Supporting Methods

Phenotypic recovery frequency calculations

To calculate the 95% confidence level for phenotypic recovery rates of various strains, in a colony with a population size of $A \approx 10^8$, a smaller representative sample size was used to make observations of size switching, and to determine a confidence interval for size switching for a given strain. Using the above method, cells from a single parent colony were plated on ten plates each. We calculated r as the percentage of all colonies that had switched sizes. The sample mean of size switching for n sets of plates of a given type of strain was given by $\bar{r} = \frac{1}{n}(r_1 + r_2 + \cdots + r_n)$. We were then able to calculate the percent of colonies that would likely develop the switched size phenotype using the equation:

$$a \in [\bar{r} - \epsilon, \bar{r} + \epsilon].$$

Then, we determined the confidence interval. The $100(1-\alpha)\%$ confidence interval was be calculated with the equation:

$$\left[\bar{r} - z_{\alpha/2}\frac{s}{\sqrt{n}}, \bar{r} + z_{\alpha/2}\frac{s}{\sqrt{n}}\right]$$

where *s* was the standard deviation, calculated using the equation:

$$s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(r_i - \bar{r})^2 = \frac{1}{n-1}\left(\sum_{i=1}^{n} {r_i}^2 - n\bar{r}^2\right)$$

and $z_{\alpha/2}$ was determined from the standard normal distribution:

$$\Pr\{|Z| \leq z_{\alpha/2}\} = 1 - \alpha.$$

For a confidence level of 95%, the corresponding $z_{\alpha/2}$ value is 1.96. This allowed us to determine the confidence level for the number of individuals with the trait within a population.

Genomic DNA extraction, library production and sequencing

For genomic DNA extraction**,** 10ml cell cultures were grown to exponential growth phase, OD_{595}≈0.5-0.8, and cells were collected by gentle centrifugation. The cells were resuspended in 400 µl DNA extraction buffer in screw-cap tubes, and 400 µl of acid-washed, 425-600 µm glass beads and 400 µl of 25: 24: 1 phenol: chloroform: isoamyl alcohol were added. The tubes were beaten in a BioSpec MiniBeadBeater-16 bead beater for 2 minutes in a cold room. An additional 200 µl of DNA extraction buffer was added to the tube, and the contents were inverted several times to mix. The samples were centrifuged for five minutes at 13,000 rcf at 4°C, and the supernatant was transferred to a clean tube and incubated with 20 µg of RNase A at 37°C for 15 minutes. After RNase treatment, an equal volume of 25: 24: 1 phenol: chloroform: isoamyl alcohol was added, the sample mixed and centrifuged, and the supernatant transferred to a new tube. Then, an equal volume of chloroform was added, the sample was mixed and centrifuged, and the supernatant was transferred to a new tube. The extracted genomic DNA was precipitated with two volumes of cold 100% ethanol in a -20°C freezer overnight, washed twice with cold 70% ethanol, and then re-suspended in 50 µl 10 mM pH 7.4 Tris Buffer.

Genomic DNA Libraries were produced for whole-genome sequencing of two *elf1Δ* P strains and five *elf1Δ* S strains using the Illumina TruSeq DNA PCR-Free LT library prep kit. The libraries were prepared from the genomic DNA from the

phenol:choloroform extraction following the manufacturer's protocols, except the shearing parameters and the bead-drying times were adjusted. 55 µl of each sample of 20 µg/µl of genomic DNA was sheared in a Covaris S220 sonicator with the duty factor set to 10%, peak power set to 175 W, 200 cycles per burst, frequency sweeping mode at 5.5°C to 6°C for 45 seconds. The manufacturer's protocol to prepare 350 bp size samples was followed, but all bead drying times were shortened to 1 to 2 minutes, never allowing the beads to fully dry. The concentrations of the resulting libraries were calculated by running qPCR using KAPA Illumina library quantification kit DNA standards and universal qPCR kit (Kit code KK4824) with triplicate samples and three dilutions for each, following the manufacturer's directions. The 16 libraries were combined into two pools and 125 bp paired ended sequencing was performed using the Illumina HiSeq2500 platform by the David H. Murdock Research Institute.

Short reads were minimally trimmed using SHEAR (https://github.com/jbpease/shear) using the following command line (all other options default):

```
shear.py --fq1 $FASTQ1 --fq2 $FASTQ2 --out1 $OUTFQ1 --out2 $OUTFQ2 \
--barcodes1 $BARCODE --platform TruSeq --trimqual 20:20 \
--trimpolyat 0 --trimambig --filterlength 50 --filterunpaired
```

Reads were mapped to the *S. pombe* reference genome v2.30 (WOOD *et al.* 2002) obtained from PomBase (ftp://ftp.ebi.ac.uk/pub/databases/pombase/pombe/Chromosome_Dumps/fasta/) using

BWA v0.7.15 (LI AND DURBIN 2009). The following command line was used (all other options default):

```
bwa mem -t 8 $GENOME $OUTFQ1 $OUTFQ2
```

Alignment SAM files were then put through GATK best practices pipeline (DEPRISTO *et al.* 2011; VAN DER AUWERA *et al.* 2013) for variant calling using GATK v3.6 (MCKENNA *et al.* 2010), PicardTools v2.5.0 (http://broadinstitute.github.io/picard), and SAMtools v1.3.1 (LI AND DURBIN 2009). The following command lines with parameters were used (all other options default):

```
java -Xmx30g -jar picard.jar AddOrReplaceReadGroups INPUT=$SAM1 \
OUTPUT=$BAMMARKED RGID=1 RGLB=lib01 RGPL=illumina \
RGPU=$BARCODE RGSM=$SAMPLENUMBER

samtools fixmate -O bam $BAMMARKED $BAMFIXED

samtools sort -O bam -o $BAMSORTED -T /home/peasejb/tmp $BAMFIXED

samtools index $BAMSORTED

java -Xmx30g -jar GenomeAnalysisTK.jar -T HaplotypeCaller \
-R $GENOME -I $BAMSORTED --genotyping_mode DISCOVERY \
-stand_emit_conf 10 -stand_call_conf 30 -o $VCFRAW
```

VCF files were compared among progenitor and mutant sequencing replicates using BCFtools v1.3.1 (LI AND DURBIN 2009). The following command lines with parameters were used (all other options default):

```
bcftools isec -n+1 $VCFRAW1 $VCFRAW2 … > common_variants.tsv
```

Variants were then cross-referenced with the current GFF3 annotation file (ftp://ftp.ebi.ac.uk/pub/databases/pombase/pombe/Chromosome_Dumps/gff3/schizosaccharomyces_pombe.chr.gff3) using a custom Python script to identify consistent SNP sites in protein-coding regions (synonymous and non-synonymous), 5′ and 3′ UTRs, and ncRNA.

Analysis of lagging chromosome

Identification of chromosome mis-segregation was performed as previously described (PIDOUX *et al.* 2000). ***elf1*Δ** P strains were streaked to individual colonies to select for small colonies. Wild-type and mutant strains were grown in EMM overnight and cell cycles were synchronized by the Hydroxyurea block-release method described above. Cells were fixed with ice-cold ethanol and washed twice with PBS and suspended in 50µl of PBS. 5µl of the suspension was added to a Poly-L-lysine coated microscope slide with 5µl of mounting media with DAPI (Vector Laboratories Inc., H1200). Cells were imaged using a Zeiss 880 laser scanning confocal microscope with a Zeiss Plan-Apichromat 63x/1.4Oil DIC oil-immersion lens (Figure 5C). Lagging chromosomes were analyzed in 200 late anaphase cells with indicated genotypes of two independent biological replicates. Results are plotted as a calculated percentage of cells with lagging chromosome to the total number of cells scored (Figure 5D). A two-

sample *t*-test was performed by comparing the percentage of cells with lagging chromosome between ***elf1***Δ and the wild-type cells.