## Supplementary notes

Bottomless 96-well plate: A square reservoir was created in the center of a Greiner bio-one clear bottomless 96-well plate (#82050, VWR, Radnor, PA) by cutting and removing the center 16 wells, leaving the surrounding 80 wells to serve as input reservoirs, followed by edge scraping to give a smooth surface across the bottom of the plate.

PDMS interface: A PDMS block containing 3-mm holes was exclusion molded from a master fabricated using 0.25" thick acrylic sheets (Small Parts, Logansport, IN) cut using a CO2 laser (Universal Laser Systems, Scottsdale, AZ). Two laser-cut acrylic sheets were assembled using a thin double adhesion mylar sheet: a 5.6" long, 3.75" wide, 0.25" thick block, and a rectangular frame with outer dimensions of 5.6"x3.75"x0.25" and inner dimensions of 4.5"x2.9"x0.25". Uncured PDMS (mixed at 1:10 crosslinker:prepolymer ratio) was poured in the rectangular master and a thin release liner sheet (3M, St. Paul, MN) was sandwiched between the master and the acrylic block. Two rectangular metal blocks were used to sandwich the full structure and a no-twist C-clamp (#5046A18, McMaster-Carr, Santa Fe Springs, CA) was used to compress the sandwich structure for exclusion molding by baking at 70 $^{o}$C for at least 4 hours. After the PDMS was cured, the sandwich structure was disassembled and the rectangular PDMS block was created by detaching the cured PDMS from the master. Next, the rectangular PDMS replica was aligned and attached to the bottom surface of a bottomless 96-well plate. A colored marker was used to mark the center of individual well reservoirs on the PDMS replica. Further, a 3-mm Harris Uni-Core biopsy punch (Ted Pella, Inc., Redding, CA) was then used to create an array of holes following the marks. Finally, a square block was cut off from the center of the PDMS replica to match the modified bottomless 96-well plate.

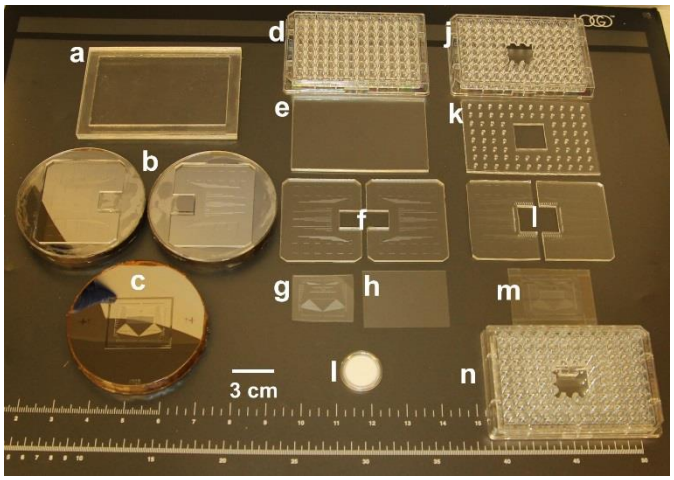
PDMS channel network: For fabrication of the PDMS channel network master molds, two silicon wafers were spin-coated with SU-8 2075 photoresist (Microchem Co., Newton, MA) at 3000 rpm to pattern microchannels using single layer photolithography. After two SU-8 masters were made, laser-cut acrylic sheets (outer dimension: 4" diameter, 0.125" thick, inner dimension: outline of the microchannel design and 0.125" thick) were adhered to the silicon wafers to define the PDMS replica shape. Exclusion molding was then used to create a uniform thickness of the PDMS replica (the thickness of the PDMS replica is defined by the thickness of the acrylic sheets). Once the PDMS channel network replicas were made, a 0.5-mm biopsy punch was used to punch through the PDMS replica to connect well reservoirs and through the PDMS channel interface and network to the microfluidic chip.

PDMS microfluidic chip: Multilayer photolithography and PDMS exclusion molding were used to fabricate the PDMS microfluidic chip. Delivery channels were patterned with a 60 µm thick layer of SU-8 2075 (spin-coated at 2000 rpm) on a silicon wafer. Next, an additional 220 µm thick layer of SU-8 was spin-coated to pattern inlets, outlet, and open channels on the silicon wafer. After photolithography, the SU-8 masters were hard baked at 200 $^{o}$C on a hotplate. Masters were then coated with fluorinated-trichlorosilane (#448931, Sigma, St. Louis, MO) using several drops of the silane solution in a desiccator under vacuum. Exclusion-molding was then used to produce a 280 µm PDMS microfluidic membrane with open inlets, outlet, and channels. The SU-8 master mold was placed on a 4" diameter, 0.5" thick steel disk. Uncured PDMS (1:10) was mixed, degassed, and then poured onto the master. A sheet of polyester film was treated with oxygen plasma and placed onto the uncured PDMS. Air bubbles trapped within the SU-8 features and uncured PDMS to be scraped off using a razor blade. Four additional sheets of polyester film and two 75 mm x 50 mm x 1 mm glass slides were stacked on top of the mold to add compliance to the stack. Finally, a steel disk was placed on top and the assembly was compressed using a no-twist C-clamp prior to baking at 70 $^{o}$C for at least 2 hours in a convection oven. After disassembly, the PDMS replica was peeled off along with the sheet of polyester film, then bonded on a 65 mm x 48 mm x 0.15 mm glass coverslip using oxygen plasma followed by removal of the polyester film. Open features (inlets, outlet and channels) were checked with a dissection tweezer under a Nikon SMZ 1500 microscopy (Nikon Instruments, Melville, NY). Scotch packaging tape (3M, St. Paul, MN) was then used to cover the glass surface area outside of the PDMS replica.

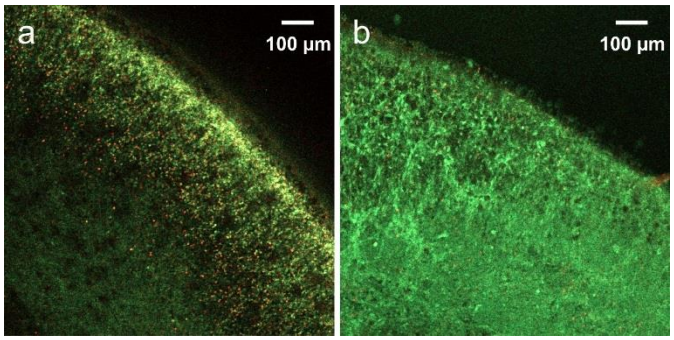
Bonding of PDMS to PDMS and to glass is done by oxygen plasma treating surfaces using 60 W at 670 mTorr for 60 s, and at 40 kHz RF from a Zepto plasma system (Diener Electronic GmBH, Ebhausen, Germany). Bonding of PDMS to PS plastic (bottomless 96-well plate) is done by silane coupling using (3-aminopropyl)triethoxysilane (#A3648, APTES, Sigma, St. Louis, MO) to modify plastic surfaces prior to bonding with oxygen plasma treated PDMS at room temperature. The order of assembly was bottomless 96-well plate and PDMS interface, followed by two PDMS channel network parts that were carefully aligned and bonded onto a PDMS microfluidic chip. These two assembled components were then bonded together to complete the four layer device. A simple outlet was created by cutting a cube of PDMS with a 0.5-mm punch followed by bonding onto the outlet port of the PDMS microfluidic chip by oxygen plasma. Finally, uncured PDMS (1:10) was carefully dripped into gaps between channel network parts to fill gaps prior to curing on a hotplate at 80 $^{o}$C for at least 1 hour.

A 96-well plate lid with a 4-mm hole above the outlet port was used as a cover for culture experiments. A 19 gauge, 0.5" long metal pin and 1 inch segment of Tygon tubing (Cole Parmer, Vernon Hills, IL) was then connected in the outlet port to complete the device.

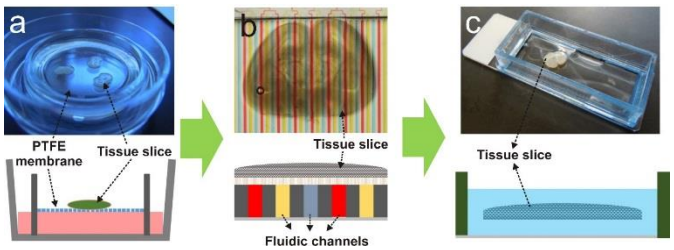
## Supplementary material



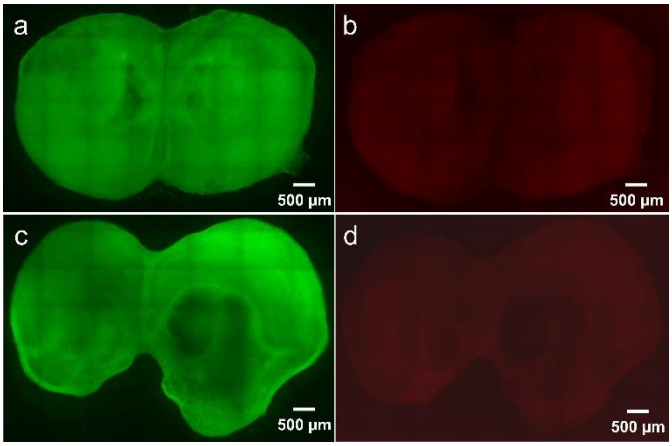

**Supplementary Figure 1.** Individual parts of masters and PDMS molds. (a) Acrylic master for molding PDMS interface layer. (b) Masters for channel network layers. (c) Master for microfluidic chip. (d) A bottomless 96-well plate. (e) A mold of a PDMS interface layer. (f) Molds of PDMS channel network layers. (g) Exclusion-molded PDMS microfluidic chip layer. (h) A glass coverslip. (i) PTFE porous membrane well insert. (j) Modified bottomless 96-well plate. (k) Modified PDMS interface layer. (l) Modified PDMS channel network layer. (m) PDMS microfluidic chip. (n) Completed 96-well plate-based microfluidic device.



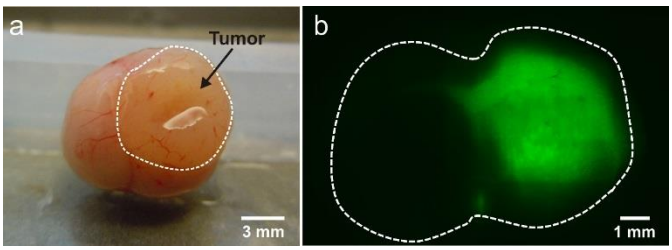

**Supplementary Figure 2.** Viability comparison of brain slice culture using PET and PTFE membrane well inserts. E18 mouse brain slices were cultured on PET porous membrane (a) and PTFE porous membrane (b) under the same culture condition. After 3 days in culture, calcein AM (green) and EthD-1 (red) were used to determine the viability of the brain slices. Confocal microscopy was used to acquire staining outcomes. The calcein/EhtD-1 ovelays above clearly show that the viability of brain slices is vastly superior in slices cultured on PTFE membranes (b) than in those cultured on PET membranes (a).



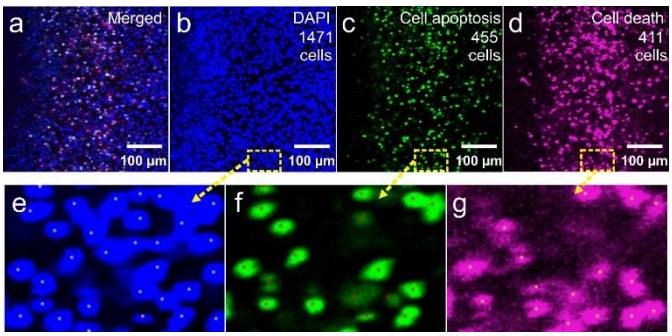

**Supplementary Figure 3.** The PTFE membrane transfer technique. The bottom schematics represent the cross-sections of the top images. (a) Tissue slices organotypically cultured above a hydrophilic PTFE porous membrane in a well insert. (b) The membrane with the tissue slice can be cut and transferred to our microfluidic device for multiplexed drug exposure. (c) The tissue slice can be removed from our device and transferred to a glass coverslip bottom chamber, where the slice can be processed for all of the standard pathology protocols of tissue staining and imaging.



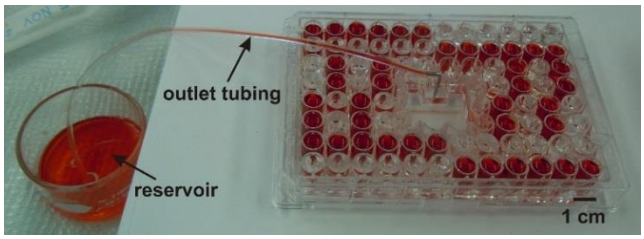

**Supplementary Figure 4.** An E18 coronal mouse brain slice was cultured in our microfluidic device with the total flow rate of 600 µL/hr for 2 days (a-b) and 150 µL/hr for 7 days (c-d), followed by staining with (a) calcein AM and (b) EthD-1,and imaging using epifluorescence microscopy. The grid patterns on both images are image stitching artifacts due to uneven illumination.



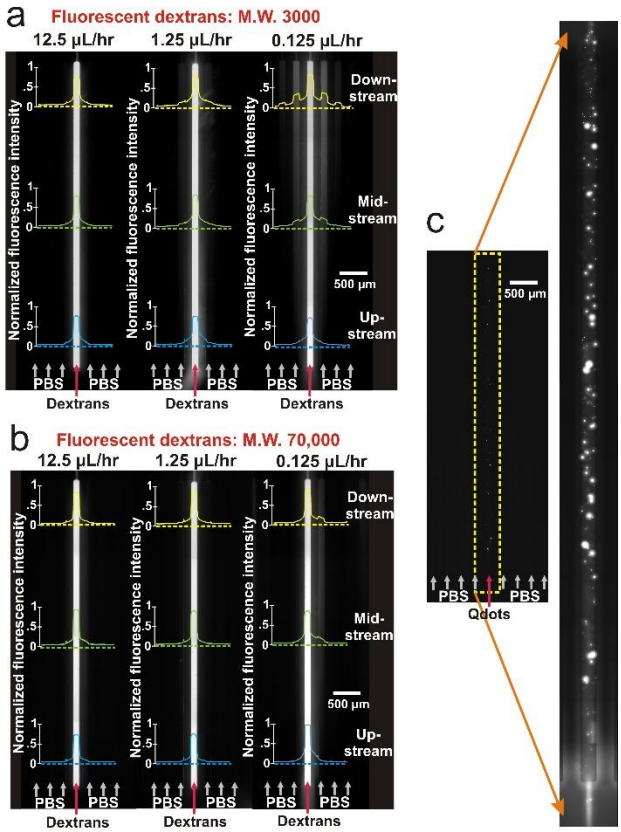

**Supplementary Figure 5.** GBM xenografts. (a) A GBM tumor grown in one hemisphere of a mouse brain (arrow). Human glioma cells were orthotopically injected in a mouse brain and grown *in vivo* for 28 days prior to the extraction of the tumor. (b) A 400 µm-thick GBM xenograft slice; the glioma cells are labelled with GFP (green).



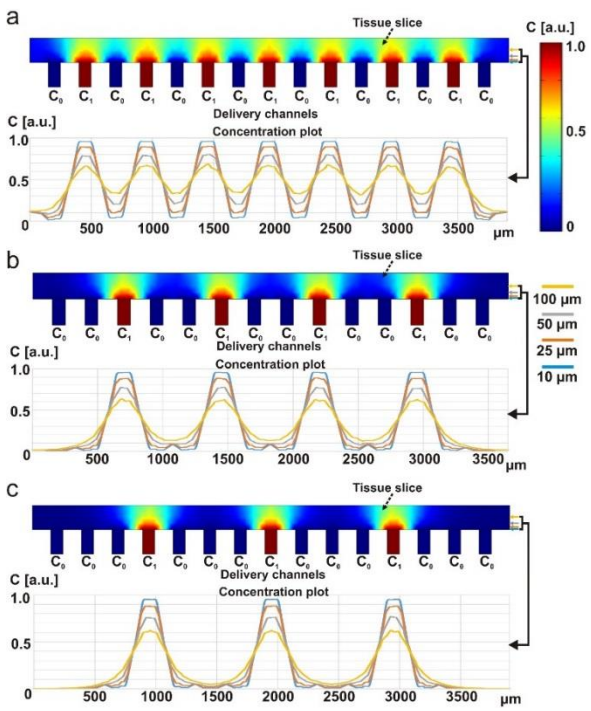

**Supplementary Figure 6.** Automated cell count. (a) 3 channel merged confocal image. (b) DAPI confocal image. (c) Cell apoptosis confocal image. (d) Cell death confocal image. (e-g) Selected regions with automated counting. Yellow dots (e,g) and red dots (f) in the middle of each cells show cell recognition.



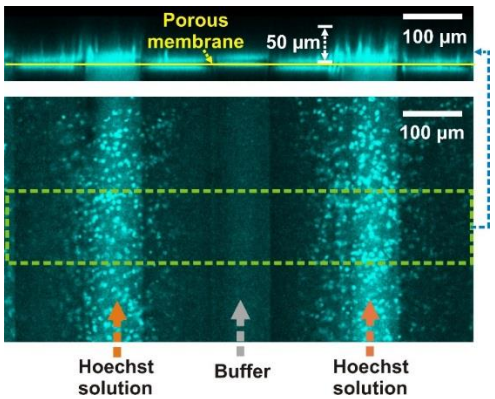
**Supplementary Figure 7.** Operation of the device by syphon. A tubing connected to the device outlet conveyed liquid down to a lower level of a reservoir.



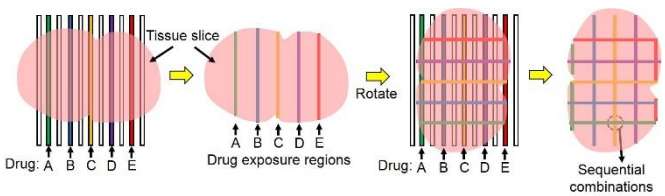

**Supplementary Figure 8.** Molecular weight-dependent behaviour reveals that fluid transport is dominated by diffusion in PTFE porous membranes. (a,b) The extent of lateral diffusion within the membrane scales with the flow velocity underneath the membrane. (c) Quantum dots with 5~20 nm in diameter flow through a microchannel in our device.



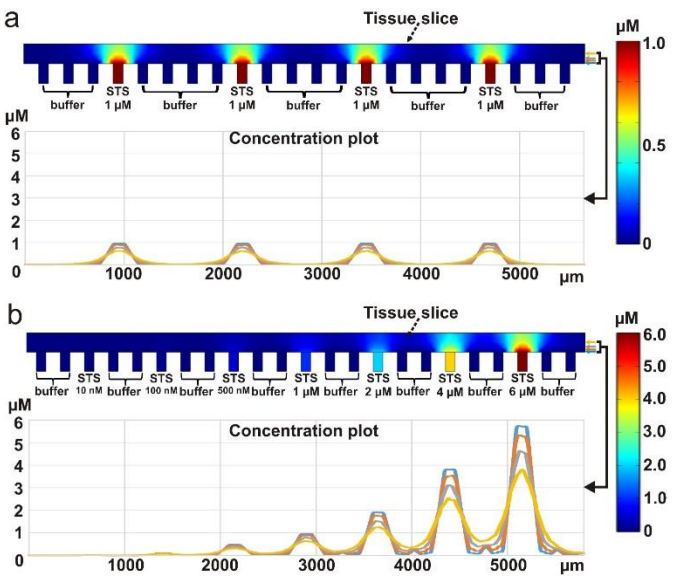

**Supplementary Figure 9.** Purely diffusive model of the expected steady-state concentration profile corresponding to a drug of MW = 466 and D = 5.7 x $10^{-10}$ $m^2s^{-1}$. Concentration plots below the 2D concentration profiles show the concentration 50 µm above the delivery channels (within the slice). Note how the buffer channels act as sinks that appear to counteract the effect of diffusion. (a) Sinks formed by a single microchannel. (b) Sinks formed by clusters of two microchannels. (c) Sinks formed by clusters of three microchannels.




**Supplementary Figure 10.** A confocal image (top: penetration in the slice; bottom: one of the optical sections) demonstrates the penetration of Hoechst dye (16 µM) into a live mouse brain slice after 1 hour of delivery. The top image shows the presence of fluorescent cells up to ~50 µm into the slice, indicating that Hoechst dye penetrated at least 50 µm.

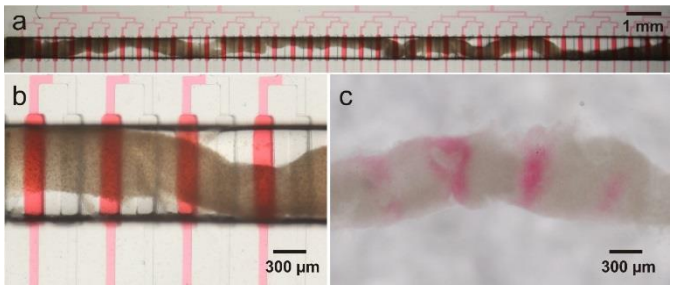



**Supplementary Figure 11.** Schematic of sequential drug delivery for drug testing on an intact tissue. After delivering the first set of drugs, the porous membrane can be rotated 90 degrees to deliver a second set of drugs. Hence, two sets of drugs can be delivered sequentially and orthogonally to a tissue slice.




**Supplementary Figure 12.** Purely diffusive model of the expected STS concentration profile corresponding to the experiments of Figure 3. (a) Concentration profile produced by four equal 1 µM/18 hr doses delivered

through intervals of four buffer channels in between one STS delivery channel. (b) Concentration profile of different doses with intervals of two buffer channels in between one STS delivery channel. The concentration plots in (a) and (b) show the concentration experienced by the cells at 10 µm, 25 µm, 50 µm, and 100 µm above the delivery channels.




**Supplementary Figure 13.** Perfusion of core tissue biopsies from mouse liver using our microfluidic device. (a) Two mouse liver biopsies were extracted and placed in our microfluidic device while the red colored streams were delivered underneath the biopsy tissues. (b) After red colored dye was flowed and flushed with HBSS, (c) the dye selectively transferred from the bottom streams into the liver biopsy.