SUPPORTING INFORMATION

Genetically Encoded Stimuli-Responsive Cytoprotective Hydrogel Capsules for Single Cells Provide Novel Genotype-Phenotype Linkage

Rosario Vanella[1,2], Alfred Bazin[1,2,3], Duy Tien Ta[1,2], and Michael A. Nash[1,2 *]

[1] Department of Chemistry, University of Basel, 4058 Basel, Switzerland.

[2] Department of Biosystems Science and Engineering, ETH Zurich, 4058 Basel, Switzerland.

[3] Institute for Chemistry, Polymers and Materials, University of Strasbourg, 67087 Strasbourg, France.

*Correspondence to michael.nash@unibas.ch

Supplementary figures

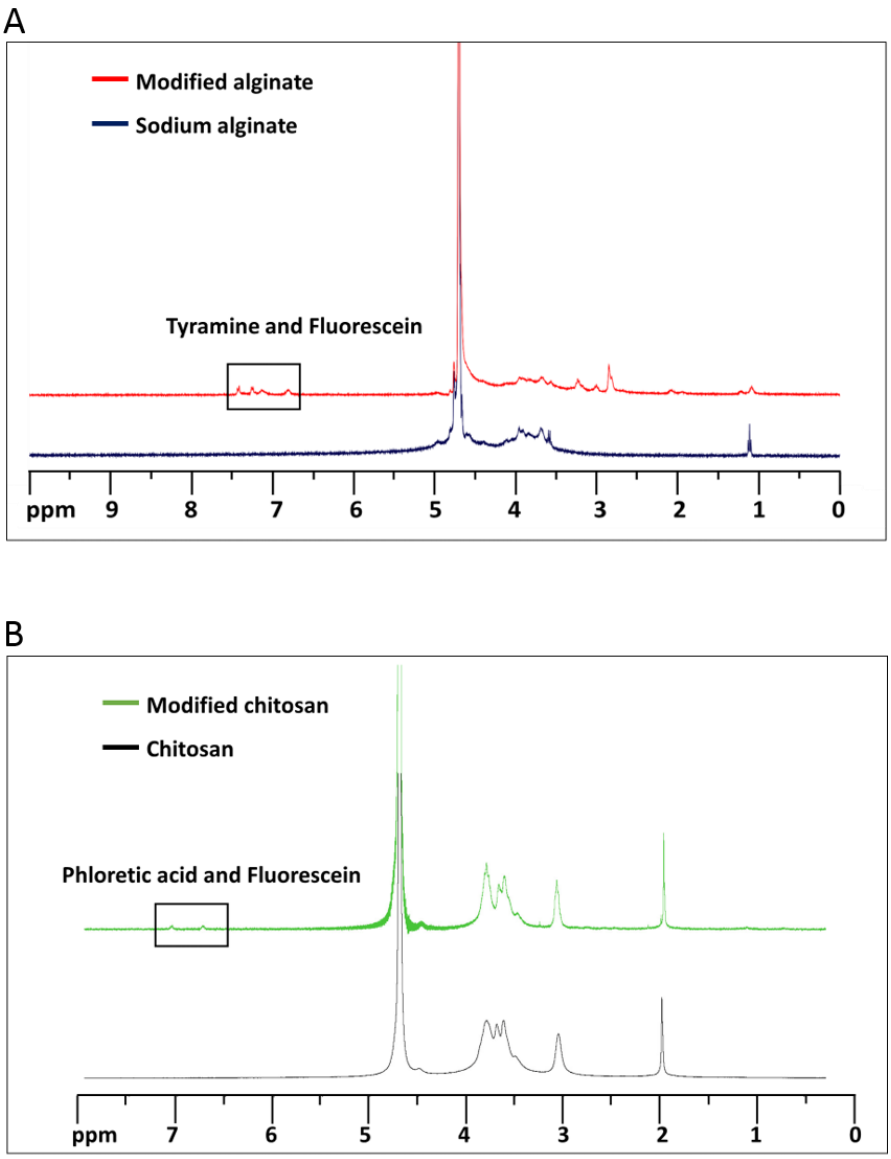

Figure S1. (A) ^{1}H-NMR spectra of sodium alginate before (blue) and after (red) conjugation with Tyramine and aminofluorescein. The incorporation of phenol and fluorescein moieties into the main alginate chain was confirmed by the group of peaks framed on the red plot that is not visible on the non-modified sodium alginate spectrum (shown in blue). (B) ^{1}H-NMR spectra of chitosan before (black) and after (green) conjugation with phloretic acid and fluorescein. The success of the reaction was confirmed by the group of peaks framed on the green spectrum not visible on the non-modified chitosan spectrum (shown in black).

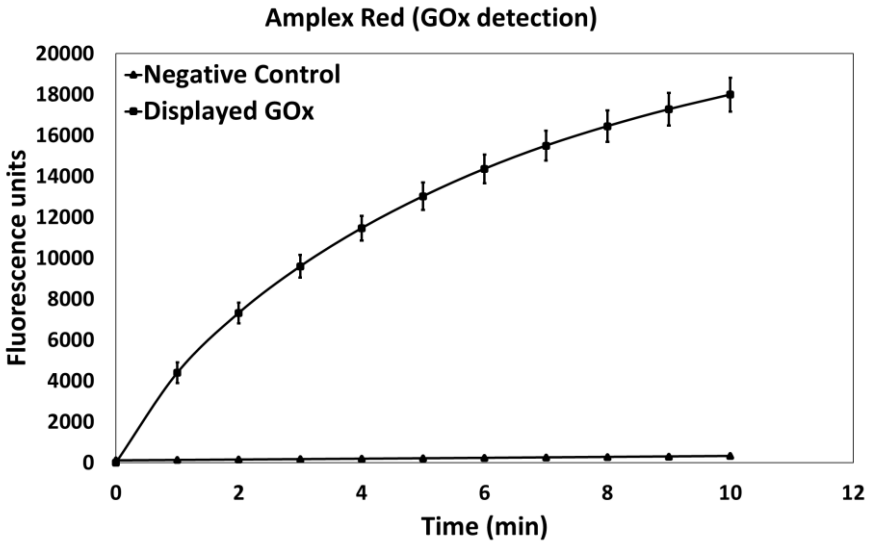

Figure S2. Yeast cells displaying Glucose Oxidase (GOx) were tested using an Amplex Red assay in order to confirm the functionality of the expressed enzyme. The reaction mixture consisted of ~10^6 yeast cells, 100 mM glucose, 4.5 µM HRP and 10 µM Amplex Red in 50 mM phosphate buffer (pH 7.4). The fluorescence was read at 590 nm every 1 min for 10 min.

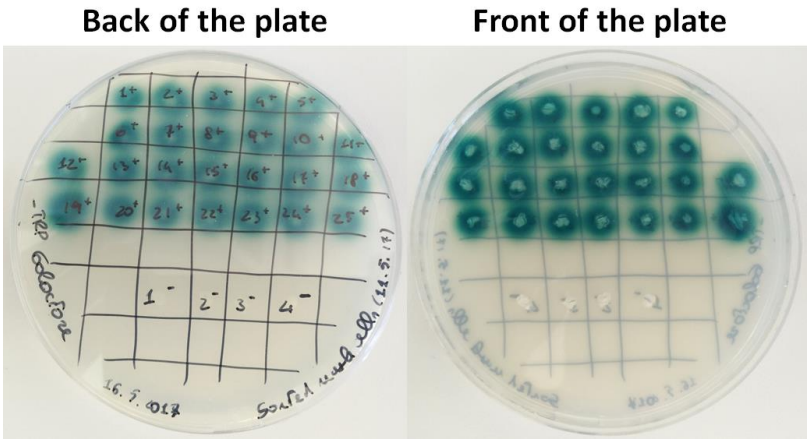

Figure S3. ABTS top agar assay for Glucose Oxidase (GOx) activity detection. A total of 25 single yeast cells were sorted after encapsulation in a fluorescent hydrogel mediated by GOx displayed on the cell wall. The cells were grown on a 2% Glucose agar plate lacking tryptophan before being transferred to a 2% Galactose plate for induction of pGAL-GOx. After 2 days at 30°C, the colonies were tested for GOx activity through ABTS top agar assay. The assay was performed by mixing 2% agar with an equal volume of reaction mixture containing 666 mM Glucose, 14 mM ABTS, and 4 U/mL HRP in 50 mM sodium phosphate buffer (pH 7.4). Green halos indicating activity of GOx were visible around all the 25 tested colonies, while no halos could be detected in proximity of GOx negative colonies used as negative control.

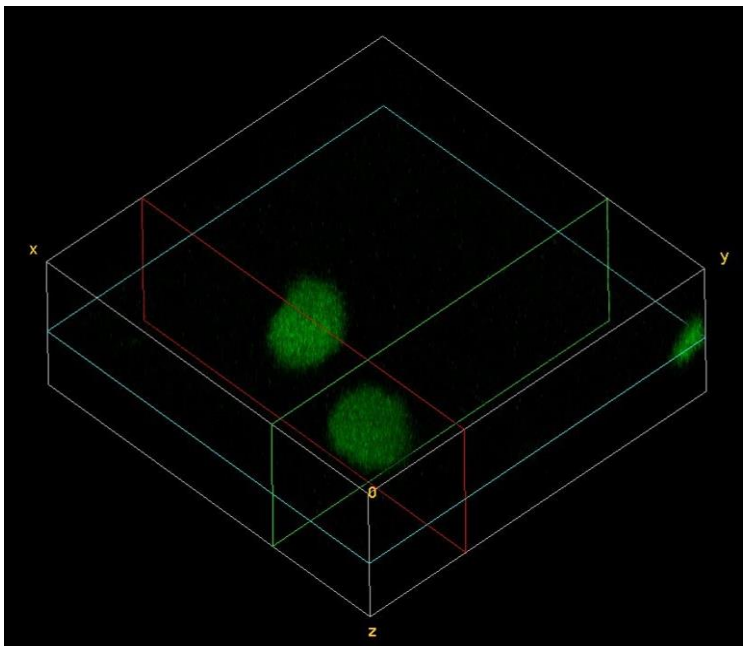

Figure S4. 3D spatial representation of encapsulated yeast cells in fluorescent alginate hydrogel. The presence of fluorescein covalently bound to the alginate macromonomer allows for the visualization through fluorescence microscopy. The cross sections of the different planes X, Y and Z used for the orthogonal view images shown in **Figure 3** of the main manuscript are displayed.

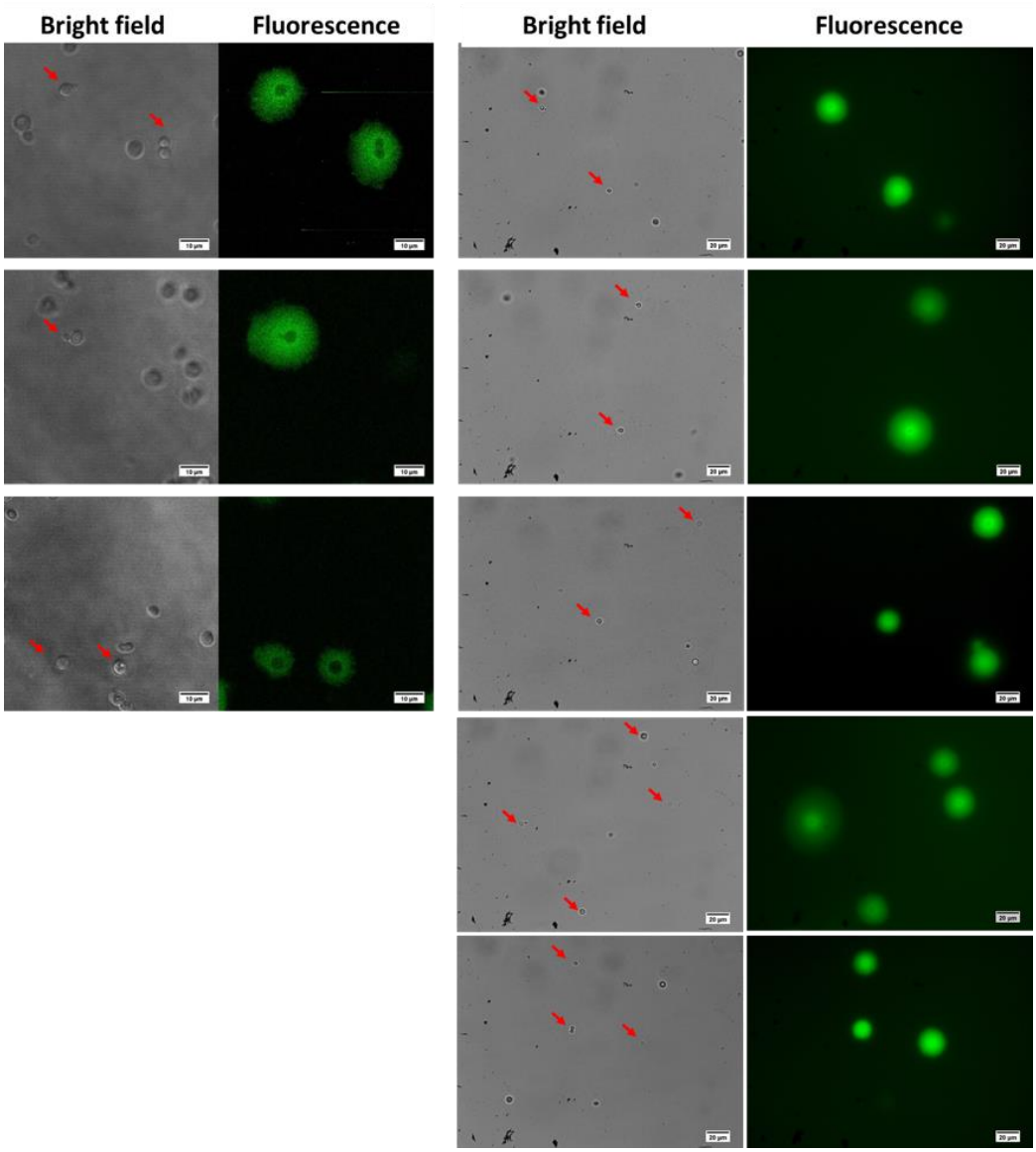

Figure S5. Bright field and fluorescence images of encapsulated cells demonstrating that cells are encapsulated as singles under standard conditions ($2x10^6$ cells/mL, 0.125% phenolated alginate). (Left) Confocal and wide field (right) fluorescence microscopy images of single yeast cells individually encapsulated in alginate hydrogel shells. Note that not 100% of cells in the mixture are encapsulated because maximally 60% are positively induced for GOx expression.

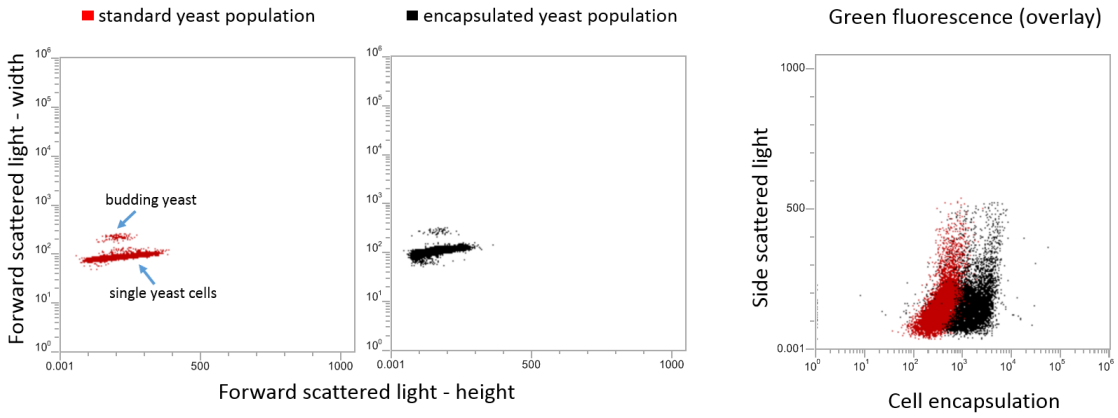

Figure S6. Flow cytometry plots demonstrating that yeast cells are encapsulated as single cells and not aggregates. (Left) Forward scatter width vs. forward scatter height for healthy EBY100 yeast cells showing two populations corresponding to single cells and budding cells. (Middle) Forward scatter width vs. forward scatter height for a yeast cell population displaying GOX and exposed to the encapsulation reagents. (Right) Side scatter vs. green fluorescence of the yeast population shown in the middle plot demonstrating that ~60% of the population was encapsulated in fluorescent alginate shells.

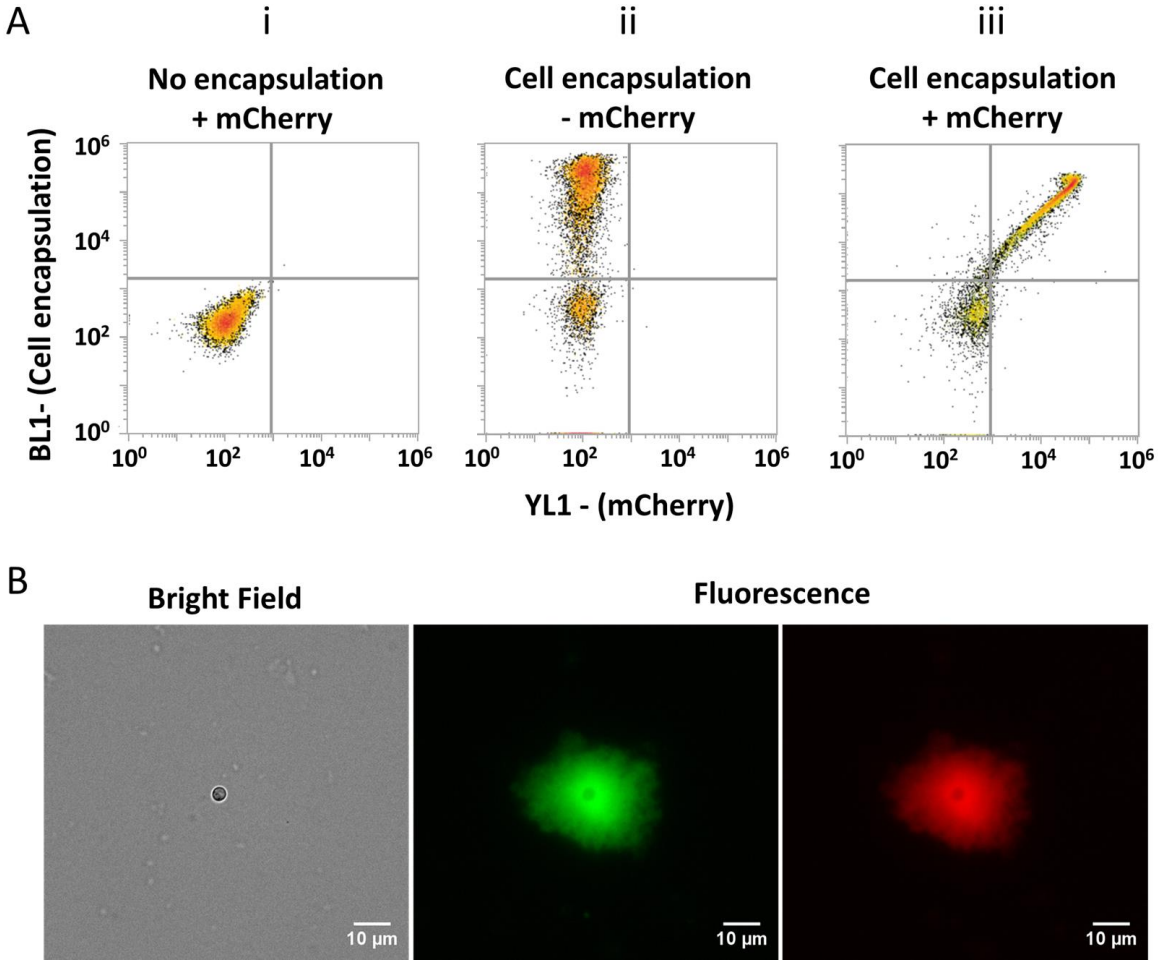

Figure S7. Flow cytometry and microscopy analyses of yeast cells enzymatically encapsulated in fluorescent hydrogel in the presence of mCherry. (A) Flow cytometry analysis of (i) a yeast population incubated with mCherry and reaction mixture without glucose, (ii) yeast cells enzymatically encapsulated in fluorescent alginate hydrogel lacking mCherry; and (iii) yeast cells encapsulated in fluorescent alginate hydrogel in presence of mCherry. (B) Bright field and fluorescence microscopy images of a yeast cell encapsulated in a fluorescent hydrogel in the presence of mCherry in the reaction mixture.

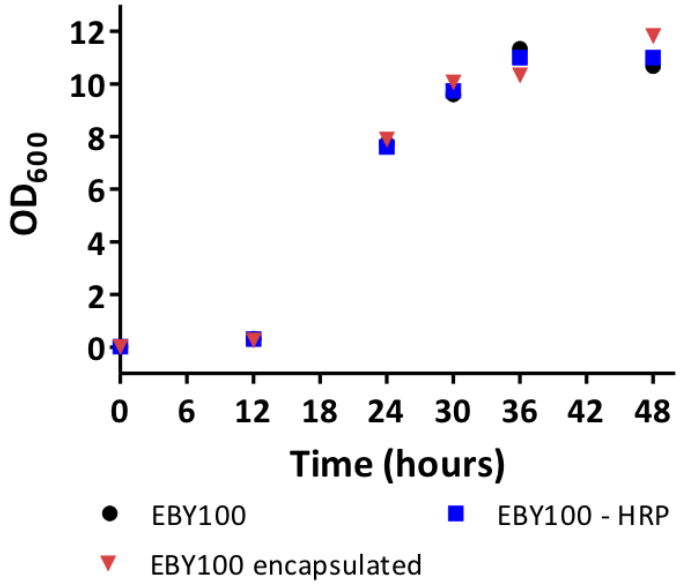

Figure S8. Encapsulation in hydrogel capsules does not inhibit the growth of yeast cells. The growth curves of various cells were measured in YPD media. The empty yeast strain containing no plasmid (EBY100, black circles), the strain carrying the Aga2-GOx plasmid exposed to the macromonomer reaction mixture lacking HRP (EBY100 -HRP), and the strain carrying the Aga2-GOx plasmid and encapsulated in alginate hydrogel all showed the same growth curve, indicating that the alginate shells do not inhibit the growth rate. The cells break out of the hydrogel shells and propagate in the media.