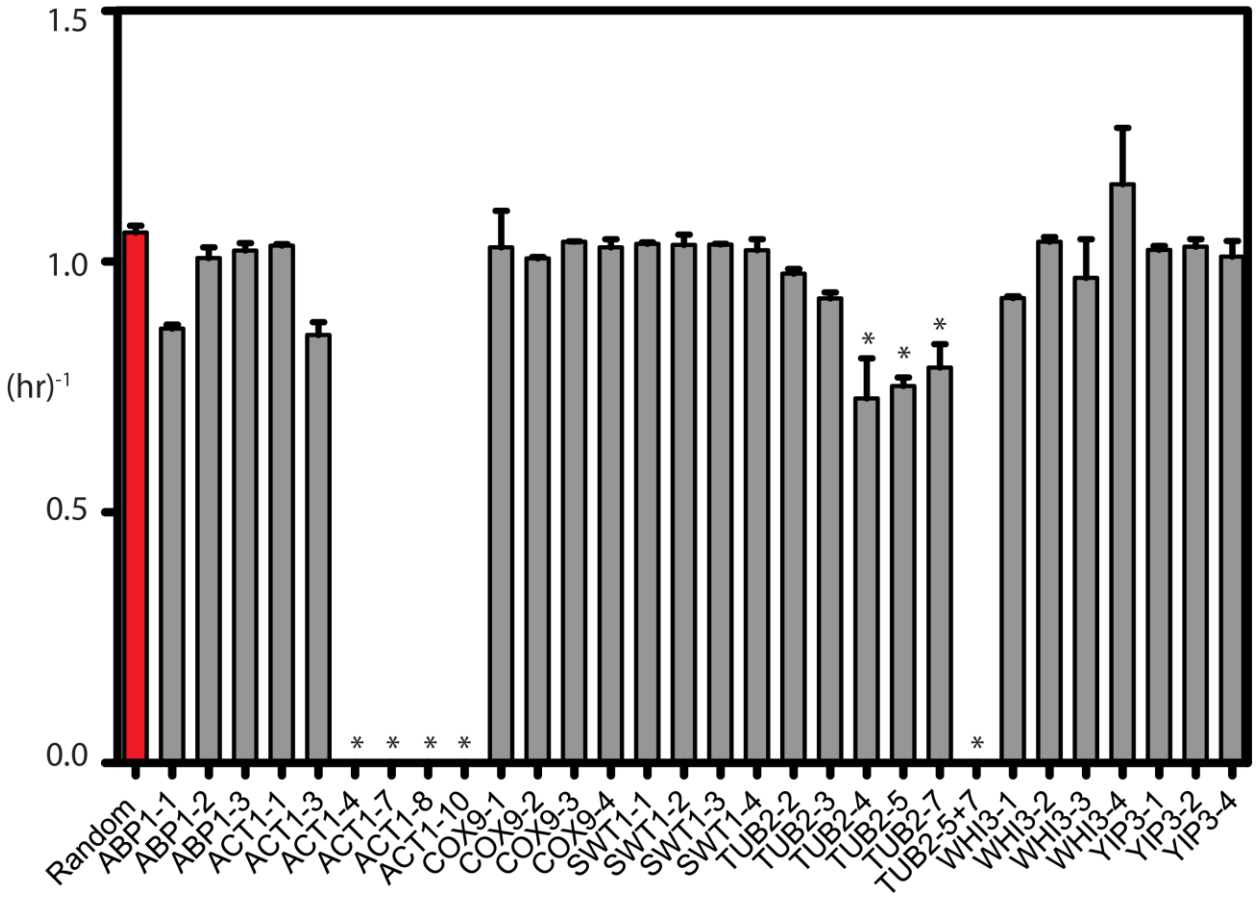
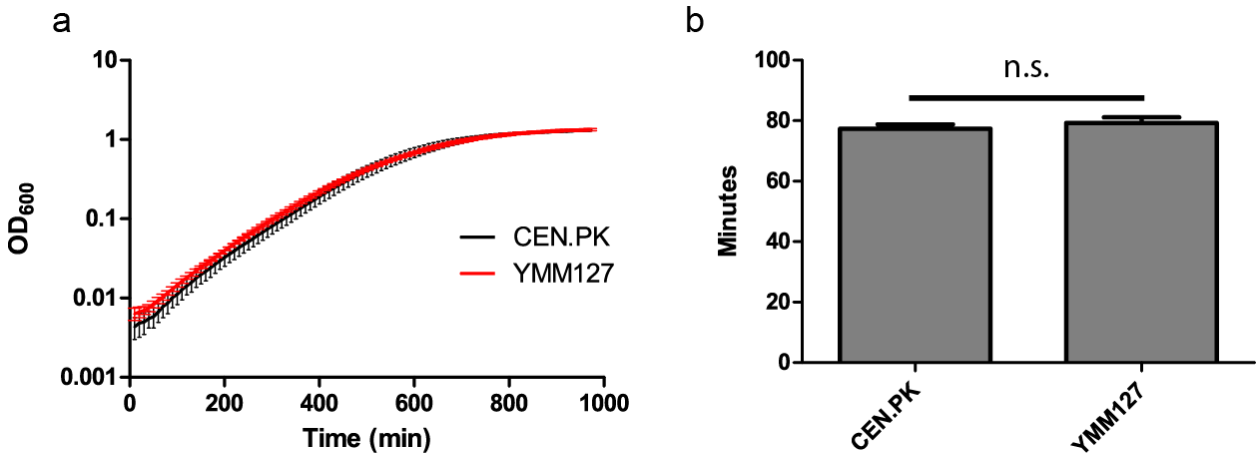
Supplementary Figure 1



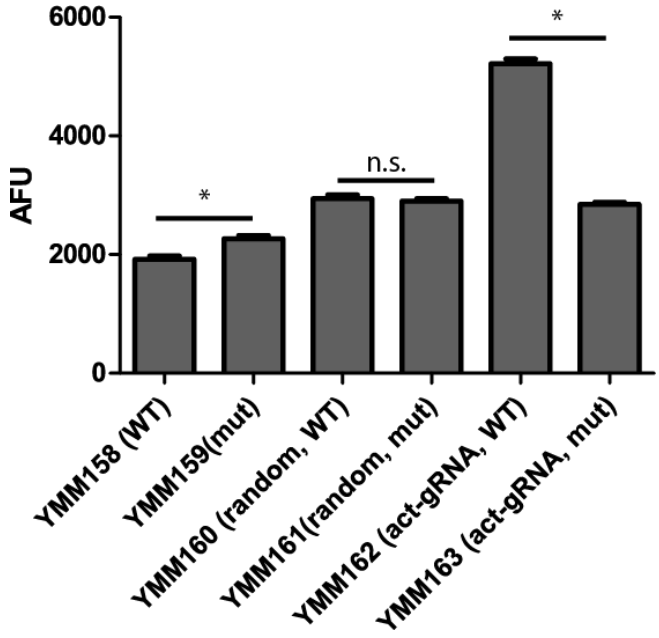
Supplementary Figure 1. | Growth rates of yeast expressing DVM targeted to promoter regions of SI candidate genes (n=2 independent transformations, mean +/- SEM). Red bar is the random sgRNA control. Asterisk indicates a statistically significant difference from the random sgRNA control (* indicates $p < .05$, one-way ANOVA and Tukey's post-test).

Supplementary Figure 2



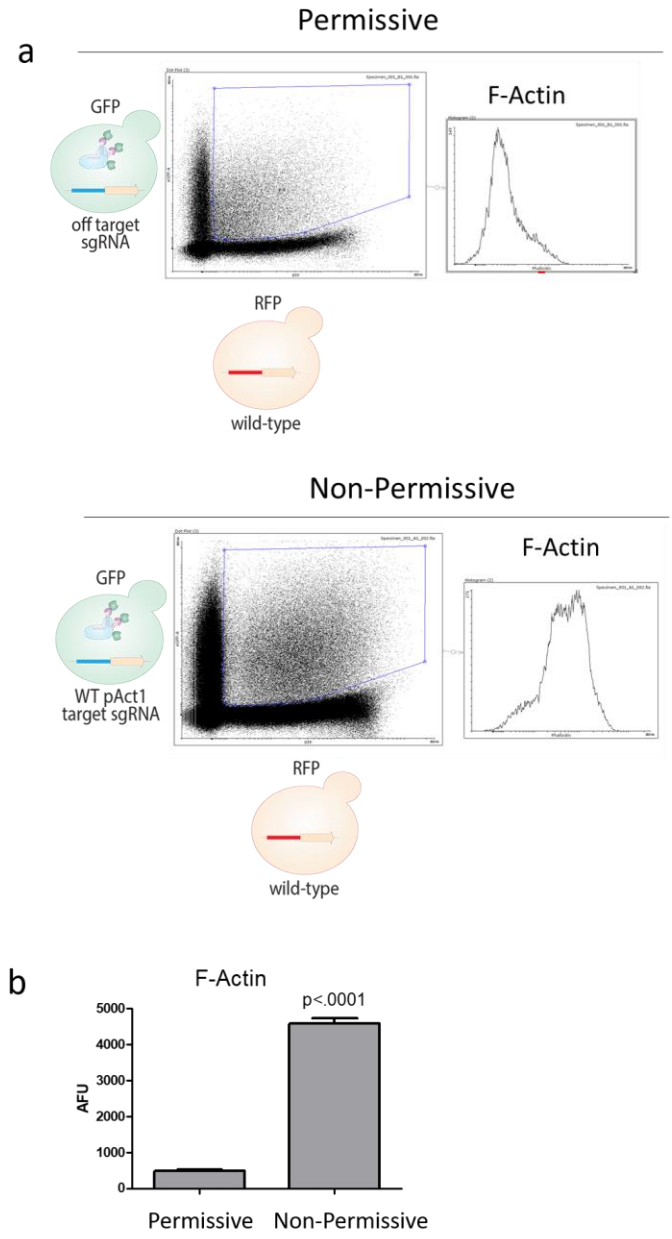
Supplementary Figure 2. Determining *ACT1* mutation's effect on growth rate | (a) Growth curves shown from strains with a wild-type (CEN.PK) and mutated (YMM127) actin promoters (n=3 independent cultures, mean +/- SEM). **(b)** Comparison of doubling time between CEN.PK and YMM127 ($p > .05$, two tailed t-test).

Supplementary Figure 3



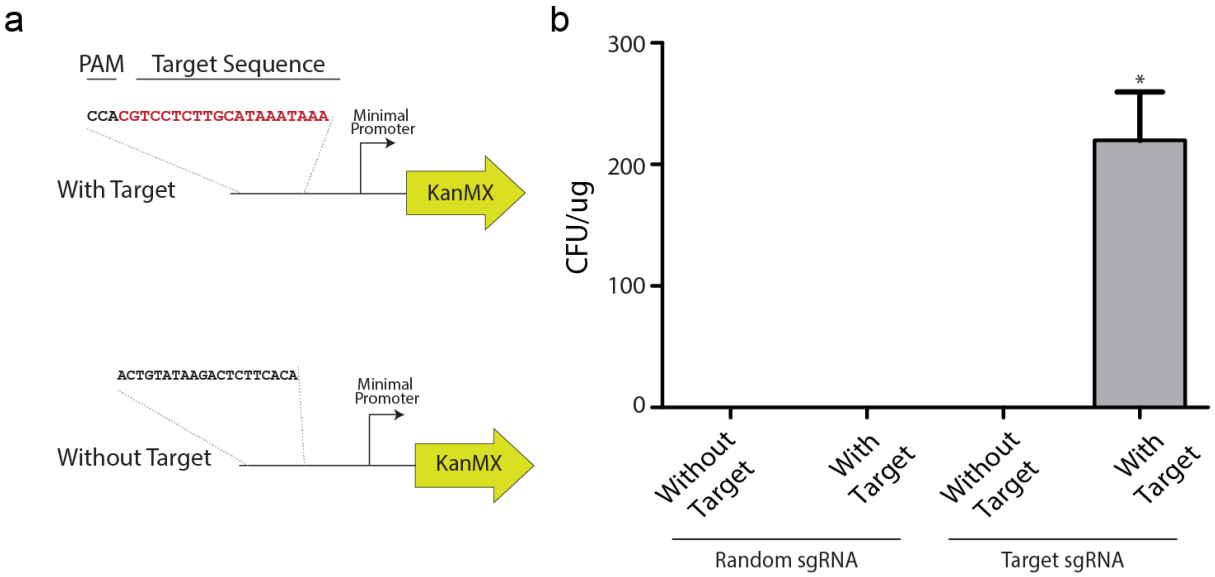
Supplementary Figure 3. Individual Cell Line Flow Cytometry Results | Average and SEM from three independent cultures. (* indicates $p < .05$, one-way ANOVA with Tukey's post-test)

Supplementary Figure 4



Supplementary Figure 4. Measuring F-Actin | (a) Representative dot plots and histograms from mating GFP and RFP expressing yeast in combinations permissive for diploid hybrids (top) and non-permissive (bottom). (b) Comparison of phalloidin stained F-actin in diploid hybrid cells. (n=3 independent matings, mean +/- SEM, two tailed t-test).

Supplementary Figure 5

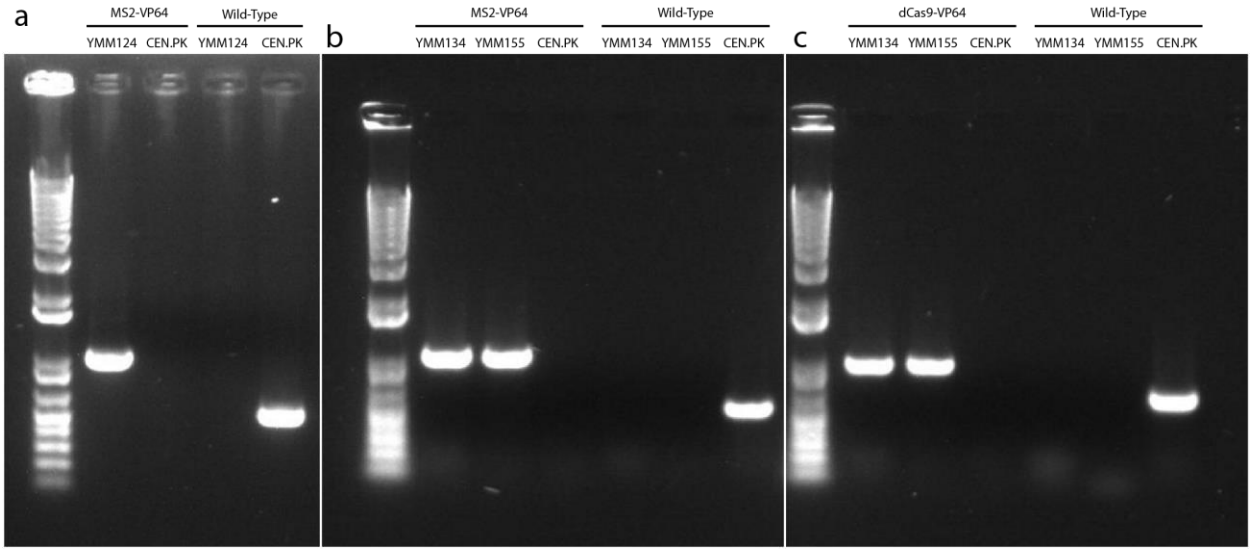


Supplementary Figure 5. Positive Selection Module | (a) Diagram of positive selection module containing the ACT1-4 target upstream of a minimal promoter and KanMX CDS. (b) Mean and SEM of colony forming units on G418 media from transforming yeast with integrating positive selection modules. Yeast expressed either DVM guided by a random sgRNA or from the ACT1-4 targeting sgRNA. (n=2 transformations. * indicates $p < .05$, one-way ANOVA with Tukey's post-test comparing far right column to all others).

Supplementary Figure 6

Supplementary Figure 6. Maps of key plasmids used in this study.

Supplementary Figure 7



Supplementary Figure 7. PCR Verification of Genomic Modifications | (a) Results from PCR analysis of *Lys2* locus in YMM124 and CEN.PK wild-type control. (b) Results from PCR analysis of *Lys2* locus in YMM134, YMM155, and CEN.PK wild-type control. (c) Results from PCR analysis of *Leu2* locus in YMM134, YMM155, and CEN.PK wild-type control.

Supplementary Table 1

Supplementary Table 1: Target Genes

Gene	Function	Overexpression Phenotype [1]
ACT1	Actin. Cytoskeletal protein [2].	Inviable [3]
ABP1	Actin Binding Protein. Cytoskeletal regulation [4].	Inviable [3]
COX9	Subunit of cytochrome c oxidase [5].	Inviable [6]
SWT1	Endoribonuclease involved in mRNA quality control [7].	Inviable [8]
TUB2	β-Tubulin. Cytoskeletal protein [9].	Inviable [3]
WHI3	Regulator of cell cycle and cell size [10].	Inviable [10]
YIP3	Vesicular transport protein [11].	Inviable [12]

Supplementary Table 2

Supplementary Table 2: Plasmids used in this study

Plasmid	Description	Reference	GenBank
pMM2-20-1	Deleterious activation screening plasmid	this study	KX981587
pMM2-20-2 to 20-XX	pMM2-20-1 with spacers 1-XXX (Supplementary Table 4)	this study	
pMM1-30A	sgRNA 2.0 cassette with a BsmbI site for oligos. BsaI releasable.	this study	KX981578
pMM1-32A	iCas9 vector. Contains destination for sgRNA cassettes.	this study	KX981579
pMM1-40A	MS2-VP64 integration cassette template.	this study	KX981582
pMM1-34Y	Integrating dCas9-VP64 cassette. sgRNA cassette destination.	this study	KX981581
pMM2-4A	Template for integrating dCas9-VP64. ACT1 sgRNA.	this study	
pMM2-22-2	Template for integrating dCas9-VP64. Random sgRNA.	this study	
pMM2-17-1	WT pACT1 driven TurboGFP	this study	KX981585
pMM2-17-2	Mutated pACT1 driven TurboGFP	this study	KX981586
pMM2-10-9	TurboRFP expression plasmid.	this study	KX981583
pMM2-10-10	TurboGFP expression plasmid	this study	KX981584
pMM2-21-5	Empty Positive selection Module Plasmid	this study	
pMM2-21-8	Positive selection module with ACT1-4 target	this study	
pMM2-21-9	Positive selection module with no target	this study	
pCM159	Minimal promoter source for positive selection	[13]	
pCRCT	iCas9 source vector. Addgene #60621	[14]	
pICSL80004	TurboRFP source vector. Addgene #50325	[15]	
pICSL80005	TurboGFP source vector. Addgene #50322	[15]	
pCORE-UK	KlURA3 and KanMX4 source vector. Addgene #72238	[16]	
M-SPn-VP64	dCas9-VP64 source[a]. Addgene #48674	[17]	
pESC-Leu	Yeast replicative vector backbone source.	Agilent	
MS2-P65-HSF1_GFP	MS2 source plasmid. Addgene #61423	[18]	

[a] BsaI sites removed in this study.

Supplementary Table 3

Supplementary Table 3: Yeast strains used in this study

Name	Genotype[a]	Description
YMM124	*MAT*a, *lys2ΔMS2-VP64 KanMX4*	Used for screening growth defects caused by DVM
YMM125	*MATα LEU2*	Wild-type *ACT1* promoter strain used for mating experiments
YMM130	*MATa ACT1-Δ1 leu2ΔdCas9-VP64 Random sgRNA*	Random sgRNA strain used for positive selection module test.
YMM131	*MATa ACT1-Δ1 leu2ΔdCas9-VP64 ACT1 sgRNA*	ACT1-4 sgRNA strain used for positive selection module test.
YMM134	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 Random sgRNA KlURA3*	Mutated *ACT1* promoter strain carrying DVM guided by random sgRNA
YMM139	*MATα LEU2* pMM2-10-9 (*TurboRFP TRP1*)	TurboRFP wild-type *ACT1* promoter strain.
YMM139b	*MATα LEU2* pMM2-10-9 (*TurboRFP TRP1*)	TurboRFP wild-type *ACT1* promoter strain.
YMM141	*MATα ACT1-Δ1 LEU2*	Mutated *ACT1* promoter strain used for mating experiments
YMM155	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3*	Synthetic incompatible strain
YMM156	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3* pMM2-10-10 (*TurboGFP TRP1*)	TurboGFP synthetic incompatible strain
YMM156b	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3* pMM2-10-10 (*TurboGFP TRP1*)	TurboGFP synthetic incompatible strain
YMM157	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3* pMM2-10-10 (*TurboGFP TRP1*)	TurboGFP mutated *ACT1* promoter strain carrying DVM guided by random sgRNA
YMM157b	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3* pMM2-10-10 (*TurboGFP TRP1*)	TurboGFP mutated *ACT1* promoter strain carrying DVM guided by random sgRNA
YMM158	*MATa* pMM2-17-1 (*pACT1- TurboGFP LEU2)*	No DVM strain with wild-type *ACT1* promoter driving TurboGFP
YMM159	*MATa* pMM2-17-2 (*pACT1-Δ1-TurboGFP LEU2)*	No DVM strain with mutated *ACT1* promoter driving TurboGFP
YMM160	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 Random sgRNA KlURA3* pMM2-17-1 (*pACT1- TurboGFP LEU2)*	Random guide DVM strain with wild-type *ACT1* promoter driving TurboGFP
YMM161	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 Random sgRNA KlURA3* pMM2-17-2 (*pACT1-Δ1-TurboGFP LEU2)*	Random guide DVM strain with mutated *ACT1* promoter driving TurboGFP
YMM162	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3* pMM2-17-1 (*pACT1- TurboGFP LEU2)*	Synthetic incompatible strain with wild-type *ACT1* promoter driving TurboGFP
YMM163	*MATa ACT1-Δ1 lys2ΔMS2-VP64 KanMX4 leu2ΔdCas9-VP64 ACT1 sgRNA KlURA3* pMM2-17-2 (*pACT1-Δ1-TurboGFP LEU2)*	Synthetic incompatible strain with mutated *ACT1* promoter driving TurboGFP

[a] All strains are derived from the CEN.PK background [19]: *ura3-52 trp1-289 leu2-3_112 his3 Δ1 MAL2-8C SUC2* except for YMM139b, YMM156b, and YMM157b which are derived from YNN216 [20]: *ura3-52 lys2-801 ade2-101.*

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