



Figure S4

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(A) *atg19Δ* and *hrr25-ts atg19Δ* cells containing HTB-Atg19 were grown to mid-log phase. Atg19 was affinity purified and subjected to quantitative mass spectrometric phosphorylation mapping (SILAC). Enlarged: phosphorylation sites; gray: lysine substitutions; green: Atg11 binding region; red: phosphorylation sites downregulated in *hrr25-ts* cells; orange: sites likely downregulated in *hrr25-ts* cells; blue: phosphorylation sites upregulated in *hrr25-ts* cells; purple: phosphorylation unchanged; underlined: sequence coverage of this SILAC experiment. (B) GFP-Atg11 wild-type, GFP-Atg11 *hrr25-ts* and GFP-Atg11 *atg19Δ* log phase cells containing BFP-Ape1 were stained with FM4-64, grown at 37°C for 2 hours and analyzed by fluorescence microscopy. Co-localization of Ape1 dots with GFP-Atg11 and the vacuole was quantified in at least 65 cells of each strain. +vac: Ape1 dot at the vacuolar membrane, -vac: Ape1 dot clearly distant to the vacuolar membrane, green bars: Ape1 dots positive for GFP-Atg11, black bars: Ape1 dots negative for GFP-Atg11. Scale bar = 3 μm. (C) Wild-type and *hrr25-ts* cells containing GFP-Atg19 were analyzed for GFP dot formation. Scale bar = 2 μm. (D) Wild-type, *atg11Δ*, GFP-Atg11 and GFP-Atg11 *hrr25-ts* cells were analyzed for Atg11 levels at 37°C by western blotting. Pgk1 serves as a loading control.