Supplementary information

Targeted permeabilization of cell wall and extraction of charged molecules from single cells in intact plant clusters using a focused electric field

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Table S1 The culture media for deep cells. Dissolve the substances in the table in distilled water and adjust pH to 5.8 with 1 M KOH and fill up to 1000 ml.

Chemical	For 1000 ml culture media
Murashige and Skoog basal salt mixture (M5524, Sigma)	4.33 g
Myo-inositol (I7508-50G Sigma) 2wt%, nicotinic acid (N0761-100G) 0.02wt%, pyridoxine hydrochloride (P6280-25G) 0.02wt%, and Thiamine hydrochloride (T1270-25G Sigma) 0.2 wt%	10 ml
2,4-Dichlorophenoxyacetic acid (D7299-100G Sigma) (stock solution: 100 mg/l)	10 ml
KH_2PO_4 (P5655-100G, Sigma) (stock solution: 100 g/l)	3.4 ml
Sucrose (196-00015, Wako)	30 g

Table S2 The culture media for BY-2 cells. Dissolve the above substances in distilled water and adjust pH to 5.8 with 1 M KOH and fill up to 1000 ml.

Chemical	For 1000 ml culture media
Murashige and Skoog basal salt mixture (392-00591, Wako)	1 liter packet
KH_2PO_4 (P5655-100G, Sigma) 80 mg/ml	2.5 ml
Thiamine hydrochloride (T1270-25G Sigma) 0.4 mg/ml, Myo-Inositol (I7508-50G Sigma) 40 mg/ml	2.5 ml
2,4-Dichlorophenoxyacetic acid (D7299-100G Sigma) (stock solution: 0.2 mg/ml)	1 ml
Sucrose (196-00015 Wako)	30 g

Table S3 voltage conditions and duration for RNA extraction from intact plant cell, protoplast and mammalian cells

	Inlet voltage, V	Waste voltage, V	Outlet voltage, V	Duration, s
Pulsed voltage for lysis	-2 to -38	120 to 2700	0	0.01 to 1
*Pushing voltage	-136 to -1364	-270 to -2700	0	0.01 to 1
*Resting pulse	0	0	0	1
Step 1 for extraction	-150	-170	0	40
Step 2 for extraction	-350	-510	0	160

* optional

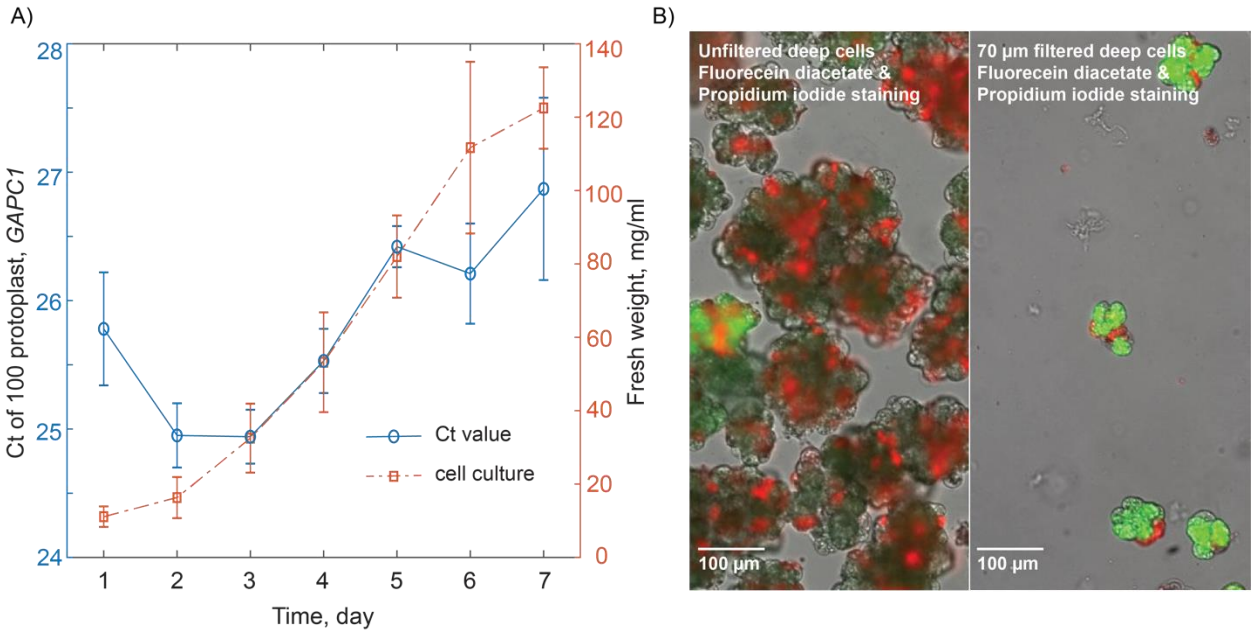

Fig. SI1: Quality control of the plant cell samples. A) Fresh weight (mg/ml) of deep cell culture and *GAPC1* expression in 100 protoplasts on different day since sub-culture. B) Effect of filtration on the viability of deep cells with a 70-µm mesh filter. Deep cells were dual stained with FDA (false color, green) and PI (false color, red) before and after filtration.

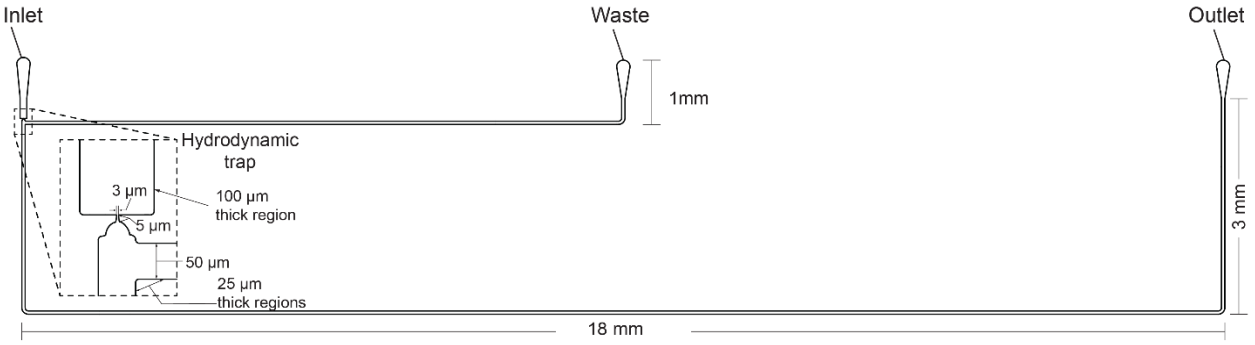

Fig. SI2: The geometry of the microchannel

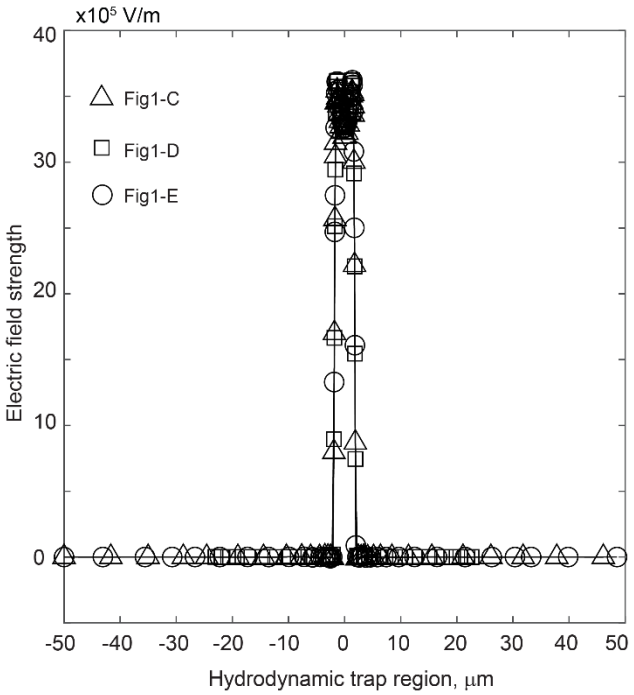

Fig. SI3: Electric field strength in the spanwise direction at the hydrodynamic trap with various geometries of the inlet channel.

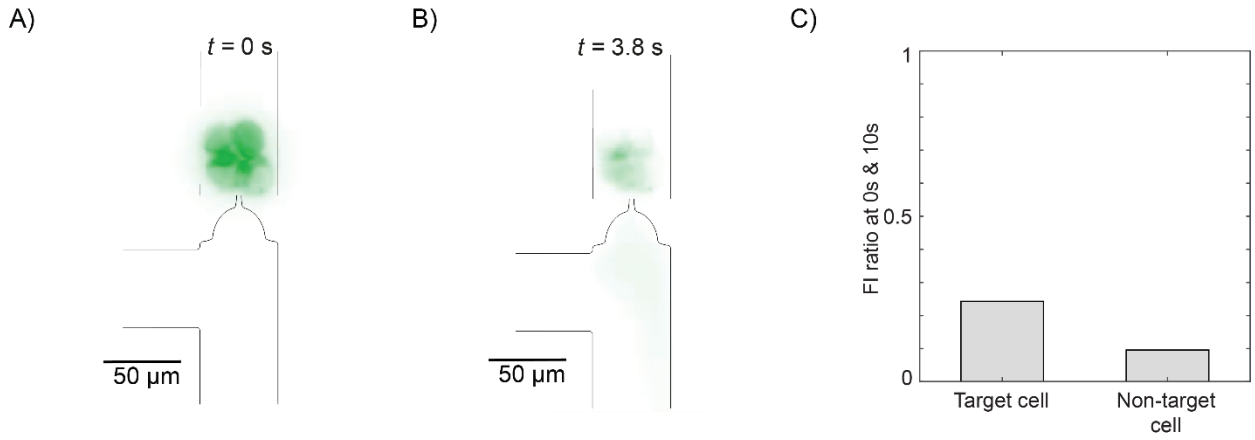

Fig. SI4: (A, B) Typical fluorescence images (false color) of deep cells in the 50-μm wide and 45-μm deep inlet channel before and after electrophoretic extraction of FDA. (C) Relative fluorescence intensities of targeted and non-targeted cells comparing before and after the application of the electric field.

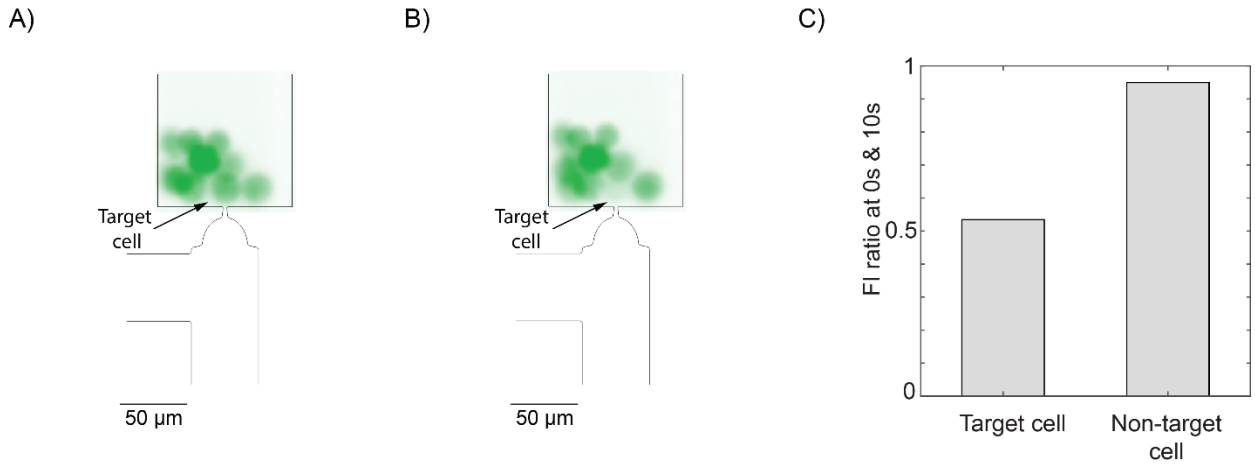

Fig. SI5: Selective extraction of calcein from a single K562 cell in a cluster of intact cells.

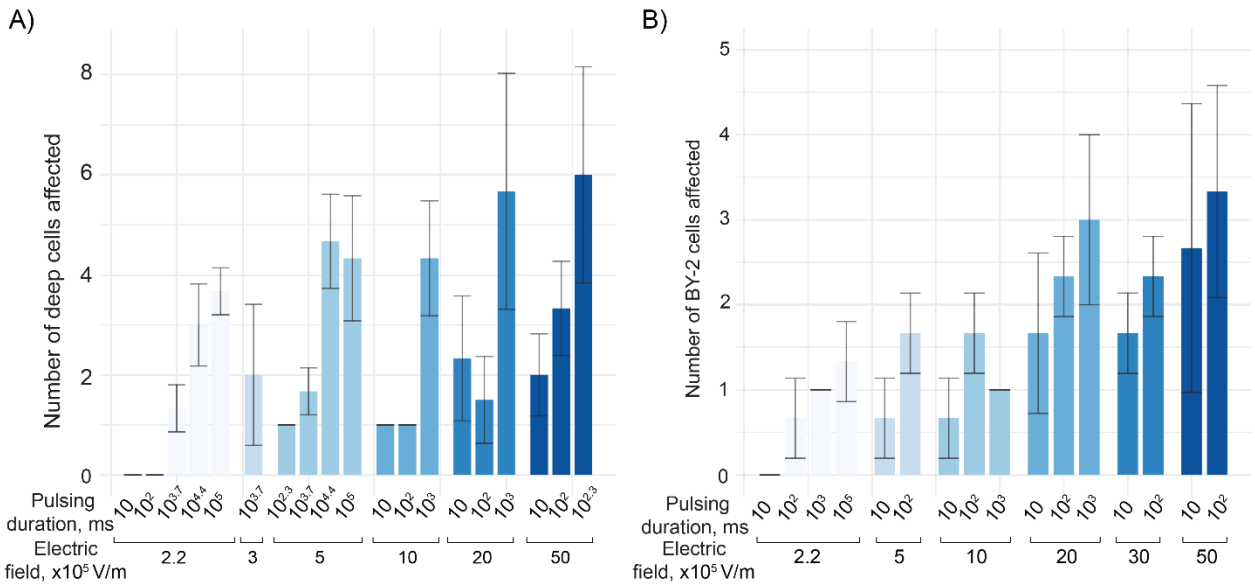

Fig. SI6: Number of cells affected in each condition: A) Deep cells and B) BY-2 cells.