



Article

Radiofrequency Treatment Attenuates Age-Related Changes in Dermal–Epidermal Junctions of Animal Skin

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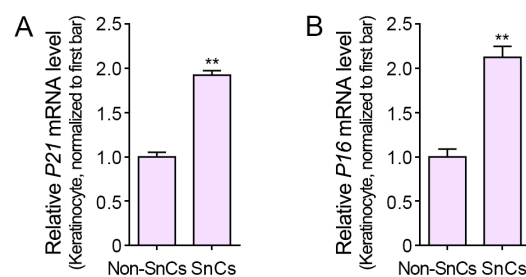


Figure S1. Expression of *P21* and *P16* of senescence marker in non-senescent and senescent keratinocytes. **(A,B)** PCR analysis of *P21* and *P16* in keratinocytes. Data are presented as mean \pm SD of three independent experiments. **, $p < 0.01$ vs first bar. CON, control; PCR, polymerase chain reaction; SnCs, senescent; SD, standard deviation.

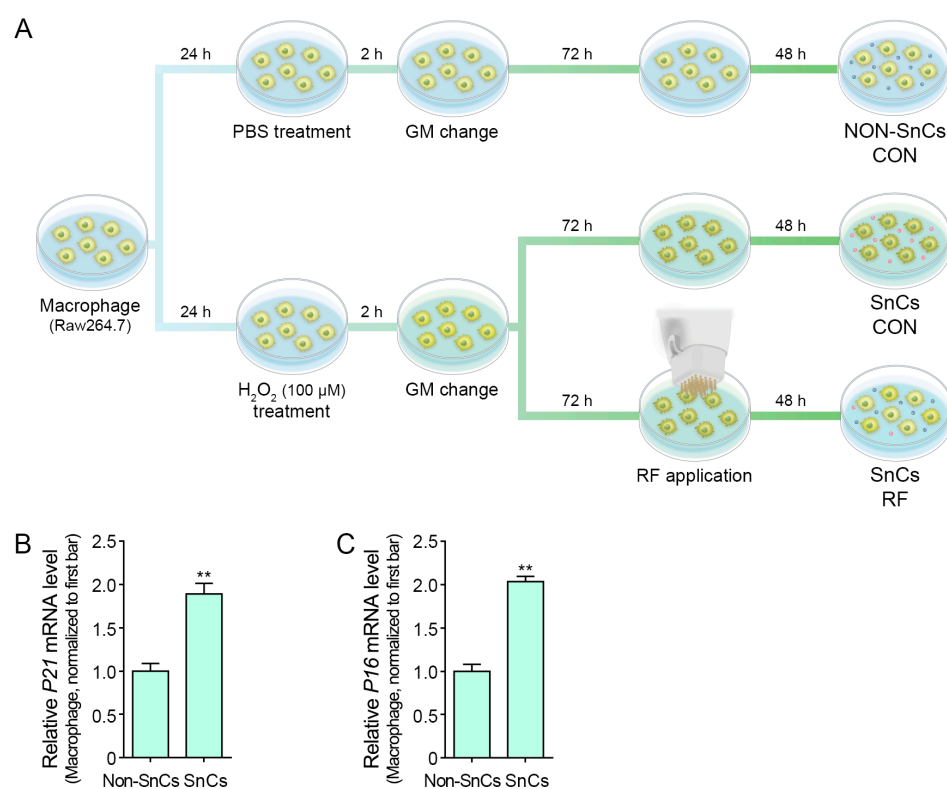


Figure S2. Schematic illustrating H₂O₂-induced senescence in macrophages for evaluation of RF treatment and expression of *P21* and *P16*. **(A)** Schematic representation of RF treatments administered to senescent macrophages. **(B,C)** PCR analysis of *P21* and *P16* in macrophages. Data are presented as mean \pm SD of three independent experiments. **, $p < 0.01$ vs first bar. CON, control; GM, growth medium; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RF, radiofrequency; SnCs, senescent; SD, standard deviation.

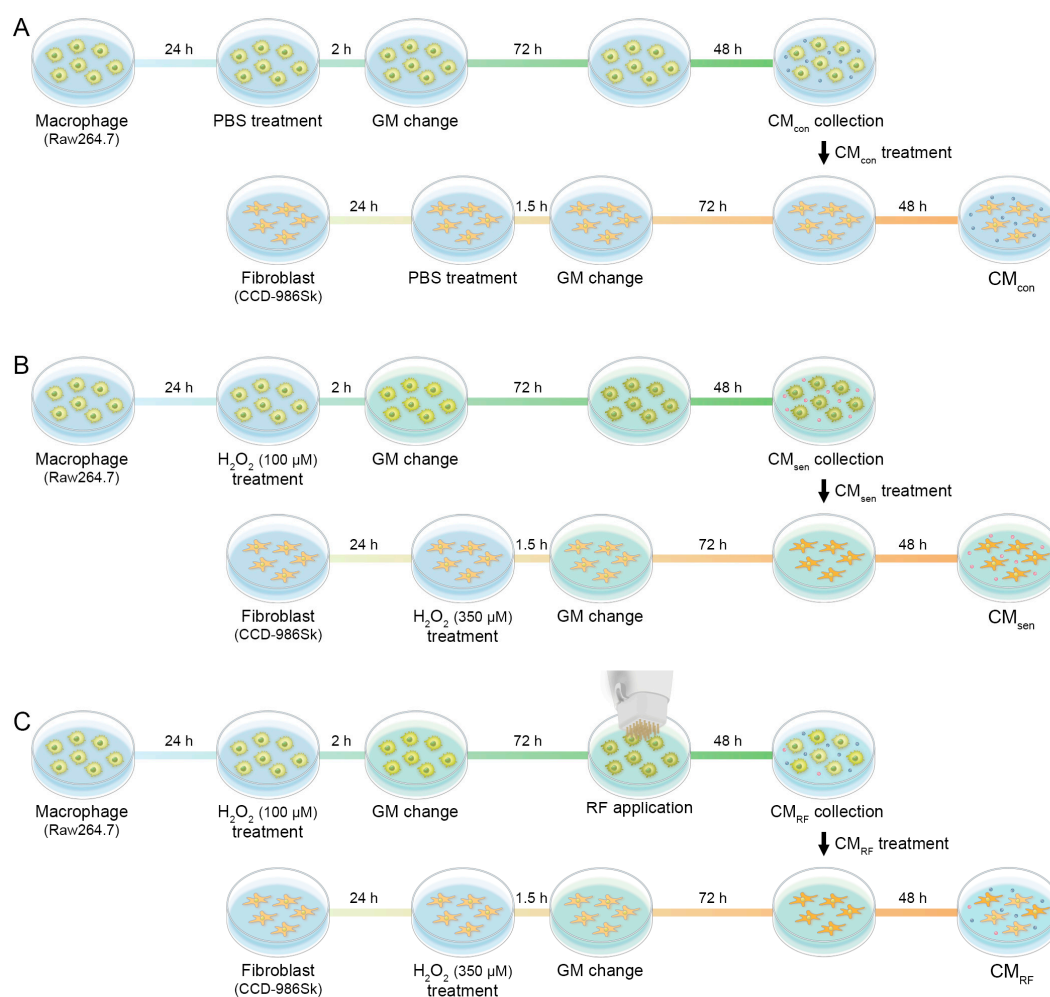


Figure S3. Schematic illustrating H₂O₂-induced senescence in fibroblasts for evaluation of RF treatment. **(A)** Schematic representation of non-senescent fibroblasts influenced by non-senescent macrophages. **(B)** Schematic representation of senescent fibroblasts influenced by senescent macrophages. **(C)** Schematic representation of senescent fibroblasts influenced by RF-treated senescent macrophages. CM_{con}, conditioned media from non-senescent macrophages; CM_{RF}, conditioned media from RF-treated senescent macrophages; CM_{sen}, conditioned media from senescent macrophages; GM, growth medium; PBS, phosphate-buffered saline; RF, radiofrequency.

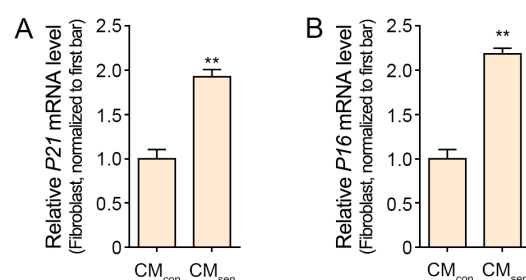


Figure S4. Expression of *P21* and *P16* of senescence marker in non-senescent and senescent fibroblasts. **(A,B)** PCR analysis of *P21* and *P16* in fibroblasts. Data are presented as mean \pm SD of three independent experiments. **, $p < 0.01$ vs first bar. CM_{con}, conditioned media from non-senescent macrophages; CM_{sen}, conditioned media from senescent macrophages; PCR, polymerase chain reaction; SnCs, senescent; SD, standard deviation.

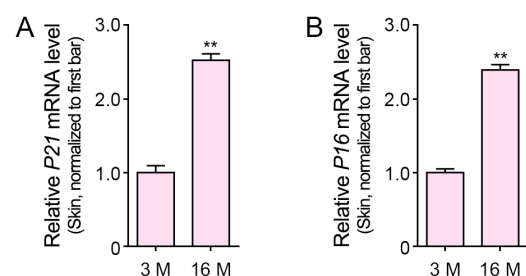


Figure S5. Expression of *P21* and *P16* of senescence marker in young and aged mice skin. **(A,B)** PCR analysis of *P21* and *P16* in 3-month-old mice and 16-month-old mice skins. Data are presented as mean \pm SD of three independent experiments. **, $p < 0.01$ vs first bar. 3 M, 3-month-old mice; 16 M, 16-month-old mice; PCR, polymerase chain reaction; SD, standard deviation.

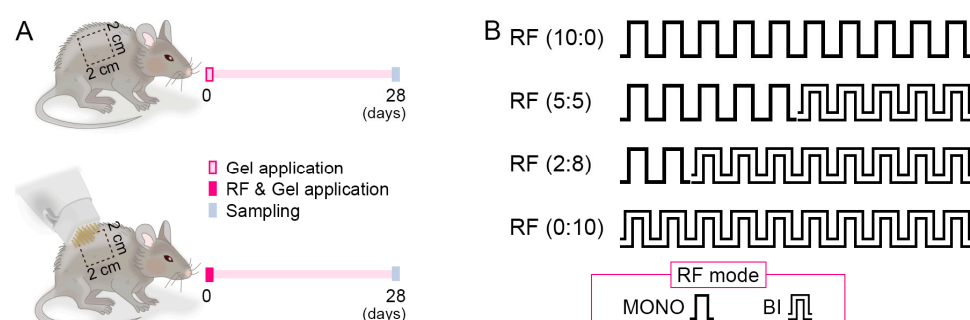


Figure S6. Schematic illustrating the experimental procedure of RF treatment in aged mice. **(A)** Schematic representation of RF treatment of 16-month-old mice. **(B)** Schematic representation of RF ratio. 10:0, 10 monopolar pulses only; 5:5, 5 monopolar pulses followed by 5 bipolar pulses; 2:8, 2 monopolar pulses followed by 8 bipolar pulses; 0:10, 10 bipolar pulses only; BI, bipolar; MONO, monopolar; RF, radiofrequency.

Table S1. List of antibodies for Western blot (WB), Enzyme-linked immunosorbent assay (ELISA), and Immunohistochemistry (IHC).

Antibody	Company	Catalog No.	Dilution rate		
			WB	ELISA	IHC
HSP47	Abcam	Ab77609	1:1,000		
HSP90	Gene Tex	GTX109753	1:5,000		
TGF- β	Abcam	Ab64715		1:5,000	
Collagen XVII	Affinity	DF2913	1:5,000		
Collagen IV	Invitrogen	PA1-28534		1:5,000	
Piezo1	Protein tech	15939-1-AP	1:500		
CD206	Santa cruz	Sc-58986	1:1,000		
IL-10	Fine Test	FNab04211		1:5,000	
pSMAD2/3	Cell signaling	8828	1:1,000		
SMAD2/3	Cell signaling	8685	1:1,000		
Nidogen	Santa cruz	sc-47773		1:5,000	1:100

Table S2. List of primers for quantitative reverse–transcription polymerase chain reaction.

Gene (Organism)	Primer sequences	
<i>ACTB (human)</i>	Forward	5'-CTC GCC TTT GCC GAT CC-3'
	Reverse	5'-TCT CCA TGT CGT CCC AGT TG-3'
<i>P21 (human)</i>	Forward	5'-GGA GAC TCT CAG GGT CGA AAA-3'
	Reverse	5'-GCT TCC TCT TGG AGA AGA TCA G-3'
<i>P16 (human)</i>	Forward	5'-CAC TTT CCT GGG CAA CAA ATA-3'
	Reverse	5'-CTT GCG GTC ATC ATC GTA GTT-3'
<i>Actb (mouse)</i>	Forward	5'-AAA GCC TAT TTC TGC CAG GAC-3'
	Reverse	5'-TCA TAG GTC ACG TAG CCC ACT-3'
<i>P21 (mouse)</i>	Forward	5'-ACC AGA GGC AGT AAC CAT GC-3'
	Reverse	5'-TGC TTC TAC AAA CCC ACA AAT G-3'
<i>P16 (mouse)</i>	Forward	5'-GGT TCT TGG TCA CTG TGA GGA T-3'
	Reverse	5'-GCA CGA ACT TCA CCA AGA AAA-3'