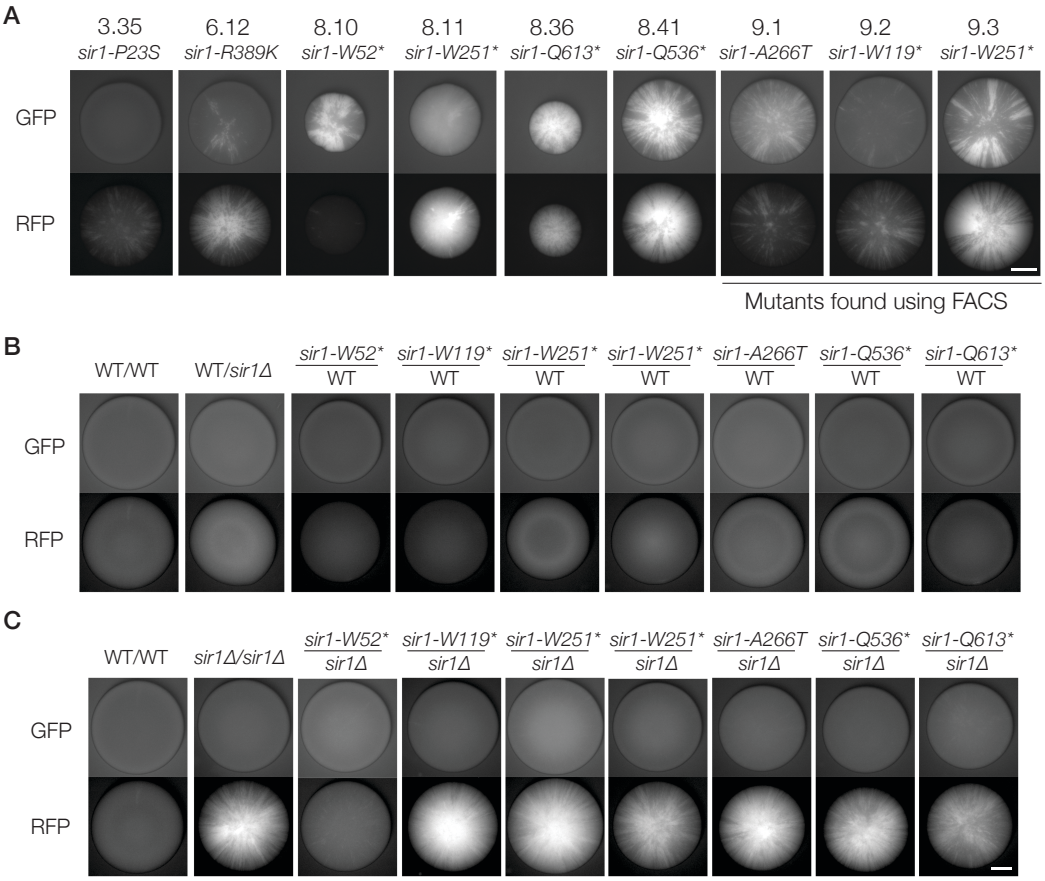

D

Mutant ID	*sir1* allele	Nucleotide position mutated	Base pair change
3.35	*sir1-P23S*	67	CCA to TCA
8.10	*sir1-W52**	155	TGG to TAG
9.2	*sir1-W119**	356	TGG to TAG
8.11	*sir1-W251**	752	TGG to TAG
9.3	*sir1-W251**	752	TGG to TAG
9.1	*sir1-A266T*	796	GCA to ACA
6.12	*sir1-R389K*	1166	AGG to AAG
8.41	*sir1-Q536**	1606	CAA to TAA
8.36	*sir1-Q613**	1837	CAA to TAA

Figure S1: Additional FLAME phenotypes and characterization of the *sir1* mutants identified. A. Colony phenotypes of the mutants identified (JRY11896, JRY11897, JRY11901, JRY11902, JRY11904, JRY11905, JRY11918-11920). Colonies were labeled with both the initial mutant ID and its associated *sir1* allele; an asterisk indicates a premature stop codon. Each mutant found during FACS sorting was from an independent mutagenized culture. B. Colony images of the dominance test diploid strains (*MATα* FLAME strain crossed with a *MATα* mutant FLAME strain) (JRY11908, JRY11909, JRY11914-11917, JRY11922-11924, JRY11955), in both the GFP and RFP channel, plated on YPD. Some variable autofluorescence was seen in the RFP channel at the colony level, however variable autofluorescence in the RFP channel was also seen in colonies without an endogenous source of RFP (data not shown). C. Colony images of the *sir1Δ* complementation test diploid strains (*MATα sir1Δ* FLAME strain crossed with a *MATα* mutant FLAME strain) (JRY11946-11957), in both the GFP and RFP channel, plated on YPD. Mutant *sir1-W52** displayed a much weaker phenotype than other colonies, yet some small sectors are seen in the RFP channel. D. Table indicating the initial mutant ID, the associated *sir1* allele, the location of the nucleotide mutation, and the resulting base pair change. Mutants 8.11 and 9.3 were isolated from independent rounds of mutagenesis and had identical *sir1* alleles, *sir-W251**. Scale bars, 2 mm.

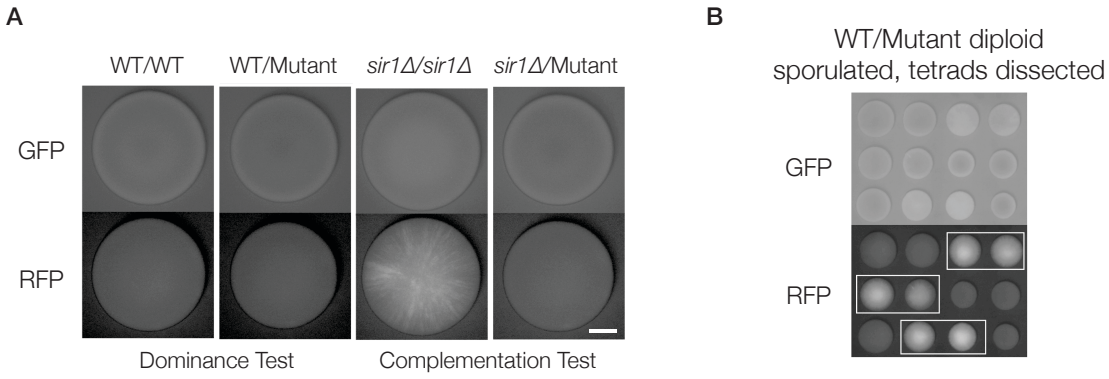

Figure S2: Genetic analysis of the mutant isolated from the second mutagenesis screen. A. Representative colony images of the diploid strains (JRY11957, JRY12476, JRY11952, JRY12477), grown on CSM. These strains were generated by mating the mutant isolated from the second mutagenesis screen (JRY12466) to *SIR+* and *sir1Δ* strains (JRY12863 and JRY12864, respectively). Scale bar, 2 mm. B. The wild-type/mutant diploid (JRY12476) was sporulated and tetrads were dissected on YPD.

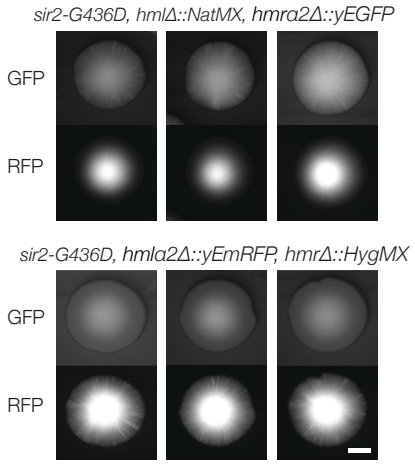

Figure S3: Overlap in GFP and RFP channels in *sir2-G436D* was not due to spectral bleedthrough. Representative colony images of *sir2-G436D, hmlΔ::NatMX, hmrα2Δ::GFP* (JRY13197) and *sir2-G436D, hmlα2Δ::RFP, hmrΔ::HygMX* (JRY13198), grown on CSM. Scale bar, 2mm.

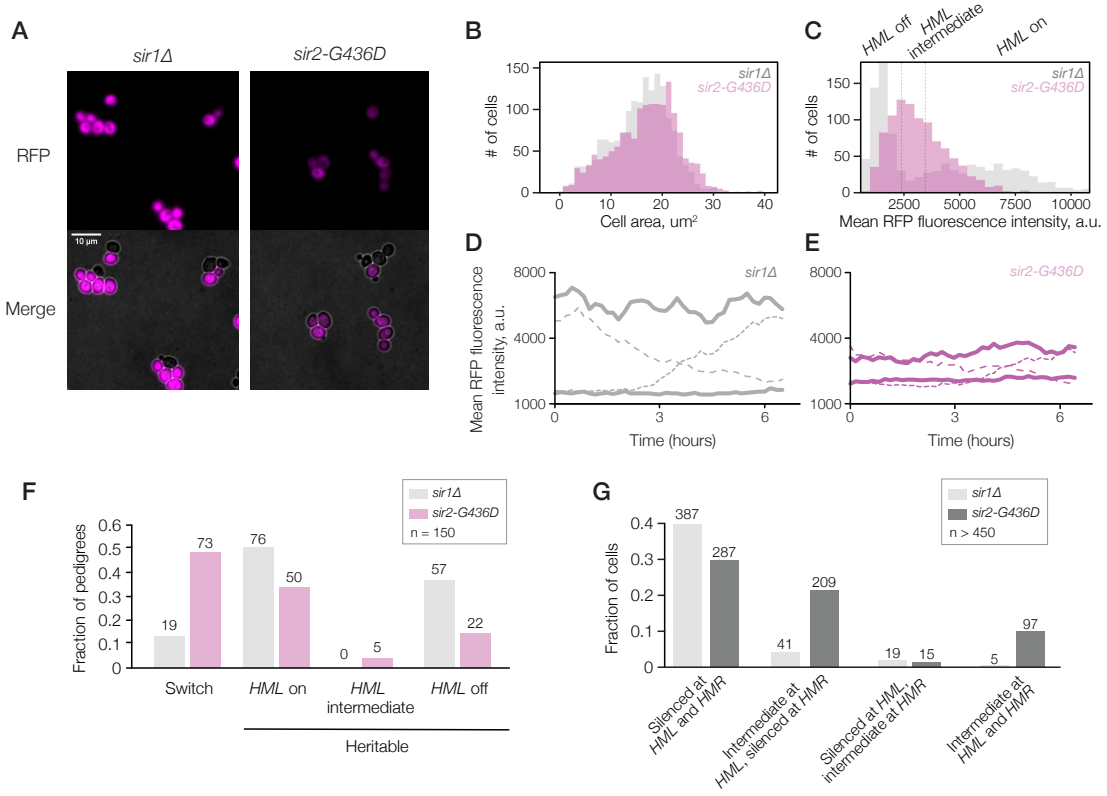

Figure S4: Live-cell imaging of *hmlα2Δ::RFP* in *sir2-G436D*. A. RFP and merged (bright-field and RFP) fluorescence microscopy images of *sir1Δ* and *sir2-G436D* cells (JRY12861, JRY12564), imaged with identical exposures. B. Distribution of the cell size for both *sir1Δ* and *sir2-G436D,* with number of cells on the y-axis and cell area in μm^2 on the x-axis. C. Distribution of the RFP mean fluorescence intensity (arbitrary units, a.u.) per cell for both *sir1Δ* and *sir2-G436D.* Dashed lines demarcate the boundaries of the three fluorescence states: *HML* off, *HML* intermediate, and *HML* on. Details on how thresholds were assigned are in *Materials and Methods*. D. RFP mean fluorescence intensity for individual *sir1Δ* cells over 6.5 hours. 12 individual cells were monitored, and 4 representative fluorescence trajectories are displayed. Each solid line represents a single cell that maintained a similar fluorescence level over the timecourse, whereas each dashed line represents a single cell that experienced a change in fluorescence. E. Same as (D), but for 4 individual *sir2-G436D* cells. F. Bar chart showing the frequency of pedigrees designated as a "switch" or "heritable", as described in Figure 3G. 150 pedigrees were observed per genotype, with the number of pedigrees per category above each bar. G. Frequency of cells exhibiting the silenced or intermediate states at *HML* and *HMR*. At least 450 cells were analyzed per genotype, with the number of cells in each category above each bar.

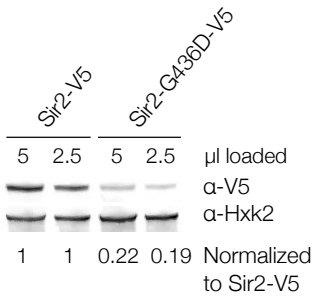

Figure S5: Sir2-G436D was present at lower levels than Sir2. Immunoblot to detect Sir2-V5, Sir2-G436D-V5, and an internal loading control Hxk2 (JRY12589, JRY12590). Protein levels were quantified, normalized to the loading control, and compared to wild-type Sir2-V5 levels.

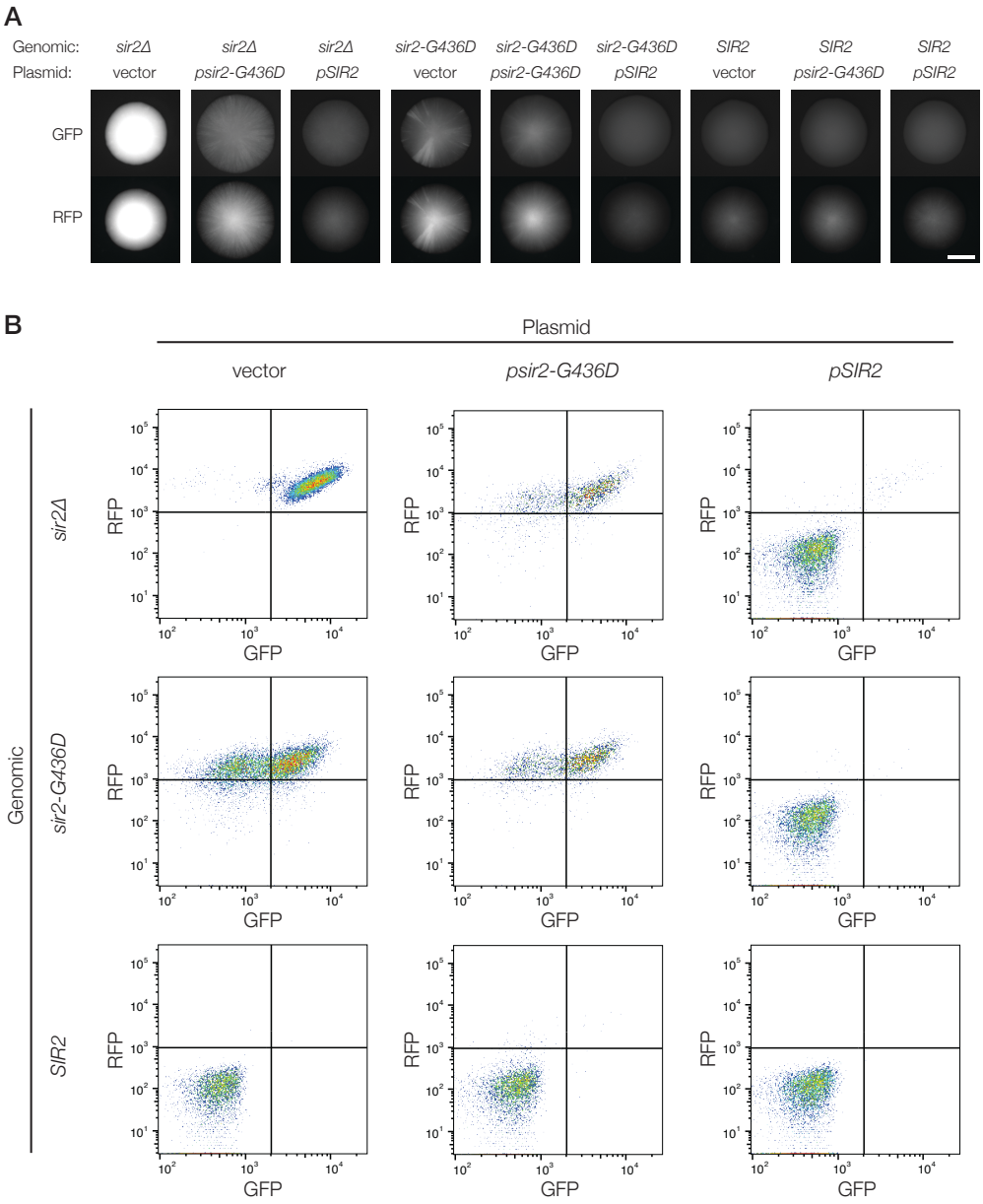

Figure S6: Effects of *SIR2* and *sir2-G436D* overexpression. A. Representative colony images of *sir2Δ* (JRY12259), *sir2-G436D* (JRY12564), and *SIR2* (JRY12860) strains with a 2 micron vector (pRS426), a 2 micron vector containing *sir2-G436D* (*psir2-G436D*) (pJR3525), or a 2 micron vector containing *SIR2* (*pSIR2*) (pJR3524). Colonies were grown on CSM -Ura to select for plasmids. Scale bar, 3 mm. B. Flow cytometry profiles of strains shown in (A). Independent cultures (n = 3 per genotype) were grown at log phase for 24 hours in CSM -Ura liquid media, fixed, and analyzed. Representative flow cytometry profiles for each strain are shown. Quadrants were established by using the fluorescence profiles of *SIR2* and *sir2Δ* cells.

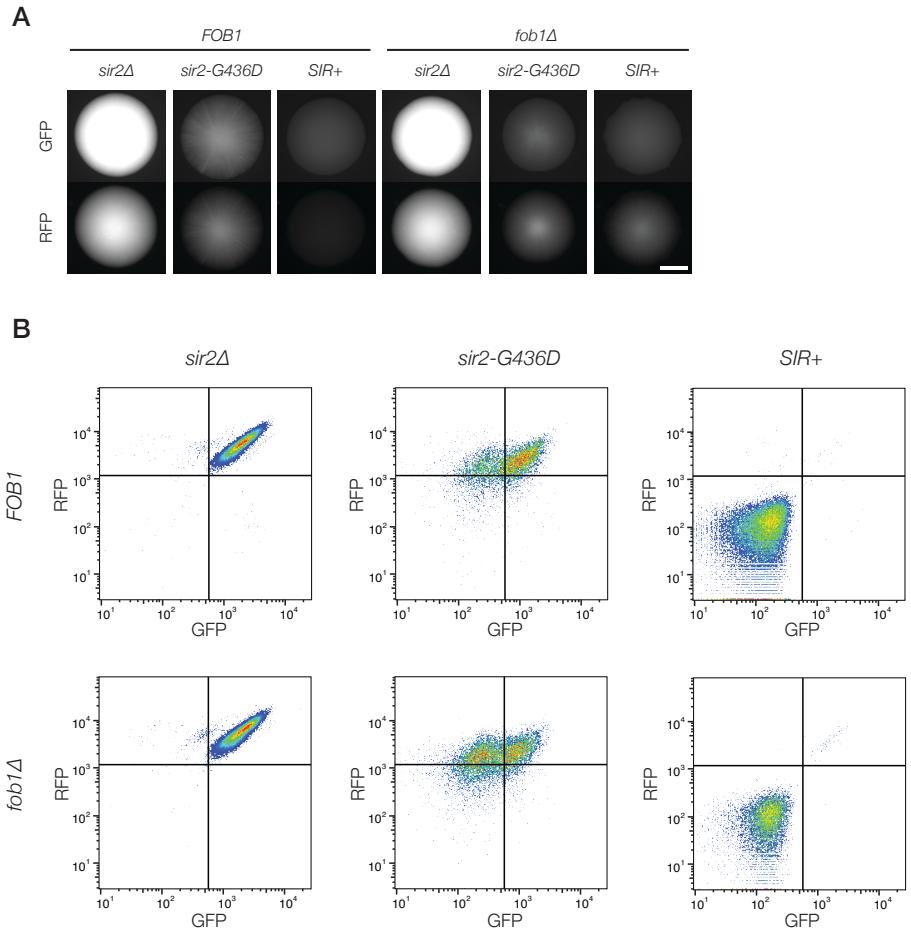

Figure S7: Effects of rDNA recombination on *sir2-G436D*. A. Representative colony images of *sir2Δ*, *sir2-G436D*, and *SIR2* with or without *FOB1* (JRY12259, JRY12564, JRY12860, JRY12900-12902), grown on CSM. Scale bar, 3 mm. B. Flow cytometry profiles of strains shown in (A). Independent cultures (n = 3 per genotype) were grown at log phase in CSM liquid media for 24 hours, fixed, and analyzed. Representative flow cytometry profiles are shown for each strain. Quadrants were established by using the fluorescence profiles of *SIR2* and *sir2Δ* cells.