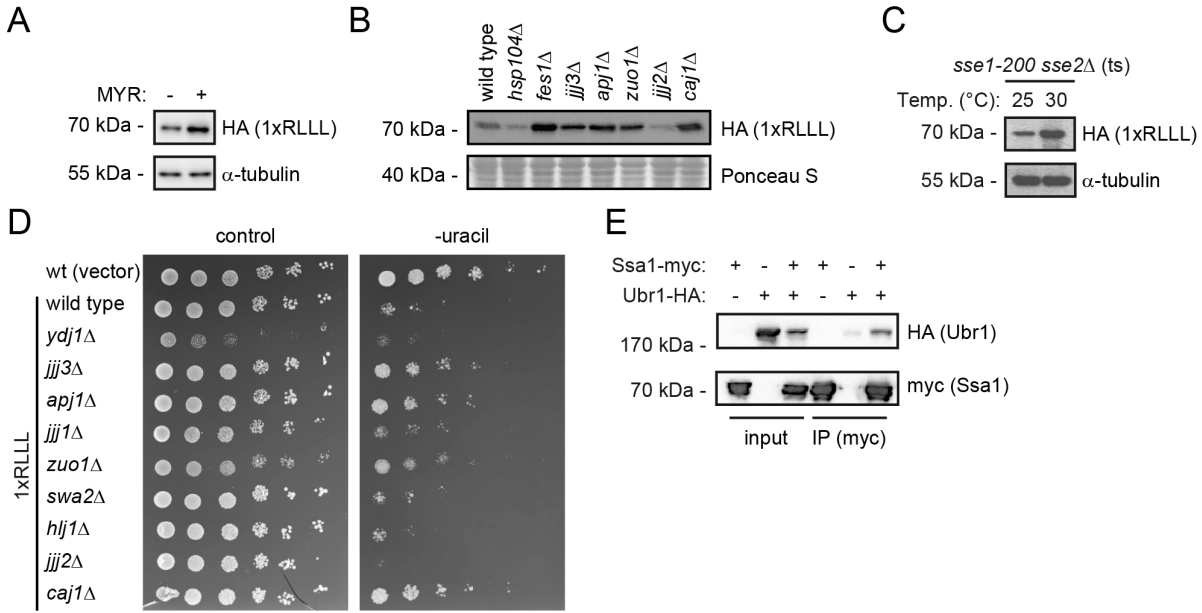


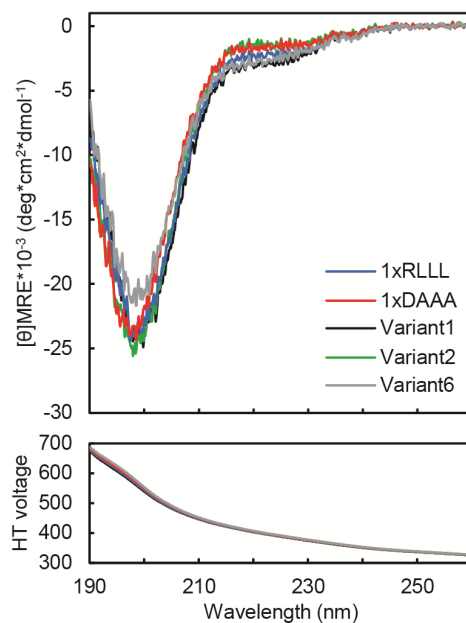
HSP70-binding motifs function as protein quality control degrons

Supplemental Material

Supplemental Figure S1 , Effect of chaperones and co-chaperones	p. 2
Supplemental Figure S2 , Circular dichroism spectroscopy of selected peptides	p. 3
Supplemental Table S1 , DNA primers used in this study	p. 4



Supplementary Figure S1 *Effect of chaperones and co-chaperones.* (A) The protein levels of the RLLL degron from cells treated (+) or untreated (-) with the Hsp70-inhibitor myricetin (MYR) for 16 hours were compared by SDS-PAGE and blotting against the HA-tag on the reporter. Tubulin served as loading control. (B) The protein levels of the RLLL degron were compared in the indicated yeast strains by SDS-PAGE and blotting for the HA-tag on the reporter. A Ponceau S staining of the membrane is included as loading control. (C) Protein levels in the temperature-sensitive (ts) Hsp110-double mutant strain (*sse1-200sse2 Δ*) at 25 $^{\circ}$ C and 30 $^{\circ}$ C were compared by blotting against the HA-tag. Tubulin served as loading control. (D) The dependence of co-chaperones for targeting the RLLL degron was analyzed by growth assays on solid media using the indicated null mutants. Wild-type cells transformed with the reporter vector alone were included for comparison. (E) Wild-type yeast cells expressing myc-tagged Ssa1 and/or HA-tagged Ubr1, as indicated, were used for immunoprecipitation (IP) with myc-trap resin. The precipitated material was analyzed by SDS-PAGE and western blotting using antibodies to myc and HA.



Supplementary Figure S2 *Circular dichroism spectroscopy of selected peptides.* The secondary structure of selected variants was analyzed by far-UV circular dichroism (CD) spectroscopy. The top panel shows molar ellipticity and the lower panel shows HT voltage.

Supplementary Table S1
DNA primers used in this study

Name	Sequence	Purpose
F1 _LEU2_1119_ fw	GCTGGTGATTATAATACCATT TAGGTGGGTTGG	Forward fragment 1 containing URA3-GFP for library construction
F1_degron_ta g_18_rv	TGATTGTAACAATGCACAGG ATCCTTCGTC	Reverse fragment 1 containing URA3-GFP for library construction
F2_degron_ta g_fw	GACGAAGGATCCTGTGCATT GTTACAATCA /iTriMix20//iTriMix20//iTriMix20/ /iTriMix20/ TCAGCACCAAGAAGAGCTGC	Forward fragment 2 containing a degron tag including four trimer 20 codon mixes for library construction
F2_rv	ATTACGCCAAGCTCGAAATT AACCC	Reverse fragment 2 containing a degron tag for library construction
F3_fw	AGTGAGGGTTAATTCGAGC TTGGC	Forward fragment 3 containing origin of replication for library construction
F3_LEU2_73 5_rv	CCAACCCACCTAAATGGTAT TATAATCACC	Reverse fragment 3 containing origin of replication for library construction
Illumina_adap ter_fw	TCGTCGGCAGCGTCAGATGT GTATAAGAGACAG cctacgatcgacgaagg	Forward containing degron tag for library sequencing
Illumina_adap ter_rv	GTCTCGTGGGCTCGGAGATG TGTATAAGAGACAG tgtggaattgtgagcggata	Reverse containing degron tag for library sequencing
VV48	GGGTGCTGATCCAGGTGTAC	qPCR HSPA1A/B forward
VV49	GTCGAAGGTCACCTCGATCT G	qPCR HSPA1A/B reverse
VV52	GGCCAGGTCATCACCATTGG	qPCR ACTB forward
VV53	CGGATGTCCACGTCACACTTC	qPCR ACTB reverse