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Supplemental Information

Molecular Time Sharing through Dynamic

Pulsing in Single Cells

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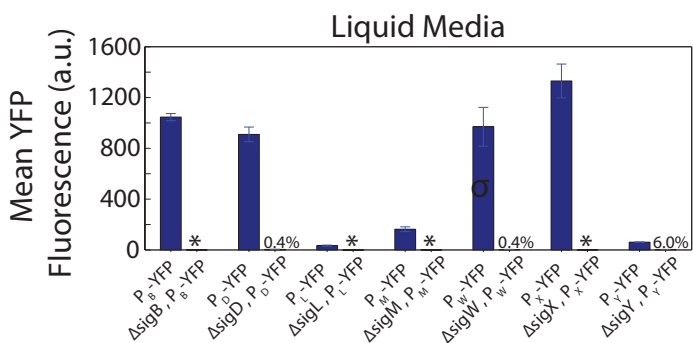
Table S1

Sigma Factor	Function	Group
σ^A	House Keeping	1
σ^H	Sporulation Initiation	3
σ^D	Chemotaxis	3
σ^B	General Stress Response	3
σ^E	Sporulation	3
σ^G	Sporulation	3
σ^F	Sporulation	3
σ^K	Sporulation	3
σ^I	Unknown	3
σ^M	Regulator of early stationary-phase genes	4
σ^W	Antibiotic resistance	4
σ^V	Cell wall protection	4
σ^X	Cell wall metabolism	4
YlaC	Oxidative stress response	4
σ^Y	Unknown	4
σ^Z	Unknown	4
σ^O	Unknown	4
σ^L	Amino acid catabolism	σ^{54} family

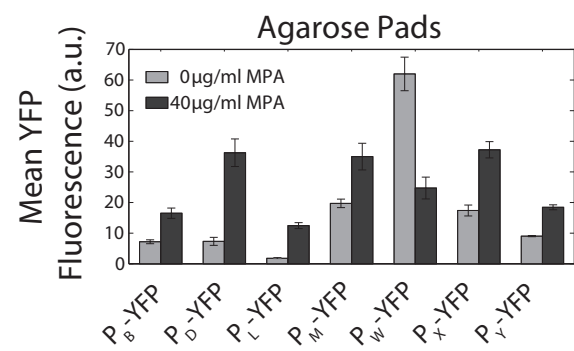
Table S1, related to Figure 1. Summary of *Bacillus subtilis* sigma factors. For each sigma factor, the process or function it has been shown to be involved in, if any, is listed. We also indicate the sigma factor structural group (Paget, M.S. et al, 2015).

Figure S1

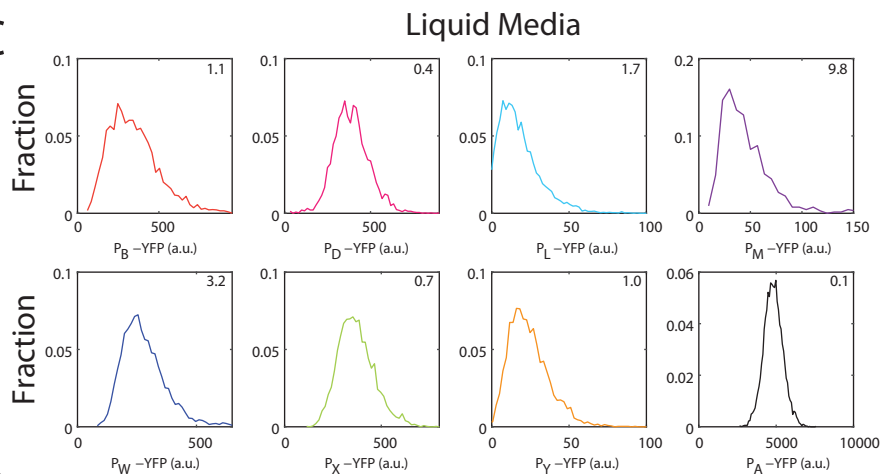
A



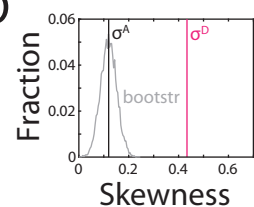
B



C



D



E

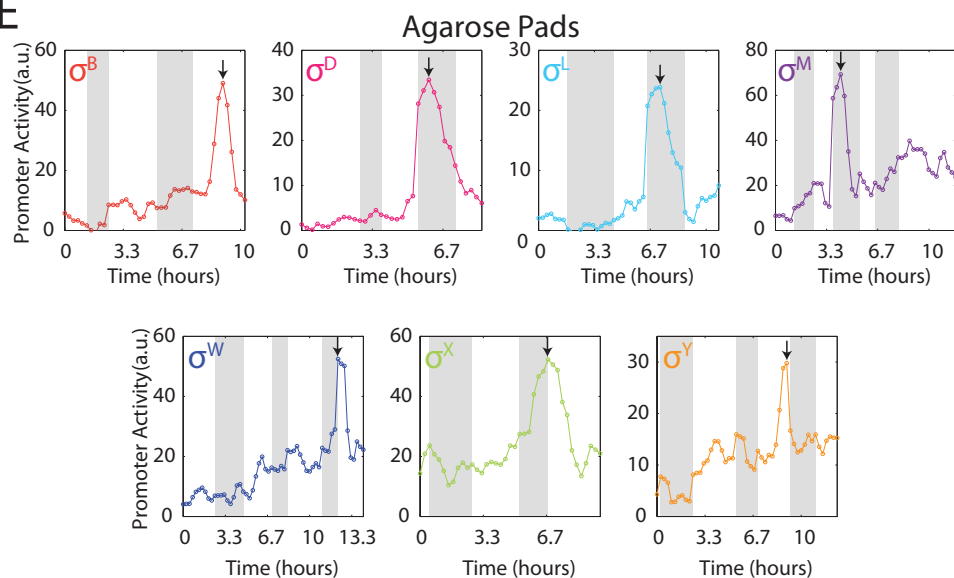


Figure S1, related to Figure 1. Behavior of Sigma Factor reporters in Liquid Culture and on Agarose Pads. (A) Sigma factor reporters are specific for their cognate sigma factor. YFP reporters for each sigma factor were analyzed in liquid media in the presence of 40 $\mu\text{g/ml}$ MPA, either in a wild-type background or a strain in which the cognate sigma factor was deleted. Bars represent the mean fluorescence of at least 300 cells, averaged over 2 independent experiments. Error bars indicate s.e.m. The percentages listed above some bars indicate the mean fluorescence of the knockout strain relative to that of the wildtype strain. * indicates fluorescence measurements indistinguishable from background.

(B) MPA activates multiple sigma factors. Sigma factor reporters were grown from single cells into small microcolonies on agarose pads of ~ 100 cells, with or without 40 $\mu\text{g/ml}$ MPA. Each bar represents the mean fluorescence of the microcolony at its final size, averaged over at least 4 separate microcolonies from 2 independent experiments. Error bars indicate s.e.m.

(C) Sigma factors activate heterogeneously in response to MPA. For each distribution, the indicated sigma factor reporter strain was grown for 3 hours in liquid culture containing 40 $\mu\text{g/ml}$ MPA. Fluorescence intensities of individual cells were analyzed by quantitative microscopy. At least 3500 cells were measured per distribution. Listed in the upper right corner of each plot is the distribution's skewness, defined as the normalized third central moment.

(D) To analyze the statistical significance of the skewness, the σ^A data from (C) were bootstrapped with replacement (50,000 trials). The distribution of bootstrap skewness values is shown in gray. The measured σ^A and σ^D skewness values are shown by the black and pink vertical lines, respectively. The skewness for σ^D is inherently higher than that of σ^A . Note that σ^D had the smallest skewness of all alternative sigmas.

(E) Automatic detection of promoter activity pulses. Time-lapse movies of seven alternative sigma factor reporter strains grown on agarose pads in the presence of 40 $\mu\text{g/ml}$ MPA were acquired. Each time trace represents the promoter activity of a single cell lineage. Alternating white and shaded areas indicate cell cycles. Black arrows denote the peak of automatically identified pulses. Only local maxima passing defined thresholds are identified as pulses, to avoid misdetection of random fluctuations (see STAR Methods).

Figure S2

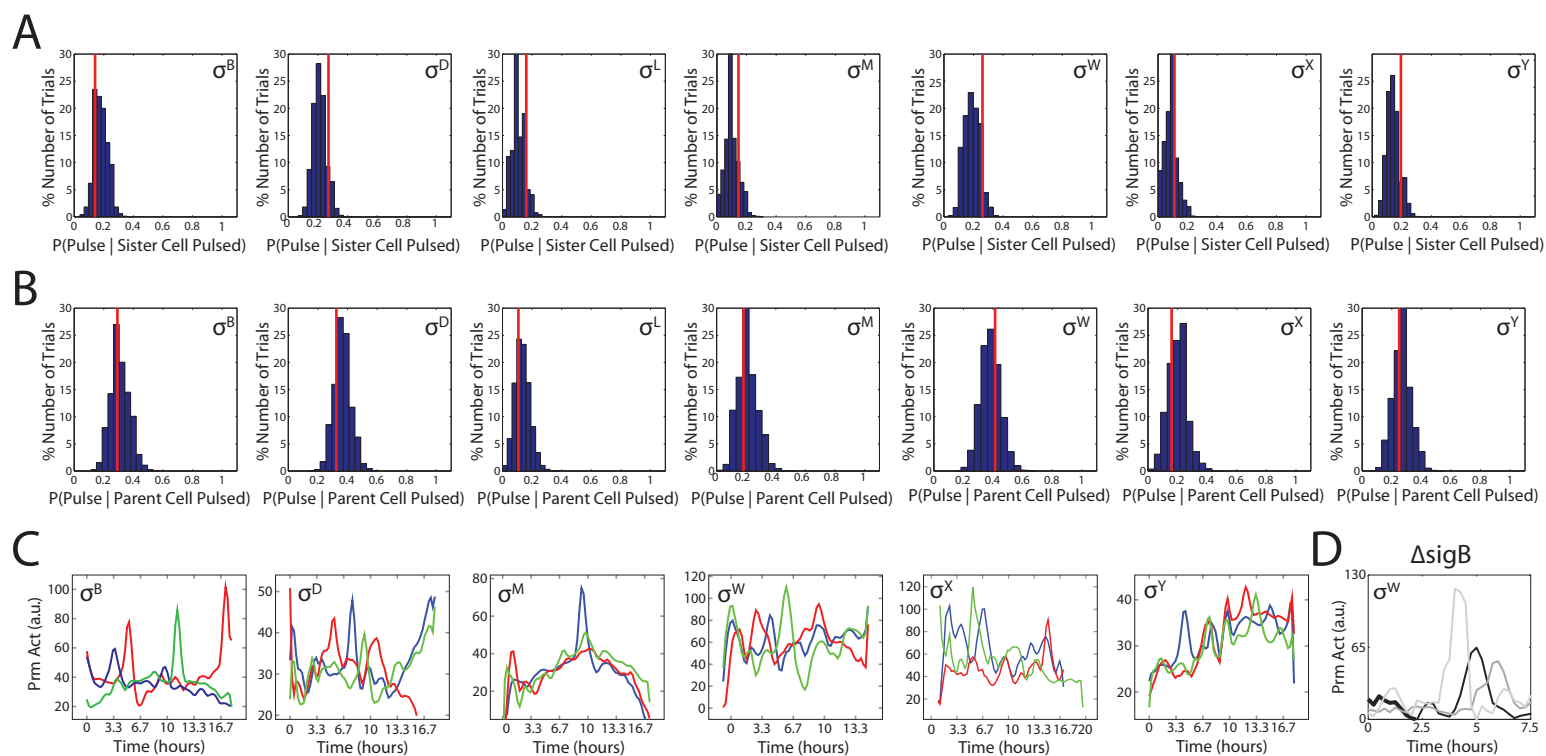


Figure S2, related to Figure 1. Sigma pulses activate stochastically, and stationary conditioned phase media activates sigma factor pulsing. (A-B) Pulse incidence between cell sisters (A) and between a parent cell and its daughter (B) is not correlated. Red lines indicate the measured frequency of two sister cells both pulsing in the same cell cycle, grown on agarose pads containing 40 $\mu\text{g/ml}$ MPA. This can be compared to the distribution of expected probabilities computed from a 'null hypothesis' in which sister or parent relationships are scrambled (blue histograms). Note that the measured values are within the distribution of values expected in the absence of a correlation. Null distributions were generated with 25,000 resamples.

(C) Multiple sigma factors pulse under stationary phase conditions. Each panel shows 3 representative traces (red, green, and blue lines) of pulsing lineages of the indicated alternative sigma factor reporter strain grown on agarose pads containing conditioned media extracted from a stationary phase culture of *Bacillus subtilis* cells grown in LB media.

(D) Alternative sigma factor pulsing does not require σ^B . 3 representative pulsing traces of a P_w -YFP reporter in a $\Delta\sigma^B$ strain grown on agarose pads containing 40 $\mu\text{g/ml}$ MPA.

Figure S3

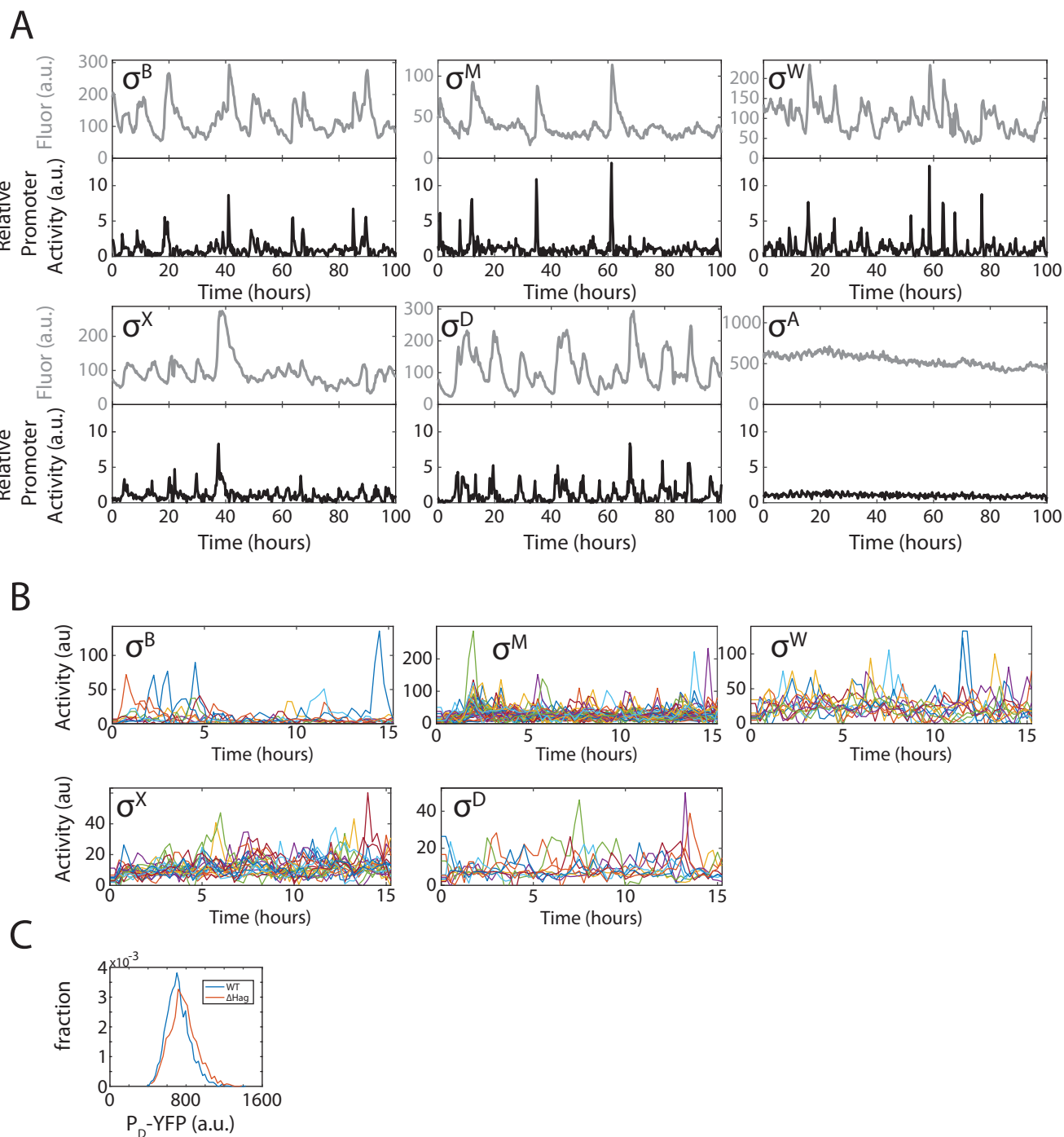


Figure S3, related to Figure 2. Sigma factor pulse behavior in the mother machine. (A) Multiple alternative sigma factors activate in pulses in the mother machine, while the housekeeping sigma factor activates constitutively. Representative timetraces are shown for five alternative sigma factors and the housekeeping sigma factor σ^A . Mean fluorescence traces are in gray, and the corresponding promoter activity traces are in black. Each trace represents the 'mother' lineage from a mother machine experiment, where cells were grown in minimal media containing 40 $\mu\text{g/ml}$ MPA. Each promoter activity trace was normalized by its own mean.

(B) Alternative sigma factors pulse without MPA. Sample traces for five alternative sigma factors strains grown in the mother machine, in minimal media that did not contain MPA. Each colored trace represents a single mother cell lineage.

(C) Deleting *hag* has minimal effect on the P_D -YFP reporter strain. The P_D -YFP reporter strain, and the P_D -YFP reporter strain carrying the *hag* deletion were grown in minimal media batch culture to OD_{600} of 0.1, and MPA was added to a final concentration of 40 $\mu\text{g/ml}$. Cells were then grown for an additional 3 hours, and the P_D -YFP reporter fluorescence was quantified by fluorescence microscopy. Plotted are the distributions of single cell fluorescence.

Figure S4

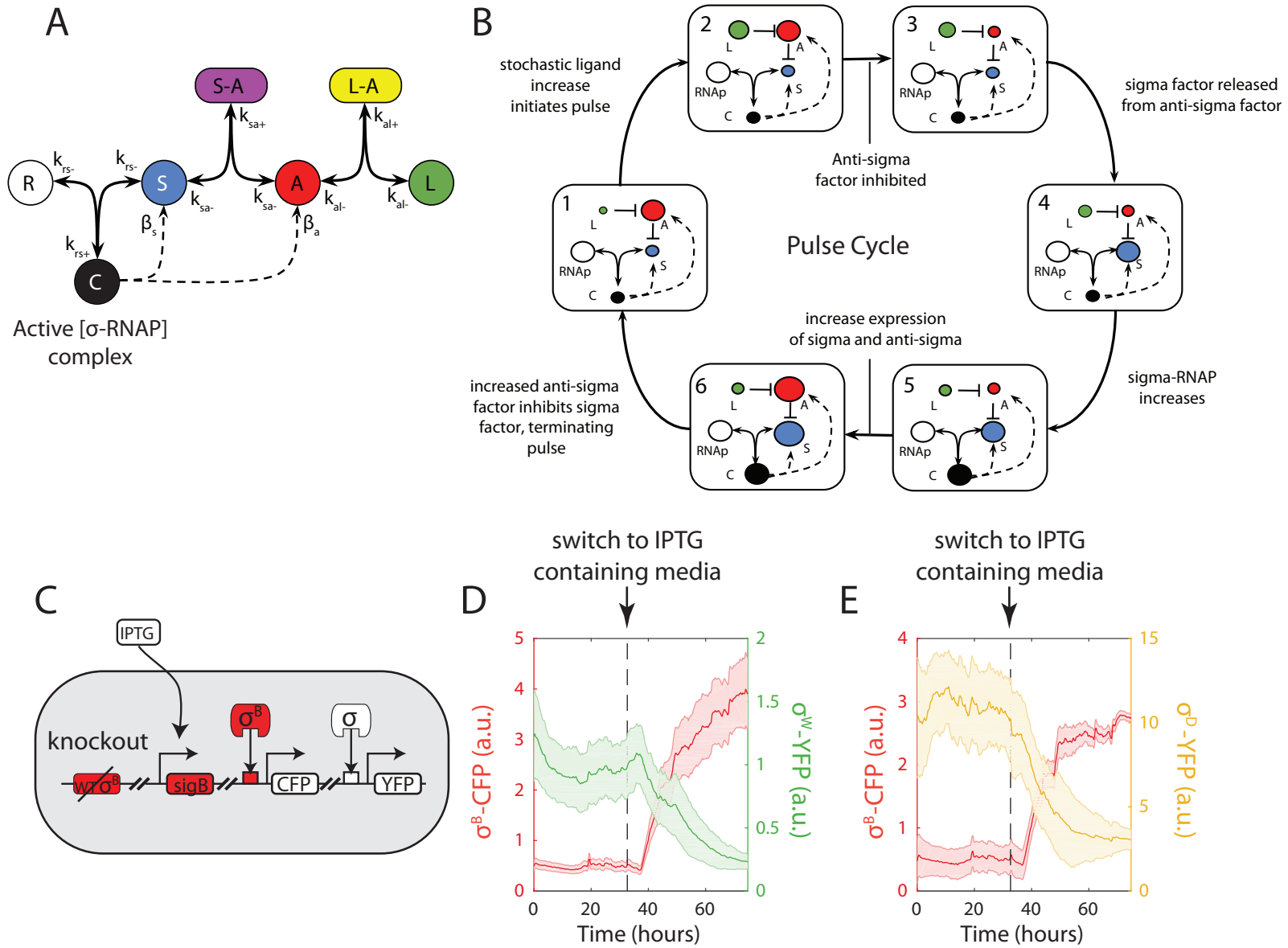


Figure S4, related to Figure 3. Pulse generation in the model and dynamic response to sigB induction.

(A) Components and interactions in the sigma factor model. Each alternative sigma factor pathway comprises the sigma factor (S), a cognate anti-sigma factor (A), and an input, represented as a regulatory ligand (L). Negative regulation occurs through sequestration of S by A in the S-A complex. Pulse activation is driven by competitive sequestration of A by L in the L-A complex. RNA polymerase (R) is shared between the σ factors, and the active σ factor-RNAP complex (C) upregulates the operon containing the σ factor and the anti- σ factor. Protein-protein interactions are represented by solid arrows, and transcriptional regulation by dashed arrows. Rate constants for each reaction are indicated.

(B) The cycle of events that occur during a pulse is indicated by consecutively numbered boxes. In each box, the size of a component indicates, schematically, its relative abundance. Cartoons are simplified compared to A.

(C) SigB induction competes with σ^W and σ^D activity. Schematic of constitutive σ^B dual reporter strain. The sigB operon was knocked out and replaced with an IPTG-inducible $P_{\text{hyperspank}}\text{-sigB}$ promoter, chromosomally integrated at the amyE locus. The strain also contained a chromosomally integrated reporter for σ^B activity, $P_B\text{-cfp}$, as well as a chromosomally integrated YFP reporter for σ^W or σ^D activity.

(D,E) These strains were grown in the mother machine with minimal media containing 40 $\mu\text{g/ml}$ MPA. At the indicated time, input syringes were switched to media that also contained 1 mM IPTG. Each trace represents the average of 50 (D) or 23 (E) traces, each one representing the mean pixel intensity of one channel. Shaded regions represent the standard deviation across all traces.

Figure S5

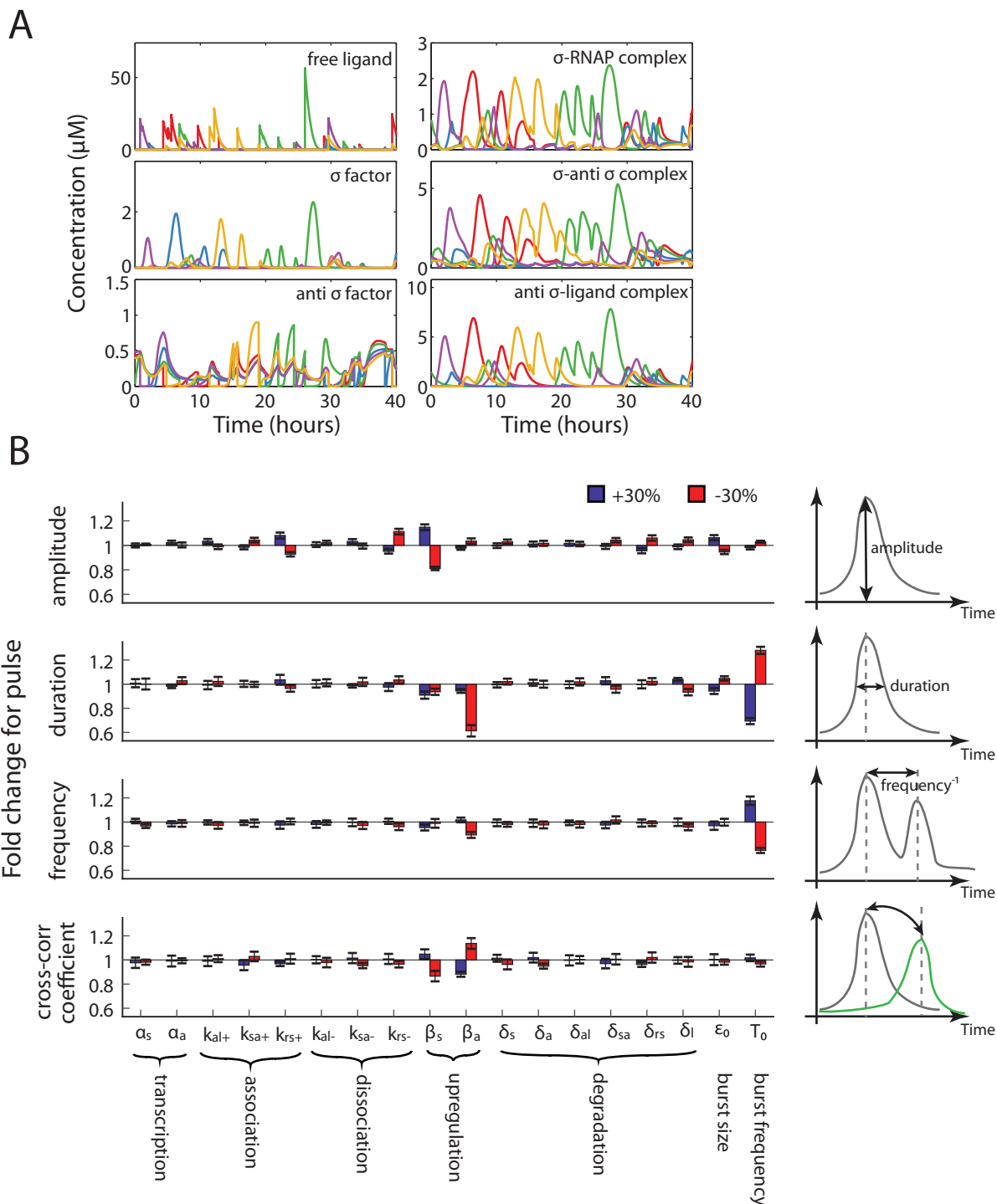


Figure S5, related to Figure 3. Simulations of five alternative sigma factor species and the housekeeping σ^A , all coupled through symmetric competition for limiting core RNAP. Simulations used parameter set A in STAR Methods.

(A) Sample traces for the five alternative sigmas and their cognate species. Stochastic ligand bursts were driven by a gamma distributed Ornstein-Uhlenbeck process (top left panel). Other panels show the indicated species over time for the same simulation.

(B) Model sensitivity analysis. Using the same parameter values as in Fig. 3D (parameter set A in Methods), we computed the fold change in indicated pulse characteristics as a result of changing the listed parameters by plus or minus 30%. Error bars show SEM for 6 repeat runs of the model.

Figure S6

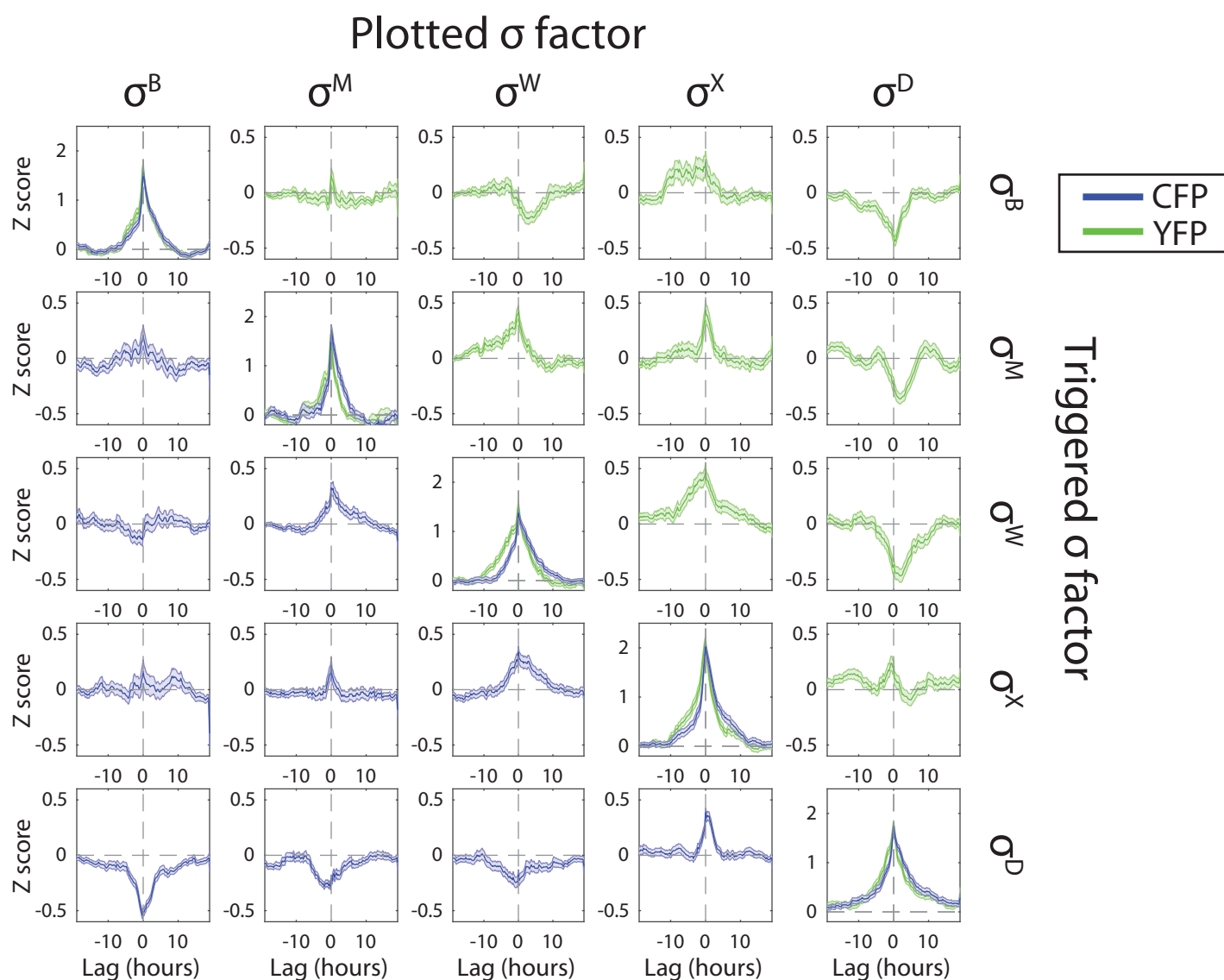


Figure S6, related to Figure 4,5. Pulse Triggered Averaging reveals dynamic correlations between sigma factors. In this technique, pulses of one sigma factor (the ‘trigger’ sigma) are identified and the dynamics of a second sigma factor in the same cell are averaged in time windows around those events (STAR Methods). The resulting pulse-triggered averages are plotted here. Each pair of sigma factors is plotted twice, once as YFP triggered on the CFP trace (green), and once as CFP triggered on the YFP trace (blue). Note the similarity in the sign of the correlation as well as the symmetry in the time delay relative to zero lag. Error bars are s.e.m. Each plot represents the average of at least 75 pulses. The underlying data set is the same as that used to calculate the cross-correlation functions (Fig. 5A).

Figure S7

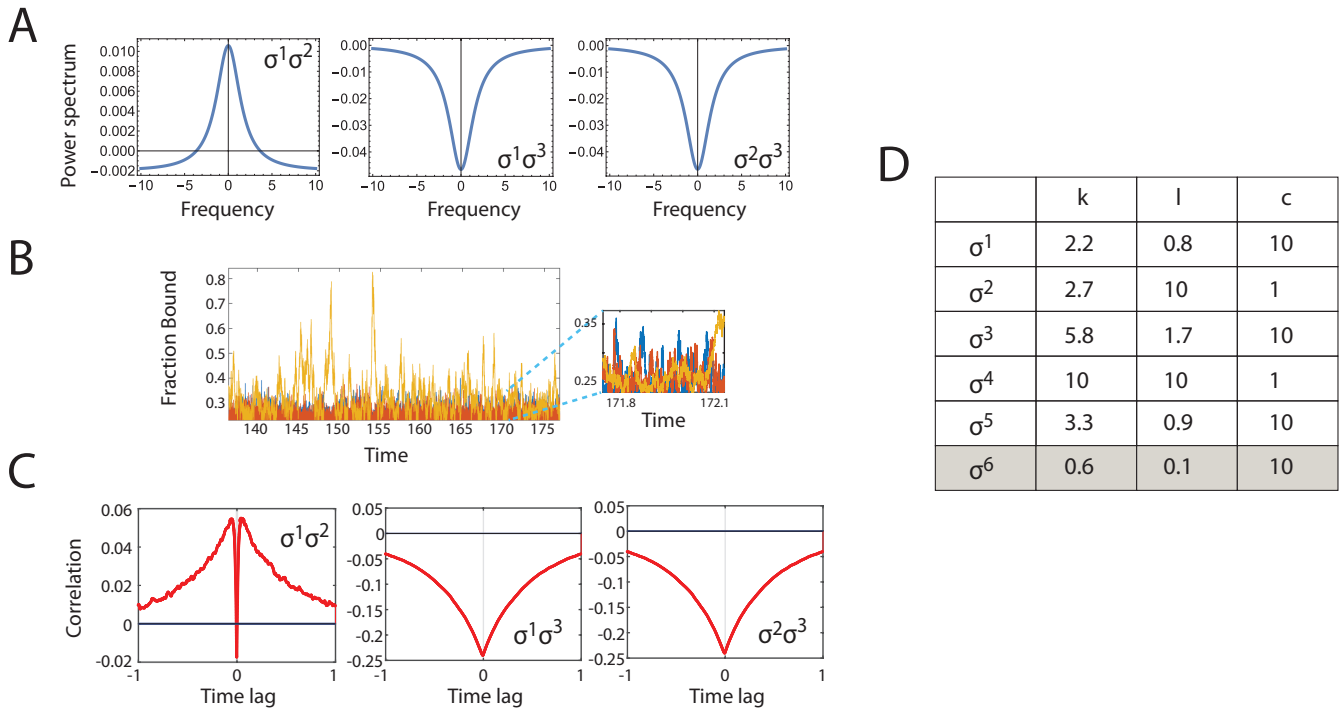


Figure S7, related to Figure 5. Positive correlations can arise from competitive interactions in a minimal model of sigma factor-RNAP interactions.

(A) For a minimal model of three sigma factors competing for binding to a limited pool of core RNAP: analytically calculated spectral densities of the cross-correlation functions between each pair of sigma factors for the choice of parameters shown in Figure 5Dii (see Supplemental Text).

(B) Simulated traces of binding fluctuations (as in Figure 5F) but with fluctuations constrained to be positive, and the magnitude of fluctuations increased by a factor of 40 for sigma factor 3 and a factor 100 for the other two sigma factors. The resulting traces are more pulse-like, similar to the experimental observations. Although this regime falls outside the assumptions of the analytical model, the resulting correlation functions computed from the simulated traces (shown in C) are consistent with the analytical correlation functions (shown in Figure 5E). In particular, the mixture of positive and negative correlations persists. Thus, it is reasonable to extend the conclusions from the analytical model to the regime of large pulse-like fluctuations.

(D) Optimal choice of parameters for the extended analytical model of 6 sigma factors (5 observed and 1 unobserved) that resulted in a 5x5 correlation matrix (amongst the 5 observed sigma factors) that exhibited a complex mixture of positive and negative correlations (Figure 5G). In order to find the optimal parameter values, we defined a cost function as the square of the difference between the entries of the generated 5x5 correlation matrix (correlations at time lag 0) and the experimentally observed correlation matrix, summed over all the entries of the matrix. This cost function was minimized by performing a particle swarm search in the 18-dimensional parameter space with each parameter constrained to a value of 0.1 and 10, and a cut-off frequency of 1 when computing the correlation functions from the power spectra (implemented in Matlab). Note that sigma factor 6 is the unobserved sigma factor.

Figure S8

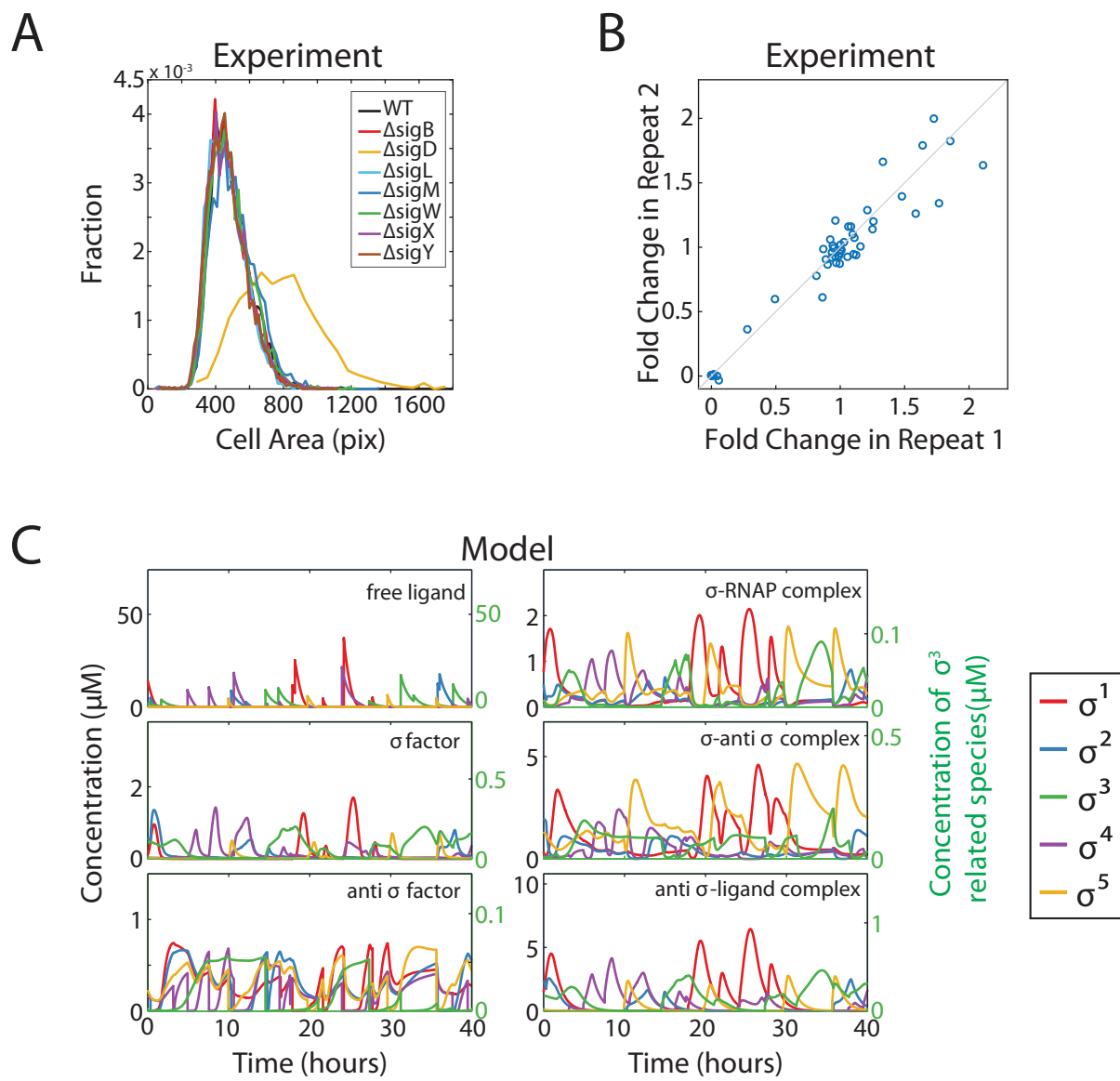


Figure S8, related to Figure 6. Experimental deletion matrix analysis and sample model simulations.

(A) Histogram of cell areas for multiple sigma factor deletion strains. Cell areas were calculated from single cell microscopy images. Each distribution represents at least 900 cells.

(B) Each entry in the deletion matrix (Figure 6A) is the average of 2 independent experiments. Plotted here is the comparison between the 2 independent experiments. The gray line is the $y=x$ diagonal, and there is good agreement between the 2 experiments.

(C) Sample traces from model simulations for the five alternative sigma factor species and their cognate species (parameter set B in Methods). σ^3 is plotted on a distinct y-axis labeled on the right side of each panel.