Supplementary Materials

Materials and Methods

Experimental Design. The developed microfluidic immobilization device satisfies three essential requirements: robust immobility, long-term viability and simplicity, making it easy to use and adopt.

The microfluidic platform permits efficient immobilization by adjusting the trap channel geometry to the size and shape of the worms, and by positioning two on-chip hydraulic valves at the front and back of the trap. Alternative immobilization strategies, such as cooling, direct pressure on the animal or chemical additives, as encountered in conventional immobilization, were rejected when designing the immobilization platform, due to their invasive nature that consistently affects sensitive developmental processes.

Long-term viability is achieved by continuously supplying a bacteria suspension to the worm, minimizing the pressure exerted on it and permitting unencumbered egg laying. Only minimal pressure is exerted to maintain the worm's position and minimize its movement. Maintenance of the correct composition of the bacterial suspension (used as a food source) is also critical for robust operation. Indeed, an insufficient concentration of bacteria severely limits long-term survival, whilst an excessive concentration may lead to device blockage and an increased likelihood that a worm will be overgrown by bacteria.

Trap channels for adult hermaphrodites (approx. 3 days after hatching) were generally chosen to have a cross-section of 45x50 µm (WxH), narrowed to approximately 30 µm toward head and tail, so as to better support these narrower regions of the worm body. Channel cross-section was chosen such that the worm fits tightly into the trap, without exerting excessive pressure. Much smaller channels did not result in improved immobilization, as much of the motion still observed in trapped worms is internal, thus not significantly affected by external fixation unless excessive amounts of pressure are applied. The channel length was chosen between 750-800 µm, such that both head and tail sit about 1/4th inside the on-chip hydraulic valves, resulting in additional fixation. Both cross-section and exact length of the trap channel vary with the age of the worms under investigation, as older adults are significantly bigger than young ones. All remaining channels were kept at 50 µm, only narrowing around the trap channel, such that worms cannot escape. A channel width of 50 µm is necessary, such that eggs can be washed out easily. Additionally length and width of the surrounding channels were chosen such that hydrodynamic pressure on-chip is kept minimal, and a pressure balance between both outlets is maintained. Roughly one third of the food flow is directed to the worm head as food, and two thirds to the bypass.

Larvae trap channels were chosen significantly smaller, cross-sections of 20x15 µm for L3s and 25x20 µm for L4s, again narrowed by 3 µm towards the head and tail. Length varied from 350-375 µm for L3s to 450-500 µm for L4s. With channel length depending on the exact age of the larvae, always chosen such that head and tail are gently held by the on-chip valves. Channels around the trap were kept 7.5 µm. All remaining channels were chosen to be 25 µm wide.

In the future device geometry will adapted such that L1 and L2 larvae can be immobilized, profiting from the advantages found in the devices presented herein, allowing the study of a variety of sensitive developmental process in these two stages. Furthermore devices may be parallelized, such that multiple worms are immobilized and imaged at the same time. Here all worms are supplied with food by the same syringe pump, and all on-chip valves connected to the same solenoid, such that the setup complexity does not increase.

Owing to its simple construction (a PDMS substrate bonded to a cover slip), the device is inherently compatible with all conventional microscopy techniques, requiring no alterations to standard imaging protocols. In addition to the microfluidic device, only a few inexpensive components are needed (costing less than $1000) and its operation can be learned in a few hours. This is of critical importance, as the adoption of many microfluidic techniques by non-specialized laboratories is hindered by the technical complexities involved in their operation. Device setup and operation is visualized in Figure 1B-E and fig.S2. A more detailed description of the setup and operation is provided in the SI as well as in Movie S1.

Device Operation. The worm loading process is shown in Fig.1B-E. First, both on-chip valves are closed and a single worm is maneuvered into the loading channel. If the worm is not in a head-first configuration it is removed and repositioned. Once ready, the first on-chip valve is opened and the worm gently pushed into the trap. Once inside the trap, the first on-chip valve is closed. The position and fluorescence signal is assessed and if found suitable, image acquisition commenced. Otherwise, the second on-chip valve is opened and the worm removed from the trap, pumping fluid from both the worm and food syringes. Once the trap chamber is empty, the loading cycle repeated.

Manipulation of the worm is performed manually using the thumbscrew on the pusher block of the syringe pump. This allows facile and gentle control over the worm position. The syringe pump containing the bacterial suspension is then initiated at a volumetric flow rate of 1µL/h for adults and 0.2µL/h for larvae. Every 30 minutes, the flow rate is transiently increased to 300µL/h (adults) or 100µL/h (larvae) for 5 seconds, to wash out eggs and debris.

It is important to note that in addition to the microfluidic device only a few inexpensive components are required, i.e. a set of syringe pumps (Aladdin 1000-220, WPI), a set of solenoid valves (MH1-A-24VDC-N-HC-8V-PR-K01-QC-AD-BD-CX-DX, Festo, Switzerland) and a homemade controller. Due to its simplicity, the entire system can be assembled within a few minutes, and mounted on any commercial microscope.

Image acquisition. Brightfield images for the long-term viability assay were acquired on a Nikon TS100F inverted microscope. Images were acquired every minute using a Nikon CFI Plan Fluor 4X objective, NA 0.13.

Epi-fluorescence and DIC images were acquired on a Nikon Ti-*S* inverted microscope equipped with an Andor Zyla 4.2 (Andor Technology Ltd., UK), an LED light source (LedHUB, Omicron Laserage Laserprodukte GmbH, Germany) and an objective piezo for Z-motorization (C Focus, Mad City Labs Inc., USA). For two-color experiments, colors were acquired simultaneously using an image splitter (Optosplit II LS Image Splitter, Cairn Research, UK).

Spinning disk confocal images were acquired on a Nikon Ti-*E* inverted microscope equipped with a Yokogawa CSU-W1 confocal scanner unit (Yokogawa, Japan) and a Hamamatsu Orca Flash 4.0 (Hamamatsu, Japan).

Image stacks were acquired at intervals of 2 - 20 minutes, at a spacing of 0.5 - 1µm. Exposure is typically set to 50 ms and the excitation light kept to a minimum (between 5 and 10% of the maximum intensity). Nikon CFI Plan Fluor 40X, NA 0.75 and Nikon CFI Plan Apo VC 60XWI, NA 1.2 objectives were used.

***C. elegans* culture and sample preparation.** All worms were cultured according to standard protocols[2] on NGM plates seeded with *E. coli* OP50. Age synchronized cultures were obtained by bleaching gravid adult animals using 20 vol% of 5 vol% NaClO and 10 vol% of 5 M NaOH in DI water. After 10 minutes, the eggs were pelleted by centrifugation at 1300 x g for 1 min, and washed once with 1 mL of fresh S-Basal. The obtained eggs were transferred onto a seeded NGM plate. Adult animals were used after 3 days, L3 larvae after 45 h and L4 larvae after 52 h.

The strains used were: Germ cell apoptosis *itIs37[pie-1p::mCherry::H2B::pie-1 3'UTR + unc-119(+)]*[3]*; xnIs87[syn-4p::GFP::syn-4::syn-4 3'UTR + unc-119(+)]*. DTC migration *qIs56[lag-2p::GFP + unc-119(+)] V*[4]. AC invasion *qyIs23[cdh-3p::mCherry::PLC deltaPH; unc-119(+)]* II; *qyIs10[lam-1p::lam-1::GFP + unc-119(+)]*[5].

Prior to an experiment, a small population of worms was washed off the plate and re-suspended in approximately 2mL of S-Basal. The worms were left to settle by gravity, the supernatant removed and fresh S-Basal added. The wash was repeated twice to remove debris. The worms were then re-suspended in approximately 0.1 mL of fresh S-Basal. It is crucial, that worms are thoroughly washed, such that pieces of agar and other contaminants originating from the NGM plate do not enter the device clogging it during loading. Provided worms are prepared in an appropriate manner no clogging is observed.

Bacteria culture and preparation. *E. coli* NA22 were cultured overnight in a shaking incubator at 37^{o}C (OD600 ~2.3). Cells were pelleted by centrifugation at 3000 x g for 2 min. The supernatant was carefully removed and the cells re-suspended in fresh S-Basal. The wash was repeated two times for a total of three washes. Finally, the bacteria were suspended in fresh S-Basal at a concentration of approximately 4.7x10^{10} cells/mL.

The bacteria concentrate (50 vol%) was subsequently mixed with OptiPrep™ (Sigma Aldrich, Switzerland) density matching liquid (35.5 vol%), S-Basal (12.9 vol%), 1M Potassium Citrate pH 6.0 (20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate, H_2O to 1 liter) (1 vol%), 1 M $CaCl_2$ (0.3 vol%) and 1 M $MgSO_4$ (0.3 vol%). This suspension was used as food source in all experiments. Control of the exact concentration of OptiPrep™ in the mix is critical, as density mismatches will affect the long-term stability of the system. If the wrong amount of OptiPrep™ is added bacteria will sink to the bottom or float to the top of the syringe, thus resulting in a gradual change of bacteria supplied to the trapped worm. Correctness of the density matching is easily verified by centrifuging the prepared solution at 3000 x f for 2 min. If the bacteria neither pellet nor float to the top the density matching is correct, resulting in a long-term stable food supply. OptiPrep™ is widely used with a variety of sensitive biological systems and has no apparent effect on the worms, with embryos left to hatch in the bacteria solution developing to full adulthood. If bacteria are prepared correctly, no clogging is observed as bacteria do not segregate or aggregate.

Viability and Egg Laying. On-chip viability was determined by scoring the egg laying rates and the motion of single worms placed in the immobilization device. Worms were deemed no longer viable once no significant head/pharynx motion was apparent and strong morphological changes became apparent (e.g. internally developing embryos/internal hatching).

Control experiments for viability and egg laying were performed both on-chip and on NGM plates. On-chip viability controls were performed in a microfluidic device consisting of a large chamber, within which a worm could crawl freely. Bacteria were supplied in the same fashion and at the same rates as in the immobilization experiments. This allowed assessment of the effect of immobilization decoupled from that of maintaining the worm in a microfluidic environment. On plate egg laying controls were performed by placing single adult worms onto small NGM plates seeded with OP50 and counting the number of eggs and worms present after 48 hours.

Germ Cell Tracking. Germ cells were tracked manually using the MTrackJ plugin for ImageJ[6]. Individual cells were identified within the acquired Z-stack and tracked forward in time, starting from the entrance to the turn of the gonad. Apoptotic cells were identified based on morphology, the fluorescence intensity of the *mCherry::H2B* marker and the disappearance of the cell membrane (at which

point the “time of death” was scored). The histone marker gains brightness once the cell starts shrinking prior to engulfment, and correlates well with the appearance of cell corpses in DIC images, thus reliably indicating apoptosis at an early stage.

After tracking, all data points were referenced to a fixed point at the turn of the gonad, further reducing motion within the dataset. All remaining motion stems from the intrinsic flexibility of the nematode’s gonad. Adult hermaphrodites for the experiment were harvested 72 hours after synchronization.

Immobilization on Agar Pads. Worms were immobilized on agar pads following conventional protocols[43]. First, a clean glass slide (Objective Slide, ThermoScientific, Switzerland) was placed in between two glass slides covered with an approximately 500 μm thick layer of adhesive tape. A drop of agarose solution (4% agarose in S-Basal) was then pipetted onto the glass slide and gently pressed using another clean glass slide. Once the agar solidified a drop of S-Basal, containing 4 mM Tetramisole was pipetted onto the agar pad and several worms of the desired age were manually picked into the droplet and covered with a cover slip. The assembly was sealed with immersion oil.

Determining DTC Migration Rates. The DTC migration rate in both L3 and L4 larvae was determined by transforming the acquired time series into kymographs (maximum intensity projection followed by transforming the time series into a kymograph). The resulting kymographs were then aligned to each other such that a common starting point was established. Average growth rates were then determined by measuring the relative distance between the two DTCs.

As a comparison, the DTC migration rate was determined on NGM plates. Single larvae were placed on NGM plates and imaged once every hour using a dissection scope (Leica MZ16 FA Fluorescence Stereomicroscope, LEICA PLANAPO 2.0X, equipped with a Nikon Coolpix 990). Likewise, DTC migration was measured in conventional agar pads. In both cases, DTC migration was scored manually. L3 and L4 larvae were harvested at 45 and 52 hours, respectively, after synchronization. Worms were loaded during the early L3 or L4 stage and imaged up to the L3/L4 or L4/adult molt respectively. Z-stacks were acquired at 20 minute intervals for a total duration of 12 hours.

Determination of the AC Invasion Rate. The invasion rate was defined as the time from the onset of invasion, i.e. stretching of the actin filaments within the AC, to the complete formation of the opening in the basement membrane. Invasion timing was scored manually.

L3 larvae in both the microfluidic and conventional immobilization experiments were harvested at 45 hours after synchronization. Worms were loaded during the early L3 stage and imaged up to the L3/L4 molt. Z-stacks were acquired at 20 minute intervals for a total duration of 12 hours.

References

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Supplementary Information – Movies and Images

Movie S1 | Worm loading process. Video representing the worm loading process. Starting from an empty trap chamber, first the left on-chip valve is opened, followed by the worm being pushed into the trap from the worm inlet. Once inside the trap chamber the first on-chip valve is closed, thus immobilizing the worm efficiently. Upon immobilization the suitability of the trapped worm for the experiment can be assessed. If found unsuited the second on-chip valve is opened and the worm gently pushed out of the trap, followed by closing the second on-chip valve, returning to the initial state. This process is then repeated until a suitable worm is found. Video acquired using a 10X objective.

Movie S2 | On-chip viability and egg laying. Video presenting the first 24 h after immobilization. During the entire duration of the experiment continuous egg laying is observed at an average rate of 1 egg per 15 minutes. Bacteria are supplied continuously and normal feeding is observed. Motion within the trap is minimal, mostly stemming from pharyngeal pumping and egg laying. This residual motion however, is necessary as normal physiological functions would otherwise be affected and viability compromised. The worm appears normal during the entire timeframe and no negative effects stemming from the immobilization are observed. Images were acquired using a 4X objective at 1 minute intervals.

Movie S3 | Germ cell migration. Video showing germ cell migration in an adult hermaphrodite. Represented is the fluorescence detected from a *mCherry::H2B* marker as maximum intensity projections of each time point. Migration of the germ cells along the gonad can be observed. A number of cells undergo apoptosis at the gonad's turn, indicated by the increase in fluorescence intensity. The remaining cells migrate further towards the turn of the gonad. After the turn the oocytes are fertilized by the spermatocytes, followed by the first few cell divisions, prior to egg laying. The gonad and the ongoing dynamic processes, such as germ cell migration, fertilization and mitosis within the fertilized embryo can clearly be followed with minimal motion in between time points. Germ cell migration, fertilization and egg laying is observed undisturbed during the entire 4 hour experiment. The represented images clearly indicate the image quality obtainable using our microfluidic immobilization device. Video was acquired using a 60X water immersion objective, Z-stacks were acquired at 2 minute intervals and at a 1 µm spacing for a total duration of 4 hours. The larger 1 µm spacing, as well as the relatively short duration of the experiment were chosen so as to minimize photodamage. Note that images have been de-convolved, but no image registration has been applied. Furthermore, increase in fluorescence intensity at the location of cell death can be observed. This stems from incomplete absorption of the mCherry histone marker during apoptosis, this however poses no negative effect on viability or germline function.

Movie S4 | Anchor cell invasion. Video showing the anchor cell invasion. Shown is an overlay of both GFP (basement membrane) and mCherry (actin within the AC) fluorescence. Stretching of the AC is initially observed, followed by retraction and the appearance of an opening in the basement membrane. Lastly, the formation of a lumen and upward migration of the AC can be observed. The entire process of AC invasion can be followed in great detail, with the actin protrusions within the AC clearly observable, as well as the basement membrane and the resulting opening within. Minimal motion is observed in between time points. Z-stacks were acquired using a 60X objective, simultaneously acquiring GFP and mCherry fluorescence using an image splitter. Represented are the in-focus planes of each Z-stack, at a 20 minute time interval. Note that images have been de-convolved, but no image registration has been applied.

Movie S5 | Long-term immobilization. Video representing a single adult hermaphrodite imaged for 121 hours. Represented are images acquired at 20 minute intervals, demonstrating normal appearance and egg laying for a large portion. Egg laying naturally halts after approximately 70 hours. The worm, as all worms imaged, was determined to no longer be viable once no significant head/pharynx motion was visible and strong morphological changes became apparent. Images were acquired using a 4X objective. Scale bar 100 µm.

Stack S1 | Multicolor time-lapse (GFP/DIC, top/bottom). Represented are the midsection of an adult hermaphrodite's gonad showing a SYN-4::GFP marker (top), and the bottom of the gonad in DIC (bottom). Excellent quality images can be acquired using both modalities, with minimal movement throughout the experiment. Z-stacks were acquired over 12 hours at 7.5 minute intervals. Scale bar 25 µm.

Supplementary Information – Setup and Operation Procedures

MATERIALS

Reagents

Fabrication of SU-8 Master

- SU-8 Photoresist (SU8 2010 and SU-8 3050, Microchem, Germany).
- Resist developer (mr-Dev 600, Microchem, Germany).
- Isopropanol.

Fabrication of Microfluidic Devices

- Chlorotrimethylsilane (TMCS; Sigma-Aldrich, cat. No. 92361).
- Polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning).

Bacteria Preparation

- LBroth (5g Yeast Extract, 10g NaCl and 10g Peptone in 1L DI water). Sterilized by autoclaving.
- SBasal (5.85g NaCl, 1g K_2HPO_4, 6g KH_2PO_4 in 1L DI water). Sterilized by autoclaving. Add 1mL cholesterol (5mg/mL in ethanol) after autoclaving.
- Optiprep Density Gradient Media (Sigma-Aldrich, cat. No. D1556).
- 1M $MgSO_4$. Sterilized by autoclaving.
- 1M $CaCl_2$. Sterilized by autoclaving.
- 1M Citrate Buffer (20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate, add water to 1 liter). Sterilize by autoclaving.
- 40 µm Cell Strainer (cat. No. 22363547, Fisher Scientific).
- Stock solution of NA22.

***C. elegans* Culture**

- NGM plates (17g agar, 2.5g Peptone, 3g NaCl, 1mL 1M $MgSO_4$, 1mL 1M $CaCl_2$, 25mL 1 M KPO4 buffer pH 6.0 (108.3 g KH2PO4, 35.6 g K2HPO4, fill to 1L with DI water), fill to 1L with DI water). Sterilized by autoclaving. Add 1mL cholesterol after autoclaving.
- 5M NaOH.
- 5% NaClO.
- SBasal.
- Stock Solution OP50.

Equipment

Fabrication of SU-8 Master

- Silicon wafer, 100 mm diameter, <100> p cut, 500 ± 5 µm thickness, one side polished.
- Photo mask.
- Spin Coater.
- Hot Plate.
- UV exposure unit.
- Surface profiler.

Fabrication of Microfluidic Devices

- 120 mm glass petri dish.
- 20-gauge hole puncher.
- Scalpel.
- Aluminum foil.
- Adhesive tape.
- Cover Slips (e.g. 24x40 mm).
- Plasma Cleaner.
- Oven (70 ^{o}C).

Hardware for Microfluidic Experiment

- Programmable syringe pump (Aladdin AI1000-220, WPI).
- Solenoid Valve (MH1-A-24VDC-N-HC-2V-PR-K01-QC-AD-BD-CX-DX, Festo, Switzerland).
- Homemade switch for solenoid valve.
- Tygon Tubing (1/16'' cat. No. 0642002, Fisher Scientific, and 1/32'' cat. No. 0641900, Fisher Scientific).

- OD. 6 mm polyurethane tubing (enough to span from the pressure supply to the microscope).
- OD. 4 mm, ID 3/32'' polyurethane tubing (~100 mm)
- Luer Adapters, Male Luer Lock to 3/32'' ID (cat. No. EW-45505-09, Cole Parmer).
- Precision pressure regulator, 1/4" ports, 2-60psi range, (cat. No. 6162K22, McMaster).
- Pressure gauge, 1/4" connection, 0-60psi range (cat. No. 4000K563, McMaster).
- 1/4" push-in fitting to 1/4" male NPT (cat. No. 5111K82 (need two per regulators), McMaster).
- 1 mL Plastic Syringe.
- 23G Blunt needle.
- 30G Blunt needle.
- 1/2'' Micro bore steel pins (G23, New England Small Tubing, USA).
- 1 mL cryovial.
- Epoxy glue.

EXPERIMENTAL PROTOCOLS

Fabrication of SU-8 Master (Timing: ~2 h)

1. Dehydrate a Silicon wafer at 200 ^{o}C for 10 minutes.
2. Spin coat the wafer according to the SU-8 datasheet. Desired feature height is 50µm for adult traps, 20µm for L4 and 15 µm for L3 traps.
3. Soft bake for 5 min at 65 ^{o}C and 10 min at 95 ^{o}C.
4. Expose the SU-8 to UV light through the photomask. Exact exposure time will vary with the UV exposure unit and the feature height.
5. Post bake for 1 min at 65 ^{o}C and 5 min at 95 ^{o}C.
6. Develop using mr-Dev 600 according to the datasheet. Development time will vary with feature height.
7. After development carefully rinse the wafer with fresh developer followed by washing with isopropanol.
8. Hard bake the wafer at 200 ^{o}C for 10 min.
9. Check the obtained master under a stereomicroscope and verify feature height.

Fabrication of PDMS devices (Timing: ~12 h)

1. To prevent adhesion of PDMS to the Silicon wafer, treat the master with TMCS for 1h in a fume hood, by placing the wafer in a vacuum desiccator along with ~1mL of TMCS. Lower the pressure until TMCS begins to boil.
2. Thoroughly mix approximately 40g of PDMS in a ratio of 20:1 (Part A:B).
3. Degas the PDMS in a vacuum desiccator (~30 min).
4. Place the wafer on a round piece of aluminum foil (120 mm diameter) and shape it into a dish.
5. Place the wafer with the aluminum dish in a 120 mm glass petri dish and pour the PDMS onto the master.
6. Degas the PDMS until all air bubbles have disappeared.
7. Cure the PDMS at 70 ^{o}C overnight.
8. Once the PDMS is cured, carefully peal it of the wafer and cut it along the cutting marks.
9. Punch access holes using the 20-gauge hole puncher.
10. Clean a cover glass and a PDMS piece using adhesive tape.
11. Expose the PDMS and cove glass to air plasma. The exact timing will depend on the machine used.
12. Following plasma activation place the PDMS onto the glass.
13. Place the device in the oven at 70 ^{o}C for several hours.

***C. elegans* Culture and Preparation** (Timing: up to 3 days, depending on strain)

Worms were grown according to standard protocols on NGM plates seeded with *E. coli* OP50. Synchronized populations were obtained as follows. All cultures were done at 20 ^{o}C.

1. Starting with a plate of gravid adult hermaphrodites (60 mm diameter).
2. Wash the plate with approximately 1 mL SBasal.
3. Take the worm suspension and aliquot to approximately 0.7 mL.
4. Add 0.2 mL 5% NaClO and 0.1 mL 5M NaOH, shake gently for 10 min.
5. Centrifuge at 1300 rcf for 1 min, decant the supernatant and add 1 mL fresh SBasal.
6. Repeat centrifugation and remove the supernatant. Place the harvested embryos on a plate seeded with OP50.
7. Once worms have reached the desired age, wash plate with 1 mL SBasal.
8. Leave worms to settle by gravity, remove the supernatant and replace with 1 mL fresh SBasal.
9. Repeat wash for a total of three times.

10. Finally, re-suspend the worms in approximately 0.1 mL SBasal.

Bacteria Culture and Preparation (Timing: ~12 h)

1. Prepare 40 mL of overnight culture (e.g. 8x5 mL of LBroth inoculated with 0.1 mL of NA22 stock each).
2. Pellet the bacteria by centrifugation (3000 rcf for 2 min) and remove the supernatant.
3. Re-suspend the bacteria in 1 mL fresh SBasal.
4. Repeat the washing for a total of 3 washes.
5. Finally, re-suspend the pellet in a total of 1 mL fresh SBasal.
6. Mix the bacteria concentrate with 0.71 mL Optiprep, 0.258 mL SBasal, 0.02 mL 1M Citrate buffer, 0.006 mL 1M $MgSO_4$ and 0.006 mL 1M $CaCl_2$. Yielding the worm food. This is can be used for several days, check for segregation and clumping. Do not store in a fridge.
7. Prior to use filter the worm food through a 40 µm cell strainer.

Setting up the Microfluidic Device

Setting up the solenoid valve (Timing: Done once ~15 min)

1. Cut the ID 3/32'' inch tubing in approximately 30 mm pieces.
2. Connect one Male Luer to tubing adapter to one side of each tubing piece.
3. Push the other end into the 4mm connector of the solenoid valve.
4. Put a G23 blunt needle on each Luer adapter.
5. Connect the OD 6mm tubing to the pressure supply. A constant supply is preferred over gas bottles. If only gas bottles are available a different pressure regulator will be needed.
6. Connect the other end of the tubing to the IN port of the pressure regulator (First connect the two 1/4'' push in fittings and the manometer to the corresponding ports).
7. Connect a second piece of tubing to the OUT port and to the upper port of the solenoid valve.
8. Open the supply pressure and set the pressure regulator to 25 psi.

Preparing the waste container (Timing: Done once ~5 min)

1. Punch three holes in the lid of a cryovial using a 23G blunt needle.
2. Place a micro bore steel pin in each hole. Two for liquid, one as ventilation.
3. Fix the pins with epoxy glue.
4. Leave the glue to harden.
5. Prior to the experiment, cut two 3cm pieces of tubing (1/16'' Tygon tubing) and connect them to two of the pins in the waste container. Connect two micro bore steel pins to the tubing and bend the end at 90^o.

Setting up the on-chip valves (Timing: ~1 min)

1. Cut two pieces of tubing (1/16'' Tygon tubing) long enough to reach from the solenoid valves to the microscope. This tubing can be reused.
2. Put one micro bore steel pins in each tubing and bend their ends at 90^o.
3. Fill the tubing with DI water and connect it to the chip.
4. Connect the other end of the tubing to the solenoid valve (Figure S2B).
5. Close and pressurize both on-chip valves (25 psi).
6. Open both valves.

Filling the chip with worm food (Timing: ~2 min)

1. Cut one piece of tubing (1/32'' Tygon tubing) and connect it to a 30G blunt needle.
2. Fill a 1 mL syringe (0.25 mL syringe in case of larval *C. elegans*) with bacteria suspension (approximately 0.5mL).
3. Connect the tubing directly to the food inlet (Figure S2C).
4. Flush the device with food until a droplet of liquid appears at each outlet (Figure S2D).
5. Plug all outlets using a steel pin blocked with a burned piece of tubing (Figure S2E).
6. Gently pressurize the device by pressing on the syringe.
7. Leave for approximately 1 min, until all air bubbles have disappeared.
8. Unplug the pins blocking the outlet.
9. Connect the waste container (Figure S2F).
10. The syringe pump containing the bacterial suspension is then initiated at a volumetric flow rate of 1µL/h for adults and 0.2µL/h for larvae. Every 30 minutes the flow rate is transiently increased to 300µL/h (adults) or 100µL/h (larvae) for 5 seconds, to wash out eggs and debris.

Connecting the worm suspension (Timing: ~1 min)

1. Cut a piece of tubing (1/16'' Tygon tubing) and connect it to a 23G blunt needle and a micro bore steel pin.
2. Fill a 1 mL syringe with SBasal (approximately 0.5 mL).
3. Connect the tubing to the syringe.
4. Place the end of the tubing in the vial holding the worms and gently suck some of the worms into the tubing.
5. Make sure the worms stay in the tubing and do not get sucked into the syringe.
6. Make sure a drop of liquid appears at the worm inlet by pushing in the syringe containing the bacteria suspension.
7. Make sure a drop of liquid appears at the end of the tubing with worms.
8. Connect the worm tubing to the chip (Figure S2G).
9. Close both on-chip valves.

On-Chip worm trapping (Timing: 5-10 min)

1. Observe worm trapping with a 4X or 10X objective.
2. With both on-chip valves closed gently push on the syringe connected to the worms.
3. Gently maneuver a single adult worm into the channel leading to the trap region (Figure 1B).
4. If the worm is oriented head first, open the first valve and gently push the worm into the trap (Figure 1C-D). Care must be taken so that the worm does not get into the food channel.
5. If the worm is oriented tail first, gently pull the worm back out of the channel by pulling on the buffer syringe.
6. Once a worm has been trapped, switch to the magnification desired for the experiment and if the worm is suitable start imaging.
7. If the worm is unsuited for the experiment open the valve next to the worm outlet and gently push out the worm by pushing on both the (buffer)worm suspension and food syringe (Figure 1E).
8. Close the valves again and repeat steps 2-8 until a suitable candidate is found.

Cleanup (Timing: 5 min)

1. Switch off the on-chip valves.
2. Remove the chip from the microscope.
3. Carefully unplug all connections. Press on the PDMS device next to the connection while unplugging so as not to break the device.
4. Remove the micro bore steel pins from the tubing and store them in ethanol for the next experiment. Make sure the ethanol is completely removed prior to reuse.
5. Remove the blunt needles, rinse them with warm water and ethanol. Dry with pressurized air and store for the next experiment.
6. Remove the tubing from the waste container. Empty the container and rinse with warm water and ethanol. Dry with pressurized air and store for the next experiment.
7. Discard all tubing and used syringes.
8. Carefully clean the bottom of the microfluidic device of all immersion oil residue using acetone.
9. Store the chip in a plastic petri dish for the next experiment.
10. Prior to reusing any of the micro bore steel pins, blunt needles or waste container, make sure they are clean and not clogged.

Supplementary Figures

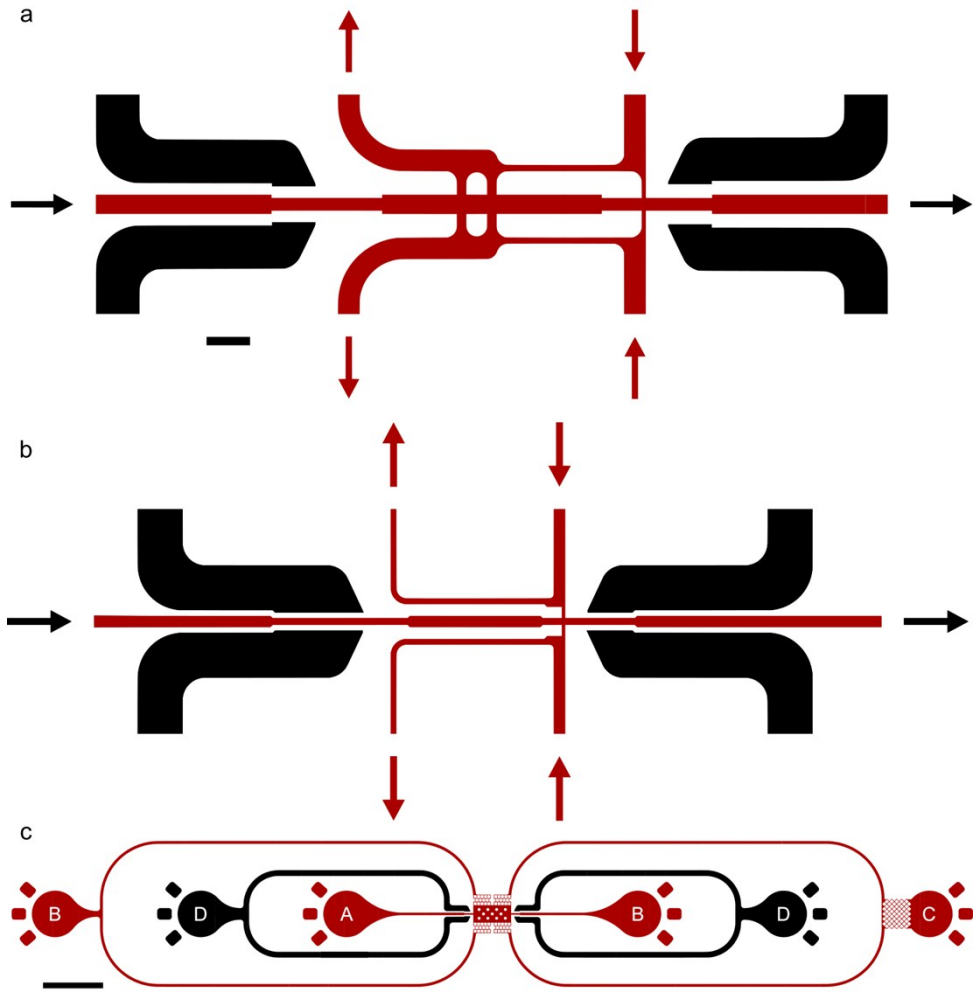

Fig.S1 | Device schematic. (a) Magnified view of the trap region designed for adult *C. elegans*. Represented in red are the fluidic channels within which the worms and bacteria are transported. Represented in black are the on-chip hydraulic valves, positioned at the front and back of the trap region, which allow for precise control over the worms entering and leaving the trap, as well as stabilizing the worm's position. Arrows indicate the flow directions: red bacteria flow, black worm flow. (b) Magnified view of the trap region designed for L4 stage larvae. The general layout follows the same design principle used in the adult device, however the size of the trap channel is adapted to the smaller size of L3 and L4 larvae, and the pillars placed along the adult trap are removed. (c) Schematic of the microfluidic device used for on-chip viability tests. In principle, the device follows the same layout as the immobilization device, with flow distribution and worm loading being identical. However instead of a narrow trap channel, the worms are kept in a large chamber, and therefore able to crawl freely. Pillars are placed inside the trap chamber so that the worm does not "swim" but "crawls". The sides of the chamber are lined by small pillars that allow undisturbed bacteria circulation, whilst keeping the worm confined. Scale bar 100 µm (a-b) 1000 µm (c).

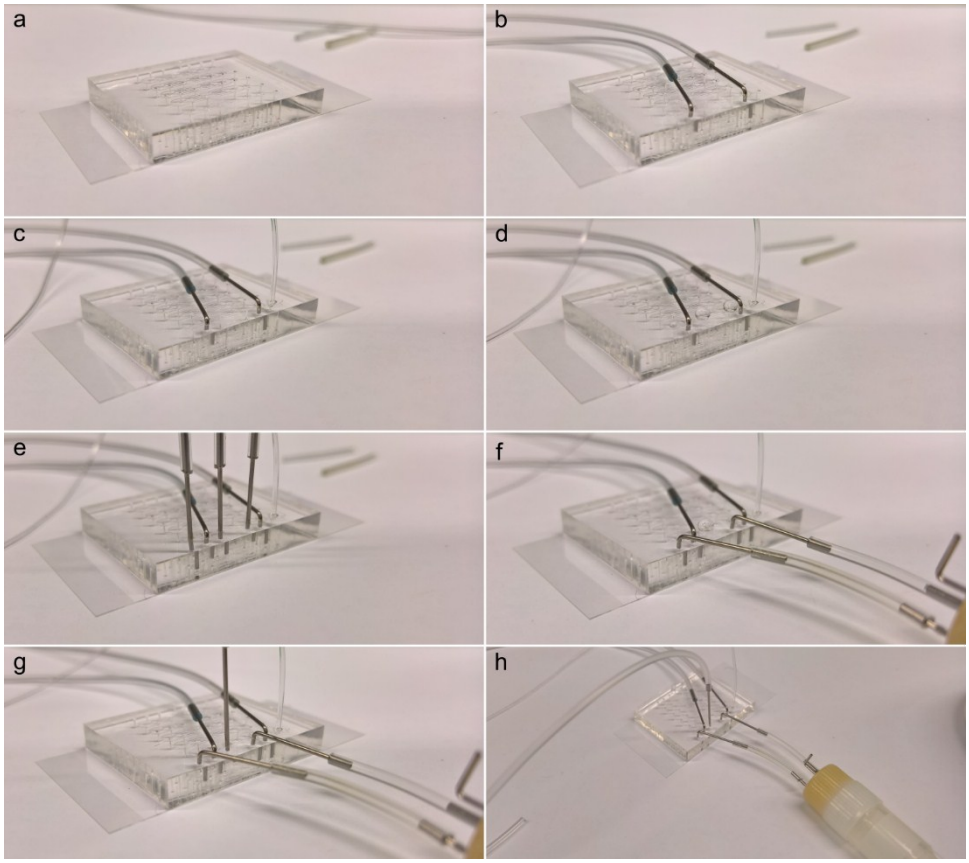

Fig.S2 | Device Setup. (a) PDMS device bonded to a coverslip. Each device has six trap chambers. (b) On-chip valves are connected to the solenoid valves via a length of tubing (1/16'' Tygon Tubing) filled with DI water. The dead end channels are pressurized (25 psi) and filled with DI water. (c) Bacteria are loaded into a 1 mL syringe and the syringe is connected to the device (1/32'' Tygon Tubing). (d) The device is filled with bacteria suspension. A droplet of liquid appears at each connection. (e) All open connections are blocked using a micro bore steel pin and the device is gently pressurized. (f) The steel pins are removed and the waste container is connected to the device. (g) The tubing with worms is connected to the device. As before prior to connecting the tubing a droplet of liquid should appear at the inlet, so as to avoid air getting into the device while connecting. (h) Enlarged view of the assembled device.

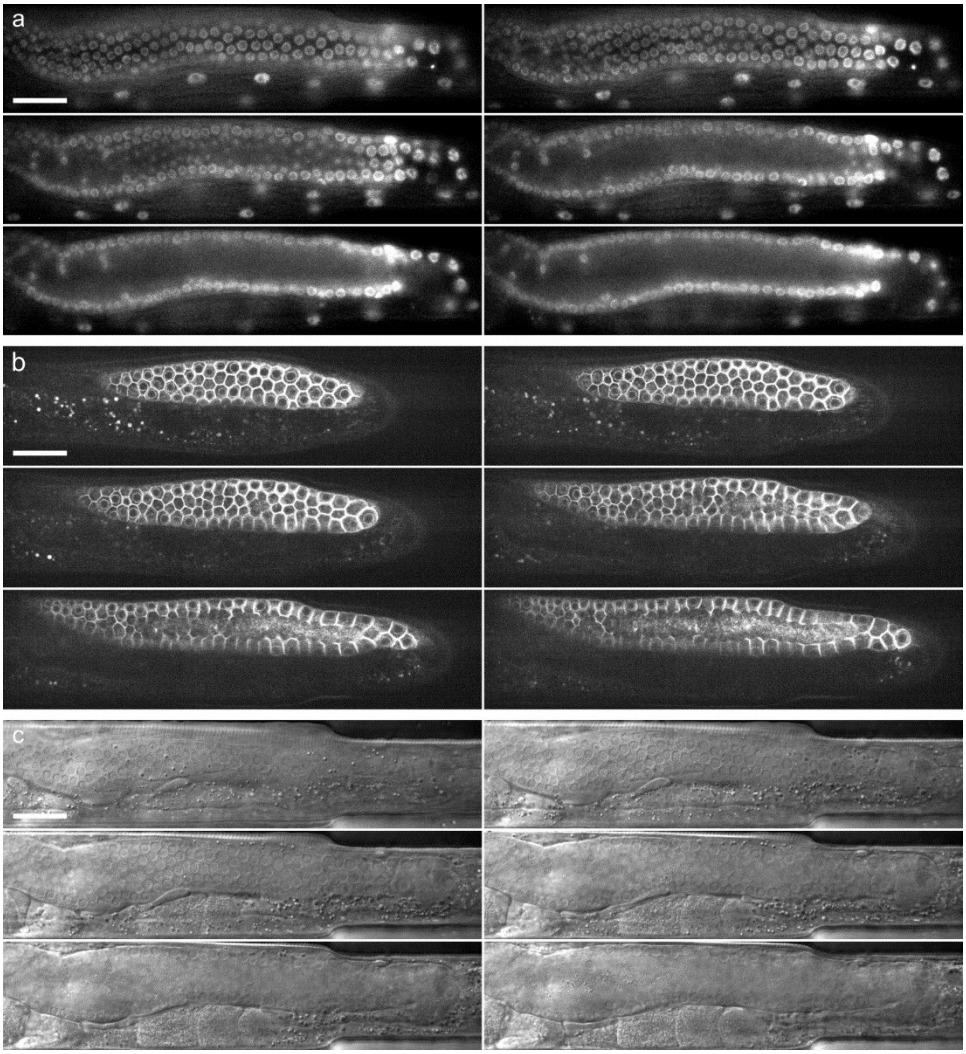

Fig.S3 | Representative z-stacks showing the bottom halve of an adult hermaphrodite's gonad. (a) Epi-fluorescence (*mCherry::H2B*), (b) Spinning disk confocal (*SYN-4::GFP*) and (c) DIC. In all three cases no motion blur is detected and image quality equal to conventional agar pads is achieved. Images were acquired using a 60X water immersion objective (NA 1.2) and are represented at 2 μm intervals.

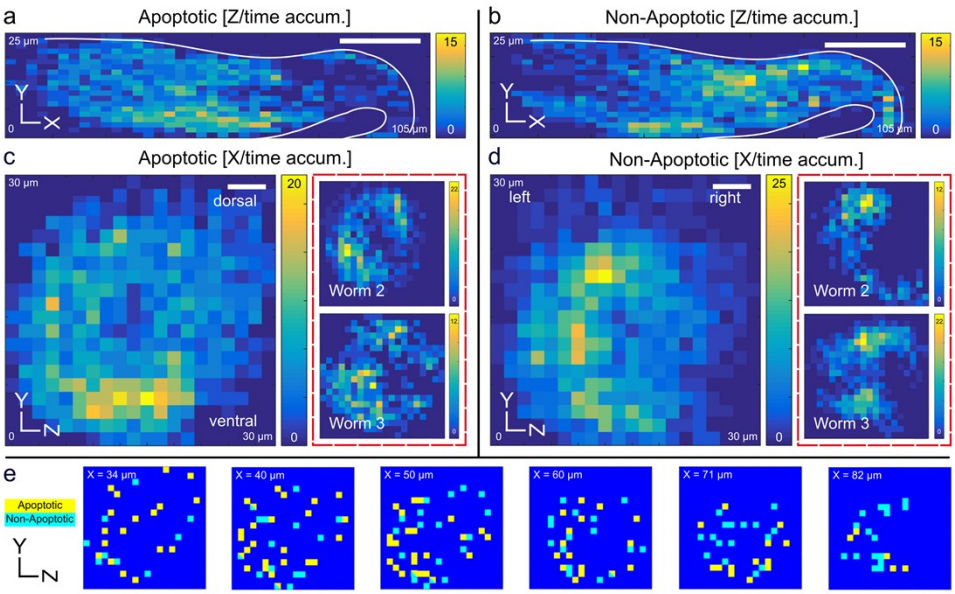

Fig.S4 | Germ cell tracking. (a-d) Gonad divided into small rectangular cuboids, color coded the accumulated number of cells passing through the cuboids during the experimental timeframe (12 h). (a, c) apoptotic and (b, d) non-apoptotic fates. Cell migration, for 100 cells in one worm, is visualized in transverse plots, where numbers are accumulated in the left-right (Z) direction (a-b) and the proximo-distal (X) direction (c-d). Insets (red boxes) show results for two additional worms (20 cells per worm) in the proximo-distal accumulated representation. Germ cells most frequently appear in two small sections on the dorsal and ventral side of the gonad and, to a lesser extent, on the left side of the gonad. (e) Representation of the cell migration positions in cross sections (YZ) at specific positions along the proximo-distal axis (X) for 100 cells. Apoptotic (yellow) and non-apoptotic (blue) fates are evenly distributed in the distal region, whereas in the region near the turn apoptosing cells drift towards the ventral-left sides and non-apoptosing cells towards the dorsal-left sides. This indicates that the position of origin of the cells does not determine their fate, but that the positon close to the turn may have a significant influence on the cell fate decision. Scale bar 25 µm (a-b), 5 µm (c-d).

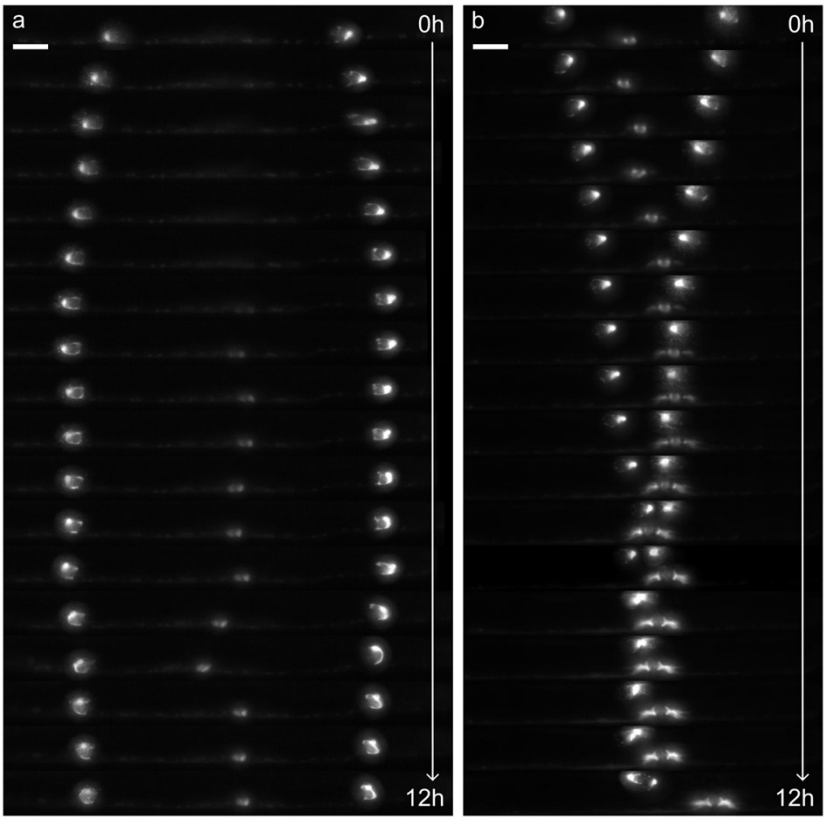

Fig.S5 | DTC migration on-chip. (a) Maximum intensity projection showing the DTC (*lag-2p::GFP*) migration in L3 larva. Clearly visible are the outward migration and the turn. Development arrests towards the end of the time series as L3/L4 molting cannot occur. (b) Maximum intensity projection showing the DTC migration in L4 larva. Both DTCs migrate towards the body center and the vulva is fully developed. Again development arrests towards the end of the time series as the L4/adult molt cannot occur. Images were acquired using a 40X air immersion objective (NA 0.6). Time interval 40 minutes, scale bar 25 µm.

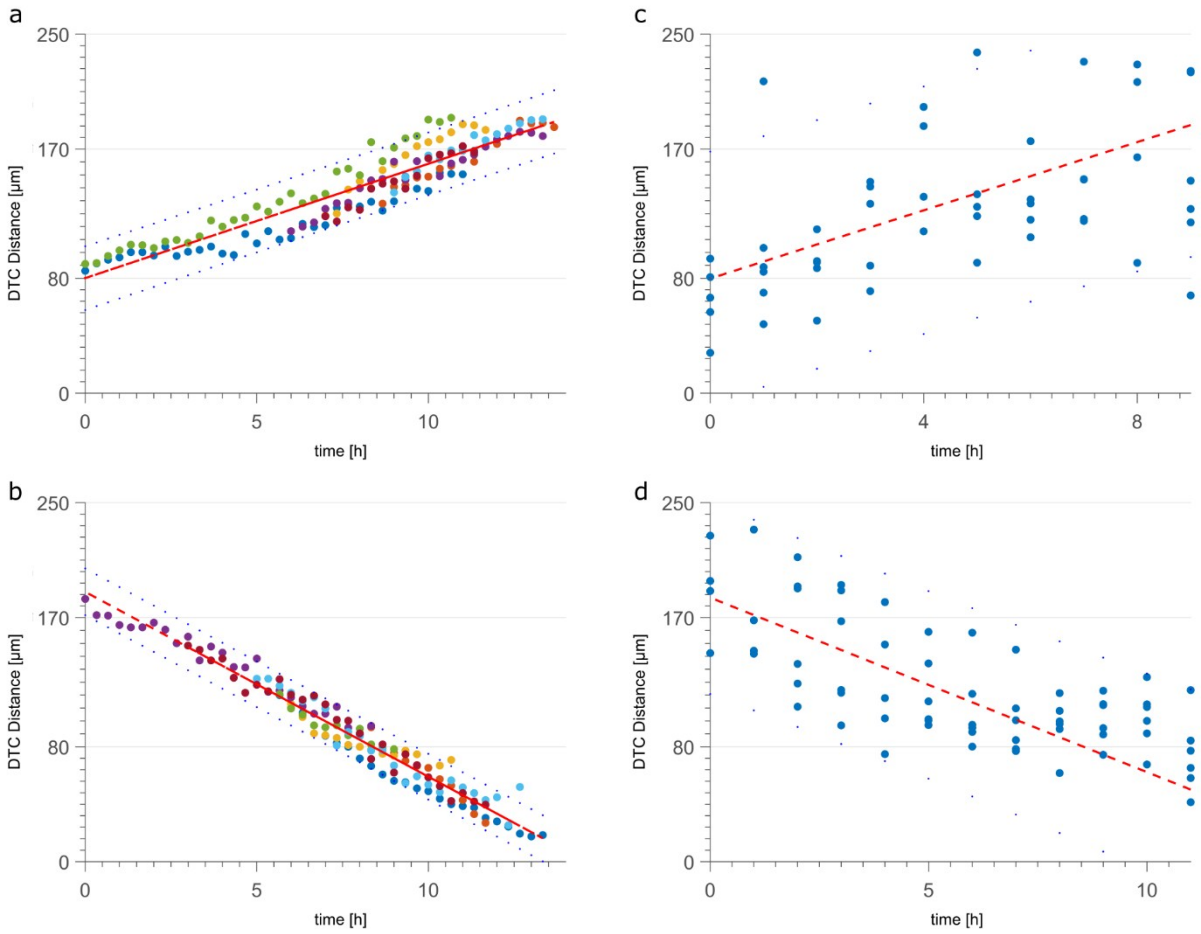

Fig.S6 | Quantification DTC migration. DTC migration on-chip L3 (a) and L4 (b). DTC migration in L3 and L4 larvae on NGM plates (c, d). Average migration rate was extracted via the slope of the linear regression, resulting in 7.96 ± 0.25 µm/h (a), 12.87 ± 0.23 µm/h (b), 11.19 ± 2.24 µm/h (c) and 12.15 ± 1.84 µm/h (d). The much broader distribution observed in (c) and (d) is mostly caused by the on plate imaging and the associated lower quality images and manual scoring of growth. Each color represents an individual worm (a-b).