

## Supplementary Figures.

**Supplementary Figure 1.** Drop recovery from the device. (a) Drops are trapped in the array of chambers. (b) By flowing oil through the device, the drops squeeze through the constrictions and are removed from the device. (c) Empty channels after drops have been removed. Scale, 40  $\mu$ m. (d) Encapsulated contents of drops are retrieved by breaking the emulsion. After recovery, yeast cells are plated and grow to form colonies on YPD plates. Scale, 10 mm.

**Supplementary Figure 2.** Cell growth in drops. (a) The total number of encapsulated cells increase as function of time, exhibiting the typical sigmoidal growth curve observed for bulk cell culture. (b) At the level of individual drops, doubling times vary significantly and increase with the number of cells initially encapsulated. During the 15 hour incubation, we monitor an array of 160 chambers and acquire images every 10 minutes. Out of these chambers, 151 are filled with drops, and 71 of these contain one or more cells. In seven drops no cell division is observed over the total 15-hour time period. When cells are grown in exponential phase and then encapsulated, cells divide more quickly (Rowat et al, unpublished data). Red bars represent the mean values for the doubling time.

**Supplementary Figure 3.** The total volume of drops containing cells decreases over time. (a) The final volume of the drops decreases with the number of initially encapsulated cells. (b) The rate of volume change of drops ( $\Delta V/\Delta t$ ) decreases with the increasing mean number of encapsulated cells: empty drops grow, while drops containing more than one cell shrink. The rate of volume change was calculated by applying a linear regression for the volume over the total time range of 900 min. Red bars represent the mean value of volume change in the corresponding interval of cell number. As the rate of volume loss is proportional to the number of initially encapsulated cells, we hypothesize that encapsulated cells consume more molecules than they produce, resulting in an osmotic imbalance between empty and cell-containing drops. Monitoring drop volume could be an interesting tool to observe metabolic rates at the single cell level.

**Supplementary Movie.** Drops are generated upstream using a flow-focusing geometry to produce an emulsion of minimal cell media in fluorocarbon oil. Frame rate, 30/s. Drop diameter, 40  $\mu\text{m}$ .