

Supplementary Information

MicroRNA-7450 regulates non-thermal plasma-induced chicken Sertoli cell apoptosis via adenosine monophosphate-activated protein kinase activation

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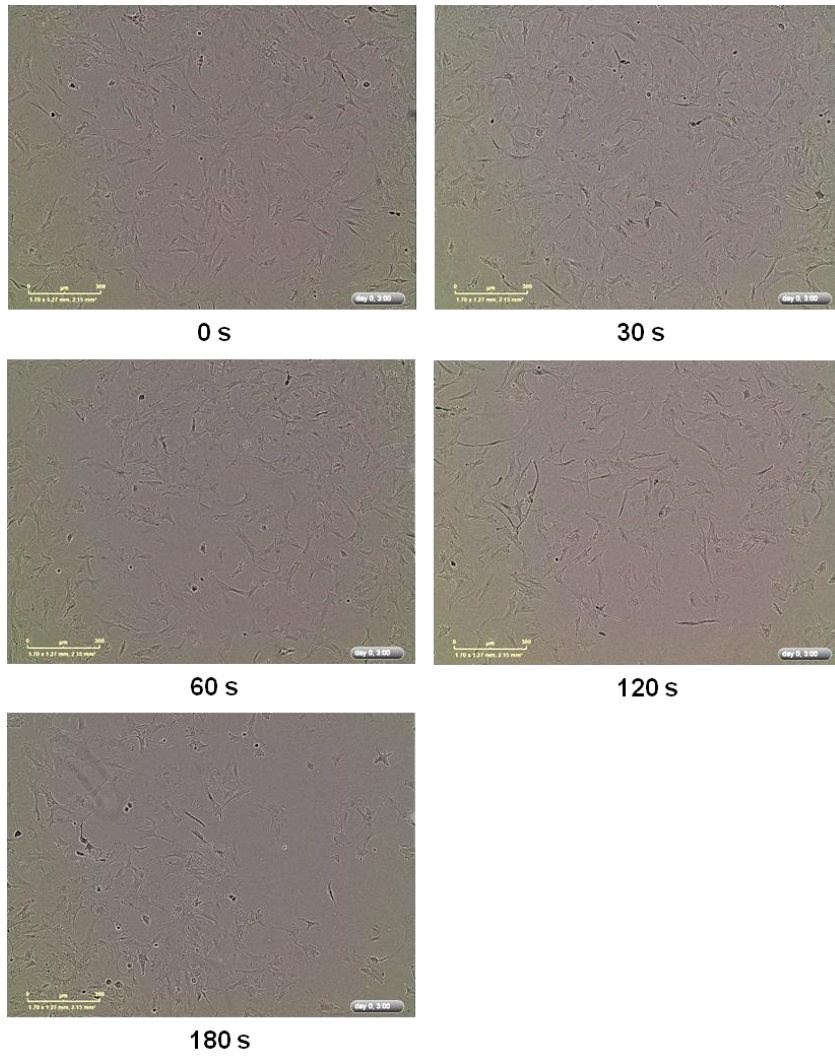
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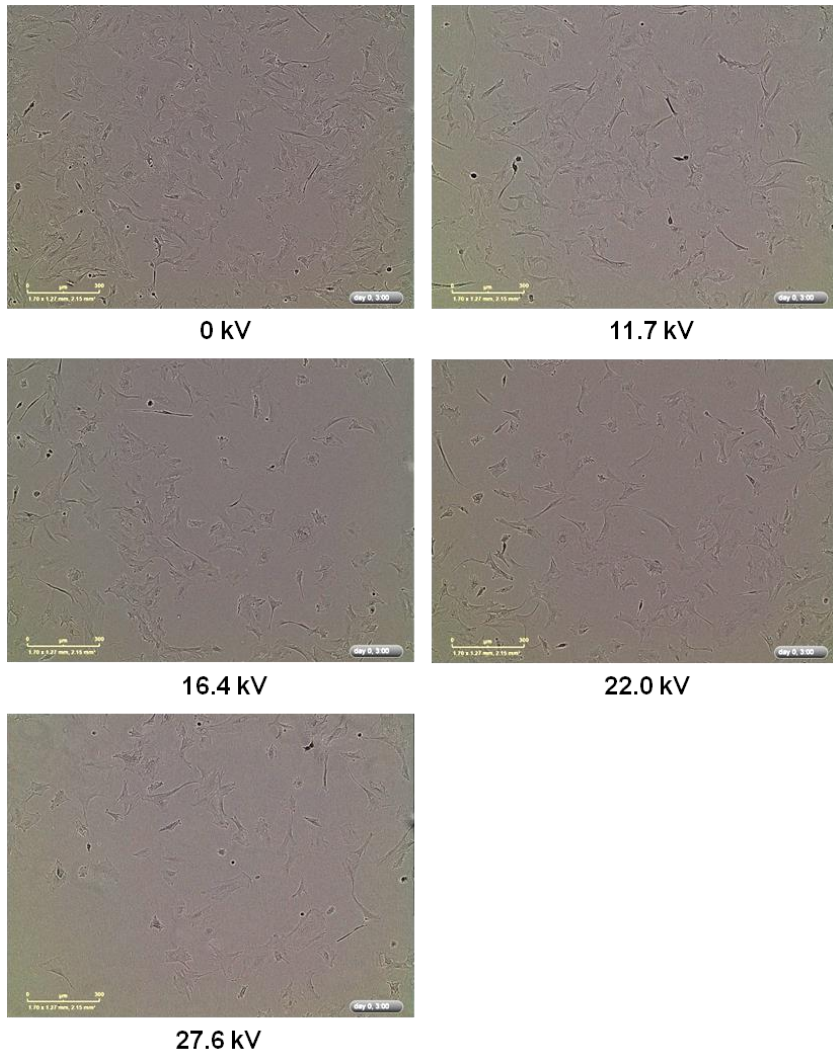
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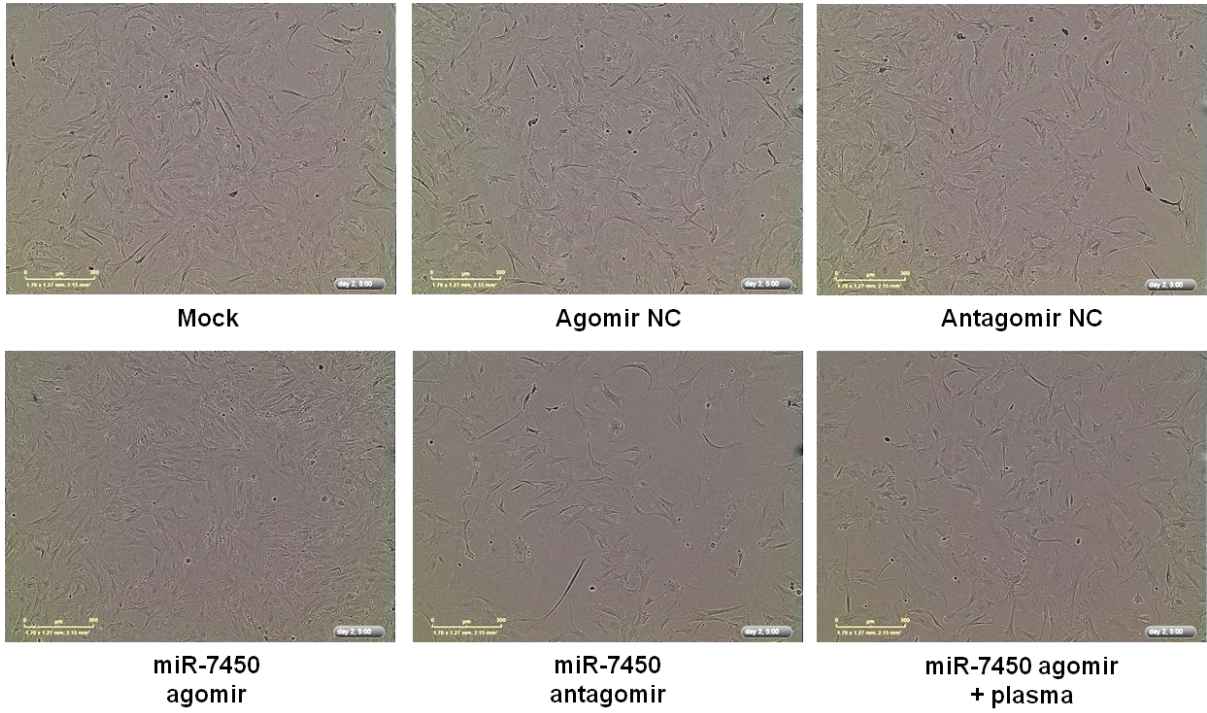
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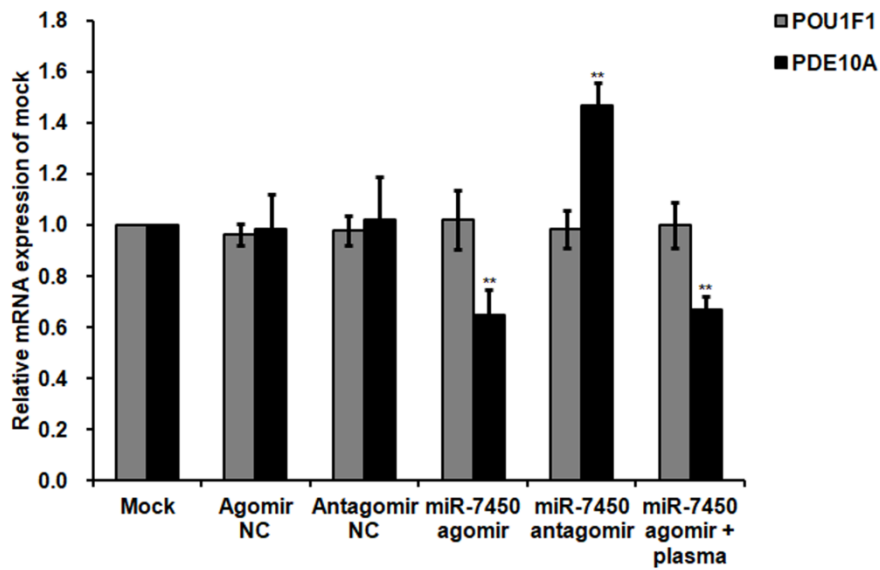
Supplementary Figure S1. Growth status of SCs exposed to 11.7 kV for different durations. Scale bar: 300 μm .



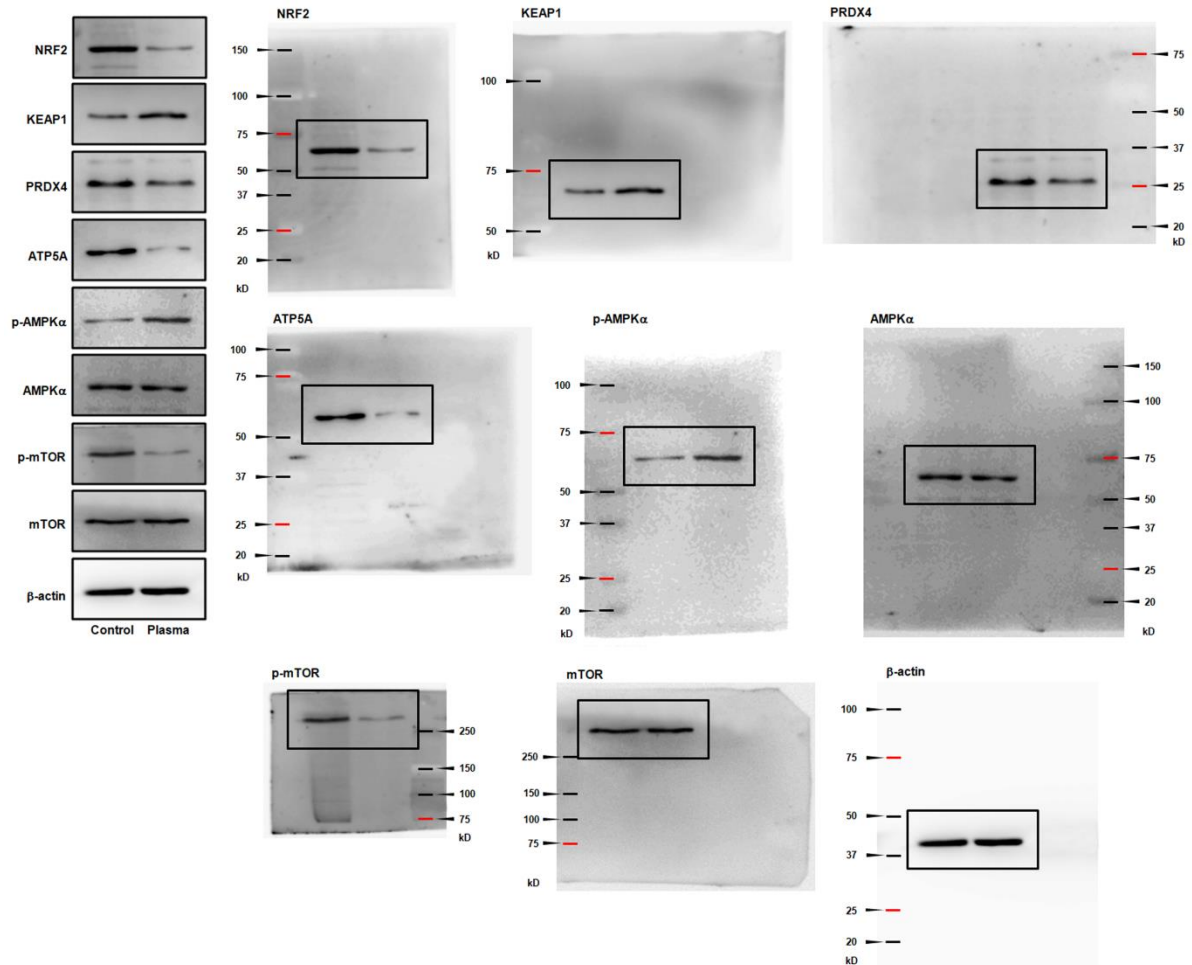
Supplementary Figure S2. Growth status of SCs exposed to different potentials for 120 s. Scale bar: 300 μm.



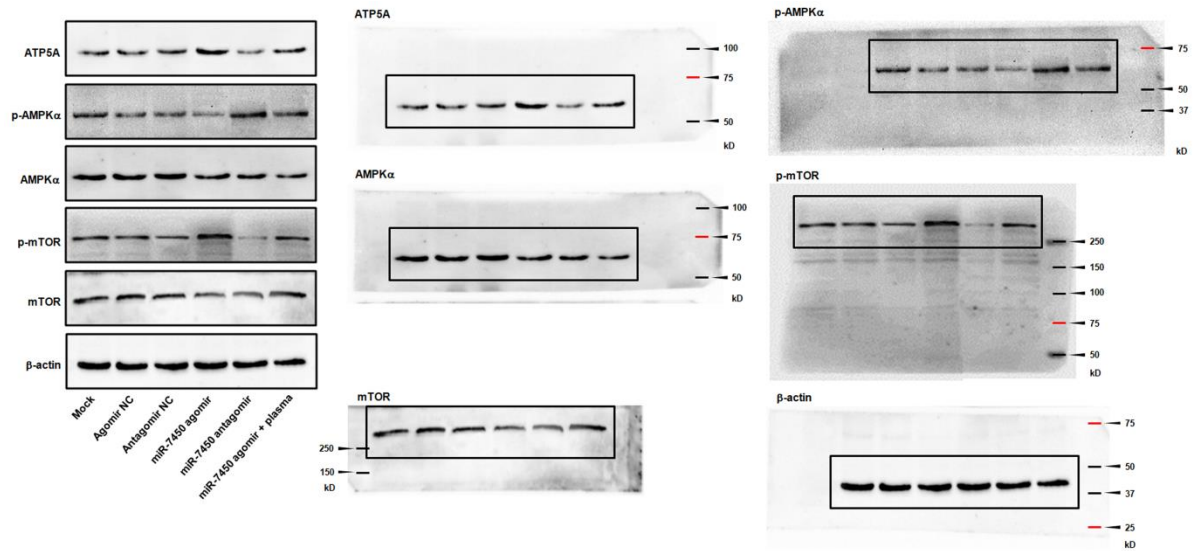
Supplementary Figure S3. Growth status of SCs transfected with miR-7450 agomir and antagomir, and miR-7450 agomir-transfected group treated with 22.0 kV of plasma for 120 s. Scale bar: 300 µm.



Supplementary Figure S4. RT-PCR analysis of a non-target gene (*POU1F1*) and an unrelated target gene (*PDE10A*) of miR-7450 in SCs transfected with miR-7450 agomir and antagomir, and miR-7450 agomir-transfected group treated with 22.0 kV of plasma for 120 s. Data are represented as the mean \pm SD (n=3 per group). *, $p < 0.05$; **, $p < 0.01$, according to one-way ANOVA and LSD test.

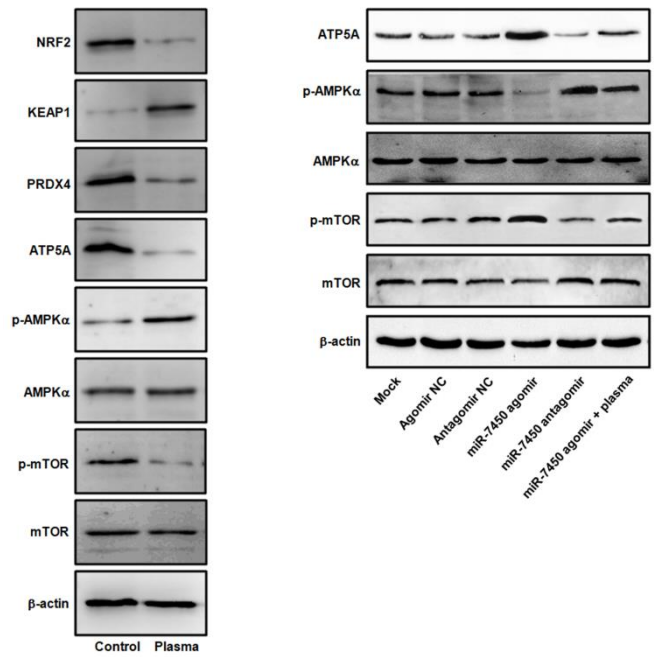


Supplementary Figure S5. Western blot analysis of the protein levels of NRF2, KEAP1, PRDX4, ATP5A, p-AMPK α , AMPK α , p-mTOR, and mTOR in SCs exposed to 22.0 kV of plasma for 120 s. Uncropped immunoblot scans for Fig. 3A. The grouping of gels/blots cropped from different gels. All blots were visualized with 5 min exposure time.



Supplementary Figure S6. Western blot analysis of the protein levels of ATP5A, p-AMPK α , AMPK α , p-mTOR, and mTOR in SCs transfected with miR-7450 agomir and antagonist, and miR-7450 agomir-transfected group treated with 22.0 kV of plasma for 120 s.

Uncropped immunoblot scans for Fig. 3F. The grouping of gels/blots cropped from different gels. All blots were visualized with 5 min exposure time.



Supplementary Figure S7. One independent replicate on chicken SC protein expression. **(A)** Western blot analysis of protein bands in SCs exposed to 22.0 kV of plasma for 120 s. **(B)** Western blot analysis of protein bands in SCs transfected with miR-7450 agomir and antagonir, and miR-7450 agomir-transfected group treated with 22.0 kV of plasma for 120 s.

Supplementary Table S1. Primer sequences for the RT-PCR

Gene	Sequence number	Sequence position	Product length (bp)	Annealing Temperature (°C)	Sequence (5'to3')
<i>β-actin</i>	NM_205518.1	625-818	194	57	F: GTGCGTGACATCAAGGAGAAGC R: CCACAGGACTCCATACCCAAGA
<i>NOX4</i>	NM_001101829.1	28-157	130	57	F: CGAGGATCTCAGAAGGTTGC R: GAGCATTACCAGATGAGCA
<i>NRF2</i>	NM_205117.1	484-619	136	57	F: AAAACGCTGAACCACCAATC R: GCTGGAGAAGCCTCATTGTC
<i>KEAP1</i>	KU321503.1	1227-1485	259	57	F: GTATCACAGCAGCGTGGAGA R: GCGTAGATGCAGTTGTTGA
<i>SOD</i>	NM_205064.1	106-278	173	55	F: ATTACCGGCTTGTCTGATGG R: CCTCCCTTTCAGTCACATT
<i>CAT</i>	NM_001031215.2	1067-1276	210	55	F: CTCATTCCAGTGGCAAGAT R: GTAGGGGCAATTCACAGGAA
<i>GPx</i>	NM_001277853.2	353-474	122	55	F: ATGTTTCGAGAAGTGCGAGGT R: ATGATGTACTGCGGGTTGGT
<i>PRDX4</i>	XM_001233999.3	595-733	139	56	F: TGCACTTAGGGGCCTTTTCA R: TTCTCCATGCTTGTCCGTGT
<i>ATP5A1</i>	NM_204286.1	1207-1364	158	57	F: GGTATCCGTCCAGCCATCAA R: GCATCCAAATCAGACCCAAACT
<i>AMPKα</i>	NM_001039605.1	726-943	218	57	F: GGAGGCGTGTTTTACATCCC R: AACTTCTCACAGACCTCCCG
<i>mTOR</i>	XM_417614.5	119-309	191	57	F: TGAAGGGGTCAAGGCAATCC R: GGCGAGCAGTGGTTGTGGAT
<i>POU1F1</i>	NM_204319.1	560-754	195	57	F: ATGTTGGCGAAGCACTGGC R: GCTTCCTCTTCCGCTCATTCA
<i>PDE10A</i>	XM_004935551.2	3258-3412	155	57	F: CCACATCAACGATGGAACAG R: ATACAGGGCAAGGTCTGTGG
U6	NR_003027.2	66-85	20	60	F: CGCAAGGATGACACGCAAAT
miR-7450	MI0024118	1-20	20	60	F: TCTGTTCTTAAGGAGGCTGA