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Supplementary information

A microfluidic Braille valve platform for on-demand production, combinatorial screening and sorting of chemically distinct droplets

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A microfluidic Braille valve platform for on-demand production, combinatorial screening and sorting of chemically distinct droplets

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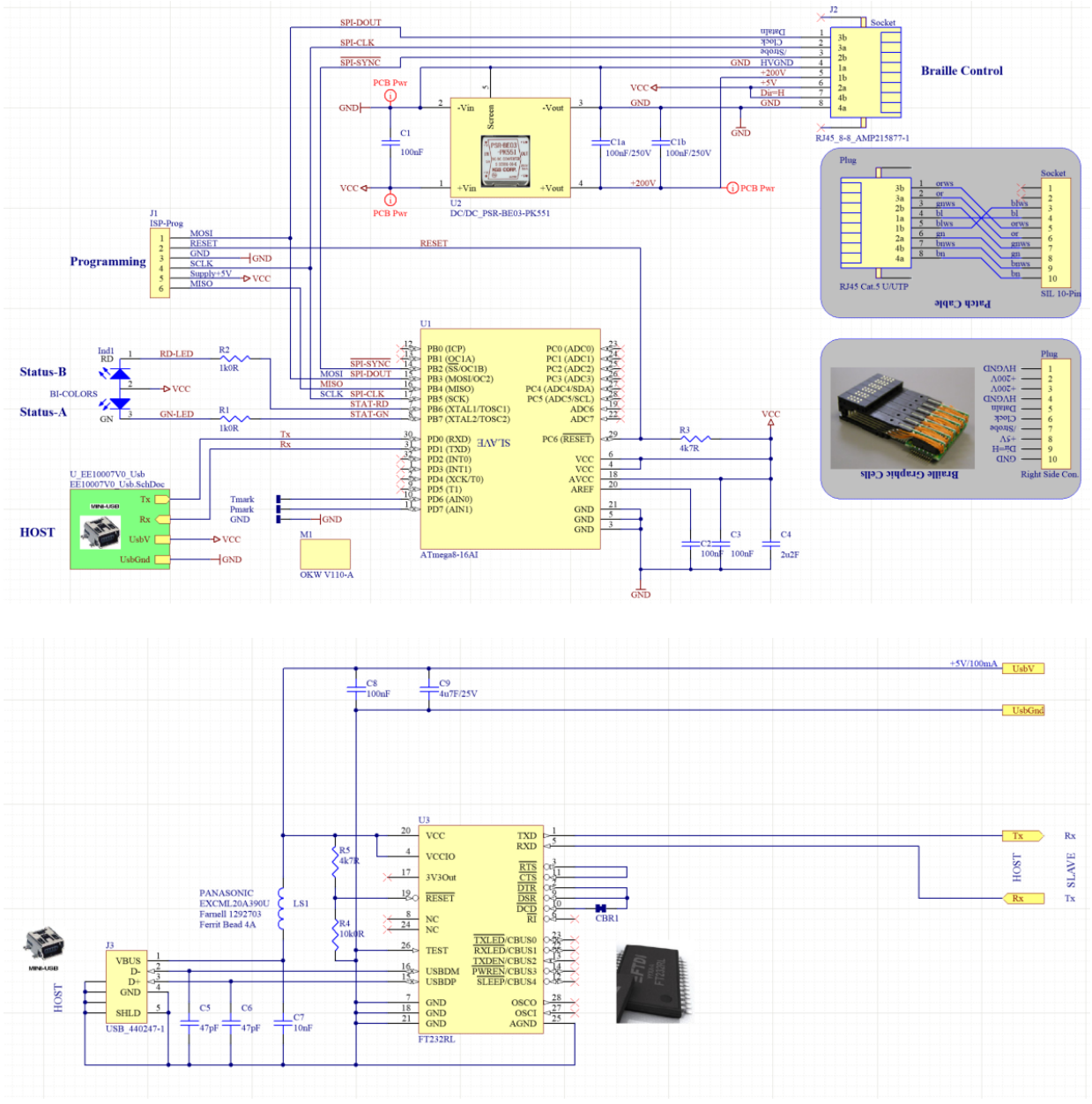
SUPPLEMENTARY INFORMATION

Contents:

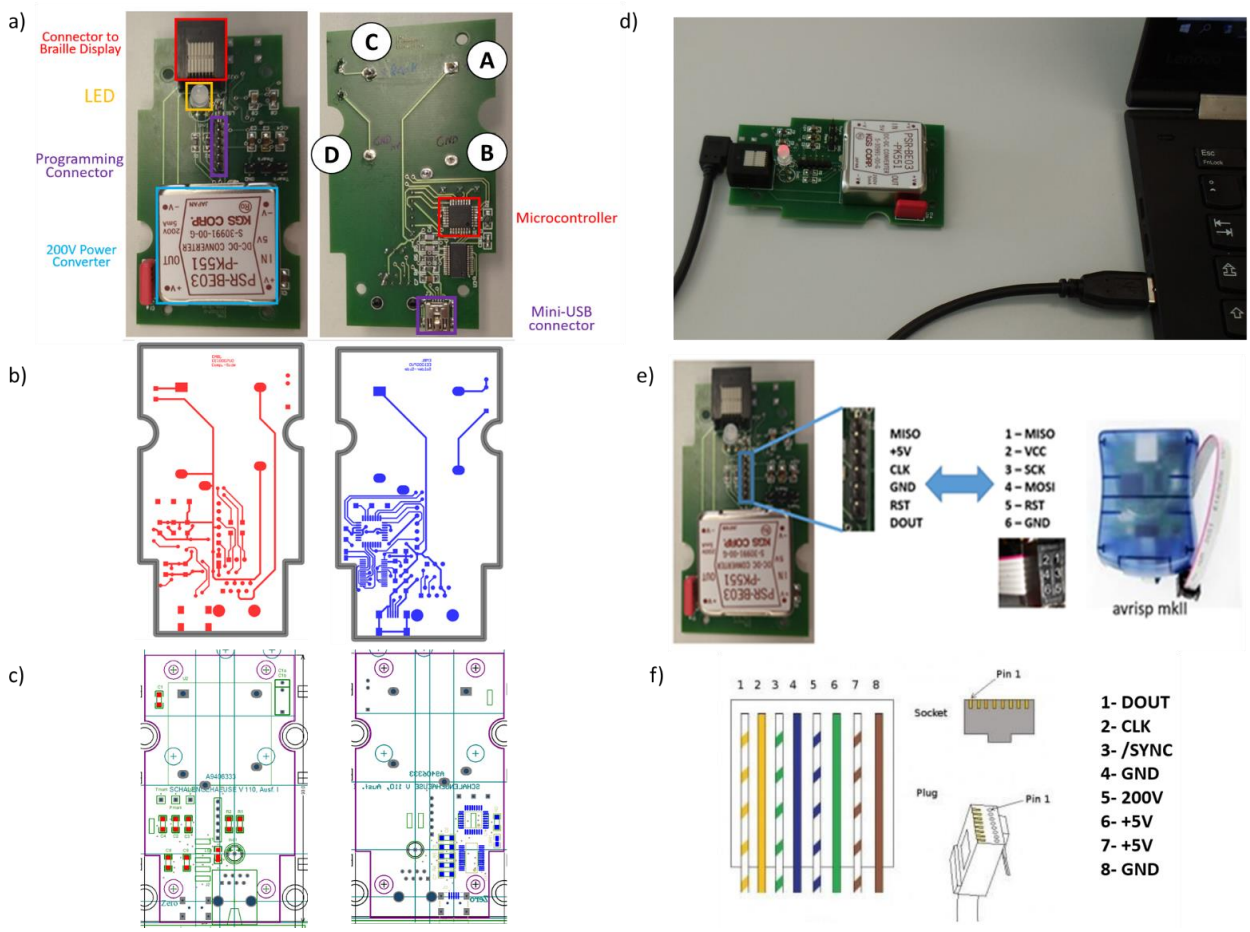
Supplementary Protocol 1 | Braille display controller

The Braille Display controller (**Supplementary Fig. 1**) is implemented in a Printed Circuit Board (PCB, **Supplementary Fig. 2)**. This task can be accomplished by either experienced electronics workshops or external service providers such as Beta Layout (https://uk.beta-layout.com/pcb/). Expert advice can also be obtained from Dr. Alejandro Gil Ortiz (e-mail: ew@embl.de)



Supplementary Figure 1 | PCB layout.

The PCB has a size of 93x52mm^2 and is based on a microcontroller that executes the commands received from a computer connected via USB to mini-USB cable and running our LabVIEW program "samples on demand", which can be found in the **Supplementary file LabVIEW ZIP-file** (for updates check www.epfl.ch/labs/lbmm/downloads/) as described in the main text **Box 1 QUICK GUIDE for the experimental setup of the combinatorial drug screening**. Since the Braille display requires 200V for operation, a power converter generates this voltage from the 5V received by the mini-USB connector, which is used not only for communication, but also to power the board. A telephone type connector is used for the connection to the Braille display. The LED is used to show the status of the display controller under operation.



Supplementary Figure 2 | Electrical connections of the BD controller a) Top (left) and bottom (right) sides of the Braille display controller board with the contact points for current measurement (A, B, C, D) **b, c)** electrical connections, pads and PCB contour. Component details for assembly can be found in Supplementary Table 1, for wiring please see **Supplementary Fig. 1** and all information about the design is provided in the **Supplementary file GerberFiles ZIP-file**. **d)** The board is directly connected to the computer in step 144, using a USB to mini-USB cable. **e)** Schematic sketch of the pinout of the RJ-45 connector to wire the cables to the corresponding pins of the Braille display and **f)** Schematic of the cables of the RJ-45 connector and the corresponding pins of the BD.

In order to fabricate and operate the Braille display controller, users have to go through the following steps:

128| Order the production of the board from a PCB manufacturer. Our prototype was manufactured by Beta Layout (https://uk.beta-layout.com/pcb/), but probably most other PCB manufacturer could do it as well, given that the PCB specifications are easy to fulfill.

For the production of the board, the Gerber files are required by the manufacturing company (see Supplementary file GerberFiles ZIP-file). Gerber files contain information about the routing, layers, vias, pads, PCB contour, etc. existing on the board design. The specific routings of the BD controller board are implemented in two layers (**Supplementary Fig. 2a and b**).

129| If the user plans to assemble the PCB by him/herself, specific components (**Supplementary Table 1** and **Supplementary file GerberFiles ZIP-file**) have to be ordered, otherwise directly proceed with **Step 114**.

Designator	Comment	Footprint	Description	Manufacturer	MPN
CBR1	JUMPER_CBR	SMD_CB/0805_S	Circuit Breaker SMD	Generic	
C1, C2, C3, C8	100nF	SMD/1206	Cap Ceramic, Keramik	Generic	
C1a, C1b	100nF/250V	SMD 1206	Poyester	Kemet	C1206C104KARACTU
C1b	100nF/250V	CAP_THD24_RM4	Poyester	Generic	
C4	2u2F	SMD/1206	Keramik	Generic	
C5, C6	47pF	SMD/1206	Cap Ceramic	Generic	
C7	10nF	SMD/1206	Cap Ceramic	Generic	
C9	4u7F/25V	SMD/1206	Cap Ceramic	generic	
LS1	EXCML20A390U -OBSOLETE	SMD/1206/0805	Ferrit-Spule	Wuerth Electronics	74279208
R1, R2	1k0R	SMD/1206	Resistor 0.125W	Generic	
R3, R5	4k7R	SMD/1206	Resistor 0.125W	Generic	
R4	10k0R	SMD/1206	Resistor 0.125W	Generic	
U1	ATmega8-16AI	32A_M	8-Bit AVR Microcontroller with 8K Bytes	Atmel	ATMEGA8-16AU
U3	FT232RL	SOP65P780X200-28N/FTDI	USB to UART converter	FTDI	FT232RL
J3	USB_440247-1	USBMINIB_440247-TH	USB 2.0, Right Angle, SMT, B Type, Receptacle, 5 Position, Black	TE connectivity	1734035-1
J1	ISP-Prog	HDR1X6	6 Pin header 2.54mm	Amphenol	G800W302018EU
J2	RJ45_8-8_AMP215877-1	AMP_215877-1	Modular Jack for Patchcable 8/8-p Shielded Buerklin 73 F 670	TE connectivity	215877-1
U2	DC/DC_PSR-BE03-PK551	PSR-BE03-PK551	DC/DC Conv 5V/200V KGS Corp.	KGS	PSR-BE03-PK551
Ind1	BI-COLORS	LED_BI-COLORS_D5	Kingbright	Reichelt	LED 5 RG-3

Supplementary Table 1 | Specific components of the Braille display controller board (as specified in the BOM file).

130| Once the board is fabricated, soldering of the components onto the board is required (**Supplementary Fig. 2c**). The necessary information to place the components can be found in **Supplementary Fig. 2** and **Supplementary Table 1**. A simple soldering station can be used, but we recommend using solder paste and a reflow oven. The components can be loaded in any order. Electrostatic discharge (ESD) protection while assembling the board is recommended.

131| Once the board is assembled, a basic test should be performed. To do so, power the PCB to a PC or a wall USB power supply (at least with 1A power capabilities) using a USB to USB-mini cable (**Supplementary Fig. 2 d**).

! CAUTION: Some pins on the board have 200V and may cause electric shocks and injuries.

Check that the main power voltages are supplied correctly to the board: 5V and 200V. To do so, take a voltmeter and set it to measure DC voltage.

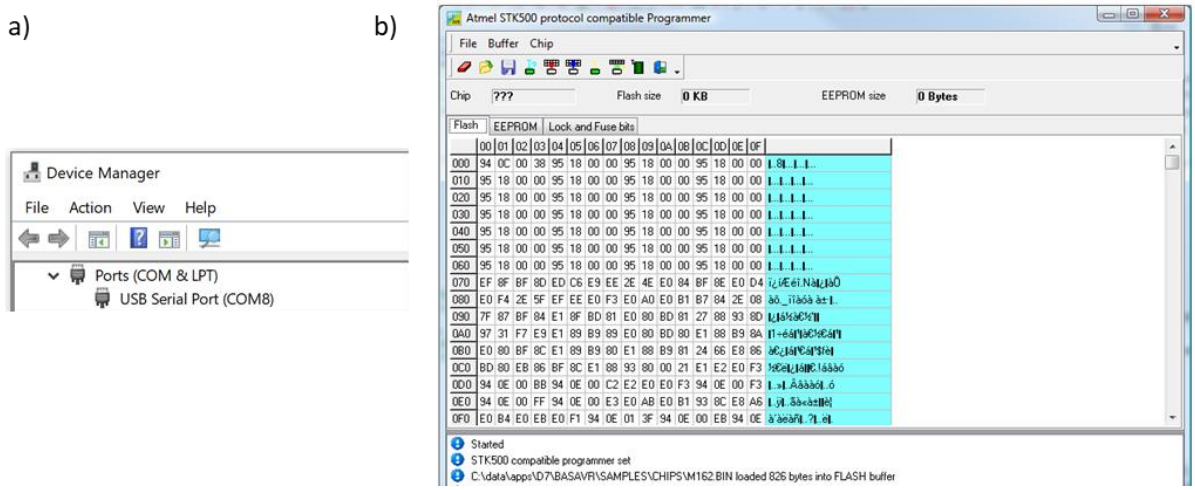
132| Turn around the PCB and locate the points A, B, C, D indicated in **Supplementary Fig. 2a**.

133| Contact point A with the red probe and B with the black probe. This measurement has to indicate 5V.

134| Repeat measuring point C (red) relative to D (black). You should obtain 200V.

! CAUTION: There might be a short circuit connection or a defect component. In this case ask a qualified technician to review the PCB. Only if the previous tests were successful proceed with Step 135.

135| Plug the PCB to a Windows computer, open the Device Manager and make sure that the USB detects a new USB serial port (**Supplementary Fig. 2d**). Windows may require to install the appropriate driver (FTDI, **Supplementary Fig. 3a**).



Supplementary Figure 3 | Screenshot of the device manager window and programmer. a) Screenshot of the device manager window visualizing the successful detection of the new USB serial port. **b)** Screenshot of the Atmel STK500 protocol compatible programmer.

136| Program the firmware of the microcontroller included in the PCB. For this the BASCOM-AVR software is needed. Download and install version 2.0.7.8 or later (https://bascom-avr.apponic.com/).

137| Open the file "ControlBrailleDisplay.BAS" (**Supplementary file gerberFiles.zip**), which contains the code for the Braille control board, using the BASCOM-AVR software.

138| Open one of the supported programmers, *e.g.* ATMEL avrisp mkII programmer to the Display Controller Board. Any other programmer supported by ATMEL would also work (https://avrhelp.mcselec.com/index.html?supported_programmers.htm). The connections from the ATMEL avrisp mkII programmer to the Display Controller Board are shown in **Supplementary Fig. 2e**).

139| Select the COM port where the board is connected.

140| Program the chip according to a pre-specified pattern (**Supplementary Fig. 3b**)

Correct programming is confirmed by specific messages (lower part of **Supplementary Fig. 3b**).

141| Assemble the box and front plate using 8 pre-defined electrical connections (**Supplementary Fig. 2f**).

142| Mount the PCB in the white box (shown in **Extended Data Fig. 1b**) with M3 screws.

143| Connect the Braille display using the pinout of the RJ-45 connector. Wire the cables to the corresponding pins of the Braille display (**Supplementary Fig. 2f)**

! CAUTION: Wrong connection may destroy the braille display and/or programmer.

144| Unplug the programmer and plug the board directly to the computer using a USB to mini-USB cable. The LED should be off.

145| Open the LabVIEW program samples on demand (which can be found in the Supplementary file LabVIEW ZIP-file (for updates check www.epfl.ch/labs/lbmm/downloads/) as described in the main text **Box 1** Experiment setup for combinatorial drug screening and barcoding, select the right port and the click Initialize. The LED should switch on in green color.

146| Use the LabVIEW program "samples on demand" as described in **Box1** and **Step 51** (in the main text).

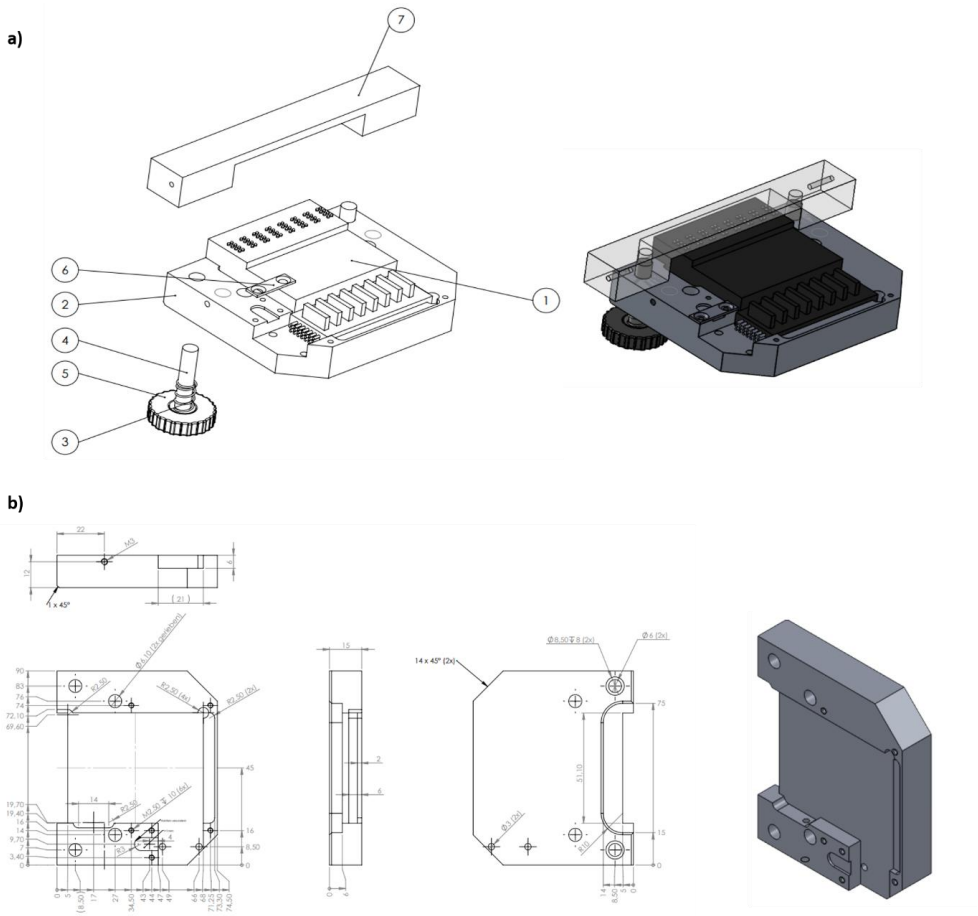
! CAUTION: Avoid contact with water. If the BD controller gets wet, unplug it immediately from the computer, let the controller dry completely (at least overnight) and make sure the PCB works by repeating the Steps 115 to 119 before re-connecting to the computer.

Supplementary Protocol 2 | Custom-made parts from the EMBL mechanical workshop

Custom-made parts from the EMBL mechanical workshop were fabricated to hold the BD and to mount and align the microfluidic PDMS chip on top of it. Readers are also pointed to previous publications, in which solutions with similar functionality have been described (https://pubs.rsc.org/en/content/articlehtml/2013/lc/c3lc51083b, https://pubs.rsc.org/en/content/articlelanding/2006/LC/B510901A).

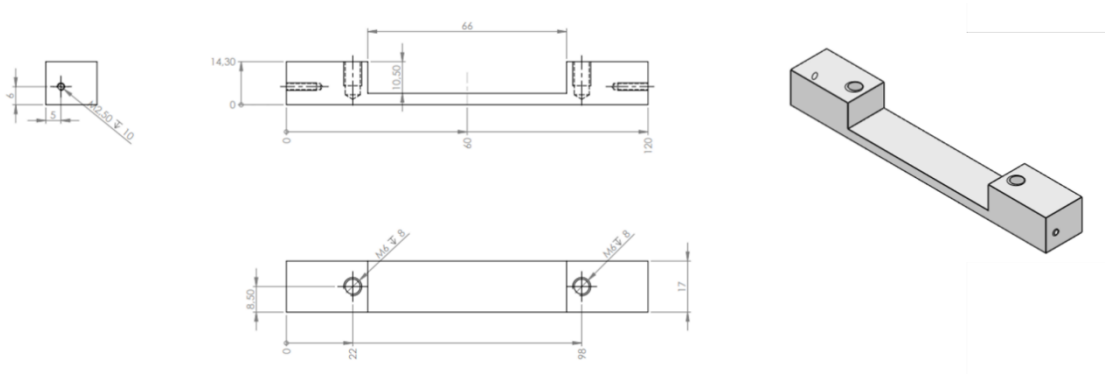
147| Order the Braille display SC-9 from KGS corporation, Japan

148| Design a CRD drawing and 3D model (**Supplementary Fig. 4a)** of the lower part of the BD holder (specific dimensions and features shown in **Supplementary Fig. 4b**) and implemented in the Solidworks 3D CAD file **Supplementary file BG-Braille-Display-controller.easm**), for milling out of an aluminum block.



Supplementary Figure 4 | Design of all parts for the BD holder. a) 1 is the BD, 2 bottom part of the holder, 3 spring, 4 and 5 screws (UNC#832x0.375´´, 9.5 mm), 6 cable clamp, 7 PMMA plate. **b)** Geometry and dimensions of the bottom part of the BD holder. For details please see the Solidworks 3D CAD file: **Supplementary file BG-Braille-Display-controller.easm**, which can be viewed with Solidworks software (https://www.edrawingsviewer.com/download-edrawings).

149| Design a CRD drawing and 3D model of the upper part of the BD holder (specific dimensions and features shown in **Supplementary Fig. 5**, and implemented in the Solidworks 3D CAD file **Supplementary file BG-Braille-Display-controller.easm**), to be made out of transparent Plexiglass (PMMA). **! CAUTION: All used materials should be resistant to corrosion and oxidation and the upper part of the BD holder must be transparent to allow monitoring Braille pin actuation and proper functioning of the valves.**

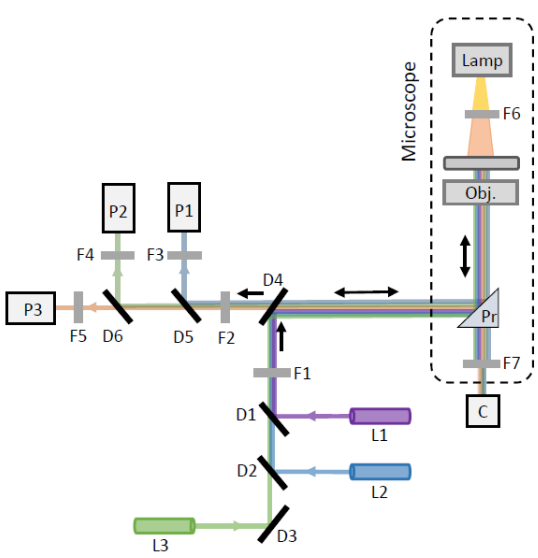


Supplementary Figure 5| Geometry and dimensions of the Plexiglass (PMMA) mounting part of the BD holder

150| In our case the production of the inhouse parts was carried out using a 5-axis milling machine (Hermle C12), but less complex machining tools or commercial CNC milling service suppliers (*e.g.* www.hubs.com; www.rapiddirect.com), can be used as well. An edge phase of 45° is recommended. The aluminum part should be anodized.

151| Insert the BD in the lower part and glue the wires permanently in place. The lower part of the BD manifold is a milled metal piece in which the BD is positioned tightly and in the correct orientation with respect to the wires which connect the BD to the power source and control unit. It is vitally important that the wires connecting to the power source and control unit are only ever plugged into the BD in the correct orientation, as applying voltage in the wrong orientation will irreparably break the BD. Lastly, fix the upper Plexiglass part of the BD holder using screws with the specified diameters (**Supplementary Fig. 4, 5** and **Supplementary file BG-Braille-Display-controller.easm**). For the upper part of the holder one should use springs in between the head of the M6 screws and the Plexiglass. This allows to nicely adjust the holding force of the PDMS chip on the BD, based on the number of rotations applied to the screw. Now the BD controller and manifold are ready to use for drug screening (**Steps 32-94**) or droplet sorting (**Steps 95 – 127**).

Detailed Optical setup



Lasers (L)
L1: 375 nm, 50 mW (Coherent, cat. no. 1178748)
L2: 488 nm, Low noise, 50 mW (Dragon Lasers Co., cat. no. 488M50)
L3: 561 nm, Low noise, 50 mW (Dragon Lasers Co., cat. no. 561LFN50)
PMTs (P)
P1: PMT (Hamamatsu Photonics, cat. no. H11903-210)
P2: PMT (Hamamatsu Photonics, cat. no. H11903-210)
P3: High dynamic range PMT (Hamamatsu Photonics, cat. no. H11903-20)
Filters (**F,** diameter: 25 mm)
F1: 390/482/563/640 Quadband Exciter (AHF, cat. no. F74-866)
F2 and F7: 446/523/600/677 Quadband Emitter (AHF, cat. no. F72-866)
F3: 445/45 Bandpass (AHF, cat. no. F37-446)
F4: 525/45 Bandpass (AHF, cat. no. F37-521)
F5: 561 Longpass (AHF, cat. no. F76-561)
F6: 633 Longpass (AHF, cat. no. F76-631)
Dichroic mirrors (**D**, dimension : 36mm x 25mm)
D1: Beamsplitter 375-415 (AHF, cat. no. F38-M01)
D2: Beamsplitter 488 (AHF, cat. no. F48-487)
D3: Silver Mirror (AHF, cat. no. F21-005)
D4: Quadband Beamsplitter 405/488/561/635 (AHF, cat. no. F73-866)
D5: Beamsplitter 485 (AHF, cat. no. F33-485A)
D6: Beamsplitter 565 (AHF, cat. no. F33-565A)
Others
Prism (**Pr**)**:** PRISME T-BP E20 L80 (cat. no. MED59040)
Camera (**C**): MotionBLITZ EoSens mini1 (Mikrotron, cat. no. 109724)
Objective (**Obj.**): CFI S-Fluor 40x/0.75 Objective (Nikon, cat. no. MRH00401)

Supplementary Figure 6 | Optical setup of the microfluidic workstation. The optical setup consists of three different excitation lasers (L1: 375 nm, L2: 488 nm, L3: 561 nm) to excite the fluorophores and the subsequent emission is collected in three separate channels using photomultiplier tubes (PMTs; P1: 445 ± 22.5 nm, P2: 525 ± 22.5 nm and P1: > 561 nm). The excitation and emission beams are filtered using various optical filters (F1-F7) and dichroic mirrors (D1-D6) as described in the table. The prism kept in the microscope allows 20 % of the emission light to pass so that the high-speed camera (C) can simultaneously image the process.

Supplementary Protocol 3 | BD screening chip and mold testing ● TIMING 2.5 h

Consistent mixing of components on the BD is critical for data collection. Whenever a new mold for the screening chip is manufactured, full functionality of chips prepared from this mold should be tested at least once. Here we describe a protocol, how to test all valves of the chip. The quality of the chip is defined by production of plugs with equal length and content. We use syringes with Fluorescein, a fluorescent dye, which are connected to each inlet. Then we prime each valve, while collecting the plugs in a tube. We record the fluorescence intensity of each plug (**Supplementary Fig. 7a**) and compare the variation of the peak length (**Supplementary Fig. 7b**) and reproducibility of the intensity (**Supplementary Fig. 7c and d**). Variations in the green fluorescence peak intensity indicate problems with the valve functions and are important for quality control.

152| Prepare syringes with barcoding solution, mineral oil and FC-40 oil with 0.5% vol/vol PFO, 5 ml each, as described in the main text and protect them from light using aluminum foil.

153| Wind collection tubing 28 times around a 500 ml glass bottle and fill the tube with PBS using a 23G syringe.

154| Use fluorescein with a concentration of 25 µM. **! CRITICAL:** Filter it with a 22 µm filter to avoid clogging.

155| Fill 13 syringes with 5 ml 25 µM Fluorescein solution and attach tubing. **! CRITICAL:** Protect syringe from light with aluminum foil to prevent photobleaching.

156| Mount fluorescein and barcode syringes on a 10- and 4-channel pump, respectively, and the oil syringes on a 2-channel pump. Make sure all tubings discharge into a waste beaker. Set all pumps to an infusion rate of 1000 µl/h and run them until there is continuous flow through all tubings. Then set the pump for oil to 200 µl/h and the fluorescein and BC pumps to 500 µl/h.

157| Start the "Samples on demand v5" LabVIEW application and click on the "Initialize BD" button. Check all valves for function.

158| Connect oil syringes, waste tubings, fluorescein tubing to the chip, BC tubing to the inlet for valve 24 and a short temporary collection tube (~30 cm) to the outlet. Open and close each valve manually and check under the microscope if the blue dye is washed out from the valve upon opening. Make sure there is no leakage at any valve by checking if no aqueous droplets form after closing the valve.

159| Change the temporary collection tube to the final one.

160| Set valve opening time for oil flow to 2 seconds, select the **Supplementary file "Priming.csv"** and run 2 cycles to check each valve for operation. If leakage occurs perform chip alignment with a new chip.

161| Run **Supplementary file "Chip_Testing.csv"** until the collection tube is filled with plugs to have uniform back pressure throughout the entire experiment.

162| To indicate the starting point of the experiment, run two cycles with the **Supplementary file "Barcoding.csv"**.

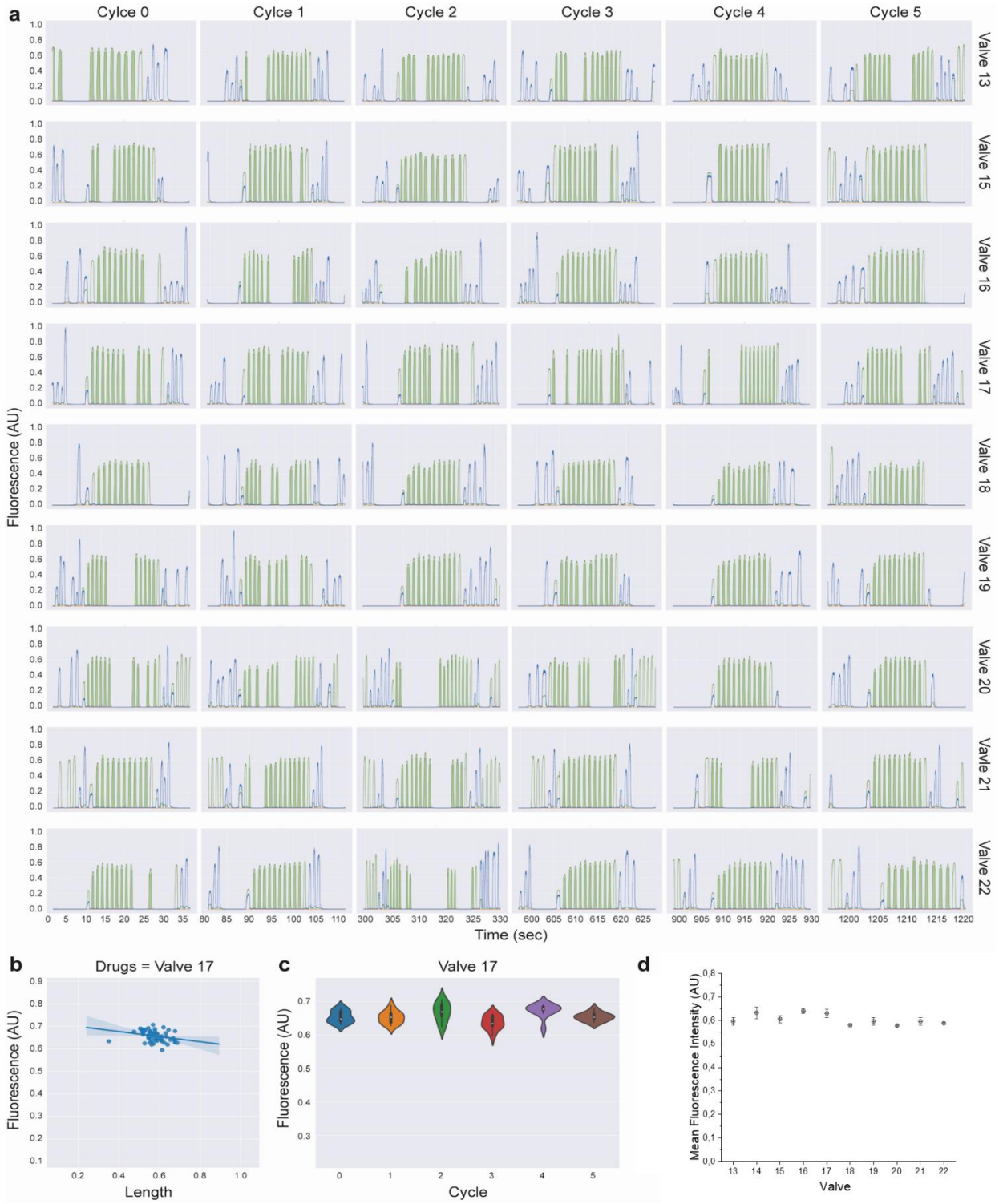
163| Produce plugs by running four cycles of **Supplementary file "Chip_Testing.csv"**. **! CRITICAL:** Protect the collection tube from light exposure with aluminum foil.

164| Run two cycles of **Supplementary file "Barcoding.csv"** to mark the end of the experiment.

165| Check for leakage by checking each valve manually and run **Supplementary file "Priming.csv"** to check each valve for operation (described in **BOX1**).

166| Do sample acquisition as described in the main text (**Steps 77-85**). No need for incubation time.

167| For data analysis use the Python script in the main text (**Steps 86-94**). Only use the mold for further experiments if 1.) all valves still open and close reliably after 6 screening cycles, 2.) no cross-contamination of blue barcoding dye in the green plugs is observed (except for the first three green plugs generated after a barcode) 3.) pure barcode plugs without green dye can be observed at least for barcodes exceeding 3 "digits" 4.) the standard deviation of the green signals does not exceed ±20%. Note that the setup described above differs significantly from the screening setup (injection of only one aqueous reagent at any given time, different flow rates, rapid photobleaching of and "wetting" caused by fluorescein), for which reason imperfections within the rules described above are tolerable. The quality of each drug screen is furthermore assessed separately based on the Zm factor (page 75 in the main text).



Supplementary Figure 7 | Chip testing for drug screening molds, to validate the valve function and signal stability over 6 cycles. **a)** Overview of the PMT signals for each valve over 6 plug generation cycles. **b)** plug length and **c)** fluorescence intensity for valve 17 are stable over all cycles of the test period. **d)** Plot illustrating the mean fluorescence intensity and standard deviation for each valve averaged over a total of 6 test cycles.

Supplementary Protocol 4 | Mold preparation for producing droplet collection chips ● TIMING 4h

The procedure is similar to that of mold preparation for screening and sorting chips (**Steps 1-31** in the main text) with minor changes in the use of material and temperature settings.

168| Take a 4 inch diameter silicon wafer, clean it with acetone and heat wafer for 5-10 minutes at 150°C to evaporate remaining solvents on the surface. Leave it to cool for 5 minutes before using it further. **! CAUTION: Acetone Is flammable. ! CRITICAL:** Wafers should never be taken directly from the hotplate onto a surface which is cold or conducts heat well, as the wafer will cool too quickly and crack. Instead, they should be put onto clean-room towels. Place wafer in the middle of the plate to avoid temperature gradients. We recommend performing all steps for mold preparation in a cleanroom.

169| Prepare the spin coater by lining the inside of the lid with aluminum foil to reduce spills of photoresist. Turn on the vacuum pump. Create a two-step-program: Step 1= 10 s at 500 rpm with acceleration ~115 rpm/s and step 2 = 40 s at 1800 rpm with acceleration ~344 rpm/s. Center silicon wafer on spin coater and apply vacuum to fix the wafer during the coating process. **! CRITICAL:** The compressed air pressure must be higher than 4 bar for the spin coater to work.

170| Pour a ~4cm diameter pool of SU-8 2150 photoresist on the center of the wafer and close the lid and run the spin coater program. Once the program has ended, carefully open the lid and move the wafer to a hotplate with a temperature of 65°C for 8 min. Subsequently move the wafer to another hotplate set to 95°C using tweezers and let it bake for 90 min. Afterwards the wafer needs to cool down to room temperature.

171| Place the photomask on the photoresist. Expose for 200 s at 350 W (mask aligner operates with approximately 10 $mJ/cm^2/sec$).

172| Carefully remove the photomask with tweezers. Post bake the wafer on a 65°C hotplate for 5 min and ramp the temperature to 95°C for 25 min before cooling down to room temperature.

173| Place a clean glass petri dish onto a shaker with the baked wafer inside, then pour 10-15 ml of SU-8 developer into the dish and start shaking immediately. After 5 min, remove the developer and wash the wafer with isopropanol (IPA) for 10 sec. Add fresh developer solution and repeat, but develop for shorter and shorter periods (e.g. 20 s, wash, then 10 s etc.) until development is complete. At this point, there should not be any white film in the developer solution. Use air gun to remove excess developer or IPA. **! CRITICAL:** The formation of white film indicates an incomplete development process.

174| Check the structures under the microscope for any visible defects such as missing channels. In absence of any defects continue with hard baking at 150 °C for 5 min and subsequent cooling to room temperature.

Supplementary Protocol 5 | Production of collection chips ● TIMING 7 h

175| Prepare 100 grams of a mixture of PDMS and cross linker in a ratio of 10:1. Mix thoroughly and degas the mixture in a desiccator for 30 minutes. Then pour this mixture into the freshly prepared mold. Subsequently the mold has to be cured in an oven for 6 h.

176| Cut the cured PDMS chip out of the mold, and punch holes for inlets and outlets using a 0.75 mm biopsy punch. Then clean the PDMS chip with a piece of scotch tape and spray with air gun to remove any dust particles.

177| Take a glass slide (~5 x 7.5 cm) and remove dust with an air gun. Afterwards place it in the oxygen plasma oven along with the PDMS chip (channels facing up) for 1.5 min, before pressing the activated surfaces together and baking the closed chip for 2 min at 65 °C to finish the bonding process.

Supplementary Protocol 6 | Statistical considerations

In case of symmetric distributions, the median and the mean are identical. Using a 300 Hz acquisition rate, and the average plug length being 1.1 seconds, we record 330 data points for each plug on average. The signals within the plugs are typically very stable, and we have no reason to assume that the distribution of individual data points within plugs is skewed. This suggests that the median and the mean should show negligible difference. Rarely it can happen that the scattering or fluorescence of particles results in a spike of the measured signal. Also, the start and end of each plug is determined based on the three channels together, but very minor asynchronicity can occur, meaning that one of the channels records baseline level values after the acquisition of the plug has started, and goes up only with a few milliseconds delay. Using the median, these high or low outliers do not impact the quantification, although their effect would likely be negligible even on the mean.

When looking at the signal variation of different plugs hosting the same sample composition, deciding between median and mean intensities is slightly more critical, as for each drug combination we have only around 7-9 separate data strings (plugs), and these also show a higher variance. Given that the median is more robust in regard to potential outliers, it seems to be the preferable metric here, but we do not expect significantly different predictions of optimal drug combination even in case readers prefer to use the mean instead.

Ave	Average	Mw	Molecular weight
BC	Barcode	**Neg-ctrl, N**	Negative control
BD	Braille Display	**PBS**	Phosphate-buffered saline
DAPI	4′,6-diamidino-2-phenylindole (DNA stain)	**PDMS**	Polydimethylsiloxane
dH2O	Distilled water	**Pen-strep**	Penicillin-Streptomycin
DMSO	Dimethyl Sulfoxide	**PFO**	1*H*,1*H*,2*H*,2*H*-Perfluor-1-octanol
DTT	DL-Dithiothreitol	**PMTs**	photomultiplier tubes
EDTA	Ethylenediaminetetraacetic acid	**Pos-ctrl, P**	Positive control
FACS	Fluorescence-activated cell sorting	**PTFE**	Polytetrafluoroethylene
FBS	Fetal bovine serum	**PVDF**	Polyvinylidene fluoride (membrane)
FC-40	3M Fluorinert Bis (nonafluorbutyl) (trifluormethyl) amin	**ROI**	Region of interest
FADS	Fluorescence-activated droplet sorting	**TP**	Throughput
FS	Freestyle medium	**Zm**	Quality factor
G	Gauge	**5-FU**	5-Fluorouracil
Hz	Herz	**σ**	Standard deviation
MA	Mask aligner	**μ**	Mean

Supplementary Table 2 | List of abbreviations.

Compound	mean_z score	std_z score	p value	p adjusted	Compound	mean_z score	std_z score	p value	p adjusted
5-FU + Cetuximab	-0,023	0,190	0,000	0,000	Irinotecan + Sorafenib	-0,202	0,287	0,000	0,000
5-FU + Irinotecan	0,149	0,216	0,000	0,000	Irinotecan+ Trametinib	0,149	0,393	0,000	0,000
5-FU + MK-2206	-0,248	0,238	0,000	0,000	Irinotecan + neg_ctrl	-0,862	0,187	0,147	1,000
5-FU + Oxaliplatin	-0,325	0,264	0,000	0,000	Irinotecan + pos_ctrl	2,590	0,770	0,000	0,000
5-FU + Pictisilib	-0,683	0,163	0,074	1,000	MK-2206 + Sorafenib	-0,265	0,244	0,000	0,000
5-FU + SP600125	-0,289	0,306	0,000	0,000	MK-2206 + neg_ctrl	-0,823	0,197	0,488	1,000
5-FU + Sorafenib	-0,699	0,167	0,110	1,000	MK-2206 + pos_ctrl	2,449	0,452	0,000	0,000
5-FU + Trametinib	-0,320	0,255	0,000	0,000	Oxaliplatin+ Cetuximab	0,314	0,362	0,000	0,000
5-FU + neg_ctrl	-1,007	0,189	0,000	0,016	Oxaliplatin+ MK-2206	0,114	0,170	0,000	0,000
5-FU + pos_ctrl	0,940	0,321	0,000	0,000	Oxaliplatin+ Pictisilib	-0,543	0,176	0,000	0,003
Cetuximab+ MK-2206	0,355	0,273	0,000	0,000	Oxaliplatin+ SP600125	0,215	0,294	0,000	0,000
Cetuximab+ Pictisilib	-0,046	0,250	0,000	0,000	Oxaliplatin+ Sorafenib	-0,366	0,253	0,000	0,000
Cetuximab+ SP600125	0,312	0,307	0,000	0,000	Oxaliplatin+ Trametinib	0,028	0,457	0,000	0,000
Cetuximab+ Sorafenib	-0,041	0,343	0,000	0,000	Oxaliplatin+ neg_ctrl	-0,626	0,187	0,007	0,443
Cetuximab+ Trametinib	0,176	0,189	0,000	0,000	Oxaliplatin+ pos_ctrl	1,658	0,388	0,000	0,000
Cetuximab+ neg_ctrl	-0,787	0,171	0,876	1,000	Pictisilib + MK-2206	-0,344	0,400	0,000	0,001
Cetuximab+ pos_ctrl	2,682	0,876	0,000	0,000	Pictisilib + SP600125	0,197	0,306	0,000	0,000
5-FU	-0,593	0,380	0,020	1,000	Pictisilib + Sorafenib	-0,554	0,217	0,000	0,026
Cetuximab	-0,119	0,285	0,000	0,000	Pictisilib + Trametinib	-0,256	0,201	0,000	0,000
Irinotecan	-0,208	0,254	0,000	0,000	Pictisilib + neg_ctrl	-0,826	0,175	0,508	1,000
MK-2206	-0,237	0,183	0,000	0,000	Pictisilib + pos_ctrl	1,448	0,562	0,000	0,000
Oxaliplatin	-0,445	0,317	0,000	0,002	SP600125 + MK-2206	0,228	0,308	0,000	0,000
Pictisilib	-0,677	0,316	0,150	1,000	SP600125 + Sorafenib	-0,031	0,352	0,000	0,000
SP600125	-0,134	0,310	0,000	0,000	SP600125 + Trametinib	0,386	0,334	0,000	0,000
Sorafenib	-0,674	0,178	0,059	1,000	SP600125 + neg_ctrl	-0,746	0,327	0,911	1,000
Trametinib	-0,368	0,240	0,000	0,000	SP600125 + pos_ctrl	3,467	1,090	0,000	0,000
neg_ctrl	-1,031	0,141	0,000	0,001	Sorafenib + neg_ctrl	-0,916	0,198	0,014	0,952
pos_ctrl	0,799	0,311	0,000	0,000	Sorafenib + pos_ctrl	1,815	0,728	0,000	0,000
Irinotecan + Cetuximab	0,222	0,282	0,000	0,000	Trametinib+ MK-2206	0,151	0,260	0,000	0,000
Irinotecan + MK-2206	0,188	0,342	0,000	0,000	Trametinib+ Sorafenib	-0,119	0,221	0,000	0,000
Irinotecan + Oxaliplatin	0,317	0,265	0,000	0,000	Trametinib + neg_ctrl	-0,702	0,161	0,083	1,000
Irinotecan + Pictisilib	-0,251	0,169	0,000	0,000	Trametinib+ pos_ctrl	2,544	0,966	0,000	0,000
Irinotecan + SP600125	0,162	0,302	0,000	0,000	neg_ctrl + pos_ctrl	-0,301	0,336	0,000	0,000

Supplementary Table 3 | Statistical analysis of drug screening data