Nanosecond Pulsed Electric Fields (nsPEFs) Regulate Phenotypes of Chondrocytes through Wnt/β-catenin Signaling Pathway

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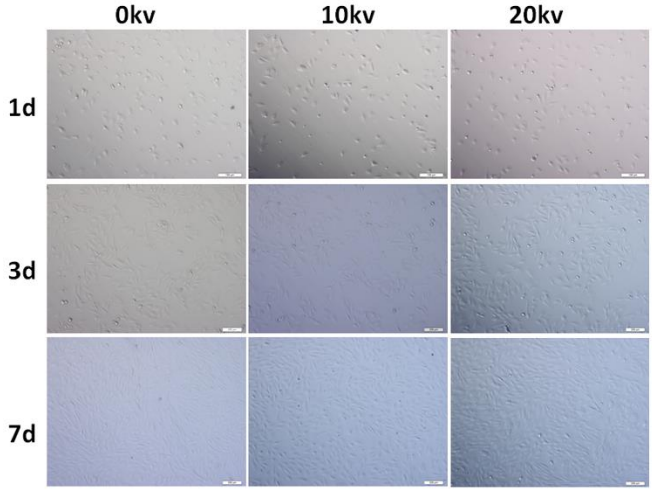
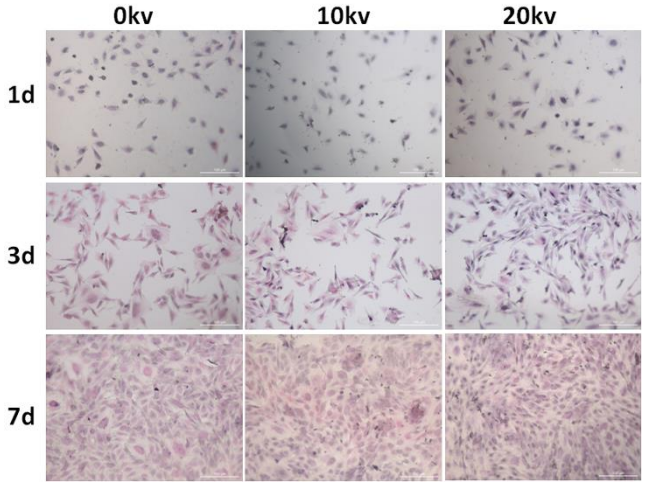
Supplementary Information



S1a



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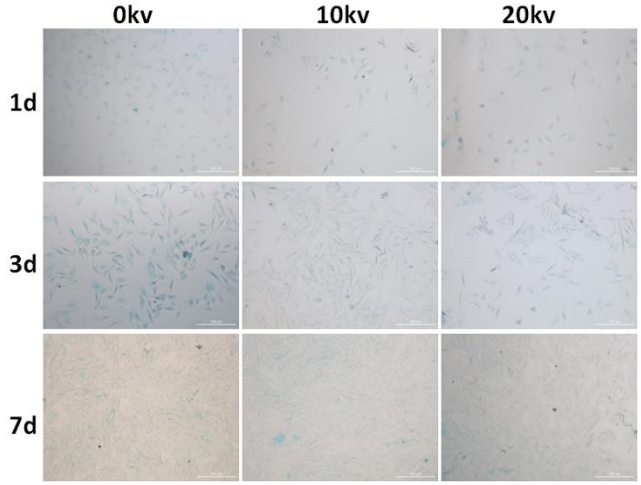

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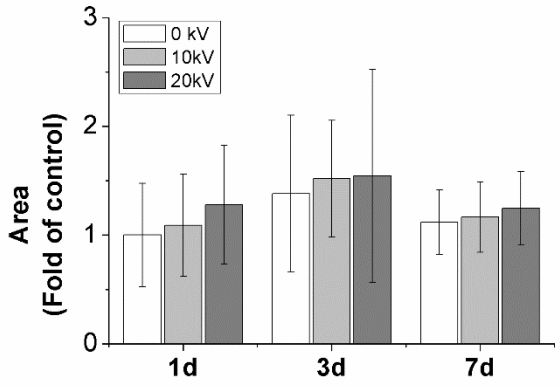

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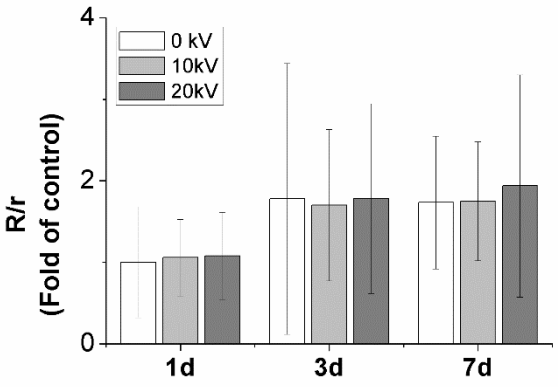
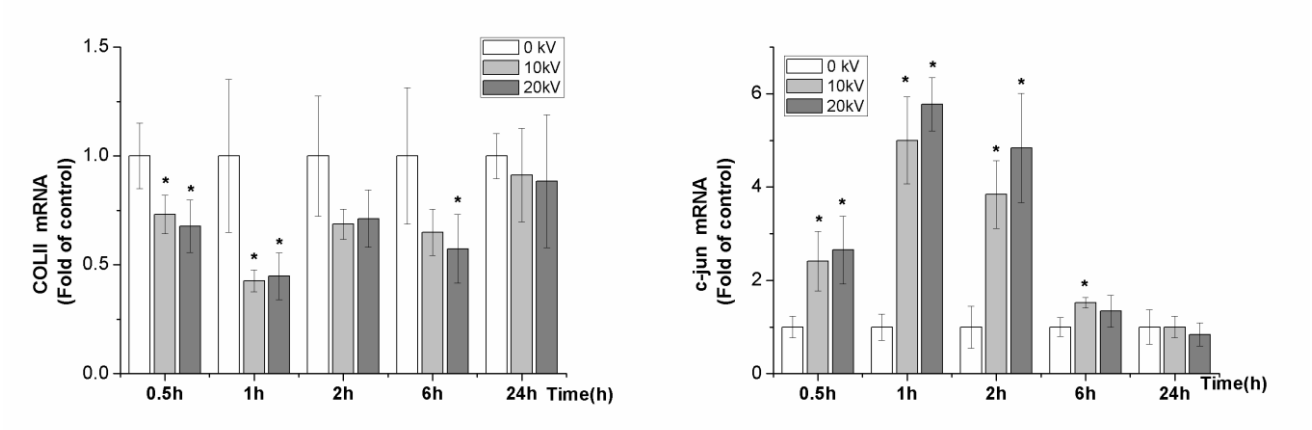


S1e

Supplementary Figure S1.

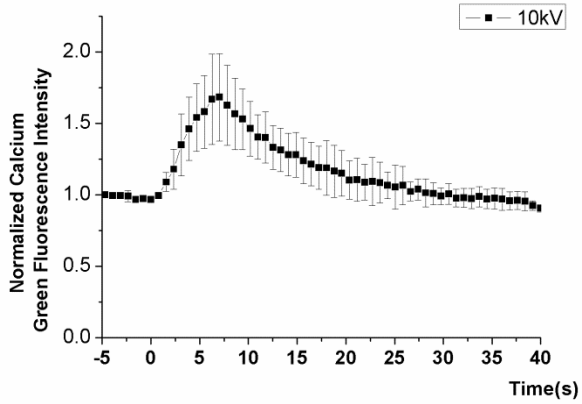
Morphology of chondrocytes after nsPEF treatment. (a) Optical microscopy images; (b) H&E staining; (c) Alcian blue staining; (d) Spreading areas of chondrocytes; (e) Rounded phenotype of chondrocytes by ratio of mean maximum (R) and minimum (r) distance between cell's centroid and contour. Data expressed as mean ± s.d. To evaluate cell morphology, chondrocytes were observed under light microscopy at days 1, 3 and 7. Cellular area and R/r were analyzed using Image-Pro Plus 6.0. Chondrocytes were fixed with 4% paraformaldehyde for 30 minutes and washed with PBS, before haematoxylin & eosin and alcian blue staining.



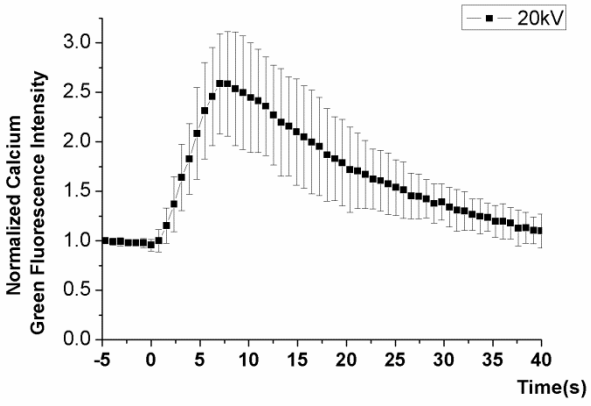
S2a **S2b**

Supplementary Figure S2

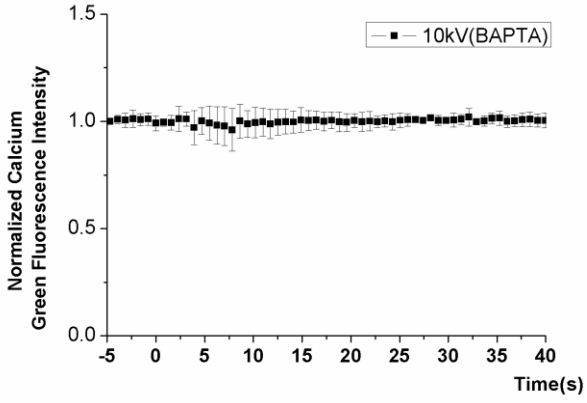
Time course analyses of gene expression of (a) COL II; (b) c-jun at 0.5, 1, 2, 6 and 24 hours after nsPEF treatment. Data expressed as mean ± s.d. *$p<0.05$.



S3a



S3b



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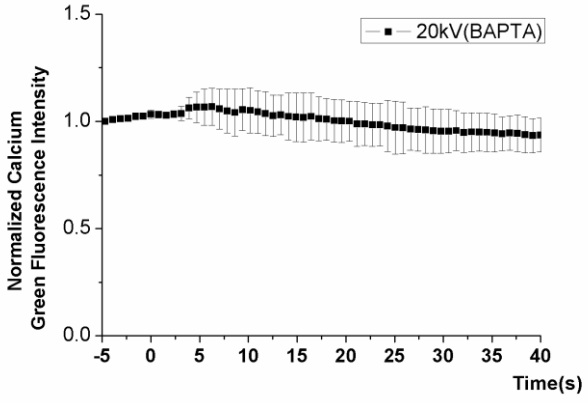

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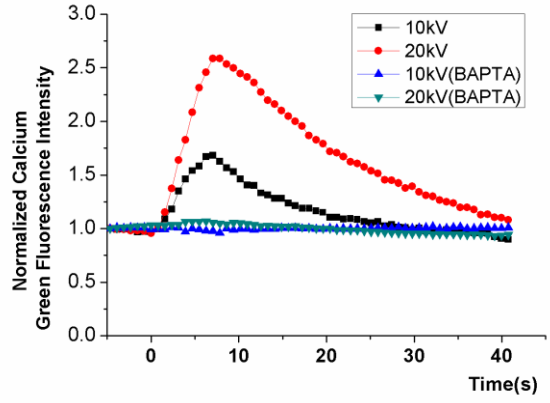

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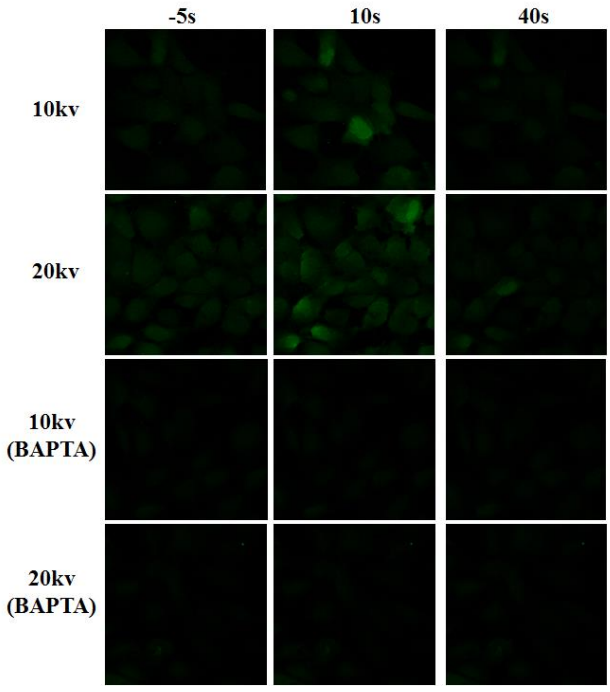
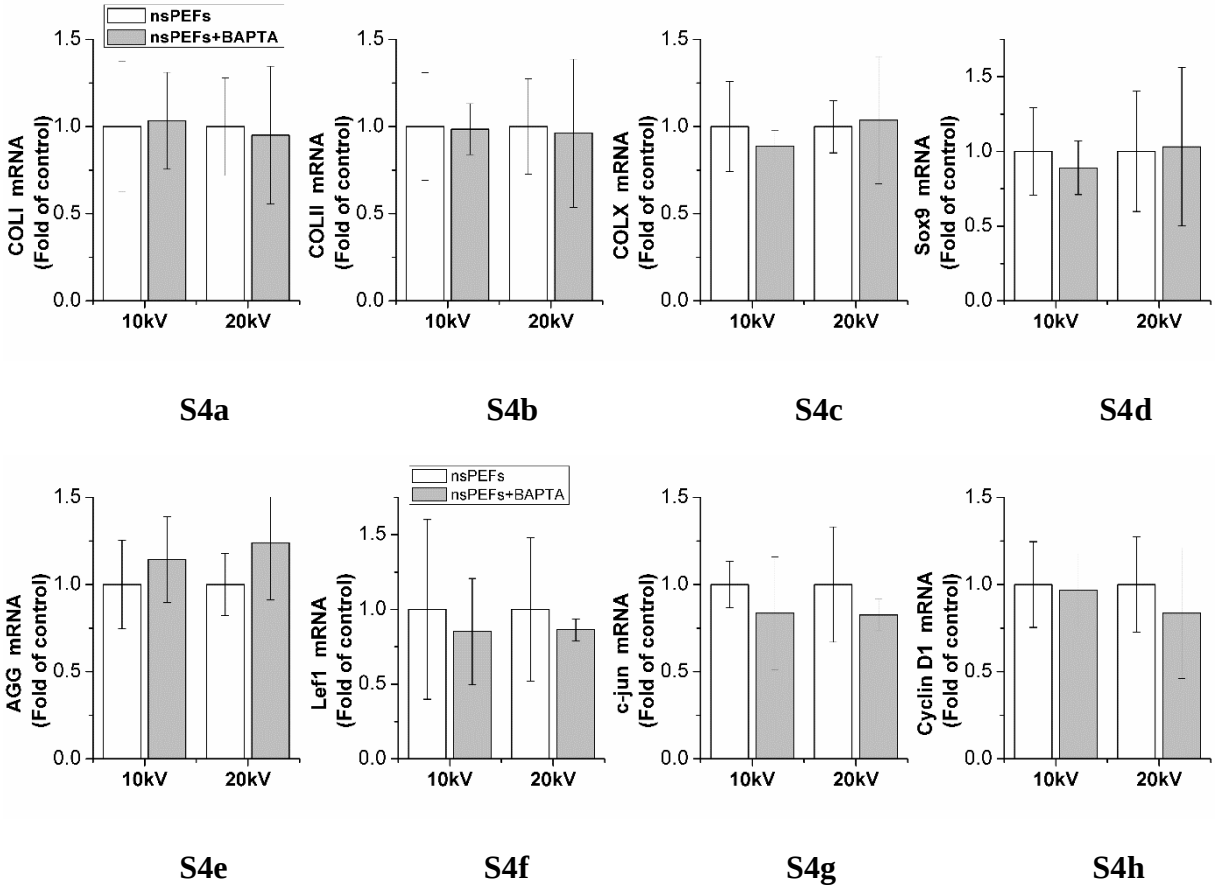


S3f

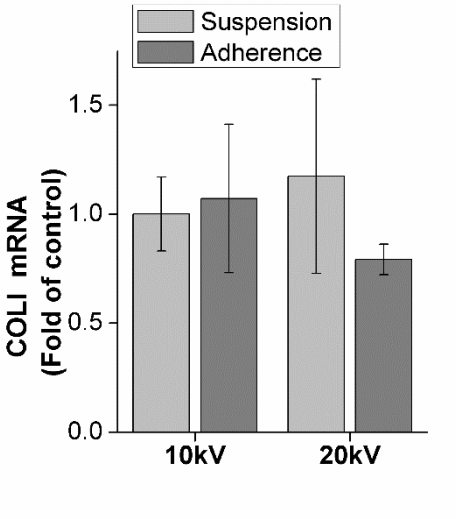
Supplementary Figure S3

Figure illustrating (a-e) Ca^{2+} fluorescence intensity; (f) confocal images after application of nsPEFs with or without co-treatment with BAPTA-AM. For chemical Ca^{2+} indicator loading, chondrocytes were incubated with 5 μM fluo-4-AM (F14201, Invitrogen) for 10 minutes at room temperature. For Ca^{2+} chelator loading, 15 μM 1,2-bis (2-aminophenoxy) ethane N,N,N',N'-tetraacetic acid (BAPTA)-AM (B1205, Invitrogen) was used to pre-treat chondrocytes for 15 minutes, followed by fluo-4-AM loading for 10 minutes at room temperature, after which nsPEFs were applied. Fluorescent imaging using confocal microscopy (LSM510, Carl Zeiss MicroImaging) was performed with line-scan mode with excitation at 488 nm and emission at 490–570 nm.

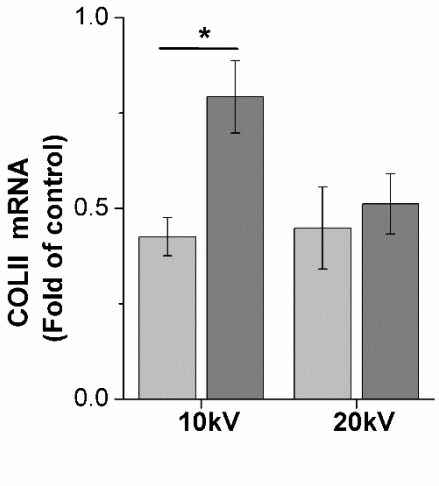


Supplementary Figure S4

Gene expression after nsPEF treatment compared to co-treatment with BAPTA-AM. (a) COL I; (b) COL II; (c) COL X; (d) Sox9; (e) AGG; (f) LEF1; (g) c-jun; (h) cyclin D1. Data expressed as mean ± s.d.



S5a



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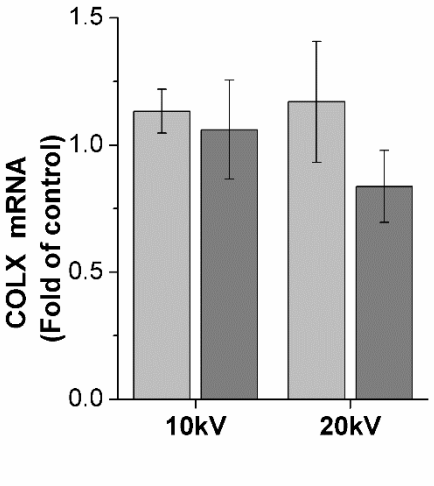

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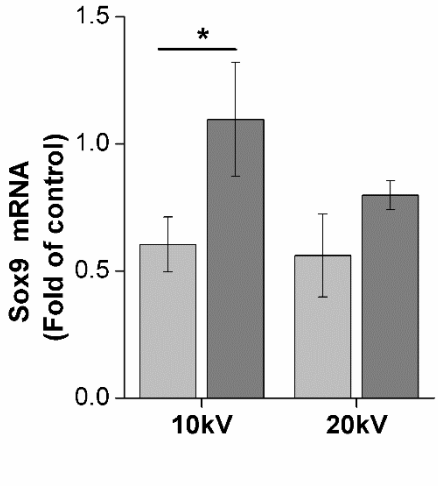

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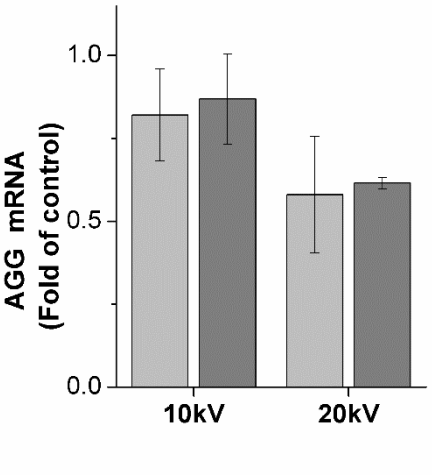

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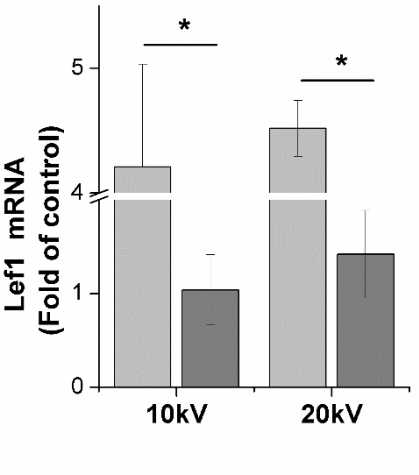

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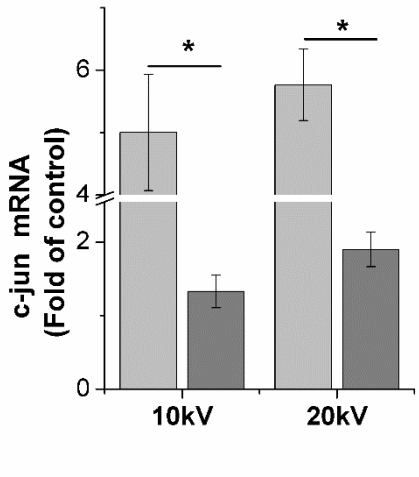

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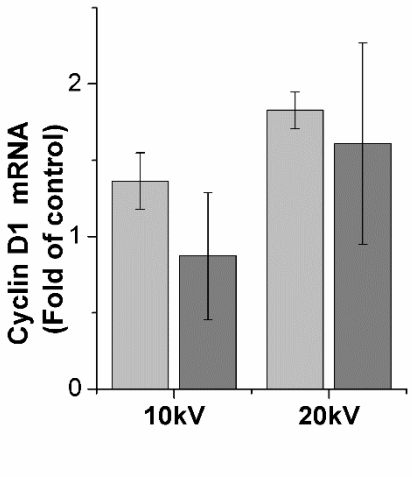

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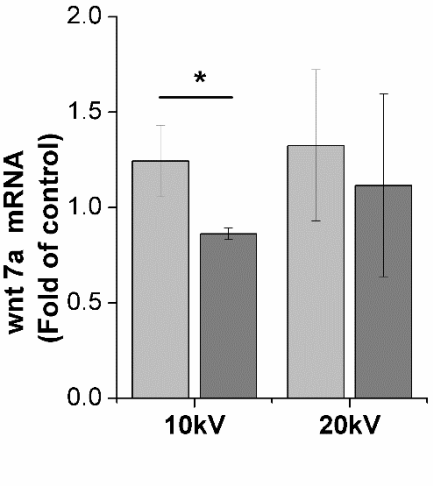

S5i

Supplementary Figure S5

Gene expression of chondrocytes in suspended and adherent states compared with untreated chondrocytes at 1 hour after 10 kV/cm or 20 kV/cm nsPEF treatment. (a) COL I; (b) COL II; (c) COL X; (d) Sox9; (e) AGG; (f) LEF1; (g) c-jun; (h) cyclin D1; (i) wnt7a. Data expressed as mean ± s.d. *$p<0.05$.