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P041

Inhibition of microRNA-221 by Estradiol Contributes to its Differential Effects on Smooth Muscle Cell Growth and Endothelial Cell Capillary Formation

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Objective

MicroRNAs play a key role in vascular remodeling associated with cardiovascular disease. MicroRNA-221 (miR-221) actively contributes to injury-induced neointima formation by inhibiting endothelial cell (EC) growth and promoting smooth muscle cell (SMC) growth. Since estradiol (E2) prevents neointimal thickening by differentially modulating EC and SMC growth, **we hypothesize that E2 mediates its vasoprotective actions by downregulating miR-221 expression and abrogating its effects on SMC and EC growth**.

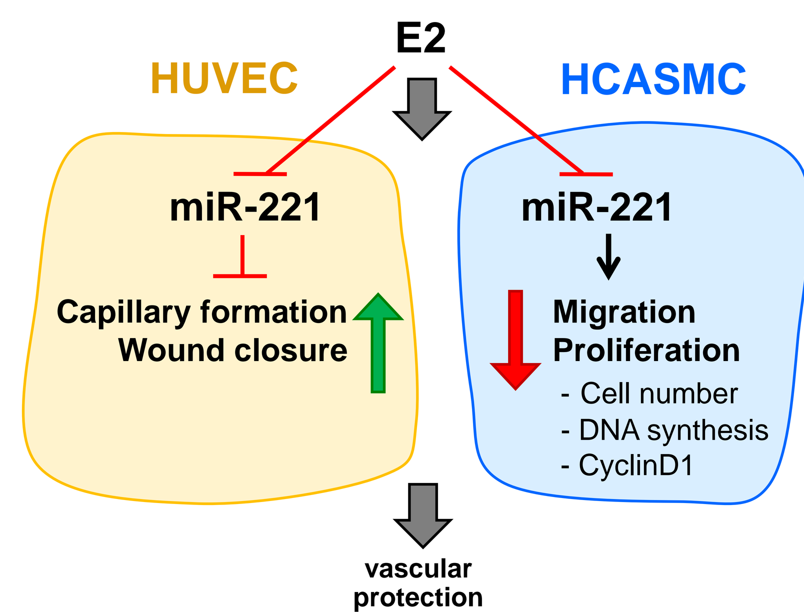
Methods

Human Umbilical Vein ECs (HUVECs) and Human Coronary Artery SMCs (HCASMCs) were treated with or without E2 (10-100nM) and PDGF-BB (20ng/ml) prior to RNA extraction and RT-qPCR. To assess the role of miR-221, both cell types were transfected with 25 nM miR-221 mimic and antimir or their respective controls. Cell counts and a BrdU ELISA kit were employed to study HCASMC proliferation. HCASMC migration was assessed using scratch wound assay. Matrigel capillary formation and scratch wound assay were used to investigate HUVEC activity. CyclinD1 protein expression was quantified by Western Blotting. Experiments were repeated three times in triplicates.

*p<.05 to respective control; §p<.05 as indicated.

Outcomes

Our findings provide the first evidence that E2 inhibits miR-221 production in HCASMCs and HUVECs and these effects contribute, in part, to the antimitogenic actions of E2 on HCASMCs and the capillary formation inducing effects of E2 in HUVECs. Modulation of miR-221 by E2 represents a novel mechanism by which E2 may mediate its differential effects on SMC and EC growth and confer vascular protection



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Results

1. miR-221 Antimir Mirrors the Actions of E2 on HUVEC Activity, whereas miR-221 Mimic has Opposite Effects

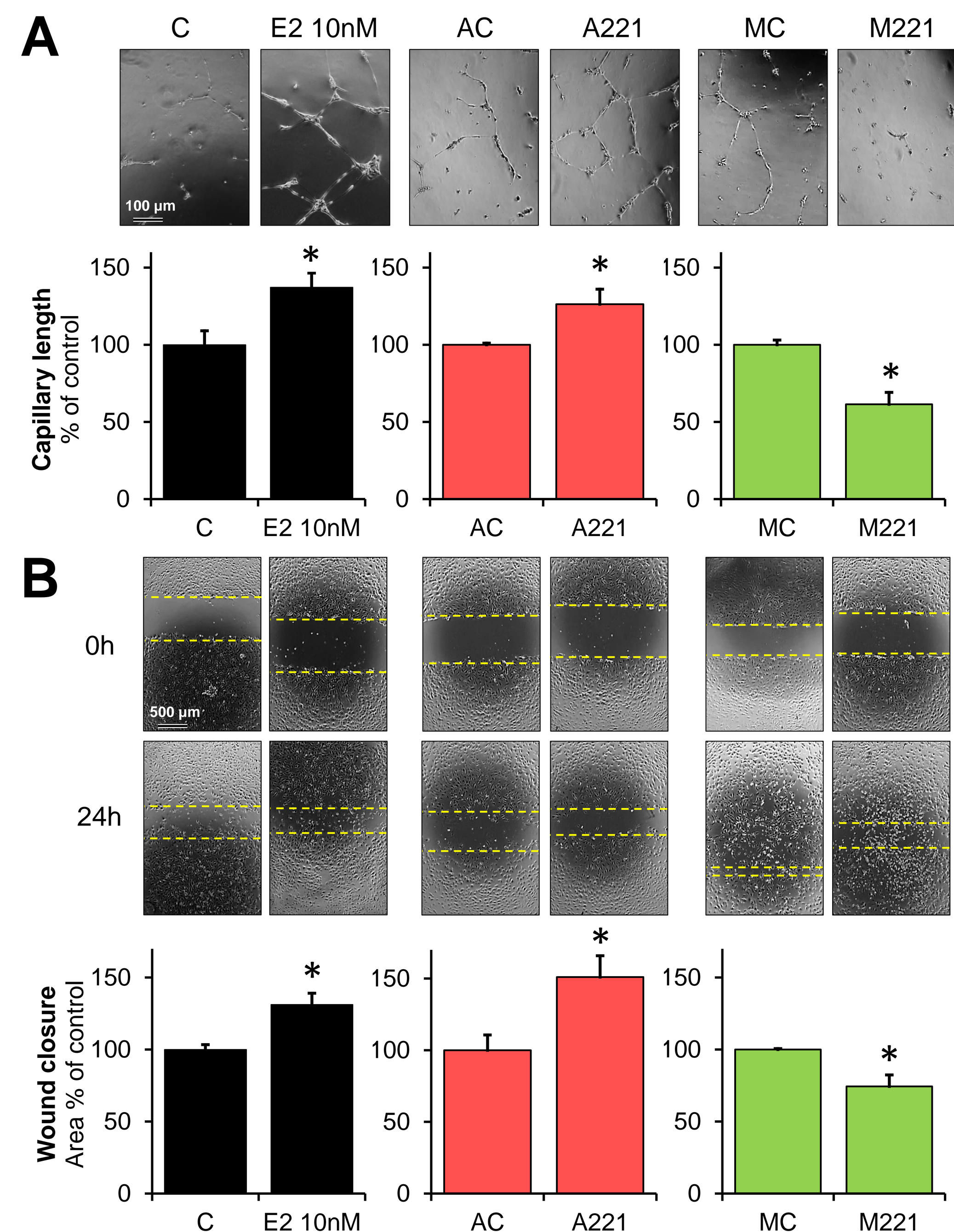

Fig. 1 Effect of E2, miR-221 antimir and miR-221 mimic on HUVEC activity. HUVECs were transfected with miR-221 antimir and mimic (A221, M221) and the respective controls (AC, MC) or treated with 10nM E2. **A.** Capillary formation: images were taken 18h after cell seeding on 2D-Matrigel, 30min after E2 treatment or 24h post-transfection. **B.** Scratch wound assay: with or without E2 or 48h after transfection.

2. miR-221 Induces HCASMC Growth Similar to PDGF-BB

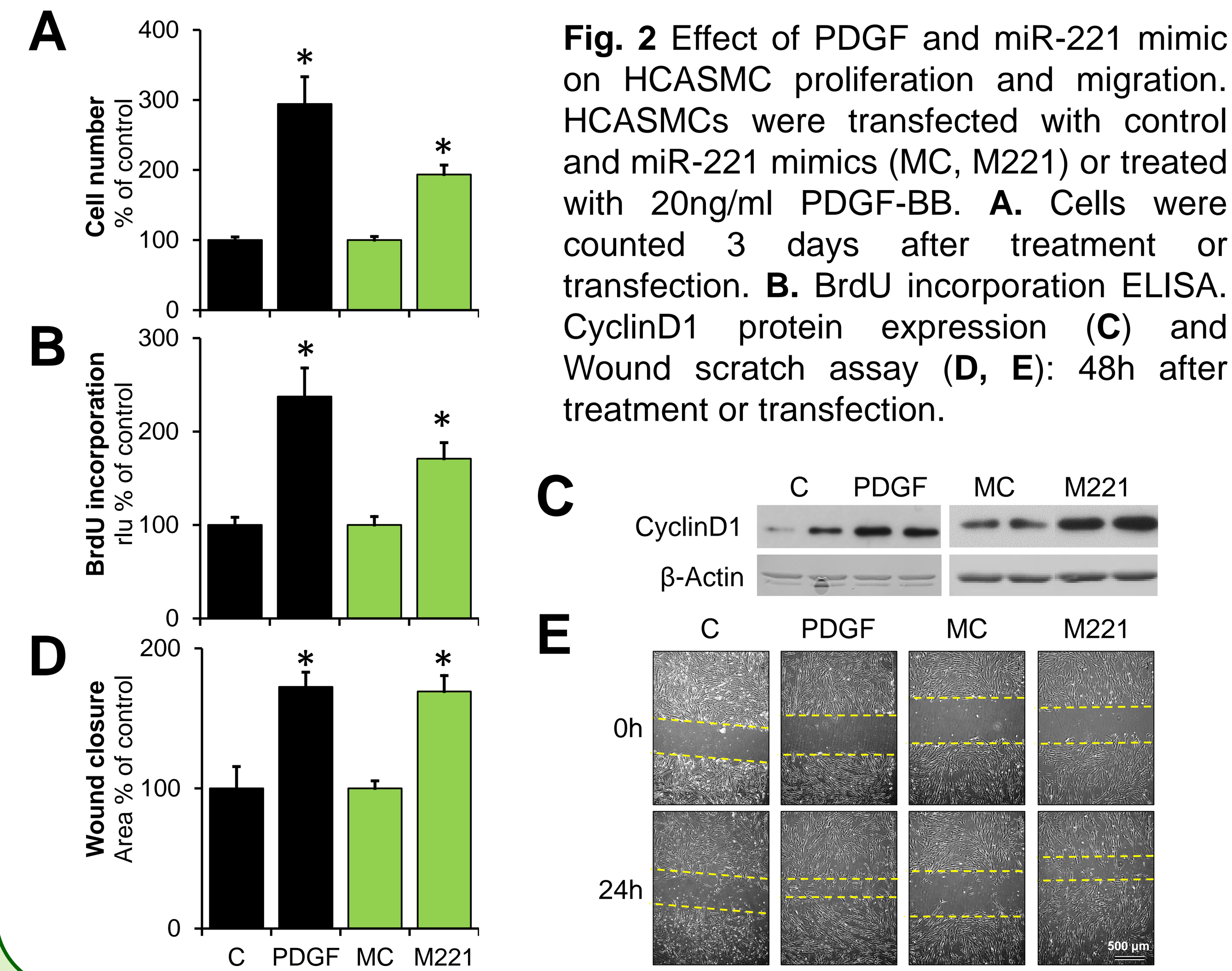

Fig. 2 Effect of PDGF and miR-221 mimic on HCASMC proliferation and migration. HCASMCs were transfected with control and miR-221 mimics (MC, M221) or treated with 20ng/ml PDGF-BB. **A.** Cells were counted 3 days after treatment or transfection. **B.** BrdU incorporation ELISA. CyclinD1 protein expression (**C**) and Wound scratch assay (**D, E**): 48h after treatment or transfection.

3. Downregulation of miR-221 Inhibits HCASMC Growth Similar to E2

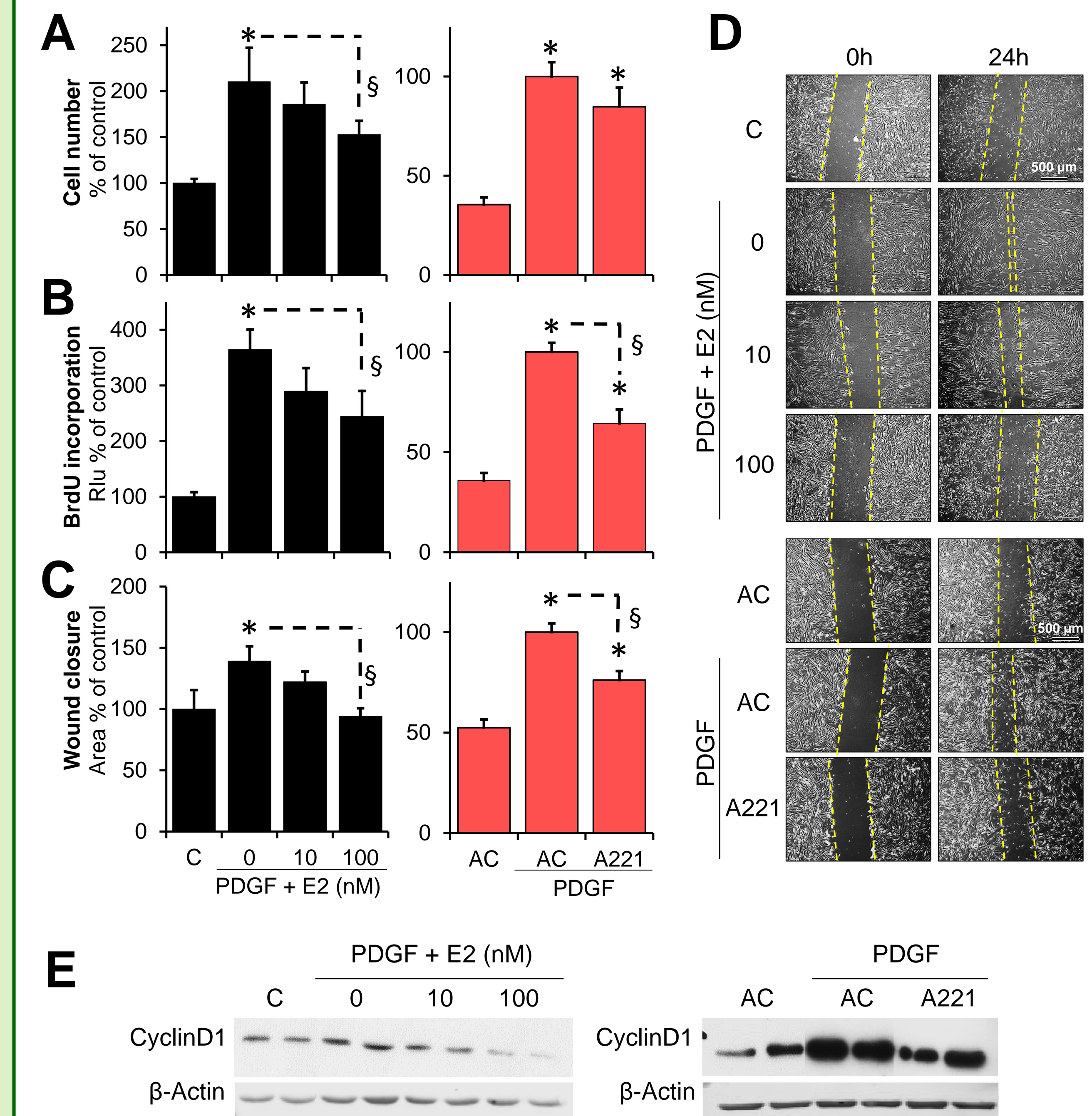

Fig. 3 Effect of miR-221 antimir and E2 on HCASMC proliferation and migration. Cells were transfected with control and miR-221 antimirs (AC, A221) or treated with or without 100nM E2 in presence of 20ng/ml PDGF-BB. **A.** Cells were counted 3 days after treatmen or transfection. **B.** BrdU incorporation ELISA. CyclinD1 protein expression (**E**) and Wound scratch assay (**C, D**): 48h after treatment or transfection.

4. E2 Inhibits miR-221 Expression in HUVECs and HCASMCs

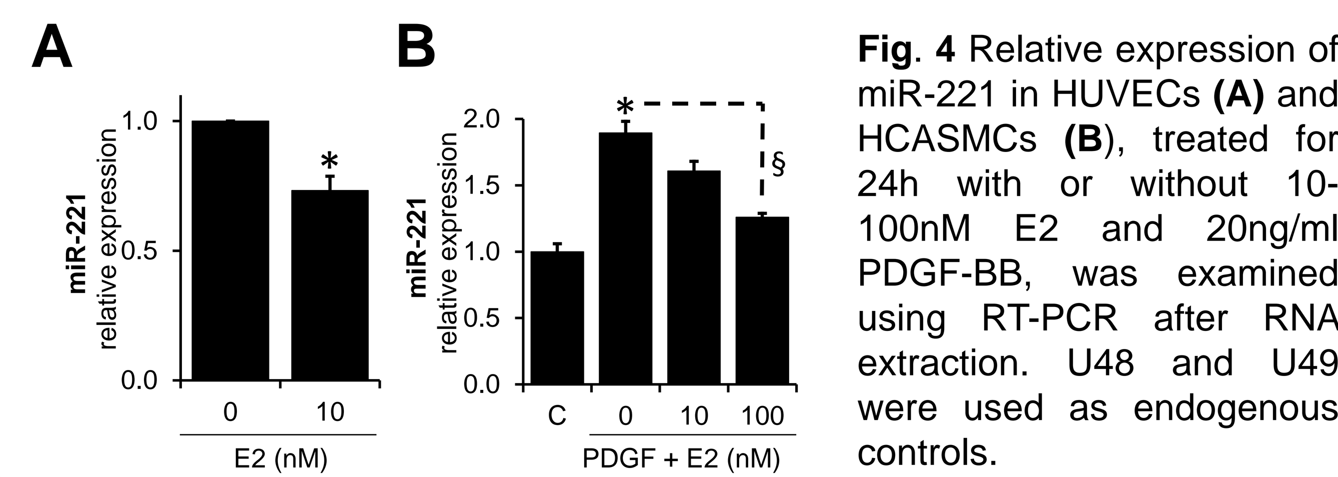

Fig. 4 Relative expression of miR-221 in HUVECs **(A)** and HCASMCs **(B)**, treated for 24h with or without 10-100nM E2 and 20ng/ml PDGF-BB, was examined using RT-PCR after RNA extraction. U48 and U49 were used as endogenous controls.

5. miR-221 Mimic Abrogates the Growth Effects of E2 in both HCASMCs and HUVECs

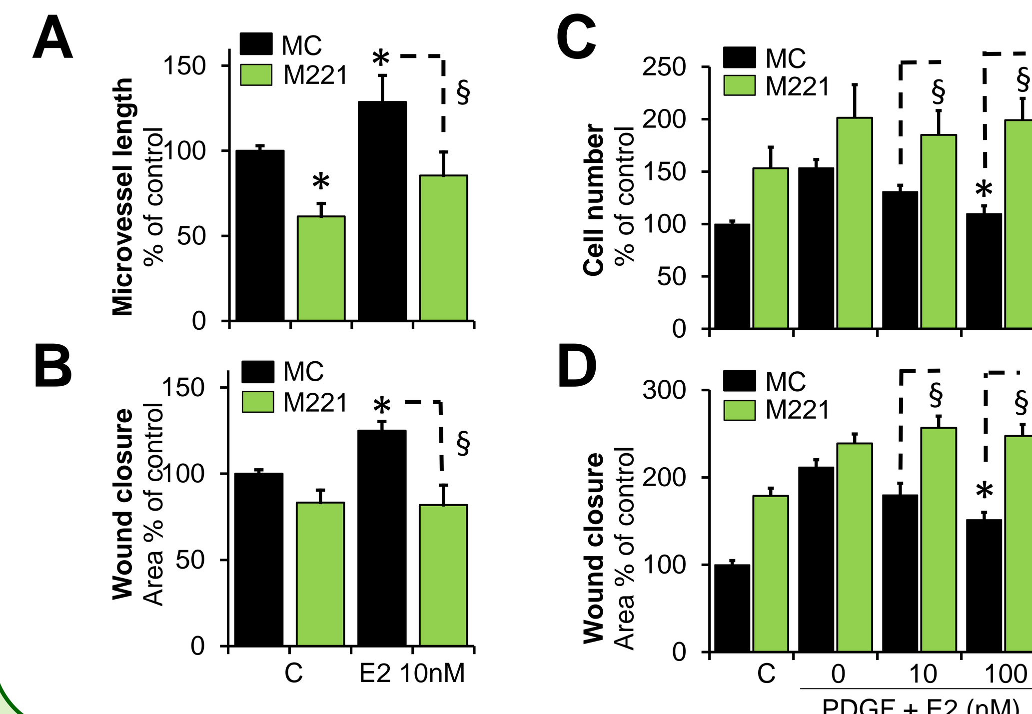

Fig. 5 Cells were transfected with miR-221 mimic (M221) and the respective control (MC) and treated with E2 simultaneously. E2-induced capillary formation (**A**) and wound closure (**B**) in HUVECs were abrogated in presence of M221. Inhibitory effects of E2 on PDGF-induced HCASMCs proliferation (**C,** cell count after 3 days) and migration (**D**, scratch wound assay, 24h) were also reversed by M221.

6. E2 Reduces miR-221 Expression via ERα

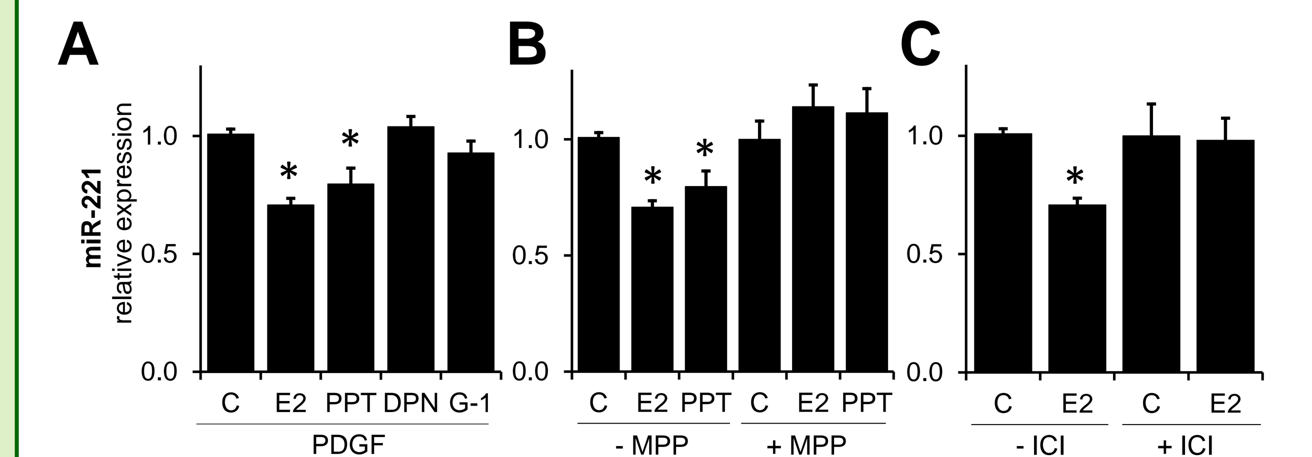

Fig. 6 Relative expression of miR-221 in HCASMCs. **A.** Treatment for 24h with 100nM E2, and Estrogen Receptor (ER) agonists PPT (ERα), DPN (ERβ) and G-1 (GPER) in presence of 20ng/ml PDGF-BB. ERα specific antagonist MPP (500nM, **B**) and unspecific ER antagonist ICI-182-782 (ICI, 1uM, **C**) were added 30min prior agonists and PDGF-BB. U48 and U49 were used as endogenous controls.