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Supplemental information

Hsa_circ_0024093 accelerates VSMC proliferation

via miR-4677-3p/miR-889-3p/USP9X/YAP1 axis

in *in vitro* model of lower extremity ASO

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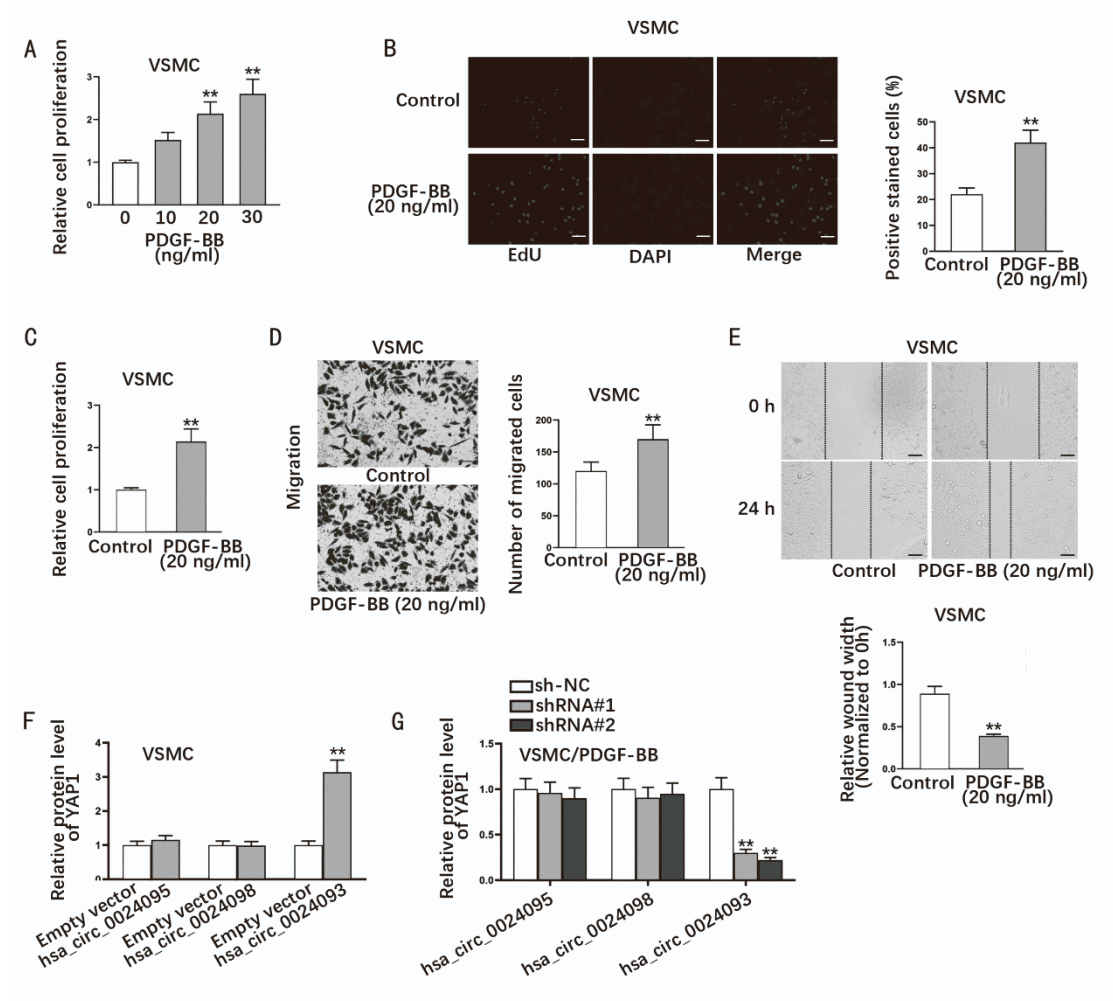


Figure S1. PDGF-BB facilitates the proliferation and migration of VSMCs.

A. The proliferation of VSMCs treated with different doses of PDGF-BB was examined. B-C. EdU (bar value = 100 μ m) and CCK-8 assays detected the proliferation of VSMCs treated with or without 20 ng/ml PDGF-BB. D-E. Transwell assay (bar value = 50 μ m) and wound healing assay (bar value = 100 μ m) examined the migration ability of VSMCs with or without PDGF-BB treatment. F-G. The quantification bar graphs of blots in Figure 1D-E were shown.

**P<0.01.

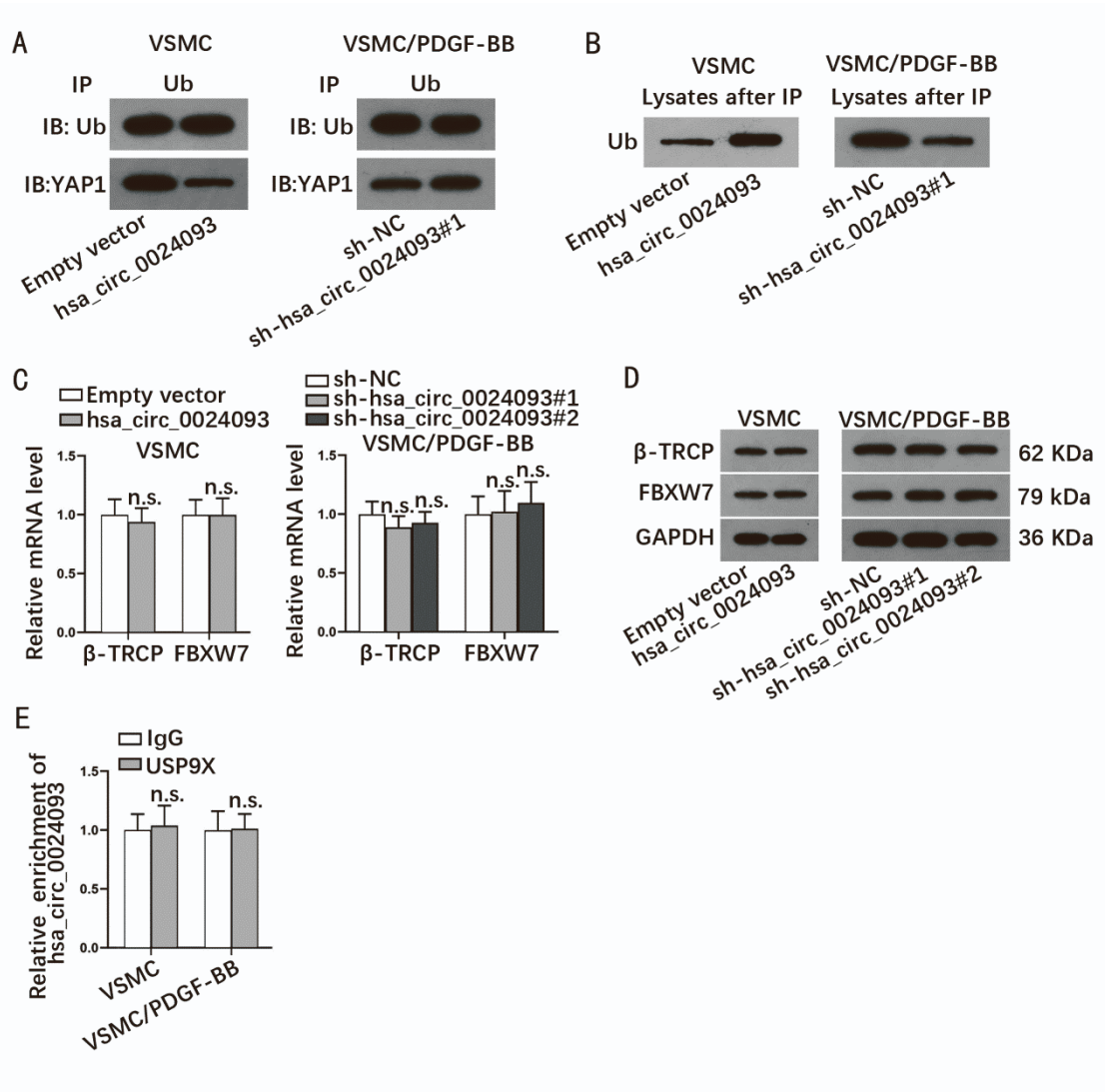


Figure S2. Hsa_circ_0024093 regulates YAP1 ubiquitination not depending on FBXW7 or β -TRCP.

A. The ubiquitination level of YAP1 in indicated VSMCs was detected. B. The level of free ubiquitin in lysates after IP was detected in indicated VSMCs. C-D. The mRNA level and protein level of FBXW7 and β -TRCP in indicated VSMCs were analyzed. E. The enrichment of hsa_circ_0024093 in USP9X groups was detected. n.s. meant no significance.

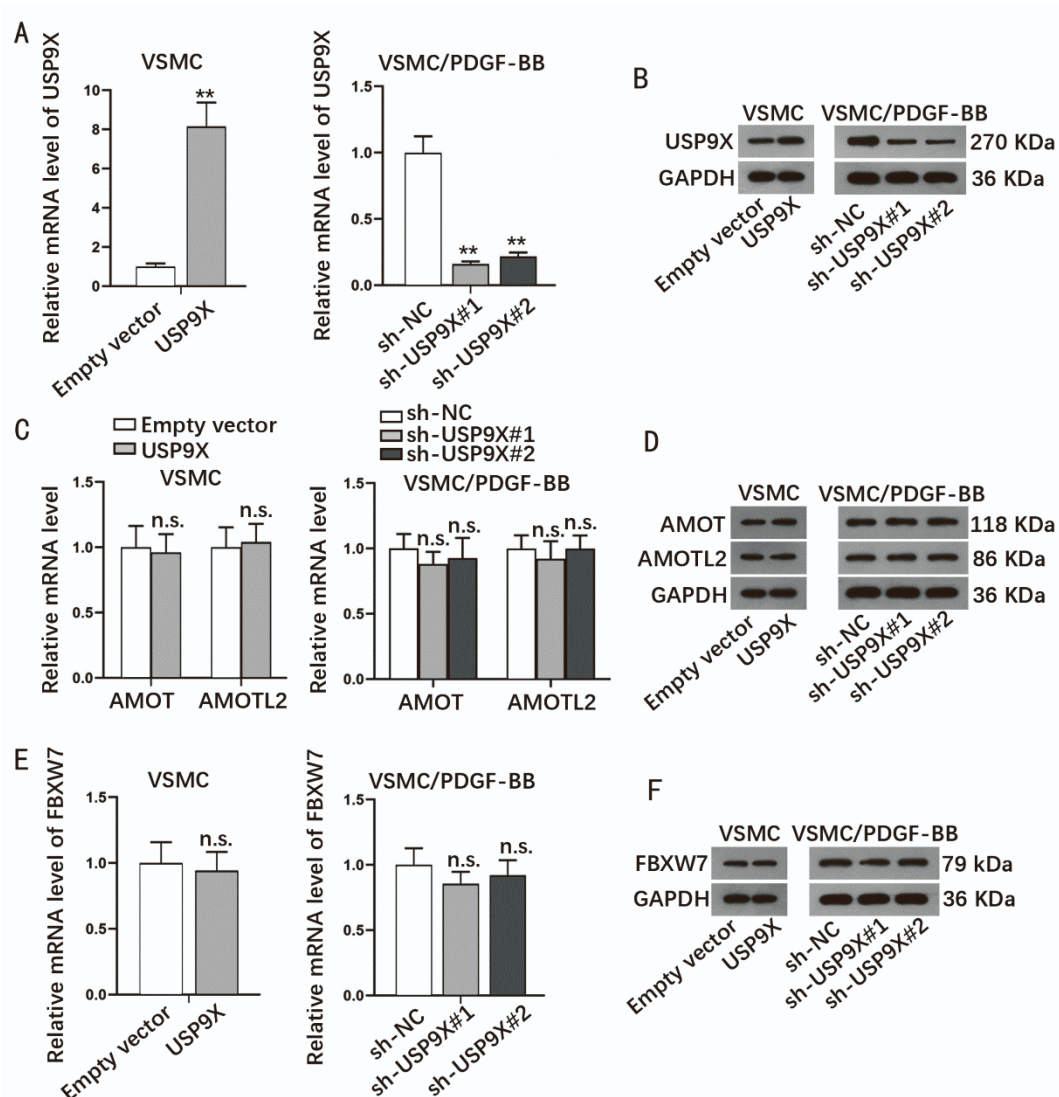


Figure S3. USP9X cannot regulate the expression of AMOT, AMOTL2 and FBXW7 in VSMCs.

A-B. USP9X expression in VSMCs with USP9X overexpression or in PDGF-BB treated VSMCs with USP9X knockdown was measured via RT-qPCR and western blot analyses. C-D. The mRNA and protein levels of AMOT and AMOTL2 were evaluated in USP9X-overexpressed VSMCs or in PDGF-BB treated VSMCs with USP9X down-regulation. E-F. The mRNA and protein levels of FBXW7 were calculated in USP9X-overexpressed VSMCs or in PDGF-BB treated VSMCs with USP9X silencing. **P<0.01, n.s. meant no significance.

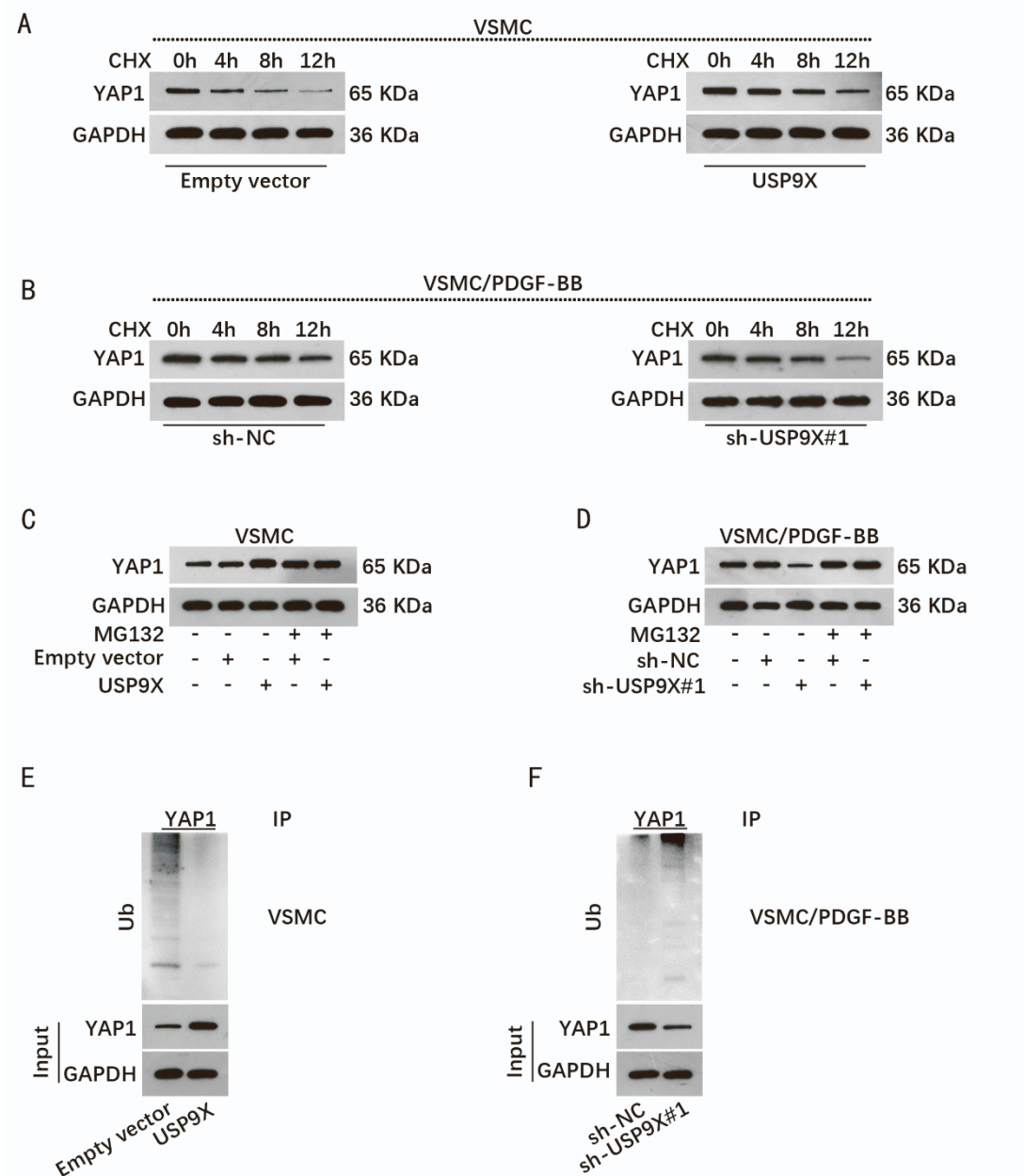


Figure S4. USP9X enhances the stability of YAP1.

A. The protein level of YAP1 was detected under CHX treatment in VSMCs in response to USP9X overexpression. B. The protein level of YAP1 under CHX treatment was calculated in PDGF-BB treated VSMCs with USP9X deficiency. C-D. YAP1 protein level in VSMCs under different treatment or transfections was investigated. E-F. The ubiquitination level of YAP1 in VSMCs under different contexts was assessed.

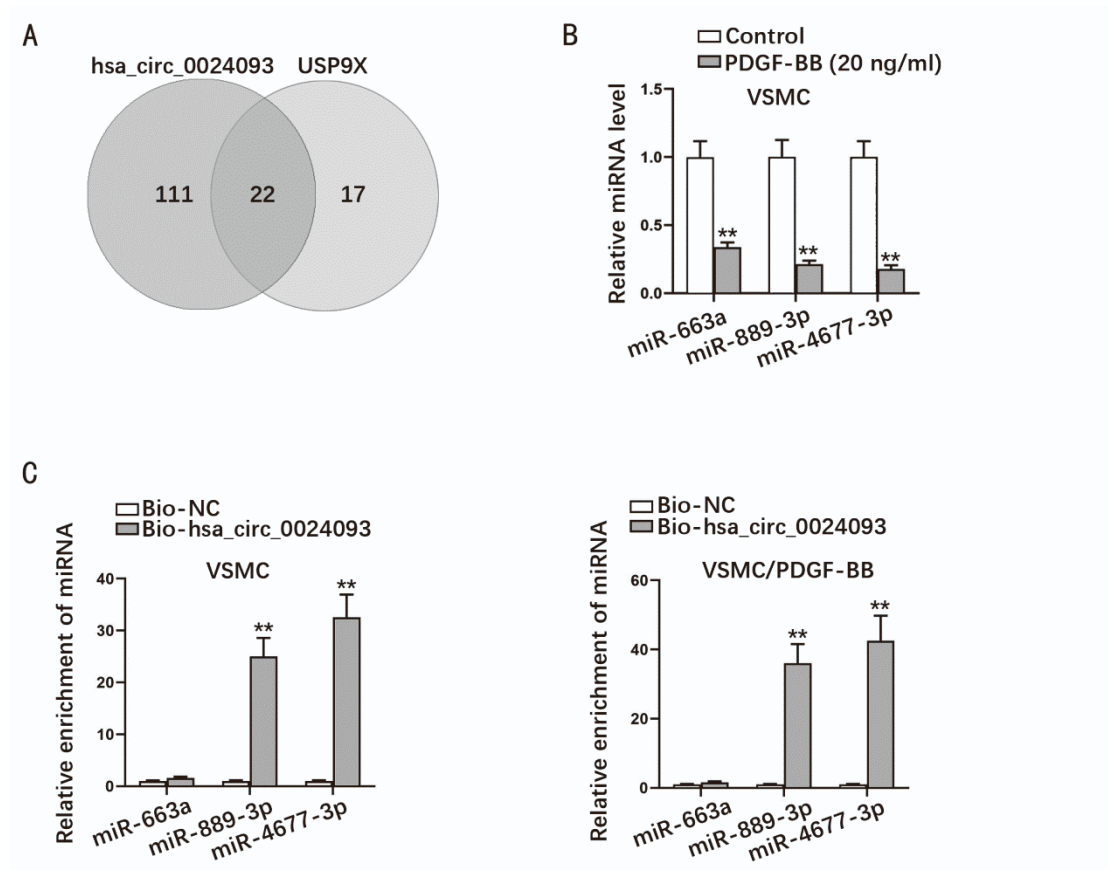


Figure S5. Hsa_circ_0024093 interacts with miR-889-3p and miR-4677-3p in VSMCs.

A. ENCORI database predicted 22 miRNAs binding with hsa_circ_0024093 and USP9X. B. The level of miR-663a, miR-889-3p and miR-4677-3p was examined in VSMCs with or without PDGF-BB treatment. C. The enrichment of miR-663a, miR-889-3p and miR-4677-3p in Bio-hsa_circ_0024093 groups was detected.

**P<0.01.