

## Expanded View Figures

**Figure EV1. With increasing stress levels, the heterogeneity in  $\sigma^V$  activation times is reduced.**

In each subpanel, a line corresponds to a single-cell trace of one mother cell in the mother machine ( $N = \sim 50$ ). The stress was added after 240 min (dashed line). Once  $P_{sigV}$ -YFP passed its half maximum the traces end.

- A, B Single-cell traces in response to 0.5  $\mu\text{g/ml}$  lysozyme.
- C, D Single-cell traces in response to 1  $\mu\text{g/ml}$  lysozyme.
- E, F Single-cell traces in response to 2  $\mu\text{g/ml}$  lysozyme.
- G, H Single-cell traces in response to 4  $\mu\text{g/ml}$  lysozyme.

Data information: The data are the same as in *n*. For more information on the number of repeats, please see Appendix Table S4.

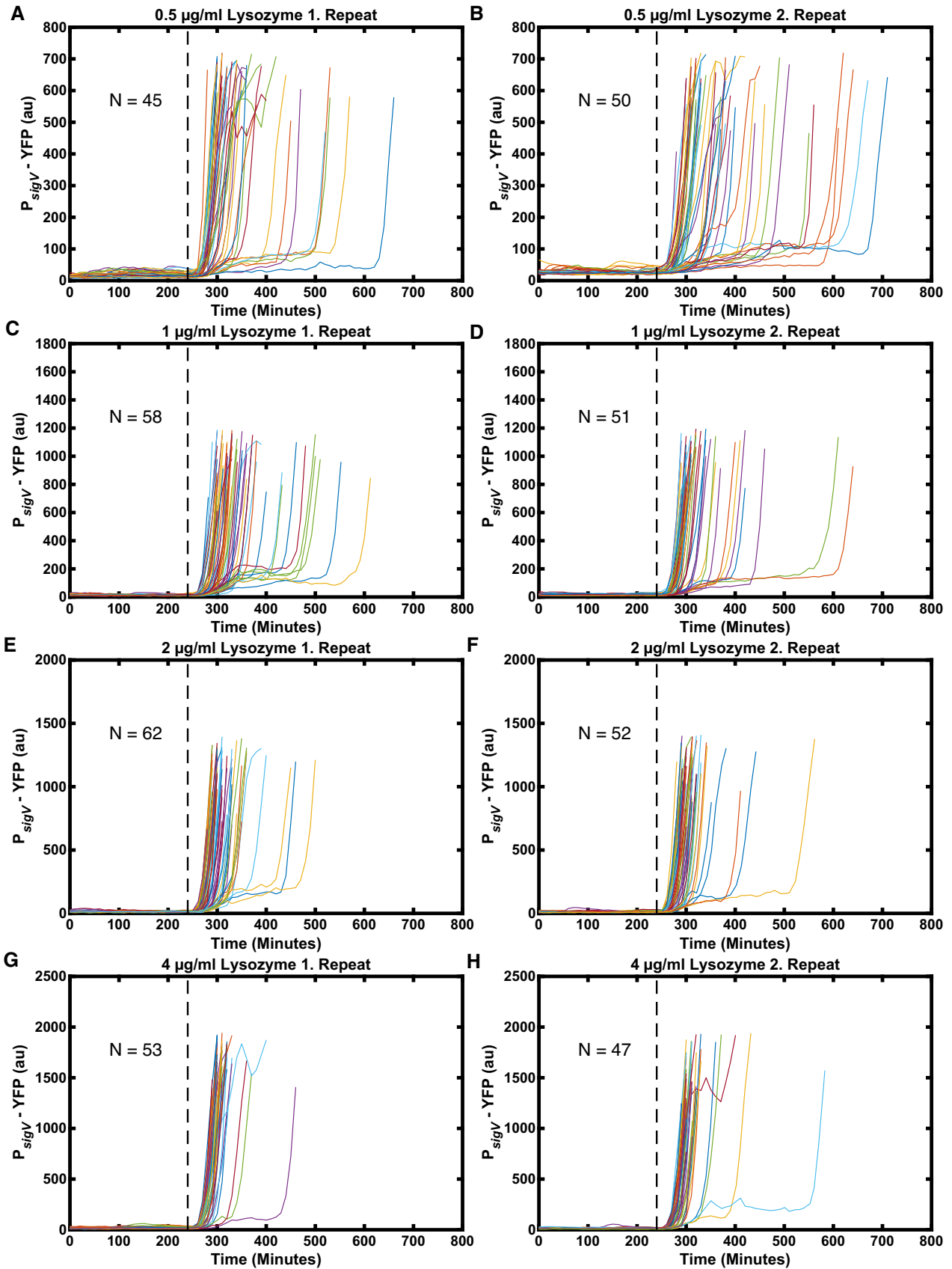
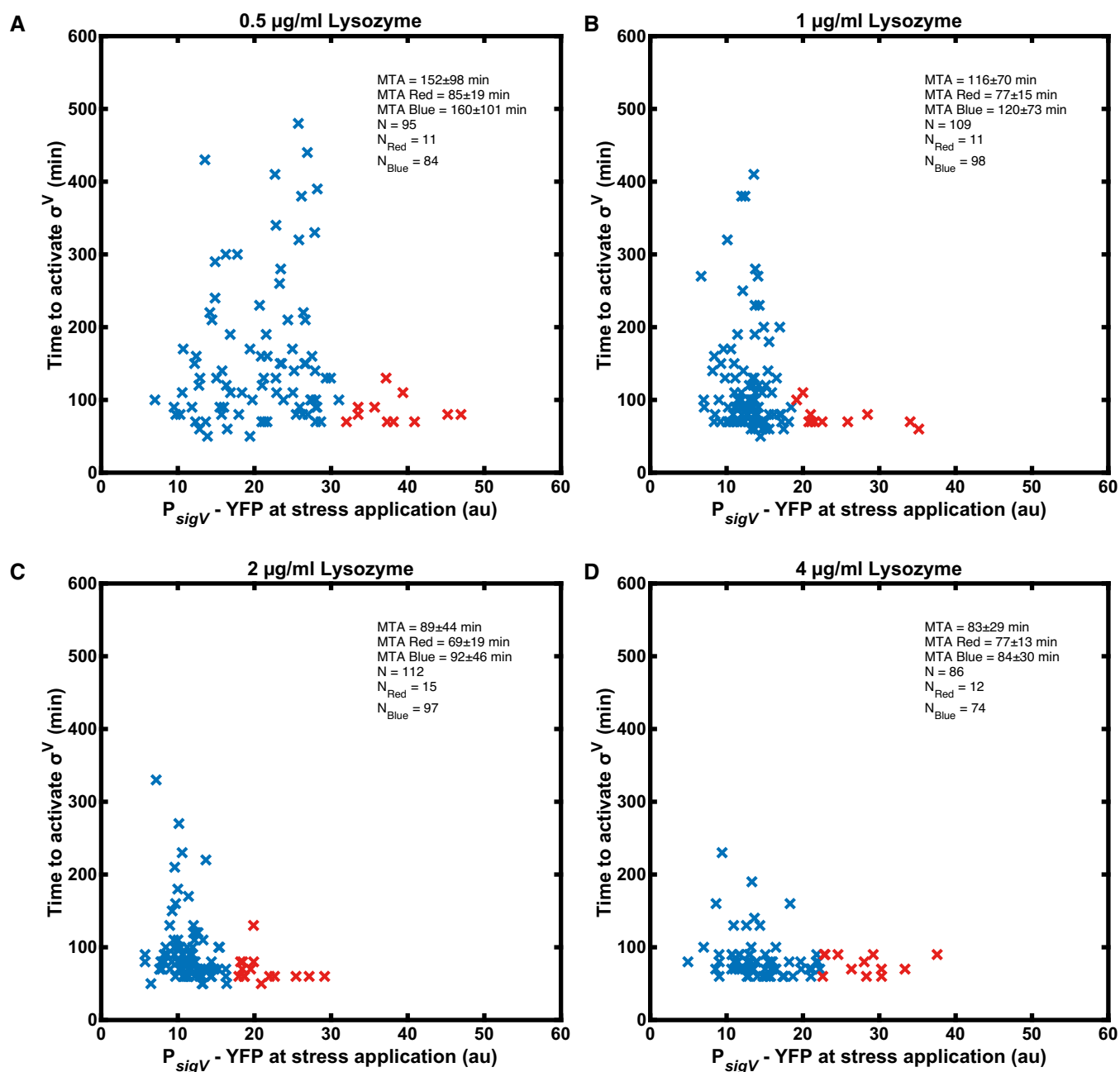


Figure EV1.

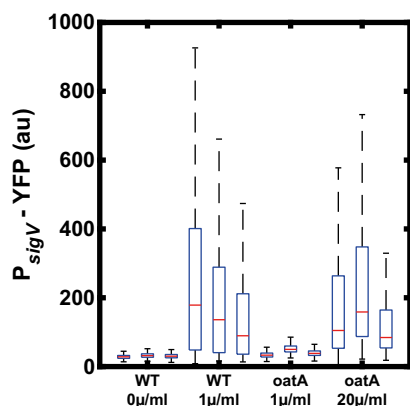


**Figure EV2. Cells with high  $P_{sigV}$ -YFP before stress activate  $\sigma^V$  rapidly.**

Figure plots  $P_{sigV}$ -YFP levels at the time of stress addition, against the time to activation. Cells with a YFP fluorescence larger than the mean + s.d. (of the pre-stress YFP distribution) are marked with red crosses. All other cells are marked with blue crosses. In the inset MTA stands for mean time to activate.

- A Activation time for 0.5  $\mu\text{g/ml}$  lysozyme.
- B Activation time for 1  $\mu\text{g/ml}$  lysozyme.
- C Activation time for 2  $\mu\text{g/ml}$  lysozyme.
- D Activation time for 4  $\mu\text{g/ml}$  lysozyme.

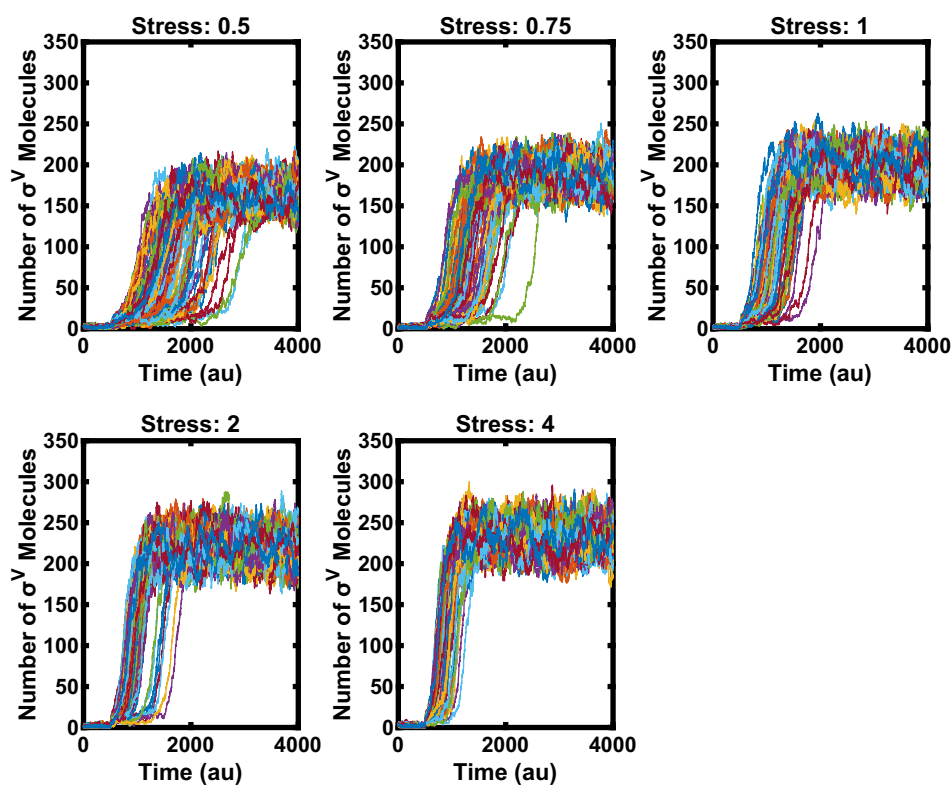
Data information: All shown data in the plots are from two biological repeats. For more information on the number of repeats, please see Appendix Table S4.



**Figure EV3. Overexpressing *oatA* shuts off  $\sigma^V$  activation.**

Increasing stress levels from 1 to 20  $\mu/ml$  recaptures the WT  $\sigma^V$  activation dynamics when *oatA* is overexpressed.  $n = 3$  biological repeats for all data shown. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively. The lower and higher whiskers of boxplot are extended to the first quartile minus 1.5 \* interquartile range and the third quartile plus 1.5 \* interquartile range, respectively.

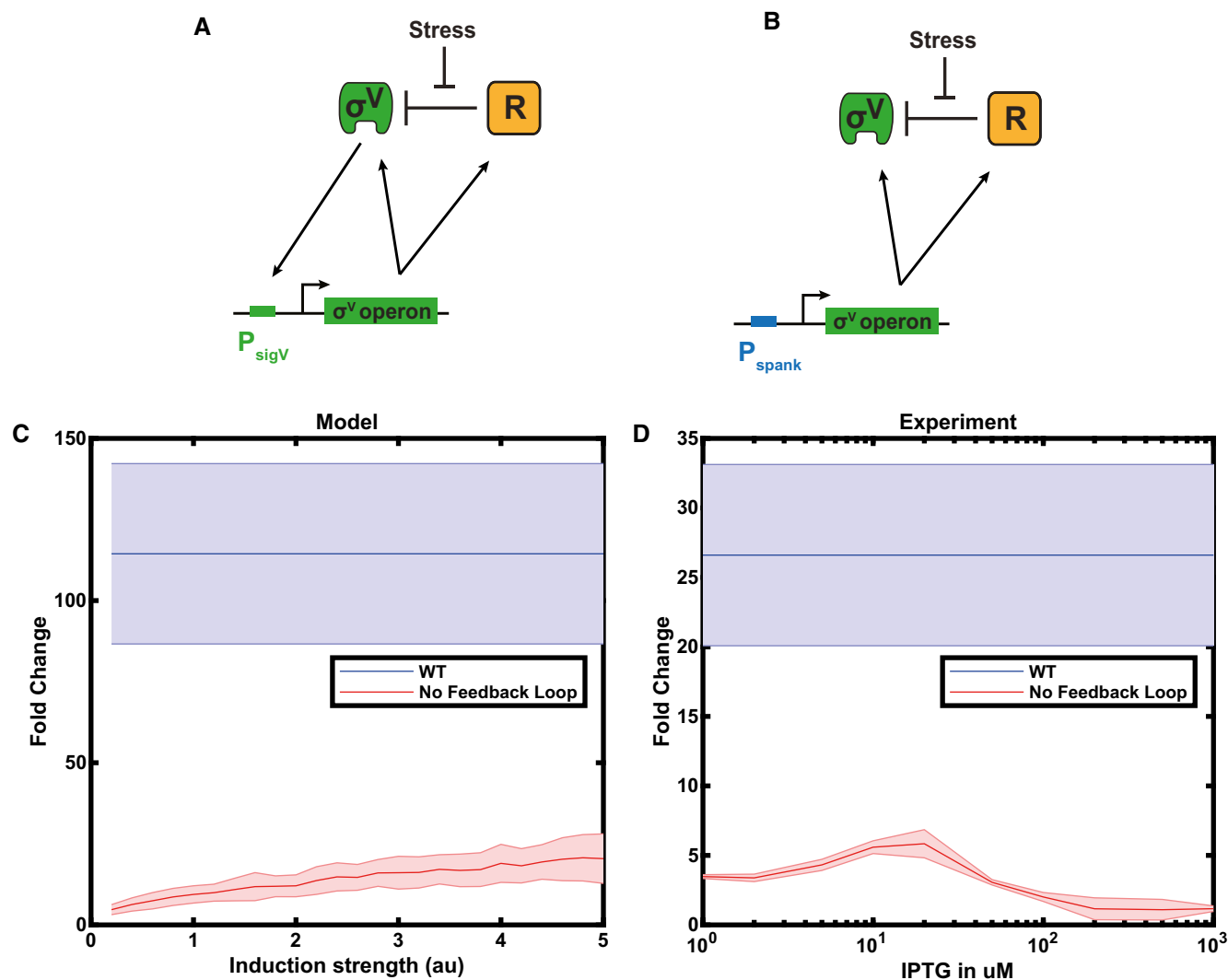
Data information: For more information on the number of repeats, please see Appendix Table S4.



**Figure EV4. With increasing stress levels, the heterogeneity in activation is reduced in the mathematical model.**

Each line corresponds to one simulated cell. The stress was added (parameter  $L$  changed from 0 to 1) at time point 500 ( $N = 100$ ).

Data information: For more information on the number of repeats, please see Appendix Table S4.



**Figure EV5. The *sigV* feedback loop increases the dynamic range of the circuit.**

A Sketch of WT *sigV* circuit. Where R stands of RsiV.

B Sketch of rewired circuit with deleted positive feedback loop. Where R stands for RsiV and  $P_{spank}$  for the IPTG inducible promoter spank.

C The fold change in *sigV* expression pre/post 1  $\mu\text{g}/\text{ml}$  lysozyme stress was calculated from model simulations of the WT system (blue line) and a feedback-broken system where the levels of the *sigV* operon are inducible (red line). The shaded areas represent one standard deviation of mean.  $N = 100$  simulation.

D The experimental fold change for the WT, and the feedback-broken strain for IPTG values between 0 to 1,000  $\mu\text{M}$ . The blue line is the WT fold change, and the red line is the fold change of  $\Delta sigVrsiVoatAyrhK P_{spank}\text{-}sigVrsiVoatAyrhK$ . The shaded areas represent one standard deviation of mean.  $n = 3$  biological repeats for all data shown.

Data information: For more information on the number of repeats, please see Appendix Table S4.