SUPPLEMENTAL MATERIAL

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ONLINE METHODS

Animal experiments

During the observation period, mice received water and food *ad libitum.* Mice were sacrificed in the acute phase (14 days) after irradiation through CO_2 inhalation, exsanguinated by heart puncture and perfused with 4°C cold phosphate-buffered saline. Organs were snap frozen in dry ice-containing ethanol and stored at -80°C. Vascular tissue from the irradiated area (carotid arteries, thoracic aorta, and aortic arch) was embedded in Optimal Cutting Temperature (OCT) Cryomount compound (Histolab, Gothenburg, Sweden), snap frozen and stored at -80°C.

RNA isolation and cDNA synthesis

Cells were lysed in 700 µL QIAzol/well (Qiagen, Hilden, Germany), scraped off cell culture plates with a cell scraper, and homogenized by pipetting up and down. Tissues were lysed in 700 µL QIAzol/tissue unit using mechanical homogenization. RNA was extracted using the Qiagen miRNeasy Mini (standard) or Micro (mouse carotid tissue and cells from 12-well plates) Kit according to the manufacturer's protocol (Qiagen). Total RNA concentration was assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). All RNA extracted from human tissue was tested for quality with a Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA). For miRNA analysis, total RNA was diluted in RNAse-free water to a concentration of 2 ng/µL. Five µL (10 ng) was used for miRNA complementary cDNA (cDNA) synthesis using the Taqman MicroRNA Reverse Transcription Kit with reverse transcriptase primers for the selected miRNAs (Online Table 1, all Thermo Fisher Scientific). For mRNA analysis, we used 300 or 1000 ng (100-200 ng for mouse carotid arteries) of total RNA for cDNA synthesis using the Taqman High Capacity cDNA Transcription Kit with random primers.

Soluble collagen assay

Cell supernatant was aspirated and stored at -80°C. Soluble collagen was measured with a soluble collagen assay kit (2BScientific, Oxfordshire, United Kingdom) according to the manufacturer's instructions.

Western blotting

Cells were washed with cold PBS (4°C), and lysed with RIPA cocktail containing RIPA lysis and extraction buffer, phosphatase- and protease inhibitors, EDTA and sodium azide (all Sigma-Aldrich, St. Louis, MO, USA). The cell lysate was aspirated and centrifuged at 14,000 rpm for 10 minutes, at 4°C. The supernatant was used for protein analysis. Protein concentrations were determined using the Bicinchoninic Acid assay (Thermo Fisher Scientific, Waltham, MA, USA). Protein samples were loaded on 12% Tris-Glycine gels (Thermo Fisher Scientific). Following electrophoresis and electrotransfer, the PVDF membrane (Bio-Rad, Hercules, CA, USA) was blocked with 5% bovine serum albumin before primary antibody incubation (detailed online table S4). After secondary antibody incubation, horseradish peroxidase activity was detected with an enhanced chemiluminescence (ECL) kit (Thermo Fisher Scientific) and analyzed using ImageJ software version 1.6.0_65.

Immunohistochemistry

We used standard biotin-streptavidin-horseradish peroxidase methods. Briefly, sections were de-paraffinized and endogenous peroxidase activity blocked in 0.3% hydrogen peroxide. For VCAM1 staining, heat-induced antigen retrieval was performed using Diva Decloaker (Biocare Medical, Concord, CA, USA). Sections were blocked for 1 hour with 1% goat normal serum (Vector Laboratories, Burlingame, CA, USA), and incubated with the primary antibody (all from Abcam, Cambridge, UK; See Online Table 4) overnight at 4°C. After secondary antibody incubation, slides were stained using the ABC Elite Kit (Vector Laboratories) followed by aminoethyl carbazole (AEC, Nichirei Biosciences, Tokyo, Japan) incubation. Nuclei were counterstained with hematoxylin (Sigma Aldrich, St. Louis, MO, USA).

Immunocytochemistry

For immunocytochemistry (ICC), cells were grown on UV-sterilized glass coverslips in 12 well plates. Cells were washed three times with cold (4°C) PBS, fixed in 4% paraformaldehyde for 10 minutes at room temperature (RT), and permeabilized by incubation with 0.2% Triton-X100 in PBS. After blocking in 3% bovine serum albumin, cells were incubated with the first antibody (anti-Caspase 3 or anti-Ki-67, both Abcam, Cambridge, UK, details see Online Table 4) at 4°C overnight. Antibodies were detected with subsequent fluorescent secondary antibodies (Alexa Fluor 488 or 594, Thermo Fisher Scientific), counterstained with Hoechst 33258 (Thermo Fisher Scientific, 1:10,000), and mounted. Cells were photographed using fluorescence microscopy on a confocal microscope (Leica SP5, Jena, Germany) equipped with a 40 × 1.4 lens and diode and argon lasers. Caspase-3 or Ki-67 positive cells were counted in six different, randomly chosen fields for each group.

Online Table 1. miRNA sequences, references, and corresponding Taqman assay ID

Name	Mature miRNA sequence	Reference	Assay ID
miR-29b	UAGCACCAUUUGAAAUCAGUGUU	[1]	000413
miR-125a	UCCCUGAGACCCUUUAACCUGUGA	[2]	002198
miR-126	UCGUACCGUGAGUAAUAAUGCG	[3]	002228
miR-143	UGAGAUGAAGCACUGUAGCUC	[4]	002249
miR-145	GUCCAGUUUUCCCAGGAAUCCCU	[4]	002278
miR-146a	UGAGAACUGAAUUCCAUGGGUU	[4, 5]	000468
miR-146b	UGAGAACUGAAUUCCAUAGGCU	[4]	001097
miR-155	UUAAUGCUAAUCGUGAUAGGGGU	[5]	002623
miR-221	AGCUACAUUGUCUGCUGGGUUUC	[6, 7]	000524
miR-222	AGCUACAUCUGGCUACUGGGU	[6, 7]	002276
miR-503	UAGCAGCGGGAACAGUUCUGCAG	[8]	001048

Online Table 2. Small RNA normalization control sequences and Taqman assay ID

Name	Sequence	Assay ID
cel-miR-39	UCACCGGGUGUAAAUCAGCUUG	000200
U6 snRNA	GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACG ATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATG ACACGCAAATTCGTGAAGCGTTCCATATTTT	001973
RNU48	GATGACCCCAGGTAACTCTGAGTGTGTCGCTGATGCCA TCACCGCAGCGCTCTGACC	001006
sno202	GCTGTACTGACTTGATGAAAGTACTTTTGAACCCTTTTC CATCTGATG	001232

Online Table 3. Genes and Taqman assay ID

Name	NCBI reference sequence	Assay ID
B4GALT5, human	NM_004776.3	Hs00408932_cn
COL1A1, human	NM_000088.3	Hs01076749_g1
DPP4, human	NM_001935	Hs00897391_m1
PTX3, human	NM_002852.3	Hs00173615_m1
RPLP0, human	NM_001002.3	Hs02992885_s1
Actb, mouse	NM_007393.5	Mm02619580_g1
Ptx3, mouse	NM_008987.3	Mm00477268_m1

Online Table 4. Abcam antibodies

Name	Catalog no.	Dilution	Secondary antibody dilution
DPP4	ab114033	1:500 (WB); 1:50 (IHC)	1:80,000 (WB); 1:200 (IHC)
KI67	ab15580	1:50 (ICC)	1:200 (ICC)
Mac2	ab53082	1:100 (IHC)	1:200 (IHC)
PTX3	ab90806	1:50 (WB, IHC)	1:10,000 (WB); 1:1,000 (IHC)
SMA	ab5694	1:100 (IHC)	1:200 (IHC)

ICC, immunocytochemistry. IHC, immunohistochemistry. WB, Western blotting.

References

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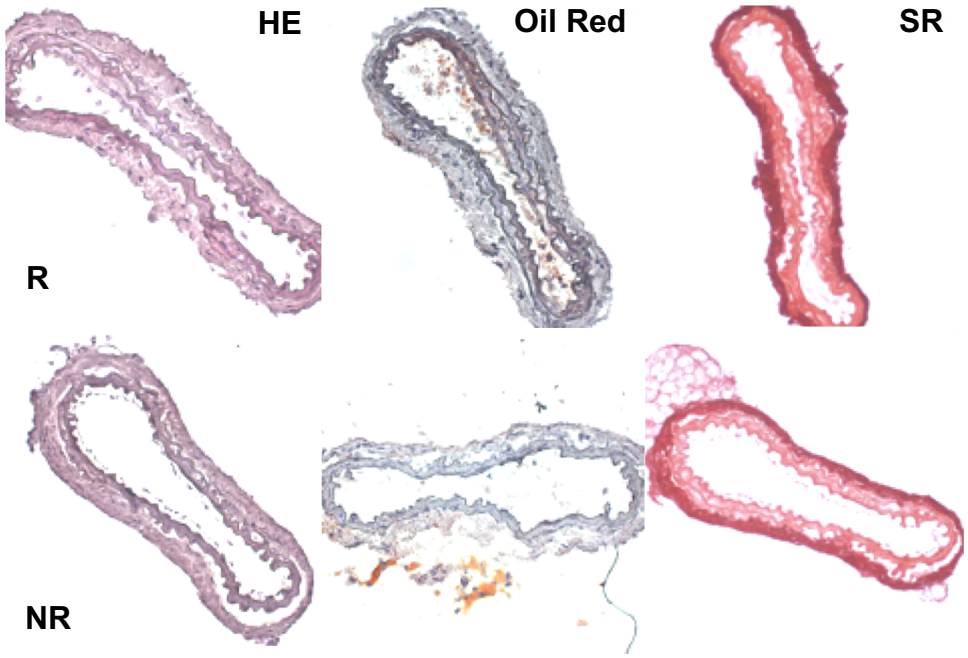
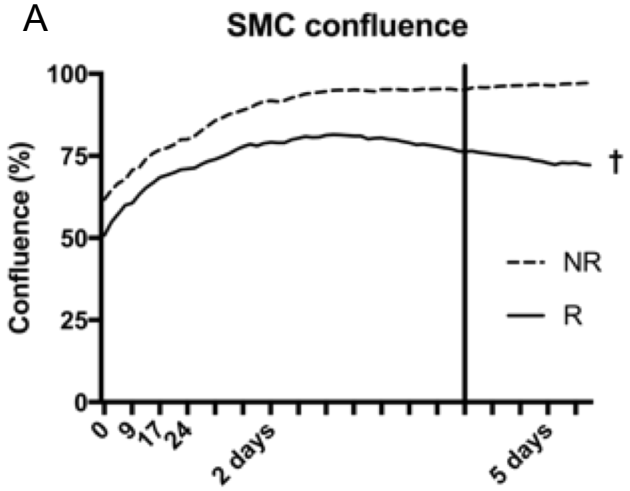
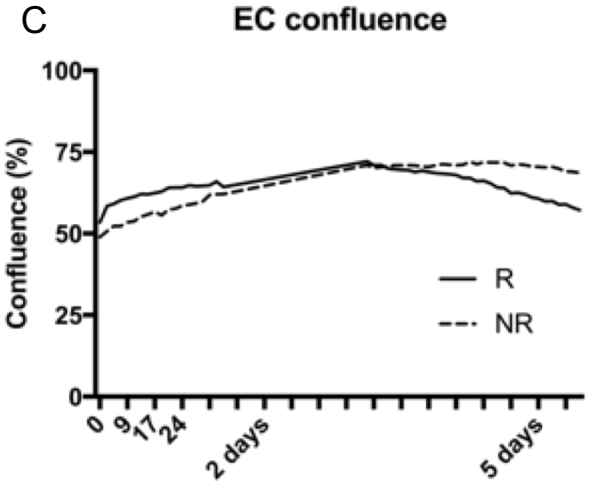
Online Figure 1.

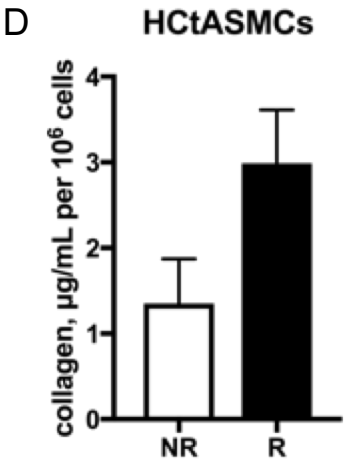


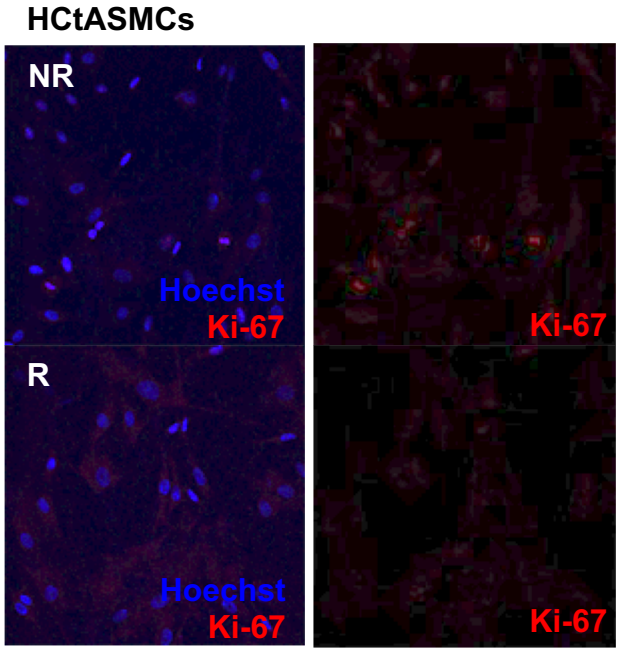
No apparent histological changes were observed in carotid arteries 10 weeks after irradiation. HE, hematoxylin and eosin; Oil Red, Oil Red O; SR, Sirius Red.

Online Figure 2.



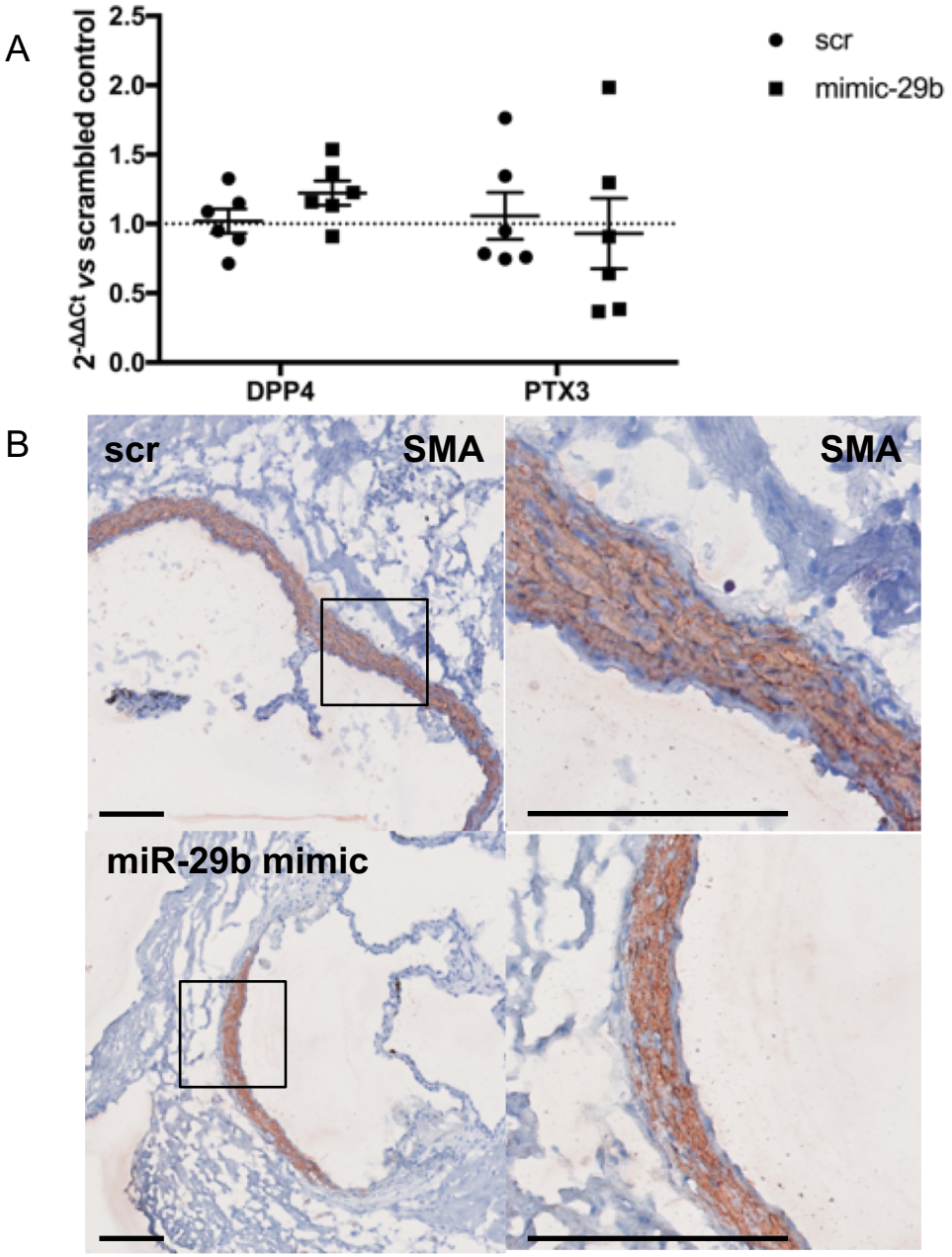






(A) Smooth muscle cell (SMC) growth was inhibited after irradiation with 2×2 Gy. On day 4 after irradiation (vertical line), the difference in confluence became significant.†$p<0.05$ in One-Way ANOVA with Holm-Šídák correction for multiple comparisons. **(B)** Irradiation hampered SMC proliferation as observed with immunocytochemical Ki-67 staining. *n*=6 in each group. Mean+SEM; **$p<0.01$ in Student's *t* test of radiated versus non-radiated. **(C)** Endothelial cell (EC) growth was not affected. **(D)** In supernatant of irradiated cells, collagen production showed an upward, albeit non-significant, trend. *n*=2 in each group. Mean+SEM. R, radiated; NR, non-radiated.

Online Figure 3.



(A) Dpp4 and Ptx3 gene expression was not significantly affected by miR-29 mimic treatment. **(B)** Smooth muscle actin (SMA) staining of aortic rings from irradiated mice receiving scrambled control (scr) or miR-29b mimics. Bars, 200 μm.