Supplementary materials

Design of the microfluidic device

1) Geometry of the chip

The microfluidic chip contains an array of twenty identical chambers of dimensions 4 um x 100 um x 200 um (depth, width, and length), connected by two parallel channels: a main channel of dimensions 20 um x 300 um x 12 mm, and a side channel of dimensions 20 um x 300 um x 4 mm (Figure 1c). The two parallel channels are also connected by two short channels of dimensions 20 um x 300 um x 200 um at the top and the bottom respectively. The fluid is pumped through the main channel, creating flows through the chambers due to a pressure difference between the two sides of the chamber. The depth of the chamber is optimized for maximum retention of the mother cells while still allowing the daughter cells to be flushed away. We have experimented with a number of different designs guided by theoretical modeling of the flux distribution (see point 3 below). The design in Figure 1 was selected for a number of advantages. First, the flow mostly passes through the main channel, while the side way fluxes through the chambers are much smaller, making it easier to achieve a small flow speed with a regular pump. Secondly, this design leads to a smooth gradient of flow speed in different chambers, which is convenient for exploring the range of flow speed for optimal mother daughter separation. Third, this design solved the problem due to occasional air bubbles emerging from the fluid, which could wipe out the cells; air bubbles are guided through the main channel, not through the chambers where the cells are loaded.

2) Manufacturing the mold

The mold for the chip was fabricated by the standard multilayer soft lithography methods[S1]. Negative photoresists Su-8 2005 and Su-8 2010 were used to fabricate the two layers of mold on silicon wafer respectively, in order to give the chambers and the channels different depth. First, the Su-8 2005 (MicroChen Corp., Newton, MA) negative photoresist was spin-coated at 5000 rpm and pre-baked according to the vendor's instruction before mask aligning. Then the coated wafer was aligned and exposed (150mJ/cm2) for 30 seconds through the mask in "direct contact" mode using a mask aligner. After exposure, the mold was post-baked and developed using Su-8 developer.

Su-8 2010 photoresists was used to fabricate the second layer, spin-coated at 3000 rpm. Another mask was aligned to the Su-8 features already patterned on the coated wafer, and the wafer was exposed (150mJ/cm2) for 50 seconds. After exposure, the mold was developed using Su-8 developer.

After the mold fabrication, PDMS was poured on the mold and allowed to cure. Access holes to the channels that allow the input and output of media were punched through the cured PDMS and the final chip was sealed to a pre-cleaned glass slide after plasma processing.

3) Theoretical modeling of flux distribution

We have experimented with chips of different geometries and different dimensions in order to reach a desirable design. The experiments were guided by theoretical modeling of flux distribution. In the model, each chamber or each segment of the connecting channel is treated as a rectangular pipe with flow Q governed by the Hagn-Poiseuille formula $\Delta P = 12Q\eta l / h^3 w$, where η is the dynamic

viscosity and l, h, and w are the channel length, height and width, and ΔP is the pressure drop. This is a reasonable approximation provided that the length of the rectangular pipe is much longer than the width and the height. The Hagn-Poiseuille formula is analogous to Ohm's law, where pressure corresponds to the voltage, flux corresponds to the current, and the resistance is given by $R = 12\eta l / h^3 w$. Using this analogy, we mapped the problem of fluid flow in a chip to current flow in an electronic circuit, and solved the flux distribution by the standard method. Supplementary Figure 1 shows the mapping of the chip design in Figure 1 to an electronic circuit and the corresponding flux distribution solved from the model.

Mother cell labeling and glass surface modification

To immobilize the mother cells, we labeled them by Sulfo-NHS-LC-biotin and coated the glass surface of the chip with biotinylated–BSA followed by neutravidin. 2 ml of cells with OD600 in the range of 0.5~1.0 were spun down and washed three times by PBS. After the wash, 0.1-0.2 mg of Sulfo-NHS-LC-biotin were added and incubated for ~15 min. The cells were then thoroughly washed and PBS was added to make a culture of OD600 3.0-4.0.

To coat the glass surface, water was first pumped in to remove any bubbles. Then 1mg/ml of biotinylated-BSA was pumped in for ~30 min, followed by washing and by 1mg/ml Neutravidin for ~15 min.

Cell loading

Typically ~15ul of cells prepared as described above was pumped into the chip. The chip was first checked under microscope to make sure that chambers were occupied by a sufficient number of cells, and then centrifuged for 5 min at 3000rpm. This latter step is necessary since the biotinylated cells can not easily bind to the avidin modified glass because of its slow diffusion rate and the large size relative to the biotin-avidin bonds. Centrifugation makes the cells contact the surface more tightly and significantly increases the retention of mother cells throughout their lifespan.

Time-lapsed imaging and quantification

Nikon TE2000 microscope was used to take time-lapsed images. Chips were mounted on the microscope in a customized holder printed by a 3D printer. We took images once every 10 minutes for all the experiments described in this paper. Fluorescent channels were recorded once every 2 hours to minimize photo bleaching and photo damage to the cell. For a single channel, about 2 positions per chamber (a total of 40 positions) were recorded under 60x/100x oil lens. On average at each position we can track 3 – 5 mother cells throughout their lifespan.

Customized version of cellseg 5.4, developed by Kaiyeung Lau (Chao Tang Lab at UCSF, unpublished software) and Zhengwei Xie, was used to segment the bright field images and to quantify the fluorescent signals.

The replicative lifespan of each individual mother cell (the number of daughters produced through its lifetime) was measured with the customized plugin of ImageJ, and the timing of an event can be saved and processed by our scripts to get the lifespan and the budding time interval profiles.

ROS staining and quantification

We used 2',7'-dichlorfluorescein-diacetate (DCFH-DA) to stain ROS in yeast cells. The amount of ROS was quantified by the fluorescence. Cells from the same culture were pumped into four parallel channels on the same chip and were stained and measured at 0, 11, 22 and 33 hours. PBS was pumped in to wash the cells, followed by DCFH-DA staining for 25 minutes. After washing by PBS, fluorescence was measured to quantify the ROS level. After that YEPD was pumped in to continue the tracking of the lifespan.

Measuring redox potential by roGFP

We used a modified GFP -- the roGFP to measure the redox potential of cells as a function of age. The oxidized and reduced forms of roGFP can be excited by light of different wavelengths, 405nm and 470nm respectively, and emit light at the same wavelength (525nm). By measuring fluorescence excited by two different wavelengths, we measured the ratio of the two different forms, which was used to quantify the redox potential.

Strains and Media

All the strains were derived from BY4741 and grown in YEPD media unless specified otherwise. The 3' GFP tagged strain was from the GFP library described by W. Hub et. al [S2] . The fob deletion mutant was taken from the mutant library described by Zheng et. al [S3]. Other strain constructs are listed below:

Strain	Genotype	Parent	Promoter Sequence	Reference
BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	S288C	--	Brachmann et al, 1998 (S4)
Hsp104 activity reporter	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, Δtrp1::[pHSP104]pCyc->Venus (HIS5)	BY4741	AAAACCTTCTGCACCATTTTTAGAAAAAAAGAAT CTACCTATTCACTTATTTATTCATTTACTTATTTATT TACATATTTATCATACATATTAACATTGAACCCTCC ATCGTGGTAGTGTTTGCTGTTCCTAACTTTTCTTT CGTTGTTCTTGTAGATATATATTTTTCCAGAATTTT CTAGAAGGGTTATTAATTACAATCTTAAACGTTCC ATAAGGGGCCGCGATTTTTTTGTTCAATTTTCAAC AGGGGGCCCATCTCAAAGAACTGCAAATTATATC ACAGTAAAAGGCAAAGGGGCGCAAACTTATGCA ACCTGCCAGATTATTATATAAGGCATTGTAATCTT GCCTCAATTCCTTCATAATTCGTTCCTTTGTCACT TGTTCCTTTTTACCCTTGAATCGAATCAGCAATAA CAAAGAAAAAAGAAATCAACTACACGTACCATA AAATATACAGAATA (on chromosome XII from coordinates 88155 to 88621)	This study
Yap1 Activity reporter	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, Δtrp1::[YAP1bs]pCyc->Venus (HIS5)	BY4741	GATCGCTATTACTAATAGCCGTTTAC(From Rpn4 promoter)	This study
Dual reporter	MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS+ Δcan1::STE2pr-spHIS5 Δlyp1::STE3pr-LEU2 cyh2 Δura3::STRE-pcyc1-mKate-HSE-pcyc1-EmGFP	BY4743	8xSTRE: AATTGGTAAGGGGCCAATTGGTAAGGGGCCAATT GGTAAGGGGCCAATTGGTAAGGGGCCTCGAGAA TTGGTAAGGGGCCAATTGGTAAGGGGCCAATTGG TAAGGGGCCAATTGGTAAGGGGCC 4xHSE: CTAGAAGCTTCTAGAAGCTTCTAGAAGCTTCTAG AAGCTTCTAGAGGATCCCCG crippled cyc1 promoter: TCGAGCAGATCCGCCAGGCGTGTATATAGCGTGG ATGGCCAGGCAACTTTAGTGCTGACACATACAGG CATATATATATGTGTGCGACGACACATGATCATATG GCATGCATGTGCTCTGTATGTATATAAAACTCTTG TTTTCTTCTTTTCTCTAAATATTCTTTCCTTATACAT TAGGTCCTTTGTAGCATAAATTACTATACTTCTATA GACACGCAAACACAAATACACACACTAAATTAAT A driving mKate2 and emerald GFP respectively.	This study
roGFP strain	MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS+	BY4743	TDH3 promoter driving roGFP: ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG	This study

	Δcan1::STE2pr-spHIS5 Δlyp1::STE3pr-LEU2 cyh2 Δura3::pTDH3-roGFP		GTGGTGCCCATCCTGGTCGAGCTGGACGGCGAC GTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG GGCGAGGGCGATGCCACCTACGGCAAGCTGACC CTGAAGTTCATCTCCACCACCGGCAAGCTGCCCG TGCCCTGGCCCACCCTCGTGACCACCCTGACCTA CGGCGTGCAGTGCTTCAGCCGCTACCCCGACCAC ATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTT CAAGGACGACGGCAACTACAAGACCCGCGCCGA GGTGAAGTTCGAGGGCGACACCCTGGTGAACCG CATCGAGCTGAAGGGCATCGACTTCAAGGAGGA CGGCAACATCCTGGGGCACAAGCTGGAGTACAA CTACAACTGCCACAACGTCTATATCATGGCCGAC AAGCAGAAGAACGGCATCAAGGTGAACTTCAAG ATCCGCCACAACATCGAGGACGGCAGCGTGCAG CTCGCCGACCACTACCAGCAGAACACCCCCATCG GCGACGGCCCCGTGCTGCTGCCCGACAACCACT ACCTGAGCACCTGCTCCGCCCTGAGCAAAGACC CCAACGAGAAGCGCGATCACATGGTCCTGCTGG AGTTCGTGACCGCCGCCGGGATCACTCTCGGCAT GGACGAGCTGTACAAG	
UPRE activity reporter	MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS+ Δcan1::STE2pr-spHIS5 Δlyp1::STE3pr-LEU2 cyh2 Δura3::UPRE-GFP-URA3	yMJ001	AGGAACTGGACAGCGTGTCGAAAAAGCTCGACA GGAACTGGACAGCGTGTCGAAAAAGCTCGACAG GAACTGGACAGCGTGTCGAAAAAGCTCGACAGG AACTGGACAGCGTGTCGAAA	Jonikas et. al. 2009 (S5)

S1. C. Luo, X. Zhu, T. Yu, X. Luo, Q. Ouyang, H, Ji, Y. Chen *Biotechnol Bioeng.* **101**, 195 (2008)
S2. W. Huh, J. Falvo, L. Gerke, A. Carroll, R. Howson, J. Weissman, E. O'Shea *Nature* **425** 686 (2003)
S3. J. Zheng, J. Benschop, M. Shales, P. Kemmeren, J. Greenblatt, G. Cagney, F. Holstege, H. Li, N. Krogen *Mol Syst Biol.* **6**, 420 (2010)
S4. C. B. Brachmann et al., *Yeast* **14**, 115 (Jan 30, 1998).
S5. M. Jonikas, S. Collins, V. Denic, E. Oh, E. Quan, V. Schmid, J. Weibezahn, B. Schwappach, P. Walter, J. Weissman, M. Schuldiner *Science* **323** 1693

Supplementary Figure Legends

Supplementary Figure 1: Theoretical modeling of the flux distribution. a) Fluid flow in the microfluidic chip in Figure 1 is mapped to current flow in an electronic circuit, with resistance given by the Hagn-Poiseuille formula. b) The calculated fluxes through the twenty chambers.

Supplementary Figure 2: More examples of single cell measurement of the activities of Msn2/4 and Hsf1 (reported by the dual reporter in Figure 3) as a function of age.

Supplementary Figure 3: Yap1 reporter activity as a function of the concentration of H2O2, showing that the reporter is responding to oxidative stress. Cells start to die at the highest concentration 1.22M.

Supplementary Figure 4: Generation versus budding time (of which the difference between two successive budding events gives the budding time interval shown in Figure 5) for individual cells from the *FOB1* deletion strain. Two sub-populations are readily visible: one with uniform budding time intervals, and the other with gradual slowing down of the budding time interval.

Supplementary Figure 5: Hac1 activity does not increase with age in most of the cells. Hac1 activity was reported by a fluorescent reporter driven by a promoter containing a UPRE.