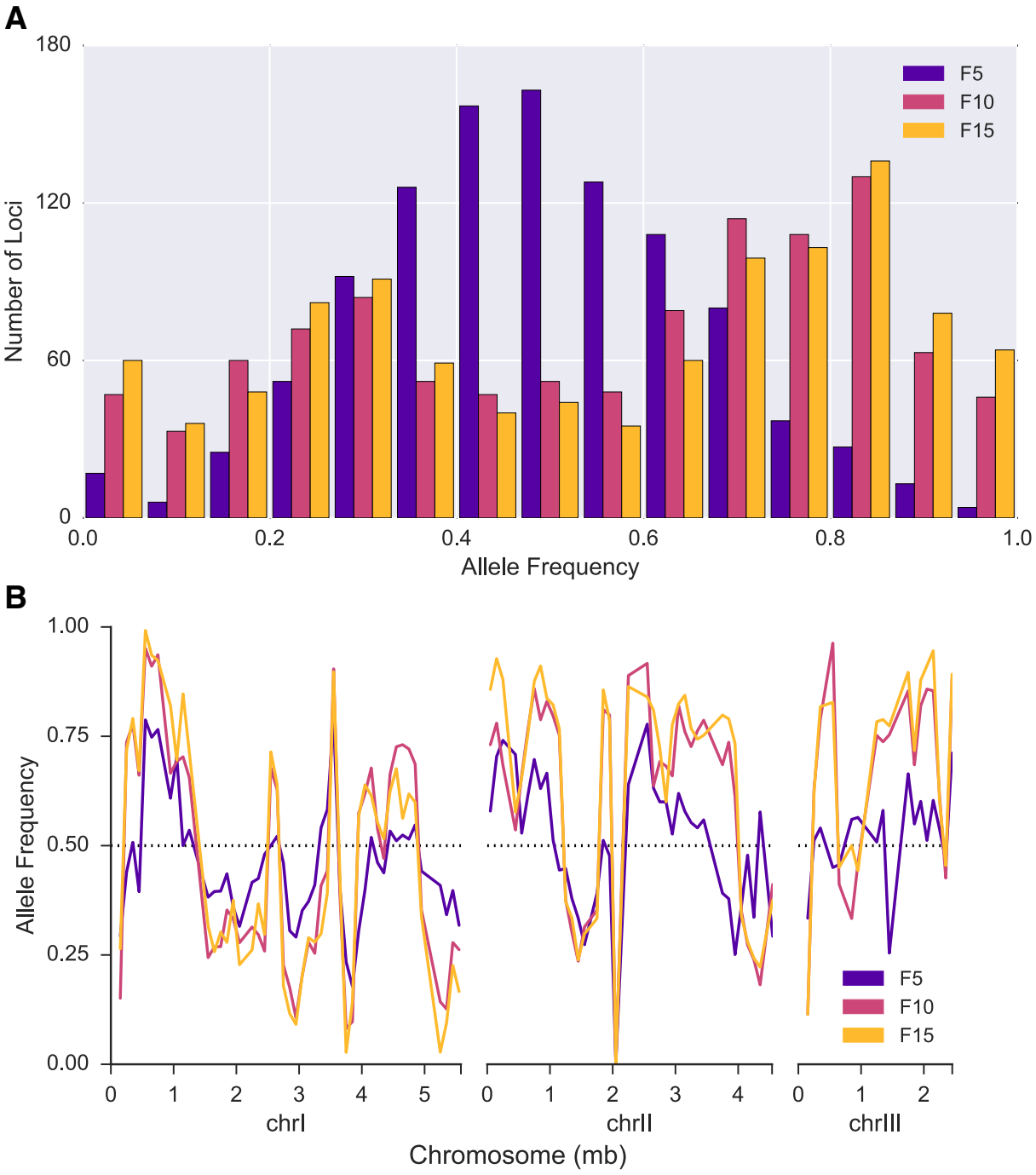
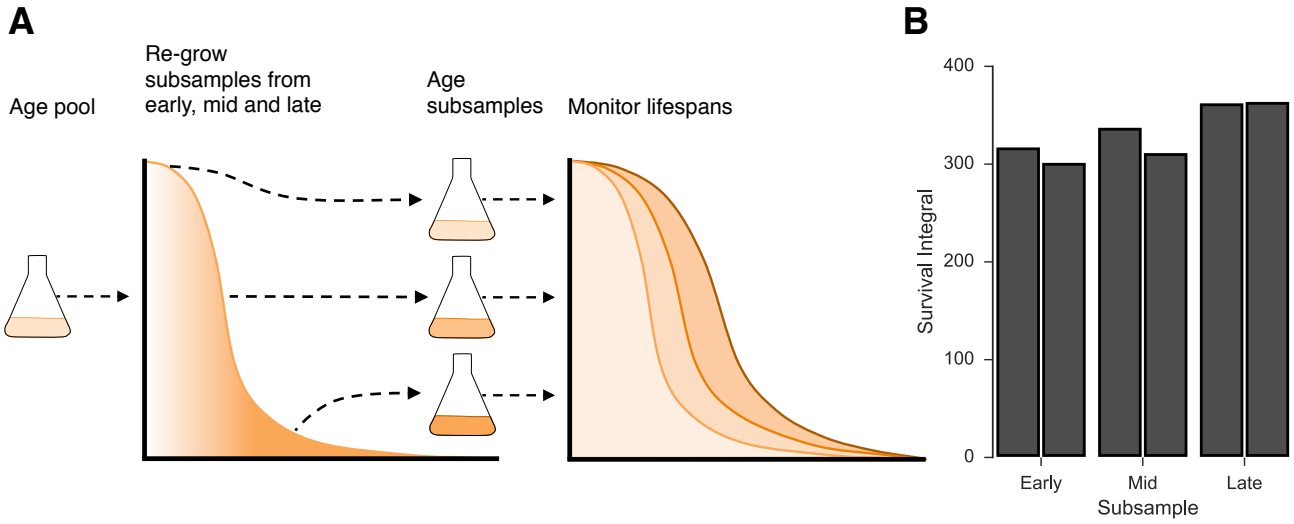


Supplementary Figure 1. Intercrossing skews allele frequencies away from 0.5, throughout the genome.

A: Number of loci falling into different bins of allele frequency at five (purple), ten (red) and fifteen (yellow) generations of intercrossing. Allele frequency refers to the frequency of the DY8531 allele, relative to the Y0036 allele, at any given locus. Note the bimodal distribution from F10 onwards.

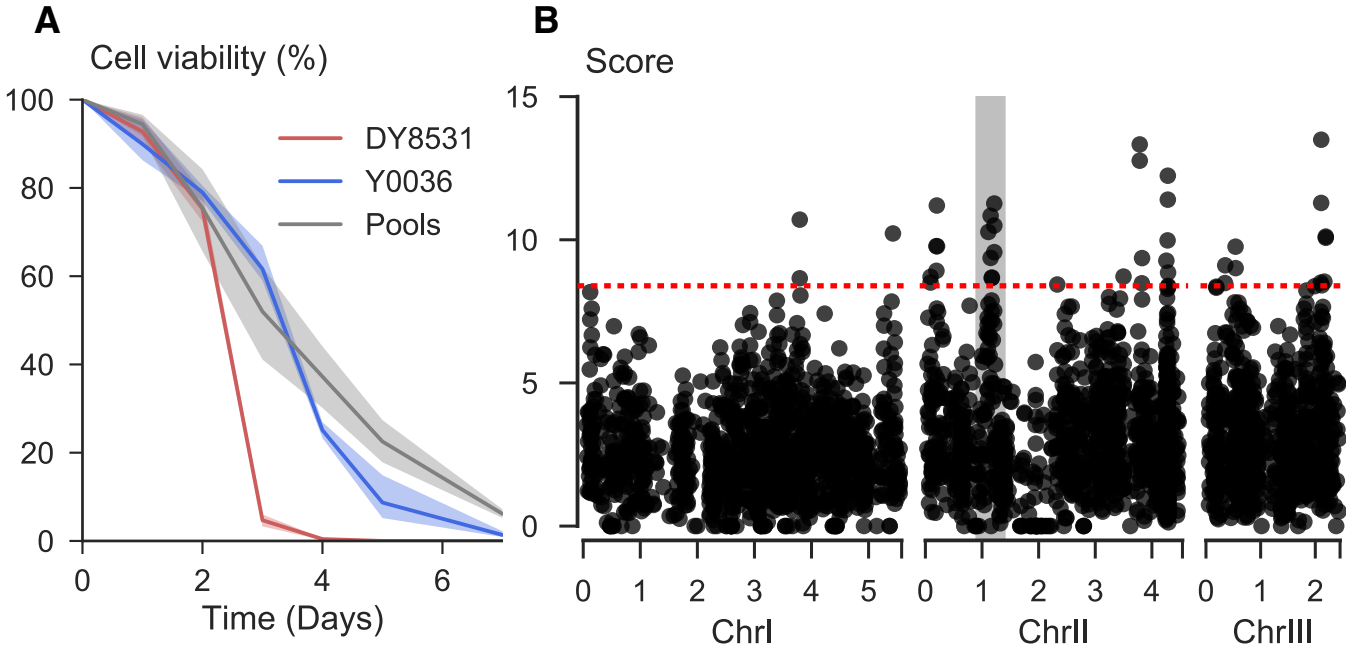
B: Sliding average of allele frequencies at loci across the genome. Note that some allele frequencies are fixed (1.00/0.00) at F15.



Supplementary Figure 2. Sampling ageing segregant pools later in time selects for more long-lived individuals.

A: Experimental design. Two independent pools were chronologically aged and sampled early (day zero), mid way through (day three) and late (day six). These subsamples were then re-grown and aged themselves. The lifespan of each subsample was recorded.

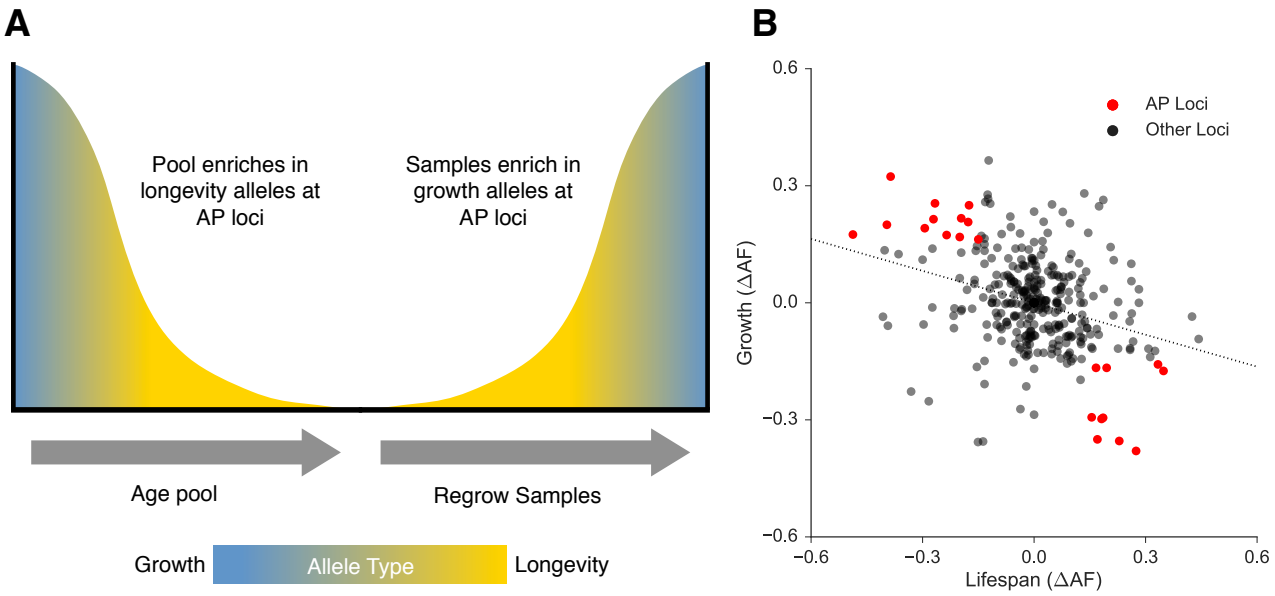
B: The survival integral (area under lifespan curve) of subsamples taken from each replicate ageing pool of segregants.



Supplementary Figure 3. Comparison of pool lifespans with parental lifespans and raw scores of allele frequency change.

A: Lifespan curves of the two parental strains - DY8531 (red, N=3) and Y0036 (blue, N=3) – compared with the pools (grey, N=8). Lines correspond to the mean ± shaded 95% confidence interval. The percentage of viable cells is measured on the Y-axis.

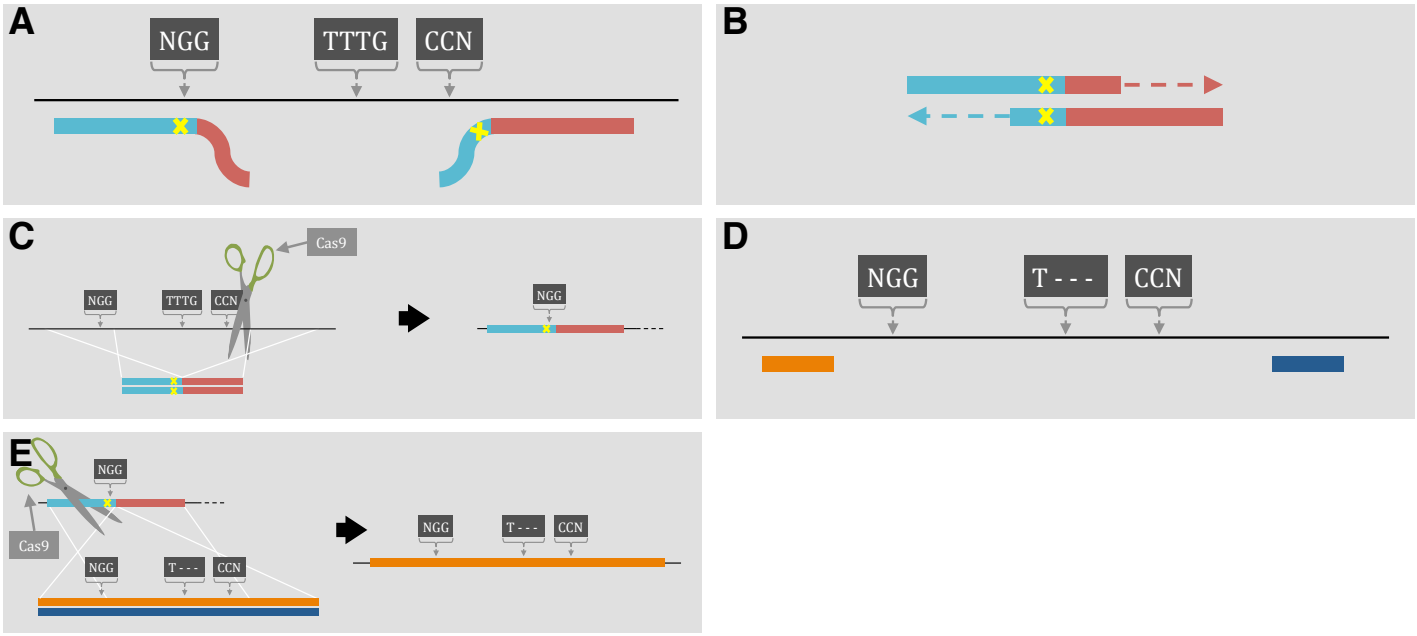
B: Raw scores of allele frequency change (mean of repeats) for each variant across the ageing time course. Score is measured on the Y-axis. Red dotted line represents the upper quartile + 1.5x inter-quartile range. Grey highlighted region corresponds to the chromosome two peak identified (see Fig. 2B).



Supplementary Figure 4. Re-growing samples leads to biasing of allele frequency at Antagonistically Pleiotropic (AP) loci.

A: Conceptualisation of experiment. As pools age, selection theoretically causes allele frequencies at AP loci to become skewed in favour of longevity alleles (yellow). When these subpopulations are allowed to re-grow, selection then favours growth alleles (blue) and the allele frequency at AP loci reverts back towards what it was before age treatment.

B: The change in allele frequency (ΔAF) during age (x-axis) and growth (y-axis) at all loci. Each point represents a locus. Red points show loci that were deemed antagonistically pleiotropic, as they exceeded a threshold of ±0.15 ΔAF during both growth and age.



Supplementary Figure 5. Cloning strategy for scar-less allele replacement in PAM-poor regions.

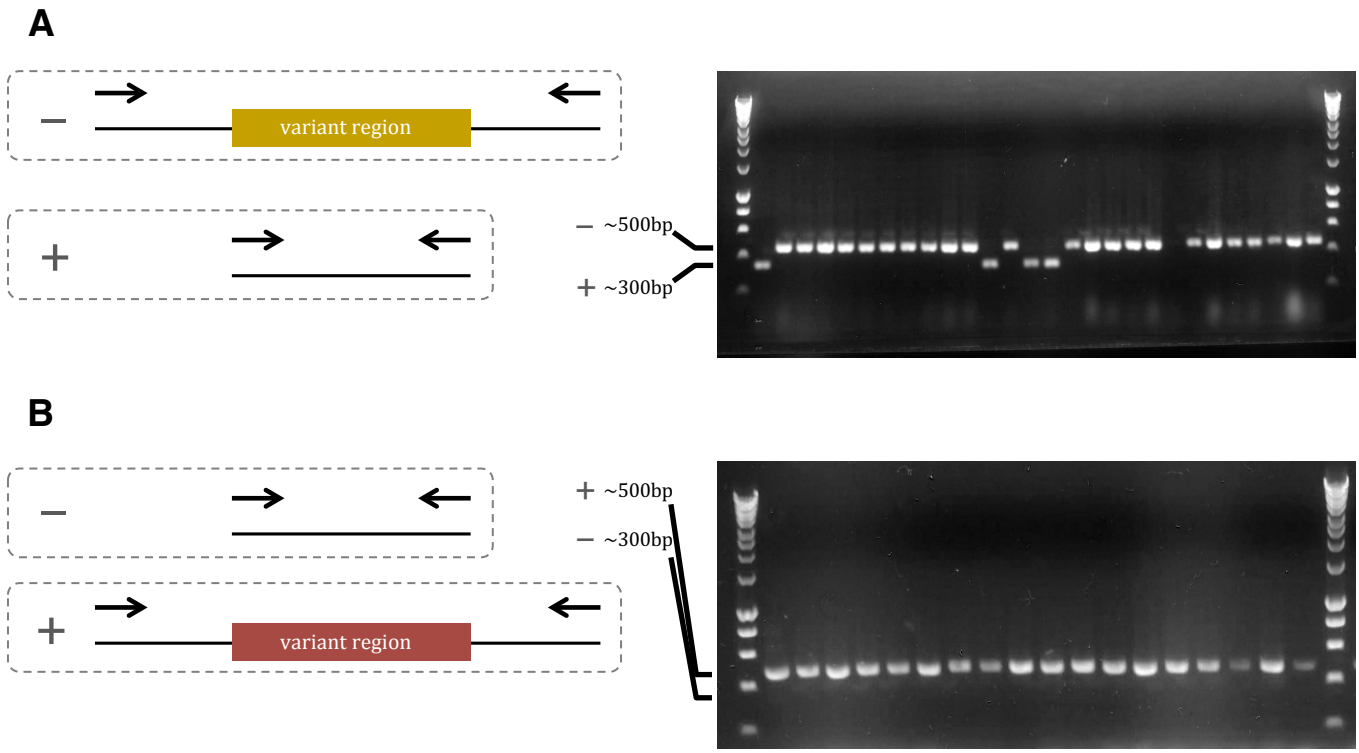
A: Amplification of a large region, including the variant site, between two PAM sites. Each primer (red/blue lines) contains an overhang complementary to the start/end of the other primer. An arbitrary modification near one of the PAM sites is introduced to one primer (yellow cross).

B: After annealing, oligos were extended to produce dsDNA templates for deletion.

C: The region is cut with Cas9, whilst simultaneously providing the dsDNA template, resulting in deletion, as well as simultaneous integration of the arbitrary modification.

D: Amplification of the region, this time from the alternative parent. Primers (orange/blue) amplify the entire region, plus ~80bp on either side for homologous recombination.

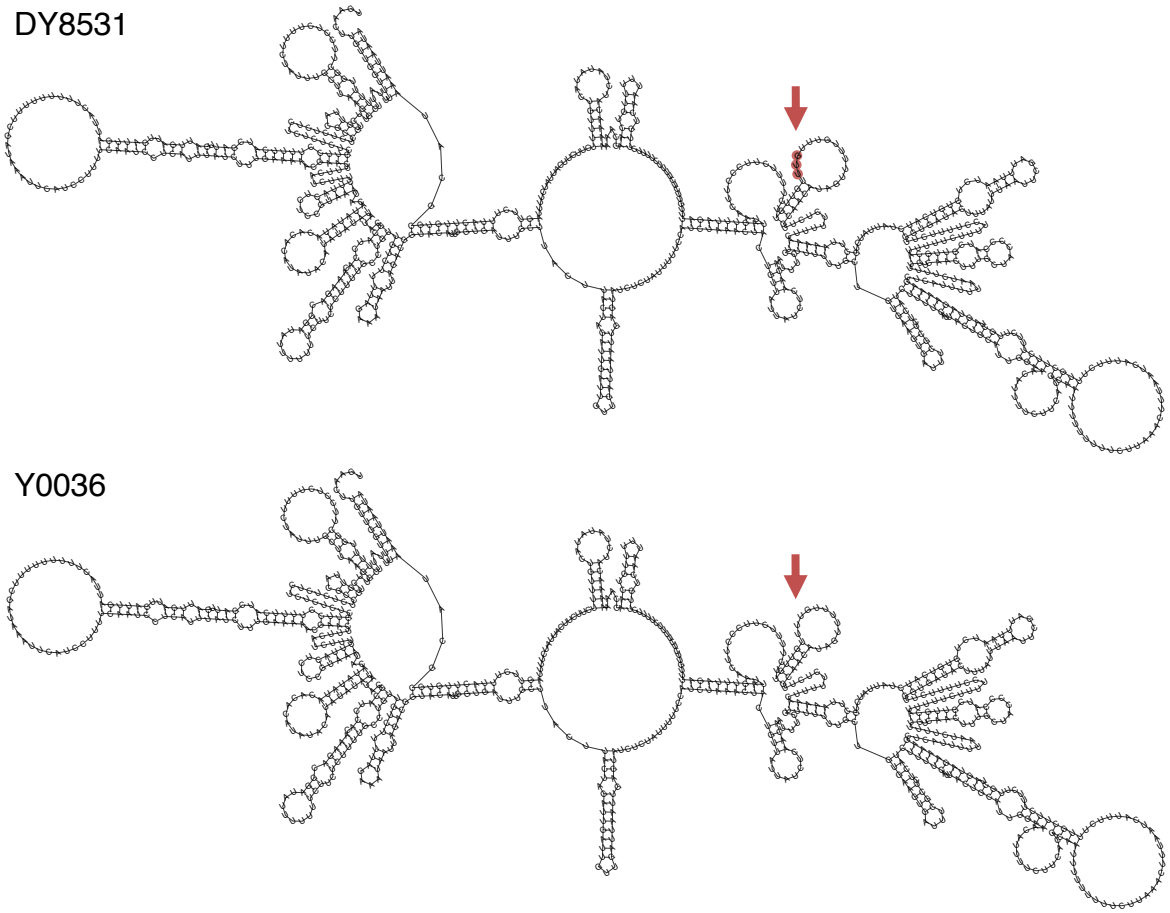
E: Cut with Cas9 providing the fragment from the PCR above. These will then be used to repair the cut site, replacing the arbitrary modification and inserting the desired parental variant.



Supplementary Figure 6. CRISPR-Cas9 mediated insertions are more easily obtained than deletions.

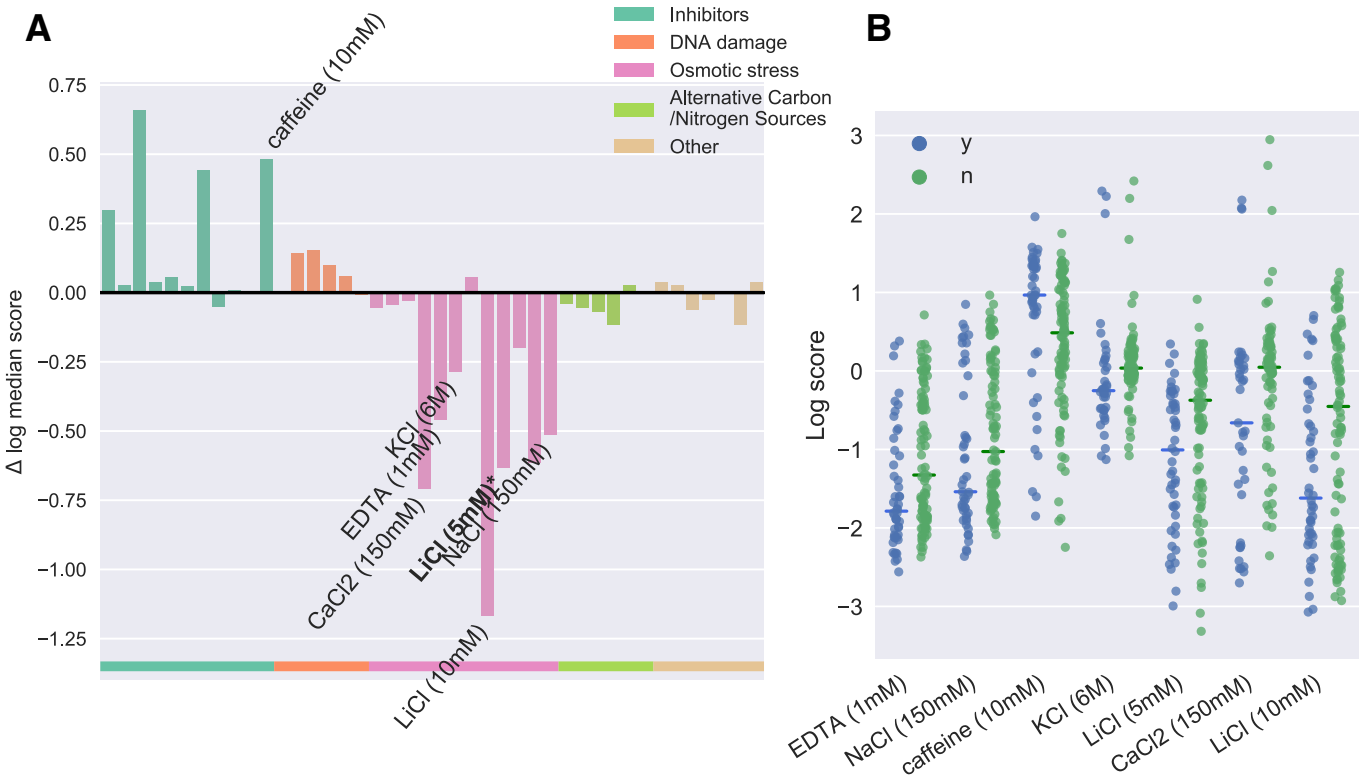
A: Deletions – schematic of primers used for PCR (left) and example results with 4/27 positive colony PCRs (right). Positive bands have an approximate size of ~300bp; negative bands have an approximate size of ~500bp.

B: Insertions – schematic of primers used for PCR (left) and example results with all (18/18) positive colony PCRs (right). Positive bands have an approximate size of ~500bp; negative bands have an approximate size of ~300bp. *S. pombe* colonies were picked at random.



Supplementary Figure 7. Predicted secondary structure of SPBC409.08 mRNA 5' UTR.

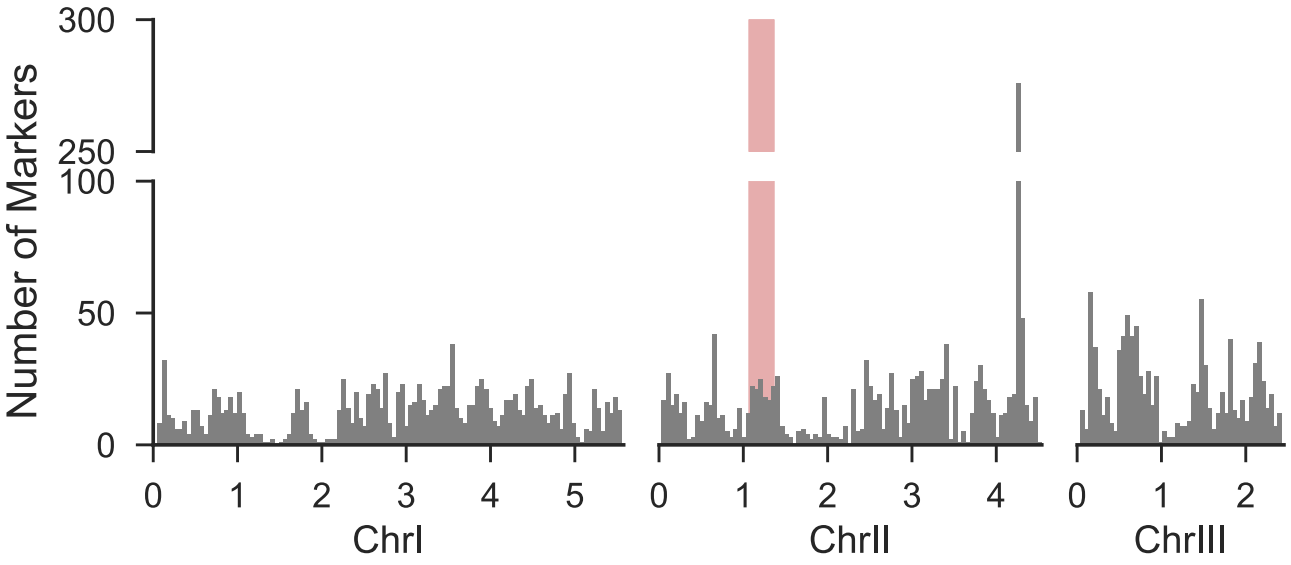
Prediction was made using the Minimum Free Energy (MFE) fold algorithm in RNA Fold (Gruber et al. 2008). Deleted bases are highlighted in red, along with a red arrow.



Supplementary Figure 8. Comparison of growth data for natural isolates with and without *ppk31* insertion.

A: Difference between the median growth score of strains with and without the *ppk31* insertion on various substrates (Δ median score). Each bar represents a single condition; Y axis plotted logarithmically for visualization. Labeled bars show conditions where there was a significant difference between the two groups before correction for multiple testing (shown in part B; see Materials & Methods). The only condition to remain significant after correction is shown in bold (5mM LiCl).

B: Raw comparison of growth data for the two groups in all seven conditions that were significant before correction. Those with the insertion are shown in blue, those without are shown in green. Y axis plotted logarithmically for visualization.



Supplementary Figure 9. Polymorphic marker distribution across 3 chromosomes. Histogram showing the number of polymorphic markers in equally sized bins across the genome. The peak region identified in this study is highlighted as a red box. Note the broken y-axis, due to the marker-dense region of Chromosome II.

Position	Annotated Region	Biological Process	Variant	Y0036 Allele	DY8531 Allele	Score
1111869	Erg27 (HSD17B7)	Lipid metabolism	SNP (CDS – Syn)	G	A	10.3
1151936	SPBC409.08	Spermidine transmemb. transport	Deletion (5' UTR)	T	TTTG	9.4
1152197	SPBC409.08	Spermidine transmemb. transport	SNP (intron)	T	A	10.8
1171666	SPBC409.18 (PLPP5) *	Lipid metabolism	SNP (CDS – Syn)	C	T	8.7
1174437	Intergenic **	-	SNP	A	T	8.7
1215851	Ppk31 (STK38)	Signal transduction	SNP (CDS – Syn)	G	T	11.3
1216499	Ppk31 (STK38)	Signal transduction	Insertion (5' UTR)	TA	T	9.6
1216900	Pex5 ***	Peroxisome organisation	SNP (5' UTR)	T	A	10.5

Supplementary Table 1. High-scoring loci in the peak region of chromosome two. Table detailing all loci with above- threshold scores in the highlighted region of chromosome two. Position relates to chromosome two. Annotated regions are according to PomBase (Wood et al. 2012, McDowall et al. 2015) with human orthologues in parentheses. Variants in coding regions are designated "Syn" for synonymous. * variant could equally affect the 3'UTR of mtx2 ** variant could equally be in the promoter of mtx2; *** variant could equally be in the promoter of ppk31.