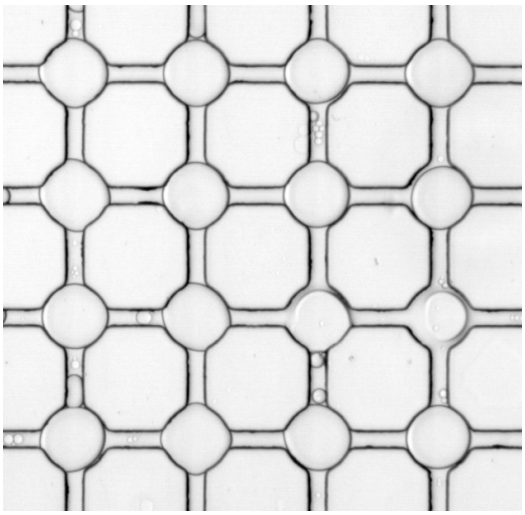
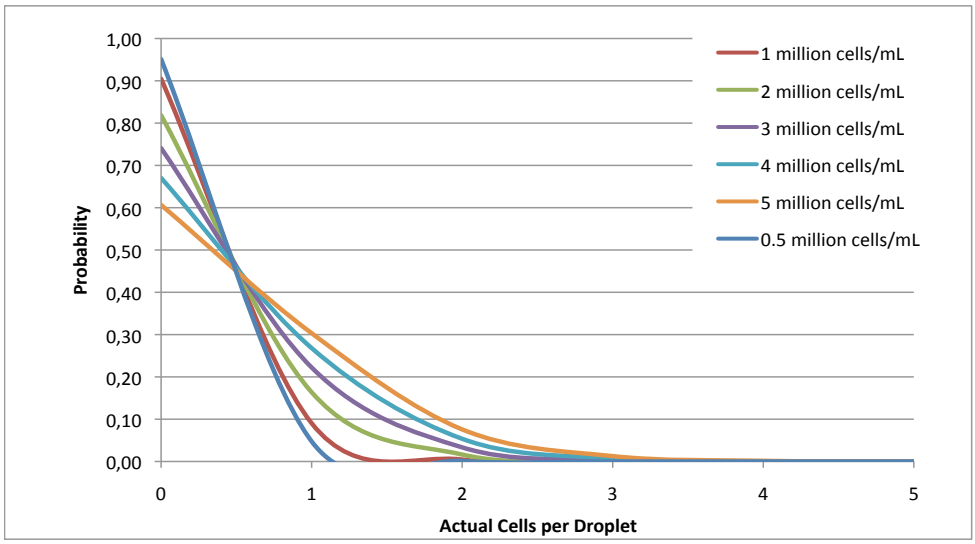


Supporting figure 1. Droplets in drop-trap

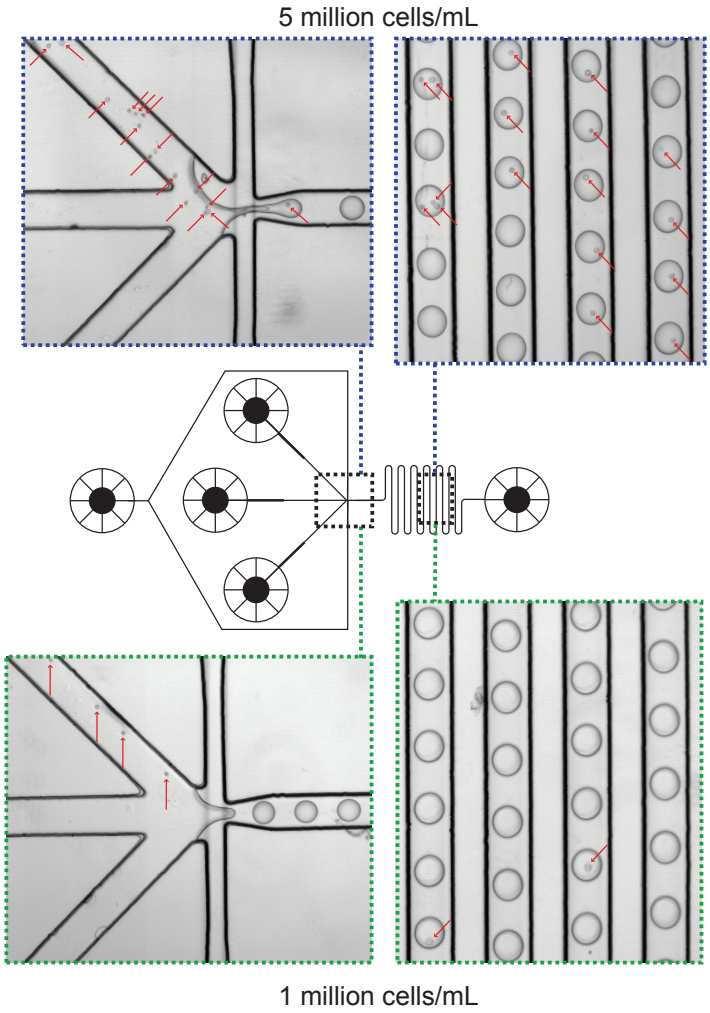
Light microscopy of drop-traps encapsulating 100 pL water-in-oil droplets. The drop-trap cavities are designed to each contain one droplet, which is spatially isolated from other droplets. Droplets are seen as round spheres in the intersections of the drop-trap grid.

Cell density (cell/mL)	5,00E+05	1,0E+06	2,0E+06	3,0E+06	4,0E+06	5,0E+06
Droplet Volume (pL)	100	100	100	100	100	100
Mean per Drop (cells/drop)	0,05	0,1	0,2	0,3	0,4	0,5
Actual cells per drop	**Probability**					
0	0,951	0,905	0,819	0,741	0,670	0,607
1	0,048	0,090	0,164	0,222	0,268	0,303
2	0,001	0,005	0,016	0,033	0,054	0,076
3	0,000	0,000	0,001	0,003	0,007	0,013
4	0,000	0,000	0,000	0,000	0,001	0,002
5	0,000	0,000	0,000	0,000	0,000	0,000
6	0,000	0,000	0,000	0,000	0,000	0,000
7	0,000	0,000	0,000	0,000	0,000	0,000
8	0,000	0,000	0,000	0,000	0,000	0,000
9	0,000	0,000	0,000	0,000	0,000	0,000
10	0,000	0,000	0,000	0,000	0,000	0,000



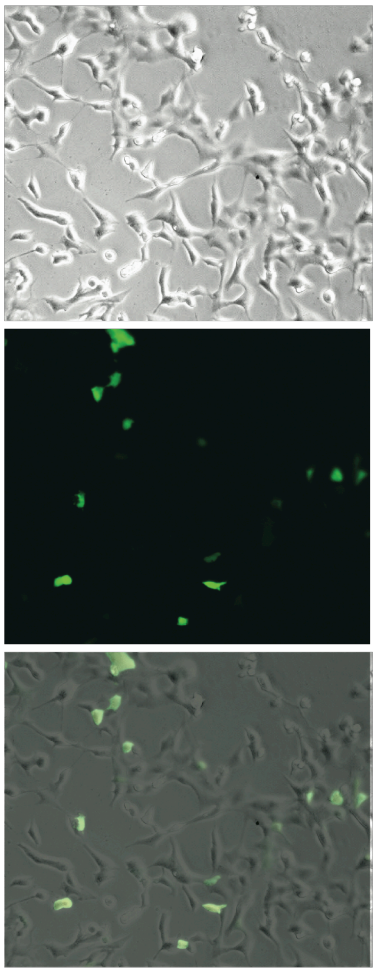
Supporting figure 2. Theoretical estimate of the amount of cells in the picoliter droplets as a function of cell density

Encapsulation of cells within the 100 pl monodisperse droplets can be estimated as a Poisson (stochastic) distribution[25] in the form of $P(N, n) = N^n \times (e^{-N}/n!)$, where N is the nominal number of encapsulated cells and n is the actual number of cells in each droplet. According to this distribution, increasing the density of cells loaded into the system from 0.5 to five million cell/mL results in an increasing of droplet occupancy. For example, when using the lowest cell density, 4.8 % of droplets are expected to contain a single cell whereas only 0.1 % of droplets are expected to contain two or more cells. This was also observed by Konry *et al.*[5]. Loading of five million cells/mL, on the other hand, will theoretically result in 30% of the droplets having single cells and 9.1% of droplets containing two or more cells.



Supporting figure 3. The density of cells loaded into the microfluidic device determines the number of cells per droplet

The middle of the image is a schematic illustration of the PDMS microfluidic device. As shown the device consists of three water phase inlets, an oil inlet, and an outlet for the generated droplets. Top panel, microscopic view of droplet entrapped cells resulting from loading HEK293 cells with a density of five million cells/mL into the microfluidic device. Consistent with the Poisson distribution (Supporting Fig. 2**)** this cell density results in approximately 40% of cell containing droplets. As evident these are not always single cells, and several cells are confined in the same droplet in approximately 9% of the cases. Note, that the percentage of droplets containing three or more cells appeared a bit higher (approximately 3-4%) than predicted from the Poisson distribution when measured experimentally. This is most probably due to a slight aggregation of cells at this high cell density. Bottom panel shows a microscopic view of the droplets resulting from loading a cell concentration of one million cells/mL into the microfluidic device. Theoretically, loading at this cell density ensures that no more than a single cell is confined in each droplet (Supporting Fig. 2**)**. Consistent with previous reports[5, 22], this was confirmed experimentally by observation of more than 5000 droplets revealing the encapsulation of one or no cells in each droplet. Note, that for the presented experiments, the substrate and lysis buffer, applied in channel two and three of the microfluidic device when performing REEAD experiments, were substituted by PBS to ensure the integrity of the cells since lysed cells cannot be detected in the light microscope used for visualization of cells and droplets in this experiment.



GFP expressing cells: both hTopI and Flp activity.
No GFP expression: only hTopI activity.

Supporting figure 4. Generation of Flp recombinase expressing HEK293 cells

HEK293 cells were transfected with the plasmid, pCAG-Flpe:GFP, expressing recombinant Flpe fused to GFP. Flpe is a Flp-recombinase variant with enhanced thermostability and activity at 37°C, making it suitable for studies in mammalian cells[26]. GFP (green fluorescent protein) was fused to Flpe to allow the number of Flpe expressing cells to be calculated by simply counting the number of green fluorescent cells. Note, that the fusion between GFP and Flpe does not affect the activity of the recombinase. Top and middle panels show a bright field image and a fluorescence image, respectively, of the transfected cells, while the bottom panel shows a merge of the bright field and fluorescence images. A transfection efficiency of 25% was determined by calculating the percentage of total cells expressing GFP.

25. Chabert, M.; Viovy, J.-L., Microfluidic high-throughput encapsulation and hydrodynamic self-sorting of single cells. *Proceedings of the National Academy of Sciences of the United States of America* 2008, *105*, 3191.
26. Buchholz, F.; Angrand, P. O.; Stewart, A. F., Improved properties of FLP recombinase evolved by cycling mutagenesis. *Nat Biotechnol* 1998, *16*, 657-662.