinovska et al. (2012); Ho et al. (2019); Miller et al. (2015a)</td>
</tr>
<tr>
<td>ΔssCG*</td>
<td>Ssa1, proteasome, Ubc4/5; Ubr1, San1, Ubp3; Tsa1; Ssa1/2/4</td>
<td>Park et al. (2007) Heck et al. (2010) Öling et al. (2014) Hanzén et al. (2016) Andersson et al. (2021)</td>
</tr>
<tr>
<td>CPY*HA</td>
<td>ER to Golgi factors</td>
<td>Taxis et al. (2002)</td>
</tr>
<tr>
<td>CTG*</td>
<td>proteasome, Ubc1/7, Hrd1, Cdc48, Ssa1, Hsp104</td>
<td>Taxis et al. (2003)</td>
</tr>
<tr>
<td>Ubc9ts-GFP</td>
<td>proteasome; Ubc4/5, JUNQ, IPOD, Q-bodies, Ubp3, Sis1, Hsp104</td>
<td>Kaganovich et al. (2008) Escusa-Toret et al. (2013) Öling et al. (2014) Malinovska et al. (2012)</td>
</tr>
<tr>
<td>dsRed-Synphilin-1</td>
<td>genes involved in cytoskeleton organization, histone modification, sister chromatid segregation, glycolipid biosynthetic process, DNA repair, and replication are required for synphilin-1 inclusion formation</td>
<td>Zhao et al. (2016)</td>
</tr>
<tr>
<td>Guk1-7-GFP</td>
<td>Ubr1, proteasome, Gim3, Sed5, Ssa1/2/4</td>
<td>Comyn et al. (2016) Babazadeh et al. (2019) Andersson et al. (2021)</td>
</tr>
</tbody>
</table>
Cell Reports

A. Bar graph showing the percentage of cells with foci before and after heat shock (HS) at 38°C for 90 min, comparing WT, Hsp104-GFP, and Hsp104-GFP sgt1-3.

B. Images of GFP-Sgt1 and Hsp82-GFP under 30°C and 42°C for 30 min, with focus on Type 1 and Type 3 foci.

C. Graph showing the percentage of cells with foci before and after 42°C for 30 min, comparing WT, hsps21, ssf1-1, hsps104a, GFP-Sgt1, and Hsp82-GFP.

D. Comparison of cells with foci under DMSO and GA treatment for GFP-Sgt1 and Hsp82-GFP at 42°C for 30 min.

E. Comparison of cells with foci under DMSO and MG132 treatment for GFP-Sgt1 and Hsp82-GFP at 42°C for 30 min.

F. Graph showing the percentage of cells with foci at 42°C for 30, 60, and 90 min, comparing Hsp104-GFP and GFP-Sgt1.

G. images showing cell morphology at 30°C and 42°C, with indications of N (nucleus), C (cytoplasm), and V (vacuole).

H. Graph showing the labelling density in gold/μm² for aggregates in the cytosol and nucleus at 30°C and 42°C.

I. Images of eGFP-SUGT1 under no HS, HS, GA, and MG132 treatments.

(legend on next page)
not appear to coalesce (Figures 3F and S3K). HAPIs seem to be unstable because they cannot be fixed with formaldehyde, which suggests that they are dynamic complexes that transiently co-localize with Hsp42-containing Q-bodies before such bodies coalesce into IPODs (Figure S3L). No foci were formed after heat shock by Skp1-GFP, the SCF complex backbone protein, suggesting no involvement of this complex in HAPI formation (Figure S3M).

Immunoelectron microscopy of cryofixed GFP-Sgt1 cells grown at 30°C (Figure 3G) showed a rather random distribution of gold particles after labeling of the sectioned cells with GFP-specific and gold-conjugated secondary antibodies (Figure 3G). However, cells that were heat shocked displayed protein aggregation that appears as electron dense clusters in the electron micrographs. We found that gold particles displayed a higher density in these areas of protein aggregation both in the cytosol and the nucleus (Figures 3G and 3H).

Because no transient foci have been described yet for Hsp90s or its co-chaperones, we tested the behavior of GFP-tagged human Sgt1 homolog SUGT1 in HeLa cells. At 37°C, GFP-SUGT1 was evenly distributed in the cytosol but formed HAPI-like shaped foci when cells were switched to 40°C. Again, cells treated with Hsp90 or proteasome inhibitor were unable to form foci (Figure S3I), demonstrating conservation of this phenomenon.

We performed pull-down experiments of GFP-Sgt1 from yeast cells growing at 30°C and cells heat shocked for 30 min at 42°C, and we used mass spectrometry to identify proteins that bind to GFP-Sgt1 at both 30°C and 42°C. In this group, components of the SCF complex and the Hsp90 machinery were strongly enriched (Table S2). Proteins enriched in binding to GFP-Sgt1 exclusively at 42°C have roles in endosome formation and trafficking, and many are found to be associated with the plasma membrane and nuclear periphery (Table S2; Figure 4A). One protein of this group is the heat-stress-induced Btr2, which plays an important role as a chaperone and sequester for misfolding proteins, a triage between refolding and degradation (Ho et al., 2019; Malinovska et al., 2012; Miller et al., 2015a). Also, Vps13 was co-purified with Sgt1 exclusively at 42°C. In human, nonfunctional Vps13 forms HAPIs, and this clearance of heat shock-induced (42°C) or shifted to 42°C was the Hsp70/Hsp90 co-chaperone Sti1 (Wolfe et al., 2013) (Table S2). With its TPR domain-containing regions, Sti1 and its mammalian ortholog HOP (Hsp70-Hsp90 organizing protein) have been shown to bind to the EEDV motives of Hsp70s and Hsp90s to form a ternary complex (Hernández et al., 2002). No HAPI formation could be observed in strains deleted for STI1 (Figure S4C), indicating the need for this Hsp70- and Hsp90-linking protein in the recruitment of the Hsp90 machinery to HAPIs.

The aggregated form of the Parkinson’s disease protein, synphilin-1, co-localizes with Sgt1 and requires Sgt1 for its clearance

When the recombinant synphilin-1 protein of Parkinson’s disease (Wakabayashi et al., 2000) is expressed in exponentially growing yeast cells, only a small fraction of synphilin-1 forms inclusions (Göttner et al., 2010). Upon shift to 42°C, all synphilin-1 formed foci, which were rapidly cleared when the temperature was lowered to 30°C, and this clearance was again dependent on a functional Hsp90 system (Figure 4D). When co-expressed with GFP-tagged Sgt1, a high number of heat-shocked cells displayed co-localization of synphilin-1 and Sgt1. This
co-localization rapidly disappeared upon return to 30°C (Figure S4D). In sgt1-3 cells, resolution of synphilin-1 foci was markedly retarded (Figure 4E), suggesting that Sgt1 needs to be fully functional to ensure rapid clearance of these inclusions.

**DISCUSSION**

The Hsp90s make up 1%–2% of total cellular protein and increase to 4%–6% in stressed cells (Prodromou, 2016), but their exact role in handling misfolded proteins and aggregates remains obscure. Although degradation of VHL was shown to be delayed in Hsp90 mutant cells (McClellan et al., 2005), degradation of variants of the misfolding cytosolic model substrate ΔssCPY* was shown to be independent of Hsp90s (Park et al., 2007). Here we show that the Hsp90 co-chaperone Sgt1 is required for degradation and together with Hsp90s is necessary for aggregate removal of misfolded proteins. Moreover, we found that Hsp90 and Sgt1 are recruited to a transient site early
the processivity of the proteasome (Crosas et al., 2006; E3/E4 ubiquitin ligase Hul5, which was shown previously to in-
partially rescued by overexpression of the proteasome-bound
somal degradation of misfolded proteins. This process can be
and reduced Sgt1 activity results in a drastically impaired protea-
formation is drastically increased in mutants of SGT1 during repli-
cative aging, and the replicative lifespan of SGT1 mutant cells is
duced aging in Sgt1-deficient cells, indicating that an interplay
between the Hsp90/Sgt1 and Hul5 systems is important for
longevity assurance.

The role of the Hsp90 co-chaperone Sgt1 in aggregate manage-
ment and protein degradation is interesting in view of data linking Hsp90 activity to neurodegenerative diseases. Several studies have proposed that Hsp90s stabilize aberrant
disease-associated proteins, and that inhibition of these chap-
erones could redirect neuronal aggregated proteins for degra-
dation (Luo et al., 2010). Other studies have demonstrated that the Hsp90 co-chaperones, CyP40 and PPS, reduce tau path-
ology but are repressed in aged and Alzheimer disease pa-
ients (Shelton et al., 2017). Interestingly, human Sgt1 concen-
trations have been shown to be drastically reduced in the
brains of Alzheimer disease patients (Bohush et al., 2019). The impact of such an increase or decrease is not known, but we show here that aggregates of the Parkinson’s disease protein, synphilin-1, co-localize with Sgt1 in HAPIs, and that Sgt1 deficiency and conditions that prevent HAPI for-
mation retard clearance of synphilin-1 aggregates. Based on
these observations, we believe the role of Hsp90s, Sgt1, and
HAPI formation deserves future attention in the field of neuro-
degeneration and PQC.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Yeast strains
  - Mammalian cell culture
- METHOD DETAILS
  - Plasmid constructions
  - Genome-wide screen for stabilizers of two misfolding
    proteins
  - Spot tests
  - Co-immuno purifications (Co-IP)
  - In vivo ubiquitination assay
  - Cycloheximide chase
  - Metabolic chase
  - Western blotting
  - Replicative lifespan assay
  - Isolation of old mother cells
  - Assessment of colony forming units (CFU)
  - Hsp104, Hsp42 and Guk1-7 protein aggregation induction
  - Sgt1, Hsp82, Vps13, Hsp42- and synphilin-1 foci for-
mation assay
  - Cell culture and transient transfection
  - Electron microscopy
  - Mass spectrometry
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.
ACKNOWLEDGMENTS

We thank the EMBL Proteomics Core Facility in Heidelberg, and in particular Mandy Rettel and Frank Stein for support with mass spectrometry and data analysis. We thank Dieter Wolf, Joris Winderickx, Per Widlund, Rebecca Andersson, and Charlie Boone for kindly sharing yeast strains and plasmids. This work was supported by grants from the Swedish Natural Research Council (VR), the Knut and Alice Wallenberg Foundation (KAW; Wallenberg Scholar), and ERC (Advanced Grant; QualiAge) to T.N.; by grants from the Swedish Cancer Society (CAN 2012/801, CAN 2015/406, and CAN 2017/643 to B.L.); and by grants from the Swedish Natural Research Council (VR 2011-5923 and VR 2015-04984 to B.L.). We thank Peter Bozhkov for supporting A.M.E.-B. during the revision process by KAW grant (DNR KAW 2018.0026).

AUTHOR CONTRIBUTIONS

F.E., A.M.E.-B., and T.N. conceived experiments and wrote the manuscript. F.E., A.M.E.-B., X.H., and L.L.B. performed experiments, A.M.E.-B. performed experiments during revision. J.L.H. and B.L. provided expertise and feedback.

DECLARATION OF INTERESTS

F.E. is now an AstraZeneca employee.

Received: June 8, 2020
Revised: November 30, 2020
Accepted: June 9, 2021
Published: June 29, 2021

REFERENCES


### KEY RESOURCES TABLE

<table>
<thead>
<tr>
<th>REAGENT or RESOURCE</th>
<th>SOURCE</th>
<th>IDENTIFIER</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibodies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse monoclonal anti-GFP</td>
<td>Roche</td>
<td>Cat# 11814460001; RRID:AB_390913</td>
</tr>
<tr>
<td>Mouse monoclonal anti-myc</td>
<td>Sigma-Aldrich</td>
<td>Cat# M4439; RRID:AB_439694</td>
</tr>
<tr>
<td>Mouse monoclonal anti-Pgk1</td>
<td>Thermo Fisher Scientific</td>
<td>Cat# 459250; RRID:AB_2532235</td>
</tr>
<tr>
<td>Mouse monoclonal anti-HA</td>
<td>Abcam</td>
<td>Cat# ab130275; RRID:AB_11156884</td>
</tr>
<tr>
<td>Rabbit polyclonal anti-Hsc82</td>
<td>Abcam</td>
<td>Cat# 30920; RRID:AB_873576</td>
</tr>
<tr>
<td>Rabbit polyclonal anti-GFP</td>
<td>Abcam</td>
<td>Cat# ab6556; RRID:AB_305564</td>
</tr>
<tr>
<td>Goat anti-Mouse IRDye 800CW</td>
<td>LI-COR</td>
<td>Cat# P/N 925-32210; RRID: AB_621842</td>
</tr>
<tr>
<td>Goat anti-Rabbit 10 nm gold</td>
<td>Electron Microscopy Sciences</td>
<td>#25108</td>
</tr>
</tbody>
</table>

| **Chemicals, peptides, and recombinant proteins** | | |
| Cycloheximide | Sigma-Aldrich | 01810 |
| Geldanamycin | Cayman chemicals | 13355 |
| MG132 | Enzo Life Sciences | BML-P100-0005 |
| cOmplete protease inhibitor cocktail | Roche | 11697498001 |
| Pefablock | Roche | 11429868001 |
| L-Azetidine-2-carboxylic acid (AZC) | Bachem | 4019045 |
| Wheat Germ Agglutinin Alexa Fluor 555 conjugate (WGA-orange) | Invitrogen | W32464 |

| **Critical commercial assays** | | |
| FuGENE HD Transfection Reagent | Promega | E2311 |
| 4-12% gradient 26 well Criterion XT Bis-Tris Protein gel | Bio-Rad | 3450125 |
| PVDF membrane | Millipore | iPFL00005 |
| Odyssey Blocking buffer in PBS | LI-COR | 927-40000 |

| **Deposited data** | | |
| Affinity-based mass spectrometry data for interactors of Sgt1 at 30°C and 42°C | This paper (Table S2) and ProteomeXchange | PXD016174 |

| **Experimental models: Cell lines** | | |
| Human: HeLa | ATCC (Manassas, VA) | ATCC Cat# CCL-2; RRID:CVCL_0030 |

| **Experimental models: Organisms/Strains** | | |
| S. cerevisiae strain Y7092 SGA query: Strain background: S288C MATα (can1::STE2pr-Sp_his5 lyp1::his3Δ1 leu2Δ0 ura3Δ0 met15Δ0) | Boone laboratory | N/A |
| S. cerevisiae strain BY4741: Strain background: S288C genotype: MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | EUROSCARF | Y00000 |
| All Saccharomyces cerevisiae yeast strains used in this study were of the S288C or BY4741 background and are listed in Table S3 | EUROSCARF or Nystrom laboratory | N/A |

| **Oligonucleotides** | | |
| VHL FWD: ATTTTTTTTTTTCTACTCAAATATACATACGCTGCATG | this paper | N/A |
| VHL REV: Cattccccggagggcggagaactgg | this paper | N/A |

(Continued on next page)
Continued

**REAGENT or RESOURCE** | **SOURCE** | **IDENTIFIER**
--- | --- | ---
VHL REV GTACACCTGGCAAAAACGACGATCTTCTTAGGGCCAGAGTTAACCACTTCCTTTAAAAGAACGACGATCTTCTTTAATGTCACCTGGCAAAACGACGATCTTCTTACATATCTCCCATCCGTGGATGTTGAC this paper N/A
HygExURA3 up: CTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAAGATAAATCATGGGTAANAAAGCTGAACACTAC this paper N/A
HygExURA3 down CTAATTTGTGAGTTTAGTATACATGCATTTACTTATACTGTTTTATTCCCTTTGCCCTCAGACCG this paper N/A
HUL5 S1 GCCTTTATAATTTAATCTCAACGCGATTCTAAAGGAGATTTCTGAC TTAAATAGCTGACCTGCTGAGCCGAC this paper N/A
HUL5 S4 GTCCTATTTCCCTAAATTGACATTTCTTCTTGTTGACCGGTGAAATTTTATCCTCTTTTGAGGAC this paper N/A
SGT1 S1 GTCTTTTTAAGGTTAAAGAGGTAGTTGTTTTAAGGAAACGAAAAGAAATGCGTACGCTGCTGAGGT this paper N/A
SGT1 S4 CTCACTATAACAAAGCTTTGTAGGCAGTTTTTAAATCTTTTTCGGAAGATGATCGAGCCCTTACGCT this paper N/A
Hsp82 S3 TGAAGAGGTTCCAGCTGACACCGAAATGGAAGAGGTAATGGACGAGCTGTACAAGTCCGGACTCAGATCTCGAGCTCAGACCTTTAAGGAGAGGTACGGTTAAGGTCTTTGGGAACAGAAACCAGATGATGTACAGTATTATTGTCAAAGAGCTTATTGTCACATTCTTTGGGAATTACCTGTGGTTGCTGTTGATGCAAAGAAGTCTCTAGAACTCAATCCAAAATATTCCACTGCTATGCTGAGAAAAGGAATATGTGAATACCATGAAAAAAGTATGACTGGATTATCAAACAGAATCTCAAGTAGTCATTACACTTATGATCAAGAATGTTCAGAAGAATGATGTAAATGTGGAATTTCAGAAAAAGGTTGTCTGCTTTGGTTAAACTTCCTTCTGGAGAGGATTACAATTGAAACTGGAACTTCTTCATCCTATAATACCAGAACAGACGTTTAAAAGTACTTTCAACAAAGATTGAAATTAAACTGAAAAAGCCAGAGGCTGTGAGATGGGAAAAGCTAGAGGGGCAAGGAGATGTGCCTACGCCAAAACATTCGTAGCAGATGTAAAGAACCTATATCCATCATCATCTTATACAAAGAAATTGGGATAAATTGGTTGGTGAGATCAAAGAAGAAGAAAAGAATGAAAAGTTGGAGGGAGATGCAGCTTTAAACAGATTATTTCAGCAGATCTATTCAGATGGTTCTGATGAAGTGAAACGTGCCATGAACAAATCCTTTATGGAGTCGGGTGGTACAGTTTTGAGTACCAACTGGTCTGATGTAGGTAAAAGGAAAGTTGAAATCAATCCTCCTGATGATATGGAATGGAAAAAGTACTAAGGGCCCGGGATCCACCGGATCTAGATAACTGATCATAATC

**Recombinant DNA**

SGT1A/ SUGT1A 333 AA short version from 1 to 1002, CAC51433_1 from 1 to 1002, 40bps overlap CATGGAGACGCTGTACAAGTCCGGACTCAGATCTCGAGCTCATTTCCCTGATCGACCAGAGCGACCAGCCAGCCAGCGCCAGCGCGCGTTAGAGGAGCTGACTAAGGCTTTGGAACAGAAACCAGATGATGTTACAGTATTATTGTCAAAGAGCTTATTGTCACATTCTTTGGGAATTACCTGTGGTTGCTGTTGATGCAAAGAAGTCTCTAGAACTCAATCCAAAATATTCCACTGCTATGCTGAGAAAAGGAATATGTGAATACCATGAAAAAAGTATGACTGGATTATCAAACAGAATCTCAAGTAGTCATTACACTTATGATCAAGAATGTTCAGAAGAATGATGTAAATGTGGAATTTCAGAAAAAGGTTGTCTGCTTTGGTTAAACTTCCTTCTGGAGAGGATTACAATTGAAACTGGAACTTCTTCATCCTATAATACCAGAACAGACGTTTAAAAGTACTTTCAACAAAGATTGAAATTAAACTGAAAAAGCCAGAGGCTGTGAGATGGGAAAAGCTAGAGGGGCAAGGAGATGTGCCTACGCCAAAACATTCGTAGCAGATGTAAAGAACCTATATCCATCATCATCTTATACAAAGAAATTGGGATAAATTGGTTGGTGAGATCAAAGAAGAAGAAAAGAATGAAAAGTTGGAGGGAGATGCAGCTTTAAACAGATTATTTCAGCAGATCTATTCAGATGGTTCTGATGAAGTGAAACGTGCCATGAACAAATCCTTTATGGAGTCGGGTGGTACAGTTTTGAGTACCAACTGGTCTGATGTAGGTAAAAGGAAAGTTGAAATCAATCCTCCTGATGATATGGAATGGAAAAAGTACTAAGGGCCCGGGATCCACCGGATCTAGATAACTGATCATAATC

gBlocks from IDT N/A

All plasmids used in this study are listed in Table S4

**Software and algorithms**

ImageJ (Schneider et al., 2012) https://imagej.nih.gov/ij/
TheCellMap (Usaj et al., 2017) https://thecellmap.org

**Other**

Zeiss Axio Observer.Z1 inverted microscope with Apotome and Axiocam 506 camera Carl Zeiss N/A
LI-COR Odyssey Infrared scanner LI-COR N/A
Criterion Cell Bio-Rad 1656001
Wet blotting system (Criterion Blotter) Bio-Rad 1704070

Cell Reports 35, 109328, June 29, 2021
RESOURCE AVAILABILITY

Lead contact
Further information and requests for reagents may be directed to, and will be fulfilled by the Lead Contact, Thomas Nyström, (thomas.nystrom@cmb.gu.se).

Materials availability
All unique reagents generated in this study are available from the Lead Contact without restriction.

Data and code availability
All datasets generated or analyzed during this study are included in the article, except for microscopy images used for quantitative analysis, which are available upon request. Affinity based mass spectrometry data are also deposited at ProteomeXchange with the accession code: PXD016174.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Yeast strains
Strains and plasmids are listed in Tables S3 and S4, respectively. The strains used in this study are derivatives of BY4741 (Brachmann et al., 1998) or haploid strains generated with the SGA methodology (Tong and Boone, 2006) (SGA library). Overexpression, N-terminal GFP tagged strains and additional deletions were generated via homologous recombination with the molecular toolbox (Janke et al., 2004). The mRuby2 tag was amplified from plasmid pFA6a-link-yomRuby2-Kan (Lee et al., 2013) via PCR with S2 and S3 primers specific for HSP82 and recombined into the C-terminus of genomic Hsp82. Overexpression of the SGT1 and HUL5 open reading frames was achieved by integration of the GPD promoter amplified from pYM-N15 with primers S1 and S4, specific to SGT1 or HUL5, respectively. GPD promoter and N-terminal EGFP tagging of SGT1 was achieved by amplification from pYM-N17 with primers SGT1 S1 and S4. Cells were selected on YPD clonNat plates and correct integration was verified by PCR. Primer sequences can be found in the Key Resources Table.

Cells were cultured at 30°C, or at 22°C in case of temperature sensitive strains, in either rich YPD medium, in complete synthetic medium (CSM) or in synthetic dropout (SD) media lacking the appropriate components (Amberg et al., 2005).

Mammalian cell culture
HeLa cells were grown in DMEM media (with 4500 mg/L glucose, 25 mM HEPES, MERCK) supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen) at 37°C in a humidified atmosphere of 5% CO2.

METHOD DETAILS

Plasmid constructions
Plasmid pFE30 expressing VHL-Leu2 was constructed by homologous recombination into plasmid pFE15 (Eisele and Wolf, 2006) expressing Δ ΔsCL*. Therefore, the sequence encoding VHL was amplified by PCR from vector pESC-LEU-GFP-VHL with primers VHL Fwd and VHL Rev. Purified PCR product was co-transformed into yeast cells with Bsu36I digested vector pFE15 and selected for growth on SD-Ura plates. The resulting plasmid pFE30 was rescued and tested by sequencing.

For replacing the URA3 selection marker in plasmid pFE15 and pFE30, the hphNT1 cassette from pFA6a–hphNT1 (Janke et al., 2004) was amplified with primers HygExURA3 up and HygExURA3 down. Purified PCR product was transformed into yeast cells harboring vector pFE15 or pFE30 and selected for growth on YPD hygromycin B plates. Resulting plasmids pFE35 and pFE36 were rescued and tested by sequencing. For construction of pSgt1 expressing human Sgt1 with a N-terminal EGFP tag for expression in animal cell lines SUGT1/ SGT1A coding double stranded DNA was synthetized flanked with 40 bps overhangs compatible to the multiple cloning site of pEGFP-C1. SGT1A coding DNA and vector were digested with Apal and SacI, ligated and transformed into DH5α cells. The resulting plasmid pSgt1 (pFE67) was tested by sequencing. Please see Key Resources Table for oligonucleotide and sequence details, Table S4 for plasmid details.

Genome-wide screen for stabilizers of two misfolding proteins
The yeast strains used (S228C background) were grown in synthetic drop-out media with corresponding antibiotics. SGA mating of query strains (Y7092) containing Δ ΔsCL* (pFE15) or VHL-Leu2 (pFE30) into the yeast deletion collection SGAV2 array and a conditional temperature sensitive (ts) allele collection (TSV5 array) (both layouts from the Boones laboratory, spotted in a 1536-spot format by using a SINGER ROTOR HDA Robot (Singer Instrument Co. Ltd.)) were performed as previously described in Tong and Boone (2006). Selected haploid crossed cells were spotted on SD/MSG-His/Arg/Lys/Ura/+canavanine/thialysine/G418 and SD/MSG-His/Arg/Lys/Ura/Leu/+canavanine/thialysine/G418. Images were taken after two days growth at 30°C and colony sizes were analyzed as previously described (Costanzo et al., 2010; Wagih et al., 2013).
Mutants displaying good growth with a score of 1 or higher are indicated in Table S1. Common hits from ΔssCL* and VHL-Leu2 screen were used for analysis with the TheCellMap.org.

**Spot tests**
For ΔssCL*, CTL*, Leu2-myc spot tests overnight pre-cultured cells expressing ΔssCL*, CTL* or Leu2-myc were diluted to an OD of A_{600} = 0.5 and spotted in a 5-fold dilution series on SD-Ura and SD-Ura-Leu media. Plates were incubated at 30 °C for 3–4 days.

For complementation assays strains were cotransformed with a plasmid expressing ΔssCL* or VHL-Leu2 with a hphNTI selection marker (pFE35 or pFE36, respectively) and with MoBY plasmids expressing wild-type alleles of the corresponding mutant strains, or the empty vector control (p5586) (Ho et al., 2009). 5-fold dilutions of cells were spotted on SD-Ura +hygromycin B and SD-Ura-Leu +hygromycin B. For complementation of the sgt1-3 allele by the GFP-SGT1 fusion allele, strains were crossed to obtain heterozygote diploid strains and tested by spot test like described below.

For heat sensitivity assays exponentially growing cells were diluted to OD_{600} = 0.5 and then spotted on YPD plates, in a tenfold dilution series. Plates were incubated at the indicated temperatures for 2–3 days prior to imaging.

**Co-immuno purifications (Co-IP)**
Cells were inoculated and grown over night in complete synthetic media (CSM), SD-Ura (if carrying pFE15 plasmid) or SD-His (pEGFP ctrl plasmid) to OD_{600} = 0.7–0.8. Fifty OD_{600} of exponentially grown cells were harvested and washed twice with ice-cold water (2 min at 3220xg, 4 °C). Cells were washed once with ice-cold IP buffer (115 mM KCl, 5 mM NaCl, 2 mM MgCl₂, 1 mM EDTA/NaOH pH 8.0, 20 mM HEPES/KOH pH 7.45) and resuspended in a final volume of 300 μL IP buffer. Cells were snap frozen and kept at –80 °C. A volume of 200 μL glass beads, 1 mM DTT, 0.5 μg/ml Pefabloc SC, 1x cOmplete (Roche) were added (calculated for 1 mL final volume) and cells were opened by 10 times vortexing for 45 s with 1 min chilling on ice between the cycles. Lysates were transferred to a new tube and buffer was added to 1 mL final volume. Lysates were incubated with 0.5% Nonidet P-40 (Roche) for 20 min on ice, followed by centrifugation at 16000xg for 20 min at 4 °C. 800 μL of pre-cleared cell lysate was incubated with 20 μL bed volume GFP-TrapA beads (Chromotek) to pull down GFP tagged proteins for 1 h at 4 °C on an over-head rotator. Beads were pelleted by centrifugation at 8000 rpm and washed 4 times with IP buffer containing 0.5% Nonidet P-40. For co-IP experiments with Hsp90 inhibition, 50 μM geldanamycin (GA) was added to growing cells 30 min prior of harvest and kept at this concentration during lysis, co-IP and washing. For immunoblot analysis beads were boiled for 5 min at 95 °C in 2x loading buffer/5% beta-mercaptoethanol. For mass spectrometry analysis beads were subjected for a final wash in 50 mM TEAB buffer followed by 2 sequential rounds of elution in 1% formic acid. Elutions were pooled and subsequently treated as described below in section sample preparation and TMT labeling.

**In vivo ubiquitination assay**
Samples were principally prepared like described in Geng and Tansey (2008). Cells harboring plasmids expressing His6-Ub and ΔssCL* or empty control vector were grown in SD-Ura hygromycin B media to early exponential growth phase. His6-Ub expression was induced by addition of 0.1 mM CuSO₄ 4 h previous to harvest. 100 OD_{600} of cells were harvested (4 min at 3220xg, 4 °C) and washed with ice cold dH₂O. 1% of the cells were used as expression level control and were lysed as described below in section western blotting. Cells were lysed with a MP FastPrep (level 5.5, 30 s, 5 min on ice, 4 cycles) in 800 μL lysis buffer. Cells were snap frozen and kept at –80 °C. A volume of 200 μL glass beads, 1 mM DTT, 0.5 μg/ml Pefabloc SC, 1x cOmplete (Roche) were added (calculated for 1 mL final volume) and cells were opened by 10 times vortexing for 45 s with 1 min chilling on ice between the cycles. Lysates were transferred to a new tube and buffer was added to 1 mL final volume. Lysates were incubated with 0.5% Nonidet P-40 (Roche) for 20 min on ice, followed by centrifugation at 16000xg for 20 min at 4 °C. 800 μL of pre-cleared cell lysate was incubated with 20 μL bed volume GFP-TrapA beads (Chromotek) to pull down GFP tagged proteins for 1 h at 4 °C on an over-head rotator. Beads were pelleted by centrifugation at 8000 rpm and washed 4 times with IP buffer containing 0.5% Nonidet P-40. For co-IP experiments with Hsp90 inhibition, 50 μM geldanamycin (GA) was added to growing cells 30 min prior of harvest and kept at this concentration during lysis, co-IP and washing. For immunoblot analysis beads were boiled for 5 min at 95 °C in 2x loading buffer/5% beta-mercaptoethanol. For mass spectrometry analysis beads were subjected for a final wash in 50 mM TEAB buffer followed by 2 sequential rounds of elution in 1% formic acid. Elutions were pooled and subsequently treated as described below in section sample preparation and TMT labeling.

**Cycloheximide chase**
Exponentially growing cells expressing ΔssCL*, CTL*, CTG* or CPY*HA were harvested and resuspended in synthetic drop out media. GFP-Sgt1-3 expressing cells were resuspended in YPD media. 50 μM geldanamycin (GA) was added to growing cells 30 min prior of chase where indicated. Protein expression was shut off by addition of cycloheximide (1.77 mM final). 2 OD_{600} of cells were harvested by adding to ice cold NaN₃ (30 mM final concentration) at indicated time-points. Protein samples for western blotting were prepared as described below.

**Metabolic chase**
Cells expressing VHL-GFP were grown in SD-Leu containing 2% (w/v) galactose. For shut-off of VHL-GFP expressing cells were shifted in exponential growth phase to SD-Leu containing 2% glucose. 2 OD_{600} of cells were harvested at indicated time points by adding to ice cold NaN₃ (30 mM final concentration). Protein samples for western blotting were prepared as described below.

**Western blotting**
Samples were principally prepared as described in Hwang et al. (2009). Cells were resuspended in 1 mL of ice cold 0.2 M NaOH and incubated for 20 min on ice. Cells were pelleted and resuspended in 100 μL urea loading buffer (8 M urea in 1x loading buffer; 50 mM
Tris/HCl pH 6.8, 2.5 mM EDTA/NaOH pH 8.0, 2% (w/v) Na-Dodecylsulfat (SDS), 0.05% (w/v) bromphenol blue). Prior to use 1% (v/v) beta-mercaptoethanol and complete protease inhibitor (Roche) was added to urea loading buffer. After incubation for 10 min at 70°C, samples were centrifuged at 13000xg for 1 min. 15 µl were loaded on a 4%–12% gradient 26 well Criterion XT Bis-Tris Protein gel (Bio-Rad). Gels were transferred on PVDF membrane (Millipore) with a wet blotting system (Criterion Blotter, Bio-Rad). The blots were incubated in Odyssey blocking buffer (LI-COR) for 1 h at room temperature prior to probing with primary antibodies in PBS-T. Membranes were washed and incubated with the appropriate secondary antibody (LI-COR, IRdye secondary antibodies). Membranes were scanned on a LI-COR Odyssey scanner, and western blots were quantified using ImageJ (NIH). Please see Key Resources Table for antibodies, reagents, and equipment details.

**Replicative lifespan assay**
Exponentially growing cells were plated on YPD plates and allowed to recover before assayed for replicative lifespan. A micromanipulator (MSM 400, Singer instruments) was used to select mother cells and to remove their daughters for assessing of replicative age.

**Isolation of old mother cells**
Old cells expressing Hsp104-GFP were isolated using the magnetabind biotin-streptavidin system according to established protocols (Hill et al., 2016; Sinclair and Guarente, 1997; Smeal et al., 1996). Biotin labeled cells were isolated after culturing for one day. The median age of the old cells was determined by counting of bud scars after formaldehyde fixation and staining cells with 10 µg/mL Wheat Germ Agglutinin Alexa Fluor® 555 conjugate (WGA, Life Technologies).

**Assessment of colony forming units (CFU)**
Cells were grown in CSM to mid-exponential phase at 30°C. Cells were shifted to 42°C for 30 min, and then allowed to recover at 30°C for 90 min. Control cells were kept growing at 30°C during that period and then adjusted to same optical density. Cells were diluted sequentially one to hundred, followed by a two or ten-fold dilution. 100 µl cell suspension were plated on CSM agar plates and number of CFU assessed after growth for 5 days at 22°C.

**Hsp104, Hsp42 and Guk1-7 protein aggregation induction**
Fluorescent protein tagged cells were grown to mid-exponential phase at 30°C in CSM and then shifted to 38°C, or to 42°C for 30 min and then back to 30°C. Samples were taken at indicated time points, fixed with formaldehyde (3.7% final concentration) for 30 min, washed three times with PBS and observed by fluorescence microscopy (see below).

**Sgt1, Hsp82, Vps13, Hsp42-, and synphilin-1 foci formation assay**
Fluorescent protein tagged cells were grown in CSM (or SD-Ura in case of dsRed-synphilin-1) to mid-exponential phase at 30°C. If cells were to be treated with 26S proteasome inhibitor MG132 (75 µM final concentration, EnzolifeSciences), SDS was added to growing cells to a final concentration of 0.003% three hours prior to addition of the drug and heat shock (Liu et al., 2007). Geldanamycin (GA) was added to a final concentration of 70 µM prior to heat shock (Theodoraki et al., 2012). For induction of HAPI or Vps13 and synphilin-1 foci, cells were shifted to 42°C water bath for 30 min. Then cells were briefly spun at 5000xg and observed with a Zeiss Axio Observer Z1 inverted fluorescence microscope, using Plan Apo 100X oil objective NA:1.4 and the following filter sets: 38 HEEgFP, 45 HQ TexasRed.

**Cell culture and transient transfection**
HeLa cells were grown on glass coverslips in DMEM (with 4500 mg/L glucose, 25 mM HEPES, MERCK) supplemented with 10% FCS, 2 mM glutamine, 100U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen) at 37°C in a humidified atmosphere of 5% CO₂. Cells were transiently transfected with eGFP-C1-SUGT1 or eGFP-C1-EV by using FuGENE HD (Promega). 18-22 h post-transfection cells were treated with MG132 (50 µM) or geldanamycin (75 µM) or DMSO as a control for 1h (Ogrodnik et al., 2014). Cells were heat shocked at 40°C for 15 min and directly observed under the microscope (see above).

**Electron microscopy**

**High pressure freezing and freeze substitution of yeast cells**
Exponentially growing GFP-Sgt1 expressing yeast cells were grown at 30°C or shifted to 42°C for 30 min prior to harvesting by filtering and high pressure freezing in a Wohland Compact 03 (M. Wohland GmbH, Sennwald, Switzerland). Freeze substitution was carried out in a Leica EM AFS2 (Leica Microsystems, Vienna, Austria) using 2% uranyl acetate dissolved in 10% methanol and 90% acetone for 1 h at –90°C (Höög et al., 2014). The temperature was raised to –50°C, 2.9°C per hour, during two washes in acetone. The cells were infiltrated with Lowicryl HM20 (Polysciences, Warrington, PA) mixed with acetone (1:4, 2:3, 1:1, 4:1) and three times with pure Lowicryl, each step lasting 2 hours. The resin was polymerized with UV light 72 h at –50°C followed by 24 hr at room temperature. 70 nm ultra-thin sections were produced using a Reichert-Jung Utlracut E Ultramicrotome (C. Reichert, Vienna, Austria) and an ultra 45° diamond knife (Diatome, Biel, Switzerland). The thin sections were placed on copper grids coated with 1% Formvar and on-section contrast stained. Micrographs were taken on a Tecnai T12 electron microscope equipped with a Ceta CMOS 16M camera (FEI Co., Eindhoven, the Netherlands) operated at 120 kV.
**Immunoelectron microscopy**

Grids were fixed in 1% paraformaldehyde in PBS for 10 min at room temperature, washed 3 × 1 min in PBS, and blocked for 1 h in 0.8% BSA + 0.1% fish skin gelatin in PBS at room temperature. For detection of GFP-Sgt1, grids were incubated in a 1 to 30 dilution of rabbit anti-GFP (ab6556, abcam, Cambridge, UK) at 4°C over-night followed by a 1 to 20 dilution of goat anti-rabbit 10 nm gold (#25108, Electron Microscopy Sciences, Hatfield, PA) for 1 h at room temperature. 3 × 20 min wash steps were carried out in PBS after incubations with each antibody. Antibodies were fixed in 1% glutaraldehyde in dH2O for 1 h and washed 3 × 1 min in dH2O. 2% uranyl acetate and Reynold’s lead citrate were used for on-section contrast staining (Reynolds, 1963). Imaging as above.

**Mass spectrometry**

**Sample preparation and TMT labeling**

Cysteines were reduced with dithiothreitol (Biomol) at 56°C for 30 min (10 mM in 50 mM HEPES (Biomol), pH 8.5), and further alkylated with iodoacetamide (Merck) at room temperature, in the dark for 30 min (20 mM in 50 mM HEPES, pH 8.5). For sample clean up and digestion, the SP3 protocol (Hughes et al., 2014) was used and trypsin (sequencing grade, Promega) was added (enzyme to protein ratio 1:20) for overnight digestion at 37°C. Next day, peptides were extracted and labeled with TMT10plex Isobaric Label Reagent (ThermoFisher) according the manufacturer’s instructions. For further sample clean up an Oasis® HLB µElution Plate (Waters) was used. Offline high pH reverse phase fractionation was carried out on an Agilent 1200 Infinity high-performance liquid chromatography system, equipped with a Gemini C18 column (3 µm, 110 Å, 100 × 1.0 mm, Phenomenex).

**Mass spectrometry data acquisition**

An UltiMate 3000 RSLC nano LC system (Dionex) fitted with a trapping cartridge (μ-Precolumn C18 PepMap 100, 5 µm, 300 µm i.d. x 5 mm, 100 Å, Thermo Fisher) and an analytical column (Acclaim PepMap 100, 75 µm x 50 cm, naoViper column, Thermo Fisher). Trapping was carried out with a constant flow of solvent A (0.1% formic acid in water) at 30 µL/min onto the trapping column for 6 min. Subsequently, peptides were eluted via the analytical column with a constant flow of 0.3 µL/min with increasing percentage of solvent B (0.1% formic acid in acetonitrile) from 2% to 4% in 4 min, from 4% to 8% in 2 min, then 8% to 28% for a further 96 min, and finally from 28% to 40% in another 10 min. The outlet of the analytical column was coupled directly to a QExactive plus (Thermo Fisher) mass spectrometer using the proxone nanoflow source in positive ion mode.

The peptides were introduced into the QExactive plus via a Pico-Tip Emitter 360 µm OD x 20 µm ID; 10 µm tip (New Objective) and an applied spray voltage of 2.3 kV. The capillary temperature was set at 320°C. Full mass scan was acquired with mass range 375-1200 m/z in profile mode in the FT with resolution of 70000. The filling time was set at maximum of 250 ms with a limitation of 3x10^6 ions. Data dependent acquisition (DDA) was performed with the resolution of the Orbitrap set to 35000, with a fill time of 120 ms and a limitation of 2x10^4 ions. A normalized collision energy of 32 was applied. A loop count of 10 with count 1 was used and a minimum AGC trigger of 2e6 was set. Dynamic exclusion time of 30 s was used. The peptide match algorithm was set to ‘preferred’ and charge exclusion ‘unassigned’, charge states 1, 5 - 8 were excluded. MS² data was acquired in profile mode.

**MS data analysis**

IsobarQuant (Franken et al., 2015) and Mascot (v2.2.07) were used to process the acquired data, which was searched against a UniProt Saccharomyces cerevisiae proteome database (UP000002311) containing common contaminants and reversed sequences. The following modifications were included into the search parameters: Carbamidomethyl (C) and TMT10 (K) (fixed modification), Acetyl (N-term), Oxidation (M) and TMT10 (N-term) (variable modifications). For the full scan (MS1) a mass error tolerance of 10 ppm and for MS/MS (MS2) spectra of 0.02 Da was set. Further parameters were set: Trypsin as protease with an allowance of maximum two missed cleavages: a minimum peptide length of seven amino acids; at least two unique peptides were required for a protein identification. The false discovery rate on peptide and protein level was set to 0.01. The protein output files of IsobarQuant were processed using the R programming language (https://www.r-project.org). As a quality filter, only proteins that were quantified with at least 2 unique peptides were used for the downstream analysis. Raw TMT reporter ion signals (signal_sum columns) were first batch-cleaned using the removeBatchEffect function from the limma package (Ritchie et al., 2015) and further normalized using the vsn package (variance stabilization normalization; Huber et al., 2002). Missing values were imputed using the knn option of the impute function using the Msnbase package (Gatto and Lilley, 2012). Proteins were tested for differential expression using limma again and called hits with a false discovery smaller 1% and a fold-change cut-off of 100%. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD016174 (See also Key Resources Table).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

To identify network regions significantly enriched for ΔssCL* and VHL-Leu2 stabilization, as well as for physical interaction with Sgt1 at 30°C and 42°C, hits were analyzed with the help of TheCellMap.org.

Lifespan assays were done in at least two replicates. Statistical analysis was performed using Logrank (Mantel-Cox test) in GraphPad Prism® 8.2.1 (Figures 2E, 2F, and S2F). Statistical analysis of Sgt1 localization by immunoelectron microscopy performed using Wilcoxon matched-pairs signed rank test in GraphPad Prism® 8.2.1 (Figure 3H). All data in the bar graphs are presented as an average of n ≥ 3 replicates ± SEM. In figures, asterisks denote statistical significance as calculated by Student’s t test *p < 0.05, **p < 0.005, ***p < 0.0005, unpaired two tailed t test using MS Excel.