Figure S1

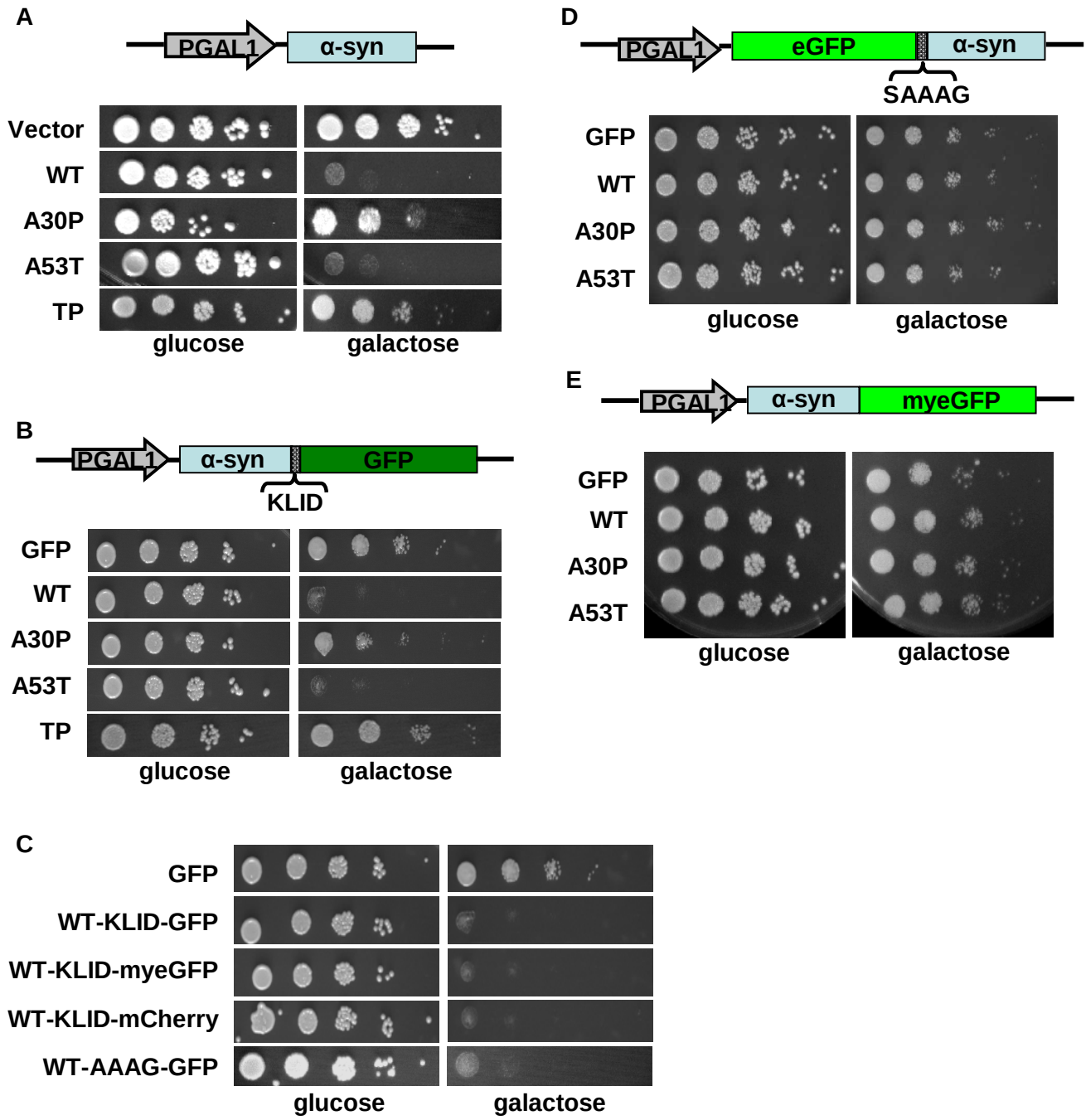

Figure S1. Tagged α-synuclein requires C-terminal GFP connected by a linker in order to reduce yeast growth. Growth was tested by spotting W303-1A *S. cerevisiae* overexpressing wild-type (WT), A53T, A30P or triple proline (TP) α-synuclein driven by the *GAL1* promoter from high-copy plasmids: (A) untagged, (B and C) C-terminally-tagged through different linkers (KLID, AAAG) with different fluorescent tags (GFP, myeGFP or mCherry), (D) N-terminally-tagged to eGFP via SAAAG linker or (E) C-terminally without linker. Linker sequences are indicated. The cells were serially spotted on inducing (galactose) and non-inducing (glucose) medium and incubated at 30°C for 2-3 days, then photographed. Cells carrying an empty vector or the vector with GFP under the *GAL1* promoter served as control.

Figure S2

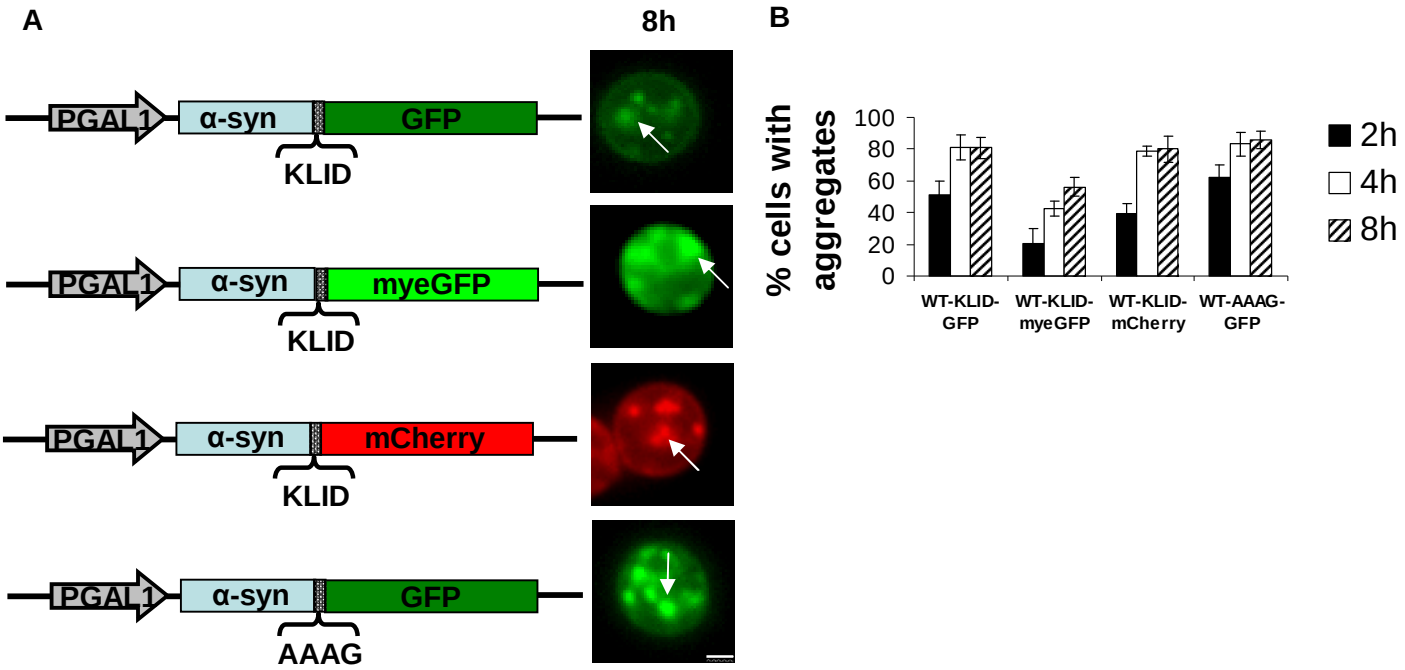

Figure S2. A C-terminal tag and a linker preserve the ability of α-synuclein to aggregate. (A) Live-cell microscopy of yeast cells expressing WT α-synuclein tagged with GFP, myeGFP and mCherry from a high-copy plasmid at 8 h of induction. White arrows point at aggregates. Scale bar = 1 µM. (B) Aggregation kinetics. The columns represent the average of 4 independent experiments.

Figure S3

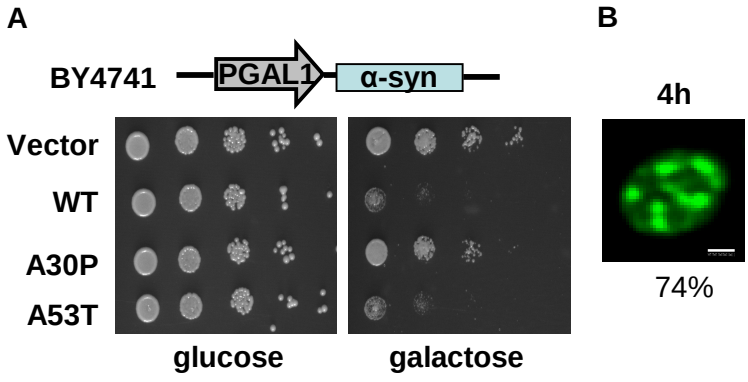

Figure S3. α-synuclein cytotoxicity and aggregation in the BY4141 background is the same as in the W303 background. (A) Growth analysis of BY4741 *S. cerevisiae* overexpressing wild-type (WT), A53T, A30P or triple proline (TP) α-synuclein driven by the *GAL1* promoter from high-copy plasmids. The cells were serially spotted on inducing (galactose) and non-inducing (glucose) medium and incubated at 30°C for 2-3 days, then photographed. Cells carrying an empty vector or the vector with GFP under the *GAL1* promoter served as control. (B) Live-cell fluorescence microscopy at 4h of α-synuclein induction. The percent of cells with aggregates at this time point is given under the picture. Scale bar = 1 µM.