Supplementary Information

Table S1: Microfabrication process overview

Layer	Height	Resist	RPM	PB, 65°	SB, 95°	Exposure	HB, 95°
DAW channels	15μm	2015	3300	1 min	3 min	150 sec	4 min
chaotic mixers	5μm	2005	3000	1 min	2 min	120 sec	3 min
main channels	40μm	2015	1000	2 min	4 min	2 doses, 120 sec ea.	10 min

RPM is rotations per minute, PB is pre-bake, SB is soft-bake, HB is hard-bake

Cell loading protocol

A 3mL syringe connected to a one foot section of PTFE #24 AWG tubing interfaced via a 23-gauge sterile luer stub is used to first wet the device channels prior to cell loading as follows. All culture media for microfluidic use is passed through a 0.22μm filter (Millipore SLGP033RS) to remove debris. A small volume ($\sim$100μL) of complete media is aspirated into the end of the tubing by using the syringe and the tubing is then connected to port 5 of the device. Pressure is applied until fluid fills all of the ports except number 1. The tubing is then removed from port 5 and wet-connected to port 2 (a wet-connect means a fluid droplet from the tubing is connected to the fluid resting on top of a port prior to applying syringe pressure in order to ensure bubbles do not enter the system). Pressure is gently applied to fill port 1 and the device channels are now fully wetted with media, except for the culture traps which remain filled with air. A new one foot section of tubing is now attached to the same syringe to replace the old tubing, and a small volume of cell suspension is aspirated. The tubing is then connected to port 1 of the device. To prepare the vacuum connection, the house laboratory vacuum is interfaced with a 20 gauge luer stub using via 0.030" ID Tygon microbore tubing and the luer end is plugged into port 3 of the air channel. Port 4 is

closed off by plugging in a luer stub fitted with a cap. The vacuum is initially OFF, and the cell suspension in the PTFE tubing is connected to port 1 and pressure is gently applied to the syringe until cells begin to fill the main perfusion channel. The flow is then stopped by ceasing to applying pressure at the syringe and the presence of a high density of cells in the main channel is confirmed visually under an inverted tissue culture microscope. At this point the vacuum is turned ON and the progress of cell loading into the culture chambers is visually monitored. Gentle tapping of the tubing containing the cells can enhance cell loading during this process by ensuring that cells move with the fluid flow into the traps rather than getting snagged on the entry-ways or due to the friction with the glass coverslip. Once the traps are completely filled, the vacuum is turned off and the line connections to the air channels are disconnected to relieve any remaining vacuum. It is now necessary to flush the untrapped cells out of the device. A new one-foot section of tubing is connected to the syringe and a small volume of complete media is aspirated and then the tubing is wet-connected to port 1 of the device. Pressure is applied to force cells in the main channel to flow out of the remaining ports of the device. Due to the extremely high resistance of the culture traps to convective flow, the cells there are effectively retained even during the high flow rate of this flush step. The device is now ready for experimental setup on a microscope stage for time-lapse imaging, or it can be precultured in a standard tissue culture incubator to allow cells to attach and proliferate for a desired amount of time.

Electronic Supplementary Material (ESI) for Lab on a Chip

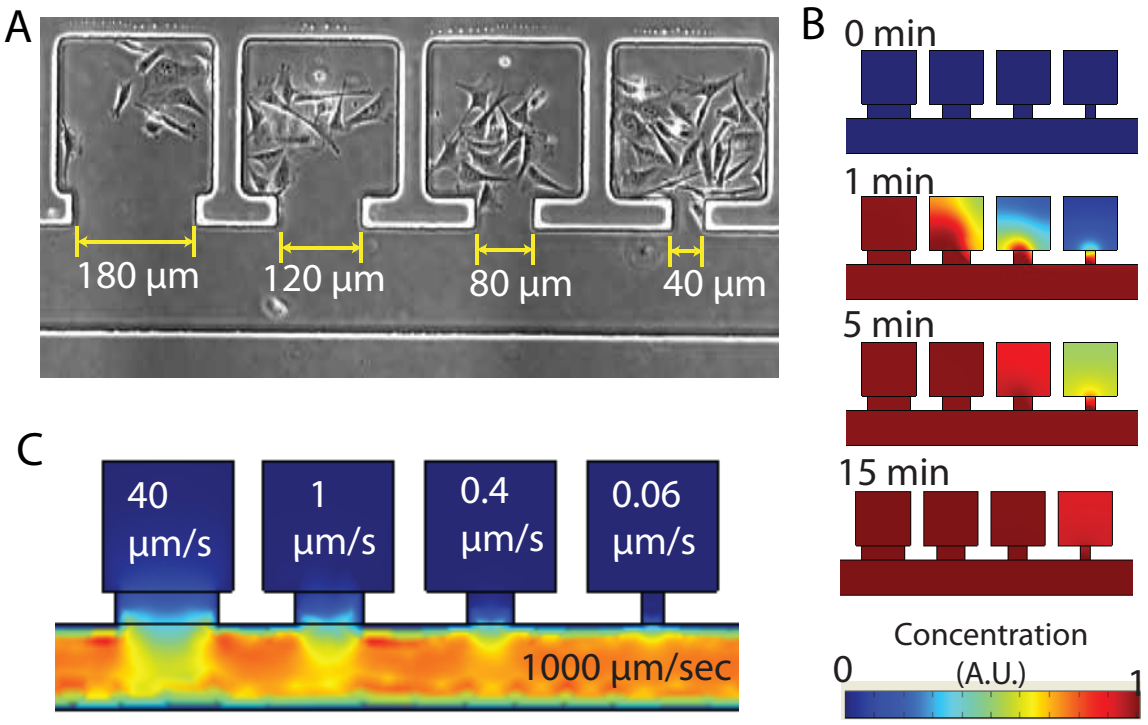

Figure S1. Tunability of mass transport. (A) Culture traps of various width openings are reliably vacuum-loaded with cells (3t3 fibroblasts shown). Larger trap openings facilitate faster diffusion of small molecules (B) as well as a higher rate of convective flow (C).

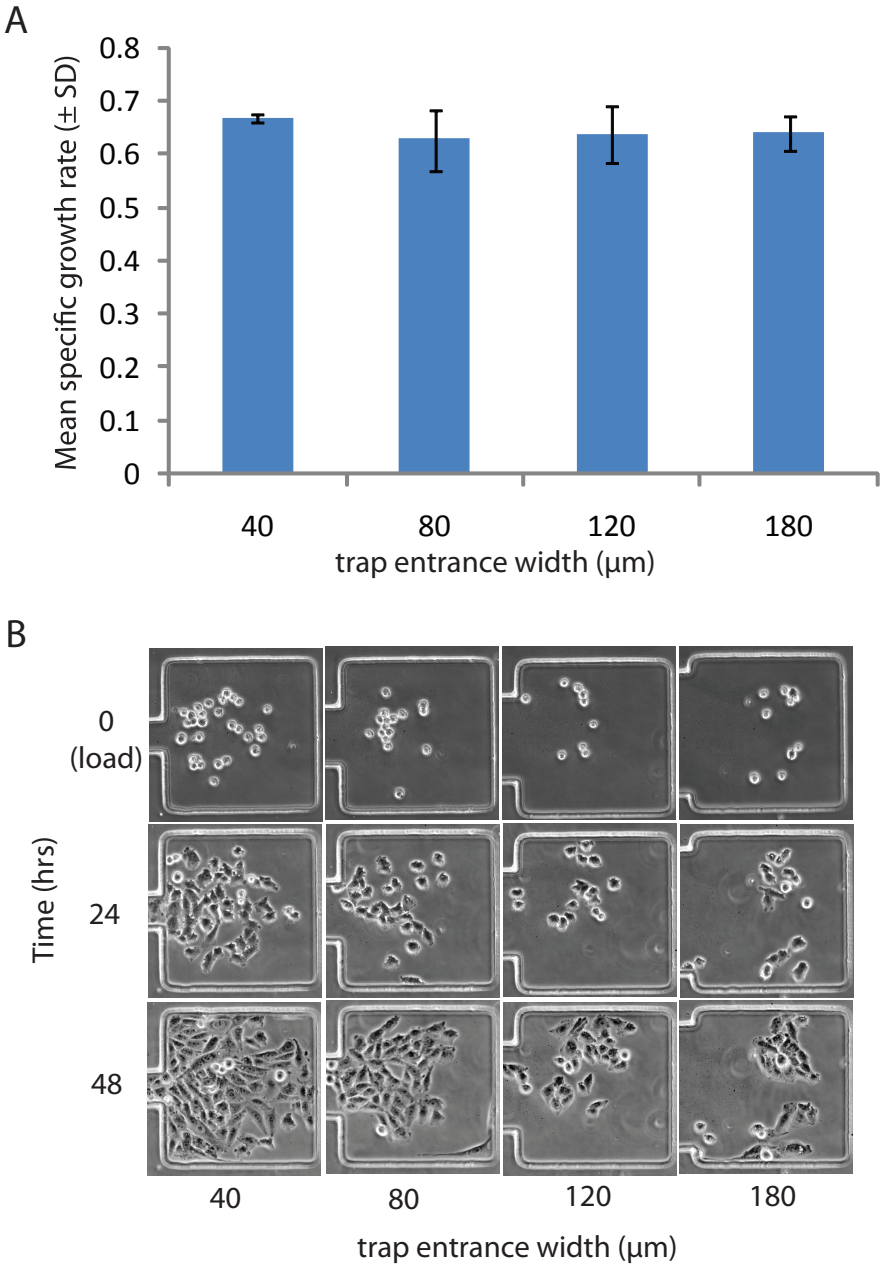

Figure S2. CHO-K1 cell growth in traps with various entrace widths. (A) The mean specific growth rate remains unchanged among the four trap entrance widths tested. The data represent the average of three independent device experiments, each containing 32 traps (8 copies of the four different trap widths). The pump perfusion flow rate for all experiments was 5μL per hr. (B) Larger trap entrance widths typically result in fewer cells loaded at the start of the experiment (top row), but cell growth for all trap sizes is characterized by normal morphology and robust proliferation.

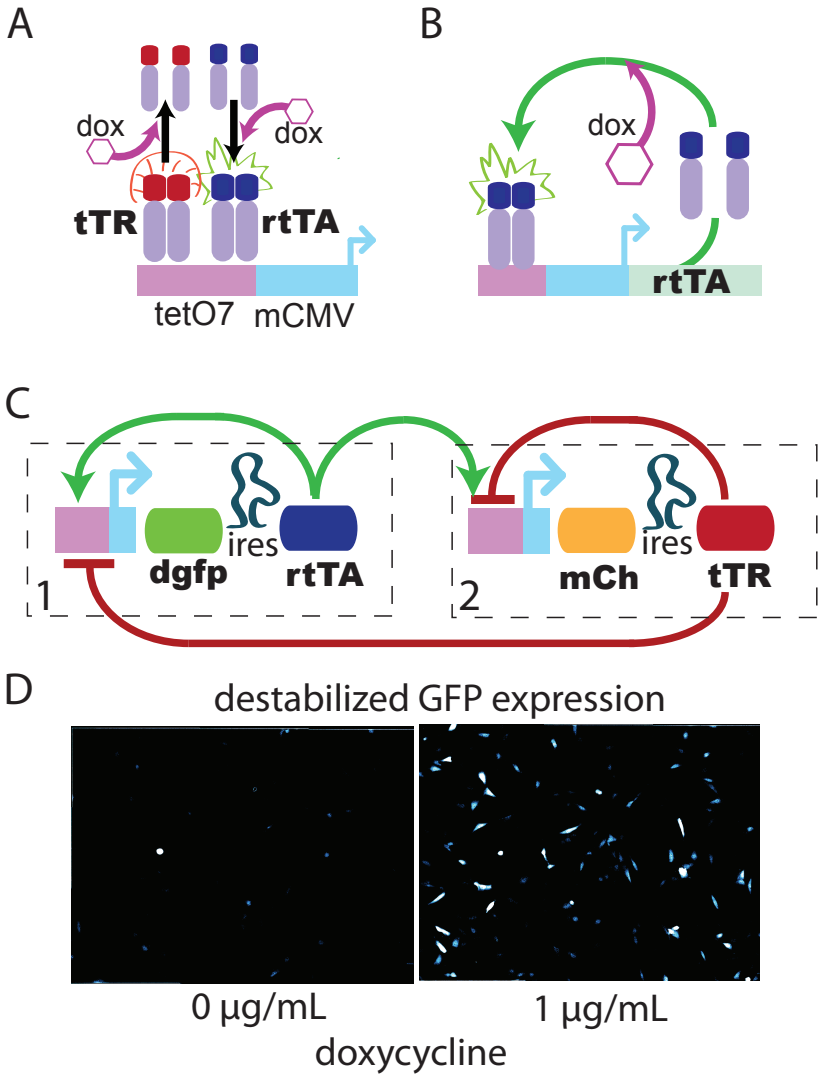

Figure S3. The fluorescent reporter cell line is a 3t3 mouse fibroblast line that has been engineered to express the reverse tet-transactivator (rtTA) and the tet-transrepressor (tTR) to control expression of destabilized GFP (Li *et al.*, 1998) from a hybrid tetO7mCMV promoter (Rossi *et al.*, 1998). (A) rtTA activation of the promoter is induced by doxycycline (a tetracycline analogue), while tTRG repression is inactivated by dox . (B) The expression of rtTA from the hybrid promoter forms a positive feedback loop whereby production of rtTA activates the promoter and makes more rtTA. (C) The reporter cell line was constructed by retroviral integration of the positive feedback portion [1] of the gene circuit and selecting for a highly expressing clone, which was then further transduced with the negative feedback component [2]. mCh is mCherry, an additional fluorescent reporter. ires stands for "internal ribosomal entry site" which allows bicistronic expression in these constructs. (D) The combined dual-feedback design allows a very low basal level of GFP expression without doxycycline, and a rapid induction to high expression in the presence of the inducer (20 hrs of induction shown).

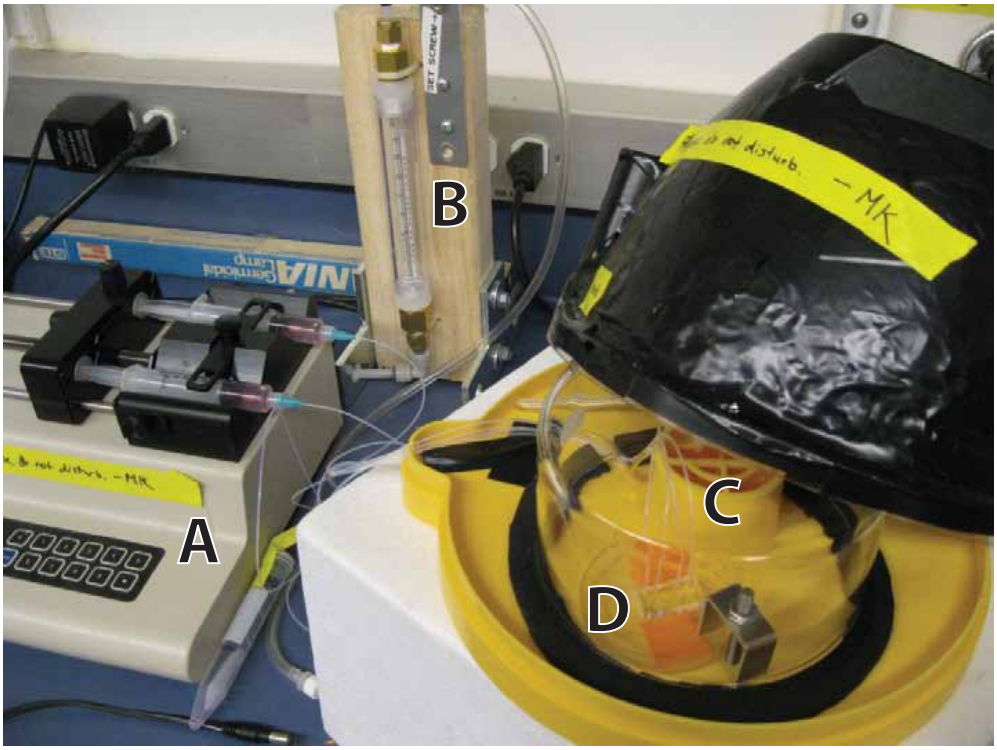

Figure S4. Custom incubator to interface with syringe pump. (A) The programmable syringe pump allows for the control of two syringes to maintain a precise flow-rate of culture medium to the devices inside the incubator. (B) A custom gas-flow regulator consisting of a flow meter (Gilmont Instruments GF-2160) coupled to a needle valve (McMaster-Carr 48965K14) allows fine-tuning of the 5% CO_2 flow rate (50mL/min) from a cylinder and regulator to the water bath in the central region of the incubator. (C) The egg incubator (Brinsea 6011260) consists of a yellow plastic base with a central water holding column to provide humidity and a heat source and sensor in the removable lid that has been covered with black electrical tape to prevent light from damaging the cells. (D) The two devices are taped to a 3cm culture dish which is held in place with a metal clamp. A glass enclosure dish (Pyrex 3140, 125 x 65 mm) surrounds the devices and sits on top of a black rubber seal to ensure the humidified CO_2 bubbling through the water column is retained.

References

Li X, Zhao X, Fang Y, Jiang X, Duong T, Fan C, Huang C, Kain S (1998) Generation of destabilized green fluorescent protein as a transcription reporter. *Journal of Biological Chemistry* **273**: 34970–34975

Rossi F, Guicherit O, Spicher A, Kringstein A, Fatyol K, Blakely B, Blau H, *et al.* (1998) Tetracycline-regulatable factors with distinct dimerization domains allow reversible growth inhibition by p16. *Nature genetics* **20**: 389–393