Supplementary information

Nano-Enhanced Electric-Field Treatment Harnessing Lightning-Rod Effect for Rapid Bacteria Inactivation

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1. Supplementary figures

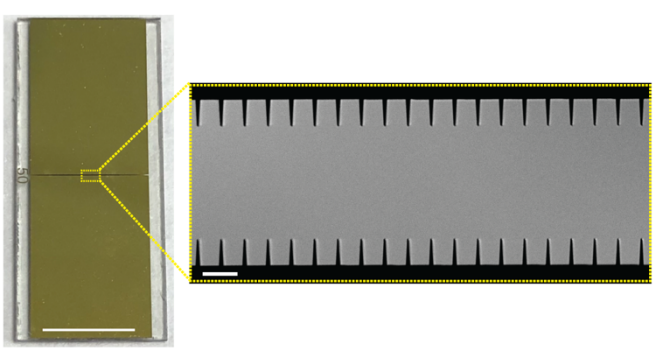

Figure S1. Digit photo of the lab-on-a-chip and the zoom-in microscopy images showing the nanowedges on the two electrodes. The scale bars are 5 mm (left) and 10 μm (right).

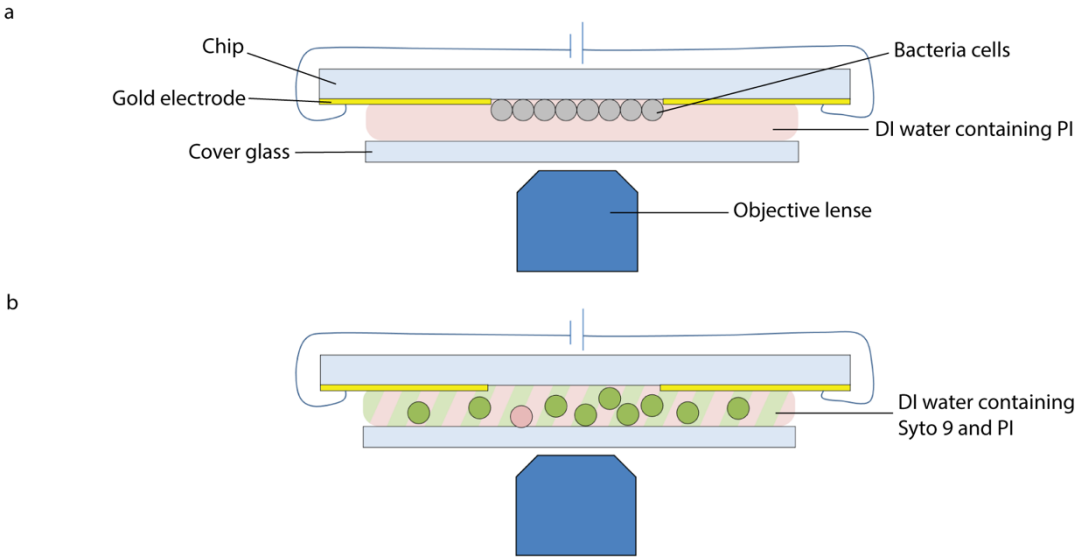

Figure S2. Experimental setup. (a) Experimental setup for immobilized cells. The medium is DI water containing PI stain. Only dead cells could be stained with PI and show red fluorescence, while live cells are not stained. (b) Experimental setup for free-moving cells. The medium is DI water containing Syto 9 and PI stain. The live cells are stained with Syto 9 and show green fluorescence. The dead cells are stained with PI and show red fluorescence.

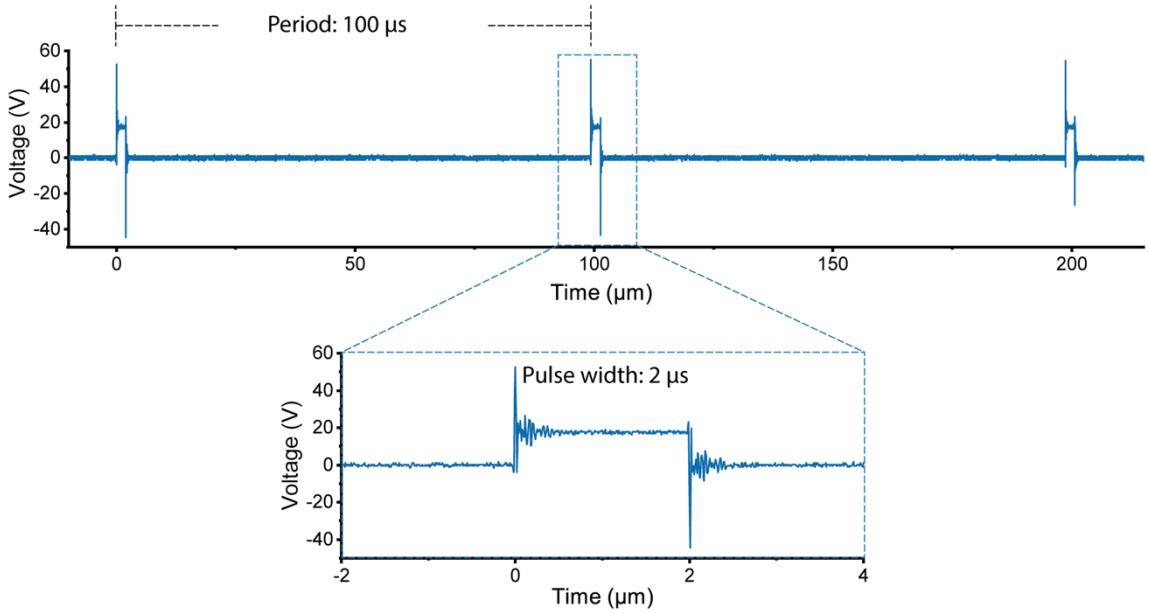

Figure S3. Waveform of the applied electric pulses at 18 V with 2 µs pulse width, 100 µs period, denoted as 18 V/2 µs/100 µs. The effective treatment time is calculated as the total time when the voltage is not 0, which equals pulse width × pulse number.

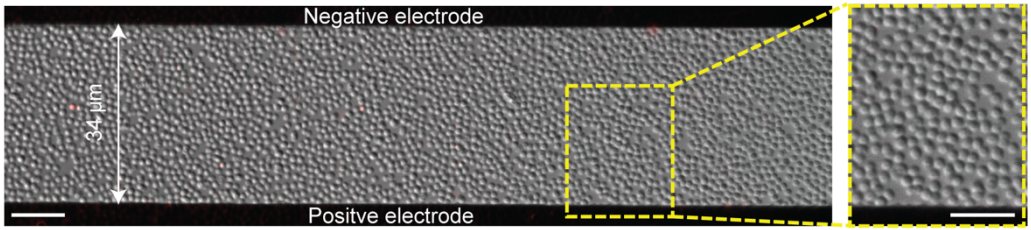

Figure S4. Electrodes that have no nanowedges but a smaller gap of 34 µm after 18 V/2 µs/100 µs/500,000 pulses treatment. The scale are is 10 µm in left image and 5 µm in zoom-in image.

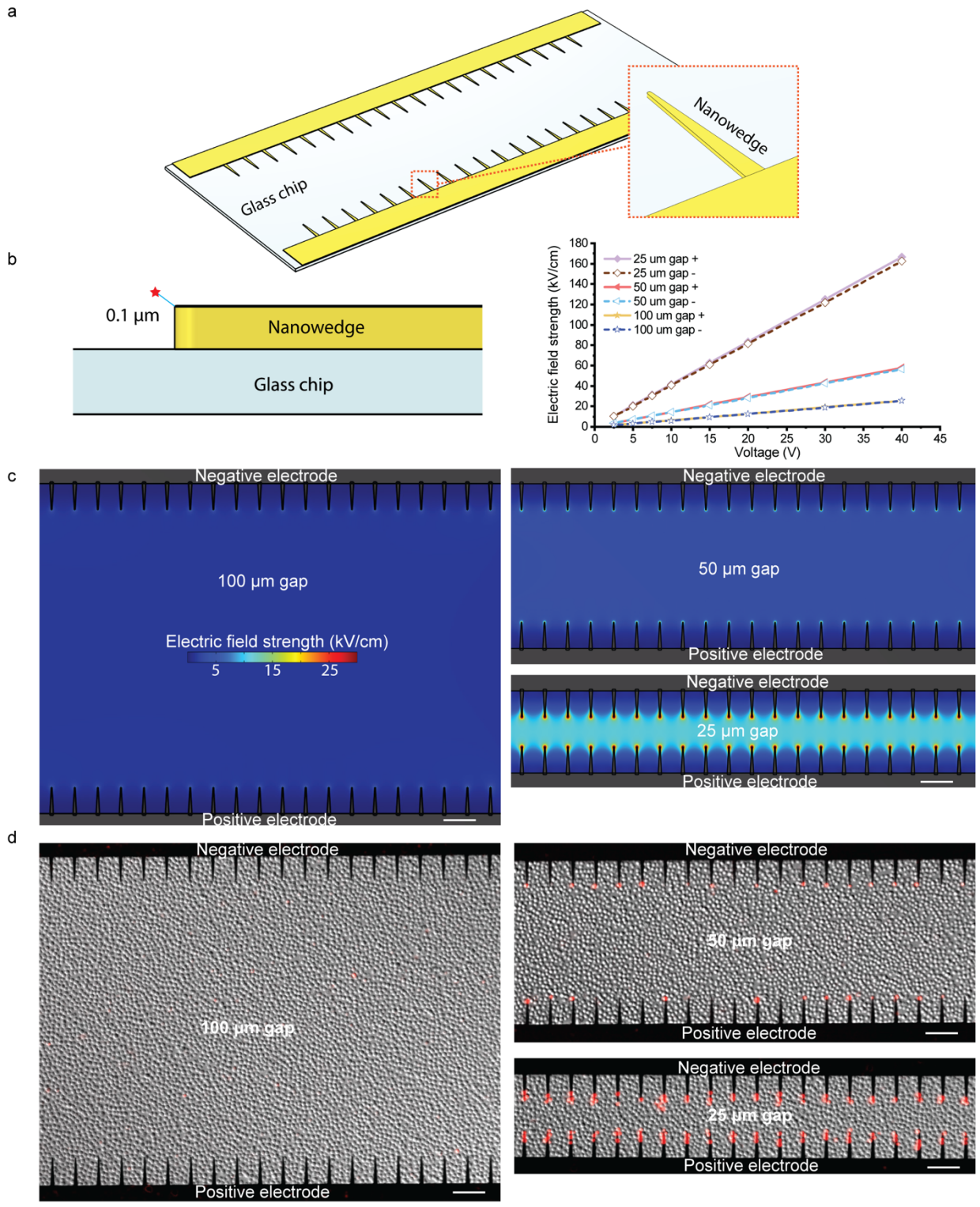

Figure S5. Chips of different gap between positive/negative electrode. (a) Simulation model setup. (b) Strength of the nano-enhanced electric field on chips with different gaps (right). The data shows the strength of the electric field at the star point which is 0.1 μm from the nanowedge tip (left). (c) Simulation results under 15 V. (d) Microscopy images after NEEFT at 15 V/2 μs/100 μs/500,000 pulses. The scale bars are 10 μm.

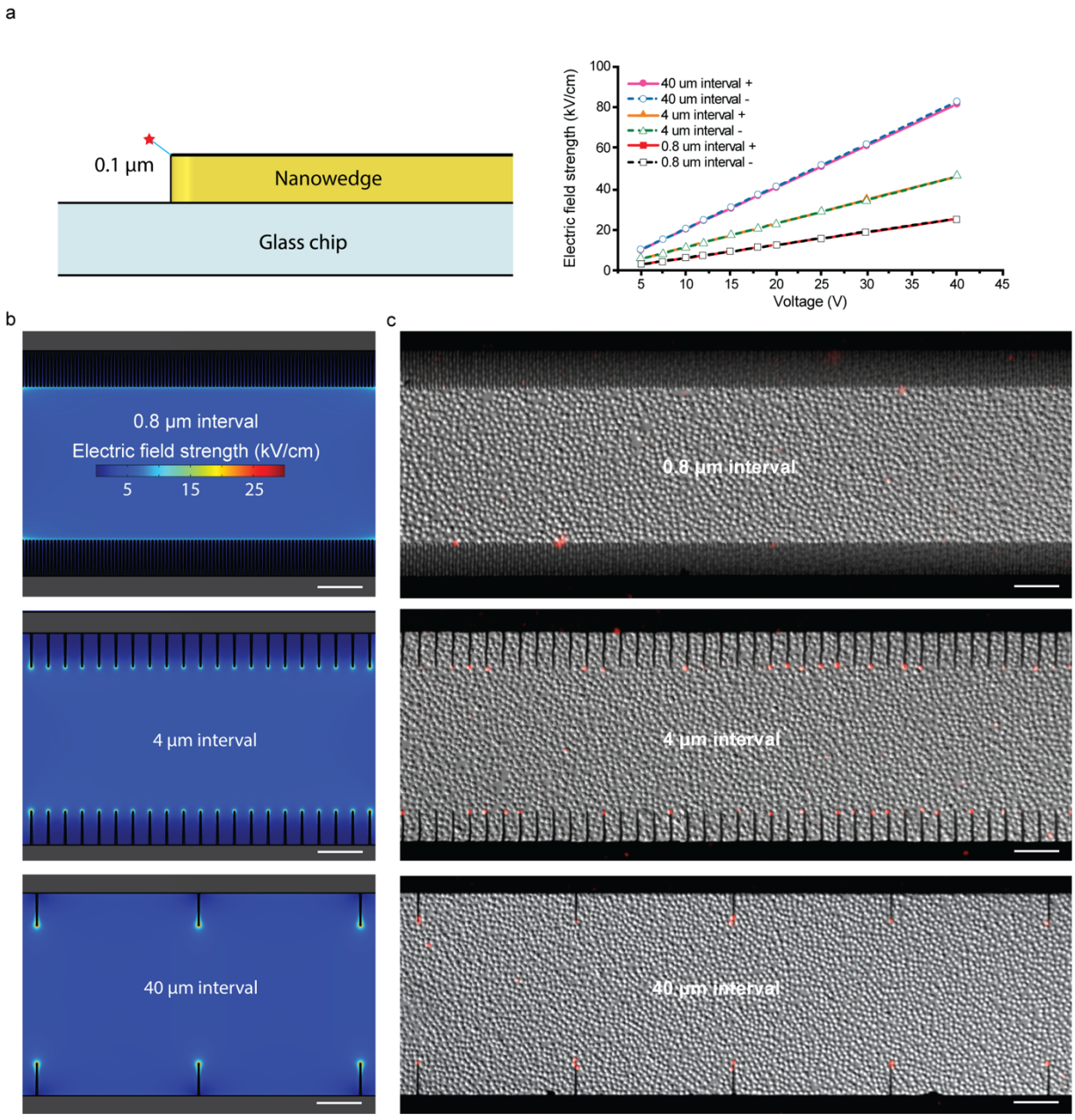

Figure S6. Chips with nanowedges of different interval. (a) Strength of the nano-enhanced electric field on the chip with nanowedges of different intervals (right). The data shows the strength of the electric field at the star point which is 0.1 µm from the nanowedge tip (left). (b) Electric field at 0.1 µm from the nanowedge tip. (c) Microscopy images after NEEFT of 20 V/2 µs/100 µs/500,000 pulses. The upper and lower electrode is the positive and negative electrode, respectively. Note that the nanowedges are 200 nm at the tip and 400 nm at the base to achieve the small interval in between. The scale bars are 10 µm.

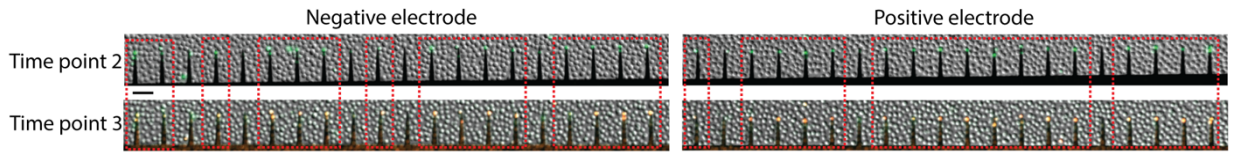

Figure S7. Microscopy images showing irreversible electroporation after 80 V/1 μs/1 ms/10 pulses. The cells inside the red frames have irreversible pores on membrane since they are stained with SYTOX Green at Time point 2 and also stained with PI at Time point 3. Scale bars is 5 μm.

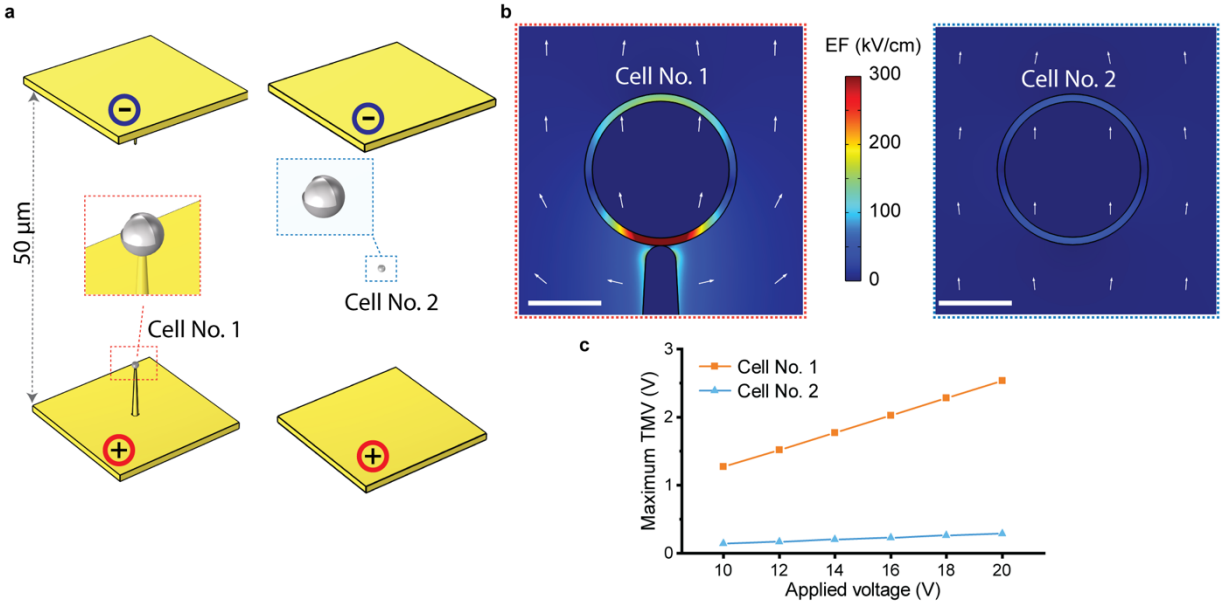

Figure S8. Theoretical analysis of cell TMV in a 3D NEEFT system with standing nanowire and bulk EFT system. (a) Simulation set up for NEEFT (left) and bulk EFT (right). (b) Voltage drop, i.e., electric field, on the cell membrane of cell No. 1 in NEEFT (left) and cell No. 2 in bulk EFT under 20 V applied voltage (right). The arrows indicate the direction of electric field. The scale bars are 0.5 μm. (c) Maximum TMV on cell No. 1 and cell No. 2 under different applied voltages. The maximum TMV of cell No. 1 is 9 times higher than that of cell No. 2.

2. Supplementary methods

2.1. Chip pre-treatment

The fabricated chips were first washed with 5.25% bleach for 30 min, and 30% H_2O_2 for 1 hour. Rinse the chips with DI water between different solutions, and store in DI water overnight after the wash steps.[1] Dry in 60 ℃ before use. To achieve bacteria immobilization, washed and dried chips were coated with positively charged poly-L-lysine (0.01%, mw 150,000-300,000). Poly-L-lysine and 2 M borate buffer was first mixed at 1:1, then about 50 μL of the mixture was added onto a chip to cover the gap between the two electrodes. After 3 hours of coating at room temperature, the chips were rinsed with DI water, and dried in 60 ℃ for 20 min. The coated chips were stored in 4 ℃ to avoid coating layer degradation. Poly-l-lysine is a positively charged polymer, which can attract negatively charged bacteria and immobilize them on the chip surface. To reuse the chips after experiments, repeat the wash and coating steps.

2.2. Electric field and transmembrane voltage (TMV) simulation.

A 3D model of the lab-on-a-chip was developed, and electric currents module in COMSOL Multiphysics is used for electric field simulation. The built-in materials glass, gold, and water are assigned to the substrate, electrodes (both bulk contact pads and nanowedges), and medium. The relative permittivity of water is 78.5. Since DI water is used in this work, the electrical conductivity is 1×10^{-4} S/m.

The cell TMV was also simulated using electric currents module in a 3D geometry. Two concentric spheres are built to represent the inner and outer surface of the bacteria cell wall. The diameter of the cell is 1 μm and cell wall thickness is 50 nm. The extracellular, intracellular, and membrane conductivity are 1×10^{-4} S/m, 0.2 S/m, and 5×10^{-7} S/m.[2]

REFERENCES

1 Maher, M., Pine, J., Wright, J. & Tai, Y.-C. The neurochip: a new multielectrode device for stimulating and recording from cultured neurons. *Journal of neuroscience methods* **87**, 45-56 (1999).

2 Boukany, P. E. *et al.* Nanochannel electroporation delivers precise amounts of biomolecules into living cells. *Nature nanotechnology* **6**, 747-754 (2011).