# Microfluidic Removal of Teratoma-forming Cells from hiPSC-derived Neural Progenitor Cells

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## ABSTRACT

Teratoma formation remains a safety concern in therapeutic cell populations derived from human-induced pluripotent stem cells (hiPSCs). Teratoma forming cells are present in small numbers and rare cell isolations are often difficult using standard flow cytometry sorting techniques. Here, we first characterized time-dependent expression of a teratoma marker stage-specific embryonic antigen (SSEA)-5, which binds the H type-1 glycan during neural differentiation of hiPSCs. We next engineered a microfluidic geometrically enhanced differential immunocapture (GEDI) device to remove SSEA5+ rare cells from hiPSC-derived neural progenitor cells. The GEDI device presents a facile tool to potentially functionalize with multiple antibodies and robustly enhance hiPSC-derived cell population safety prior to therapeutic transplantation.

## 1 Introduction

Pluripotent stem cells (PSCs) represent a highly promising strategy to derive cells for therapeutic transplantation[1–5]. Reprogramming of somatic cells to human-induced PSCs (hiPSCs)[6] has emerged at a powerful technology as a tool for modeling diseases as well as generating patient-specific cells for therapeutic purposes. Differentiated cells derived from patient-specific hiPSCs are thought to be less immunogenic than tissue derived from non-allogenic human embryonic stem cells (hESCs)[7–9],circumvent ethics of using hESCs, and self-renew indefinitely, enabling derivation of the large number of cells required for therapeutic transplantation[10]. Of tissue derived from hiPSCs, neural stem and progenitor cells are a promising therapy for treating chronic neurodegenerative diseases, for example Alzheimer's disease[11] and Parkinson's disease[12, 13]. Neural progenitor cells (NPCs) are a promising strategy to treat Alzheimer's disease and Parkinson's disease due to a widespread loss of neurons[14–16]. Cell transplant therapies that enhance neurogenesis or replace lost neurons may delay the progression of neurodegenerative diseases. NPC grafts onto rodent models of AD demonstrate increased hippocampal synaptic density and increased cognitive function[17]. Additionally, cellular therapies that used fetal ventral midbrain tissue as a source of dopaminergic neurons have reported varying degrees of success[18]. However, cellular transplants utilizing fetal ventral midbrain tissue are limited due to ethical concerns of harvesting cells from donors and the inability to obtain adequate numbers of cells for transplant. hiPSC-derived NPCs (hiPSC-NPCs) are poised to overcome these obstacles and have demonstrated cell integration into host tissue[19]. In addition to chronic neurodegenerative diseases, acute neurodegeneration may result from traumatic brain injury (TBI) 20or spinal cord injury[20–22]. Human NPC transplantation has shown promising results to treat TBI and has demonstrated neuroprotection and improved cognitive outcomes following TBI[23]. Taken together, these results demonstrate that NPCs are a promising method to treat acute neurodegeneration in humans. Despite the promise of hiPSCs derived cells to treat disease, tumorigenicity remains a concern[24, 25]. At present, numerous techniques have been applied to remove teratoma-forming potential from hiPSC-derived products, such as pretreatment with antimitotic agents and delayed transplant[26], sorting with FACS[27], MACS[28], introduction of inducible suicide genes[29], and complete differentiation into mature dopaminergic neurons[30]. Unfortunately, each of these methods have limitations. Pretreatment with antimitotic agents and delayed transplant or complete differentiation into mature dopaminergic neurons increases turn-around time to derive therapeutic cells, and could make it difficult to transplant cells during the short optimal timing of transplantation in the subacute phase[31]. Although inducible suicide genes respond in 95% of cells , they are unlikely to eliminate teratoma because as few as 2 embryonic stem cells in two million non-neoplastic cells are capable of forming tumors in 60% of the transplants[26, 29]. Additionally, FACS and MACS have thus far proven insufficient to eliminate teratoma-forming potential and both require pre-labeling cells with antibodies, which could interfere with proliferation and differentiation[27, 28]. However, microfluidic devices represent a potential avenue to remove rare, teratoma-forming cells. Microfluidic devices have shown effective capture of rare circulating tumor cells (CTCs) from whole blood[32], indicating their potential to remove rare cells

from a cell population. Further, microfluidic devices have demonstrated enrichment and high survivability of hiPSCs and derived neural stem cells[3]. To this end we have engineered a geometrically enhanced differential immunocapture (GEDI) device to capture and remove rare, teratoma-forming cells from a hiPSC-derived NPC population by exploiting the expression of stage-specific embryonic antigen (SSEA)-5, which binds the H type-1 glycan highly and specifically expressed on hPSCs. The GEDI device exploits size of cells to maximize the number of collisions a cell experiences with an antibody functionalized post (**Figure 1**). Device geometry is designed such that cells above a set size follow a pathline through the device that causes repeated collision with the antibody functionalized posts. To remove cells from a hiPSC-derived NPC population, cells are first differentiated on a culture plate, resuspended as a single-cell suspension, then teratoma-forming cells are captured by antibodies on the chip surface, and a purified population is obtained in the effluent (**Figure 1d**). We believe microfluidic immunocapture represents a promising tool to address the tumorigenic potential of hiPSC-derived cell cultures.

## 2 Results and Discussion

### 2.1 Time-dependent expressiono of SSEA-5 on hiPSCs and NPCs

To design a device that removes teratoma-forming cells from a hiPSC-derived NPCs we first sought to characterize the differentiation dynamics of hiPSCs. We sought to characterize how SSEA-5 expression changed during neural differentiation, as SSEA-5 was our principal marker for cells with high teratoma-forming potential than those simply expressing TRA-1-60. We used flow cytometry to measure single-cell SSEA-5 expression at different time points of hiPSC differentiation and measured the percent of the population SSEA-5+ at each time point of differentiation (**Figure 2a**). During differentiation we noted that SSEA-5 expression decreased dramatically throughout the 12-day differentiation period (**Figure 2b,c**). Further, we noted that SSEA-5+ population drops at discrete intervals rather than continuously during the differentiation process. SSEA-5 expression was roughly constant during the first 3 days of neural differentiation and then dramatically reduced at day 4. SSEA-5 expression remained constant again through day 6 and reduced further between day 6 and day 9. After 9 days, SSEA-5 expression levelled off, indicating that most cells have completed differentiation.

However, despite the drop in average SSEA-5 expression, we sought to investigate a potential residual population of pluripotent or partially-differentiated cells with teratoma-forming potential. For each differentiation time point, we observed the percent of cell population with SSEA-5 expression above the bottom 5% of undifferentiated hiPSCs. We designated this fraction as SSEA-5+ (**Figure 2d**). Strikingly, we note that on day 12 of differentiation, 1% of the cells were still SSEA-5+ after a full time-course of differentiation. It is unlikely this small fraction of cells represents noise in the flow cytometry results because control non-stem cells (BxPC3 cell line) stained for SSEA-5 only showed 0.04% of the population being SSEA-5+. Similar numbers were obtained with the use of a single antibody conjugated to a fluorophore to measure SSEA-5 expression (**Supplementary Figure S1a**). This suggests that the observed differences were not measurement errors but indeed there is a residual population of hiPSC-NPCs with a pluripotency signature and thus teratoma-forming potential. To further investigate these cell populations we plotted the skew of SSEA-5 expression at each time point of differentiation. Interestingly, the differentiating cells were initially skewed towards low SSEA-5 expression (negative skew), indicating a few cells lose pluripotency faster than the rest of the population. Later on, however, at day 9 and day 12 (**Figure 2e**), the skew became positive (skewed towards high SSEA-5). This indicates that at the later stages of differentiation there are cells that slowly differentiate and retain pluripotency expression. Finally, to corroborate flow cytometry data with immunofluorescence (IF) images (**Figure 2f**), we stained undifferentiated hiPSCs and day 9 hiPSC-NPCs with Oct4, a master regulator of pluripotency, SSEA-5, and Nestin (a marker of neural differentiation 3). Strikingly, IF images corroborate flow data, clearly indicating that pockets of cells with high SSEA-5 expression remain on day 9, though with weak Oct4 expression, suggesting the cells are not fully pluripotent, but may retain some pluripotential.

### 2.2 Microfluidic immunocapture of teratoma-forming cells

Having confirmed the presence of residual cells with expression of pluripotency markers and thus teratoma-forming potential, we sought to employ a microfluidic GEDI device to remove the teratoma-forming cells from hiPSC-NPCs. To demonstrate SSEA-5 as a pluripotency surface marker capable of capturing hiPSCs on a GEDI device, we first qualitatively compared capture on glass coverslips functionalized with anti-SSEA-5, anti-TRA-1-60, and normal mouse IgG antibodies (isotype control) (to test for steric hindrance (**Figure 3a**). Qualitatively, we noticed similar numbers of cells on the anti-SSEA-5 and anti-TRA-1-60 coverslips compared to the isotype control cover slips. Knowing that anti-SSEA-5 and anti-TRA-1-60 coverslips capture hiPSC, we tested functionalized GEDI chips with each of the three above antibody functionalizations respectively, and a combination of anti-SSEA-5 plus anti-TRA-1-60 functionalized on the same GEDI chip. Undifferentiated hiPSCs were pumped through the GEDI chips at approximately 300 cells/mL. A pass-through rate was then calculated based on the number of cells counted in the effluent of each chip (ratio of number of cells in effluent to number of loaded cells). Observed pass-through rates are indicated in **Figure 3b**. Whereas anti-SSEA-5 and anti-TRA-1-60 GEDI chips had similar pass-through rates, the isotype-control GEDI chips had a much higher pass-through rate, indicating both anti-SSEA-5 and anti-TRA-1-60 GEDI chips

captured hiPSCs preferentially compared to the control. Given the anti-SSEA-5 GEDI chips had the lowest overall pass-through rate, we chose to focus on this antibody for future experiments.

### 2.3 Capture based on SSEA-5 expression

After confirming SSEA-5 expression exists in differentiated hiPSC-NPCs, we sought to confirm our device's ability to remove SSEA-5+ cells from articficially mixed cell population containing SSEA5+ and SSEA-5- cells, in a manner proportional to SSEA-5 expression level of the target population. To demonstrate this capability, we spiked hiPSC-NPCs (300 cells/mL) with a population of 300,000 BxPC3s/mL. We then calculated the passed through rate for each day of differentiation (**Figure 3c**), anticipating that with differentiation, number of SSEA5+ cells will reduce and less hiPSC-NPC will get captured on the microfluidic pillars. Indeed, we found that as the hiPSC-NPCs became further differentiated, our removal specificity reduced because there were few SSEA5+ cells to bind to SSEA5 antibodies on GEDI pillars (cells in effluent for day 9 and 12 is similar to isotype control). This suggests the GEDI device captures based on SSEA-5 expression.

To confirm the cells that were captured on the GEDI chip were pluripotent stem cells with teratoma-forming potential, we processed day 12 hiPSC-NPCs through an anti-SSEA-5 GEDI device and also tested purified NPCs that were maintained in proliferating cultures for 2 passages (P2 NPCs). We stained cells caught on the GEDI chip for Oct4, DAPI, and SSEA-3 (a stand in for SSEA-5, due to the inability to use a secondary fluorescent antibody against SSEA-5 on chip (textbfsupplementary figure 1b)) (**Figure 3d**). Strikingly, nearly all of the cells, irrespective of P2-NPC or hiPSC-NPC, caught on GEDI chip co-expressed DAPI and SSEA-3. However, in the day 12 hiPSC-NPC sample, a few OCT4+ cells were captured, which were not seen in the P2 sample, indicating further differentiation in the P2 sample, as expected.

### 2.4 Removal of SSEA-5+ Cells

Having confirmed our device captures cells proportional to SSEA-5 expression and having confirmed the presence of rare teratoma-forming cells in differentiated populations, we quantified the effectiveness of the GEDI device in removing teratoma-forming cells from a day 9 hiPSC-NPC population by imaging cells in the effluent of anti-SSEA-5 and isotype control functionalized GEDI chips. The fraction of cells positive for pluripotency marker SSEA-3 were lower in the effluent obtained from anti-SSEA-5 GEDI chip than the isotype control chip for all thresholds (**Figure 4b**), indicating removal of pluripotent cells from the day 9 hiPSC-NPCs. Interestingly the fraction of OCT4+ cells were similar at low to moderate thresholds however were drastically lower for the SSEA-5 GEDI chip effluent at higher thresholds (**Figure 4c**). Further, the absolute fraction of OCT+ cells were higher in day 9 hiPSC-NPCsin an absolute sense compared to SSEA-3. A plausible explanation for device performance is that at low thresholds bonafide NPCs are counted as OCT4+ cells, but at higher thresholds fewer bonafide NPCs are counted as positive, and thus residual pluripotent cells make up a larger fraction of the OCT4+ cells, of which there are more in the control GEDI chip effluent. Further, if we aggregate the average fluorescence intensity per nuclei area for the anti-SSEA-5 GEDI chips and isotype control GEDI chips and then normalize fluorescence intensity by the respective secondary control, we find reduced pluripotency marker expression in the anti-SSEA-5 GEDI chip compared to the isotype control chip (**Figure 4a**). This indicates the anti-SSEA- 5 GEDI chips can sucessfully remove teratoma-forming cells from the day 9 hiPSC-NPC population and the effluent cells can then be potentially used for therapeutic purposes.

## 3 Conclusion

In summary, we have demonstrated that a small residual population of cells that express pluripotency markers, and therefore markers indicative of teratoma-forming potential, exist even after prolonged periods of neural differentiation of hiPSCs. To this end, we have demonstrated proof-of-concept study wherea SSEA4 antibody-functionalized GEDI device can successfully reduce this teratoma-forming potential by filtering teratoma-forming cells out of the hiPSC-NPC population. Future work would require transplanting GEDI-sorted cells into a mouse model. We believe this work represents the first report on eliminating rare cells with teratoma-forming potential in hiPSC-derived neural stem cells with therapeutic poetntial. We believe that the GEDI technology is easily adaptable to other hiPSC-dervied cells populations, could purify differentiated population in a label free manner, and could be used to increase turn-around time required to derive safe-therapeutic cell populations. Further we have demonstrated that hiPSCs lose SSEA-5 expression in a more discrete than continuous process, as indicated by drops in fluorescence intensity between day 3 and 6 cells and day 6 and day 9 cells. This suggests there may be optimal time-points at which to filter or sort cells prior to therapeutic transplantation, if one envisions a clinical setting in which therapeutic cells are routinely derived from hiPSCs.

## 4 Methods

### 4.1 Device fabrication and functionalization

Devices were fabricated by A.M. Fitzgerald  Associates (Burlingame, CA) according to previously described specifications[32, 33]. The silicon device surfaces were functionalized with a previously described protocol using 3-mercaptopropyltrimethoxysilane

(MPTMS) and N-gamma-maleimidobutyryloxysuccinimide ester (GMBS) NeutrAvidin-biotin chemistry (Thermo Fisher Scientific, Rockford, IL)[32, 33].The devices were functionalized with primary antibodies via a biotinylation linkage. NeutrAvidin-functionalized devices were incubated with either (1) 2 $\mu$g/ml biotinylated mouse anti-human anti-SSEA-5 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) (2) 10 $\mu$g/ml mouse anti-human anti-TRA-1-60 antibody (ThermoFisher Scientific, Waltham, MA), (3) 2 $\mu$g/ml of (1) and 10 $\mu$g/ml of (2). Control devices were functionalized with biotinylated normal (non-specific) mouse IgG antibodies ((Miltenyi Biotec, Bergisch Gladbach, Germany) at 2 $\mu$g/ml. All antibodies were prepared in 2% BSA with 1.3-1.5mM EDTA in PBS. Polydimethysiloxane (PDMS) sheets (5:1 base:curing agent), approximately 3 mm thick, were polymerize for 18 hr at 60°C and trimmed to form covers for the GEDI device. A PDMS sheet was clamped to the top of the post arrays. Inlet and outlet holes were created with a biopsy punch, and tygon tubing was inserted into the PDMS gasket to connect GEDI chip inlet to a syringe pump and outlet to a microcentrifuge tube for effluent collection. Devices were primed with a 50/50 Ethanol/DI water mixture, and then flushed with DI water water and PBS before experiments.

### 4.2 Cell Culture

The human iPSCs were a generous gift from Dr. Jan Lammerding at Cornell university, through a collaboration with Silvia G Priori[34, 34, 35]. BxPC3 cells were obtained ATCC (Manassas, VA). All cell lines were cultured in humidified incubators (37 C and 5% CO2) using TeSR-E8 media (StemCell Technologies, Vancouver, Canada) or STEMdiff Neural Induction Medium (StemCell Technologies, Vancouver, Canada) for hiPSCs and hiPSC-NPCs respectively. hiPSC, hiPSC-NPCs, and NPCs were supplemented with 10 $\mu$M Y-27632 ROCK Inhibitor for the first day after passaging. DMEM supplemented with 10% FBS and 1% Antibiotic antimycotic for BxPC3s. hiPSCs were harvested after 3–5 days of culture at 60–80% confluency, and differentiating cells were harvested on the stated day.

### 4.3 Flow cytometry

hiPSCs or hiPSC-NPCs were trypsinized for 5–7 minutes at 37 C and then, fixed with 4% v/v paraformaldehyde in PBS for 10-15 minutes. Cells were then permeabilized with 0.1% w/w Saponin in PBS for 5 minutes. Staining was performed with a 1:250 dilution of primary antibody and fluorescently conjugated isotype control antibody in 10% FBS in PBS blocking buffer overnight in the dark at 4C. Secondary fluorescent antibody was incubated for 1hr at room temperature. For data in which flow cytometry was performed directly after GEDI, cells were stained in primary antibody for 1 hour, then washed. Analysis was performed using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) and Flowjo software. Cells were gated using FlowJo v10 first by identifying cells based on SSC:H vs FSC:H. Single cells were then identified by plotting FSC:A vs FSC:H and gating out cells that did not fit the main linear trend of this plot. 3 biological replicates of undifferentiated hiPSCs were then fit to a normal distribution, and a cutoff threshold for counting cells as SSEA5-+ was set at just above the bottom 5% of undifferentiated hiPSCs.

### 4.4 Capture Experiments

Cells were labeled with 10$\mu$M CellTracker Dye, either Green CMFDA or Red CMPTX (Thermo Fisher Scientific, Rockford, IL) for 45 minutes, trypsinized for 5–7 minutes at 37°C and resuspended in carrier solution (2% BSA, 1.3–1.5 mM EDTA in PBS) at approximately 300 cells/ml. Precise counts for each set of experiments were obtained by counting by hand 200ul samples of each cell-suspension-running-buffer mixture. In experiments in which cells were spiked into BxPC3 populations, cultures were trypsinized for 5–7 minutes at 37°C and resuspended in carrier solution at approximately 300,000 cells/ml. Functionalized silicon devices were mounted with Tygon tubing inlets and outlets in a custom PMMA holder. Cell capture was achieved by flowing 0.5 ml of cell suspension through the device at 1 ml/h followed by manual cell enumeration using fluorescence microscopy.

### 4.5 On-Chip Immunofluorescence

Cells were trypsinized for 5–7 minutes at 37°C and resuspended in carrier solution (2% BSA, 1.3–1.5 mM EDTA in PBS) at approximately 800,000-1,000,000 cells/ml. 0.5 ml of cell suspension was processed through GEDI devices functionalized with anti-SSEA-5 or normal mouse IgG (control) antibodies. Samples were fixed in 2% PFA in 50% PHEM buffer (60 mM PIPES, 25 mM HEPES, 10 mM EGTA and 2 mM MgCl2) for 15 minutes and blocked in 10% FBS in PBS for 1 hour. After staining of surface markers, the samples were permeabilized with 0.1% (w/w) Saponin. The samples were stained with, for Oct4, a primary (Abcam, ab60720, ) and an AlexaFluor-488-conjugated secondary antibody (Invitrogen), for SSEA-3, a primary Rat monoclonal to SSEA-3 (Abcam, Ab16286, Lot 1987272) and an AlexaFluor-555-conjugated secondary goat anti-rat antibody (Abcam, A21434, Lot GR3225471-1) and, for DNA/nuclei, DAPI (Invitrogen). Samples were processed, fixed and stained within 48 h of collection.

### 4.6 GEDI Effluent ImmunoFluorescence

3 60mm plates (Corning, CLS430166) of Day 9 hiPSC-NPCs were mixed and pumped through anti-SSEA-5 and isotype control functionalized GEDI chips at 1ml/hr for 30 min. The effluent of each chip (3 of each functionalization) was collected and immeditately fixed for 12 min in 4% w/v PFA. 70% of cells were then stained for Oct4, Nestin, and SSEA-3. 30% of cells received the corresponding secondary fluorescent antibody only. After staining, cells were pipeted and mounted onto glass coverslips coated in alginate hydrogel, to stick the fixed cells to the glass. During mounting, 2 of the secondary control slides (1 SSEA-5 and 1 isotype conrol) were damaged. Cells were then located on slides via the DAPI signal. Each slide was imaged in 3-4 locations on a Zeiss Z710 confocal microscope.

#### *4.6.1 CellProfiler Image Analysis*

Quantitative data was obtained by using DAPI signal to locate cells as primary objects in CellProfiler. Secondary objects were then identified using DAPI as seed locations for secondary objects. The total intensity of the secondary object (ie: Oct4 or SSEA-3 intensity) was calculated. Mean Oct4 and SSEA-3 intensity across each of the four conditions (anti-SSEA-5/positive stain, anti-SSEA-5/control stain, isotype control/positive stain, and isotype control/negative stain) for all objects was calculated, and divided by mean DAPI object area for the same condition. Secondary object size was normalized by DAPI area, due to the fact that secondary object area had high variance in the negative stain, due to poorly defined object borders (as expected). Mean secondary object positive stain fluorescence intensity normalized by mean secondary object negative stain fluorescence intensity is reported in **Figure 4**. **Figure 4** also reports the fraction of secondary objects in the positive stain that are a specified fraction brighter than the mean negative stain corresponding secondary object (ie: a threshold of 1.5 means the object is counted as positive if its integrated intensity is at least twice 1.5 times as bright as the average corresponding negative stain object.

### 4.7 Statistics

All statistical tests were performed in GraphPad 8.0 using either a one-way ANOVa test using either Tukey's multiple comparison test or Sidak's multiple comparison test, or Dunnett's multiple comparison test, where specified. Box and whiskers plots show all points, min to max, middle bar represents mean.

## References


1. Nishikawa, S.-i., Goldstein, R. A. & Nierras, C. R. The promise of human induced pluripotent stem cells for research and therapy. **9**, 725–729, DOI: 10.1038/nrm2466 (2008).

2. Robinton, D. A. & Daley, G. Q. The promise of induced pluripotent stem cells in research and therapy. *Nature* **481**, 295–305, DOI: 10.1038/nature10761 (2012). nature10761.

3. Singh, A. *et al.* Adhesion strength-based, label-free isolation of human pluripotent stem cells. *Nat. Methods* **10**, 438–444, DOI: 10.1038/nmeth.2437 (2013).

4. Takahashi, K. & Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **126**, 663–676, DOI: 10.1016/j.cell.2006.07.024 (2006). NIHMS150003.

5. Takahashi, K. *et al.* Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **131**, 861–872, DOI: 10.1016/j.cell.2007.11.019 (2007).

6. June, C. & Page, S. E. E. L. Corrected 5 june 2009; see last page. *Science* **324**, 797–802, DOI: 10.1126/science.1151526 (2009). /www.ncbi.nlm.nih.gov/pubmed/18029452.

7. Chhabra, A. Inherent immunogenicity or lack thereof of pluripotent stem cells: implications for cell replacement therapy. *Front. Immunol.* **8**, 1–11, DOI: 10.3389/fimmu.2017.00993 (2017).

8. Araki, R. *et al.* Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature* **494**, 100–104, DOI: 10.1038/nature11807 (2013).

9. Guha, P., Morgan, J. W., Mostoslavsky, G., Rodrigues, N. P. & Boyd, A. S. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell Stem Cell* **12**, 407–412, DOI: 10.1016/j.stem.2013.01.006 (2013).

10. Serra, M., Brito, C., Correia, C. & Alves, P. M. Process engineering of human pluripotent stem cells for clinical application. *Trends Biotechnol.* **30**, 350–359, DOI: 10.1016/j.tibtech.2012.03.003 (2012).

**11.** Penney, J., Ralvenius, W. T. & Tsai, L.-H. Modeling Alzheimer's disease with iPSC-derived brain cells. *Mol. Psychiatry* DOI: 10.1038/s41380-019-0468-3 (2019).

**12.** Kikuchi, T. *et al.* Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. *Nature* **548**, 592–596, DOI: 10.1038/nature23664 (2017).

**13.** Morizane, A. *et al.* MHC matching improves engraftment of iPSC-derived neurons in non-human primates. *Nat. Commun.* **8**, 1–12, DOI: 10.1038/s41467-017-00926-5 (2017).

**14.** Tuszynski, M. H. *et al.* A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat. Medicine* **11**, 551–555, DOI: 10.1038/nm1239 (2005).

**15.** MP, M. Pathways towards and away from Alzheimer's disease. *Nature* **430**, 631–9 (2004).

**16.** Surmeier, D. J., Obeso, J. A. & Halliday, G. M. Selective neuronal vulnerability in Parkinson disease. *Nat. Rev. Neurosci.* **18**, 101–113, DOI: 10.1038/nrn.2016.178 (2017).

**17.** Blurton-jones, M. *et al.* Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. **106**, 1–6, DOI: 10.1073/pnas.0901402106 (2009).

**18.** Brundin, P., Barker, R. A. & Parmar, M. Neural grafting in Parkinson's disease. Problems and possibilities. *Prog. Brain Res.* **184**, 265–294, DOI: 10.1016/S0079-6123(10)84014-2 (2010).

**19.** Hargus, G. *et al.* Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc. Natl. Acad. Sci.* **107**, 15921–15926, DOI: 10.1073/pnas.1010209107 (2010). arXiv:1408.1149.

**20.** Khazaei, M., Ahuja, C. S. & Fehlings, M. G. Induced pluripotent stem cells for traumatic spinal cord injury. *Front. Cell Dev. Biol.* **4**, 1–9, DOI: 10.3389/fcell.2016.00152 (2017).

**21.** Kobayashi, Y. *et al.* Pre-Evaluated Safe Human iPSC-Derived Neural Stem Cells Promote Functional Recovery after Spinal Cord Injury in Common Marmoset without Tumorigenicity. *PLoS ONE* **7**, 1–13, DOI: 10.1371/journal.pone.0052787 (2012).

**22.** Butts, J. C. *et al.* Differentiation of V2a interneurons from human pluripotent stem cells. *Proc. Natl. Acad. Sci. United States Am.* **114**, 4969–4974, DOI: 10.1073/pnas.1608254114 (2017).

**23.** Blaya, M., Tsoulfas, P., Bramlett, H. & Dietrich, W. Neural progenitor cell transplantation promotes neuroprotection, enhances hippocampal neurogenesis, and improves cognitive outcomes after traumatic brain injury. *Exp. Neurol.* **264**, 67–81, DOI: 10.1016/j.expneurol.2014.11.014 (2015).

**24.** Stevens, K. R. & Murry, C. E. Human Pluripotent Stem Cell-Derived Engineered Tissues: Clinical Considerations. *Cell Stem Cell* **22**, 294–297, DOI: 10.1016/j.stem.2018.01.015 (2018).

**25.** Barker, R. A., Parmar, M., Studer, L. & Takahashi, J. Human Trials of Stem Cell-Derived Dopamine Neurons for Parkinson's Disease: Dawn of a New Era. *Cell Stem Cell* **21**, 569–573, DOI: 10.1016/j.stem.2017.09.014 (2017).

**26.** Magown, P., Brownstone, R. M. & Rafuse, V. F. Tumor prevention facilitates delayed transplant of stem cell-derived motoneurons. *Annals Clin. Transl. Neurol.* **3**, 637–649, DOI: 10.1002/acn3.327 (2016).

**27.** Tang, C. *et al.* An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. *Nat. biotechnology* **29**, 829–34, DOI: 10.1038/nbt.1947 (2011). NIHMS150003.

**28.** Fong, C. Y., Peh, G. S., Gauthaman, K. & Bongso, A. Separation of SSEA-4 and TRA-1-60 labelled undifferentiated human embryonic stem cells from a heterogeneous cell population using magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS). *Stem Cell Rev. Reports* **5**, 72–80, DOI: 10.1007/s12015-009-9054-4 (2009).

**29.** Itakura, G. *et al.* Fail-Safe System against Potential Tumorigenicity after Transplantation of iPSC Derivatives. *Stem Cell Reports* **8**, 673–684, DOI: 10.1016/j.stemcr.2017.02.003 (2017).

**30.** Doi, D. *et al.* Prolonged maturation culture favors a reduction in the tumorigenicity and the dopaminergic function of human ESC-derived neural cells in a primate model of Parkinson's disease. *Stem Cells* **30**, 935–945, DOI: 10.1002/stem.1060 (2012).

**31.** Nagoshi, N. & Okano, H. Applications of induced pluripotent stem cell technologies in spinal cord injury. *J. Neurochem.* **141**, 848–860, DOI: 10.1111/jnc.13986 (2017).

**32.** Kirby, B. J. *et al.* Functional characterization of circulating tumor cells with a prostate-cancer-specific microfluidic device. *PLoS ONE* **7**, 1–10, DOI: 10.1371/journal.pone.0035976 (2012).

**33.** Gleghorn, J. P. *et al.* Capture of circulating tumor cells from whole blood of prostate cancer patients using geometrically enhanced differential immunocapture (GEDI) and a prostate-specific antibody. *Lab on a Chip* **10**, 27–29, DOI: 10.1039/b917959c (2010).

**34.** Di Pasquale, E., Song, B. & Condorelli, G. Generation of Human Cardiomyocytes: A Differentiation Protocol from Feeder-free Human Induced Pluripotent Stem Cells. *J. Vis. Exp.* 1–6, DOI: 10.3791/50429 (2013).

**35.** Di Pasquale, E. *et al.* CaMKII inhibition rectifies arrhythmic phenotype in a patient-specific model of catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis.* **4**, e843–11, DOI: 10.1038/cddis.2013.369 (2013).

## Conflicts of interest

There are no conflicts to declare.

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## Author contributions statement

All experiments were performed by K.W. Passed-through experiments were performed by K.W. and C.L. Data was analyzed by K.W., A.S., and B.K. Experiments were conceived by K.W., A.S., and B.K. K.W., A.S, and B.K. wrote the paper.

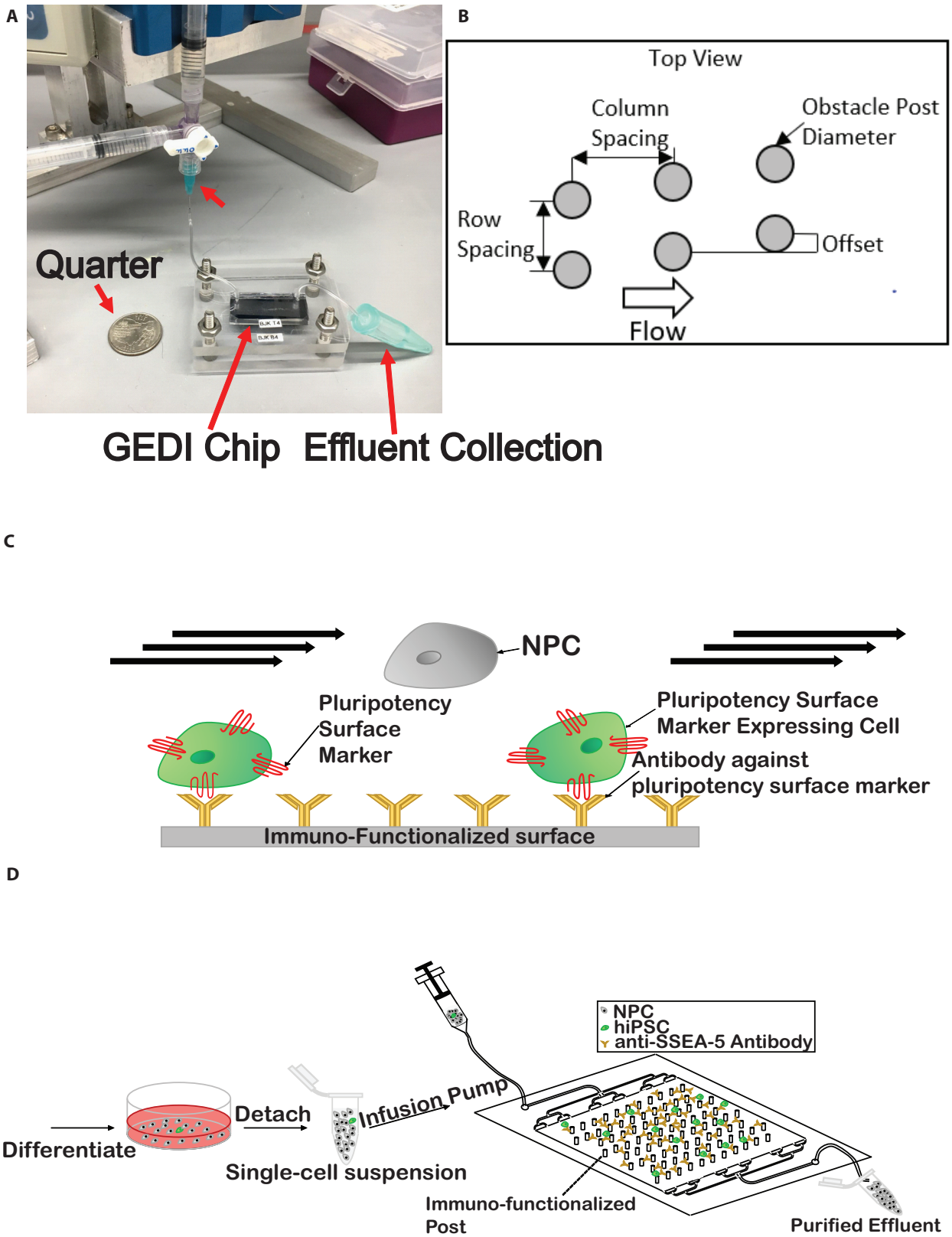
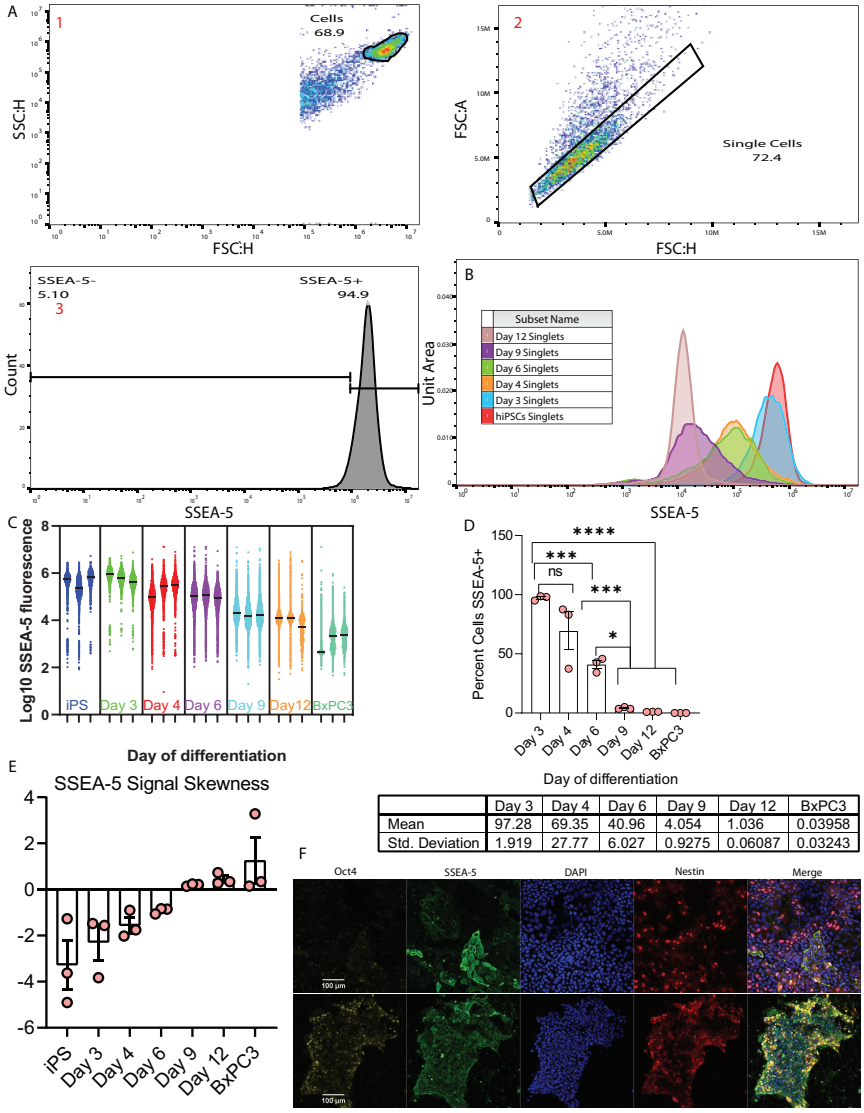



**Figure 1.** Cartoon Schematic of GEDI device. (**A**) Picture of GEDI chip setup. (**B**) GEDI device schematic (**C**) Cartoon depiction of surface functionalization. (**D**) Overview of GEDI running procedure.




| | Day 3 | Day 4 | Day 6 | Day 9 | Day 12 | BxPC3 |
|---|---|---|---|---|---|---|
| Mean | 97.28 | 69.35 | 40.96 | 4.054 | 1.036 | 0.03958 |
| Std. Deviation | 1.919 | 27.77 | 6.027 | 0.9275 | 0.06087 | 0.03243 |

**Figure 2.** Temporal expression of SSEA-5 on hiPSCs and hiPSC-NPCs. (**A**) Gating of single-cells from SSC:H vs. FSC:H to identify cells, FSC:A vs FSC:H to identify single-cells, and thresholding of hiPSCs to identify SSEA-5+ cells (averaged over 3 hiPSCs populations. (**B**) SSEA-5 expression histograms for different time points of differentiation. (**C**) Log10 SSEA-5 Expression for 3 biological replicates at each time point. (**D**) Percent of cells SSEA-5$^{+}$ at each time point (bar represents mean), *p=.0206, ***p<.0008, ****p<.0001. (**E**) Skew of SSEA-5 expression at each time point (error bars represent mean with SEM). (**F**) IF Images of day 9 hiPSC-NPCs (top) and undifferentiated hiPSCs (bottom) (10x)(scale bar: 100$\mu m$).

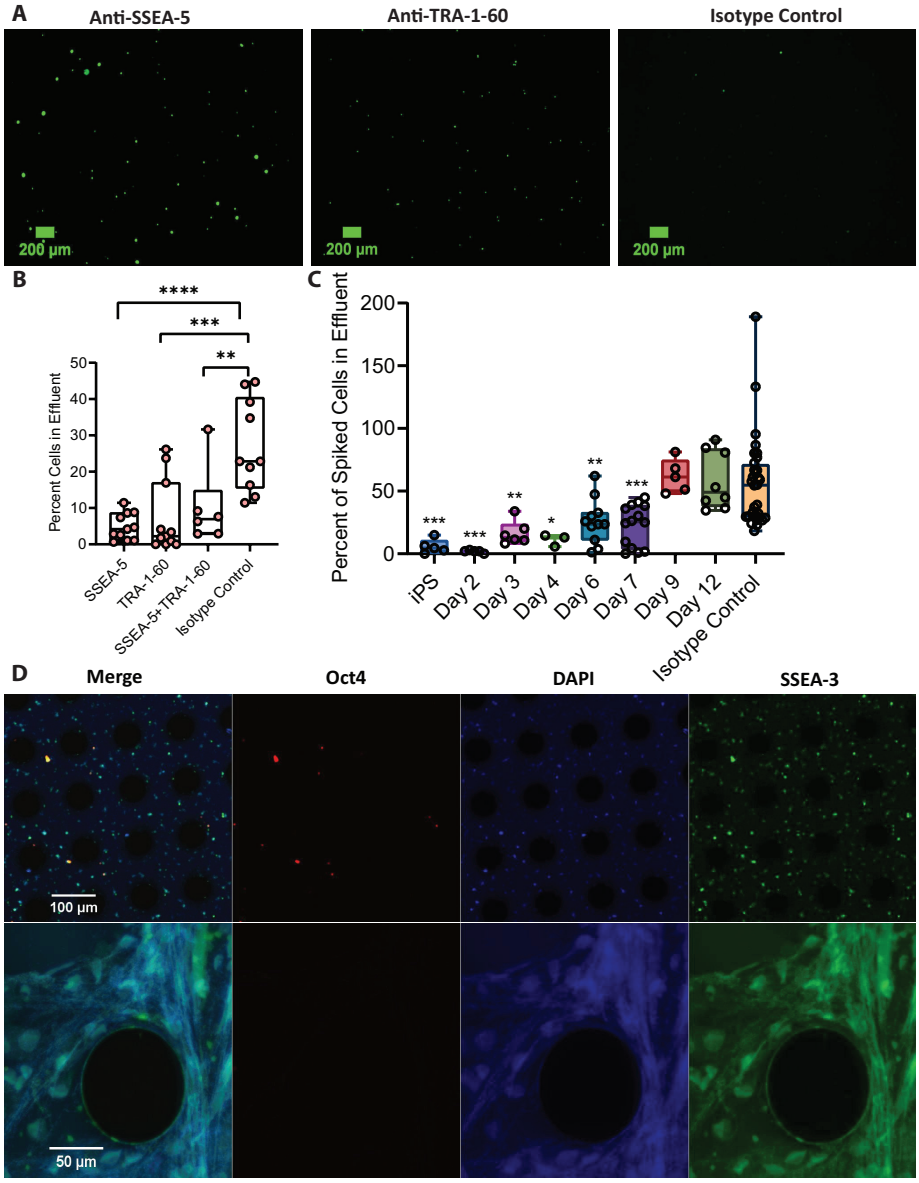



**Figure 3.** Teratoma-Forming-Cell Capture. (**A**) hiPSCs captured on glass cover-slips with designated antibody surface functionalization. (**B**) Passed through rates for undifferentiated hiPSCs alone in suspension (approx 300 cells/ml sent through) ****p<0.0001, ***p=0.0002, **p=0.0088, Tukey's multiple comparison test. (**C**) Passed through rates for hiPSC-NPCs spiked into a larger BxPC3 population at different time points of differentiaiton. (**D**) Immunofluorescence images of cells captured on anti-SSEA-5 GEDI chips. Top: 10x, Day 12 NPCs; Bot: 40x, P2 NPCs (hiPSC differentiated for 5 days, passaged, differentiated for 3 more days, then proliferated through 2 passages in a neural proliferation media.

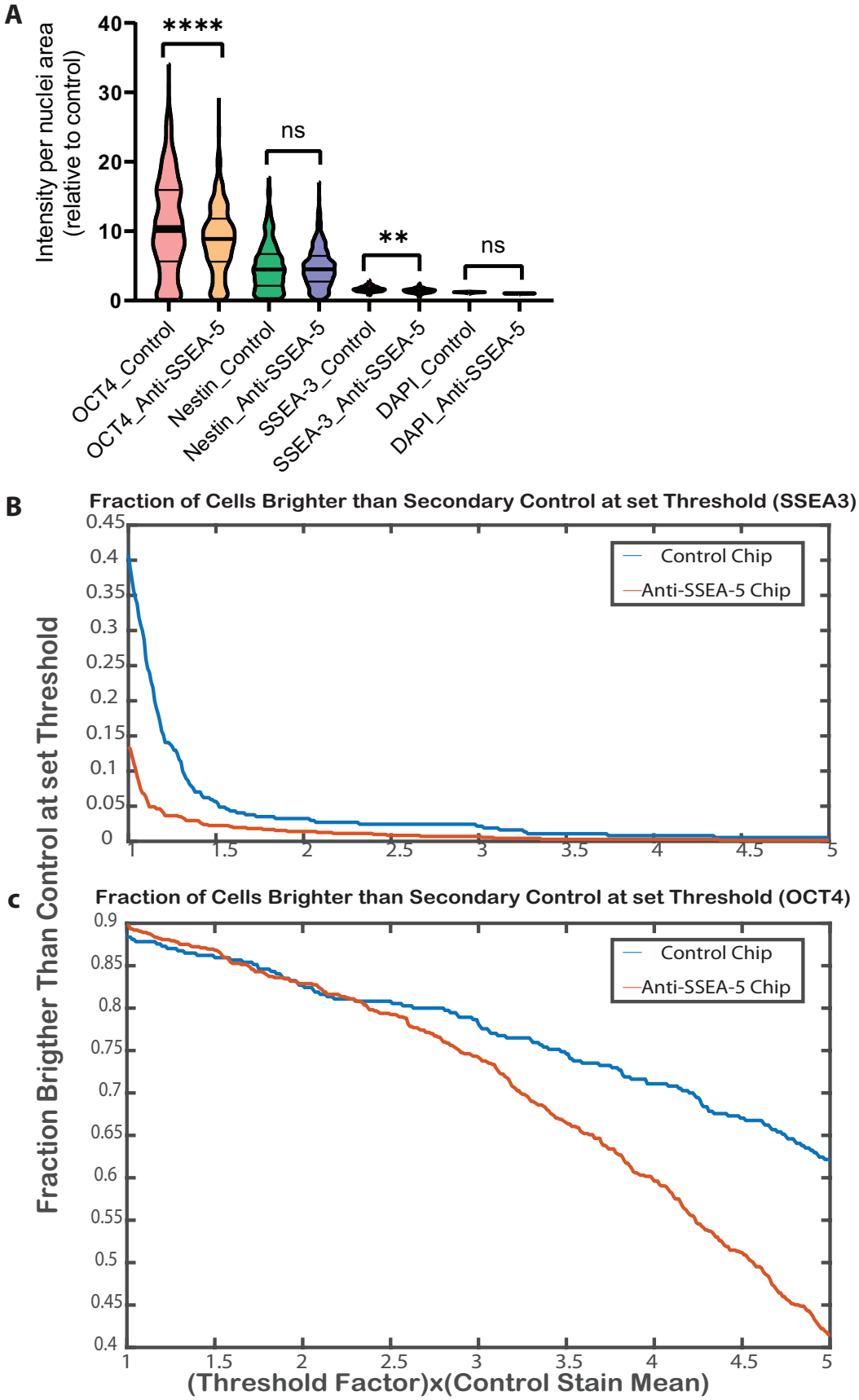
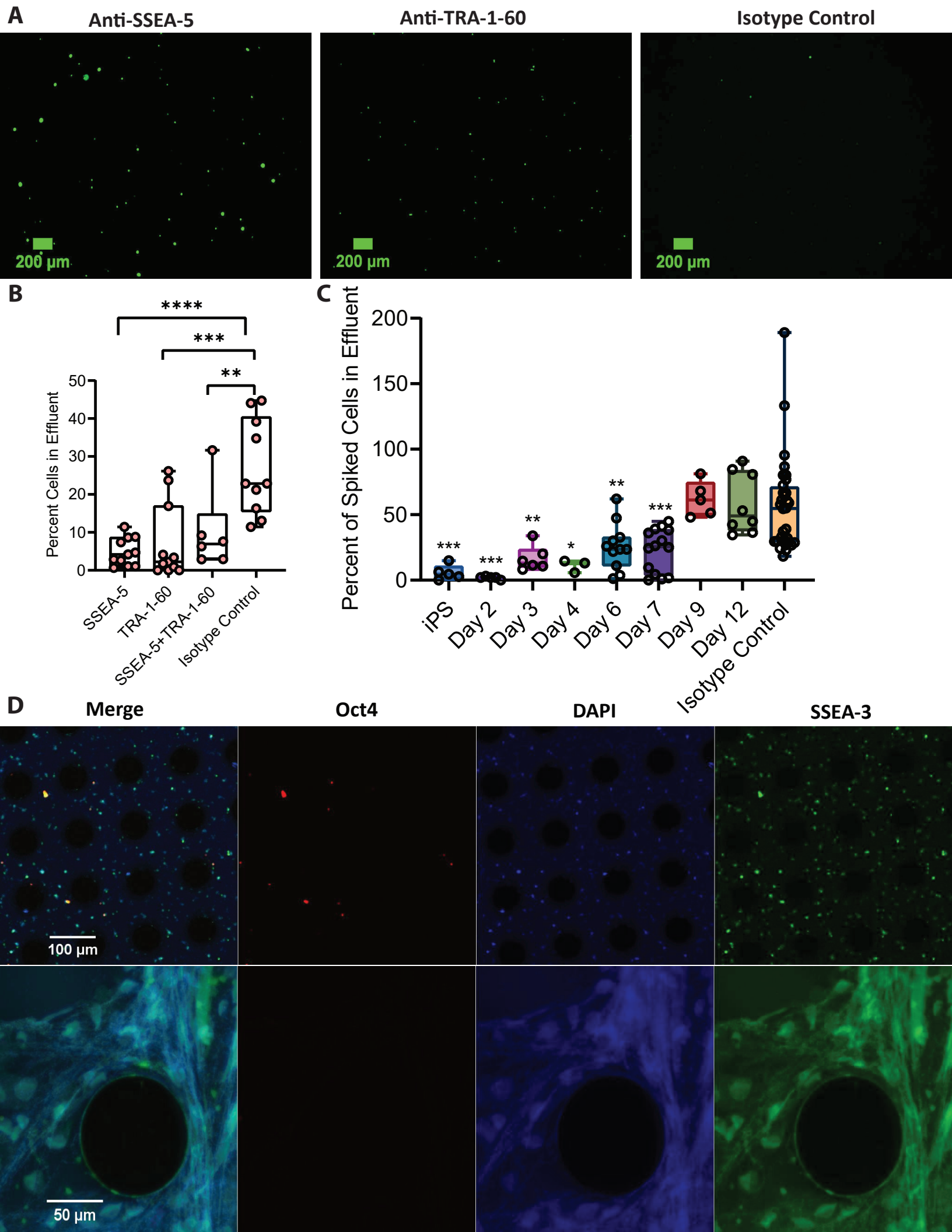
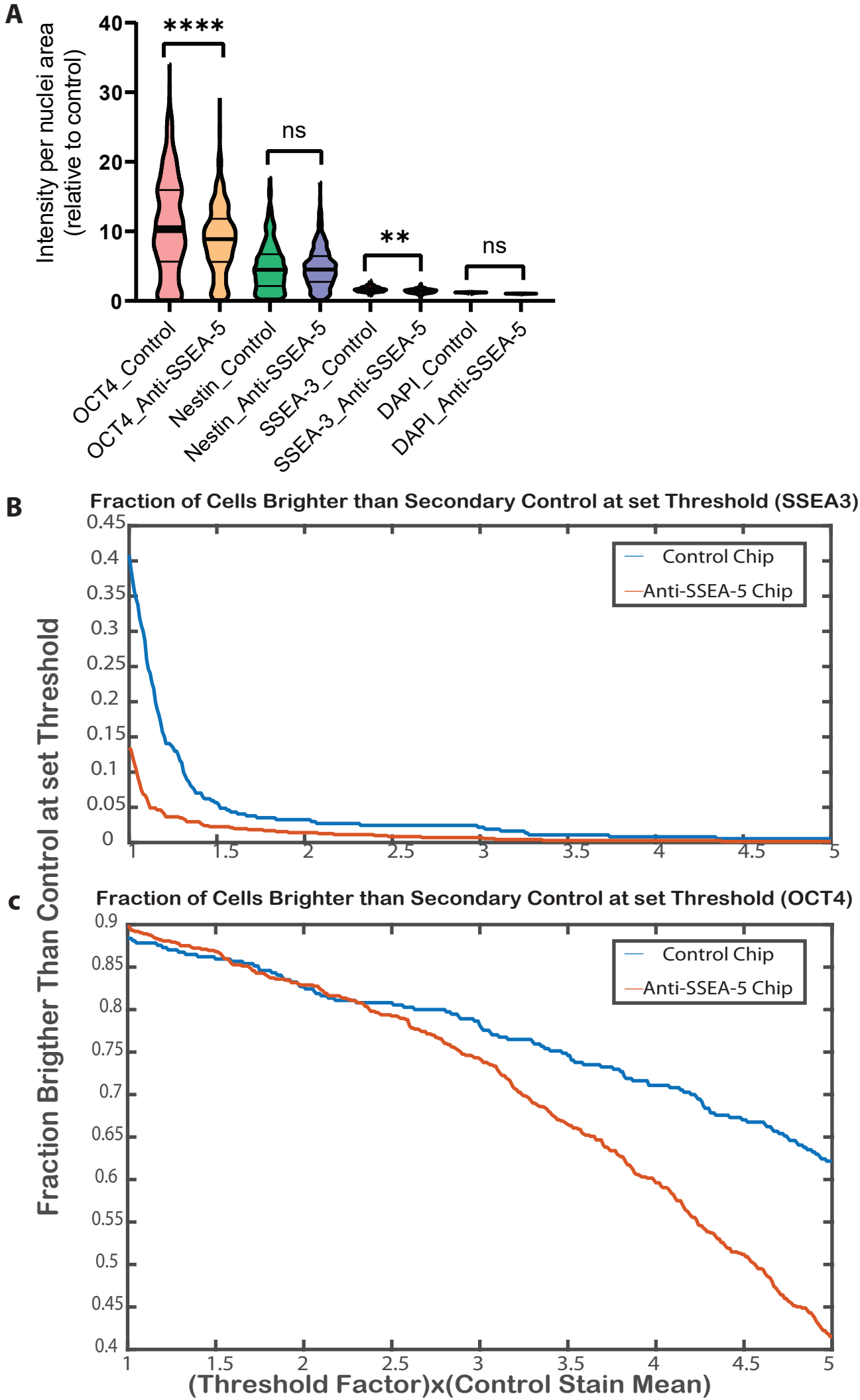
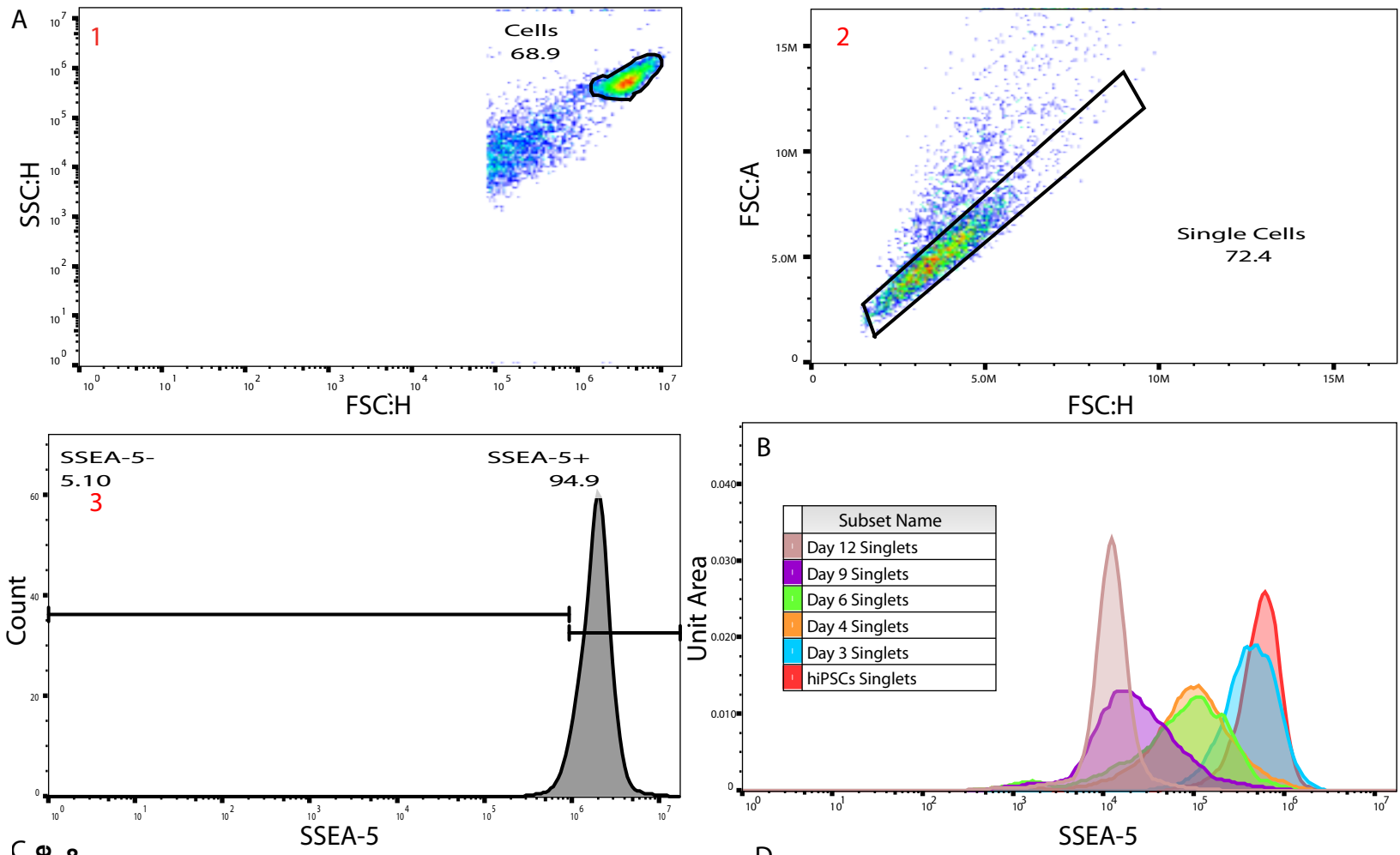
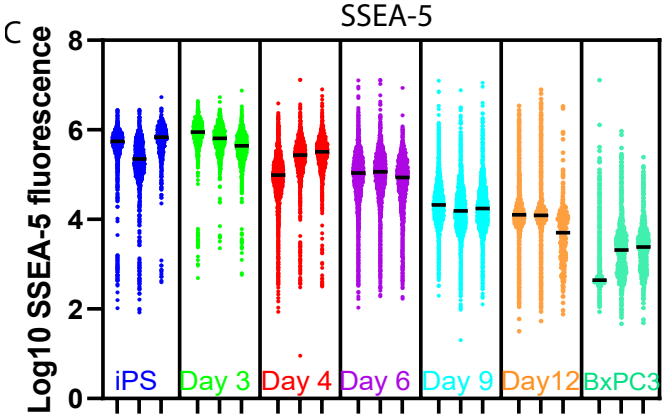


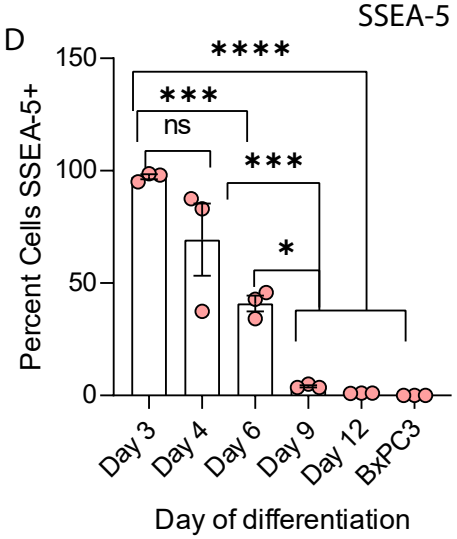

**Figure 4.** Residual Teratoma-Forming-Cells. (**A**) Expression intensity per nuclei area relative to mean of secondary control for individual cells (117 Outliers removed by ROUT Q=0.1%), ****p<0.0001, **p=0.6625, Sidak's multiple comparison test. (**B**) Fraction of day 9 hiPSC-NPCs (a) OCT4$^{+}$ and (b) SSEA-3$^{+}$ in effluent of anti-SSEA-5 and isotype control GEDI chip at varying threshold.

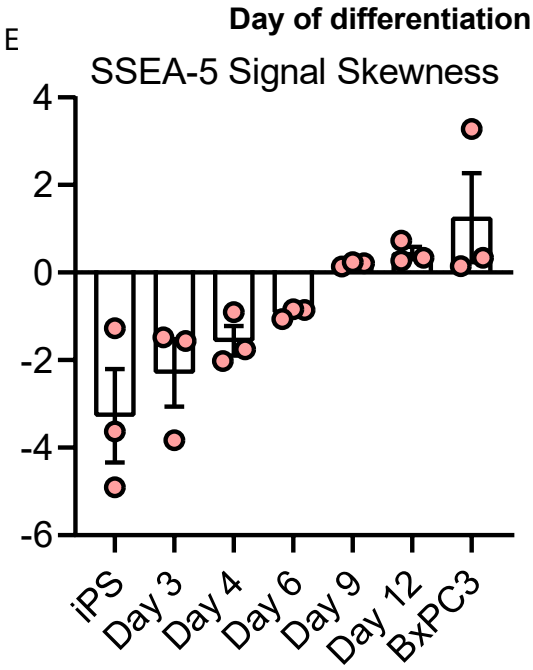

A
Anti-SSEA-5
Anti-TRA-1-60
Isotype Control
200 µm
200 µm
200 µm
B
****
***
**
Percent Cells in Effluent
50
40
30
20
10
0
SSEA-5
TRA-1-60
SSEA-5+TRA-1-60
Isotype Control
C
Percent of Spiked Cells in Effluent
200
150
100
50
0
***
***
**
*
**
***
iPS
Day 2
Day 3
Day 4
Day 6
Day 7
Day 9
Day 12
Isotype Control
D
Merge
Oct4
DAPI
SSEA-3
100 µm
50 µm


A
Intensity per nuclei area (relative to control)
40
30
20
10
0
****
ns
**
ns
OCT4_Control
OCT4_Anti-SSEA-5
Nestin_Control
Nestin_Anti-SSEA-5
SSEA-3_Control
SSEA-3_Anti-SSEA-5
DAPI_Control
DAPI_Anti-SSEA-5
B
Fraction of Cells Brighter than Secondary Control at set Threshold (SSEA3)
Control Chip
Anti-SSEA-5 Chip
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
1.5
2
2.5
3
3.5
4
4.5
5
c
Fraction of Cells Brighter than Secondary Control at set Threshold (OCT4)
Fraction Brigther Than Control at set Threshold
Control Chip
Anti-SSEA-5 Chip
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
1
1.5
2
2.5
3
3.5
4
4.5
5
(Threshold Factor)x(Control Stain Mean)




| | Day 3 | Day 4 | Day 6 | Day 9 | Day 12 | BxPC3 |
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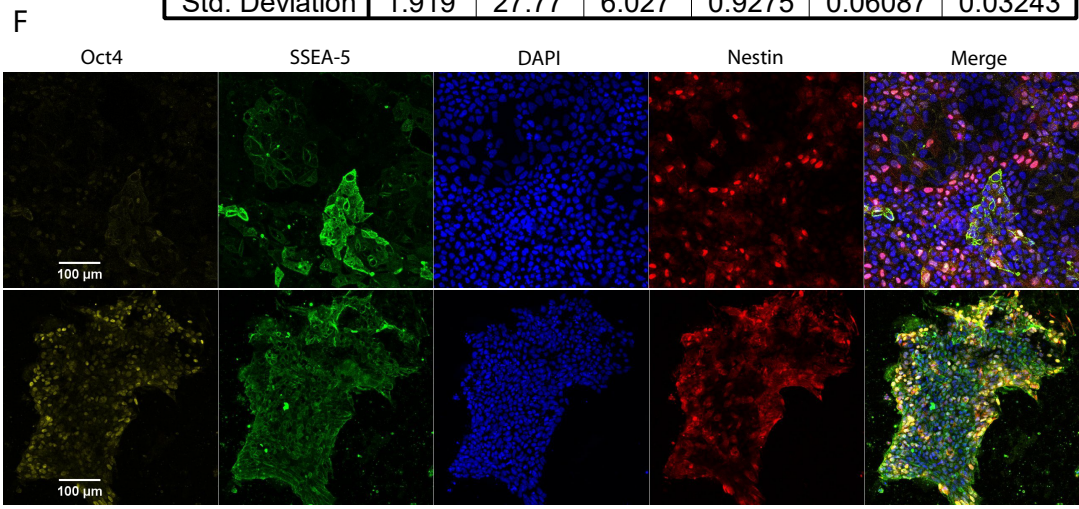
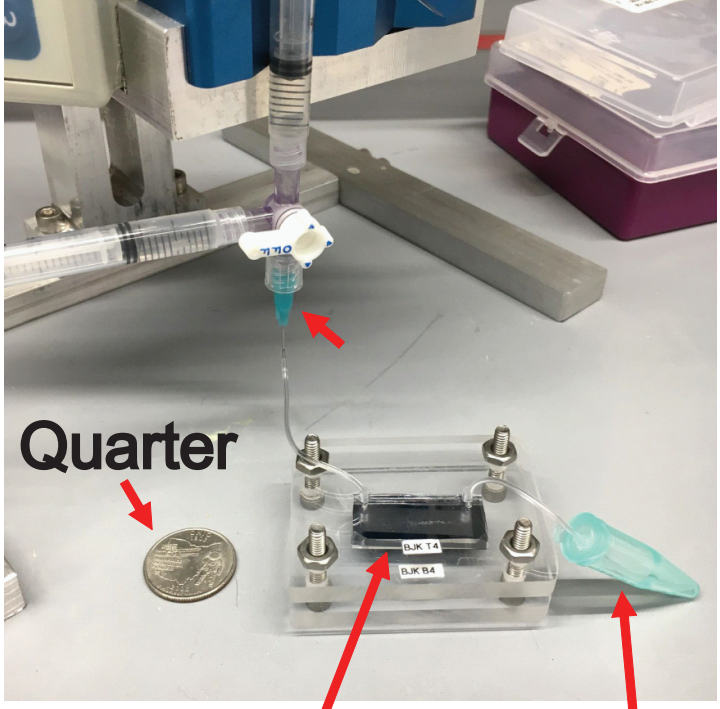
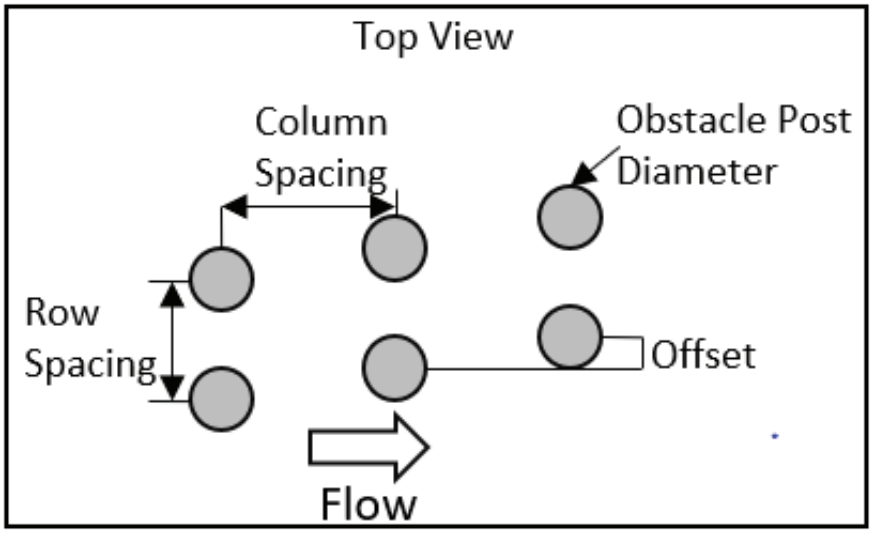


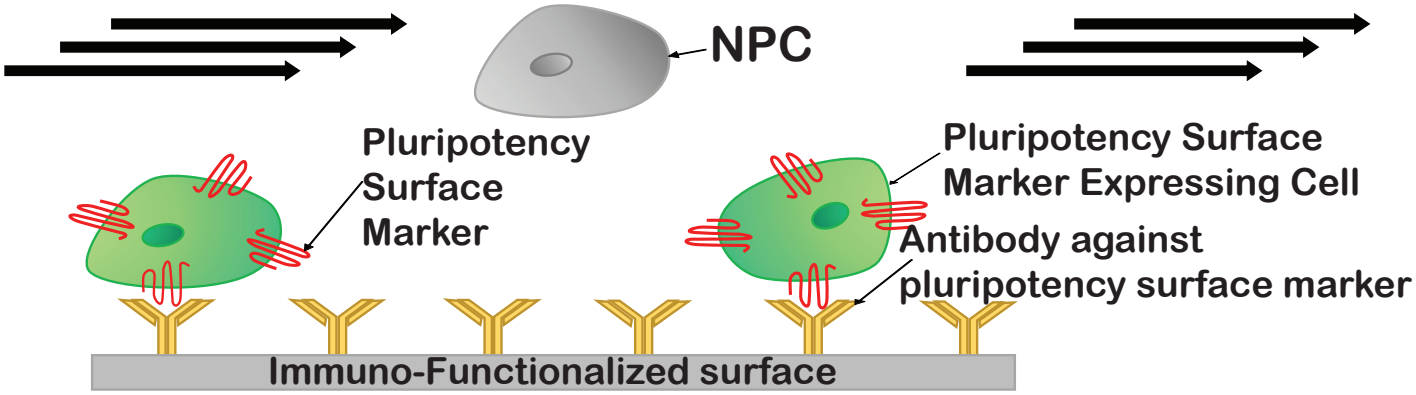
A


Quarter
GEDI Chip
Effluent Collection

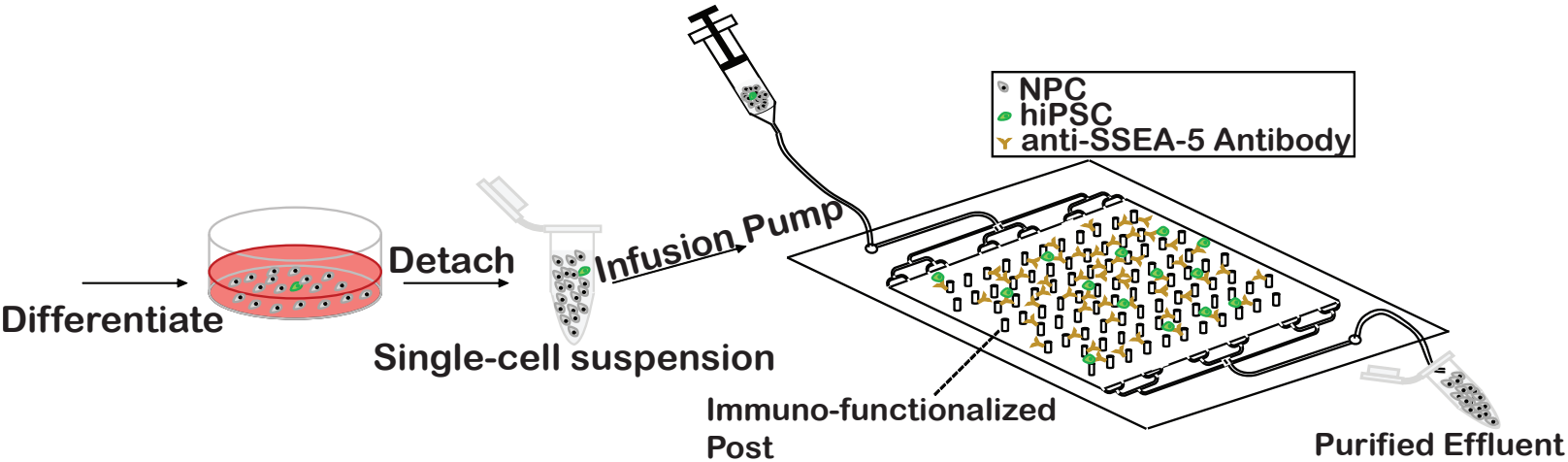
B


Top View
Column Spacing
Obstacle Post Diameter
Row Spacing
Offset
Flow

C


NPC
Pluripotency Surface Marker
Pluripotency Surface Marker Expressing Cell
Antibody against pluripotency surface marker
Immuno-Functionalized surface

D


NPC
hiPSC
anti-SSEA-5 Antibody
Differentiate
Detach
Single-cell suspension
Infusion Pump
Immuno-functionalized Post
Purified Effluent