**Table S1: Experimental protocols of Center 1 and Center 2. Related to Experimental Procedures.**

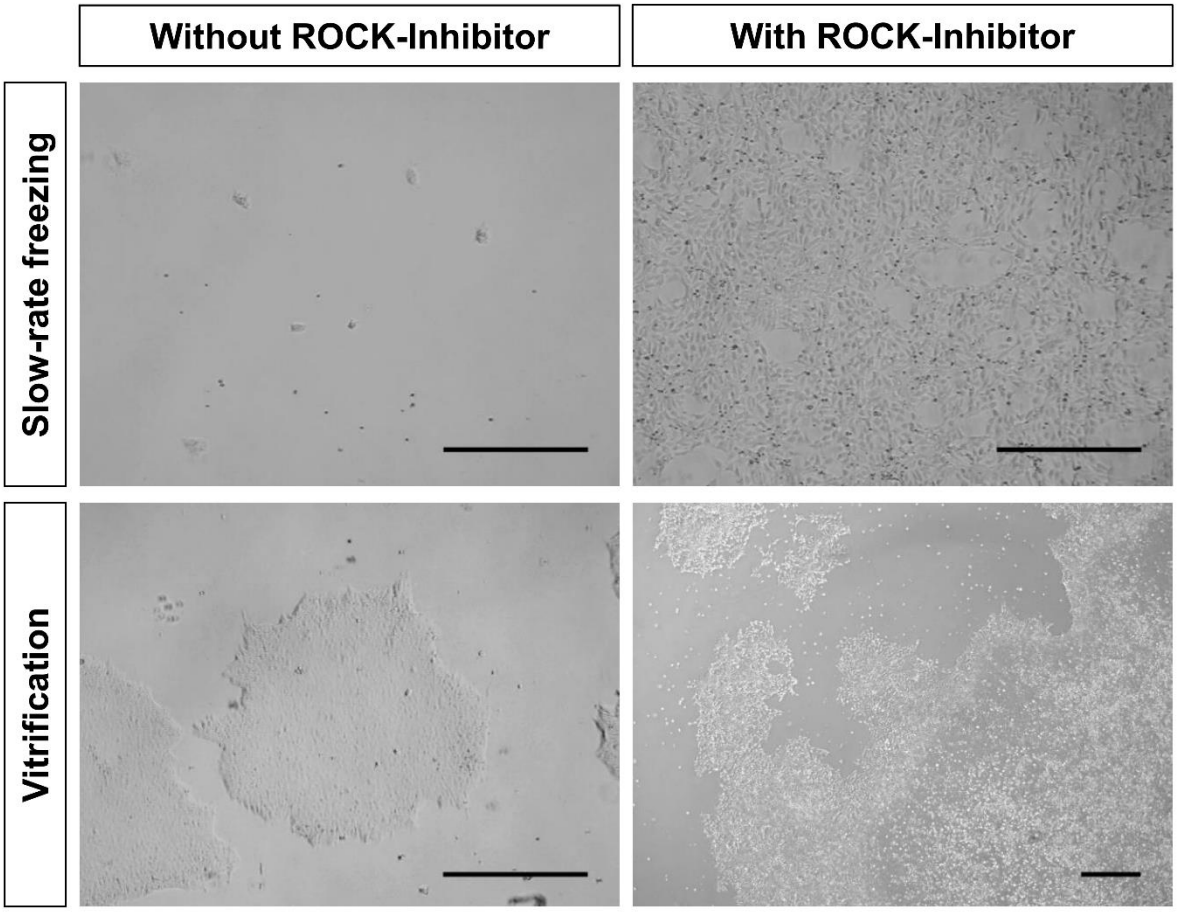
| | **Center 1** | **Center 2** |
|---|---|---|
| **Coating** | Matrigel (Corning) | Geltrex (Gibco) |
| **Detachment of hiPSCs** | EDTA (Thermo Fisher Scientific) | Gentle Cell Dissociation (STEMCELL Technologies) |
| **Single cell dissociation** | TrypLE (Thermo Fisher Scientific) | Accutase (Thermo Fisher Scientific) |
| **Fixation of cells** | BD Cytofix (BD Biosciences) | 4% PFA (Carl Roth) |
| **Blocking for immunocytochemistry** | PBS (Thermo Fisher Scientific)<br>+ 2% BSA (Sigma Aldrich)<br>+ 0.2% Tween-20 (Sigma Aldrich) | PBS (Thermo Fisher Scientific)<br>+ 3% Donkey serum (PAN Biotech)<br>+ 0.1% Triton X-100 (Sigma Aldrich) |
| **Microscopy** | Leica DMI6000 (Leica) | Axio Observer.Z1 (Carl Zeiss) |
| **Flow cytometry** | BD FACS Aria III (BD Biosciences) | Cytoflex (Beckman Coulter) |
| **Cell counting** | NucleoCounter NC-200 (Chemometec) | LUNA-FL™ Dual Fluorescence Cell Counter (Logos Biosystems) |

**Table S2: Experimental performed at Center 1 and Center 2 using human induced pluripotent stem cells (hiPSCs) and small molecule neural precursor cells (smNPCs).**

| Cell type | Experiment | Center 1 | Center 2 |
|---|---|---|---|
| hiPSCs | Cell counting | X | X |
| | Cell viability | X | X |
| | Confluency | X | X |
| | ICC | X | X |
| | FACS | X | X |
| | RNA-Seq | | X |
| | SEM | X | |
| smNPCs | Cell counting | | X |
| | Cell viability | | X |
| | ICC | | X |
| | FACS | | X |

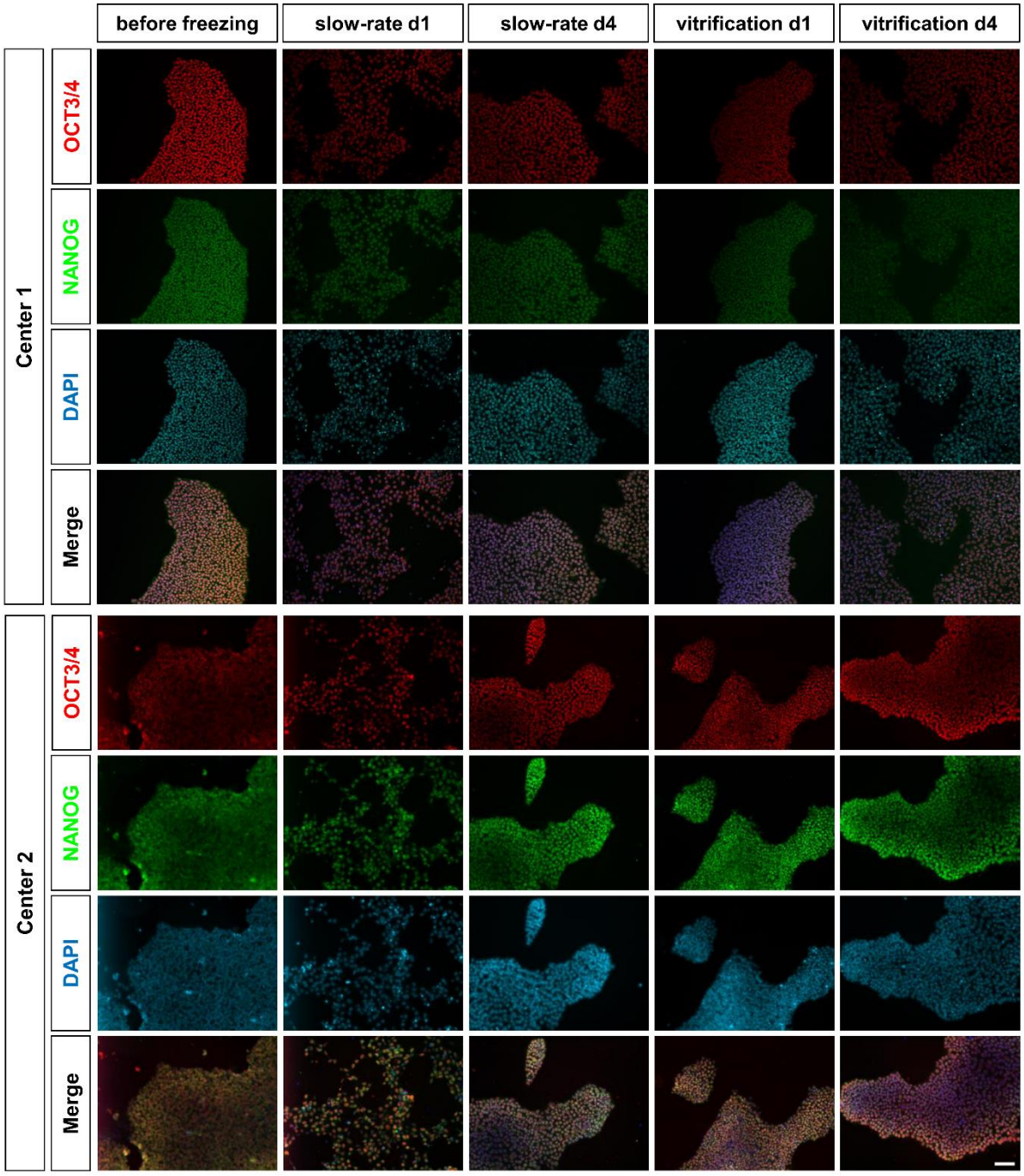
ICC = immunocytochemistry, FACS = fluorescence-activated cell sorting, RNA-Seq = RNA-Sequencing, SEM = scanning electron microscopy.

**Supplemental Figure 1: Rho-associated protein kinase (ROCK) inhibitor (RI) is redundant after cryopreservation of human induced pluripotent stem cells (hiPSCs) via adherent vitrification.**




HiPSCs were cryopreserved via slow-rate freezing and vitrification and thawed with or without RI. We observed that RI inhibitor is crucial for thawing of slow-rate frozen hiPSCs, but redundant for thawing after freezing via adherent vitrification. Scale bars 500µm. Related to Figure 2.

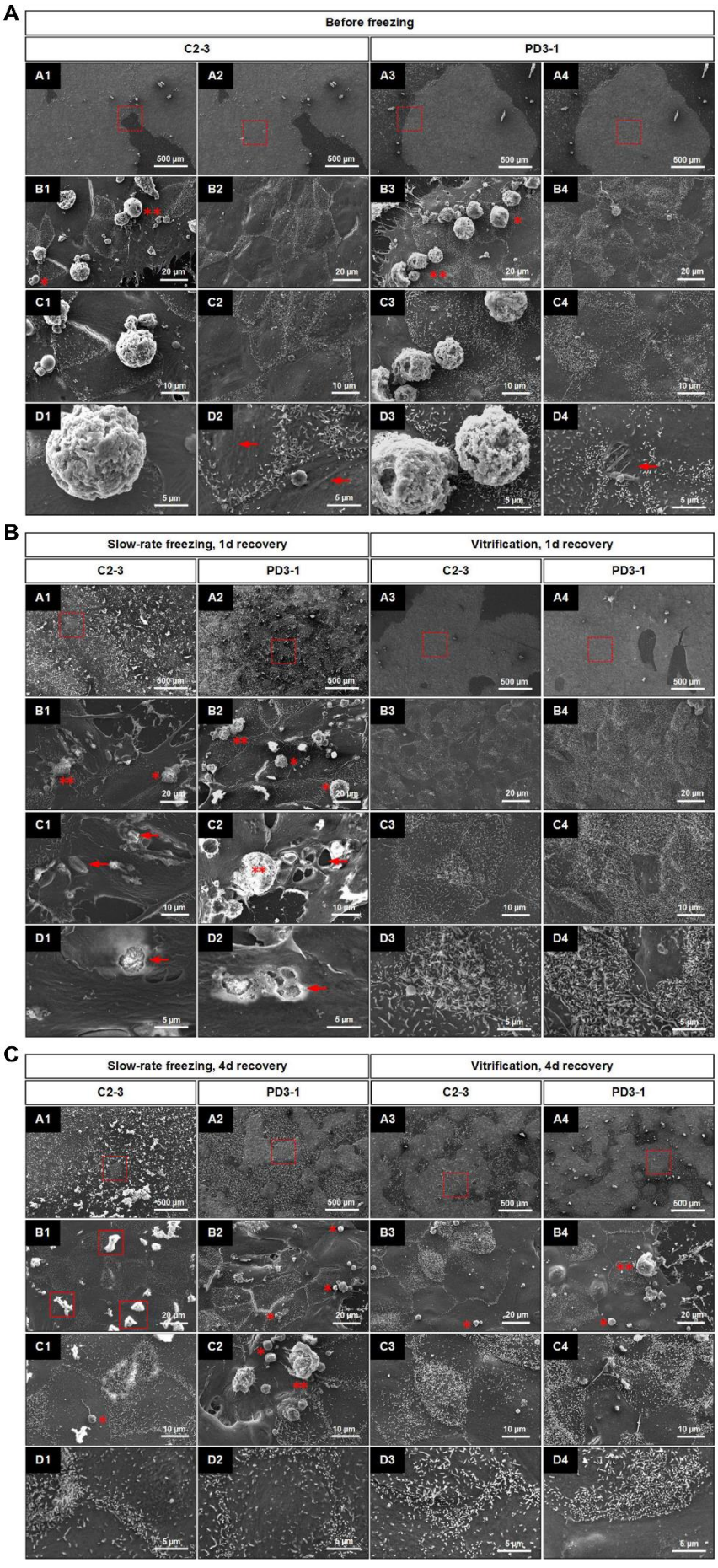
**Supplemental Figure 2: Strong expression of pluripotency markers in human induced pluripotent stem cells (hiPSCs) before and after cryopreservation.**




Respective immunocytochemistry (ICC) images of pluripotency markers OCT3/4 (red) and NANOG (green) staining for both freezing methods. ICC revealed strong expression of both markers in the majority of the cells. Scale bar 100µm. Related to Figure 3.

**Supplemental Figure 3: Scanning electron microscopy (SEM) revealed preservation of cell-cell contacts of human induced pluripotent stem cells (hiPSCs) by adherent vitrification.**

**(A)** SEM images of hiPSCs before cryopreservation. Cells within colonies displayed numerous microvilli and intact cell-cell adhesions (regions of interest, arrows). Round and damaged cells were only detected at colony borders (asterisks). **(B)** SEM images at day 1 after thawing. Slow-rate frozen hiPSC colonies were decreased in size (regions of interest), showed large holes and disruption of colony integrity (arrows). Round cells with undamaged and damaged membrane were detected (asterisks and double asterisks, respectively). Adherent vitrification maintained large hiPSC colonies. Cells were covered with numerous microvilli (regions of interest). **(C)** SEM images at day 4 after thawing. Slow-rate frozen hiPSCs increased in size, displayed microvilli and few round detached or damaged cells were detected (asterisks). Artefacts of the extracellular matrix (ECM) coating were visible. Vitrified hiPSCs showed intact cell-cell adhesions and only few round detached and damaged cells (asterisks). Related to Figure 5.


A
Before freezing
C2-3
PD3-1
A1
A2
A3
A4
B1
B2
B3
B4
C1
C2
C3
C4
D1
D2
D3
D4
500 μm
20 μm
10 μm
5 μm
B
Slow-rate freezing, 1d recovery
Vitrification, 1d recovery
C2-3
PD3-1
C2-3
PD3-1
C
Slow-rate freezing, 4d recovery
Vitrification, 4d recovery
C2-3
PD3-1
C2-3
PD3-1