

Electronic Supplementary Information

Computational Modeling

Computational simulations of flow streamlines within microchannels were performed using a finite element solver (COMSOL multiphysics) (Figure S1). An incompressible Navier-Stokes equation was solved with no-slip boundary conditions at the channel and pillar walls. Constant volume flow rates were assumed at the inlets while the outlet reservoirs were fixed at atmospheric pressure. The simulations were utilized to design the geometry and spacing of the pillars; by changing these parameters, the resulting modifications in the streamlines could be observed. Visualization of the streamlines enabled prediction of the flow pathway of different sized particles, as well as optimization of channel configuration. Simulations were also performed to design the inlet channels for optimized flow focusing with varying flow rates of the sample and sheath solutions. Maintaining a properly focused flow ensures that the particles and cells are effectively positioned along the upper channel wall prior to separation, which is critical for obtaining high separation efficiencies.

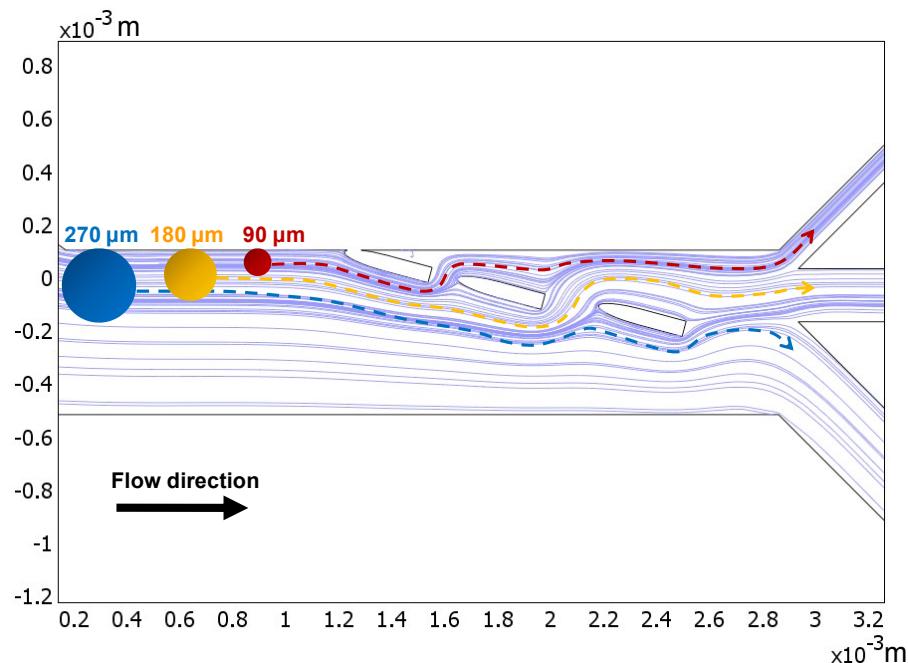


Figure S1. Computational simulation results showing the streamlines within a microchannel for separation into three size groups of 0 – 100 μm , 100 – 200 μm and 200 – 300 μm . Particles are superimposed to indicate the individual pathlines followed (dashed lines).

Materials

Silicon wafers were purchased from Techgophers (Los Angeles, CA) and microscope glass slides were purchased from Fischer Scientific (Tustin, CA). PDMS prepolymer and curing agents (Sylgard 184) were obtained from Dow Corning (Midland, MI). Food coloring used for flow visualization was obtained by Tone Brothers (Ankeny, IA). AZ 4620 photoresist (Shipley Corporation), hexamethyldisilazane (HDMS) (Shin-Etsu MicroSi), acetone, isopropanol and methanol (Gallade Chemical), piranha solution and deionized water were provided by the Nanoelectronics Research Facility at the University of California, Los Angeles. Polybead® microbeads were purchased from Polyscience (Warrington, PA). Phosphate buffered saline (PBS), bovine serum albumin (BSA), glycerol and Tween 20 were purchased from Sigma (St. Louis, MO).

Device Fabrication

The fabrication of our device is based on well-established methods for creating PDMS chips²⁷ wherein a glass substrate is joined together with a microfabricated PDMS cast. Briefly, a silicon wafer was first cleaned in a Piranha

bath to ensure proper adhesion of the resist layer. Next, photolithography was performed to pattern AZ photoresist as an etch mask. The wafer was then etched using Deep Reactive Ion Etching (Unaxis Versaline), resulting in smooth, near vertical sidewalls. Once the mold was fabricated, PDMS was mixed and degassed to remove bubbles. The mixture was poured onto the Si mold and cured for 2 hours at 80°C. Once the PDMS hardened, individual chips were cut and inlet and outlet holes were punched. Prior to bonding, PDMS chips and glass slides were cleaned in a Harrick air plasma cleaner/sterilizer (Ithaca, NY).

EB Preparation

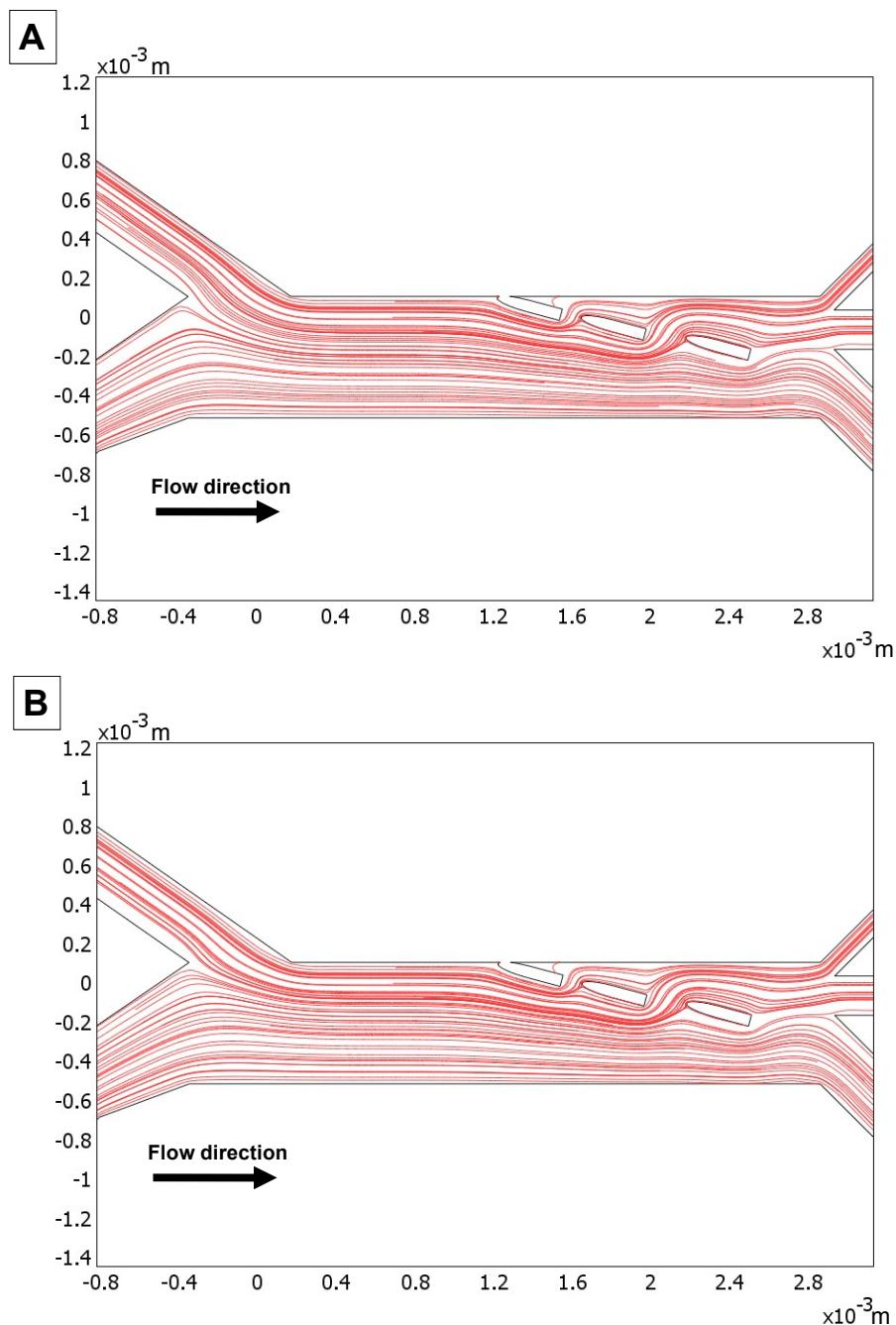
Mouse ES cells were cultured on irradiated mouse embryonic fibroblast (MEF) cells in Knock-Out Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA) supplemented with 15% fetal calf serum (HyClone, Logan, UT), 1000 U/mL leukemia inhibitory factor (Millipore, Temecula, CA), 100 µM 2- mercaptoethanol (Sigma-Aldrich), 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.1 mM nonessential amino acid (all purchased from Invitrogen). Prior to EB formation, ES cells were transferred to gelatin-coated tissue culture dishes and cultured for two passages to eliminate residual MEF cells. For the generation of EBs, ES cells were trypsinized and plated (10^5 cells/well) in Ultra Low Attachment 6-well plates (Corning, Corning, NY). Culture of EBs was carried out in Knock-Out Dulbecco's Modified Eagle Medium supplemented with 15% fetal calf serum (Omega Scientific, Tarzana, CA), 2 mM L-glutamine, 100 µM 2-mercaptopethanol, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.1 mM non-essential amino acid. EBs were fed with fresh medium every other day and cultured for 4 days before separation experiments. All cultures were maintained in a 37°C humidified incubator supplemented with 5% CO₂.

Experimental Setup

The experiments were performed under a Leica inverted microscope equipped with a MotionPro MP1000 high speed camera (Redlake MASD, Tucson, AZ). Videos were captured using Redlake MiDAS software at a speed of 30 frames/second. The particle and buffer solutions were transferred into 1 mL glass syringes (Hamilton, Reno, NV) and driven by two syringe pumps (Harvard Apparatus, Holliston, MA) at constant flow rates. Tygon tubing (Fischer Scientific, Tustin, CA) of 0.5 mm inner diameter was used to transfer the solutions to both inlets of the device. Prior to running the experiments, a PBS/BSA solution was pumped into the device and incubated for 30 min to aid channel priming and to adsorb BSA onto the surfaces to minimize particle/cell adhesion. The sheath buffer was a solution of EB culture media and 1% Tween 20, which was added to reduce bubble formation during experimentation.

Cell Viability Analysis

Viability of EBs was analyzed by using 7-AAD staining solution (BD Biosciences, San Jose, CA). Briefly, sorted EBs and EBs maintained under the standard culture condition were collected immediately after and 48 hours after sorting experiments, dissociated into single cells by using trypsin digestion, and centrifuged for 5 minutes at 200×g. After removal of supernatant, cell pellets were resuspended in 350 µl of Hank's Buffered Salt Solution (HBSS) containing 4% serum, incubated with 7 µl of 7-AAD solution for 5 minutes, and kept on ice until FACS analysis. Cell viability data was acquired and analyzed with BD FACS Canto II Flow Cytometry System (BD Biosciences, San Jose, CA). To determine non-viable population, unstained mES cells were used to define threshold for 7-AAD level (PerCP-Cy5-5-A channel) as shown in Fig. S5. For each condition, 30,000 cells were sampled.

COMSOL simulations for device optimization

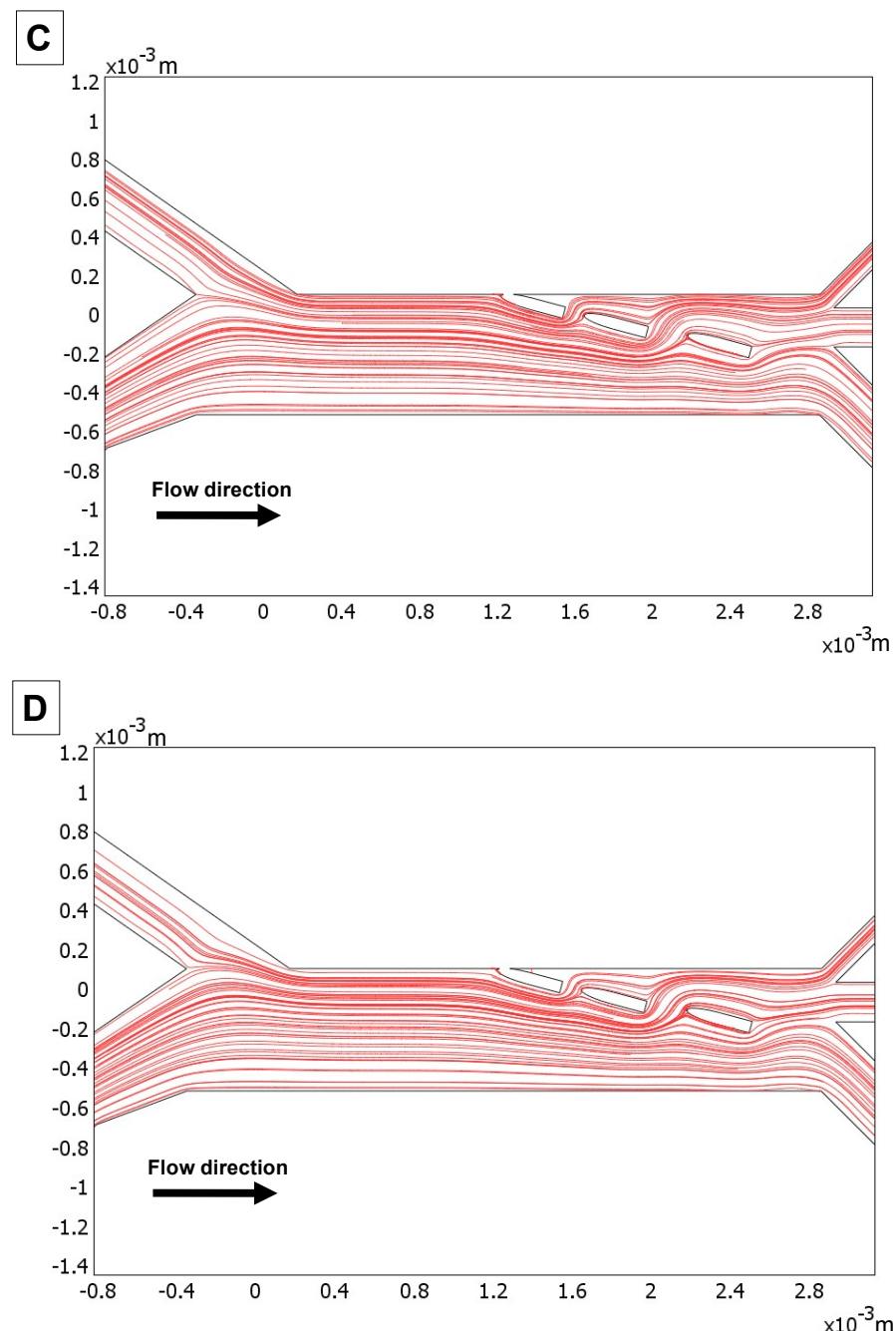


Figure S2. Computational simulation results showing the streamlines for various flow rates. The flow rates of the sample (upper branch) and the sheath (lower branch) solutions were optimized to enhance particle alignment for improved separation efficiency. For each simulation, the sample flow was fixed at 1 $\mu\text{L}/\text{min}$ while the sheath flow was changed: [A] 1 $\mu\text{L}/\text{min}$, [B] 2 $\mu\text{L}/\text{min}$, [C] 4 $\mu\text{L}/\text{min}$ and [D] 12 $\mu\text{L}/\text{min}$.

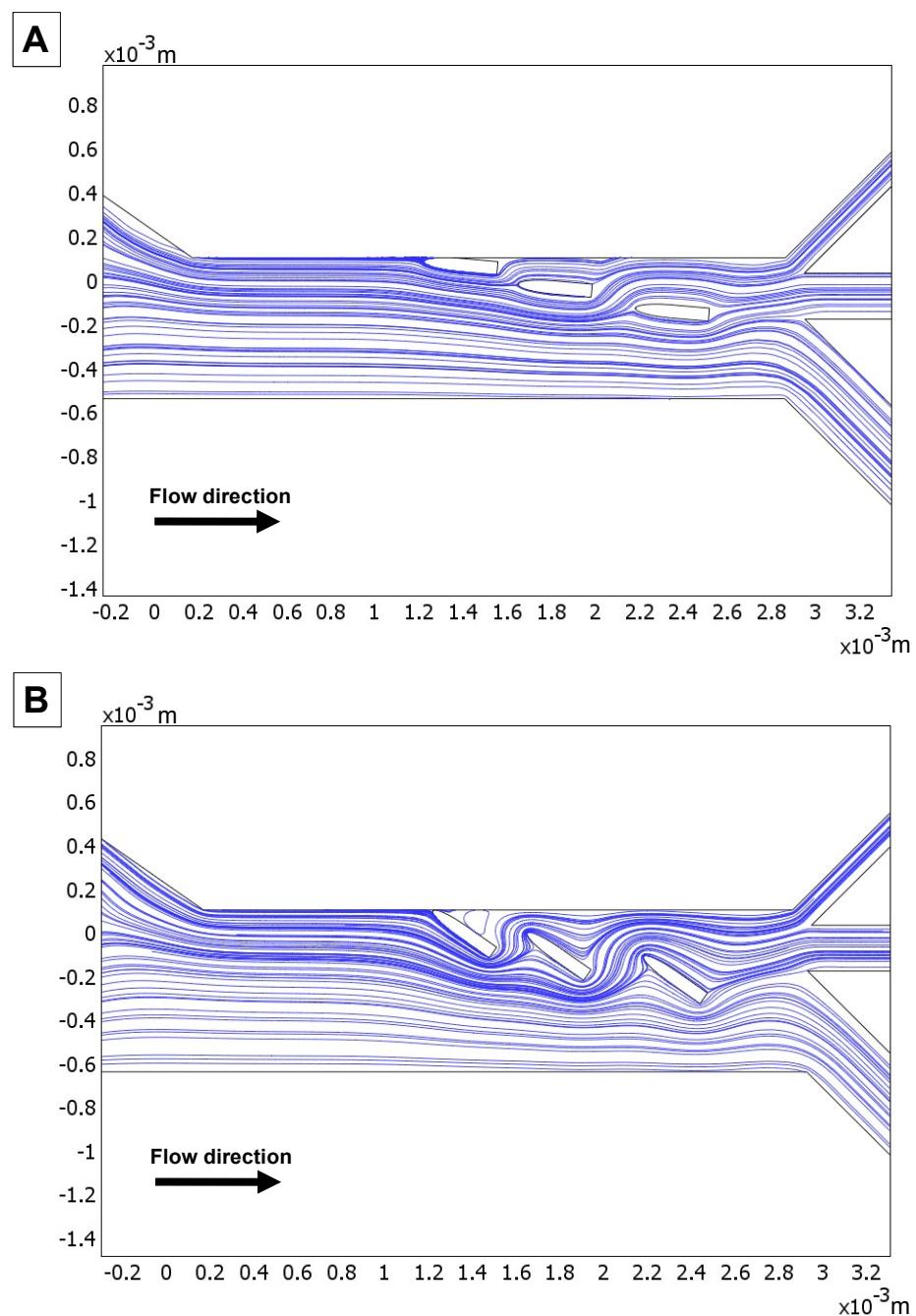


Figure S3. Computational simulation results showing the streamlines for two pillar angles; [A] 5 degrees and [B] 35 degrees. The pillar angle is measured with respect to the upper channel wall and the inter-pillar spacing is unchanged. The flow rates of the sample and sheath flows are 1 $\mu\text{L}/\text{min}$ and 4 $\mu\text{L}/\text{min}$ respectively.

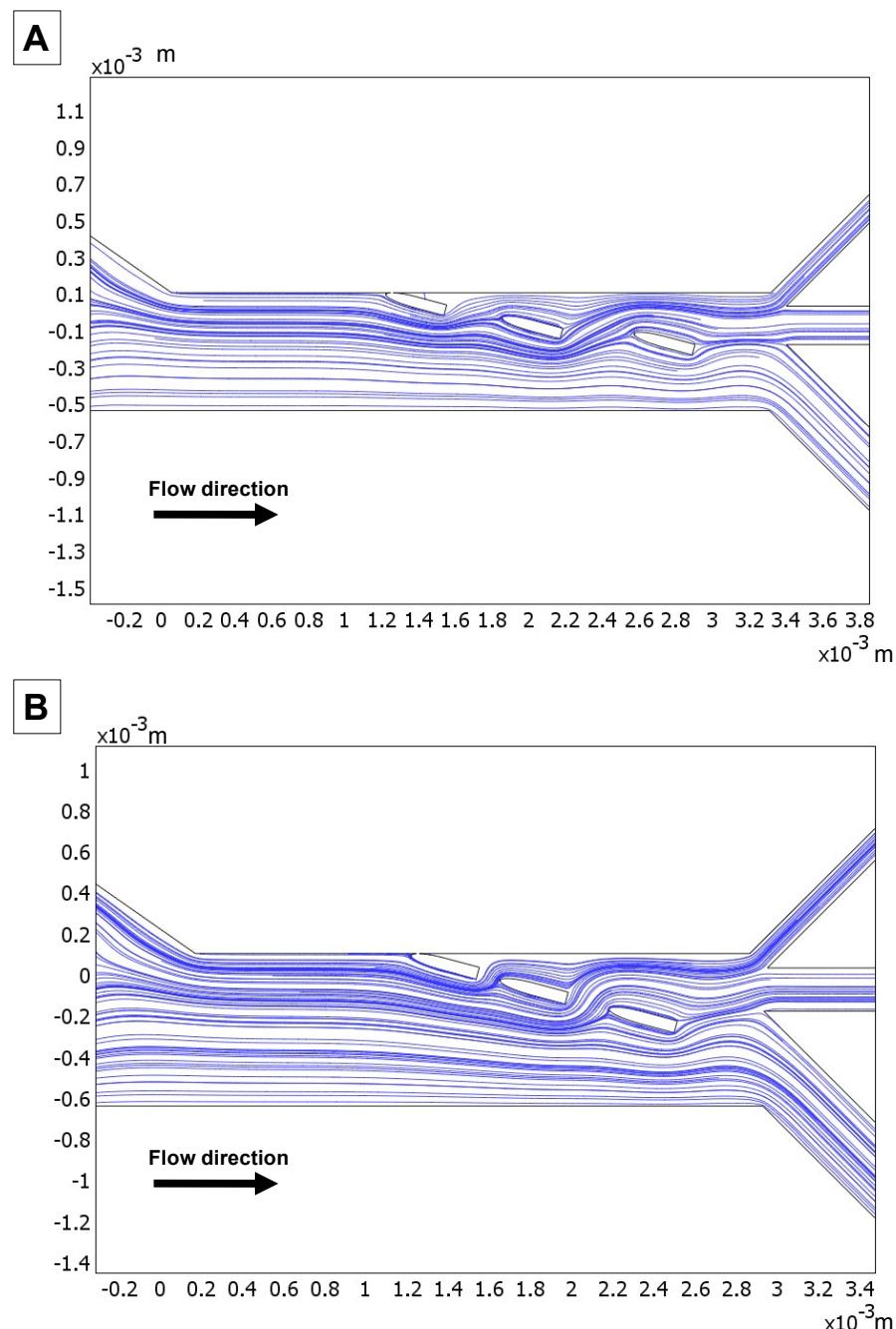


Figure S4. Computational simulation results showing the streamlines for various pillar configurations. [A] The spacing between the first and second set of pillars is 300 μm and 390 μm respectively, approximately 3x longer than the proposed design. [B] The pillars are aligned along their central axes leaving the inter-pillar spacing unchanged. The pillar angle is 15 degrees and the flow rates of the sample and sheath flows are 1 $\mu\text{L}/\text{min}$ and 4 $\mu\text{L}/\text{min}$ respectively.

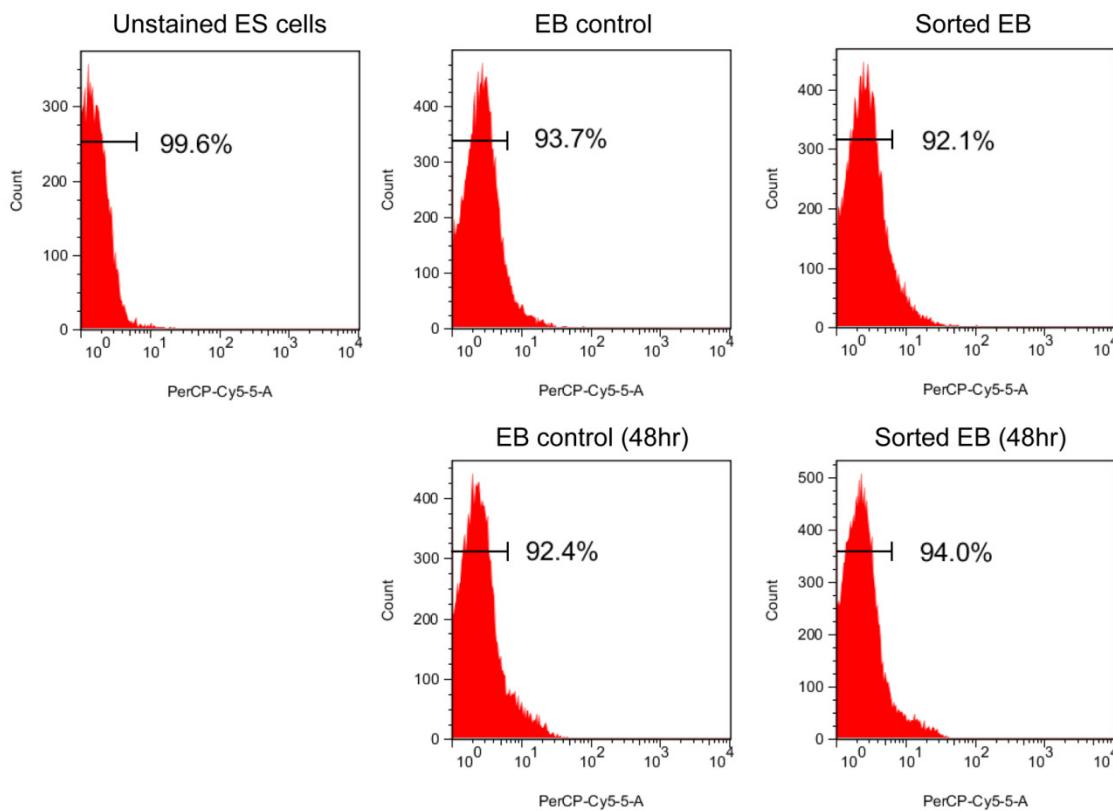
EB viability

Figure S5. Viability of sorted and control (unsorted) populations of EBs. Unstained mES cells were used to define a threshold of viable cells for all samples (top left panel). Histograms of 7-AAD staining intensity of control EBs (top center panel), EBs immediately after sorting (top right panel), control EBs 48 hours later (bottom center panel), and sorted EBs kept with fresh media in the incubator for 48 hours (bottom left panel). 30,000 cells were sampled for each condition.