Breker et al., http://www.jcb.org/cgi/content/full/jcb.201301120/DC1

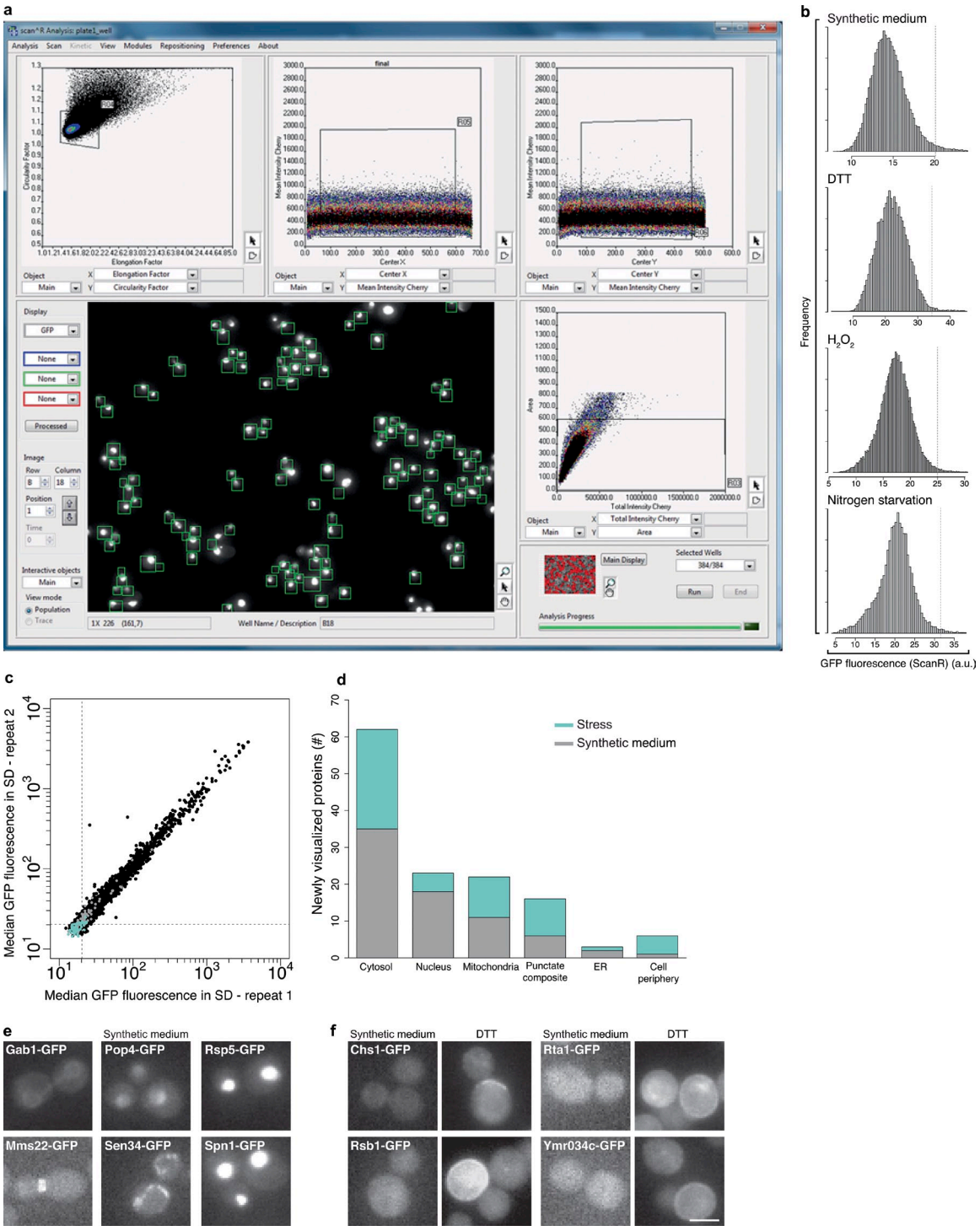


Figure S1. **Single-cell proteomics of stress responses.** (a) Using mCherry to segment images into single-cell objects allows gating on homogenous populations. Each strain in the manipulated library harbors one ORF fused C-terminally to a GFP tag and a constitutively expressed cytosolic mCherry. By choosing a standard gate according to shape, size, and intensity parameters, it is possible to reach an analysis of a homogenous population of only live cells. (b) Threshold of detection above autofluorescence background can be calculated based on quantitative analysis of a strain with no GFP expression. Thousands of measurements of WT cells (with no GFP cassette) in SD and exposed to DTT, H_2O_2, or nitrogen starvation are normally distributed (Shapiro-Wilk test, $P < 0.05$). The threshold of detection that is >99% likely to represent biological GFP fluorescence was set as the $Mean_{condition} + 2.58\sigma_{condition}$. Strains below this cutoff are marked as "below threshold" in the localization category. This experiment was performed once on at least 12,000 cells per sample. (c–f) Integration of localization and abundance analysis allows the detection of 120 proteins that have not been previously visualized. (c) A scatter plot ($r^2 = 0.97$) of median fluorescence values measured for each of 5,330 strains of the yeast GFP library in two independent experiments in SD. a.u., arbitrary units. Gray and cyan dots represent newly visualized strains under SD and under the three stresses, respectively. (d) Newly visualized strains categorized according to their subcellular localization. (e) Representative examples of six newly assigned essential proteins visualized under SD. (f) Examples of four cell periphery proteins expressed specifically under one of the stress conditions, DTT. Bars, 5 µm.

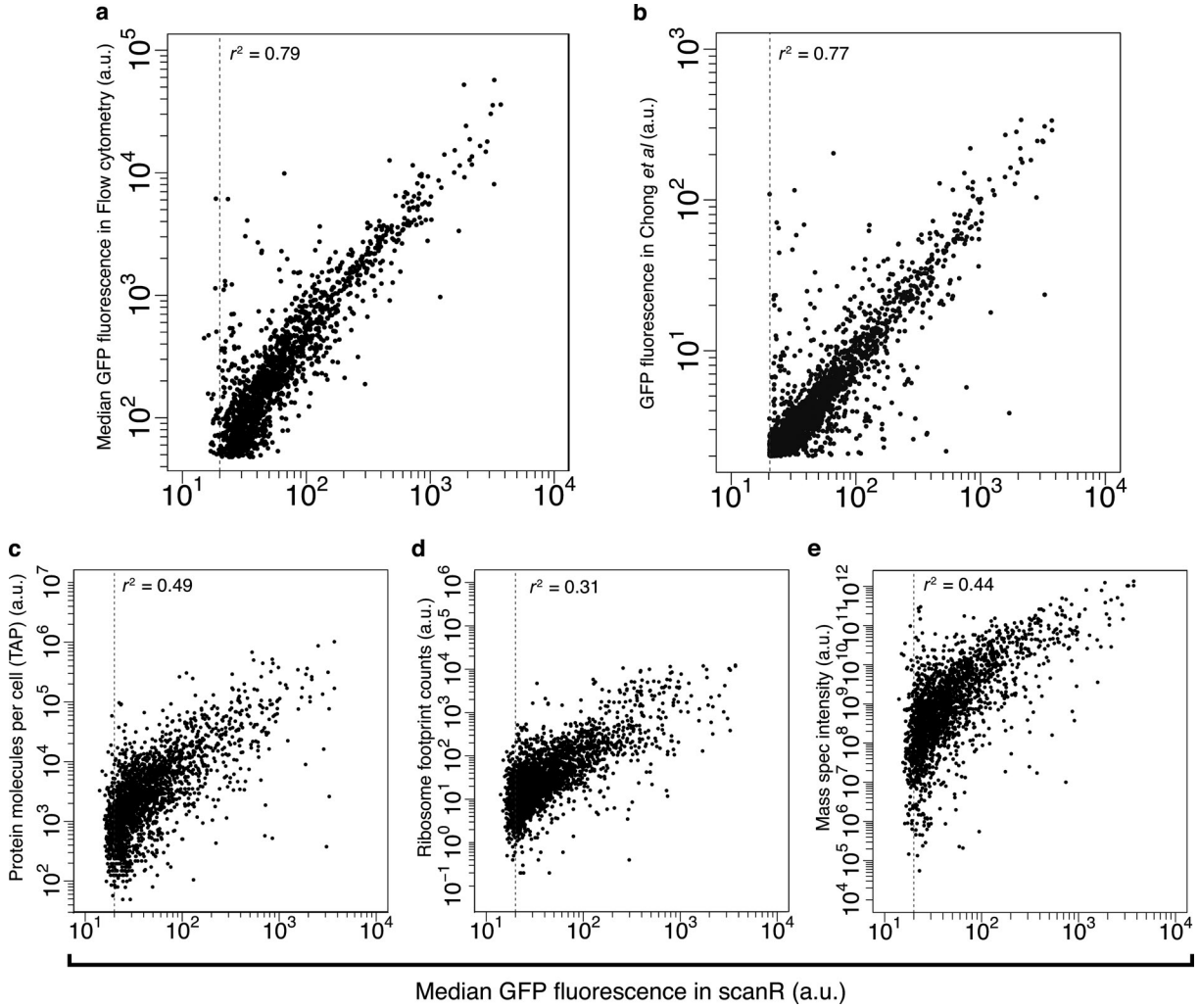


Figure S2. **Comparison of our intensity measurements (scanR) to published datasets validates high-throughput microscopy for proteomic measurements.** (a) A comparative scatter plot of median fluorescence values measured in this study versus strains of the same yeast GFP library previously measured by flow cytometry (Newman et al., 2006). (b) Comparison to an independent microscopy measurement of the yeast GFP library (Andrews, B., personal communication). (c) Comparison to protein intensity measurements performed by Western blotting on the TAP-tagged protein collection (Ghaemmaghami et al., 2003). (d) Comparison to protein levels inferred from ribosome footprint counts (Ingolia et al., 2009). (e) Comparison to protein levels inferred from whole-proteome mass spectrometry analysis (Walther et al., 2010).

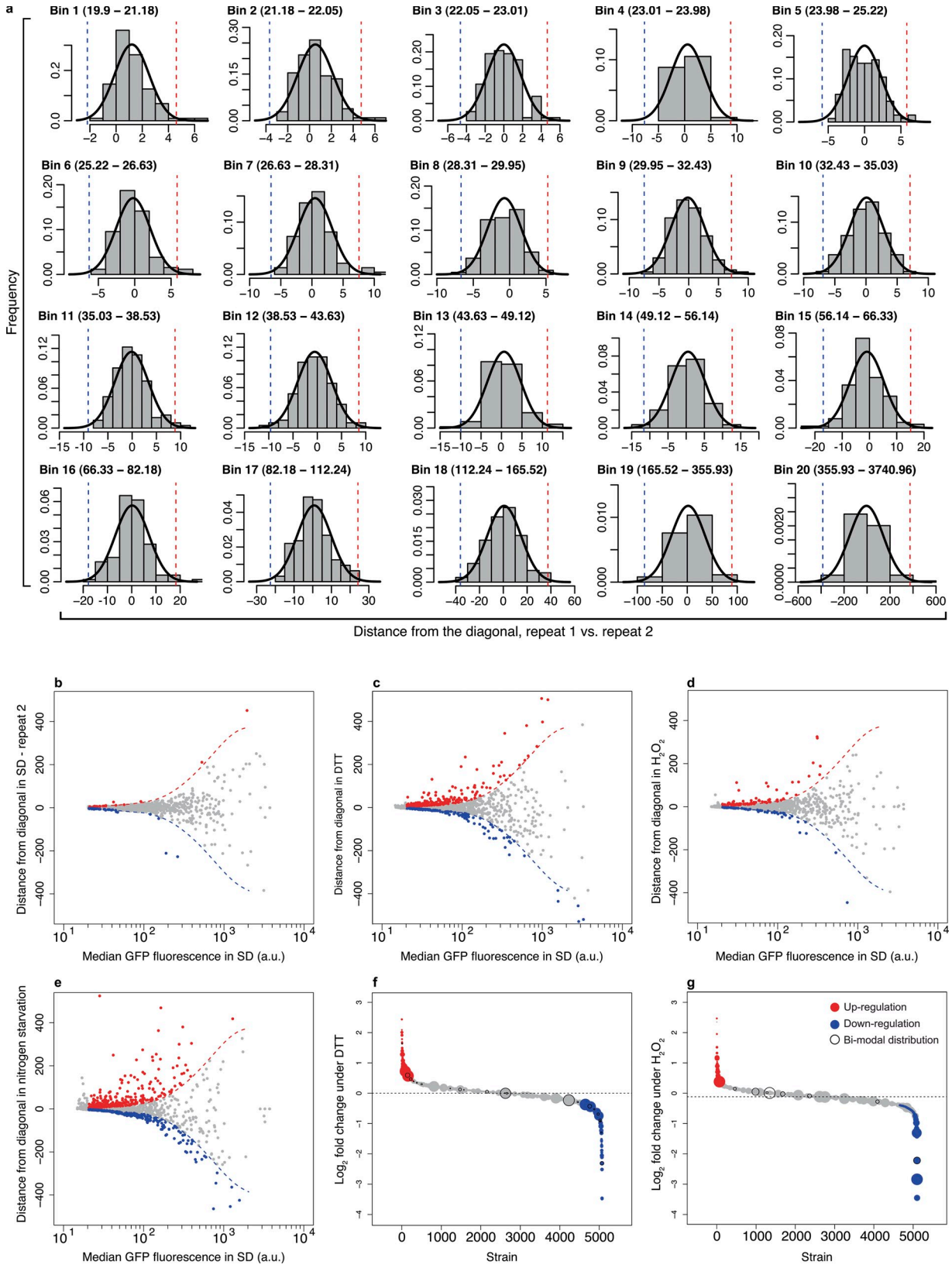


Figure S3. **Detection of significant abundance change thresholds and events for each stress.** (a) Intensity detection range was binned by abundance (5% of the strains in each bin), and distance from diagonal in the scatter plot of replicate 1 versus replicate 2 under SD was measured in each of the bins and plotted separately. A Shapiro-Wilk test for each of the bins did not reject normality ($P < 0.05$), and empirical thresholds for significance of distance from the diagonal were set ($P < 0.01$) from each side. The top of each plot gives the abundance values in that bin. The red and blue broken lines show the threshold distances for up-regulation and down-regulation, respectively. Histograms give the distribution of distances from the diagonal in each bin, and black smoothed lines give the best-fit normal approximation. (b) Volcano plot representing the up-regulated and down-regulated proteins in an independent replicate grown under the same conditions as the control (SD). Broken lines demonstrate thresholds of significance for each of the bins (for calculation see Fig. S6). (c) As in b, but for measurements performed on the library during growth in DTT. (d) As for b, but for measurements performed in H_2O_2. (e) As in b, but for measurements performed on the library during growth in nitrogen starvation medium. (f) All strains sorted by $\log_2$ ratio of abundance changes in DTT relative to SD. Significant abundance change events are marked, red for up-regulation and blue for down-regulation, and black circles mark bi-modally distributed strains, tested by a Hartigan's dip test for unimodality ($P < 0.05$). (g) As in f but for measurements during growth in H_2O_2. For measurements during starvation, see Fig. 1. Exact names and fold changes for each protein can be found in Tables S3–S5.

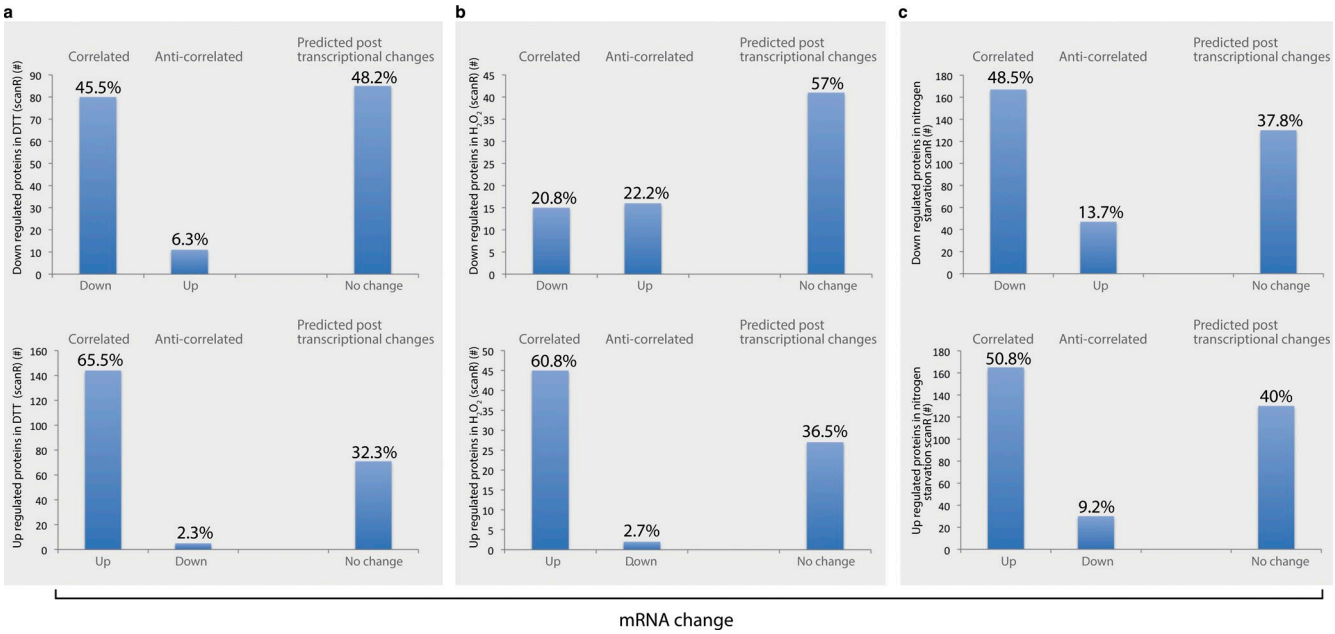


Figure S4. **Detection of potential posttranscriptional regulation events by comparison to published microarray datasets.** Down-regulated proteins (top) and up-regulated proteins (bottom) as measured by our system were compared with published mRNA data. mRNA was assigned as "Up" or "Down" if at any of the time points measured for this stress it had changed significantly more than twofold, and as "No change" if there was no significant change in any of the time points. (a) Our abundance measurements in DTT (180 min) were compared with Travers et al. (2000) in all four time points measured (15, 30, 60, and 120 min). (b) Our abundance measurements in H_2O_2 (60 min) were compared with Gasch et al. (2000) in all 10 time points measured (10, 20, 30, 40, 50, 60, 80, 100, 120, 140, and 160 min). (c) Our abundance measurements during nitrogen starvation (24 h) were compared with Gasch et al. (2000) in all seven time points measured (0.5, 1, 2, 4, 8, 12, and 24 h).

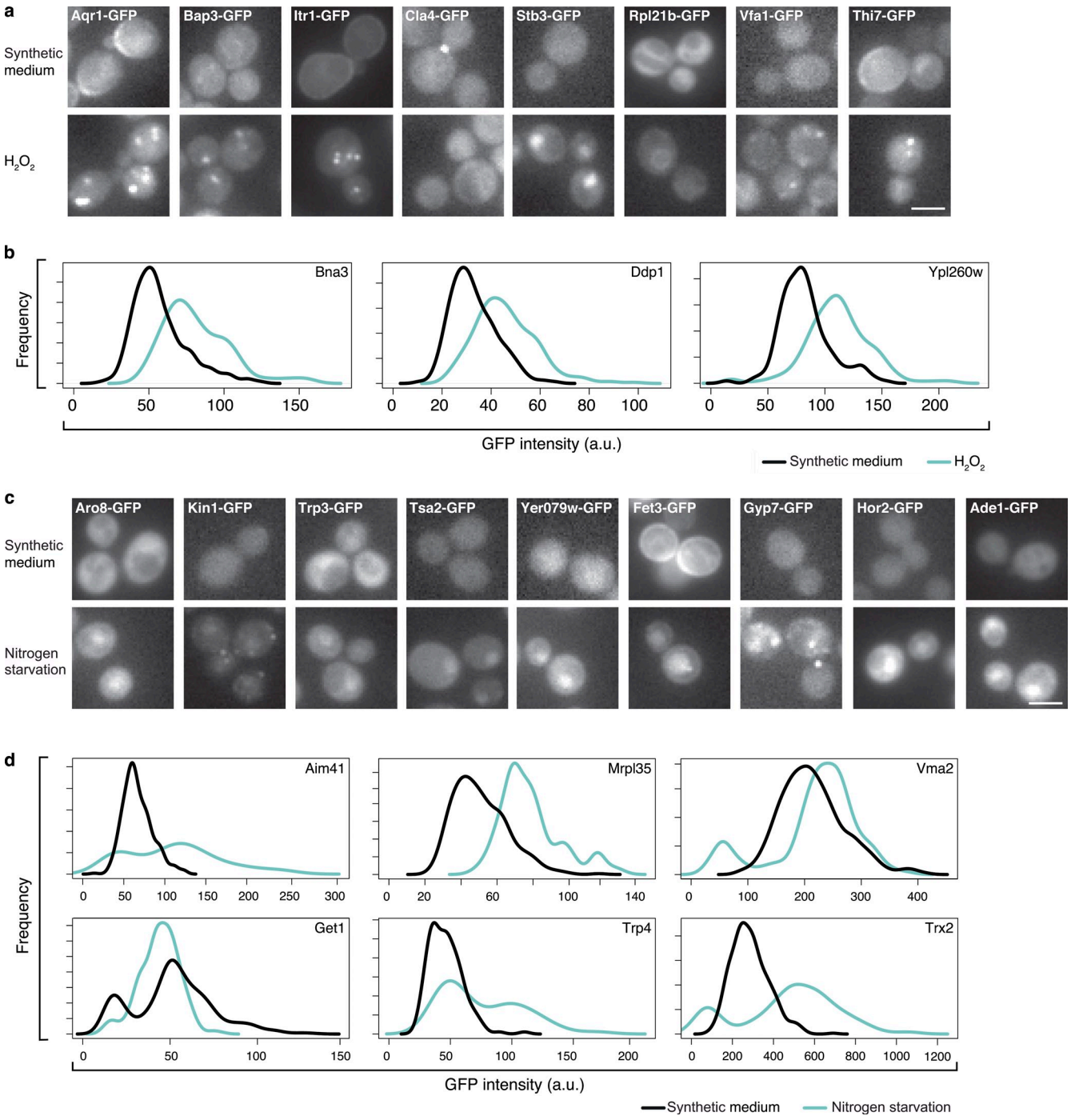
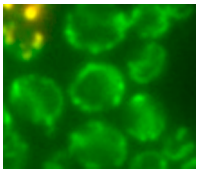
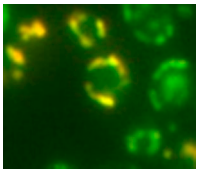


Figure S5. **Proteins change abundance or localization during growth in H_2O_2 or nitrogen starvation and are essential during this growth condition.** (a) Eight representative examples (of 20) of proteins that changed localization under H_2O_2 and were essential uniquely under H_2O_2 but not in SD (Hillenmeyer et al., 2008). (b) Three examples (out of 13) of abundance changes in proteins essential under H_2O_2 (Hillenmeyer et al., 2008). (c) Nine representative examples (of 27) of proteins that changed localization under nitrogen starvation and are essential uniquely under this condition (Hillenmeyer et al., 2008) but not in synthetic medium. (d) Six representative examples (out of 44) for abundance changes for essential proteins under starvation (Hillenmeyer et al., 2008).



Video 1. **A cell expressing Aim37-GFP that changes its localization from mitochondria to vacuole membrane when grown in H_2O_2.** During 5 h of time-lapse microscopy, Aim37-GFP (green) and MTS-dsRed (red) were tracked every 10 min under H_2O_2 exposure. The video shows 3 frames per second. Aim37-GFP is seen in the vacuole membrane accompanied by accumulation of MTS-dsRed in the vacuole lumen. This phenomenon occurs for ∼30% of the population.



Video 2. **A cell expressing Aim37-GFP that does not change its localization when grown in H_2O_2.** During 5 h of time-lapse microscopy, Aim37-GFP (green) and MTS-dsRed (red) were tracked every 10 min under H_2O_2 exposure. The video shows 3 frames per second. Aim37-GFP remains in mitochondria as does the MTS-dsRed. This phenomenon occurs for ∼70% of the population.

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Tables S1–S13 are available as Excel files.