Supporting Information

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SI Materials and Methods

Materials. Simvastatin was obtained from Sigma-Aldrich. Fluconazole was obtained from VWR.

Yeast Strains and Growth Conditions. All experiments were performed in the W303 background unless otherwise noted. Secretion assays used a *bar1* mutant strain, which was a gift from the Fink laboratory (Whitehead Institute, Cambridge, MA). The standard lithium acetate transformation protocol was used for all transformations (1). To select for single integrants, ~50 ng of cut plasmid DNA was used in the transformation, resulting in a mix of single and tandem integrants. To select for tandem integrants, ~500 ng of cut plasmid DNA was used in the transformation. Although all data used fusions with fluorescent proteins, untagged α-synuclein (α-syn) splice isoforms were retested in multiple experiments and behaved similarly.

Standard conditions were used for all yeast growth. For galactose induction, unless otherwise noted, cells were grown to log phase for 6–8 h in synthetic medium containing glucose before being diluted into synthetic medium containing raffinose for overnight growth. Log-phase cells then were diluted into synthetic medium containing galactose for induction. Unless otherwise specified, cells were induced for 6 h.

For screening suppressors and enhancers, a standard lithium acetate transformation protocol was adapted for use with 96-well plates (1, 2). After 2 d of growth, galactose plates were scanned, and the density of the spot was analyzed using ImageQuant TL. This process was repeated at least three times for each strain.

Synthetic medium included 0.67% yeast nitrogen base without amino acids (Fischer Scientific) supplemented with amino acids as needed (MP Biomedicals) and 2% (wt/vol) sugar. Yeast extract/peptone medium included 1% yeast extract, 2% peptone, 2% glucose adjusted to pH 7.0, and 2% sugar. Plates included 2% agar.

Plasmid Construction. A seventh exon of α-syn was found recently. Therefore, in many previous works, α-synΔ4 is referred to as "α-syn lacking exon 3" or "α-syn126," and α-synΔ6 is referred to as "α-syn lacking exon 5" or "α-syn112" (3). α-Syn splice isoforms were generated using overlap-extension PCR with Pfu Turbo (Agilent Technologies). The second round of PCR also added the sequences required for subcloning via BP reaction into pDONR221 (Invitrogen) (4, 5). The resulting entry clone then was used in an LR reaction to move the insert to a variety of necessary entry vectors, all from the pAG series (6).

For screening previously established genetic modifiers of α-syn–induced toxicity, the hits were cherry-picked from the Yeast FLEXGene collection (7). Any gene used in low throughput was cherry-picked from the original library and sequence-verified. If required in a different backbone, the gene was moved to pDONR221 by BP reaction and then to a vector of the pAG series by LR reaction. mKate-*CHC1* and mKate-*VPS21* were inserted with the same protocol into pRS-GPD-mKate-ccdB.

Single-Insertion PCR Analysis. Colony PCR was used to screen all strains for those with a single insertion in the correct locus (Fig. S1*A*). Correct strains were those that PCR-amplified bands with primers A+B and C+D but not A+D and B+C. Those that were correct by this first screening were analyzed by long-extension PCR (Roche).

Microscopy Processing. Cells were induced following standard galactose induction before visualization by fluorescent microscopy. Cells were spun down, washed, and resuspended in 1× PBS before being viewed with a Plan Apochromat 100×/1.40 NA oil objective lens on a Nikon Eclipse Ti microscope at room temperature. Images were taken with a CoolSNAP HQ camera (Photometrics). Z stacks were taken above and below the plane of focus and then were deconvoluted by using the 3D deconvolution algorithm in the NIS-Elements HR software.

1. Gietz RD, Schiestl RH, Willems AR, Woods RA (1995) Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. *Yeast* 11:355–360.
2. Cooper AA, et al. (2006) Alpha-synuclein blocks ER-golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 313(5785):324–328.
3. Beyer K, Ariza A (2013) α-Synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. *Mol Neurobiol* 47(2):509–524.
4. Hartley JL, Temple GF, Brasch MA (2000) DNA cloning using in vitro site-specific recombination. *Genome Res* 10:1788–1795.
5. Walhout AJ, et al. (2000) GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol* 328:575–592.
6. Alberti S, Gitler AD, Lindquist S (2007) A suite of Gateway cloning vectors for high-throughput genetic analysis in *Saccharomyces cerevisiae*. *Yeast* 24(10):913–919.
7. Hu Y, et al. (2007) Approaching a complete repository of sequence-verified protein-encoding clones for *Saccharomyces cerevisiae*. *Genome Res* 17:536–543.

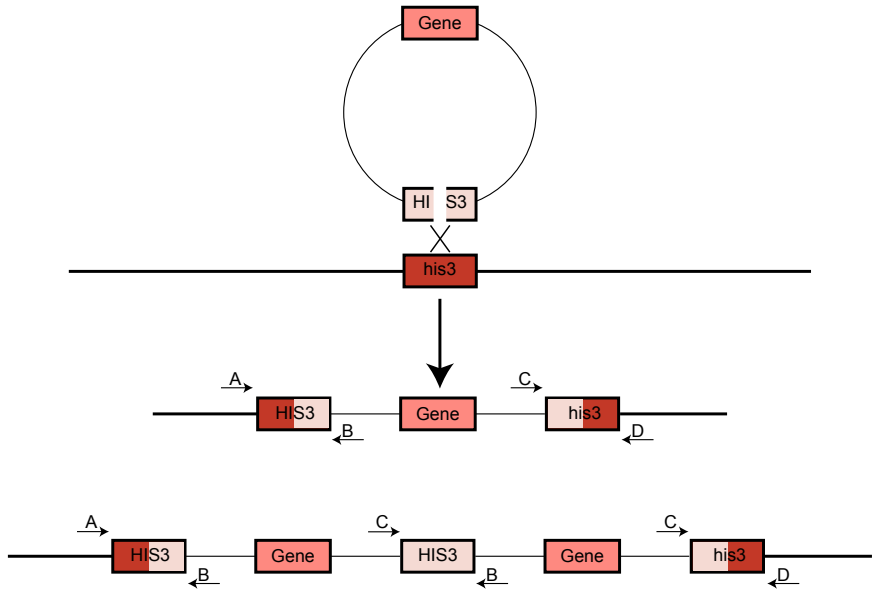

Fig. S1. Diagram showing the primer placement for testing for single integration of pRS plasmids. Primers were designed to be used regardless of the identity of the inserted gene. Colony PCR was used with primer sets A+B, C+D, A+D, and B+C. A+B and C+D would result in a band if there was a correct insertion; A+D would result in a band if there was no correct insertion, and B+C would result in a band if there were tandem insertions. After this screening, long-extension PCR was used with primers A+D to confirm the results.

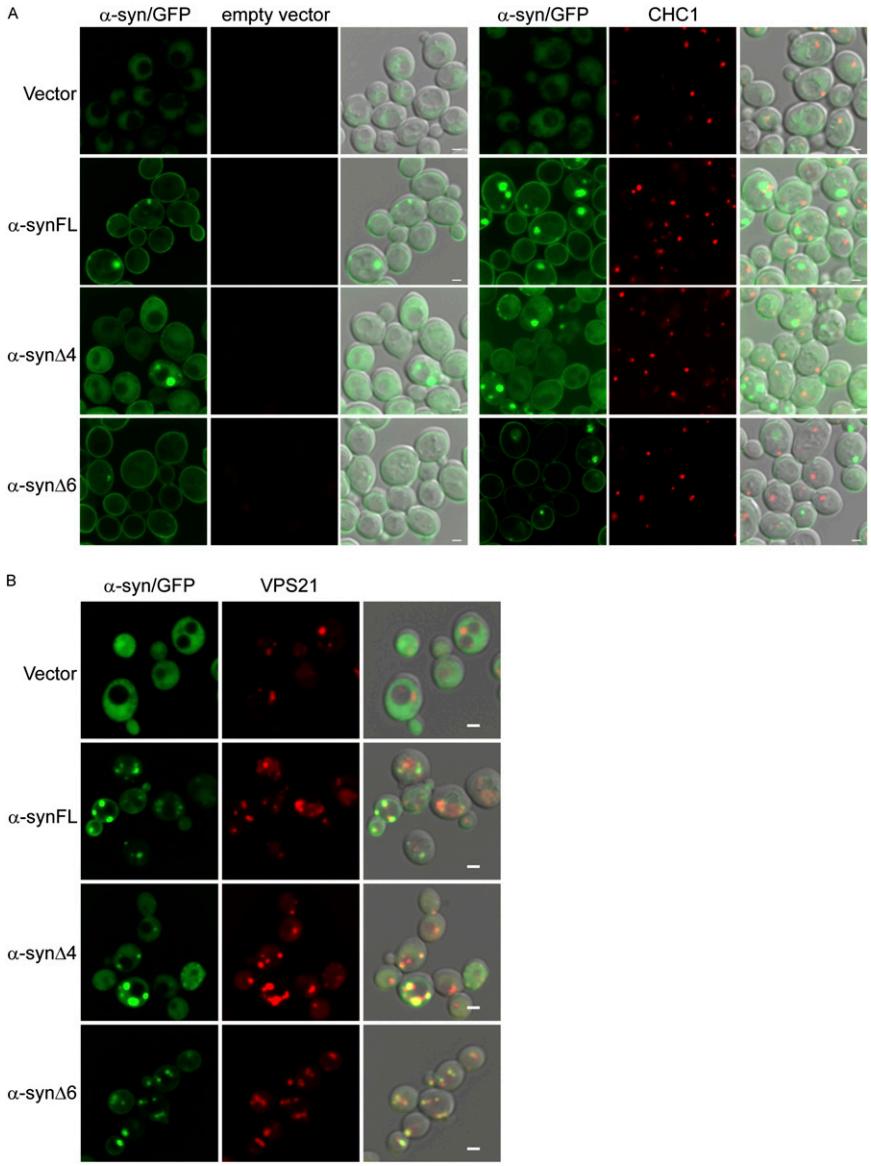

Fig. S2. (*A*) Fluorescent microscopy of α-syn-GFP–expressing cells with the addition of an empty vector or mKate-clathrin heavy chain 1 (Chc1p). (Scale bars, 2 μm.) (*B*) Fluorescent microscopy showing colocalization of α-syn-GFP and mKate-vacuolar protein sorting 21 (Vps21p). (Scale bars, 2 μm.)

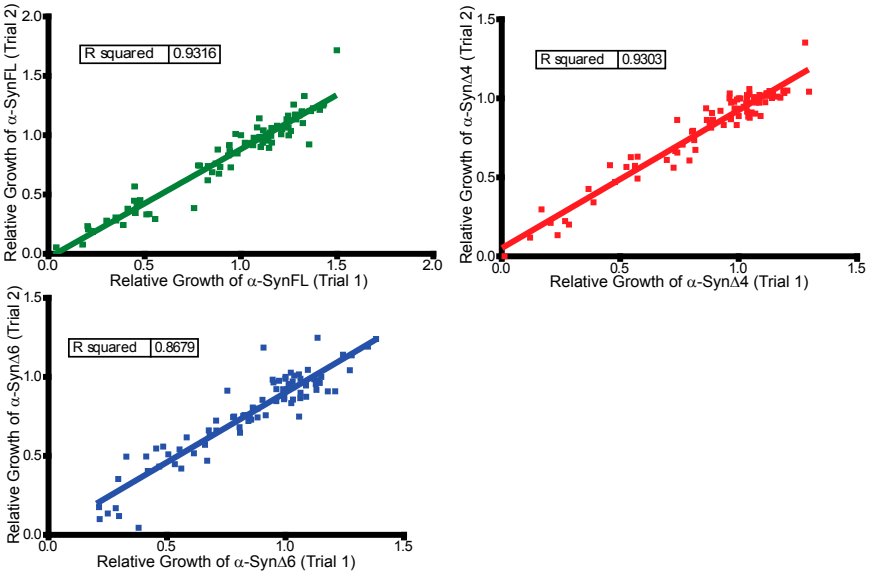

Fig. S3. High-throughput transformation of splice isoforms with genetic modifiers of α-syn–induced toxicity produced reproducible results. Each spot on each graph represents the rate of growth of one splice isoform of α-syn strain transformed with one genetic modifier on two separate trials.

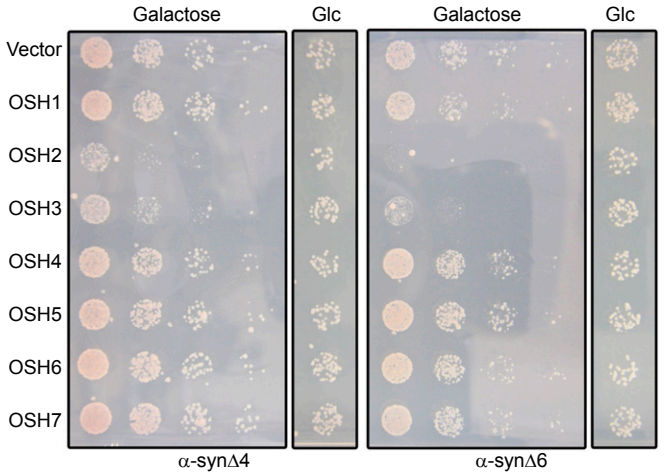

Fig. S4. Spot test analysis of the α-syn splice isoform–expressing strains in the presence of all seven OSH family members. Glc, glucose.

Table S1. Strain list

Strain	Genotype
1× α-synFL GFP	W303 mat a pAG306 Gal α-synFL GFP
2× α-synFL GFP	W303 mat a pAG305 Gal α-synFL GFP pAG306 Gal α-synFL GFP
3× α-synFL GFP	W303 mat a pAG304 Gal α-synFL GFP pAG305 Gal α-synFL GFP pAG306 Gal α-synFL GFP
4× α-synFL GFP	W303 mat a pAG303 Gal α-synFL GFP pAG304 Gal α-synFL GFP pAG305 Gal α-synFL GFP pAG306 Gal α-synFL GFP
4× α-synFL DsRed	W303 mat a pAG303 Gal α-synFL DsRed pAG304 Gal α-synFL DsRed pAG305 Gal α-synFL DsRed pAG306 Gal α-synFL DsRed
1× α-synΔ4 GFP	W303 mat a pAG306 Gal α-synΔ4 GFP
4× α-synΔ4 GFP	W303 mat a pAG303 Gal α-synΔ4 GFP pAG304 Gal α-synΔ4 GFP pAG305 Gal α-synΔ4 GFP pAG306 Gal α-synΔ4 GFP
1× α-synΔ6 GFP	W303 mat a pAG306 Gal α-synΔ6 GFP
4× α-synΔ6 GFP	W303 mat a pAG303 Gal α-synΔ6 GFP pAG304 Gal α-synΔ6 GFP pAG305 Gal α-synΔ6 GFP pAG306 Gal α-synΔ6 GFP
2× α-synFL DsRed	W303 mat a pAG303 Gal α-synΔ4 GFP pAG304 Gal α-synFL
2× α-synΔ4 GFP	DsRed pAG305 Gal α-synFL DsRed pAG306 Gal α-synΔ4 GFP
2× α-synFL DsRed	W303 mat a pAG303 Gal α-synΔ6 GFP pAG304 Gal α-synFL
2× α-synΔ6 GFP	DsRed pAG305 Gal α-synFL DsRed pAG306 Gal α-synΔ6 GFP
Vector a	W303 mat a pAG303 Gal GFP pAG304 Gal GFP
α-synFL multicopy a	W303 mat a pAG303 Gal α-synFL GFP pAG304 Gal α-synFL GFP
α-synΔ4 multicopy a	W303 mat a pAG303 Gal α-synΔ4 GFP pAG304 Gal α-synΔ4 GFP
α-synΔ6 multicopy a	W303 mat a pAG303 Gal α-synΔ6 GFP pAG304 Gal α-synΔ6 GFP
Vector α	W303 mat α pAG303 Gal GFP pAG304 Gal GFP
α-synFL multicopy α	W303 mat α pAG303 Gal α-synFL GFP pAG304 Gal α-synFL GFP
α-synΔ4 multicopy α	W303 mat α pAG303 Gal α-synΔ4 GFP pAG304 Gal α-synΔ4 GFP
α-synΔ6 multicopy α	W303 mat α pAG303 Gal α-synΔ6 GFP pAG304 Gal α-synΔ6 GFP
Aβ	W303 mat α pAG305 Gal Aβ
bar1	mat a bar1 leu2 ura3 trp1 his2 ade1

Table S2. Genes tested in high-throughput analysis of α-syn suppressors and enhancers

Yeast gene	Effect with α-synFL	Effect with α-synΔ4	Effect with α-synΔ6
Amino acid transport			
AVT4	Suppressor	Suppressor	Suppressor
DIP5	Suppressor	Suppressor	Suppressor
LST8	Suppressor	Suppressor	Suppressor
Autophagy			
NVJ1	Suppressor	Suppressor	Suppressor
Cytoskeleton			
ICY1	Suppressor	Suppressor	Suppressor
ICY2	Suppressor	Suppressor	Suppressor
Manganese transport			
CCC1	Suppressor	Suppressor	Suppressor
PMR1	Enhancer	Enhancer	Enhancer
Protein phosphorylation			
IME2	Suppressor	Suppressor	Suppressor
PTP2	Suppressor	Suppressor	Suppressor
GIP2	Suppressor	Suppressor	Suppressor
YCK3	Suppressor	Suppressor	Suppressor
RCK1	Suppressor	Suppressor	Suppressor
CDC5	Suppressor	Suppressor	Suppressor
PTC4	Suppressor	Suppressor	Suppressor
SIT4	Enhancer	Enhancer	Enhancer
CAX4	Enhancer	Enhancer	Enhancer
PPZ2	Enhancer	Enhancer	Enhancer
PPZ1	Enhancer	Enhancer	Enhancer
Transcription/translation			
CUP9	Suppressor	Suppressor	Suppressor
HAP4	Suppressor	Suppressor	Suppressor
FZF1	Suppressor	Suppressor	Suppressor
MGA2	Suppressor	Suppressor	Suppressor
MKS1	Enhancer	Enhancer	Enhancer
VHR1	Suppressor	Suppressor	Suppressor
JSN1	Suppressor	Suppressor	Suppressor
SUT2	Enhancer	Enhancer	Enhancer
TIF4632	Suppressor	Suppressor	Suppressor
STB3	Suppressor	Suppressor	Suppressor
MATALPHA1	Enhancer	Less enhancement	Less enhancement
Trehalose biosynthesis			
UGP1	Suppressor	Suppressor	Suppressor
TPS3	Suppressor	Suppressor	Suppressor
NTH1	Suppressor	Suppressor	Suppressor
Ubiquitin-related			
CDC4	Suppressor	Suppressor	Suppressor
UIP5	Suppressor	Suppressor	Suppressor
HRD1	Suppressor	Suppressor	Suppressor
UBP11	Enhancer	Enhancer	Enhancer
UBP7	Enhancer	Enhancer	Enhancer
Vesicular transport, ER-Golgi			
YPT1	Suppressor	Suppressor	Suppressor
YKT6	Suppressor	Suppressor	Suppressor
BRE5	Suppressor	Suppressor	Suppressor
SEC21	Suppressor	Suppressor	Suppressor
UBP3	Suppressor	Suppressor	Suppressor
ERV29	Suppressor	Suppressor	Suppressor
SEC28	Suppressor	Suppressor	Suppressor
SFT1	Suppressor	Suppressor	Suppressor
GLO3	Enhancer	Enhancer	Enhancer
TRS120	Enhancer	Enhancer	Enhancer
GYP8	Enhancer	Enhancer	Enhancer
YIP3	Enhancer	Enhancer	Enhancer
BET4	Enhancer	Enhancer	Enhancer
SLY41	Enhancer	Enhancer	Enhancer
GOS1	Enhancer	Enhancer	Enhancer

Table S2. Cont.

Yeast gene	Effect with α-synFL	Effect with α-synΔ4	Effect with α-synΔ6
SEC31	Enhancer	Enhancer	Enhancer
Other cellular processes			
PFS1	Suppressor	Suppressor	Suppressor
PDE2	Suppressor	Suppressor	Suppressor
MUM2	Suppressor	Suppressor	Suppressor
OSH2	Suppressor	Enhancer	Enhancer
OSH3	Suppressor	Enhancer	Enhancer
PHO80	Suppressor	Suppressor	Enhancer
ISN1	Suppressor	Suppressor	Suppressor
EPS1	Enhancer	Enhancer	Enhancer
IDS2	Enhancer	Enhancer	Enhancer
TPO4	Enhancer	Enhancer	Enhancer
QDR3	Suppressor	Less enhancement	Less enhancement
IZH3	Enhancer	Enhancer	Enhancer
Newly characterized ORFs			
YKL088W (CAB3)	Suppressor	Suppressor	Suppressor
YDL121C	Suppressor	Suppressor	Suppressor
YBR030W (RKM3)	Suppressor	Suppressor	Suppressor
YOR129C (AFI1)	Suppressor	Suppressor	Suppressor
YOR291W (YPK9)	Suppressor	Suppressor	Suppressor
Unknown function			
YKL063C	Suppressor	Suppressor	Suppressor
YML081W (TDA9)	Suppressor	Suppressor	Suppressor
YNR014W	Suppressor	Suppressor	Suppressor
YML083C	Suppressor	Suppressor	Suppressor
YDR374C	Suppressor	Suppressor	Suppressor
YMR111C	Suppressor	Suppressor	Suppressor

The genes in this table were identified in refs. 1 and 2. Yellow highlighting marks effects that were different between FL a-syn and the splice isoforms.

1. Yeger-Lotem E, et al. (2009) Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. *Nat Genet* 41(3):316–323.
2. Cooper AA, et al. (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 313(5785):324–328.