
Supplementary information

Cryomicroneedles for transdermal cell delivery

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Video S3. Thawing behavior of CryoMNs removed from -196 °C (liquid nitrogen) and placed on human figure

Video S4. Thawing behavior of CryoMNs removed from -80 °C and placed at RT (~24 °C).

1. Supplementary Materials and Methods

Materials

Stainless steel master mold of microneedles (10 × 10 array, 400 µm base diameter, 700µm pitch, 5 µm tip radius, and 1200 µm height) was purchased from Micropoint Technologies Pte Ltd (Singapore). Sylgard® 184 silicone elastomer kit was purchased from Dow Corning (USA). dimethyl sulfoxide (DMSO), sucrose, bovine serum albumin (BSA), cell strainer (70 µm), ovalbumin (from chicken egg white, OVA), lipopolysaccharides (from Escherichia coli O111:B4, LPS) and ALT Activity Assay kit were purchased from Sigma-Aldrich (USA). Dulbecco's modified eagle medium (DMEM), DMEM: Nutrient Mixture F-12 medium (DMEM/F12), Medium 254, Human Melanocyte Growth Supplement-2 (PMA-free, HMGS-2), fetal bovine serum (FBS), 10000 U/mL penicillin-streptomycin (P/S), trypsin-EDTA, alamarBlue™ cell viability reagent, LIVE/DEAD™ viability/cytotoxicity kit, 2-Mercaptoethanol, MEM non-essential amino acids solution, ACK Lysing Buffer, anti-mouse MHC Class II antibody (APC-conjugated, AF6-120.1, 17-5320-82), anti-mouse CD86 antibody (APC-conjugated, GL1, 17-0862-82), Click-iT™ Plus TUNEL Assay, and CyQUANT™ LDH Cytotoxicity Assay Kit were purchased from ThermoFisher Scientific (USA). DELFIA® EuTDA Cytotoxicity Reagents was purchased from Sciencewerke (Singapore). ImmunoCult™-XF T Cell Expansion Medium, ImmunoCult™ Human CD3/CD28 T Cell Activator, Anti-Mouse CD11c Antibody (FITC conjugated, Clone: N418, 60002FI), and Mouse IFN-gamma ELISA Kit were purchased from the StemCell Pte. Ltd. (Singapore) Phosphate buffered saline (1X) (PBS) was purchased from Lonza (Singapore). RPMI-1640 medium (+ 2.05mmol/L L-glutamine), sodium pyruvate, HEPES were purchased HyClone (USA). from Recombinant murine granulocyte-macrophage colony stimulating factor (GM-CSF) and Recombinant murine IL-4 were purchased from Peprotech (USA). H&E Stain Kit (Hematoxylin and Eosin, ab245880) was purchased from Abcam (USA).

Cell culture

Human Hela-Red fluorescent protein (RFP) stable cell line (RFP-Hela), human immortalized keratinocytes (HaCaT) and normal human dermal fibroblasts (NDF), B16 melanoma cell line transfected with ovalbumin (B16-OVA) were cultured in DMEM supplemented with 10% FBS and 1% P/S in culture dish at 37 °C and 5% CO₂. Human mesenchymal stem cells (MSCs) were cultured in DMEM/F12 supplemented with 10% FBS and 1% P/S in culture dish at 37 °C and 5% CO₂. Human epidermal melanocytes from darkly pigmented donor (ThermoFisher Scientific, USA) were cultured in Medium 254 supplemented with HMGS-2 (5 mL for 500 mL medium), 10% FBS, and 1% P/S in culture dish at 37 °C and 5% CO₂. Human Peripheral Blood CD8⁺CD45RA⁺ T Cells were cultured in ImmunoCult™-XF T Cell Expansion Medium supplemented with ImmunoCult™ Human CD3/CD28 T Cell Activator (25 µL/mL) and 1% P/S in culture dish at 37 °C and 5% CO₂.

The MSCs, melanocytes T Cells were used for experiments between 3 and 10 passages. The culture medium was changed every 2 or 3 days and culture dish with 80 ~ 90% confluence were used for further cell experiments.

Optimization of cryogenic medium

The matrix solutions with various formulations were prepared by dissolving DMSO and sucrose in PBS at the designated concentrations (0, 0.5, 1, 2.5, 5 or 10 % (v/v) for DMSO, 0, 50, 100, or 200 mM for sucrose). The RFP-Hela, HaCaT, NDF, MSCs, melanocytes and T-cells were detached and resuspended in 1 mL freezing solution (1 × 10⁶ cells/mL). After 15 min incubation, 1 mL of cell suspensions were added into cryovials. The cryovials were directly put into -20 °C refrigerator. After 4h, the cryovials were transferred to -80 °C refrigerator. After 24h, the cryovials were taken out and thawed in a water bath at 37 °C. The thawed cells were seeded on the 48-well plate supplemented with fresh culture media. After culturing for 24h, the cells were imaged by phase contrast microscope.

Furthermore, alamarBlue™ cell viability assay was conducted according to the provided protocol and the fluorescence (excitation:540nm, emission 580nm) of the incubated media containing 10% alamarBlue™ reagent (~4 h) was measured using a plate reader (SpectraMax M5, Molecular Devices). The viability of cells frozen in 10 % (v/v) DMSO was used as positive control. The relative viability was calculated by dividing the viability of cells frozen under experimental conditions from the positive control.

To evaluate the toxicity of DMSO to the skin cells, HaCaT, NDF, MSCs and melanocytes were cultured in the appropriate media supplemented with different concentration of DMSO (i.e. 0%, 1%, 2.5 %, 5 % and 10%) for 24h. Then alamarBlue™ cell viability assay was conducted according to the provided protocol.

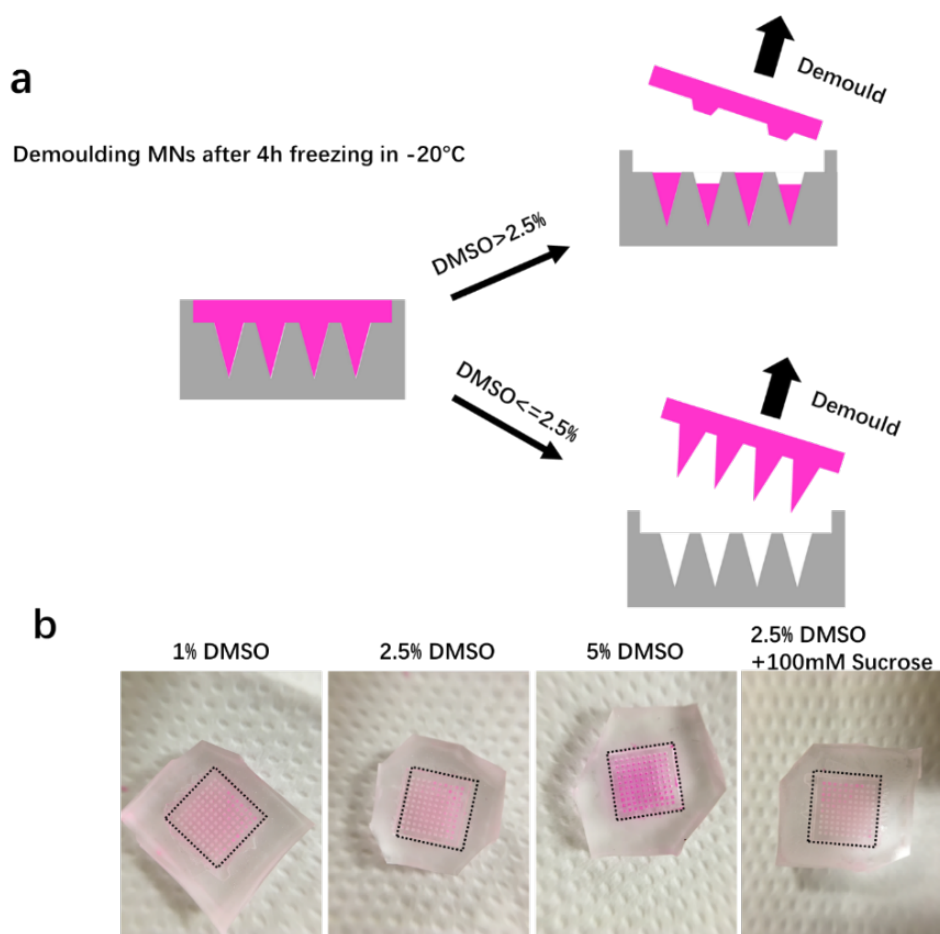
Generation and stimulation of bone marrow-derived dendritic cells (BMDCs)

BMDCs were isolated from femur bones of C57BL/6 mice according to previously reported procedures. Briefly, femur bones were removed from the mouse and the bone marrow was flushed by using RPMI 1640 with a sterile syringe (26-28 gauge needle). the marrow cells were then collected with cell strainer (70 µm) and were collected by centrifugation at 1200 rpm for 5 min. 5 mL ACK lysing buffer were added to lyse blood cells and remaining cells were collected by centrifugation at 1200 rpm for 5 min. The cells were seeded into 6 well plate at the concentration of 2×10^6 cells/ml and cultured in RPMI 1640 supplemented with 10% FBS, 2.05mmol/L L-glutamine, 0.1 nmol/L non-essential amino acids Solution, 1 mmol/L sodium pyruvate, 1 mmol/L HEPES, 1% P/S, 20 ng/mL of GM-CSF (PeproTech), 10 ng/mL of IL-4 (PeproTech) and 0.056mmol/L 2-Mercaptoethanol. The culture medium was changed every 2 days. On the 7 day, all the non-adherent cells were collected for the further experiments. The percentage of positive DCs was evaluated by flow cytometer after surface staining by anti-mouse CD11c antibody.

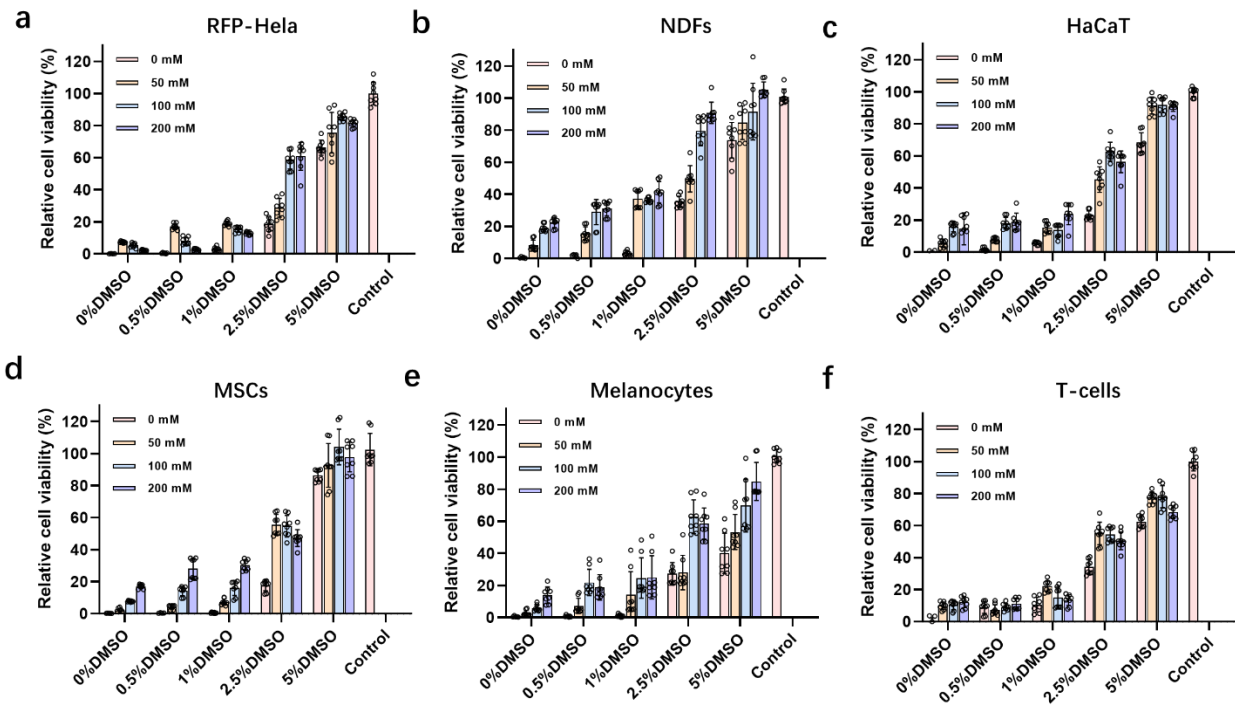
Optimization of vaccination with OVA-DCs-cryoMNs. The male C57BL/6 mice (male, 6-8 weeks, 20-25g) were purchased from InVivos Co., Ltd. (Singapore). To prepare the ovalbumin loaded DCs (OVA-DCs), DCs were incubated with 50 µg/mL OVA for 24 h. To determine the best application frequency for cryoMN vaccination, mice were grouped by different application frequency with 4×10^5 OVA-DCs each time. Four groups of mice received either 0, 1, 2, or 3 times cryoMNs vaccination per week lasting for four weeks. To optimize OVA-DCs dosage for cryoMNs vaccination, the mice were divided into four groups with each group of mice receiving twice vaccinations per week of OVA-DCs dosage of either 0 (blank cryoMNs), 2×10^5 cells (200k OVA-DCs loaded cryoMNs), 4×10^5 cells (400k OVA-DCs loaded cryoMNs), or 6×10^5 cells (600k OVA-DCs loaded cryoMNs) over four-week duration.

At day 28 of vaccine application, mice were euthanized to evaluate immune stimulation. The spleen and lymph nodes were harvested and processed into the single cell suspension. The cells extracted from lymph nodes were stained with anti-CD11c, anti-CD86, and anti-MHCII antibodies to evaluate the homing effect of mature DCs. The proliferation and IFN-γ secretion of splenocytes were quantified using alamarBlue™ cell viability assay and IFN-γ ELISA Kit accordingly. The weights of the mice were monitored through the vaccination period. At day 28, the livers were also harvested and the level of Alanine Aminotransferase (ALT) were conducted with the ALT Activity Assay Kit (Sigma).

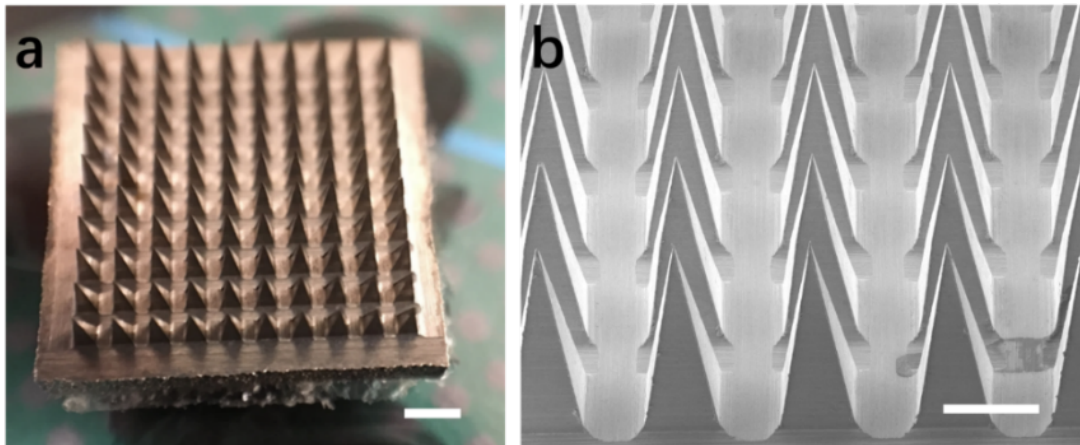
2. Supplementary Figures (S1 – S27)



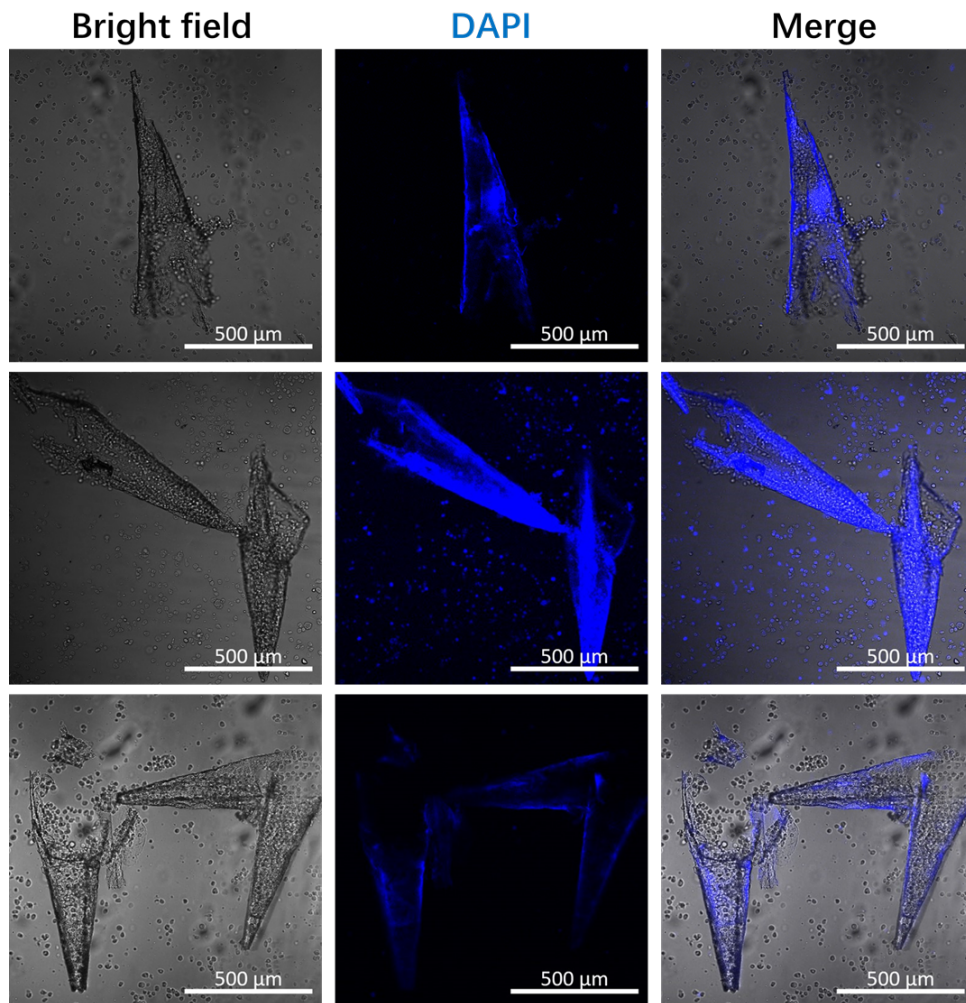
Supplementary Figure 1. Demoulding of cryoMNs. **a**, Schematic illustration of the influence of DMSO concentration (% , v/v) on the demoulding process of cryoMNs from PDMS mold. **b**, Images of the PDMS mold after demoulding the cryoMNs with different concentration of DMSO. The rhodamine B was added into the cryogenic medium and used as the indicator to illustrate the needle tip left in the PDMS mold after demoulding.



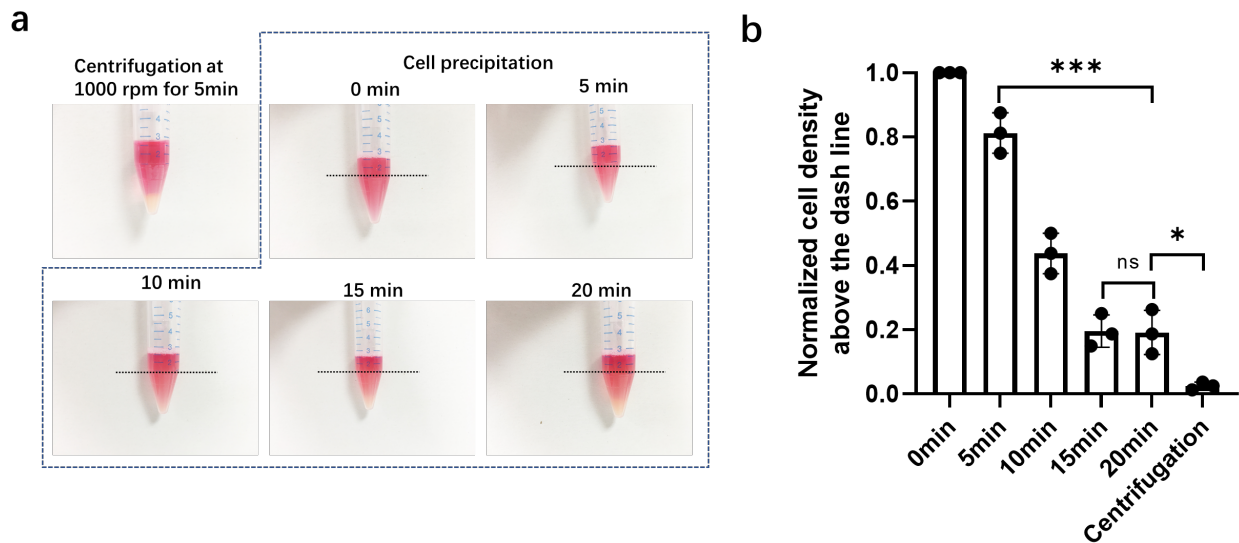
Supplementary Figure 2. Influence of different cryogenic medium on cell viability. Cell viability of RFP-Hela (a), normal dermal fibroblasts (NDFs) (b), HaCaT (c), MSCs (d), melanocytes (e) and T-cells (f) after being frozen in various formulation of cryogenic medium (PBS as base medium supplemented with DMSO and sucrose). The data was obtained from alamarBlue™ assay. The PBS with supplement of 10 % (v/v) DMSO was used as control for reference. In this optimization experiment, cells were frozen in the cryovials. Data are presented as mean $\pm$ s.d. (n = 8 biologically independent samples).



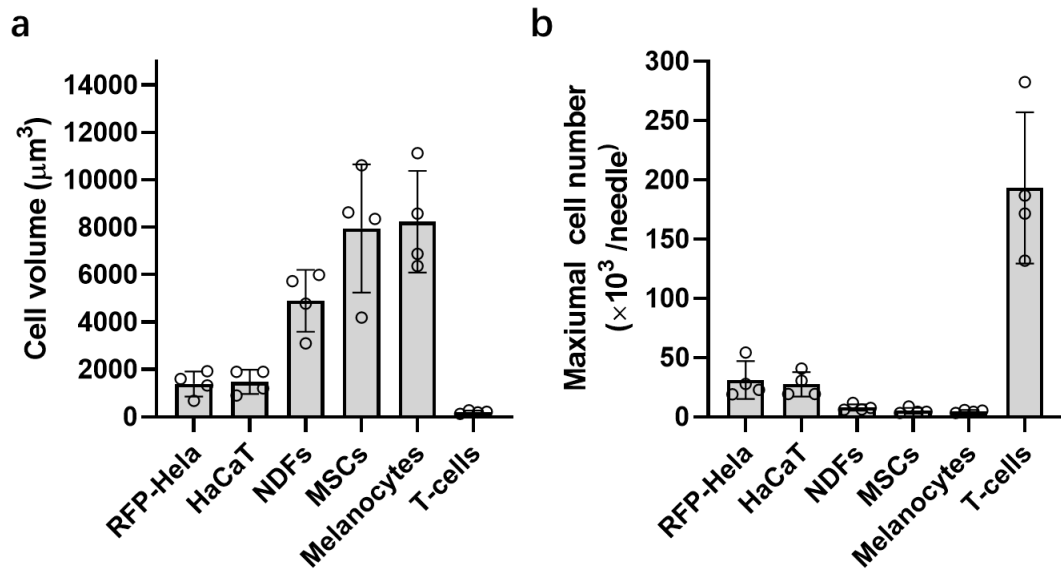
Supplementary Figure 3. Morphology of stainless steel microneedle template used in this study. **a**, Photograph of the template. Scale bar, 1000 μm . **b**, Image of scanning electron microscope (SEM) of the template. Scale bar, 500 μm . All experiments were repeated independently for two times with similar results.



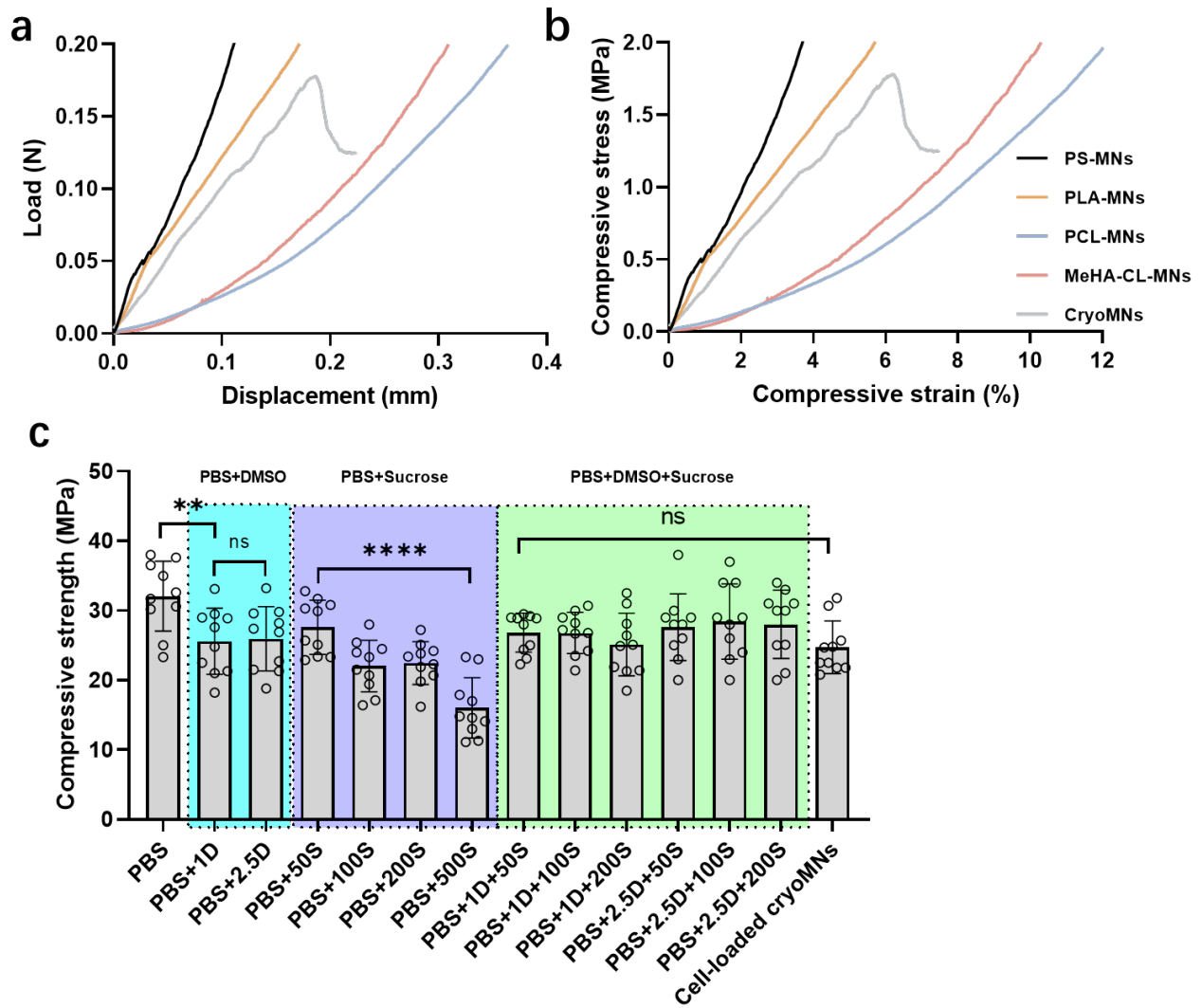
Supplementary Figure 4. Additional images of a single MN loaded with cells that were stained with NucBlue™ Live ReadyProbes™ Reagent. Scale bar, 500 μ m. All experiments were repeated independently for two times with similar results.



Supplementary Figure 5. Influence of time on the number of cells that can precipitate into the bottom. **a**, Photographs of cell precipitation in cell culture media by either conventional centrifugation or self-precipitation after different time. **b**, Normalized density of cells that cannot precipitate to the bottom of tube. Data are normalized to the cell density without precipitation (0 min). Data are presented as mean $\pm$ s.d. ($n = 3$ biologically independent samples). Two-tailed Student's t -test was used to for comparing 20 min precipitation with other groups ($***P = 0.0003$ versus 5 min precipitation; ns: $P = 0.9367$ versus 15 min precipitation; $*P = 0.0145$)

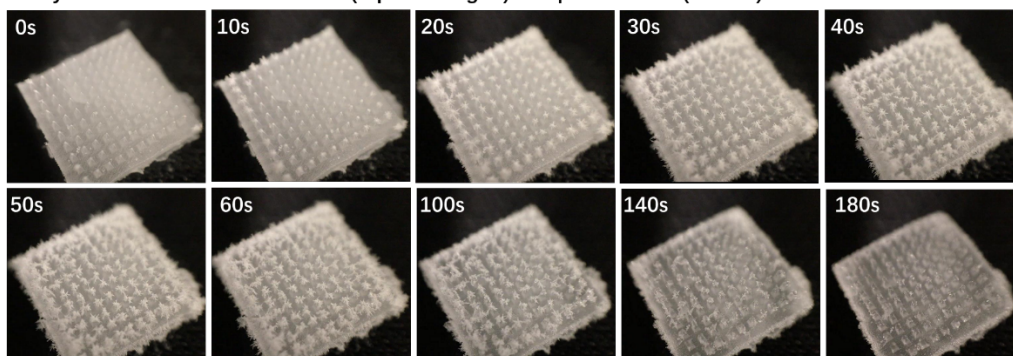


Supplementary Figure 6. Cell volume and loading capacity of cryoMNs. **a**, Volume of different types of cells calculated from the fluorescent images in Live/Dead staining assay. Data are presented as mean $\pm$ s.d. ($n = 4$ biologically independent samples). **b**, The maximum loading capacity of the present cryoMNs for different types of cells. Data are presented as mean $\pm$ s.d. ($n = 4$ biologically independent samples).

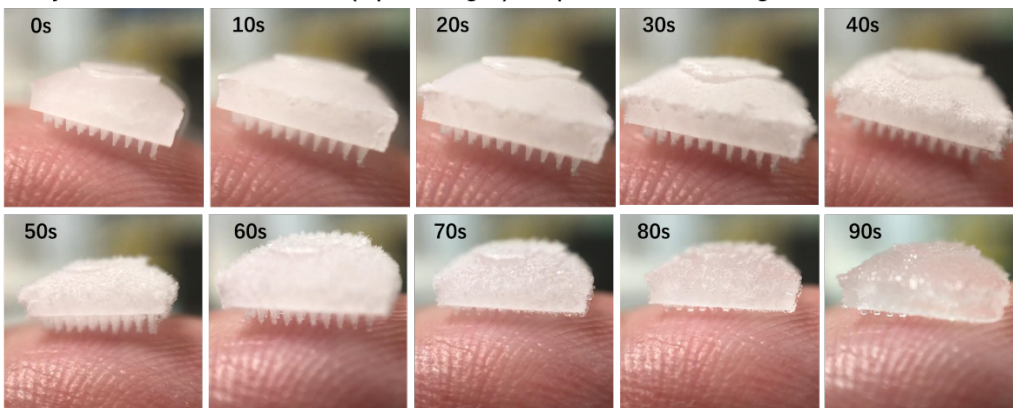


Supplementary Figure 7. Mechanical tests of cryoMNs and conventional polymeric microneedles by Instron 5543 Tensile Meter. **a, b**, Profile of load versus displacement (**a**) and compressive stress versus strain (**b**) of cryoMNs, polystyrene MNs (PS-MNs), polylactic acid MNs (PLA-MNs), polycaprolactone MNs (PCL-MNs) and crosslinked hyaluronic acid MNs (MeHA-CL-MNs). A compressive force in the longitudinal direction of the MNs was generated by a moving transducer at a speed of 0.5 mm/min. **c**, Compressive strength of the cryoMNs made from different formulations of cryogenic medium. Data are presented as mean $\pm$ s.d. ($n = 10$ independent samples). Two-tailed Student's t -test was used for comparison ($**P = 0.0084$ for PBS versus PBS+1D; ns: $P = 0.8691$ for PBS+1D versus PBS+2.5D). Original one-way ANOVA was used for comparison of multiple columns ($****P = 1.02966 \times 10^{-6}$; ns: $P = 0.3798$).

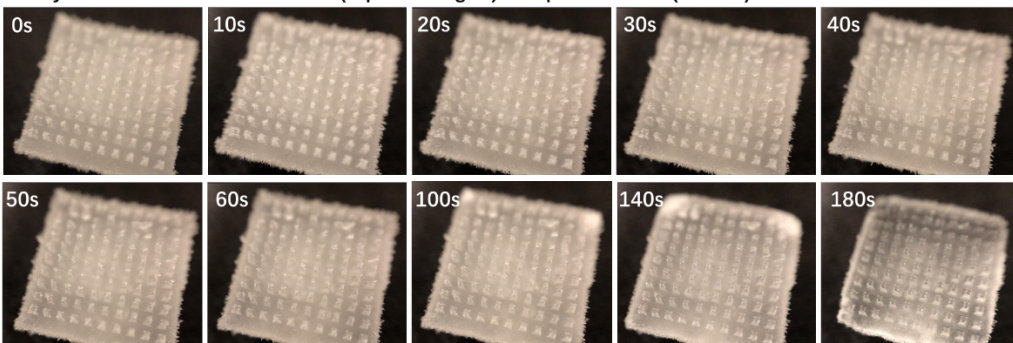
a. CryoMNs removed from -196 °C (liquid nitrogen) and placed at RT (~24 °C)



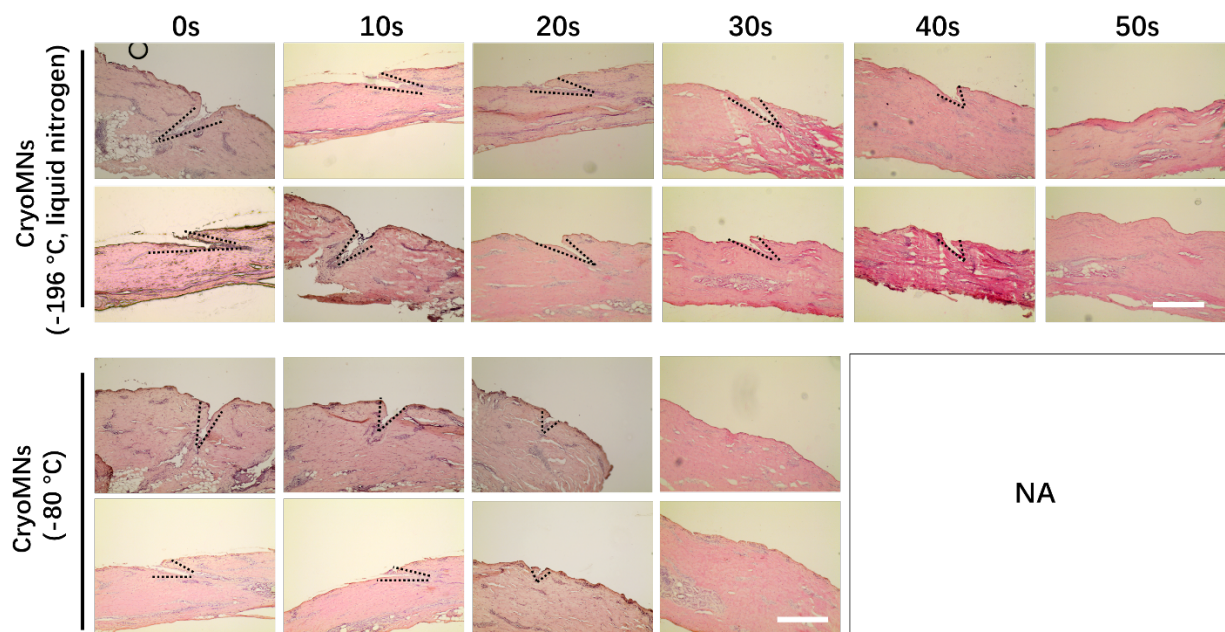
b. CryoMNs removed from -196 °C (liquid nitrogen) and placed on human finger



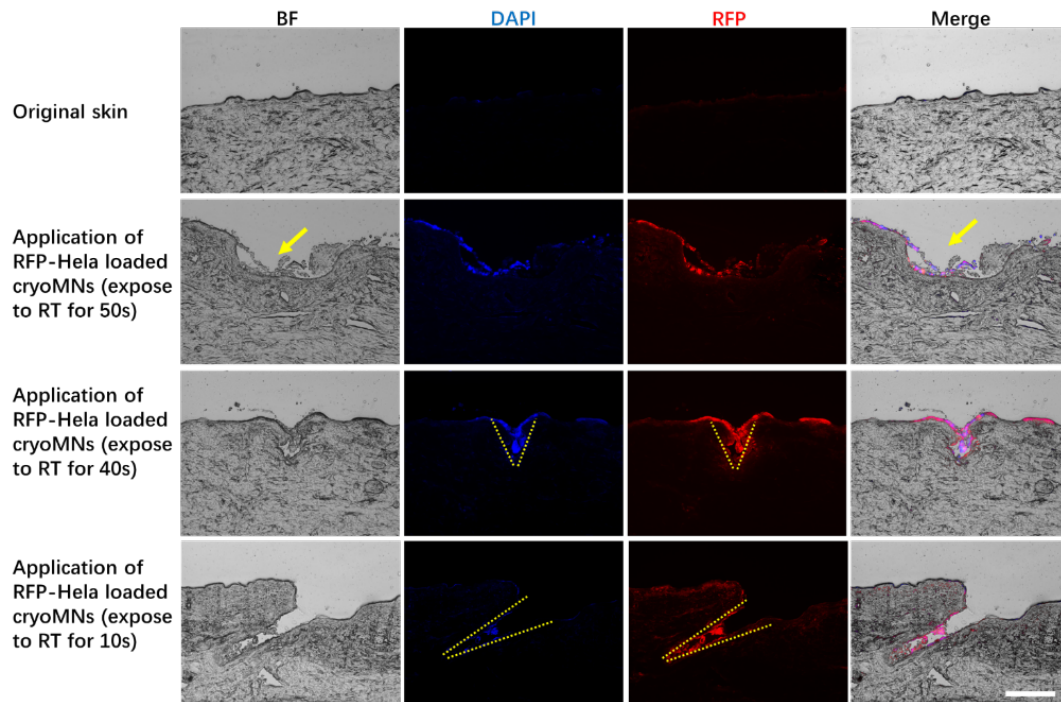
c. CryoMNs removed from -80 °C (liquid nitrogen) and placed at RT (~24 °C)



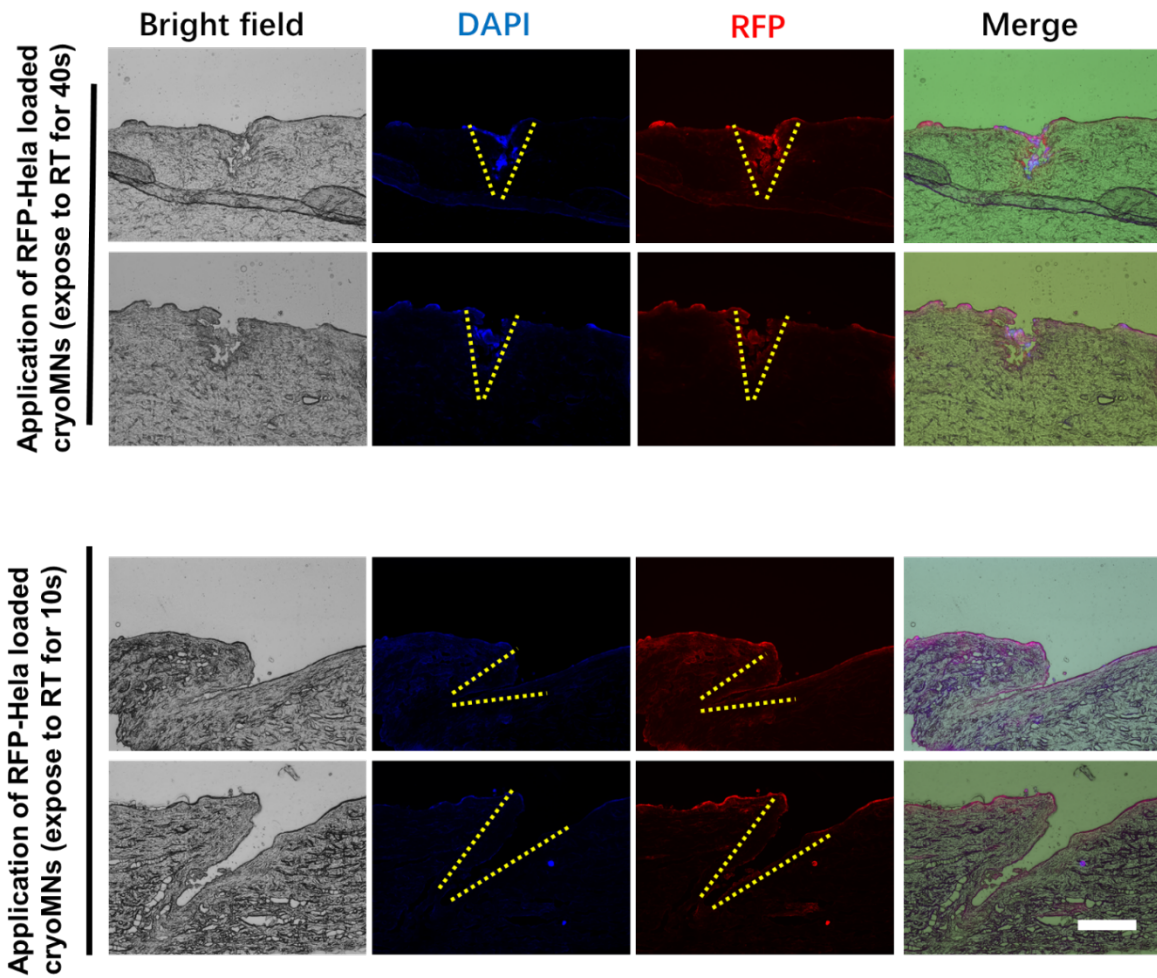
Supplementary Figure 8. Thawing behavior of cryoMNs. Thawing behavior of CryoMNs (-196 °C liquid nitrogen) after being exposed to room temperature (24°C) **(a)** and placed on human finger **(b)**. **c**, Thawing behavior of CryoMNs (-80°C) after being exposed to room temperature (24°C).



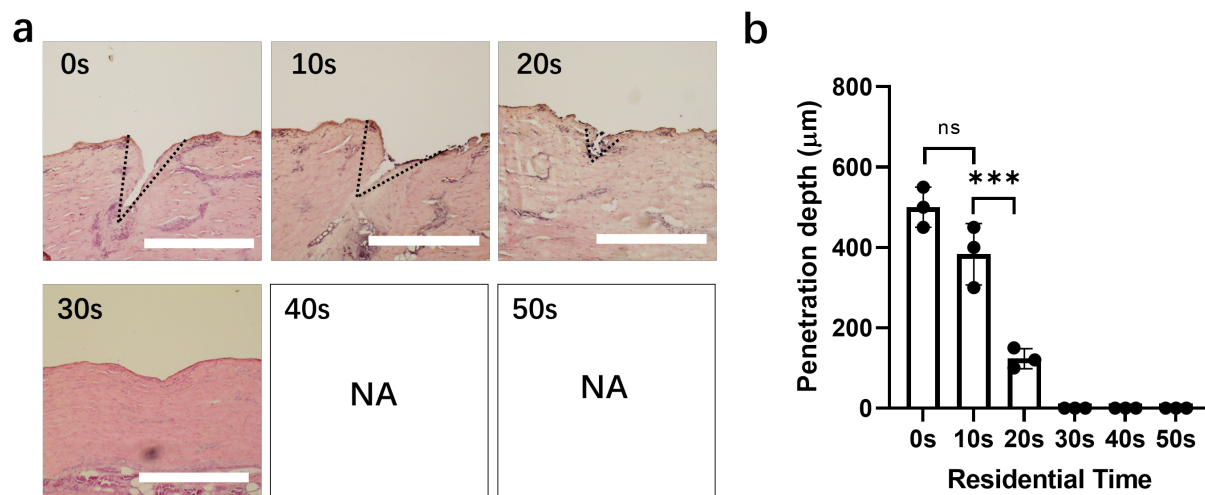
Supplementary Figure 9. Additional H&E staining images of porcine skin inserted by cryoMNs. The cryoMNs were removed from their cryopreservation environment (-196 °C, liquid nitrogen or -80°C) and placed at RT (~24 °C) for 0, 10, 20, 30, 40, and 50 s before skin penetration. Scale bar, 500 μ m. All experiments were independently repeated two times with similar results.



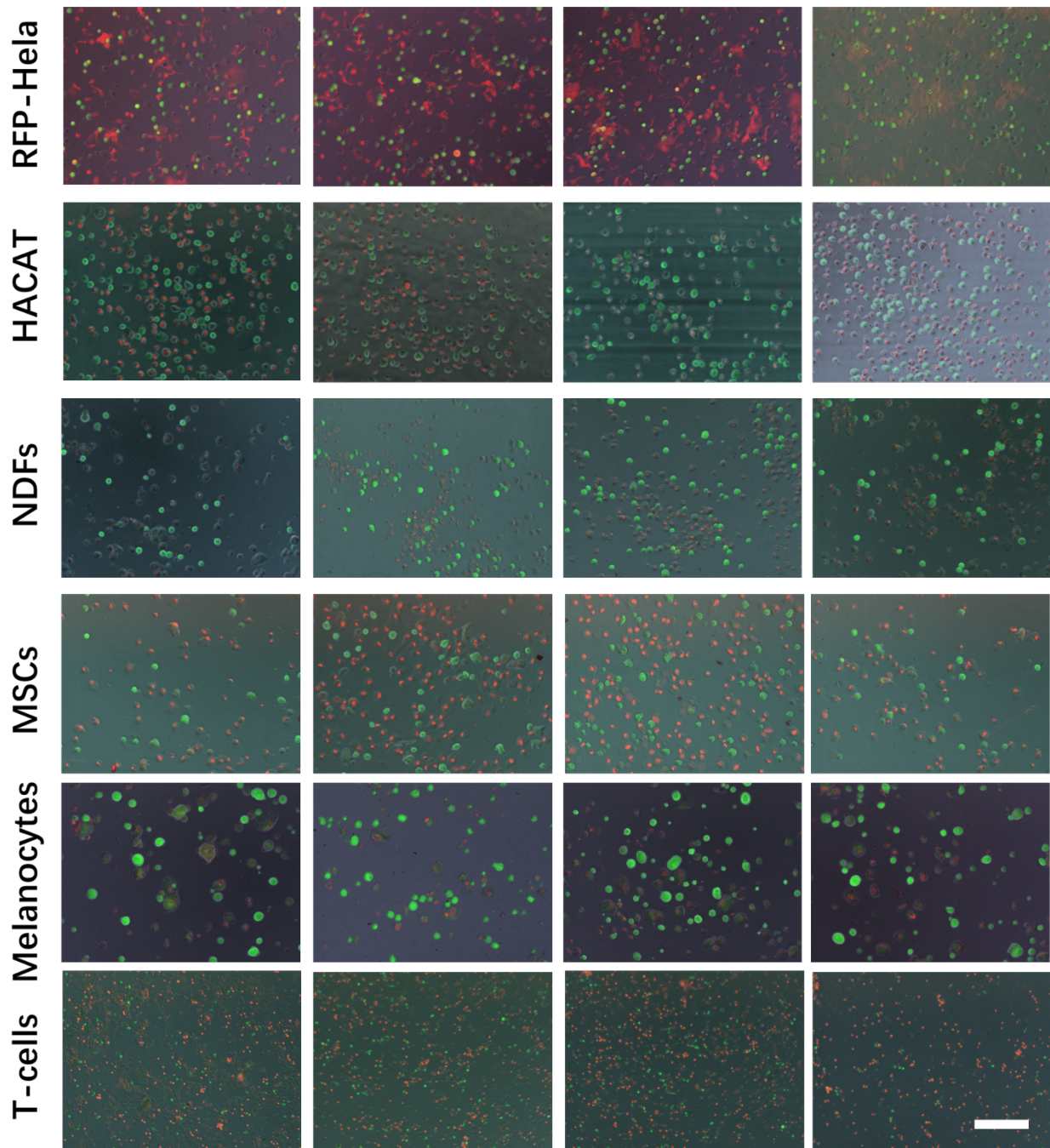
Supplementary Figure 10. Fluorescent images of cryosection of original porcine skin, porcine skin applied with RFP-Hela loaded cryoMNs (-196 °C, liquid nitrogen) after different exposure time under room temperature. The needle shape was outlined with dash line. The arrow refers to the cells which were left on the surface of skin due to the failure penetration of cryoMNs. Scale bar, 200 μ m. 2 images were taken for each group. All experiment were repeated independently for two times with similar results.



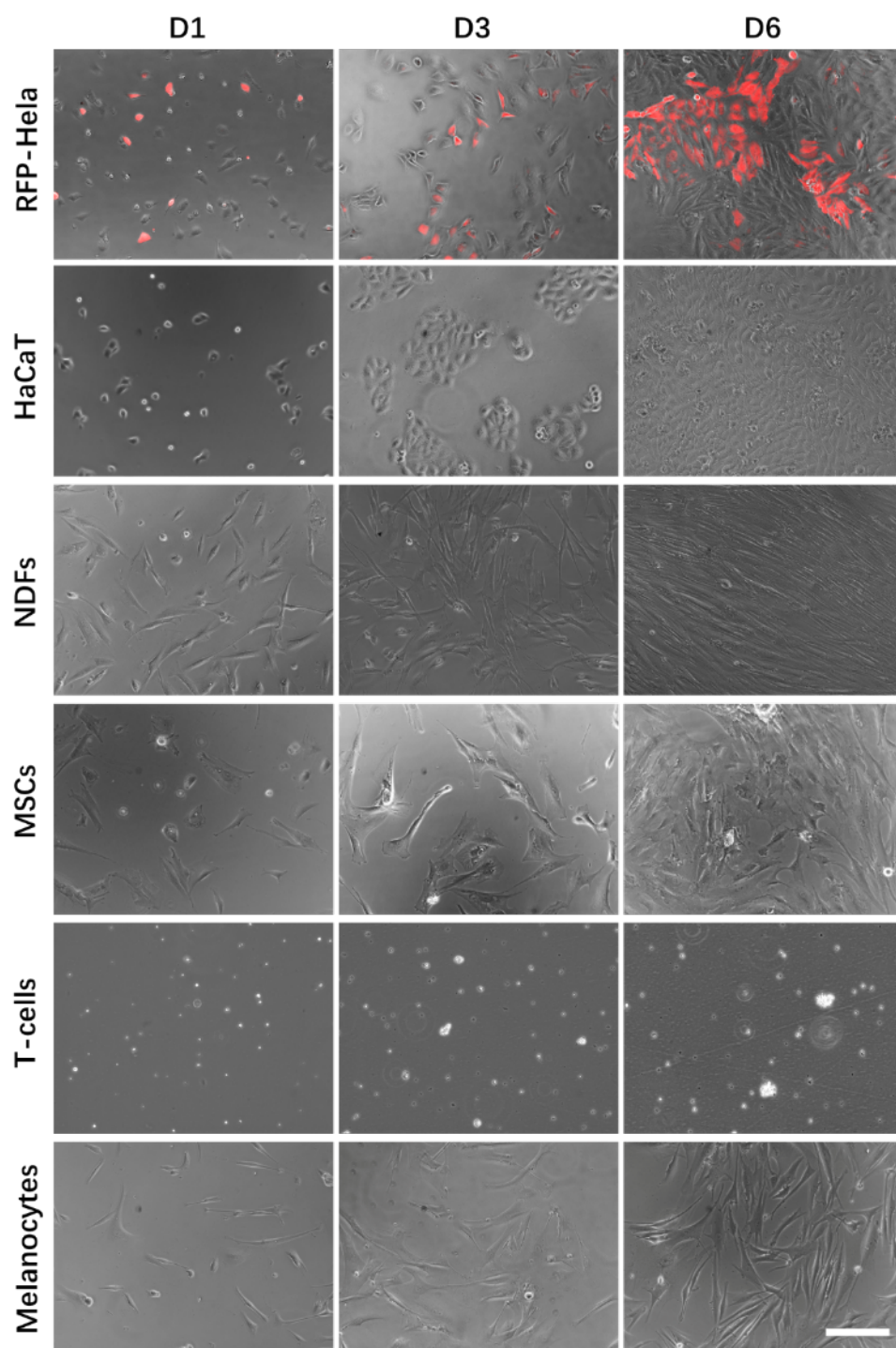
Supplementary Figure 11. Additional fluorescent images of cryosection of original porcine skin, porcine skin applied with RFP-Hela loaded cryoMNs (-196 °C, liquid nitrogen) after different exposure 10s and 40s under room temperature. Scale bar, 200µm. All experiment were repeated independently for two times with similar results.



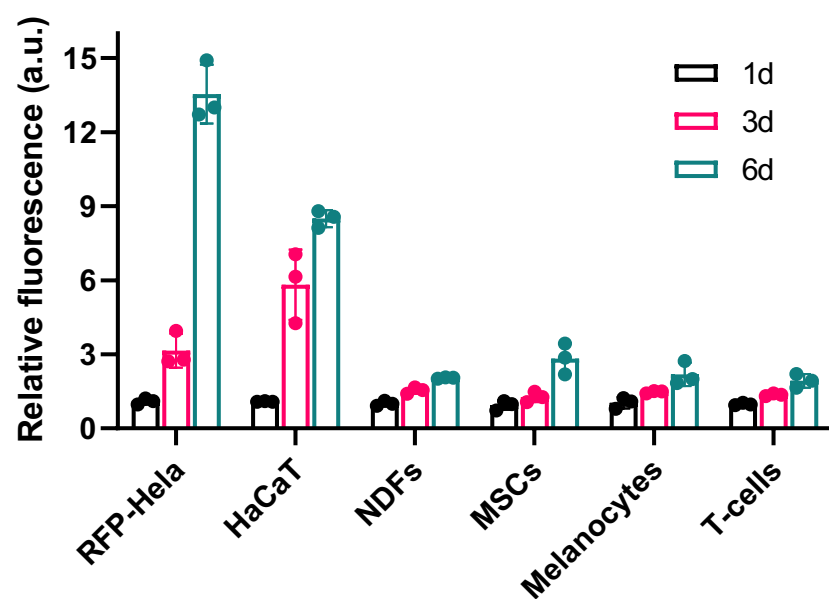
Supplementary Figure 12. Characterization of skin penetration ability of cryoMNs which were taken out from -80 °C freezer. **a**, Representative H&E staining images of porcine skin inserted by cryoMNs. The cryoMNs were removed from their cryopreservation environment (-80 °C) and placed at RT (~24 °C) for 0, 10, 20, 30, 40, and 50 s before skin penetration. Scale bar, 500 μm. 3 images were taken for each group. All experiments were repeated independently for two times with similar results. **b**, Quantitative data of the influence of residence time on skin penetration depth. Data are presented as mean ± s.d. (n = 3 independent samples). Two-tailed Student's *t*-test was used to for comparing penetration depth after different residence time. (ns: *P* = 0.0913 for 0s versus 10s; ****P* = 0.0003 for 10s versus 20s).



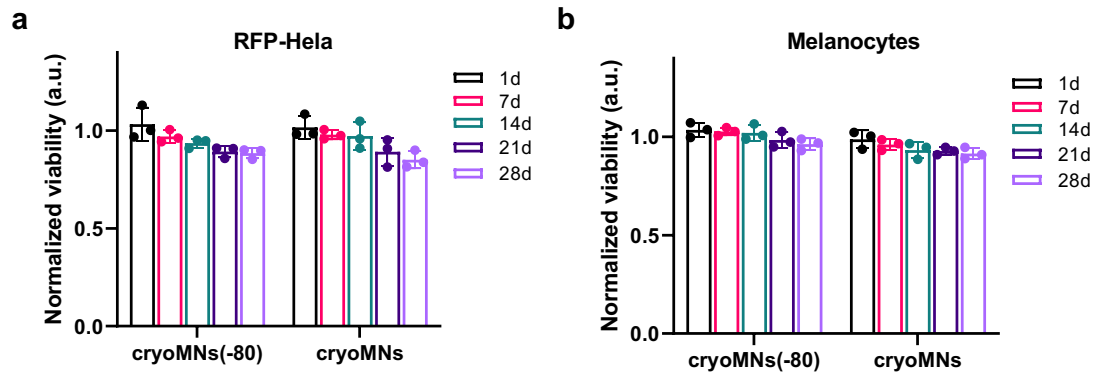
Supplementary Figure 13. Additional live (green)/dead (red) staining images of the cells loaded in cryoMNs. Green channel, red channel and bright field were merged together. Scale bar, 200 μ m. All experiments were repeated independently for three times with similar results.



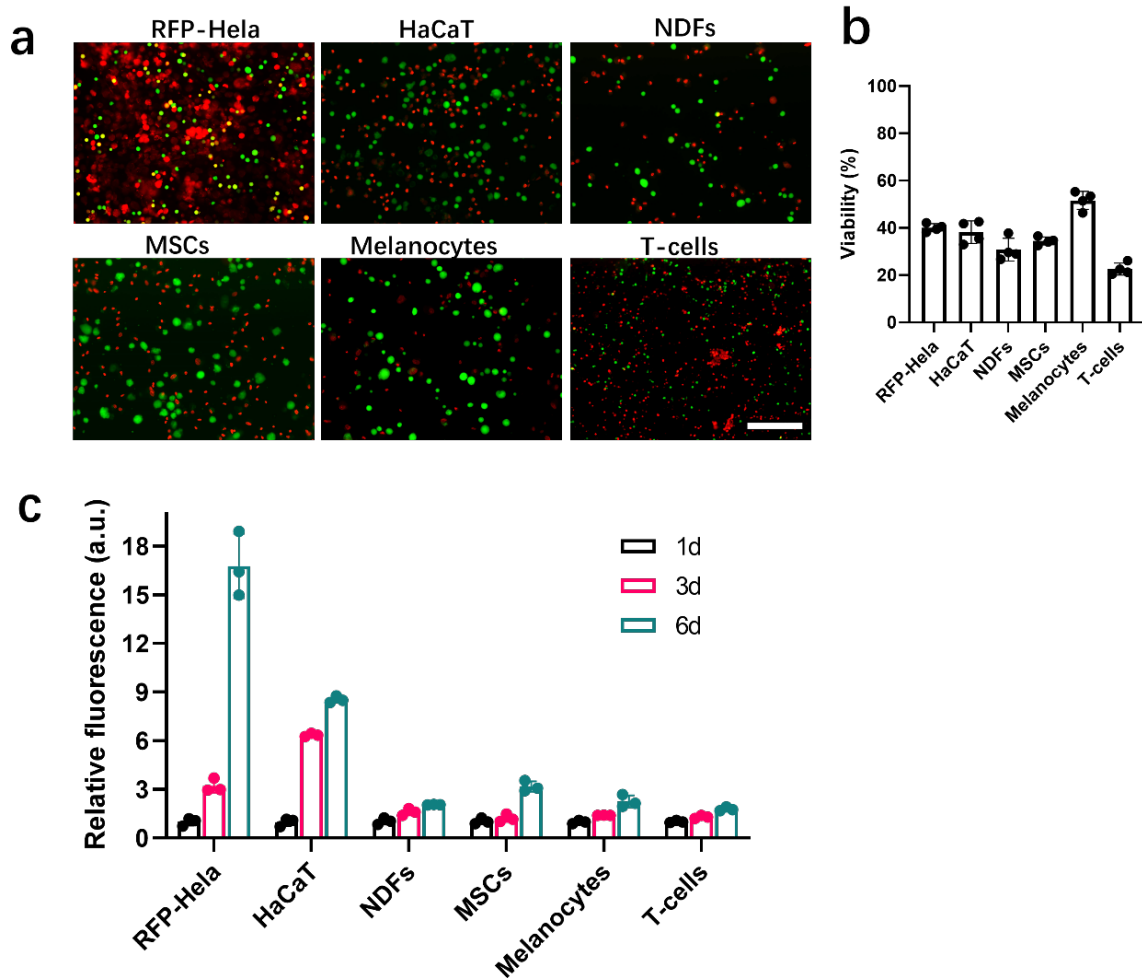
Supplementary Figure 14. Representative images of cell proliferation after being released from cryoMNs (-196 °C, liquid nitrogen). Scale bar, 200µm. 3 images were taken for each group. All experiment were repeated independently for three times with similar results.



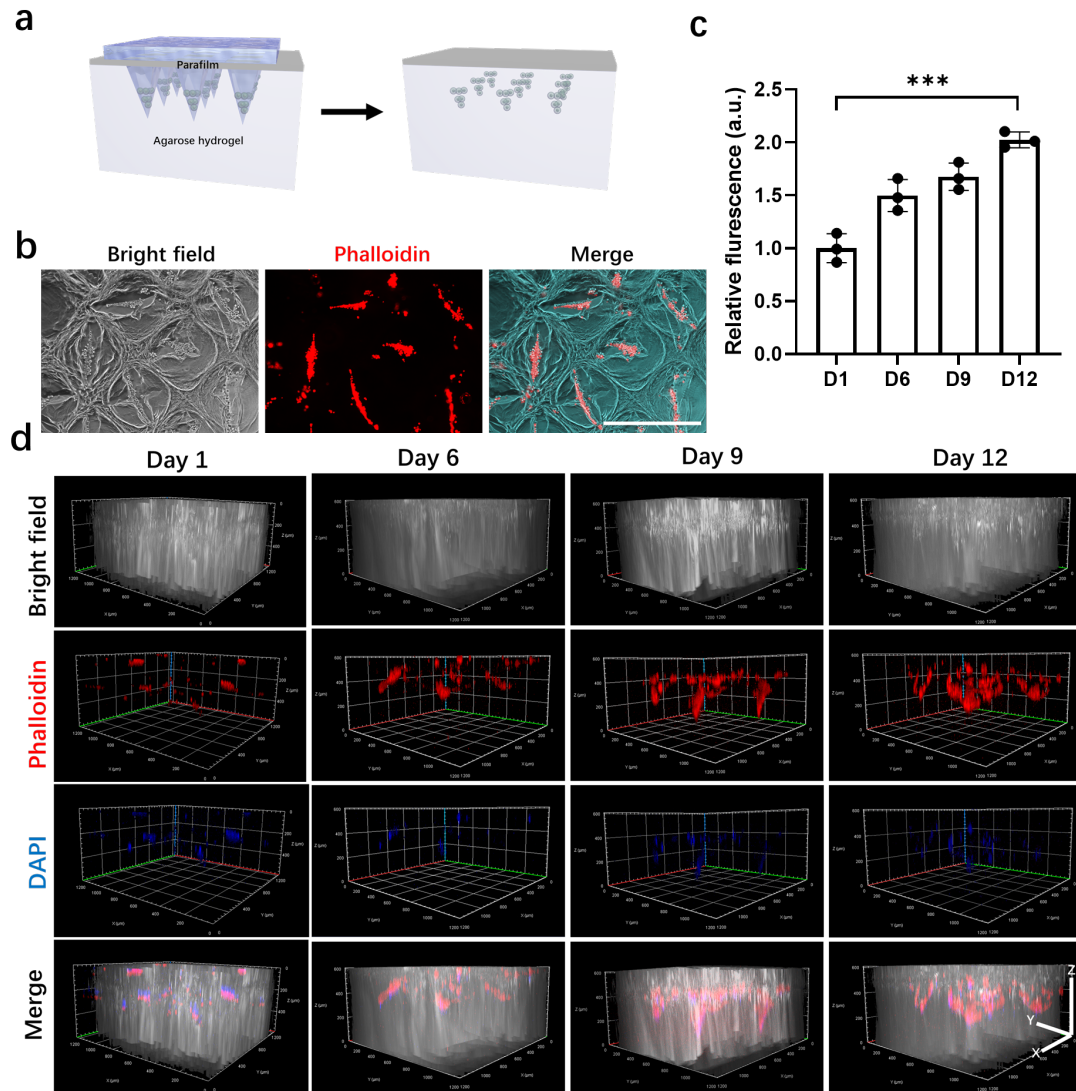
Supplementary Figure 15. Proliferation of the cells without treatment and cultured for 1, 3 and 6 days. Data were obtained by using the alamarBlue™ assay and the fluorescent intensity on the 1st day was used as reference. Data are presented as mean $\pm$ s.d. (n = 3 biologically independent samples)



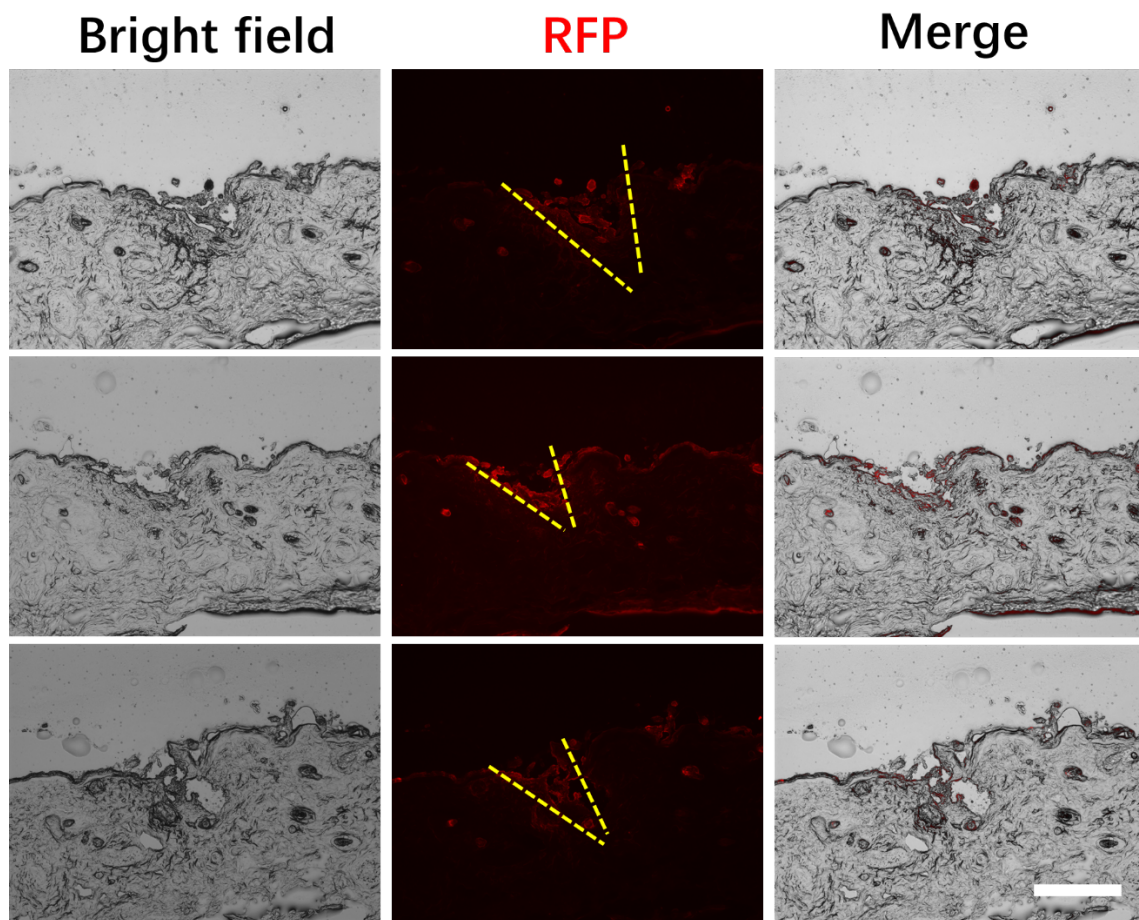
Supplementary Figure 16. Normalized viability of cells in cryoMNs (from either -80 °C or -196°C) after long-term storage. **a)** is for RFP-Hela **b)** is for melanocytes. Data were obtained by amaraBlue™ assay. The normalized viability was calculated by normalizing the fluorescence at each time point to the fluorescence at day 0. Data are presented as mean $\pm$ s.d. (n = 3 biologically independent samples).



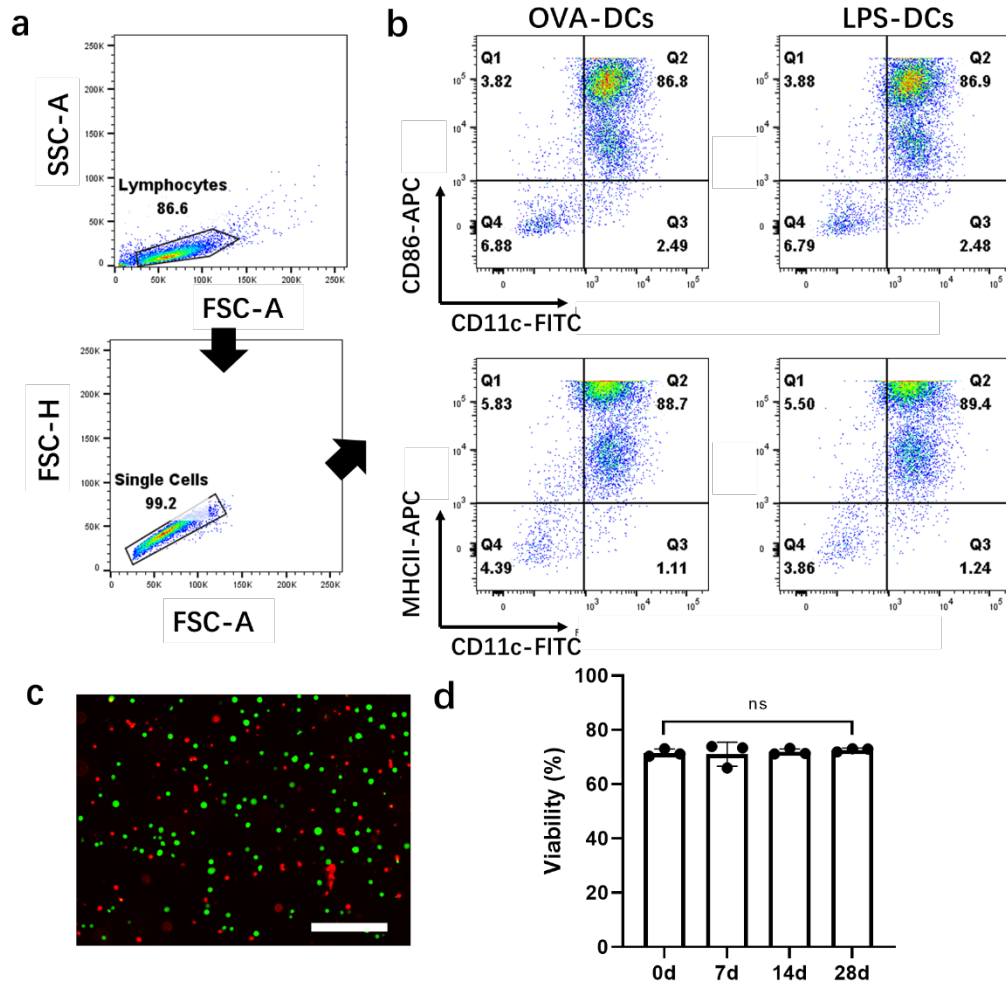
Supplementary Figure 17. Cell viability and proliferation after release from cryoMNs after being taken out from -80 °C. **a**, Live (green)/dead (red) staining images of the cells loaded in cryoMNs. Scale bar, 200 μ m. 4 images were taken for each group and all experiments were repeated independently for three times with similar results. **b**, Quantitative data of cell viability calculated from live (green)/dead (red) staining by dividing number of living cells (green) over the total number of cells. Data are presented as mean $\pm$ s.d. ($n = 4$ biologically independent samples). **c**, Proliferation of the cells after being released from cryoMNs and cultured for 1, 3 and 6 days. Data were obtained by using the alamarBlue™ assay and the fluorescence intensity obtained on the 1st day was used as reference. Data are presented as mean $\pm$ s.d. ($n = 3$ biologically independent samples)



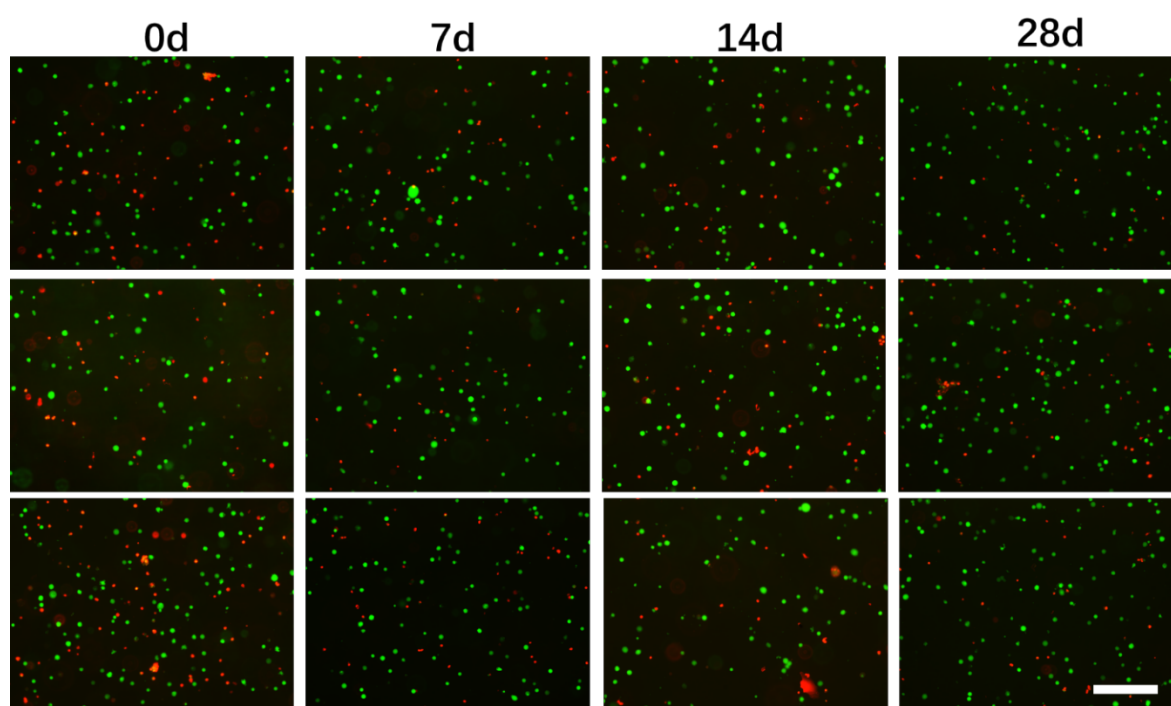
Supplementary Figure 18. Delivery of mesenchymal stem cells (MSCs) in 3D skin model with cryoMNs (-196 °C, liquid nitrogen). **a**, Schematic illustration of MSCs delivered into 3D skin model with cryoMNs. The skin model was established by coating the agarose hydrogel (mimicking dermis with interstitial fluid) with a parafilm layer (representing the water impermeable stratum corneum and epidermis). **b**, Representative Images of top view of the agarose hydrogel after MSCs were delivered inside by cryoMNs. Scale bar, 1000μm. 3 images were taken for each group and all experiments were repeated independently for three times with similar results. **c**, Proliferation of the cells after being released from cryoMNs and cultured in hydrogel for 1, 6, 9 and 12 days. Data were obtained by using the alamarBlue™ assay and the fluorescent intensity on the 1st day was used as reference. Data are presented as mean ± s.d. (n = 3 biologically independent samples). Two-tailed Student's *t*-test was used for comparing relative fluorescence at day 12 with relative fluorescence at day 1 (***P* = 0.0003). **d**, Fluorescent 3D images of MSCs inside skin model after culturing for 1, 6, 9, and 12 days. At each time point, MSCs were stained with phalloidin (red) and DAPI (blue). Scale bar (x, y, z), 500μm.



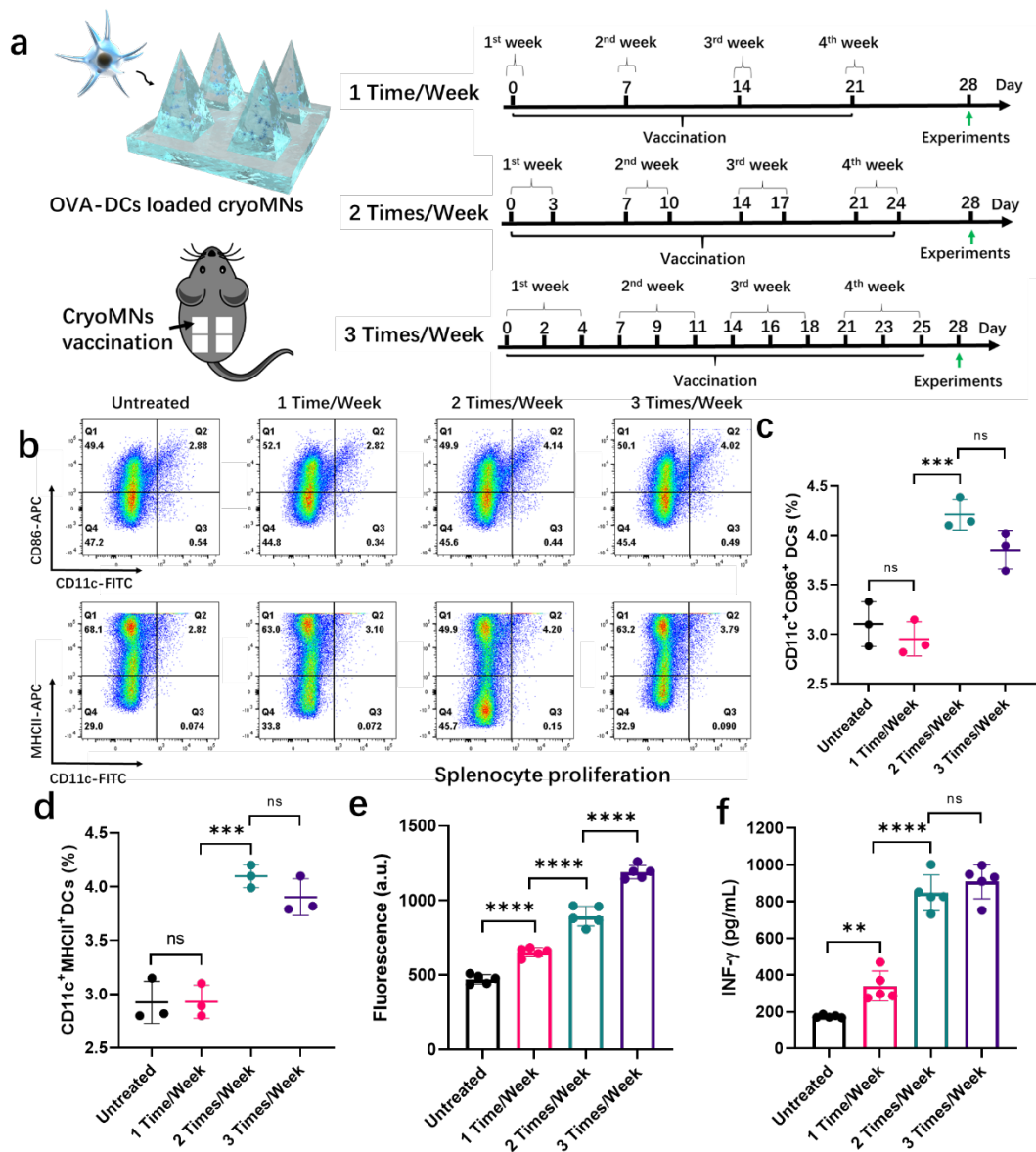
Supplementary Figure 19. Additional fluorescent images of cryosections of mouse skin treated with RFP-HeLa cell-loaded cryoMNs. Skin can induce very light auto-fluorescence. Scale bar, 200 μ m. All experiments were repeated independently for two times with similar results.



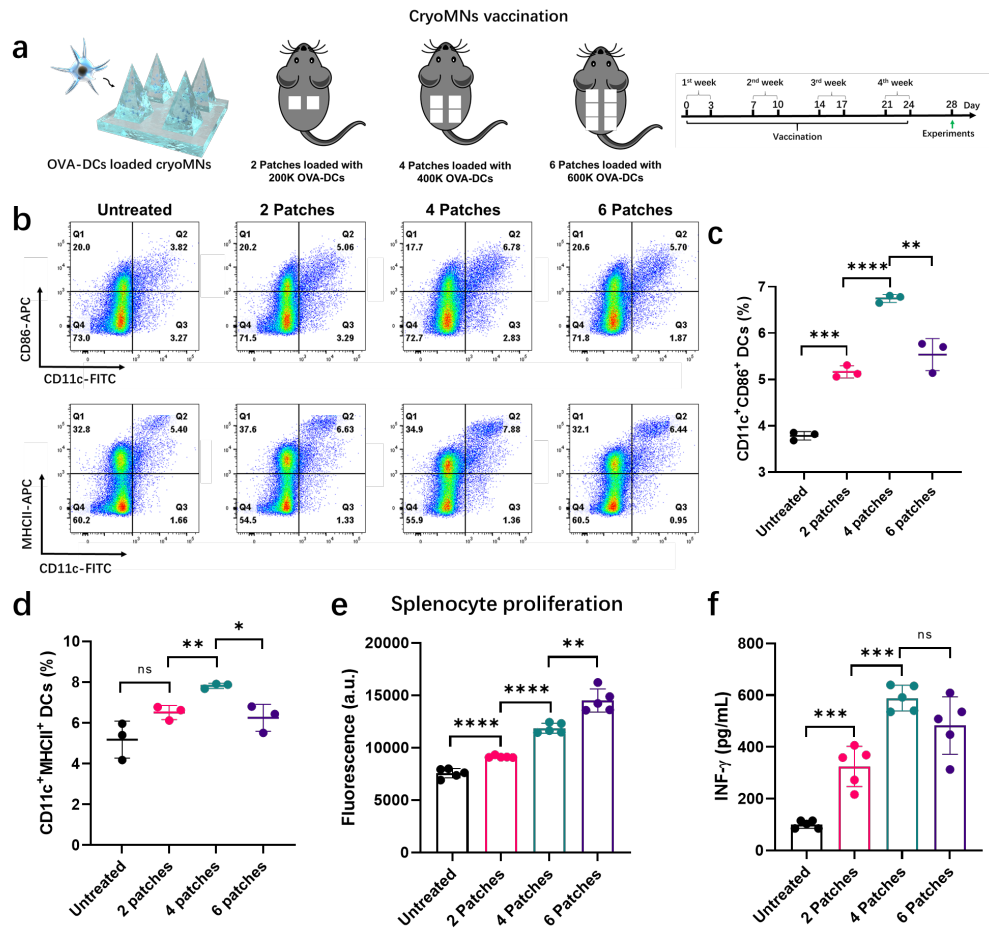
Supplementary Figure 20. Evaluation of DC viability in cryoMNs. **a**, Gating strategy for determining single cell populations for subsequent analysis. **b**, Representative plots of the percentage of CD11c⁺CD86⁺ DCs and CD11c⁺MHCII⁺ DCs after pulsing with OVA and LPS analyzed by the flow cytometry. **c**, Representative live (green)/dead (red) staining images of the OVA-DCs after release from cryoMNs. Scale bar, 200 μ m. 3 images were taken for each group and all experiments were repeated independently for two times with similar results. **d**, Viability of OVA-DCs in cryoMNs after storage in liquid nitrogen for 0, 7, 14, 28 days. Quantitative data were calculated from live (green)/dead (red) staining by dividing number of living cells (green) over the total number of cells. Data are presented as mean $\pm$ s.d. ($n = 3$ biologically independent samples). Two-tailed Student's t -test was used for comparing viability of OVA-DCs in new-fabricated cryoMNs (storage for 0 day) and the viability in cryoMNs after storage for 28 days (ns: $P = 0.2787$)



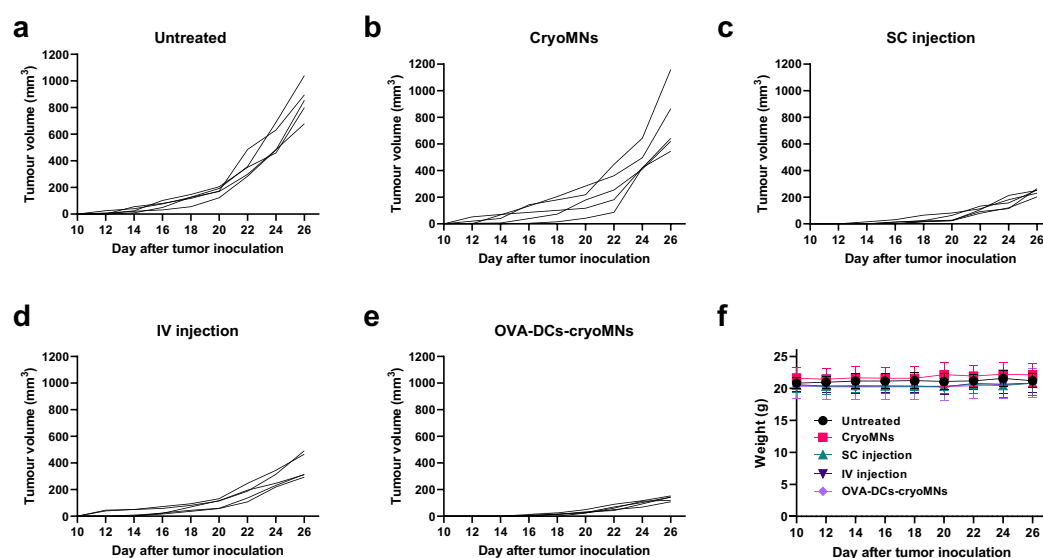
Supplementary Figure 21. Additional live (green)/dead (red) staining images of the OVA-DCs released from cryoMNs after storage in liquid nitrogen for 0, 7, 14 and 28 days. Scale bar, 200 μ m. All experiment were repeated independently for two times with similar results



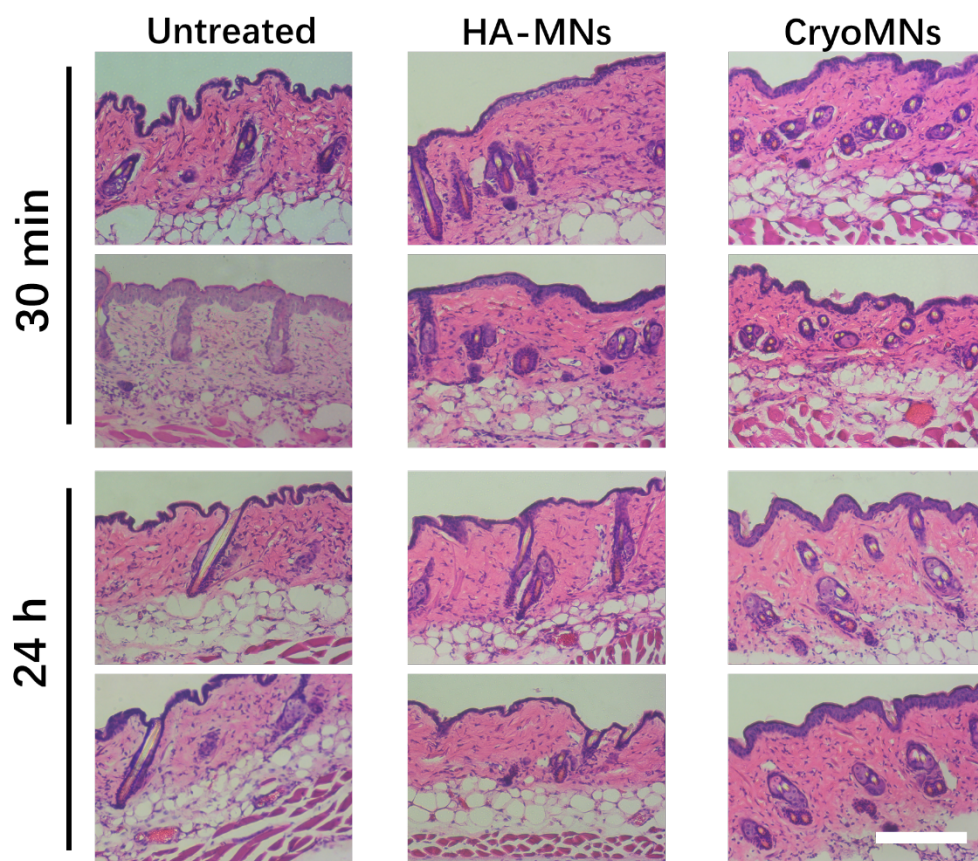
Supplementary Figure 22. Optimization of administration frequency for vaccination with OVA-DCs loaded cryoMNs. **a**, Schematic graph of the administration protocol. **b**, Representative plots of the percentage of CD11c⁺CD86⁺ DCs and CD11c⁺MHCII⁺ DCs in draining lymph nodes (dLNs) of mice in different treatment groups analyzed by the flow cytometry. **c**, **d** Quantification of CD11c⁺CD86⁺ DCs (**c**) and CD11c⁺MHCII⁺ DCs (**d**) in different treatment groups. Data are presented as mean ± s.d. (n = 3 independent animals). **e**, *In vitro* proliferation of extracted splenocytes restimulated with 50 µg/ml antigen (OVA). The fluorescence intensity was obtained by using the alamarBlue™ assay. Data are presented as mean ± s.d. (n = 5 independent animals). **f**, The secretion level of IFN-γ by splenocytes in the culture supernatants. Data are presented as mean ± s.d. (n = 5 independent animals). In **c-f**, two-tailed Student's *t*-test was used for comparing different administration frequency. In **c**, ns: *P* = 0.4126 for Untreated versus 1 Time/Week; ****P* = 0.0007 for 1 Time/Week versus 2 Time/Week; ns: *P* = 0.0688 for 2 Time/Week versus 3 Time/Week. In **d**, ns: *P* = 0.9653 for Untreated versus 1 Time/Week; ****P* = 0.0004 for 1 Time/Week versus 2 Time/Week; ns: *P* = 0.1705 for 2 Time/Week versus 3 Time/Week. In **e**, *****P* = 1.32568 × 10⁻⁵ for Untreated versus 1 Time/Week; *****P* = 8.81395 × 10⁻⁵ for 1 Time/Week versus 2 Time/Week; *****P* = 3.63806 × 10⁻⁵ for 2 Time/Week versus 3 Time/Week. In **f**, ***P* = 0.0020 for Untreated versus 1 Time/Week; *****P* = 1.98038 × 10⁻⁵ for 1 Time/Week versus 2 Time/Week; ns: *P* = 0.3467 for 2 Time/Week versus 3 Time/Week.



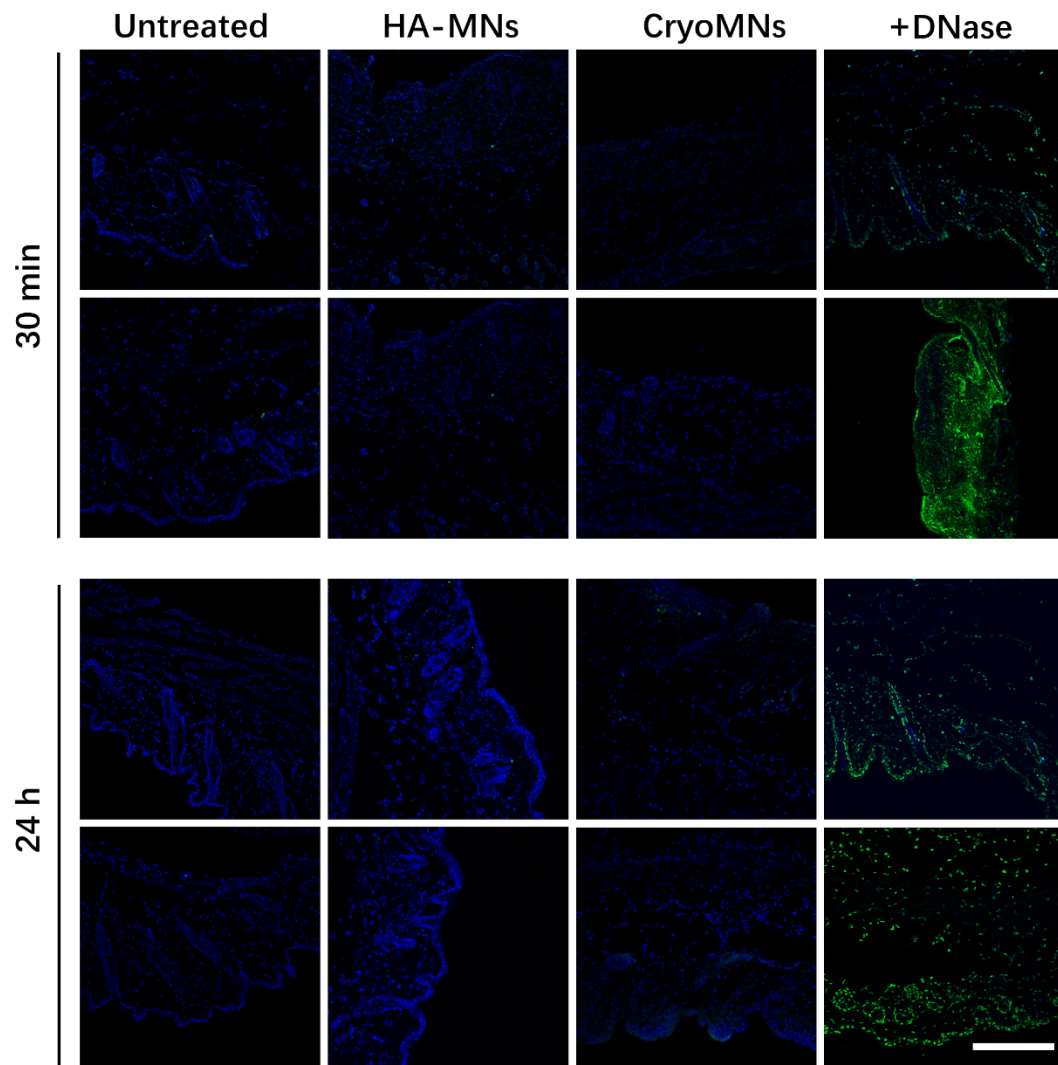
Supplementary Figure 23. Optimization of administration dosage for vaccination with OVA-DCs loaded cryoMNs. One patch of cryoMNs loaded with 100K OVA-DCs **a**, Schematic illustration of the administration protocol and dosage used for vaccination. **b**, Representative plots of the percentage of CD11c⁺CD86⁺ DCs and CD11c⁺MHCII⁺ DCs in draining lymph nodes (dLNs) of mice in different treatment groups analyzed by the flow cytometry. **c**, **d** Quantification of CD11c⁺CD86⁺ DCs (**c**) and CD11c⁺MHCII⁺ DCs (**d**) in different treatment groups. Data are presented as mean $\pm$ s.d. ($n = 3$ independent animals). **e**, *In vitro* proliferation of extracted splenocytes restimulated with 50 μ g/ml antigen (OVA). The fluorescence intensity was obtained by using the alamarBlueTM assay. Data are presented as mean $\pm$ s.d. ($n = 5$ independent animals). **f**, The secretion level of INF- γ by splenocytes in the culture supernatants. Data are presented as mean $\pm$ s.d. ($n = 5$ independent animals). In **c-f**, two-tailed Student's *t*-test was used for comparing different dosage. In **c**, $^*P = 0.0001$ for Untreated versus 2 Patches; $^{****}P = 6.12084 \times 10^{-5}$ for 2 Patches versus 4 Patches; $^{**}P = 0.0041$ for 4 Patches versus 6 Patches. In **d**, ns: $P = 0.0772$ for Untreated versus 2 Patches; $^{**}P = 0.0035$ for 2 Patches versus 4 Patches; $^*P = 0.0158$ for 4 Patches versus 6 Patches. In **e**, $^{****}P = 5.90603 \times 10^{-5}$ for Untreated versus 2 Patches; $^{****}P = 1.56978 \times 10^{-6}$ for 2 Patches versus 4 Patches; $^{**}P = 0.0011$ for 4 Patches versus 6 Patches. In **f**, $^{***}P = 0.0002$ for Untreated versus 2 Patches; $^{**}P = 0.0002$ for 2 Patches versus 4 Patches; ns: $P = 0.0879$ for 4 Patches versus 6 Patches



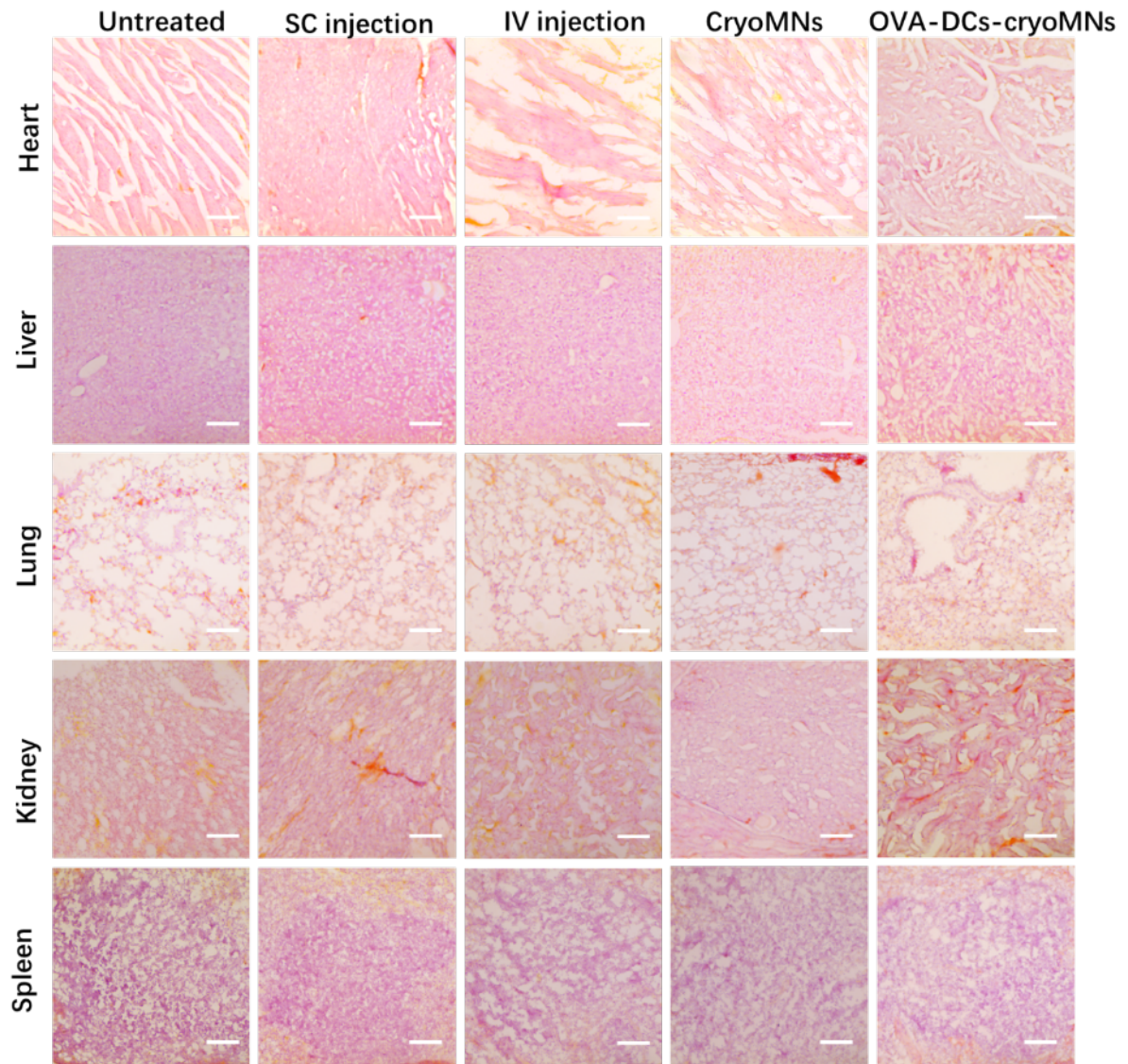
Supplementary Figure 24 Tumour and weight monitoring. Individual tumour-growth curves of mice without treatment (a), with vaccination of cryoMNs (b), subcutaneous (SC) injection of OVA-DCs (c), intravenous (IV) injection of OVA-DCs (d) and OVA-DCs-cryoMNs (e). f, Weight of mouse in different treatment group after tumour inoculation. Data are presented as mean $\pm$ s.d. (n = 5 independent animals)



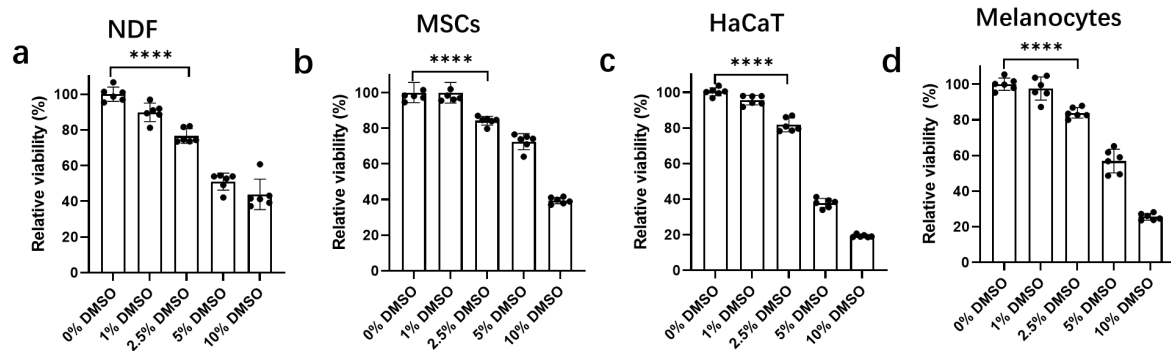
Supplementary Figure 25. Additional images of H&E staining of skins 30 min or 24 hours after MN application. Scale bar: 200 μ m. All experiment were repeated independently for two times with similar results.



Supplementary Figure 26. Additional images of skins stained with TUNEL assay (green) and Hoechst (blue) after MN applications. Skin samples treated with DNase served as a positive control. Scale bar: 200 μm . All experiments were repeated independently for two times with similar results.



Supplementary Figure 27. Systemic toxicity of OVA-DCs vaccination via different methods, including subcutaneous (SC) injection, intravenous (IV) injection and OVA-DCs loaded cryoMNs (OVA-DCs-cryoMNs). H&E analysis for mouse major organs after vaccination. Scale bar, 100 μ m. 3 images were taken for each group and all experiments were repeated independently for two times with similar results.



Supplementary Figure 28. Influence of DMSO on cell viability. Relative viability of fibroblasts (NDF) (**a**), MSCs (**b**), HaCaT (**c**), and melanocytes (**d**) after culturing in the media supplemented with different concentration of DMSO for 24 h. The data were obtained from alamaBlue™ viability assay. The value was calculated by dividing fluorescence intensity of groups with different concentration of DMSO from fluorescence intensity of control group (0% DMSO) and presented as percentage (%). Data are presented as mean $\pm$ s.d. ($n = 6$ biologically independent samples). In **a-d**, two-tailed Student's t -test was used for comparing viability in 2.5% DMSO media with 0% DMSO media. In **a**, **** $P = 1.58955 \times 10^{-6}$; In **b**, **** $P = 8.65873 \times 10^{-6}$. In **c**, **** $P = 1.32532 \times 10^{-6}$ In **d**, **** $P = 5.52692 \times 10^{-6}$.

3. Supplementary Movies (S1 – S4)

Movie S1. Establishment of mechanical test of cryoMNs

Movie S2. Thawing behavior of CryoMNs removed from -196 °C (liquid nitrogen) and placed at RT (~24 °C)

Movie S3. Thawing behavior of CryoMNs removed from -196 °C (liquid nitrogen) and placed on human figure

Movie S4. Thawing behavior of CryoMNs removed from -80 °C and placed at RT (~24 °C).