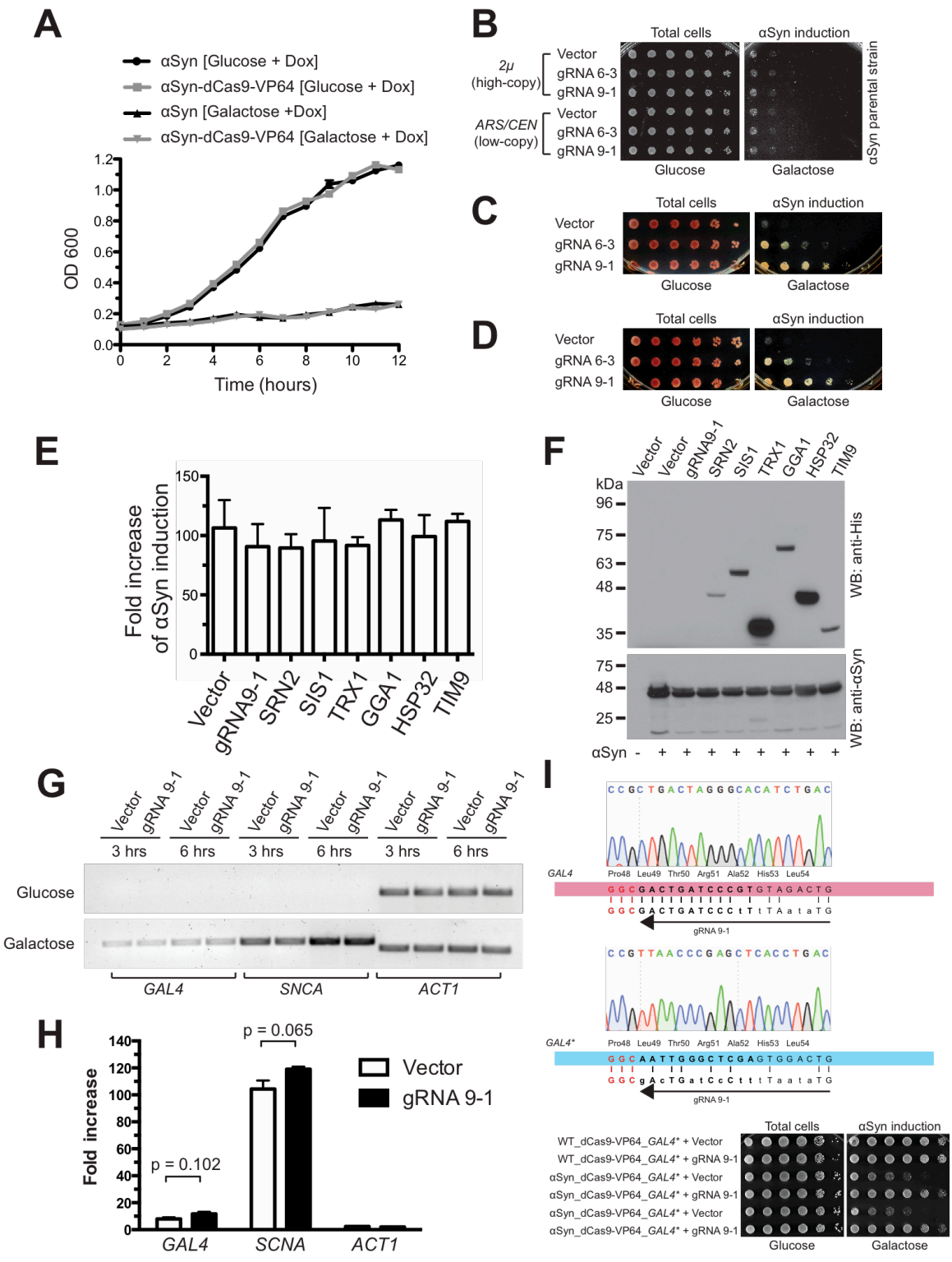

A
αSyn [Glucose + Dox]
αSyn-dCas9-VP64 [Glucose + Dox]
αSyn [Galactose +Dox]
αSyn-dCas9-VP64 [Galactose + Dox]
OD 600
Time (hours)
B
Total cells
αSyn induction
2μ (high-copy)
ARS/CEN (low-copy)
Vector
gRNA 6-3
gRNA 9-1
αSyn parental strain
Glucose
Galactose
C
D
E
Fold increase of αSyn induction
Vector
gRNA9-1
SRN2
SIS1
TRX1
GGA1
HSP32
TIM9
F
kDa
WB: anti-His
WB: anti-αSyn
αSyn
G
3 hrs
6 hrs
Glucose
Galactose
GAL4
SNCA
ACT1
H
p = 0.065
p = 0.102
Fold increase
I
GAL4
GAL4*
gRNA 9-1
WT_dCas9-VP64_GAL4* + Vector
WT_dCas9-VP64_GAL4* + gRNA 9-1
αSyn_dCas9-VP64_GAL4* + Vector
αSyn_dCas9-VP64_GAL4* + gRNA 9-1

Figure S1. gRNA-mediated suppression of αSyn toxicity, related to Figure 1, Figure 2, and Table S1.

(A) Growth profiles of the αSyn-expressing parental yeast strain (black lines) as well as the αSyn and crisprTF (dCas9-VP64)-expressing screen strain (gray lines) were determined in glucose and galactose media (which induces αSyn expression), and in the presence of Dox for dCas9-VP64 induction. These cells did not contain any gRNAs. Cell density was measured by OD_{600} at indicated time points. Parental and screen strains exhibited similar growth profiles in glucose media and both showed severe growth defects upon αSyn induction by galactose, suggesting that expression of dCas9-VP64 by itself does not affect αSyn-mediated toxicity. Error bars represent the standard error of three independent biological replicates.

(B) gRNA 6-3 and gRNA 9-1 were tested individually in the αSyn-expressing parental yeast strain, which does not express dCas9-VP64 (crisprTF). Neither of the two gRNAs were able to suppress αSyn toxicity in the absence of dCas9-VP64 (crisprTF). These results, along with the data presented in Figure 1B, demonstrate that the protective effect of gRNA 6-3 and gRNA 9-1 in αSyn suppression depends on the expression of dCas9-VP64.

(C-D) With integrated crisprTF, both gRNAs also rescued yeast viability in the other two high αSyn-expressing strains, HiTox (middle) and 4xαSynTox (bottom), which were published by Dr. Lindquist's group (Cooper et al., 2006; Gitler et al., 2009; Outeiro and Lindquist, 2003; Yeger-Lotem et al., 2009).

(E) Overnight cultures of the screen strain overexpressing the indicated genes were induced in Scm-Ura+Gal+Dox for 18 hours. The expression level of αSyn-YFP was quantified by flow cytometry (using LSR Fortessa II flow cytometer equipped with 488/FITC laser/filter set) and normalized to the non-induced control (Scm-Ura+Glucose+Dox). Data are presented as mean ± SEM of three biological replicates.

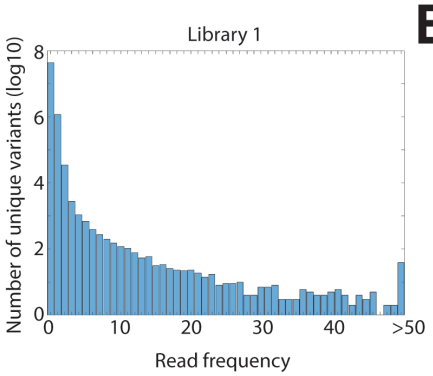
(F) The expression of αSyn-YFP and individual genes was further validated by Western blotting of yeast whole cell lysates.

(G) Overnight cultures of screen yeast cells harboring no gRNA ('Vector') or gRNA 9-1 ('gRNA 9-1') were grown in glucose and galactose media for 3 and 6 hours. Total RNA was extracted from these samples, and the expression level of *GAL4*, *SNCA* (αSyn) and *ACT1* were analyzed by RT-PCR using gene-specific primers. Representative data from two independent experiments are shown.

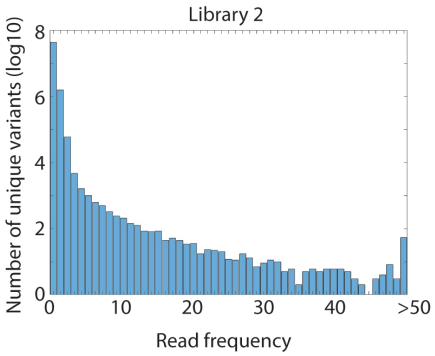
(H) Quantitative real-time PCR was performed with the same primers in (G) and normalized to the gene expression in glucose cultures (6 hours, n =4). Primers are listed in Table S5.

(I) One of the potential binding sites of gRNA 9-1 was located within the *GAL4* ORF (Table S1). To investigate the effect of gRNA 9-1/crisprTF on *GAL4* expression and exclude the possibility that gRNA 9-1's protective effect was mediated by repressing *GAL4* expression (which acts as the activator of *GAL1* promoter, which drives the expression of αSyn), the potential gRNA 9-1 binding site in the *GAL4* ORF in the screen strain was removed by replacing six synonymous codons from Leu49 to Leu54 (this modified *GAL4* is designated as *GAL4**). Compared with the vector control, gRNA 9-1 consistently achieved αSyn-toxicity suppression in two independent *GAL4** screen strains, indicating that the rescued growth phenotype by gRNA 9-1/crisprTF was independent of the interaction between *GAL4* and gRNA 9-1.

A



B

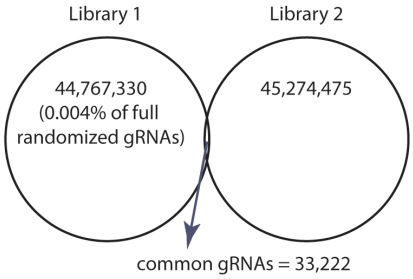


C

Replicate	Number of total reads	Number of variants	Variants per total reads
Library 1	46,053,460	44,767,330	0.982
Library 2	46,993,730	45,274,475	0.979

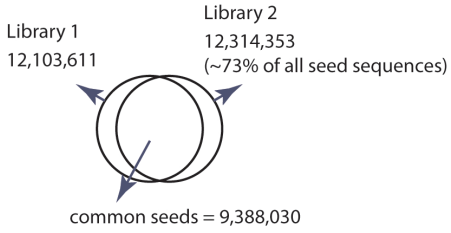
D

Number of unique gRNAs



E

Number of unique seeds



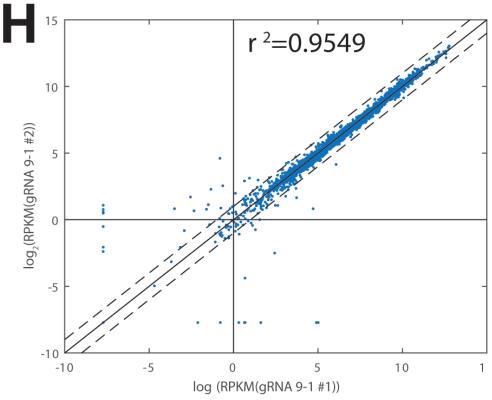
F

Library 1	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
G	20.11	20.30	21.11	21.51	20.45	19.17	18.74	18.37	18.27	18.73
A	33.62	31.76	30.18	29.78	29.82	30.42	30.49	30.65	30.76	30.64
T	38.17	32.83	33.71	33.80	34.58	34.91	35.28	35.48	35.48	35.00
C	8.11	15.11	15.00	14.91	15.15	15.50	15.50	15.50	15.49	15.64
Library 1	**N11**	**N12**	**N13**	**N14**	**N15**	**N16**	**N17**	**N18**	**N19**	**N20**
G	19.53	19.93	20.08	20.25	19.63	18.70	18.43	18.34	17.70	23.09
A	29.83	28.91	28.30	28.65	29.62	30.99	31.30	28.27	26.96	24.93
T	34.54	34.38	34.20	33.63	33.64	34.03	34.83	36.57	37.49	36.86
C	16.10	16.77	17.43	17.47	17.11	16.27	15.44	16.82	17.84	15.12

G

Library 2	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
G	20.34	21.02	21.99	22.48	21.02	19.35	18.52	17.94	17.82	18.55
A	33.65	31.17	29.64	29.50	29.80	30.74	31.13	31.35	31.19	30.76
T	38.12	32.00	32.58	32.58	33.63	34.22	35.10	35.62	35.81	35.10
C	7.88	15.81	15.78	15.44	15.54	15.68	15.25	15.09	15.17	15.59
Library 2	**N11**	**N12**	**N13**	**N14**	**N15**	**N16**	**N17**	**N18**	**N19**	**N20**
G	19.81	20.72	21.18	21.42	20.55	19.29	18.54	17.90	17.09	22.23
A	29.55	28.35	27.52	27.87	29.10	30.96	31.43	28.78	27.61	25.55
T	34.19	33.40	32.85	32.20	32.46	33.09	34.47	36.70	37.81	37.18
C	16.45	17.54	18.45	18.51	17.90	16.66	15.55	16.62	17.49	15.04

H



I

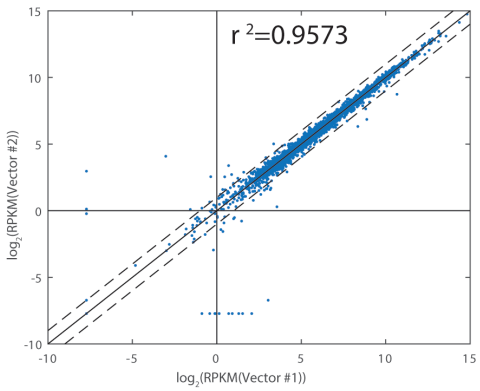


Figure S2. Randomized gRNA libraries for crisprTF-based screening, related to Figure 1 and Table S2.

(A-B) The numbers of unique gRNAs were quantitated by pooled Illumina sequencing after introduction of the library into the yeast screen strain. The two biological replicates (Library 1 and 2) were transformed and built from the same DNA library described in Detailed Protocols.

(C) About 98% of reads obtained from Illumina sequencing were unique variants when sequenced at a depth of ~45 million reads.

(D) Due to the large oligo library (4^{12} = ~ 1 trillion) used to build the libraries prior to yeast transformation, only about 0.004% of the theoretical diversity of the 20-nucleotide randomized gRNAs was covered in each transformation reaction. Therefore, gRNAs that overlapped between two libraries (that were transformed from the same oligo stock) were rare (<0.1%).

(E) The experimental coverage for the seed sequences was ~73% of the theoretical diversity (~12 million unique seed sequences were detected in either library out of a theoretical diversity of 4^{12}). About 56% of all possible seed sequences were present in both of our transformed libraries (~9.4 million out of 4^{12}).

(F-G) The DNA library used to encode randomized gRNAs was synthesized to be AT-rich (A = 32%, T = 32%, C =18%, and G =18% at each position) to match the average GC-content of yeast promoters. The frequency of the randomized 20-mer nucleotides was analyzed by two deep sequencing replicates. The normalized values match the designed GC-content of the randomized synthetic oligos, indicating that the library was accurately re-constructed from the randomized oligos *in vivo*.

(H) Scatter plot of the RNA-Seq data demonstrates that most of the gRNA 9-1 modulated genes had a concordant change in the levels of expression between two biological replicates, $r^2 = 0.9549$.

(I) Linear regression between two sequencing replicates carrying the vector control, r^2 = 0.9573. Mean of RPKM values of the biological replicates for gRNA 9-1 and vector control were compared and plotted as a Volcano plot (Figure 1C); there were numerous outliers (FDR-adjusted p-value ≤ 0.1, fold-change ≥ 2, indicating that a specific group of genes (detailed in Table S2) was differentially modulated by gRNA 9-1.

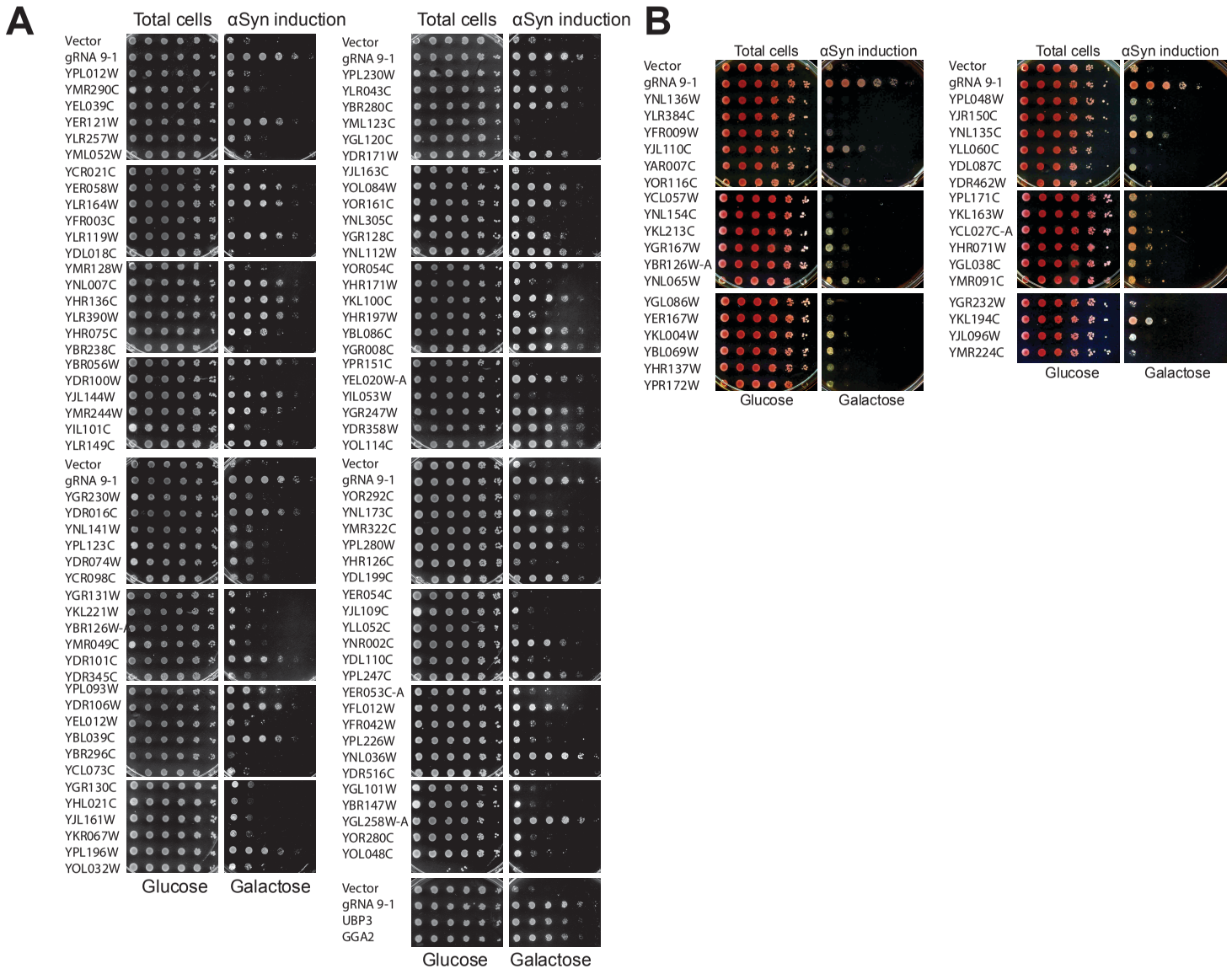
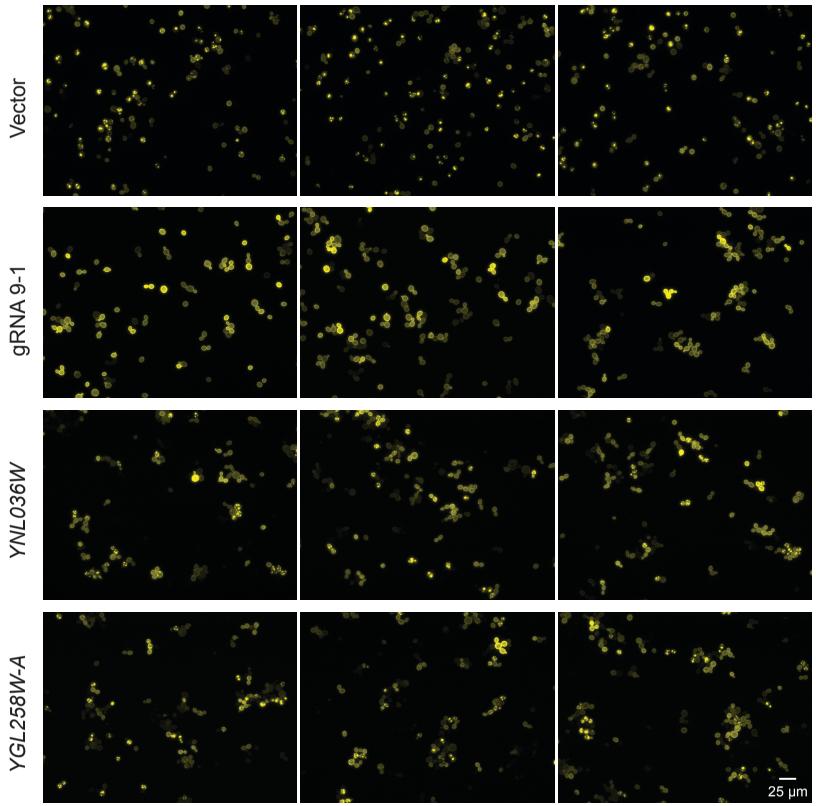

A
Total cells
αSyn induction
Vector
gRNA 9-1
YPL012W
YMR290C
YEL039C
YER121W
YLR257W
YML052W
YCR021C
YER058W
YLR164W
YFR003C
YLR119W
YDL018C
YMR128W
YNL007C
YHR136C
YLR390W
YHR075C
YBR238C
YBR056W
YDR100W
YJL144W
YMR244W
YIL101C
YLR149C
YGR230W
YDR016C
YNL141W
YPL123C
YDR074W
YCR098C
YGR131W
YKL221W
YBR126W-A
YMR049C
YDR101C
YDR345C
YPL093W
YDR106W
YEL012W
YBL039C
YBR296C
YCL073C
YGR130C
YHL021C
YJL161W
YKR067W
YPL196W
YOL032W
Glucose
Galactose
YPL230W
YLR043C
YBR280C
YML123C
YGL120C
YDR171W
YJL163C
YOL084W
YOR161C
YNL305C
YGR128C
YNL112W
YOR054C
YHR171W
YKL100C
YHR197W
YBL086C
YGR008C
YPR151C
YEL020W-A
YIL053W
YGR247W
YDR358W
YOL114C
YOR292C
YNL173C
YMR322C
YPL280W
YHR126C
YDL199C
YER054C
YJL109C
YLL052C
YNR002C
YDL110C
YPL247C
YER053C-A
YFL012W
YFR042W
YPL226W
YNL036W
YDR516C
YGL101W
YBR147W
YGL258W-A
YOR280C
YOL048C
UBP3
GGA2
B
YNL136W
YLR384C
YFR009W
YJL110C
YAR007C
YOR116C
YCL057W
YNL154C
YKL213C
YGR167W
YBR126W-A
YNL065W
YGL086W
YER167W
YKL004W
YBL069W
YHR137W
YPR172W
YPL048W
YJR150C
YNL135C
YLL060C
YDL087C
YDR462W
YPL171C
YKL163W
YCL027C-A
YHR071W
YGL038C
YMR091C
YGR232W
YKL194C
YJL096W
YMR224C

Figure S3. Systematic overexpression of genes modulated by gRNA 9-1 and evaluation of their effects on αSyn toxicity in yeast, related to Figure 2, Table S2, and Table S4.

(A) Plasmids containing genes that are modulated by gRNA 9-1 were obtained from the yeast ORF library (Open Biosystems Yeast ORF Collection) and transformed into the screen strain. Cells expressing individual genes were spotted onto galactose plates and scored for the suppression of αSyn toxicity in comparison to cells expressing dCas9-VP64 and gRNA 9-1 as well as those expressing dCas9-VP64 and vector control. *UBP3* (a known suppressor of αSyn toxicity) was used as a positive control. A complete list of differentially expressed genes and annotations as well as associated scores are presented in Table S2.

(B) Examination of αSyn-toxicity suppression by a randomly selected set of overexpressed genes from the yeast ORF library. Thirty-four yeast genes were randomly chosen from the yeast ORF library (Open Biosystems Yeast ORF Collection) and transformed into the screen strain. Cell survival in the presence of αSyn induction was measured by spotting assays and compared to survival of cells expressing dCas9-VP64 and gRNA 9-1 ('gRNA 9-1'; scored as 6) as well as those expressing dCas9-VP64 and vector control ('Vector'; scored as 1). Only five genes (*YJL110C*, *YOR116C*, *YNL065W*, *YNL135C*, and *YKL194C*) out of 34 genes scored greater than or equal to 3. A complete list of genes and annotations as well as associated scores are presented in Table S4.

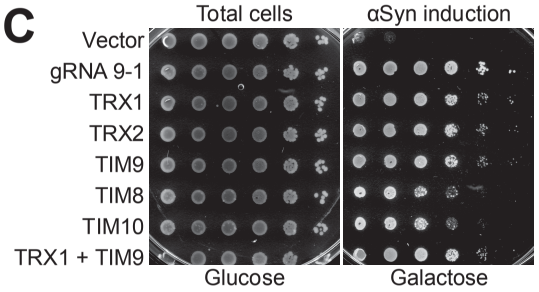
A



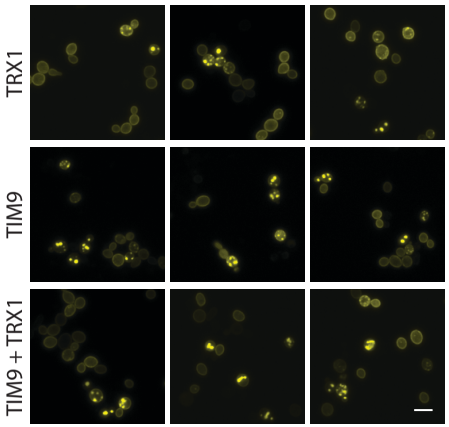
B

Overexpression	RNA-Seq (Log_2)	Cells with αSyn aggregates	Score of αSyn Suppression	Biological Function
Vector Control		91.7%	1	
gRNA 9-1		6.5%	10	
YNL036W	1.213	42.8%	6	Carbonic anhydrase
YGL258W-A	1.044	58.4%	5	Unknown

C



D



E

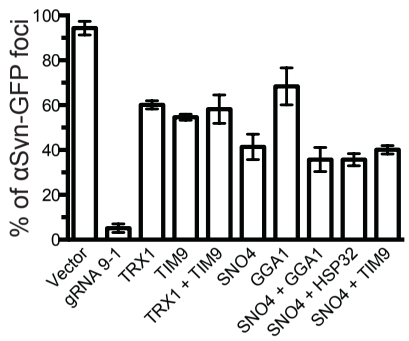


Figure S4. gRNA 9-1/crisprTF is the strongest modulator of αSyn-induced phenotypes, related to Figure 2, Table 1, and Table S2.

(A-B) We systematically tested the protective effects of the individual genes that were differentially controlled by gRNA 9-1/crisprTF. Fluorescent microscopy clearly showed that gRNA 9-1/crisprTF was the strongest suppressor of αSyn aggregates compared with any individual genes found in this study or previous genome-wide screens. αSyn-YFP foci were observed in only 6.5% of cells that overexpressed gRNA 9-1/crisprTF (scored as 10). This significantly outperformed cells overexpressing *YNL036W* (42.8% cells with αSyn-YFP foci; scored as 6), *YGL258W-A* (58.4% cells with αSyn-YFP foci; scored as 5), and other individual genes in Figure 2 (summarized in Table 1 and Table S2).

(C) Yeast *TRX* and *TIM* family proteins function together to protect mitochondria from oxidative stresses (Durigon et al., 2012). Genes in both families were highlighted in gRNA 9-1 expression profiling. Cells harboring individual genes from *TRX* (*TRX1* and *TRX2*) and *TIM* (*TIM8*, *TIM9*, and *TIM10*) families were overexpressed in the yeast screen strain to test for αSyn-toxicity protection. All these proteins strongly suppressed αSyn toxicity when overexpressed. We did not observe synergistic protective effects in yeast assays when *TRX1* and *TIM9* were co-expressed, in contrast to our results in human cells.

(D) Representative images of αSyn-YFP foci in screen yeast cells overexpressing *TRX1*, *TIM9* or both. Bar = 10 μm.

(E) Other co-expressed gene pairs (*SNO4* + *GGA1, SNO4* + *HSP32,* and *SNO4* + *TIM9*) were tested in αSyn-YFP foci assays. None of them demonstrated synergistic αSyn protection compared to single gene expression.

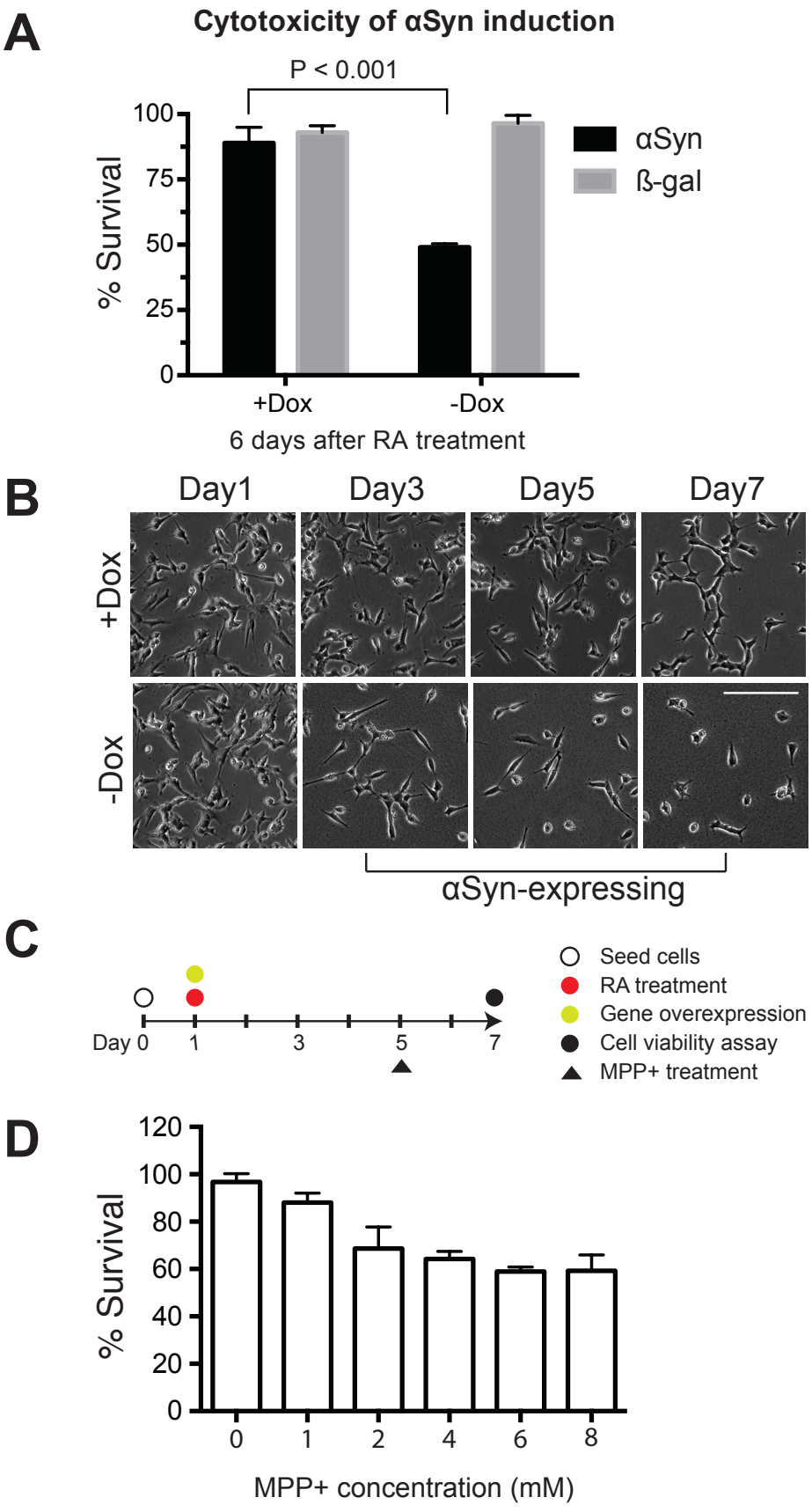

A
Cytotoxicity of αSyn induction
P < 0.001
100
75
50
25
0
% Survival
αSyn
ß-gal
+Dox
-Dox
6 days after RA treatment
B
Day1
Day3
Day5
Day7
+Dox
-Dox
αSyn-expressing
C
Seed cells
RA treatment
Gene overexpression
Cell viability assay
MPP+ treatment
Day 0
1
3
5
7
D
120
100
80
60
40
20
0
% Survival
0
1
2
4
6
8
MPP+ concentration (mM)

Figure S5. Inducible expression of αSyn in the human neuronal model of PD, related to Figure 3.

(A) αSyn and ß-gal (non-toxic negative control) expression were induced in human SH-SY5Y neuroblastoma cells by removal of Dox from media. αSyn-expressing cells significantly lost viability at the 6th day post-differentiation (retinoic acid treatment).

(B) Representative images showing retraction of neuritic processes, membrane blebbing, and cell death in αSyn-expressing cells (-Dox condition). Bar = 10 μm.

(C) Schematic of the experimental procedure used to study the effect of MPP+, a known inducer of neuronal cell death, on differentiated SH-SY5Y cells.

(D) A series of titration treatments were performed to identify minimal concentration of MPP+ that result in maximal toxicity. Cells were treated with different concentrations of MPP+ for 48 hours and cell viability was measured by CellTiter-Glo luminescent assay and normalized to the non-MPP+ treatment (n = 3). 6 mM MPP+ was found to be the optimal concentration for maximal toxicity and therefore was used in the survival assay.

Protocols: Randomized gRNA Library Construction and Screening

1. Prepare yeast competent cells
    a. Grow 100 mL overnight culture of the yeast screen strain in YPD media.

    b. Collect cells by spinning down for 3 minutes at 5000 RPM. Wash twice with 40 mL distilled water with centrifugation for 3 minutes at 5000 RPM and collect cells again.

    c. Resuspend cells in 800 μL distilled water and transfer to a fresh 2 mL microcentrifuge tube. Spin down for 1 minute at 5000 RPM. Remove water and resuspend cells in 1 mL filtered 0.1 M Lithium Acetate (Sigma) by pipetting.

    d. Incubate cells for 30 minutes at 30°C.

    e. Prepare the transformation master mix. For each reaction:

Reagents	**Volume (μL)**
1 M Lithium Acetate (Sigma)	40
10 mg/mL Denatured Salmon Sperm DNA (Life Technologies)	5
50% Polyethylene Glycol 3350 (Sigma)	240
Distilled Water	65
Total	**350**

2. Construct and screen randomized gRNA library
    a. Linearize the gRNA backbone vector (pRS426-gRNA-HindIII-EcoRI) by HindIII-HF and EcoRI-HF double digestion for 2 hour at 37 °C.

Reagents	**Volume (μL)**
Vector DNA (1 μg/μl)	2
HindIII-HF (New England Biolabs)	1
EcoRI-HF (New England Biolabs)	1
10X CutSmart Buffer (New England Biolabs)	5
Distilled Water	41
Total	**50**

    b. The linearized DNA (5761bp) was purified and cleaned by QIAquick Gel Extraction Kit (Qiagen). Elute DNA in 40 μL distilled water in the final step, followed by DNA concentration.

    c. The randomized oligo library was synthesized by IDT using the following template: 5'-GCTGGGAACGAAACTCTGGGAGCTGCGATTGGCAG(N1:32181832)(N1)(

N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N1)(N
1)GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3', where N1 indicates the hand-mixed nucleotide with the following ratio: A:C:G:T = 32:18:18:32.

d. Mix linearized vector DNA, randomized oligos, 50 μL yeast competent cells, and 350μL transformation mix. Vortex briefly to mix thoroughly.

Reagents	Volume (μL)
Linearized Vector DNA (1 μg/μl)	1
Randomized Oligo Library (100 μM)	3
Yeast Competent Cells	50
Transformation Master Mix	350

e. Incubate at 30°C for 20 minutes. Invert tubes several times at 10 minutes after the start of incubation to mix contents well. Incubate an addition 20 minutes at 42°C.

f. Collect cells by spinning down for 3 minutes at 5000 RPM. Wash with 1 mL distilled water with centrifugation for 3 minutes at 5000 RPM and collect cells again.

g. Resuspend and grow the transformed cells in 100 mL YPD media for 2 hours.

h. Plate 100 μL of YDP culture on Scm−Ura+Glucose plates to determine the library transformation efficiency (with triplicates).

i. Collect remaining cells by spinning down for 3 minutes at 5000 RPM. Wash twice with 40 mL distilled water with centrifugation for 3 minutes at 5000 RPM and collect cells again.

j. Grow cells in 200 mL Scm−Ura+Glucose media in the presence of 1 μg/mL Doxycycline overnight (~12 hours) to induce crisprTF expression.

k. Spin down cells for 3 minutes at 5000 RPM. Resuspend cells in 5 mL distilled water and plate on Scm−Ura+Galactose+Doxycycline plates (100 μL per 10-cm agar plate). Spread cells using sterile glass beads.

l. Plates were incubated at 30 °C for 2-3 days.

3. Characterization and validation of protective gRNAs

a. Pick up surviving colonies from Scm−Ura+Galactose+Doxycycline plates.

b. gRNA sequences of surviving colonies were directly PCR-amplified by using KAPA2G Robust PCR Kit (Kapa Biosystems) with T3-promoter (5’-

AATTAACCCTCACTAAAGG-3') and T7-promoter (5'-TAATACGACTCACTATAGG-3') primers.

c. PCR-amplified gRNA fragments (1034bp) were purified by QIAquick Gel Extraction Kit (Qiagen), and then were cloned back in the linearized pRS426-gRNA-HindIII-EcoRI vector by using Gibson assembly. Independent clones were randomly chosen for Sanger sequencing.

d. Verified gRNA constructs were individually transformed in both the parental and screen yeast strains to test the suppression of αSyn toxicity.