https://doi.org/10.1038/s41467-023-37897-9

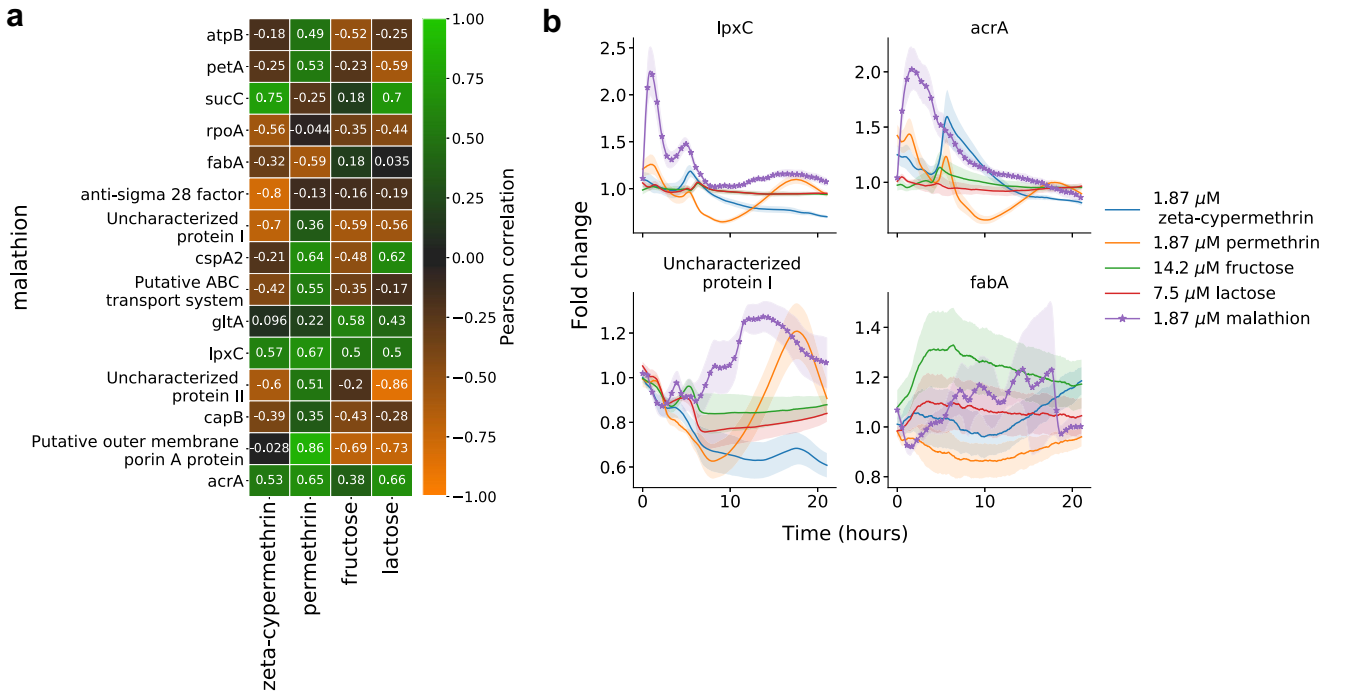

Fig. 5 | The 15-dimensional genetic reporter cell state provides a unique response to malathion. a For each genetic reporter, the heatmap depicts the Pearson correlation of the malathion fold change response (rows) with the fold change response to zeta-cypermethrine, permethrin, fructose, or lactose (columns). **b** The fold change response (reporter + compound with respect to reporter + no compound) of four reporters – two with highest overall correlation and two with lowest overall correlation across compounds. The error bars represent the propagated standard deviations of each of the individual responses across three biological replicates. Source data are provided as a Source Data file.

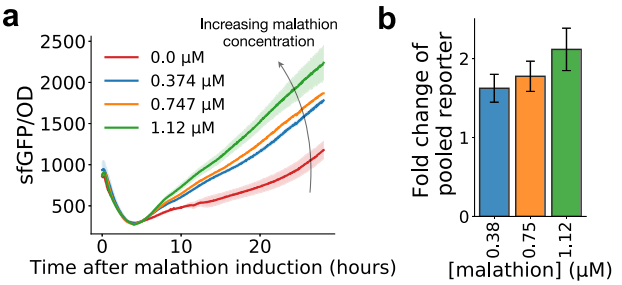

Fig. 6 | Pooling all 15 malathion reporters results in enhanced reporting for environmental monitoring. a Time-lapse response after pooling all 15 malathion reporters into a single well and inducing with malathion. Error bars represent the sample standard deviation across three biological replicates. **b** The fold change at 24 h of the pooled reporter with malathion induction with respect to the pooled reporter without malathion induction. The error bars represent the propogated standard deviations of each of the individual responses across three biological replicates and the bar height represents the fold change calculated using the mean sfGFP/OD signal from malathion and from control conditions. Source data are provided as a Source Data file.

by any individual reporter at the same concentration is 2.3 and is a transient response (*lpxC*, see Fig. 5b). The maximum fold change corresponding with a sustained response is 1.5 obtained by *cspA2*. For sustained response to malathion, the pooled reporter provides more salient response than any individual reporter alone.

Our experiments confirm the usefulness of the malathion reporters outside of the laboratory and in field environments. A potential strategy for environmental malathion monitoring would be to collect a soil sample, culture the pooled reporters from a media made from the sample, then measure the *sfGFP* response. Though this strategy is enticing, there may be growth competition effects between strains that we have not addressed. Next, we aim to understand if it is possible to detect malathion in environmental samples from our individual reporters.

Detecting malathion in environmental samples

The malathion reporter library, selected through observability analysis, has only been examined in an ideal laboratory scenario with either pure or processed malathion whose mass spectrum has been analyzed; it is not yet known if the reporters will be able to sense malathion when induced with actual environmental water samples that have been treated with the insecticide. In the previous section we showed that pooled reporters act as salient malathion sensors. However, confounding factors may be present in the environmental sample such as other small compounds that may make it difficult to deconvolve malathion response from the response due to the confounder. Therefore, in this section we describe an experiment to assess whether or not the malathion concentration can be deduced from our individual reporters treated with environmental insecticide samples.

In order to test if the genetic reporters can sense malathion from environmental samples, irrigation water was collected from three crops after being sprayed with a mixture of Spectracide (50% malathion) and water (Fig. 7a). The concentration of the mixture sprayed was either 0, 1, or 8 times the maximum recommended working concentration of Spectracide – 1 fluid ounce per gallon of water. To rid the solution of unwanted microbes and particles, the irrigation water was strained and filtered prior to to the induction of the genetic reporters (see "Methods" section). The growth and induction protocols all remain the same as for the samples treated with Spectracide in Fig. 4c, d.

We found that a total 9 out of the 15 of the reporters were activated by induction of the irrigation water containing malathion. Figure 7a shows the average per cell fluorescence 24 h after induction of