SUPPLEMENTARY INFORMATION

Cell Cycle Synchronization by Nutrient Modulation

Yuan Tian[1, 2], Chunxiong Luo[1, 2†], Yuheng Lu[3], Chao Tang[2, 4], Qi Ouyang[1, 2†]

Microfluidic Chip Fabrication

The chip mold was constructed by patterning photoresist (SU8, Clariant Corp.) on silicon wafer in a classical overlay lithography procedure. The photoresist relief mold consists of two parts: the higher (about 10 μm) main channel and lower (3.5μm) growth chambers which distributed along main channel and connected to main channel with fine necks (40μm wide, also 3.5μm high), see Figure S1. The chip body was fabricated by molding PDMS (polydimethylsiloxane, RTV615, GE Toshiba silicones Co. Ltd. catalyst: base = 1:8) against mold, then cured in an oven at75□ for half an hour, peeled away from master and sealed with a cover slip. The chip bodies are soft, when infused culture medium with syringe pumps, the ceiling of the growth chamber rises a little, so budding yeasts, whose dimension is about 5μm，could be clamped in chambers. There are four equivalent modules in one chip, so four different experiments can be carried out in one chip simultaneously.

G_1 index oscillations under different modulation schemes

As described in the main text, modulating colonies with 60 min poor + 90 min rich medium or 75 min poor + 75 min rich medium alternately can induce the most effective synchronization.

Figs. S2A-G show the G_1 indices of individual colonies used to construct Fig. 4. Here G_1 index curves of four or more colonies (indicated by different colors) correspond to every modulation scheme are plotted, they scatter at the beginning of the experiment then mingle with each other when modulated by 3 or more rounds of starvation.

The steady state phase distribution of budding yeast colonies supplied with rich medium was examined in control experiments; the results are shown in Fig. S2H. During the incubation course of 492 min., 27 cells from 9 different colonies multiplied to a total number of 706, the total G_1 index, however, reached a stationary value between 0.3 and 0.4. Note that the G_1 indices for the individual colonies do not show any obvious oscillatory behavior either (Fig. S2H).

Computational Model

I. The stochastic model used in simulation

Saccharomyces cerevisiae has a cell cycle consisting of three phases: G_1, S and M. It is widely accepted that G_1 phase is the nutrient sensitive phase of the budding yeast -- when subject to poor nutrient conditions, G_1 phase is lengthened more than other phases and cells tend to accumulate in G_1 phase.

To get a quantitative assessment on the lengths and variability of the cell cycle phases for mother/daughter cells and under the rich/poor nutrient conditions used in our synchronization experiments, we cultured cells in the same rich and poor nutrient conditions and measured the time lengths of different cell cycle phases. The results are summarized in Table S1. In our modulation experiments, poor and rich medium are supplied alternately, so we are interested in the behaviors of cell under nutrient switch but not in a continuous environment. Specifically, to get the phase duration for cells in rich medium, we first treated the yeast colonies with poor medium for one and a half hours, and then photographically recorded their growth in rich medium for the following three hours. Every complete G_1 or non-G_1 process in this 3 hours' cultivation, if started in the first 1.5 hours of rich medium, is considered as a sample that can reflect the cell state in the rich medium period of the modulation experiments. We show the distributions of these samples in Fig. 5 (left column), and the mean values and standard deviations in Table S1 (middle row). In the case of non-G_1 phase, all samples were calculated together without distinguishing daughter or mother cells for they are highly similar (the mean length and standard deviation of daughter non-G_1 samples are 66.94 min and 13.64 min, compared to the total mean of 71.48 min).

For the case of poor medium, similar experiment and data analysis were performed except that cells were cultivated in poor medium for 8 hours (no prior rich medium treatment was introduced because cells had been supplied with adequate nutrient during hours of experimental preparation) and the start time limitation of non-G_1 samples was enlarged half hour (poor medium caused G_1 phase accumulation, non-G_1 samples were rare close to nutrient switch). The results are shown in Fig. 5 (right column) and Table S1 (lower row). The mean length and standard deviation of daughter non-G_1 samples are 110.5 min and 54.69 min, compared to the total mean of 107.8min.

	G_1	Non-G_1

	Mother	Daughter	
Rich nutrient	15.92(4.06)	44.85(20.68)	71.48(19.18)
Poor nutrient	128.9(73.66)	173.8(80.45)	107.8(48.34)

Table S1: Mean duration and standard variation (in brackets) of cell cycle phases.

The unit is in minutes.

According to our observations, there is a sizable variation in all the phase lengths. So one of the key issues in our modeling is to capture the variations in the cell cycle behavior in a population. We use the following model to fulfill this goal.

Each cell cycle phase is divided into k steps, where k depends on cell type, nutrient condition and the cell cycle phase. Every cell in a population at any given time is at one of the steps. In every round of evolution (in our simulation time step = 10 sec), cells can finish their current step and enter the next one irreversibly with a probability p (which depends on cell type, cell cycle phase and nutrient conditions). So the progression through the cell cycle is regarded as a sequence of events with a transition probability from one event to the subsequent event.

According to the probability theory, the time j (in terms of simulation rounds) needed by a cell to finish a cell cycle phase consisted of k steps satisfies the negative binomial distribution:

$$N(j) = C_{j-1}^{k-1} p^k (1-p)^{j-k} \qquad (1)$$

To derive the parameter k_i and p_i (where i denotes different conditions: mother/daughter cell, rich/poor medium, G_1/non-G_1 phase), we fitted the negative binomial distribution to measured phase length data with the help of Matlab Statistics Toolbox. Table S2 summaries our model parameters derived in this way.

	G_1		Non-G_1
	Mother	Daughter	
Rich nutrient	k_1=9, p_1=0.1098	k_2=5, p_2=0.0197	k_3=14 p_3=0.0338

Poor nutrient	k_4=4, p_4=0.00527	k_5=5, p_5=0.00486	k_6=6 p_6=0.009492

Table S2: Model parameters derived from the experimental data. The transition probability is per 10 sec.

□. Simulation of the dynamical course of cell cycle synchronization

Stochastic simulations of the model were carried out to demonstrate that the synchrony is due to the nutrient modulation and to capture the population behavior when various modulation schemes were applied. The initial G_1 index of the simulation run is set to 33% and the cells are distributed uniformly on different steps. When a cell finishes all the *k* steps in non-G_1 phase, a daughter cell is born into the population. The G_1 index was calculated at the interval of 5 min.

If the medium is switched in the middle of a cell cycle phase, it may be mapped to a non-integer position of the corresponding course of the counterpart nutrient environment. In this condition, we round the non-integer step number with probability. For example, when the nutrient switched from poor to rich, a mother cell who finished 3 steps of its G_1 phase will be mapped to the 6.75 ($9\times3/4$) steps of the rich medium G_1 course. Then we set the step number to 6 with a chance of 25% or to 7 with a chance of 75%.

Synchronization of the Schizosaccharomyces pombe

To demonstrate the generality of our synchronization method, the similar modulation schemes were also applied to fission yeast populations **(**The rich medium used in fission yeast experiments was YE5S, the poor medium was 2.5% YE5S aqueous solution**)**.

The cell cycle of fission yeast can be divided into four phases: G_1, S, G_2, M. Prevoius studies suggested that the fission yeast uses two size thresholds to coordinate its growth and division, one at the entrance of the mitosis, which plays the major role in the nutrient-rich environment; the other at the end of the G_1 phase, which will be in action when the cells are in poor medium (1, 2). So a reasonable period of starvation will result in an accumulation in G_1 phase, which can potentially synchronize the cell cycle (3, 4). Here we considered the separation of the two daughter cells but not the division of nucleus as the beginning of the cell cycle, so the increase of the total cell number in a colony can be calculated by counting newly cleaved septa. Then we

introduced septum index—the fraction of cells with septa, which can be regarded as a mitotic index—to measure the synchronization degree of a fission yeast colony (5). As in the case of budding yeast, the G_1 cell fraction should arise during the poor medium periods, this means that the septum index culminates at the end of rich medium stages, which is confirmed by our experiments (Fig. S3).

REFERENCES

1. Nurse P, Thuriaux P (1980) Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. *Genetics* 96:627-637

2. Nurse P (1975) Genetic control of cell size at cell division in yeast. *Nature* 256:547-551

3. Sando N (1963) Biochemical studies on the synchronized culture of schizosaccharomyces pombe. *J Gen Appl Microbiol* 9:233-241.

4. Simanis V, Hayles J, Nurse P (1987) Control over the onset of DNA synthesis in fission yeast. *Phil Trans R Soc Lond B* 317:507-516.

5. Mitchison J (1970) in *Physiological and cytological methods for Schizosaccharomyces pombe* (Academic Press, London, UK).

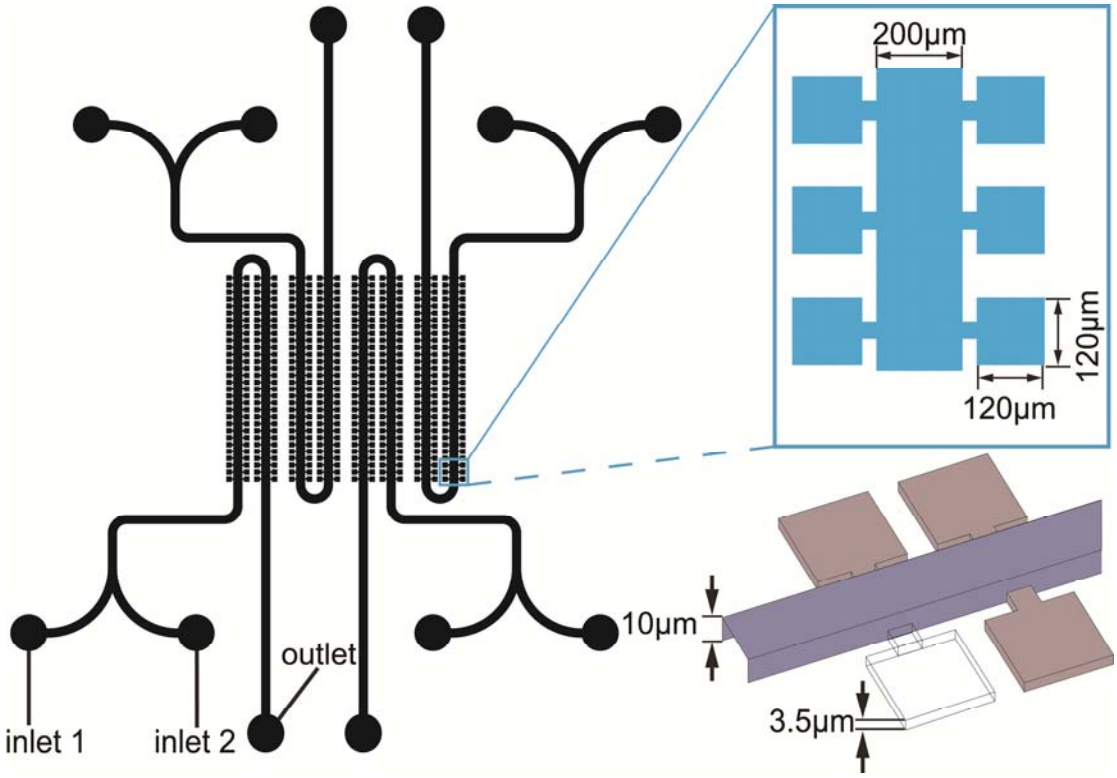

Figure S1. The chip layout planar graph and 3D structure. Left, the profile of the microfluidic chips used in this work. **Top right,** the dimensions of the main channels and the growth chambers. **Bottom right,** the 3D structures and vertical dimensions of the main channels and the growth chambers.

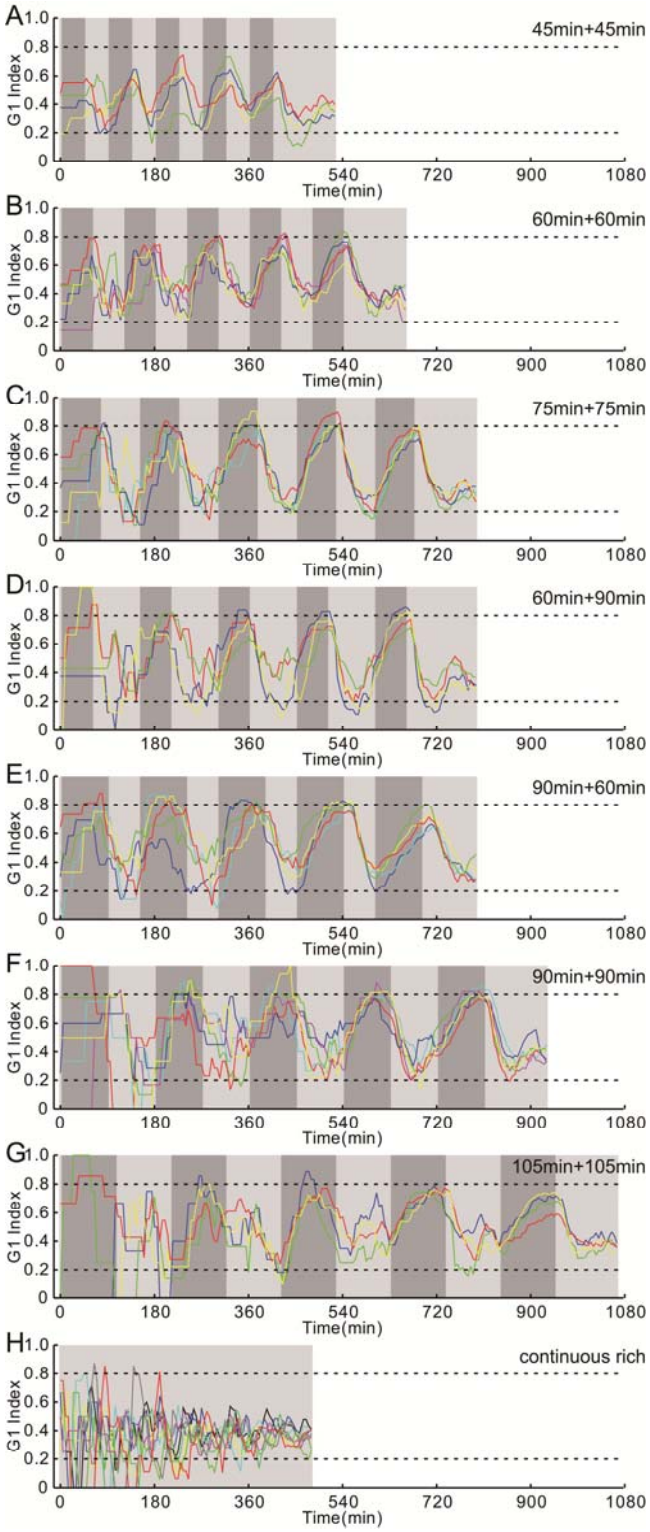

Figure S2. G_1 index oscillations of different colonies under different modulation scheme (Dark grey areas indicate poor medium stages and light grey areas rich medium)**: A,** 45 min. poor medium (p) + 45 min. rich medium (r). **B,** 60 min. p + 60 min. r. **C,** 75 min. p + 75 min. r. **D,** 60 min. p + 90 min. r. **E,** 90 min. p + 60 min. r. **F,** 90 min. p + 90 min. r. **G,** 105 min. p + 105 min. r. **H,** continuous rich medium.

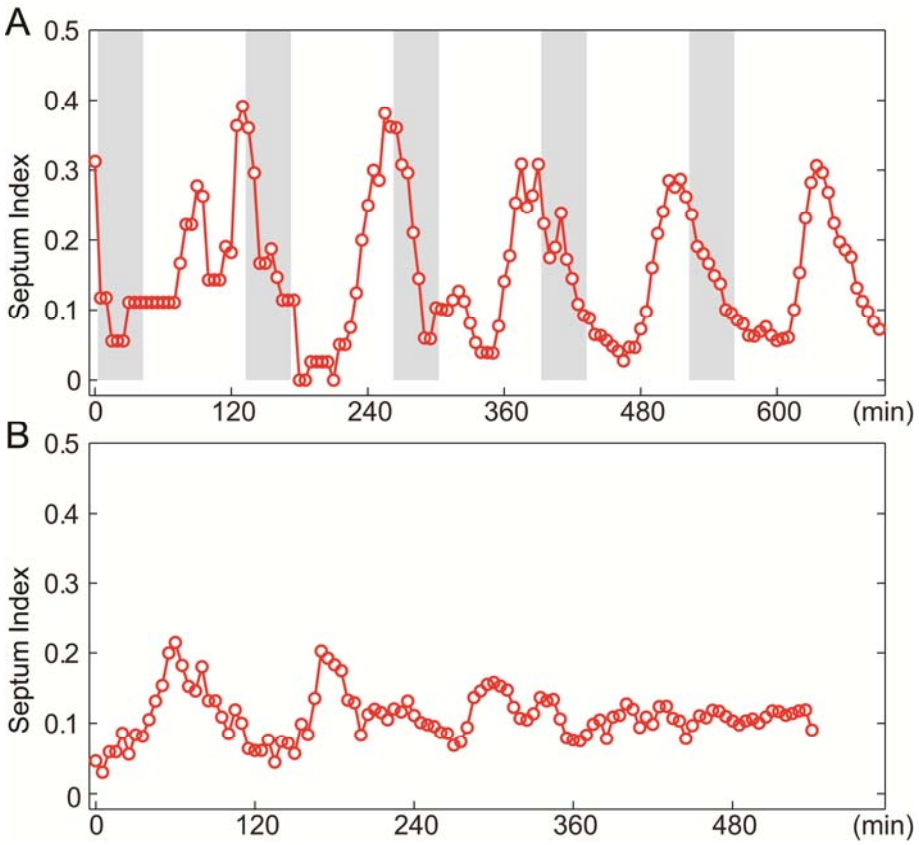

Figure S3. The septum index of fission yeast colonies under the modulation of an optimized scheme (40 min. poor1.5% medium + 90 min. rich medium), grey background indicate poor medium stages. a, the overall statistics of fission cells from 5 different chambers modulated with the same scheme (the total cell number at the end of the curve is 513). **b,** the overall statistics of fission yeast cells from 6 different chambers with continuous rich medium supply (the total cell number at the end of the curve is 544).