Expanded View Figures

Figure EV1. The estimation of "Dex-trade-off" adaptive mutations.

A The parental SNP polymorphism of *L. kluyveri AZF1* in the JYL1897 background (the wild-type of our lab) in comparison with the published reference genome of CBS 3082.

B The *AZF1* mutational spectrum of 30 more randomly picked heat stress-evolved *sef1*Δ suppressors (five clones each from 37, 38, and 39°C-Evo *sef1*Δ suppressors in both MATa and MATα lineages). The mutations were identified by Sanger sequencing. "N.D." means that there is no mutation detected within the coding region. Whether the clones that carry no *azf1* mutation displayed "Dex-trade-off" phenotypes or not is indicated on the right side of each table.

C The "Dex-trade-off" phenotypic spectrum of 156 heat stress-evolved *sef1*Δ suppressors. The "Dex-trade-off" phenotypes of each clone are displayed according to the simple fitness scores under the YPD_37°C condition (Dataset EV3). Clones with score 1 (with fitness worse than *sef1*Δ strain) were defined as "with Dex-trade-off" (please also see Appendix Fig S3B for the simple fitness score definition). About 14.74% (23/156) of clones in this batch showed no clear "Dex-trade-off."

A

AZF1 (JYL1897 background)
864T>C

B

MATa lines

Strain ID	*AZF1* MutType	
37a-6		N.D.
37a-7	686dupG	Frame-shift
37a-8	686dupG	Frame-shift
37a-9	1351C>T	STOP-gain
37a-10		N.D.
38a-6	1972C>T	STOP-gain
38a-7	1855G>T	STOP-gain
38a-8	301_302delTC	Frame-shift
38a-9	929C>G	STOP-gain
38a-10	686dupG	Frame-shift
39a-6	1972C>T	STOP-gain
39a-7	1855G>T	STOP-gain
39a-8		N.D.
39a-9		N.D.
39a-10		N.D.

No Dex-trade-off

No Dex-trade-off

With Dex-trade-off

MATα lines

Strain ID	*AZF1* MutType	
37alpha-6	1316_1317delAT	Frame-shift
37alpha-7	877G>T	STOP-gain
37alpha-8	1705C>T	STOP-gain
37alpha-9	1705C>T	STOP-gain
37alpha-10	686dupG	Frame-shift
38alpha-6	1159C>T	STOP-gain
38alpha-7	929C>A	STOP-gain
38alpha-8	961C>T	STOP-gain
38alpha-9	1705C>T	STOP-gain
38alpha-10	686delG	Frame-shift
39alpha-6		N.D.
39alpha-7	1348C>T	STOP-gain
39alpha-8	686delG	Frame-shift
39alpha-9	301_302delTC	Frame-shift
39alpha-10	1096C>T	STOP-gain

With Dex-trade-off

C

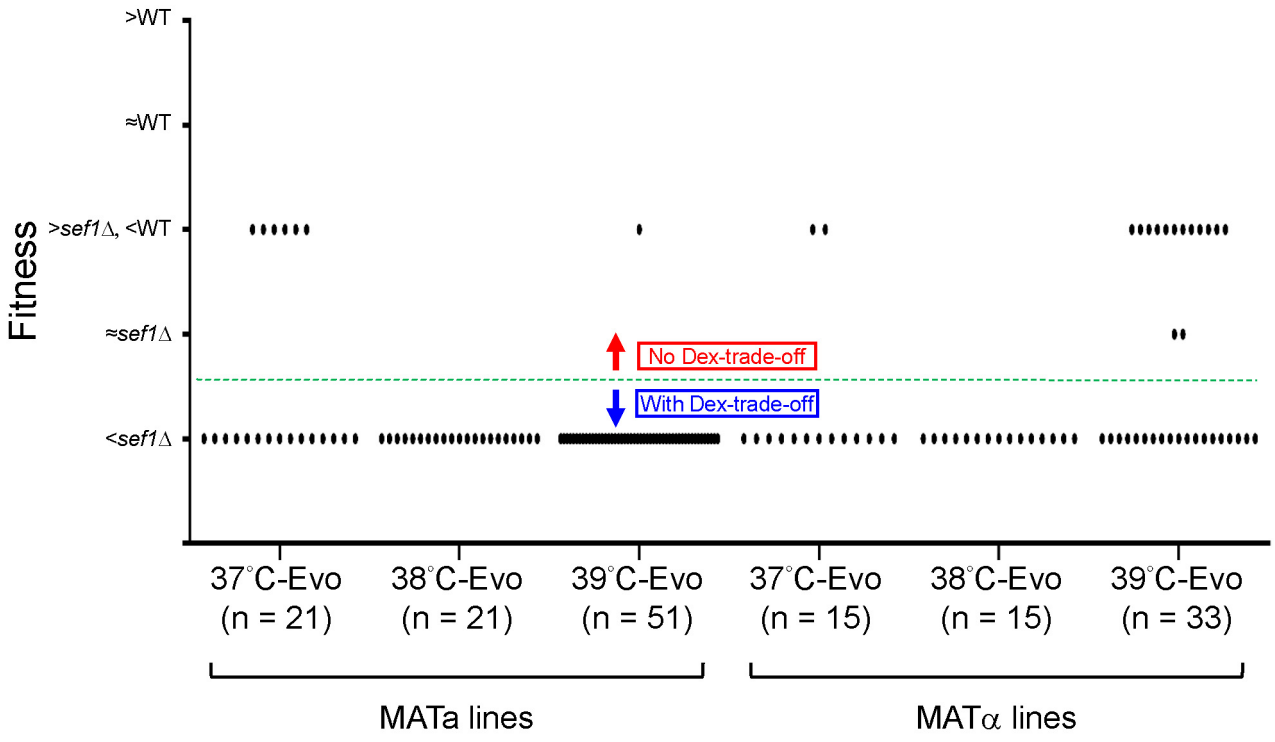

Figure EV1.

Figure EV2. The estimation of "*ira1*-related" adaptive mutations.

A The parental SNP and indel polymorphisms of *L. kluyveri IRA1* in the JYL1897 background (the wild-type of our lab) in comparison with the published reference genome of CBS 3082.

B The *IRA1* mutational spectrum of 10 more randomly picked 28°C-Evo *sef1*Δ suppressors (5 clones each in both MATa and MATα lineages). The mutations were identified by Sanger sequencing. "NonSyn" means non-synonymous mutation. Whether the clones that carry no *ira1* mutation displayed "desiccation hypersensitivity" phenotypes or not is indicated on the right side of the table.

C Specific desiccation hypersensitivity of *ira1*Δ in both wild-type and *sef1*Δ backgrounds. The cells grown overnight in YPD were harvested and desiccated by air-dry at 28°C for 20 h. The post-desiccation viabilities were determined by spot assays compared to the non-desiccated controls. The *azf1*Δ is not sensitive to desiccation.

D The desiccation hypersensitivity of 28°C-Evo *sef1*Δ suppressors by taking 28°C-Evo-N3, -N4, and -N5 in the MATα lines as examples. Tested strains carrying different types of *ira1* mutations showed different levels of desiccation hypersensitivity.

E The "desiccation hypersensitivity" phenotypic spectrum of 84 28°C-evolved *sef1*Δ suppressors. The desiccation sensitivities of each clone are displayed according to the qualitative viabilities (Dataset EV3). Clones with a score ≥ 3 (with post-desiccation viability ≤ 1% range) were defined as "hypersensitive" to desiccation (please also see Dataset EV3 for the rank definition). About only 7.14% (6/84) of clones in this batch showed no "hypersensitivity."

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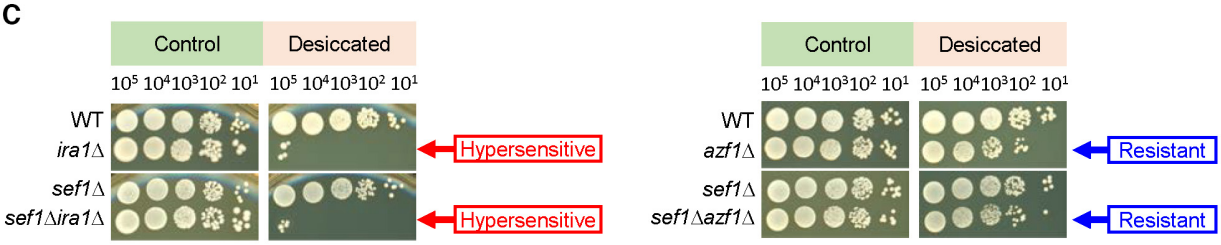
A

IRA1 (JYL1897 background)
4949A>G
269_292delGCACAAGCACAAGCACAAGCACAA
2704_2705insGCATGCACAGCATGCACA

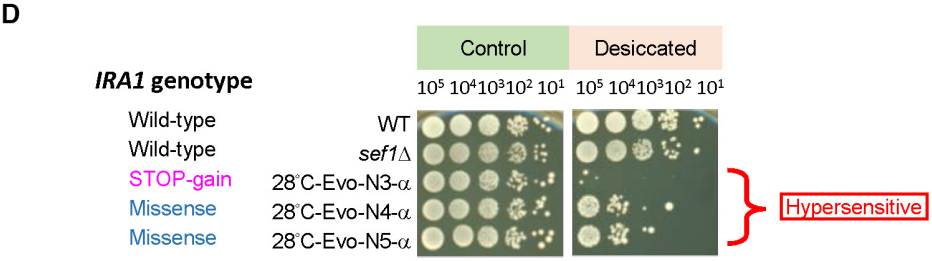
B

Strain ID	*IRA1* MutType		
28a-16	7031C>G	STOP-gain	
28a-17	5490_5491insACCGG	Frame-shift	
28a-18	6374_6397delACGACGTTGTAGAGATCAACTTCA	Inframe_deletion	
28a-19	6374_6397delACGACGTTGTAGAGATCAACTTCA	Inframe_deletion	
28a-20	6650G>A	NonSyn	
28alpha-16		N.D.	Hypersensitive
28alpha-17		N.D.	
28alpha-18	7776G>C 7818C>T	Syn	Desiccation-resistant
28alpha-19		N.D.	Hypersensitive
28alpha-20	5698C>T	STOP-gain	

C



D



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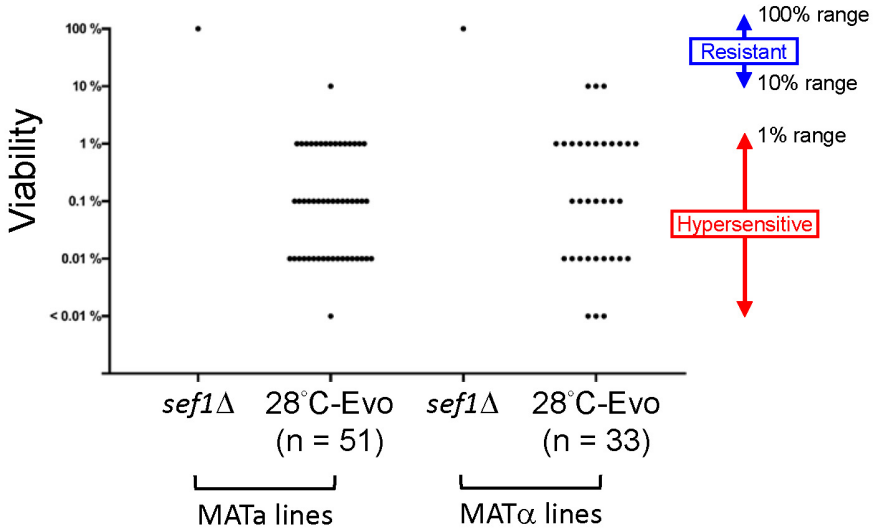

Figure EV2.

Figure EV3. Positive epistasis in gene expression changes between *sef1*Δ and *azf1*Δ genotypes.

A The compensation of defective TCA cycle gene expression by *azf1*Δ mutation under the YPGly condition in either wild-type or *sef1*Δ background. A total of 21 genes (Fig 5C) were included.
B The epistasis effect of *azf1*Δ-induced upregulation of TCA cycle genes in the *sef1*Δ background compared with the wild-type.
C The enhanced transcriptional remodeling of glycerol utilization and auxiliary pathways by *azf1*Δ mutation under the YPGly condition in either wild-type or *sef1*Δ background. A total of 51 genes (Fig 5D) were included.
D The epistasis effect of *azf1*Δ-induced differential gene expression of glycerol utilization and auxiliary pathways in the *sef1*Δ background compared with the wild-type.
E The epistasis effect of *azf1*Δ-induced upregulation of stress responsive genes in the *sef1*Δ background compared with the wild-type. A total of 186 genes (Appendix Fig S12A) were included.

Data information: For (A) and (C), the gene expression changes are displayed as the $\log_2$ values of the mean TPM (from RNA-seq data) ratios between *azf1*Δ and *AZF1* genotypes under either wild-type or *sef1*Δ background. In the *Y*-axis, positive values indicate gene upregulation while negative values indicate downregulation. For (B), (D), and (E), the $\log_{10}$ values of the relative mean TPM ratios (the "*sef1*Δ*azf1*Δ/*sef1*Δ" group relative to the "*azf1*Δ/wild type" group) were used to determine the epistasis effects between *sef1*Δ and *azf1*Δ genotypes (positive or negative as indicated as "+" or "−"). The dots labeled with red and green indicate that the respective upregulated and downregulated genes show higher *azf1*Δ-induced expression changes under the *sef1*Δ than the wild-type background (i.e., positive epistasis). The dark blue lines represent the equal relative mean TPM ratio (i.e., there is no detectable epistasis).

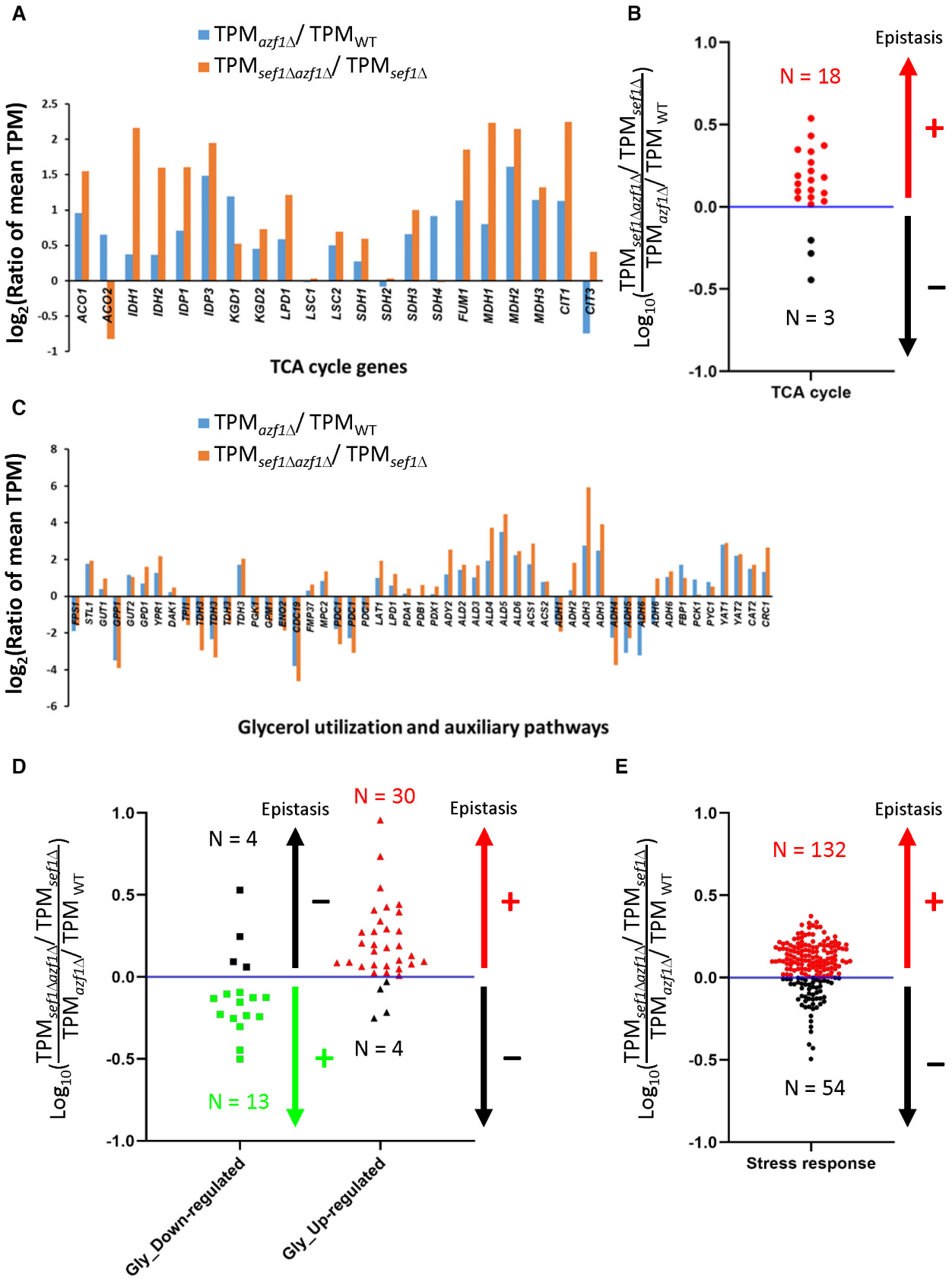

Figure EV3.

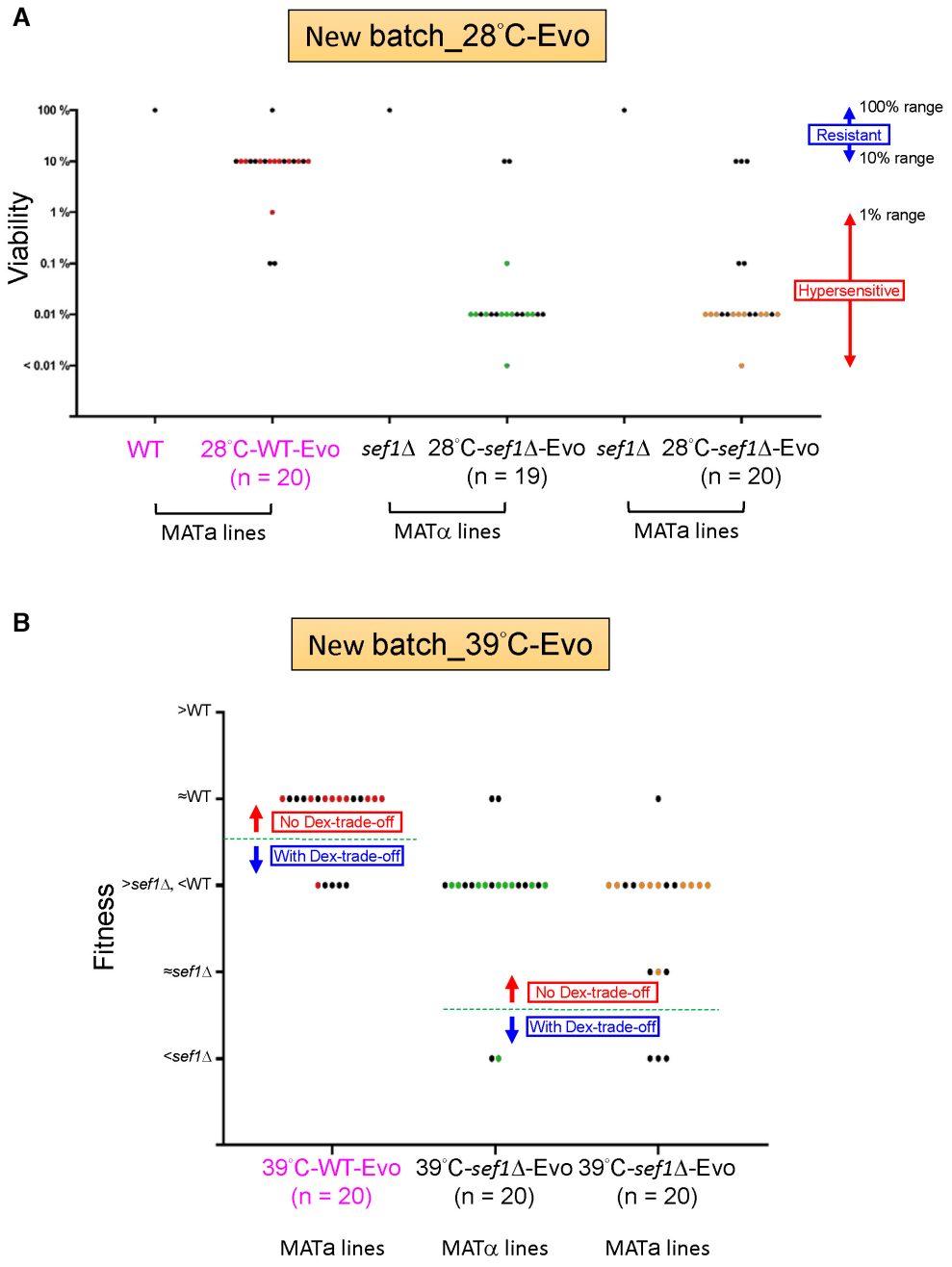

Figure EV4. Phenotypic surveys for the clones from a new batch of suppressor development including the wild-type controls.

A The "desiccation hypersensitivity" phenotypic spectrum of 20 each 28°C-evolved new batch clones (one clone of the new batch MATα 28°C-*sef1*Δ-Evo was removed from the collection due to contamination). The desiccation sensitivities of each clone are displayed according to the qualitative viabilities (Dataset EV16) as described above. The *sef1*Δ background seems more prone to generate adaptive mutations with desiccation hypersensitivity (18/19 in MATα and 17/20 in MATa lines) than the wild-type background (3/20).

B The "Dex-trade-off" phenotypic spectrum of 20 each 39°C-evolved new batch clones. The "Dex-trade-off" phenotypes of each clone are displayed according to the simple fitness scores under the YPD_37°C condition (Dataset EV16) as described above. Clones with score 1 (with fitness worse than *sef1*Δ strain) were defined as "with Dex-trade-off" for the new batch 28°C-*sef1*Δ-Evo while a score ≤ 3 (with fitness worse than the wild-type strain) for the 28°C-WT (wild-type)-Evo. About 14.74% (23/156) of clones in this batch showed no "Dex-trade-off." Unlike observed in the first batch of suppressor development, there is no clear preference in any genetic background to generate a higher frequency of adaptive mutations with "Dex-trade-off" (5/20 in the wild-type, 2/20 in MATα, and 3/20 in MATa lines).

Data information: For (A) and (B), the dots labeled with colors (red, green, or orange in different lines) stand for clones isolated from the same population in each line while the dots labeled with black from different populations.

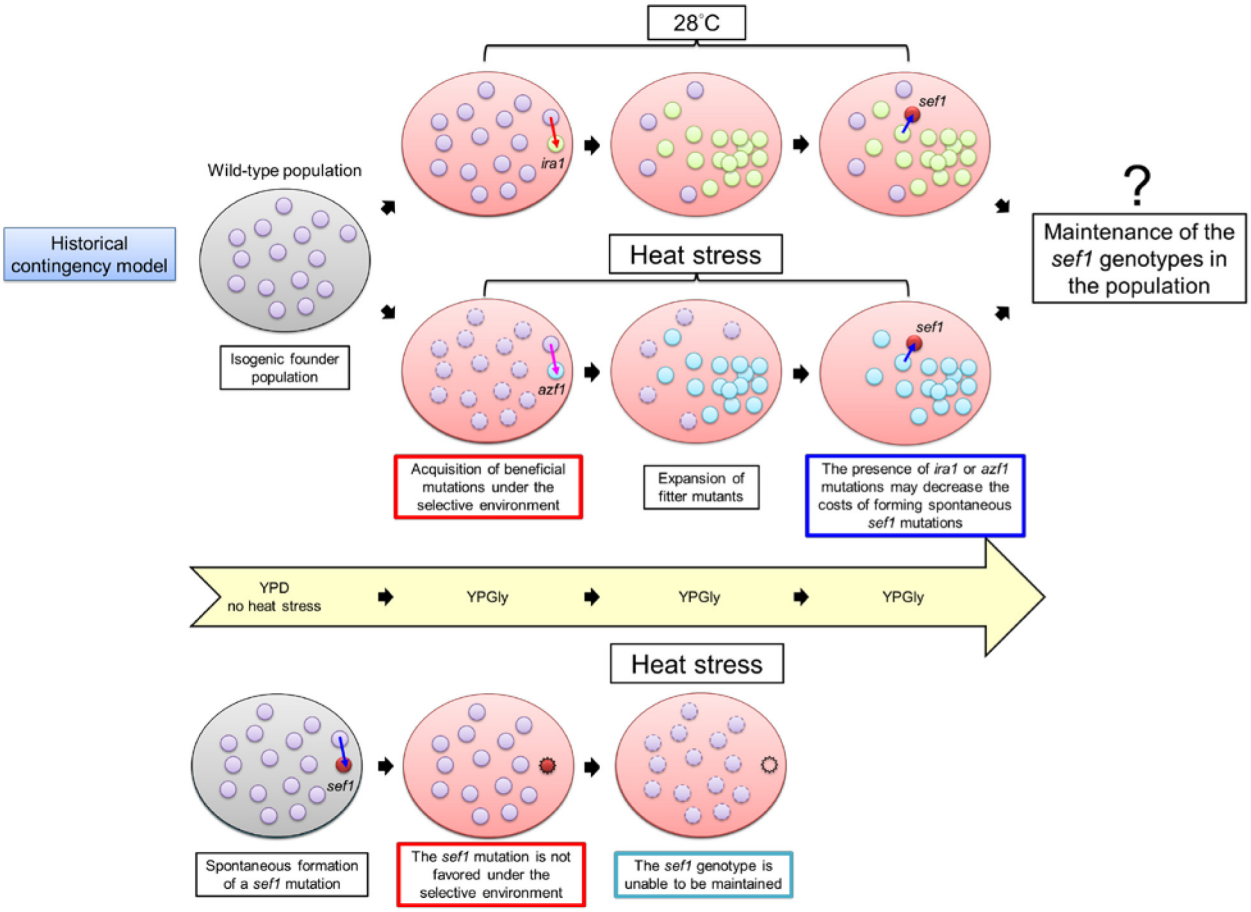

Figure EV5. The historical contingency model for the fates of spontaneous deleterious *sef1* loss-of-function mutations.

In this hypothetical model, the upper panel shows that the subsequent formation of the *sef1* loss-of-function mutations under mild (28°C) or harsh (heat stress) selective conditions are preserved by the presence of pre-existing primary beneficial mutations (e.g., *ira1* or *azf1*) in the founder population (e.g., the wild-type population). The primary mutations alleviate the deleterious effects of *sef1* mutations. The loss-of-function *sef1* genotypes are unable to be preserved in the population without the primary mutations (bottom panel). However, whether the secondarily formed *sef1* subpopulation can compete with the primary population and then expand is not guaranteed, possibly depending on their pleiotropies and the selections by changing environments in the future.

