



Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2018 February ; 38(2): 335–343. doi:10.1161/ATVBAHA.117.310491.

Vascular Semaphorin 7A upregulation by disturbed flow promotes atherosclerosis through endothelial β 1 integrin

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Abstract

Objective—Accumulating evidence suggests a role of semaphorins in vascular homeostasis. Here we investigate the role of semaphorin 7A (Sema7A) in atherosclerosis and its underlying mechanism.

Approach and Results—Using genetically-engineered *Sema7A*^{-/-}*ApoE*^{-/-} mice, we showed that deletion of Sema7A attenuates atherosclerotic plaque formation primarily in the aorta of *ApoE*^{-/-} mice on a high fat diet. A higher level of Sema7A in the atheroprone lesser curvature suggests a correlation of Sema7A with disturbed flow (d-flow). This notion is supported by elevated Sema7A expression in human umbilical venous endothelial cells (HUVECs) either subjected to oscillatory shear stress or treated with the PKA/CREB inhibitor H89. Further studies using the partial carotid artery ligation model showed that d-flow in the left carotid artery of *Sema7A*^{+/+}*ApoE*^{-/-} mice promoted the expression of endothelial Sema7A and cell adhesion molecules (CAMs), leukocyte adhesion, and plaque formation, while such changes were attenuated in *Sema7A*^{-/-}*ApoE*^{-/-} mice. Further studies showed that blockage of β 1 integrin, a known Sema7A receptor, or inhibition of FAK, MEK1/2 or NF κ B, significantly reduced the expression of CAMs and THP-1 monocyte adhesion in Sema7A overexpressing HUVECs. Studies

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Disclosures

None.

using chimeric mice suggest that vascular, most likely endothelial Sema7A plays a major role in atherogenesis.

Conclusions—Our findings indicate a significant role of Sema7A in atherosclerosis by mediating endothelial dysfunction in a β 1 integrin-dependent manner.

Keywords

Atherogenesis; Endothelial phenotypic changes; Disturbed blood flow; Leukocyte recruitment; Semaphorin 7A

Journal Subject Code

Vascular Disease

Introduction

Endothelial cells (ECs) sense and respond to various pathophysiological stimuli in maintaining vascular integrity and homeostasis.^{1–4} When exposed to disturbed blood flow (d-flow), these cells undergo major phenotypic changes that lead to increased vascular permeability, cytokine release and leukocyte adhesion.^{4–6} Such structural and functional changes in vessel wall increase the susceptibility to atherosclerosis.^{7–10} Previous studies looking into mechanisms underlying the hemodynamic regulation of vascular endothelium have unveiled an array of flow responsive candidates, including membrane receptors, adhesion molecules, glycocalyxes, and cytoskeleton proteins that are considered as promising therapeutic targets for atherosclerotic disease.^{8, 11, 12}

Semaphorins are originally identified as regulators of neuronal growth, while individual family members have been shown to play roles in other physiological and pathological conditions, including immune responses, organogenesis, angiogenesis, vascular development and disorders^{13, 14}. Semaphorin 7A (Sema7A) is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein¹⁵ with an N-terminal seven-bladed β -propeller Sema domain, a plexin-semaphorin-integrin domain, an immunoglobulin-like domain and a C-terminal GPI anchoring domain.¹⁶ The Sema domain contains an RGD motif commonly found in integrin-binding proteins.¹⁷ Expression of Sema7A and its known membrane receptors, integrin β 1 and plexin C1, were found in neurons, endothelial cells, platelets, monocytes, T cells, dendritic cells (DCs) and cancer cells.¹⁸ Previous studies showed that Sema7A is involved in the regulation of inflammatory response, cancer progression, and neuron growth.^{17, 19, 20} Recently, lung EC Sema7A was reported to be upregulated by HIF-1 α , an oxygen sensitive transcription factor, in the setting of acute inflammation induced by hypoxia, LPS or seawater.^{21–23} However, there has been no report whether Sema7A participates in the development of atherosclerosis.

We have previously shown that disruption of Sema4D, another member of semaphorin family, protects against the development of atherosclerosis.²⁴ In this study, we utilized a partial carotid artery ligation (PCL) model in *ApoE*^{-/-} mice fed with a high fat diet (HFD), and showed that endothelial Sema7A expression is increased by surgically induced d-flow,

while genetic deletion of *Sema7A* ameliorated atherosclerosis in *ApoE*^{-/-} mice. *Sema7A* appeared to cause endothelial cell dysfunction through endothelial $\beta 1$ integrin/FAK/MEK1/2/NF κ B pathway, acting as a proatherogenic molecule.

Materials and methods

Materials and methods are available in the online-only Data Supplement.

Results

Genetic deletion of *Sema7A* reduces plaque size in the aorta of *ApoE*^{-/-} mice

To investigate the role of *Sema7A* in atherosclerosis, we generated *Sema7A*^{-/-}*ApoE*^{-/-} mice and fed them the HFD to induce atherosclerosis. After 12 w, the mice were sacrificed and their aortas were stained with Sudan IV. Atherosclerotic lesion size was 51.5% smaller in *Sema7A*^{-/-}*ApoE*^{-/-} mice than in *Sema7A*^{+/+}*ApoE*^{-/-} mice ($5.0 \pm 1.0\%$ of the total aorta area vs $10.3 \pm 1.1\%$, $P < 0.01$) and the reduction of the lesion size was most prominent in the aortic arch (59.2%) and less in the descending aorta (41.5%) (Figure 1A, B). Histological studies showed that areas of lipid accumulation in the aortic root were 51.8% less in *Sema7A*^{-/-}*ApoE*^{-/-} mice than in *Sema7A*^{+/+}*ApoE*^{-/-} mice ($18.6 \pm 3.1\%$ vs $38.6 \pm 3.8\%$, $P < 0.001$) (Figure 1C, D). Similar serum lipid profiles and body weights were found in *Sema7A*^{-/-}*ApoE*^{-/-} and *Sema7A*^{+/+}*ApoE*^{-/-} mice (Table II in the online-only Data Supplement). In addition, immunostaining also showed high levels of *Sema7A* protein in human atherosclerotic carotid tissues compared with human healthy abdominal aorta (Figure 1E). These results indicate that *Sema7A* participates in atherosclerosis development.

D-flow upregulates vascular endothelial *Sema7A* expression

Sema7A deletion primarily reduces plaque formation in aortic arch, suggesting a higher expression level of *Sema7A* in this region. Indeed, higher expression levels of *Sema7A* mRNA and protein in the aortic arch (AA) were observed compared with that in the descending aorta (DA) (Figure 2A, B). The lesser curvature (LC) of the aorta is an atheroprone region that encounters d-flow as compared to the greater curvature (GC) (Figure 2C). Further studies showed that *Sema7A* mRNA and protein levels were higher in LC than in GC (Figure 2D, E). To test if hemodynamic stress increases *Sema7A* expression *in vivo*, we induced d-flow by PCL in WT mice²⁵ (Figure 2C and Figure I in the online-only Data Supplement). Compared with the untied right common carotid artery (RCA) with steady flow (s-flow), left common carotid artery (LCA) showed a 4-fold increase in *Sema7A* mRNA levels 48 h after the ligation (Figure 2F). To explore the cellular source for the increased *Sema7A* expression under d-flow, we examined *Sema7A* protein expression in carotid artery endothelium after the PCL by *en face* staining. Prominent endothelial *Sema7A* protein was detected in the ligated LCA, as shown by co-staining of *Sema7A* and CD31, whereas little *Sema7A* staining was found in the control RCA (Figure 2G). Consistently, *Sema7A* mRNA level in cultured human umbilical vein endothelial cells (HUVECs) exposed to oscillatory shear stress (OS) was significantly higher than that in the cells exposed to laminar shear stress (LS) (Figure 2H). These data indicate that d-flow upregulates vascular endothelial *Sema7A* expression.

To understand how d-flow upregulates endothelial Sema7A expression, we performed a *silico* promoter analysis and identified potential binding sites in human and mouse Sema7A promoter regions for cAMP response element-binding protein (CREB), a transcription factor known to respond to shear stress^{26, 27} (Figure II A, B in the online-only Data Supplement). As PKA is a canonical CREB upstream regulator in flow-mediated signaling, we examined Sema7A expression in HUVECs after inhibiting the PKA-CREB signaling using the PKA inhibitor H89. Results showed that H89 reduced CREB phosphorylation and increased Sema7A mRNA and protein expression (Figure 2I-L). These data suggest that suppression of PKA/CREB signaling may be an underlying mechanism in d-flow-induced endothelial Sema7A expression.

Sema7A-deficiency reduces d-flow induced endothelial ICAM-1 and VCAM-1 expression, leukocyte adhesion, and plaque formation

Endothelial adhesion molecules ICAM-1 and VCAM-1 are upregulated by d-flow and contribute to leukocyte recruitment, endothelial inflammation and dysfunction.²⁸ To determine whether d-flow-induced Sema7A expression contributes to endothelial inflammation, we assessed ICAM-1 and VCAM-1 levels in carotid arteries in *Sema7A*^{+/+} and *Sema7A*^{-/-} mice following PCL surgery. Compared to the control RCA, ICAM-1 and VCAM-1 mRNA levels were increased by 2.81- and 5.24-fold ($P < 0.0001$), respectively, in PCL-treated LCA from *Sema7A*^{+/+} mice. In contrast, such increases were abrogated in *Sema7A*^{-/-} mice (Figure 3A, D). Carotid P-selectin mRNA levels were also reduced in LCA of *Sema7A*^{-/-} mice compared to *Sema7A*^{+/+} mice, whereas E-selectin mRNA levels in PCL-treated LCA were similar in *Sema7A*^{+/+} and *Sema7A*^{-/-} mice (Figure III in the online-only Data Supplement), indicating selective regulation by Sema7A on d-flow induced expression of adhesion molecules. Consistently, *en face* immunostaining showed higher endothelial ICAM-1 and VCAM-1 expression in LCA from *Sema7A*^{+/+} mice compared with that from *Sema7A*^{-/-} mice (Figure 3B, C, E, F).

Leukocyte rolling and adhesion, mediated by selectins and adhesion molecules, are crucial for monocyte recruitment and accumulation in atherogenesis.²⁹ Given the role of Sema7A in upregulating the expression of ICAM-1 and VCAM-1, as well as P-selectin, we examined the functional relevance of Sema7A in endothelial inflammation. Using a real-time intravital microscopy, we monitored leukocyte rolling and adhesion on mouse cremaster venule endothelium primed by *TNF- α* .³⁰ Leukocyte rolling and adhesion were decreased by 45.2% (28.5 ± 3.7 cells/min vs 15.6 ± 1.6 cells/min, $P < 0.01$) and 68.2% (12.4 ± 1.2 cells/min vs 4.0 ± 0.6 cells/min, $P < 0.0001$), respectively, in *Sema7A*^{-/-} mice compared to *Sema7A*^{+/+} mice (Figure 3G, H, Supplemental Video I in the online-only Data Supplement). Using a modified PCL model, in which F4/80⁺ leukocytes adhered on the endothelium of ligated carotid artery were quantified, we showed that leukocyte adhesion was decreased by 67.2% (19.0 ± 2.5 cells/field vs 57.9 ± 4.3 cells/field, $P < 0.0001$) in *Sema7A*^{-/-} mice compared to *Sema7A*^{+/+} mice (Figure 3I, J). These results indicate that Sema7A deficiency reduces d-flow induced upregulation of adhesion molecules and the consequent leukocyte adhesion in mice.

Reduced leukocyte recruitment is expected to inhibit atherogenesis. To evaluate the effect of *Sema7A* deletion on d-flow induced plaque formation *in vivo* we established an extended PCL model, in which *Sema7A*^{-/-}*ApoE*^{-/-} and *Sema7A*^{+/+}*ApoE*^{-/-} mice were subjected to PCL and fed HFD for 2 w, followed by *in situ* measurements of the plaque area. Although PCL induced atherosclerotic lesions developed in the LCA of both groups, the average lesion size in LCA from *Sema7A*^{-/-}*ApoE*^{-/-} mice was significantly reduced compared with that from *Sema7A*^{+/+}*ApoE*^{-/-} mice (17.8 ± 3.7% vs 52.1 ± 4.0%, *P* < 0.0001) (Figure 3K, L). These findings suggest that *Sema7A* promotes d-flow induced atherosclerotic plaque formation.

***Sema7A* overexpression enhances ICAM-1 and VCAM-1 expression in HUVECs and monocyte-endothelial cell interaction via β 1 integrin pathway**

To investigate the mechanism by which *Sema7A* regulates ICAM-1 and VCAM-1 expression and leukocyte adhesion in atherosclerosis, we generated a *Sema7A*-overexpressing endothelial cell line by transducing lentiviral vectors expressing human *Sema7A* with a GFP tag (Lenti-h*Sema7A*-GFP) into HUVECs (Figure IVA in the online-only Data Supplement). Quantitative PCR, Western blotting and immunostaining were used to determine *Sema7A* expression in the cell line (Figure 4A, B and Figure IVB in the online-only Data Supplement). Compared to HUVECs transduced with the control virus (Lenti-pCDH-GFP), HUVECs transduced with Lenti-h*Sema7A*-GFP expressed significantly higher levels of ICAM-1 and VCAM-1 (Figure 4C, D and Figure VC, D in the online-only Data Supplement).

β 1 integrin is a *Sema7A* receptor that mediates cell-cell interaction in axon outgrowth and T cell response.^{17, 20} Given the presence of β 1 integrin in endothelial cells, we hypothesized that *Sema7A* may mediate endothelial dysfunction through β 1 integrin. Flow cytometry showed that β 1 integrin blockage by a monoclonal antibody (P5D2) significantly reduced ICAM-1 expression compared to an isotypic control (IgG) (Figure 4E). Similarly, pre-incubation with a blocking antibody against integrin α 1 (5E8D9) subunit reduced ICAM-1 expression in *Sema7A* overexpressing HUVECs (Figure 4E), suggesting that *Sema7A* mediates endothelial phenotypic changes, at least in part, through the α 1 β 1 heterodimer.

Previous studies showed that activation of focal adhesion kinase (FAK) by *Sema7A* binding to β 1 integrin activates downstream mitogen-activated protein kinase (MAPK).¹⁷ NF κ B, the downstream molecule to MAPK, is a canonical switch for the expression of pro-inflammatory molecules including ICAM-1 and VCAM-1.³¹ We analyzed FAK/MAPK/NF κ B signaling in *Sema7A*-overexpressing HUVECs. As expected, FAK phosphorylation was increased by *Sema7A* overexpression (Figure 4F). Treatment of PF573228 (10 μ M), a specific inhibitor for FAK, U0126 (10 μ M), a potent inhibitor for both MEK1 and MEK2, or BMS-345541 (20 μ M), a highly selective inhibitor for the catalytic subunits of IKK-2 and IKK-1, attenuated ICAM-1 expression in *Sema7A*-overexpressing HUVECs (Figure 4G). Downstream NF κ B P65 phosphorylation was also inhibited by U0126 (10 μ M) and BMS-345541 (20 μ M) (Figure 4H). These findings indicate that FAK/MEK1/2/NF κ B may act downstream of the *Sema7A*-integrin β 1 axis to mediate vascular endothelial dysfunction.

To determine the involvement of $\beta 1$ integrin and the downstream FAK/MEK1/2/NF κ B signaling in leukocyte-endothelial interaction, an *in vitro* monocyte-endothelial cell adhesion assay was performed. *Sema7A*-overexpressing HUVECs had a 1.1-fold increase in THP-1 cell adhesion compared with control HUVECs (60.4 ± 1.8 cells/field vs 28.7 ± 0.9 cells/field, $P < 0.0001$). However, the increased monocyte adhesion was attenuated by antibody blockage of integrin $\beta 1$ or $\alpha 1$ subunits, or by inhibition of FAK, MEK1/2 or NF κ B with small molecule inhibitors (Figure 4I, J). These results indicate that *Sema7A* enhances the expression of adhesion molecules and leukocyte adhesion on endothelial cells in a $\beta 1$ integrin-dependent manner that involves downstream FAK/MEK1/2/NF κ B signaling.

Endothelial *Sema7A* plays a major role in promoting leukocyte adhesion and plaque formation in *ApoE*^{-/-} mice

Sema7A is expressed in many cell types.¹⁸ To distinguish the role of vascular vs blood cell-derived *Sema7A* in leukocyte adhesion, we performed an *ex vivo* leukocyte adhesion assay, in which aortic endothelium from *Sema7A*^{+/+}*ApoE*^{-/-} or *Sema7A*^{-/-}*ApoE*^{-/-} mice were treated with LPS and incubated with leukocytes from these mice. As shown in Figure 5A, although adhered monocytes on LPS-treated *Sema7A*^{+/+} aortic endothelium were ~30% less when incubated with *Sema7A*^{-/-} leukocytes compared with *Sema7A*^{+/+} leukocytes ($P < 0.05$), monocyte adhesion was virtually blocked when LPS-treated *Sema7A*^{-/-} endothelium was incubated with either *Sema7A*^{+/+} or *Sema7A*^{-/-} leukocytes, indicating that vascular, most likely endothelial *Sema7A* plays a major role in monocyte adhesion in the aorta upon inflammatory stimulation.

To distinguish the role of vascular vs blood cell-derived *Sema7A* in d-flow induced atherogenesis *in vivo*, we generated chimeric mice of which blood cells of *Sema7A*^{+/+}*ApoE*^{-/-} or *Sema7A*^{-/-}*ApoE*^{-/-} mice were replaced with bone marrow-derived cells from these mice through bone marrow transplantation (BMT). A modified PCL was performed 4 w after BMT following with HFD for another 2 w. As shown in Figure 5B, atherosclerotic lesion size was significantly reduced in *Sema7A*^{-/-}*ApoE*^{-/-} mice compared with that in *Sema7A*^{+/+}*ApoE*^{-/-} mice when transplanted with either *Sema7A*^{-/-}*ApoE*^{-/-} or *Sema7A*^{+/+}*ApoE*^{-/-} blood cells. These results support the notions that *Sema7A*-deficiency reduces atherosclerotic lesion size, particularly in the aortic arch exposed to d-flow and that most likely endothelial, but not blood cell-derived, *Sema7A* plays a major role in the atherogenesis.

Discussion

In this study, we examined the role of *Sema7A*, an EC expressed semaphorin family member, in d-flow-induced vascular inflammatory changes, leukocyte adhesion, and atherosclerotic plaque formation and found that upregulation of *Sema7A* by d-flow in large arteries contributes to endothelial dysfunction via endothelial integrin $\beta 1$, whereas deficiency of *Sema7A* in *ApoE*^{-/-} mice confers significant protection against atherosclerosis.

Members of semaphorin family are known to promote atherosclerosis by enhancing monocyte-endothelial adhesion, promoting macrophage retention and neovascularization,

^{32–34} while studies indicate that Sema3A expression is reduced in response to proatherogenic conditions.³⁵ To extend our knowledge on the role of semaphorin family members in atherosclerosis, we measured their expression at distinct aortic regions exposed to d-flow and s-flow. Compared with the descending aorta (s-flow), the aortic arch (d-flow) showed significantly increased Sema7A level but reduced expression of Sema3A, Sema3C, Sema3D, Sema3F and Sema6A (Figure VA in the online-only Data Supplement). Given the fact that the causative role of d-flow in atherosclerosis may not be directly estimated due to variations of leukocyte mediated inflammation, genetic background, and diverse embryonic origin of the aorta in the conventional HFD-fed dyslipidemic mouse model^{36, 37}, we employed a PCL model that allows a more accurate delineation of d-flow mediated endothelial dysfunction during atherosclerosis.^{25, 38–40} In this modified model, increased Sema7A expression was found in mouse LCA subjected to PCL compared with the control RCA, suggesting that Sema7A expression is regulated by d-flow induced shear stress (Figure VA, B in the online-only Data Supplement). Using the atherosclerotic-onset mouse model, *LDLR*^{-/-} mice fed a Western diet for 2 weeks, the expression of more semaphorins have been shown to be affected at sites of altered hemodynamic stress.³⁵ In this analysis Sema7A mRNA expression did not show a significant difference between the lesser and greater curvature of the aortic arch, however whole aorta and not just endothelial cells were analysed. In addition, it will be interesting to examine if hyperlipidemic conditions alters Sema7A expression in endothelial cells.

D-flow antagonizes the protective effect of s-flow on blood vessel, leading to chronic inflammation, EC dysfunction, and atherosclerosis.^{41, 42} Interestingly, Sema7A is hardly detected in ECs cultured under static or s-flow conditions. In contrast, exposure to d-flow markedly increased Sema7A mRNA and protein levels in the endothelium. To determine how Sema7A transcription is elevated in response to d-flow we analyzed the Sema7A promoter sequence and identified conserved binding sites (CREs) for the CREB transcription factor. Activated by s-flow or laminar shear stress, PKA/CREB pathway reduces the expression of pro-inflammatory genes and maintains endothelial homeostasis.²⁶ These findings collectively indicate Sema7A as a potential transcriptional target to the shear responsive transcription factor CREB. This notion is supported by our observation that EC Sema7A expression was increased by H89, an inhibitor of PKA/CREB signaling. Sema7A promoter analysis further indicated the presence of consensus binding sites for other shear responsive elements, including NFκB, IRF-1 and KLF4, implying that a comprehensive network of transcription factors may be involved in hemodynamic regulation of Sema7A. The regulation of Sema7A by d-flow may also be achieved through epigenetic DNA methylation in its promoter.⁴⁰ Future studies are warranted to address the effect of these factors, and to provide more mechanistic insights on how Sema7A is regulated under proatherogenic hemodynamics.

Our findings reveal a key role of endothelial Sema7A/β1 integrin axis in d-flow induced EC dysfunction, inflammation and atherogenesis. As shown in Figure 5C, first, endothelial Sema7A was upregulated by d-flow, while Sema7A deletion attenuated leukocyte adhesion induced by d-flow and atherosclerotic plaque formation in *ApoE*^{-/-} mice. Second, integrin β1 ligation by Sema7A activates FAK/MEK1/2/NFκB signaling in ECs. Third, overexpression of Sema7A in ECs promotes adhesion molecule expression and monocyte-

endothelial interaction. Consistently, Sema7A has been shown to mediate acute inflammation in lung ECs via activating integrin $\beta 1$. The other known receptor for Sema7A is Plexin C1, which has been reported to mediate EC cytoskeleton change and swelling under seawater stimulation.²¹ Whether plexin C1 is involved in atherosclerosis remains to be elucidated.

In summary, our data showed a significant role of Sema7A in d-flow mediated EC dysfunction and atherosclerotic plaque formation. Integrin $\beta 1$ appears to play a crucial role in mediating the proatherogenic effect of Sema7A that activates a downstream FAK/MAPK/ $\text{NF}\kappa\text{B}$ pathway, thus promoting leukocyte adhesion and infiltration, and the development of atherosclerosis. These findings suggest new strategies in the prevention and treatments for atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

This work was supported in part by grants from the Natural Science Foundation of China (81620108001, 81370373, and 91439112 to L.Z. and 31300781 and 81670134 to C.T.), the Netherlands Heart Foundation (2013T127 to H.Z. and J.M.G.), the National Institutes of Health (HL119798 and HL095070 to HJ) and the Priority Academic Program Development of Jiangsu Higher Education Institutions of China.

Non-standard Abbreviations and Acronyms

PCL	Partial carotid artery ligation
HUVECs	human umbilical venous endothelial cells
ICAM-1	intercellular adhesion molecule 1
VCAM-1	vascular cell adhesion molecule 1
HFD	high-fat diet
LPS	lipopolysaccharide
AA	aortic arch
DA	descending aorta
LC	lesser curvature
GC	greater curvature
LCA	left common carotid artery
RCA	right common carotid artery
CREB	cAMP response element-binding protein

FAK	focal adhesion kinase
MAPK	mitogen-activated protein kinase

References

1. Davies PF, Zilberberg J, Helmke BP. Spatial microstimuli in endothelial mechanosignaling. *Circ Res.* 2003; 92:359–70. DOI: 10.1161/01.RES.0000060201.41923.88 [PubMed: 12623874]
2. Feaver RE, Gelfand BD, Blackman BR. Human haemodynamic frequency harmonics regulate the inflammatory phenotype of vascular endothelial cells. *Nat Commun.* 2013; 4:1525.doi: 10.1038/ncomms2530 [PubMed: 23443553]
3. Giannotta M, Trani M, Dejana E. VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *Dev Cell.* 2013; 26:441–54. DOI: 10.1016/j.devcel.2013.08.020 [PubMed: 24044891]
4. Gimbrone MA. The Gordon Wilson lecture. Understanding vascular endothelium: a pilgrim's progress. Endothelial dysfunction, biomechanical forces and the pathobiology of atherosclerosis. *Trans Am Clin Climatol Assoc.* 2010; 121:115–27. discussion 127. [PubMed: 20697555]
5. Garcia-Cardena G, Comander J, Anderson KR, Blackman BR, Gimbrone MA Jr. Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. *Proc Natl Acad Sci U S A.* 2001; 98:4478–85. DOI: 10.1073/pnas.071052598 [PubMed: 11296290]
6. Ni CW, Qiu H, Rezvan A, Kwon K, Nam D, Son DJ, Visvader JE, Jo H. Discovery of novel mechanosensitive genes in vivo using mouse carotid artery endothelium exposed to disturbed flow. *Blood.* 2010; 116:e66–73. DOI: 10.1182/blood-2010-04-278192 [PubMed: 20551377]
7. Tarbell JM, Shi ZD, Dunn J, Jo H. Fluid Mechanics, Arterial Disease, and Gene Expression. *Annu Rev Fluid Mech.* 2014; 46:591–614. DOI: 10.1146/annurev-fluid-010313-141309 [PubMed: 25360054]
8. Chatterjee S, Fisher AB. Mechanotransduction in the endothelium: role of membrane proteins and reactive oxygen species in sensing, transduction, and transmission of the signal with altered blood flow. *Antioxid Redox Signal.* 2014; 20:899–913. DOI: 10.1089/ars.2013.5624 [PubMed: 24328670]
9. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol Rev.* 2011; 91:327–87. DOI: 10.1152/physrev.00047.2009 [PubMed: 21248169]
10. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat Clin Pract Cardiovasc Med.* 2009; 6:16–26. DOI: 10.1038/ncpcardio1397 [PubMed: 19029993]
11. Simmons RD, Kumar S, Jo H. The role of endothelial mechanosensitive genes in atherosclerosis and omics approaches. *Arch Biochem Biophys.* 2016; 591:111–31. DOI: 10.1016/j.abb.2015.11.005 [PubMed: 26686737]
12. Nigro P, Abe J, Berk BC. Flow shear stress and atherosclerosis: a matter of site specificity. *Antioxid Redox Signal.* 2011; 15:1405–14. DOI: 10.1089/ars.2010.3679 [PubMed: 21050140]
13. Wannemacher KM, Wang L, Zhu L, Brass LF. The role of semaphorins and their receptors in platelets: Lessons learned from neuronal and immune synapses. *Platelets.* 2011; 22:461–5. DOI: 10.3109/09537104.2011.561891 [PubMed: 21668292]
14. Suzuki K, Kumanogoh A, Kikutani H. Semaphorins and their receptors in immune cell interactions. *Nat Immunol.* 2008; 9:17–23. DOI: 10.1038/ni1553 [PubMed: 18087252]
15. Xu X, Ng S, Wu ZL, Nguyen D, Homburger S, Seidel-Dugan C, Ebens A, Luo Y. Human semaphorin K1 is glycosylphosphatidylinositol-linked and defines a new subfamily of viral-related semaphorins. *J Biol Chem.* 1998; 273:22428–34. [PubMed: 9712866]
16. Liu H, Juo ZS, Shim AH, Focia PJ, Chen X, Garcia KC, He X. Structural basis of semaphorin-plexin recognition and viral mimicry from Sema7A and A39R complexes with PlexinC1. *Cell.* 2010; 142:749–61. DOI: 10.1016/j.cell.2010.07.040 [PubMed: 20727575]
17. Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature.* 2003; 424:398–405. DOI: 10.1038/nature01790 [PubMed: 12879062]

18. Garcia-Areas R, Libreros S, Iragavarapu-Charyulu V. Semaphorin7A: branching beyond axonal guidance and into immunity. *Immunol Res.* 2013; 57:81–5. DOI: 10.1007/s12026-013-8460-5 [PubMed: 24222277]
19. Ma B, Herzog EL, Lee CG, Peng X, Lee CM, Chen X, Rockwell S, Koo JS, Kluger H, Herbst RS, Sznol M, Elias JA. Role of chitinase 3-like-1 and semaphorin 7a in pulmonary melanoma metastasis. *Cancer Res.* 2015; 75:487–96. DOI: 10.1158/0008-5472.CAN-13-3339 [PubMed: 25511377]
20. Suzuki K, Okuno T, Yamamoto M, Pasterkamp RJ, Takegahara N, Takamatsu H, Kitao T, Takagi J, Rennert PD, Kolodkin AL, Kumanogoh A, Kikutani H. Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha beta 1 integrin. *Nature.* 2007; 446:680–4. DOI: 10.1038/nature05652 [PubMed: 17377534]
21. Zhang M, Yan X, Liu W, Sun R, Xie Y, Jin F. Endothelial semaphorin 7A promotes seawater aspiration-induced acute lung injury through plexin C1 and beta1 integrin. *Mol Med Rep.* 2017; doi: 10.3892/mmr.2017.7097
22. Zhang M, Wang L, Dong M, Li Z, Jin F. Endothelial Semaphorin 7A promotes inflammation in seawater aspiration-induced acute lung injury. *Int J Mol Sci.* 2014; 15:19650–61. DOI: 10.3390/ijms151119650 [PubMed: 25353180]
23. Morote-Garcia JC, Napiwotzky D, Kohler D, Rosenberger P. Endothelial Semaphorin 7A promotes neutrophil migration during hypoxia. *Proc Natl Acad Sci U S A.* 2012; 109:14146–51. DOI: 10.1073/pnas.1202165109 [PubMed: 22891341]
24. Zhu L, Stalker TJ, Fong KP, Jiang H, Tran A, Crichton I, Lee EK, Neeves KB, Maloney SF, Kikutani H, Kumanogoh A, Pure E, Diamond SL, Brass LF. Disruption of SEMA4D ameliorates platelet hypersensitivity in dyslipidemia and confers protection against the development of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2009; 29:1039–45. DOI: 10.1161/ATVBAHA.109.185405 [PubMed: 19390055]
25. Nam D, Ni CW, Rezvan A, Suo J, Budzyn K, Llanos A, Harrison D, Giddens D, Jo H. Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. *Am J Physiol Heart Circ Physiol.* 2009; 297:H1535–43. DOI: 10.1152/ajpheart.00510.2009 [PubMed: 19684185]
26. Csiszar A, Labinskyy N, Smith KE, Rivera A, Bakker EN, Jo H, Gardner J, Orosz Z, Ungvari Z. Downregulation of bone morphogenetic protein 4 expression in coronary arterial endothelial cells: role of shear stress and the cAMP/protein kinase A pathway. *Arterioscler Thromb Vasc Biol.* 2007; 27:776–82. DOI: 10.1161/01.ATV.0000259355.77388.13 [PubMed: 17272757]
27. Kim PG, Nakano H, Das PP, Chen MJ, Rowe RG, Chou SS, Ross SJ, Sakamoto KM, Zon LI, Schlaeger TM, Orkin SH, Nakano A, Daley GQ. Flow-induced protein kinase A-CREB pathway acts via BMP signaling to promote HSC emergence. *J Exp Med.* 2015; 212:633–48. DOI: 10.1084/jem.20141514 [PubMed: 25870201]
28. Albaugh G, Bellavance E, Strande L, Heinburger S, Hewitt CW, Alexander JB. Nicotine induces mononuclear leukocyte adhesion and expression of adhesion molecules, VCAM and ICAM, in endothelial cells in vitro. *Ann Vasc Surg.* 2004; 18:302–7. DOI: 10.1007/s10016-004-0030-9 [PubMed: 15354631]
29. Lusis AJ. Atherosclerosis. *Nature.* 2000; 407:233–41. DOI: 10.1038/35025203 [PubMed: 11001066]
30. Gavins FN, Chatterjee BE. Intravital microscopy for the study of mouse microcirculation in anti-inflammatory drug research: focus on the mesentery and cremaster preparations. *J Pharmacol Toxicol Methods.* 2004; 49:1–14. DOI: 10.1016/S1056-8719(03)00057-1 [PubMed: 14670689]
31. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med.* 1997; 336:1066–71. DOI: 10.1056/NEJM199704103361506 [PubMed: 9091804]
32. Luque MC, Gutierrez PS, Debbas V, Kalil J, Stolf BS. CD100 and plexins B2 and B1 mediate monocyte-endothelial cell adhesion and might take part in atherogenesis. *Mol Immunol.* 2015; 67:559–67. DOI: 10.1016/j.molimm.2015.07.028 [PubMed: 26275342]
33. Yukawa K, Tanaka T, Kishino M, Yoshida K, Takeuchi N, Ito T, Takamatsu H, Kikutani H, Kumanogoh A. Deletion of Sema4D gene reduces intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Int J Mol Med.* 2010; 26:39–44. [PubMed: 20514420]

34. Wanschel A, Seibert T, Hewing B, Ramkhalawon B, Ray TD, van Gils JM, Rayner KJ, Feig JE, O'Brien ER, Fisher EA, Moore KJ. Neuroimmune guidance cue Semaphorin 3E is expressed in atherosclerotic plaques and regulates macrophage retention. *Arterioscler Thromb Vasc Biol.* 2013; 33:886–93. DOI: 10.1161/ATVBAHA.112.300941 [PubMed: 23430613]
35. van Gils JM, Ramkhalawon B, Fernandes L, et al. Endothelial expression of guidance cues in vessel wall homeostasis dysregulation under proatherosclerotic conditions. *Arterioscler Thromb Vasc Biol.* 2013; 33:911–9. DOI: 10.1161/ATVBAHA.112.301155 [PubMed: 23430612]
36. Awgulewitsch A, Majesky MW. Interpreting inflammation: smooth muscle positional identity and nuclear factor-kappaB signaling. *Arterioscler Thromb Vasc Biol.* 2013; 33:1113–5. DOI: 10.1161/ATVBAHA.113.301407 [PubMed: 23677878]
37. Trigueros-Motos L, Gonzalez-Granado JM, Cheung C, Fernandez P, Sanchez-Cabo F, Dopazo A, Sinha S, Andres V. Embryological-origin-dependent differences in homeobox expression in adult aorta: role in regional phenotypic variability and regulation of NF-kappaB activity. *Arterioscler Thromb Vasc Biol.* 2013; 33:1248–56. DOI: 10.1161/ATVBAHA.112.300539 [PubMed: 23448971]
38. Heo KS, Le NT, Cushman HJ, Giancursio CJ, Chang E, Woo CH, Sullivan MA, Taunton J, Yeh ET, Fujiwara K, Abe J. Disturbed flow-activated p90RSK kinase accelerates atherosclerosis by inhibiting SENP2 function. *J Clin Invest.* 2015; 125:1299–310. DOI: 10.1172/JCI76453 [PubMed: 25689261]
39. Merino H, Parthasarathy S, Singla DK. Partial ligation-induced carotid artery occlusion induces leukocyte recruitment and lipid accumulation—a shear stress model of atherosclerosis. *Mol Cell Biochem.* 2013; 372:267–73. DOI: 10.1007/s11010-012-1468-7 [PubMed: 23054191]
40. Dunn J, Qiu H, Kim S, Jjingo D, Hoffman R, Kim CW, Jang I, Son DJ, Kim D, Pan C, Fan Y, Jordan IK, Jo H. Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis. *J Clin Invest.* 2014; 124:3187–99. DOI: 10.1172/JCI74792 [PubMed: 24865430]
41. Davies PF, Civelek M, Fang Y, Fleming I. The atherosusceptible endothelium: endothelial phenotypes in complex haemodynamic shear stress regions in vivo. *Cardiovasc Res.* 2013; 99:315–27. DOI: 10.1093/cvr/cvt101 [PubMed: 23619421]
42. Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, Pritchard WF, Powell S, Chang GY, Stoeckert CJ Jr, Davies PF. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *Proc Natl Acad Sci U S A.* 2004; 101:2482–7. [PubMed: 14983035]
43. Seltsam A, Strigens S, Levene C, Yahalom V, Moulds M, Moulds JJ, Hustinx H, Weisbach V, Figueroa D, Bade-Doeding C, DeLuca DS, Blasczyk R. The molecular diversity of Sema7A, the semaphorin that carries the JMH blood group antigens. *Transfusion.* 2007; 47:133–46. DOI: 10.1111/j.1537-2995.2007.01076.x [PubMed: 17207242]
44. Fong KP, Barry C, Tran AN, Traxler EA, Wannemacher KM, Tang HY, Speicher KD, Blair IA, Speicher DW, Grosser T, Brass LF. Deciphering the human platelet sheddome. *Blood.* 2011; 117:e15–26. DOI: 10.1182/blood-2010-05-283838 [PubMed: 20962327]

Highlights

1. Endothelial Sema7A is upregulated by disturbed blood flow potentially via PKA/CREB pathway.
2. Sema7A mediates disturbed blood flow induced vascular endothelial dysfunction and leukocyte infiltration in a β 1 integrin-dependent manner.
3. Deletion of Sema7A ameliorates atherosclerotic plaque formation in mice.

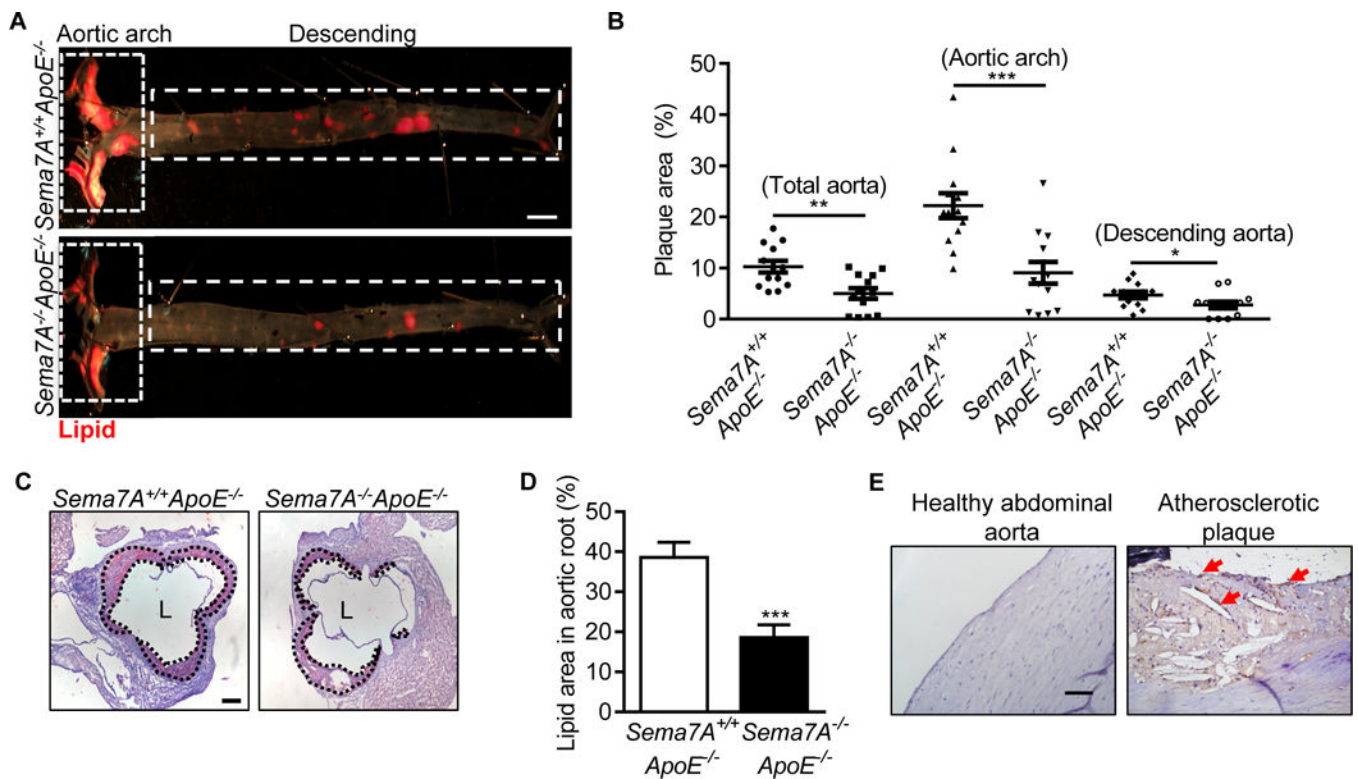


Figure 1.

Sema7A deletion reduces lipid deposition in the aorta of *ApoE*^{-/-} mice and vascular *Sema7A* is up-regulated during atherogenesis (A) Aortic plaque areas in *Sema7A*^{+/+}*ApoE*^{-/-} and *Sema7A*^{-/-}*ApoE*^{-/-} mice on HFD for 12 w were analyzed by *en face* Sudan IV-staining (Bar=2 mm). (B) Quantitative analysis for total aorta, aortic arch and descending aorta are shown. Data are mean ± SEM (n = 10 mice per group). **P*<0.05; ***P*<0.01; ****P*<0.001. (C, D) Aortic root sections from *Sema7A*^{+/+}*ApoE*^{-/-} and *Sema7A*^{-/-}*ApoE*^{-/-} mice on HFD for 12 w were stained with Oil Red O. Plaque area were demarcated by dotted lines (Bar=200 μm). (E) *Sema7A* expression in human atherosclerotic plaques was detected by immunostaining in brown (arrows). Nuclei were stained by hematoxylin (Bar=100 μm).

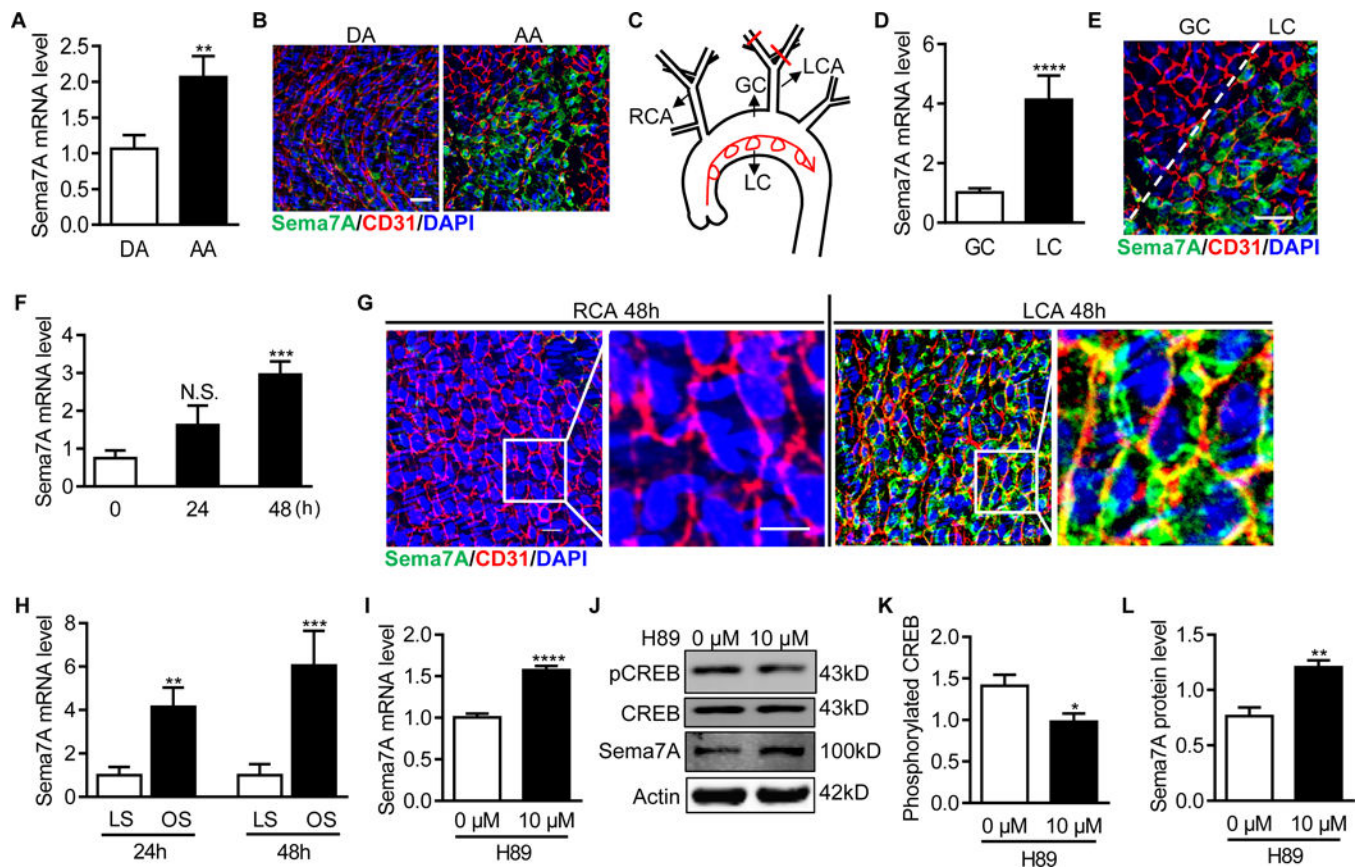


Figure 2.

Disturbed blood flow up-regulates vascular endothelial Sema7A expression. (A, D) Total RNAs in the aortic arch (AA) or the descending aorta (DA) (A), and in the lesser (LC) or greater (GC) curvature regions of the aortic arch (D) from C57BL/6J mice were isolated. Sema7A mRNA expression was analyzed by qPCR normalized to GAPDH. Fold-changes are shown. Data are mean \pm SEM. ** P <0.01; **** P <0.0001. Results are representative of 3 independent experiments. (B, E) *En face* staining for CD31 (red) and Sema7A (green) on the aortic arch (AA) or the descending aorta (DA) (B) and the lesser (LC) or greater (GC) curvature (E) was shown. Nuclei were stained with DAPI (blue). Bar = 50 μ m. The data are representative of 3 independent experiments. (C) The diagram of aortic arch and carotid arteries. (F) Total RNAs of LCA and the control RCA from C57BL/6J mice were isolated 24h and 48 h after PCL. The ratio of LCA to RCA was calculated (n = 5 mice per group). Data are mean \pm SEM. *** P <0.001. The bar graphs represent the results from 3 independent experiments (one-way ANOVA with non-parametric multiple comparison test) (G) Carotid arteries from C57BL/6J 48 h after PCL were *en face* stained for CD31 (red) and Sema7A (green). Nuclei were stained with DAPI (blue). Bar = 10 μ m. (H) Sema7A mRNA levels in HUVECs exposed to LS (15 dyn/cm²) or OS (\pm 5 dyn/cm², at 1 Hz) were analyzed by qPCR. Data are mean \pm SEM, n=3, ** P <0.01, *** P <0.001. (I-L) HUVECs treated with H89 for 24 h and Sema7A mRNA expression was analysed by qPCR (I) Sema7A protein expression (J, L) and CREB phosphorylation were analyzed by Western blotting (J, K). Data are mean \pm SEM, n=3, * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001. RCA: right common carotid

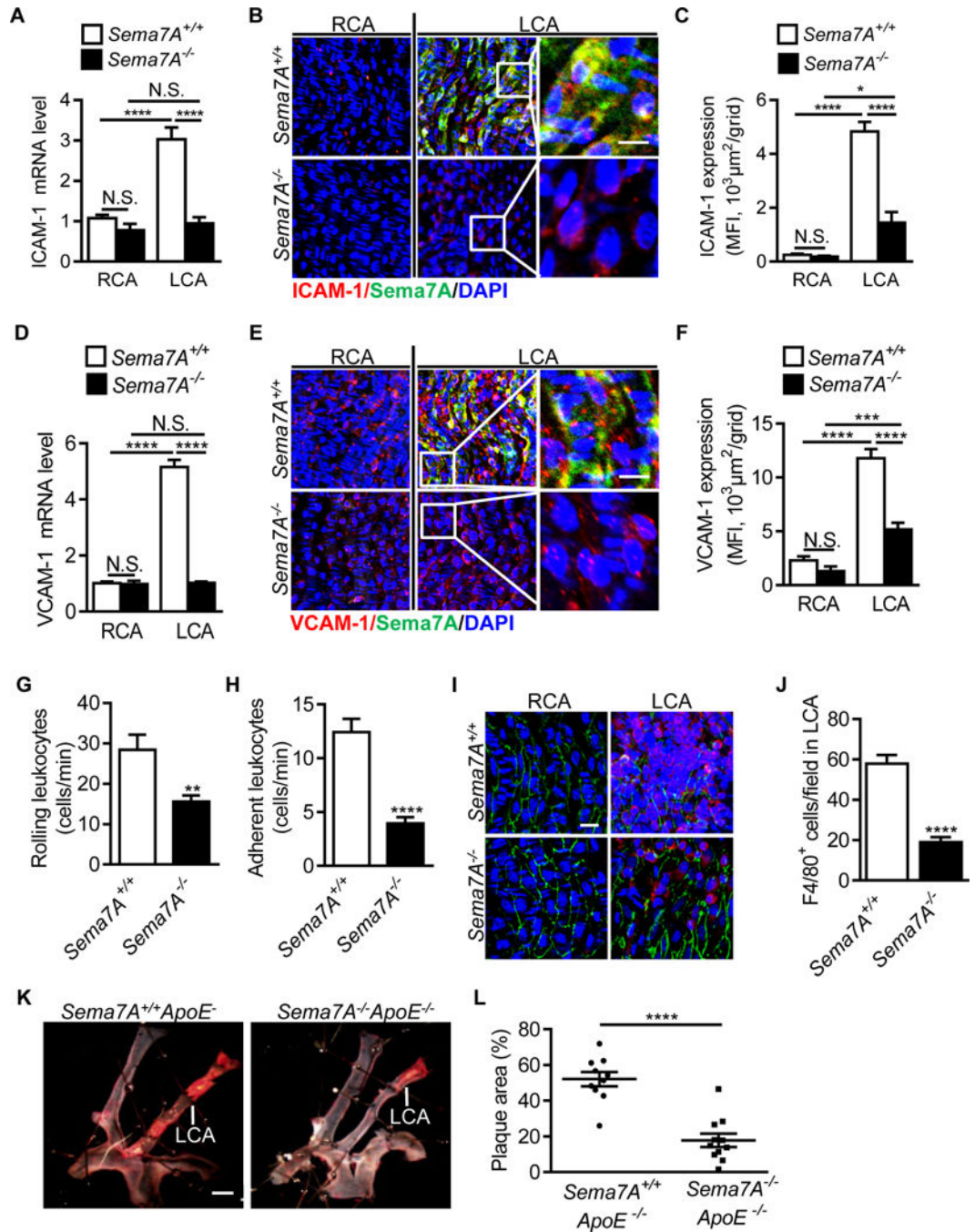
artery; LCA: left common carotid artery; DA: descending aorta; AA: aortic arch; GC: greater curvature; LC: lesser curvature; LS: laminar shear stress; OS: oscillatory shear stress.

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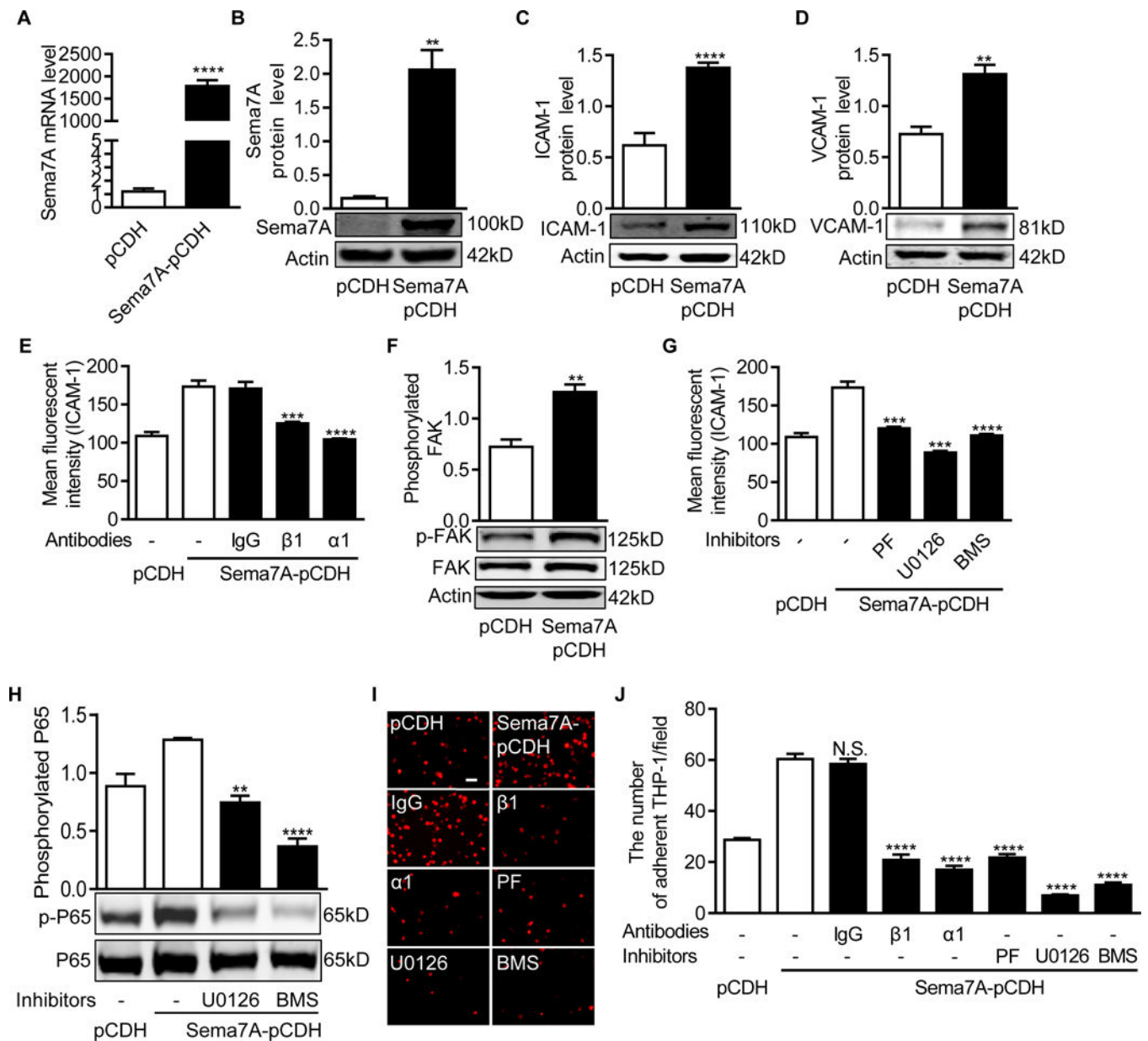
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**Figure 3.**

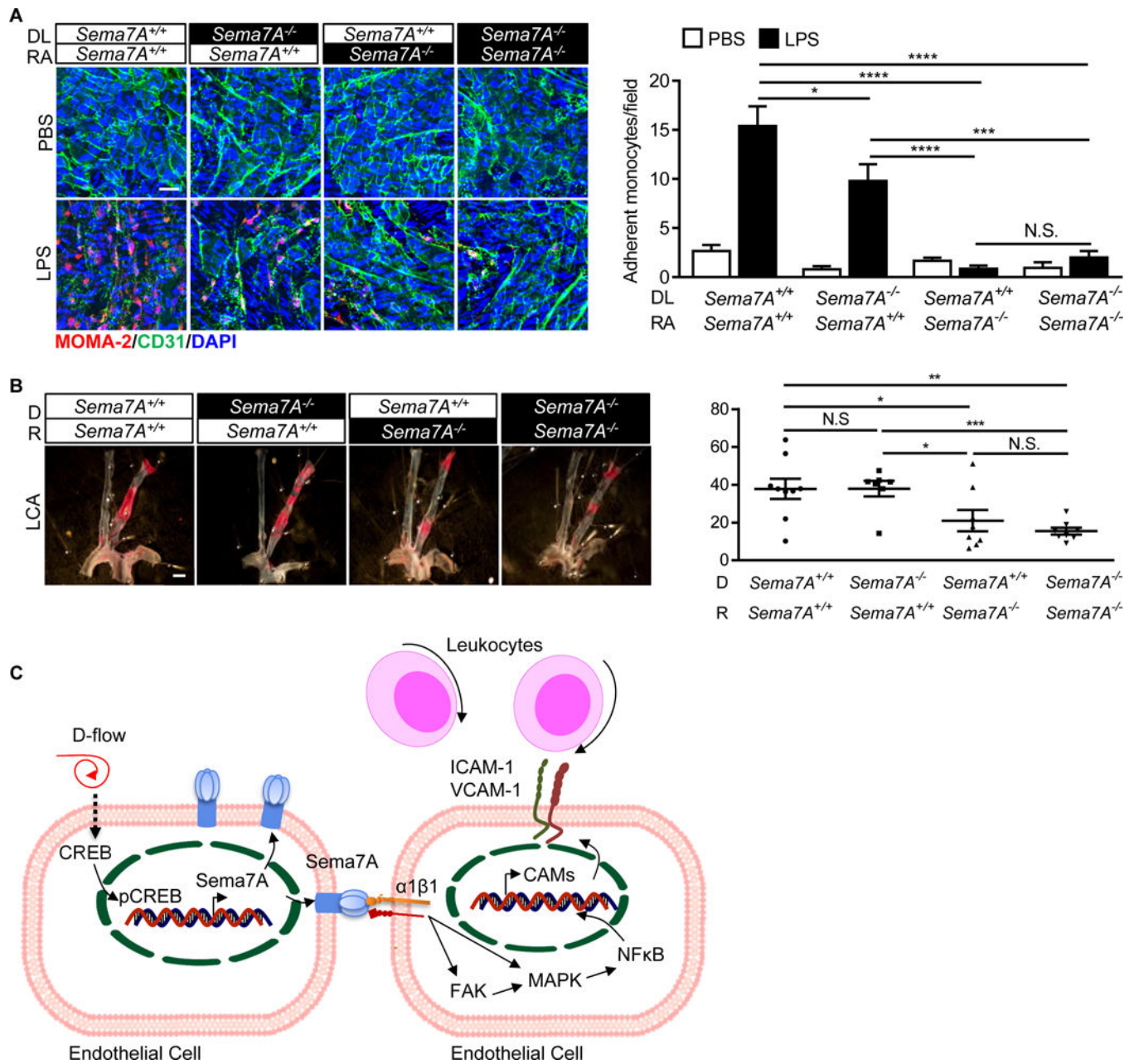
Sema7A deficiency reduces d-flow induced endothelial expression of ICAM-1 and VCAM-1, leukocyte adhesion and plaque formation. ICAM-1 and VCAM-1 mRNA (A, D) and protein (B, E) level in LCA and RCA in *Sema7A*^{+/+} and *Sema7A*^{-/-} mice after PCL were analyzed by qPCR (n=9 mice per group) and *en face* immunostaining of aortic luminal surface (red: ICAM-1 or VCAM-1; green: Sema7A; blue: nuclei. Bar = 10 μm). Data are representative of 3 independent experiments. Mean fluorescent intensity for ICAM-1 (C) and VCAM-1 (F) was used for the statistical analysis of panels B and E, respectively. Each

result includes 15-30 fields from 3 mice. Data are mean \pm SEM. * P <0.05; *** P <0.001; **** P <0.0001. (G, H) Mice were injected with *TNF- α* around the cremaster muscle. Rolling and adhesion of leukocytes on cremaster venules (4-5 vessels per mouse) were analyzed under a real-time intravital microscope and counted visually as numbers of rolling (G) or adherent cells (H) on the vascular surface per min (n=6 mice per group). Data are mean \pm SEM. ** P <0.01; **** P <0.0001. Results are representative of 3 independent experiments. (I, J) Adhesion of F4/80⁺ leukocytes in partially ligated LCA was observed under a confocal microscope and recorded as numbers of adherent cells on the vascular surface per field (Bar = 20 μ m). Data are mean \pm SEM (n = 9 per group). **** P <0.0001. Results are representative of 3 independent experiments. (K) *Sema7A*^{+/+}*ApoE*^{-/-} and *Sema7A*^{-/-}*ApoE*^{-/-} mice were subjected to PCL. After 2 w on HFD, lipid deposition (in red) was analyzed by *en face* Sudan IV-staining. Bar=1 mm. The lesion surface areas were quantified and displayed as the percentage area of the LCA (L). Data are mean \pm SEM (n = 9 mice per group). **** P <0.0001. RCA: right common carotid artery; LCA: left common carotid artery.

**Figure 4.**

Sema7A overexpression enhances ICAM-1 and VCAM-1 expression in HUVECs and monocyte-endothelial cell interaction via $\beta 1$ integrin and the downstream FAK/MEK1/2/NF κ B pathway (A) Sema7A mRNA expression in the Lenti-pCDH-hSema7A-GFP-transduced HUVECs was analyzed by qPCR (n = 5 per group). Data are mean \pm SEM. *** P <0.001. (B-D) Sema7A (B), ICAM-1 (C) and VCAM-1 (D) proteins in Lenti-pCDH-hSema7A-GFP-transduced HUVECs were analyzed by Western blotting normalized to β -actin and displayed as fold-changes relative to Lenti-pCDH-GFP-transduced control HUVECs. Data are mean \pm SEM. Results are representative of 3 independent experiments. ** P <0.01; *** P <0.001; **** P <0.0001. (E) Sema7A-overexpressing and control HUVECs were pre-treated with blocking antibodies against $\beta 1$ (P5D2) or $\alpha 1$ (5E8D9) integrin

subunits for 24 h and analyzed for ICAM-1 expression by flow cytometry. Data are mean \pm SEM. **** P <0.0001. Results are representative of 3 independent experiments. (F) FAK phosphorylation in Sema7A-overexpressing HUVECs was analyzed by Western blotting normalized to total FAK, and displayed as fold-changes relative to the control HUVECs. Data are mean \pm SEM. ** P <0.01. Results are representative of 3 independent experiments. (G) Sema7A-overexpressing and control HUVECs were pre-treated with PF573228 (10 μ M), U0126 (10 μ M) and BMS-345541 (20 μ M) for 24 h. The cells were analyzed by flow cytometry for ICAM-1 expression. (H) Cells were treated with U0126 (10 μ M) and BMS-345541 (20 μ M), and the lysates were analyzed by Western blotting for NF κ B (p65) phosphorylation. Data are mean \pm SEM. ** P <0.01; **** P <0.0001. Results are representative of 3 independent experiments. (I, J) THP-1 cells were stained by DiI and incubated with HUVECs for 30 min at 37°C. HUVECs were also pretreated with blocking antibodies against β 1 or α 1 integrin subunits or inhibitors for FAK, MAPK/MEK1/2 or NF κ B for 24 h. Adherent THP-1 cells (red) on the monolayer of Sema7A-overexpressing or control HUVECs were visualized under a microscope (I) and statistically analyzed (J). Bar=20 μ m. Data are mean \pm SEM. **** P <0.0001. Results are representative of 3 independent experiments. PF: PF573228, FAK inhibitor; U0126: MAPK/MEK1/2 inhibitor; BMS: BMS-345541, NF κ B inhibitor.

**Figure 5.**

Endothelial Sema7A plays a major role in promoting leukocyte adhesion and plaque formation in *ApoE*^{-/-} mice (A) Mouse thoracic aortas were isolated from *Sema7A*^{-/-}*ApoE*^{-/-} and *Sema7A*^{+/+}*ApoE*^{-/-} mice (8-w-old). The exposed endothelium was treated with LPS (5 μg in 2 mL medium per well) for 4 h and cultured with leukocytes from *Sema7A*^{-/-}*ApoE*^{-/-} or *Sema7A*^{+/+}*ApoE*^{-/-} mice for 30 min at 37°C. Adhered monocytes on the aortic endothelium were stained with a fluorescence-labeled anti-MOMA-2 antibody (red). Bar = 20 μm. Data are mean ± SEM. Results are representative of 3 independent experiments. **P*<0.05; ****P*<0.001; *****P*<0.0001. (B) *Sema7A*^{+/+}*ApoE*^{-/-} and *Sema7A*^{-/-}*ApoE*^{-/-} mice were transplanted with *Sema7A*^{+/+}*ApoE*^{-/-} and *Sema7A*^{-/-}*ApoE*^{-/-} bone marrow cells

and recovered for the following 4 w before subjected to PCL. After additional 2 w on HFD, atherosclerotic lipid deposition (in red) was analyzed by *en face* Sudan IV-staining. Bar=1 mm. The lesion surface areas were quantified and displayed as the percentage area of the LCA. Data are mean \pm SEM (n = 7 mice per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. DL: donor leukocyte; RA: recipient aorta; D: donor; R: recipient; LCA: left common carotid artery. (C). A proposed model for the role of Sema7A in d-flow-induced endothelial phenotypic changes and leukocyte recruitment in atherogenesis. When exposed to d-flow, endothelial Sema7A expression is upregulated, potentially by the cAMP/CREB pathway. Endothelial Sema7A activates the transcription of ICAM-1/VCAM-1 genes through binding to its receptor integrin $\alpha 1\beta 1$, resulting in FAK/MAPK/NF κ B signaling pathway activation and the recruitment of leukocytes into atherosclerotic lesions. D-flow: disturbed flow.