Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPLEMENTARY INFORMATION

Ultrathin SU-8 Membrane for Highly Efficient Tunable Cell Patterning and Massively Parallel Large Biomolecular Delivery

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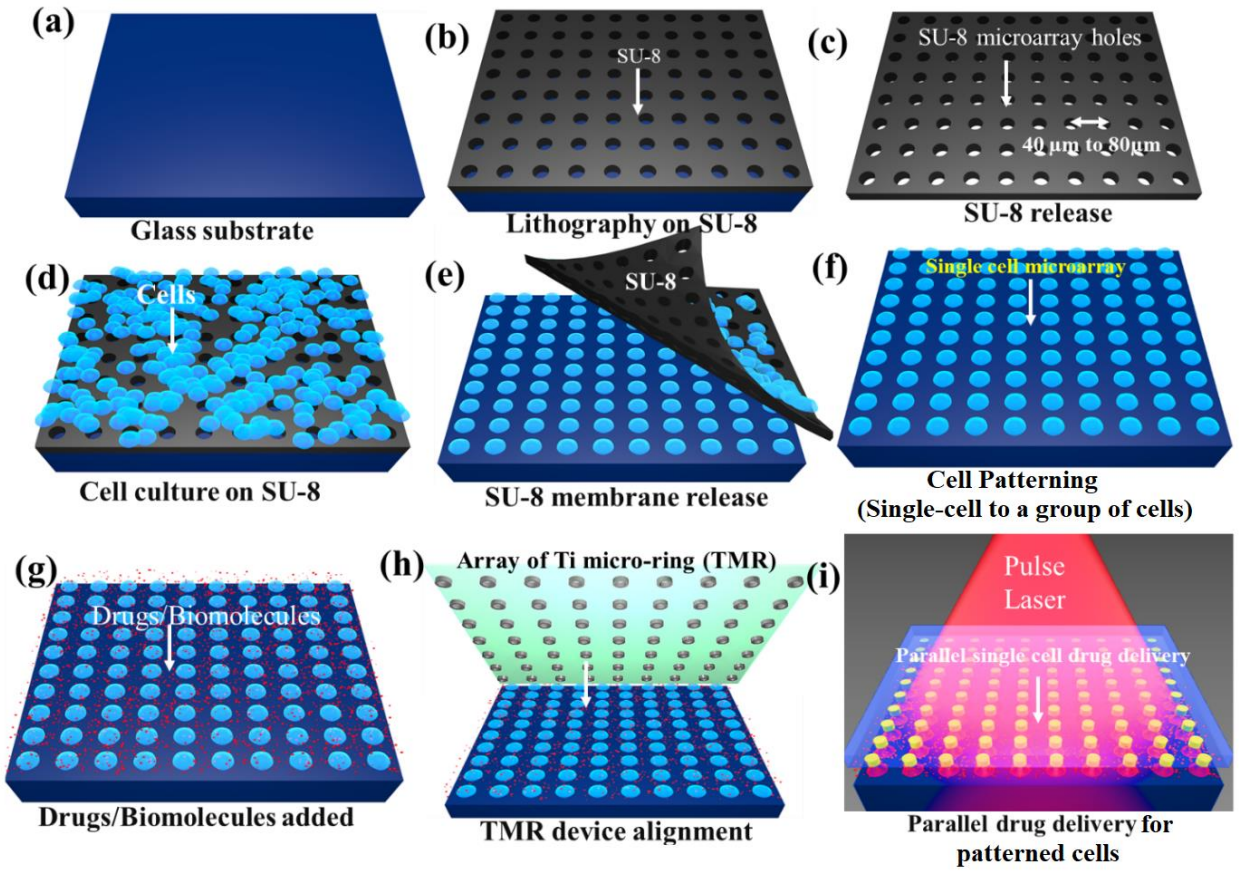

Figure S1. Schematic representation of the use of SU-8 membrane micro stencil for cell patterning and massively parallel intracellular delivery using TMR-assisted photoporation technique. (a) Clean glass substrate; (b) Fabrication of SU-8 membrane on a glass substrate; (c) Release of standalone SU-8 membrane from the glass substrate; (d) Cell culture on top of SU-8 membrane laid on the petri dish; (e) Peeling off of the SU-8 membrane after 24 hours of

cell seeding; (f) Obtain an array of cell pattern (single-cell to a group of cells) on the petri dish; (g) Addition of biomolecules for intracellular delivery; (h) The alignment of TMR device across the cell pattern; (i) Exposure of an IR pulse laser, creating transient membrane pores to deliver biomolecules into patterned cells.

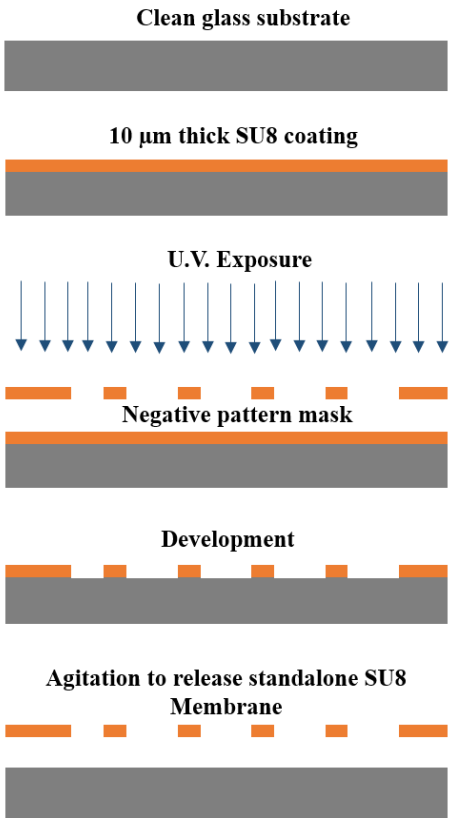

Figure S2. Schematic representation of the step-by-step procedure to fabricate ultrathin SU-8 standalone micro stencil membrane.

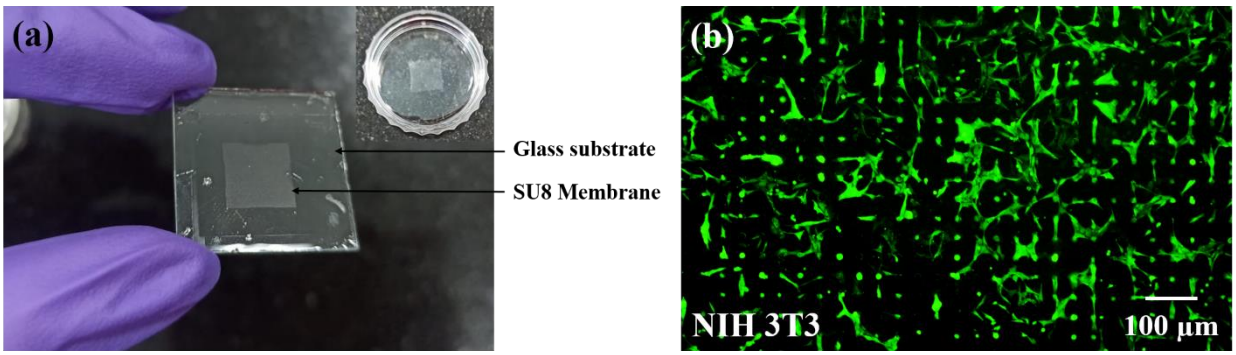

Figure S3. (a) Picture of fabricated standalone SU8 membrane placed on the substrate across a patterning area; (b) Microscopic bright-field image of the NIH3T3 cells seeded on top of SU-8 membrane confirming its biocompatibility.

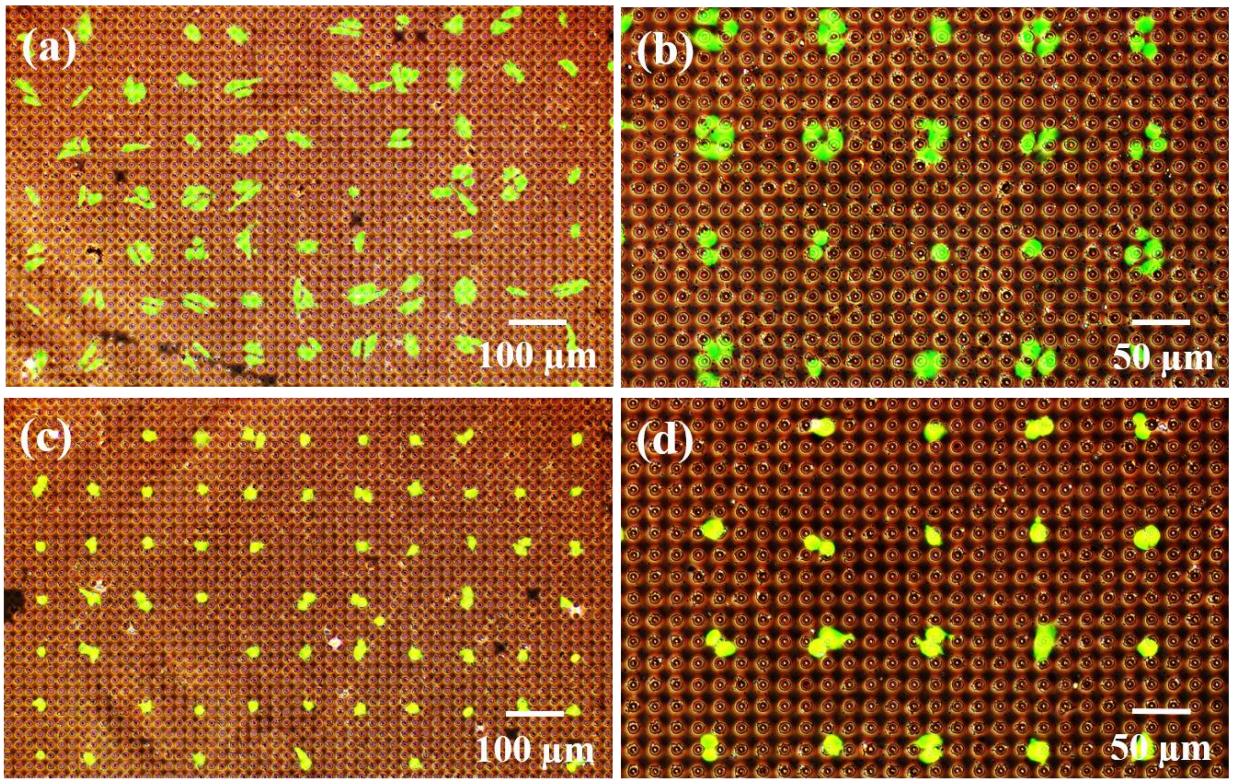

Figure S4. Alignment of TMR device over the patterned cells – (a, b) SiHa cells; (c, d) L929 cells.

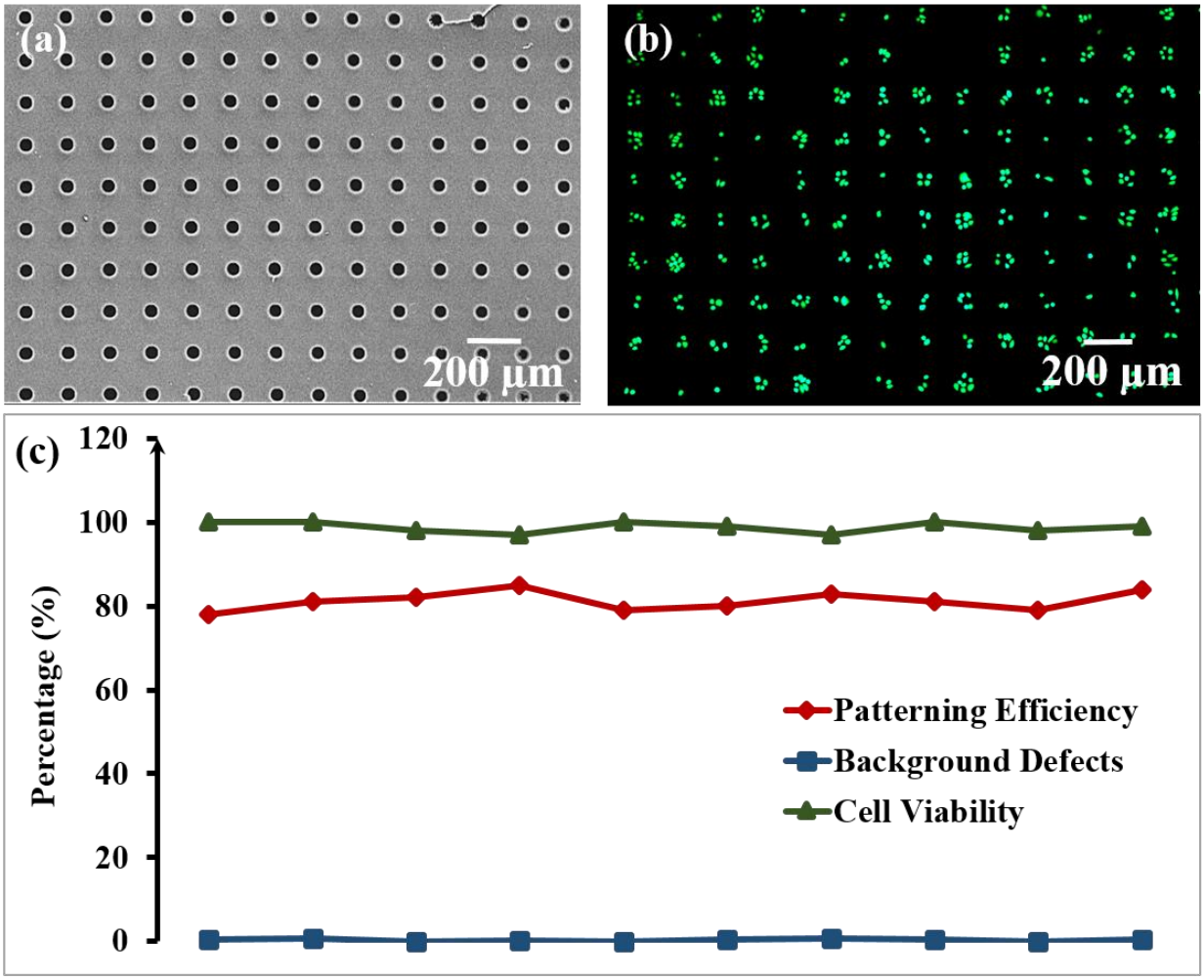

Figure S5. SiHa cells patterning efficiency - (a) SEM image of the final 3D SU-8 standalone membrane kept on a glass substrate showing an array of through-holes. (b) A representative fluorescent microscopic image of SiHa cells stained by Calcein AM after lift-off of SU-8 membrane. (c) Recorded data from 10 randomly selected locations of the SU8 membrane after lift-off to estimate the patterning performance.

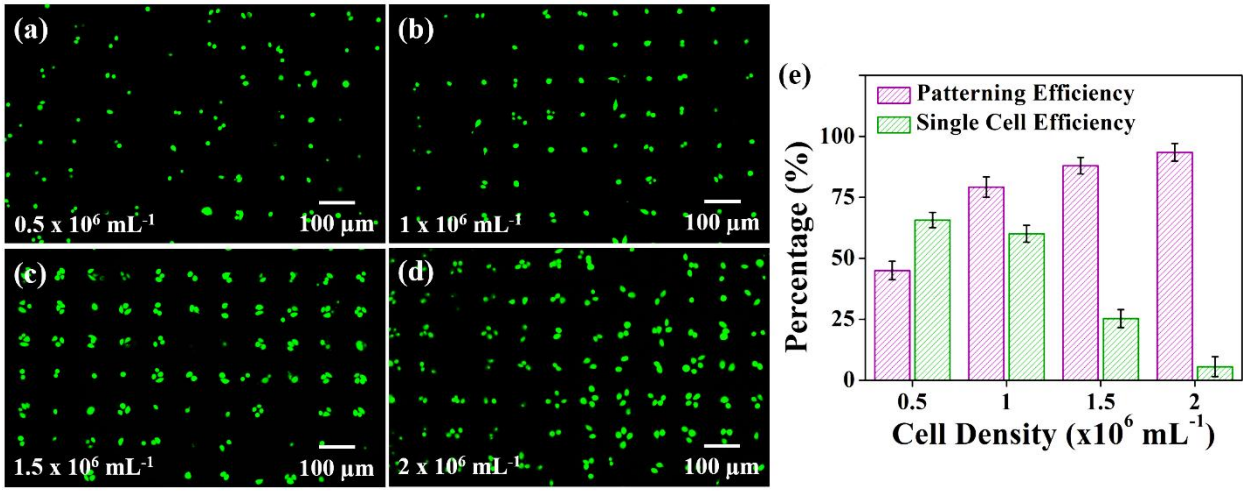

Figure S6. Fluorescent images showing SU-8 membrane-assisted patterning of L929 cells seeded with varying concentration – (a) 0.5 × 10^6 mL^{-1}; (b) 1 × 10^6 mL^{-1}; (c) 1.5 × 10^6 mL^{-1}; (d) 2 × 10^6 mL^{-1}. (e) Graph of cell patterning efficiency and single-cell efficiency using 45 µm SU-8 membrane for L929 cells at various cell seeding concentrations (n=3 replicate).

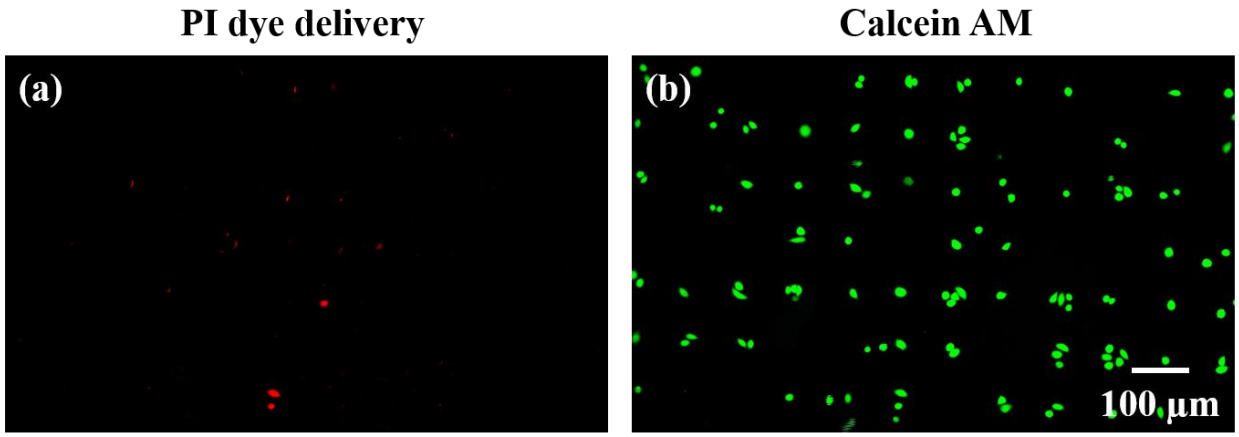

Figure S7. Fluorescent images for SU-8 membrane patterned SiHa cells after the TMR-assisted photoporation (without any media between patterned cells and TMR device) – (a) PI dye delivery; (b) Calcein AM stained.

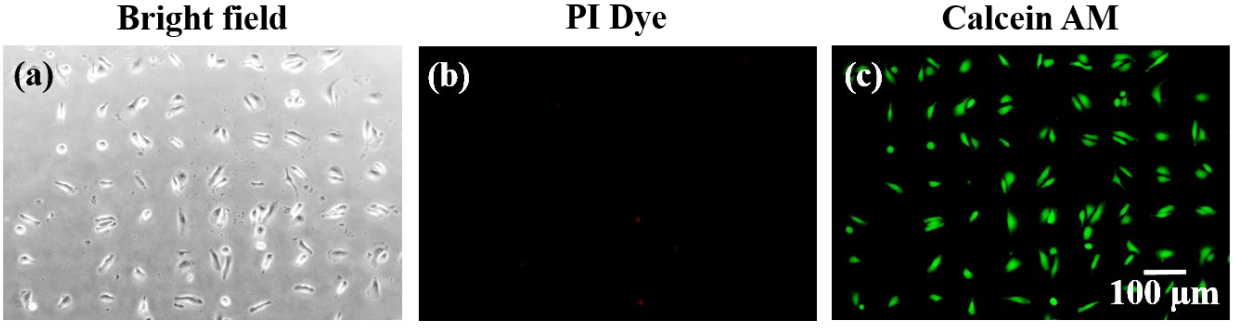

Figure S8. Control experiment on SU-8 membrane-assisted patterned SiHa cells using TMR platform. (a) bright-field image; (b) PI dye staining on the nucleus of dead cells (No laser exposure); (c) cell viability test using calcein-AM confirmed that all cells are live (green color).

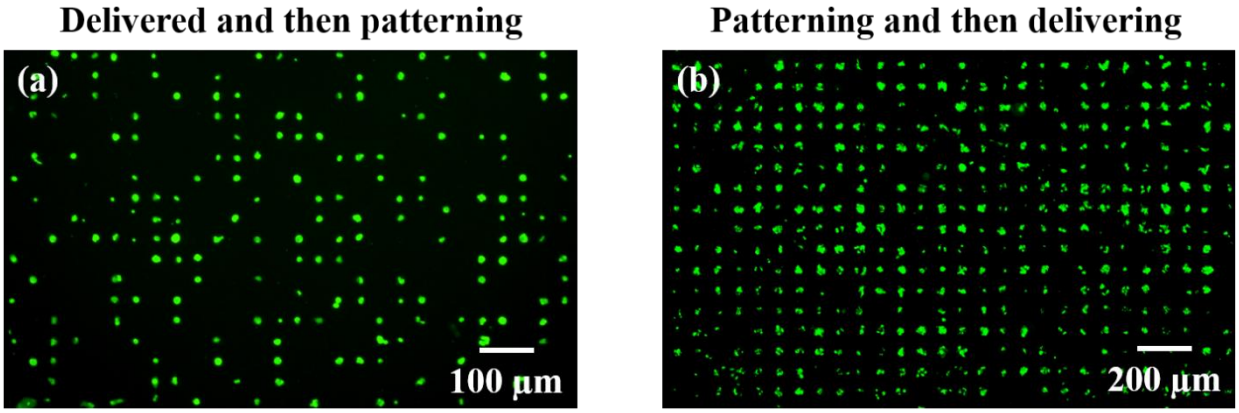

Figure S9. (a) L929 cells (Calcein AM stained) were first delivered with PI dye (668 Da) biomolecule and then patterned using SU-8 membrane 45 µm hole with patterning efficiency ~ 30%. (b) L929 cells (Calcein AM stained) were first patterned with an efficiency of ~ 81% and then delivered with the dextran biomolecule.

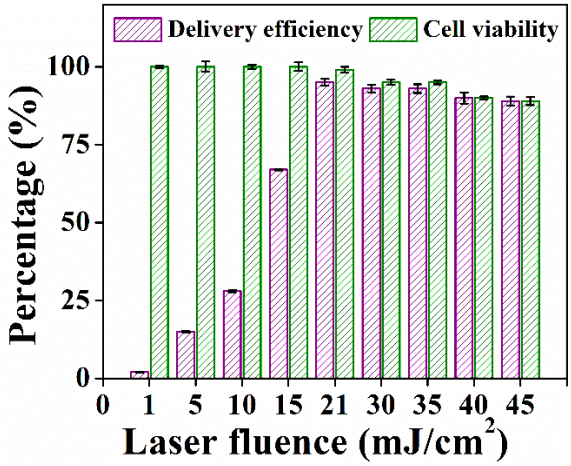

Figure S10. Quantification of PI dye delivery in SiHa cells at various laser fluence (n=3 replicate, data presented as mean ± S.D.).

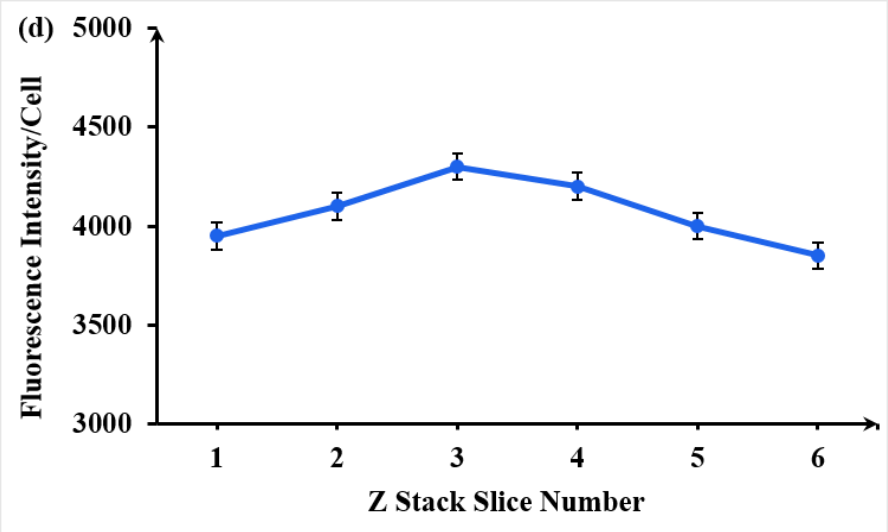

Figure S11. Quantifying fluorescence intensity of merged images on a per-cell basis indicates uniform delivery and cell viability (n=3 replicate, data presented as mean ± S.D).

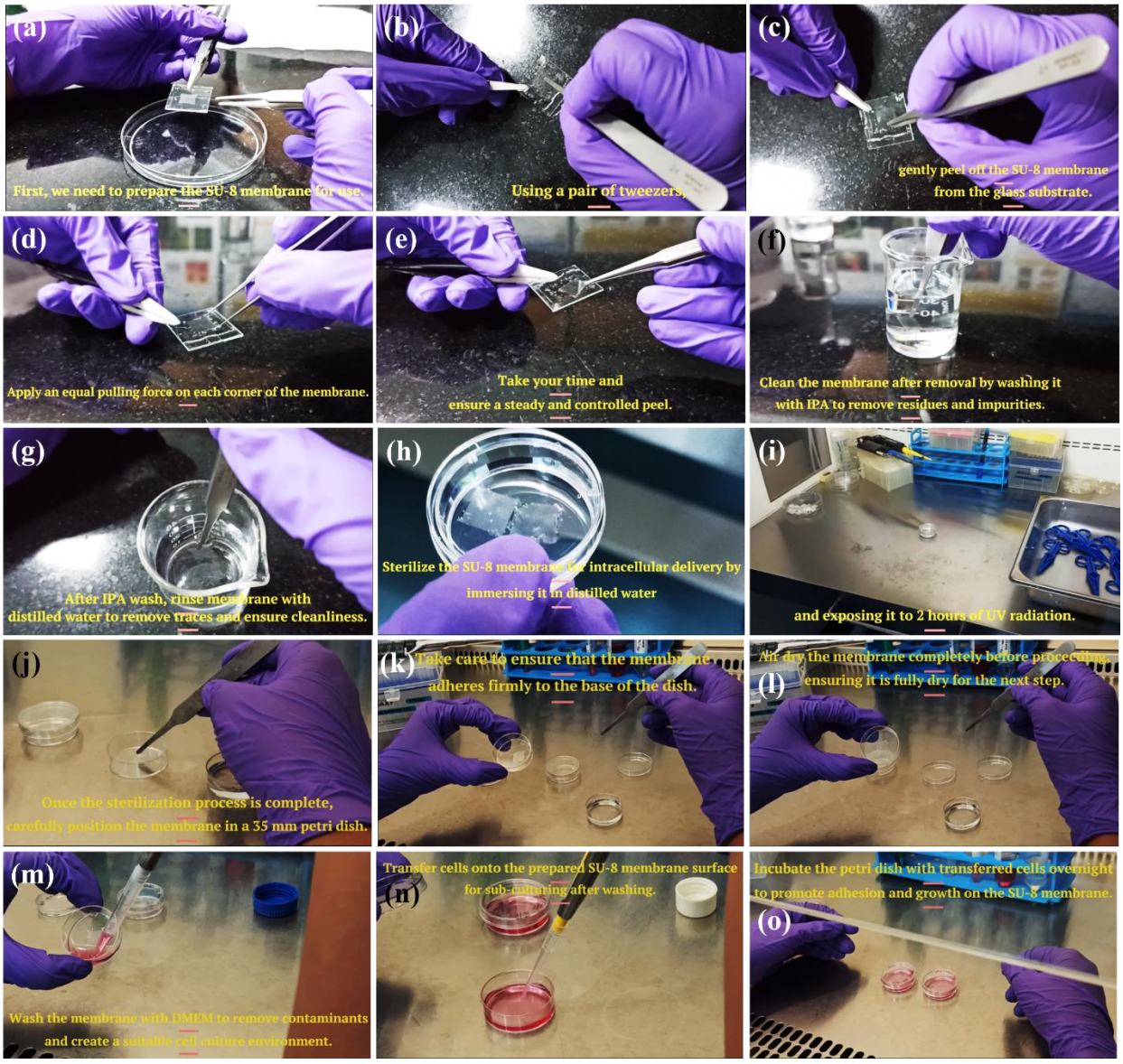

Figure S12. Step-by-step guide: Transferring a SU-8 membrane from a glass substrate to a cell culture dish.