Olfactomedin 2 Regulates Smooth Muscle Phenotypic Modulation and Vascular Remodeling Through Mediating Runx2 Binding to SRF

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Online Supplemental Figures

Supplemental Figure I

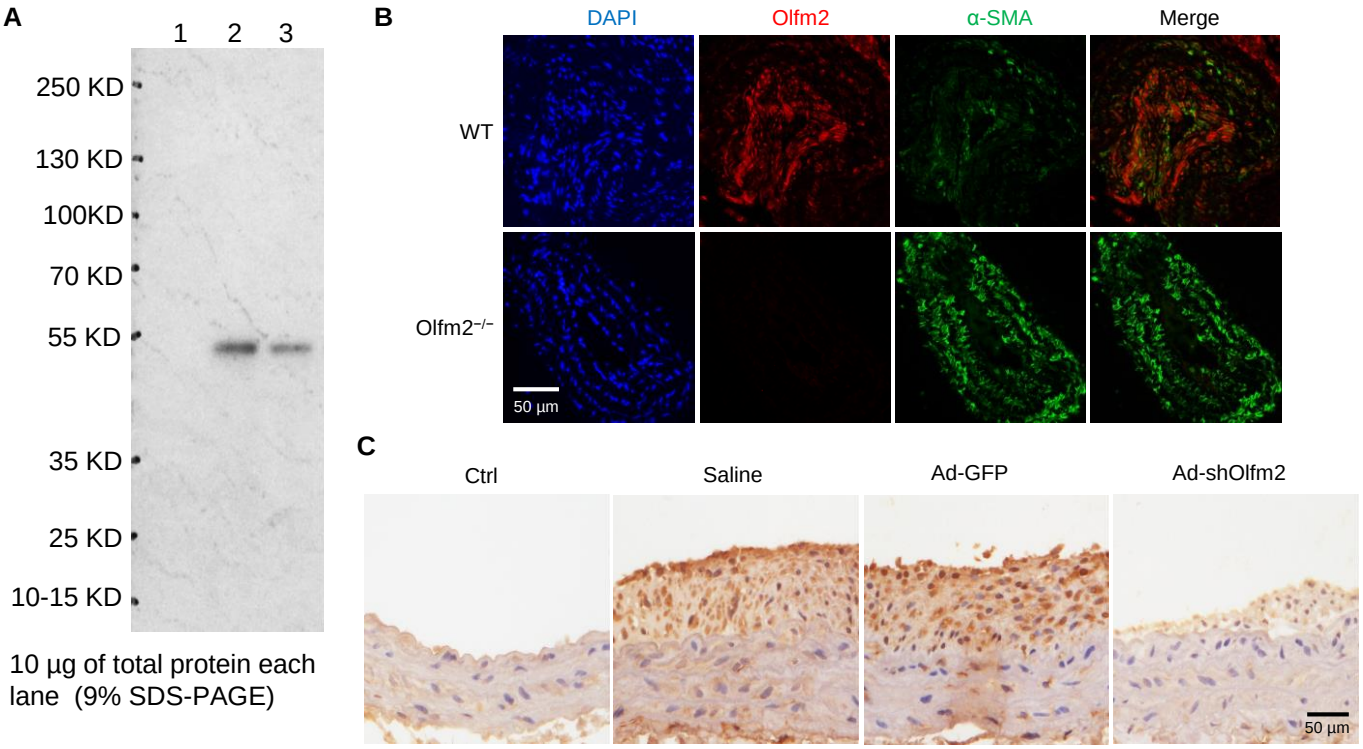

Figure I. Olfm2 expression is normal or injured arteries. A, Olfm2 antibody did not detect Olfm2 expression in carotid arteries of Olfm2-/- mice (Lane 1), but detected its expression in SMCs isolated from aorta of WT mouse (Lane 2) and rat (Lane 3). B, Co-immunoflurescent staining showed that Olfm2 was mainly expressed in neointimal and medial SMCs of WT, but not Olfm2-/- mouse arteries with injury. C, Injury-induced Olfm2 expression was effectively blocked by its shRNA delivered by adenoviral vector. The balloon-injured arteries were incubated with sterile saline solution, adenovirus expressing control (Ad-GFP) or Olfm2 shRNA (Ad-shOlfm2 as indicated. 14 days after the injury, the artery samples were collected, and artery sections were immunostained for Olfm2. The Olfm2 expression was visualized using DAB substrate (3, 3'-diaminobenzidine).

Supplemental Figure II

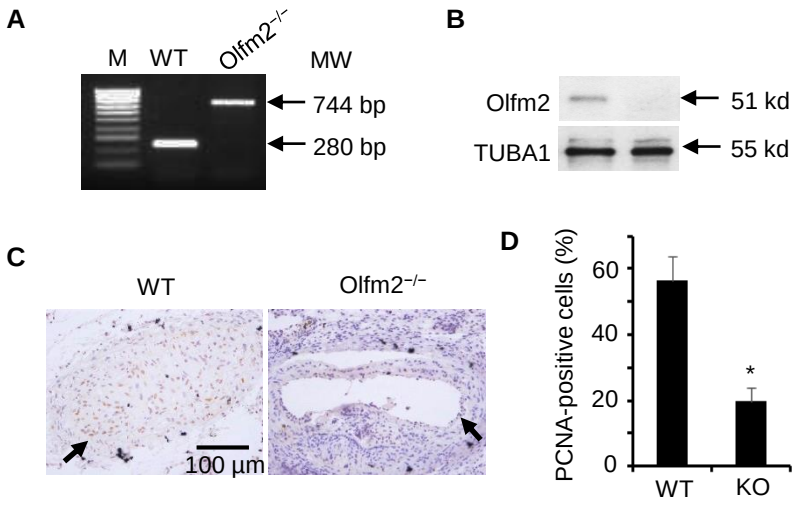

Figure II. Olfm2 knockout inhibited SMC proliferation in vivo. A, Olfm2 was not expressed in carotid arteries of Olfm2−/− mice. Genomic DNA was extracted from mouse tails and PCR genotyping was performed to identify wild type (WT) and Olfm2−/− mice. M: Marker; MW: Molecular Weight. B, Carotid arteries were isolated from WT and Olfm2−/− mice, and Western blot was performed to detect Olfm2. TUBA1 was used as an internal control. C, IHC staining of PCNA was performed to detect PCNA-positive SMCs in injured carotid arteries of WT or Olfm2-/- mice. Arrows point to the internal elastic lamina. D, The percentage of PCNA-positive cells were calculated using Image-Pro plus 6.0 software. *, $P < 0.01$ vs. WT groups (n=5).

Supplemental Figure III

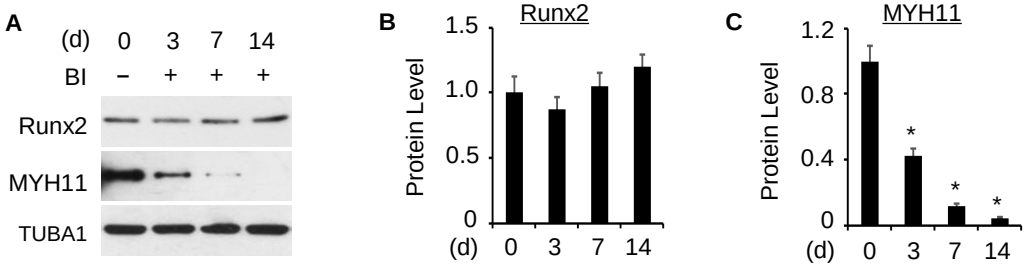

Figure III. Runx2 was not significantly induced in rat carotid arteries by balloon-injury (BI). A, Western blotting was performed to examine time-course expression of Runx2 and SMC marker MYH11 in rat carotid arteries with balloon-injury (BI). B and C, Quantification of Runx2 (B) and MYH11 (C) expression by normalizing to the TUBA1 level. *, *P*< 0.01 vs. uninjured group (d 0) (n=3).

Supplemental Figure IV

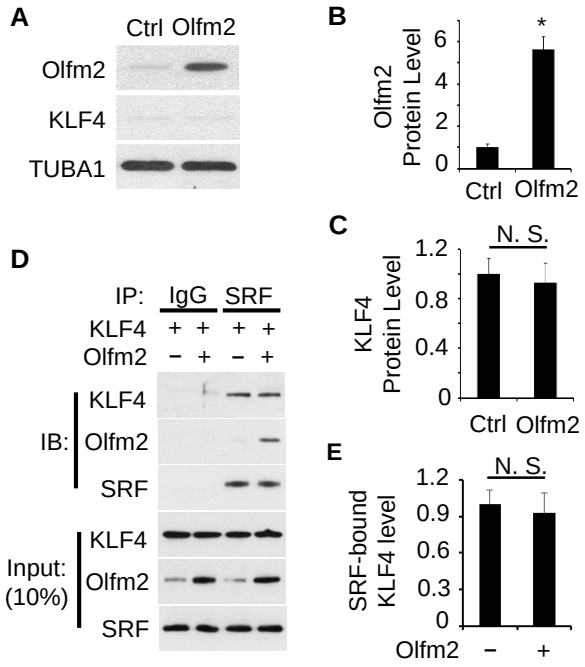

Figure IV. Olfm2 did not affect KLF4 expression and KLF4-SRF interaction. RASMCs were transfected with control, Olfm2 and KLF4 plasmids. A, Western blotting was performed to detect Olfm2, KLF4 expression. B-C, Quantification of Olfm2 (B) and KLF4 (C) expression shown in A by normalizing to the TUBA1 level. *, $P < 0.01$ vs. control groups (n=3). D, Co-IP assays were performed to detect SRF-KLF4 interaction. IP: Immunoprecipitation; IB: Immunoblotting. E, Quantification of SRF-bound KLF4 by normalizing to the input SRF level shown in D. N.S.: non-significant (n=3).

Supplemental Figure V

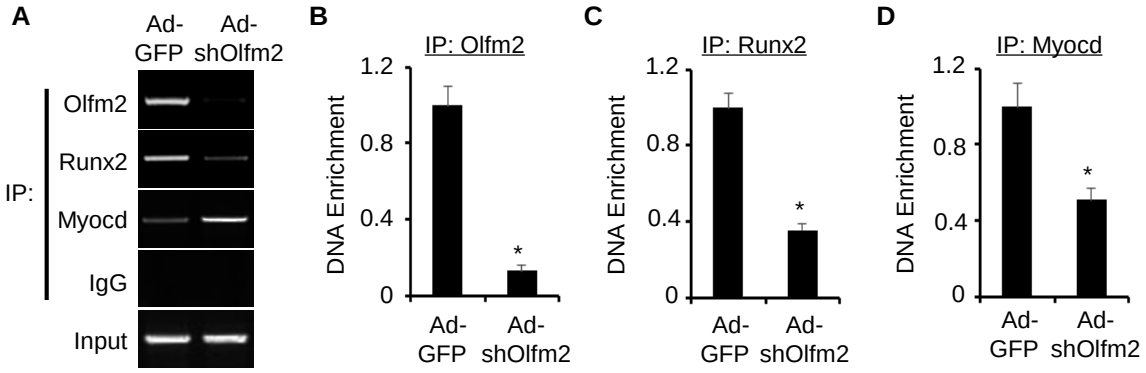

Figure V. Olfm2 knockdown inhibited Runx2 while promoted Myocd association with the CArG box in MYH11 promoter in the chromatin setting. RASMCs were transduced with Ad-GFP and Ad-shOlfm2 followed by PDGF-BB treatment (20 ng/ml) for 24 h. ChIP assays were performed to detect the association of proteins indicated with the MYH11 promoter CArG box using corresponding antibodies as indicated for coimmunoprecipitation (IP). IgG was used as a negative control. The CArG box surrounding DNA enrichment was detected by semi-quantitative PCR (A) or real-time quantitative PCR (B-D). *, $P < 0.01$ vs. Ad-GFP-transduced group (n=5).

Supplemental Figure VI

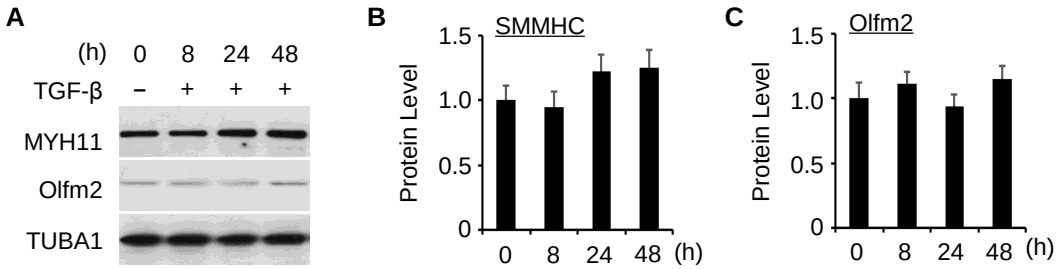

Figure VI. Olfm2 was not induced in TGF-β-treated RASMCs. A, Serum-starved RASMCs were treated with vehicle (-) and TGF-β (+, 5 ng/ml) for the times as indicated. Cell lysates were collected and Western blotting was performed to examine MHY11 and Olfm2 expression. B-C, Quantification of protein expression shown in A by normalized to the TUBA1 level. There is no significant difference in MYH11 and Olfm2 expression between vehicle- and TGF-β-treated groups.