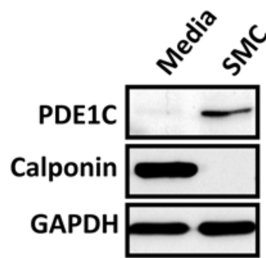
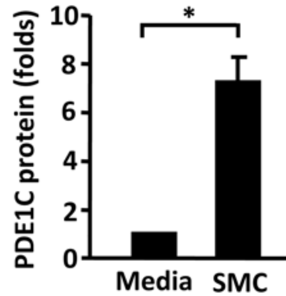
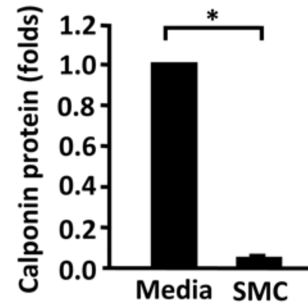
Supplemental Figure S1

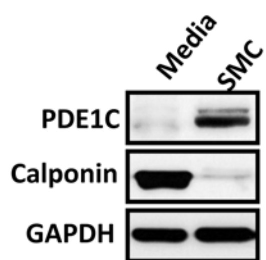
A Rat Aorta

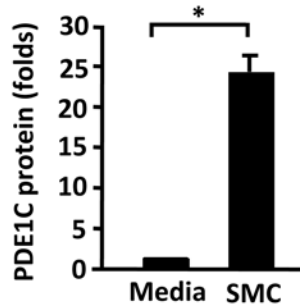


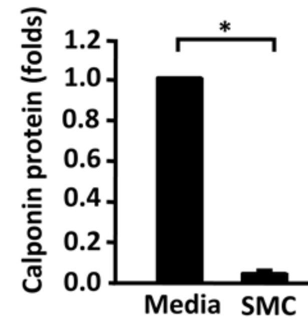




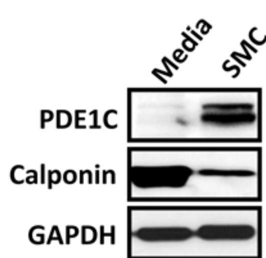
B Human Aorta

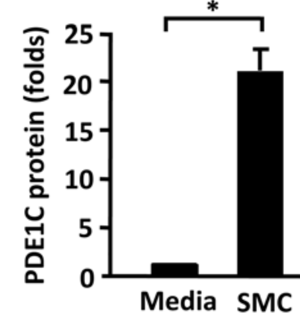


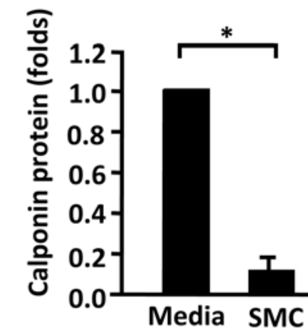




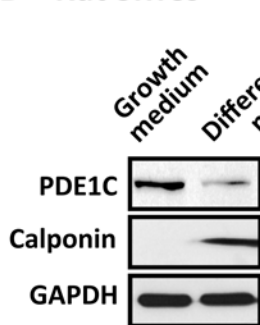
C Human Saphenous Vein

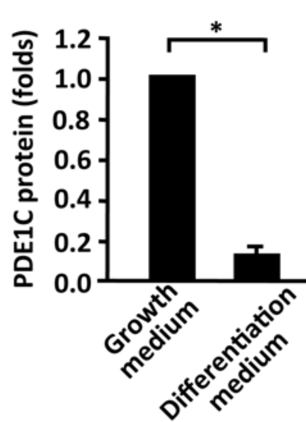






D Rat SMCs





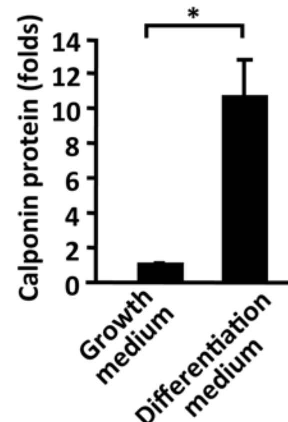
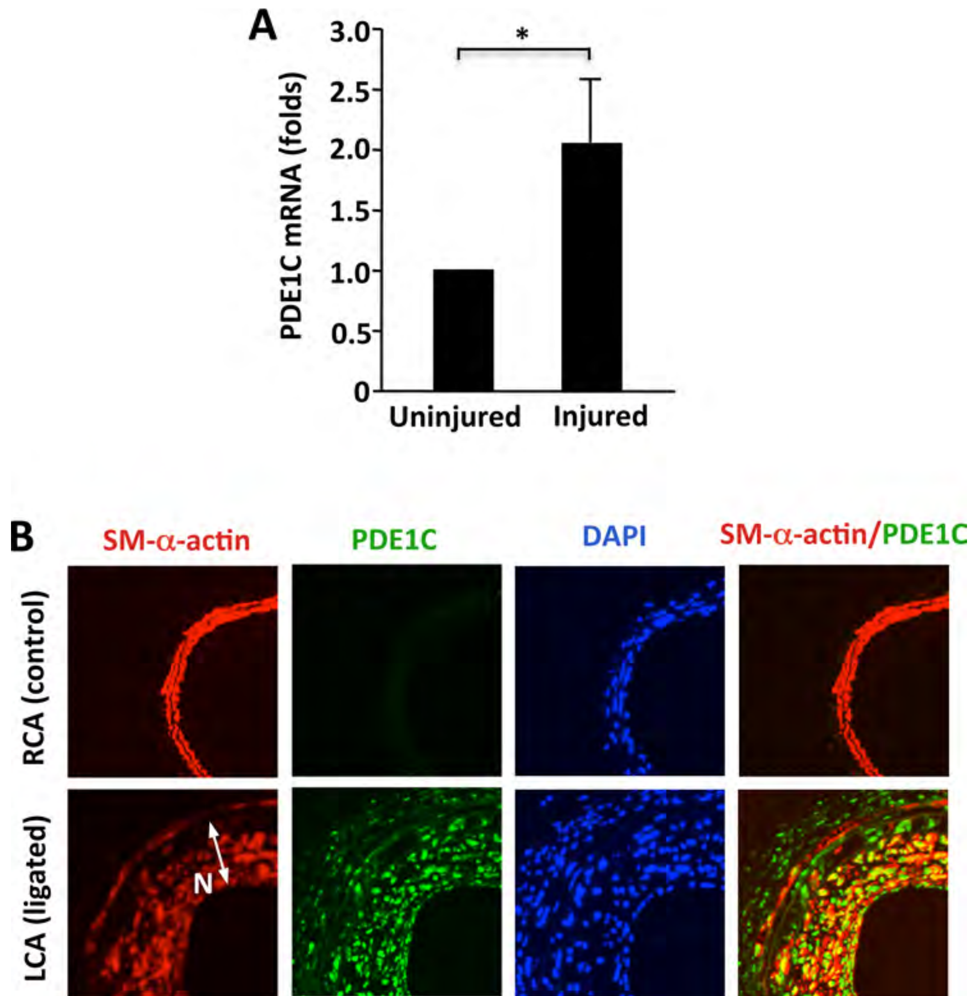


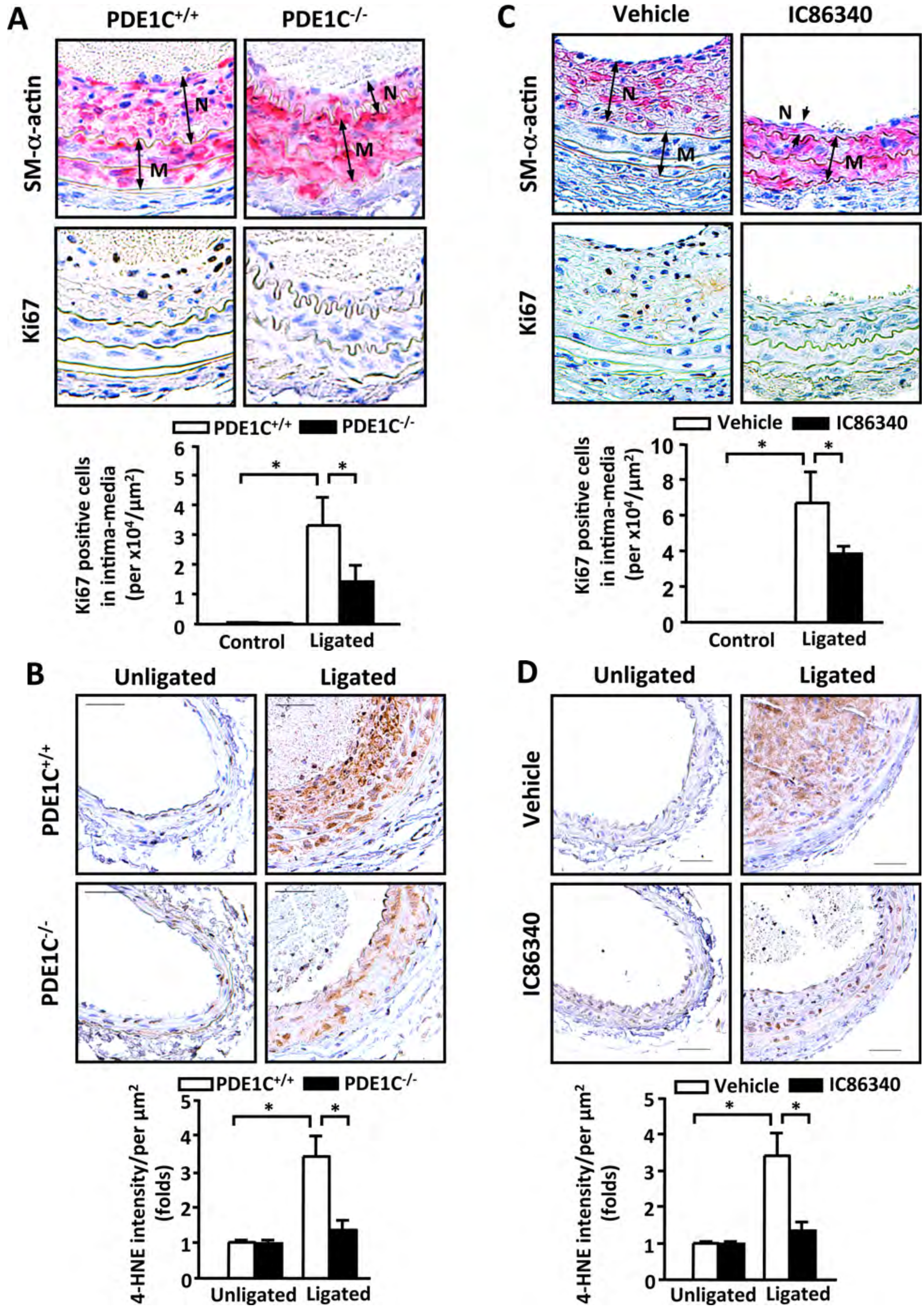
Figure S1. PDE1C protein level is increased in synthetic SMCs. Western blotting results showing protein levels of PDE1C and calponin in contractile SMCs (freshly isolated medial layers) and corresponding synthetic SMCs (cultured SMCs) from rat aortas (**A**), human aortas (**B**), and human saphenous veins (**C**). Contractile SMCs are freshly isolated medial tissues procured by removing endothelial cells and pealing off adventitial layers. Synthetic SMCs are cultured growing SMCs isolated from the corresponding vessel with the explant method. (**D**) Cultured rat aortic SMCs were in differentiation medium (medium 231 supplemented with Smooth Muscle Growth S (SMGS), from Cascade Biologics) or growth medium (medium 231 supplemented with Smooth Muscle Differentiation Supplement (SMDS), from Cascade Biologics) for 2 days. SM-MHC and calponin are used as contractile SMC markers. Values are mean ± SD of triplicate experiments. *P < 0.05.

Supplemental Figure S2



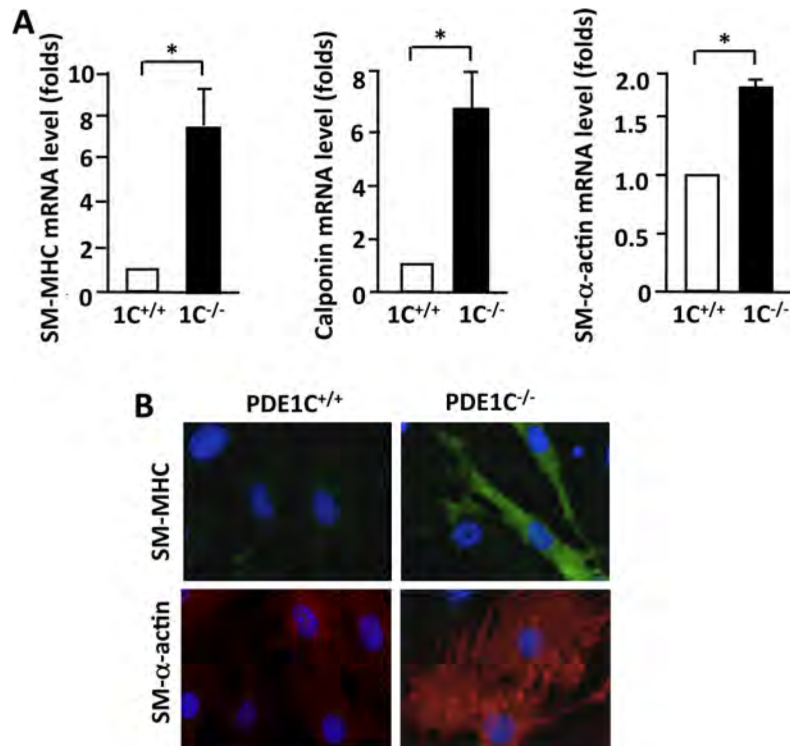
Supplemental Figure S2. (**A**) **PDE1C is elevated in femoral arteries after wire injury.** qRT-PCR showing the PDE1C mRNA levels of in uninjured (right) and injured (left) femoral arteries. C57BL/6J mice were subjected to femoral artery wire injury for 28 days, total RNA were isolated from right uninjured and left injured femoral arteries. The levels of PDE1C mRNA were assessed by qPCR. Values are means ± SD of triplicate (three arteries are pooled together) . $^*P < 0.05$. (**B**) **PDE1C are induced in SMC-like cells.** Representative images of carotid artery sections subjected to immunofluorescent double staining with SM-α-actin (red) and PDE1C (green), Nuclei were stained wit DAPI (blue). FVB mice were subjected to left common carotid artery (LCA) ligation for 14 days. Right common carotid artery (RAC) was used as the control vessel. The merged images (right panels) show that PDE1C-positive cells are largely overlapped with SM-α-actin-positive cells in the neointimal region. N: neointima.

Supplemental Figure S3



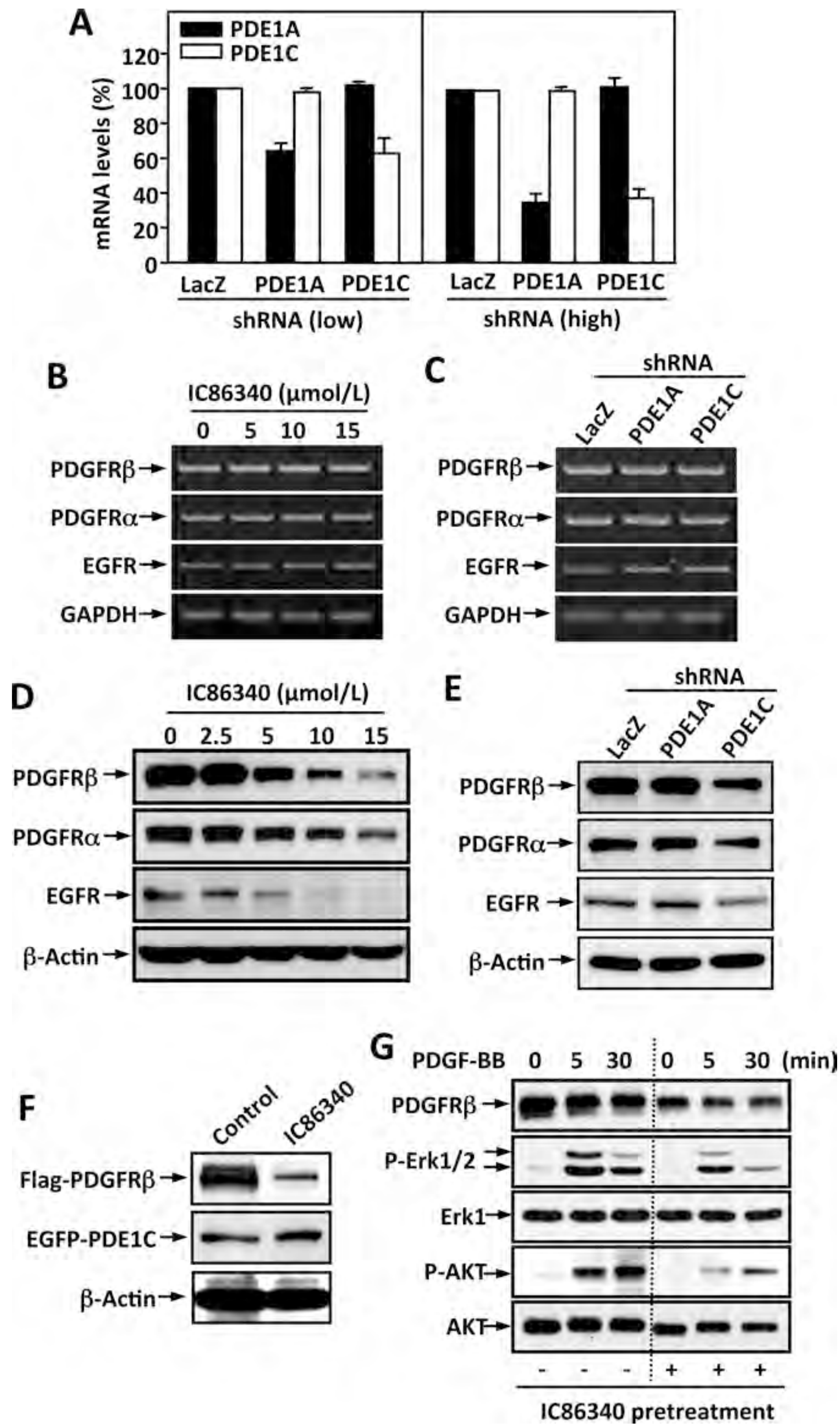
Supplemental Figure S3. (**A and C**) **PDE1C deficiency or PDE1 inhibition attenuates SMC proliferation in response to vascular injury.** Top panels: representative images of carotid artery sections immunostained with SM-α-actin (pink) or Ki67 (brown). Bottom panel: quantitative data of Ki67 positive cells in intima and media. A, PDE1C$^{+/+}$ and PDE1C$^{-/-}$ mice or were subjected to left common carotid artery ligation for 14 days. C, FVB mice were subjected to left common carotid artery ligation for 14 days in the presence of vehicle or 30 µmol/L IC86340 applied perivascularly via pluronic gel. SM-α-actin and Ki67 immunostaining were performed in cross-sections of carotid arteries. Ki-67-positive cells in the intima and the media were calculated. Values are means ± SEM (n=3). *P < 0.05. N: neointima; M: media. **(B-D) PDE1C deficiency suppresses ROS production in carotid arteries after vascular injury.** Top panels: Representative images of carotid arteries immunostained with 4-HNE (an oxidative stress marker). C, PDE1C+/+ and PDE1C-/- mice were subjected to left common carotid artery ligation for 14 days. D, FVB mice were subjected to left common carotid artery ligation for 14 days in the presence of vehicle or 30 µmol/L IC86340 applied perivascularly via pluronic gel. 4-HNE immunostaining were performed in cross-sections of carotid arteries. Quantification were performed using Image Pro Plus software. Values are means ± SEM (n=3). *P < 0.05.

Supplemental Figure S4



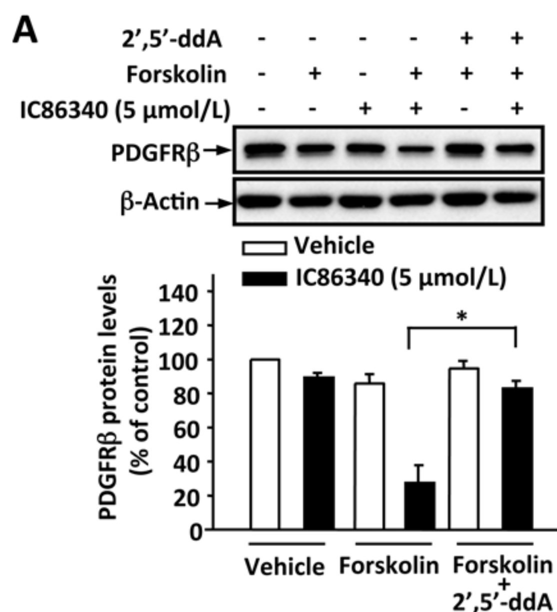
Supplemental Figure S4. (**A**) Real-time RT-PCR showing the mRNA expression of SM-MHC, calponin, and SM-α-actin in low-passage aortic SMCs isolated for PDE1C$^{+/+}$ and PDE1C$^{-/-}$ mice. (**B**) Immanofluorescence staining of SM-MHC or SM-α-actin in PDE1C$^{+/+}$ and PDE1C$^{-/-}$ SMCs. Green: SM-MHC; Red: SM-α-actin; Blue: nuclei.

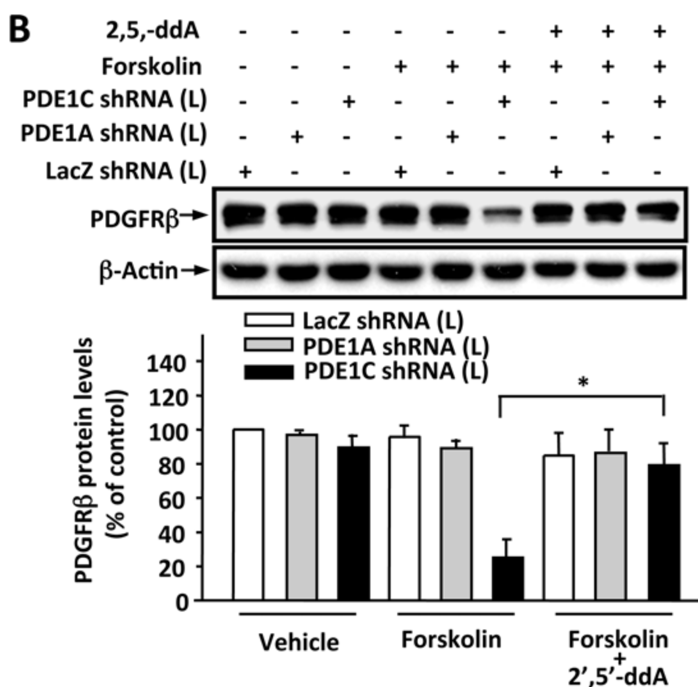
Supplemental Figure S5

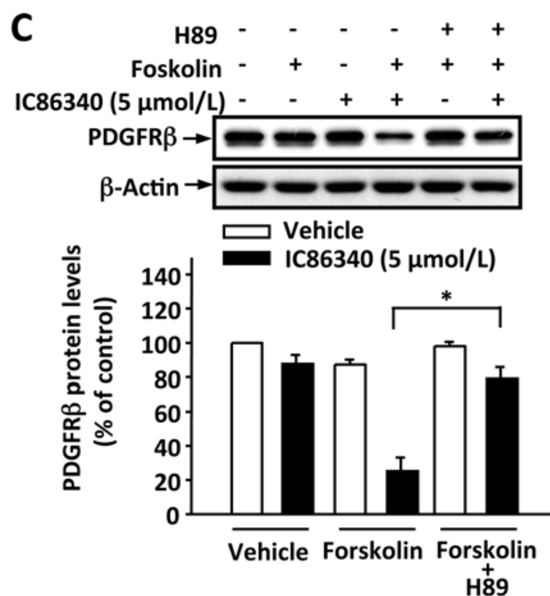


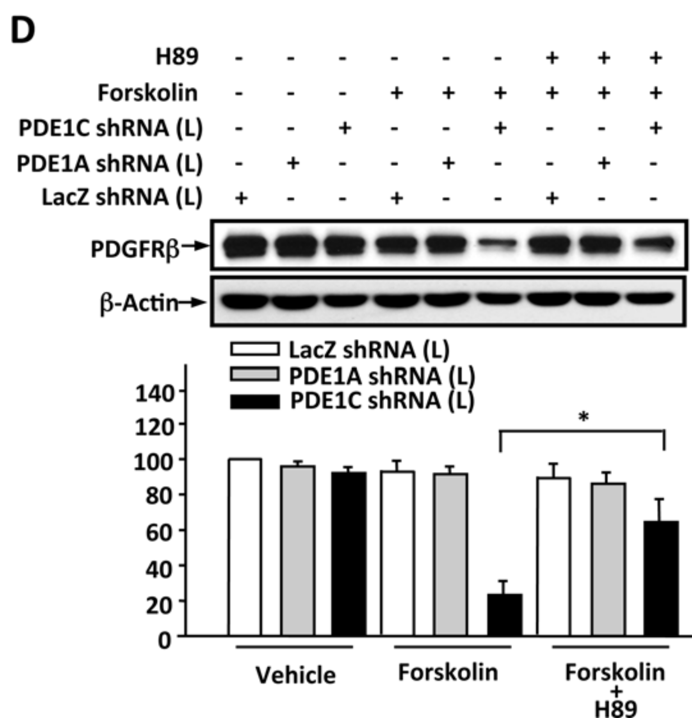
Supplemental Figure S5. Role of PDE1C in regulating PDGFRβ levels. (**A**) PDE1A and PDE1C mRNA levels in rat SMCs treated with a high dose or a low dose of adenovirus encoding LacZ shRNA, PDE1A shRNA or PDE1C shRNA. (**B-C**) PDE1 inhibitor IC86340 or PDE1C shRNA does not affect mRNA levels of multiple growth factor receptors including PDGFRβ, PDGFRα, and EGFR. Levels of mRNA were determined by RT-PCR. (**D-E**) PDE1 inhibitor IC86340 and PDE1C shRNA reduced the protein levels of multiple growth factor receptors. The protein levels of PDGFRβ, PDGFRα, and EGFR were assessed by immunoblotting. Rat aortic SMCs were treated with indicated doses of PDE1 inhibitor IC86340 for 24 h or transduced with adenovirus encoding shRNA against LacZ, PDE1A,or PDE1C shRNA. (**F**) PDE1 inhibitor IC86340 reduced exogenously expressed PDGFRβ protein but not PDE1C protein. Rat aortic SMCs were transfected with Flag-PDGFRβ or EGFP-PDE1C via electropolation for 2 days and then treated with 15 μmol/L IC86340 for 24 h. PDGFRβ and PDE1C were detected by anti-Flag and anti-GFP antibodies, respectively. (**G**) PDGF-stimulated Erk1/2 and AKT activation was attenuated when PDGFR was downregulated. Rat aortic SMCs were treated with 15 μmol/L IC86340 in DMEM with 0.1% FBS for 24 h, washed, and stimulated with 10 ng/ml PDGF-BB for 5 or 30 min. Phospho-Erk1/2, Erk 1, phospho-AKT, and AKT were measured by immunoblotting.

Supplemental Figure S6



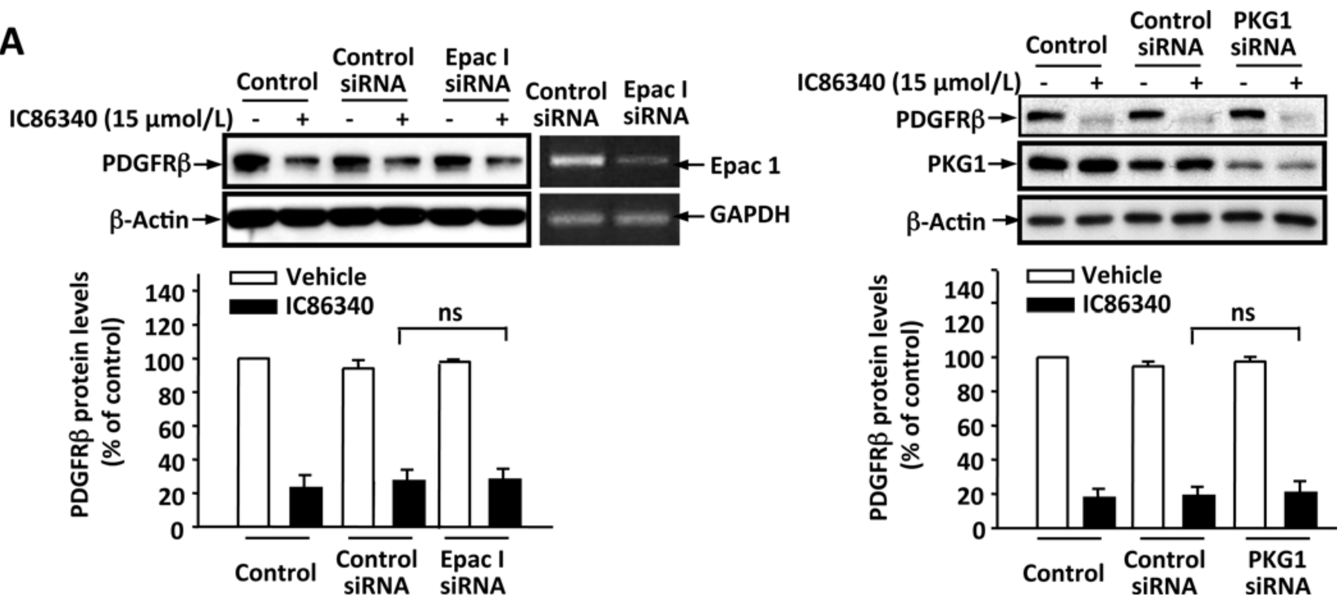


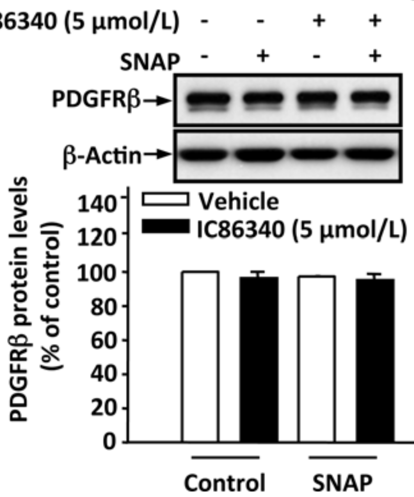


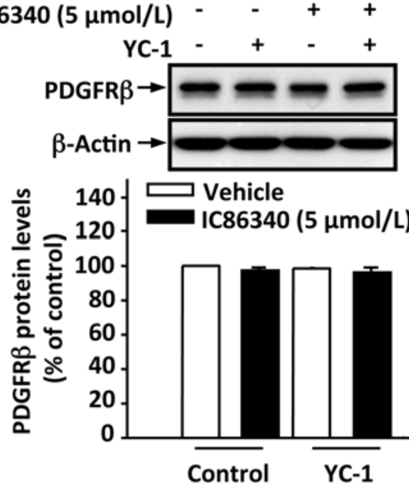


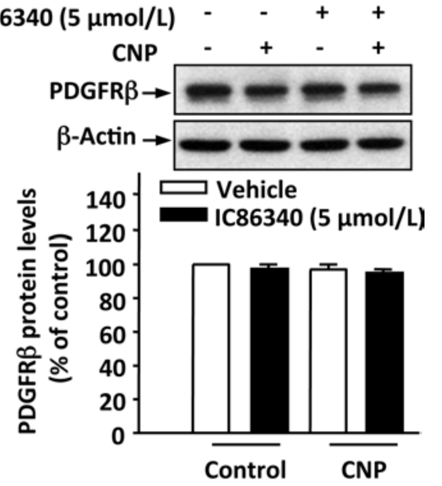
Supplemental Figure S6. Role of tmAC-cAMP signaling in PDE1C-mediated regulation of PDGFRβ. (A) The tmAC inhibitor blocked the synergistic effect of IC86340 and forskolin on PDGFRβ protein reduction. Rat aortic SMCs were treated with 5 µmol/L IC86340, 10 µmol/L forskolin, or both in the presence or absence of tmAC inhibitor 2',5'-dideoxyadenosine (2'5'-ddA,10 µmol/L) for 24 h in DMEM containing 0.1% FBS. (**B**) The tmAC inhibitor blocked the synergistic effect of PDE1C shRNA and forskolin on PDGFRβ protein reduction. Rat aortic SMCs were transfected with a low dose of adenovirus expressing LacZ-shRNA, PDE1A-shRNA, or PDE1C-shRNA for 3 days, followed by treatment with 10 µmol/L forskolin in the presence or absence of 10 µmol/L 2',5'-ddA for 24 h in DMEM containing 0.1% FBS. (**C**) PKA inhibitor H89 blocked the synergistic effect of IC86340 and forskolin on PDGFRβ protein reduction. Rat aortic SMCs were treated with 5 µmol/L IC86340, or 10 µmol/L forskolin, or both in the presence or absence of 5 µmol/L H89 for 24 h in DMEM containing 0.1% FBS. (**D**) PKA inhibition by H89 blocked the synergistic effect of PDE1C shRNA and forskolin on PDGFRβ protein reduction. Rat aortic SMCs were transfected with a low dose of adenovirus expressing LacZ-shRNA, PDE1A-shRNA, or PDE1C-shRNA for 3 days, and then treated with 10 µmol/L forskolin in the presence of absence of 5 µmol/L H89 for 24 h in DMEM containing 0.1% FBS. Percentile changes normalized to the left lane. Values are mean ± SD (n= 3). $^*p<0.05$.

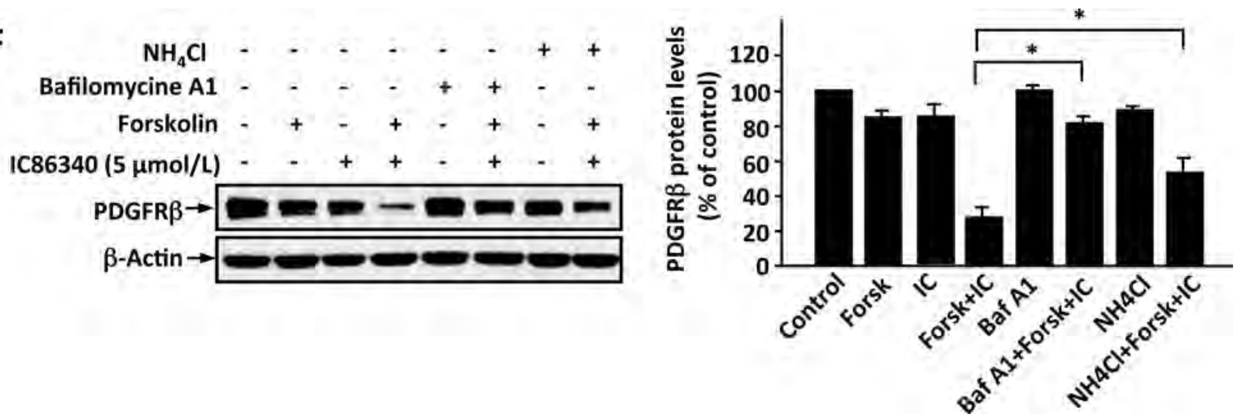
Supplemental Figure S7





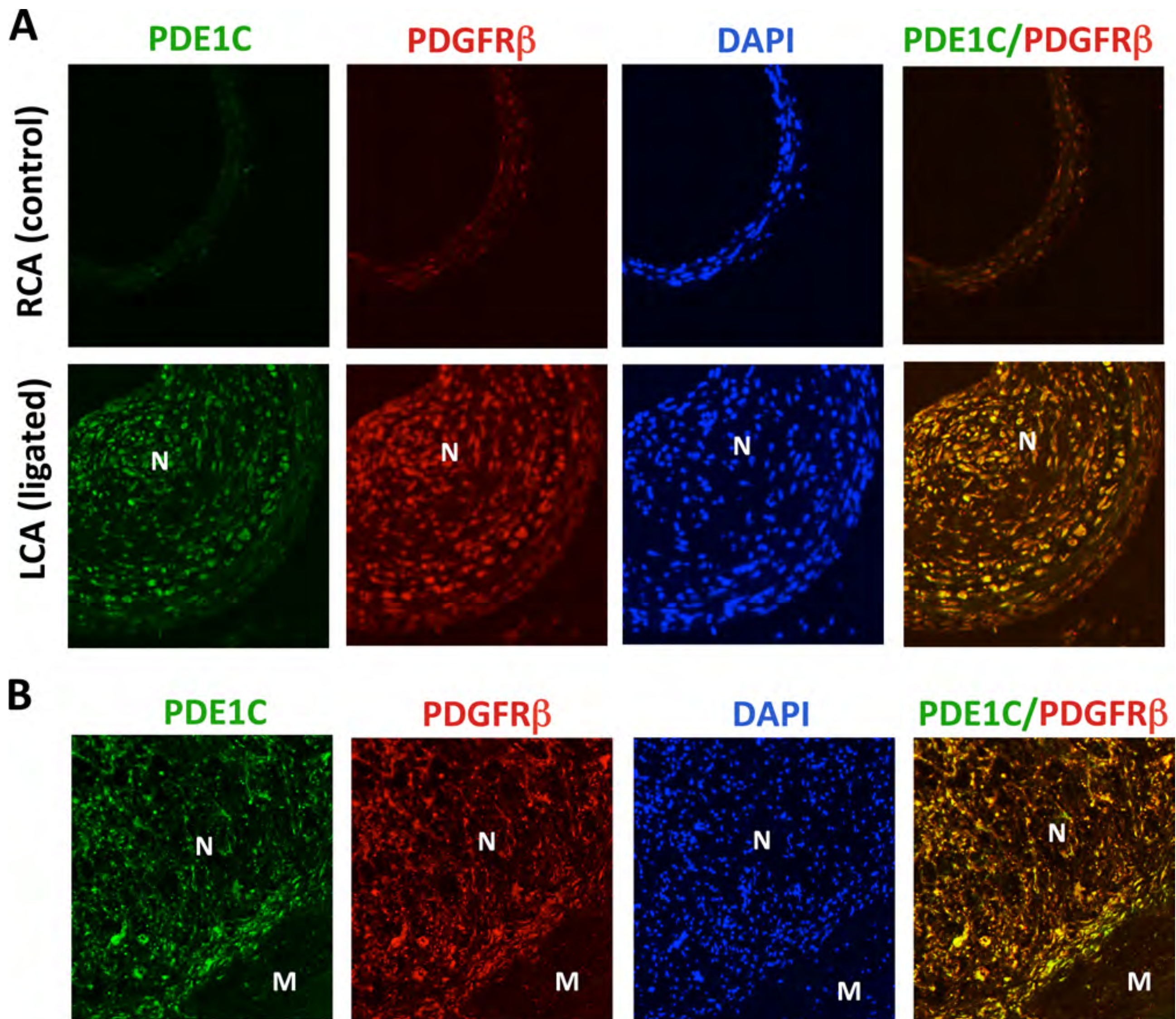






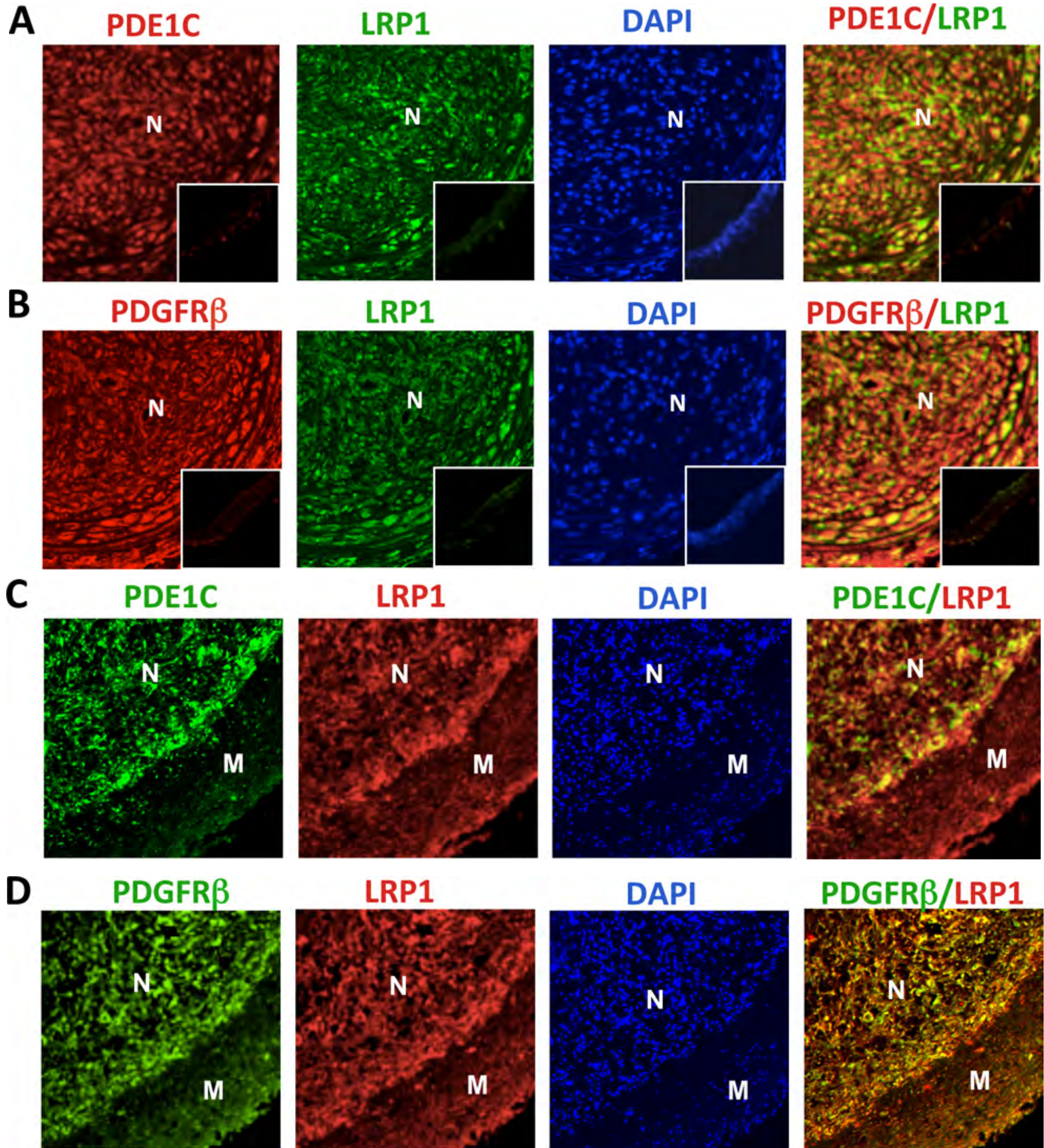
Supplemental Figure S7. (**A**) **Epac was not involved in PDE1 inhibition-induced PDGFRβ protein reduction.** Rat aortic SMCs were transfected with 50 nmol/L control siRNA or Epac siRNA for 3 days, and then treated with 15 µmol/L IC86340 for 24 h in DMEM containing 0.1% FBS. Right panel showed Epac knockdown by specific siRNA using RT-PCR. (**B**) **PKG1 was not involved in PDE1 inhibition-induced the PDGFRβ protein reduction.** Rat aortic SMCs were transfected with 50 nmol/L control siRNA or PKG1 siRNA for 3 days, and treated with 15 µmol/L IC86340 for 24 h in DMEM containing 0.1% FBS. (**C-E**) **cGMP and PDE1 inhibition does not have synergistic effect on PDGFRβ.** Rat aortic SMCs were treated with either 5 µmol/L IC86340 alone, one of cGMP elevators (100 µmol/L NO donor SNAP, 10 µmol/L sGC activator YC-1, and 100 nmol/L CNP), or both of IC86340 and a cGMP elevator for 24 h in DMEM containing 0.1% FBS. (**F**) **Lysosome inhibitors abrogated enhanced effect of forskolin and IC86340 on PDGFRβ protein reduction.** Rat aortic SMCs were treated with 5 µmol/L IC86340 and 10 µmol/L forskolin in the presence or absence of 20 mmol/L NH_4Cl or 50 nmol/L Bafilomycine A for 24 h in DMEM containing 0.1% FBS. Protein levels of PDGFRβ and β-actin equal loading were determined by immunoblotting. PDGFRβ protein and equal loading β-actin were determined by immnoblotting. Quantitative data show percentile changes normalized to the 1st lane. Values are mean ± SD (n= 3). *$p<0.05$. ns: no significant difference.

Supplemental Figure S8



Supplemental Figure S8. PDE1C and PDGFRβ co-localization in neointimal lesions. (**A**) Representative images of carotid artery sections subjected to co-immunofluorescent staining of PDE1C (green) and PDGFRβ (red), and nuclei are stained with DAPI (blue). FVB mice were subjected to left common carotid artery (LCA) ligation for 14 days. Right common carotid artery (RAC) was used as the control vessel. N: neointima. (**B)** Reprehensive co-immunofluorescent staining images of human coronary artery with neointimal lesions. Green: PDE1C, Red: PDGFRβ, Blue: nuclei stained with DAPI. The merged images (right panels) show that PDE1C-positive cells are largely overlapped with SM-α-actin-positive cells in the neointimal region. M: media, N: neointima.

Supplemental Figure S9



Supplemental Figure S9. Co-expression of LRP1 with PDE1C or PDGFRβ in neointimal lesions. (**A-B**) Representative images of carotid artery sections subjected to co-immunofluorescent staining of LRP1 (green) together with PDE1C (red) or PDGFRβ (red), and nuclei are stained with DAPI (blue). FVB mice were subjected to left common carotid artery (LCA) ligation for 14 days. Right common carotid artery (RAC) was used as the control vessel (inset). N: neointima. (**C-D)** Reprehensive co-immunofluorescent staining images of human coronary artery with neointimal lesions. Green: LRP1, Red: PDE1C or PDGFRβ, Blue: nuclei stained with DAPI. The merged images (right panels) show that LRP1-postive cells are largely overlapped with PDE1C- or PDGFRβ-positive cells in the neointimal region. M: media, N: neointima.