Supplementary Information 1 – Device Fabrication and Operation

As shown in Fig. 1c, the perfusion culture device consisted of: (1) a commercial PS bottomless 96-well plate (Model 655-000, Greiner Bio-One), (2) 3 PDMS layers with each layer being about 1 mm-thick, (3) PC membrane discs with 6 mm in diameter and 11 μm in thickness, (4) a 1.2 mm-thick glass layer, and (5) PDMS plugs. As illustrated in Fig. 1b, the PDMS layers were used to: (1) provide a fluidic channel of 2 mm thick and 6 mm wide between the inlet and outlet chambers and (2) anchor the placement of the PC membrane discs in the culture chamber during the device assembly. The PC membrane discs were cut and punched out from PC membrane sheets (PCT1047100, Sterlitech) using a hole puncher (3/8-Inch, EK Tools Circle Punch). The 1.2 mm-thick large glass slide (260230, Ted Pella) was used without any modification.

As illustrated in Supplementary Fig. 1, PDMS Layer 1 was prepared using the following procedural steps: (1) mixing the PDMS precursor and the curing agent (Sylgard® 184 silicone elastomer kit, Dow Corning Corp.) at a 10:1 ratio (~30 g total); (2) transferring the mixture on the well plate lid; (3) degassing the mixture in a vacuum desiccator; (4) placing the well plate onto the PDMS mixture placed on the plate cover while continuing to degas until no bubble was observed; and (5) curing the assembly in an oven at 70℃ for 5 h. These procedures produced the PDMS structure molded into the well plate shape while leaving a ~1 mm-thick layer between the well plate and the plate cover. The plate cover was removed from the PDMS/well plate assembly. PDMS Layers 2 and 3 were prepared in a similar manner except that 10 g PDMS precursor mixture was placed onto the outer side of plate cover and was kept horizontal to mold the mixture into the 1 mm-thick flat layer upon curing. The PDMS layer was then peeled off from the plate cover.

As illustrated in Supplementary Fig. 2, PDMS layers were modified by punching holes before assembly. PDMS Layers 1, 2 and 3 were physically aligned and stacked. A hole puncher (33-36-SH, Miltex) was used to punch out holes through the three PDMS layers using a stainless punch die with a diameter of 6 mm (i.e., same as the diameter of wells). In order to make 6-mm wide microfluidic channels between inlet and outlet chambers, the channel areas of the PDMS Layer 3 were cut using a razor blade.

As illustrated in Supplementary Fig. 3, the PDMS layers were then separated to: (1) place PC membrane discs onto the bottom surface of PDMS Layer 1 and (2) bond PDMS Layer 1 and PDMS Layer 2 using oxygen plasma (PDC-001, Harrick Plasma) treatment. Subsequently, PDMS Layer 3 was bonded to the bottom surface of PDMS Layer 2 using plasma treatment. Finally, the large glass slide was bonded to the assembly using plasma treatment.

As shown in Supplementary Fig. 1, PDMS plugs were made in a manner similar to the molding of PDMS Layer 1 into the well plate, but without using the degassing step. The PDMS structures molded into wells were separated from the flat PDMS layer by cutting with a blade and use the separated structures as plugs. In order to provide external fluidic connections, holes were punched through the center of the plugs using a hand press (Schmidt Technology) and a stainless steel round punch (CR0320245N21R4, Technical Innovations) with 0.60 mm inner diameter. Stainless steel pins (NE-1300-01, New England Small Tube) with the outer diameter of 0.63 mm were used to connect the plugs and PE tubing with the inner diameter of 0.58 mm (BB31695-PE/3, Scientific Commodities). For each culture chamber, the flow rate of culture medium was controlled using a syringe pump (NE-1800, New Era Pump Systems. Inc.) and syringes (148232A, BD) or a peristaltic pump (C.P. 78023-22, Ismatec).

The stainless steel pins were autoclaved prior to culture. To sterilize the internal areas of the assembled device, 70% isopropanol (IPA) was pipetted into outlet chamber wells to fill and wash the entire fluidic passage within the chambers and between the chambers to be utilized for cell culture. After 3 times washing with IPA, the fluidic passage was rinsed off 5 times with PBS. After rinsing, the superfluous PBS above the membrane was replaced with correspondence cell culture medium by pipetting. Then the device was seeded with cells and external fluidic connections were made. The device was placed in an incubator during culture while the pump was placed outside the syringe incubator.

Supplementary Information 2 – Materials and Methods for Perfusion Effect on Interactions between Human Multiple Myeloma Cells and Osteoblasts

Materials: BMMCs were previously isolated from multiple myeloma patients' bone marrow aspirate by Ficoll-Paque gradient centrifugation, were frozen, and were stored in liquid nitrogen. Prior to use, BMMCs were unfrozen and labeled with Carboxyfluorescein succinimidyl ester (CFSE, C34554, CellTrace). Bone marrow culture medium was prepared using RPMI with L-glutamine, 6.2×10^{-4} M of $CaCl_2$, 1×10^{-6} M sodium succinate, 1×10^{-6} M hydrocortisone, and 1 unit/ml heparin. The RPMI medium was supplemented with 10% plasma pooled from 10 patients. These procedures were conducted in accordance with the Institutional Review Board–approved protocols at Hackensack University Medical Center and in accordance with the Declaration of Helsinki. Human OSB cell line (hFOB 1.19) was used. For plate reading, OSBs were modified to express green fluorescent protein (GFP-OSBs). A non-target vector (CSHCTR001-HIV-H1, Genecopoeia) contained a coding GFP sequence for easy identification of transduced cells was used. Transduction of hFOB1.19 was performed by spinoculation of viral particles per manufacture's direction (1 h, 25℃, at 800 × g). For flow cytometry, OSBs were used without the GFP modification. To differentiate from GFP-OSBs, BMMCs used in drug evaluation were labeled with a cell proliferation dye (eFluor®670, eBioscience).

Culture Procedures: Prior to OSB seeding, 30 μL of 0.1 mg/ml fibronectin (CB-40008, BD Bioscience) was used to coat the PC membrane in the culture wells for 1 h and washed 3 times with PBS. $2x10^4$ OSB were seeded and cultured in each culture chamber for 4 days using the OSB growth medium which consisted of: (1) 1:1 mixture of Ham's F12 Medium Dulbecco's Modified Eagle's Medium(12634-010, Life Technology) with 2.5 mM L-glutamine (25030-081, Life Technology), (2) 10% fetal bovine serum (FBS, S11150, Atlanta Biologicals), and (3) 0.3 mg/ml Geneticin (G418, 11811023, Life Technology). The culture device was kept in a humidified incubator at 34°C in 5% CO_2. The medium flow rate into each culture chamber was controlled by a syringe pump. After 4 days of OSB culture, $4\sim8x10^4$ BMMCs were added to each chamber by opening the PDMS plugs. The culture medium flow was stopped for 4 h to allow settling of the cells, and then was switched from the OSB growth medium to the bone marrow culture medium. The culture device was switched from the 34°C incubator to a humidified 37°C in 5% CO_2 incubator. To evaluate the effect of the medium flow rate on MMC-OSB interactions, six treatment groups (triplicated per group) were tested in 1 week culture with: (1) two cell populations (OSB alone and OSB+BMMC) and (2) three flow rates (0.25 μL/min, 0.8 μL/min, 2.5 μL/min). To show the effect of BMMCs on the viability of GFP-OSBs during co-culture, two cell populations (OSB alone and OSB+BMMC, triplicated per group) were cultured for 0, 7 and 21 days and the fluorescent intensity of GFP-OSBs was measured by a plate reader at 528 nm (excitation at 485 nm). All GFP-positive OSBs were considered to be alive. The following equation was used to correlate the fluorescent intensity (x) of OSBs to the number of GFP-OSBs (y): $\log(y) = 0.9678 \log(x) + 1.2916$.

For drug evaluation, CFZ was applied on eight groups (triplicated per group) with: (1) two cell populations (OSB alone and OSB+BMMC) and (2) four CFZ concentrations in the bone marrow culture medium (0 nM, 0.5 nM, 5 nM and 50 nM) for 6 h. The culture groups were allowed to recover in the bone marrow medium for 1 week after the drug treatment. Using plate reading, the fluorescent intensity of GFP-OSBs was measured at 528 nm (excitation at 485nm) and the fluorescent intensity of eFluor®670-labeled BMMCs was measured at 690 nm (excitation at 660 nm). Relative BMMC fluorescent intensity changes in co-cultures were obtained by subtracting the background fluorescent intensity from OSB-only culture.

Flow Cytometry: Cells were isolated from the culture wells at the conclusion of the culture by trypsinization. The harvested samples were dissociated by grinding in microcentrifuge tubes. Single cell suspensions were prepared by filtering the ground samples through a 40 μm cell strainer. The cells were stained in fluorescence-activated cell sorting (FACS) buffer (phosphate buffered saline (PBS, P2194, Sigma- Aldrich) containing 1% bovine serum albumin (BSA, A2058, Sigma- Aldrich) and 0.02% sodium azide) with the following fluorochrome-conjugated monoclonal antibodies: (1) anti-CD138 APC (clone: B-B4, Miltenyi Biotec), anti-CD38 PE-Cy5 (303508, Biolegend) or anti-CD38 PE-Cy7 (303516, Biolegend), anti-CD56-PE-Vio770 (clone: REA196, Miltenyi Biotec) for MM cell identification and (2) CD13 PE (301704, Biolegend) to differentiate OSBs from BMMCs. $CFSE^+CD38^+CD56^+$, $CFSE^+CD38^+CD138^+$, and $CFSE^+$ $CD56^+CD138^+$ cells were gated and identified as MMC subpopulations. Also, the harvested cells were stained with 7-aad (00-6993-50, eBioscience) to identify dead cells. The proliferation of the MMC subpopulations was determined by analyzing the CFSE intensity, as each cell division results in a 50% decrease in the fluorescent intensity of this dye. $CFSE^-CD13^+7AAD^+$ were gated and identified as dead OSB. A minimum of 10,000 events were acquired and analyzed using a Beckman Coulter FC500 flow cytometer and the CXP cytometer analysis software (Beckman Coulter).

Plate Reading: The fluorescent intensity of GFP-OSBs in the culture wells was read at the excitation wavelength of 485/20 nm, and filtered at the emission wavelength with 528/20 nm using a plate reader (Synergy HTX multi-mode reader, Biotek) at Days 0, 7 and 21.

Supplementary Information 3 – Microbeads-Guided Reconstruction of 3D Cellular Network with Primary Murine Osteocytes

Materials: Primary osteocytes were isolated from long bones of 20-week old C57BL/ByJ (B10.BR) mice using the procedures given in Stern et al. In brief, the bones were: (1) harvested, (2) cleaned, (3) diced into small chips of 1-2 mm long and 0.5 mm wide, and (4) digested 7 times using a collagenase(9001-12-1, Sigma- Aldrich) solution (300 active U/ml in α-MEM [11900-024, Life Technology]) and an ethylenediaminetetraacetic acid (EDTA, E9884, Sigma-Aldrich) solution (5 mM in 1% BSA). The digested chips were then placed in a collagen-coated 12-well plate with osteogenic α-minimal essential medium (α-MEM) containing 10% FBS, 1% penicillin- streptomycin (PS, 15140-122, Life Technology), 3 mM β-glycerophosphate (154804-51-0, Sigma- Aldrich), and 50 μg/ml L-ascorbic acid (50-81-7, Sigma- Aldrich) at 37˚C and 5% CO_2 incubator conditions. After 13 days of post-digestion, cells migrated out of the bone chips were suspended using trypsin, removed using a micropipette from the well plate, and used for the 3D reconstruction experiment. Spray-dry/sintered BCP microbeads were purchased from CaP Biomaterials LLC. The microbeads were sieved to a size range of 20-25 μm, coated with collagen type I (9007-34-5, Sigma-Aldrich) using 10 mg/mL collagen/hexafluoroisopropanol (920-66-1, Sigma-Aldrich) for 2 h, and washed with PBS three times.

Culture Procedures: Isolated cells and BCP microbeads (10^7 cells/mL and 10^7 microbeads/mL) were thoroughly mixed. 10 μL of the mixture was placed in each culture chamber of the well plate-based perfusion device using a micropipette onto the top of the membrane to form a ~200 μm-thick tissue sample. After waiting for 12 h for the cells to attach to the microbead surface, the tissue samples were cultured up to 13 days at a flow rate of 0.8 μL/min using osteogenic α-MEM medium.

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Histology and Microscopy: On Day 13, the 3D tissues were harvested and fixed with 4% formaldehyde (50-00-0, Sigma-Aldrich)/PBS. They were then dehydrated in sequential ethanol solutions with increasing concentrations from 50% to 100%. Some samples were embedded in an optimal cutting temperature compound (Tissue-Tek), and cut into histological sections of 20 μm in thickness. The sections were stained with (1) H&E (03972, Sigma) to examine cell morphology and distribution, (2) co-stained with 4',6-diamidino-2-phenylindole (DAPI, 28718-90-3, Sigma-Aldrich) and goat anti-mouse sclerostin antibody (AF1589, R&D systems) followed by rabbit anti-goat TRITC-conjugated secondary antibody (305-025-006, Jackson Immuno Research Laboratory) to detect sclerostin production, and (3) co-stained with DAPI and goat anti-mouse ALP antibody (AF2910, R&D systems) followed by rabbit anti-goat TRITC-conjugated secondary antibody to detect ALP production. After staining, all the section samples were observed under a fluorescent microscope (Nikon Ti-E).

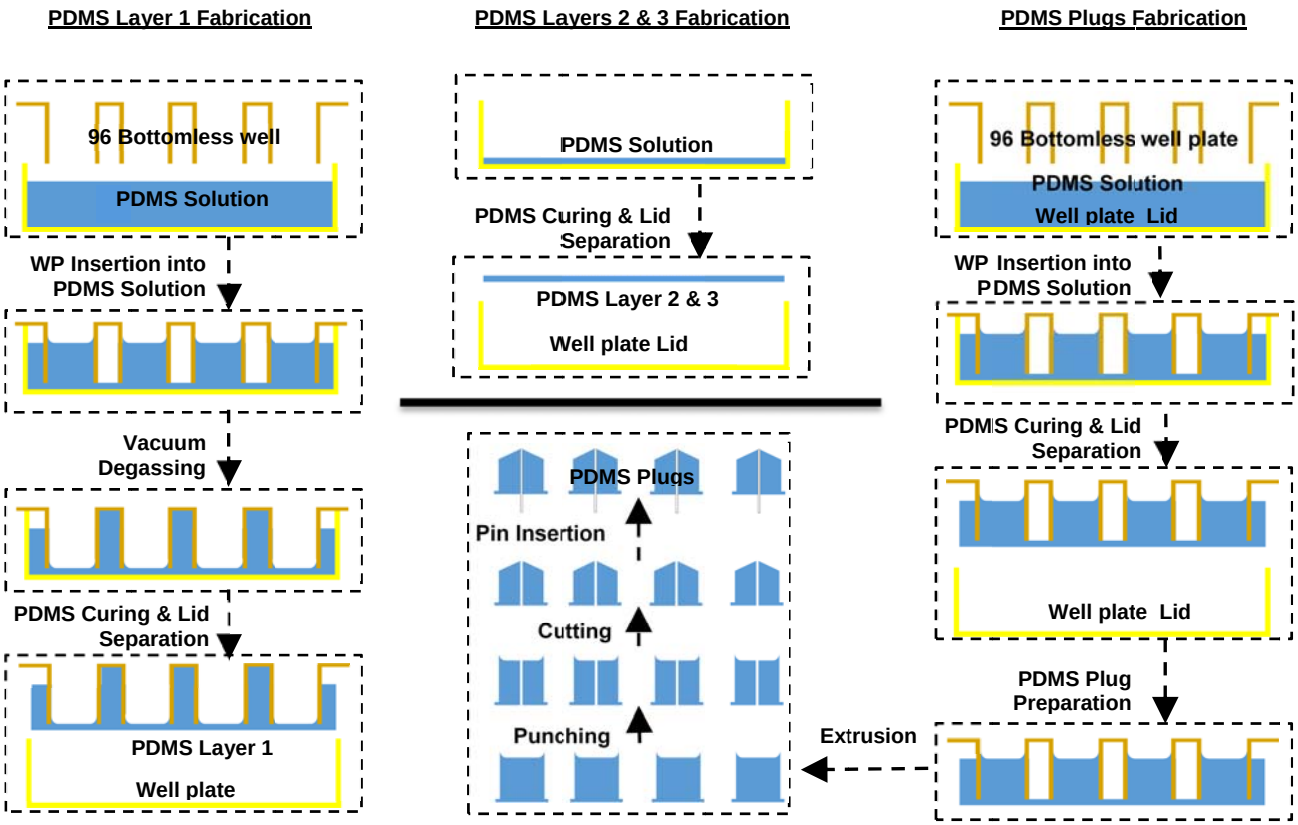
Supplementary Information 4 – Materials and Methods for Circulation of Primary Murine T Cells through Primary Murine Intestine Epithelial Cells

Materials: PC membrane discs with an average pore size of 10 μm were coated with electrospun PCL/collagen nanofibrous mesh. Using the previously established electrospinning method, basemembrane-like fibrous meshes with random fiber organization were prepared. To obtain stable and strong nanofibers, slow degradable, biocompatible PCL was used as the fiber matrix phase in which Type I collagen (representing ECM molecules) was dispersed. eGFP T cells harvested from an eGFP transgenic B6 mouse were used. The cells were filtered through a 40 μm filter prior to use. IECs were harvested from the small intestine of a B6 mouse, and were expanded by conditional cell reprogramming using the procedures described by Liu et al. RPMI complete was used as the common culture medium was used to support the culture of both T cells and IECs (RPMI 1640, 10% FBS, 1% L – glutamine, 1% Hepes, 0.5% 2-Mercaptoethanol, and 1% PS,).

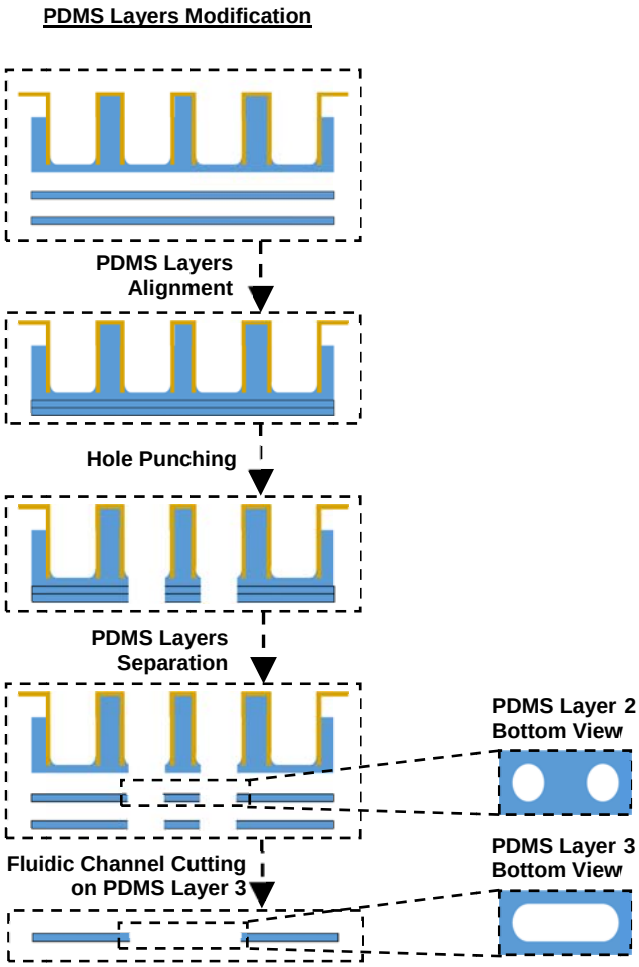
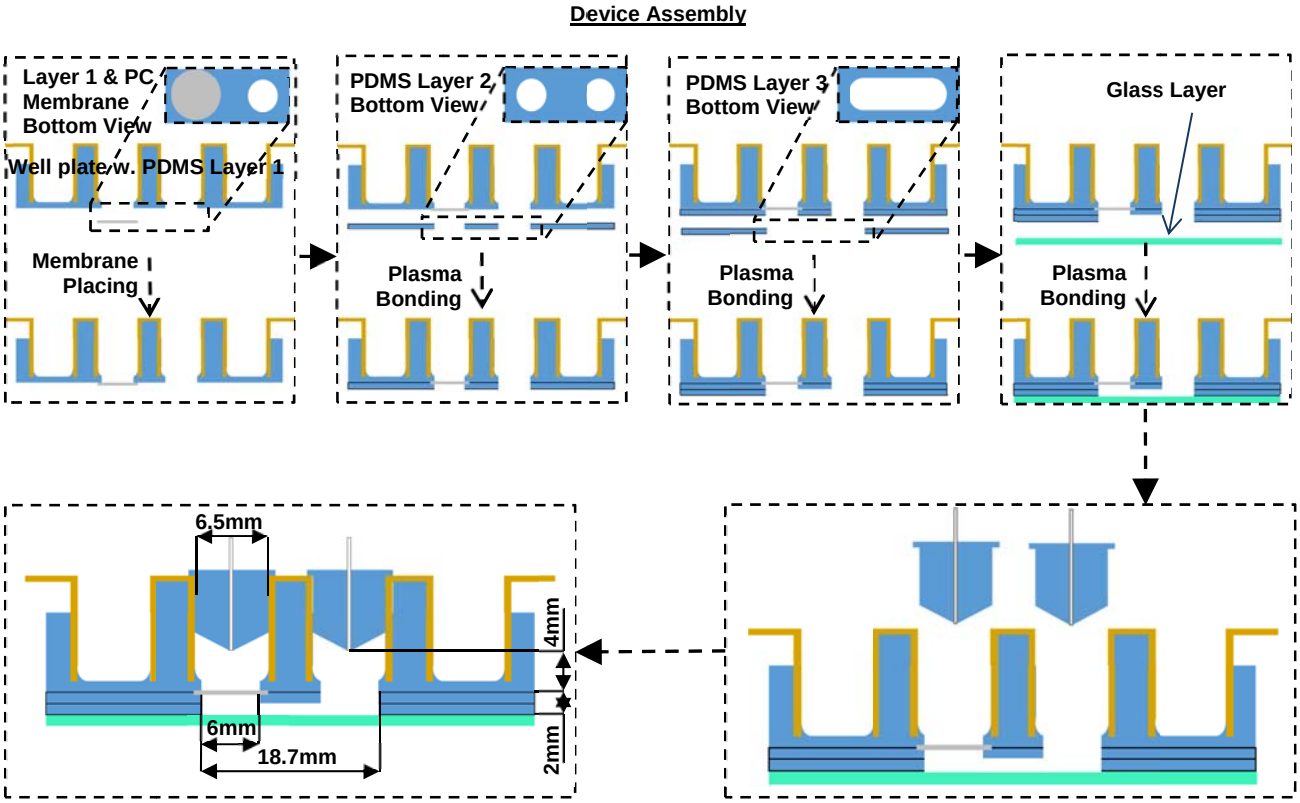
Culture Procedures: $2.5x10^4$ primary murine B6-SIINFEKL conditionally reprogrammed IECs were seeded onto the PC membrane coated with the PCL/collagen nanofibrous mesh in the culture chamber. The device was placed in an incubator maintained at 5% CO_2 and 37℃. The cells were allowed to settle and spread without any medium flow for 4 day. RPMI complete culture medium was changed every day in the first 4 days. On Day 5, the circulation of the culture medium stored in the medium reservoir was started through the inlet and the outlet chambers using a peristaltic pump (ISMATEC) at the rate of 7.6 μL/min. The outlet was connected to a silcone septa glass vial (SE-06-718-679, Restek™) which was used as a reservoir to introduce T cells (see Fig. 4a for the circulation configuration). To being circulation, $2.5x10^5$ T cells were added to the medium reservoir. In order to avoid the settling of T cell in the reservoir, a magnetic stirrer was used to keep T cells suspended in the medium IEC and T cell were co-cultured up to 3 days at the rate of 7.6 μL/min in RPMI complete medium.

Cell Counting and Microscopy: At culture times of 1 h, 3 h, 6, 24 h, 48 h and 72 h, 10 μL of T cell suspension was taken out of the reservoir to assess T viability by counting live and dead T cell numbers using a hematocytometer (1475, Hausser Scientific) and an optical microscope. Dead T cells were identified by trypan blue staining (15250-061, Life Technology). On day 7, the IEC monoculture and the membrane were removed from culture well and stained with ActinRed™ 555 (R37112, Life Technology) and DAPI (28718-90-3, Sigma-Aldrich), fluorescent images were taken using an inverted fluorescent microscope (Nikon Ti-E). After 6 h of culture, the cells and the membrane were removed from the culture wells, bright flied images were taken using an inverted microscope.

Supplementary Fig. 1. Schematic illustrations of fabricating PDMS parts for microfluidic connections



Supplementary Fig. 2. Schematic illustration of modifying PDMS parts



Supplementary Fig. 3. Schematic illustration of device assembly