cancers

Supplementary Materials: Metformin Treatment Suppresses Melanoma Cell Growth and Motility Through Modulation of microRNA Expression

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Table S1. Summary of sequence reads and the detected miRNAs in four libraries.

Sample	Total Reads	Clead Reads(%)	miRNAs
A2058-C	11909638	11734194 (98.53%)	1155
A2058-Met	13141031	12904655 (98.20%)	1261
A375-C	12376430	1194227 (96.53%)	1143
A375-Met	14184012	13965787 (98.46%)	1229

Table S2. Target candidates of miR-192-5p and miR-584-3p were identified using a microarray approach and a bioinformation approach.

Targets of miR-192-5p	Targets of miR-584-3p
EFEMP1	SCAMP3
CTH	PSMB1
RPL4	TM4SF19
PPP1CA	CABP7
SDS	LPCAT3
KIAA1467	IMP4
ATF3	TYR
PABPC4	BAX
GOT1	GDF15
CSF1	RPL38
PIM1	HLA-DQB2
COPS7A	EI24
PDCD7	DHRS12
SUPT4H1	PPP1R3F
CMTM6	

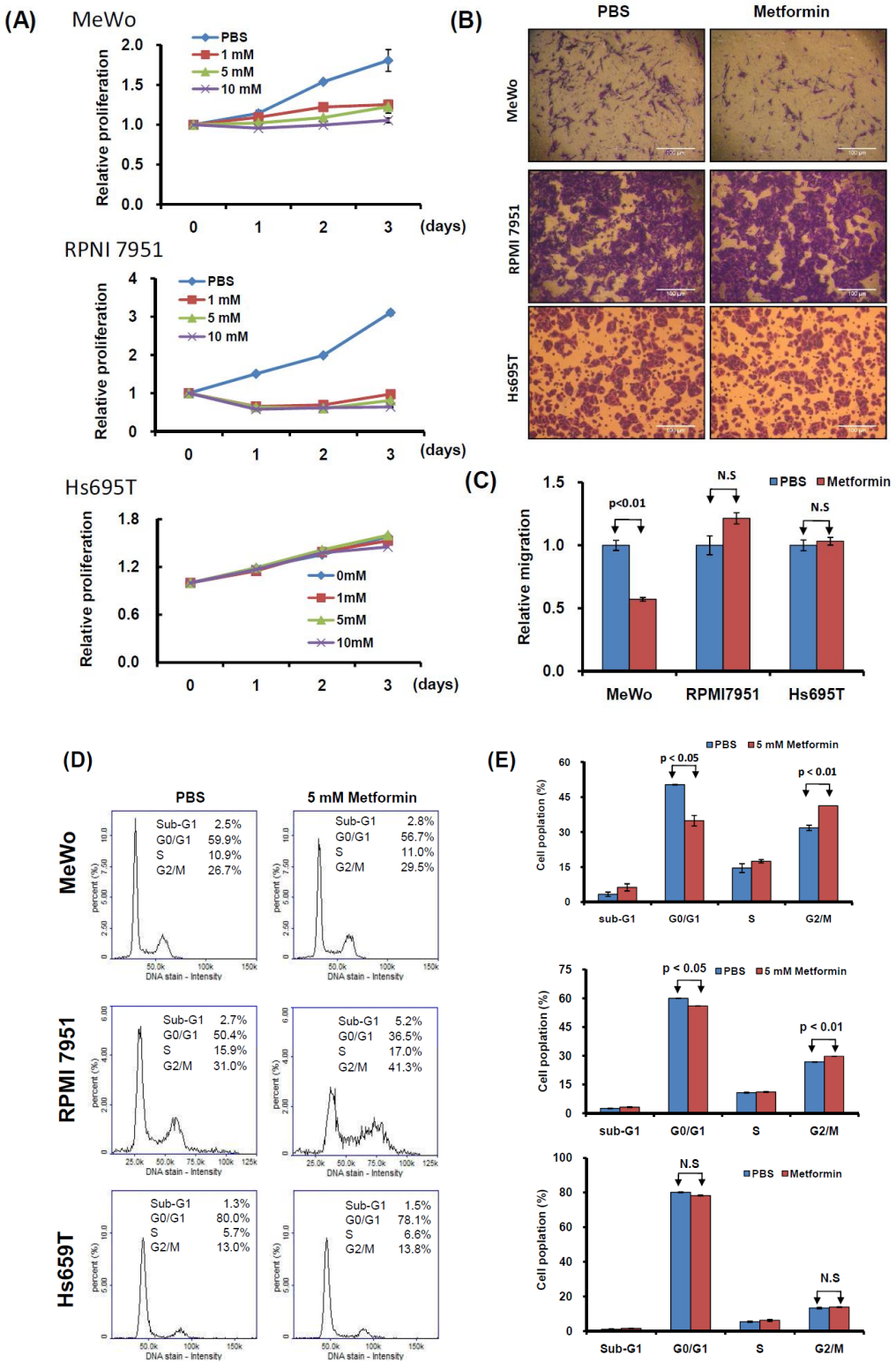

Figure S1. Proliferation and motility of melanoma cells were suppressed after metformin treatment. (**A**) Cell growth was examined in the MeWo, RPMI7951, and Hs695T cells after metformin treatment by using the CellTiter-Glo One solution assay. (**B**) and (**C**) Cell migration assay was used to examine and quantify the MeWo, RPMI7951, and Hs695T cells treated with or without metformin (5 mM) for three days. (**D**) and (**E**) Cell cycle progression was examined and quantified in the MeWo, RPMI7951, and Hs695T cells treated with or without metformin.

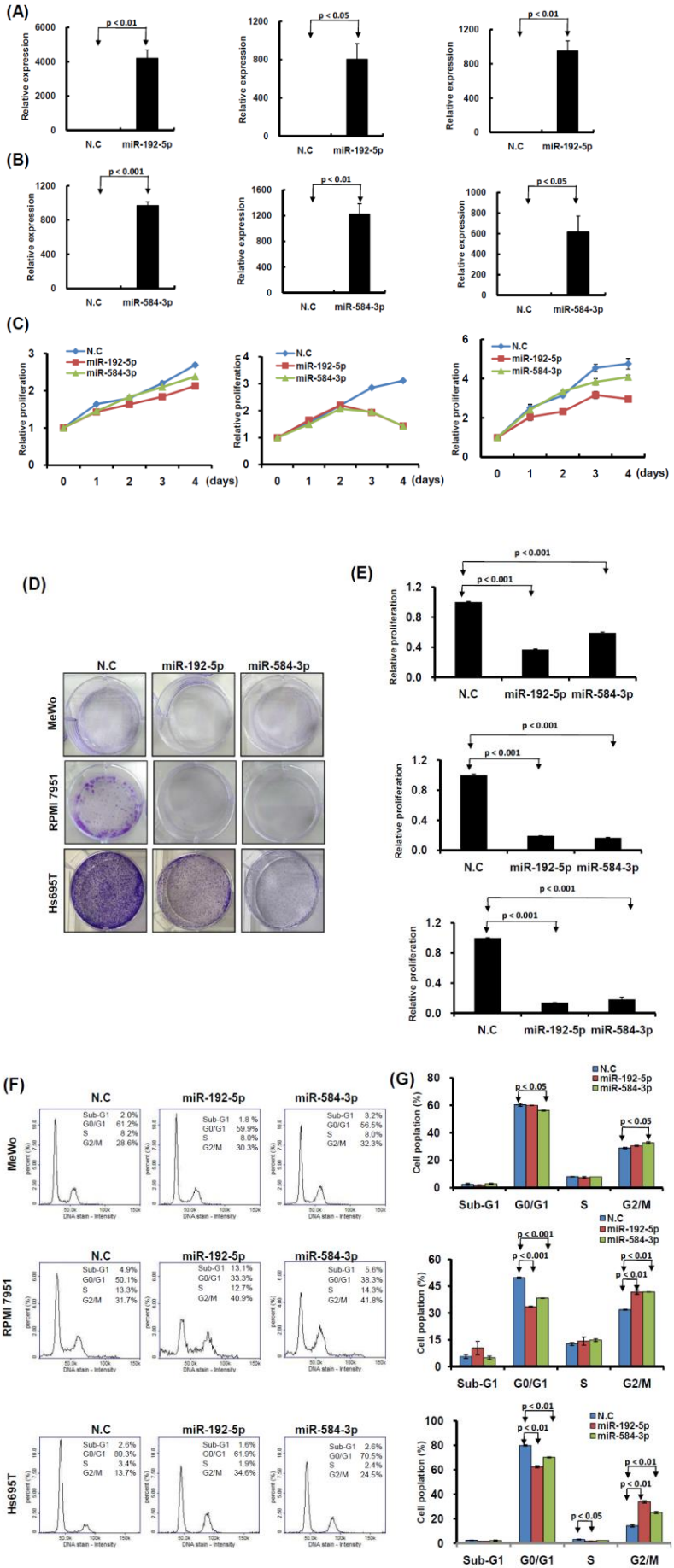
(A)
Relative expression
N.C
miR-192-5p
p < 0.01
p < 0.05
(B)
miR-584-3p
p < 0.001
(C)
Relative proliferation
(days)
(D)
MeWo
RPMI 7951
Hs695T
(E)
(F)
Sub-G1
G0/G1
S
G2/M
(G)
Cell poplation (%)

Figure S2. Growth of melanoma cells was suppressed after transfection of miR-192-5p and miR-584-3p mimic candidates. (**A**) and (**B**) After transfection of miRNA mimics, the relative expression levels of miR-192-5p and miR-584-3p were examined in the MeWo, RPMI7951, and Hs695T cells through real-time PCR. (**C**) After miR-192-5p, miR-584-3p, and control, respectively, were transfected into the melanoma cells, cell proliferation was assessed. (**D**) and (**E**) Colony formation was examined with crystal violet and quantified using OD595 nm. (**F**) and (**G**) Cell cycle progression was examined and quantified in the MeWo, RPMI7951, and Hs695T cells after miR-192-5p or miR-584-3p transfection.

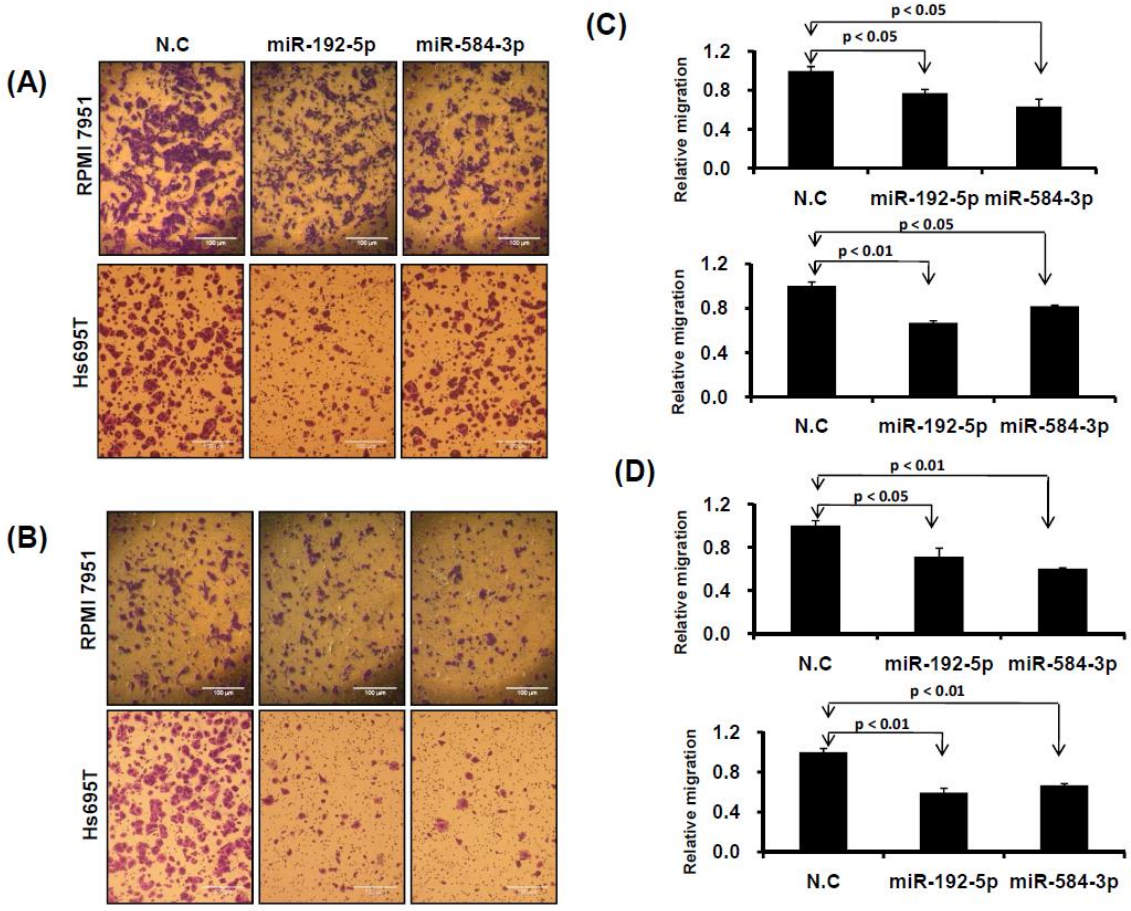

Figure S3. Motility of melanoma cells was suppressed after transfection with miR-192-5p and miR-584-3p mimic candidates. (**A**) and (**B**) The cell migration and invasion ability were examined in three melanoma cells, MeWo, RPMI7951, and Hs695T, after miR-192-5p or miR-584-3p transfection. (**C**) and (**D**) Then, the numbers of migrating or invading cells were quantified by counting three different fields under a phase-contrast microscope. The cell photographs from a representative experiment are presented, and the graph data were quantified using Ascent software. Data are reported as colonies compared with control (mean ± SD).

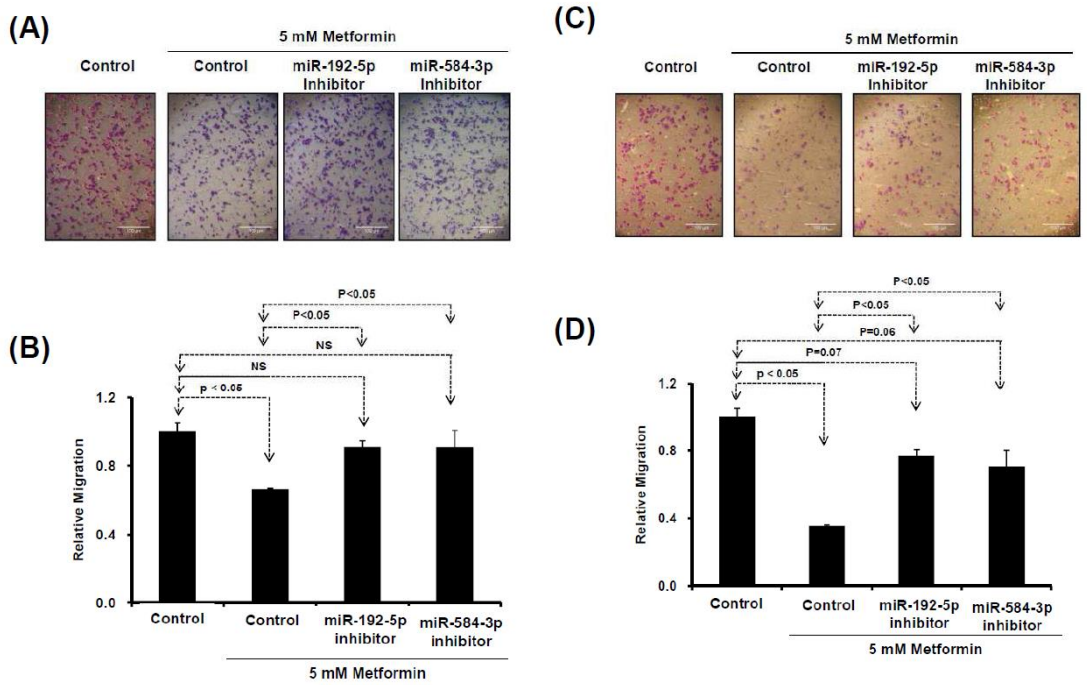

Figure S4. Partial improvement made by miR-192-5p and miR-584-3p inhibitors in the metformin-induced suppression of melanoma cell motility. (**A**) and (**C**) Migration ability was examined using a Transwell assay in A2058 and A375 cells with miR-192-5p and miR-584-3p inhibitor transfection. After transfection with the miR-192-5p inhibitor, miR-584-3p inhibitor, or scramble control for 24 h, the cells were treated with or without metformin and then subjected to a Transwell assay. Migrating cells were stained with a crystal violet solution. (**B**) and (**D**) Number of migrating cells was determined by counting three fields under a phase-contrast microscope. Photographs of the cells from a representative experiment are presented, and the graph data were quantified using Ascent software. Data are reported as colonies compared with the control (mean ± SD).

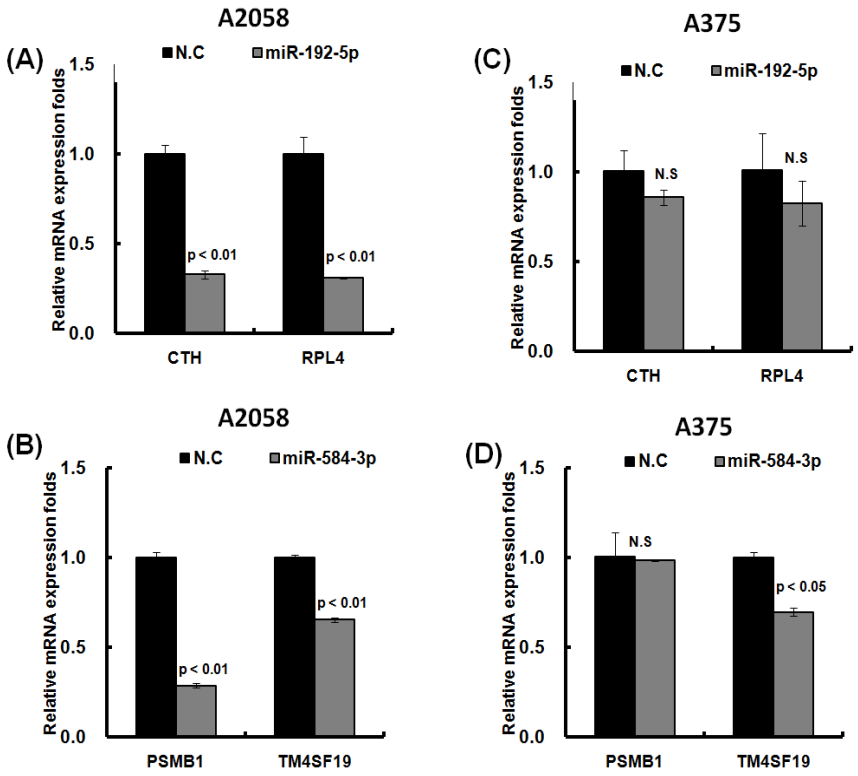

Figure S5. Expression levels of CTH, RPL4, PSMB1, and TM4SF19 were examined through real-time PCR in two melanoma cells with miR-192-5p (**A** and **B**) and miR-584-3p (**C** and **D**) transfection.