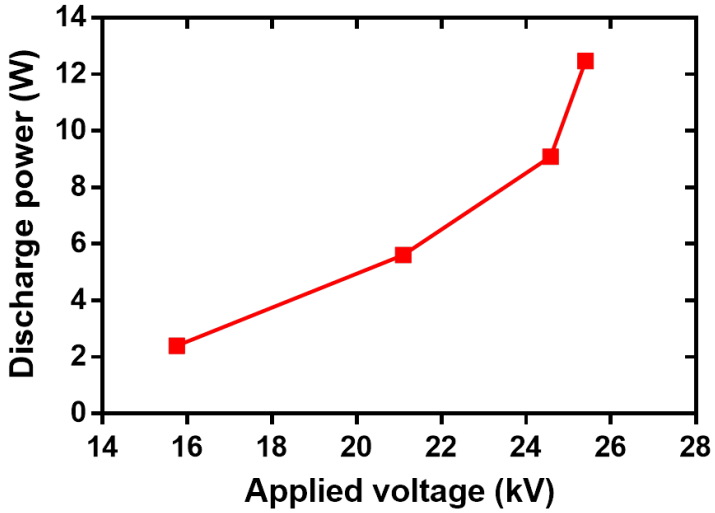
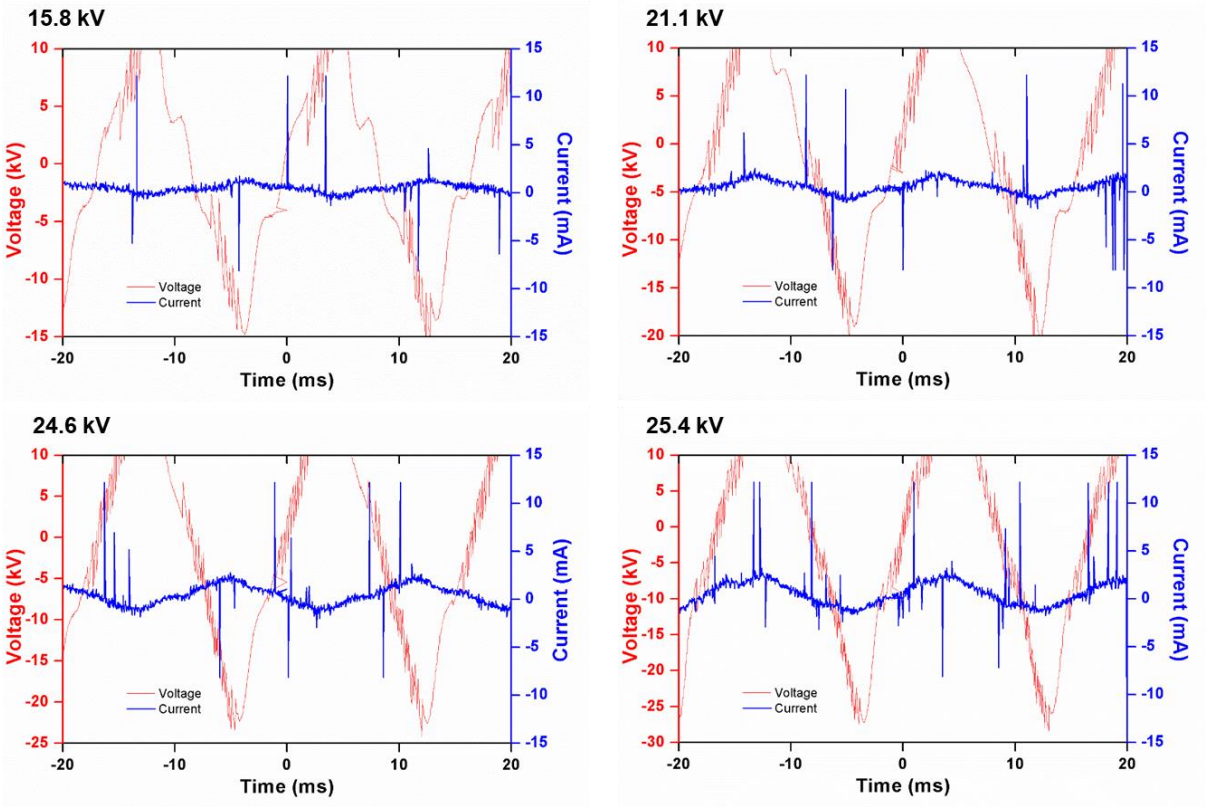


Appendix A Cold plasma discharge power and applied voltage



Appendix B Voltage and current waveforms obtained at different applied voltages of cold plasma

Appendix C Relative viability of Sertoli cells (SCs) exposed at different discharge powers for 20, 40, and 60 s (Exp. 2 (1))

Discharge power (W)	Exposure duration (s)	Relative cell viability of control (%)
Control [1)]	0	100.00 ± 0.00*
2.4 [1)]	20	105.42 ± 5.63[a]
	40	103.72 ± 6.49[a]
	60	84.45 ± 4.00[b]
5.6 [1)]	20	86.35 ± 4.04[b]
	40	72.73 ± 1.83[c]
	60	68.47 ± 3.19[cd]
9.1 [1)]	20	69.40 ± 4.17[cd]
	40	57.11 ± 3.10[ef]
	60	53.41 ± 3.43[efg]
12.5 [1)]	20	61.37 ± 5.55[de]
	40	50.31 ± 2.47[fg]
	60	47.55 ± 3.97[g]
Discharge power (W) [2)]		
2.4		97.86 ± 11.64
5.6		75.85 ± 8.75
9.1		59.97 ± 8.19
12.5		53.08 ± 7.74
Exposure duration (s) [3)]		
20		80.64 ± 18.39
40		70.97 ± 21.88
60		63.47 ± 15.43
P-value		
Discharge power		<0.0001
Exposure duration		0.095
Power × Duration		<0.0001

[1)] Data are represented as the mean ± SD of 3 replicates (n=3).

[2)] Data are represented as the mean ± SD of 9 replicates (n=9).

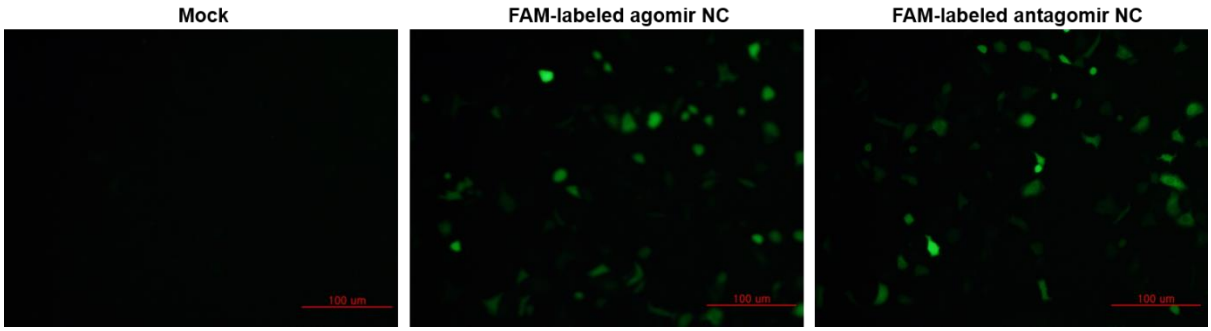
[3)] Data are represented as the mean ± SD of 12 replicates (n=12).

Within the same column, different lowercase letters indicate significant differences (P<0.05). *, different from all treatment groups (P<0.05).

Appendix D Sequences for microRNA (miRNA) agomir and antagomir

MiRNA name	Sequence (5'to3')
Agomir NC	F: UUCUCCGAACGUGUCACGUTT R: ACGUGACACGUUCGGAGAATT
Antagomir NC	F: CAGUACUUUUGUGUAGUACAA
miR-7450 agomir	F: UCUGUUCUUAAGGAGGCUGAGGC R: CUCAGCCUCCUUAAGAACAGAUU
miR-7450 antagomir	F: GCCUCAGCCUCCUUAAGAACAGA
miR-100 agomir	F: AACCCGUAGAUCCGAACUUGUG R: CAAGUUCGGAUCUACGGGUUUU
miR-100 antagomir	F: CACAAGUUCGGAUCUACGGGUU

NC, negative control.



Appendix E miRNA transfection efficiency in SCs. Chicken SCs were transfected with mock (Lipofectamine® RNAiMAX Regent only), carboxyfluorescein (FAM)-labeled miRNA agomir NC, and FAM-labeled antagomir NC. Imaging of SCs with the green fluorescence showed successful transfected cells. Scale bar: 100 μm.

Appendix F Primer sequences for the RT-PCR

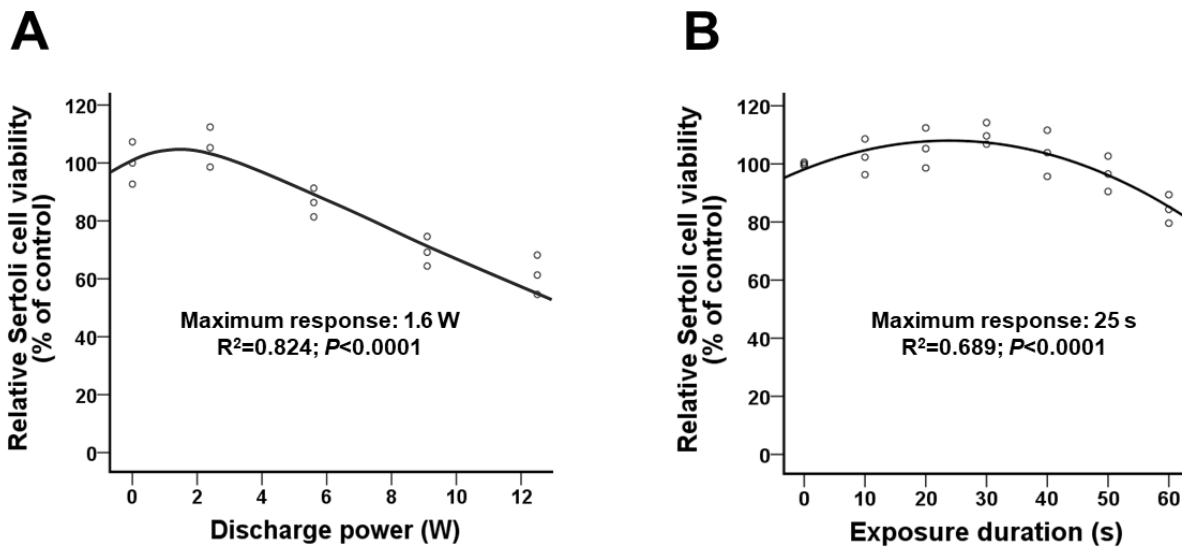
Gene	Sequence number	Sequence position	Product length (bp)	Annealing Temperature (°C)	Sequence (5'to3')
β-actin	NM_205518.1	625-818	194	57	F: GTGCGTGACATCAAGGAGAAGC R: CCACAGGACTCCATACCCAAGA
NOX4	NM_001101829.1	28-157	130	57	F: CGAGGATCTCAGAAGGTTGC R: GAGCATTCACCAGATGAGCA
NRF2	NM_205117.1	484-619	136	57	F: AAAACGCTGAACCACCAATC R: GCTGGAGAAGCCTCATTGTC
KEAP1	KU321503.1	1227-1485	259	57	F: GTATCACAGCAGCGTGGAGA R: GGCGTAGATGCAGTTGTTGA
SOD	NM_205064.1	106-278	173	55	F: ATTACCGGCTTGTCTGATGG R: CCTCCCTTTGCAGTCACATT
CAT	NM_001031215.2	1067-1276	210	55	F: CTCATTCCAGTGGGCAAGAT R: GTAGGGGCAATTCACAGGAA
GPx	NM_001277853.2	353-474	122	55	F: ATGTTCGAGAAGTGCGAGGT R: ATGATGTACTGCGGGTTGGT
PRDX1	NM_001271932.1	358-545	188	56	F: ACAAGGTGGTTTGGGCACTA R: TCTCATCAACAGAACGGCCA
PRDX3	XM_426543.5	414-551	138	56	F: TTTCACCTTTGTGTGCCCCA R: TTGCGCGGGGTATTTATCCA
PRDX4	XM_001233999.3	595-733	139	56	F: TGCACTTAGGGGCCTTTTCA R: TTCTCCATGCTTGTCCGTGT
PRDX6	NM_001039329.2	189-340	152	58	F: TGAGTTCAGCAAACGCAACG R: GCTCTCGGTCCTTATCAGCG
ATP5A1	NM_204286.1	1207-1364	158	57	F: GGTATCCGTCCAGCCATCAA R: GCATCCAAATCAGACCCAAACT
ATP5B	NM_001031391.2	482-637	156	57	F: GCCCCATCACAACGAAACAG R: CGCCTCCAAACAAACCAATC
ATP5C1	NM_001278096.1	272-411	140	57	F: ATTAAGGCACCCGAGGACAA R: ACTTCCTTCCCTGCATTGGA
AMPKα1	NM_001039603.1	1443-1632	190	58	F: AATCATTGAAACGAAGTCTGGGA R: TGTATGACTGCCTGGTCTTGGA
mTOR	XM_417614.4	119-309	191	57	F: TGAAGGGGTCAAGGCAATCC R: GGCGAGCAGTGGTTGTGGAT
U6	NR_003027.2	66-85	20	60	F: CGCAAGGATGACACGCAAAT
miR-7450	MI0024118	1-20	20	60	F: TCTGTTCTTAAGGAGGCTGA
miR-100	MI0001258	13-32	20	60	F: AACCCGTAGATCCGAACTTG

NOX4, nicotinamide adenine dinucleotide phosphate oxidase 4; *NRF2*, nuclear factor erythroid 2-related factor 2; *KEAP1*, kelch-like ECH-associated protein 1; *SOD*, superoxide dismutase; *CAT*,

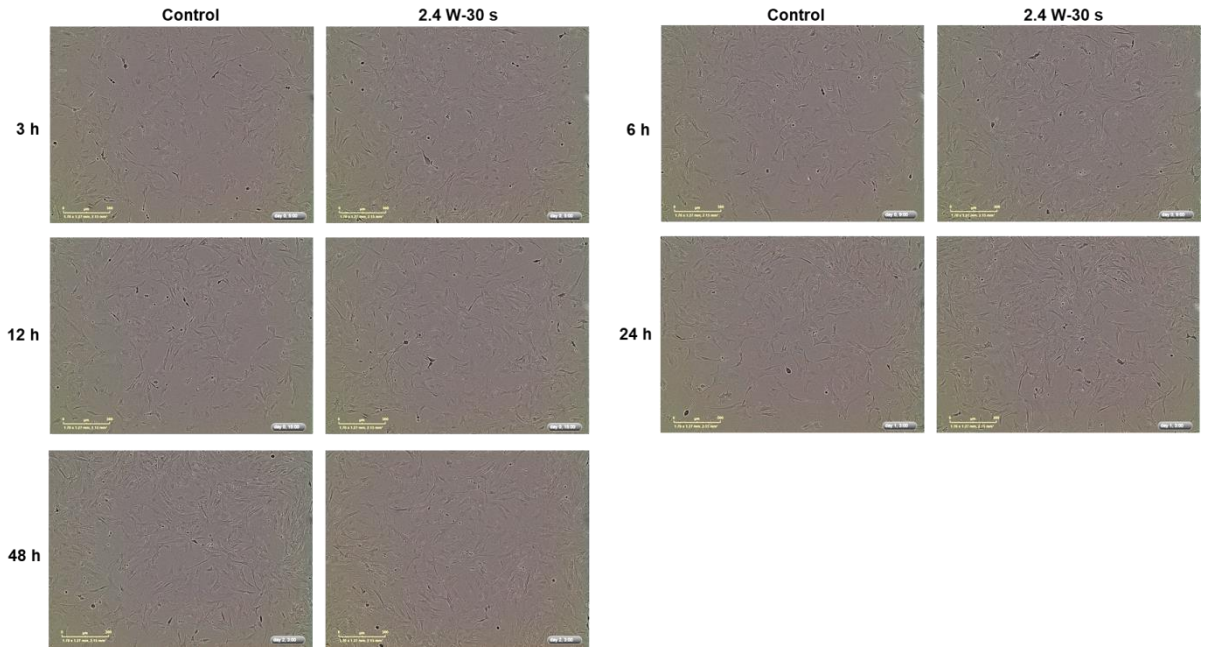
catalase; *GPx*, glutathione peroxidase; *PRDX*, peroxiredoxin; *ATP5A1*, ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1; *ATP5B*, ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide; *ATP5C1*, ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1; *AMPKα1*, adenosine monophosphate-activated protein kinase α1; *mTOR*, mammalian target of rapamycin.

Appendix G Primary and secondary antibodies used in study and their respective dilutions

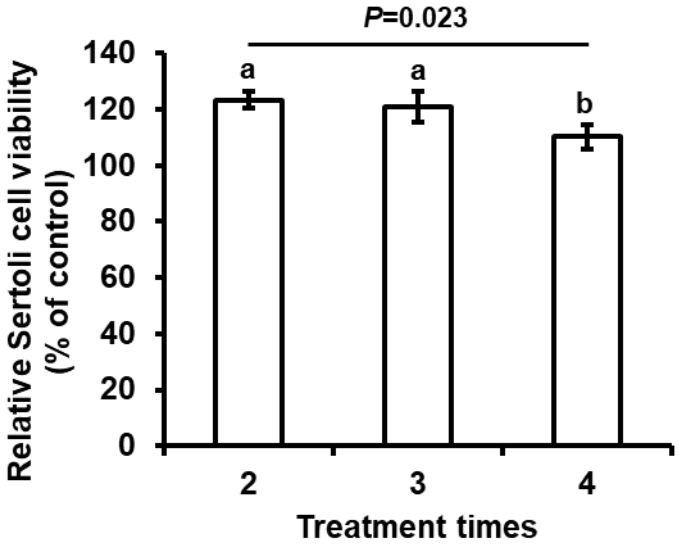
	Target	Name of antibody	Source and reference	Species raised in; clonality	Dilution used
Primary antibodies	NRF2	Anti-NRF2	Santa Cruz Biotechnology, Dallas, Texas, USA	Mouse; monoclonal	1:200
	KEAP1	Anti-KEAP1	Santa Cruz Biotechnology	Mouse; monoclonal	1:200
	PRDX4	Peroxiredoxin 4	LifeSpan BioSciences, Seattle, WA, USA	Mouse; monoclonal	1:200
	ATP5A	Anti-ATP5A	Abcam	Mouse; monoclonal	1:250
	p-AMPKα1	Anti-phospho-AMPK alpha1 (Thr172)	Cell Signaling Technology, Danvers, MA, USA	Rabbit; polyclonal	1:1,000
	AMPKα1	Anti-AMPK alpha1	Cell Signaling Technology	Rabbit; polyclonal	1:1,000
	p-mTOR	Anti-phospho-mTOR (Ser2448)	Abcam	Rabbit; polyclonal	1:1,000
	mTOR	Anti-mTOR	Abcam	Rabbit; polyclonal	1:1,000
	Beta-actin	Anti-beta actin	Santa Cruz Biotechnology	Rabbit; polyclonal	1:1,000
Secondary antibodies	Goat IgG	Anti-rabbit IgG H&L (HRP)	Abcam	Goat; polyclonal	1: 5,000
	Goat IgG	Anti-mouse IgG-HRP	Santa Cruz Biotechnology	Goat; polyclonal	1: 5,000



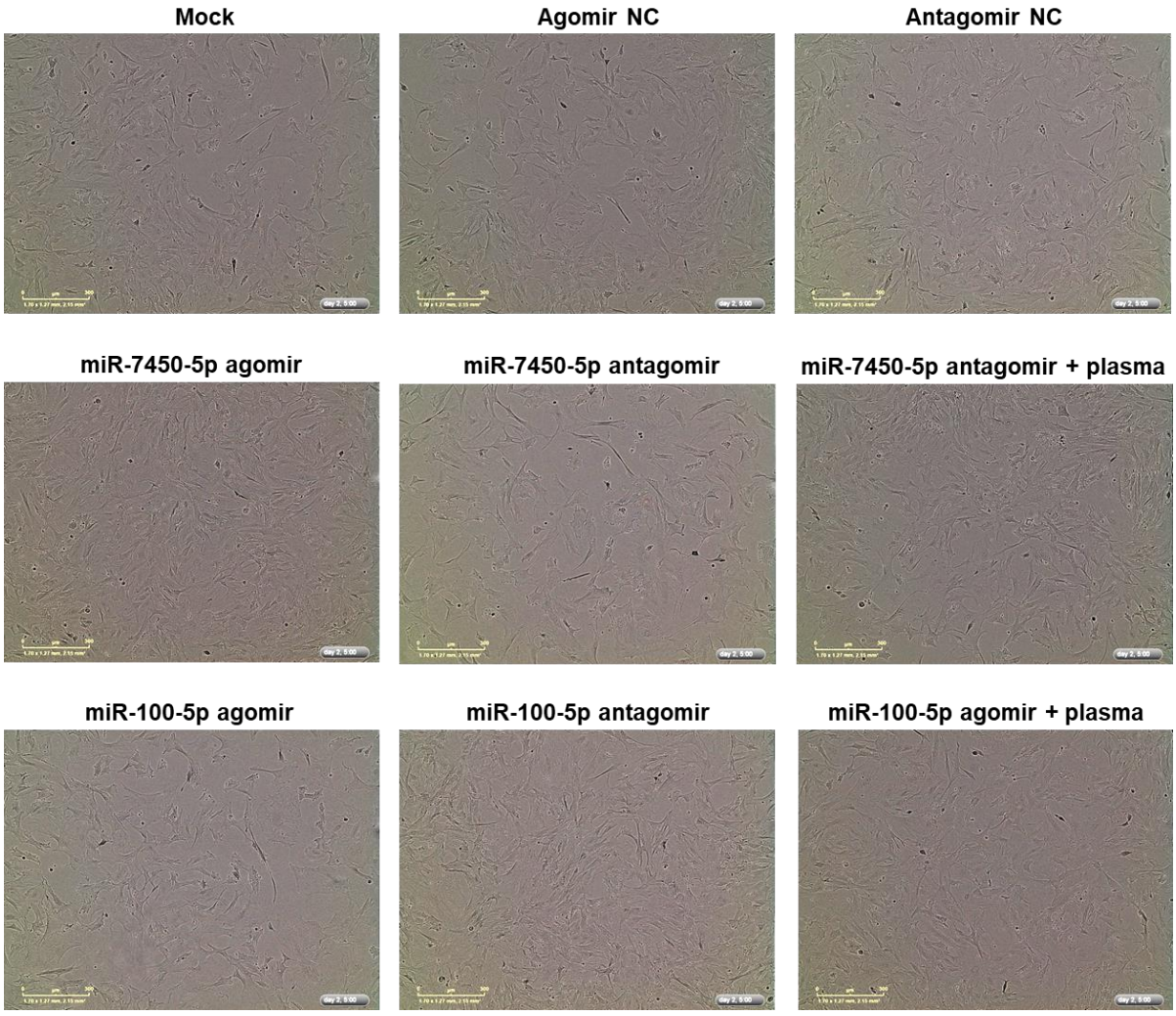
Appendix H Relations between cold plasma exposure and relative SC viability (Exp. 2 (2) and (3)). (A) Relations between discharge power and relative SC viability. (B) Relations between exposure duration and relative SC viability.



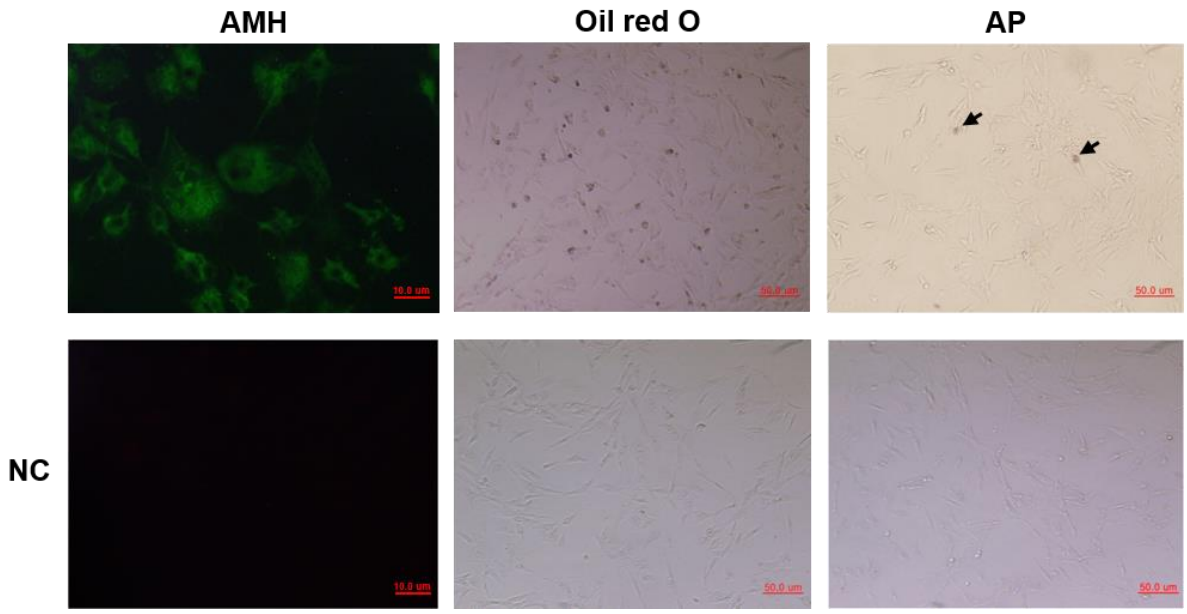
Appendix I Growth status of SCs exposed at 2.4 W of cold plasma for 30 s twice with different interval times (Exp. 2 (5)). Scale bar: 300 μm.



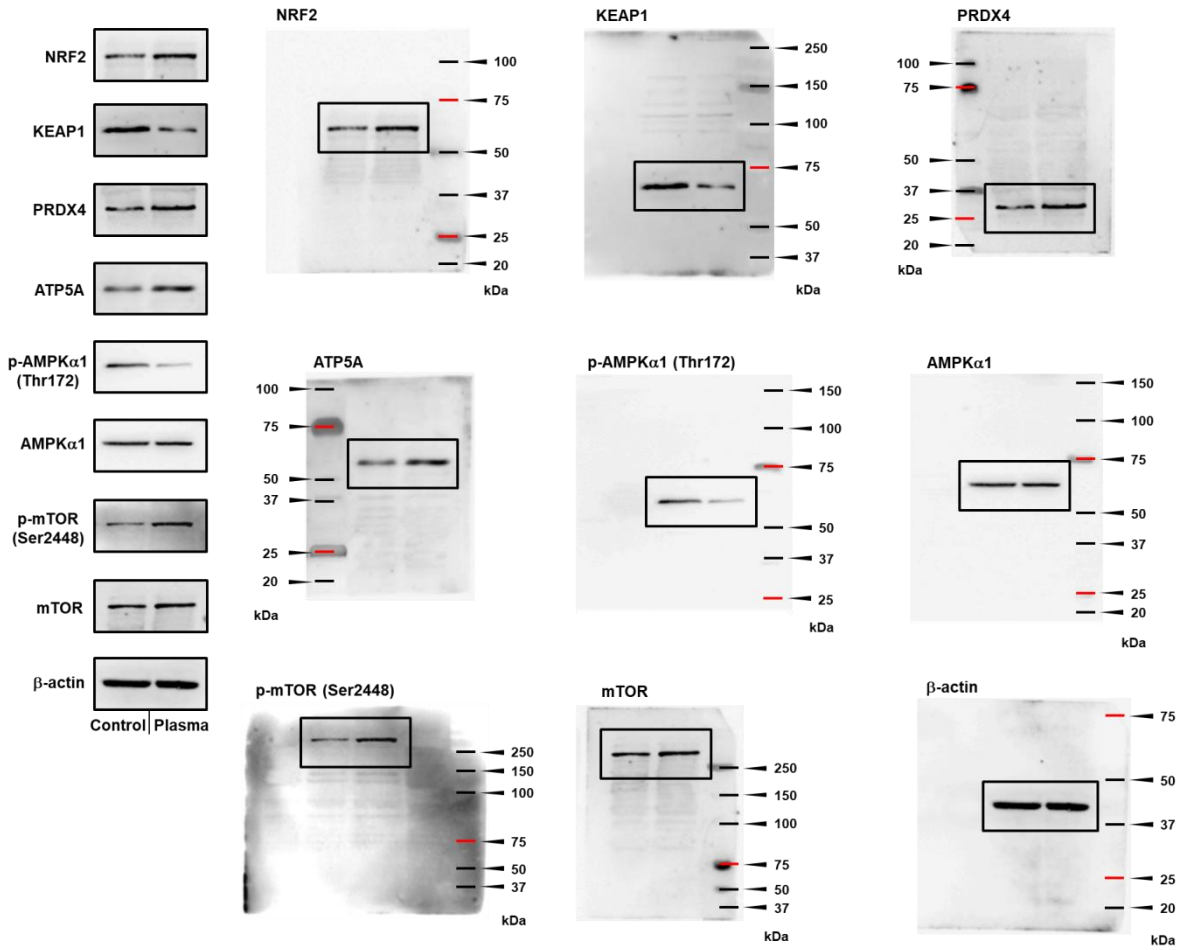
Appendix J Relative viability of SCs exposed to 2.4 W of cold plasma for 30 s with an interval time of 6 h twice, three times or four times (Exp. 2 (6)). Data are represented as the mean ± SD of 3 replicates (n=3). Different lowercase letters indicate significant differences (P<0.05).



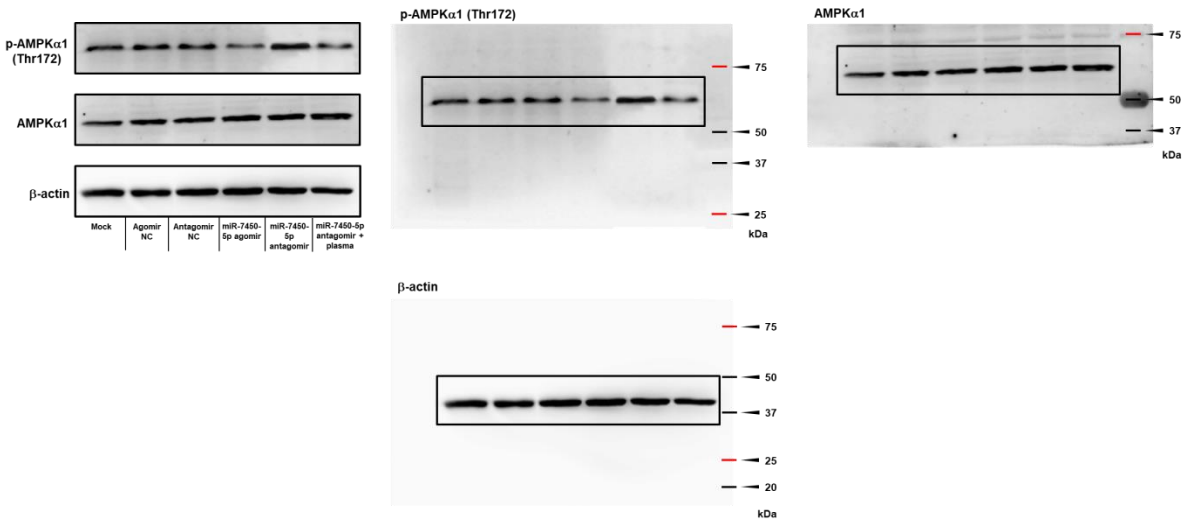
Appendix K Growth status of SCs transfected with agomir and antagomir of miR-7450-5p and miR-100-5p, and miR-7450-5p antagomir and miR-100-5p agomir groups treated with 2.4 W of cold plasma for 30 s twice with an interval time of 6 h (Exp. 6). Scale bar: 300 μm.



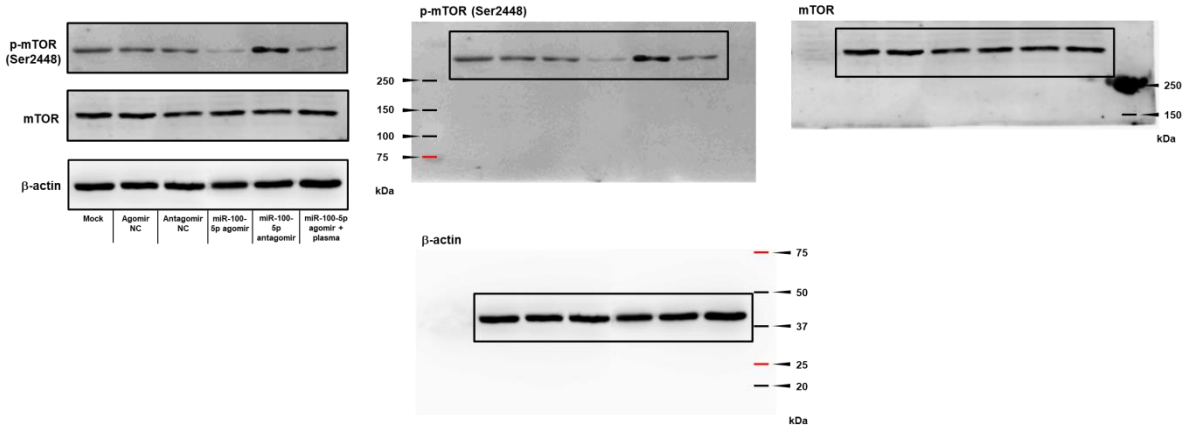
Appendix L Anti-Mullerian hormone (AMH), oil red O, and alkaline phosphatase (AP) staining of immature chicken SCs cultured *in vitro* and their negative controls (Exp. 1). For AMH staining, scale bar: 10 μm; for oil red O staining, scale bar: 50 μm; for AP staining, scale bar: 50 μm.



Appendix M Western blot analysis of the protein levels of NRF2, KEAP1, PRDX4, ATP5A, p-AMPKα1 (Thr172), AMPKα1, p-mTOR (Ser2448), and mTOR in SCs exposed to 2.4 W of cold plasma for 30 s twice with an interval time of 6 h (Exp. 3, 4, 5). Uncropped immunoblot scans for Fig.5-A. The grouping of gels/blots cropped from different gels. All blots were visualized with 5 min exposure time.



Appendix N Western blot analysis of the protein levels of p-AMPKα1 (Thr172) and AMPKα1 in SCs trasfected with miR-7450-5p agomir and antagomir in the presence or absence of double cold plasma treatment at 2.4 W for 30 s with an interval time of 6 h (Exp. 8). Uncropped immunoblot scans for Fig.11-A. The grouping of gels/blots cropped from different gels. All blots were visualized with 5 min exposure time.



Appendix O Western blot analysis of the protein levels of p-mTOR (Ser2448) and mTOR in SCs transfected with agomir and antagomir of miR-100-5p, and miR-100-5p agomir group treated with 2.4 W of cold plasma for 30 s twice with an interval time of 6 h (Exp. 8). Uncropped immunoblot scans for Fig.11-C. The grouping of gels/blots cropped from different gels. All blots were visualized with 5 min exposure time.