Supplementary Material

Dataset S1. Log$_2$fold changes of the 1737 genes that were differentially expressed at 42ºC relative to 37ºC. The grey cells correspond to significant changes in GE ($q <$ 0.001).

Dataset S2. Genes with restored, novel and reinforced expression shared between single mutants.

Supplementary Figures

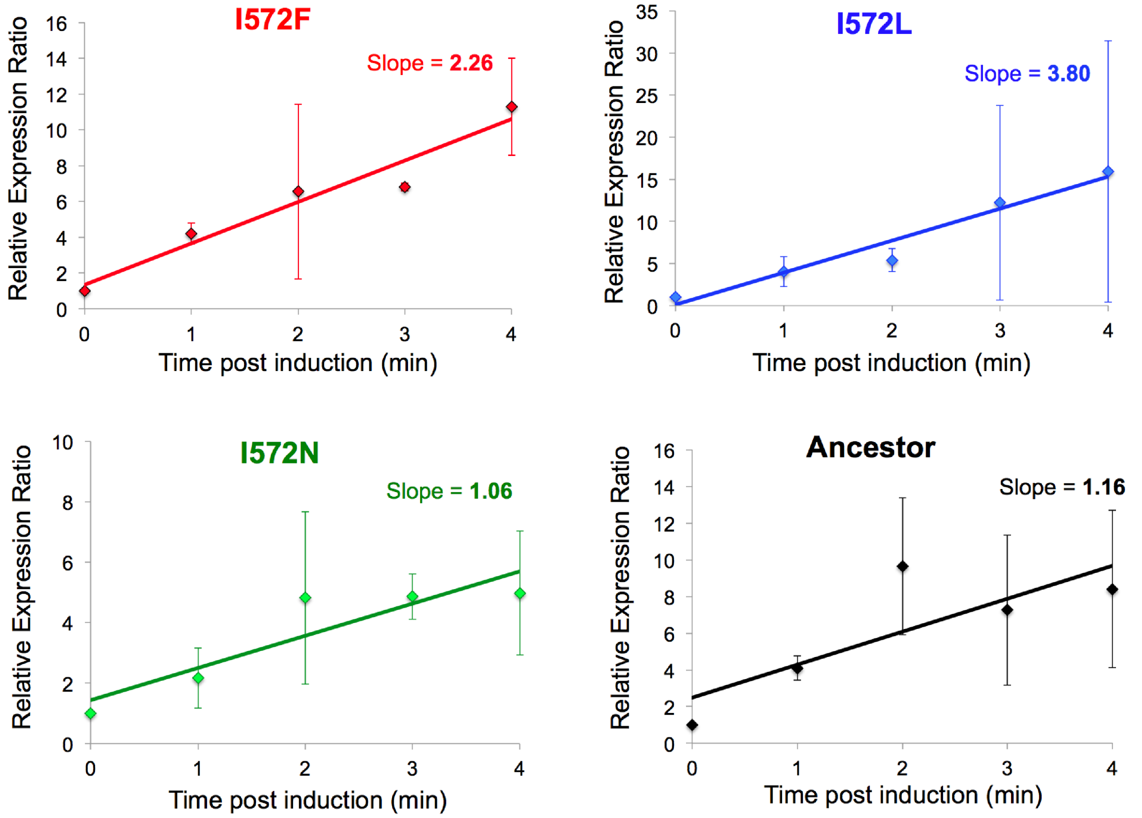


Fig S1. Transcription efficiency of the mutants and the ancestor at 42ºC. Each diamond represents the mean of three independent measurements and the bar indicates the associated standard deviation. The slope of the fitted regression represents the overall transcription efficiency of RNAP. Based on a test of equality of regression parameters, we did not detect statistical differences between the transcription efficiency of each mutant and the transcription efficiency of the ancestor.

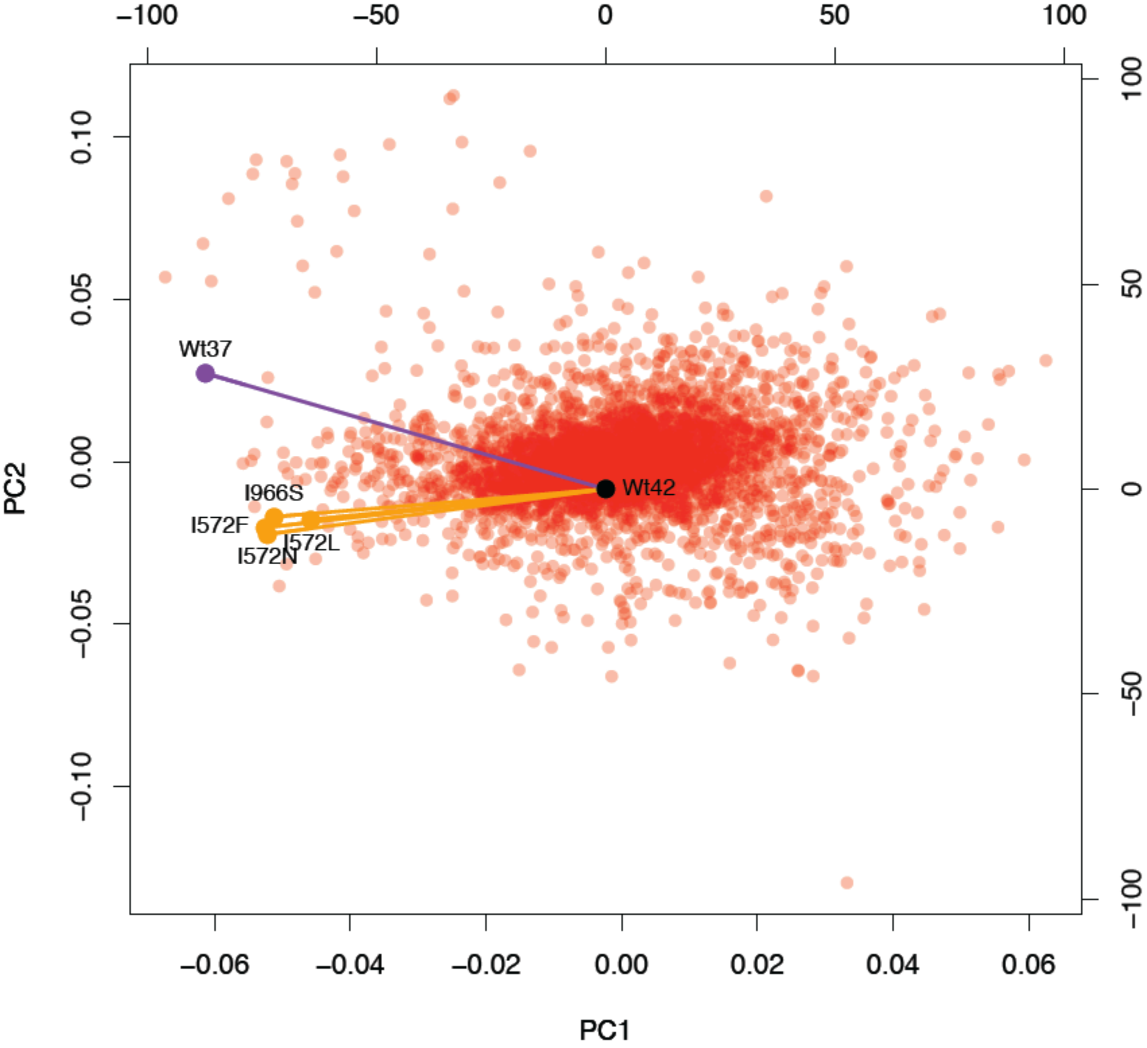
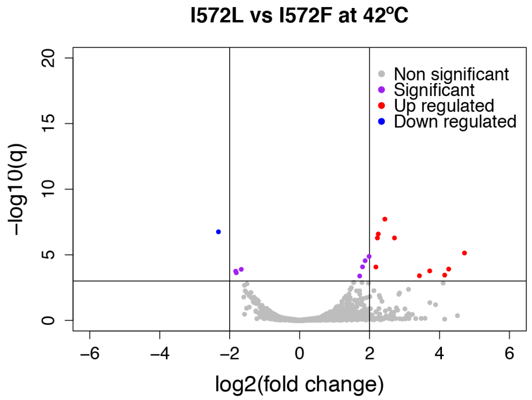
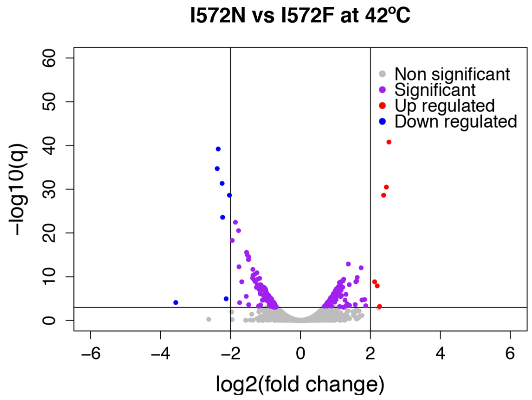
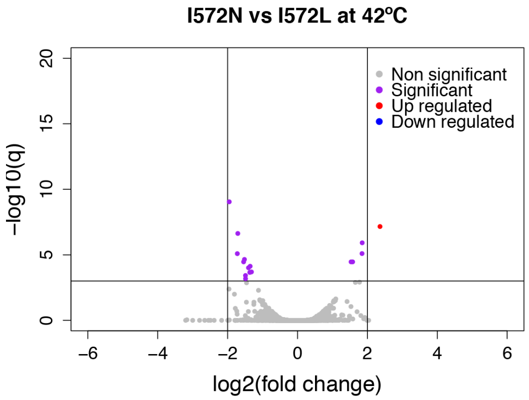
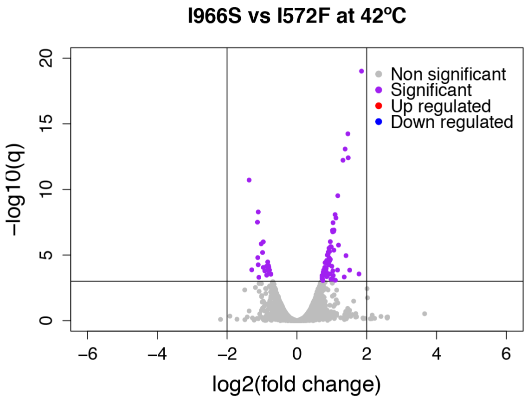


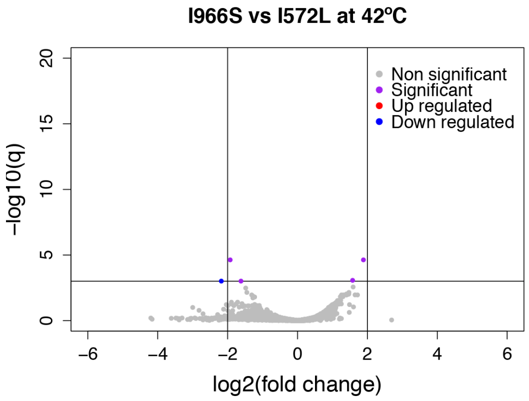
Fig S2. Principal component analysis. For the PCA analysis the $\log_2$fold change were computed for each mutant and condition against the ancestral expression at 42ºC and calculated using the *prcomp* function in R version 3.0.2 (Team 2013). Red dots represent genes, and the lines represent the overall direction and magnitude of differences from the ancestor or at 42ºC (wt42). The ancestor at 37ºC is wt37; the gold lines represent clones with single mutations.

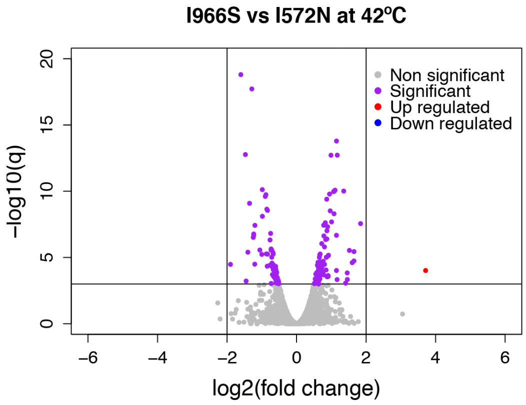

I572L vs I572F at 42°C


I572N vs I572F at 42°C


I572N vs I572L at 42°C


I966S vs I572F at 42°C


I966S vs I572L at 42°C


I966S vs I572N at 42°C

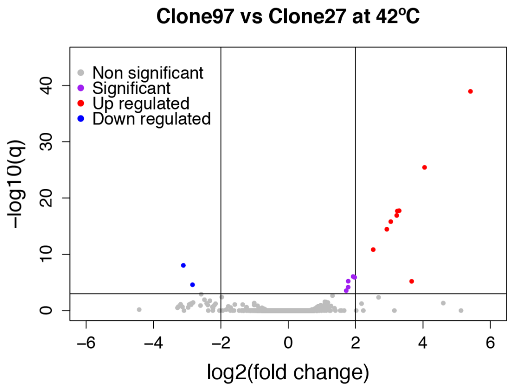

Clone97 vs Clone27 at 42°C

Fig S3. GE differences between mutants and clones. Volcano plot showing the global differential expression of genes (represented as dots) pairwise comparisons between mutants and between high-temperature adapted clones. Colors represent status with respect to 2-fold expression difference, represented by two vertical lines, and a significance at *q* = 0.001, represented by an horizontal line.

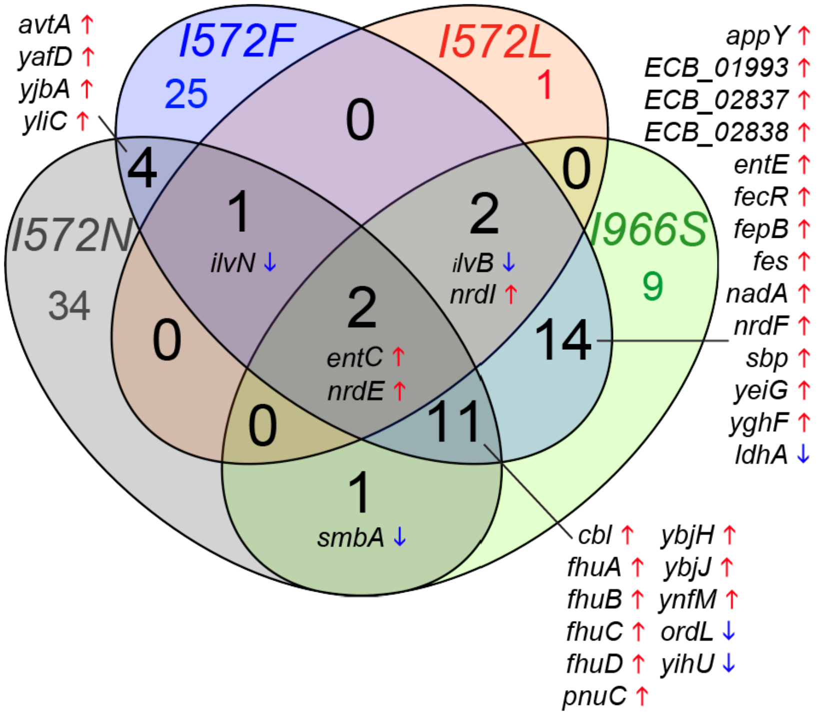


Fig S4. Convergence of genes with novel expression in single mutants. Number of shared genes with novel expression in the four mutants.

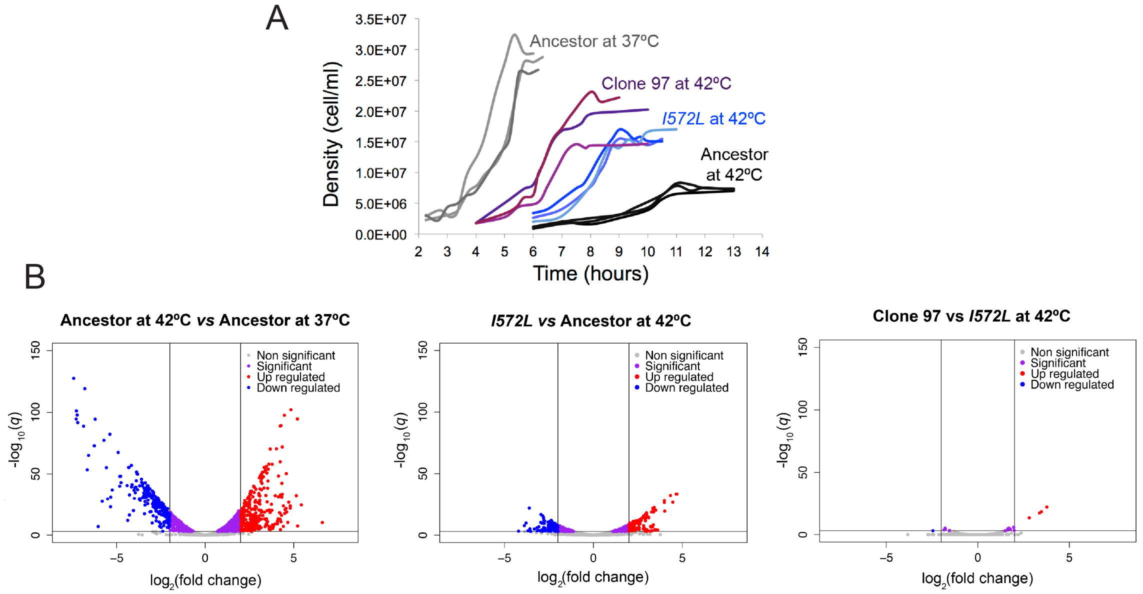


Fig S5. Phenotypic changes during thermal stress adaptation (clone 97). (A) Growth improvements during the heat stress adaptive walk. (B) Changes in GE during the acclimation and adaptive response.

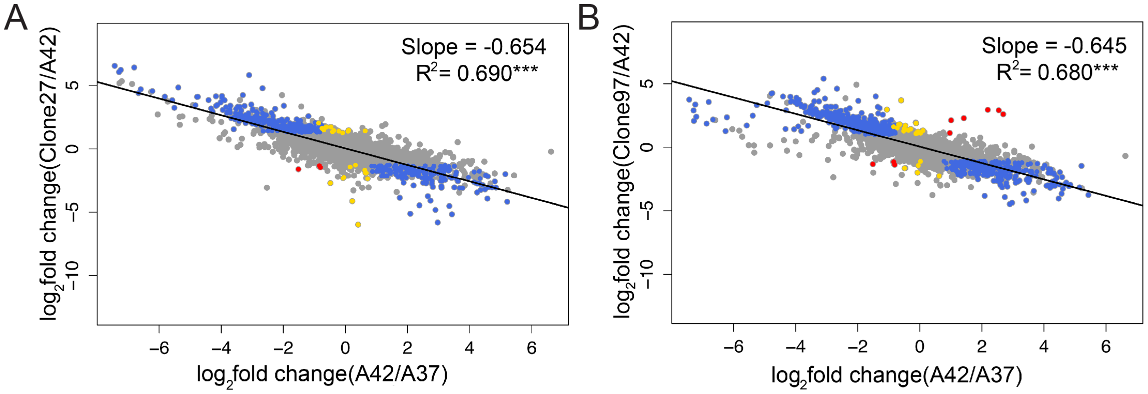


Fig S6. Global changes in GE during the acclimation and adaptive response. In all the graphs the *x*-axis represents the acclimation changes (ancestor grown at 42ºC *vs* ancestor grown at 37ºC). The *y*-axis represents the changes during thermal adaptation: clone 27 *vs* ancestor grown at 42ºC (A) and clone 97 *vs* ancestor grown at 42ºC (B). We used the same color than in figure 3.

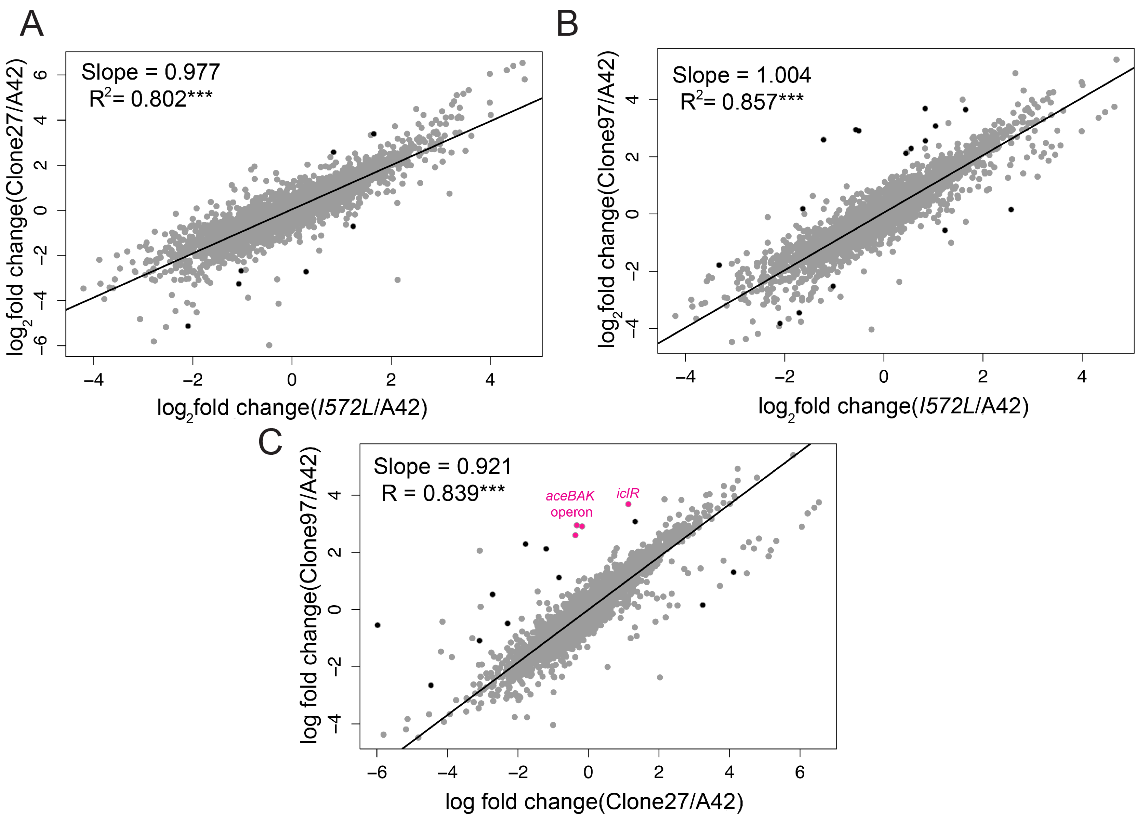


Fig S7. Similarities in global changes in GE between the mutation *I572L* and two high-temperature adapted clones. The black dots in (A) and (B) correspond to the differentially expressed genes between the clone 27 or 97 *vs* the mutation *I572L*. (C) Similarities in global changes in GE between the two high-temperature adapted clones. The pink dots correspond to the differentially expressed genes (clone 27 *vs* clone 97) possibly explained by the *iclR* mutation in clone 97 (supplementary table S5). Finally, the black dots correspond to the rest of the genes differentially expressed between clone 27 and clone 97.