# Supporting Information

## Debs et al. 10.1073/pnas.1204514109

### SI Text

**SI Materials and Methods.** ***Microfluidic device fabrication.*** Standard soft-lithography techniques (1) were used for the fabrication of microfluidic devices from polydimethylsiloxane (PDMS). Channel patterns were printed as negatives on a photolithography mask (Selba SA) and projected onto a silicon wafer (Siltronix) coated with SU-8 photoresist (MicroChem Corp.) using UV light (MJB3 contact mask aligner; SUSS MicroTec). In consequence, only regions of the SU-8 photoresist below transparent regions of the mask (corresponding to the channels) polymerized. Subsequently, nonpolymerized SU-8 photoresist was removed using SU-8 developer (MicroChem Corp.). A mixture of 90% PDMS and 10% curing agent (wt/wt) (Sylgard 184 silicone elastomer kit; Dow Corning Corporation) was poured over the SU-8 mold, degassed and cross-linked at 65 °C overnight. Polymerized PDMS was peeled off the mold and inlets and outlets were punched with Harris Uni-Core punches (Electron Microscopy Sciences). The structured side of the PDMS chip was treated with oxygen plasma and bonded to an indium tin oxide (ITO)-coated glass slide (Delta technologies). Subsequently the electrodes were casted by melting 51In/32.5Bi/16.5Sn low temperature solder (Idium Corporation) and introducing it into the corresponding microfluidic channels (2). Short pieces of electrical wires (Radiospares) were glued (Loctite UV Glue; Henkel Corporation) to the solder electrodes and served as electrical connections. To minimize wetting, the channel surfaces were rendered hydrophobic, by flushing the device with Aquapel (PPG Industries) and purging with $N_2$ prior to use.

***Cell culture and purification of antibodies.*** The 4E3 hybridoma cells (3–5) and Elec-403 hybridoma cells (6) were grown in HyClone Roswell Park Memorial Institute (RPMI) 1640 media (Thermo Fischer), supplemented with 10% hybridoma-tested FBS (Sigma-Aldrich), 100 μM nonessential amino acids (Gibco), 1 mM sodium pyruvate (Sigma), 1 × RPMI 1640 vitamins (Sigma-Aldrich), and 100 μM β-mercapthoethanol. Cell cultures were split every 2 to 3 d and new media was added to the cells 12 h prior to encapsulation into drops.

For the purification of antibodies, $1.5 \times 10^6$ hybridoma cells were transferred into 175 $cm^2$ flasks in 25 mL of hybridoma medium (HyClone) and cultivated for 1 wk at 37 °C under a 5% $CO_2$ atmosphere saturated with water. Subsequently the supernatant was filtered using 0.22 μm filters (Millipore). Antibodies were purified using the Prosep-A kit (Millipore). The purified antibody solution was concentrated using Amicon 30,000 Da filters (Millipore). For each 25 mL of hybridoma supernatant approximately 250 μL of antibody solution at a concentration of approximately 2 mg/mL was obtained.

***Encapsulation of hybridoma cells.*** Hybridoma cells were harvested immediately before encapsulation, centrifuged, and washed twice with PBS to remove all secreted antibodies. The cell density was adjusted to $1.25 \times 10^6$ cells/mL in RPMI 1640 media containing 1.6 ng/mL angiotensin converting enzyme 1 (ACE-1) (R&D Systems). This cell suspension was loaded in a 5 mL polyethylene syringe (Fisher Bioblock), cooled with an ice bag, stirred at 200 rpm using an 8 mm magnetic stir bar (Roth), and injected via Polytetrafluoroethylene (PTFE) tubing (0.56 mm × 1.07 mm internal/external diameter; Fisher Bioblock) into the microfluidic device (Fig. 1*C*) using a syringe pump (PHD 2000, Harvard Apparatus) at a flow rate of 2,000 μL/h. Drops were generated by flow focusing this continuous phase using perfluorinated oil (HFE-7500), containing 2% (wt/wt) EA-Surfactant (7), at a flow rate of 4,000 μL/h. The resulting emulsion was collected in a 1 mL polyethylene open syringe (without the plunger inserted) by connecting it to the outlet of the microfluidic device via PTFE tubing (0.56 mm × 1.07 mm internal/external diameter; Fisher Bioblock). After the desired incubation time at 37 °C under a 5% $CO_2$ atmosphere, mineral oil was added to fill the syringe completely before inserting the plunger and reinjecting the emulsion into the integrated microfluidic chip (Fig. 1*D*).

***Sorting and recovery of hybridoma cells.*** Emulsions containing hybridoma cells were reinjected into the integrated chip (Fig. 1*D*) allowing the droplets to fuse with a second droplet species containing the fluorogenic substrate for ACE-1 (Fig. 1*B*). Fused drops were flushed through a delay line (8), where approximately 80% of the total volume was removed through two outlets connected to syringe pumps operating in withdraw mode, resulting in slower movement of the drops in the delay line and hence an incubation time of about 40 min. Drops were spaced out at the sorting module by injecting additional oil. Further downstream the sorting of drops was performed using embedded electrodes adjacent to the Y-junction of the sorting module, where one of the outlets (waste outlet) was connected to a syringe pump operating in withdraw mode aspirating at 70% of the incoming flow rate, thus forcing the drops into this outlet in absence of any electric field. An AC-voltage of 3,000 V was applied to the electrodes at a frequency of 6,000 Hz and a pulse length of 40 ms when the green fluorescence crossed a predefined threshold. Electrode activity was controlled using sorting gates (as in fluorescence-activated cell sorting) defined by an in-house LabView 8.2 program. The flow rates used on the integrated chip were, as follows: reinjected emulsion (2% EA-Surfactant in HFE-7500), 50 μL/h; separating oil (0.5% EA-Surfactant in FC40), 1,000 μL/h; ACE-1 substrate, 50–80 μL/h; oil for ACE-1 substrate drops (pure FC40), 250–300 μL/h; suction upstream of the delay line, 600–650 μL/h; oil separating droplets of the fused emulsion (0.5% EA-Surfactant in FC40), 700–800 μL/h; and aspiration from the waste outlet, 550 μL/h. Sorted drops were collected in a 1.5 mL Eppendorf tube held on ice and subsequently broken by adding an equal volume of 1H,1H,2H,2H-Perfluoro-1-octanol (Aldrich) and shaking for around 5 s. One milliliter of fresh RPMI 1640 media was added, the oil phase was removed using a pipette, and cells were centrifuged and cultivated in 96-well plates.

***Determination of antibody concentration.*** ELISAs against ACE-1 were performed by coating 96-well plates with 1 μg/mL ACE-1 overnight at 4 °C. Subsequently, the plates were washed using PBS supplemented with 0.05% Tween 20. Forty microliters of hybridoma supernatant or 50 μL of purified 4E3 antibody solutions were added and incubated at room temperature (RT) for 3 h. After another washing step, secondary goat polyclonal antibody against mouse IgG coupled to alkaline phosphatase (Sigma) was added and incubated for 2 h at RT before washing again. A chromogenic substrate of alkaline phosphatase, *p*-nitrophenylphosphate (pNPP; Sigma-Aldrich), was added to the wells and incubated for 30 min. Subsequently, the OD was measured at 405 nm using a Spectramax M5 plate reader (Molecular Devices). Total IgG concentrations were determined using the Easy-Titer IgG assay kit (Thermo Scientific) according to the manufacturer's instructions.

***Monitoring of ACE-1 activity.*** ACE-1 was added to hybridoma supernatants at a concentration of 1.4 ng/mL to assess the ACE-1-inhibitory effect of hybridoma supernatants. After 3 h of incubation at 37 °C, a custom synthesized fluorogenic substrate for ACE-1 (Bachem; Fig. 1*B*), was added at a concentration of 15 μM and the fluorescence kinetics followed ($\lambda_{ex} = 488$ nm; $\lambda_{em} = 520$ nm) in a Spectramax M5 plate reader over 1 h.

***Biochemical characterization of single hybridoma cell clones.*** Single hybridoma cells recovered from droplets within specific sorting gates were individually pipetted using gel loading pipette tips (epTIPS; Eppendorf), and seeded in 96-well plates containing 200 μL of fresh RPMI 1640 media per well (Fig. S5). After expansion for 14 d, 500 cells from each well were seeded again in 200 μL of media and incubated for 5 d. Subsequently supernatants were analyzed for the total concentration of IgG, the 4E3 antibody concentration, and for the inhibitory effect on ACE-1. To obtain average values for each population, the same procedure was carried out in parallel using 500 pooled cells from the respective gates. After their expansion, 500 cells were seeded again into microtiter plates to allow for the same characterization.


1. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM (1998) Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anall Chem* 70:4974–4984.
2. Siegel AC, Bruzewicz DA, Weibel DB, Whitesides GM (2007) Microsolidics: Fabrication of three-dimensional metallic microstructures in poly(dimethylsiloxane). *Adv Mater* 19:727–733.
3. Skirgello OE, *et al.* (2006) Inhibitory antibodies to human angiotensin-converting enzyme: Fine epitope mapping and mechanism of action. *Biochemistry* 45:4831–4847.
4. Naperova IA, *et al.* (2008) Characteristics of monoclonal antibody binding with the C domain of human angiotensin converting enzyme. *Bioorg Khim* 34:358–364.
5. Naperova IA, *et al.* (2008) Mapping of conformational mAb epitopes to the C domain of human angiotensin I-converting enzyme. *J Proteome Res* 7:3396–3411.
6. Remy MH, Frobert Y, Grassi J (1995) Characterization of monoclonal antibodies that strongly inhibit *Electrophorus electricus* acetylcholinesterase. *Eur J Biochem* 231:651–658.
7. Holtze C, *et al.* (2008) Biocompatible surfactants for water-in-fluorocarbon emulsions. *Lab Chip* 8:1632–1639.
8. Frenz L, Blank K, Brouzes E, Griffiths AD (2009) Reliable microfluidic on-chip incubation of droplets in delay-lines. *Lab Chip* 9:1344–1348.

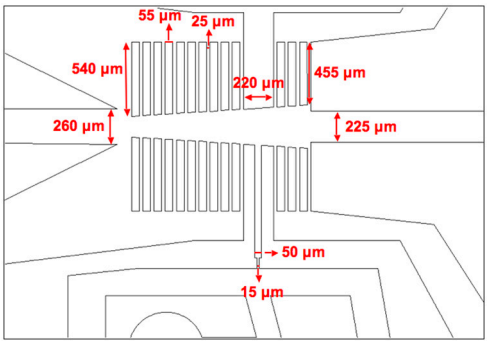




**Fig. S1.** Dimensions of the fusion chamber in the integrated chip.

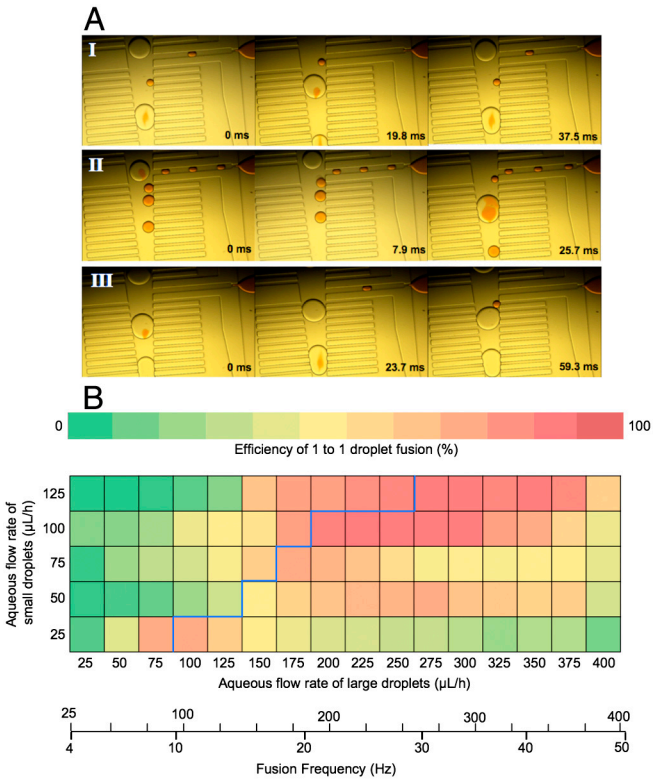



**Fig. S2.** Pillar-induced droplet fusion of two different droplet species. (*A*) Regime I, optimal 1:1 droplet fusion; regime II, failure of the droplet fusion process due to the release of nonfused small droplets from the pillar chamber, as observed for high aqueous flow rates of the small droplets; and regime III, failure of the droplet fusion process due to the passage of big droplets without fusion occurring, as observed for high aqueous flow rates of the large droplets. (*B*) Heat map depicting percentage (color coded) of droplets undergoing optimal 1:1 droplet fusion (regime I in *A*) for different aqueous flow rates for large and small droplets (at constant oil flow rates). More than 2,000 fusion events were monitored, respectively, based on video analysis and fluorescence measurements [using different concentrations of fluorescein in the small (100 μM) and the large (10 μM) droplets]. For low aqueous flow rates of the large droplets, the fraction of droplets that did not undergo 1:1 fusion passes the chamber according to regime II (*Left*), for high aqueous flow rates it passes the chamber according to regime III (*Right*). The transition between these two scenarios is indicated by the blue line. The frequency of droplet fusion relative to the aqueous flow rate of the large drops is indicated on the second parallel *x* axis.

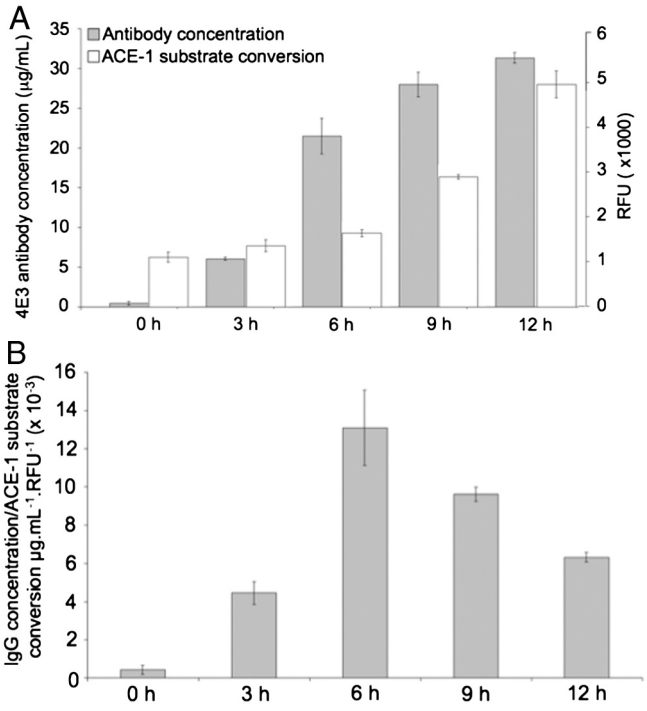



**Fig. S3.** Kinetic analysis of antibody secretion from individually encapsulated hybridoma cells. The 4E3 hybridoma cells were encapsulated in 660 pL drops at a cell density of $1.25 \times 10^6$ cells/mL and incubated at 37 °C. (*A*) The emulsion was broken at different time points and supernatants were analyzed for 4E3 antibody concentration by ELISA and for the conversion of the fluorogenic substrate of ACE-1 (15 µM) in the absence of any ACE-1 enzyme. (*B*) Ratio between 4E3 antibody secretion and substrate conversion in the absence of ACE-1. RFU, relative fluorescence unit. Error bars correspond to ±1 standard deviation.

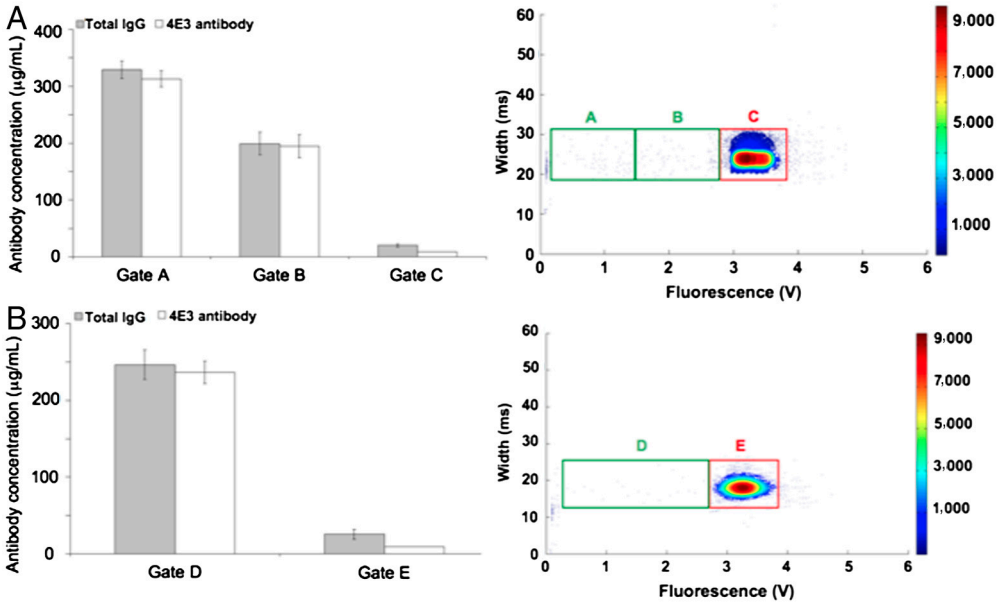



**Fig. S4.** Concentrations of antibodies in droplets. Concentrations of total IgG (gray) and 4E3 antibody (white) present in droplets within different fluorescence gates (*A*–*E* in the plots on the right) for the 1:1,000 (*A*) and 1:10,000 (*B*) mixture of 4E3 and Elec-403 cells. Error bars correspond to ±1 standard deviation.

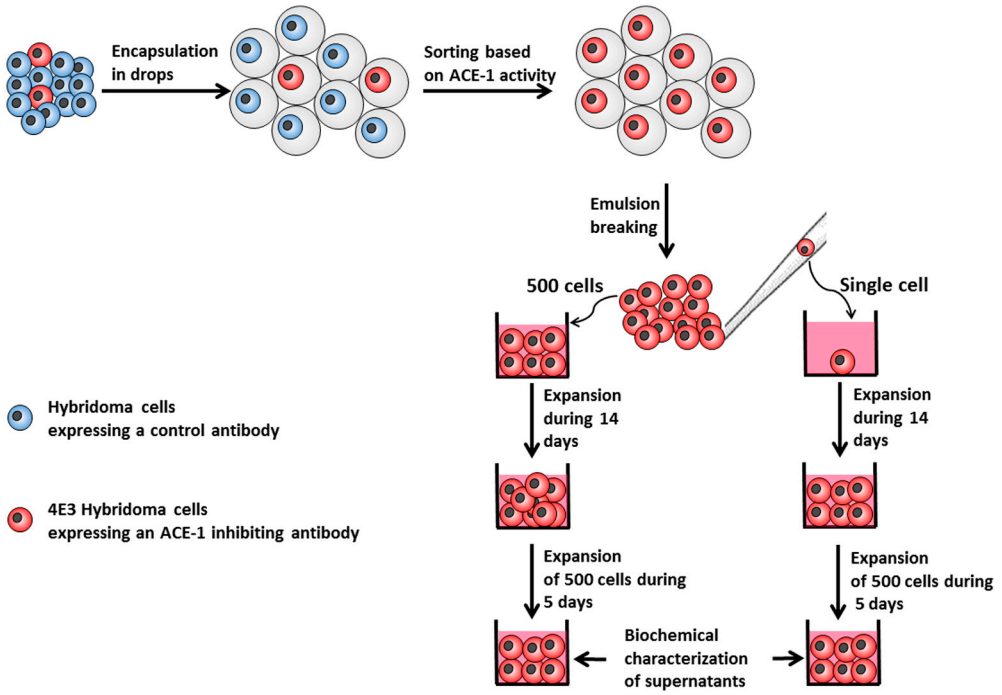



**Fig. S5.** Workflow of the screening procedure including the recultivation of sorted hybridoma cells. Starting from a heterogeneous population, hybridoma cells were encapsulated into droplets and sorted for the expression of 4E3 antibodies (inhibiting ACE-1). Subsequently, the droplets were broken chemically and individual cells, as well as a pool of 500 cells, were pipetted into the wells of a microtiter plate using gel loading pipette tips. After 14 d of expansion, 500 cells from each well were cultivated in fresh media for 5 d, followed by a biochemical characterization of the supernatants.

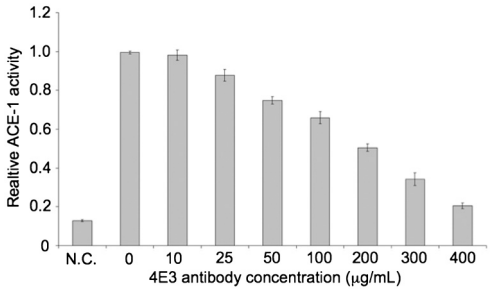
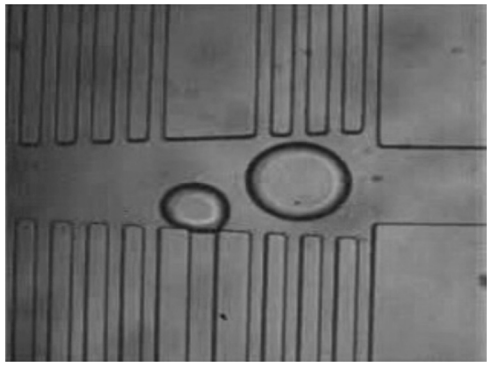



**Fig. S6.** Activity of ACE-1 in the presence of increasing concentrations of 4E3 antibody. ACE-1 enzyme in a concentration of 1.6 ng/mL was incubated for 3 h with different concentrations of purified 4E3 antibody in RPMI media, before adding 15 μM of the fluorogenic substrate of ACE-1. N.C., negative control (in the absence of ACE-1). Error bars correspond to ±1 standard deviation.

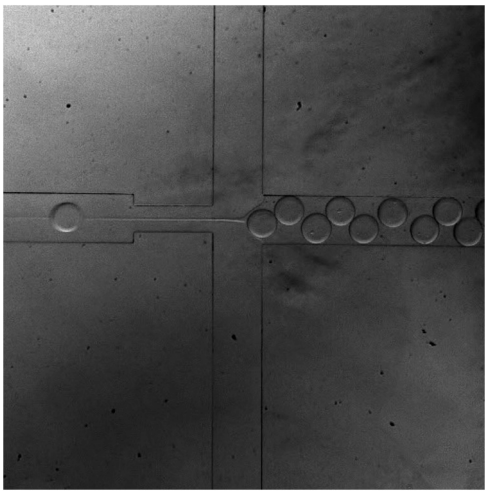


**Movie S1.** Pillar-induced droplet fusion of two different droplet species. Optimal 1:1 droplet fusion (Regime I in Fig. S2*A*).

Movie S1 (AVI)

**Movie S2.** Encapsulation of hybridoma cells in 660 pL drops.

Movie S2 (AVI)



**Movie S3.** Reinjection of 660 pL drops containing single hybridoma cells and ACE-1.

Movie S3 (AVI)

**Movie S4.** Fluorescence-based sorting of fused drops based on ACE-1 activity.

Movie S4 (AVI)