Transient intracellular acidification regulates the core transcriptional heat shock response

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Supplemental figures

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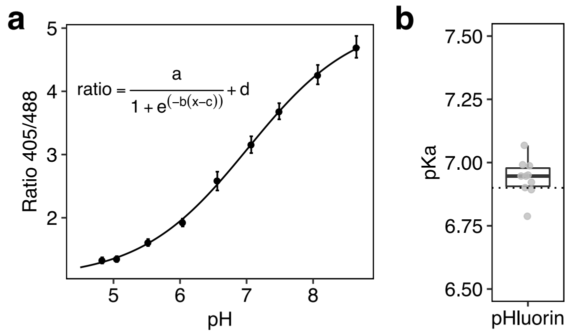

Figure S1. Figure Supplement: pHluorin calibration. a) A representative pHluorin calibration curve showing the relationship between intracellular pH and fluorescence ratio. Error bars are the standard deviation of the population of cells measured. **b)** Comparison between calibration curves taken on different days. The curves are compared by solving for the apparent pK_a of pHluorin (see Methods section for equation); while absolute values of the ratio vary by day and instrument, the pK_a should be constant. The *in vitro* pK_a as calculated in Bagar 2009[82] is shown with the dashed line.

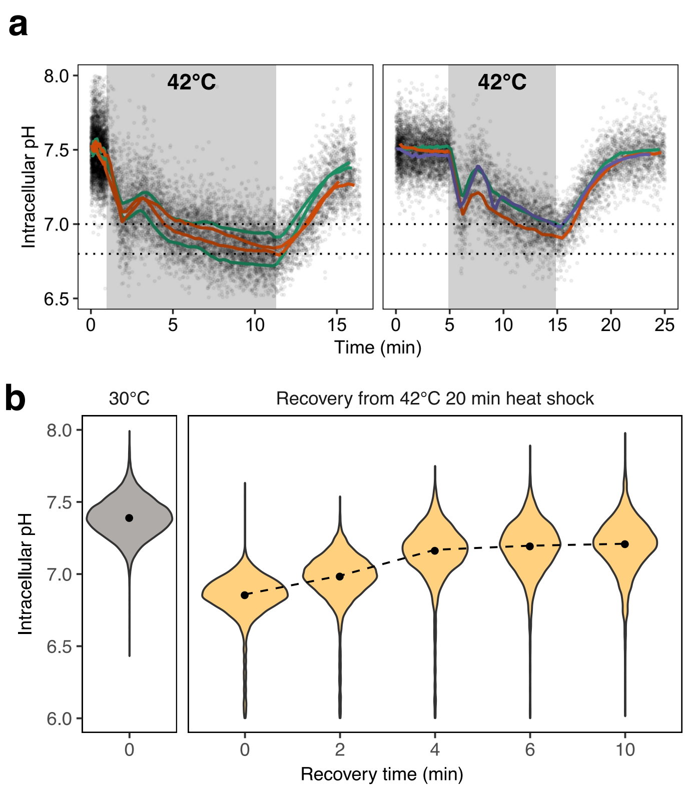

Figure S2. Figure Supplement: Yeast cells respond to stress with intracellular pH changes and production of heat shock proteins which can be tracked at the single-cell level. a) Replicates of dynamic pH change measurements. **b)** Intracellular pH recovery timecourse for cells stressed at 42°C for 20 minutes

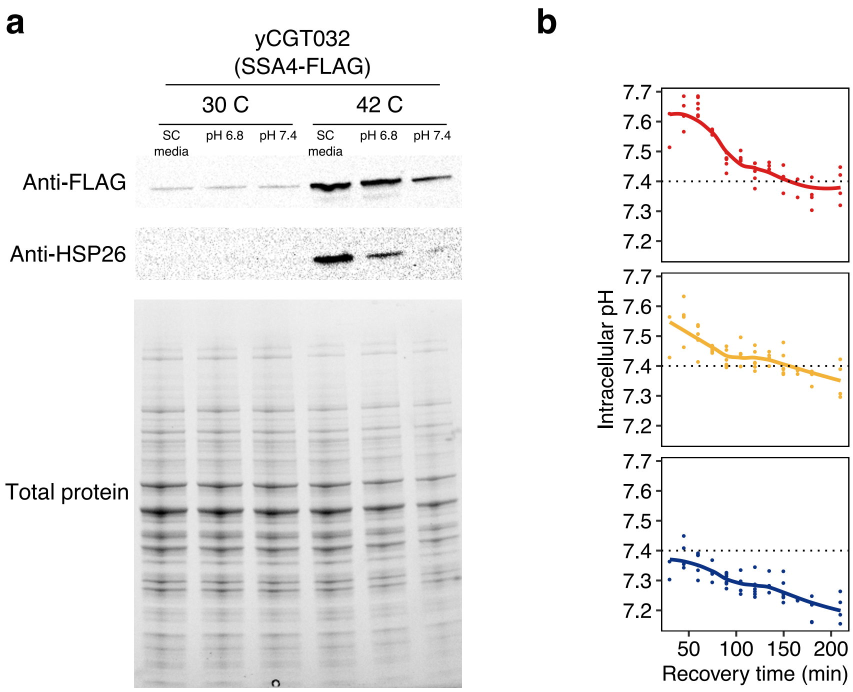

Figure S3. Figure Supplement: Acidic intracellular pH during stress is necessary for rapid production of a heat shock protein. a) Western blot and total protein gels for two strains of yeast heat stressed with and without ionophore treatment, as described in Growth Conditions above. Samples were taken 1 hour after stress. **b)** The intracellular pH during recovery in SC media buffered to pH 7.4 following pH-manipulated stress. Colors refer to the pH during stress; red: 5.0, yellow: 6.8, blue: 7.5

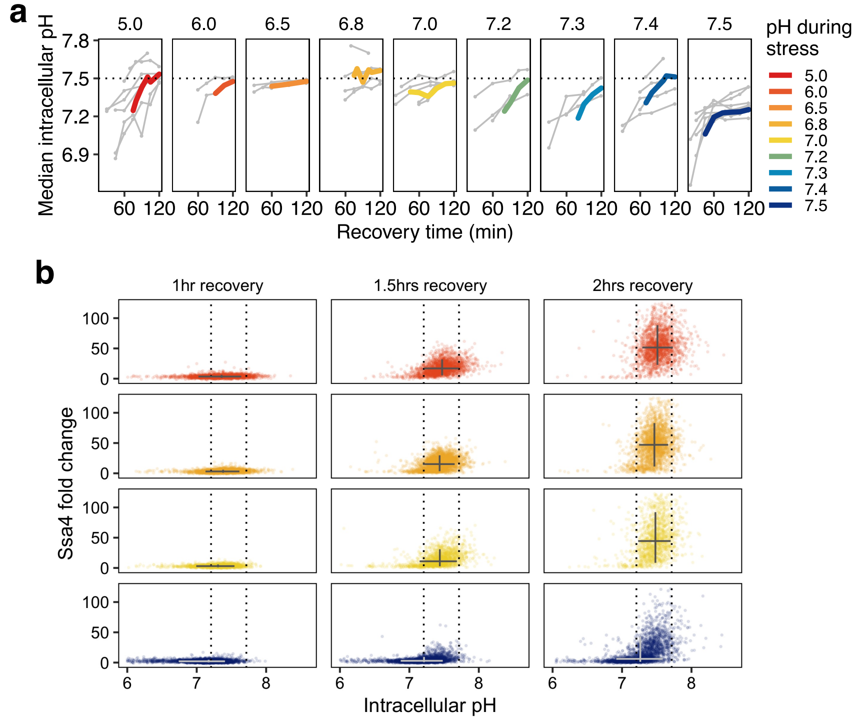

Figure S4. Figure Supplement: Preventing acidification during stress causes acidification and pH misregulation during recovery; this misregulation is correlated with low Ssa4 expression. a) Intracellular pH recovery after stress at different intracellular pHs. **b)** Recovery of intracellular pH is correlated with high Ssa4 levels on the single-cell level. Cells that are stressed at the resting pH have a large proportion of cells that do not recover intracellular pH and do not produce high levels of Ssa4.

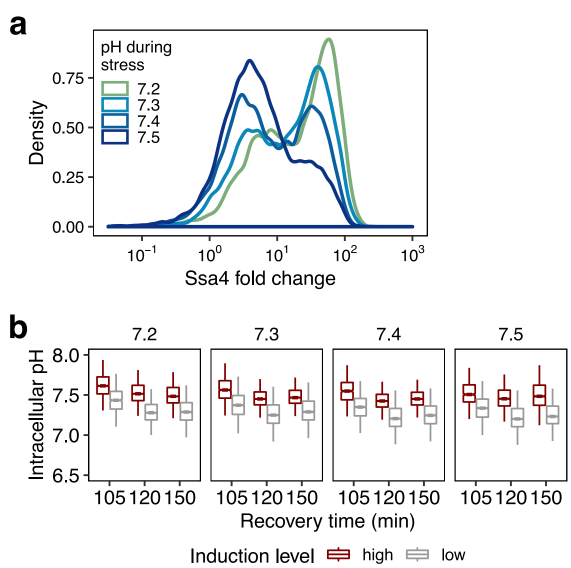

Figure S5. Figure Supplement: Preventing acidification during stress causes acidification and pH misregulation during recovery; this misregulation is correlated with low Ssa4 expression. a) Bimodal distribution of Ssa4 fold-change in cells stressed close to the resting pH. **b)** Intracellular pH distributions for both high-expressing (red) and low-expressing (gray) cells for all conditions shown in c.

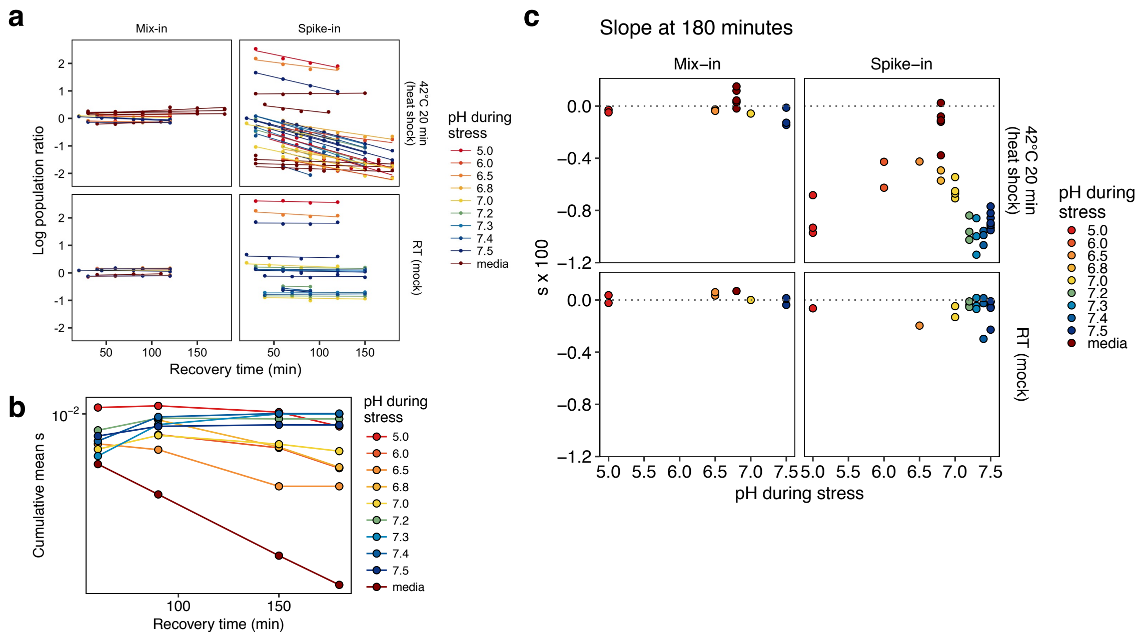

Figure S6. Figure Supplement: Populations that experience the native pH during stress have the smallest fitness deficit during recovery a) All fitness data with fits. The slopes of these lines are shown in Figure 4b **b)** Evolution of the growth rate difference as a function of time: cumulative mean for each timepoint shown for all stress pH values. The wild-type behavior is exemplified in the dark red curve ('media') where the mean fitness loss decreases as a function of time as cells resume growing at the unstressed rate during recovery. **c)** Relative growth rates (derived from slopes in a) plotted as a function of pH for both heat shock and mock treatments, and for both fitness measurement schemes.

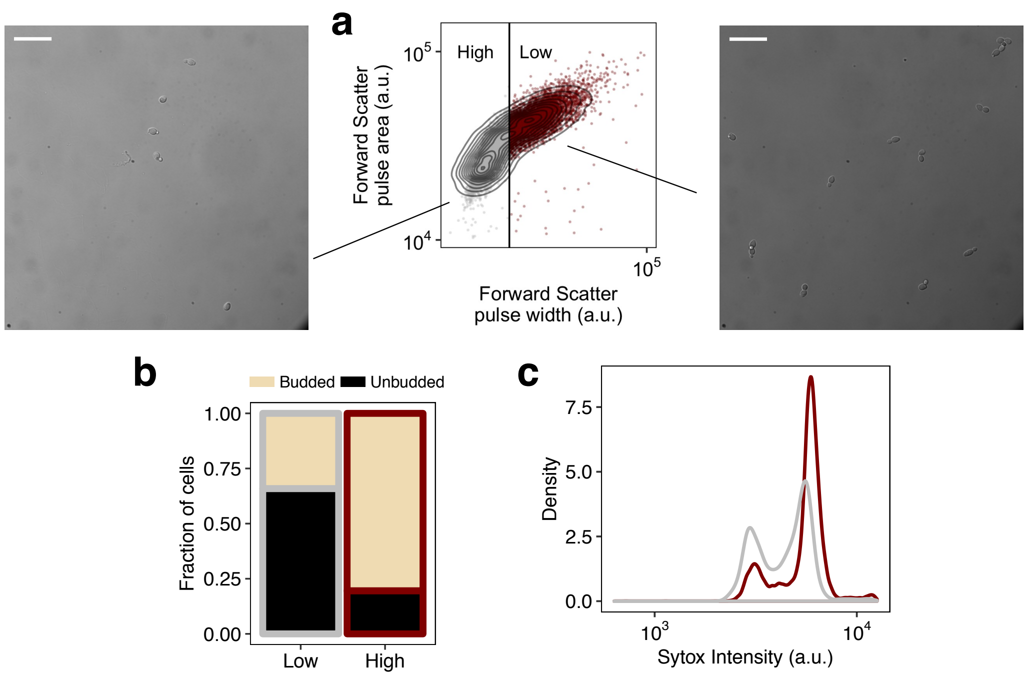

Figure S7. Figure Supplement: Fitness, intracellular pH, and heat shock protein production during recovery is correlated in single cells a) Cells were partitioned into two categories in the forward scatter width channel and were sorted based on this partioning. Sorted cells were fixed and visualized by microscopy. Representative images for each population are shown. Scale bar is 25 μm. **b)** Quantification of microscopy data; N = 217 cells scored. **c)** Fixed cells were stained with Sytox and analyzed by flow cytometry to assess DNA content. The relative heights of the lower and higher peak reflect the proportion of cells in each population that have doubled their DNA, and are thus actively growing.

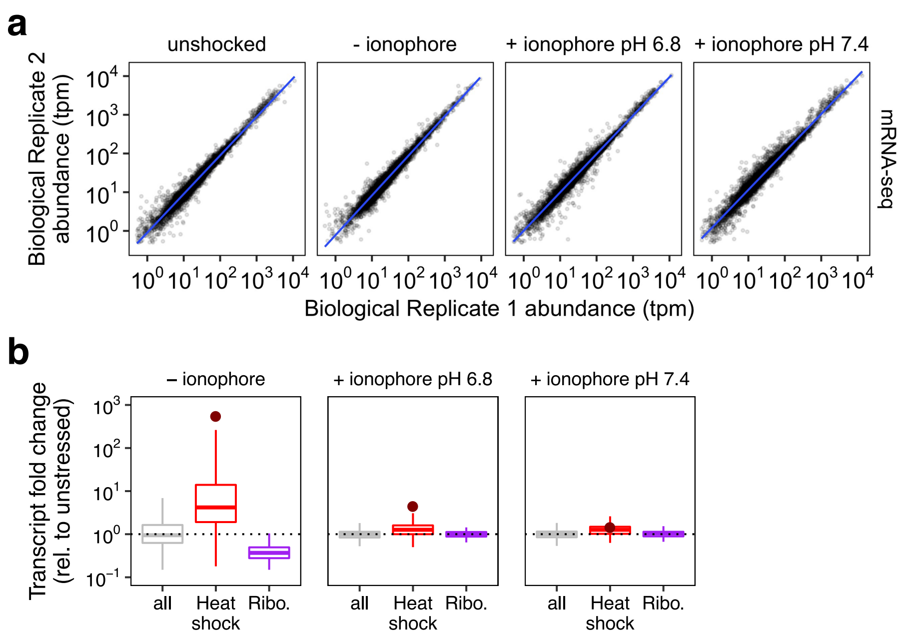

Figure S8. Figure Supplement: Failure to acidify during stress specifically represses Hsf1-activated genes. a) Correlation between mRNA-Seq biological replicates. **b)** Induction (fold change in stressed cells relative to unstressed cells) for different pHs during stress and different categories of genes.