**Chen et al \_Supplemental Materials**

**Supplemental Figures and Figure Legends**

Figure S1, related to Figure 1

Figure S2, related to Figure1 and Figure 3

Figure S3, related to supplemental Experimental Procedures

Movie S1, related to Figure 3

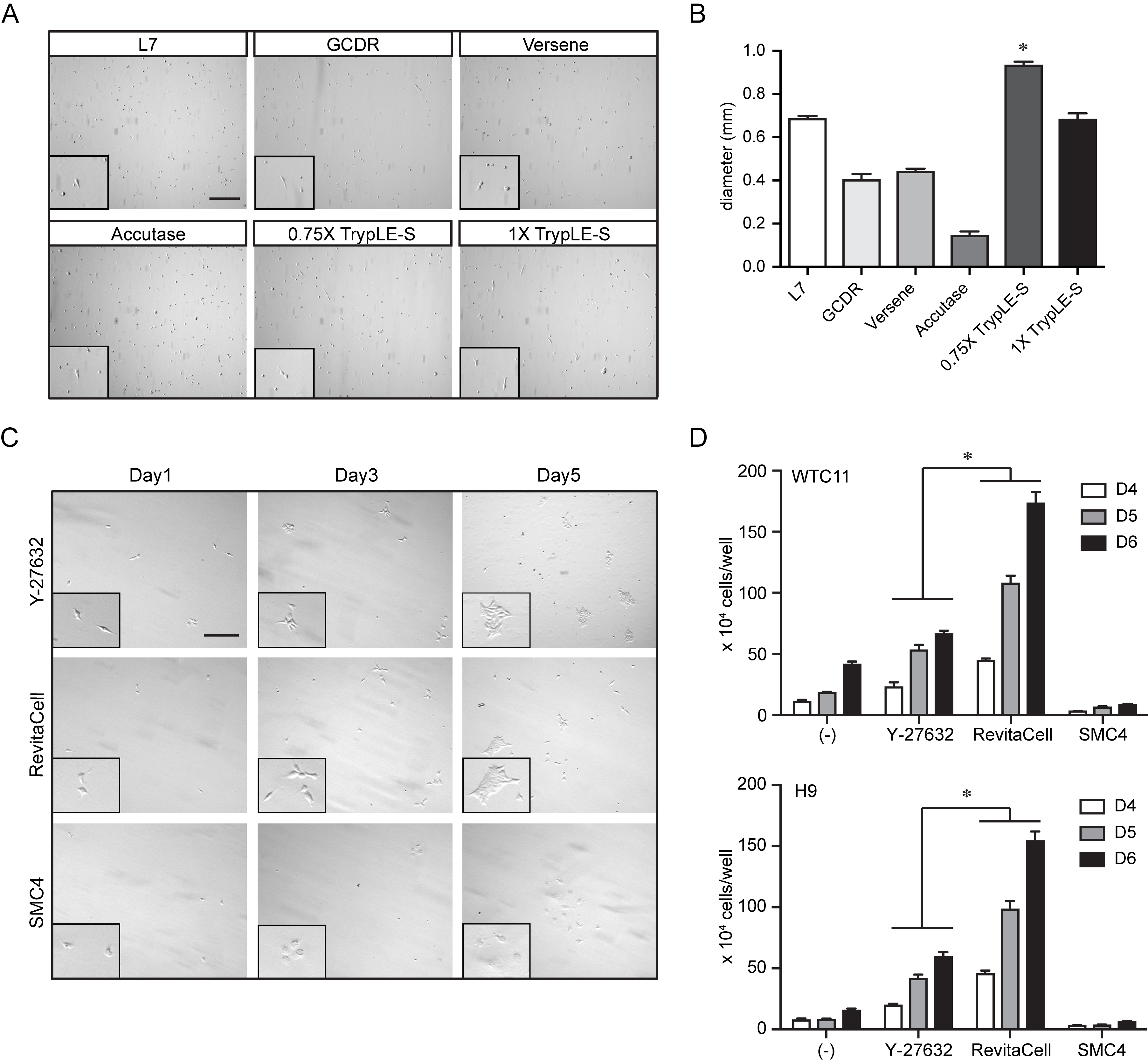
**Supplemental Tables**

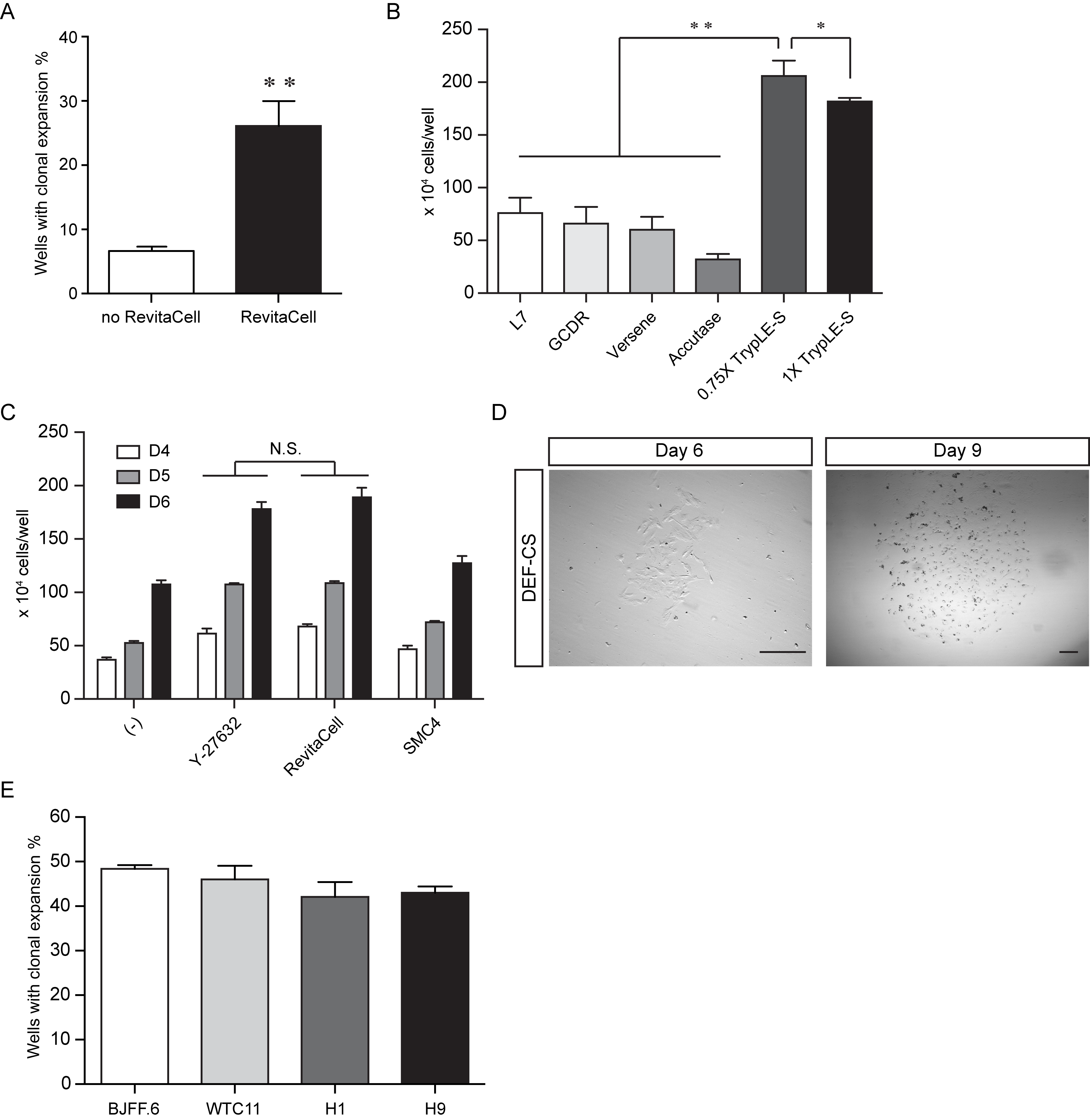
Table S1. Genome editing projects using E8-Flex, RevitaCell, and Geltrex.

Table S2. Genome editing projects using Stem-Flex, RevitaCell, and Geltrex.

Table S3. Antibodies.

**Supplemental Experimental Procedures**

**Figure S1. TrypLE-Select supports single cell survival.** (A)Representative images for single cell dissociation in each reagent were shown 24 hrs after plating.(B)hPSCs dissociated with TrypLE-Select showed great recovery in the size of hPSC colonies without addition of ROCKi (n=3). (C) BJFF.6 iPSCs were dissociated with 0.75X TrypLE-S and seeded as single cells. Representative images for colonies in each supplemented media were shown at day 1, 3 and 5 post seeding. (D) RevitaCell outperformed Y-27632 in improving single cell survival (n=3). Error bars denote the mean ± SEM. (\*) p<0.01. Scale bar, 100 μm.



**Figure S2.** **Different culture systems.** (A) BJFF.6 iPSCs were single-cell sorted into 96-well plates supplement with DEF-CS single-cell clonal medium with or without RevitaCell. RevitaCell-supplemented medium exhibited increased clonal efficiency in DEF-CS culture system (n=3). Improved cell survival and growth was observed in hPSCs dissociated with TrypLE-Select (B) and in Stem-Flex: Matrigel supplemented with ROCK inhibitor (Y-27632 or RevitaCell) (C) (n=3). (D) Representative image of a single colony grown in DEF-CS culture medium at day 6 and 9 post single-cell sorting. (E) Single-cell cloning efficiency of hPSCs in laminin-521-coated 96-well plate using Stem-Flex with RevitaCell (n=3). Error bars denote the mean ± SEM. (\*) p<0.05, (\*\*) p<0.01. Scale bar, 50 μm.

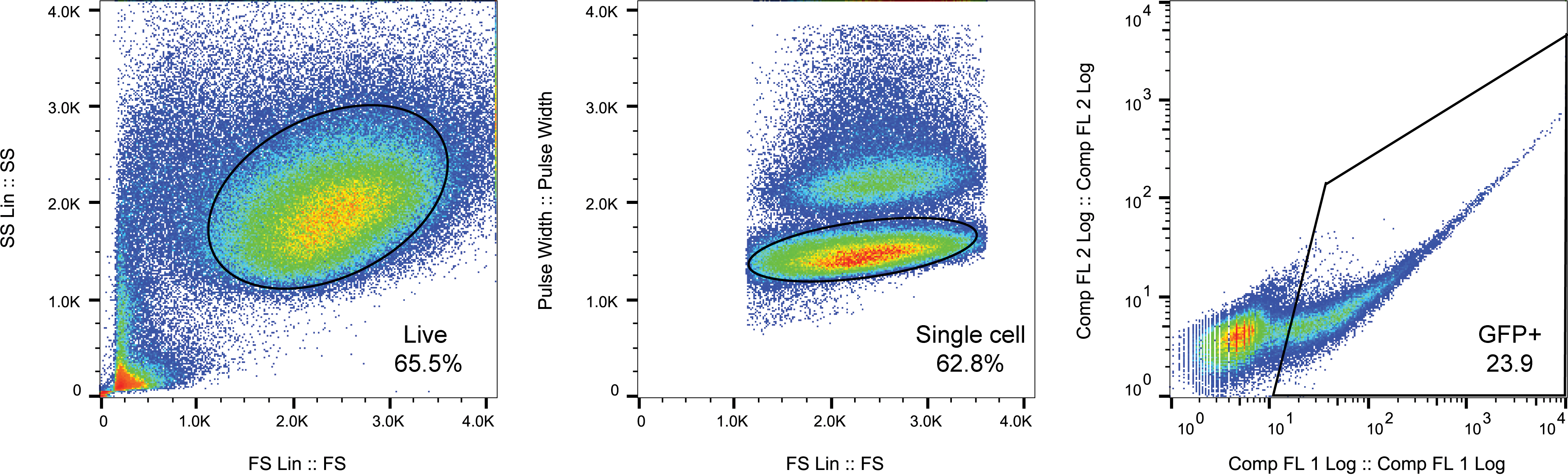
**Figure S3. Flow cytometry cell sorting (FACS).** Left panel (SSC vs FCS density plot): Gate on the plot represents live population. Middle panel (Pulse width vs FCS density plot): Gate on the plot represents single-cell population in live population. Right panel (FL1 vs FL2 density plot with compensation): Gate on the plot represents GFP-positive cells in live, single-cell population.

Table S1. Genome editing projects using E8-Flex, RevitaCell, and Geltrex.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parental cells** | **Cell type** | **Culture method** | **Reprogramming method** | **Source** | **Modification** | **Donor type** | **Average**  **clonability** |
| 85599.4 | iPSC | E8-Flex | Sendai virus | patient sample | Correction | ssODN | 32% |
| 9677-4 | iPSC | E8-Flex | Sendai virus | patient sample | Correction | ssODN | 23% |
| ANF4Cr1 | iPSC | E8-Flex | Lentivirus | patient sample | Correction | ssODN | 40% |
| ANF4Cr1 | iPSC | E8-Flex | Lentivirus | patient sample | Correction | ssODN | 40% |
| ANF4Cr1 | iPSC | E8-Flex | Lentivirus | patient sample | Correction | ssODN | 42% |
| HT112F | iPSC | E8-Flex | Sendai virus | external collaborator | Correction | ssODN | 5% |
| H1 | hESC | E8-Flex |  | WiCell | deletion |  | 38% |
| H9 | hESC | E8-Flex |  | WiCell | deletion |  | 43% |
| H1 | hESC | E8-Flex |  | WiCell | Knock-in | plasmid donor | 35% |
| H1 | hESC | E8-Flex |  | WiCell | Knock-in | plasmid donor | 38% |
| 1016SeVA | iPSC | E8-Flex | Sendai virus | patient sample | Knock-in reporter | plasmid donor | 42% |
| 1016SeVA | iPSC | E8-Flex | Sendai virus | patient sample | Knock-in reporter | plasmid donor | 47% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 38% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 42% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 38% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 46% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 43% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 40% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 37% |
| H9 | hESC | E8-Flex |  | WiCell | Knock-in reporter | plasmid donor | 38% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | Knock-in reporter | plasmid donor | 35% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | Knock-in reporter | plasmid donor | 41% |
| 1016SeVA | iPSC | E8-Flex | Sendai virus | patient sample | Knock-out |  | 35% |
| PMD-114D | iPSC | E8-Flex | Lentivirus | external collaborator | Knock-out |  | 32% |
| PMD-114D | iPSC | E8-Flex | Lentivirus | external collaborator | Knock-out |  | 26% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-out |  | 38% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-out |  | 35% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-out |  | 33% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-out |  | 40% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | Knock-out |  | 36% |
| H1 | hESC | E8-Flex |  | WiCell | Knock-out |  | 37% |
| H9 | hESC | E8-Flex |  | WiCell | Knock-out |  | 38% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | Knock-out |  | 42% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 42% |
| F10336.3 | iPSC | E8-Flex | Sendai virus | patient sample | point mutation | ssODN | 15% |
| F10336.3 | iPSC | E8-Flex | Sendai virus | patient sample | point mutation | ssODN | 18% |
| H1 | hESC | E8-Flex |  | WiCell | point mutation | ssODN | 43% |
| H1 | hESC | E8-Flex |  | WiCell | point mutation | ssODN | 38% |
| H1 | hESC | E8-Flex |  | WiCell | point mutation | ssODN | 42% |
| H1 | hESC | E8-Flex |  | WiCell | point mutation | ssODN | 35% |
| H1 | hESC | E8-Flex |  | WiCell | point mutation | ssODN | 33% |
| H9 | hESC | E8-Flex |  | WiCell | point mutation | ssODN | 35% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 42% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 38% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 40% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 35% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 42% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 33% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 35% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 40% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | point mutation | ssODN | 38% |
| Moshi-15-FLV | iPSC | E8-Flex | Sendai virus | patient sample | point mutation | ssODN | 42% |
| Moshi-15-FLV | iPSC | E8-Flex | Sendai virus | patient sample | point mutation | ssODN | 25% |
| Moshi-15-FLV | iPSC | E8-Flex | Sendai virus | patient sample | point mutation | ssODN | 35% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | point mutation | ssODN | 36% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | point mutation | ssODN | 40% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | point mutation | ssODN | 37% |
| WTC11 | iPSC | E8-Flex | Episomal | Coriell institute | point mutation | ssODN | 35% |
| BJFF.6 | iPSC | E8-Flex | Sendai virus | GEiC | small deletion | ssODN | 38% |

Table S2. Genome editing projects using Stem-Flex, RevitaCell, and Geltrex.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parental cells** | **Cell type** | **Culture method** | **Reprogramming method** | **Source** | **Modification** | **Donor type** | **Average**  **clonability** |
| B12-106.1 | iPSC | Stem-Flex | Sendai virus | patient sample | Correction | ssODN | 33% |
| 153231-11 | iPSC | Stem-Flex | Sendai virus | patient sample | Correction | ssODN | 38% |
| 153231-11 | iPSC | Stem-Flex | Sendai virus | patient sample | Correction | ssODN | 40% |
| 153231-11 | iPSC | Stem-Flex | Sendai virus | patient sample | Correction | ssODN | 39% |
| 153231-19 | iPSC | Stem-Flex | Sendai virus | patient sample | Correction | ssODN | 32% |
| H1 | hESC | Stem-Flex |  | WiCell | deletion |  | 35% |
| H1 | hESC | Stem-Flex |  | WiCell | deletion |  | 27% |
| H9 | hESC | Stem-Flex |  | WiCell | deletion |  | 45% |
| iPSC4259 | iPSC | Stem-Flex | Sendai virus | external collaborator | deletion |  | 40% |
| iPSC4676 | iPSC | Stem-Flex | Sendai virus | external collaborator | deletion |  | 38% |
| WTC11 | iPSC | Stem-Flex | Episomal | Coriell institute | deletion |  | 44% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 36% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 48% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 46% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 40% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 45% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-in reporter | plasmid donor | 40% |
| H9 | hESC | Stem-Flex |  | WiCell | Knock-in reporter | plasmid donor | 37% |
| IPSC0028 | iPSC | Stem-Flex | Retrovirus | external collaborator | Knock-in reporter | plasmid donor | 43% |
| IPSC0028 | iPSC | Stem-Flex | Retrovirus | external collaborator | Knock-in reporter | plasmid donor | 43% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 40% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 40% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 45% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 43% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 35% |
| H9 | hESC | Stem-Flex |  | WiCell | Knock-out |  | 46% |
| H9 | hESC | Stem-Flex |  | WiCell | Knock-out |  | 48% |
| H9 | hESC | Stem-Flex |  | WiCell | Knock-out |  | 45% |
| IPSC0028 | iPSC | Stem-Flex | Retrovirus | external collaborator | Knock-out |  | 45% |
| Moshi-15-FLV | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 42% |
| Moshi-15-FLV | iPSC | Stem-Flex | Sendai virus | GEiC | Knock-out |  | 37% |
| ALF2-CR1 | iPSC | Stem-Flex | Lentivirus | patient sample | point mutation | ssODN | 28% |
| BIO-Ni037A | iPSC | Stem-Flex | Episomal | external collaborator | point mutation | ssODN | 43% |
| BIO-Ni037A | iPSC | Stem-Flex | Episomal | external collaborator | point mutation | ssODN | 38% |
| BIO-Ni037A | iPSC | Stem-Flex | Episomal | external collaborator | point mutation | ssODN | 40% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 36% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 45% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 44% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 42% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 40% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 42% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 44% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 35% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 42% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 40% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 43% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | point mutation | ssODN | 38% |
| F10336.3 | iPSC | Stem-Flex | Sendai virus | patient sample | point mutation | ssODN | 22% |
| H1 | hESC | Stem-Flex |  | WiCell | point mutation | ssODN | 38% |
| H1 | hESC | Stem-Flex |  | WiCell | point mutation | ssODN | 42% |
| H9 | hESC | Stem-Flex |  | WiCell | point mutation | ssODN | 47% |
| H9 | hESC | Stem-Flex |  | WiCell | point mutation | ssODN | 42% |
| P301S(bi) | iPSC | Stem-Flex | Retrovirus | external collaborator | point mutation | ssODN | 42% |
| Wolf13 | iPSC | Stem-Flex | Sendai virus | patient sample | point mutation | ssODN | 45% |
| Wolf4 | iPSC | Stem-Flex | Sendai virus | patient sample | point mutation | ssODN | 42% |
| WTC11 | iPSC | Stem-Flex | Episomal | Coriell institute | point mutation | ssODN | 38% |
| WTC11 | iPSC | Stem-Flex | Episomal | Coriell institute | point mutation | ssODN | 42% |
| WTC11 | iPSC | Stem-Flex | Episomal | Coriell institute | point mutation | ssODN | 44% |
| WTC11 | iPSC | Stem-Flex | Episomal | Coriell institute | point mutation | ssODN | 45% |
| 4194 | iPSC | Stem-Flex | Sendai virus | external collaborator | tagging | ssODN | 35% |
| BJFF.6 | iPSC | Stem-Flex | Sendai virus | GEiC | tagging | ssODN | 43% |
| H9 | hESC | Stem-Flex |  | WiCell | tagging | ssODN | 45% |
| IPSC0028 | iPSC | Stem-Flex | Retrovirus | external collaborator | tagging | ssODN | 47% |
| IPSC0028 | iPSC | Stem-Flex | Retrovirus | external collaborator | tagging | ssODN | 45% |

**Table S3. Antibodies.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibody** | **Host** | **Source** | **Dilution** |
| OCT4 (ICC) | Rabbit | Invitrogen (part no. A25532) | 1:400 |
| SSEA4 (ICC) | Mouse | Invitrogen (part no. A25530) | 1:100 |
| SOX2 (ICC) | Rat | Invitrogen (part no. A24759) | 1:100 |
| TRA-1-60 (ICC) | Mouse | Invitrogen (part no. A24868) | 1:100 |
| TUJ1 (ICC) | Rabbit | Invitrogen (part no. A25532) | 1:500 |
| AFP (ICC) | Mouse | Invitrogen (part no. A25530) | 1:200 |
| SMA (ICC) | Mouse | Invitrogen (part no. A25531) | 1:200 |

ICC: Immunocytochemistry

**Supplemental Experimental Procedures**

**Cell culture**. The hPSC lines were maintained on Matrigel (Corning) coating plate in E8-Flex medium (ThermoFisher). Matrigel coating was performed by diluting concentrated matrix in ice-cold DMEM/F12 medium following manufacturer’s instructions. The solution was allowed to coat for at least 1h at RT. For passaging, hPSCs were washed once with DPBS and were dissociated using non-enzymatic L7 passaging solution (Lonza) into small clumps, collected and dilution passaged according to the initial density, ranging from 1:4 to 1:8. Cell cultures were maintained in a humidified incubator set at 37°C and 5% CO2.

**Generation of genome-engineered iPSC.** hPSCs used for genome editing projects are ranging between passage 20 to 30. Before single cell dissociation, hPSCs were pretreated with ROCK inhibitor (Y-27632 or RevitaCell) for 1hour. 6-well plate is coated with Matrigel and incubates RT for at least 1 hour. Culture medium plus ROCKi (2ml/well) was added to cultureware and incubate at 37˚C prior to seeding cells. hPSCs are dissociated into single cells with 0.75X TrypLE-select reagent. Cells from cultureware was harvested with culture medium and spin down at 120g for 2 mins. Approximately 1 to 1.5 × 106 hPSCs were washed in DPBS and resuspended in P3 primary buffer with 1 μg gRNA, 1.5 μg Cas9 vectors and 0.5 ug GFP expression construct and then electroporated using a 4D-Nucleofector (Lonza) using CA-137 program. Following nucleofection, cells were plated in 6-well plate with warm medium supplemented with ROCKi for 24 hrs. Next day, the medium was replaced with fresh medium without ROCKi and cells were further recovered for another 1-2 days. After recovery, nucleofected pool could be screened with specific assays to confirm modifications. Modified pool sample could then be FACS sorted into 96-well plate using Moflo cell sorter (1 cell/well). Mature hPSC colonies could be harvest for downstream analysis between day 11 to 12.

**Single-cell dissociation and FACS single-cell sorting**. Different culture systems and supplements have been applied for the single-cell passing and cloning. Media mTeSR1, TeSR-E8 (StemCell Technologies), DEF-CS (TaKaRa, Clontech), Essential 8-Flex (E8-Flex) and Stem-Flex (ThermoFisher) were used following user manuals. hPSCs were adapted in different culture media for at least 3 passages before performing single-cell passaging. Coated 96-well plates were prepare a day before and kept at 37°C incubator using different coating reagents following manufacturer’s instructions. For Vitronectin XF (VN-XF), non-tissue culture-treated cultureware was used; for the other matrices, tissue culture-treated cultureware was used. VN-XF coated plates were specifically used for mTeSR1 and TeSR-E8 culture systems. Briefly, VN-XF coating was performed by diluting VN-XF (StemCell Technologies) in CellAdhere Dilution Buffer (StemCell Technologies) to a final concentration of 10 μg/mL, then immediately using the solution to coat non-tissue culture-treated cultureware at least 1 h at RT or overnight (ON) at 4 C. Before singe-cell dissociation, hPSCs were pre-treated with different inhibitors or supplements including 10μM Y-27632 (ROCK inhibitor, ROCKi, Stem Cell Technologies), 1X RevitaCell (ThermoFisher), or SMC4 (Biovision) for at least 1h. During the pre-treatment, 100μl/well supplemented media was added to 96-well plates, and plates were equilibrated in a 37°C incubator prior to single-cell sorting. After pretreatment, hPSCs were washed once with DPBS and incubated with 0.75X TrypLE-Select (mix 1xTrypLE-S and 0.5 mM EDTA/DPBS(-/-) at 3:1 ratio) for 3 min at 37°C. Then, the solution was removed and single-cell suspension was mixed with regular culture medium, centrifuged at 120g for 2 min and resuspended in media supplemented with different additives. For single-cell sorting, live single hPSCs were subjected to FSC/SSC gate and then flow-sorted using a Beckman Coulter (Indianapolis, IN) MoFlo HTS/Propel Labs (Ft. Collins, CO) sorter with a 100um nozzle and low sheath pressures (17.2 PSI) to minimize mechanical stress to the hPSCs. No cell strainer or propidium iodide was used. A representative nucleofected pool was subjected to MoFlo cell sorter and dot plots with gating were shown in Figure S3. After sorting, plates were centrifuged at 120g for 2 mins and maintained in a humidified incubator set at 37°C and 5% CO2.

**Crystal violet attachment assay**. The hPSCs were dissociated with different dissociation reagents, L7 (Lonza), GCDR (Stem Cell Technologies), accutase (Innovative Cell Technologies), Versene or TrypLE-Select (ThermoFisher). Approximately 10,000 cells per well of 6-well plate were seeded. For supplements, ROCKi, RevitaCell or SMC4 was also added for the first 72 h after seeding, and reduced to 1/2X of original concentration for another 4 days. Media was replaced with fresh media and supplement, the day after plating and every 2 days afterward. After the specified time for attachment and/or growth, total cell number was assessed. Briefly, the plates were washed twice with PBS to remove non-attached cells and fixed using ice-cold methanol for 10min, and subsequently stained with 0.5% w/v crystal violet in methanol for 5 min. The crystal violet was then thoroughly washed away using distilled water, and the plates were dried overnight at RT, before quantification was performed. Optical density was measured by ImageJ. All cell-number quantification experiments were performed in triplicates.

**Immunofluorescence staining**. Pluripotentcy staining was performed by using Pluripotent Stem Cell 4-Marker Immunocytochemistry kit (A25526, ThermoFisher) following the manufacturer’s recommend protocol. For EB formation, hPSCs were single-cell dissociated with 0.75X TrypLE-Select and resuspended in differentiation medium containing DMEM/F12 (Gibco), 20% fetal bovine serum (Gibco), 1% non-essential amino acids (Gibco), 2 mM L-glutamine (Gibco) and 100 mM β-mercaptoethanol. 5,000 cells/100ul were seeded in V-bottom 96-well non-tissue culture plate (Corning) and centrifuge at 200g for 2 min. At day 3, ball-like clumps were transfer to ultra-low binding 6-well plate (Corning). After 7 days, EBs were transferred to Matrigel-coated chamber. After 3 weeks in culture, spontaneously differentiated embryoid bodies were stained for the three embryonic layers with the 3-Germ Layer Immunocytochemistry kit (A25538, Thermofisher). Images of the stained cells were captured using the Nikon fluorescence microscope and CCD camera.

**Statistical analysis.** Each experiment was performed with samples from at least three independent groups. All data were processed and graphed in Prism GraphPad 6.0 with descriptive statistics calculated. The difference between experimental groups was assessed by one-way ANOVA. All tests were two-sided with a significance level of 5% unless otherwise noted.