

Fibroblast Activation Protein Mediates Vascular Restenosis After Surgery for Peripheral Arterial Disease

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Abstract

Postoperative vascular restenosis is a major obstacle to the long-term efficacy of surgery for peripheral arterial disease (PAD). Abnormal migration and proliferation of vascular smooth muscle cells (VSMCs) trigger neointimal hyperplasia, the core pathology of restenosis, while relevant molecular regulators remain poorly defined. This study investigated the expression, function and mechanism of fibroblast activation protein (FAP). Bioinformatics analysis of GEO datasets identified FAP as a candidate factor. FAP showed low expression in normal arteries but was obviously elevated in injured vessels and synthetic VSMCs, and its expression correlated positively with VSMC migration. FAP knockdown restrained VSMC migration in vitro and mitigated neointimal hyperplasia in vivo. RNA sequencing suggested FAP may be involved in regulating the Net1–RhoA–ROCK signaling axis. Collectively, FAP is upregulated in injured arterial tissues and promotes VSMC migration to facilitate neointimal hyperplasia and restenosis, indicating its potential as a biomarker and therapeutic target for PAD-associated restenosis.

1. Introduction

Peripheral arterial disease (PAD) is pathologically characterized by atherosclerotic stenosis or occlusion of the arteries supplying the extremities, predominantly the lower limbs¹. It imposes a substantial global burden, with prevalence estimates ranging from 113 million² to 200 million³, being higher in females than in males and increasing markedly with age⁴. PAD frequently leads to limb pain⁵, impaired health-related quality of life, and loss of mobility and it confers elevated risks of major adverse cardiovascular events, amputation, and all-cause mortality^{6–8}. Percutaneous transluminal angioplasty (PTA) and its advanced modalities such as drug-coated balloon angioplasty serve as pivotal interventional strategies^{9,10} that effectively improve walking capacity and quality of life in patients with intermittent claudication, while reducing target lesion revascularization and major adverse limb events^{1,2,11}. Restenosis remains a major adverse complication following PTA, substantially limiting its long-term efficacy¹². Despite these advances, restenosis remains a major complication, with 1-year rates of 25%–63% in femoropopliteal lesions and approximately 60% in infrapopliteal lesions, significantly limiting long-term efficacy and clinical outcomes¹³.

Neointimal hyperplasia is the predominant cause of mid-term restenosis following PTA and stent implantation¹². In normal vessels, vascular smooth muscle cells (VSMCs) maintain a quiescent contractile phenotype, expressing markers such as α -SMA, SM22 α , and CNN1^{14–16}. Vascular injury triggers inflammatory responses and growth factors, particularly platelet-derived growth factor (PDGF), which drive VSMCs toward a synthetic phenotype with enhanced proliferation, migration, and extracellular matrix production^{17,18}, ultimately leading to neointimal hyperplasia and luminal restenosis.

Current prevention of restenosis relies on drug-coated balloons, drug-eluting stents, and covered stents during primary PTA, which suppress neointimal hyperplasia and improve patency^{1,2}. However, their

clinical benefits remain limited in complex in-stent restenosis, particularly in cases involving cross-junctional lesions, severe calcification, or those associated with severe stent fracture or displacement^{19–21}. Management of established ISR remains challenging, requiring secondary interventions such as atherectomy, repeat PTA, or bypass²⁰. Oral anticoagulant and antiplatelet therapies reduce thrombotic ISR but show limited efficacy against neointimal hyperplasia-driven ISR. Therefore, therapies specifically targeting neointimal formation are lacking; identifying novel molecular targets for excessive neointimal hyperplasia could provide new strategies for the prevention and management of lower extremity ISR.

To address the pressing need for novel molecular targets, our study employed bioinformatic approaches and identified fibroblast activation protein (FAP) as a promising candidate. FAP is a transmembrane serine protease that participates critically in extracellular matrix remodeling and tissue fibrosis, processes that share mechanistic similarities with neointimal hyperplasia^{22,23}. Although FAP has been extensively studied in tumor stroma^{24,25}, fibrotic diseases^{24,26} and aortic artery aneurysm²⁷, its potential role in the pathogenesis of lower extremity arterial restenosis remains completely unexplored. This study, therefore, sought to characterize the expression and functional relevance of FAP in neointimal formation following PTA.

2. Material and Methods

2.1 Gene Expression Omnibus (GEO) Dataset Acquisition and Data Preprocessing

2.1.1 Dataset Acquisition

Transcriptomic datasets related to neointimal hyperplasia were retrieved from the Gene Expression Omnibus (GEO) database using the keyword “neointimal”. Duplicate datasets and subgroup datasets were excluded. Additional exclusion criteria included non-peripheral arterial tissue samples, single-cell sequencing datasets, studies lacking paired control and experimental groups, datasets with tissue collection time points ≤ 7 days or ≥ 21 days, and datasets containing fewer than 200 differentially expressed genes (DEGs). Following screening, two datasets (GSE164050 and GSE220512) were selected for subsequent analyses (Fig. 1A).

2.1.2 Data Preprocessing

Transcriptomic analysis and functional enrichment analysis were performed using the selected datasets. DEGs were identified in each dataset, and overlapping DEGs between datasets were determined by intersection analysis. Commonly upregulated genes were subsequently subjected to Gene Ontology (GO) enrichment analysis, including biological process (BP), cellular component (CC), and molecular function (MF) analyses, to identify candidate genes potentially involved in neointimal hyperplasia.

2.2 Expression and Distribution of FAP In Vitro and In Vivo

2.2.1 In Vitro Experiments

Human primary cells representing different cellular components of the arterial wall, including human umbilical vein endothelial cells (HUVECs), human VSMCs, primary human adventitial fibroblasts (AFs), and THP-1-derived macrophages, were isolated from human aortic tissue specimens to assess the distribution pattern of FAP under physiological conditions. Total RNA was extracted from isolated cells and reverse-transcribed into cDNA for quantitative real-time polymerase chain reaction (qRT-PCR) analysis (Fig. 2A).

The study protocol using human primary cells and commercially available human cell lines was approved by the Ethics Committee of Chinese PLA General Hospital (PLAGH-2025-C-088). Written informed consent was obtained from all patients and their legal guardians for the isolation of primary human cells. All experiments involving human-derived materials (primary cells and cell lines) were carried out in strict accordance with relevant official guidelines and ethical regulations.

Rat primary VSMCs were subsequently stimulated with either 10% fetal bovine serum (FBS) or platelet-derived growth factor-BB (PDGF-BB) for 24 h to induce phenotypic transformation. Cell migratory capacity was evaluated using a scratch wound-healing assay. Protein expression levels of FAP and VSMC contractile markers (α -SMA, SM22 α , and CNN1) were determined by Western blot analysis.

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Institute of Model Animals of Chinese PLA General Hospital and the Animal Care and Use Committee of Chinese PLA General Hospital (PLAGH 0792).

At the experimental endpoint, mice were euthanized by intraperitoneal injection of an overdose of tribromoethanol (Avertin; Sigma-Aldrich, St. Louis, MO, USA) at 500 mg/kg. Following loss of respiratory and cardiac function confirmed by cessation of spontaneous breathing and lack of palpable pulse, thoracotomy was immediately performed as a secondary physical euthanasia procedure to ensure complete death.

2.2.2 In Vivo Experiments

Animal models of vascular injury were established, and arterial tissues were harvested at 4 weeks after intervention. Normal carotid arteries, as well as neointimal tissues obtained from the carotid artery wire injury model (CAWIM)²⁸ and the partial carotid ligation (PCL) model²⁹, were collected. Immunofluorescence staining was performed on carotid artery sections to examine the localization and expression pattern of FAP in normal and injured vascular tissues. Isotype IgG staining was included as a negative control to assess staining specificity.

2.3 Experimental Validation of FAP Expression and Distribution In Vitro and In Vivo

2.3.1 In Vitro Experiments

Small interfering RNA (siRNA) targeting FAP was selected and transfected into vascular smooth muscle cells (VSMCs) to suppress endogenous FAP expression. Three siRNA sequences targeting rat FAP (siFap-1, siFap-2, and siFap-3) were designed, with their corresponding sense and antisense strands listed (Table S1). Following transfection, VSMCs were stimulated with 10% FBS for 24 h. Cell migratory capacity was assessed using a scratch wound-healing assay. Western blot analysis was performed to verify FAP knockdown efficiency and assess the expression levels of VSMC contractile markers to evaluate VSMC phenotypic switching.

2.3.2 In Vivo Experiments

Ten-week-old male C57BL/6J mice were randomly assigned to receive one of three AAV9-mediated FAP RNA interference constructs (AAV-Fap-1, AAV-Fap-2, or AAV-Fap-3) before vascular injury procedures. The construct showing the highest knockdown efficiency was selected for subsequent experiments. Subsequently, carotid artery wire injury and partial carotid ligation (PCL) models were established. Carotid artery tissues were harvested at 4 weeks after injury for histological and immunofluorescence analyses. Hematoxylin and eosin (HE) staining was performed to evaluate neointimal thickness and luminal morphology, whereas immunofluorescence staining was used to examine FAP expression within neointimal lesions.

2.4 Transcriptomic Analysis and Functional Enrichment Analysis

Primary rat vascular smooth muscle cells (VSMCs) were assigned to four experimental groups for Transcriptome sequencing (RNA-seq): (1) siRNA-mediated FAP-knockdown VSMCs under non-stimulated conditions (SiNs); (2) siRNA-mediated FAP-knockdown VSMCs stimulated with 10% FBS (SiFBS); (3) control VSMCs under non-stimulated conditions (NcNs); and (4) control VSMCs stimulated with 10% FBS (NcFBS). Following treatment, total RNA was extracted from each group for RNA-seq (Fig. 4A). Differentially expressed genes (DEGs) were identified, followed by Gene Ontology (GO) enrichment analysis to characterize the biological processes and functional changes associated with different experimental conditions.

3. Results

3.1.1 Screening and Bioinformatic Analysis of GEO Datasets

A total of 82 records were initially retrieved from the GEO database. Following sequential screening according to the predefined inclusion and exclusion criteria, two datasets (GSE164050 and GSE220512) were selected for subsequent analysis (Figure 1B-C). Intersection analysis of DEGs identified 256 commonly upregulated genes and 34 commonly downregulated genes across the two datasets (Fig. 1E). Due to the relatively small number of downregulated genes, only the 256 commonly upregulated genes were subjected to further functional enrichment analysis.

3.1.2 GO Enrichment Analysis and Identification of FAP as a Candidate Hub Gene

GO enrichment analysis was performed on the 256 commonly upregulated genes. Molecular Function (MF) analysis identified 17 enriched genes within the top three functional categories, including integrin binding, cell adhesion molecule binding, and immune receptor activity (Fig. 1D). Among these genes, FAP was the only gene significantly enriched in MF categories, whereas no significant enrichment involving FAP was observed in the GO Biological Process (BP) or Cellular Component (CC) categories (Figure S1A-B). The remaining genes were primarily associated with inflammatory responses and leukocyte-related pathways. These findings suggested that FAP may represent a candidate regulator involved in vascular neointimal hyperplasia.

3.2 Expression and Distribution Characteristics of FAP in Vascular Tissues and Cells

3.2.1 Distribution of FAP in multiple vascular wall-derived cells

qRT-PCR analysis showed that FAP transcripts were abundantly expressed in VSMCs and fibroblasts, while HUVECs and THP-1-derived macrophages exhibited lower expression levels (Fig. 2B). These results indicate a cell type-specific pattern of FAP expression in primary human arterial wall cells.

Stimulation with FBS or PDGF-BB increased the migratory capacity of VSMCs, as assessed by scratch wound-healing assays ($P < 0.001$) (Fig. 2C,2G). Western blot analysis demonstrated that FAP protein expression was increased in stimulated VSMCs ($P < 0.001$), whereas only minimal expression was detected under basal conditions (Fig. 2D,2F, S2A-B). Although no significant change in α -SMA expression was observed ($P > 0.05$), the expression levels of SM22 α and CNN1 were markedly decreased following stimulation ($P < 0.001$) (Fig. 2E,2H, S2C-D), suggesting an early phenotypic transition from the contractile to the synthetic state.

3.2.2 FAP expression is elevated in injured carotid arteries

To characterize the expression pattern of FAP in vascular neointimal lesions, the CAWIM model was established. FAP expression was increased in neointimal tissues following arterial injury, whereas minimal fluorescence was observed in the IgG negative control (Fig. 2I). In this model, neointimal hyperplasia was primarily localized to the injured vascular region and was accompanied by a relatively thin neointimal layer and a tendency toward thrombus formation.

To further validate the expression pattern of FAP, the PCL model was established to induce neointima formation through disturbed blood flow (d-flow). Consistent with the findings in the CAWIM model ($P > 0.05$), robust FAP expression was observed throughout neointimal tissues in the PCL model (Fig. 2I,2J). Notably, the CAWIM model frequently developed thrombotic occlusion and exhibited substantial variability in neointimal thickness among animals, whereas the PCL model showed more reproducible lesion formation. Therefore, the PCL model was selected for all subsequent in vivo experiments.

3.3 Regulatory Effects of FAP on VSMC Function and Neointimal Hyperplasia

3.3.1 FAP knockdown suppresses VSMC migration and preserves the contractile phenotype

To investigate the functional role of FAP in VSMCs, siRNA-mediated FAP knockdown was performed in PDGF-BB-stimulated cells. Among the three siRNA (siFap-1, siFap-2, and siFap-3) sequences tested, siFap-2 showed the highest knockdown efficiency and was selected for subsequent experiments, as confirmed by Western blot analysis ($P < 0.01$) (Fig. 3A-B, S2E-F).

After efficient siRNA-mediated silencing of FAP, the migratory capacity of VSMCs was evaluated using a scratch wound-healing assay. No significant difference in wound closure was observed between groups at 12 h ($P > 0.05$). However, at 24 h, FAP-silenced VSMCs exhibited markedly reduced migration capacity and impaired wound closure compared with the control group ($P < 0.001$), indicating that FAP knockdown attenuated VSMC migration under stimulatory conditions (Fig. 3C-D).

Analysis of VSMC phenotypic markers showed no significant change in α -SMA expression ($P > 0.05$), whereas SM22 α and CNN1 expression levels were significantly increased in FAP-silenced cells compared with controls ($P < 0.001$) (Fig. 3E-F, S2G). These findings suggest that FAP knockdown was accompanied by a shift toward a more contractile VSMC phenotype.

3.3.2 In vivo effects of FAP knockdown on neointimal hyperplasia in vascular injury models

As FAP is reported to exhibit extremely low basal expression in normal mouse organs²³, the knockdown efficiency of FAP in C57BL mice was evaluated using adeno-associated virus-mediated RNA interference (AAV-Fap-1, AAV-Fap-2, and AAV-Fap-3), based on the immunofluorescence intensity in neointimal tissues.

Among the three constructs, AAV-Fap-RNA1 effectively reduced FAP expression in mouse neointimal tissues, as confirmed by immunofluorescence analysis (Fig. 3G-H). HE staining revealed that FAP knockdown via AAV transfection decreased both the intima-to-media (I/M) area ratio and the degree of luminal stenosis in the common carotid artery (Fig. 3I-J). These findings indicate that FAP plays a critical role in promoting neointimal formation following vascular injury, whereas suppression of FAP expression markedly attenuates neointimal hyperplasia and vascular restenosis.

3.4 RNA-seq Analysis of VSMCs with FAP Deficiency

3.4.1 Differential gene expression profiles in four experimental groups

RNA-seq was performed in FAP-knockdown and control VSMCs under both serum-stimulated and non-stimulated conditions. Comparison between the SiFBS and NcFBS groups identified 349 differentially expressed genes (DEGs), including 284 upregulated and 65 downregulated genes ($\log_2FC \geq 1$, $P \leq 0.05$) (Fig. 4B). In contrast, comparison between the SiNs and NcNs groups identified 182 DEGs, including 98 upregulated and 84 downregulated genes (Figure S3A). FAP expression was markedly reduced in both SiFBS and SiNs cells, confirming the effectiveness of siRNA-mediated knockdown at the transcriptomic level ($\log_2FC < -2.2$, $p < 0.0001$ for both comparisons).

The top 30 DEGs with the lowest adjusted P values ($padj$) were identified across two comparisons. Results for the serum-stimulated group (SiFBS vs NcFBS) are shown (Fig. 4C, Table S2), while those for the non-stimulated group (SiNs vs NcNs) are presented (Figure S3B, Table S3).

Beyond the top 30 DEGs, where *Net1* ($\log_2FC = -1.08$, $padj < 0.0001$) was significantly downregulated, further inspection of the DEG list identified several other genes associated with the Net1-RhoA signaling axis. Compared with NcFBS group, SiFBS group exhibited significantly decreased expression of *RhoA* ($\log_2FC = -0.32$, $padj < 0.01$), *Arhgef2* ($\log_2FC = -0.33$, $padj < 0.0001$) and *Rock1* ($\log_2FC = -0.26$, $padj < 0.05$), and increased expression of *Arhgap6* ($\log_2FC = 0.45$, $padj < 0.0001$) and *Rock2* ($\log_2FC = 0.29$, $padj < 0.001$).

3.4.2 Functional enrichment analysis of DEGs induced by FAP knockdown and serum stimulation

To clarify the biological functions affected by FAP deficiency, GO enrichment analysis was performed on the identified differentially expressed genes, focusing only on statistically significant terms ($padj < 0.05$).

In the SiFBS versus NcFBS comparison, significantly enriched BP included G-protein-coupled receptor signaling pathway ($padj < 0.05$); CC analysis revealed no statistically significant enrichment of any functional terms ($padj > 0.05$); whereas MF analysis revealed significant enrichment of signaling receptor activity, molecular transducer activity ($padj < 0.001$), G-protein-coupled receptor activity, and transmembrane signaling receptor activity ($padj < 0.05$) (Fig. 4D, Table S4).

In the SiNs versus NcNs comparison, the significantly enriched BP was cell adhesion ($padj < 0.05$), whereas no CC or MF terms were significantly enriched in the DEGs ($padj > 0.05$) (Figure S3C, Table S5).

4. Discussion

The present study uncovers a significant role of FAP in vascular restenosis. We found that FAP expression was markedly upregulated in injured arteries and in synthetic VSMCs, and that FAP knockdown significantly attenuated VSMC migration in vitro and reduced neointimal hyperplasia in vivo. These findings extend the current understanding of FAP in the cardiovascular system, which has so far focused on its proteolytic activity in extracellular matrix remodeling and fibrous cap reconstruction within atherosclerotic plaques^{30,31} as well as its function as a signaling molecule that mediates

fibroblast–macrophage crosstalk to amplify pro-inflammatory responses^{32,33}. Our results demonstrate that FAP critically contributes to vascular remodeling after injury and highlight its potential as a novel therapeutic target for restenosis.

Low basal expression of FAP is normally maintained under physiological conditions³⁴, whereas markedly elevated FAP expression has been widely observed in pathological tissues associated with various cardiovascular diseases, including the fibrous caps of coronary atherosclerotic plaques³⁵, as well as tissues affected by myocardial infarction, myocardial fibrosis³⁶ and aortic dissection³⁰. In the present study, FAP was found to exhibit extremely low basal expression in normal rat VSMCs and mouse tissues, which is consistent with previous findings. Notably, our transcriptomic analysis revealed that FAP knockdown induced only mild transcriptional alterations in the cell adhesion BP pathway under non-stimulated conditions but led to widespread alterations after serum stimulation. These findings support a context-dependent role for FAP, wherein its biological functions become prominent only upon VSMC activation and phenotypic modulation, rather than in the quiescent state.

To further validate the functional role of FAP, we performed siRNA-mediated silencing (siFap-2, the most efficient construct) and observed that FAP knockdown significantly impaired wound closure in scratch assays and preserved the contractile phenotype, as evidenced by increased SM22 α and CNN1 expression without altering α -SMA levels. These in vitro findings were corroborated in two complementary in vivo models: the carotid artery wire injury model (CAWIM) and the partial carotid ligation (PCL) model. Both models showed abundant FAP expression in neointimal lesions, and AAV9-mediated FAP knockdown (AAV-Fap-RNA1) reduced the intima-to-media area ratio and luminal stenosis. Together, these loss-of-function experiments establish that FAP is required for efficient VSMC migration and neointimal hyperplasia following arterial injury. These convergent lines of evidence from cellular and animal studies provide a strong rationale for targeting FAP as an anti-restenosis strategy.

Transcriptomic profiling of VSMCs following FAP deficiency has not been reported. Previous studies have suggested that FAP is involved in extracellular matrix remodeling and multiple signaling pathways associated with cellular migration and tissue remodeling^{27,31}. Our RNA-seq analyses in FAP-silenced cells highlighted numerous genes related to cytoskeletal organization, cell motility, and vascular remodeling, providing a foundation to identify specific candidate genes mediating migratory changes.

Among the migration-related genes altered by FAP knockdown, *Net1* was of particular interest. *Net1* is a RhoA-specific guanine nucleotide exchange factor (GEF) that activates RhoA signaling to regulate actin cytoskeleton remodeling, focal adhesion maturation, and cell migration^{37,38}. Previous studies have shown that Net1-mediated RhoA activation enhances stress fiber formation, myosin light chain phosphorylation, and cell motility³⁷. Activated RhoA further binds and stimulates its canonical downstream effector ROCK, which consists of two highly homologous isoforms, ROCK1 and ROCK2^{39,40}. Accumulating evidence has revealed that the RhoA/ROCK cascade acts as a core regulator of VSMC

contraction and phenotypic switching in multiple hypertensive models, and is tightly linked to increased peripheral resistance and vascular wall thickening⁴¹.

RNA-seq analysis revealed significant downregulation of both *Net1* and *RhoA* in FAP-deficient VSMCs under serum-stimulated conditions. Notably, expression of *Arhgef2*, another RhoA-activating factor^{42,43}, was also reduced, whereas *Arhgap6*, a GTPase-activating protein that negatively regulates RhoA activity^{44,45}, was upregulated. For the downstream effector ROCK, its two isoforms exhibited opposite expression trends: *ROCK1* was downregulated and *ROCK2* was upregulated. Overall, these molecular changes suggest that the Net1-RhoA-ROCK signaling axis is at least substantially perturbed and ultimately suppressed in FAP-knockdown VSMCs. Further investigation into whether FAP directly interacts with or regulates the stability/activity of Net1, RhoA, or ROCK isoforms will be critical to fully understand how FAP orchestrates this signaling axis to promote VSMC migration and neointimal hyperplasia.

Although GO enrichment analysis revealed upregulation of several migration-related genes and pathways, including *Cxcr4*, and signaling processes such as G-protein coupled receptor and receptor-ligand activity, the magnitude and completeness of these changes were smaller than those observed for the Net1-RhoA-ROCK axis. The scattered upregulation of individual migration-related genes, such as *Cxcr4*, may represent a compensatory response rather than a primary mechanism driving VSMC migration.

In summary, this study provides the first evidence that FAP is actively involved in the development of neointimal hyperplasia and vascular restenosis. FAP expression was markedly increased in injured arteries and synthetic VSMCs, while its suppression attenuated VSMC migration and reduced neointimal formation in vivo. Mechanistically, transcriptomic analyses suggest that inhibition of the Net1–RhoA–ROCK signaling axis may underlie these protective effects. Collectively, these findings expand the current understanding of FAP beyond its established roles in extracellular matrix remodeling and fibrosis, and identify FAP as a potential molecular target for the prevention of restenosis after peripheral arterial revascularization.

Declarations

Acknowledgment

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Declaration of Interest

The authors have nothing to disclose regarding financial and personal relationships that could inappropriately influence or bias the work.

Approval for animal experiments

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Institute of Model Animals of Chinese PLA General Hospital and the Animal Care and Use Committee of Chinese PLA General Hospital (PLAGH 0792).

Approval for human experiments

The study protocol using human primary cells and commercially available human cell lines was approved by the Ethics Committee of Chinese PLA General Hospital (PLAGH-2025-C-088). Written informed consent was obtained from all patients and their legal guardians for the isolation of primary human cells. All experiments involving human-derived materials (primary cells and cell lines) were carried out in strict accordance with relevant official guidelines and ethical regulations.

Author contributions statements

Jiashu Ge and Tianfeng Ma conceived and designed the research framework, completed the main experiments, collected and analyzed the experimental data, and drafted the original manuscript. Zeyu Cai performed key experiments, analyzed data, and participated in manuscript drafting. Dan Rong supervised the study design, interpreted experimental results, and revised the manuscript. Wei Guo oversaw the entire research project, acquired funding, finalized the manuscript, and took full responsibility for the published work. All authors have read, understood, and fully agreed upon the author sequence and the publication of this article, with no conflicts of authorship to declare.

Data availability

All datasets analyzed in this study, including GSE164050 and GSE220512, are publicly available from the Gene Expression Omnibus (GEO) database. If necessary, we will apply for permission from the relevant departments of the Chinese PLA General Hospital. The original data covering transcriptomic sequencing and basic experiments can be made public after their approval is obtained.

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Figures

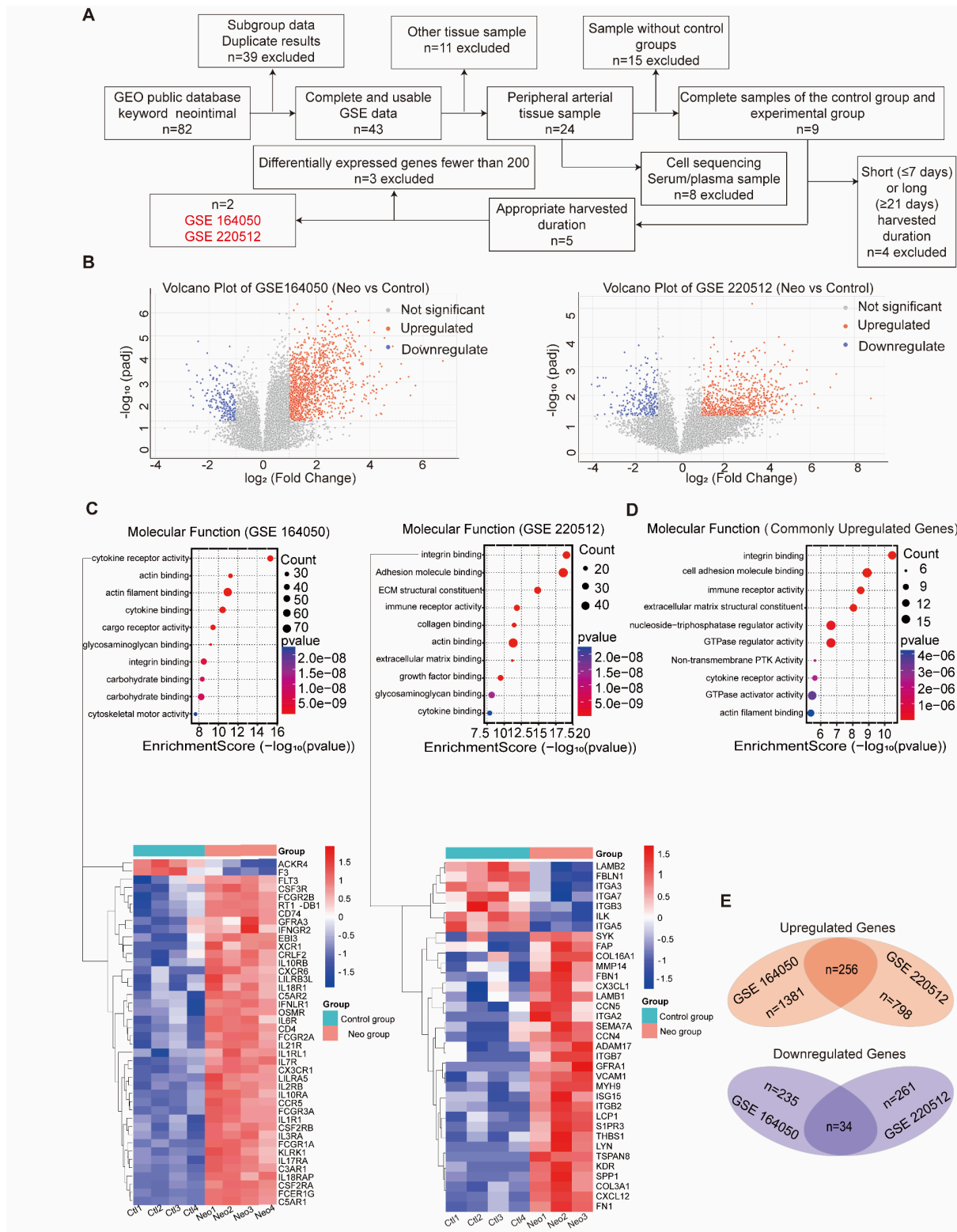


Figure 1

Identification of differentially expressed genes and functional enrichment from GEO datasets

(A) Workflow of GEO dataset selection and screening. Two datasets (GSE164050 and GSE220512) were selected from 82 initial “neointimal”-related datasets according to predefined inclusion/exclusion criteria.

(B) Volcano plots showing differentially expressed genes (DEGs) in GSE164050 (left) and GSE220512 (right). Red dots indicate upregulated genes, blue dots indicate downregulated genes, and gray dots indicate non-significant genes.

(C) Molecular Function (MF) enrichment analysis for DEGs in GSE164050 (left) and GSE220512 (right). Dot size indicates the number of enriched genes, and color represents enrichment significance ($-\log_{10}$ P value).

(D) MF enrichment analysis of 256 commonly upregulated genes across both datasets.

(E) Venn diagrams showing the overlap of upregulated (top) and downregulated (bottom) genes between GSE164050 and GSE220512.

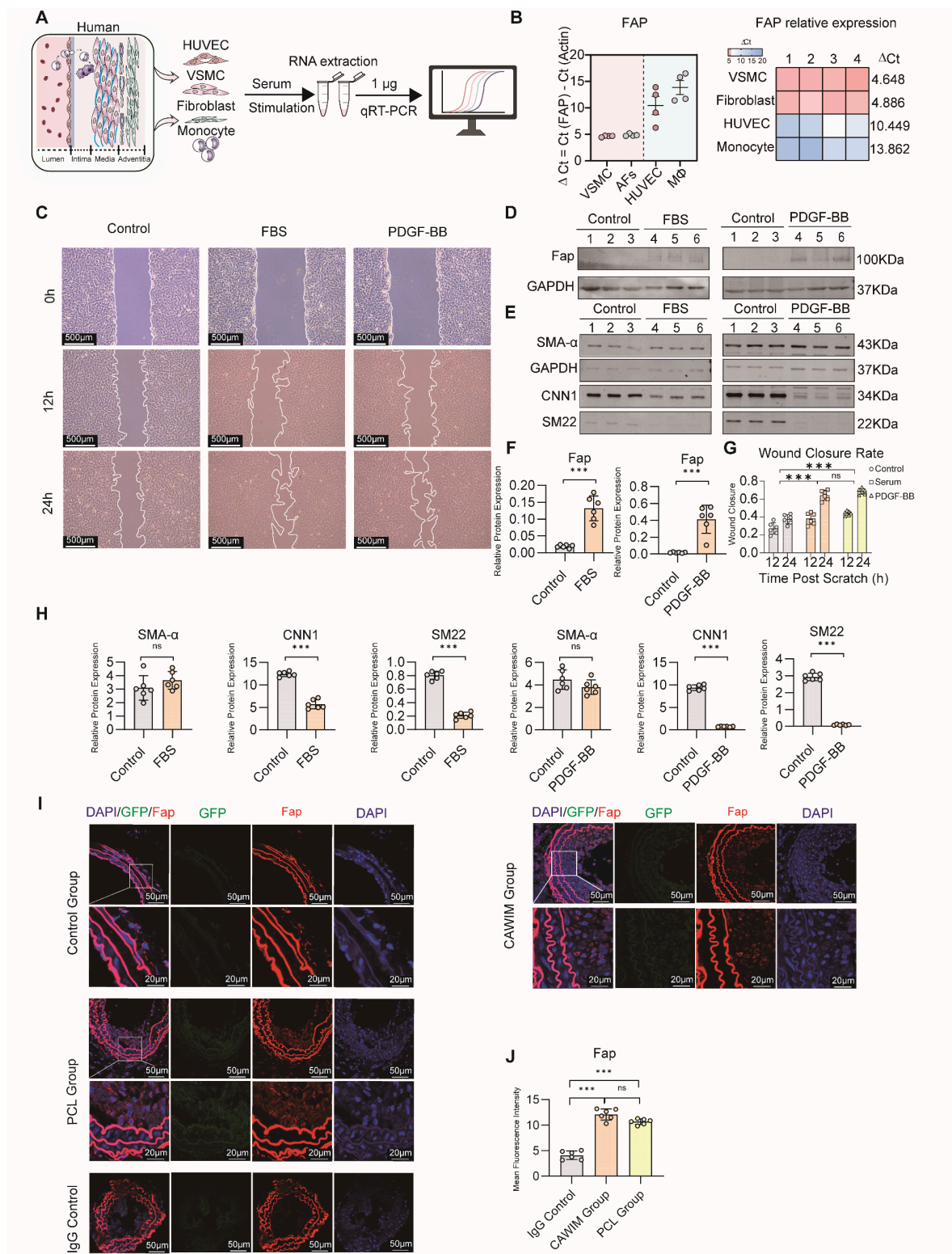


Figure 2

FAP expression and distribution in vascular cells and injured arteries

(A) Experimental design for in vitro analysis of FAP expression in primary human vascular cells, including VSMCs, adventitial fibroblasts (AFs), HUVECs, and monocytes. Cells were subjected to RNA extraction and qRT-PCR.

(B) Relative FAP mRNA expression in human VSMCs, AFs, HUVECs, and monocytes. Heatmap of ΔCt values is shown.

(C, G) Representative scratch wound-healing images and quantification of VSMCs under control, 10% FBS, or PDGF-BB stimulation at 0, 12, and 24 h.

(D, F) Western blot analysis and quantification of FAP protein expression in VSMCs after 24 h stimulation with 10% FBS or PDGF-BB. Full-length uncropped blots are provided in Figure **S2A–B**.

(E, H) Western blot analysis and quantification of contractile markers (α -SMA, SM22 α , CNN1) in VSMCs after 24 h stimulation with 10% FBS or PDGF-BB. Full-length uncropped blots are provided in Figure **S2C–D**.

(I) Representative immunofluorescence images showing FAP expression in carotid arteries from control, CAWIM, and PCL groups. GFP marks transduced cells, and DAPI stains nuclei. Insets show high-magnification regions.

(J) Quantification of mean FAP fluorescence intensity in neointimal lesions. Data are mean $\pm$ SD. *** $P < 0.001$; ns, not significant.

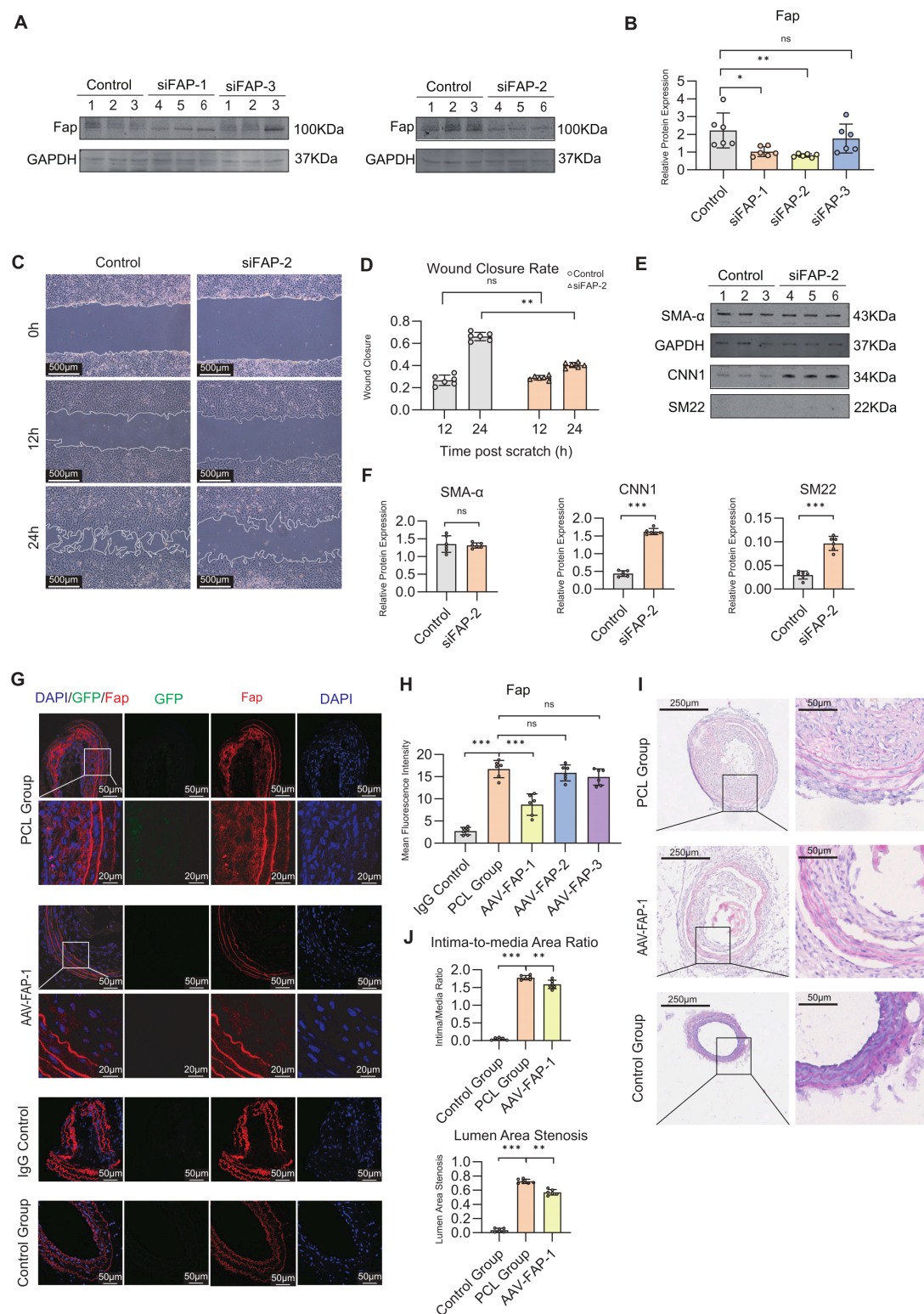


Figure 3

FAP knockdown suppresses VSMC migration and affects contractile marker expression

(A) Western blot analysis of FAP protein expression after transfection with three different siRNAs (siFAP-1, siFAP-2, siFAP-3) in VSMCs. GAPDH served as loading control. Full-length uncropped blots are provided in Figure **S2E–F**.

(B) Quantification of FAP protein levels normalized to GAPDH. Data are mean $\pm$ SD. Statistical significance: ns, not significant; *, $P < 0.05$; **, $P < 0.01$.

(C) Representative scratch wound-healing images of VSMCs transfected with control or siFAP-2 at 0, 12, and 24 h. Scale bar, 500 μ m.

(D) Quantification of wound closure rate over 24 h in control and siFAP-2 VSMCs. Data are mean $\pm$ SD. Statistical significance: ns, not significant; **, $P < 0.01$.

(E–F) Western blot analysis and quantification of contractile markers (α -SMA, SM22 α , CNN1) in VSMCs 24 h after siFAP-2 transfection. GAPDH was used as a loading control. Full-length uncropped blots are provided in Figure **S2G**.

(G–H) Representative immunofluorescence images and quantification of FAP expression in neointimal lesions following AAV-FAP-1 or AAV-FAP-2 transduction in PCL-treated carotid arteries. GFP marks transduced cells, and DAPI stains nuclei. Insets show high-magnification regions. Scale bars: 50 μ m (low) and 20 μ m (high).

(I–J) Hematoxylin-eosin staining of carotid arteries and quantification of intima-to-media (I/M) ratio and lumen area stenosis after FAP knockdown or AAV-FAP-1 transduction. Data are mean $\pm$ SD. **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

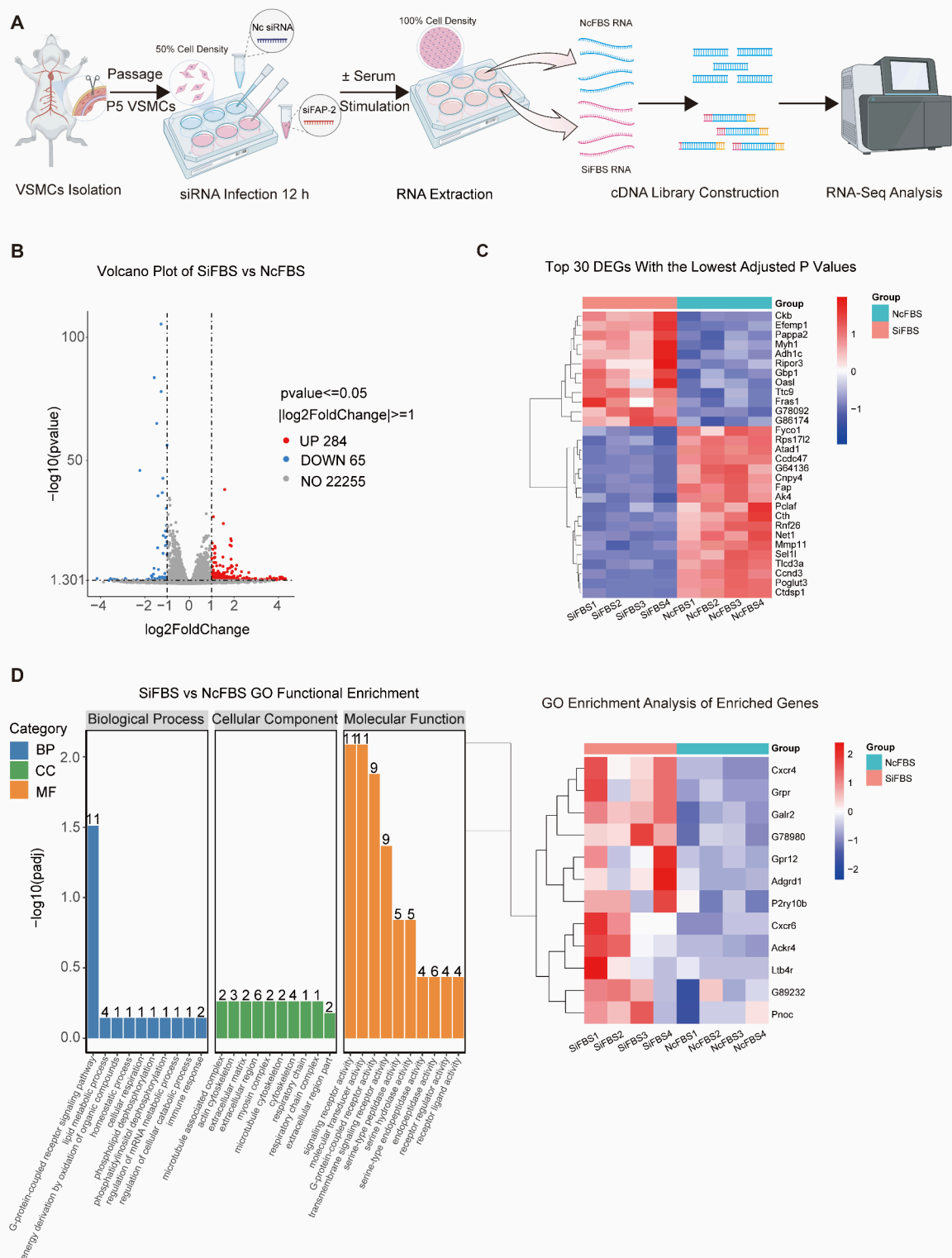


Figure 4

Transcriptome profiling and GO functional enrichment analysis of FAP-deficient VSMCs under different culture conditions

(A) Schematic overview of the four experimental groups for RNA-seq analysis in primary rat VSMCs.

- (B)** Volcano plots showing differentially expressed genes (DEGs) between the SiFBS and NcFBS groups. Red dots represent upregulated genes, and blue dots represent downregulated genes.
- (C)** Heatmap displaying the top 30 DEGs ranked by padj in the SiFBS vs NcFBS comparison.
- (D)** Bubble plot of significantly enriched GO terms (BP and MF) for DEGs from the SiFBS vs NcFBS group. Bubble size indicates the number of enriched genes, and color represents padj.

Supplementary Files

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