Figure S1. Heat-Shock does not Induce Changes in the Molecular State of Ypt1p<sup>G80D</sup>. Mutant ypt1-<i>G80D</i> cells were grown in YPD medium (1 × 10<sup>8</sup> cells/ml) and incubated at 27°C or 45°C for 45 min. Subsequently, total cytosolic extracts of the cells were subjected to SEC analysis. The upper panel shows traces of the resolved protein peaks in the SEC analysis (upper image), and the lower panel shows immunoblot detection of Ypt1p<sup>G80D</sup> in the corresponding fractions after SDS-PAGE. A 2.5 mg aliquot of total protein was applied to the SEC column, and 30 µl of each fraction was subjected to SDS-PAGE. Ypt1p<sup>G80D</sup> was detected with a polyclonal anti-Ypt1p antibody.
Figure S2. Ypt1p has Molecular Chaperone Activity, but Ypt1p<sup>G80D</sup> does not. For the chaperone activity assay, light scattering was monitored at 340 nm over a 15 min incubation period. (C) Solutions of 1 µM CS alone (○-) or with 8.35 µM GST (●-), Ypt1p (▲-), or Ypt1p<sup>G80D</sup> (■-) in 50 mM HEPES (pH 8.0) were incubated in a spectrophotometer cell at 43°C. Shown are representative data out of at least three independent experiments.
Figure S3. Experimental Workflow for the LC/MS Analysis Performed to Identify Putative Targets of Ypt1p Chaperone Activity.
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PSM, peptide-spectrum match. MW, molecular weight.